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Optimization of fermentation conditions for improving bread quality from enzyme glucose oxidase

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Abstract: In the process of making bread, different ingredients are added to enhance the development of continuous protein networks that are necessary for bread quality. In this study, glucose oxidase derived from Aspergillus niger additions produced higher bread volume and promoted a longer life of the finished product. By the Box – Wilson method, optimization conditions were determined as follow: the optimal temperature of fermentation was 31°C, the time of fermentation was 103 mins, and ratio ES was 0.03%. The results confirmed that the glucose oxidase addition significantly improved bread quality. Under the optimal fermentation conditions, the volume of bread (328.6 cm^3) was higher than the control sample (159.7 cm^3).

Keywords: Glucose oxidase, Enzyme, Bread, Box – Wilson, Fermentation.

I. INTRODUCTION

Bread is one of the important foods in the world, especially in the Western world and Greater Middle East. There are many types of breads such as bagels, baguettes, brioche, The bread was made from flour, water, yeast, salt and additives,… [1]. In bakery, potassium bromate is commonly used to increase bread volume and texture. However, it is a nephrotoxic and carcinogenic substance used in food industry. According the US Food and Drug Agency,the maximum concentration of potassium bromate allowed in bread (FDA) is 0.02µg/g (0.02mg/kg) [2]. Nowaday, the organic food is developping. Chemical additives have shown ill effects and was no safe for human health [3].

The industry bakery applied and used several enzymes to improve the quality of bread. For example, the α- and β-amylases are the common enzymes in bread making. The α-amylases can improve the textural properties of bread as well as reduce elasticity; the amyloglucosidase is used to produce glucose from starch to enhance the fermentation process [3].

Glucose oxidase used in the food industry such as removing oxygen from fruit juice; reducing glucose in egg whites; improve color, smell of food materials; and prevent rancidity, bad smell of mayonnaise. Moreover, glucose oxidase can improve the volume of bread [4]. According to the reaction mechanism, hydrogen peroxide produced will perform the oxidation process, linking the sulfide bridges of gluten in the dough to create strong network connections of protein molecules.

The production cost of any process can be considerably reduced by process optimization. To answer this problem, we analized and optimized three parameters (factors) for obtained the best bread volume including:

- Z1 (%): Ratio ES
- Z2 (minutes): The time of fermentation
- Z3 (°C): The temperature of fermentation
- y: the volume of bread after baking.

By using the statistical model, we carried out and determined the optimal conditions. The experimental data used and analyzed in the preparation of this model were obtained according to the use of a two-level, factorial experimental design. After obtained the parameters of the statistical model, we applied Box–Wilson's steepest ascent method to determine the optimal conditions [5][6][7].

II. MATERIAL AND METHODS

2.1 Material

Wheat flour used in the process of experimenting from Danang company, Vietnam (humidity <14%, protein 11.8%, gluten 32.2% , good elasticity and moderate tension).

Enzyme glucosidase from *Aspergilus niger* purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

2.2 Bread making procedure

Bread making tests were performed using the mould bread. Dough formulation: 1000 gram wheat flour, 1.2 % compressed yeast, 1 % salt and 55.5 % water. Compressed yeast and salt were pre-dissolved in water, separately. The ingredients were mixed and kneaded for 15 min. The resulting dough (28^{o}C) was proofed for 75 min (30^{o}C and 97 % RH) (first proof), with two intermediate punches (partial loss of gas) at 45 and 55 min. The bulk dough was then sheeted and divided into 135 g pieces, molded into a loaf shape and returned to the fermentation cabinet for 60 min at 30^{o}C and 97 % RH (second proof). Finally, the pieces were baked at 220^{o}C for 10 min in a forced convection oven. The baked loaves were cooled at room temperature about 2 h and then were stored in polyethylene bags at 20^{o}C until analysis.

Bread quality parameters

Bread volume was determined according to AACC-method 10-05 (AACC 2000). Specific volume of bread (SVB) was expressed as volume/weight ratio [8].

Experimental design

Using the Box – Wilson optimization method to design and build the mathematical model about relationships between y and technological factors effect on the volume bread in bread making procedure (Z_1, Z_2, Z_3). The mathematical model of y was described as follow:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \quad (1)$$

In the equation [1], x_i (i=1…n)are the code values of ratio ES, time and temperature of fermentation and b_i (i=0…n) is the model coefficient. Y is the volume of bread after baking. In two-level, factorial experimental design there are n variables (factors). The factors have two different levels including: minimum and maximum levels. For these designs, we studied in a dimensionless coordinate system using following definitions.

The design matrix has orthogonal properties, the coefficient matrix were calculated by the following equation:

$$b_j = \frac{1}{N}\sum_{i=1}^{N} x_{ji} y_i \quad (2)$$

For a dimensionless coordinate system, we have: the maximum level is +1, minimum level is -1, and the central experiment is zero. Besides, the number of experiments is 2^n. In this study, 2^3= 8 experiments (3 variables) and five experiments at central points were carried out to identify the regression model.

These variables x_1, x_2, x_3 were coded by variables of Z_1, Z_2, Z_3 presented as follow:

$$x_j = \frac{Z_j - Z_{jo}}{\lambda_j} \;;\; j = 1,2,3 \quad (3)$$

Where,

$$Z_j^o = (Z_j^{max} + Z_j^{min})/2$$
$$\Delta Z_j = (Z_j^{max} - Z_j^{min})/2 \quad (4)$$
$$Z_j^{min} \le Z_j \le Z_j^{max};\; j = 1 \text{ to } 3$$

The experimental number is determined:

$$N = 2^k + n_o = 13 \quad (5)$$

With: k=3, n_o=5

III. RESULTS AND DISCUSSION

3.1 Develop the mathematical models of the volume of bread

The design matrix included coded values of the independent and coded dependent variables.The interrelation between the coded independent variables presented in Table 1.The constituent objective function for bread volume (y) depended on the parameters (factors), including: ratio ES (Z1, %), time of fermentation (Z2, min) and temperature of fermentation (Z3, ^{o}C). The experimental results presented thatbread volume (y) has related to Z_1, Z_2, Z_3. The experiments were performed with the levels of three parameters in Table 1 to determine the volume of bread. With the statistical model, the design matrix showed in Table 2 and Table 3.

Table 1. Levels of independent variables

Parameters	Levels			Deviation ΔZ_1
	Low -1	Central 0	High +1	
	-1	0	+1	
Ratio ES (%)	0.025	0.03	0.035	0.005
T (min)	90	100	110	10
t (^{o}C)	28	30	32	2

Table 2. Optimal design matrix (k=3, n_o=5)

Experiment	Value of real variables			Value of coded variables							Value of objective function
N_0	Z_1	Z_2	Z_3	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	Y
1	0,025	90	28	- 1	- 1	- 1	+ 1	+ 1	+ 1	- 1	247,2
2	0,035	90	28	+ 1	- 1	- 1	- 1	- 1	+ 1	+ 1	250,3
3	0,025	110	28	- 1	+ 1	- 1	- 1	+ 1	- 1	+ 1	253,9
4	0,035	110	28	+ 1	+ 1	- 1	+ 1	- 1	- 1	- 1	256,7
5	0,025	90	32	- 1	- 1	+ 1	+ 1	- 1	- 1	+ 1	268,9
6	0,035	90	32	+ 1	- 1	+ 1	- 1	+ 1	- 1	- 1	277,7
7	0,025	110	32	- 1	+ 1	+ 1	- 1	- 1	+ 1	- 1	315,9
8	0,035	110	32	+ 1	+1	+ 1	+ 1	+ 1	+ 1	+ 1	318,2
T1	0,03	100	30	0	0	0	0	0	0	0	317,4
T2	0,03	100	30	0	0	0	0	0	0	0	319,2
T3	0,03	100	30	0	0	0	0	0	0	0	316,9
T4	0,03	100	30	0	0	0	0	0	0	0	318,1
T5	0,03	100	30	0	0	0	0	0	0	0	317,0

Table 3. Matrix planning for value of code variables

No	$\mathbf{x_0}$	$\mathbf{x_1}$	$\mathbf{x_2}$	$\mathbf{x_3}$	$\mathbf{x_1x_2}$	$\mathbf{x_1x_3}$	$\mathbf{x_2x_3}$	$\mathbf{x_1x_2x_3}$	**Y**
1	+ 1	- 1	- 1	- 1	+ 1	+ 1	+ 1	- 1	y_1
2	+ 1	+ 1	- 1	- 1	- 1	- 1	+ 1	+ 1	y_2
3	+ 1	- 1	+ 1	- 1	- 1	+ 1	- 1	+ 1	y_3
4	+ 1	+ 1	+ 1	- 1	+ 1	- 1	- 1	- 1	y_4
5	+ 1	- 1	- 1	+ 1	+ 1	- 1	- 1	+ 1	y_5
6	+ 1	+ 1	- 1	+ 1	- 1	+ 1	- 1	- 1	y_6
7	+ 1	- 1	+ 1	+ 1	- 1	- 1	+ 1	- 1	y_7
8	+ 1	+ 1	+ 1	+ 1	+ 1	+ 1	+ 1	+ 1	y_8
T1	0	0	0	0	0	0	0	0	Y_9

No	x_0	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	**Y**
T2	0	0	0	0	0	0	0	0	Y_{10}
T3	0	0	0	0	0	0	0	0	Y_{11}
T4	0	0	0	0	0	0	0	0	Y_{12}
T5	0	0	0	0	0	0	0	0	Y_{13}

By applying the design matrix presented in Table 2 and Table 3, the mathematical model of regression equations (6) were calculated after processing the experimental data, determining the coefficients (Table 4), testing the significance of the coefficients by the Student test (Table 5), and testing the regression equations for the fitness of the experimental results by Fisher test (Table 6) [9]:

$$y= 273,6+2,125x_1 +12,575x_2 +21,575x_3– 0,85x_1x_2 + 0,65x_1x_3 +9,3x_2x_3 -0.775x_1x_2x_3 \quad (6)$$

Table 4. The value of the coefficients b

b_0	273.6	b_{12}	-0.85
b_1	2.125	b_{13}	0.65
b_2	12.575	b_{23}	9.3
b_3	21.575	b_{123}	-0.775

Table 5. The value of the coefficients by the Student test

t_0	639.2523	t_{12}	1.985981
t_1	4.964953	t_{13}	1.518692
t_2	29.38084	t_{23}	21.72897
t_3	50.40888	t_{123}	1.81075

Table 6. The regression equations for the fitness of the experimental results by Fisher test

STN	y_u	$\overline{y_u}$	$\left\lvert y_u - \overline{y}_u \right\rvert^2$
1	247,2	246,625	0,3306
2	250,3	250,875	0,3306
3	253,9	253,175	0,5256
4	256,7	257,425	0,5256
5	268,9	271,175	5,1756
6	277,7	275,425	5,1756
7	315,9	314,925	0,9506
8	318,2	319,175	0,9506

From the results, the error mean square was determined from Eq. (7) as 1.463. The tabulated value of the Student's t distribution $F_p(f_1f_2)$ was 19.13 for a significance level of P =0.05 and f_1 = 4, f_2 =2. Besides, p= 0.05, f=2, the value $t_p(f)$ determined 4.3.

$$S_{du}^2 = \frac{\sum_{i=1}^{8}\left(y_u - y_u^-\right)^2}{N - N'} = 3{,}491 \qquad (7)$$

$$S_{th}^2 = 1{,}463$$

Since the t_{12}, t_{13} , t_{123} were less than tabulated t value, constants of b_{12} , b_{13} , b_{123}were omitted from the regression equations. Moreover, the application of Fisher's F test determined the estimated regression equation fits the experimental data (8).

$$y = 273.6 + 2.125x_1 + 12.575x_2 + 21.575x_3 + 9.3x_1x_3 \qquad (8)$$

From Eq. (8) and the identification of the statistical model, Box– Wilson's steepest ascent optimization method was utilized. After optimisation, the results presented in Table 7.

Table 7. Values predicted statistically according to optimal parameters and values obtained after validation

Parameters	Ratio ES (%)	T (min)	t (^oC)	Prediction V (cm^3)	Value obtained after experiment V (cm^3)
Content	0.0303	103	31	329.7	328.6

From Table 7, bread volume improved and enhanced at the optimal conditions. The volume of bread was 328.6 (cm^3), increased about 2 times than the control sample (without enzyme, 159.7 cm^3). The structure of samples presented in Fig.1. The optimal sample had the good distribution of the air in the size holes. Consequently, the structures of holes were strong, hold CO_2, improved the volume of bread.

Control sample (without enzyme)

Optimal sample (glucose oxidase)

Fig. 1. Bread quality after utilized enzyme glucose oxidase and optimized the parameters.

IV. CONCLUSION

In this study, the mathematical models (6) and (8) described the relationship between the temperature of fermentation; the time of fermentation; the ratio ES with bread volume. The bread quality was improved and enhanced after utilized enzyme glucose oxidase. We can apply glucose oxidase and use for replacement potassium bromate for bakery products.

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