# INFLUENCE OF THREE DIFFERENT HEMICELLULASES ON THE QUALITY OF BREAD OBTAINED FROM WHITE "SHORT" GLUTEN FLOUR

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***Abstract***
*Since a century ago, enzymes were used in bread making industry. In recent years, the range of enzymes used to obtain bread was increased with enzymes that have as substrates the non-starch polysaccharides from wheat and rye flours, beeing known the fact that supplementation with hemicellulases of flours rich in this substances leads to an improved quality of bread.*
*The aim of this study is to verify if the supplementation of white flour, with "short" gluten, with lower hemicelluloses content may be the substrate for positive action of hemicellulases. For this purpose, three hemicellulolytic enzymes were tested - a fungal hemicellulase from Aspergillus oryzae, a fungal pentosanase from Humicola insolens and a bacterial xylanase from Bacillus subtilis. Analyses were focused on the characterization of flour used in the experiments by some parameters such as moisture, ash content, wet gluten content, gluten deformation index, Alveograph characteristics and determination of some quality parameters of the results bread - volume, porosity and elasticity. The results showed that for the analyzed flour, the supplementation with hemicellulolytic enzymes may be a technological solution to improve the physical indices of bread, so its quality. Percentage increases were compared to control sample, beeing: 17 % for volume, 5,5 % for porosity and 8,5 % for elasticity. Due to the specific conditions in which the baking tests take place, it can be concluded that the use of these enzymes can be successfully applied in pan bread technology.*

**Keywords:** hemicelluloses, "short" gluten flour, hemicellulase, pentosanase, xylanase.

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## 1. INTRODUCTION

Hemicelluloses, together with cellulose, forms the non-starch polysaccharides group of wheat flours. The term hemicelluloses refers to a group of homo- and heteropolymers, mostly composed from chains whose main structural units are xilopyranose, mannopyranose, galactopyranose and glucopyranose linked by 1,4- (and occasionally 1.3-) β glycosidic bonds, to which are attached various substituents. In plant cell walls, hemicelluloses are found generally associated with cellulose (*Jeffries, 1994*). The main hemicelluloses from wheat flours (known as pentosans) are arabinoxylans and arabinogalactans. Arabinoxylans generally consist of between 1500 - 5000 residues (*Chaplin, 2004*).
Hemicellulases term designates a large group of enzymes, including endo-and exoxylanases, α-L-arabinofuranosidases and ferulic acid esterases. All members of this group are able to split the wheat flour pentosans but the impact of their action on dough and baking process varies very widely. Hemicellulases are classified according to the substrate, links which are split and hydrolysis products (*Jeffries, 1994*).
Although they are present in minor amounts in flour - 2-2.5% (*Cauvain and Young, 2007*), pentosans are a very important functional component, covering about 30% of water binding capacity of wheat flour (*Courtin and Delcour, 2002*). There is a classification into soluble and insoluble in water arabinoxylans (*Bordei et al., 2000*), but more recent classification refers to water extractable arabinoxylan (WE-AX) and water-unextractable arabinoxylans (WU-AX) (*Courtin and Delcour, 2002*). It is supposed that WE-AX have a positive influence in bread making and WU-AX a negative effect (*Whitehurst and van Oort, 2010*). Flour pentosans influence the bread making process in at least two ways: by their significant water retention capacity which affects water

distribution in dough and altering the rheological properties of dough for increase the gas retention (*Eliasson, 2006*).

It is believed that the addition of hemicellulases in dough, especially in high fiber dough, has a positive effect on bread quality through partial solubilisation of bran fragments that interrupt the continuity of gluten network (*Poutanen, 1997*). However, the mechanism by which hemicellulases exerts their effects are still limited, probably because of wheat flour pentosans that differ from one variety to another and enzymes specificity that is, also, changes depending on different substrates (*Steffolani et al., 2010*).

The aim of study is to verify if supplementation of white flour, with "short" gluten, with lower hemicelluloses content may be the substrate for positive action of hemicellulases. For this purpose, three hemicellulolitic enzymes, from different origins and different mode of action were tested.

## 2. MATERIALS AND METHODS

### 2.1. Materials

*Flour* was purchased from SC COMPAN S.A. Targoviste. The quality indices of flour and rheological characteristics of dough are summarized in Table 1.

The used *enzymes* included a fungal hemicellulase from *Aspergillus oryzae* (*S.C. Enzymes & Derivates România S.A.*), a fungal pentosanase from *Humicola insolens (Pentopan 500 BG - Novozymes)* and bacterial xylanase from *Bacillus subtilis* (*S.C. Enzymes & Derivates România S.A.*).

In baking tests there were used a *compressed yeast* from Pakmaya (SC Rompak Paşcani LLC) and salt (sodium chloride).

### 2.2. Methods

***Flour analysis***. For determining flour moisture, ash content, wet gluten content, gluten deformation index, there were used the Romanian standardized methods. Glutenic index was determined using the method proposed by ICA Bucharest. The Alveograph characteristics (P, L, P/L, W) of dough obtained from used flour was measured with Chopin Alveograph (AACC Method 54-30A).

***Bread making procedure***. For baking tests it was used the straight dough method and the baking bread Moulinex machines The dough formulation (expressed for 100g flour) included 100g flour, compressed yeast-3g (3%), salt-1,5g (1.5%), water-60g (60%), hemicellulolytic enzymes (different doses). For a loaf, the quantity of flour used was 300 g.

***Bread analysis***. Three hours after baking the loaves were weighed. The loaf volume was determined by rapeseed displacement, using the Fornet apparatus. Porosity and elasticity of bread crumb was determined using the Romanian standardized methods (STAS 91-83).

## 3. RESULTS AND DISCUSSION

The figures 1, 2 and 3 proves that even strong white flours can be improved in terms of volume by the addition of hemicellulases. From graphs, it can be concluded that the increase of enzymes doses (whether it is the hemicellulase from *Aspergillus oryzae*, the pentosanase from *Humicola insolens* or the xylanase from *Bacillus subtilis*), up to a certain amount, produces an increase in bread volume. Then, by increasing the dose, it can be observed a downward slope, steeper for fungal pentosanase.

**Table 1. Quality indices of flour used in experiments**

| Moisture (%) | Ash content (% dry weight) | Wet gluten content (%) | Gluten deformation index (mm) | Glutenic index | Alveograph characteristics |
|---|---|---|---|---|---|
| 13,53 | 0,64 | 27,60 | 4 | 48,02 | P =184mm$H_2O$ |
| | | | | | L = 21mm |
| | | | | | P/L = 8,76 |
| | | | | | W = 174×10e$^{-4}$J |

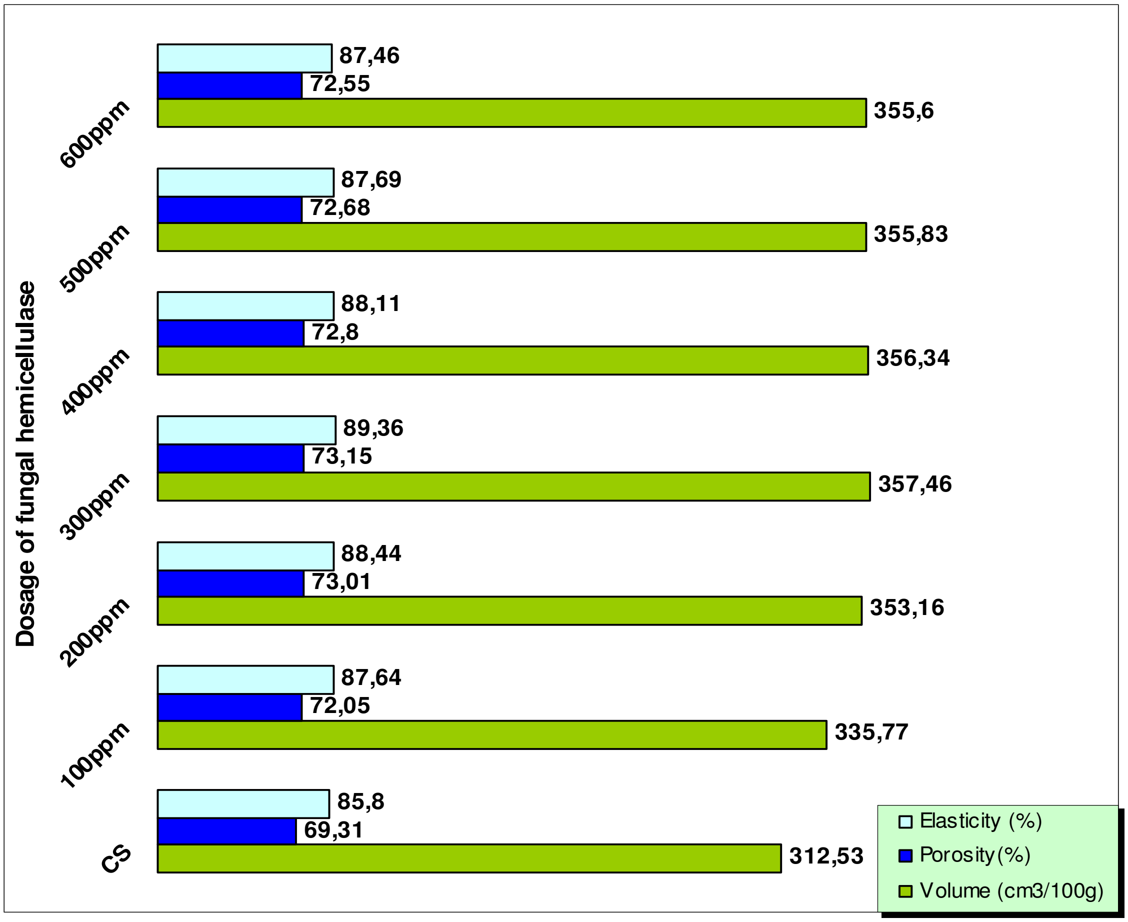


**Figure 1. Comparative changes of volume, porosity and elasticity of bread supplemented with fungal hemicellulase.**

Positive effect on loaf volume decreases in the order - fungal pentosanase, fungal hemicellulase and bacterial xylanase.

The positive effect of these enzymes on bread volume is due, in part, to water redistribution from the non-starch polysaccharides fraction, affected by hydrolysis, to gluten fraction. By increasing the volume of gluten fraction increases its extensibility and, consequently, the ability of dough to retain the gas resulting from alcoholic fermentation. In addition, volume growth is probably due to the fact that the hydrolytic action of the enzymes (separately) disaggregates secondary network of arabinoxylans which was a support for dough strong tendency to oppose to volume expansion under pressure of CO2 released from yeasts fermentation. The decrease of loaf volume in case of overcome optimal dosage of enzymes correlates with the decrease of supplemented dough ability to retain gas.

This can be explained by the fact that flour contains relatively small amounts of hemicelluloses and byproducts resulting from the initial degradation are substrates for hydrolytic enzymes action causing the formation of simple compounds with low water-binding capacity that can not create a high viscosity in the aqueous phase of the dough.

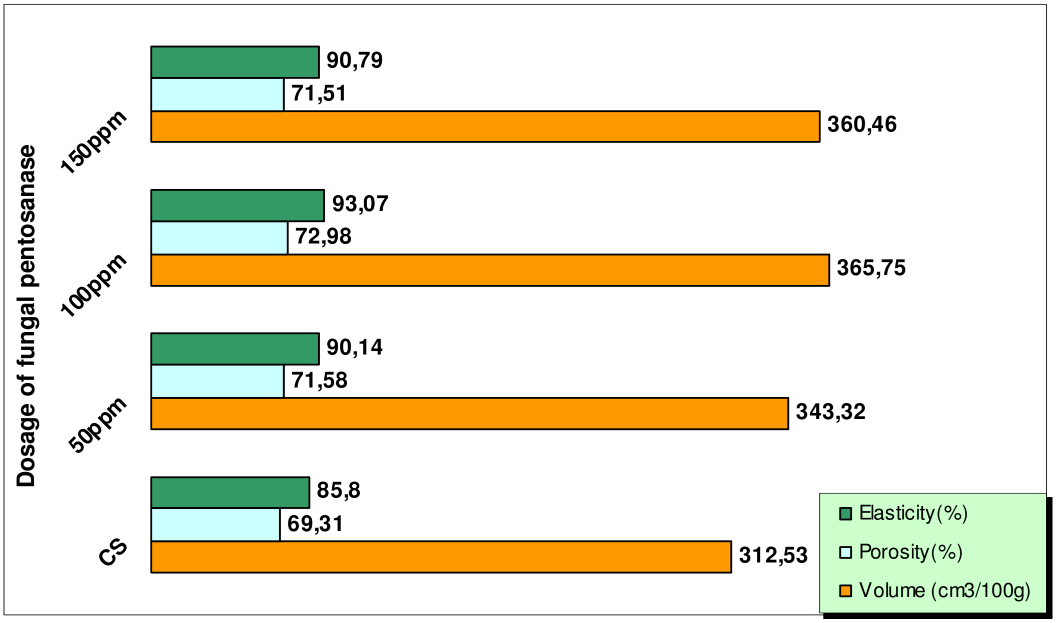


**Figure 2. Comparative changes of volume, porosity and elasticity of bread supplemented with fungal pentosanase.**

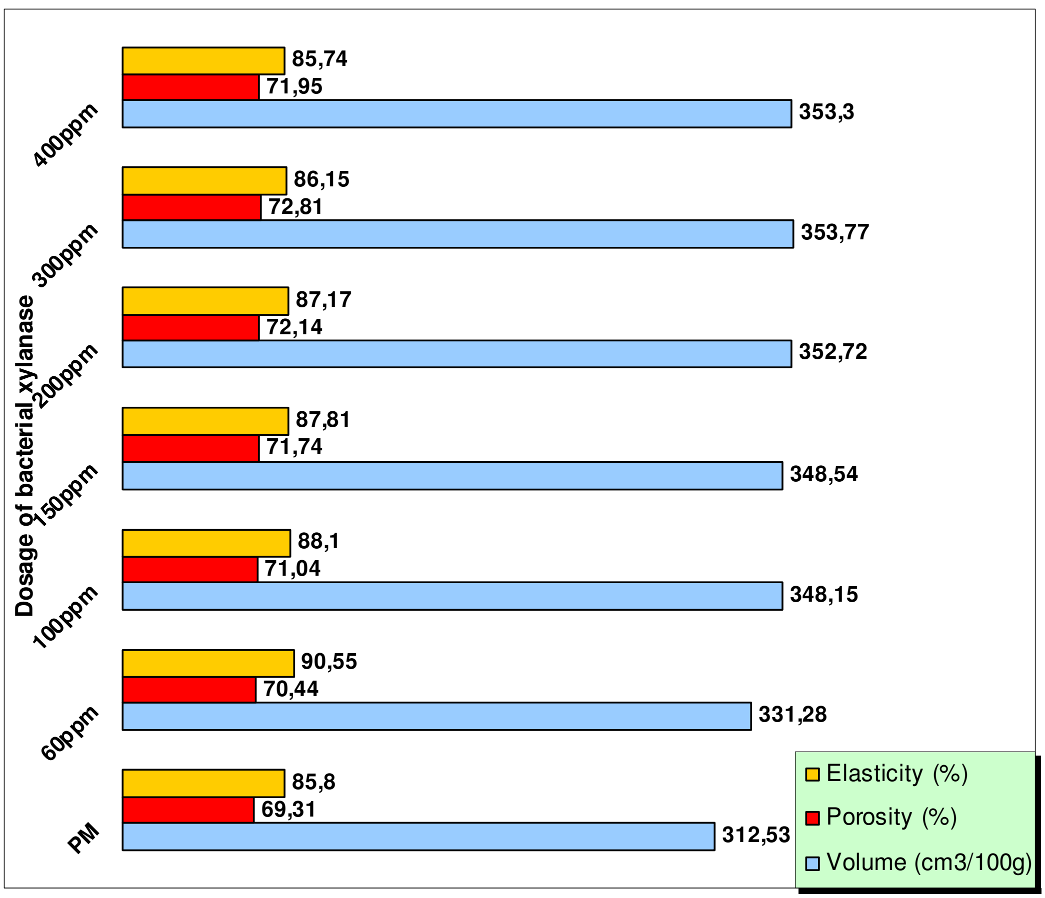


**Figure 3. Comparative changes of volume, porosity and elasticity of bread supplemented with bacterial xylanase.**

**Table 2. Percentual variation of bread volume, porosity and elasticity depending on the dose of hemicellulolytic enzymes added.**

| Percentual variation of: | Fungal Hemicellulase | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 ppm | 100 ppm | 200 ppm | 300 ppm | 400 ppm | 500 ppm | 600 ppm |
| **Volume** | 0 | +7,43 | +13,00 | **+14,37** | +14,01 | +13,85 | +13,78 |
| **Porosity** | 0 | +3,95 | +5,33 | **+5,54** | +5,03 | +4,86 | +4,67 |
| **Elasticity** | 0 | +2,14 | +3,07 | **+4,14** | +2,69 | +2,20 | +1,93 |

| | Fungal Pentosanase | | | |
|---|---|---|---|---|
| | 0 ppm | 50 ppm | 100 ppm | 150 ppm |
| **Volume** | 0 | +9,85 | **+17,02** | +15,33 |
| **Porosity** | 0 | +3,27 | **+5,29** | +3,17 |
| **Elasticity** | 0 | +5,05 | **+8,47** | +5,81 |

| | Bacterial Xylanase | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 ppm | 60 ppm | 100 ppm | 150 ppm | 200 ppm | 300 ppm | 400 ppm |
| **Volume** | 0 | +5,99 | +11,39 | +11,52 | +12,85 | **+13,19** | +13,04 |
| **Porosity** | 0 | +1,63 | +2,49 | +3,50 | +4,08 | **+5,04** | +3,80 |
| **Elasticity** | 0 | **+5,53** | +2,68 | +2,34 | +1,59 | +0,40 | -0,06 |

The increase of free water content in the dough, above a certain limit, leads, in this case, to reduce gas retention by the system.

The table 2, which presents the increase (in percentage) for volume, porosity and elasticity due to the addition of hemicellulolytic enzymes for products made from the used flour, shows that the most significant increases of bread volume are: 17.02% for fungal pentosanase, 14.37% for fungal hemicellulase and 13.19% for bacterial xylanase.

The values obtained for bread porosity, when the three enzymes are added, show that is an increase comparative with control sample. In this case, the samples supplemented with bacterial xylanase have the lowest values. Regarding the porosity, the increase is relatively small and within the range from 5% to 5.5%.

Bread crumb elasticity is also improved more significantly when fungal pentosanase was added in dough. Improvements of elasticity varies from one enzyme to another, within wider range than the porosity (8.47% for fungal pentosanase, 5.53% for bacterial xylanase and 4.14% for fungal hemicellulase). For increased doses of hemicellulase and xylanase, elasticity decreases around the value obtained for control sample.

## 4. CONCLUSIONS

Addition of hemicellulolytic enzymes in strong white flour doughs can promote improved physical properties of obtained bread, the maximum loaf volume increase beeing around 17%. Porosity was increased with maximum 5,5 % and elasticity with maximum 8,5 %.

For the specific conditions in which the baking tests take place, bacterial xylanase exhibited no obvious effect to improve bread quality compared to the other tested enzymes, so the recommendation regarding the use of these enzymes in the bread-making processes refers mainly to the fungal hemicellulolytic enzymes, especially in pan bread technology.

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