

OLIVERA D. ŠIMURINA<sup>1</sup>  
STEVAN D. POPOV<sup>2</sup>  
BOJANA V. FILIPČEV<sup>1</sup>  
JELENA M. DODIĆ<sup>2</sup>  
MARIJA I.  
BODROŽA-SOLAROV<sup>1</sup>  
MIRJANA DEMIN<sup>3</sup>  
ZVONKO B. NJEŽIĆ<sup>1</sup>

<sup>1</sup>Institute of Food Technology,  
University of Novi Sad, Novi Sad,  
Serbia

<sup>2</sup>Faculty of Technology, University  
of Novi Sad, Novi Sad, Serbia

<sup>3</sup>Faculty of Agriculture, University  
of Belgrade, Zemun, Serbia

SCIENTIFIC PAPER

UDC 633.11:664.641.12/.654.3:66

DOI 10.2298/CICEQ121127029S

## MODELLING THE EFFECTS OF TRANSGLUTAMINASE AND L-ASCORBIC ACID ON SUBSTANDARD QUALITY WHEAT FLOUR BY RESPONSE SURFACE METHODOLOGY

### Article Highlights

- Dough and bread quality of substandard flour can be improved by using TG and L-AA
- RSM was applied in the analysis of the individual and interactive effect of TG and L-AA
- TG has more linear effect in the fermentation process, on the crumb quality than L-AA
- L-AA has a much greater effect on the specific volume of bread than TG
- Desirability function defines optimum dose of TG and L-AA for sample of flour substandard quality

### Abstract

*Over the past decade, extreme variations in climatic conditions have been observed, which in combination with inadequate agro techniques lead to decreased quality of mercantile wheat, i.e. flour. The application of improvers can optimise the quality of substandard wheat flour. This paper focuses on systematic analysis of individual and interaction effects of ascorbic acid and transglutaminase as dough strengthening improvers. The effects were investigated using response surface methodology. Transglutaminase had much higher linear effect on the rheological and fermentative properties of dough from substandard flour than L-ascorbic acid. Both transglutaminase and L-ascorbic acid additions had a significant linear effect on the increase of bread specific volume. Effects of transglutaminase and ascorbic acid are dependent on the applied concentrations and it is necessary to determine the optimal concentration in order to achieve the maximum quality of the dough and bread. Optimal levels of tested improvers were determined using appropriate statistical techniques, which applied the desirability function. It was found that the combination of 30 mg/kg of transglutaminase and 75.8 mg/kg of L-ascorbic acid achieved positive synergistic effects on rheological and fermentative wheat dough properties, as well on textural properties and specific volume of bread made from substandard quality flour.*

*Keywords: substandard quality flour, transglutaminase, L-ascorbic acid, optimization.*

Over the past decade, an appreciable stagnation of quality and yield of mercantile wheat at the global, regional and local levels has occurred [1-4]. This phenomenon is the consequence of more

expressed climate changes that are manifested in different ways [5]. Unfavourable climate conditions and inadequate agro-techniques decrease the level of usability of wheat genetic potential. The processing industry is faced with the fact that in certain production years the quality of mercantile wheat is below average required values. Wheat flour produced from wheat of low technological quality is also of substandard quality.

Correspondence: O.D. Šimurina, Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.

E-mail: [olivera.simurina@fins.uns.ac.rs](mailto:olivera.simurina@fins.uns.ac.rs)

Paper received: 27 November, 2012

Paper revised: 4 April, 2013

Paper accepted: 25 July, 2013

Flour quality can be improved by chemical, enzymatic or physical treatment [6]. The most frequently applied method of protein modification in wheat processing industry is protein cross-linking. Nowadays, it is common practice to add improving agents to wheat flour to overcome quality deficiencies [7]. The investigations showed that L-ascorbic acid (L-AA) and transglutaminase (TG) have important role in wheat protein cross-linking.

L-AA changes the spatial structure of gluten by oxidation of the sulfhydryl group of the cysteine residue forming inter- and intra-molecular disulfide bridges [8]. L-AA increases dough strength, reduces dough stickiness [9] and gives greater tolerance to excessive addition [10] than other oxidants, as supplementation beyond the optimal concentration does not decrease loaf volume [7]. L-AA has the greatest effect in weak flours, and added in the quantity of 50 to 100 mg/kg achieves bread volume increase even up to 18% [11,12]. TG (EC 2.3.2.13, protein-glutamine  $\gamma$ -glutamyltransferase) catalyzes reaction of transfer of acyl group between  $\gamma$ -carboxamide group from protein bound glutamine residue (acyl donors) and different primary amines (acyl acceptors). Acyl acceptors are most frequently  $\epsilon$ -amino groups of lysine residues in a protein or peptide. As a result of the reaction, inter- or intra-molecular covalent cross-linking occurs [13,14]. In the case of gluten, this enzyme induced the formation of high molecular weight polymers despite the low lysine content in gluten proteins. The formation of these polymers results in strengthening of the gluten network [14]. The investigations showed that TG can improve dough elasticity and may produce beneficial effects during breadmaking that are similar to oxidizing improvers [13]. Tseng and Lai [15] have investigated and compared the effects of TG and L-AA in three flours with different compositions and rheological properties; however, no study has investigated the combined effects of these two ingredients in substandard wheat flour. The innovative character of this study lies in the fact that so far there has been no research examining the effects of ascorbic acid and transglutaminase both

separately and combined in flour of substandard quality. This paper features experiments applied to low quality flour and defines optimum doses of these two dough strengtheners in order to achieve the set goals. These goals are related to increasing the maximum height of gaseous release (H'm), specific volume of bread and crumb resilience.

By applying response surface methodology (RSM) it is possible to analyze all the effects of independent or combined factors. Many researchers apply RSM as a mathematical modelling tool in bioprocess optimization [16,17]. By using RSM, process variables could be controlled together to result in maximum product properties with desired characteristics [18,19].

The purposes of this paper were the following:

1) The analysis of individual and interaction effects of TG and L-AA on rheological and fermentative properties of dough and bread quality produced from substandard wheat flour.

2) The analysis of the adequacy of regression models, *i.e.*, establishing whether the suggested models are in accordance with the data (through values of coefficient determination  $R^2$  and lack of fit importance), as well as whether there is sufficient precision of models in predictions (through F-test).

3) The optimization of the concentration of TG and L-AA by the application of desirability function with the aim of improving certain rheological and fermentative properties of dough, as well as bread quality.

## EXPERIMENT

### Materials and methods

In this study we used commercial refined soft wheat flour. As displayed in Table 1, quality parameters of flour were well below the optimum value required for bread making [20–22]. Therefore, the tested flour could be graded as substandard quality flour.

Protein content of the tested wheat flour (Nx6.25) was analysed using a Kjeldahl procedure (ICC Standard No 105/2) [23] and gluten index values were determined using the Glutomatic 2200 system

Table 1. The criteria of optimal wheat flour quality for bread production [26–28] and the characteristics of the tested wheat flour

Quality parameter	Optimal quality of the refined wheat flour for bread production	Quality characteristic of the tested wheat flour
Protein, % dry weight	11.5–13.5	10.7
Gluten index, %	60–90	52.0
Extensograph area, cm <sup>2</sup>	>75	30.0
Resistance of extension, BU	200–400	120.0
Alveograph, $W \times 10^{-4}$ , J	160–200	140.0
Alveograph ratio, <i>PL</i>	<0.6	1.1

(ICC Standard No 155) [24]. Rheological properties were determined using a Brabender extensograph (ICC Standard No 114/1) [25] and a Chopin alveograph (ICC Standard No 121) [26].

The applied TG was a commercial preparation Veron TG containing 100 transglutaminase units per g, manufactured by ABF Ingredients Company, Germany. The recommended dosage was 10–30 mg/kg. In the experiment the applied doses of enzyme were 15 and 30 mg/kg of flour. L-AA was also a commercial preparation produced by BASF, Germany, and was applied in a dose of 50 and 100 mg/kg of flour, based on the results of previous investigations [27]. Enzyme and acid were added according to the experimental design presented in Table 2.

### Uniaxial extension test

A texture analyzer (Texture Technologies, Stable Micro Systems, Surrey, UK) equipped with a Kieffer dough and gluten extensibility rig was used to perform a small scale uniaxial extension test. Dough samples were prepared in a farinograph mixing bowl using 300 g flour, 6 g salt and water according to the design given in Table 2. For all samples, dough consistency was 450 B.U. A piece of dough (25 g) was placed to rest in a thermostat at controlled temperature ( $30 \pm 1$  °C) and relative humidity (85%) for 20 min. The sample was then rolled by hand into a cylindrical shape, placed in the standard Kieffer mold and compressed with a lubricated top plate. The sample was rested for 40 min in the thermostat under the same conditions. Before the start of the test, the sample was clamped between the plates of the Kieffer rig. The setting values were the following: pre-test speed of 2.0 mm/s, test speed of 3.3 mm/s, post-test speed of 10.0 mm/s, distance between 100 mm, and an automatic trigger force of 5 g by using a 5 kg load cell. The parameters recorded were the resistance to extension ( $R_{\max}$ ) and extensibility until dough rupture ( $E$ ).

The results shown are the mean values of at least seven measurements.

### Dough development and gas retention tests

The Chopin rheofermentometer F3 (Villeneuve-la-Garenne, France) was used to measure several parameters of dough development: maximum height of gaseous release ( $H'm$ , mm), and volume of CO<sub>2</sub> retention (ml). Dough was prepared in a high-speed Diosna mixer (Dierks & Söhne Maschinenfabrik, Osnabrück, Germany) with low speed of 85 rpm for 1 min and high speed of 120 rpm for 7 min. Dough was prepared with compressed yeast (4% flour weight basis), salt (2% flour weight basis), the amounts of TG and L-AA were added according to the experimental design (Table 2). The dough weight  $315 \pm 1$  g was placed in the removable basket of the gasometer and with the load weight of 2000 g directly on the dough. The cover was fitted with an optical sensor and the test was run for 3 h at  $30 \pm 1$  °C. All the experiments were replicated three times.

### Bread preparation

The bread dough formula was: flour (100 g), compressed yeast (4 g/100 g flour), salt (2.0 g/100 g flour), vegetable fat (1 g/100 g), enzyme TG (dose: 0–30 mg/kg flour, according to Table 2) and L-AA (dose: 0–100 mg/kg, according to Table 2). The amounts of added water were adapted for each treatment point to keep dough consistency at 450 BU.

Dough was mixed for 1 min at low speed of 85 rpm and for 7 min at high speed of 120 rpm in a laboratory Diosna mixer. Bulk proofing was conducted for 45 min at 85% relative humidity and  $30 \pm 1$  °C. Dough was scaled to  $350 \pm 1$  g and manually rounded, rolled, and put into tin pans. Baking was done as described by Filipčev *et al.* [28]. All the experiments were replicated three times.

Table 2. Results of experimental design for quality properties of wheat dough and bread made from substandard wheat flour;  $R_{\max}$ , resistance to extension;  $E$ , extensibility until dough rupture;  $H'm$ , maximum height of gaseous release;  $SV$ , specific volume

TG, mg/kg	L-AA, mg/kg	$R_{\max}$ / g	$E$ / mm	$H'm$ / mm	CO <sub>2</sub> retention, ml	Resilience, %	Firmness, g	$SV$ / ml g <sup>-1</sup>
0 (-1)	0 (-1)	23.6	38.3	64.8	1289	33.6	672.3	4.1
15 (0)	0 (-1)	26.2	27.8	70.9	1331	33.9	638.8	4.4
30 (1)	0 (-1)	27.0	25.3	68.8	1424	37.0	473.4	4.5
0 (-1)	50 (0)	30.4	24.6	64.8	1362	33.8	539.7	4.5
0 (-1)	100 (1)	40.7	22.2	67.0	1441	34.9	522.7	4.6
15 (0)	50 (0)	29.2	19.6	73.7	1485	34.2	519.0	4.8
15 (0)	50 (0)	28.8	19.8	73.8	1484	34.8	519.1	4.8
15 (0)	100 (1)	40.7	17.9	80.2	1568	35.0	510.8	5.1
30 (1)	50 (0)	32.6	18.4	83.6	1520	38.4	340.5	5.2
30 (1)	100 (1)	34.9	25.0	84.2	1662	36.9	502.2	5.3

## Bread evaluation

Quality analysis of bread samples was carried out by measuring weight, volume and instrumental textural properties. Bread volume was determined by seed displacement method 2 h after baking. Specific volume (ml/g) of bread was calculated from loaf volume and weight. Three breads from each batch were measured and the results were averaged. Bread samples were then packed in plastic bags and stored for 24 h at room temperature until evaluation for bread instrumental textural attributes. Crumb firmness and resilience were evaluated using the Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK), according to a modified AACC method 74-10A, described by Filipčev *et al.* [28].

## Statistical analysis

Experimental data was processed using Design-Expert 8.1 software (Stat-Ease Corporation, Minneapolis, MN, USA). The experiment was conducted as a full factorial design  $3^2$  with two replicates at a central point (Table 2).

The effects of TG and L-AA on the investigated response functions were established in the model in the form of polynomial equations (1):

$$Y = b_0 + b_1TG + b_2L-AA + b_{11}TG^2 + b_{22}L-AA^2 + b_{12}TG \times L-AA \quad (1)$$

where  $Y$  is the response function of the experimental data, TG and L-AA are the independent variables, and the  $b$  parameters are the coefficients. The adequacy of all the models was tested by dispersive analysis, and the parameters that describe the adequacy are the following: the coefficient of determination  $R^2$ ,  $F$  values of model and  $P$  values of lack-of-fit.

Aiming at the determination of optimal TG and L-AA concentrations in order to improve dough properties and quality of bread made from substandard flour, a desirability function was applied. The desirability function was used because of its additional benefits - user flexibility in selecting optimum conditions for analysis of a variety of samples and more effective use of resources [19,29].

## RESULTS AND DISCUSSION

### Models of effects of transglutaminase and L-ascorbic acid on the rheological and fermentative properties of dough and on the quality of bread from substandard flour

Rheological properties according to Kieffer extensibility dough/gluten rig (TA) relating to the maximum resistance to extension ( $R_{max}$ ) and extensibility

from start until dough rupture ( $E$ ) are shown in Table 2. Minimum value for  $R_{max}$  (23.6 g) was achieved in non-treated wheat dough, while treatment with 100 mg/kg L-AA or 15 mg/kg TG and 100 mg/kg L-AA had identical values, which were also the highest ones (40.7 g) for  $R_{max}$ . By the addition of L-AA and TG, dough extensibility decreased and ranged from 38.3 mm in the control sample to 17.9 mm in the sample that underwent treatment with 15 mg/kg TG and 100 mg/kg L-AA.

Dough parameters ( $H'm$ ,  $CO_2$  retention) during fermentation are presented in Table 2. The treatments with TG and L-AA and their mixtures increased the maximum height of gaseous release ( $H'm$ ) and the volume of  $CO_2$  retention was achieved at maximum concentrations of TG and L-AA.

Experimental values for firmness, resilience and specific volume of bread obtained by the application of treatments are also presented in Table 2. Bread without any additions had the highest value for firmness (672.3 g). The lowest firmness (340.5 g) was registered in bread with the application of 30 mg/kg of transglutaminase and 50 mg/kg of ascorbic acid. The same treatment also influenced resilience improvement. The highest specific volume (5.3 ml/g) was determined for bread sample with maximum TG dose (30 mg/kg) and maximum L-AA dose (100 mg/kg).

Effects of models obtained by RSM analysis, after removing outliers and terms with insignificant coefficients, are presented in Table 3. Models take into consideration the directions and intensity of the individual and interactive effects of TG and L-AA on the rheological and fermentative properties of dough and textural properties and specific volume of bread from substandard wheat flour.

According to the results given in Table 3, TG and L-AA exhibited significant effects ( $P < 0.05$ ) on all the analysed rheological and fermentative parameters of dough. TG exerted significant linear effect on the decrease of extensibility values, whereas all the other dough properties were increased. This effect is the consequence of the influence of TG on protein chains networking and strengthening of protein dough structure. The dough improves its elasticity characteristics and decreases its viscosity characteristics. Extensibility is a criterion of dough viscosity and it decreases with TG addition, whereas resistance is a criterion of its elasticity and increases with TG addition. The increased dough elasticity improves its characteristics during fermentation, which affects the increase of the values of the investigated parameters ( $H'm$  and  $CO_2$  ret). Generally speaking, a positive effect of TG to dough is also transferred to bread, which leads to

Table 3. Models of the effects of TG and L-AA on quality properties of wheat dough and bread made from substandard flour and assessment of their adequacy

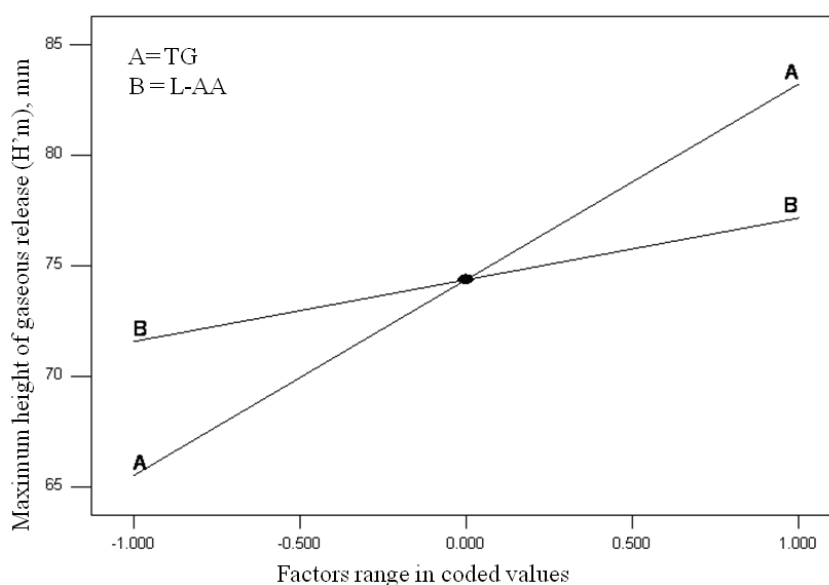
Model of response	ANOVA for each model of responses			
	$R^2$	$F_{cal.}$ value of model	$F_{tab.}$ value of model	$P$ value of lack of fit
$R_{max} = 3.41 + 0.15TG + 0.18L-AA + 3.11 \times 10^{-3}TG \times L-AA$	0.92	23.48	4.76	0.027 (s)
$E = 5.13 - 0.59TG - 0.26L-AA - 1.67 \times 10^{-3}TG \times L-AA$	0.99	176.67	19.30	0.631 (ns)
$H'm = 10.14 + 0.36TG + 0.02L-AA + 4.73 \times 10^{-3}TG \times L-AA$	0.98	82.27	6.56	0.194 (ns)
$CO_2 \text{ ret.} = 17.97 + 5.03TG + 2.29L-AA$	0.98	126.09	5.14	0.023 (s)
$R = 18.56 + 1.04TG$	0.95	10.74	4.74	0.593 (ns)
$F = 7.23 + 4.90TG - 3.35L-AA - 0.38TG^2 + 0.02L-AA^2 + 3.34 \times 10^{-4}TG \times L-AA$	0.98	31.40	9.01	0.811 (ns)
$SV = 15.14 + 0.02TG + 9.98L-AA + 5.43TG \times L-AA$	0.99	213.35	5.14	0.999 (ns)

the increase of volume, elasticity and crumb stability. Based on the results given in Table 3, it can be stated that TG had a much higher linear effect on the rheological properties of dough from substandard flour than L-AA. Such an expressed TG effect is the consequence of dough strengthening due to inter- and intra-molecular cross-linking of protein by covalent bonds and the formation of polymers [14]. However, with square increase of the concentration, neither addition had significant effect on the dough parameters at all. This could be explained by the fact that the polymerization degree depends on the number of available reactive glutamine and cysteine residues located on the molecule surface [30]. At initial TG and L-AA doses, reactions are intensive and exert considerable effects on dough properties. With square increase of TG and L-AA doses, significant effects are missing due to limited number of accessible reactive residues. Besides, saturation of protein component in dough may occur, and, as a consequence, increasing

TG and L-AA doses would neither increase polymerization degree in dough nor affect the rheological and fermentative properties of dough [31].

During fermentation, both improvers showed similar effects. They increased maximum height of gaseous release ( $H'm$ ) and  $CO_2$  retention. It is in accordance with previous investigations, which state that TG enzyme has similar effect on the properties of dough as well as oxidizing improvers [13]. The experimental results showed that the linear effect of TG on fermentative properties of dough is several orders of magnitude higher than the L-AA effect in the tested doses. This trend can be seen in the perturbation graph of the maximum height of gaseous release  $H'm$  (Figure 1).

As a linear term, TG and L-AA had twice as strong effect on  $CO_2$  retention volume in relation to L-AA. Further increase of TG and L-AA concentrations had no significant effect on either of the investigated fermentative dough properties.

Figure 1. The perturbation graph showing the effect of TG and L-AA on the maximum height of gaseous release ( $H'm$ ).

The presented results led us to the conclusion that TG and L-AA exhibit a dough strengthening effect, but the intensity of their effect is different and dose-dependent. The effect of TG was higher than L-AA, but it lost its significance with square increase of the dose. TG catalyses the formation of inter- and intra-molecular  $\epsilon$ -( $\gamma$ glutamyl) lysine cross-links (G-L bonds) and has longer time action due to cumulative effect, in contrast to L-AA which is a mediator in an oxidative process in dough by forming of S-S bonds within a relatively short period [13,32].

TG and L-AA had a significant ( $P < 0.05$ ) synergistic effect of on the increase of maximum resistance, decrease of dough extensibility and increase of maximum height of gaseous release. This synergistic effect will have a significant impact on bread attributes, especially on firmness and specific volume (discussed further below).

After analyzing the models of regression regarding the effect on textural properties and specific volume of bread, it was noted that TG significantly influenced these parameters ( $P < 0.05$ ). The significant and positive linear effect of TG was noted on bread crumb resilience, in contrast to L-AA which showed no statistically significant effect on the change of this parameter. The perturbation diagram clearly shows the passivity of L-AA to bread crumb resilience, as well as significant contribution of TG to its improvement (Figure 2).

TG had a significant ( $P < 0.05$ ) linear effect on the increase of bread crumb firmness, which can be considered as a positive property which contributes to

crumb strength. Crumb strength is a measure of the ability of sliced bread to withstand handling. In some countries, lack of crumb strength is a common complaint amongst consumers [13,33]. However, by square increase of TG concentration, crumb firmness of bread is significantly ( $P < 0.05$ ) decreased. On the other hand, L-AA had a significant linear effect ( $P < 0.05$ ) to the decrease of crumb firmness, but with square increase of concentration, crumb firmness of bread was significantly ( $P < 0.05$ ) increased, but to a lesser extent. TG and L-AA affected bread crumb firmness in opposite directions, depending on the applied concentration, which can be clearly seen in the perturbation diagram (Figure 3). The effect of TG and L-AA on bread firmness increase was significant ( $P < 0.05$ ). This could be the result of the synergistic effect of TG and L-AA on the increase of dough resilience which stabilized bread crumb.

Both TG and L-AA additions had a significant ( $P < 0.05$ ) linear effect on the increase of bread specific volume, with L-AA exerting higher effect in comparison to TG (Table 3). However, the increasing levels of enzyme did not cause significant improving effects on bread specific volume values, which is in accordance with previous investigations [34]. There was a significant interaction ( $P < 0.05$ ) between TG and L-AA on the increase of bread specific volume (Figure 4).

Relatively high values of determination coefficient ( $R^2$ ) point to an adequate fitting of the experimental results with the proposed method. Calculated  $F$ -values are higher than tabulated values [35] in all

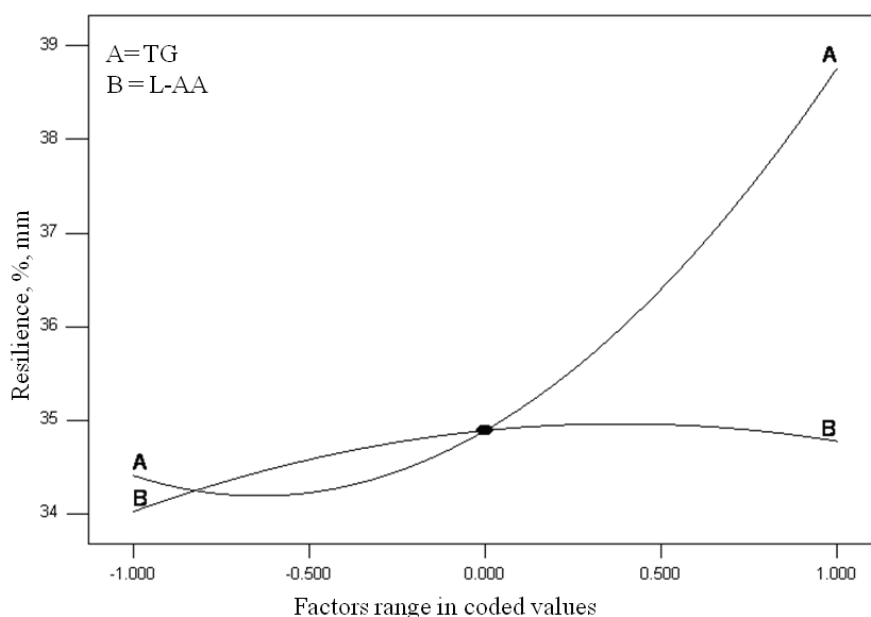


Figure 2. The perturbation graph showing the varying intensity effect of TG and L-AA on the resilience of crumb bread made from substandard flour.

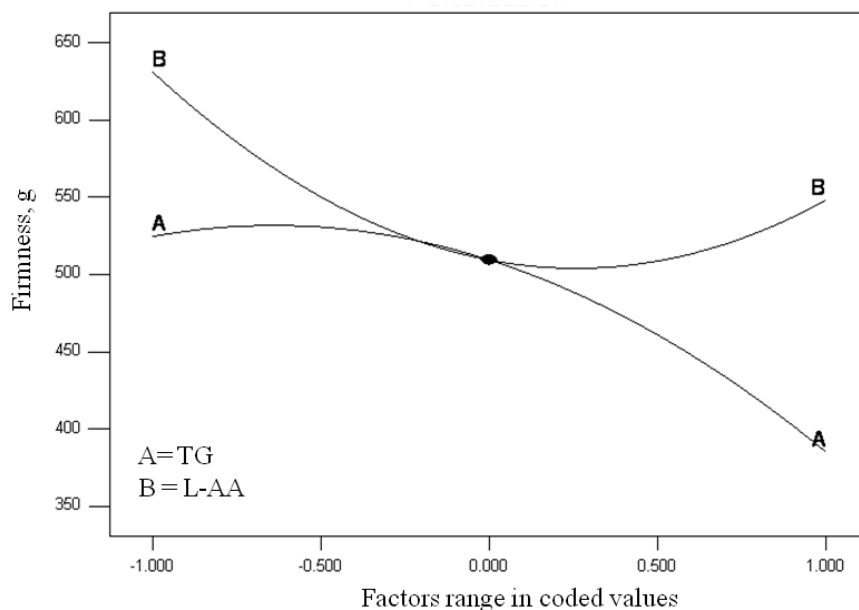


Figure 3. The perturbation graph showing the opposite effect of TG and L-AA on the firmness of crumb bread made from substandard flour.

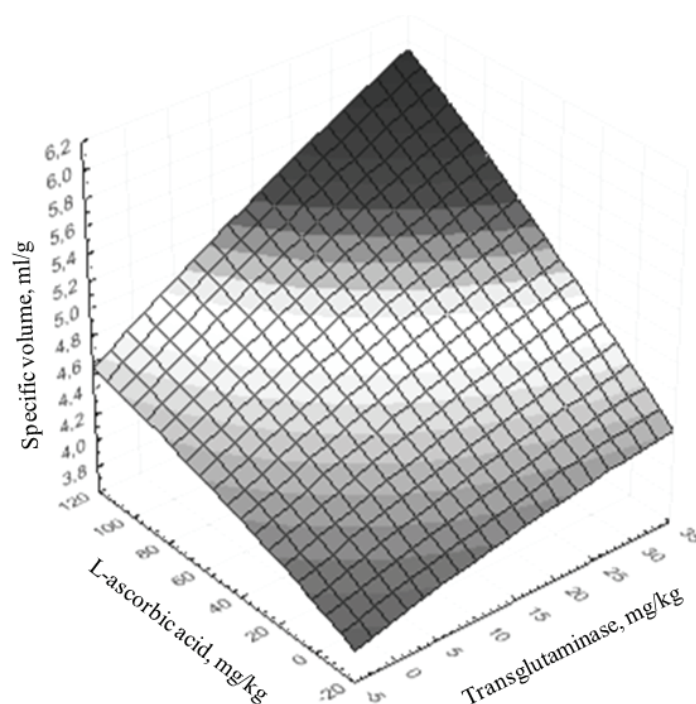


Figure 4. Three-dimensional graph showing the effect of TG and L-AA concentration on specific volume of bread made from substandard flour.

the responses, from which it is possible to conclude that the proposed model provides a good prediction of the experimental data. For some responses ( $R_{\max}$ ,  $\text{CO}_2$  retention) the models have  $P$  value of lack of fit  $< 0.05$ , therefore the lack of fit was significant. It is considered that in such cases the models are not opposed to the data (high  $R^2$  values and  $F_{\text{calc.}} > F_{\text{tab.}}$ ),

but do not fulfill all the diagnostic measures of adequacy and all the predictions obtained by such models will have great variations [36]. The statistical analysis indicates that the proposed models referring to dough extensibility ( $E$ ) and maximum height of gaseous release ( $H'm$ ) in dough during fermentation were adequate to high  $R^2$  values and no significant



lack of fit. Models constructed for textural properties and bread specific volume have also insignificant lack of fit, which indicated that they are adequate and important. Therefore, these the models were further implemented into the optimization process.

#### The optimization of TG and L-AA concentration by the application of desirability function

The intensity and direction of individual and interactive effects of the TG and L-AA in the investigated range depends on the applied dosage. Desirability function approach was used to locate the optimal doses of TG and L-AA that produce adequate responses regarding the quality of dough and bread made from substandard flour. The proposition of the criteria necessary for the determination of optimal experimental conditions was based upon preliminary experiments and the literature data and it is shown in Table 4. The solutions for the proposed criteria in the optimization of the TG and L-AA doses are also shown in Table 4.

Following optimization, four solutions (A, B, C and D) were obtained with predicted values for *E* (21.23–23.94 mm) and *H'm* (84.52–88.42 mm) of dough and for *R* (37.99–38.17 %), *F* (410.88–463.18 g), and *SV* (5.31–5.51 ml/g) of bread (Table 4). To obtain the given ranges of the observed responses, it was necessary that TG doses reach 30 mg/kg and L-AA doses range from 75.8 to 98.9 mg/kg. Having in mind the fact that the variation coefficient is for each solution below 10% (Table 4), the dispersion of the results is within limits. This means that by the application of each of the proposed solutions it would be possible to achieve the optimal quality of dough and bread made from the tested substandard flour. Despite lower calculated desirability in comparison to sol-

utions A and B, the solutions C seems to be the best one particularly judging from the point of view of economic justification. Therefore, this solution was subsequently analyzed to compare the predicted responses with measured values in a new experiment with optimal TG (30 mg/kg) and L-AA (75.8 mg/kg) doses. The results of comparison are also given in Table 4.

The predicted results (21.64 mm, 84.52 mm, 37.99%, 410.88 g and 5.31 ml/g) for *E*, *H'm*, *R*, *F* and *SV*, respectively, obtained under the optimum conditions were close to the experimental results (22.41 mm, 86.01 mm, 412.56 g, 38.34% and 5.45 ml/g). Tests performed for each response showed low values of relative error (less than 10%), indicating that the models were adequate to predict these responses.

#### CONCLUSION

Response surface methodology was successfully applied for the analysis of individual and interactive effects of transglutaminase and L-ascorbic acid on rheological and fermentative dough properties and quality of bread produced of substandard flour. The linear effect of transglutaminase on dough extensibility established by Kieffer dough and gluten extensibility rig was higher than that of L-ascorbic acid. Similarly, higher effect of transglutaminase than L-ascorbic acid was recorded on the properties of dough during fermentation, especially on the maximum height of gaseous release and CO<sub>2</sub> retention. The transglutaminase had significant and positive linear effect on crumb resilience, whereas L-ascorbic acid did not significantly affect this parameter. With square increase of both improver doses, the effect on the tested responses was either reduced or lost. Significant interactive effect of transglutaminase and

Table 4. Optimization solutions of TG and L-AA concentration for the set requirements of dough and bread quality and verification of the selected solution; *R*, resilience of crumb; *F*, firmness of crumb; *SV*, specific volume of bread; *D*, desirability value

Goals	Factors		Responses					D
	TG, mg/kg	L-AA, mg/kg	E, mm	H'm, mm	R, %	F, g	SV, ml/g	
	In range	In range	In range	Max	Max	In range	Max	
Solutions of the factors			Prediction values of the responses					
A	30.0	82.8	21.40	85.70	38.05	423.36	5.37	0.849
B	30.0	81.4	21.23	85.46	38.04	420.59	5.36	0.847
C	30.0	75.8	21.64	84.52	37.99	410.88	5.31	0.839
D	30.0	98.9	23.94	88.42	38.17	463.18	5.51	0.835
Variation coefficient, %			5.75	1.95	5.37	0.20	1.55	-
Selected solution			Experimental values of the solution C					
C	30.0	75.8	22.41	86.01	412.56	38.34	5.45	0.836
Relative error between predicted and experimental values, %			7.32	8.54	9.12	7.15	1.03	-



L-ascorbic acid contributed to the increase of maximum resistance and decrease of dough extensibility in that way, improving dough elasticity from substandard flour. It is also important their synergistic effect on bread specific volume increase. Since single and interaction effects of transglutaminase and L-ascorbic acid on rheological and fermentative properties and bread textural properties were dose-dependent, the improver doses were optimized in order to maximise the quality of dough and bread. Mixture of transglutaminase in 30 mg/kg dose and L-ascorbic acid in 75.8 mg/kg dose yielded better dough properties, as well as higher quality of bread made from substandard wheat flour compared to dough and bread without these additives.

### Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project III No.46005). The authors would like to thank ABF Ingredients Company (Darmstadt, Germany) for donating the sample of enzyme and S. Šolaja from Barentz d.o.o. (Belgrade, Serbia) for his engagement in sample procurement.

### REFERENCES

- [1] Y. Pomeranz, *Wheat Chemistry and Technology*, Volume II, AACC, St. Paul, MN, 1971, p.69
- [2] V. Đurić, A. Kondić-Špika, N. Hristov, J. Popov-Raljić, *Chem. Ind. Chem. Eng. Q.* **16** (2010) 73-78
- [3] L. Unbehend, G. Unbehend, M.G. Lindhauer, *Nahrung* **47** (2003) 140-144
- [4] K. Balla, M. Rakszegi, Z. Li, F. Bekes, S. Bencze, O. Veisz, *Czech J. Food Sci.* **29** (2011) 117-128
- [5] G.G. Mikhaylenko, Z. Czuchajowska, B.-K. Baik, K.K. Kidwell, *Cereal Chem.* **77** (2000) 507-511
- [6] D. Panyam, A. Kilara, *Trends Food Sci. Tech.* **7** (1996) 120-125
- [7] I.J. Joye, B. Lagrain, J.A. Delcour, *J. Cereal Sci.* **50** (2009) 11-21
- [8] M. Nakamura, T. Kurata, *Cereal Chem.* **74** (1997) 647-650
- [9] A. Aamodt, E.M. Magnus, E. M. Faergestad, *J. Food Sci.* **68** (2003) 2201-2210.
- [10] Y. Yamada, K.R. Preston, *Cereal Chem.* **71** (1994) 297-300
- [11] Šimurina, O., Mastilović, J., Milošević, S., Filipčev, B. *Cereal-Bread* **33** (2006) 61-68
- [12] V.O. Selomulyo, W. Zhou, *J. Cereal Sci.* **45** (2007) 1-17
- [13] J.A. Gerrard, S.E. Fayle, A.J. Wilson, M.P. Newberry, M. Ross, S. Kavale, *J. Food Sci.* **63** (1998) 472-475
- [14] C. Larré, S. Denery-Papini, Y. Popineau, G. Deshayes, C. Desserme, J. Lefebvre, *Cereal Chem.* **77** (2000) 32-38
- [15] C.S. Tseng, H.M. Lai, *J. Food Sci.* **67** (2002) 750-755
- [16] S. Popov, J. Ranković, J. Dodić, S. Dodić, A. Jokić, *Food Technol. Biotechnol.* **48** (2010) 376-383
- [17] P. Rathakrishnan, P. Nagarajan, R. Rajesh Kannan, *Chem. Ind. Chem. Eng. Q.* **17** (2012) 215-222
- [18] L. Flander, M. Salmenkallio-Marttila, T. Suortti, K. Autio, *LWT-Food Sci. Technol.* **40** (2007) 860-870
- [19] G.S. Vieira, L.M. Pereira, M.D. Hubinger, *Int. J. Food Sci. Tech.* **47** (2012) 132-140
- [20] H. Perten, *Cereal Foods World* **35** (1990) 401-402
- [21] M.A. Pagani, M. Lucisano, M. Mariotti: Italian bakery, in: *Bakery products quality and technology*, Y.H. Hui (Ed.), Blackwell Publishing, Ames, IA, 2006, p. 527
- [22] Z. Pótsa: Grain quality requirements in heat industry, in: *A, Pannon quality - graded wheat grain*, Z. Bedő (Ed.) *Agroinform Kiadó és Nyomda Kft*, Budapest, 2008, p. 103-107
- [23] ICC-Standard No 105/2, Determination of crude protein in cereals and cereals products for food and feed, approved 1980, revised 1994, International Association for Cereal Science and Technology, Wien, 1994
- [24] ICC-Standard No 155, Determination of wet gluten quantity and quality (Gluten Index ac. To Perten) of whole wheat meal and wheat flour (*Triticum aestivum*), approved 1994, International Association for Cereal Science and Technology, Wien, 1994
- [25] ICC Standard No. 114/1, Method for Using the Brabender Extensograph, approved 1972, revised 1992, International Association for Cereal Science and Technology, Wien, 1992
- [26] ICC Standard No. 121, Method for Using the Chopin Alveograph, approved 1972, revised 1992, International Association for Cereal Science and Technology, Wien, 1992
- [27] O. Šimurina, J.Mastilović, B. Filipčev, S. Milošević, A. Novaković, in: *Proceedings of the 1<sup>st</sup> International Congress "Food Technology, Quality and Safety"*, Novi Sad, Serbia, 2007, Institute of Food Technology in Novi Sad (FINS), 2007, p. 185-191
- [28] B. Filipčev, L.J. Lević, M. Bodroža-Solarov, N. Mišljenović, G. Koprivica, *Int. J. Food Prop.* **13** (2010) 1035-1053
- [29] G. Derringer, R. Suich, *J. Quality Technol.* **12** (1980) 214-219
- [30] P.C. Lorenzen, *Food Res Int.* **40** (2007) 700-708
- [31] G.A.H. Jong, S.J. Koppelman, *J. Food Sci.* **67** (2002) 2798-2806
- [32] C.E. Stauffer, Oxidants, in: *Functional additives for bakery foods*, C.E. Stauffer (Ed), Van Nostrand Reinhold, New York, 1990, p. 1
- [33] Z. Nježić, J. Živković, B. Cvetković, *Chem. Ind. Hem. Eng. Q.* **16** (2010) 399-403
- [34] A. Basman, H. Köksel, K.W. Ng Perry, *Eur. Food Res. Technol.* **215** (2002) 419-424
- [35] NIST/SEMATECH e-Handbook of Statistical Methods, Commerce Department's U.S. Technology Administration, 2003, <http://www.itl.nist.gov/div898/handbook/> (accessed 15 September 2012)
- [36] J.T. Shelton, A.I. Khuri, J.A. Cornell, *Technometrics* **25** (1983) 357-365.

OLIVERA D. ŠIMURINA<sup>1</sup>  
STEVAN D. POPOV<sup>2</sup>  
BOJANA V. FILIPČEV<sup>1</sup>  
JELENA M. DODIĆ<sup>2</sup>  
MARIJA I.  
BODROŽA-SOLAROV<sup>1</sup>  
MIRJANA DEMIN<sup>3</sup>  
ZVONKO B. NJEŽIĆ<sup>1</sup>

<sup>1</sup>Institut za prehrambene tehnologije,  
Univerzitet u Novom Sadu, Novi Sad,  
Srbija

<sup>2</sup>Tehnološki fakultet, Univerzitet u  
Novom Sadu, Novi Sad, Srbija

<sup>3</sup>Poljoprivredni fakultet, Univerzitet u  
Beogradu, Zemun, Srbija

NAUČNI RAD

## MODELOVANJE EFEKATA TRANSGLUTAMINAZE I L-ASKORBINSKE KISELINE NA BRAŠNO SUBSTANDARDNOG KVALITETA METODOM ODZIVNE POVRŠINE

*U poslednjoj deceniji uočene su ekstremne varijacije klimatskih uslova, koje u kombinaciji sa neadekvatnim agrotehničkim merama uzrokuju smanjenje kvaliteta merkantilne pšenice, odnosno pšeničnog brašna. Ova pojava u pojedinim proizvodnim godinama poprima regionalni karakter. U tom slučaju, brašno substandardnog kvaliteta treba tretirati poboljšivačima, koji imaju potencijal da njegov kvalitet podignu do optimalnog nivoa pogodnog za preradu. Ovaj rad se fokusira na sistematskoj analizi individualnih i interaktivnih efekata L-askorbinske kiseline i transglutaminaze kao ojačivača testa. Efekti su utvrđeni primenom metode odzivne površine. Transglutaminaza postiže veći linearni efekat od L-askorbinske kiseline na reološke i fermentativne osobine testa od brašna substandardnog kvaliteta. Oba dodatka imaju značajan linearni efekat na povećanje specifične zapremine hleba. Utvrđeno je da se primenom kombinacije od 30 mg/kg transglutaminaze i 75,8 mg/kg L-askorbinske kiseline postiže pozitivan sinergistički efekat na reološke i fermentativne osobine pšeničnog testa, kao i dobra teksturalna svojstva i specifična zapremina hleba od brašna substandardnog kvaliteta.*

*Ključne reči: brašno substandardnog kvaliteta, transglutaminaza, L-askorbinska kiselina, optimizacija.*