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# The Potential of Wheat Biofortified with Sulfur and Nitrogen

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## HIGHLIGHTS

- Interference of agronomic biofortification of wheat in the ability to form gluten.
- There is no difference in color between biofortified and non-biofortified wheat flour.
- There was no statistical differences among the values of sulfur amino acids.
- The realization of new experiments with different dosages of N and S will be necessary.

**Abstract:** The objective of this work was to verify if the nutrients sulfur and nitrogen, once applied to wheat through the process of agronomic biofortification, contribute to the formation of proteins and sulfur amino acids, increasing, consequently, the strength of semolina wheat flour and its ability to form gluten. The chemical, colorimetric, rheological, and enzymatic analyses of biofortified and non-biofortified semolina wheat flour were carried out and submitted to analysis of variance. Regarding the color, there was no difference among the samples, which present a light color. The average protein value was appropriate in both flours, with an average content of 11.32g 100g<sup>-1</sup>. The wet gluten, the dry gluten, and the content of sulfur amino acids also did not present significant differences among the samples. According to the alveography analysis, the samples presented differences in the analyzed parameters. Among the quality characteristics measured through the farinography, the falling number and the valorimeter value were lower in the biofortified sample, indicating that it weakens quicker throughout the mixture process. The enzymatic activity of the flours were different between them. The semolina wheat flours studied are classified as improver what. With the obtained results, it will be necessary the realization of new experiments with appropriate dosages in order to verify if the biofortification really alters the amount of proteins, as well as sulfur amino acids and other parameters.

**Keywords:** sulfur amino acids; agronomic biofortification; semolina wheat flour; gluten; protein.

## INTRODUCTION

Wheat is among the world's most consumed cereals, specially in the form of flours obtained through the grinding of grains. In Brazil, it is one of the feedstocks that stands out the most due to the high commercialization, consume, and application in the production of multiple bakery products as well as other segments of the food industry. Unlike other cereals, wheat has a viscoelastic characteristic. Such characteristic is desirable to bakery products since it leads to the ideal texture and volume as well as conferring other attributes relevant in the technological and sensory points of view, such as extensibility, tension resistance and mixing tolerance to the dough, providing good crumb structure in bread.

The unique characteristics that make the wheat stand out are related to gluten. Gluten is a protein compound formed by the insoluble proteins gliadin and glutenin, which grant to the dough extensibility and elasticity, attributes essential in bakery. The formation takes place when water is added to the wheat flour with proper mechanical work, allowing the development of the gluten network and resulting in a viscoelastic dough.

Weak gluten is indicated for the production of cakes and cookies. The average gluten is indicated for the production of cracker biscuits and pizzas. The flours with strong connections in the gluten network are fitted for the production of bread, and flours with very strong connections are used for the production of pasta. In Brazil, this last type of flour has a higher value since the country does not have an adequate climate for its production, hence the need to import it.

The use of elements such as nitrogen (N) and sulfur (S), applied through proper techniques during the planting, help in the obtaining of foods with higher nutrient content. Nitrogen available in the right amount to the plant is an important factor to determine the potential productivity of wheat. It occupies an important function since this nutrient is found in high concentrations in the vegetative tissues of plants as well as grains. It is required in high amounts by the wheat plant [1]. Nitrogen is very important in protein production, becoming part of them. The more N in the wheat, the higher the chances of higher protein values. A field trial examined the effects of three urea-based fertilizers and four N rates on various traits of wheat flour grown in a no-till system. The results showed no differences between the three fertilizers, but the highest doses of N produced changes, such as an increase in the protein content of the grain and the gluten content of the flour [2]

Sulphur, in ideal amounts in the soil, favors the development of the wheat agriculture since the right availability of this element increases the efficiency of the use of N. Besides, it increases the technological quality of the wheat grain, promoting higher commercial value in the resulting product [3]. This improvement in quality is related to the sulfur amino acids, methionine, cysteine, and cystine, which present disulfide bonds between the molecules. During the formation process of the gluten network, these sulfur bonds interact and strengthen themselves, leading to the formation of stronger gluten.

The biosynthesis of the gluten proteins, gliadin and glutenin, is connected to S once they are constituted by the amino acids methionine, cysteine, and cystine. These amino acids present strong intermolecular and intramolecular disulfide bonds as well as free sulfhydryl groups in the formation of gluten networks [4]. Thus, wheat plants with lower S content lead to the production of flours with less disulfide bonds, favoring the formation of weak gluten [1].

The content of minerals such as N and S can be increased in the grains of wheat and other vegetables through biofortification. According to Beasley and coauthors [5], biofortification is an intervention of micronutrients destined to increase the density and the biodisponibility of essential vitamins and minerals in basic cultures. Biofortification can be genetic or agronomic. In the first case, it happens through genetic enhancement in the vegetable [6]. In agronomic biofortification, fertilizers are applied to the plants during planting and/or development. This fertilizing is applied directly on the soil, on the leaves, or on both [7].

Taking into consideration what has been presented, this work was developed with the objective of verifying if the nutrients N and S, applied in the process of agronomic biofortification in wheat, adds to the formation of proteins and sulfur amino acids, increasing, consequently, the strength of the wheat flour and its ability to form gluten.

## MATERIAL AND METHODS

### Obtaining the wheat grains

The commercial fields of wheat, cultivated in the southern region of Brazil, in the states of Rio Grande do Sul and Santa Catarina, were selected for the conduction of the study. The handling of the crops followed the conventional recommendations for the culture.

When carrying out the study, the commercial wheat fields were either supplied or not supplied with extra N and S. The amount of nutrients applied by hectare corresponded to 2,640 kg of N in the form of the

commercial liquid fertilizer Nitamin® (8 L ha<sup>-1</sup>), formulated with a polymer of amide nitrogen and 150.3 g N + 340 g of S in the form of the commercial liquid fertilizer SupaS® (1 L ha<sup>-1</sup>), formulated with ammonium thiosulphate. The application of the nutrients took place through foliar spraying, in the phenological stage of booting.

### **Obtaining the semolina wheat flours**

In order to obtain the semolina wheat flours, the material first went through the stages of reception and storage of grains. Then, the cleaning was carried out, that is, the removal of impurities, rocks, dirt, and other foreign bodies.

The obtaining of the semolina wheat flours was carried out in an experimental mill (Labormill, 4. RB, R. Bona SRL, Monza, Italy) located in the city of Varginha, in Minas Gerais, Brazil. In this stage of the milling, there was a separation of the parts that constitute the wheat grains, with the semolina being considered the innermost part of the endosperm. After milling, two semolina wheat flours were obtained: a control sample, without the addition of any nutrient, and a sample biofortified with N and S. The samples were properly conditioned and identified.

### **Analyses performed in the semolina wheat flours**

#### **Color analysis**

The color was determined in the Colorimeter Minolta, model CR - 410, using the system CIEL\*a\*b\*, with the illuminant D<sub>65</sub> and a reading angle of 10°, taking into consideration the characters clarity (L\*), which varies between zero (black) and 100 (white), and chromaticity coordinates a\* and b\*, which varies between - 60\* (green) and + 60\* (red), and between - 60\* (blue) and + 60\* (yellow).

#### **Protein content**

The protein analysis was carried out in triplicate in the wheat flour samples through the micro-Kjeldahl method [8]. The digestion was performed in a Solab digester block, model SL 25/40. The distillation was carried out in the Tecnal nitrogen distiller model TE 0364. Finally, the titration to quantify the percentage of nitrogen in the samples was also carried out. The conversion factor used was 5.83, which is a standard for wheat.

#### **Dry and wet gluten content**

The wet and dry gluten tests were carried out in triplicate in accordance to the method described by AACC [9].

This methodology was carried out manually. At first, porcelain capsules were put in the greenhouse at 95 °C in order to remove the humidity. Then, they were put in the desiccator to cool down. Later, 25 g of wheat flour was weighed and added with approximately 15 mL of distilled water, considering the flour's ability to absorb water. Finally, the dough rounding was carried out in order to form the protein network named gluten.

After an hour, this ball was carefully washed in running water and compressed so there would be no gluten loss. When the drained liquid was completely clean, the washing was stopped, and the wet gluten was weighed. Then, this gluten was taken to the greenhouse at 105 °C until it reached constant weight. After that, it was cooled out in a desiccator. Finally, the dry gluten was weighed.

#### **Content of sulfur amino acids**

In this study, the sulfur amino acids analyses were cystine, methionine, and cysteine. In order to carry out the analysis, it was used the ion chromatography, and Biochrom and Pickering equipment, which were manufactured in the United Kingdom and the United States, respectively.

The samples were oxidized with hydrogen peroxide and formic acid at cold temperature. Then, it was performed acid hydrolysis using aqueous hydrochloric acid. The process oxidized the methionine and the cysteine, avoiding losses during hydrolysis and the break of peptide bonds in the sample. After hydrolysis, the sample was adjusted to a pH between 2 and 2.5, and filtered.

The amino acids were split in an amino acid analyzer, and the detection was performed using post-column derivatization with ninhydrin reagent and a reading in 440 nm and 570 nm. For quantification, a calibration was used. In order to guarantee the quality, an internal standard, pet food, was analyzed in each execution.

## Alveography

The procedure was performed in the Chopin water column alveograph and carried out in accordance with the methodology described by the manufacturer [10] in duplicate.

The viscoelastic characteristics of the dough were analyzed through different alveography characters such as gluten strength (W), tenacity (P), dough extensibility (L), and the relation between tenacity/extensibility (P/L) [11].

The area values (W) or gluten strength were calculated based on the multiplication of the factor 6.54 and the area under the curve in cm<sup>2</sup>.

## Farinography

The farinography was performed in duplicate in the Brabender Farinograph, with a 50 g vat, model - Mod. N<sup>o</sup> 8101045001; Typ. 820604. The wheat flour dough mixture properties were determined in accordance with AACCC's method n<sup>o</sup> 54-21.01 [12]. The farinograph characters considered were water absorption (AA), dough development time (TDM), stability (EST), and index of tolerance to the mixture (ITM).

## Falling number

This analysis was performed in the Perten Instruments Falling Number (FN) model 1700. The samples were prepared. Weighed, and put in viscometer tubes. Later, distilled water was added to the tube, and it was stirred in order to obtain an homogeneous suspension. Then, the tubes were put in hot water baths, with the measurement taking place after one minute. The total time in seconds was counted after the start of the analysis until the stirrer would fall. This time was measured and registered by the equipment [13].

## Classification of semolina wheat flours

According to the Normative Instruction n. 38, from November 30, 2010 [14], the wheat is classified as Soft Wheat, Bread, Improver, Wheat for other uses and Durum, set accordingly to the analytical determinations of alveography (W), falling number and stability (minutes). Thus, with results obtained from the analyses mentioned previously, which were performed in the control flour and in the biofortified flour, it was possible to classify these products in classes.

## Statistical analysis

The results were analyzed through analysis of variance and F-test. The software used for the study was Sisvar version 5.6 [15].

## RESULTS

In Table 1, the results obtained in the chemical, rheological, and colorimetric analysis performed in the control and biofortified wheat flours, as well as their enzymatic activity, are shown.

**Table 1.** Result of the analyses of protein, sulfur amino acids, gluten, color, alveography and falling number of the control and biofortified semolina wheat flours

Analyses	TREATMENTS							
	Control semolina wheat flour				Biofortified semolina wheat flour			
Protein (g 100g <sup>-1</sup> )	11,56				11,08			
Sulfur amino acids (mg 100g <sup>-1</sup> )	Cysteine+Cystine		Methionine		Cysteine+Cystine		Methionine	
	274		206		265		203	
Gluten (g 100g <sup>-1</sup> )	Wet gluten		Dry gluten		Wet gluten		Dry gluten	
	28,8156		12,3160		26,5861		9,0485	
Color	L*	a*	b*		L*	a*	b*	
	90,22	0,88	10,44		90,14	0,86	10,78	
Alveography	W (J)	P (m/m)	L (m/m)	P/L	W (J)	P (m/m)	L (m/m)	P/L
	315,82 <sup>a</sup>	129,36 <sup>b</sup>	52,60 <sup>a</sup>	2,46 <sup>b</sup>	365,85 <sup>b</sup>	120,78 <sup>a</sup>	66,40 <sup>b</sup>	1,82 <sup>a</sup>
Falling number (Seconds)	319 <sup>a</sup>				329 <sup>b</sup>			

Averages followed by different letters for the same characteristic should be considered different by the F-Test ( $p < 0,05$ ). With W=gluten strength, P=tenacity, L=extensibility, P/L=tenacity/extensibility.

**Table 2.** Result of the farinography analysis in the control and biofortified wheat flours

Duplicate	Control semolina wheat flour						Biofortified semolina wheat flour					
	AA	TDM	EST	ITM	TQ	VV	AA	TDM	EST	ITM	TQ	VV
First	66,0	7,0	6,4	104	8,7 <sup>b</sup>	87 <sup>b</sup>	66,1	6,0	5,9	114	7,5 <sup>a</sup>	75 <sup>a</sup>
Second	65,9	6,9	6,9	98	8,8 <sup>b</sup>	88 <sup>b</sup>	66,2	5,7	5,9	105	7,4 <sup>a</sup>	74 <sup>a</sup>

Averages followed by different letters for the same characteristic should be considered different by the F-Test ( $p \leq 0,05$ ). With AA= water absorption; TDM= dough development time; EST= stability; ITM= index of tolerance to the mixture; TQ= falling time; VV= valorimeter value.

The protein and sulfur amino acid contents in the studied samples did not present significant differences ( $p > 0,05$ ) through the F-test.

The amounts of wet gluten and dry gluten did not present significant difference through the test performed. The samples presented and average value of 27.71 g 100g<sup>-1</sup> of wet gluten and 10.65 g 100g<sup>-1</sup> of dry gluten.

None of the characters analyzed by the colorimeter, L\*, a\*, and b\*, presented statistical difference among them through the F-test, at 5% probability. In relation to the attribute L\*, both results indicate the the color of the flours tend to a lighter color, once this analysis varies between zero (black) and 100 (white). Meanwhile, the chromaticity coordinates a\* and b\* had averages of 0.86 and 10.61, respectively. They are related to the follow characteristics accordingly: from - 60\* (green) to + 60\* (red) and from -60\* (blue) to +60\* (yellow). Thus, they had values close to the “gray area” of the CieLab System color graphic, but with lighter color. These last two results indicate color normality, that being, white.

All the properties evaluated in the alveography presented significant difference at the level of 5% probability. The parameters W and L were higher for the flour coming from the biofortified wheat. Meanwhile, P and the relation P/L were higher in the non-biofortified wheat flour.

In this study, the values of the falling number were 319 seconds for the control flour and 329 seconds for the biofortified sample, presenting significant different in the F-test performed.

The characters evaluated in the farinography analysis, shown in Table 2, presented average water absorption values of 66.55%, dough development time of 6.4 minutes, stability of 6.8 minutes, and index of tolerance to the mixture of 105.25 UF, with no significant differences among them at the level of 5% probability. However, the falling time (TQ) and the valorimeter value (VV) presented significant differences among the samples: 8.8 minutes in the control flour and 7.4 minutes in the biofortified sample, and 88 for the control and 74 for the biofortified flour, respectively.

The wheat flours are classified in accordance with Annex III of the Brazilian Normative Instruction n. 38, from November 30, 2010 [14], which establishes the wheat classes in the relation to the reference values of gluten strength (W) obtained through alvenography, stability obtained through farinography, and falling number.

The flours must fit in at least two of the three characters established in the Table 3. Thus, the control and biofortified flours are classified as improver, in accordance with the results obtained, present in Table 4.

**Table 3.** Classification of wheat in Group II destined to milling and other purposes.

Classes	Gluten strength (Minimum value expressed in 10 <sup>-4</sup> J)	Stability (minutes)	Falling number (seconds)
Improver	300	14	250
Bread	220	10	220
Domestic	160	6	220
Basic	100	3	200
Other Uses	Any	Any	Any

Source: Ministério da Agricultura Pecuária e Abastecimento [14].

**Table 4.** Average values of the samples in relation to gluten strength and falling number.

Treatment	Gluten strength W (J)	Falling number
Control semolina wheat flour	315,82	319
Biofortified semolina wheat flour	365,85	329

## DISCUSSION

The protein content indicates which product in the food industry the wheat flour can be used for. The wheat flour used to make bread must contain at least 11% good quality protein. Meanwhile, for the production of cakes and cookies, the content can be lower, between 8.5% and 11% [16]. The protein content found in the work of Zhang and coauthors [17], in 12 samples of wheat flour with different properties and characteristics, coming from four pasta factories, was between 9.1 and 12.7g 100g<sup>-1</sup>. Wheat cultivars grown in three different locations in northern India were evaluated for their variability in grain and flour characteristics in the work of Siddiqi and coauthors [18]. Protein content varied significantly ( $p \leq 0.05$ ) from 9.32 to 12.60%. These values indicate that the flours analyzed in this work are in accordance with the average of 11.32 g 100g<sup>-1</sup>. The flours analyzed in this work, according with the protein content, can be used in the production of bread, cake, and cookies.

The amino acids form the basic units of proteins and polypeptides [19]. In this work, the amino acids analyzed were cystine, methionine and cysteine. They present disulfide bonds in their composition. Such characteristic may favor the formation of the gluten network. It was expected an increase in the values of cystine, methionine and cysteine due to the agronomic biofortification with N and S. It is believed that the amount of N and S applied was not enough to change the values of sulfur amino acids. New researches, with different dosages of N and S, must be carried out in order to prove the influence of biofortification in changing the amount of amino acids.

Gluten is the main storage protein of wheat grains. Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. Gluten has fundamental role in determining the rheological properties of dough [20]. During the gluten washing test, the water soluble proteins, albumin and globulin, are washed alongside the starch. The proteins glutenin and gliadin, which are insoluble and responsible for the formation of gluten, present, however, high water absorption ability (two thirds of the wet dough mass equals the absorbed water) [16]. In order for the flour to be useful for bread production, it must contain at least 26.0 g 100g<sup>-1</sup> of wet gluten and at least 8.5 g 100g<sup>-1</sup> of dry gluten [9]. The wet and dry gluten contents of wheat flours grown at three different locations in India varied significantly ( $p \leq 0.05$ ) from 23.46 to 43.04% and from 8.28 to 15.00%, respectively [18]. The samples analyzed fit the requirements for wet and dry gluten, and, therefore, are considered useful for the production of bread. The wheat biofortification did not generate differences in the gluten values, nor did it change the amount of protein and sulfur amino acids.

Differences in the color of wheat grains are caused by the presence of polyphenols, tannins, anthocyanins and carotenoids. In different tissues of the grain there are different amounts of colored substances that affect their content in bran and flour and, therefore, their content in bakery products and biscuits [21]. Color is an essential quality characteristic in flour. Although consumers prefer whiter flours, it does not indicate them to be of better quality [11]. The values of L, a\*, and b\* found in the samples studied indicate color normality, that is, white color. This means it will probably not be rejected by the consumers. The biofortification did not change the flour color. Thus, it still is a good characteristic for consumer acceptance.

The alveography is a method established for the characterization of flours, evaluating the performance of the dough during fermentation and promoting the formation of the alveoli generated by carbon dioxide. Other properties may also be examined such as gluten strength (W), which indicated how fitting for bread production the flour is; tenacity (P), higher pressure required to grow the dough; dough extensibility (L); the relation between tenacity and extensibility (P/L), which evaluates the dough stability, characters related to the rheological factors of the dough [11]. The alveography test allows, thus, the analysis of the deformation of the dough during fermentation and growth in the oven [16]. The results present in Table 2 show that the wheat biofortification cause changes in the alveographic characteristics.

The index of dough resistance, W, is an alveographic parameter intimately connected with the technological success of bakery products, and the optimal values vary accordingly with the type of product [22]. The parameter W represents the behavior of the dough throughout the fermentation process. The gluten strength (W) may present variations in the values in the range of 100 or above 300 x 10<sup>-4</sup>J. Lanzarini and coauthors [23] evaluated different wheat flours intended for baking, the W values varied between 225.66 and 384.33 x 10<sup>-4</sup> J. The values found in the present work are in the range found in the work mentioned previously, presenting high values for strength of gluten, being considered strong. The higher the gluten strength, the

higher the flour strength [22]. The gluten strength was higher in the biofortified flour. The possible change in the content of damaged starch, caused in the milling process, for example, may result in an increase in the value of  $W$  [24]. This might explain the changes of  $W$  among the samples.

The parameter  $P$  is considered an indicator of the dough resistance to deformation [25]. In this study, the flour without biofortification was considered the most resistant to deformation due to the value found. The opposite was expected since the  $N$  and  $S$  added to the plant in the biofortification process should elevate the content of sulfur amino acids.

The parameter  $L$  is called biaxial extensibility of dough or, simply, extensibility since it is a measure of how much the dough can be extended before rupture [26]. In this study, the biofortified flour has more extensibility. The wheat flour dough extensibility is, among other factors, associated to the structure of the gluten network and to the distribution of the molecular sizes of the gluten polymers. The polymer entanglement network theory was suggested to explain the extensibility at the molecular level. Biofortification did not change the protein, sulfur amino acids, and wet and dry gluten contents. According to Jødal and Larsen [26], the maximum over-pressure  $P$  and the length of the curve  $L$  are significantly negatively correlated.

The relation between tenacity and extensibility is represented by  $P/L$ . Values under 0.60 result in extensible gluten, between 0.61 and 1.20 in balanced gluten, and above 1.21 in tenacious gluten, which indicates resistance [27]. Despite different statistical values, the gluten in both flours are considered tenacious, similar to the work of Lanzarini and coauthors [23] who evaluated type 1 wheat flour, produced in different mills in the state of Paraná (Brazil).

With the results obtained for the characters  $P/L$  and  $W$ , the wheat flour analyzed are considered resistant and are indicated for pasta production.

The falling number allows the determination of the fermentation capacity of the wheat flour dough. When the value found is higher, it means less activity of the alpha-amylase enzyme. This factor impedes the industrial processing. The enzyme content in the flour interferes in quality of the bread. Amylase activity between 200 and 350 seconds results in a bread with a firm crumb, high volume, and soft texture. On the other hand, high enzymatic activity (< 200 seconds) or low (above 350 seconds) results in undesirable aspects in the product [28]. Thus, according to these authors, the flours analyzed in this research result in product of desirable quality.

The determination of rheological properties of the dough is essential for the estimation of the processing since it indicates how the dough behaves during the mixture, fermentation, and baking in order to obtain high quality products [29]. The stability is one of the most relevant characters. It is defined as the time interval given in minutes in which the apex of the curve is always above the 500 UB line, referring to points of the obtained graph. It equals the difference between departure and arrival time. Arrival time corresponds to the beginning of the formation of the gluten network. The stability time informs how long the wheat flour can be mixed without the viscoelastic dough bonds breaking. It is the maximum time value in which the dough should be mixed before the gluten network breaks. This last phenomenon is called departure time since it is the time in which the band apex leaves the 500 UF line, after the start of water addition [16]. Besides these factors, the dough stability indicated the degree of resistance to kneading and better technological quality. The values of dough stability come from, usually, the number of crossed bonds between the protein molecules in the gluten as well as the strength of these bonds [30]. According to Nitzke and Thys [31], the flours must present water absorption above 55% once a lower value does not favor the necessary conditions for the development of the dough. Also, the final product does not reach the desirable quality. Stability is an attribute related to the time of kneading. Flours with values under 12 minutes resist to lower kneading time, and this period must be respected since it can lead to the break of the gluten network. According to these same authors, the analyzed materials can classify the wheat flour quality. Both the semolina wheat flours are classified as average/weak strength, indicated for the production of fermented cookies. The water absorption in both the control flour and the biofortified flour makes evident the use of this feedstock for the production of dough in the food industry. The characters falling time and valorimeter value presented significant differences among them, indicating that the biofortified flour weakens quickly during the mixture process when compared to the control flour.

In relation to the classification of the flours studied, according to the Tables 3 and 4, they are considered improvers since they have high values of gluten strength and falling number. Therefore, regarding the flour classification, there was no influence of the agronomic biofortification at the level it was used. In the work of Cazetta and coauthors [32] nitrogen fertilization in top dressing increased the overall gluten strength ( $W$ ), as well as the flour protein content and reduced the  $P/L$  ratio, positively influencing the quality of flour for baking.

## CONCLUSION

Biofortification with N and S, at the levels used in this study, did not change characteristics such as color, wet and dry gluten content, protein content, sulfur amino acids, and classification of wheat flours, with both being classified as improver wheat. This absence in change of the characters mentioned previously might be related to the low degree of freedom used in the research, which limited the verification of the influence of biofortification. The alveographic analysis presented significant differences in the analyzed parameters. The parameters TQ and VV, from the farinographic analysis, were considered lower for the biofortified flour, showing that it weakens far quicker than the control flour.

It would be interesting, in future researches, to try agronomic biofortification with higher contents of the nutrients N and S.

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## REFERENCES

1. Bona FD. [Nutritional management of wheat crops]. International plant nutrition institute. Agronomic Information, 154, 2016.
2. Rodrighero MB, Caires EF, Lopes RB, Zielinski AA, Granato D, Demiate IM. Wheat technological quality as affected by nitrogen fertilization under a no-till system. *Acta Sci. Technol.*, 2015 Jan;37:175-181. Doi: 10.4025/actascitechnol.v37i1.21113
3. Byers M, Franklin J, Smith SJ. The nitrogen and sulphur nutrition of wheat and its effect on the composition and baking quality of the grain. *Aspects of Applied Biology, Cereal Quality*, 1987;15:337-44.
4. Wieser H. Chemistry of gluten proteins. *Food Microbiol.* 2007 Apr; 24:115-9. Doi: 10.1016/j.fm.2006.07.004
5. Beasley JT, Bonneau JP, Sánchez-Palacios JT, Moreno-Moyano LT, Callahan DL, Tako E, et al. Metabolic engineering of bread wheat improves grain iron concentration and bioavailability. *Plant Biotechnol. J.* 2019 Aug; 17:1514-26.
6. Pascoalino JAL, Reis AR, Moraes MF. [Integration of genetic and agronomic biofortification of crops with selenium]. *SBCS.* 2016 May;42:33-5.
7. Welch RM. Linkages between trace elements in food crops and human health. In: Alloway BJ (Ed.). *Micronutrient deficiencies in global crop production*. New York: Springer, 287-309, 2008.
8. Association of Official Analytical Chemists. *Official methods of analysis of AOAC International*. 17 ed. Gaithersburg, 2000.1094p.
9. American Association of Cereal Chemists. *Approved methods*. 10 ed. Saint Paul, 2000. Available in: <<http://methods.aaccnet.org/about.aspx>>.
10. Chopin SA. *Manufacturer*. France. 1993.
11. Empresa Brasileira de Pesquisa Agropecuária -Trigo. [Description of methods used to assess wheat quality]. Passo Fundo, 2009.
12. *Approved Methods of Analysis (AACC) International*, 11 Ed. Saint Paul, MN, U. S. A. Method 54-60.01. Rheological behavior of flour by farinograph: constant flour weight procedure. Approved Jan 6, 2011.
13. Perten Instruments. [The Falling Number method]. 2018. Available from: <<https://www.perten.com/Products/Falling-Number/The-Falling-Number-Method/>>.
14. Brazil. [Ministry of Agriculture, Livestock and Supply]. Normative Instruction number 38, of November 30, 2010.
15. Ferreira DF. [Statistical analysis and experiment planning program]. Version 5.6. Lavras - MG, 2006.
16. Montenegro FM, Ormenese RCSC. *Institute of Food Technology. [Assessment of the Technological Quality of Wheat Flour]*. Campinas, 2005.
17. Zhang YR, Wang YH, Shen JQ, Guo YY, Fei YX, Yu XY, et al. Effects of wheat flour with different farinograph and pasting characteristics on the surface tackiness of frozen cooked noodles. *Cereal Chem.* 2022 Jul; 99:1205-17. Doi: 10.1002/cche.10583
18. Siddiqi RA, Singh TP, Rani M, Sogi DS, Bhat MA. Diversity in grain, flour, amino acid composition, protein profiling, and proportion of total flour proteins of different wheat cultivars of North India. *Front. Nutr.* 2020 Sep; 7:1-16. Doi: 10.3389/fnut.2020.00141
19. Ustunol Z. Amino acids, peptides and proteins. *Applied food protein chemistry*. 2014 Dec; 2014 11-21. Doi: 10.1002/9781118860588.ch3
20. Biesiekierski JR. What is gluten? *J Gastroenterol Hepatol.* 2017 Mar; 32, Suppl 1, 78-81. Doi: 10.1111/jgh.13703.
21. Martinek P, Jirsa O, Vaculová K, Chrpová J, Watanabe N, Burešová V, et al. Use of wheat gene resources with different grain colour in breeding. *Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs.* 2013 Nov; 75-8.



22. Oro T. [Adaptation of methods for evaluating the technological quality of whole wheat flour]. [Doctoral thesis]. Florianópolis (SC): Universidade Federal de Santa Catarina; 2013.
23. Lanzarini DP, Martins RC, Rauhen TG, Bravo CEC, Pinto EP. [Quality control applied to breadmaking wheat flour produced in mills in the state of Paraná]. *Braz. J. Dev.* 2020; 6, 16919-29. Doi:10.34117/bjdv6n4-020
24. Preston KR, Kilborn RH, Dexter JE. Effects of starch damage and water absorption on the alveograph properties of Canadian hard red spring wheats. *Can. Inst. Food Sci. Technol. J.* 1987 Apr; 20, 75-80. Doi: 10.1016/S0315-5463(87)71093-1
25. Dubois M, Dubat A, Launay B. *The AlveoConsistograph Handbook*. (AACC Internacional, 2008).
26. Jødal ASS, Larsen KL. Investigation of the relationships between the alveograph parameters. *Sci. Rep.* 2021; 11, 5349.
27. Guarienti EM. [Wheat quality: technological and sanitary aspects]. *Agricultural Report, Belo Horizonte*, v. 34, 274, p. 72-82, 2013.
28. Gutkoski LC, Durigon A, Mazzutti S, Silva ACTD, Elias MC. [Effect of grain maturation period on physical and rheological properties of wheat]. *Food Sci. Technol.* 2008 Dec; 28,888–94. Doi: 10.1590/S0101-20612008000400019
29. Torbica A, Blažek KM, Belović M, Hajnal EJ. Quality prediction of bread made from composite flours using different parameters of empirical rheology. *J. Cereal Sci.* 2019 Sep; 89. Doi: 10.1016/j.jcs.2019.102812
30. Costa MDGD, Souza ELD, Stamford TLM, Andrade SAC. [Technological quality of national and imported wheat grains and flour]. *Ciencia Tecnol Alime.* 2008 Mar; 28, 220–5. Doi: 10.1590/S0101-20612008000100031
31. Nitzke JA, Thys RCS. [Assessment of the technological/industrial quality of wheat flour]. Instituto de Ciência e Tecnologia de Alimentos – ICTA. Rio Grande do Sul, 2011. Available in: <<https://lume-re-demonstracao.ufrgs.br/avaliacao-qualidade/2a.php>>. Accessed on: May 8, 2024.
32. Cazetta DA, Fornasieri Filho D, Arf O, Germani R. Industrial quality in cultivars of wheat and triticale submitted to the nitrogen fertilization in no-till system. *Bragantia.* 2008; 67, 741-50. Doi: 10.1590/S0006-87052008000300024



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