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IMPROVEMENT OF WHEAT FLOUR DOUGH RHEOLOGY BY ALPHA -AMYLASE AND PROTEASE COMBINATION

***Georgiana Gabriela CODINĂ[1], Silvia MIRONEASA[1]**

[1]*Faculty of Food Engineering, Stefan cel Mare University, Suceava, Romania,*
codina@fia.usv.ro ; silviam@fia.usv.ro
*Corresponding author
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Abstract: *The objective of this study was to determine the optimum combination of α-amylase and protease on Alveograph wheat flour dough rheological properties. Response Surface Methodology (RSM) was used to relate the enzymes used like independent variables on Alveograph rheological parameters maximum pressure (P), extensibility (L), ratio configuration (P/L), index of swelling (G) and deformation energy (W). The wheat flour used in the research as control sample was a strong one with a low α amylase content. Each independent variable was tested at five levels: 0.000, 0.020, 0.040, 0.060, 0.080% for α-amylase and 0.000, 0.002, 0.004, 0.006 and 0,008 % for protease respectively. The results showed that the enzyme combinations weakened the dough (by increasing dough extensibility and reducing dough tenacity and deformation energy). Among the enzymes used like variables, protease has a more effective effect on weakening the dough as compared to α-amylase. The best results obtained on Alveograph rheological parameters were for 0.060% α-amylase and 0.001% protease combination.*

Key words: *wheat flour, α-amylase, protease, Alveograph, optimization*

1. Introduction

Wheat flour contains enzymes like amylases, proteases, lipases, phosphatases and oxidases [1]. From these enzymes the main role corresponds to the amyllolytic and proteolytic enzymes.

Normally, wheat flour contains enough amounts of β-amylase but little or no α-amylase. β-amylase can produce some maltose without the presence of α-amylase, but the amount produced is relatively small, so α-amylase must be added to optimize the amylase activity. The hydrolysis of starch carried out by α-amylase favor the production of low molecular weight dextrins, degraded by the endogenous β-amylase in maltose. During baking, dextrins won't form gels, will increase the amount of free water from the bread crumb, will conduct to a softer crumb, will reduce the starch ability to retrogradate and will increase the shelf life of the finished backery products [2, 3, 4]. However an activity too intense of α-amylase may conduct to a sticky mass form by dextrins which will conduct to a stickiness bread crumb with a wet, and unbaked aspect. Therefore, in wheat flour dough α-amylase activity must be an optimum one, not too moderate and not too intense.

Proteases may be used to reduce the mixing time, to decrease dough consistency, to reduce gluten strength and to improve the bread flavor [5]. Therefore it may be adding in bread making in order to improve dough extensibility, machining properties, gas retention, mixing time and bread sensorial characteristics [6]. It should be used only for processing strong gluten flours with high resistance and elasticity and low extensibility [7].

The practice of adding different enzymes in bread technology is a very common one in order to optimize the wheat flour dough rheological properties and therefore to improve the quality characteristics of the finished bakery products.

In order to improve dough rheological properties the effect of α-amylase and protease enzymes was evaluated on strong wheat flour with a low α-amylase activity in a single and in different combinations by using Response Surface Methodology in order to establish an optimized enzyme combination.

2. Materials and methods

2.1. Basic ingredients

The chemical composition of the flour was determined according to the specific Romanian standards: moisture content (SR ISO 711:1999), wet gluten content (SR 90:2007), gluten deformation index (SR 90:2007), ash content (SR EN ISO 2171:2010), and the falling number (SE EN ISO 3093:2010).

Enzymatic agents (commercial names Clarase G Plus and Belpan BI) commercialized by S.C. Enzymes & Derivates Romania was used. The effect of enzymatic agents was evaluated at five levels: 0.000, 0.020, 0.040, 0.060, 0.080% for *α*-amylase and 0.000, 0.002, 0.004, 0.006 and 0,008 % for protease respectively.

Clarase G Plus is a fungal *α*-amylase derived from a selected strain of *Aspergillus oryzae* and Belpan BI is a standard protease derived from a selected strain of *Bacillus subtillus* purchased from Enzymes & Derivates (Neamt, Romania).

2.2. Rheological tests

The Alveograph parameters were determined according to Romanian standard SR EN ISO 27971:2008. Each Alveograph curve was analyzed for the fallowing parameters: P, the maximum pressure needed to blow the dough bubble, expresses dough resistance; L, length of the curve, expresses dough extensibility; P/L, configuration ratio of the Alveograph curve; G, index of swelling; W, baking strength (surface area of the curve) [8, 9].

2.3. Statistical analysis

To study the effects of the enzymatic agents on wheat flour dough rheological properties, the two types of this, the most used in the bread making technology namely *α*-amylase (C_GP) and protease (B_BI) were used as independent variables. Levels of enzymes added in wheat flour were selected at a level range from 0.000 to 0.080 % for *α*-amylase and a level range from 0.000 to 0.008 % for protease, respectively. Response surface methodology (RSM) and central composite design (CCD) with two factors and five different levels was generated by the Stat Ease Design Expert 7.0.0 software package (trial version). The complete experimental design required 11 experimental runs that consist of 4 factorial points, 3 centre points and 4 axial. The centre point in the design was repeated three times to allow the adequate estimation of the model. The experimental results were analyzed by multiple regression method. The following equation (1) was proposed for each of the response variables:

$$Y = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_{11} \cdot X_1^2 + b_{22} \cdot X_2^2 + b_{12} \cdot X_{12} \quad (1)$$

where Y is the predicted response, b_0 a constant, b_1, b_2 - coefficient of linear effects, b_{11}, b_{22} - coefficient of quadratic effects, b_{12} - coefficient of the interaction effects and X_1, X_2 are the independent variables.

The statistical significance of the coefficients in the regression equations and the quality of the models' fitness was evaluated by analysis of variance (ANOVA). The fit of each model to the experimental data was given by the lack of fit test, coefficient of determination, R^2 and adjusted coefficient of determination, adjusted-R^2 and coefficient of variation, CV.

2.4. Optimization procedure

After the surface-response results, optimization of the mix of enzymes of *α*-amylase and protease in wheat flour was performed using a multiple response method called desirability [10]. The desirability functions involve transformation of each predicted response into an individual desirability function, d_n, which includes the desires and researcher's priorities to build an optimum procedure for each of the independent variables. The value of individual desirability function, d_n ranges between *0*, for a completely undesired response, and *1*, for a fully desired response.

For simultaneous optimization of the Alveograph rheological parameters: maximum pressure (P), extensibility (L), ratio configuration (P/L), index of swelling (G) and deformation energy (W) were used following modified desired function (2) developed by Derringer and Suich [11].

$$d_n = 0 \quad \text{if} \quad Y_n < A$$
$$d_n = \left(\frac{Y_n - A}{B - A}\right)^s \quad \text{if} \quad A \le Y_n \le B \qquad (2)$$
$$d_n = 1 \quad \text{if} \quad Y_n \le B$$

where *A* and *B* are the constraints of the response, respectively the lowest and the highest values of Y_n and *s* is a weighing factor.

The individual desirability functions are then combined into a single composite response, namely total desirability function, D ($0 \le D \le 1$) defined as the geometric mean of the individual desirability function d_n, which can be expressed as:

$$D = (d_1 \cdot d_2 \cdot \ldots \cdot d_n)^{(1/k)} \qquad (3)$$

where d_n, $n = 1, 2, \ldots\ k$ is the individual desirability function for each response. A high value of D indicates the more desirable and the best combination of enzymes doses, which is considered as the optimal solution of this formulation mix that generated the best results for Alveograph rheological characteristics.

3. Results and discussion

3.1. Analytical characteristics

The wheat flour used in this study presents the following chemical characteristics: moisture content 14.30%, wet gluten content 29.10%, gluten deformation index 2.00 mm, ash content 0.65% and falling number 462 s. The Alveograph wheat flour rheological properties of the control sample are: maximum pressure (P) 138.00 mm, extensibility (L) 67.00 mm, ratio configuration (P/L) 2.06, swelling index (G) 18.20 mm; deformation energy (W) $342.00 \cdot 10^{-4}$ J. According to the chemical and rheological characteristics of the control sample it represents a strong wheat flour quality for bread making with low alpha-amylase content.

3.2. Effect of process variables on the Alveograph rheological characteristics

The experimental design consisted of five levels for each of the variables, *α*-amylase and protease used in this study, and two replications for each experimental condition. A Central Composite Design (CCD) was employed in this study to perform the experiments by varying simultaneously all the variables (Table 1).

Table 1.
Central composite design: coded and actual values of independent variables

Experimental design points	Process variables			
	C_GP (%)		B_BI (%)	
	Coded	Actual	Coded	Actual
1	-1	0.020	-1	0.002
2	1	0.060	-1	0.002
3	-1	0.020	1	0.006
4	1	0.060	1	0.006
5	-α	0.000	0	0.004
6	α	0.080	0	0.004
7	0	0.040	- α	0.000
8	0	0.040	α	0.008
9*	0	0.040	0	0.004
10*	0	0.040	0	0.004
11*	0	0.040	0	0.004

*- centre point repeated 3 times; C_GP - α-amylase Clarase G Plus; B_BI – protease Belpan BI

The estimated regression coefficients of the models for de response variables, maximum pressure (P), extensibility (L), ratio configuration (P/L), index of swelling (G) and deformation energy (W), are shown in Table 2.

Analysis of variance (ANOVA) for each of the response variables shows that the two factor interaction (FI) models for P, W and P/L ratio, and quadratic models for L and G Alveograph parameters adequately represented the data obtained for wheat flour dough rheological properties.

Table 2.
Significant coefficients (95% confidence interval) of the design factors of the regression fitting model for Alveograph rheological parameters

Factor	P (mm)	L (mm)	P/L	G (mm)	W (10^{-4}J)
Constant	85.18	83.00	1.00	20.30	239.36
C_GP	- 8.66	13.92	- 0.24	1.61	ns
B_BI	- 14.60	8.57	- 0.24	0.99	- 33.98
C_GP · B_BI	ns	ns	ns	ns	ns
C_GP^2	-	5.06	-	0.54	-
B_BI^2	-	ns	-	ns	-
R^2	0.95	0.96	0.98	0.97	0.88
Adjusted-R^2	0.93	0.93	0.97	0.94	0.84
Lack of fit	ns	ns	ns	ns	ns
CV %	4.77	4.39	4.96	2.06	5.57

ns - no significant effect at the 5% level; P - maximum pressure; L – extensibility; P/L - ratio configuration; G - index of swelling; W - deformation energy; C_GP - α-amylase, Clarase G Plus; B_BI – protease, Belpan BI; C_GP · B_BI - interaction between α-amylase, Clarase G Plus and protease, Belpan BI; C_GP^2 - quadratic effect of α-amylase, Clarase G Plus; B_BI^2 - quadratic effect of protease, Belpan BI; R^2 - coefficient of determination; *Adj.* R^2 - adjusted coefficient of determination; *CV %* - coefficient of variation.

The regression models were highly significant for all Alveograph parameters with coefficients of determination (R^2) exceeding 85%, which indicates that a high proportion of variability is well explained by the each model. The lack-of-fit tests did not result in a significant F-value, indicating that the models obtained for Alveograph parameters are sufficiently accurate for predicting the wheat flour dough rheological properties obtained by blending with these enzymes. The coefficients of variation (CV) for each Alveograph rheological parameter show a small value, giving a better reproducibility.

The response surfaces of the maximum pressure (P) as functions of the doses of *α*-amylase (C_GP) and protease (B_BI) showed that the *α*-amylase and protease had a negative effect on P value, while the interaction between this had a non-significant effect on the P value. As can be seen in Fig. 1a the P parameter decreased with the increased dose of *α*-amylase and protease, but the effect of interaction

between α-amylase and protease is non-significant ($p > 0.05$) (Table 2).
Positive linear effect of the used enzymes was observed on L (Table 2). These effects might be attributed to both enzymes used in combinations due to the hydrolyze effect on the peptide bonds of the protease enzymes and to the intensification of the starch degradation process of the amylase enzymes. The statistical analysis of the coefficients of the model for L parameter revealed that the interaction coefficients were non-significant. It indicated that independent variables individually affected the response variable, L. Also, the quadratic effect of protease on the L was insignificant ($p > 0.05$). Fig. 1b shows the response and contour plot for effect of *α*-amylase and protease on L parameter.
Negative linear effect of *α*-amylase and protease, respectively was observed on P/L Alveograph ratio (Table 2), while the interaction between these had a non-significant effect on the P/L value. The effect of the *α*-amylase and protease added in wheat flour on P/L ratio is shown in Fig. 1c. An increase of *α*-amylase and protease level in wheat flour conducted to a decrease in the Alveograph ratio value P/L.
As can be seen in Table 2, the effect of quadratic term of protease on index of swelling (G) is insignificant, but the quadratic term of *α*-amylase has a positive significant influence on G, possibly due to the hydrolyze effect on the starch polymer which will exerts a hydrolytic release of water from the gluten substrate, which reduces the dough viscosity [12].
G was significantly affected ($p < 0.05$) by the linear terms of α-amylase and protease concentrations added in wheat flour. The interaction term between α-amylase and protease (C_GP · B_BI) was found to be non-significant ($p > 0.05$).
Increasing concentrations of *α*-amylase and protease in wheat flour caused an increase in the index of swelling of dough (Fig. 1d).
As shown in Fig. 1e, with the addition of protease (B_BI), the deformation energy of dough significantly decreased. The results of the recent study support previous reports of the Caballero et al. [13] showing that protease addition in wheat flour dough decrease Alveograph deformation energy. It was observed in Table 2 that protease added in wheat flour had significant negative linear effect ($p < 0.05$) on W, while the linear term of *α*-amylase and the interaction term between *α*-amylase and protease had a non significant effect ($p > 0.05$).

3.3. Optimized solution for the formulation

Multiple response optimizations were performed to determine the optimum levels of enzymatic agent's *α*-amylase (Clarase G Plus) and protease (Belpan BI) to achieve the desired response goals. Simultaneous optimization was performed by imposing some constraints for the Alveograph parameters according to Banu et al. [14] like: the optimal values for maximum pressure (P) in range 80-100 mm and for deformation energy (W) in range from 250 mm to 300 mm, while for extensibility (L) the target value of 100 mm is desirable.
The best combinations between these enzymes used in this study in order to obtain optimum values for Alveograph rheological parameters were extracted by Design Expert software that performs from the thousands of iterations and calculation the maximum desirability value and the conditions on which it was arrived. On the basis of these calculations, a total desirability value (D) of 0.811 was obtained for the following concentrations of enzymes used in wheat flour: 0.060% α-amylase and 0.001% protease (Table 3).

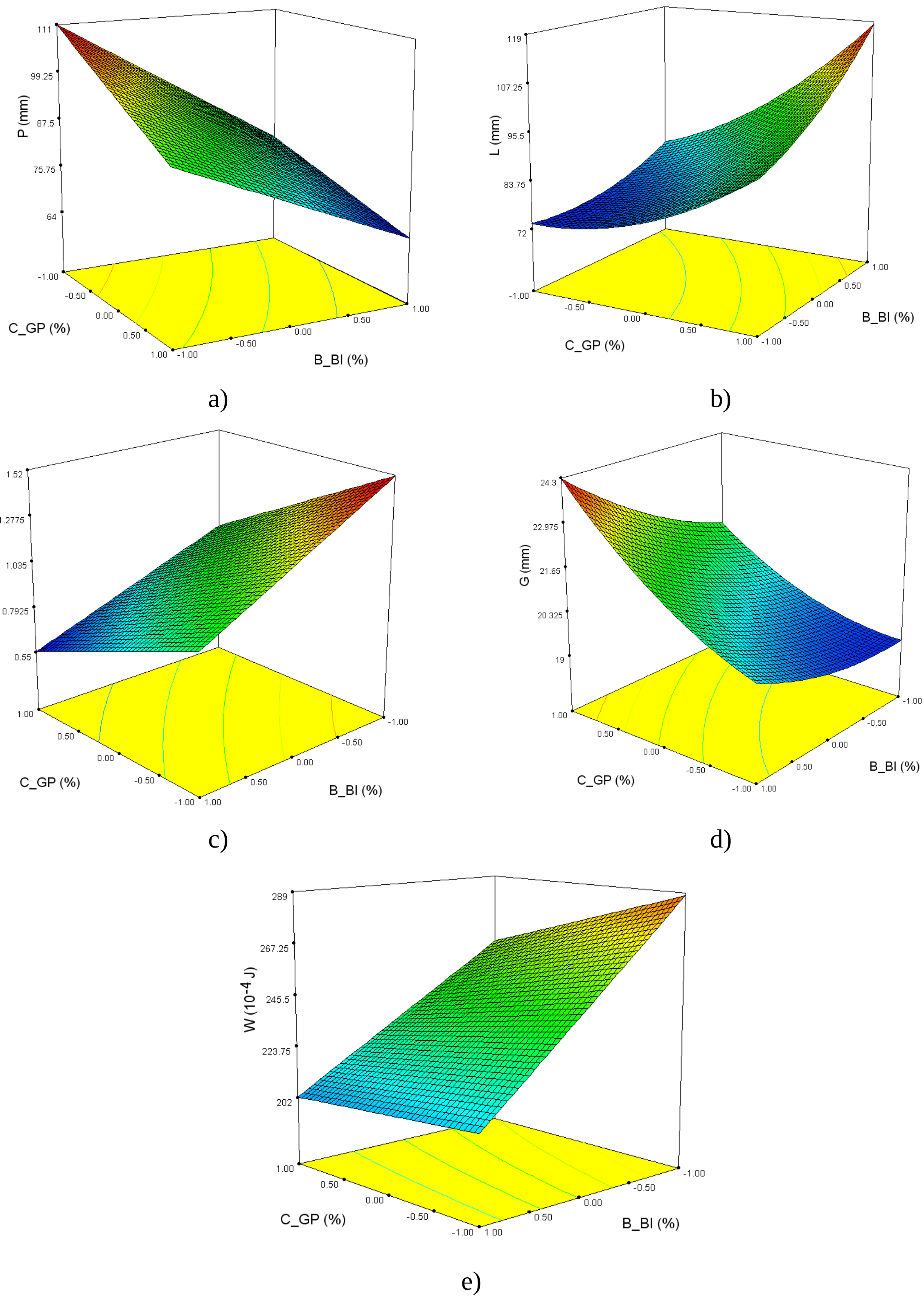

Fig. 1. Response surface plots showing the combined effects of enzymes α-amylase Clarase G Plus (C_GP) and protease Belpan BI (B_BI) on Alveograph rheological parameters

The response-surface plot corresponding to this D value is represented in Fig. 3, where the best combination of enzymes used in this study is obtained at the top of the graph. The coordinates of D value represent the optimum conditions and the corresponding predicted responses for rheological parameters are shown in Table 3.

Table 3.

Simultaneous optimization of proves variables by desirability approch

Independent process variables	Actual levels	P (mm)	L (mm)	P/L	G (mm)	W (10^{-4} J)	Total desirability value
α-amylase (C_CP), (%) Protease (B_BI), (%)	0.060 0.001	84.910	94.523	0.905	21.607	250.000	0.811

C_CP - α-amylase Clarase G Plus; B_BI – protease Belpan BI; P - maximum pressure; L – extensibility; P/L – Alveographic ratio; G - index of swelling; W - deformation energy.

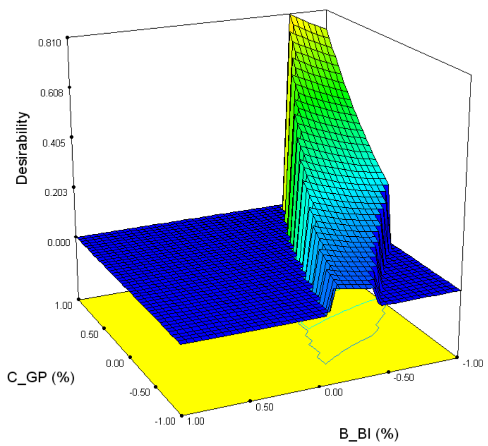

Fig. 2. Response surface plot of the total desirability function

4. Conclusions

In the present study, Response surface methodology was found to be effective in predicting models for Alveograph rheological parameters and to establish a relationship between processing variables - *α*-amylase Clarase G Plus and protease Belpan BI and responses - maximum pressure (P), extensibility (L), index of swelling (G), deformation energy (W) and P/L ratio of wheat flour dough rheology. The results showed that, *α*-amylase and protease have a significant effect on the P, L, G and P/L, while W is especially significantly influenced by protease addition. By using simultaneous optimization of the multi-response method by desirability function in order to obtain an optimum of *α*-amylase and protease combination we concluded that the best results on the Alveograph rheological properties were obtained for a formulation of 0.060% *α*-amylase and 0.001% protease.

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