

INFLUENCE OF THE ADDITION OF DIFFERENT ADDITIVES ON PHYSICO-CHEMICAL PROPERTIES OF WHEAT FLOUR AND RHEOLOGICAL PROPERTIES OF DOUGH WITH FARINOGRAPH

Durim ALIJA^{1*}, Daniela NIKOLOVKSA NEDELKOSKA², Tatjana KALEVSKA², Gafur XHABIRI¹, Viktorija STAMATOVSKA², Eljesa ZIBERI¹

^{1*} Faculty of Food Technology and Nutrition, University of Tetova, North Macedonia

² Faculty of Technology and Technical Science – Veles, University St. Clement of Ohrid - Bitola, North Macedonia

*Corresponding author e-mail: durim.alija@unite.edu.mk

Abstract

This paper aims to evaluate the physicochemical properties and investigate rheological analyzes with the Farinograph of wheat flours without additives and with several different additives. The rheological aspects of dough samples made from flour of three varieties of wheat and their combination with Fungal α -Amylase and Glycolipase-Xylanase were investigated by rheological methods using the Farinograph and Dynamic Rheology device. The results from the Farinograph showed that the best rheological qualities of the dough produced from different types of flour with additive mixture have the test from the wheat variety Rakon (T 400S), while the test from the wheat variety Novosadska Rana (T 400) and Zvezdana (T500) have lower rheological quality. Comparing the results obtained from Physico-chemical analyzes, we notice that the protein and gluten content has the highest values in the Hungarian variety Rakon. It is worth noting that the capacity for water absorption is increased in all types of mixtures, while the stability of the dough, as one of the important indicators of the Farinograph, has different values after mixing different types of flour with additives. These results are useful for bakery manufacturers to develop new products by mixing flour with additives to produce bread with high gluten content. The addition of Fungal α -Amylase and Glycolipase-Xylanase additives can have a strong effect on dough rheology, by increasing the nutritional value of bakery products.

Keywords: rheological properties, Fungal α -Amylase, Glycolipase-Xylanase, Physico-chemical analysis, kneading dough

1. Introduction

Wheat is an important crop in bread production. According to the botanical classification, it belongs to the class of monocotyledons, of the family Gramineae and the genus *Triticum*; and starch is the main ingredient. The spread of new wheat varieties with high productivity, their scientific treatment, and other agro-technical measures are important factors that in recent years have significantly influenced the achievement of high stabilized yields (Bach, et al., 2017).

Cereals today play an essential role in increasing the demand for food associated with the food chain. More than 50% of energy from food comes from cereals. For this reason, the productive typology, form, and consumption of cereal-based foods are committed to important innovations in cereal-based food technology. Wheat is the most widespread crop in the world. Bread made from its flour feeds about 70% of the country's population because in terms of caloric content it is higher than bread prepared from other cereals. So one kilogram of wheat bread contains 2000-2500 calories. (Sinani, et al., 2008).

In terms of the conditions that our country offers, with climatic variability and diversity of soils, the spread of the most suitable types of wheat is one of the most important tasks of agriculture. The research problem is based on the study and verification of the impact of food additives on flour and its products, as well as raising public awareness on this issue.

An evaluation of the impact of the improvers (food additives) on dough production, the impact on their

rheology and bakery products, as well as the study of different wheat varieties to determine the quality of the flour was also done. The main factors related to changes in the quality of bakery products, especially in the volume of bread and the structure of the dough, such as the protein content of flour and the impact of food additives have also been studied. Quantitative structures and formations of gluten proteins determine the qualities of the dough and the quality of the product by evaluating different types of wheat (Sinani, et al., 2008).

The processing of wheat flour into the dough is an important step in the bakery industry. Wheat flour dough is a complex multi-phase system in which continuous physicals, chemicals, and biochemicals modifications occur at the molecular and microstructural levels (Esselink, et al., 2003). It is very important to investigate the effect of the added ingredients (additives), in this case, Fungal α -Amylase and Glycolipase-Xylanase, on the rheological behavior of wheat flour. Various methods have been developed to evaluate the rheological properties of wheat flour dough (Rao & Rao, 1993; Peressini, 2009).

This paper aims to evaluate the physicochemical and technological properties and sensory characteristics of flour and its products as well as the comparison between flour products with additives and without them. This study also aims to inform consumers about the impact of additives on flour and its products and to add the right amount of additives to the kneading process.

The hypothesis that the addition of additives in appropriate doses has a positive effect on flour and its products is elaborated, while there is no negative impact on the health of the consumers if used by the standard and regulations for the use of food additives (Cox, et al., 2021).

2. Material and methods

2.1. Materials: For the performing of the physicochemical, sensory, rheological, and kneading properties of the dough, different varieties of wheat were used, respectively: Novisadska Rana (variety from Serbia, T400, also the reference flour), Zvezdana (variety from Serbia, T500) and Rakon (variety from Hungary, T400S).

To determine the most suitable flour for bread production, two types of additives (enzymes) from two different manufacturers were used, ie four types of additives, listed below:

- ✓ **Fungal α -Amylase (Muhlenchemia) – M.H**
- ✓ **Fungal α -Amylase (Danisko) – D**
- ✓ **Glycolipase- Xylanase- (GLU-XY) – (Muhlenchemia) – M.H**
- ✓ **Glycolipase- Xylanase - (GLU-XY) (Danisko) – D**

By adding additives to the above types of flour, a total of 15 different mixtures were obtained. To perform the analysis, the mass of 100 kg was divided into 1 kg, from which it follows that each kg of flour must be added:

Fungal α -Amylase additives - 0.15 g / kg flour; 150 ppm

Glycolipase-Xylanase Additives (GLU-XY) - 0.10 g / kg flour; 100 ppm

2.2. Methods: After performing the mixtures (flour with additives, where 15 combinations are obtained) the analyzes are determined according to the official methods of analysis by the Association of Official Analytical Chemists, ISO standard methods, and valid standards, as follows:

1. Moisture - standard drying method ISO 712: 2009 (ISO-1415-1, 2006)
2. Proteins - Method KJELDAH ISO 20483: 2013, (ISO-20483, 2013)
3. Starch - Everest method POLARIMETRIC
4. GLUTEN - Manual method ISO 21415-1: 2006 (ISO-1415-1, 2006)
5. Sediment - Standard method according to ZELANY, ISO 5529: 2007 (ISO-5529, 2007)
6. HECTOLITER - Standard method
7. FOSS Infratec™ 1241 Grain Analyzer - water and ash absorption
8. Rheological analyzes of flour and flour-additive mixtures - Farinograph apparatus (Brabender

2.2.1. Production of bread: The production of bread was performed in the laboratories of the Faculty of Food Technology and Nutrition, at the University of Tetovo. We took 1 kg of flour from each variety, where then the same amount of all additives was added to the recipe. After mixing the reference flours with the additive, we added: 1.7-1.8% table salt (Tuzla Salt - Bosnia and Herzegovina), 2.1% Bread Yeast - Digo (Damex), and 52% water from the total mass of flour, approximately 520 ml of water was used. The temperature of the water used for kneading the dough was in the range of 25-30 ° C, and it was performed with the help of the mixer - SILVER CREST. The dough was then divided into 570 g sizes and shaped, using two baking parallels. Fermentation is done for 60 min at 30 ° C (BINDER incubator) and finally baked at 225-230 ° C for 25-30 min (baking oven - Memert Sterilizer).

2.2.2. Sensory analysis: Various sensory parameters were evaluated, such as shape, the color of the bark, cracking of the bark, porosity, characteristic taste, consistency, smell, and texture.

2.2.3. Statistical analysis: For the analysis of certain groups of results, statistical methods are used according to Duncan and student (t) test at $p < 0.05$ through the SPSS software program.

3. Results and discussion

For the process of making bread, Physico-chemical analyzes have a wide range of importance. For that purpose, a comparison of Physico-chemical analysis was made between the above varieties of wheat. The Physico-chemical characteristics of wheat varieties are given in the following Table 1.

Table 1. Physico-chemical characteristics of different varieties of wheat

Physico-chemical characteristics of different varieties of wheat			
Parameters	Novisadska Rana	Rakon	Zvezdana
Proteins	12.70% a	15.70 \pm 2.12% b	12.2 \pm 0.35% a
Moisture	14.40% a	13.70 \pm 0.49% a	13.90 \pm 0.35% ab
Starch	69.80% ab	67.10 \pm 1.91% a	69.50 \pm 0.21% b
Bran	3.10% a	3.50 \pm 0.28 a	4.40 \pm 0.92 b
Gluten	29.30% a	35.50 \pm 4.38% b	28 \pm 0.92% a
Sediment	34.70% a	64.50 \pm 21.07% b	37 \pm 1.63% a
Hectoliter	85.20% ab	77 \pm 5.8% b	69.80 \pm 10.89% a

*Different letters in the same rows show significant differences ($p < 0.05$)
 \pm SD value*

Compared to the reference variety (Novisadska Rana), Rakon and Zvezdana varieties have significant differences ($p < 0.05$), in all parameters except moisture content, starch, and hectoliter weight. Table (1) shows that we have the lowest percentage of proteins in the Zvezdana variety with a value of 12.2% \pm 0.35%. Approximately the same value of proteins was conducted in the study (Shekularac, 2018) with a value of 12.88%. While the highest percentage of protein is in the variety Rakon with 15.7 \pm 2.12%. The highest amount of gluten has the variety Rakon 35.5 \pm 4.38%, while the lowest is in the variety Zvezdana with 28 \pm 0.92%, which is similar to the value of (Shekularac, 2018) with 28.2%. The sediment value determines the quality class of wheat, where the first class is determined by the sediment value of at least 38%, wherein in our case we have the variety Rakon with 64.5 \pm 21.07% (first class). Less than 30% is characterized by the second class,

which includes the other two varieties of wheat Zvezdana $37 \pm 1.63\%$ and Novisadska Rana 34.7% . The highest hectoliter weight has the wheat variety Novisadska Rana 85.2% , and the lowest Zvezdana $69.8 \pm 10.89\%$.

Table 2 Presented Physico-chemical characteristics of flour from the same selected wheat varieties.

Table 2. Physico-chemical characteristics of flour

Physico-chemical characteristics of flour from different varieties of wheat			
Parameters	T-400 Novisadska Rana	T-400 S Rakon	T-500 Zvezdana
Proteins	10.80 a	14.20 ± 2.4 b	11.80 ± 0.71 a
Moisture	14.70 b	14.40 ± 0.21 a	14.40 ± 0.21 ab
Ash	0.4 a	0.5 ± 0.07 a	0.5 ± 0.07 a
Water absorption	51.90 a	55.70 ± 2.69 b	53.70 ± 1.27 a
Gluten	26.70 a	34.20 ± 5.3 b	26.30 ± 0.28 a

*Different letters in the same rows show significant differences ($p < 0.05$)
 \pm SD value*

From table (2) it can be noticed that in all types of flour the percentage of moisture is within the limits of the standard (max 14%). All three types of flour of different varieties have significant differences ($p < 0.05$), except in the ash content (because of the functional performance). The average content of proteins in the flour is 13%, wherein in our case we find TIP 400S- Rakon with a higher percentage of protein 14.2 ± 2.4 , while the lowest value has the flour TIP 400 Novisadska Rana with 10.8%. The highest amount of gluten has flour T-400S with 34.2 ± 5.3 , then the flour T-400 with 26.7%, and with the lowest amount of gluten is the flour T-500 with 26.3 ± 0.28 .

After performing the mixtures (flour with additives) we continued with the Physico-chemical analysis, to monitor the differences between the appropriate type of flour (reference) and mixtures with additives. Also, we monitored the differences between the same additives produced by different manufacturers (Tables 3,4, and 5).

Table 3. Physico-chemical analysis in flour T-400

Physico-chemical analysis in flour T=400					
INDICATORS	Reference	Reference + Fungal α -Amylase MH	Reference + Fungal α -Amylase - D	Reference+ GLU-XY MH	Reference + GLU-XY Danisko
Proteins	10.80 a	11 ± 0.14 b	10.90 ± 0.07 ab	11 ± 0.14 b	10.9 ± 0.07 ab
Moisture	14.70 a	14.90 ± 0.14 ab	15 ± 0.21 b	14.90 ± 0.14 ab	15.10 ± 0.28 b
Ash	0.4 a	0.4 ± 0 a	0.4 ± 0 a	0.4 ± 0 a	0.4 ± 0 a
Water absorption	51.90 a	52.80 ± 0.64 b	52.20 ± 0.21 ab	52.60 ± 0.49 b	52.7 ± 0.57 b
Gluten	26.70 b	26.60 ± 0.07 a	26.80 ± 0.07 c	26.80 ± 0.07 c	26.70 ± 0 b

*Different letters in the same rows show significant differences ($p < 0.05$)
 \pm SD value*

The protein content has a slight change in the ratio of reference (T400 Novisadska Rana) and mixtures with the additive (0.07 ± 0.14), where the highest and equal protein content has the additives of the manufacturer MH (Fungal α -Amylase and GLU-XY). It is worth noting that there are significant differences in all parameters

($p < 0.05$), except the ash parameter. It's important to mention that the percentage of ash is not affected regardless of the additives used. The moisture and water absorption from the reference with increasing values, where the maximum humidity reaches the mixture Reference + GLU-XY Danisko (15.1 ± 0.28) while the water absorption in the mixture Reference + Fungal α -Amylase MH (52.8 ± 0.64). In the amount of gluten, the percentages are closely correlated from 26.6% to 26.8%.

Table 4 shows the results of the Physico-chemical analysis of the T-400S flour enriched with an additive.

Table 4. Physico-chemical analysis in flour T-400 S

Physico-chemical analysis in flour T-400 S					
Parameters	Reference	Reference + Fungal α -Amylase MH	Reference + Fungal α -Amylase - D	Reference+ GLU-XY MH	Reference + GLU-XY Danisko
Proteins	14.20 b	14.10 ± 0.07 a	14.20 ± 0 b	14.20 ± 0 b	14.20 ± 0 b
Humidity	14.40 a	14.50 ± 0.07 b	14.50 ± 0.07 b	14.50 ± 0.07 b	14.50 ± 0.07 b
Ash	0.5 a	0.5 ± 0 a	0.5 ± 0 a	0.50 ± 0 a	0.5 ± 0 a
Water absorption	55.70 b	55.80 ± 0.07 c	55.60 ± 0.07 a	55.70 ± 0 b	55.60 ± 0.07 a
Gluten	34.20 b	34.10 ± 0.07 b	34.10 ± 0.07 b	33.90 ± 0.21 b	33.20 ± 0.71 a

*Different letters in the same rows show significant differences ($p < 0.05$)
 \pm SD value*

In the T-400 S flour, the amount of protein does not change at all except in the case of the Reference + Fungal α -Amylase MH mixture where the amount of protein is reduced from 14.2 to 14.1 ± 0.07 . The moisture is increased by 0.1 compared to the reference sample. The ash content is not affected by the added additives. The water absorption parameter is increased when additives from the manufacturer MH are used (Reference + Fungal α -Amylase MH with 55.8 ± 0.07 and Reference + GLU-XY MH with 55.7 ± 0) while when additives from manufacturer Danisko we have a reduction (Reference + Fungal α -Amylase - D with 55.6 ± 0.07 and Reference + GLU-XY D with 55.6 ± 0.07). It is worth noting that there are significant differences in all parameters ($p < 0.05$), except the ash parameter. The amount of gluten is reduced when additives are used, whereas during the use of Fungal α Amylase the amount of gluten is reduced by 0.1 compared to the reference sample (34.2). But the reduction of gluten continued, even more, when we used the GLU-XY, where for Reference + GLU-XY MH the result is 33.9 ± 0.21 while for Reference + GLU-XY Danisko is 33.2 ± 0.71 . This phenomenon is directly related to the quality of the flour which varies from year to year because the activity of enzymes is significantly influenced by climatic conditions both during the biological maturation of wheat grains and during harvest. Thus, wheat grain flour harvested at high temperatures (dry grains) is poor in enzyme content, and those harvested in humid climates (germinated grain) are richer in enzymes (alpha-amylase activity is 10-200 times higher than normal flour) (Banu, 2009).

Table 5 shows the results of the Physico-chemical analysis of the T-500 flour enriched with an additive.

Table 5. Physico-chemical analysis in flour T-500

Physico-chemical analysis in flour T-500					
Parameters	Reference	Reference + Fungal α -Amylase MH	Reference + Fungal α -Amylase - D	Reference+ GLU-XY MH	Reference + GLU-XY Danisko
Proteins	11.80 b	11.70 \pm 0.07 a	11.80 \pm 0 b	11.70 \pm 0.07 a	11.70 \pm 0.07 a
Moisture	14.40 a	14.50 \pm 0.07 b	14.50 \pm 0.07 b	14.50 \pm 0.07 b	14.50 \pm 0.07 b
Ash	0.5 a	0.5 \pm 0 a	0.5 \pm 0 a	0.5 \pm 0 a	0.5 \pm 0 a
Water absorption	53.70 ab	53.90 \pm 0.14 bc	54.10 \pm 0.28 c	53.60 \pm 0.07 a	53.90 \pm 0.14 bc
Gluten	26.30 a	27.40 \pm 0.78 b	26.90 \pm 0.42 ab	27.10 \pm 0.57 ab	27.20 \pm 0.64 b

*Different letters in the same rows show significant differences ($p < 0.05$)
 \pm SD value*

In T-500 we do not notice a change in the values of the proteins from the reference value (11.8) only when the mixture Reference + Fungal α Amylase D is used, while in the other three cases there is a decrease in this value by 0.1. The moisture parameter indicates an increase from the reference value (14.4) of four identical mixtures, with a value of 14.5. 0.07. The ash content even in this case remains constant. Water absorption decreases from the reference value (53.7) when using the additive GLU-XY MH with a value of 53.6. 0.07. While in other cases there is an increase where it reaches the maximum in the use of the additive Fungal α Amylase - D with a value of 54.1 \pm 0.28. Gluten in this case increases in each mixture where it reaches its maximum when using the additive Fungal α -Amylase MH with a value of 27.4 \pm 0.78. In Table 5, significant differences ($p < 0.05$) were observed in all parameters except ash content.

Indicators given by farinography are water absorption (%); dough development (min); dough stability (min.); the degree of softening of the dough and quality number/quality class.

Water absorption (%)

The highest value of water absorption was found in flour T-400S with 54.1%, followed by T-500 at 52.6%, and T-400 at 51.1%. With the addition of additives in all types of flour, the water absorption gradually increases, except for the flour T-400. The highest absorption in percent shows the mixture T-400S + Fungal α -Amylase MH with a value of 54.3% where from this correlation we notice that there is an increase up to 0.2%.

In the flour mixtures, T-500 with additive is detected with the highest increase in water absorption, where the value of the percentage of water absorption without additive is 52.6%, while that of the mixture with Fungal α -Amylase MH reaches 54.3% which means we have an increase of up to 1.5%. These results correlate with the results obtained by (Hopek, et al., 2006), who use different types of flour treated with Fungal α Amylase, wherein in certain quantities there is an increase in water absorption capacity from 59.2% to 60.8%.

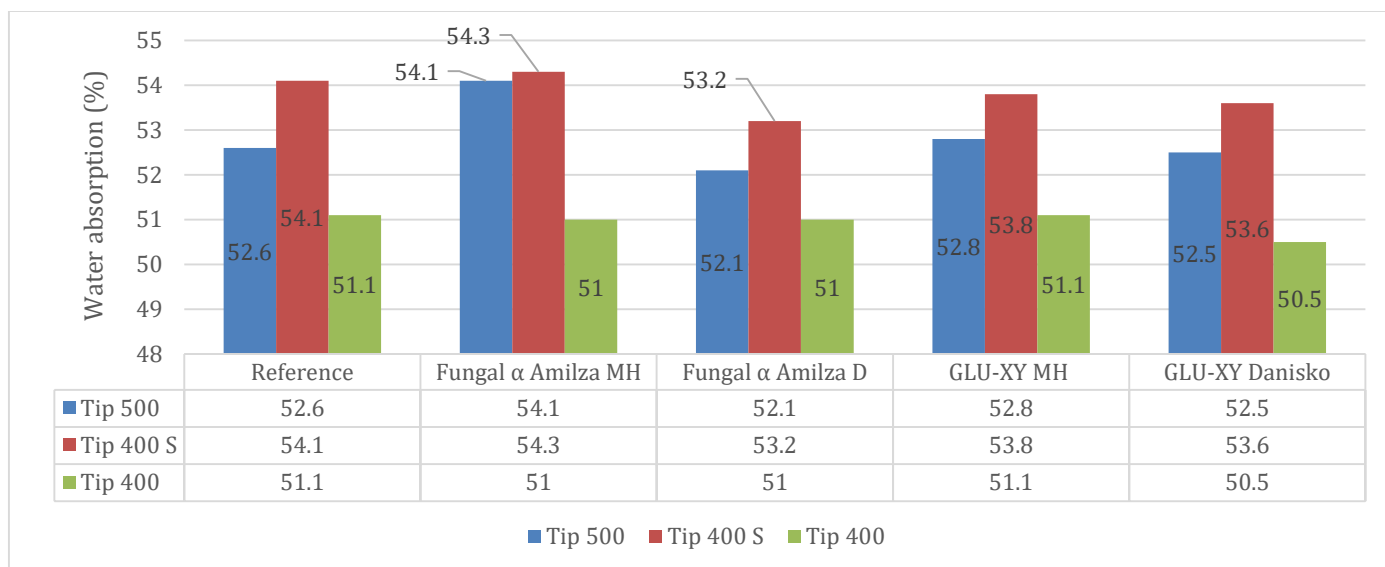


Fig1. Water absorption (%), flour-additive mixtures

Dough development

Dough development - all flours, after treatment with additives have reduced dough development. It should be noted that only in the case of the mixture Reference + GLU-XY MH there is an increase in the dough development time from 2:26 min to 2:34 min, the same represents the maximum. An increase in dough development time is also presented in the T-400 flour where from 0.5 min (Reference) it reached 2.11 min (GLU-XY MH). These results correlate with the results obtained by Antonietta & Carmela (2011), who use different types of flour treated with α Amylase, where in certain quantities there is a reduction in dough development (as in our mixture T-400 Reference + Fungal α -Amylase MH) from 1.6 min to 1.3 min. Taking the reference flour, the highest development of the dough is at a flour TIP 400S with 2.26 min, followed by T-500 with 1.38 min and T-400 with 0.5 min, while mixtures with additives caused a decrease in values, similarly to in the study (Ananingsih, et al., 2013).

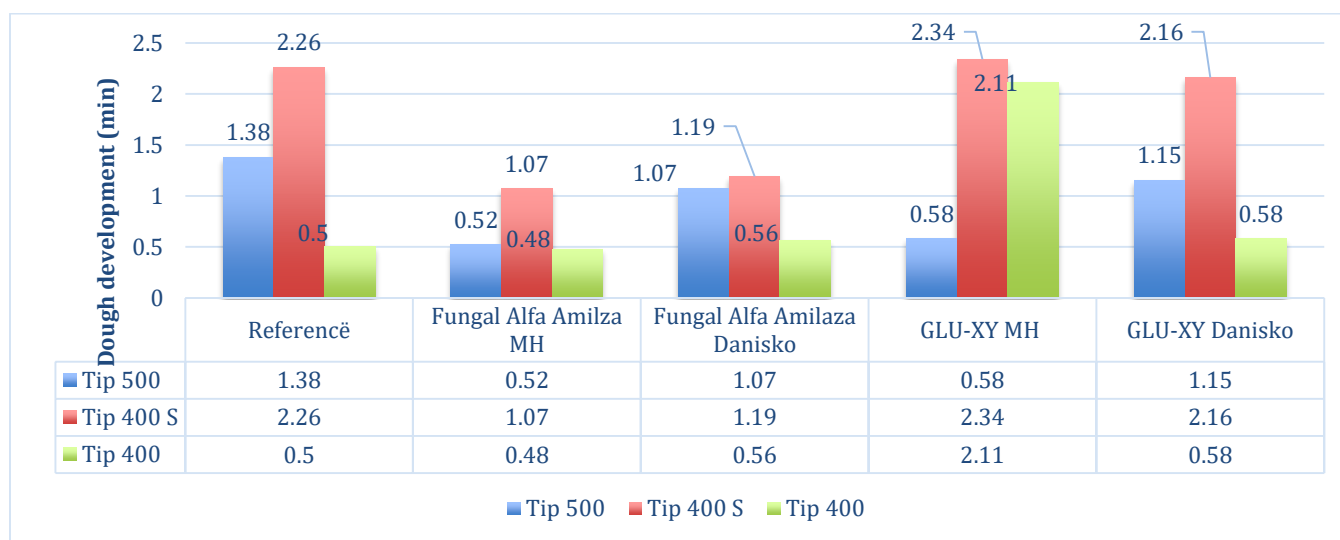


Fig2. Development of dough (min), mixtures of flour-additives

Dough stability (min)

Dough stability - the best dough stability is founded in T-400S flour with 1.44 min, while the lowest on T-400 flour with only 0.5 min. With the addition of additives, the stability of the dough is changed, thus the maximum reaches the mixture Reference (T-400S) + GLU-XY MH with 5.46 min. and the mixture Reference (T 500) + GLU-XY MH with 2.07 min. We have lower stability in flour mixtures T-400 + Fungal α -Amylase MH with only 0.06 min and T-500 + Fungal α -Amylase MH with 0.05 min, similar to the study (Ananingsih, et al., 2013).

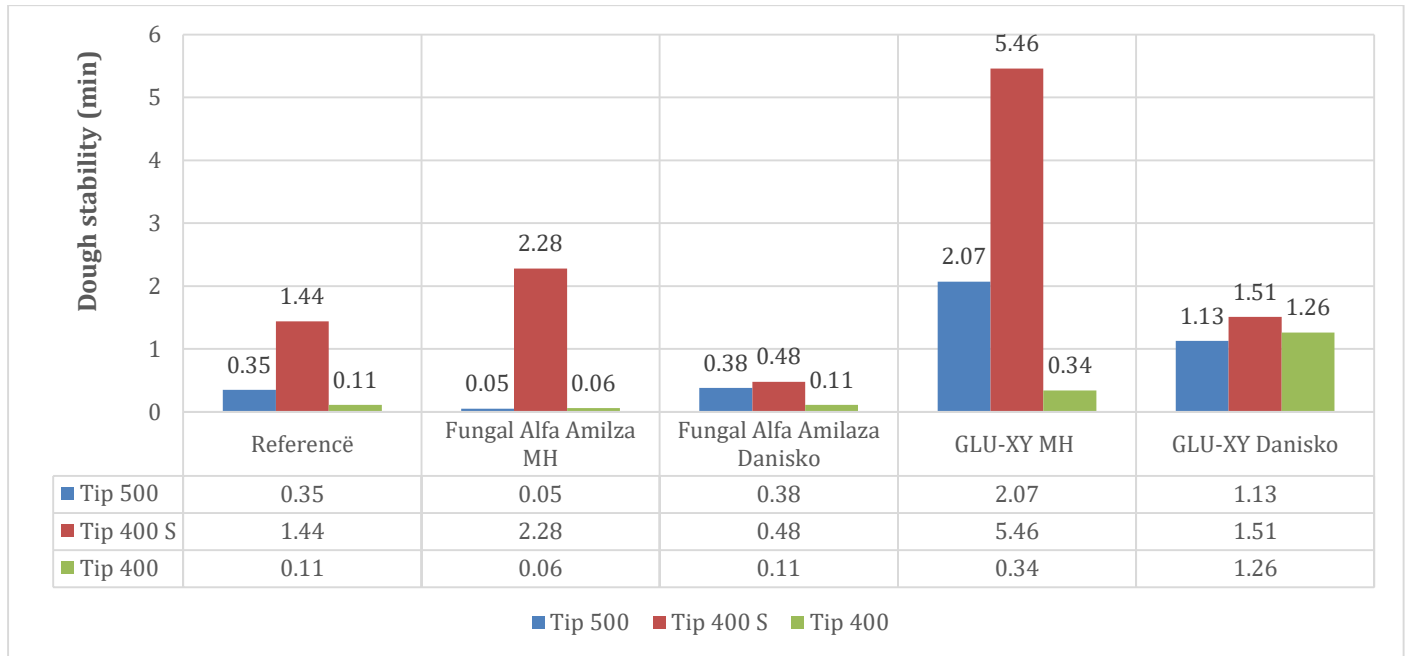


Fig3. Stability of the dough (min), mixtures of flour-additives

Antonietta & Carmela, (2011) have obtained similar results in terms of reducing the value of stability, using different types of flour treated with α -Amylase, where in certain quantities there is a decrease in stability (as in our case T-400S Reference + Fungal α -Amylase D) from 5.8 min to 1.9 min.

The degree of softening of the dough

The flour of T-400 with a value of 79 UF has a better degree of softening dough (UF), while the lowest flour T-400S with only 13 UF. By adding additives, the softening speed of the dough is improved due to the slower ability of water absorption from additives, reaching the maximum in the mixture Reference (T 500) + Fungal α -Amylase MH with a value of 225 UF, followed by Reference (T 400S) + Fungal α -Amylase D with a value of 117 UF and Reference (T 400) + Fungal α -Amylase MH with a value of 105 UF. In their study, Antonietta & Carmela (2011) also obtained an increase in the degree of softening dough (UF). Their study used different types of flour treated with α Amylase, where in certain quantities there is an increase in the degree of softening dough. All of this affects the development time of the dough, the stability of the dough, the speed of softening of the dough as well as the determination of the quality class of the flour. It should be noted that the mixture with GLU-XY Dansiko with different types of flour results in a significant reduction in the softening degree of the dough.

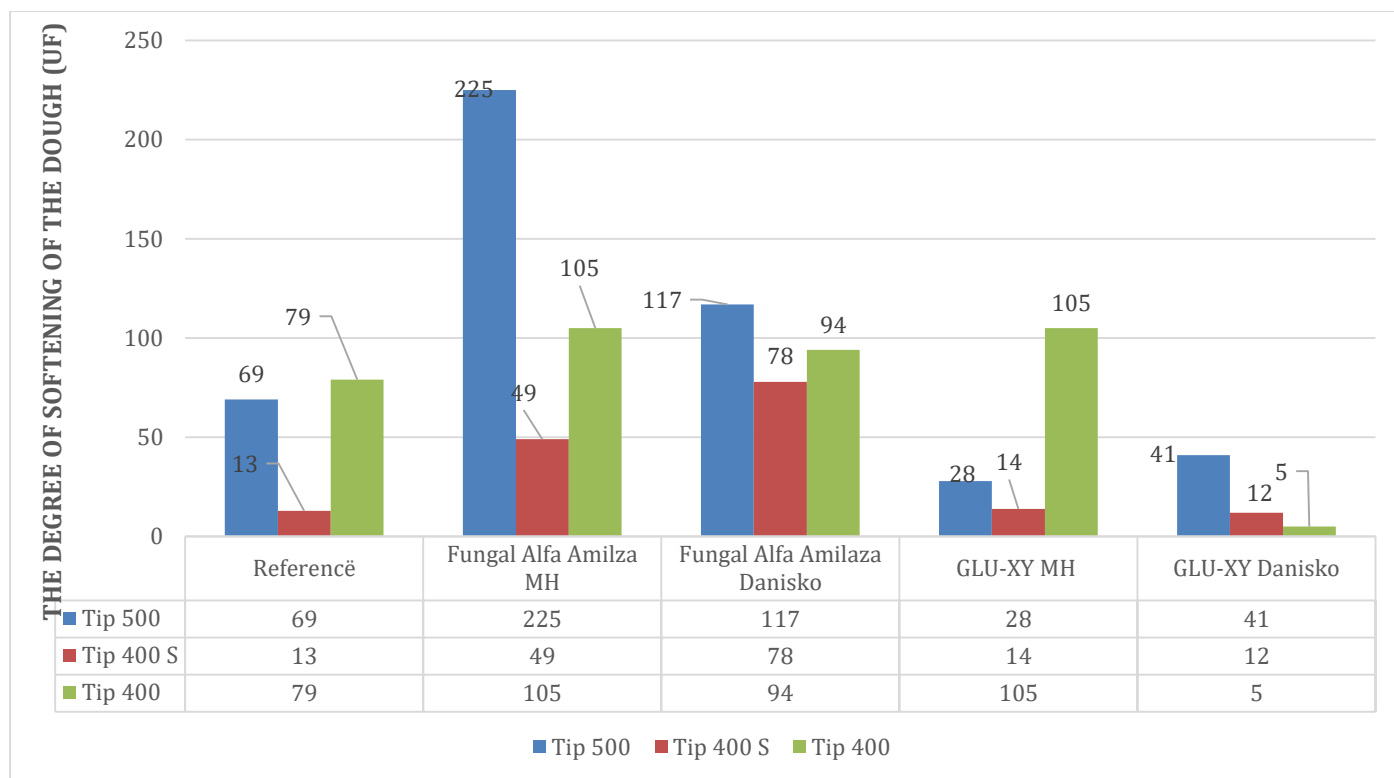


Fig4. The degree of softening of the dough, mixtures of flour-additives

Qualitative Number / Qualitative Class

The flour T-400S has a better qualitative number 82, ie qualitative class A2¹, but for the production of bread, a qualitative class B is needed, which shows that more mixes have this class, except the flour T-400S. From the graph, we see that the mixtures of flour with additives GLU-XY MH and GLU-XY D significantly improve the qualitative number by increasing it, thus the maximum reached the mixture Reference (T-400S) + GLU-XY MH with a value of 87 (class A1), while mixtures with the additives Fungal α -Amylase MH and Fungal α -Amylase D reduce the qualitative number as in the case of the mixture Reference (T 500) + Fungal α -Amylase MH with value 11 (class C2).

¹ Qualitative number (Hankoczy-it):

A1 100 - 85.3; A2 84.7 - 70.2

B1 69.9 - 55.1; B2 54.8 - 45.0

C1 44.8 - 30.1; C2 30.0 - 0.0

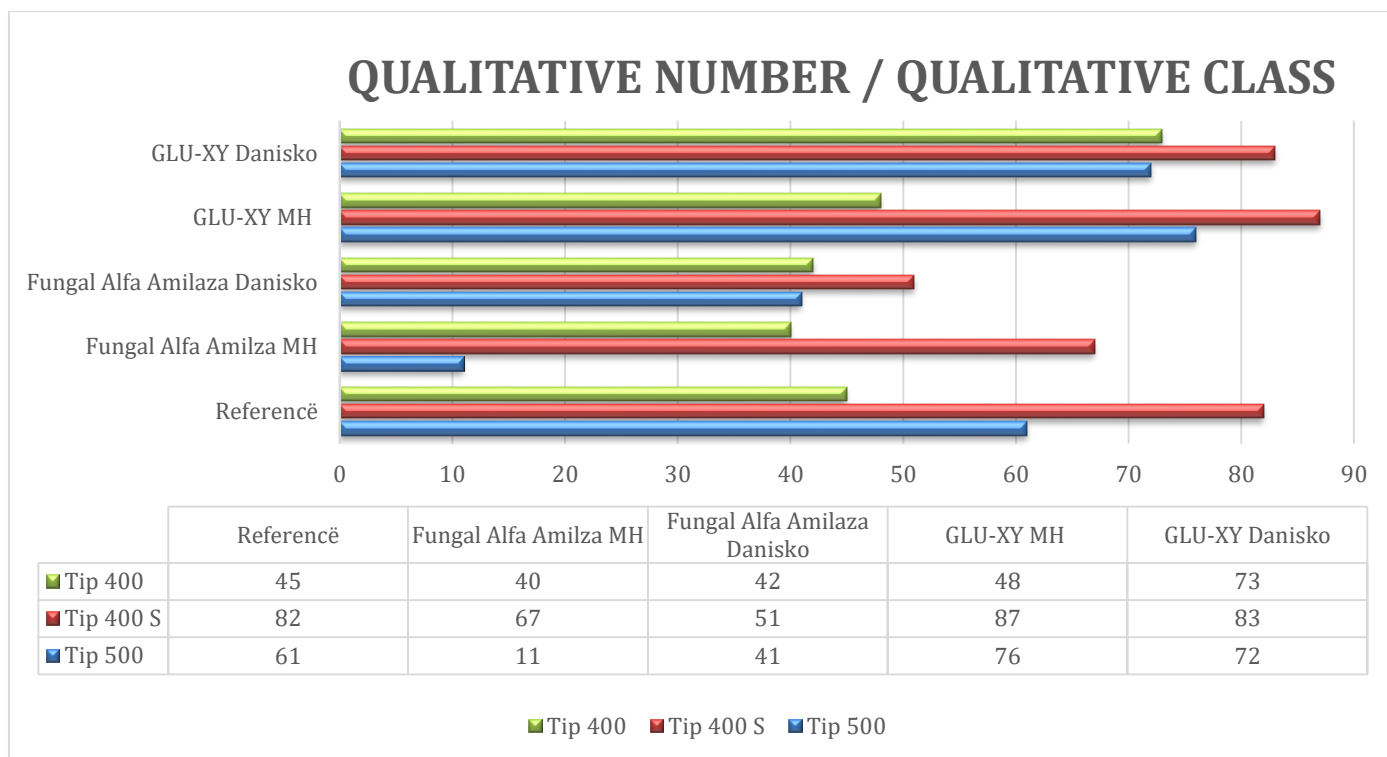


Fig 5. Qualitative number / qualitative class, mixtures of flour-additives

3.1. Sensory characteristics of bread made of flour with additives (enzymes): Several sensory parameters are evaluated, such as shape, bark color, bark cracking, porosity, characteristic taste, consistency, odor, and texture.

From the sensory characteristics of bread made from flour with additives, (Figure 6), we can say that the color of the bread crust with the addition of additives is even more highlighted, all bread has a normal shape and cracking of the crust; the degree of porosity of the bread is generally normal, while the connection of the crust with the dough, no matter of the addition of the type of additive, is very good. Bread made from flour T-400, and T-400S with a mixture of GLU-XY additives generally have a more pronounced granulation rate compared to bread produced from T-500.

Sensory characteristics

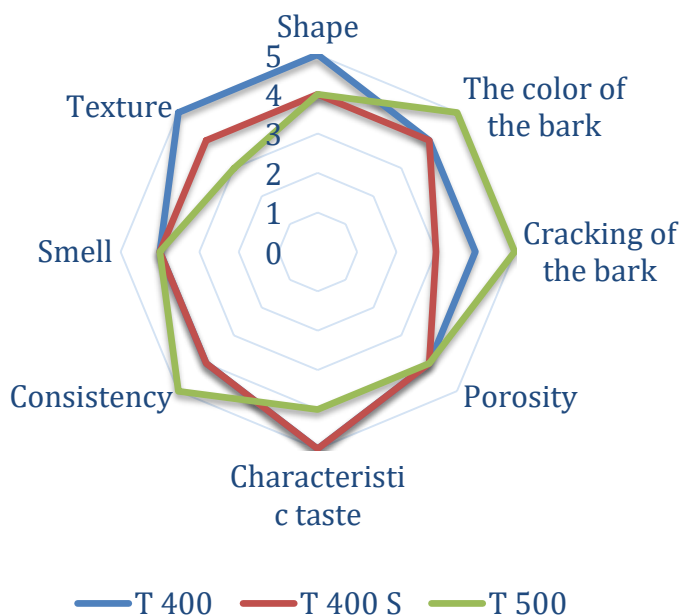




Fig6. Sensory characteristics, mixtures of flour-additives

4. Conclusions

Considering the principles of use of Fungal α -Amylase and Glycolipase-Xylanase, they do not represent any health risk, when we respect the legal regulation, recommended by the manufacturer of the additive. Their role in the dough is to improve rheological qualities. There is a correlation between the amount of water absorption and the mixing time, i.e., the time of dough development. From the obtained results we conclude that to increase the water absorption capacity, the softening degree of the dough, and the concentration of gluten, it is recommended to use additives from Fungal α Amylase (M.H and D), because it stimulates the fermentation of the yeast, thus increases the volume of the bread, and contributes to good crumb quality.

Even in the baking process, Fungal α -Amylase has a very important effect, reducing the concentration of sugars that are created during mixing and fermentation, where because of all this occurs the reaction of Maillard, which is responsible for non-enzymatic staining of bread crust and generating the characteristics of the bread, including smell and taste. To get good bakery products - bread, mixtures of flour T-400 and T-500 with additives are more suitable, due to their physicochemical and rheological characteristics, which give these products better development of the dough as well as quality class B, especially the mixture with Glycolipase-Xylanase (M.H and D). Mixtures of flour T-400 S with additives, based on the obtained results are intended for the production of pie, because the concentration of protein and the amount of gluten is higher, compared to the aforementioned flours.

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