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INFLUENCE OF MIXING PROCEDURE OF WHEAT DOUGH WITH ADDED OXIDATIVE IMPROVERS ON THE TEXTURE AND COLOR OF WHEAT BREAD

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Abstract

The importance of oxidative improvers and dough mixing, knowledge of their interdependence on achieving the optimal quality of wheat bread properties is still actual problem. In this experiment influence of the mixing procedure on the wheat bread quality with added oxidative improvers is determined.

Dough is prepared from wheat flour obtained with addition of oxidative improvers at fast and intensive mixing. Oxidative improvers added to wheat flour are: commercial pure L-ascorbic acid (0.005; 0.008, and 0.011%), hydrogen peroxide (0.001; 0.002 and 0.003%) and glucose oxidise (0.002; 0.004 and 0.006%).

In order to clearly establish relations between added oxidative improver and mixing procedure, texture and color measurement is performed. Hardness of bread crumb is measured by means of Texture Profile Analysis - TPA. Color is measured by CIELAB color system and specific volume of bread is determined by millet seed displacement.

It was found that all added oxidative improvers influence in a different way texture and colour of wheat bread crumb, respectively the finished products obtained from them. Samples with added glucose oxidase exhibit lower parameter of hardness as compared with the other two oxidative improvers. Glucose oxidase cause greatest changes in crumb colour as well as negative effect on specific volume of samples, similar to hydrogen peroxide, observed accordingly to fast mixing procedure. Intensive mixing procedure produce positive effect on specific volume and causes lower parameter of hardness, compared to fast mixing when samples of same oxidative improvers are compared.

Thus depending on the type of oxidative improver and added quantity of oxidative improver, the quality of produced wheat bread is different. On the basis of the results obtained, glucose oxidase used with L-ascorbic acid should provide the optimal improvement of investigated parameters when their ratio is adjusted to the mixing procedure used.

***Key words**: Color, Mixing, Oxidative improver, Texture, Wheat.*

1. Introduction

The first significant process in the preparation of wheat bread is blending together of the ingredients. During this stage, a number of significant changes take place. Water plays a key role in solubilisation, hydration and dispersal of used ingredients. All these processes depend on the mixing action employed. In past, mixing would have been done by hand; nowadays it is most commonly performed by some type of mechanical mixing device. Energy transfer and incorporation of air during process of mixing are fundamental to the manufacture of wheat bread. During process of dough mixing air is incorporated in dough structure that is captured with formation of gluten network. This process is strongly related with mixing intensity. Transfer of energy during mixing process is essential for developing a gluten network of wheat bread. Transfer of energy to dough is of such importance that it might be considered as an ingredient in itself. In general terms, the greater the transfer of energy to the dough in process of mixing the greater the improvement in dough gas incorporation and retention and therefore the greater the bread volume. Characteristic highly preferred by customers. Eventually, depending on wheat dough characteristics, a point is reached when transferring more energy confers no extra gas incorporation and retention and even deteriorates dough.

Today, it is common practice to use oxidative improvers in the preparation of wheat dough products in order to optimize the quality of gluten network and retention of air. Oxygen is natural oxidative dough improver. Oxygen reacts in all process of dough formation: flour storage, mixing, dough forming, and dough maturation. Mixing wheat dough in atmosphere of oxygen, oppositely from mixing of atmosphere of nitrogen, leads to an increase in resistance and decrease in the elasticity of dough [1].

Reduction of free sulfhydryl groups (–SH groups) effects of various oxidative improvers on the process of protein networking is experimentally proved demonstrated [1, 2, 3, 4]. Selomylio and Zhou, [5] point our that oxidative 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 an 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 0.001% - 0.005%, but it can be as high as 0.01%.

During development of intensive mixing process, without fermentation in mass, it is noticed that use of L-ascorbic acid can reduce energy that is needed for dough formation. By recommendation of "Chorleywood Bread Process" 40 kJ of energy per kilogram of dough is needed for no longer than 5 minutes.

Glucose oxidase, given its enzymatic nature, among consumers has the attribute of "naturalness" and therefore safety. Influence that glucose oxidase manifest upon the dough and the quality of the finished product should be quantified and compared with the most frequently used oxidizing improver, L-ascorbic acid.

Limiting factor of reaction is presence of oxygen while in dough's with yeast, presence of glucose plays important role.

Since glucose oxidase catalyzes the reaction in which a hydrogen peroxide is generated and which exhibits its oxidizing effect on dough, hydrogen peroxide was used to examine differences in the activities of added hydrogen peroxide solution and adding glucose oxidase.

According to World Health Organization (WHO) there is no evidence that peroxidase manifest carcinogenic effect [6]. When present in wheat dough hydrogen peroxide is active manifest oxidative activity, in dough it can be introduced or created by yeast activity or enzymes

Yeasts use about half of present hydrogen peroxide, while other half manifest oxidative activity on dough (Liao *et al*. 1998). Research has shown that presence of hydrogen peroxide in dough leads to protein denaturation and to agglomeration of protein molecules up to 53 kDa [7]. According to results of Toyosaki, addition of hydrogen peroxide and hydrogen peroxide plus hydrogen peroxidase strongly influence process of creation of dityrosine links in dough [8].

It is known that air incorporated in process of mixing wheat dough plays an important role in activation of oxidative improvers and scope of their action. Oxygen is needed for oxidative effects of L-ascorbic acid. In absence o oxygen L-ascorbic acid manifest a reduction activity [9].

The aim of this research, presented in this paper, is to investigate the effects of addition of oxidative improvers depending on the mixing procedure used on the wheat bread quality.

2. Materials 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 InfratecTM 1241 Grain Analyzer is used for flour composition analysis. Accuracy and precision of determination are validated by interlaboratory tests.

Oxidation improvers used in the experiment are:

- L-ascorbic acid (Weifang Ensign Industry Co., Ltd., China), given that it is in the form of white crystalline powder, it is added as a water solution. 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, it is added as a water solution. Three dosages are used (0.002%; 0.004%; 0.006% based on flour);
- Hydrogen peroxide (98%) is added as a water solution. Three dosages are used: (0.001; 0.002; 0.003% 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 Novi Sad is used.

Preparation of bread

Baking test is performed using basic formula (expressed in baker's percentage): 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, on that consistency wheat dough gives the best possible quality of wheat bread as it is known by empirical rheology experience.

Fast mixing process

Baking test is conducted with the use of a mixer Diosna (Dierks & Söhne Maschinenfabrik, Osnabrück, Germany). Dough is mixed at 80 rotations per minute for 300 sec.

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 ^{0}C and 80% relative humidity for a period of 75 minutes, baking is performed for 15 minutes at 220 ^{0}C with the addition of steam for 3 seconds in bakery oven (Thermodynamics, Croatia). After baking, bread is cooled up to room temperature for 2hr and then for the next 22h stored in a climate chamber at 22 ^{0}C with a relative humidity of 65%.

Intensive mixing process

Baking test is conducted with the use of a mixer Diosna (Dierks & Söhne Maschinenfabrik, Osnabrück, Germany). Dough is mixed at 1400 rotation per minute for 100 sec. Dough fermented in the mass for 15 minutes, kneading is done by hand and 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 ^{0}C and 80% relative humidity for a period of 75 minutes, baking is performed for 15 min at 220 ^{0}C with the addition of steam for 3 seconds in bakery oven (Thermodynamics, Croatia). After baking, bread is cooled up to room temperature for 2 h and then for the next 22 hr stored in a climate chamber at 22 ^{0}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. Specific bread volume (cm^3/g) is calculated as ratio of the mean value of volume and mass of four bread samples.

Texture analysis

Hardness of bread crumb is measured 24hr after baking 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 includes the following adjustments:

- Speed probe before the test 1.0 mm/sec.
- Speed probe during the test 5.0 mm/sec.
- Speed probe after the test 5.0 mm/sec.
- 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.

Determination of bread color

Bread color is determined 24 hr after baking in five replicates using a colorimeter Minolta Chroma Meter CR-400 (Konica Minolta Sensing Inc., Japan) with a diameter of contact area of 8 mm. Before the measurement, instrument calibration is carried out with standard white color. Mean value are presented according to CIELAB color system. L * lightness of color-coordinate (where 0 indicates black and 100 white color), a* - red-green coordinate (where a* + indicates red and a*- indicates green color) and b* yellow-blue coordinate (where b*+ indicates yellow b* - blue color).

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.

3. Results and Discussion

Specific volume: Value of wheat bread specific volume with addition of oxidative improvers depends upon applied concentration, added oxidative improver and of the mixing process used.

In Table 1, specific wheat bread volumes are presented. Values of specific volumes are compared on the basic of added oxidative improver as well on mixing process applied.

By comparison it is found that mixing process exhibit great effect on wheat bread specific volume in all cases studied.

Lowest and highest dose of L-ascorbic acid, increased specific volume of wheat bread, when dough is produced with fast mixing process, compared to control sample. Medium dose of L-ascorbic acid shows negative effect on specific volume of wheat bread. This means that increase of L-ascorbic acid dose will not necessarily result in proportionally increased specific volume of wheat bread.

Additions of glucose oxidase and hydrogen peroxide exhibit negative effect on specific volume in all samples produced with fast mixing process.

Table 1. Effect of mixing process applied on the specific volume of wheat bread with addition of the studied oxidative improvers

Sample	Specific volume (cm^3/g)	Standard dev.	Specific volume (cm^3/g)	Standard dev.
Control	3.530^{ab}	0.060	-	-
L-ascorbic acid				
0.005%	3.603^{b}	0.064	4,321^{c}	0,216
0.008%	3.432^{ab}	0.190	4,692^{e}	0,150
0.011%	3.605^{b}	0.105	4,252^{c}	0,184
Glucose oxidase				
0.002%	3.442^{ab}	0.167	3,727^{ab}	0,081
0.004%	3.404^{a}	0.044	3,647^{ab}	0,038
0.006%	3.385^{a}	0.107	3,065^{d}	0,102
Hydrogen peroxide				
0.001%	3,375^{a}	0,021	3,816^{b}	0,083
0.002%	3,464a^{b}	0,036	3,600^{a}	0,024
0.003%	3,445^{a}	0,036	3,820^{b}	0,099
Fast mixing process			*Intensive mixing process*	

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

Lowest dose of glucose oxidase is statistically similar to the control sample and medium dose of L-ascorbic acid.

Different authors claim different effects of glucose oxidase on bread specific volume. Martínez-Anaya and Jiménez [10] 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 noticeable [11, 12].

Negative effect of glucose oxidase and hydrogen peroxide on specific volume of wheat bread produced with fast mixing process compared to the control sample are due to too strong gluten network with insufficient extensibility that produce rigid dough and bread with lower specific volume which is unfavorable characteristics by consumer. This means that application of oxidative improvers in fast mixing process of wheat dough will have small positive effect on specific volume of wheat bread.

When wheat dough is produced by intensive mixing process all oxidative improvers exhibit greater positive effect on specific volume of wheat bread. All samples, except that with highest dose of glucose oxidase, have significantly increased specific volumes.

Statistically, samples with added L-ascorbic acid are significantly different from samples of L-ascorbic acid produced with fast mixing process and exhibit greatest increase of wheat bread specific volume compared with other two oxidative improvers studied. This shows importance of energy transfer to wheat dough in mixing process and its effect on gas incorporation and retention. Transferred energy stretch gluten network and incorporates air, while oxidative improvers improve wheat dough elasticity. Incorporated air acts as oxidative improves as well and exhibit positive effect on activity of applied L-ascorbic acid.

Texture analysis: On the basic of data presented in Table 2 it can be observed that with the addition of all *studied* oxidation improvers, bread crumb hardness is statistically significantly modified, compared with the control sample and on the basic of mixing process applied.

Table 2. Effect of mixing process applied on the wheat bread crumb hardness with addition of the studied oxidative improvers

Sample	Hardness (g)	Standard dev.	Hardness (g)	Standard dev.
Control	5332^{a}	905	-	-
L-ascorbic acid				
0.005%	7717^{abcd}	822	5017^{ab}	1022
0.008%	10095de	344	4444^{a}	443
0.011%	11168^{e}	2922	4895^{ab}	1252
Glucose oxidase				
0.002%	6749^{ab}	1135	11239^{c}	1245
0.004%	11454^{e}	1090	11309^{c}	656
0.006%	8087^{bcd}	921	21109^{d}	2775
Hydrogen peroxide				
0.001%	7504^{abc}	953	6171^{ab}	510
0.002%	7713^{abcd}	497	11577^{c}	987
0.003%	9295^{cde}	1245	7332^{b}	500
Fast mixing process			*Intensive mixing process*	

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

Change in hardness of wheat bread crumb is good indicator of wheat bread quality and staling. During storage time of wheat bread, hardness of crumb will

increase [13]. Crumb hardness increase occurs due to loss of moisture and due to phenomena of starch retrogradation [14].

Addition of glucose oxidase in doses over 0.004%, L-ascorbic acid in doses over 0.008% and hydrogen peroxide in all doses studied increase hardness of breads significantly compared to the control sample.

According to the results, addition of all oxidative improvers in all concentrations has led to increase of hardness, compared with the control sample. This finding is not in accordance with research of Bonet *et al*. [15], 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 increase of bread crumb hardness, with fast mixing process applied. This effect could be associated with flour quality as well as with laboratory baking test procedure and it should be investigated further.

Crumb hardness of samples obtained by intensive mixing process and addition of L-ascorbic acid is smaller than hardness of control sample while samples width added glucose oxidase are significantly different from samples with glucose oxidase obtained by fast mixing process. Samples of hydrogen peroxide exhibit values of crumb hardness between samples of L-ascorbic acid and glucose oxidase. This effect of mixing process on bread crumb hardness is due to fact that energy transferred to wheat dough stretch gluten network and proportionally to increased specific volume decrease specific weight of bread.

Determination of bread color: Effects of added oxidative improvers and process of wheat dough mixing on color of wheat bread are presented in Table 3.

With addition of L-ascorbic acid and hydrogen peroxide wheat bread with lighter color is produced. Addition of glucose oxidase creates opposite effect and all samples are with darker color. Similar effect are observed in samples produced with fast mixing process and intensive mixing process, but it must be noted that smallest dose of L-ascorbic acid and medium dose of hydrogen peroxide in intensive mixing process produce bread that is darker then control sample and in general, samples obtained with intensive mixing process are poses darker color.

Effects of added oxidative improvers and process of wheat dough mixing on color of wheat bread crumb are presented in Table 4.

All oxidative improvers regardless of mixing process applied modified red-green color coordinate of wheat bread crumb. Red-green coordinate indicates that bread crumb poses pronounced green tone compared to control sample and samples. Smallest effect is observed with addition of L-ascorbic acid and application of intensive mixing process. Addition of L-ascorbic acid and application of intensive mixing process produces wheat bread with lighter crumb compared to samples of same oxidative improver obtained by fast mixing process.

Modification of yellow-blue color coordinate of wheat bread crumb is insignificant in all samples regardless of mixing process applied except for medium and highest dose of hydrogen peroxide.

Table 3. Effect of mixing process applied on the wheat bread colour with addition of the studied oxidative improvers

Sample	L* (D65)	a* (D65)	b* (D65)	L* (D65)	a* (D65)	b* (D65)
Control	51,779^{ab}	15,051^{a}	30,639^{abd}	51,779^{ac}	15,051^{a}	30,639^{cd}
L-ascorbic acid						
0.005%	54,149^{a}	14,758^{a}	32,766^{b}	45,494b^{a}	14,864^{b}	24,035^{a}
0.008%	53,539^{ab}	13,678^{a}	31,393^{ab}	51,982a^{c}	14,249^{ab}	29,619^{bc}
0.011%	50,512^{ab}	15,541^{a}	30,526^{ab}	54,773^{c}	13,401^{a}	30,939^{c}
Glucose oxidase						
0.002%	49,699^{ab}	14,774^{a}	27,868a^{cd}	48,684^{ab}	14,643^{ab}	27,034^{ab}
0.004%	49,533^{ab}	14,700^{a}	28,036^{cd}	47,692^{ab}	13,859^{ab}	24,103^{a}
0.006%	47,145^{ab}	15,091^{a}	25,547^{acd}	49,764^{ab}	13,695^{ab}	26,27^{ab}
Hydrogen peroxide						
0.001%	53,468^{a}	14,281^{a}	31,249^{ab}	50,074^{abc}	14,542^{ab}	26,362^{ab}
0.002%	50,143^{b}	15,362^{a}	29,295^{c}	49,535^{ab}	14,532^{ab}	27,224^{ab}
0.003%	52,958^{ab}	13,67^{a}	30,521ab^{cd}	52,700^{ac}	13,717^{ab}	29,118^{bc}
Fast mixing process				*Intensive mixing process*		

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

Table 4. Effect of mixing process applied on the colour of wheat bread crumb with addition of the studied oxidative improvers

Sample	L* (D65)	a* (D65)	b* (D65)	L* (D65)	a* (D65)	b* (D65)
Control	74,66^{ac}	-1,042^{b}	17,082^{b}	-	-	-
L-ascorbic acid						
0.005%	75,85^{b}	-0,868^{ab}	17,714^{ab}	76,932^{cd}	-1,026^{a}	16,89^{a}
0.008%	75,368^{ab}	-0,826^{ac}	17,642^{ab}	76,972^{cd}	-1,026^{a}	17,14^{abc}
0.011%	74,842^{abc}	-0,970^{ab}	17,848^{ab}	77,418^{d}	-1,036^{a}	16,982^{ab}
Glucose oxidase						
0.002%	75,85^{b}	-0,868^{ab}	17,714^{ab}	76,932^{cd}	-1,026^{a}	16,89^{a}
0.004%	75,368^{ab}	-0,826^{ac}	17,642^{ab}	76,972^{cd}	-1,026^{a}	17,14^{abc}
0.006%	74,842^{abc}	-0,970^{ab}	17,848^{ab}	77,418^{d}	-1,036^{a}	16,982^{ab}
Hydrogen peroxide						
0.001%	74,858^{abc}	-0,784^{acde}	18,084^{a}	75,764^{a}	-0,934^{ad}	17,35^{abc}
0.002%	75,114^{abc}	-0,766^{acde}	18,026^{a}	76,08^{ac}	-0,714^{bc}	17,57^{c}
0.003%	73,994^{c}	-0,834^{a}	17,9^{a}	75,19^{ab}	-0,834^{cd}	17,414^{bc}
Fast mixing process				*Intensive mixing process*		

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

4. Conclusions

- The use of oxidative improvers and fast mixing process can produce to rigid wheat dough with negative effect on specific volume of bread compared to control sample. This is true for glucose oxidase and hydrogen peroxide, only L-ascorbic acid improve specific volume characteristic when fast mixing process and only in certain examined doses.

- Use of oxidative improvers and intensive mixing process increased specific volume in all cases studied except for highest added dose of glucose oxidase compared to control sample. Energy transferred in process of intensive mixing of wheat dough stretch gluten network and incorporates air and results with positive effect on wheat bread specific volume. The biggest positive effect is generated when L-ascorbic acid is used as an oxidative improver in intensive mixing process of wheat dough.

- Crumb hardness is increased in all samples by fast mixing process. Smallest increase is observed by L-ascorbic acid compared to control sample. The biggest positive effect on crumb hardness is observed when L-ascorbic acid is used in intensive mixing process as an oxidative improver. All studied doses of L-ascorbic acid and intensive mixing process decreased crumb hardness compared to control sample. Negative effect of hydrogen peroxide is minimized while negative effect of glucose oxidase is increased by intensive mixing process.

- Effect of studied oxidative improvers on expression of bread color and color of bread crumb is not of mayor importance. L-ascorbic acid and hydrogen peroxide produce lighter samples while glucose oxidase produced darker. This effect is probably closely associated with production of reducing sugars by glucose oxidase and the Maillard reaction and not with its effect on gluten network.

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