Impact of lipase, glucose oxidase and transglutaminase on physical and qualitative properties of pan bread

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Summary
Quality problems, such as poor texture and undesirable sensory properties, are occasionally encountered in pan bread. In this study, wheat-based pan bread was produced on a pilot scale by adding lipase, glucose oxidase and transglutaminase to flour to improve the flour rheology and bread quality. Response surface methodology was used to investigate the effect of these enzymes at various contents on specific volume of pan bread. The specific volume of pan bread ranged from 6.12×10^{-3} $m^3 \cdot kg^{-1}$ to 7.29×10^{-3} $m^3 \cdot kg^{-1}$. Textural properties of the bread produced with the optimum enzyme formulation were superior to the control bread, and its sensory properties were also more appreciated. The results obtained in this study showed that the use of enzymes improved technological properties of pan bread and allowed the production of a fresher and more consumer-friendly pan bread.

Keywords
enzyme; lipase; glucose oxidase; transglutaminase; pan bread; response surface methodology

Bread is at the forefront of cereal-based foods in meeting the nutrients, minerals, vitamins, and dietary fibre need in the human diet. Since flour, the main ingredient for making bread, may not always have the desired properties, it is fortified using external agents called flour improvers or additives [1]. Especially the low quality of gluten in wheat grains greatly affects the rheological properties of bread. The additives include emulsifiers, oxidizing agents and other compounds such as wheat gluten, preservatives, flavourings, non-fat dry milk or fat [2, 3]. Emulsifiers (sodium and calcium stearoyl lactylate, diacetyl tartaric acid esters of mono- and diglycerides, ethoxylated mono- and diglycerides) form an aggregate that causes the gluten to become more elastic and extensible, thereby strengthening the dough [3]. Oxidizing agents such as ascorbic acid provide disulfide bond formation between polypeptide chains, forming larger molecular aggregates [2]. Alternatively, problems with rheological properties of dough and bread quality can be solved by the addition of enzymes [1, 4]. Enzymes also play a role in delaying staling [5]. Enzymes are considered safe, making them a good alternative to chemical additives and are therefore used to optimize dough properties, end product quality and stability during storage [6, 7]. Enzymes are also useful for modifying wheat proteins to better retain the gas generated during fermentation and for improving the rheological properties of the dough of weak flour and bread [5].

Various enzymes have been used in the baking industry for various purposes. These included amylases (α-amylase and β-amylase), xylanase, transglutaminase (TG), glucose oxidase (GOD) and lipase [3, 8, 9]. α-Amylase (EC 3.2.1.1) is the most frequently used enzyme in bakeries [10]. α-Amylases are preferred for their positive influence on bread volume, crumb grain, crust and crumb colour, flavour development and anti-staling effect [6]. There is also evidence that amylases have a positive effect on dough development such as increasing the resistance, elasticity and softness of the dough [11] and decreasing crumb firmness and hardness. However, disadvantages, such as

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reducing dough extensibility or stability, were also reported [12]. Xylanases are well-known dough conditioners. They have reportedly been used to increase loaf volume through improved dough processability as well as having an anti-staling effect [6]. Glucose oxidase (GOD; β-D-glucose: oxygen 1-oxidoreductase; glucose aerodehydrogenase; E.C. 1.1.3.4.) is a currently preferred enzyme alternative to chemical oxidizing agents used for dough improvement by increasing the resistance and decreasing the extensibility of the dough [13, 14]. It increases the bread volume and improves the crumb grain of bread [13]. Lipases strengthen dough stability and increase bread volume, texture, and shelf-life [15]. However, a high concentration of lipase decreases the volume, producing a stiffer dough, leading to reduction in the bread volume [16]. Transglutaminases lead to strengthening, increase stability and resistance of the dough, consequently improving the volume, texture and shelf-life of the bread [5].

The pan bread can easily be cut into equal slices hence it is preferred by the catering industry. However, these products have poor texture profiles such as low volume and high hardness, together with undesirable sensory properties such as bitter taste. Since bakers prefer to use only wheat flour in the production of pan bread, it is not possible to combine wheat flour with flour obtained from other crops to improve the technological properties of bread. At the same time, since consumers do not want chemical additives in bread, the possibilities of using enzymes to improve flour rheology and bread quality were investigated in this study, as well as in standard bread production. The preliminary findings of the study showed that using various enzymes gave positive results in pan bread. The study aimed to determine the effect of these enzymes, namely lipase, GOD and TG, on specific volume, volume and density of pan bread. Their levels were optimized using response surface methodology (RSM) with a regression equation model. In addition to textural analyses, sensory evaluation was also performed.

MATERIALS AND METHODS

Materials

Wheat flour, salt and beet sugar used in bread-making were purchased from the market in Istanbul, Turkey. Pan bread was produced on a pilot scale by using flours of the same characteristics, supplied from the same batch. Dry yeast (OZyeast, Istanbul, Turkey) used in bread-making was kept in the refrigerator during the experiments. Potable tap water was used in the experiments. Before using the flours, they were stored at 20 °C for 2 weeks and the quality characteristics of the flours were determined. Lipase (Lipomill S), glucose oxidase (Qximill QP) and transglutaminase (T-Glutamill) were supplied by Mirpain (Istanbul, Turkey).

Characterization of flour

Gluten and gluten index were measured according to the methods AACC 38-12.02 [17] using Gluten washer 6000 – Glutomatic System (Bastak Instruments, Ankara, Turkey) and Gluten index 2100 (Bastak Instruments), respectively. Moisture level and falling number were determined by moisture analyser LJ16 (Mettler Toledo, Columbus, Ohio, USA) and enzyme measurement Number of Drops 5000 (Bastak Instruments), respectively, according to AACC 56-81B [18]. Baking quality (hydrated) and performance of flour were measured using Mixolab (Chopin Technologies, Villeneuve-la-Garenne, France) according to AACC 54-60.01 [19]. The rheological behaviour of flours (extention properties of dough) was determined by alveograph and Mixolab (both Chopin Technologies) according to AACC 54-30.02 [20]. All experiments were performed in three replications.

Bread-making process

Dough samples were prepared in spiral mixers with the same features using 1 kg flour, 0.600 kg water, 0.020 kg sugar, 0.012 kg salt, 0.006 kg dry yeast and appropriate amounts of an enzyme or enzyme combinations in quantities suitable for experimental design points. The ingredients were mixed for 5 min at 3 Hz and for 6.5 min at 3.3 Hz. After mixing, the dough was pre-fermented on the bench for 10 min at 25 ± 2 °C, then cut to 0.500 kg pieces and placed in pan bread moulds (1 loaf per mould). Bread was fermented at 30 °C for 120 min in a fermentation cabinet at 80% humidity. At the end of the fermentation, the bread was baked in a layered oven at 230 °C for 36 min. At the beginning of baking, the oven was steamed. After baking, the breads were removed from the oven and left to cool.

Experimental design and optimization

Response surface methodology (RSM) using three-level three factor Box-Behnken experimental design was used to investigate the main effect of the independent process variables such as content of lipase (X_1), the content of GOD (X_2), and content of TG (X_3) on selected dependent variables, namely, specific volume (Y_1), volume

Tab. 1. Experimental design and observed responses.

Formulation	Independent variables			Dependent variables*		
	X_1: Lipase [mg·kg^{-1}]	X_2: Glucose oxidase [mg·kg^{-1}]	X_3: Transglutaminase [mg·kg^{-1}]	Y_1: Specific volume [10^{-3} m^3·kg^{-1}]	Y_2: Volume [10^{-4} m^3]	Y_3: Density [kg·m^{-3}]
F1	3	1	30	6.39 ± 0.02	27.83 ± 0.02	156.54 ± 0.40
F2	6	2.5	30	6.21 ± 0.08	27.22 ± 0.47	160.92 ± 1.94
F3	3	4	30	6.28 ± 0.11	26.56 ± 0.37	159.27 ± 2.87
F4	6	1	50	7.29 ± 0.17	30.55 ± 0.11	137.32 ± 4.59
F5	3	2.5	10	6.60 ± 0.21	28.47 ± 0.61	151.59 ± 4.86
F6	6	2.5	30	6.34 ± 0.09	28.02 ± 0.80	157.74 ± 4.40
F7	3	2.5	50	6.78 ± 0.13	28.95 ± 0.50	147.54 ± 2.83
F8	6	4	50	6.64 ± 0.20	28.43 ± 0.36	150.71 ± 2.10
F9	6	1	10	6.43 ± 0.11	27.01 ± 0.29	155.52 ± 2.62
F10	6	4	10	6.63 ± 0.18	28.49 ± 0.53	150.83 ± 4.09
F11	6	2.5	30	6.12 ± 0.07	26.85 ± 0.83	163.52 ± 3.18
F12	9	1	30	7.27 ± 0.10	30.80 ± 0.70	137.58 ± 4.68
F13	9	2.5	10	6.80 ± 0.21	29.75 ± 0.42	147.20 ± 2.36
F14	9	4	30	6.93 ± 0.22	30.10 ± 0.12	144.30 ± 1.07
F15	9	2.5	50	7.15 ± 0.11	31.08 ± 0.24	139.86 ± 1.85
F16	6	2.5	30	6.19 ± 0.04	27.14 ± 0.26	161.64 ± 1.96

* – experimental results are the average of triplicates (mean ± standard deviation).

(Y_2) and density (Y_3). The levels of the process variables were determined according to the enzymes common to the bakery industry and the preliminary studies. The three levels of process variables were coded –1, 0 and 1. Coded levels for the process variables are presented in Tab. 1.

The Box-Behnken design comprising 16 experiments with 3 replicates was applied to optimize the enzyme content for the bread-making process. It was designed using Stat-Ease Design Expert 11.0.0 (Stat-Ease, Minneapolis, Minnesota, USA). Data were modelled by multiple regression analysis adopting backward elimination (after adding hierarchical model terms) and only the variables significant at $p < 0.05$ levels were selected for the model construction. Results of the Box-Behnken Design experiments were studied by non-linear multiple regression to fit the following second order polynomial regression model, or its reduced form defined as Eq. 1

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum \sum_{i<j} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 \quad (1)$$

where y represents the response variable, β_0 is intercept, while β_i, β_{ij}, and β_{ii} are linear, interaction and second order regression coefficients, respectively. A positive sign of coefficient β indicated a synergistic effect, while a negative sign indicated an antagonistic effect of the independent variables.

Statistical significance of the terms in the regression equations was also examined. The significant terms ($p < 0.05$) in the model were found by variance analysis (ANOVA) for each response. Evaluation of the model qualification was based on coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and predicted coefficient of determination (R^2_{pred}) values of the models. Results were expressed as average of triplicate measurements at each significance level. Terms that were not significant ($p > 0.05$) were removed from the model by backward elimination regression at the stage of ensuring model suitability and the obtained models were used to predict the response for optimal extraction conditions. By keeping one variable at the central point, three-dimensional plots of two factors versus evaluated properties were drawn and the corresponding contour plots were obtained.

Numerical optimization of process variables based on multiple responses was performed using Design-Expert 11.0 software (Stat-Ease). The de-

sirability function methodology was performed for simultaneous optimization to find the optimum conditions of the selected variables to obtain the optimal bread formulation. The main objective of the optimization study was to maximize specific volume and volume of bread while minimizing its density. The optimization criteria for all independent variables were selected within the ranges (lipase 3–9 mg·kg^{-1}, glucose oxidase 1–4 mg·kg^{-1} and transglutaminase 10–50 mg·kg^{-1}).

Determination of physical and textural properties

Specific volume of bread (*SVB*), volume of bread (*VB*) and density of bread (*DB*) were measured utilizing a volume measurement device Volscian Profiler 600 (Stable Micro Systems, Godalming, United Kingdom) which uses a 3-dimensional laser system. Crumb hardness was determined by experimental design using texture analyser TA.XT Plus (Stable Micro Systems) in bread prepared from flour containing an optimum amount of enzyme by experimental design. In the study, the shelf-life of the bread was planned to be 7 days. The baked bread was left to cool, it was sliced with a slicing machine and packaged as one slice of bread in one bag under aseptic conditions. The measurements were carried out for 1 week.

Sensory analysis

Sensory analyses were performed on bread prepared from flours containing an optimum amount of enzyme determined by experimental design. The bread scoring test was performed according to AACC 10-12.01 [21]. The bread was incubated at room temperature (25 ± 2 °C) and kept in sterile bags for 12 h before scoring. In the scoring test, bread shape and appearance, crust colour, crumb structure, crumb colour, crumb texture, flavour (sourness-sweetness) and mouthfeel features were evaluated on a scale with a maximum of 10 points for each parameter for each panelist. The sensory evaluation was performed by taking the average of the scores given by the panelists for each parameter. A number of 6–10 panelists were involved in the evaluation.

Statistical analysis

One-way ANOVA followed by Tukey's test ($p < 0.05$) was performed using SPSS software (IMB SPSS Statistics 19, SPSS, Chicago, Illinois, USA) to determine significant differences ($p < 0.05$) between the textural properties of bread samples of the optimum enzyme formulation and the non-enzymatic treatment.

RESULTS AND DISCUSSION

Characteristics of bread

The quality characteristics of the flour used in bread making were determined as gluten 30.6 %, gluten index 91.0 %, moisture content 14 % and falling number 435 s. Mixolab parameters showing the baking quality and performance of flour were determined as protein reduction 0.5 Nm, starch gelatinization 1.9 Nm, amylase activity 1.7 Nm and starch gelling 2.5 Nm. Alveograph parameters showing rheological behaviour of the flour were determined as tenacity 105.9 mm, elasticity 84.0 mm, deformation energy 305.0 × 10^{-4} Nm.

Fitting the model

The experimental results for the independent process variables (X_1: lipase content, X_2: GOD content and X_3: TG content) and dependent variables (Y_1: *SVB*, Y_2: *VB* and Y_3: *DB*) are presented in Tab. 1. The experimental values were fitted to a second-order polynomial model and the equations of the model were constructed for each independent variable (Tab. 2). *SVB*, *VB* and *DB* response values of bread prepared with different enzyme formulations are given in Tab. 1. *SVB* ranged from 6.12 × 10^{-3} m^3·kg^{-1} to 7.29 × 10^{-3} m^3·kg^{-1}. *VB* results changed between 26.56 × 10^{-4} m^3 to 31.08 × 10^{-4} m^3. *DB* ranged from 137.32 kg·m^{-3} to 163.52 kg·m^{-3}. For each response (Y_1, Y_2 and Y_3), a quadratic equation was obtained as shown in Eq. 2, Eq. 3 and Eq. 4.

$$Y_1 = 6.21 + 0.26X_1 - 0.11X_2 + 0.17X_3 - \\ - 0.21X_2X_3 + 0.29{X_1}^2 + 0.21{X_2}^2 + 0.32{X_3}^2 \quad (2)$$

$$Y_2 = \left(27.45 + 1.24X_1 - 0.32X_2 + 0.66X_3 - \\ - 0.90X_2X_3 + 1.23{X_1}^2 + 1.03{X_3}^2\right) \times 10^{-4} \quad (3)$$

$$Y_3 = 160.95 - 5.75X_1 + 2.27X_2 - 3.71X_3 + \\ + 4.52X_2X_3 - 6.79{X_1}^2 - 4.74{X_2}^2 - 7.62{X_3}^2 \quad (4)$$

All three models were defined as quadratic models and a quadratic identification was determined as the best model method to describe the variables ($p < 0.0001$). A high R^2 value is desired for models and it should be close to 1. It is expected that there will be an appropriate level of agreement of R^2 and R^2_{adj} [22]. The R^2 values for *SVB*, *VB* and *DB* models were 0.84, 0.83 and 0.84, the R^2_{adj} values approximately 0.81, 0.81 and 0.81, while the R^2_{pred} values 0.74, 0.74 and 0.75, respectively. The significant lack-of-fit value is an indication that the model does not represent the data well. If the model does not represent the data well, the lack-of-fit becomes significant [23]. A p value

Tab. 2. ANOVA results and second order polynomial equation for responses (after backward elimination regression).

	Specific volume of the bread					Volume of the bread					Density of the bread				
	Coefficient	*SS*	*df*	*F* value	*P* value	Coefficient	*SS*	*df*	*F* value	*P* value	Coefficient	*SS*	*df*	*F* value	*P* value
Model	+ 6.21	6.04	7	28.97	< 0.0001	$+27.45\times10^{-4}$	9.03×10^{7}	6	32.36	< 0.0001	+ 160.95	3011.61	7	29.04	< 0.0001
X_1	+ 0.26	1.66	1	55.72	< 0.0001	$+1.24\times10^{-4}$	3.69×10^{7}	1	79.30	< 0.0001	- 5.75	793.28	1	53.54	< 0.0001
X_2	− 0.11	0.29	1	9.91	0.0032	-0.32×10^{-4}	2.53×10^{8}	1	4.44	0.0249	+ 2.27	123.68	1	8.35	0.0063
X_3	+ 0.17	0.73	1	24.37	< 0.0001	$+0.66\times10^{-4}$	1.05×10^{7}	1	22.57	< 0.0001	- 3.71	330.69	1	22.32	< 0.0001
X_1X_2															
X_1X_3															
X_2X_3	− 0.21	0.54	1	18.09	0.0001	-0.90×10^{-4}	9.72×10^{8}	1	20.88	< 0.0001	+ 4.52	245.00	1	16.54	0.0002
X_1^2	+ 0.29	1.04	1	34.92	< 0.0001	$+1.23\times10^{-4}$	1.81×10^{7}	1	38.80	< 0.0001	- 6.79	553.07	1	37.33	< 0.0001
X_2^2	+ 0.21	0.52	1	17.65	0.0002						- 4.74	269.66	1	18.20	0.0001
X_3^2	+ 0.32	1.26	1	42.16	< 0.0001	$+1.03\times10^{-4}$	1.26×10^{7}	1	27.16	< 0.0001	- 7.62	696.23	1	46.99	< 0.0001
Residual		1.13	38				1.82×10^{7}	39				563.02	38		
Lack of fit		1.01	29	2.69	0.0606		1.53×10^{7}	30	1.63	0.2239		485.59	29	1.95	0.1475
Pure error		0.12	9				2.82×10^{9}	9				77.42	9		
Core total		7.21	47				1.09×10^{6}	47				3597.61	47		
R^2	0.8422					0.8327					0.8425				
R^2_{adj}	0.8131					0.8070					0.8135				
R^2_{pred}	0.7441					0.7434					0.7465				

SS – sum of squares, *df* – degrees of freedom, *F* value – Fisher value, *P* value – probability value, X_1 – lipase, X_2 – glucose oxidase, X_3 – transglutaminase, X_1X_2 – interaction of lipase and glucose oxidase, X_1X_3 – interaction of lipase and transglutaminase, X_2X_3 – interaction of glucose oxidase and transglutaminase, X_1^2 – quadratic effect of lipase, X_2^2 – quadratic effect of glucose oxidase, X_3^2 – quadratic effect of transglutaminase, R^2 – coefficient of determination, R^2_{adj} – adjusted coefficient of determination, R^2_{pred} – predicted coefficient of determination.

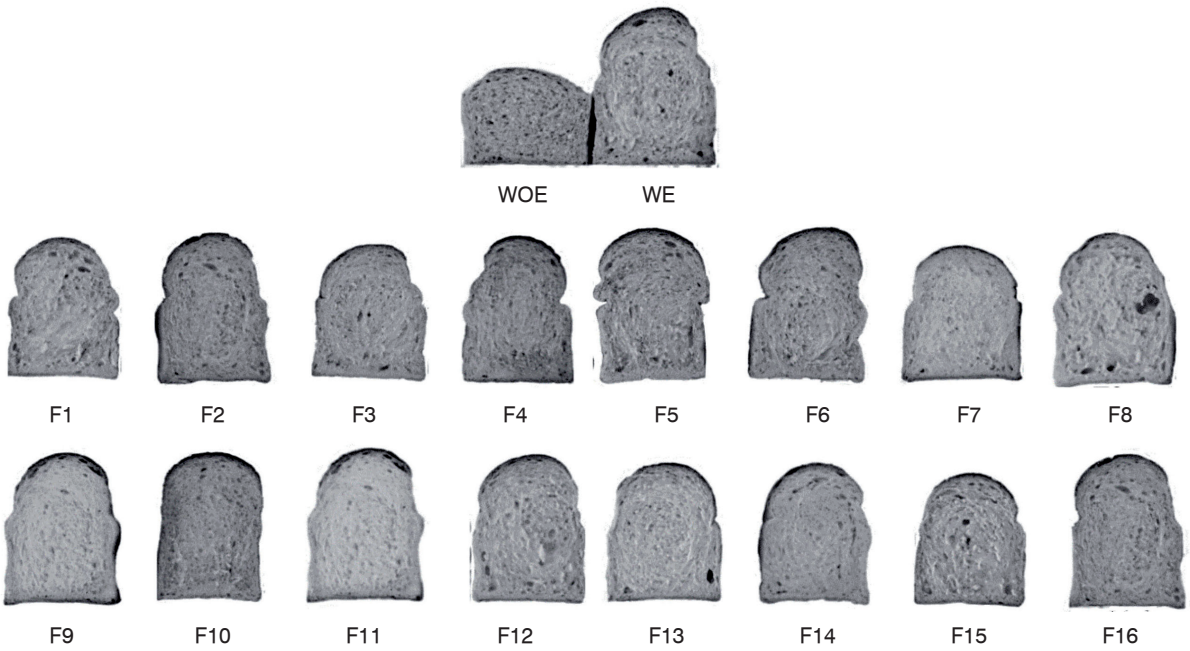

Fig. 1. Slices of bread produced in the trials.

WOE – bread without enzymes, WE – bread with optimum enzyme formulation (lipase, glucose oxidase and transglutaminase of 8.945, 1.875 and 49.847 mg·kg^{-1}, respectively), (F1–F16) – formulations according to the experimental design given in Tab. 1.

of lack-of-fit for *SVB*, *VB* and *DB* models was found to be statistically not significant as 0.06, 0.22 and 0.14, respectively. Diagnostic plans, including estimated values and experimental values, help us to understand whether there is an appropriate agreement between the values and hence evaluate whether the model is suitable or not [24]. When the R^2 and R^2_{adj} values were examined, it was observed that there was a good agreement between the estimated and experimental values. In other words, it is seen that the obtained models can explain the data well. Although the enzymes were added to bread in small quantities, with many internal and environmental factors interacting with them, we believe that suitable models were obtained to study the effects of these enzymes.

Effect of enzyme formulations on specific volume of bread

SVB was determined to vary between 6.12×10^{-3} m^3·kg^{-1} and 7.29×10^{-3} m^3·kg^{-1} in bread produced with 16 formulations, F1–F16, applied in the trials (Tab. 1). Bread slices produced in the trials are shown in Fig. 1. An increase in lipase content ($p < 0.0001$) and TG content ($p < 0.0001$) was significantly effective on *SVB*. These results are similar to those of SCHOENLECHNER et al. [25], who used a small amount of TG (0.3 mg·kg^{-1}) and observed an increase in *SVB*. In this study, 10–50 mg·kg^{-1} TG was used and it was determined that the *SVB* increased as the TG content increased.

The 3D response surface plot for the interaction effects of enzymes (X_2X_3) on *SVB* (Y_2) is given in Fig. 2. Similar to TG, an increase in the *SVB* was observed with the increase in lipase content ($p < 0.0001$). However, a negative relationship was observed between the increase in GOD content ($p = 0.0032$) and *SVB*, and this relationship was found to be significant. STEFFOLANI et al. [7] reported that the decrease in *SVB* with increasing GOD content was a result of increased dough strength. BONET et al. [13] found that the combined use of GOD and ascorbic acid at low contents increased specific volume of bread due to oxidation effect on the gluten network in bread, while the use of high contents decreased the *SVB* due to excessive oxidation. In this study, the effect of two enzymes on *SVB* was also evaluated. There was no significant effect on *SVB* neither with the use of lipase and GOD together nor with the use lipase and TG together. However, the use of GOD and TG together showed an antagonistic effect ($p = 0.0005$) and caused a decrease in *SVB* (Fig. 2A). In addition, the quadratic effects of lipase, GOD and TG were statistically significant ($p < 0.0001$, $p = 0.0002$ and $p < 0.0001$, respectively). A statistically significant ($p = 0.0001$)

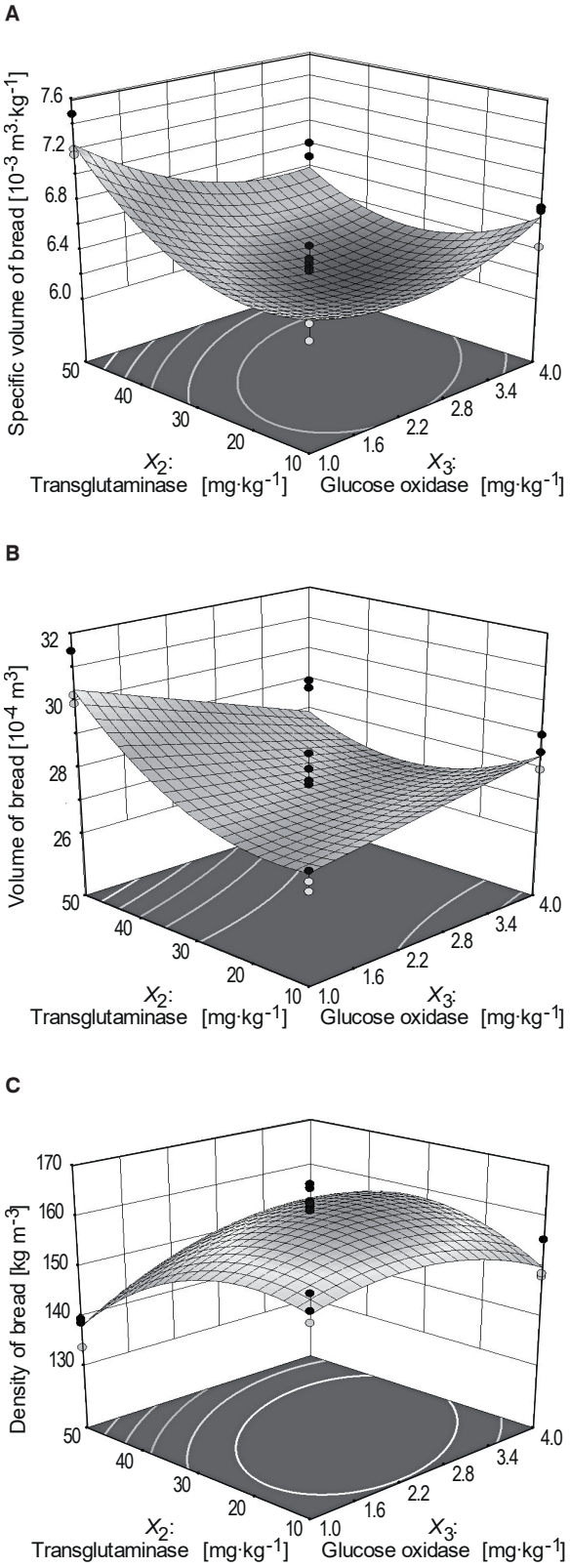

Fig. 2. Response surface plots.

A – effect of glucose oxidase and transglutaminase on specific volume of bread, B – effect of glucose oxidase and transglutaminase on volume of bread, C – effect of glucose oxidase and transglutaminase on density of bread.

model was established for *SVB* and the significant terms in the model were found as $X_1, X_2, X_3, X_2X_3, X_1^2, X_2^2, X_3^2$. The value of R^2_{pred} was in reasonable agreement with R^2 (Tab. 2).

Effect of enzyme formulations on volume of bread

The *VB* varied between 26.56×10^{-4} m^3 to 31.08×10^{-4} m^3 for bread produced with 16 formulations, F1–F16, applied in the trials (Tab. 1). The enzymes that positively affected *VB* were lipase ($p < 0.0001$) and TG ($p < 0.0001$). However, GOD had a negative effect ($p = 0.0249$) on *VB*. The 3D response surface plot for the interaction effects of enzymes (X_2X_3) on *VB* (Y_2) is given in Fig. 2. The supplementation by GOD and TG had a significantly negative effect ($p = 0.0002$) on *VB* (Fig. 2B). While TG alone increased the *VB*, its use together with GOD had a negative effect on the *VB*. Similar to the present study, STEFFOLANI et al. [7] found that the increase in protein cross-linking through isopeptide bonds (by TG treatment) and S-S bond (by GOD treatment) produced very strong dough and low *VB* when these enzymes were added to Argentinian high-protein flour. However, SCHOENLECHNER et al. [25] observed that *VB* increased statistically significantly due to the interaction of TG and xylanase. In the present study, there was no significant interaction between lipase with GOD and lipase with TG. A statistically significant model ($p < 0.0001$) was established for *VB* and the significant terms in the model were found as $X_1, X_2, X_3, X_2X_3, X_1^2, X_3^2$. The value of R^2_{pred} was in reasonable agreement with R^2 (Tab. 2).

Effect of enzyme formulation on density of bread

DB varied between 137.32 kg·m^{-3} to 163.52 kg·m^{-3} in bread produced with 16 formulations, F1–F16, applied in the trials (Tab. 1). Lipase content ($p < 0.0001$) and TG content ($p < 0.0001$) had a significantly negative effect on DB. However, an increase in GOD had a significant positive effect ($p = 0.0063$) on *DB*, despite its content being lower than other enzymes. The 3D response surface plot for the interaction effects of enzymes (X_2X_3) on *DB* (Y_3) is given in Fig. 2A significant increase in *DB* was observed with supplementation with GOD and TG together ($p = 0.0002$) and these two enzymes had a synergistic effect (Fig. 2C). However, there was no significant effect on *DB* at the use of combinations of lipase with GOD and lipase with TG. A statistically significant model ($p < 0.0001$) was established for *DB* and the significant terms in the model were found as $X_1, X_2, X_3, X_2X_3, X_1^2, X_2^2,$

X_3^2. The value of R^2_{pred} was in reasonable agreement with R^2 (Tab. 2).

Optimization and verification of the model

The optimization was aimed at maximizing *SVB* and *VB*, which are the most important parameters in bread quality. The response (dependent) variables were optimized using the desirability function. The suitability and usability of the equations found for the optimum response values of the models were analysed using the most suitable conditions found as a result of optimization. The maximum possible desirability was 1 for enzyme formulation used in bread-making. For bread-making, the optimum enzyme formulation was as follows: lipase 8.945 mg·kg^{-1}, GOD 1.875 mg·kg^{-1} and TG 49.847 mg·kg^{-1}. Triplicate analyses were performed for optimum conditions to verify the validity of the models. Experimental results of *SVB*, *VB* and *DB* obtained for bread produced in the pilot plant using optimum enzyme contents were found to be 7.38×10^{-3} m^3·kg^{-1}, 31.29×10^{-4} m^3 and 135.49 kg·m^{-3}, respectively. The predicted results obtained from the mathematical model were found to be 7.48×10^{-3} m^3·kg^{-1}, 32.08×10^{-4} m^3 and 132.73 kg·m^{-3} for outputs *SVB*, *VB* and *DB* respectively. The fact that the values estimated by the models were very close to the experimental data showed that the usability of RSM models was high.

Textural properties of bread samples

Texture is one of the most important features in the consumer's preference for food. The main consideration for bakers and consumers has been bread crumb freshness. Crumb freshness is directly related to bread specific structure and mechanical properties [26]. Therefore, the results of the texture analysis of the bread give information about the freshness. Bread, which is a bakery product

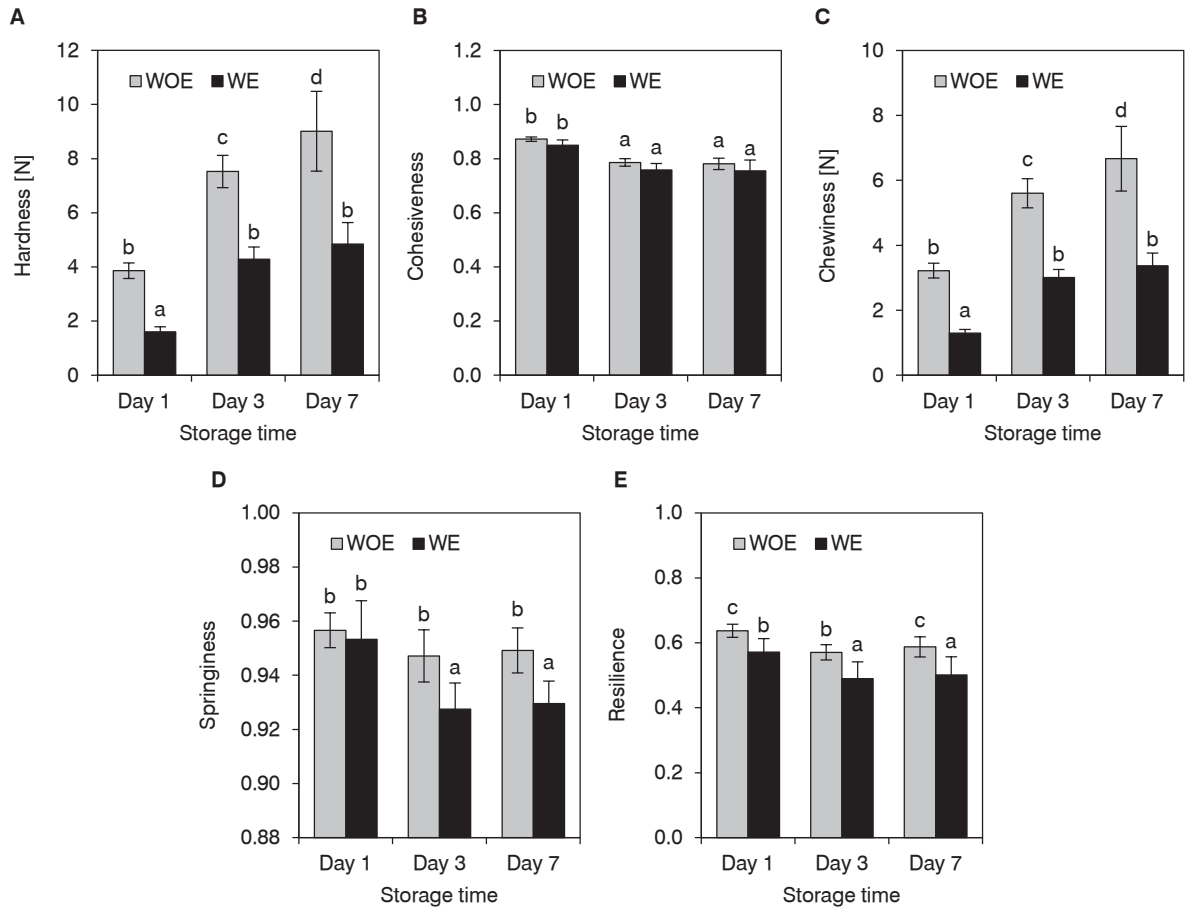

Fig. 3. Textural properties of enzyme-added bread during storage.

A – hardness, B – cohesiveness, C – chewiness, D – springiness, E – resilience.
The values with different superscripts in the same parameter groups (graphs) are significantly different ($p < 0.05$).
WOE – bread without enzymes, WE – bread with optimum enzyme formulation (lipase, glucose oxidase and transglutaminase of 8.945, 1.875 and 49.847 mg·kg^{-1}, respectively).

with a short shelf-life in general, is affected by the production process, storage and baking conditions. One of the most important indicators of bread stale is the firmness of the bread crumb [27]. As seen in Fig. 3, the addition of enzyme significantly reduced hardness, chewiness and resilience of the bread during storage compared to the control sample. However, there was no statistically significant effect on cohesiveness. Since chewiness is a hardness-derived parameter, compatibility between them is significant. GOESAERT et al. [10] found that an increase in the firmness of bread as a result of 6-day storage reduced the durability of bread. It was stated that the reason for this is the decrease in crumb flexibility caused by the less flexible gluten network. The results obtained in this study showed that enzyme supplementation had a positive effect on textural properties of the bread compared to the control bread, depending on the storage time.

Sensory properties

The sensory scores of foods provide important information about their acceptability and marketability. Sensory evaluation is a way of examining the physical and chemical properties of bread, relating them to the behaviour of the bread in the mouth and is a decisive analysis of the overall bread quality [28]. The sensory quality of bread is perceived by the consumer's senses of sight, smell, taste, hearing and touch [29, 30]. Loaves of bread were evaluated in terms of 7 characteristics in the discriminative tests performed with trained panelists. The bread prepared with enzyme additives was preferred over the control bread in terms of shape and appearance, crust colour, crumb structure, crumb colour, crumb texture, flavour and mouthfeel. A significant correlation between sensory analysis for bread freshness and instrumental measurements of mechanical deformation was reported by LASSOUED et al. [31]. The results of texture analysis and sensory analysis in the present study were in harmony. As a result, the bread supplemented with enzymes was preferred by the consumers in terms of sensory properties compared to the control bread.

CONCLUSIONS

In recent years, bread consumption has been decreasing with the changes in people's eating habits in some countries. However, bread continues to be an integral part of the diet in many regions. Since the flavour and texture of bread, in other words, its sensory properties and quality, are largely dependent on the flour used, weak (low-protein) flours should be strengthened with additives or flour improvers. An alternative approach to chemical additives is represented by using certain enzymes, which are considered safe and are widely used in the bakery industry. The type and amount of enzyme to be used varies depending on the characteristics of the flour, the type and the production method of bread. Although the recommendations of the manufacturer are considered in determining the enzyme amount to be used, this information may not be sufficient for the production of the bread of the desired quality. Using RSM textural properties, which are very important regarding bread, were improved with the help of added enzymes, thus producing bread that preserved its freshness for a longer period and fulfilled the requirements of consumer's taste in terms of texture and sensory properties.

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