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# The monitoring of enzyme activity of protease on the bread dough

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***Abstract***

This study presents the action of an exogenous enzyme: protease in different dosages, in the bread dough. The determination of the rheological characteristics of the dough is obtained by alveographic method. The addition of proteolytic enzymes allows adjustment of the rheological characteristics of dough according to the needs of the technological process. The specific effect of proteases on the physical properties of gluten shows that by proteolysis there is a break and a reorientation of protein chains that form the gluten network. The positive influence of protease enzyme is shown on the volume and porosity of the bread, also on reducing the kneading process of the dough and the energy consumption for the technological process.

***Keywords***: bread, protease, alveograph method

## 1. Introduction

Bread is the most comun traditional food product in the entire world. It has a high nutritive value due to the content of easily retainable sugars, lipids and proteins [1].

Enzymes applications have grown to be a common practice in the baking industry with advantage of being considered as natural additives. The exogenous enzymes are being used in the baking industry to improve dough-handling properties. The synthetically additives can be replaced with natural additives, as enzymes [1,2].

Proteolytic enzymes are used for processing strong gluten flours with high resistance and elasticity and low extensibility. The dough obtained from strong gluten flours cannot expand under pressure of the gase fermentation which show that it has little capacity to retain the gas. Dough elasticity is improved at low doses of protease and it is reduced at higher doses. A limited action of proteases causes weakening of the gluten network, while a strong action destroys this network, completely loses its elasticity and the dough becomes sticky. Research conducted with alveograph method at optimal concentrations of proteases shows that the enzyme improves the plastics proprieties of the dough, which makes the dough easier to handle during the technological process [1-4].

The enzyme addition 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 [1].

## 2. Materials and Method

*2.1. Sample preparation.* Materials used for the preparation of the dough samples are wheat flour 650 with normal bread making properties (moisture

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13.30%, protein content 12.75%), salt, water, yeast and protease.

The protease used is: Alphamalt B10020 – enzyme preparation witch contains protease

A sample of 250g of flour is mixed with a solution of salt, yeast and protease in a laboratory mixer 15 min to form dough. The amount of water was adjusted according to the water absorption capacity of flour.

The first dough sample MARTOR contained 95% flour, 1.7% salt, 1.7% yeast and does not any protease.

The second dough sample F1 contained 95% flour, 1.7% salt, 1.7% yeast and 1g/100kg protease.

The third dough sample F2 contained 95% flour, 1.7% salt, 1.7% yeast and 2g/100kg protease.

The fourth dough sample F3 contained 95% flour, 1.7% salt, 1.7% yeast and 3g/100kg protease.

Each dough sample is divided in five circular consecutive dough patties witch are rested 20 min in the alveograph in a temperature-regulated compartment at 25 °C. Each dough patty is tested individually and the result is the average of the five dough patties.

*2.2. Methods of analysis.* The determination of the rheological characteristics of the dough was obtained by alveographic method.The alveographic method relies on measuring the resistance to biaxial stretch under air pressure of a dough sample prepared in standard conditions [5].

The dough patty is placed on the alveograph, witch blows air into it. The dough patty expands into a bubble that eventually breaks. The pressure inside the bubble is recorded as a curve on graph paper. The alveograph determines the gluten strength of dough by measuring the force required to blow and break a bubble of dough. The results include P Value, L Value, and W Value. Stronger dough requires more force to blow and break the bubble (higher P value). A bigger bubble means the dough can stretch to a very thin membrane before breaking. A bigger bubble indicates the dough has higher extensibility; that is, its ability to stretch before breaking (L value). A bigger bubble requires more force and will have a greater area under the curve (W value).

From the alveogram the following indicators were obtained:

• P Value is the force required to blow the bubble of dough. It is indicated by the maximum height of the curve and is expressed in millimeters (mm). It is also known as the viscosity or the value of maximum pressure that is in relationship to the resistance of the deforming dough (mm $H_2O$)
• L Value is the extensibility of the dough before the bubble breaks. It is indicated by 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 and is expressed in millimeters (mm).

• G Value is the 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 ($cm^3$) used to stretch the dough under bubble form.
• P/L Ratio is the balance between dough strength and extensibility. It is the rapport of configuration of the curve.

• W Value is the area under the curve. It is a combination of dough strength (P value) and extensibility (L value) and is expressed in joules. It represents 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 $mm^3$; L- the average abscise at breaking point in mm; S- surface of the curve, $cm^2$.

• Ie – elasticity index, represents the raport between the measured pressures, expressed in mm $H_2O$ to form bubbles after the insufflations of 200 $cm^3$ 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.

## 3. Results and Discussion

The dough samples alevograms are represented in Figures 1, 2, 3, and 4. Each dough sample alveorgam show the five dough patties tested (marked with different colors) and the parameters registered at the testing moment. The results of the samples are represented by the average value obtained from the values of the dough patties tests for each dough sample.

In Figure 1 the dough sample MARTOR alveogram represents the dough sample that does not contain protease. This sample is considered the standard blank sample. The alveogram's characteristics for flour used for bread have the fallowing values: P = [65 – 75mm], L = [130 – 150mm], G = [20 – 30], P/L = [0,5 – 0,6] and W > 180 $10^{-4}$J. The values for dough sample MARTOR, regarding the resistance of the deforming dough (P) and the balance between dough strength and extensibility (P/L ratio) are higher than the normal values. The values regarding the dough extensibility (L), expansion index (G) and the total quantity of absorbed energy during the dough deformation (W) are very low, therefore the dough is sensitive to stretch and can easily brake. It cannot be used for bread making.

**Sample MARTOR** (no protease)

Parameters
Lab. temp: 25 ºC
Moisture: 14.40%
Falling number (FN): 307 s

Results

P = 58 mm$H_2O$
L = 60 mm
G = 16.3
W = 163 $10^{-4}$J
P/L = 0,96
Ie = 54.55 %

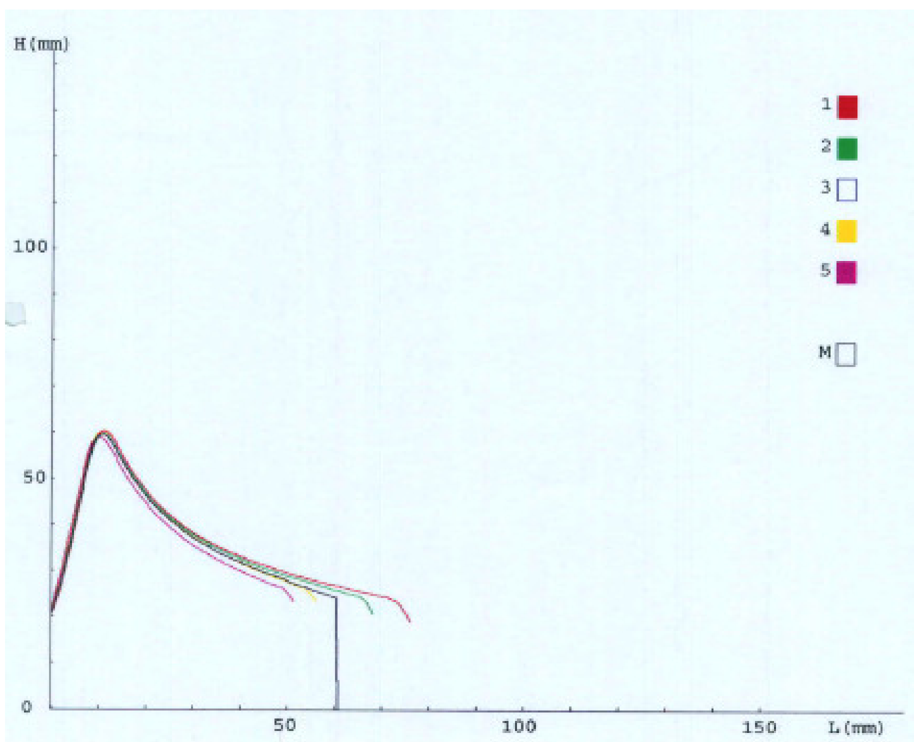


**Figure 1.** "MARTOR" (no protease) sample alveogram

**Sample F 1** (protease 1g/100kg)

Parameters
Lab. temp: 25 ºC
Moisture: 14.40%
Falling number (FN): 338 s

Results

P = 62 $mmH_2O$
L = 88 mm
G = 19.8
W = 179 $10^{-4}$ J
P/L = 0.70
Ie = 39.78%

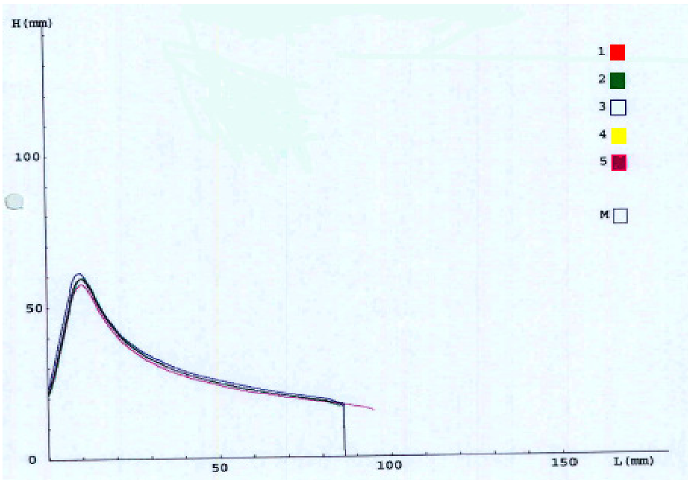


**Figure 2.** F 1 (protease 1g/100kg) sample alveogram

**Sample F 2** (protease 2g/100kg)

Parameters

Lab. temp: 25 ºC
Moisture: 13.30%
Falling number (FN): 350 s

Results

P = 69 $mmH_2O$
L = 129 mm
G = 23.5
W = 215 $10^{-4}$ J
P/L = 0.53
Ie = 30.11 %

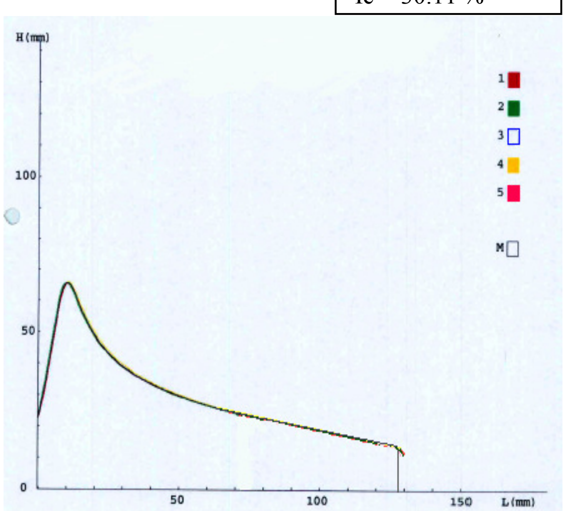


**Figure 3.** F 2 (protease 2g/100kg) sample alveogram

**Sample F 3** (protease 3g/100kg)

Parameters

Lab. temp: 25 ºC
Moisture: 13.30%
Falling number (FN): 292 s

Results

P = 55 $mmH_2O$
L = 63 mm
G = 18.2
W = 160 $10^{-4}$J
P/L = 0.87
Ie = 49.44 %

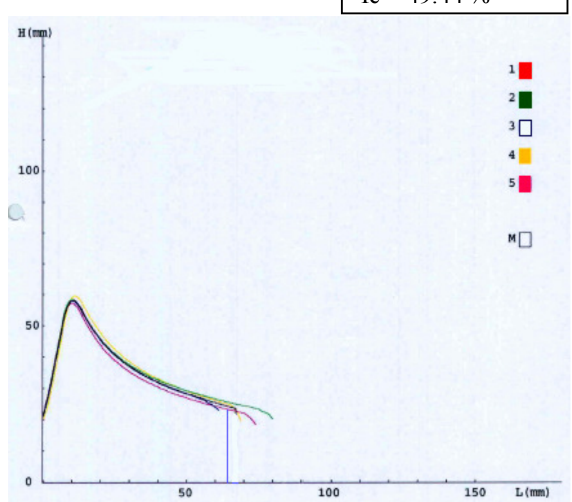


**Figure 4.** F 3 (protease 3g/100kg) sample alveogram

In Figure 2 is represented the alveogram of dough sample F 1 (protease 1g/100kg) that has in composition 1g/100kg protease. Compared with the blank sample MARTOR there are small differents regarding the dough strength (P) that increased only with 4 $mmH_2O$, but looking at the extensibility characteristics and the absorbed energy during the dough deformation we can see, obviously, the dough quality improvements. Only the P/L ratio and the Ie value have decrease because of the dough strength and the dough extensibility. Using 1g/100kg protease improves the dough elasticity and causes weakening of the gluten network.

In Figure 3 the alveogram of dough sample F 2 (protease 2g/100kg) represents the dough sample that contains 2g/100kg protease. There is a noticeable increase in all the indicators that suggests the improvement of the dough. The dought resistants to deformation (P) has increased and the dough extensibility characteristics (L and G) are higher than the value of dough sample MARTOR. Moreover, the elasticity index (Ie) has decreased and the total quantity of absorbed energy during the dough deformation (W) is with 52 $10^{-4}$ J higher then the value of dough sample MARTOR. This result expresses advantages of using protease in the preparation of the dough for bakery. The reduction of dough's consistency through the addition of proteases leads to the increasing of extensive character and the resistance of dough.

In Figure 4 is the alveogram for the dough sample F3 (protease 3g/100kg) that contains 3g/100kg protease. Addition of this dosage of protease, decreased the dough strength (P) with 3 $mmH_2O$ compared to the sample MARTOR. The absorbed energy during the dough deformation (W) is reduced and the P/L ratio are higher. There is a small increase of the extensibility characteristics. Dough with a high content of protease cannot be used for bread making because the dough elasticity is reduced and the overdose of protease destroys the gluten network, which completely loses its elasticity and the dough becomes sticky.

In Tabel 1 there are presented the characteristics of dough samples obtained by alveographic method.

The dough sample F 2 (2g/100kg protease) has the highest value for the following indicators, the dough extensibility characteristics (L and G), the total quantity of absorbed energy during the dough deformation (W) compared to the dough samples F 1 (1g/100kg protease) and F 3 (3g/100kg protease).

The dough sample F 1 (1g/100kg protease) presents improvments of the dough extensibility characteristics compared to the MARTOR sample. The dosage of 1g/100kg protease improves the dough elasticity and the processing quality but it does not achive the standard parametrs for bread.

The dough sample F 3 (3g/100kg protease) has the worst values for all the indicator compared to the dough sample F 1 (1g/100kg protease) and dough sample F 2 (2g/100kg protease). This dosage shows that the dough extensibility characteristics (L and G) and the total quantity of absorbed energy during the dough deformation (W) have decrease significantly.

**Table 1.** Alveograph results of the dough samples: MARTOR (no protease), F 1 (protease 1g/100kg), F 2 (protease 2g/100kg), F 3 (protease 3g/100kg)

| Sample | MARTOR (no protease) | F 1 (protease 1g/100kg) | F 2 (protease 2g/100kg) | F 3 (protease 3g/100kg) |
|---|---|---|---|---|
| **P($mmH_2O$)** | 58 | 62 | 69 | 55 |
| **L(mm)** | 60 | 88 | 129 | 63 |
| **G** | 16.3 | 19.8 | 23.5 | 18.2 |
| **W(10E-4J)** | 163 | 179 | 215 | 160 |
| **P/L** | 0.96 | 0.70 | 0.53 | 0.87 |
| **Ie(%)** | 54.55 | 39.78 | 30.11 | 49.44 |

## 4. Conclusion

The additive actions of complex enzymes as ameliorator on flour have positive effects on the rheological characteristics of dough. The technological characteristics of the flour and the nutritive value of the bread are characterized by the following variables: initial volume, fermentation time, flexibility, the dough condition to fermentation, water retention, maximum resistance, extensibility, final rise to baking, final volume of the bread, nutritive value, and energy value In order to improve these variables, different additives and substances are used in the bread manufacture, some of these being native components of the flour. The alveograph test provides results that are common specifications used by flour millers and processors to ensure a more consistent process and product. The alveograph is well suited for measuring the dough characteristics of weak gluten wheat. Weak gluten flour with low P value (strength of gluten) and long L value (extensibility), is preferred for cakes and other confectionery products. Strong gluten flour will have high P values amylase and is preferred for breads.

Addition of the correct dosage of protease in dough can improve the extension of freshness, increases of the quantity of fermentation sugars that can make finite products with a more pronounce color of crust.

A lower dosage of protease does not have a big improvement effect on the quality of the dough and it is not relevant for the technological process.

The overdose of protease leads to a wet and sticky content of the dough which affects the dough handling during the technological process and an abnormal volume and porosity.

Selecting a correct dosage of protease 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 bakery products with "clean label", more natural, this products being the product that enjoys the greatest interest from consumers.

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