

# Studies about Obtaining Safe and Healthy Bakery Products Using the Beneficial Properties of Enzymes

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**Abstract:** In present, most mills from our country obtain brown wheat flour in intermediate extraction, besides white flour, and its quality is mostly inadequate and unconstant. Our paper's purpose was the optimization of the brown wheat flour's quality with the help of enzymes, using some of its beneficial properties. Enzymes are natural compounds of many ingredients used in bakery, which are destroyed in the process and maintain the image of "clean product" of the finished product. Using the sinergetical of the following enzymes – laccase,  $\alpha$ -amylase and phytase we improved the dough's rheological properties and brown wheat flour in bread. By adding phytase we obtained an increase in bioavailability of minerals ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ) as a result of the enzyme's action on phytine.

**Key words:** brown flour, enzymes, minerals bioavailability, Mixolab

## 1. Introduction

Most of the processing technologies applied in the modern bakery-industry determine a decrease in nutritional substances of the raw-materials used in the process. Mostly, the resulted products have an increased energetical value and a decreased value in bio-active compounds. Either the inadequate intake of energy, but mostly of bio-active compounds, determines metabolic unbalances that are transformed in pathology. As a result, the quality of the product is very important. The interaction between food and health, of the potential novice action of food on the human body, and the solution through alimentation is a fact, and in the interest of certain specialists groups of nutrition [2].

In Romania, the population has an average annual consumption of bread 110 kg, and 66 kg in the Western states, and 83 kg in the Eastern states. In the context of major nutritional problems of certain population groups from our country, bakery product makers should think about developing new products able to respond the people's nutritional necessity. The Government has identified as a solution in the anemia control from the iron and folic acid deficit, white wheat flour strengthens. We consider that the consumers' education and obtaining attractive brown wheat and whole-wheat flour products are the alterative solution. In present, most mills from our country obtain brown wheat flour in intermediate extraction, besides white flour, and its quality is mostly inadequate and unconstant.

Our paper's purpose was the optimization of brown wheat flour's quality with the help of enzymes. Enzymes are natural compounds of many ingredients used in bakery. If an enzyme is added, it's sometimes destroyed by heat in the baking process. In both situations, the producers can obtain the beneficial effects of enzymes, while maintaining the image of "clean product" of the finished product. Enzymes are very specifically for certain tasks, eliminating undesirables effects. In this paper we've studied the effect

of laccase,  $\alpha$ -amylase and phytase on the rheological properties of dough from brown wheat flour and on the quality of the bread obtained. Rheological determination was made using Mixolab Chopin apparatus [7, 8]. The bread's quality was determined using the bakery tests. To point out the phytase effect on the minerals' bioavailability we determined the  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  quantity from bread.

## 2. Materials and methods

### Methods

The physical-chemical determinations were performed using the following methods:

- the moisture content was determined through the SR ISO 712:2005 [8],
- the ash content through the SR ISO 2171:2002,
- the gluten index through the SR ISO 21415-2:2007 (Sistem Glutomatic 2200, Perten Instruments AB),
- the wet gluten content through the SR ISO 21415-2:2007 (Sistem Glutomatic 2200, Perten Instruments AB),
- the Zeleny test through the ISO 5529 (Sadkiewicz Instruments – Poland, model SWD),
- the falling number value through the ICC 107/1 [6] (Falling Number, model 1400PT, Perten Instruments AB),
- granularity through the sieve (no. 0.5 and VIII),
- acidity through the SR 90:2007.

The rheological tests were done using the Mixolab Chopin.

The following parameters were recorded: water absorption, development time, stability at mixing, dough weakening (C2 and  $\alpha$ ), starch gelatinization (C3 and  $\beta$ ), amylase activity (C4 and  $\gamma$ ), starch gelling (C5).

### The baking test

The one-stage method was used to prepare the dough using a laboratory mixer. The dough was prepared at 29°C by mixing flour (100%), water (according to the water absorption capacity indicated by the Mixolab), salt (1.5%) and yeast (2%). The dough was fermented at 30°C for 120 min in a laboratory leavener and a intermediate re-kneading of 30 s was performed after 60 min. The dough was then divided in two pieces which were modeled and placed in trays. After a final leavening of 30 min, the trays were introduced into the oven. The baking was made at 230°C, for 35 min (when placing them into the oven, a steam tap was turned on for 10-15 s). After cooling (for 2 hours) the samples were weighed and analysed [1].

The  $\text{Ca}^{2+}$  ions were determined through the complexometric method with murexide, the  $\text{Mg}^{2+}$  ions through the Mann and Yoe colorimetric method and the  $\text{Fe}^{2+}$  through the method with bathophenanthroline [1].

### Materials

The experiments was achieved at the Arcada Mill (3300 kg/h).

Wheat (hectoliter weight was 78.4 kg/hl) had at first break rollers 16.5% moisture content. The wheat had 24.3% wet gluten content, 97.5% gluten index and the falling number was 427 s.

The brown flour was obtained through the complementary extraction, the fractions component are showed in table 1.

TABLE 1. The quality indices of the brown flour used in the determinations as witness sample

Parameters	Values
Fractions	FD, Div1, C4I, B3I, C1I, C1II, C1III, B4I, C9I, C9II, B5f, C6I, T100
Mositure content, %	13,7
Ash content, %	1.25
Wet gluten content, %	26.7
Gluten index, %	94.6
Test Zeleny, ml	23
Falling number, s	422
Acidity, grade	4
Granularity	Rest sieve 0.5 – 0.5% / Pass through sieve VIII – 76,4%

The investigated samples were obtained by mixing the flour with different additives: fungal  $\alpha$ -amylase (from *Aspergillus oryzae*) 5 g/100 kg flour, fungal laccase (from *Aspergillus niger*) 5 g/100 kg flour, fungal phytase (from *Aspergillus niger*) 5 g/100 kg flour.

### 3. Experimental design

For the analyses of the way in which the enzymes adding improve the dough and bread's quality we've established a factorial experimental plan in which the variables (the enzymes dosage used) have two values. This experimental plan is represented by several combinations (8 experiments) that form an experimental matrix, presented in table 2.

TABLE 2. Factorial experimental plan with 3 independent variables ( $2^3$ )

Experiment	Independent variables			Determination made
	1 phytase	2 laccase	3 $\alpha$ -amylase	
1	-	-	-	- Mixolab rheology - Baking Tests - Quantity of $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Fe}^{2+}$ in bread
2	+	-	-	
3	-	+	-	
4	-	-	+	
5	+	+	-	
6	+	-	+	
7	-	+	+	
8	+	+	+	

### 4. Results and discussion

The wheat from Romania's harvest of 2007, so the flour obtained from that are characterised through a low  $\alpha$ -amylase activity. Brown wheat flour used as a witness in our study had a falling number of 422 s. By  $\alpha$ -amylase add we aimed for the improvement of this index. The Falling Number value registered in the flour sample, analysed in the experiments made are presented in table 3.

TABLE 3. Falling Number for the samples of flour analysed in the experiment

Experiment	Adding made	Falling Number, s
1	witness	422
2	with phytase	433
3	with laccase	429
4	with $\alpha$ -amylase	350
5	with phytase and Laccase	438
6	with phytase and $\alpha$ -amylase	346
7	with laccase and $\alpha$ -amylase	329
8	with phytase, laccase and $\alpha$ -amylase	330

The  $\alpha$ -amylase add determined a slight decrease of the flour's water absorption capacity, while the laccase and phytase determined the increase of this index at 61,4-61.5%, in comparison with 60.4% registered in the witness sample's case.

The first part of the curve registered with the Mixolab is the one suitable for the dough's development, at 30°C and is similar to the one registered with the Farinograf. The flour's adding change the dough's development rate, expressed by the time in which the dough reach maximum resistance (C1), from 4.01 min. for the witness sample, 3.25-3.34 min for the samples with enzymes adding.

On the plateau that develops at constant temperature (30°C), after obtaining the value C1 (Nm), a stability of the dough's at deformation is registered. The period in which the couple and temperature are constant determine the dough's stability. The dough's stability at the samples added with laccase and  $\alpha$ -amylase has improved, while the samples with phytase add, the stability decreased (Fig 1.).

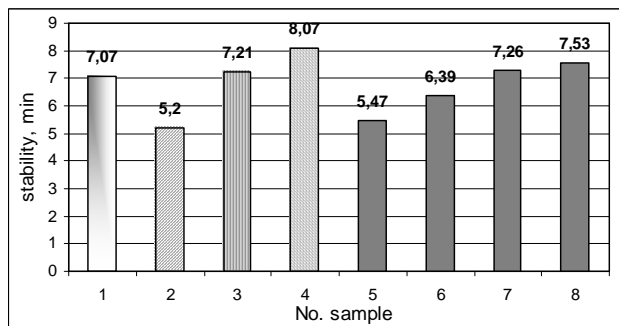


Figure 1. Dough's stability for the wheat samples investigated

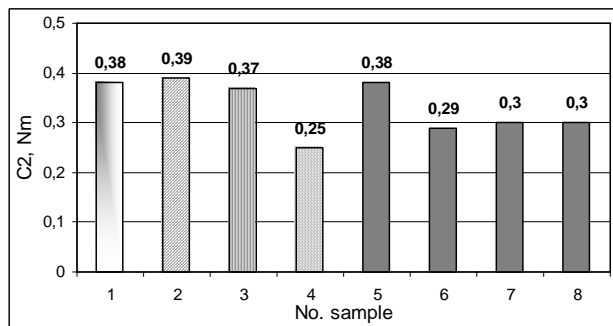


Fig 2. Couple C2, Nm, dough's for the wheat samples investigated

After the dough's stability period a couple decrease is registered, represented by the dough's mechanical soak, and explained by unfolding of the proteic-chain under the mechanical-pressure force. The increase of dough's temperature in the Mixolab determines the protein coagulation along with the release of most of the water at kneading (beginning at 45-50°C), that stresses the dough's soaking, that reaches a minimum value at a certain time [5]. The minimum forces couple at C2 from the Mixolab curve was register between 58-60.1°C, where some changes o the protein during heating can be hidden by the modifying of the starch's physico-chemical properties [3]. The lowest values for C2 were represent by the added samples with  $\alpha$ -amylase, 0.25 Nm, compared with 0.38 Nm for the witness sample and for samples with laccase and phytase (Fig 3.). That leads to larger values for the  $\alpha$ -slope. The proteolytic enzymes' activity is characterised in Mixolab by the  $\alpha$  slope.

Analysing the quality of the bread obtained at the baking test, we observed that the bread's volume was correlated with the decrease of the couple C2 (correlation coefficient- 0.76,  $p < 0.028769$ ).

The largest volume was obtained at the samples with  $\alpha$ -amylase add, phytase and  $\alpha$ -amylase add, laccase and  $\alpha$ -amylase add, and the sample with all the enzymes add, as observed in Fig 2., showed lower C2 values than in the other samples (table 4.).

As the temperature increases, the quantity of water in the dough also increases, because the water released from the proteins that coagulate. The released water determines the swelling and gelling starch, that leads to an increase in the dough's consistence. As the starch's gelling power increases and the  $\alpha$ -amylase activity decreases, the maximum consistence of the dough will be higher. The  $\alpha$ -amylase adding determines the decrease of the couple C3. So, at the samples with  $\alpha$ -amylase add, C3 showed values of 1.53 Nm, and 1.62 Nm at the sample with all the enzymes, in comparison with 1.85 Nm at the witness sample (Fig 3.).

On the second plateau with constant temperature, the dough's consistence decreases as the flour has an increased  $\alpha$ -amylase activity. So, the lowest consistence (C4) was obtained at the sample with  $\alpha$ -amylase, 1.34 Nm, in comparison with 1.39 Nm registered at the witness sample. In the other samples, the couple C4 has higher values as the witness sample.

TABLE 4. Quality parameters of bread obtained at the baking tests

Sample	Speciifc volume cm <sup>3</sup> /100 g bread x100	Porosity %	Elasticity %
P1	219	60.1	81.8
P2	242	62.0	93.1
P3	223	65.8	94.5
P4	267	68.4	89.3
P5	236	59.0	90.0
P6	269	69.5	94.2
P7	240	62.1	91.4
P8	239	62.8	96.6

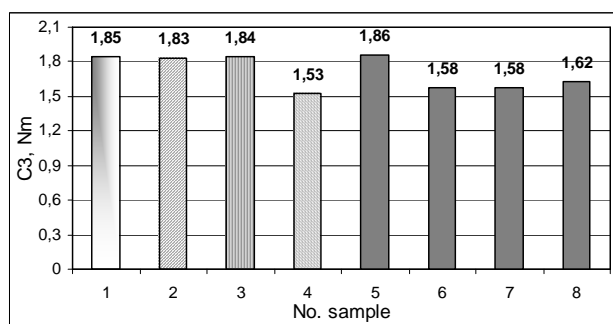


Figure 4. Couple C3, Nm, dough's for the samples of flour analysed

The last stage registered at the Mixolab takes place during the dough's cooling. The starch's gelling and retrogradation begins, that leads to the starch's increase in consistence. The starch's retrogradation is marked with Mixolab by the value of the couple C5, and has lower values at the sample with add the  $\alpha$ -amylase 1.99 Nm, compared with the witness- 2.53 Nm. We have to mention that at all the samples; C5 has lower values than the witness sample. The adding  $\alpha$ -amylase allows decrease of the retrogradation [4].

The curve segment obtained at the Mixolab that describes the retrogradation and gelling process was strongly affected by add the  $\alpha$ -amylase in the dough. In our research, this was more obvious because the witness sample had a low  $\alpha$ -amylase activity, because of the high value of the Falling Number, 422 s. In Fig 4. the comparison of the Mixolab curve registered for the samples that contain  $\alpha$ -amylase and the witness sample. As we can see from the witness sample, as in the other samples without  $\alpha$ -amylase add, C4 is registered in an area of slope,

and in the  $\alpha$ -amylase added samples that area doesn't exist. The phytase add determines a decrease of C5. This can be explained because the phytase releases Ca ions from the phytates that becomes available, as a co-factor for the  $\alpha$ -amylase activity.

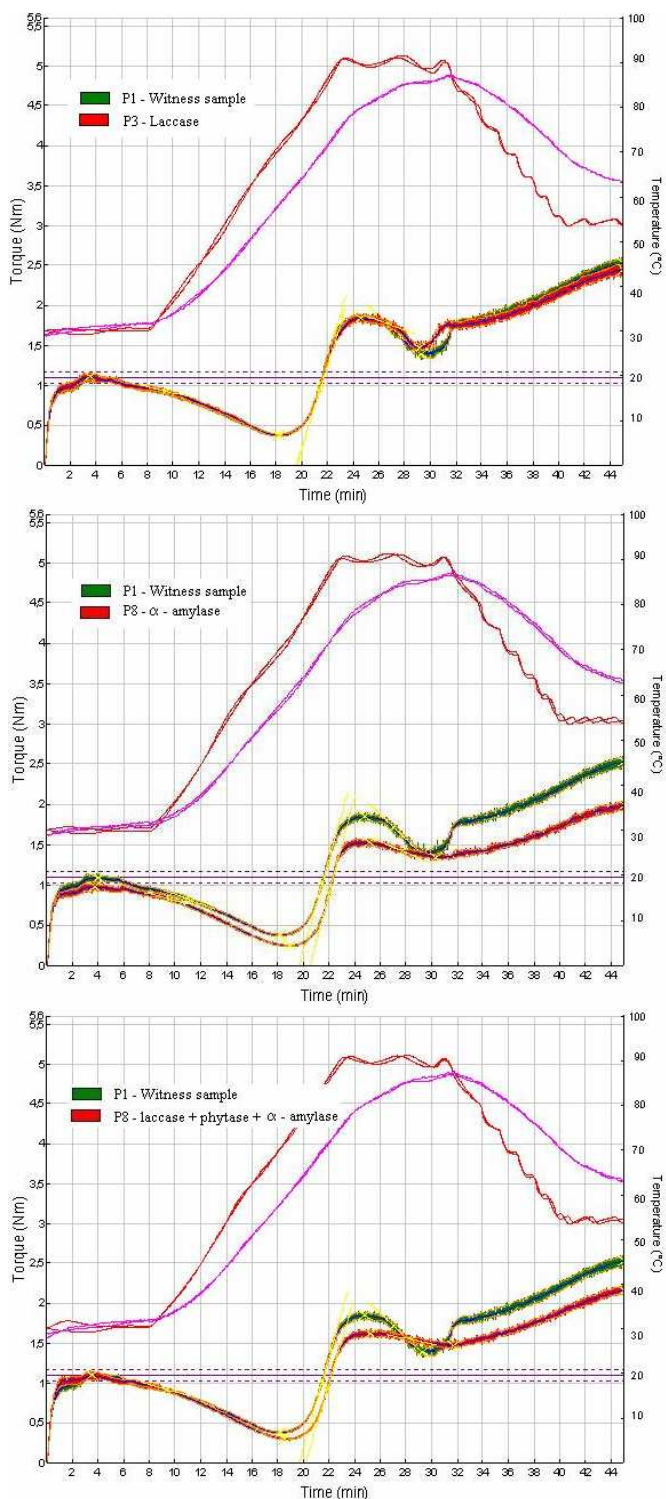


Figure 4. Curves registered with Mixolab

Lower values for C4 and C5 for the added samples are correlated with higher value of the bread.

The best results are registered for the difference C5-C4 that indicates the retrogradation decrease of the starch. As

shown in Fig. 5, C5-C4 presents, for the analysed samples three stages: 0.65-0.73 Nm for the flour with  $\alpha$ -amylase and phytase/laccase added, 0.98-1.04 Nm for the flour with phytase/laccase and 1.14 Nm for the witness sample.

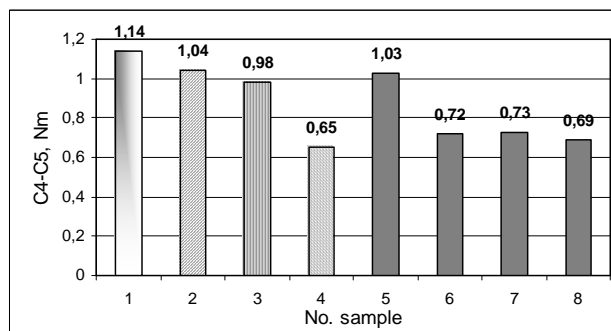


Figure 5. Retrogradation of the starch, C5-C4, for the samples of flour investigated

The flour samples that displayed the lowest values of the C5-C4 difference had a better quality of the core-freshness, porosity and resilience (table 4.). We've observed a significant indirect correlation ( $-0.73$ ) at ( $p < 0.038405$ ) between the specific volume of the bread and C5-C4.

The bread obtained from brown wheat flour with laccase and  $\alpha$ -amylase displayed an increased volume, without the problem of soaking during processing, compared with the bread obtained from the witness sample. This is explained by the laccase's capacity to oxidate the ferulic acid linked to the pentosan and tyrosine from the proteins and form covalent bridges, thus modifying the dough's viscosity.

Very good results were obtained at the brown wheat flour with phytase and  $\alpha$ -amylase. So, the bread had a specific volume, an increased porosity and viscosity than the samples obtained with  $\alpha$ -amylase or laccase and  $\alpha$ -amylase. This can be explained by the activation of the  $\alpha$ -amylase (low in the analysed flour, and very probable the adding of the  $\alpha$ -amylase, 5 g/100kg flour, wasn't enough), because the phytase releases Ca ions, that favour this enzyme's activity. We could say that the phytase add has a double effect: it improves the baking proprieties of flour by activating the endogenous  $\alpha$ -amylase and improves the nutritional proprieties of the products by releasing the bivalent ions - Ca, Fe and Mg from the phytate. For the samples with phytase add the Mg concentration increased by 2.4 times, the Fe by 1.4 and the Ca by 2.2 times, compared with the sample without phytase.

## 5. Conclusions

Using the synergic effect of enzymes - laccase,  $\alpha$ -amylase and phytase we've improved the rheological proprieties of the dough and the quality of the bread obtained from brown wheat flour. By adding phytase we have obtained an increase of bioavailability of the Ca, Fe and Mg ions, as a result of the enzyme's action on the phytine.

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