Effects of Some Bi-enzymatic Mixtures in Breadmaking Biotechnology I

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This paper presents the research results on using some bi-enzymatic mixtures as unique additives to improve the bread quality. Addition of some enzymes can intensify their actions during the breadmaking. Thus, one can use together glucose oxidase and hemicellulase, xylanase and α-amylase, xylanase and cellulase, amylloglucosidase and α-amylase, amylloglucosidase and glucose oxidase, and, of course, others mixtures. The aim of this study is to determine some of these bi-enzymatic mixtures on the quality of bread with and without the addition of wheat bran. The obtained results prove the positive effect of using some bi-enzymatic mixtures as additives, thus reducing or eliminating the use of chemical additives in the breadmaking technology.

Key words: bread technology, bi-enzymatic mixtures, bread quality

The aim of exogenous enzymes used in breadmaking is to improve the product quality, leading to an increase of its volume, a reduced stickiness and staling, and an increased shelf life. Enzymes can substitute the addition of different emulsifiers and other chemical additives used in bread production. There is strong evidence in literature which can sustain such a procedure. Due to changes in baking industry and to an increased demand for more natural products, enzymes have gained real importance in breadmaking, where they improve dough and bread quality leading to an improved dough flexibility, machinability, stability, loaf volume and crumb structure [1, 2].

The use of xylanase in combination with other enzymes presents a synergistic effect, leading to better results compared to its solely use [3]. By adding a mixture of glucose oxidase and hemicellulase in bread, the latter intensifies the action of glucose oxidase. Glucose oxidase improves the dough’s handling properties which were degraded by the addition of hemicellulase (it should be noted that hemicellulases increase dough stickiness) [4].

α – Amylase and hemicellulase added together lead to a reduction of the dough resistance and to an increase of its extensibility, but an improved elasticity, the effects of the two enzymes being synergistic [5]. The results show that the use of hemicellulase in combination with α-amylase has beneficial synergistic effects, improving the rheological characteristics of dough compared to their separate utilization. The substitution of hemicellulase with xylanase, in a certain proportion has the same effect on the dough rheology.

When glucose oxidase and α-amyrase were used in combination during the breadmaking, a synergistic effect was observed as a result of cooperation of the two enzymes in the dough [6]. It has also to be noticed the improvement of the external appearance of bread crust obtained by combining the two enzymes in comparison with the case of using only glucose oxidase.

The positive effects of hydrolytic enzymes have been related to both nonspecific and specific action of enzymes. The former appears to be associated with the release of water due to hydrolysis of the polymeric substrates to smaller components with a lower water capacity. For certain enzyme levels, the result is softer dough with superior handling properties and an improved bread quality. Both common and the specific mechanisms of enzymes were used to explain the effects of hydrolytic enzymes on the bread quality [7].

Several researchers suggested that the improvement in machining properties and bread quality can be, at least partially, attributed to the release of water that occurs when appropriate natural substrates (starch, protein, pentosan, or cellulose) are sufficiently hydrolyzed to reduce their water binding capacity. The released water can reduce dough viscosity resulting in a softer dough with better machining properties, and to an increased spring in the oven [8, 9].

Adding wheat bran to regular wheat flour has a detrimental effect on dough structure and bread quality. However it must be noted that flour strength (defined as the ability of the flour to produce quality dough with suitable behavior in breadmaking process) and the rheological properties of Einkorn wheat are low; bran particles being reported to have a negative impact on bread volume and crumb elasticity [10]. It has been proved that the addition of individual enzymes and/or emulsifiers (or a combination of these) can significantly improve bread volume and crumb firmness in whole-grain einkorn breads. Crumb porosity characteristics, however, were not affected. Synergetic interactions between dough quality improvers are implied [10].

This paper is concerned with the results derived from the following bi-enzymatic mixtures: glucose oxidase / hemicellulase on one hand and xylanase / α-amylase on the other. These were added to bran free breads and breads with added bran alike. A future paper will discuss results obtained from other bi-enzymatic mixtures.

Experimental part

Materials

Regular commercial white flour with the following properties: 13.58% water content, 59.17% hydration percentage, mineral substances 0.65%, 13.42% proteins, wet gluten content 28%, gluten index* 33, extensibility index** under 30cm, deformation index*** 12mm.

*The gluten index is defined as the percentage of wet gluten that passes through the special sieve of the Gluten Index Centrifuge. The index characterizes gluten quality.
as weak or strong. The gluten index parameter can take values between 0 and 100. The optimal value is considered to be between 65 and 80.

Wheat gluten extensibility is measured by the gluten extensibility index. A high degree of extensibility is usually desirable. The gluten extensibility index is represented by the length of a wet gluten wick extended until it reaches its breaking point. Measurements are made in centimeters. A distinction is made between weakly extensible (under 25 cm) and elastically extensible gluten (over 35 cm).

The deformation index of wheat gluten is measured through the shape change of a wet gluten ball on an horizontal plane at 30°C, by determining the difference between diameters before and after an 1 hour period. The optimal values for the deformation index range between 6-13 mm. A value lower than 6 mm shows evidence for a strong, tenacious type of gluten and a value larger than 20 mm defines a weaker gluten, characterized by a very rapid process of proteolytic degradation.

The wheat bran (B) used for the experiment had a 13.15% water content and 3.2% particles smaller than 660 μm defines a weaker gluten, characterized by a very rapid process of proteolytic degradation.

The following enzyme combinations were used:
- **Fermizyme GO1500 (G)** – an enzyme extracted from *Aspergillus niger* which contains glucose oxidase with an enzymatic activity of 1500 SARRET UNIT /g (Overseas Bakery & Ingredients Romania SRL București);
- **Fermizyme I (H)** – an enzyme extracted from the *Trichoderma viride* mold, which comprises hemicellulase with an enzymatic activity of 4100 XTU /g (Overseas Bakery & Ingredients Romania SRL București);
- **Bet’AsC (X)** – a pure concentrate of bacterial xylanase (Beldem Food Ingredients Romania);
- **Alpha Amylase (α-A)** – a pure concentrate of α-amylase (Puratos Romania).

**Baking Test**

The recipe used for making bran free bread consisted of: 800g of flour, 13g of yeast, 13g of salt, 473g of water according to the water absorption determined by farinograph, and the established level of enzyme mixture. The dough was kneaded with the aid of a laboratory mixer for 3 min. After 60 min of fermentation at 25-30°C, 1000g dough pieces were hand shaped into elongated forms and were left in the leavening chamber for 60 min at 25-30°C. The end product was baked for 35 min at 250°C.

The recipe for bread with added bran consisted of: 570g of flour, 30g of bran (5%), 10g of yeast, 10g of salt, 337g of water according to the water absorption determined by farinograph, and the established level of enzyme mixture. The dough was kneaded with the aid of a laboratory mixer for 5 min. After 60 min of fermentation time at 25-30°C, 660g dough pieces were hand shaped into elongated forms and they were left in the leavening chamber for 75 min at 30-35°C. The end product was baked for 35 min. at 250°C.

The usage limits for the enzyme preparations were determined through a trial - error process and only intervals in which the enzymes registered effects were chosen. Addition levels for each enzyme preparations represent arithmetical averages of the double trials results. Only levels with maximum impact on sample quality were chosen (i.e. maximum sample volume, porosity and elasticity of the crumb, suitable crust color). Bi-enzymatic mixtures were achieved by combining the addition levels of singular enzyme preparations.

**Analysis methods:**

Flour and bran quality analysis was carried out in accordance with STAS 90-77 and STAS 6283/1-83 regulations. Bread quality analysis was made according to STAS 91-83 [11] regulations.

**Results and discussions**

The Effect of the Glucose Oxidase and Hemicellulase Mixture on Bran Free Bread (G+H)

Six samples have been prepared: an enzyme free control sample, M, and 5 samples which contained combinations of different levels of glucose oxidase and hemicellulase, corresponding to their best results (maximum sample volume, maximum crumb porosity and elasticity in addition to a suitable crust color).

The best results for hemicellulase were achieved using 9g and respectively 11g of Fermizyme I/100kg of flour [12]; in the case of glucose oxidase the best results were obtained using 14, 18 and 22g of Fermizyme GO1500/100kg of flour [4].

The bi-enzymatic mixture contained: P1 - 11g H and 14g G/100g of flour; P2 - 11g H and18g G/100 g of flour; P3 - 11g H and 22 g G/100g of flour; P4 - 9g H and14g G/100 g of flour; P5 - 9g H and22g G/100 g of flour.

The stickiness of the dough containing the aforementioned bi-enzymatic composite shows different results according to the amount of hemicellulase present. Dough stickiness increases and decreases in direct relation to the amount of hemicellulase present, more hemicellulase leading to increased stickiness [13]. When the level of hemicellulase is constant stickiness is reduced when raising the level of glucose oxidase.

Additionally the dough mixing, fermentation and shaping tolerance is directly influenced by the level of glucose oxidase present in the mix. The prevalence of the glucose oxidase level over the level of hemicellulase positively influences the above mentioned dough proprieties [4].

All the samples containing enzymes presented an increased volume and improved elasticity/porosity compared to the control sample. The hemicellulase intensifies the effects of glucose oxidase [4], as shown in the graphics bellow (no.1 and 2).

The best result appears in case of sample P1 which presents a weight ratio of H/G=0.78, shows a 30% volume increase compared to the control sample and a porosity increase of 9.2%.

The Effect of the Glucose Oxidase and Hemicellulase Mixture on Bread with Added Bran (G+H+B)

Six samples have been prepared: a control sample without enzymes, M, and 5 samples which contained the same combinations of glucose oxidase and hemicellulase as previously detailed.

The addition of glucose oxidase and hemicellulase mixture produces the same results as with the bran free bread. The dough handling properties are improved through reduced stickiness and increased mixing, fermentation and shaping tolerances.

From a qualitative perspective all samples that benefited from the presence of enzymes featured an increased volume and improved crumb porosity/elasticity. Moreover by adding glucose oxidase with hemicellulase a noticeably lesser crust color intensity has been achieved. The explanation lies in the fact that glucose oxidase consumes part of simple carbohydrates.

The results are shown in figures 3 and 4.

The best result appears in case of sample P1 which presents a weight ratio of H/G=0.41, shows a 29.2% volume increase...
The Effect of Xylanase and α-Amilase Mixture on Bran Free Bread (X+α-A)

Six samples have been prepared: a control sample without enzymes, M, and 5 samples which contained various enzyme mixtures in different quantities that are representative for the best result obtained (volume, maximum porosity and elasticity, proper crust color).

The best results for xylanase correspond to 10, 11, 12, 13 and 14g Bel’ Ase C /100kg of flour [14], and for α-amilase the best values are 1, 2, 3, 4 and 5g Alpha Amylase /100kg of flour. The bi-enzymatic mixture contained: P1 - 10g X and 1g α-A /100g flour; P2 - 11g X and 2g α-A /100 g flour; P3 - 12g X and 3g α-A /100g flour; P4 - 13g X and 4g α-A /100 g flour; P5 - 14g X and 5g α-A /100 g flour.

The addition of the xylanase and α-amilase mixture prompted an increase in dough stickiness which is directly proportional to the quantity of enzymes present and tributary to the presence of α-amilase. Still the dough did not become hardly to handle. The qualitative features of samples containing this bi-enzymatic mixture are better than those of the control sample, as reflected by figures 5 and 6.

The best result appears in the case of sample P5 which presents a weight ratio of α-A/X =0.36, a 20% volume increase compared to the sample and a 6.6% porosity coefficient increase.

Control sample crust color is more intense compared to samples benefiting from the presence of xylanase alone [14]. The presence of α-amilase also appears to positively influence the bread staling process. It becomes obvious that a mixture of xylanase and α-amilase is more efficient than just using xylanase alone (13% volume and 2.6% porosity increase compared to the control sample [14]).

The reason lies in the fact that the α-amilase increases the amount of available fermentable carbohydrates and also acts upon dough stability within the fermentation process, in addition to influencing loaf volume and crust colour.

The Effect of Xylanase and α-Amilase Mixture on Bread with Added Bran (X+α-A+B)

Six samples were prepared: one control sample without enzymes, M, and 5 samples which contained the same combinations of enzymes showed before.

Results of the quality control check conducted on samples containing the bi-enzymatic mixture are shown in figures 7 and 8.

Akin to the previous case, in the presence of α-amilase, dough stickiness increases in direct relation to the enzyme level without altering the dough handling properties. All samples containing the bi-enzymatic mixture showed better volume and crumb porosity properties compared to the control sample.

The P5 sample registered the best values: for a weight ratio of α-A/X =0.36, a 9.8% volume and a 6.9% porosity increase was noticed compared to the control sample. By comparing these results with those obtained from the xylanase alone (with a 17.9% volume and a 9.7% porosity increase reported to the control sample [14]) it becomes
obvious that quality indicators have a lower value. Therefore it can be argued that \( \alpha \)-amilase does not improve the effect of xylanase in the case of bread with added bran.

## Conclusions

By adding glucose oxidase and hemicellulase together, the dough used for both bread types showed improved handling properties. These are reflected by a reduced stickiness and an increasing in the mixing, fermentation and shaping tolerances. All samples containing bi-enzymatic mixture developed better volumes, crumb porosity and elasticity in comparison to the control sample. Crust color became less intense due to fact that glucose oxidase consumed part of the simple carbohydrates. It appears that hemicellulase intensifies the effects of glucose oxidase which in turn corrects the negative effects of hemicellulase (for example dough stickiness).

The effects of the xylanase and \( \alpha \)-amilase mixture demonstrate that dough stickiness increases in direct relation to the enzyme level in both types of bread, due to presence of \( \alpha \)-amilase. However, the dough maintains its handling properties.

In the case of the bran free bread, the mixture of xylanase and \( \alpha \)-amilase proves to be more efficient than the presence of xylanase alone. The reason lies in the fact that \( \alpha \)-amilase increases the amount of available fermentescible glucides, the stability of the dough within the fermentative process as well as volume and crust color. At the same time, in the case of bran added bread, the mixture of xylanase and \( \alpha \)-amilase proves to be not more efficient than the presence of xylanase alone.

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