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## Research Article

### Use of enzyme preparations for improvement of the flour baking properties

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#### Abstract

The main reason for the wheat flour poor quality is a deviation in the enzymatic complex and the biopolymers state due to poor agricultural and technical conditions, pest damage to grain stocks, unfavorable storage conditions. The use of various bakery improvements allows to make the technological process and the quality of bread better. The article presents research on the stabilization of the quality of bakery flour with low amylolytic activity (PE - 426 s) and strong gluten (the quality of gluten on the DIG (deformation index of gluten) device is 40 conditional units. Enzyme preparations with amylase and hemicellulase activity and sulfur-containing amino acid cysteine were used as improvers. The effect of each improver on the baking properties of the flour was determined by the results of the laboratory baking test. Using a complex of these baking improvers makes it possible to increase the efficiency of each component due to the synergy of their action. The complex of improvers in medium dosages showed best results of bread quality, organoleptic and physical parameters of bread improved, the specific volume of bread having increased from 2.4 to 4.3 cm<sup>3</sup>/g, i.e. in 1.8 times.

**Practical applications.** The use of enzyme preparations directly at the mills allows us to maximize the potential of raw materials, stabilize the quality of flour, produce flour with specified quality parameters and meet the needs of the bakery and confectionery industry.

**Keywords:** wheat flour, enzyme preparations, gluten content, amylolytic activity, hemicellulase activity, pentosans

#### Abbreviations:

DIG – deformation index of gluten

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## **Introduction**

The population growth poses a challenge to grain producers to increase gross yield which is related to a more intensive use of agricultural land and a new high yielding varieties introduction.

The disadvantage of this lies in the increase in grain quantity with low protein content and low baking properties. Therefore, it is necessary to correct the flour properties. In many countries the widespread use of exogenous enzymes in flour directly in mills makes it possible to obtain high quality flour from low quality raw materials (Kapreglianz 2009). One of the main problems of the milling and bakery industry is the production of high consumer properties products. However, differences in wheat types and varieties, in climatic and agro-technical conditions for growing and harvesting, in grain storage and processing technology determine a different quality.

Improvement and correction of flour properties are necessary to ensure their standardized quality for production of bread and pastry products.

According to numerous studies abroad it has been found that the flour baking properties depend on two major structural complexes: protein-carbohydrate proteinase and amylase. The rate of change in these complexes has an effect on the final product quality.

Therefore, one of the options to regulate baking in order to produce products with the desired high quality is the use of specific improvers that act on the protein and carbohydrate-amylase complexes (Gerasimova et al. 2004; Matveeva 2007).

Unlike bakery and pastry products a miller has the other requirements for the bread improvers use:

1. Bread improvers must not disturb the organoleptic and technological properties of flour. Accordingly, the additives must be in form of a dry powder with a flour particle size, ash content and moisture content that does not impair flour indicators and does not produce strong side odors and flavors (Meleshkina 2005).

2. When the food additive is added to the flour during production the problem with the high dosing and mixing must be resolved within a short period. Consequently, an important property of the preparation is the mealiness (Meleshkina 2005; Drobot et al. 2001).

3. The specificity of bread improvers being applied during milling is that they start to work in the liquid phase without affecting the flour in dry state. Determination of standard quality indicators may not show improvement in baking properties so it is advisable to assess the quality with a laboratory baking test.

4. An important requirement for the improver is the long shelf life since flour is a product that is exposed to longer storage.

5. The most important requirement for the quality of bread improvers is safety and that it should not have a negative effect on the human organism (Meleshkina 2005; Kondratiev and Kondratiev 2002).

Based on the above-mentioned requirements for the improver it was decided to use enzyme preparations of fungal origin that were inactivated during baking and thus did not have a negative effect on the human organism.

## **Materials and Methods**

### **Raw materials**

**Flour.** For this study a high-quality variety of flour from Ukrainian producer was used which was obtained under a shortened scheme of the technological process. The flour quality indicators are in accordance with the industry standard ISTU 46.004-99 “Wheat Flour. Technical conditions”.

**Improvers.** There were used enzymatic preparations with amylase and hemicellulase activity as well as sulfur-containing amino acid cysteine.

Enzyme preparations – improvers whose function consists of biochemical processes accelerating during dough fermentation and catalyzed by the enzymes contained in them (Popper 2010).

Wheat dough contains components, upon the enzymatic action, in which a change in dough properties can be achieved and the quality of the final product can be improved. The most important of these are starch, proteins, lipids, cellulose, hemicellulose, pentosans (Dubreil 2002).

$\alpha$ -Amylase is an endo-acting enzyme that randomly hydrolyzes the  $\alpha$ -1,4 glucosidic linkages in polysaccharides, resulting in short chain

dextrins. The  $\alpha$ -amylases degrade damaged starch in wheat flour into small dextrins thus allowing yeast to work continuously during dough fermentation, proofing and the early stage of baking. This results in improved bread volume and crumb texture. In addition, the small oligosaccharides and sugars such as glucose and maltose produced by amylases enhance the reactions for the browning of the crust and baked flavour. If the amylase content is low, this leads to low dextrin production and poor gas production. This in turn results in inferior quality bread with reduced size and poor crust colour (Hoseney 1994). However, this is not the only effect of  $\alpha$ -amylases one of the main effects is the reduction of dough viscosity during starch gelatinization (Pritchard 1986). Gelatinization of non-damaged starch granules starts at 55°C. This leads to amylose leaking out of the granules and initial melting of amylopectin crystallites. These events lead to a sharp increase in dough viscosity, which terminates oven spring. When  $\alpha$ -amylases attack gelatinized starch, this will result in a prolonged oven spring and thus larger volume (Kragh 2002). Wheat and wheat flour contain endogenous enzymes, of which amylases take an important part. However, the level of  $\alpha$ -amylase in some flour is sometimes very low and thus there is a need for wheat flours to be supplemented with  $\alpha$ -amylase van (Dam and Hille 1992).

Enzyme preparations with amylase activity are presented by Fungamyl 2500 SG. Fungamyl 2500 SG is  $\alpha$ -amylase of fungal origin that is produced from *Aspergillus oryzae*. Fungamyl hydrolyzes the 1,4 -glucosidic bonds of amylose and amylopectin from starch to form maltose and dextrins. This helps to increase the gas formation properties of the flour and to intensify the dough fermentation in bread production (Drobot et al. 2001).

Numerous studies have been performed to demonstrate the positive effects of pentosans-modifying enzymes, which are presented by industry as pentosanases, xylanases, arabinoxylanases and/or hemicellulases, here further referred to as xylanases (Hamer 1991; Rouau and Moreua 1986). Another effect ascribed to hemicellulases is to offset reduced gluten coagulation caused by pentosans by hydrolyzing

the pentosans to an extent whereby this effect is not longer occurring. It was shown in the paper, reported that the use of hemicellulases in a batter significantly improved gluten coagulation. The resulting gluten also was shown to have a much better bread-making quality. This effect was explained by the absence of any detectable pentosans in the remaining gluten, whereas normally 2-3% pentosans were attached to gluten. These gluten-linked pentosans were considered to have a steric hindrance effect on gluten coagulation (Collins and Gerday 2003).

Hemicellulases are broadly used in bread making, and depending on the application there is generally an appropriate hemicellulase or a mix of different hemicellulases that gives the desired properties (Hoseney and Faubion 1981), stability and oven spring and volume. This immediately indicates that there is not one single hemicellulase giving all desired effects in any application, but that the hemicellulase type(s), usage and dose rates need to be optimized in each case (van Oort 2010).

Enzyme preparations with the hemicellulase activity are presented by Pentopan 500 BG. which is purified from the enzyme obtained from the cultivation of *Humicola insolens*. This enzyme exhibits pentosanase activity (optimal pH 5-6). The use of this enzyme helps to stabilize the dough's properties, to increase the bread loaf volume, to improve the structure of the bread crumb, to extend the period within which the final products retain their freshness (Drobot et al. 2001; Kondratiev and Kondratiev 2002).

Cysteine hydrochloride (L-cysteine) – is a curative-improver that allows to regulate the dough rheological properties in the processing of flour with too low elastic gluten. Bread quality improvement by adding cysteine helps to keep a better bread fresh appearance. But an insignificant overdose of cysteine hydrochloride can degrade the dough properties.

**Experience staging.** The effect of the improvers presented by the enzymes Fungamyl 2500 SG, Pentopan 500 BG and the amino acid cysteine hydrochloride was evaluated through a laboratory baking test at different quantities.

**Determination of flour quality.** According to the requirements of the industry standard GSTU 46.004-99 “Wheat flour. Technical conditions” a good flour must be following these parameters: humidity – not more than 15 %, gluten content not less than 24 %, gluten quality not less than group II, ash content – not more than 0.55 %, whiteness – not less than 54 units. Falling Number – not less than 160 s.

All quality parameters have been determined in accordance with the methods of Ukrainian standards: standard GOST 9404-88. “Flour and bran. Methods for: Determination of humidity”; standard GOST 27839-88 “Wheat flour. Determination of the quantity and quality of gluten”; standard GOST 27494-87 “Flour and bran. Determination of ash content”; standard GOST 26361-84 “Flour. Determination of flour color”; GOST 27676-88 “Grain and grain products. Determination of the “Falling Number” according to Hagberg Perten.

**Evaluation of flour quality with a laboratory baking test.**

Trial laboratory bread baking was carried out for the form bread. The amount of water needed to mix the dough was determined based on the moisture content of the flour. According to the recipe for 100 g flour need 3 g yeast, 4 g sugar and 1.3 g salt. Three secondary kneading’s were made after the 90, 150 and 180 min from the beginning of the fermentation. The fermentation of the dough is carried out in a thermostat at a temperature of  $31 \pm 1^\circ\text{C}$ . After the completion of the fermentation the samples were molded manually. The end of the dough rising was determined by the organoleptic method. The baking was carried out in a humidified oven at  $220\text{-}230^\circ\text{C}$  for 20-25min. Bread quality should be evaluated not earlier than 4 hours after baking but not later than 24 hours after baking. The main indicators of bread quality are: organoleptic evaluation (bread appearance, crust surface and color, crumb shape, type of pores), loaf volume, porosity and specific loaf volume.

**Results and Discussions**

**Determination of flour quality indicators.** Flour quality indicators are: humidity – 15.0%; gluten content – 25.9%; gluten quality as measured by the EDG device for measuring the gluten quality – 40 units; whiteness – 58 units; ash content – 0.53%; Falling Number – 426 s. This flour has a low baking quality because of the strong gluten (40 units) and the low amylolytic activity (FN 426 s). The laboratory baking test showed that the control sample had a uniform golden crust but low loaf volume ( $350\text{cm}^3$ ), low porosity (70%) and average elasticity of bread crumb which could be explained by the low enzyme activity of the starting flour sample. In order to improve the flour quality it was decided to use the enzymes Fungamyl 2500 SG. Pentopan 500 BG and the amino acid L-cysteine hydrochloride.

**Effect of the enzyme Fungamyl 2500 SG on bread quality.** According to the manufacturer’s recommendations, the added quantity is  $0.002 - 0.01 \text{ g.kg}^{-1}$ . To test these recommendations the enzyme preparation was added into the test flour as follows: sample 1 – control sample; sample 2 –  $0.002 \text{ g.kg}^{-1}$  (minimum recommended quantity); sample 3 –  $0.005 \text{ g.kg}^{-1}$  (average recommended quantity); sample 4 –  $0.01 \text{ g.kg}^{-1}$  (maximum recommended quantity); sample 5 –  $0.02 \text{ g.kg}^{-1}$  (2 times the maximum recommended quantity).

**Table 1.** Indicators of bread quality with enzyme Fungamyl 2500 SG in different quantities

Sample	Bread improver	Quantity	Bread (loaf) volume, $\text{cm}^3$	Specific (loaf) volume, $\text{cm}^3.\text{g}^{-1}$	Porosity, %
Sample 1	Fungamyl 2500 SG	control	350	2.3	75
Sample 2		min	420	2.7	77
Sample 3		average	425	2.8	78
Sample 4		max	450	2.9	78
Sample 5		2 max	460	2.9	80

With the addition of minimum recommended quantity of Fungamyl 2500 SG the bread loaf volume was increased from 350 to 420 cm<sup>3</sup> (Table 1). This indicates that the enzyme preparation increases the amylolytic activity of the flour even in a low quantity. Additional quantity increase up to 0.005; 0.01 and 0.02 g.kg<sup>-1</sup> resulted in a gradual increase of the specific loaf volume by 70-100 cm<sup>3</sup> for each quantity.

With the increasing the bread specific loaf volume the physical properties of bread are also changing: the porosity increases from 75 to 80% and the pores size increases also. With a high quantity of 0.02 g.kg<sup>-1</sup> (2 times the maximum recommended quantity) the organoleptic properties of the bread are decreased, the crust surface is uneven and the porosity is uneven having large pores. For this sample, flour optimal quantity is 0.005 g.kg<sup>-1</sup>.

**Effect of the enzyme Pentopan 500 on bread quality.** The recommended quantity by the manufacturer is within the range of 0.02-0.10 g.kg<sup>-1</sup>. The action of the preparation was checked by the following quantities: sample 1 – control sample; sample 2-0.02 g.kg<sup>-1</sup> (minimum recommended quantity); sample 3-0.06 g.kg<sup>-1</sup> (average recommended quantity); sample 4-0.10 g.kg<sup>-1</sup> (maximum recommended quantity). The results from the laboratory baking test are presented in Table 2.

**Table 2.** Indicators of bread quality with enzyme Pentopan 500 BG in different quantities

Sample	Bread improver	Quantity	Bread (loaf) volume, cm <sup>3</sup>	Specific (loaf) volume, cm <sup>3</sup> .g <sup>-1</sup>	Porosity, %
Sample 1	Pentopan 500 BG	control	355	2.4	73
Sample 2		min	450	3.1	77
Sample 3		average	525	3.6	79
Sample 4		max	550	3.8	81

The bread with Pentopan 500 BG had a brighter crust and a larger loaf volume compared to the control sample. With the addition of 0.06 g.kg<sup>-1</sup> there was a significant increase in the bread loaf volume from 355 to 525 cm<sup>3</sup> and the porosity was

increased from 73 to 79%, the crust became thin and uniform.

With the next quantity increase the enzyme preparation increased the bread loaf volume to 550 cm<sup>3</sup> and the porosity up to 81%. These results suggest the possibility of using the enzyme preparation in an average quantity (0.06g.kg<sup>-1</sup>) to improve the bread quality.

Cysteine hydrochloride (L-cysteine) was added into the flour in the following quantities: minimum –0.05g.kg<sup>-1</sup>; average–0.1g.kg<sup>-1</sup>; maximum–0.2 g.kg<sup>-1</sup>. Amino acid L-cysteine did not significantly affect the bread quality. With the addition of the maximum quantity the bread loaf volume even decreased from 360 to 320cm<sup>3</sup> (Table 3).

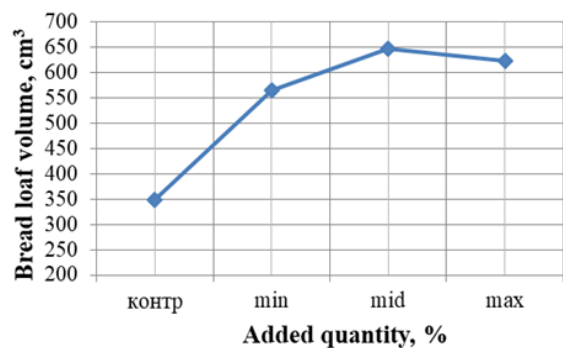
These quality changes are related to the low gluten (protein) content and low gas retention properties of the examined flour sample, which are further reduced when gluten is attenuated.

From the results obtained it was decided to use several enzyme preparations with a different principle of action as well as the amino acid cysteine to determine their complex influence on bread quality.

**Table 3.** Indicators of bread quality with enzyme cysteine hydrochloride in different quantities

Sample	Bread improver	Quantity	Bread (loaf) volume. cm <sup>3</sup>	Specific (loaf) volume. cm <sup>3</sup> /g	Porosity. %
Sample 1	Cysteine hydrochloride	control	360	2.4	73
Sample 2		min	310	2.0	72
Sample 3		average	320	2.1	70
Sample 4		max	320	2.0	70

**Effect of the enzyme preparations complex with amylase. Hemicellulose activity and sulfur-containing amino acid.** To improve the quality of the bread a complex of Fungamyl 2500 SG. Pentopan 500 BG and cysteine hydrochloride were prepared in minimal, average and maximum quantity. Fig. 1 shows the change in bread loaf volume with different quantities of a complex from bread improvers.



**Figure 1.** Bread loaf volume with different quantities of a complex from bread improvers

There was a significant increase in the bread loaf volume from 350 to 565cm<sup>3</sup> with a minimum quantity of the improver. The bread loaf volume increased to 650cm<sup>3</sup> with an average quantity of the improver. The maximum quantity resulted in a decrease in the loaf volume and an increase of porosity with uneven pores (Table 4).

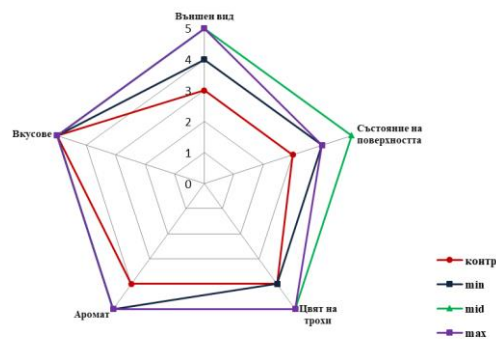
**Table 4.** Indicators of bread quality with a complex bread improver

Sample	Bread improver	Quantity	Bread (loaf) volume. cm <sup>3</sup>	Specific (loaf) volume. cm <sup>3</sup> /g	Porosity. %
Sample 1	Bread improver complex (Fungamyl 500 SG + Pentopan 500 BG + Cysteine hydrochloride)	control	350	2.4	74
Sample 2		min	565	3.9	83
Sample 3		average	650	4.5	81
Sample 4		max	625	4.3	84

The best results were shown with the use of the complex improver in an average quantity – the bread specific loaf volume increased from 2.4 to 4.3 cm<sup>3</sup>.g<sup>-1</sup> (in 1.8 cm<sup>3</sup> time).

Sensory profile of bread (Bread point estimate) is shown in Fig. 2.

The bread had a regular shape with a smooth crust surface, the color of crumb became lighter compared to the control sample, with a homogeneous color and good elasticity. Bread has a characteristic taste without lateral aftertaste.



**Figure 2.** Sensory profile of bread (Bread point estimate)

Enzyme preparations with a different principle of action allow the regulation of the dough alcoholic fermentation, the improvement of gas formation properties, the increase in flour water absorption and the intensity of the dough maturation.

Fungamyl 2500 SG compensates the lack of α-amylase in the flour. increases the dextrin accumulation, increases the gas formation and sugar formation properties of the flour and intensifies the technological process As for the flour obtained from the concise process scheme - the presence of high quantities of pentosans decreases the quality of the bread.

Pentopan 500 BG is an enzyme preparation with hemicellulose activity that affects insoluble high molecular pentosanes and increases the proportion of low molecular weight pentosanes which helps to form a more stable gluten structure. The addition of preparations with hemicellulose activity contributes to an increase of the proportion of the associated moisture in the dough. This leads to an increase in the water absorption properties of the semi-finished products and to improvement of the structural and mechanical dough characteristics.

L-cysteine positively affects the strong gluten, relaxes it and accelerates the dough maturation. The weakening of gluten and the increase in the elasticity with the addition of cysteine is explained by the change in the proportion of sulfhydryl groups and S-S bonds in proteins.



## Conclusions

Enzyme preparations with  $\alpha$ -amylase (Fungamyl 2500 SG) and hemicellulose (Pentopan 500 BG) activity in quantity of 0.005 g.kg<sup>-1</sup> and 0.06 g.kg<sup>-1</sup> respectively improve the quality and the bread loaf volume. In the creation and co-integration of a complex from enzyme preparations characterized by hemicellulose and amylolytic activity their synergistic effect is manifested – the bread loaf volume is increased and the organoleptic characteristics are improved.

The practical use of a complex from enzyme preparations is of great importance and finds application in the development of optimal bread improvers. The flour obtained from the concise scheme is with low amylolytic activity process (FN – 426 s) and strong gluten (40 units) and it is appropriate to use a complex of enzyme preparations aimed at improving of the bread quality.

Based on the studies in order to improve the baking properties of the flour it is recommended that a complex of enzyme preparations bread improver should be added in the following quantities: Fungamyl 2500 SG – 0.005 g.kg<sup>-1</sup> Pentopan 50 BG – 0.06 g.kg<sup>-1</sup>; Cysteine hydrochloride – 0.1 g.kg<sup>-1</sup>.

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