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# Influence of a fungal protease on the physical properties of bread made from short gluten flours

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***Abstract***

The paper's aims is to demonstrate the positive impact that the addition of a fungal protease has on some physical properties of bread (volume, porosity, elasticity) obtained from "short" gluten flours, with a protein network which is characterized by a high elasticity and resistance.

Fungal protease, by its ability to cleavage the peptidic bonds from polypeptidic chains of protein, may be a solution to improve the quality of bread made from strong flours (replacing the chemical additives) and creates the necessary preconditions for reducing energy kneading dough. It can be assimilated into modern methods of obtaining pan bread (because of specific conditions which have been made by baking tests) which use reduced fermentation and processing times, shortening of the technological process having positive repercussions in the economic efficiency domain, too.

***Keywords***: fungal protease, wheat flour, gluten, bread.

## 1. Introduction

The wheat dough is a complex system composed mainly of starch, water, proteins and pentosans (arabinoxylans). The gluten obtained from hydrated storage wheat proteins plays the dominant role in dough rheology but the other components are, also, significantly involved.

Romanian flours are characterized by a very good protein content and present a deformation index of gluten which is predominantly situated on the left of interval 5-15 mm [1] in which flours are very good/good for bread production. Due to the low deformation index which most often (and especially if procedures for obtaining the bread are with short duration of fermentation) not allow to doughs to develop the optimum capacity to retain the fermentation gases, it is necessary to supplement dough with additives which may decrease the high resistance of gluten. For this purpose, currently in the breadmaking it is used a series of chemical agents, mainly L-cysteine which, through the reduction of gluten proteins disulfide bonds, decreases the dough tenacity and elasticity. Another possibility could be use of proteases from different origins, although their mechanism of action in the dough is different compared with L-cysteine. Proteolytic enzymes hydrolyze gluten proteins, reducing their average molecular weight and the elasticity of the dough [2,3].

## 2. Materials and Method

***Materials***

*Flour.* In experiments, strong flour, with short gluten from SC COMPAN S.A. Targoviste (FA3) was used. The flour's determined characteristics are summarized in Table 1. The flour's quality indices refer to the protein content (expressed by wet gluten content), moisture, the elastico-plastic characteristics of dough, purity and content of non-starch polysaccharides (judged by the ash content) and α-amylase activity.

*Fungal protease* (from *Genencor International*). The preparation derived from *Aspergillus oryzae* contains enzymes

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that are typically used in neutral and acid pH applications. On gluten, they are slow acting relative to other proteases. The activity of enzyme preparation is 500.000 HUT/g.

*Compressed yeast.* In baking tests it has been used the compressed baking yeast from Pakmaya (SC Rompak Paşcani LLC).

*Salt (sodium chloride)* - having the characteristics from STAS 1465-72.

***Methods***

*Determination of flour moisture using drying method (ICC Method No 110/1).* The setting of humidity was done by the indirect method, by drying. Analyzed flour was maintained at a certain temperature (classical method - at $105^0$C for 4 hours; rapid method - at 130± $2^0$C for one hour) until all the free water evaporates and other secondary effects that alter the chemical components not take place.

*Determination of flour ash content using the burning method at 900-920$^0$C.* Ash is defined as the quantity of mineral materials wich remains, after applying the burning methods, as incombustible residue of the analyzed sample. The result is expressed as a percentage by reporting the mass of the residue at the dry matter of the analyzed sample.

*Determination of the flour wet gluten content.* The method is based on separation of gluten by washing the dough made from flour with a solution of NaCl, concentration of 2%. The result is expressed as a percentage gained by reporting the weight of the wet gluten at the weight of meal flour taken into consideration.

*Determination of deformation gluten index.* The method involves the maintaining of a wet gluten sphere (5g) for one hour at a temperature of $30^0$C and determination of the deformation by measuring two medium horizontally diameters (in mm) - before and after the rest - and calculating the difference between them.

*Determination of α-amylase activity in flours by the "Falling Number" (AACC 56-81B).* The method, developed by *Hagberg-Perten*, is based on a rapid gelification of an aqueous suspension of flour in boiling water and measurement of liquefaction produced by α-amylase to starch gel obtained from sample of flour. The Falling Number is defined as the time (expressed in seconds) needed to an agitator to fall into gel flour heated, in a viscosimeter.

*Alveographical method for determining the rheological properties of dough (AACC 54-30A).* Produced by Chopin, the Alveograph is an instrument that gives valuable information about the rheological dough sample by measuring the pressures attained during the inflation of sample dough into a bubble. The alveogram characteristics are: P - known as the overpressure, P is the maximum pressure ($mmH_2O$), measured as the maximum height (h) in mm on the alveogram and multiplied by a factor of 1.1, P value beeing usually used as an indicator of dough tenacity and resistance to deformation; L - is the average length (mm) of the curve from the point where the dough bubble starts to inflate to the point where the bubble bursts and the pressure drops suddenly, L being commonly used as a measure of dough extensibility; P/L – configuration curve ratio is thought to indicate general gluten performance; W - represents the energy required to inflate the dough bubble until rupture and generally indicates the baking strength of the sample [4-6].

*The baking test.* In experiments, baking bread Moulinex machines have been used, which carry out all the process operations - mixing-kneading,re-kneading,fermentation, final proof, baking - in the same room in which operations parameters (temperature, time) are strictly controlled based on program, offering the possibility to compare corectly the obtained results. The dough was prepared using the direct method and the next recipe (exprimed for 100g flour):100g flour, yeast-3g (3%), salt-1,5g (1.5%), water – 60g (60%), additives - different doses reported to flour weight.

*Determination of bread volume by the method with the Fornet apparatus.* The principle of this method is measuring the volume of rape seeds replaced by the bread using the Fornet apparatus, the results being exprimated at 100g product.

*Determination of bread porosity.* The method consists in determination of the total volume of pores of a known volume of crumb, knowing its mass and density. To obtain an average of porosity, bread was transverse sectioned, removing the crust and cut from crumb three cylinders, from three different areas, which were subjected to measurement method.

*Determination of bread elasticity.* The method consists of pressing a piece of crumb cylinder from one minute and measure the return to the original position, after removing the force and after a rest for one minute. To achieve the analysis, it were used the crumb cylinders from the porosity determination.

**Table 1.** Characteristics of flour used in experiments

| **Flour code** | **Moisture (%)** | **Ash (% dry weight basis)** | **Wet gluten (%)** | **Gluten deformation index (mm)** | **Glutenic index** | **Alveogram parameters** | **Falling Number (sec.)** |
|---|---|---|---|---|---|---|---|
| FA3 | 13,53 | 0,64 | 27,60 | 4 | 48,02 | P =184mm$H_2O$ | 251 |
| | | | | | | L = 21mm | |
| | | | | | | P/L = 8,76 | |
| | | | | | | W = 174×10e$^{-4}$J | |

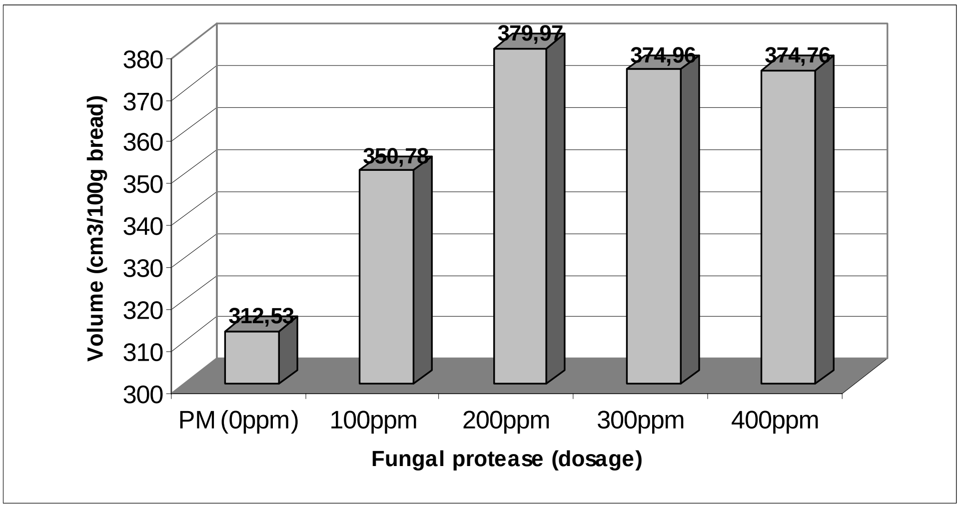


***Figure 1.*** Variation of bread volume depending on the dose of added fungal protease.

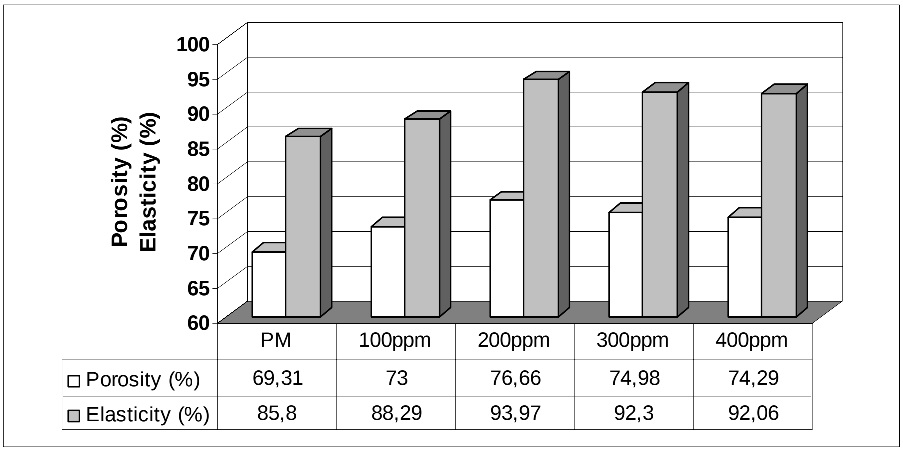


***Figure 2.*** Variation of bread porosity and elasticity depending on the dosage of added fungal protease.

## 3. Results and Discussion

By supplementing the FA3 flour with fungal protease it may observed that, along with increasing the dose of enzyme (up to optimum value), is a corresponding increase in the volume of bread (Fig. 1). This is due to an alteration of resistant gluten structure through a cleavage (in a limited number) of the constituent polypeptidic chains.

Increasing the addition of fungal protease over the optimal dose, for the specific conditions of making the test baking, bread volume declines as a result of diminishing the ability of the dough to retain gas fermentation. A similar trend with the variation of volume - increase until a certain amount, followed by a decrease - records the other physical characteristics of bread - porosity and elasticity, issues presented in Fig. 2.

The low volume of witness sample (and, accordingly, low porosity and elasticity) is caused by a high resistance of gluten network that make the dough to oppose an appreciable resistance to increasing pressure gas derived from the fermentation.

From Table 2 it is found that supplementing FA3 flour with a fungal protease may provide a maximum increase of the volume of bread around 20%, of porosity around 10% and of elasticity around 10%, these values indicating a good potential improvement of this enzyme for bread obtained from flours which have a gluten with high resistance and low deformability.

***Table 2.*** The percentual variation of the bread volume, porosity and elasticity depending on the dosage of fungal protease added.

| **Percentual variation of:** | **FA3** | **Fungal protease** | | | |
|---|---|---|---|---|---|
| | 0 ppm (PM) | 100 ppm | 200 ppm | 300 ppm | 400 ppm |
| Volume | 0 | +12,23 | +21,57 | +19,97 | +19,91 |
| Porosity | 0 | +5,32 | +10,60 | +8,18 | +7,18 |
| Elasticity | 0 | +2,90 | +9,52 | +7,57 | +7,29 |

## Conclusions

The involvement of fungal proteases in the breadmaking processes can be a successful alternative to the use of L-cysteine. The supplementation of dough with this enzyme leads to significant increases in volume (~20%), porosity (~10%) and elasticity (~10%) of the result bread.

The use of fungal protease minimizes the resistance and tenacity of the dough, thus reducing the dough mixing energy requirements. It can be assimilated into modern methods of obtaining pan bread from flours with strong gluten (because of specific conditions which have been made by baking tests). Also, the decreasing of fermentation and processing times induced by using of fungal protease presents positive economic effects, increasing the productivity.

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