INFLUENCE OF A FUNGAL GLUCOSE OXIDASE ON QUALITY OF BREAD MADE FROM WEAK WHEAT FLOURS

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Abstract
There is a need to reduce the use of chemical oxidizing agents (potassium bromate, potassium iodate, benzoyl peroxide, calcium peroxide, azodicarbonamide, ascorbic acid) in the bread making industry. In order to meet the consumer demand for additives-free products, the baking industry is deeply involved in research for alternatives to these chemical improvers. For example, some enzyme preparations, especially oxidoreductases, may have the same positive effects on bread quality. Based on these considerations it was appreciated as being useful to make a study to reveal the influence of glucose oxidase addition on the quality of bread made from weak wheat flours, characterized by high deformation indices.
After its addition to dough, fungal glucose oxidase improved the physical properties of bread made with flour showing poor bread potential (fragile gluten network). The observed improve for bread volume was maximum 14,42%, for porosity maximum 5,48% and for elasticity maximum 6,25%, comparing with reference bread. The improving effect of the fungal glucose oxidase was at the same level to that obtained with ascorbic acid. The maximum score obtained for bread supplemented with fungal glucose oxidase (95,81 points) was superior that the reference bread (88,13 points) and almost at the same level as bread supplemented with ascorbic acid (95,93 points). From this point of view it can be said that fungal glucose oxidase may successfully replace chemical oxidizing agents in trials to improve the quality of bread obtained from weak wheat flours.

Keywords: glucose oxidase, gluten network, weak flours, sulphhydryl groups.

1. INTRODUCTION
Chemical oxidants (potassium bromate, potassium iodate, benzoyl peroxide, calcium peroxide, azodicarbonamide, ascorbic acid) are frequently added to flour to improve its breadmaking performance. Substituting these oxidants by enzymes such as glucose oxidase is a very interesting option [1]. One effect of glucose oxidase in the dough is to oxidize glucose to form gluconic acid with the aid of atmospheric oxygen, but the slight souring that occurs in the process is negligible; its other effect is to transform water into hydrogen peroxide. This oxidizing agent may act on the thiol groups of the gluten, either directly or via several pathways, inducing formation of disulphide bonds and thus tightening of the protein network [2, 3, 4]. Addition of glucose oxidase led to a stiffer and less extensible dough.

Based on these assessments, it was considered to be useful to undertake a study to reveal the influence of glucose oxidase addition on the quality of bread made from weak flours characterized by high gluten deformation indexes. It was also examined the influence of the duration of processing and products weight on characteristics of bread supplemented with glucose oxidase.

2. MATERIALS AND METHODS
2.1. Materials
Flours. In experiments, a weak white flour from SC COMPAN S.A. Targoviste (FA1) was used. The flour’s determined characteristics are summarized in Table 1. The flour’s quality indexes refer to the protein content (expressed by wet gluten content), moisture, the elastic-plastic characteristics of dough, purity and
content of non-starch polysaccharides (judged by the ash content) and α-amylase activity.

<table>
<thead>
<tr>
<th>Flour code</th>
<th>Moisture (%)</th>
<th>Ash (% dry weight basis)</th>
<th>Wet gluten content (%)</th>
<th>Gluten deformation index (mm)</th>
<th>Glutenc index</th>
<th>Alveogram parameters</th>
<th>Falling Number (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>13.30</td>
<td>0.51</td>
<td>27.68</td>
<td>24</td>
<td>12.18</td>
<td>P = 86mmH2O</td>
<td>298</td>
</tr>
</tbody>
</table>

It was used a fungal glucose oxidase (trade name OXY GO - provided by Enzymes & Derivates Romania s.a.) derived from Aspergillus niger, presented in the form of green-yellow powder.

Ascorbic acid (provided by Enzymes & Derivates Romania s.a.) is presented in the form of white crystalline powder, having a concentration of ascorbic acid 99.5-100%.

Compressed yeast. In baking tests, compressed baking yeast from Pakmaya (SC Rompak Pașcani LLC) has been used.

Salt (sodium chloride) - having the characteristics in accordance with STAS 1465-72.

2.1. Methods

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

Determination of flour ash content using the burning method at 900-920°C. Ash is defined (ICC Standard No. 104/1) as the quantity of mineral materials which 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 relating the weight of the wet gluten to the weight of meal flour taken into consideration.

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

Determination of α-amylase activity in flours by the "Falling Number" (ICC Method 106/1, AACC 56-81B). The Falling Number is defined as the time in seconds required to stir and to allow a viscometer stirrer to fall a fixed distance through a hot aqueous flour suspension undergoing liquefaction due to the presence of α-amylase activity. The higher the α-amylase activity level, the faster the stirrer will fall through the suspension.

Alveographic method for determining the rheological properties of dough (ICC Method No.121, AACC 54-30A, ISO No 5530/4). Produced by Chopin, the Alveograph is an instrument that gives valuable information about the rheological properties of dough sample by measuring the pressures attained during the inflation of dough into a bubble. The alveogram characteristics are: P - known as the overpressure, P is the maximum pressure (mmH2O), measured as the maximum height (h) in mm on the alveogram and multiplied by a factor of 1.1, P value being 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 [5, 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 relying on the program, offering the possibility to correctly compare the obtained results. The dough was prepared using the direct method and the recipe (expressed for 100g flour):100g flour, yeast-3g (3%), salt-1.5g (1.5%), water – 60g (60%), additives - different doses related to flour weight. To determine the influence that the duration of process and the product weight have on bread quality, we used the technological regimes program P1 (180 minutes) and P2 (115 minutes) of Moulinex machines. The quantities flour used were 350 grams 300 grams respectively.

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 expressed for 100g product.

Determination of bread porosity - STAS 91-83 method. 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 cross-sectioned, removing the crust and crumb-shaped in three cylinders, from three different areas, which were subjected to measurement method.

Determination of bread elasticity - STAS 91-83 method. The method consists of pressing a piece of crumb cylinder for one minute and measure its return to the original position, after removing the force and after a rest for one minute. To achieve the analysis, crumb cylinders from the porosity test were used.

After completion of the baking test and determination of the physical properties of finished products (volume, porosity and elasticity), the authors proceed to establish a score based on the values obtained for these characteristics.

3. RESULTS AND DISCUSSION

As shown in Fig. 1, in both cases, the supplementation of FA1 flour with glucose oxidase leads to an increase in the volume of obtained bread. This means an increase in dough capacity to retain CO₂ resulted from the fermentation due to the indirect action of glucose oxidase that strengthens the gluten network and secondary arabinoxylan network as a result of involvement of hydrogen peroxide which, in turn, activates endogenous peroxidases.

The poor quality of gluten from FA1 flour may be due to an intense proteolytic activity and to the presence of high quantities of proteolysis activators that also contribute to matrix gluten weakening. Most endogenous proteases and the proteolysis activators contain sulphydryl as active groups. Their oxidation diminishes the negative effect on gluten network, reducing the tendency of dough deformation.

As for reference breads and those supplemented with glucose oxidase the value of specific volume is significantly lower in the case of longer technological regime. The limiting factor in process is the availability of oxygen. Besides other chemical reactions that consume oxygen, yeast needs oxygen before starting the actual fermentation, as it initially breathes instead of fermenting. Glucose oxidase, needing oxygen for work to strengthen the gluten, can act only in the early stages of kneading and fermentation; then, oxygen is almost absent from the dough because its consumption by yeasts. By increasing the duration of fermentation, the remaining active endogenous proteases, free sulphydryl group substances but also other hydrolytic enzymes have more time to determine the dough
weakening. Thus, the initial effect of strengthening realised by glucose oxidase is reduced. As a result, the dough retention gases ability will decrease by extending the duration of fermentation and the obtained bread will have a lower specific volume. Also, as the weight of dough piece increases, the specific volume decreases. This is due to additional weight which gluten network must support.

![Graph showing comparative changes in volume of bread supplemented with glucose oxidase](image1)

**Figure 1.** Comparative changes in the volume of bread supplemented with glucose oxidase in two different variants of the technological scheme for different masses of the pieces of dough (FA1 flour).

![Graph showing comparative changes in porosity of bread supplemented with glucose oxidase](image2)

**Figure 2.** Comparative changes in the porosity of bread supplemented with glucose oxidase in two different variants of the technological scheme for different masses of the pieces of dough (FA1 flour).

In doughs with greater weight, ameliorative effect exerted by the enzyme may be weaker because the surface/volume ratio becomes increasingly smaller with increasing weight of dough.

However, the adoption of longer regime require the use of higher doses of glucose oxidase.
(almost twice than the other case) to achieve maximum specific volume of bread.
Fig. 2. shows that the bread porosity reaches higher values when the period of dough fermentation, consecutive of the strengthening action exerted by glucose oxidase, is less, time available for negative action of endogenous proteolytic enzymes on gluten being lower.

In both cases, maximum values of elasticity (Fig. 3) are achieved at the use of enzyme doses higher than those for which specific volume and porosity maximum dose are obtained.
The level of the physical characteristics of bread gives preeminence (mainly in terms of volume and porosity) to short process (Fig. 1, 2 and 3).

![Graph showing comparative changes in the elasticity of bread supplemented with glucose oxidase in two different variants of the technological scheme for different masses of the pieces of dough (FA1 flour).]

**Table 2.** The score obtained for physical properties of bread made from FA1 flour according to the dosage of glucose oxidase or L-ascorbic acid added and the technological regime adopted (P1, P2).

<table>
<thead>
<tr>
<th>FA1 (P1)</th>
<th>Glucose oxidase</th>
<th>FA1 (P1)</th>
<th>Ascorbic acid</th>
<th>FA1 (P2)</th>
<th>Glucose oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0ppm (PM)</td>
<td></td>
<td></td>
<td>88,13 p</td>
<td></td>
<td>88,13 p</td>
</tr>
<tr>
<td>1ppm</td>
<td>92,68 p</td>
<td></td>
<td>92,79 p</td>
<td>1ppm</td>
<td>94,07 p</td>
</tr>
<tr>
<td>3ppm</td>
<td>92,13 p</td>
<td></td>
<td>94,39 p</td>
<td>3ppm</td>
<td>94,84 p</td>
</tr>
<tr>
<td>4ppm</td>
<td>92,75 p</td>
<td></td>
<td>94,1 p</td>
<td>4ppm</td>
<td>95,1 p</td>
</tr>
<tr>
<td>5ppm</td>
<td>95,05 p</td>
<td></td>
<td>95,1 p</td>
<td>5ppm</td>
<td>94,33 p</td>
</tr>
<tr>
<td>7ppm</td>
<td>91,07 p</td>
<td></td>
<td>94,33 p</td>
<td>7ppm</td>
<td></td>
</tr>
</tbody>
</table>

From table 2, comparing the obtained bread scores in case of using glucose oxidase or ascorbic acid, it can be concluded that, by using the longer regime (P1), if supplementation with the two additives, the maximum score is at approximately the same level. In the case of application of short process, the calculated values are higher, both for the observable reference bread and for the ones supplemented with glucose oxidase and the dose of enzyme required to obtain the maximum score is lower.

**4. CONCLUSIONS**

Glucose oxidase is a suitable enzyme to improve the quality of bread made from poor
quality flours that form doughs with high deformability. Adding glucose oxidase to dough can lead to various physicochemical changes including cross-linking of wheat proteins. Consequently, the dough shows better viscoelastic/rheological characteristics and the baked bread has larger volume, porosity and elasticity. The effect is likely caused by the H₂O₂ produced by the enzyme.

The effect of dough strengthening is developed in the first part of breadmaking process (when the oxygen in dough is available for reaction catalyzed by enzyme). Technological regimes that give the best results are those with lower durations of fermentation that do not provide sufficient time for endogenous proteolytic enzymes negative action performed on the structure of gluten.

Improvement effects are more obvious when the weight of dough pieces is lower.

5. REFERENCES