Food Sci. Biotechnol. 25(S): 77-84 (2016)
DOI 10.1007/s10068-016-0101-z

Food Science
and
Biotechnology

Determination of the Optimum Mixture of Transglutaminase, L-Ascorbic Acid and Xylanase for the Quality and Consumer Acceptability of Bread using Response Surface Methodology

Mi Jeong Kim and Sang Sook Kim*

Division of Functional Food Research, Research Group of Cognition and Sensory Perception, Korea Food Research Institute, Seongnam, Gyeonggi 13539, Korea

Received December 12, 2015
Revised February 16, 2016
Accepted March 7, 2016
Published online March 31, 2016

*Corresponding Author
Tel: +82-31-780-9042
Fax: +82-31-780-9059
E-mail: sskim@kfri.re.kr

pISSN 1226-7708
eISSN 2092-6456

Abstract The optimum levels of transglutaminase (TGase), L-ascorbic acid (L-AA), and xylanase (Xyl) were determined using response surface methodology to improve quality and consumer acceptability of bread made with wheat flour. A Box-Behnken design with three independent variables (TGase, L-AA, and Xyl) and three levels was used to develop models for the different responses (peak time, mixing tolerance, extensibility, resistance, specific volume, hardness, and consumer acceptability). Overall, L-AA and Xyl improved dough and bread properties, whereas the addition of TGase positively affected to texture and overall acceptability by consumer test. The optimal formulation for dough and bread properties and consumer acceptability were identified and the optimal value was 0.36 g/100 g TGase, 0.026 g/100 g Xyl, and 0.005 g/100 g L-AA. The results demonstrate that the addition of optimum amounts of TGase, Xyl, and L-AA improves the baking quality of the flour by enhancing dough properties and increase the consumer acceptability of the bread.

Keywords: bread quality, transglutaminase, xylanase, L-ascorbic acid, response surface methodology

Introduction

The bread is one of widely consumed wheat products and its quality is commonly affected by the protein quantity and quality of wheat flour (1). For the production of yeast-leavened bread, flour with a protein content of at least 11% is usually preferred (2). However, climatic conditions prevent the production of wheat with this protein content level, resulting in import wheat with high protein content by many countries (2). Fluctuations in international wheat prices due to unexpected natural disasters or abnormal weather create a burden for the national economy and affect food security. Thus, the alternatives for utilizing wheat flour with low quality or low quantity of protein in wheat bread should be proposed for bakery industry.

Wheat flour with low breadmaking quality can be improved by chemical oxidizing agents or exogenous enzymes that improve the gluten network (3). L-Ascorbic acid (L-AA), one of the most widely used flour improvers, increases dough strength and improves bread volume and crumb structure (4). The L-AA enhances the rheological properties of dough is primarily via its electron-donating capacity and the formation of reactive products such as O_2^- (5). The concentration of L-AA commonly used for breadmaking is in the range of 20-150 ppm and the appropriate concentration is different depending on the type of cultivar, flour, and bread (6). As an enzyme that enhances the functional properties of elasticity and water holding capacity, transglutaminase (TGase, EC 2.3.2.13, protein-glutamine γ-glutamyltransferase) is used in various food products and catalyzes the formation of a crosslink between a free amine group and the γ-carboxamide group of protein-bound glutamine (7). When it is used in breadmaking, TGase can improve the functionality of flour proteins by forming large insoluble polymers (8). In addition, xylanase (Xyl, endo-β-1,4-xylanase EC 3.2.1.8) is frequently used in breadmaking industry to enhance bread quality (9). Xylanase hydrolyzes arabinoxylans that interfere with the formation of a gluten network (10).

Combinations of chemical oxidizing agents and crosslinking enzymes have frequently been used to improve the quality of flour and the end product (4,11-13). Simurina *et al.* investigated the effect of a combination of TGase and L-AA on substandard quality wheat flour and observed an improvement in dough properties as well as the quality of bread prepared from substandard wheat flour compared to dough and bread without these additives. Steffolani *et al.* (11), Filipcev *et al.* (12), and Shafisoltani *et al.* (13) examined the synergistic effects of Xyl and other enzymes or oxidizing agents on dough and

bread properties. Despite extensive study of the application of various dough improvers and their combinations in commercial wheat flour, optimal combinations of L-AA, TGase, and Xyl for wheat flours containing a low quality or quantity of protein have not been established. In addition, optimal study of chemical oxidizing agents and enzymes to improve consumer acceptability was limited.

Therefore, this study aimed to develop a new combination of enzymes (TGase and Xyl) and chemical oxidizing agent (L-AA) to improve the dough properties and bread quality of flours with low protein contents using a Box-Behnken design. The consumer acceptability of the breads was also evaluated to determine the optimal combination of TGase, Xyl, and L-AA.

Materials and Methods

Materials The commercial wheat flour used in this study was provided by CJ Jeiljedang Corporation (Seoul, Korea). The protein, crude fat, ash, and gluten contents of the flour were 8.92±0.09, 0.78±0.11, 0.43±0.04, and 24.5±1.36% based on 14% moisture content, respectively. The enzymes used in this study were TGase (TG-F102; Shanghai Dongsheng Food Co., Ltd., Shanghai, China) and Xyl (Pentopan mono BG; Novozymes, Bagsvaerd, Denmark), and the both were provided from Double U Co., Ltd. (Yongin, Korea). The TGase was a commercial prepared powder and 20 units of enzyme activity per gram. The Xyl contained 2,500 fungal xylanase units (FXU) per gram of powdered preparation. In addition, L-AA (ES Ingredients Co., Ltd., Gunpo, Korea) was used as a chemical oxidizing agent.

Dough properties The peak time and mixing tolerance of dough were determined using a 10 g mixograph (National Mfg. Co., Lincoln, NE, USA) according to AACC approved method 54-40.02 (14). Dough extensibility and resistance were determined using a texture analyzer (TA-HD plus; Stable Micro Systems Ltd., Godalming, England) according to the procedure described by Barros *et al.* (15) with modifications to the sample preparation. Wheat flour samples and 2% NaCl solution (57% of flour weights) were mixed to form dough and were then incubated for 30 min at 30°C and RH 70%. Strips (8x50x5 mm, width x length x height) were prepared from the dough. The shaped strips were placed on a flat metal plate, and the resistance and extensibility were measured using a probe (Kieffer dough and gluten extensibility rig; Stable Micro Systems Ltd.) in tension mode at a pre-test speed of 2.0 mm/s, a test speed of 3.3 mm/s, a post-test speed of 10.0 mm/s, and a distance of 150 mm.

Preparation of yeast-leavened bread Yeast-leavened bread was prepared using AACC Method 10-10B (14) with modification of the ingredients as follows: flour, 100%; dry yeast, 2.12%; salt, 6%; baking improver, 0.2%; shortening, 3%; and water, 67% based on flour weight, fwb.

Table 1. Box-Behnken design for the optimization of transglutaminase, xylanase, and L-ascorbic acid addition

Combination no.	Coded level			Actual levels (g/100 g of flour)		
	TGase[1)]	Xyl[2)]	L-AA[3)]	TGase	Xyl	L-AA
1	1	1	0	0.5	0.05	0.005
2	-1	1	0	0	0.05	0.005
3	1	-1	0	0.5	0	0.005
4	-1	-1	0	0	0	0.005
5	0	1	1	0.1	0.05	0.025
6	0	1	-1	0.1	0.05	0
7	0	-1	1	0.1	0	0.025
8	0	-1	-1	0.1	0	0
9	1	0	1	0.5	0.01	0.025
10	1	0	-1	0.5	0.01	0
11	-1	0	1	0	0.01	0.025
12	-1	0	-1	0	0.01	0
13	0	0	0	0.1	0.01	0.005
14	0	0	0	0.1	0.01	0.005
15	0	0	0	0.1	0.01	0.005

[1)]TGase is transglutaminase containing 20 units/g.
[2)]Xyl is xylanase containing 2,500 FXU/g.
[3)]L-AA is L-ascorbic acid.

Specific volume and hardness of bread The specific volume (mL/g) of bread was determined on the day of baking using a Volscan profiler (Stable Micro Systems Ltd.) and was calculated by dividing the volume of the bread by its weight. The hardness of the bread was measured as described by Bourne (16). To determine the hardness of the bread, a crumb of bread (25x25x19 mm) was compressed with a plunger (50 mm diameter) at a crosshead speed of 10 mm/s using a TA (TA-XT plus; Stable Micro Systems Ltd.).

Consumer test of bread A total of 100 consumers participated in a consumer test of the breads. The consumer acceptability of the bread samples prepared from flour and the 15 combinations listed in Table 1 was assessed over two days at the Korea Food Research Institute (Seongnam, Korea). In the first, the consumers received a pre-score card, which consisted of questionnaires to assess consumer concepts of ideal intensities of 11 bread characteristics (crumb color, air cell size, uniformity, milk flavor, butter flavor, yeast flavor, moistness, softness, cohesiveness, chewiness, and adhesiveness) on a 7-point category scale (1=too weak, 4=neither strong nor weak, and 7=too strong). After that, the consumers evaluated the appearance, odor, taste, texture, and overall acceptability of the breads using a 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, and 9=like extremely). In addition, the consumers evaluated the intensity of 11 bread characteristics (crumb color, air cell size, uniformity, milk flavor, butter flavor, yeast flavor, moistness, softness, cohesiveness, chewiness, and adhesiveness) of the bread samples on a 7-point category scale as for the pre-score card. For consumer testing, four pieces of bread samples (19x20x20 mm) were presented on a dish (14 cm, diameter) with a three-digit random code. All of consumer tests were conducted in individual booths. Each consumer

evaluated a total of 15 breads for 2 days. On the each day, the panelists evaluated seven or eight samples, which were presented randomly and in a monadic manner. Water was provided to panelists for rinsing the mouth before tasting a sample and in between samples and two minutes was given before presenting next samples to minimize sensory fatigue.

Experimental design Response surface methodology (RSM) was used to optimize the formulation of TGase, Xyl, and L-AA in flours containing low quantity or quality of protein. A three-level three-factor experimental design with three replicates at the center point was used to investigate the effects of the three independent variables (TGase, Xyl, and L-AA) on dough properties, bread, and consumer acceptability (17). The concentrations of the two enzymes and L-AA were selected based on preliminary experiments or manufacturer recommendations (Table 1). The complete experimental design consisted of 15 combinations.

Data analysis The effects of the three independent variables on the responses (Y) were modeled using the response surface regression (RSREG) procedure of Statistical Analysis Software (SAS, version 9.00; SAS Institute Inc., Cary, NC, USA). The second-order response function for the experiments was predicted by the following equation:

$$Y=\beta_0+\sum_{i=3}^{3}\beta_i X_i+\sum_{i=3}^{3}\beta_{ii}X_i^2+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j \quad (1)$$

where β_0 is a constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. X_i is level of the independent variables. RIDGE MAX/MIN in the RSREG SAS output was used to compute the estimated ridge of maximum or minimum response for increasing radii from the center of the original design (18). Analysis of variance (ANOVA) was performed using SAS to assess differences among the samples. When a difference among samples was identified, the Student Newan-Keul's (SNK) multiple comparison was performed to separate the means. Minitab Statistical Software (Version 17; Minitab Inc., State College, PA, USA) was used to create response plots by holding constant one variable of the second-order polynomial equation. To obtain the final optimized formula for quality and consumer acceptability of bread, superimposed contour plots of significant response variables were applied using Minitab Statistical Software. In addition, principal component analysis (PCA) was performed using XLSTAT software (Addinsoft, Paris, France) to summarize the relationship between the dough and bread properties of wheat flour and the consumer perception of breads prepared with and without TGase, Xyl, and L-AA.

Results and Discussion

Effects of TGase, Xyl, and L-AA on dough properties Breadmaking quality of wheat flours was affected by dough strength and extensibility (19). In this study, peak time, mixing tolerance, extensibility, and resistance of the wheat flours containing different levels of TGase,

Table 2. Experimental responses of dough properties, bread and consumer acceptability of breads

	Dough properties				Bread		Consumer acceptability[2)]				
Combination no.[3)]	Peak time (min)	Mixing Tolerance **[5)] (mm)	Resistance *** (g)	Extensibility *** (mm)	Specific Volume *** (mg/mL)	Hardness *** (g)	Flavor	Appearance	Taste	Texture**	Overall**
1	3.87[1)]	6.40[b4)]	65[b]	71[d]	4.35[efg]	681[c]	5.85	6.16	5.55	5.56[abc]	5.60[ab]
2	3.33	8.00[ab]	76[ab]	71[d]	4.86[a]	533[def]	5.73	5.97	5.62	5.59[abc]	5.71[ab]
3	4.41	8.57[ab]	78[ab]	91[bc]	3.92[h]	772[b]	5.85	6.26	6.17	5.83[abc]	6.08[ab]
4	4.01	8.23[ab]	39[c]	104[b]	4.22[g]	589[cde]	5.57	5.79	5.42	5.35[abc]	5.44[ab]
5	4.13	9.50[a]	31[c]	122[a]	4.75[abc]	589[cde]	5.55	5.79	5.57	5.56[abc]	5.73[ab]
6	3.84	8.60[ab]	27[c]	125[a]	4.82[ab]	541[def]	5.46	5.73	5.54	5.38[abc]	5.59[ab]
7	4.30	10.97[a]	42[c]	102[b]	4.20[g]	653[c]	5.91	5.79	5.66	5.63[abc]	5.74[ab]
8	4.43	8.93[ab]	32[c]	117[a]	4.29[fg]	788[b]	5.56	5.77	5.40	5.32[abc]	5.47[ab]
9	4.78	10.13[a]	86[a]	79[cd]	4.46[def]	998[a]	5.76	5.93	5.72	5.59[abc]	5.80[ab]
10	4.56	8.83[ab]	59[b]	85[cd]	4.20[g]	801[b]	6.20	6.15	6.00	5.87[ab]	6.16[a]
11	4.45	9.67[a]	63[b]	76[cd]	4.51[cdef]	549[def]	5.70	5.76	5.42	5.18[bc]	5.32[b]
12	4.19	8.13[ab]	31[c]	120[a]	4.60[bcde]	626[cd]	5.78	5.96	5.86	5.79[abc]	5.93[ab]
13	4.43	9.30[ab]	30[c]	129[a]	4.48[def]	534[def]	5.62	5.83	5.62	5.56[abc]	5.68[ab]
14	4.49	9.80[a]	32[c]	123[a]	4.55[cdef]	472[f]	5.58	5.60	5.52	5.04[c]	5.42[ab]
15	4.54	10.03[a]	30[c]	129[a]	4.63[abcd]	489[ef]	5.52	5.85	5.66	5.61[abc]	5.60[ab]

[1)]Data are means of 3 replications.
[2)]Consumer test was participated by 100 consumers.
[3)]Identification of combination no. is described in Table 1.
[4)a-h]Values with same alphabet within a column are not significantly different.
[5)]**, ***significantly differ at $p<0.01$ and $p<0.001$.

Table 3. Regression coefficients and *R* square values of the predicted second order polynomial models for the response variables

Regression Coefficients[1)]	Dough properties				Bread		Consumer acceptability				
	Peak time	Mixing tolerance	Extensibility	Resistance	Specific volume	Hardness	Odor	Appearance	Taste	Texture	Overall
β_0	4.75	10.71	116.48	44.75	4.76	399	5.51	5.71	5.61	5.34	5.52
β_1	0.22	-0.19	-4.44	8.10	-0.14	144	0.06	0.10	0.07	0.08	0.09
β_2	-0.21	-0.77	-0.67	-3.39	0.25	-49.9	-0.06	0.0004	-0.09	-0.02	-0.05
β_3	0.11	0.64	-3.61	7.10	0.03	35.6	-0.06	-0.05	-0.07	-0.04	-0.07
β_{12}	0.03	-0.56	2.05	-9.62	-0.05	0.37	-0.12	-0.03	-0.17	-0.09	-0.16
β_{23}	0.01	-0.24	10.16	-7.37	-0.01	23.9	-0.05	0.02	0.008	0.04	0.04
β_{13}	0.09	-0.02	6.32	0.31	0.09	81	-0.12	-0.05	-0.06	-0.02	-0.03
β_{11}	-0.37	-1.79	-36.6	29.28	-0.08	87	0.21	0.25	0.10	0.15	0.11
β_{22}	-0.47	-0.76	1.47	-1.26	-0.34	111	-0.05	0.06	-0.07	0.03	-0.02
β_{33}	0.15	0.09	9.22	-15.93	0.03	187	0.12	-0.04	0.004	0.09	-0.15
R^2	0.969	0.969	0.828	0.883	0.985	0.956	0.730	0.823	0.705	0.380	0.644

[1)]β_0, Constant; β_1, β_2, and β_3 are linear coefficients of transglutaminase (X_1), xylanases (X_2), and L-ascorbic acid (X_3), respectively; β_{12}, β_{23}, and β_{13} are interaction coefficients of X_1xX_2, X_2xX_3, and X_1xX_3, respectively; β_{11}, β_{22}, and β_{33} are quadratic coefficients of X_1^2, X_2^2, and X_3^2, respectively

Xyl, and L-AA are summarized in Table 2. The estimated values of regression coefficients are given in Table 3, and the regression equation for peak time, mixing tolerance, extensibility, and resistance (Y) with TGase (X_1), Xyl (X_2), and L-AA (X_3) can be described as below:

Peak time

$$Y=4.75+0.22X_1-0.21X_2+0.11X_3+0.03X_1X_2+0.01X_2X_3+0.09X_1X_3 -0.37X_1^2-0.47X_2^2+0.15X_3^2 \quad (2)$$

Mixing tolerance

$$Y=10.71-0.19X_1-0.77X_2+0.64X_3-0.56X_1X_2-0.24X_2X_3-0.02X_1X_3 -1.79X_1^2-0.76X_2^2+0.09X_3^2 \quad (3)$$

Extensibility

$$Y=116.48-4.44X_1-0.67X_2-3.61X_3+2.05X_1X_2+10.16X_2X_3+6.32X_1X_3 -36.6X_1^2+1.47X_2^2+9.22X_3^2 \quad (4)$$

Resistance

$$Y=44.75+8.10X_1-3.39X_2+7.10X_3-9.62X_1X_2-7.37X_2X_3+0.31X_1X_3 +29.28X_1^2-1.26X_2^2-15.93X_3^2 \quad (5)$$

Peak time and mixing tolerance measured with mixograph were related with dough strength. Peak time indicates optimum dough develop time, referred to as time to peak resistance (20). As shown in Table 2, the peak time of the 15 combinations was in the range of 3.33-4.78 min. The peak time did not differ significantly among the 15 combination samples but was short for dough with high levels of Xyl (0.05 g/100 g flour) (Table 2). Additionally, as indicated by the regression coefficients in Table 3, Xyl (β_2= −0.21) had a linear negative effect on peak time. This result is consistent with Shafisoltani *et al.* (13), who reported that Xyl decrease the development time of dough due to a reduction of the viscosity in dough. It was known that a reduction of the viscosity in dough is due to Xyl hydrolyzes insoluble arabinoxylans to water soluble arabinoxylans (21). The mixing tolerances of the 15 combinations differed significantly (p<0.01) and ranged from 6.4 to 10.97 mm (Table 2). The mixing tolerance measures mixograph bandwidth at 7 min, referred to as tolerance (20). Higher mixing tolerance values were indicated higher dough strength. In general, the dough containing high levels of L-AA (0.025 g/100 g flour) exhibited high mixing tolerance (Table 2 and Fig. 1A). As shown in Table 3, L-AA (β_3=0.64) had a linear positive effect on the mixing tolerance of dough, whereas TGase (β_1= −0.19) and Xyl (β_2= −0.77) had a negative linear effect (R^2=0.969). These results are supported by previous studies demonstrating that oxidants such as L-AA were known to strengthen the gluten network by creating disulfide bonds (5,22).

The extensibility and resistance of the 15 combinations differed significantly (both p<0.001) and were in the range of 71-129 mm and 27-86 g, respectively. The extensibility of combinations 13, 14, and 15 (TGase: 0.1 g/100 g flour; Xyl: 0.01 g/100 g flour; L-AA: 0.005 g/100 g flour) were higher than those of the other samples. The effects of these combinations (TGase, Xyl, and L-AA) on dough extensibility might be explained by the regression coefficients of the predicted second-order polynomial models and three dimensional response surface plots for the response variables (Table 3 and Fig. 1B). As shown in Table 3, TGase (β_1= −4.44), Xyl (β_2= −0.67), and L-AA (β_2= −3.61) had a linear negative effect on dough extensibility. Rosell *et al.* (23), reported that TGase decreases dough extensibility and might lead to an undesirable reduction in loaf volume at high concentrations. These reports were explained by study of Bauer *et al.* (24), who found that the high level of TGase caused excessive cross-linking of the gluten proteins leading to decrease the extensibility of the dough and mechanical damage of the gluten network. While the interacting effects of TGase and Xyl (β_{12}=2.05) or Xyl and L-AA (β_{23}=10.16) or TGase and L-AA (β_{13}=6.32) positively increased the extensibility of the dough, indicating synergistic effects of dough properties by combinations of TGase, Xyl, or L-AA. By contrast, dough resistance was lower in combinations 13, 14, and 15 than in others. Based on the regression model, Xyl (β_2= −3.39) exhibited a linear negative

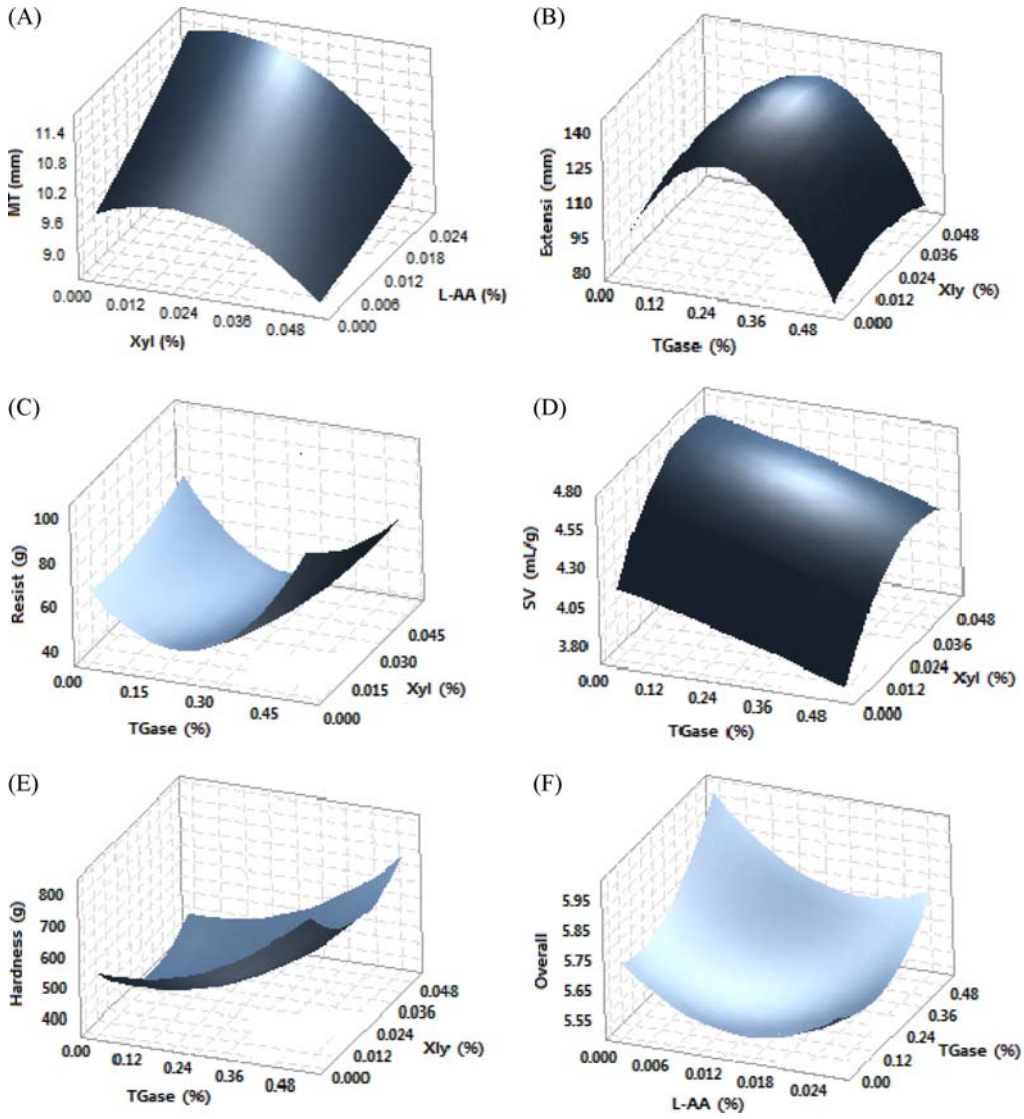

Fig. 1. Response surface plot of mixing tolerance, extensibility and resistance of dough, specific volume and hardness of breads, and overall acceptability of combinations of transglutaminase, xylanase, and L-ascorbic acid. (A) response surface plot of mixing tolerance. (B) response surface plot of extensibility. (C) response surface plot of resistance. (D) response surface plot of specific volume. (E) response surface plot of hardness. (F) response surface plot of overall acceptability. (MT, mixing tolerance; SV, specific volume; Overall, overall acceptability; TGase, transglutaminase containing 20 units/g; Xyl, xylanases containing 2,500 FXU/g; L-AA, L-ascorbic acid; % was calculated by g/100 g flour.)

effect, indicating that Xyl decreases dough resistance (Fig. 1C). In addition, the interactive effects of TGase and Xyl (β_{12}= –9.62) or Xyl and L-AA (β_{23}= –7.34), when TGase or L-AA was combined with Xyl, resulted in lower dough resistance compared to the linear effects of these additives (TGase: β_1=8.10 or L-AA: β_3=7.10).

Effects of TGase, Xyl, and L-AA on SV and hardness of breads The specific volume and hardness of breads prepared from flour and the 15 combinations are presented in Table 2. The breads prepared from the 15 combination samples differed significantly in specific volume (p<0.001) and hardness (p<0.001). The specific volume and hardness of the bread samples were in the range of 3.92-4.86 mg/mL and 472-998 g, respectively. The regression coefficients and three dimensional response surface plots for specific volume and hardness of breads are presented in Table 3 and Fig. 1. The regression equation for specific volume and hardness of bread with TGase (X_1), Xyl (X_2), and L-AA (X_3) can be described as below:

Specific volume

$$Y=4.76-0.14X_1+0.25X_2+0.03X_3-0.05X_1X_2-0.01X_2X_3+0.09X_1X_3 -0.08X_1^2-0.34X_2^2+0.03X_3^2 \quad (6)$$

Hardness

$$Y=399+144X_1-49.9X_2+35.6X_3+0.37X_1X_2+23.9X_2X_3+81X_1X_3 +87X_1^2+111X_2^2+187X_3^2 \quad (7)$$

In Table 3, the regression coefficients for the specific volume

indicated a positive linear effect (β_2=0.25) of Xyl. The results in this study are comparable with the results of Filipcev *et al.* (12), who reported a positive effect of xylanase on the loaf volume of bread. By contrast, the quadratic coefficient (β_{22}= −034) of Xyl revealed a negative effect on the specific volume of bread. These results may explain the improvement of the specific volume of bread by low levels of Xyl (<0.03 g/100 g), in contrast to the decreased in specific volume observed at high levels of Xyl (>0.03 g/100 g flours) (Fig. 1D). Simurina *et al.* (4) reported that the combination of TGase (0.3 g/100 g flour) and L-AA (0.75 g/100 g) produced superior dough properties and bread quality compared to the control flour (without these additives). As reported by Simurina *et al.* (4), in this study, the interaction of TGase and L-AA (β_{13}=0.09) positively affected the specific volume (Table 3). Crumb hardness is a commonly used as an index of bread quality because it is directly associated with consumer acceptance and the loaf volume of bread (25,26). In this study, the hardness of breads prepared from combinations 13, 14, and 15 was low compared to the other samples. Caballero *et al.* (1) reported that bread with added Xyl and TGase exhibited significantly decreased hardness compared with the control bread. Primo-Martin *et al.* (27) reported that the interactive effect of TGase and Xyl in improving the hardness of breads was due to rheological changes and the release of pentosan from the gluten network (27). In this study, as shown in Table 3 and Fig. 1E, the interactive effects of TGase and Xyl decreased the hardness of bread.

Use of TGase, L-AA, and Xyl mixture resulted in decreased crumb hardness and increased specific volume of bread as shown in Fig. 1. Previous studies have reported a negative correlation between the specific volume and crumb hardness of breads (25,26). In this study, similar results were obtained; bread with a high specific volume exhibited low hardness, as shown in Fig. 1D and Fig. 1E.

Effects of TGase, Xyl, and L-AA on consumer acceptability The experimental responses of appearance, odor, taste, texture, and overall acceptability of breads prepared from the 15 combinations are presented in Table 2. Consumer evaluated ideal characteristics of bread before evaluating 15 bread samples and the result exhibited that consumers preferred the bread with low air cell size and yeast flavor and high moistness, milk flavor, and butter flavor. The air cell size (p<0.001) and moistness (p<0.05) differed significantly among the 15 bread samples (data not shown). The texture (p<0.01) and overall acceptability (p<0.01) differed significantly among the 15 breads, whereas no differences were observed in the odor, appearance, and taste acceptability. Overall, the addition of TGase, Xyl, or L-AA to flour affected the texture and overall acceptability of the breads but not odor, appearance, or taste acceptability. Bread prepared using combination 10 (TGase: 0.5 g/100 g flour; Xyl: 0.01 g/100 g flour; L-AA: 0 g/100 g flour) had the highest overall acceptability among the samples. By contrast, bread prepared using combination 11 (TGase: 0.1 g/100 g flour; Xyl: 0.01 g/100 g flour; L-AA: 0.025 g/100 g flour) had the lowest overall acceptability. These results suggest that the addition of TGase positively affected to overall acceptability, whereas the addition of L-AA did not affect overall acceptability. The results in this study were comparable with the study by Yamazaki *et al*. (28) who reported the bread with added TGase increased sensory attributes as acceptability and moistness although bread with high concentration of TGase resulted in decreasing specific volume. Also, combination 12, which was contained only 0.01 g/100 g flour of Xyl, also exhibited relatively high texture acceptability, as reported by Shafisoltani *et al*. (13). Shafisoltani *et al*. (13) demonstrated that Xyl improves bread texture by forming water soluble arabinoxylans, which have a positive effect on dough properties and bread quality in dough networks (29). The response surface plot of overall acceptability is presented in Fig. 1F. Increasing TGase and decreasing L-AA in bread were associated with high overall acceptability. The regression coefficients and *R* square values of the polynomial models for consumer acceptability are presented in Table 3. The linear and quadratic coefficients of TGase were positive, implying improved consumer acceptability.

Optimization of TGase, Xyl, and L-AA The optimization goal in this study was to improve the quality and consumer acceptability of bread. The optimum levels of TGase, Xyl, and L-AA for dough properties, bread, and consumer acceptability of wheat flour containing protein of low quality were determined by RSREG. Extensibility and specific volume have been used widely to study flour quality and the effect of specific additives in breadmaking (30). Thus, the optimal formulations for dough extensibility, bread specific volume, and consumer acceptability identified by RSREG were compared with the control bread. The side shapes and cross-sections of breads prepared with and without the formulations for dough, bread, and consumer acceptability are presented in Fig. 2A. As shown in Fig. 2A, volumes of bread samples prepared with optimum mixture conditions determined in this study were higher than that of control bread. The optimal dough extensibility was achieved for using the combination of TGase 0.222, Xyl 0.015, and L-AA 0.001 g/100 g flour, and the optimal specific volume of bread was achieved using the combination of TGase 0.07, Xyl 0.03, and L-AA 0.005 g/100 g flour. For the overall acceptability of bread, the optimal formulations were TGase 0.426, Xyl 0.016, and L-AA 0.005 g/100 g flour. Because the properties of dough or bread and consumer acceptability exhibited different trends, the final optimized formula was obtained by superimposed contour plots of significant responses. Response contour plots for mixing tolerance, extensibility and resistance of dough, specific volume and hardness of bread, and overall acceptability by consumer test were overlaid and the results of superimposed contour plots are presented in Fig. 2B. The gray regions of Fig. 2B represented a combination value of TGase, Xyl, and L-AA for the final optimized formula. The values of TGase, Xyl, and L-AA in superimposed contour plots were ranged in 0.33-0.39, 0.02-0.032, and 0.0025-0.0075 g/100 g, respectively. In this study, the optimum concentrations of TGase, Xyl, and L-AA were identified at the centroid of the gray regions and

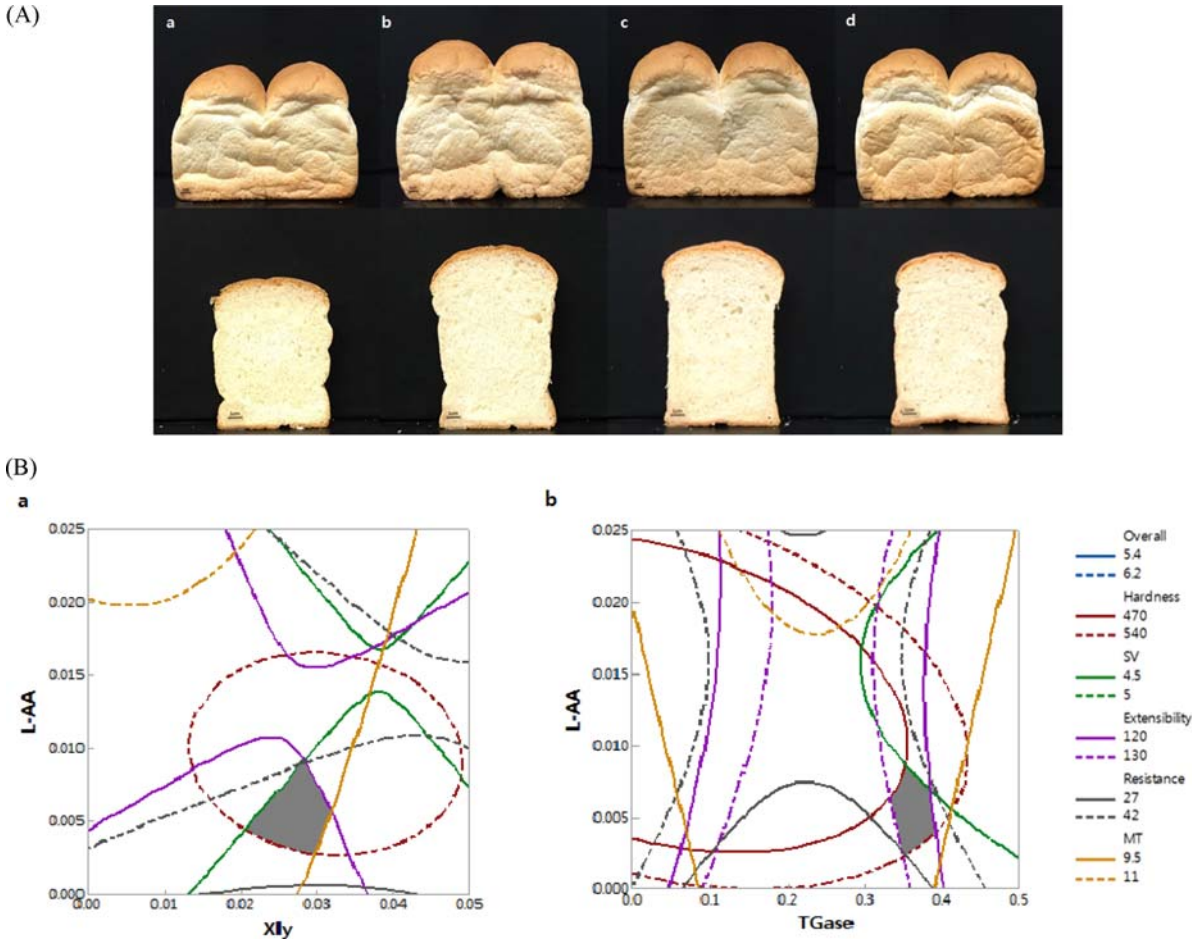

Fig. 2. (A) Side shape and cross-sections of breads prepared with and without optimized formulations and (B) superimposed contour plots of significant response variables as affected by the percentages of TGase, Xyl, and L-AA incorporated. (A): a, control; b, dough extensibility; c, bread specific volume; d, overall acceptability of consumer test, respectively. (B): Gray regions of a and b represent a combination value of TGase, Xyl, and L-AA for optimum values of MT, extensibility, resistance, SV, hardness, and overall acceptability. Overall, overall acceptability of consumer test; SV, specific volume; MT, mixing tolerance; TGase, transglutaminase containing 20 units/g; Xyl, xylanases containing 2500 FXU/g; L-AA, L-ascorbic acid; % was calculated by g/100 g flour).

the value of final optimized formula was 0.36 g/100 g TGase, 0.026 g/100 g Xyl, and 0.005 g/100 g L-AA.

Principal component analysis (PCA) Principal component analysis (PCA) was conducted to determine how the 15 combinations affected dough properties and the specific volume, hardness, and consumer test of breads (Fig. 3). Principal component (PC) 1 (x-axis) and PC 2 (y-axis) accounted for 40.04 and 20.12% of the total variance, respectively. The samples, positively loaded on PC1, were relatively high in acceptability and moistness of bread by consumer test, peak time and resistance of dough, bread hardness, and TGase compared to the samples negatively loaded on PC1. By contrast, samples negatively loaded on PC1 were high in extensibility and mixing tolerance of dough, the specific volume of the breads, the air cell size of bread by consumer test, and Xyl. As shown in Fig. 3, the properties of dough or bread and consumer acceptability exhibited different trends. Combinations 3, 9, and 10 contained high levels (0.5 g/100 g flour) of TGase and had low dough extensibility and specific volume, indicating a negative effect on baking quality. However, the breads prepared from these combinations were rated relatively high in consumer acceptability, indicating that TGase might improve consumer acceptability of breads. Even though high specific volume and extensibility are desirable characteristics of bread, consumer acceptability can be affected by not only specific volume but also other characteristics such as moistness of breads, as reported by Yamazaki *et al*. (28). In study of Yamazaki *et al*. (28), the high specific volume resulted in low moistness of breads. As exhibited in Fig. 3, the high specific volume of bread resulted in low moistness and big air cell size of bread crumb in this study.

In summary, the effects of combination of TGase, Xyl, and L-AA in wheat flour, which contains 9% protein, was investigated on dough properties, bread quality, and consumer acceptability of yeast-leavened bread using response surface methodology. Results of this study implied that quality of flour could be improved by adding a

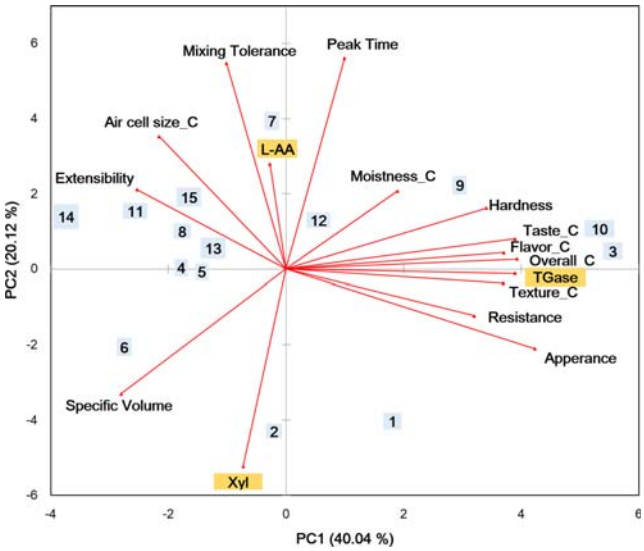

Fig. 3. Loading plot of dough properties, bread quality, and consumer test of breads prepared with 15 combinations, TGase, Xyl, and L-AA on principal component 1 (x-axis) and principal component 2 (y-axis). (TGase, transglutaminase containing 20 units/g; Xyl, xylanases containing 2,500 FXU/g; L-AA, L-ascorbic acid, Air cell size_C, air cell size by consumer test; Moistness_C, moistness by consumer test; Taste_C, taste acceptability; Flavor_C, flavor acceptability; Overall_C, overall acceptability; Texture_C, texture acceptability; Appearance_C. appearance acceptability).

combination of TGase, Xyl, and L-AA to weak flour, resulting in increase of dough extensibility and specific volume of bread. In addition, the higher TGase resulted in the higher consumer acceptability of bread while the highest level of TGase used in this study affected negatively on dough and bread qualities. Consequently, this study could inform the utilization of wheat flour with low quality or low quantity of protein in the baking industry.

Acknowledgments This study was supported by a grant from Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries in Gyeonggi-Do, Korea (323049-04-3-SB010).

Disclosure The authors declare no conflict of interest.

References

1. Caballero PA, Gomez M, Rosell CM. Improvement of dough rheology, bread quality and bread shelf-life by enzymes combination. J. Food Eng. 81: 42-53 (2007)
2. Pomeranz Y. Wheat: Chemistry and Technology. 3rd ed. American Association of Cereal Chemist, St. Paul, MN, USA. pp. 30 (1988)
3. Joye IJ, Lagrain B, Delcour JA. Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking-A review. J. Cereal Sci. 50: 11-21 (2009)
4. Simurina OD, Popov SD, Filipcev BV, Dodic JM, Bodroza-Solarov MI, Demin M, Njezic ZB. Modeling the effects of transglutaminase and L-ascorbic acid on substandard quality wheat flour by response surface methodology. Chem. Ind. Chem. Eng. Q. 20: 471-480 (2014)
5. Nakamura M, Kurata T. Effect of L-ascorbic acid on the rheological properties of wheat flour-water dough. Cereal Chem. 74: 647-650 (1997)
6. Koehler P. Concentration of low and high molecular weight thiols in wheat dough as affected by different concentrations of ascorbic acid. J. Agr. Food Chem. 51: 4948-4953 (2003)
7. Zhang D, Zhu Y, Chen J. Microbial transglutaminase production: Understanding the mechanism. Biotechnol. Genet. Eng. 26: 205-222 (2009)
8. Larre C, Denery-Papini S, Popineau Y, Deshayes G, Dsserme C, Lefebvre J. Biochemical analysis and rheological properties of gluten modified by transglutaminase. Cereal Chem. 77: 121-127 (2000)
9. Primo-Martin C, Martinez-Anaya MA. Influence of pentosansase and oxidases on water-extractable pentosans during a straight breadmaking process. J. Food Sci. 68: 31-41 (2003)
10. Collins T, Hoyoux A, Dutron A, Georis J, Genot B, DauvrinT, Arnaut F, Gerday C, Feller G. Use of glycoside hydrolase family 8 xylanases in baking. J. Cereal Sci. 43: 79-84 (2006)
11. Steffolani ME, Ribotta PD, Perez GT, Leon AE. Combinations of glucose oxidase, a-amylase and xylanase affect dough properties and bread quality. Int. J. Food Sci. Tech. 47: 525-534 (2012)
12. Filipcev B, Simurina O, Bodroza-Solarov M. Combined effect of xylanase, ascorbic and citric acid in regulating the quality of bread made from organically grown spelt cultivars. J. Food Quality 37: 185-195 (2014)
13. Shafisoltani M, Salehifar M, Hashemi M. Effects of enzymatic treatment using response surface methodology on the quality of bread flour. Food Chem. 148: 176-183 (2014)
14. AACC. Approved Method of the AACC. 10th ed. Methods 54-40.02 and 10-10B. American Association of Cereal Chemists, St. Paul, MN, USA (2000)
15. Barros F, Alviola JN, Tilley M, Chen YR, Pierucci VRM, Rooney LW. Predicting hot-press wheat tortilla quality using flour, dough and gluten properties. J. Cereal Sci. 52: 288-294 (2010)
16. Bourne MC. Texture profile analysis. Food Technol. 32: 62-66 (1978)
17. Box GEP, Behnken DW. Some new three level designs for the study of quantitative variables. Technometrics 2: 455-475 (1960)
18. Myers RH, Montgomery DC. Response Surface Methodology: Process and Product Optimization using Designed Experiments, 2nd ed. John Wiley & Son Inc., New York City, NY, USA. pp. 235-302 (2002)
19. Branlard G, Rousset M, Loisel W, Autran JC. Comparison of 46 technological parameters used in breeding in bread wheat quality evaluation. J. Genet. Breed. 45: 263-280 (1991)
20. Nash D, Lanning SP, Fox P, Martin JM, Blake NK, Souza E, Graybosch RA, Giroux MJ, Talbert LE. Relationship of dough extensibility to dough strength in a spring wheat cross. Cereal Chem. 83: 225-258 (2006)
21. Butt MS, Tahir-Nadeem M, Ahmad Z, Sultan MT. Xylanases and their applications in baking industry. Food Technol. Biotech. 46: 22-31 (2008)
22. Selomulyo VO, Zhou W. Frozen bread dough: Effects of freezing storage and dough improvers. J. Cereal Sci. 45: 1-17 (2007)
23. Rosell CM, Wang J, Aja S, Bean S, Lookhart G. Wheat flour proteins as affected by transglutaminase and glucose oxidase. Cereal Chem. 80: 52-55 (2003)
24. Bauer N, Koehler P, Wieser H, Schieberle P. Studies on effects of microbialtransglutaminase on gluten proteins of wheat. II. Rheological properties. Cereal Chem. 80: 787-790 (2003)
25. Spices R. Application of rheology in the bread industry. pp. 343-361. In: Dough Rheology and Baked Product Texture. Faridi H, Faubion JM (eds). Van Nostrand Reinhold Press Inc., New York City, NY, USA (1990)
26. Rozylo R. Effect of process modifications in two cycles of dough mixing on physical properties of wheat bread baked from weak flour. Food Bioprocess Tech. 7: 774-783 (2014)
27. Primo-Martin C, Valera R, Martinez-Anaya MA. Effect of pentosansase and oxidases on the characteristics of doughs and the glutenin macropolymer (GMP). J. Agr. Food Chem. 51: 4673-4679 (2003)
28. Yamazaki K, Naruto Y, Nakamura H, Takahashi K. Improving the breaking properties and oil absorption of breadcrumbs by microbial transglutaminase. Food Sci. Technol. Res. 13: 28-34 (2007)
29. Martinez-Anaya MA, Jimenez T. Functionality of enzymes that hydrolyses starch and non-starch polysaccharide in breadmaking. Z. Lebensm. Unters. Forsch. 205: 209-214 (1997)
30. Delcour J, Hoseney RC. Principles of Cereal Science and Technology. American Association of Cereal Chemist, St. Paul, MN, USA. pp. 89 (2010)