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ALVEOGRAPH AND BREAD MAKING QUALITY OF WHEAT DOUGH AS AFFECTED BY ADDED GLUCOSE OXIDASE

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Abstract: Although there is considerable interest in glucose oxidase, little is known about its activity on rheological characteristics of wheat dough and with bread making quality. Influence that the glucose oxidase exerts on rheological properties of the wheat dough is investigated on the basis of rheological determinations obtained with alveograph. Influence of glucose oxidase on bread making properties is determined on the basis of results obtained from the laboratory scale baking tests. In order to clearly establish relations between added dose of glucose oxidase (0.002; 0.004; 0.006 %) and rheological properties of wheat dough, as well as with bread making quality, analysis of dough with and without glucose oxidase (control sample) is performed and results are discussed with comparison with L-ascorbic acid as the most commonly used oxidative improver. Results show that glucose oxidase causes greatest increase of dough tenacity and causal reduction of extensibility. This leads to a negative effect on specific volume. Glucose oxidase causes increase of crumb hardness in smaller extent than L-ascorbic acid.

Keywords: bread-making, glucose oxidase, improver, alveograph, rheology

Introduction

Today, it is common practice to use oxidizing improvers in the preparation of bakery products in order to optimize the quality of wheat flour. Reduction of free sulfhydryl groups (*-SH groups*) effects of various oxidizing improvers on the process of protein networking is experimentally proved demonstrated (Sokol, Mecham and Pence, 1960; Hird and Yates, 1961; Bloksma, 1963; Tsen and Hlynka, 1963). Selomylio and Zhou, (2007) point that oxidizing improvers cause a significant increase in bread volume and optimization of all bread sensory quality characteristics that a customer takes into account in order to select a product. L-ascorbic acid is most used oxidative improver in practice. It is concluded that by traditional way of preparing the wheat dough, depending on wheat flour quality, the usual dose of L-ascorbic acid is 10-50 ppm but it can be as high as 100 ppm.

Glucose oxidase is an enzyme with oxidizing effect due to the release of hydrogen peroxide in catalyzed reactions. In the presence of oxygen, glucose oxidase catalyzes the oxidation of β -D-glucose to the α -D-glukonlaktone and hydrogen peroxide (Steffolani, Ribotta, Pérez and León, 2010). Due to these facts and release of hydrogen peroxide, glucose oxidase can be used in baking as oxidative improver, alone or in synergy with L-ascorbic acid.

The aim of this research, presented in this paper, is to investigate the effects of addition of glucose oxidase on the rheological properties and bread quality in comparison with the effects of L-ascorbic acid as the commonly used oxidation improver.

Material and Methods

Wheat flour used in the experiment is obtained by laboratory milling of wheat variety Pobeda. Flour had the following chemical parameters: protein: 12.6% (d.m.); moisture: 13.2%, ash: 0.518% (d.m.); water

absorption: 64.95%, wet gluten: 32.81%. FOSS Infratec™ 1241 Grain Analyzer is used for flour composition analysis. Accuracy and precision of determination are validated by interlaboratory tests.

Oxidation improvers added in the experiment are:

- **L-ascorbic acid** (Weifang Ensign Industry Co., Ltd., China), given that it is in the form of white crystalline powder, after dilution in water it is dosed. Three dosages are used (0.005%; 0.008%; 0.011% based on flour);
- **Glucose oxidase** (Bakezyme® GO 1500 BG - Royal DSM, Netherlands), given that it is in the form of brown crystalline powder, after dilution in water it is dosed. Three dosages are used (0.002%; 0.004%; 0.006% based on flour);

Other ingredients used in this work were: Salt (Salt product, Serbia), yeast (Kvas Ltd., Croatia), all were purchased at the local supermarket. For preparation of dough, drinking water of city of Novi Sad is used.

Biaxial extension: Parameters of wheat dough quality are defined according to modified method Chopin Alveograph® ICC Standard No 121 (ICC Standards, 1996).

Modification is reflected in the reduction of the amount of water added in salt solution for the amount of water added through the solution of water and oxidation improvers. The amount of salt is calculated according to the total water.

According to Seyhun, Sumnu and Sahin (2005) change in the hardness of bread crumb is a good indicator of bread aging process because there is an increase of hardness during storage. Increasing in the hardness of bread crumb is due to the reduction of moisture as well as due to the phenomenon of retrogradation (Biliaderis, Izydorczyk and Rattan, 1995).

Texture analysis: Hardness of bread crumb is measured on TA.XT2 Texture Analyzer (Stable Micro Systems, UK), using TPA (Texture Profile Analysis). Flat disk with compression diameter of 75 mm is used (accessory P/75).

Working conditions include the following adjustments:

- Speed probe before the test 1.0 mm / second;
- Speed probe during the test 5.0 mm / second;
- Speed probe after the test 5.0 mm / second;
- Deformation of 75%.

The first three slices, from both ends of the loaf are not used, for determination of bread crumb hardness middle slice is used. Measurement is performed in three replicates with slice thickness of 35 mm.

Preparation of bread

Baking test is performed using basic formula (expressed in % of the flour): wheat flour (100%), yeast (2%), salt (2%), pork fat (0.7%). Oxidative improvers are added in the form of solutions, each oxidative improver is added in three concentrations, the amount of added water is reduced by the amount of water added in solution of oxidative improver, so that the total amount of added water, provide dough consistency of 500 farinograph units, that is consistency of wheat dough for which it is known by empirical rheology experience that it gives the best possible quality of wheat bread. Baking test is conducted with the use of a mixer Diosna (Dierks & Söhne Maschinenfabrik, Osnabrück, Nemačka).

Dough fermented in the mass for 60 minutes, kneading is done by hand and then the dough is left to ferment for another 60 minutes, after which kneading is performed again. Dough ferment for another 30 minutes, then it is divided into pieces weighing 150 g, shaped into loaves and placed in bread molds with

following dimensions (24.5 x 9 x 6.5). Final fermentation is carried out at 30°C and 80% relative humidity for a period of 75 minutes, baking is performed for 15 minutes at 220°C with the addition of steam for 3 seconds in bakery oven (Thermodynamics, Croatia). After baking, bread is cooled up to room temperature for 2h and then for the next 22h stored in a climate chamber at 22°C with a relative humidity of 65%.

Specific volume: Two hours after baking bread, mass and volume are measured, volume measurement is conducted by displacement procedure of millet seeds (Kaluđerski and Filipović, 1998). Specific bread volume (cm³/g) is calculated as ratio of the mean value of volume and mass of four bread

Statistical analysis: Analysis of variance was performed using the software Statistica 9.0 (Statsoft, Tulsa, USA). Mean values were considered significantly different at P values ≤ 0.05.

Results and Discussion

The rheological characterisation of dough has been performed on the basis of the determinations carried out by the Chopin alveograph. The phases of the Alveograph method tries to simulate phases of dough processing (sheeting, rounding and molding) during baking process.

Generally, alveograph parameter (P) is considered as an indicator of the dough's tenacity. It is maximum pressure inside the dough bubble that cause rupture of bubble. Aldovrandi and Vitali (1995) indicate that values of parameter P for standard wheat dough quality range 60-80 mm H₂O, very good wheat dough quality 80-100 mm H₂O, whilst extra strong wheats dough are characterized by (P) value higher than 100 mm H₂O. Lowest value of parameter P and therefore dough's tenacity is found for the control sample. Due to activity of L-ascorbic acid, dough samples containing mention oxidizing improver, demonstrate greatest maximum pressure that dough bubble can retain, that means that L-ascorbic acid exhibit improving effect on strength of the gluten network.

Parameter of extensibility (L) of 100 mm is generally considered as good for bakery production. All investigated samples with L-ascorbic acid have (L) values similar to the (L) value of control sample. Parameter (L) of control sample is not in optimal range for bakery production. From results it can be concluded that samples with added glucose oxidase poses lower extensibility than samples with added L-ascorbic acid or control sample and that both oxidising improvers degraded desirable property of extensibility. Swelling index (G) represents measure of dough extensibility. As well as parameter L, swelling index is lower in all samples with added oxidising improvers.

The ratio P/L of 0.50 indicates either resistant and very extensible wheat dough or moderately extensible and less resistant wheat dough. Value of 1.50 indicates very strong and moderately extensible wheat dough, whilst wheat dough with P/L value in the range 0.40-0.80 is suitable for bakery production. Ratio of P/L increased with the increase of added ascorbic acid dose. P/L ratio of samples with glucose oxidase shows same tendency as with addition of L-ascorbic acid. Compared to the control sample all samples with added oxidising improvers posses bigger P/L ratio, addition of glucose oxidase in dose of 0,006% show greatest increase of P/L ratio.

Standard quality wheat dough is characterized by (W) value in range 160-200, whilst good quality wheat dough is characterized by (W) value in the range 220-300 and higher than 300, respectively (Bordes, Branlard, Oury, Charmet and Balfourier, 2008). Parametar of energy (W) is higher in all samples treated with oxidising improvers compared with control sample, samples with added glucose oxidase exhibit the greatest enlargement. L-ascorbic acid increase elasticity index (Ie) which is indicator of dough elasticity, all samples with glucose oxidase show increased rigidity of dough.

Remarkable fact is that glucose oxidase creates some effects as L-ascorbic acid, but their magnitude is greater. All samples with doses of glucose oxidase have higher maximum pressure (P), P/L ratio and

lower (L) and (G) values. While some parameters and these are dough energy (E) and elasticity index (Ie) have lower values. Results are shown in table 1.

Table 1. The parameters obtained by the alveograph for the control dough and dough with different doses of L-ascorbic acid and glucose oxidase

Sample Dose (%)	P	L	G	W	P/L	Ie
Control	92	77	19.5	236	1.19	52.2
L-ascorbic acid						
0.005%	98	75	19.3	252	1.31	53.3
0.008%	99	78	19.7	267	1.27	54.7
0.011%	99	73	19.0	257	1.36	55.5
Glucose oxidase						
0.002%	105	69	18.5	253	1.52	53.1
0.004%	109	66	18.1	249	1.65	50.3
0.006%	174	34	13.0	245	5.12	0.0

P-maximum pressure (mm H₂O), L-extensibility (mm), G-swelling index (mm), W-energy (J 10⁻⁴).

P/L- ratio, Ie- elasticity index (%)

Strengthening effects of glucose oxidase is associated with gluten protein crosslinking via disulfide bonds (Primo Martin, De Beukelear, Hamer and Van Vliet, 2003). But unlike L-ascorbic acid effects are associated and with oxidative gelation of soluble pentazans (Vemulapalli, Miller and Hosney, 1998).

Evaluation of L-ascorbic acid and glucose oxidase as oxidative improvers is complemented with baking tests. The variations of bread quality parameters is shown in tables 2 and 3. It is obvious that the specific volume depends on both, dose and used oxidative improver.

Lowest and highest dose of L-ascorbic acid, increased specific volume of bread compared to control sample. Statistically this two samples are most similar to one another and different from medium and highest dose of glucose oxidase. Medium dose of L-ascorbic acid shows negative effect on specific volume of bread. This means that increase of L-ascorbic acid dose will not necessary result in increased specific volume of bread.

Table 2. Effect of adition of glucose oxidase and L-ascorbic acid on the specific volume of bread

Sample	Specific volume (cm ³ /g)	Standard dev.
Control	3.530 ^{ab}	0.060
L-ascorbic acid		
0.005%	3.603 ^b	0.064
0.008%	3.432 ^{ab}	0.190
0.011%	3.605 ^b	0.105
Glucose oxidase		
0.002%	3.442 ^{ab}	0.167
0.004%	3.404 ^a	0.044
0.006%	3.385 ^a	0.107

Results are the mean values of four replicates, values labeled with identical uppercase letter in column are not significantly different ($P \leq 0.05$)

Addition of glucose oxidase exhibit negative effect on specific volume in all doses. Lowest dose of glucose oxidase is statistically similar to the control sample and medium dose of L-ascorbic acid. Negative effect on specific volume of bread compared to the control sample are due to too strong gluten network with insufficient extensibility which produce rigid dough and bread with lower specific volume which is unfavorable characteristics by consumer.

Different authors claim different effects of glucose oxidase on bread specific volume. Martínez-Anaya and Jiménez (1998) claim that addition of glucose oxidase produce positive effect on specific volume while some authors claim that effect of glucose oxidase on specific volume is negative or not noticable (Risiah, Suttom, Low, Lin and Gerrard, 2005; Vemulapalli et al., 1998).

On the basis of data presented in table 3 it can be observed that with the addition of both oxidation improvers, bread crumb hardness is statistically significantly modified, compared with the control sample. Addition of glucose oxidase in doses over 0.004% and L-ascorbic acid in doses over 0.008% increase hardness of breads significantly compared to the control sample.

Table 3. Effect of addition of glucose oxidase and L-ascorbic acid on the bread crumb hardness

Sample	Hardness (g)	Standard dev.
Control	5332 ^a	905.722
L-ascorbic acid		
0.005%	7717 ^{abcd}	822.009
0.008%	10095 ^{de}	344.411
0.011%	11168 ^e	2922.411
Glucose oxidase		
0.002%	6749 ^{ab}	1135.613
0.004%	11454 ^e	1090.786
0.006%	8087 ^{bcd}	921.457

Results are the mean values of three replicates, values labeled with identical uppercase letter in column are not significantly different ($P \leq 0.05$)

According to the results, glucose oxidase added in all concentrations has led to increase of hardness, compared with the control sample. This finding is not in accordance with research of Bonet, Rosell, Caballero, Gomez, Perez-Manuera and Liuch, (2006), they are of opinion that glucose oxidase added in doses less than 0.005% creates softer bread crumb compared to the control sample. Sample with glucose oxidase added in a minimum concentration of 0.002% provided a minimum hardness of sample bread crumb, with added oxidising improver.

This effect could be associated with the ability of pentazans to absorb large amounts of water in the process of gelation under the influence of glucose oxidase, flour quality as well as with laboratory baking test procedure and it should be investigated futher.

Conclusions

In general, three major parameters of wheat flour for bakery production are characterized by medium tenacity (P), medium or high energy (W) and medium (L) values.

Investigation of the activity of glucose oxidase show greatest increase of dough tenacity and reduction of extensibility for all samples of wheat dough, compared with control sample and samples with

L-ascorbic acid. Relationship of this two parameters is not within the desired limits, i.e., strong and short wheat is obtained.

The use of glucose oxidase in higher doses leads to a large networking of gluten, with consequent increased rigidity of dough.

Largest improvement of bread quality, in terms of increase of specific volume is observed in sample with highest dose of L-ascorbic acid, statistically there is no difference between highest and lowest dose. Addition of glucose oxidase in all doses exhibit negative effects on the specific volume. This observation is in accordance with claims of some authors.

Addition of both oxidative improvers resulted in the increase of bread crumb hardness with significant changes compared to the control sample. Crumb hardness of sample with lowest dose of glucose oxidase exhibit statistically greatest similarity with control sample. All samples with glucose oxidase exhibit lower parameter of hardness compared to the corresponding dose of L-ascorbic acid.

On the basis of the results obtained, glucose oxidase should be used with L-ascorbic acid and could provide the improvement of alveograph parameters, as well as crumb hardness.

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