# INFLUENCE OF ENZYMES AS AMYLASE, HEMYCELLULARE AND XYLANASE ON THE RHEOLOGICAL CHARACTERISTICS OF TYPE 650 WHEAT FLOUR

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**Abstract**: The effect of enzymes differs depending on the characteristics of flour and that is why they should be known very well, their effects in dough and in bread. In this paper, some combinations of enzymes  $\alpha$  – amylase, xylanase, hemycellulase and ascorbic acid are tested and the rheological properties using alveograms are analysed. The results show that the use of hemicellulases in combination with  $\alpha$  – amylase has beneficial synergy effects, improving the rheological characteristics of dough that for their separate utilization. The substitution of hemicellulase with xylanase in a certain proportion has the same effect on the rheological characteristics of dough.

Keywords: a - amylase, hemicellulase, xylanase, ascorbic acid, synergy sffects, alveogram

#### **INTRODUCTION**

The ameliorative used in the bread industry are chosen depending on what should be ameliorated, generally, where certain defects of flour can be improved. The synthetically additives can be replaced with natural additives, as enzymes. The enzyme additivation of flours presents the advantage of constant quality flour, which does not modify the technological process, does not affect the health of consumers. The enzymes are used in small quantities and do not influence to a great extent the price of bread. They can be successfully used in the place of chemical additives for synthesis.

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Depending on the substrate where enzymes action, they can be: amylolithical  $(\alpha - \text{amylase fungic and bacteria})$ , gluco amylases, carbohydracids, proteases, hemicellulases, phentoxanasis, xylanasis, phospholipids, oxidizes.

The amylolithic enzymes control the level of fermentation sugar in dough, the characteristic of the part from the middle and the relegation of the starch, with high influences on increasing the bread freshness.

The addition of  $\alpha$  – amylase leads to:

- the extension of freshness
- the increase of the quantity of fermentation sugars, capable of forming gases during the entire period of the technical process inside the chains of amylopectine (Bordei, 2005).
- the obtaining of finite products with a more pronounce color of crust, by increasing the quantity of fermentation sugar.
- the increase of carbon dioxide quantity.

The dextrins in normal quantity have a favorable effect on dough, by increasing the capacity of water retention and improving the aspect of the middle part (soft, fluffy) (Banu, 2000). The alpha amylases help to the forming of dextrins, by deforming the starch chains that suffered a weak degradation (Florea, 2003). The beta dextrine formed is contributing to the increasing of dough viscosity. The water connection energy decreases with the increasing of proteins content, increasing damaged starch content and the content of soluble penthozan (Hugo, 2000).

The reduction of dough's consistency through the addition of alpha amylases leads to the increasing of extensive character and decreasing of the resistance of dough. This behaviour is due to the fact that the maltose obtained by starch hydrolysis realizes a dehydrating action on gluten. The quantity of free water in dough will increase, reducing consistency (Takeda and Matsumura, 2001).

The effect of hemicellulases on dough is the hydrolysis of the soluble and insoluble penthosane in water with the formation of olygomers with high reactive molecular mass (Dinu, 2002). They have as effect the neutralisation of the negative action of soluble penthosane on bread volume, improving the stability of dough and its tolerance for fermentation, the improvement of the dough capacity to retain fermentation gases, the decrease of dough viscosity and the improvement of the processing quality (Diaconescu, 2004).

The action of the hemicellulases contributes to a better gluten structure (extensibility). The capacity of hydratation of penthosans is reduced; the water resulting through hydrolysis (34%) can be absorbed and redistributed

in the process of gluten formation (Bordei, 2005). The ameliorative effect of hemicellulases is based on this.

The optimal results are obtained when hemicellulases together with alpha – amylases are used, having synergy effect.

Xylanase is a penthosane which catalyses the hydrolysis of the  $\beta$ - xilan bounds, either of the connections  $\beta$ -(1,4) from the inside of the main chain of xylan, forming oygomers with diverse molecular mass, either at the ends of the chain putting into freedom more simple products. In dough, xylanases action not only on xylans and arabixylans, but also on the protein complex. The positive action resides in the liberation of water, which becomes available to gluten (Boredi, 2005). The xylanases improve the stability of dough and the fermentation tolerance, increase the dough capacity to retain gases, the volume of bread. So, the bread obtained has an improved and uniform interior structure. The elasticity remains unchained.

Ascorbic acid is a good agent of cyclic oxide – reduction that is not consumed during reactions in dough. Its use is efficient because it moves the thermal optimum of activity of alpha amylase in the direction of some decreased temperatures and inactivates the starch hydrolytic enzymes, reducing the gathering of dextrine in the process of baking bread.

The ascorbic acid in the presence of the oxygen included in kneading, reduces the reactive SH groups, in the first minutes from starting the action, these will be oxidized at disulphide connections S-S-. This fact leads to the improvement of the dough resistance, of its elasticity in the case of weak and medium flour; for strong flour, the effects are not advantageous, but in combination with the effects of  $\alpha$ -amylase, hemicelulase, xilanase, will be obtained optimal results on improving flour.

#### MATERIALS AND METHODS

Flour 650 type from the production of 2004 was used.

The enzyme named clarase (alpha amylase from the fungus *Aspergillus oryzae*), hemicellulase and xylanase were used. The enzymes were gently provided by SC EDR Romania SA, Neamt.

The experiments have been realized in the laboratory for quality flour analysis at SC MOARA CIBIN SA Sibiu. The rheological properties of flours were analysed using alveograms. For the obtaining of alveograms, the Alevograph apparatus NG Consistograph – CHOPIN was used. The tests were realized in conformity with the CE Directives.

From the alveogram the following indicators were obtained:

- P viscosity or the value of maximum pressure that is in relationship to the resistance of the deforming dough (mm H<sub>2</sub>O);
- L extensibility (the length of the curve that begins from the origin until the perpendicular point that corresponds to decreasing pressure due to rupture of air bubble) (mm);
- G expansion index G being the average of the expansion index on the graphic of cellules and corresponds to breaking the abscise L, G = 2.226L, where L air volume (cm<sup>2</sup>) used to strech the dought under bubble form.
- W the action of deformation of the dough, based on a gram of dough, evaluated at 10 E - 4 joule, calculated as follows: W= 1.32 x (V/L) x S, where V- air volume in mm<sup>3</sup>; L- the average abscise at breaking point in mm; S- surface of the curve, cm<sup>2</sup>;
- P/L rapport of configuration of the curbe
- Ie elasticity index, represents the rapport between the measured pressures, expressed in mm H<sub>2</sub>O to form bubbles after the insufflations of 200 cm<sup>3</sup> of air in dough form, that correspond to a length L of 40 mm or an index of expansion G from 14,1 and the maximum of the curve P: Ie %= P200/Pmax.
- FN Falling Number was determined with the device PERTEN-HAGBERG. FN is defined as being the total period, in seconds, from the immersion of the tube in the water bath until falling from a distance established in a gelatin suspension.

The enyzmes activity was determined in flour samples with and without (blind) additives.

Two tests were realized:

- **Test 1:** the analysis of the effect of enzymes alpha amylase, hemycellulase, xylanase combined two or three on the rheological characteristics of flour 650. Ascorbic acid was also added in one sample. Five samples were analysed:
  - Blind sample with flour F650 (M)
  - F650 with 3% α-amilase,12% hemicellulose (P1)
  - F650 with 3%  $\alpha$ -amilase,3% xilanase (P2)
  - F650 with 3% α-amilase, 1.5% xilanase, 6% hemicellulase (P3)
  - F650 with 5% α-amilase,12% hemicellulose, 8% ascorbic acid (P4)

The deformation index of blind sample (flour 650) was calculated in conformity with the standard method and has the value ID = 8 mm.

• **Test 2:** the analysis of the effect of mixtures of enzymes alpha – amylase, hemycellulase, xylanase, combined with ascorbic acid on the rheological characteristics of flour 650. Three samples were analysed:

- Blind sample with flour F650 (M)
- F650 with 2g  $\alpha$   $\alpha$ -amylase , 10 hemicellulose , 3g ascorbic acid (P1)
- F650 with 2g  $\alpha$ -amylase, 5g hemicellulose, 1,5 g xylanase, 3g ascorbic acid(P2)

The quantity of enzymes was expressed in grams and was reported to 100 kg of flor.

The deforming index of blind sample (flour 650) was calculated taking into account the value of 12 mm, the value of quantitative gluten was determined and is of 28 l, g, humidity of flour u = 14,5% and the laboratory temperature was 25° C.

## **RESULT AND DISCUSSIONS**

The results obtained at Test 1 are presented in Figure 1.



Figure 1: Variation diagram for test 1of the characteristics of the alveogram (resistance, extensibility, energy for kneading, rapport of configuration of the curve, elasticity index) and the values of FN for blind sample (M) and samples additived with enzymes (P1, P2, P3, P4)

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It can be observed that tests P1 and P2 are similar for the deforming resistance (P), extensibility (L), at the energy for kneading and the elasticity index. From the initial ameliorative was replaced the hemycellulase with xylanase in proportion of 4:1 and was obtained test P2.

At test 3 (P3), half of the quantity of hemycellulose was replaced with xylanase in the same proportion, obtaining the same value of resistance at deformation P, but a higher value of estensibility L and a greater value of the deforming energy and the same value of the elasticity index and the one for falling FN.

The action of xylanase is similar to hemicellulase, in combination with  $\alpha$  - amylase, at a rapport xylanase: hemicellulose = 1:4 for values of P, W, Ie and FN. It can be proved that the two enzymes, hemycellulase and xylanase have synergetic effects (gathered). When half of the quantity of hemicellulase is replaced with xylanase that might have the same effect (1:4), the same effect as in the blind sample is obtained.

The use of hemycelulase determines an increase of dough viscosity, effect eliminated at the addition of xylanase.

Improved values for the characteristics elasticity and stretching energy of samples with a great quantity of  $\alpha$ - amylase, together with hemicellulase and ascorbic acid, can be obtained. The ascorbic acid has a role of oxidant improving the resistance and elasticity properties of dough (Ie).

The results obtained at Test 2 are presented in Figure 2.

At the substitution of half of the quantity of hemicellulase with xylanase in proportion of 1:4 in the complex  $\alpha$  – amylase, hemicellulase, ascorbic acid the same effect will be obtained, for the resistance to extensibility, elasticity of dough and for the deforming energy.

The effects of ascorbic acid are benefic to a additive action together with  $\alpha$  – amylase and hemycellulase.



Figure 2: Variation diagram for test 2 of the characteristics of the alveogram (resistance, extensibility, energy for kneading, rapport of configuration of the curve, elasticity index) and the values of FN for blind sample (M) and samples additived with enzymes and ascorbic acid (P1, P2)

#### CONCLUSIONS

The additive actions of complex enzymes as ameliorator on flour have positive effects on the rheological characteristics of dough.

The mixing  $\alpha$  – amylase and hemicellulase determines obtaining a weak flour, diminishing the dough resistance and the increasing of extensibility, but an improvement of dough elasticity, the effects of the 2 enzymes being synergic. The falling number (N) increases this additivation of flour.

The additivation with acid ascorbic causes an improvement of the other parameters that will determine and improvement of the resistance and extensibility of flour, as well as increasing energy for deforming the dough. It will be observed a decreasing of falling number (FN)

More, the amelioration effect of hemicellulase can be totally or partially substituted (at half) with the improvement effect of xynalase in quantitative rapport of hemycellulase s: xylanase = 4:1 and addition of some complex additive with  $\alpha$  – amylase, ascorbic acide.

Selecting a correct enzyme preparation will be made in conformity with the rheological characteristics of dough and the proportions from the dough will be added so that they would be maximal.

The enzyme preparations are used to obtain a bread with "clean label", more natural, bread being the product that enjoys the greatest interest from consumers.

#### REFERENCES

- 1. Banu, C. (coordinator), "Aditivi si ingrediente pentru industria alimentara", Tehnica Ed., Bucharest, 2000
- 2. Bordei., D., "Tehnologia moderna a panificatiei", AGIR Editure, Bucharest, 2005
- Dinu., C., "Enzime pectiolitice de origine microbiana:, www. unibuc.ro/en/publicatii\_2002/eBooks/biologie/ Progrese Volume 1/ Chapter 6/ Chapter 6.doc, 2002
- 4. Diaconescu., D., Ivan., E., "Amelioratori enzimatici folositi in panificatie", 2004, Informative bulletine of ,miiling and bread making, vol. 15, no. 3
- 5. Florea., T., "Chimia alimentelor", Editura Academica, Galati, vol 1,2,3, 2001
- Jascanu., V., Stefoane., E, D, "Influence of Clarase and Hemicellulase over 650 Flour Type", 2006, "Acta Universitatis Cibiniensis", green house F, chemia 9 (1), 73-80
- 7. Hugo., L.F., Rooney., L.W., Taylor, JRN, "Malted Sorghum as a functional Ingredient in Composite Bread", Cereal Chemistry, 77 (4): 428-432.
- 8. Takeda, K., Matsumura., Y., Shumuzu., M., "Emulsifying and Surface Properties of weat Gluten under Acidic Conditions", 2001, Journal of food science, vol.66, no. 3, pag 393-399.