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BOX- BEHNKEN EXPERIMENTAL DESIGN IN FACTORIAL EXPERIMENTS: THE IMPORTANCE OF BREAD FOR NUTRITION AND HEALTH

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ABSTRACT

BACKROUND: In this study, a BOX-BEHNKEN design (BBD) of response surface methodology was used to investigate the effects of the amount of bran, the amount of yeast and the fermentation time on the amount of phytic acid in bread. The goal of first order factorial experiment is to identify the optimum levels of independent variables for the dependent variable. In this study, the implementation of first order response surface model and interpretation of the results were based on a 3^k Box- Benhnken (BBD) experimental design with one replicate. RESULTS: The calculation of the data for the first-order response surface model revealed that R^2 was 99,5% and that the model described most of the variance in the dependent variable (phytic acid). CONCLUSION: According to the results of the Box-Behnken experimental design (BBD), it was found that the amount of bran and the fermentation time had highest effect on phytic acid and that the amount of yeast, either alone or in any interaction, had no effect on the amount of phytic acid. Thus, it was concluded that optimal use of the amount of bran and fermentation time in the production of high-quality bread could prevent several diseases in future.

Keywords: Box-Behnken Experimental Design; Fermentation Time; Phytic Acid; Response Surface Method; The Amount Of Bran; The Amount Of Yeast

INTRODUCTION

Phytic acid, which is naturally present in grains, is found in wheat flour and particularly in the external layer of wheat kernel. Phytic acid has antioxidant and anti-cancerogenic properties, reduces the risk of heart disease and is a potent inhibitor of iron absorbtion (Febles et al., 2002).

Phytic acid is a phosphorus storage compound found in particularly legumes, nuts, roots and tubers(Lasztity and Lasztity, 1990). The positive and negative effects of this compound, which has been well-documented to be important for plant physiology, have been largely debated. It has been suggested that, besides its antioxidant effect, phytic acid also reduces excessive Fe load in the body through chealating effect and decreases the risk for colon cancer (Harland and Morris, 1995), and prevents the kidney stone formation by binding excessive calcium in the body (Ohkawa et al., 1984; Zhou and Erdman, 1995; March et al., 1998; March et al., 2001; Grases et al., 2004). However, phytic acid leads to some nutritional disorders by forming a complex with several essential minerals such as Fe, Zn, and Mn and interacting with protein and amino acids (Zhou and Erdman, 1995; Egli et al., 2002; Hurrel, 2004).

In recent years, it has been debated to what extent the amount of phytic acid in bread is influenced by the wheat bran added to bread and other foods to enrich the diet in fiber affects. It is beyond doubt that, the complete elucidation of these issues will significantly contribute to our understanding of the importance of bran bread for health.

Factorial experiments are commonly used in all research fields, particularly in health since health-related conditions are influenced by multiple factors. Therefore, the effects should be evaluated together in a health-related condition in order to obtain a more accurate result. In factorial experiments, different levels of multiple factors are investigated simultaneously and one factor can be examined at different levels of the other factor or factors.

In this study, optimal levels of three variables, namely, the amount of bran, the amount of yeast and the fermentation time were determined using Box Behnken experimental design in order for the amount of phytic acid to be within the range defined previously.

MATERIALS AND METHODS

Response surface methodology (RSM) aims at building a regression model (approximation) that is closest to the true regression model. The true regression model is usually never known. The model to be built is based on observation data and the model is empirical. Multiple regression analysis should be used in response surface methodology(Kocabaş, 2001). For example; first order response surface model for the case of three independent variables is expressed as follows:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \varepsilon \qquad (2.1)$$

Sample response surface model is expressed as follows:

$$\hat{Y} = b_o + b_1 x_1 + b_2 x_2 + b_3 x_3 + e \qquad (2.2)$$

In equation 2.1;

Y=response variable (dependent variable)

x_i = predictor variable (independent variable)

β_o = constant

β_1, β_2, β_3= partial regression coefficients

ε ,e= error

In this situation, $x_1 = x_2 = x_3$ represent the mean value of Y. b_1, b_2, b_3 are regression coefficients.For example; where b_2, x_1 ve x_3 variables remain the same, x_2 exerts effect on the dependent variable Y. Therefore, it is called partial regression coefficient. Thus, once the effects of the other variables were removed, b_2 gives the amount of change in the dependent variable caused by a one-unit change in the independent variable (Şıklar, 2000).

It is called a “multiple linear regression model with k regressor variables”.

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \varepsilon \qquad (2.3)$$

The model can be further complicated by including interaction terms in first order response surface model. A first order response surface model with two predictors and interaction term is as follows:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \varepsilon \qquad (2.4)$$

The equation (2.4) can be called the linear regression model. Similarly, second-order response surface models can also be developed. A second-order response surface model with two predictor variables is written as:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon \qquad (2.5)$$

The experiment investigates the effects of various combinations of two or more factors and their levels on the response variable. In factorial experiments, the main effects of the factors and their interaction are assessed (Şıklar, 2000).

The factor effect is defined as the change in the response variable for certain levels of the factors.

The selection of the factors and their levels requires deep competent knowledge under time and cost constraints in factorial experiments. The assignment of the factor levels to the experimental units is under the control of the researcher. The levels of factors can be qualitative or quantitative. Quantitative factors are usually constructed from continuous variables such as temperature or drug dosage. Qualitative factors are usually described by ordered numerical values.

A BBD should consist of an equal number of replicates of all possible combinations of factor levels.

In some experiments, when the difference in the response between the levels of one factor is not the same for all levels of another factor, we say we have an interaction between the factors (Montgomery, 2001; Box and Draper, 1987).

A model including an interaction where both factors are quantitative is expressed as follows:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \varepsilon \qquad (2.6)$$

In the equation (2.6), Y represents the response, β’s represent the parameters, x_1 is a variable that represents factor A, x_2 is a variable that represents factor B and ε is an error term. The x_1 and x_2 are coded variables ranging between -1 and +1. The $x_1 x_2$ shows the interaction between x_1 and x_2. Parameter estimates in the regression model are transformed into response estimates (Montgomery, 2001; Box and Draper, 1987).

Advantages of factorial experiments

The simplest response surface model is the 2^2 experimental design. Suppose that there are two factors, A and B, each with two levels. The A^-, A^+ and B^-, B^+ represent the levels of the factors. The effect of changing factor A is given by $A^+B^- - A^-B^-$ whereas the effect of changing factor B is given by $A^-B^+ - A^-B^-$. Because experimental error is present, it is desirable to take two observations at each treatment combination and estimate the effects of the factors using average responses. Thus, at least six observations are required (Montgomery, 2001; Box and Draper, 1987; Dobson, 1990).

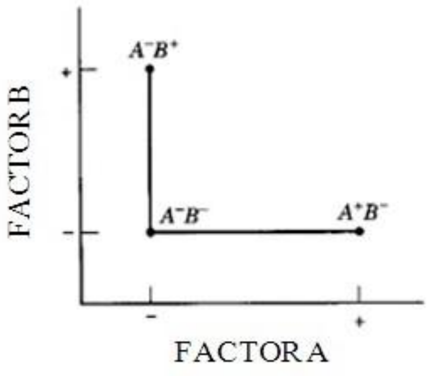

Figure 1. The interaction of the factors A and B

If a factorial experiment is performed, an additional treatment combination A^+B^+ is taken. Two estimates of the A effect can be made: $A^+B^- - A^-B^-$ and $A^+B^+ - A^-B^+$, and similarly, two estimates of the B effect can be made. These response estimates obtained are as precise as those from the single-factor experiment. However, only four observations are required, which suggests that relative efficiency of the factorial design to the one-factor-at-a-time experiment is (6/4)=1.5. As shown in Figure 2.2, this relative efficiency increases as the number of the factors increases (Box and Draper, 1987).

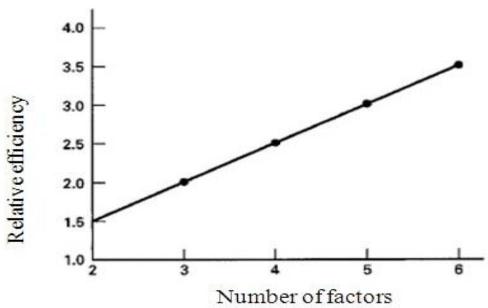

Figure 2. The relationship between the number of factors and relative efficiency

Suppose that there is an interaction. If the one-factor-at-a-time design indicates that A^-B^+ and A^+B^- gives better responses than A^-B^-, it can be concluded logically that A^+B^+ is better. However, if there is an interaction this conclusion may be seriously in error.

In summary, factorial designs have important advantages and more effecient than one-factor-at-a-time experiments. In addition, a factorial design should be used when interactions may be present to avoid misleading results. In conclusion, factorial designs are used to estimate the effects of a factor at several levels of other factors and valid conclusions are obtained (Dobson, 1990).

Until this section is a first-order response surface model will be discussed how to create and test. The generated data are obtained with the help of this model is quite easy to learn about the structure of the system. In some studies, objective information about the system, and the response range of the variable, or to determine the point of maximum or minimum. The steepest ascent in the maximum of the method is first-order response surface model point, the steepest descent in the minimum point in the method used to demonstrate the mathematical methods.

The path to be followed in the calculation of steepest ascent/descent is as follows:

Suppose that $x = x_2 = ... = x_j = 0$ is the starting point (Myers and Montgomery, 2002).

i) The variable x_j with the highest information gain or the greatest regression coefficient in absolute value is used to determine the step width.

ii) where the step width for the other variables is the difference in the levels of the selected factor Δx_j and the mid-point;

$$\Delta x_j = \frac{b_j}{b_j \Delta x_j}, \text{ (j= 1,2,…,k) } i \neq j \qquad (2.7)$$

iii) Δx_j are transformed from coded form to actual form.

Besides, the 3^k design, which is one of the simplest factorial designs, has three levels for each ‘k’ factors. In the response surface methodology, BBD is highly effective in 3^k factorial experiments. Even though there is no limitation for the number of variables “k”, two or three k’s are preferred in practice. For example, the corresponding 3^k design where k=4 requires 81 design points, however, the number of model coefficients to be estimated is 15. (constant term, four first-order coefficients, four second-order coefficients and six interaction term coefficients).

Additionally, there is a relationship between the number of variables and the total cost of the experiment. As the number of variables increases, so does the number of design points required. When there are more than 3 variables, fractional replication of 3^k factorial designs can be preferred. The levels of the factors are usually equally-spaced and quantitative in 3^k factorial designs that will be used in response surface studies. The factor levels can be coded as 0,1 and 2 (Box and Draper, 1987; Myers and Montgomery, 2002).

Box-Behnken experimental design

The Box-Behnken experimental design, developed by Box and Behnken in 1980, is a useful method for developing second-order response surface models. The

Box-Behnken design is based on the construction of balanced incompleted block designs and requires at least three levels for each factor. We will try to explain the structure of Box Behnken design with three-factors. In Box-Behnken experimental design, the level of one of the factors is fixed at the center level while combinations of all levels of the other factors are applied(Kocabaş 2001; Myers and Montgomery 2002). As shown in Table 1, the level of the factor C was fixed and then, the combinations of all levels of the factors A and B were applied and subsequently, the same procedures were performed for the factors B and A, respectively. The last column of the design matrix contains center point values.

Table 1. Three-factor Box-Behnken experimental designs

Rank	Box-Behnken Experimental Design		
	A	**B**	**C**
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

Taguchi's robust parameter design

The selection of optimal levels for control factors in a system is called parameter design. The system can be a process or a product. Those factors that are within our control are called control factors. Additionally, there is a second group of factors that cause most variation in the response variable. Those factors are called noise factors and are uncontrollable factors in the system. The uncontrollability of these factors increases the variation in the response variable. The objective of robust parameter design is to create a design insensitive to the noise factors whose variation can not be eliminated or controlled. Noise factors are usually functions of environmental conditions. For example, the temperature and humidity of the room where the design is developed, if uncontrollable, are noise factors (Box and Draper, 1987; Dobson, 1990; Lucas, 1994; Myers and Montgomery, 2002).

In summary, robust parameter designs are experimental designs that are not influenced by changes in the noise parameters. They minimize variability in the response transmitted from noise factors. In such a situation, squared error loss criterion is given by :

$$L = \sum_{i=1}^{n} (y - \bar{y})^2 \qquad (2.8)$$

the levels of the factors where the value of equation 2.8 is minimum meet the desired conditions.

In this equation, $\bar{y}$ represents the target value for the response variable.

Taguchi method makes use of orthogonal designs where an orthogonal array involving control variables is crossed with an orthogonal array involving noise variables for robust parameter design. Thus, there is a cross array. For example, for 2^2x2^2 cross array, 2^2 for the control variables is called the inner array and 2^2 for the noise variables is called the outer array. The cross array is shown in Figure 3(Box and Draper, 1987; Dobson, 1990; Lucas 1994; Myers and Montgomery, 2002).

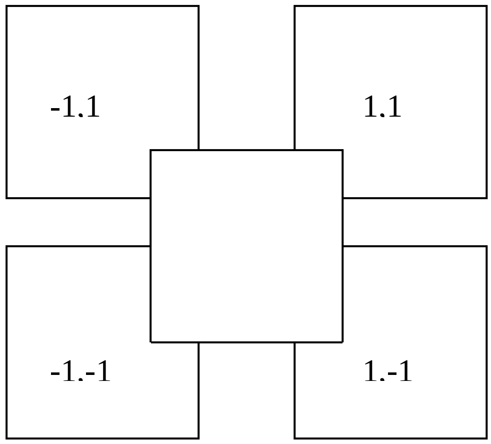

Figure 3. 2^2 x2^2 cross array

The values at the corners of the inner and outer arrays are for control variables. Each point in the outer array represents the arrangement of the observations. Taguchi suggested that one may summarize the observations in each outer array using a summary statistic that provide information about the mean and variance. This summary statistic is called a signal-to-noise ratio (SNR). The statistical analysis is performed by using SNR as the response variable. There are three special SNRs specified by Taguchi and used in response surface methodology (Myers and Montgomery, 2002).

Smaller the better

The purpose of the smaller the better type is to reach the minimum response so the zero point is assumed to be the target value.

Squared-error loss function is given by $E_2(y-0)^2$.

$$SNR_s = -10 \log \sum_{i=1}^{n} \frac{y^2}{n} \qquad (2.9)$$

The above ratio is used to determine the χ value that gives the levels of the control variables that minimize the expected value of squared error loss E_2y^2.

$\sum_{i=1}^{n}$: is the sum of the values of n response variables in the outer array. The SNR value is calculated for each inner array point.

Larger the Better

The purpose of the 'larger the better' type is to reach the maximum response. It is treated similarly with the 'smaller the better' approach. The y value in equation 2.11 is replaced by $\frac{1}{y}$.

The expected squared error loss function is defined by ;

$$E_2\left(\frac{1}{y}\right)^2 \qquad (2.10)$$

and

$$SNR_s = -10\log\sum_{i=1}^{n}\frac{1/y^2}{n} \qquad (2.11)$$

Nominal the best

The purpose of the 'nominal the best' type is to find the χ value that gives the levels of the control variables which bring the response variable to the the target. In this situation, two different SNRs are considered. The selection of the SNR to be used depends on the structure of the system. According to Taguchi, if the mean and variance of the response variable can be changed independently, one or more response variables may be used to eliminate bias. These adjustment variables enable the researcher to change the mean without changing the variance. This analysis is a two- stage process. First, the adjustment factors that enable the response variable to meet the target value are chosen and subsequently, the levels of the other control factors that minimize the SNR are identified. In this situation, the expected squared error loss function is defined by:

$$E_2(y-t)^2 \qquad (2.12)$$

and

$$SNR_{T1} = -10\log s^2 \qquad (2.13)$$

s^2 represents the sample variance computed from the design points on the outer array of the cross array and is calculated by using the equation below:

$$s^2 = \frac{\sum_{i=1}^{n}(y_i-\bar{y})^2}{n-1} \qquad (2.14)$$

When the levels of the factors maximizing the SNR are applied the target value for the response variable is reached.

A different SNR has been suggested in cases where the standard error of the response variable is related to the mean values, which can be optimally applied when the relationship is linear (Box and Draper, 1987; Dobson, 1990; Lucas 1994; Myers and Montgomery, 2002).

Data Set

In this study, breads of 400 g were made by adding 0%, 10% and 20% wheat bran (having an ash content of 5,82% in dry matter, a protein content(N* 5,7) of 13,7% in dry matter, a raw celullose content of 6,8% in dry matter) and 2%, 4% and 6% yeast into 12,4% flour (having an ash content of 0,51% in dry matter and a protein content of (N*5,7) 12,4% in dry matter) and three different fermentation procedures were carried out (20-10-30= 60 min, 30-20-40 = 90 min, 30-30-60=120 min.). The method of (Özkaya and Kahveci, 1990) was used in experimental baking.

In this study, the effects of the amount of bran, the amount of yeast and the fermentation time on the amount of phytic acid during bread making were investigated. A Box-Behnken Experimental Design of response surface methodology was used. Minitab 15 statistical software package was used for the analysis. In this program, an experimental model was developed coincidentally. The analysis was carried out according to the predetermined levels of the experimental design.

RESULTS

Table 2 below introduces the levels of the factors investigated and the amount of phytic acid at these levels. The normal distribution of residuals, which is the precondition of Box-Behnken experimental design, was checked and the normal distribution of residuals was verified.

Table 3. The regression analysis on 3^3 BBD

Factors	T	p
Constant	39,229	0,000(**)
Amount of bran	32,984	0,000(**)
Amount of yeast	-1,739	0,142
Fermentation time	-2,318	0,047(*)
Amount of bran^2	-0,945	0,388
Amount of yeast^2	-0,786	0,467
Fermantation time ^{2}	-1,761	0,139
Amount of bran^2	-0,404	0,703
Amount of bran^2	-0,064	0,951
Amount of yeast^2	-0,681	0,526

R^2 = 99,5% R^2 (adj) = 98,7%
(*)p<0,05 (**)p<0,01

Table 2. The amount of phytic acid according to the amount of bran, the amount of yeast and the fermentation time.

The amount of bran (%)	The amount of yeast (%)	The fermentation time (*) (min.)	The amount of phytic acid (**) (mg/100g)
0	2	60	156,9
		90	151,5
		120	142,3
	4	60	154,8
		90	142,3
		120	133,0
	6	60	152,1
		90	144,3
		120	123,4
10	2	60	334,9
		90	329,1
		120	321,2
	4	60	362,2
		90	323,0
		120	303,5
	6	60	321,7
		90	309,2
		120	287,8
20	2	60	511,9
		90	507,1
		120	481,9
	4	60	498,3
		90	489,3
		120	474,6
	6	60	495,4
		90	487,9
		120	459,0

(*): 20 min.-10 min.-30 min.=60 min.; 30 min.-20 min.-40min.= 90 min;
The fermentation procedure was applied for 30 min.-30 min.-60 min.=120 min.
(**): The results are expressed on dry matter basis (Özkaya and Kahveci, 1990).

Table 3. presents the t statistic and p values of the regression coefficients of the independent variables and their interaction in the regression analysis on the BBD. The table reveals that the main effects with respect to the amount of bran and the fermantation time are statistically significant. A R^2 (corrected) of 98,7% indicates that the model developed explains 98,7% of the variance in the dependent variable (the amount of phytic acid).

Table 4 shows the results of variance analysis for the 3^3 BBD.

Table 4. Analysis of variance for the 3^3 BDD

Sources of variation	Degrees of freedom	Sum of square	Mean of square	F	p
Regression	9	242 330	26 925,6	122,30	0,000*
Linear	3	241 285	80 428,5	365,31	0,000*
Quadratic	3	906	302	1,37	0,353
Interaction	3	139	46,3	0,21	0,885

*p<0,01

The table shows that the linear regression equation is significant. Below is the contour plot and response graphic by using the ‘nominal the best’ (medium level) type in line with our purpose;

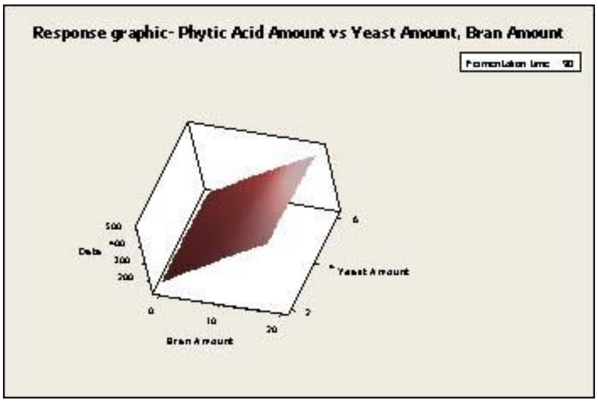

Figure 4. The response graphic for the ‘nominal the best’ type in BBD

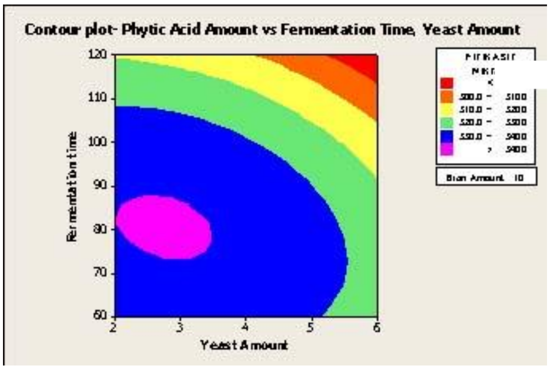

Figure 5. The contour plot for the ‘nominal the best’ type in BBD

Figure 4 and figure 5 demonstrate that if the ‘nominal the best’ model is used and the amount of bran is selected to be 10% the targeted amount of phytic acid will be 300-340 (mg/100g)

Below is the contour plot and response graphic using the ‘smaller the better’ type in line with our purpose;

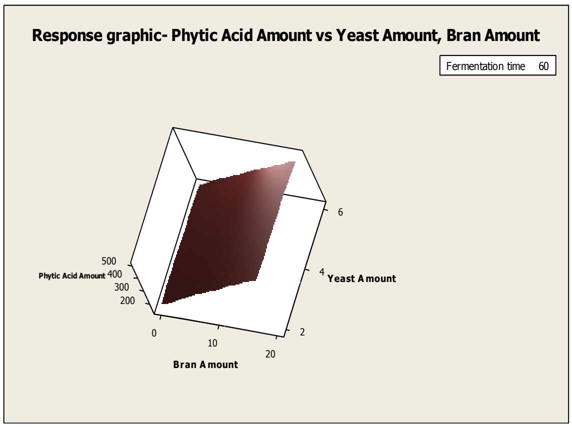

Figure 6. The response graphic for the ‘smaller the better’ type in BBD

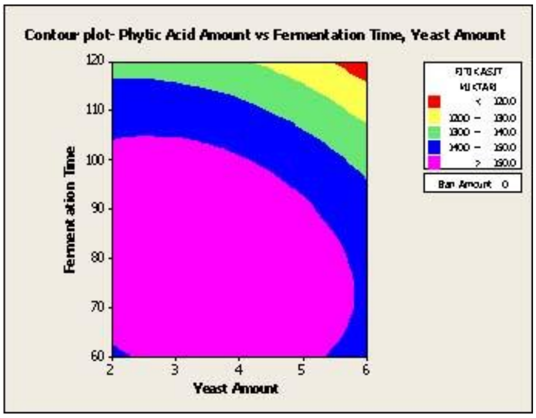

Figure 7. The contour plot for the ‘smaller the better’ type in BBD

Figure 6 and Figure 7 show that if the ‘smaller the better’ type is used and the amount of bran selected to be 0% the amount of phytic acid will be 120-150 (mg/100g).

Below is the contour plot and response graphics using the ‘bigger the better’ type in line with our purpose:

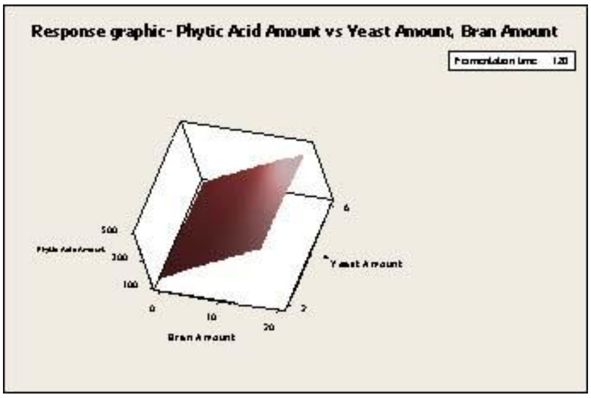

Figure 8. The response graphic for the ‘bigger the better’ type in BBD

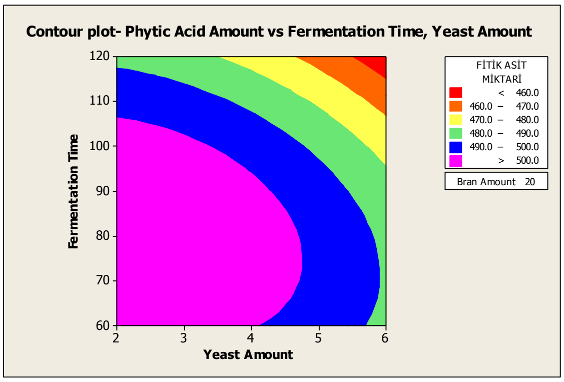

Figure 9. The contour plot for the ‘bigger the better’ type in BBD

Figure 8 and figure 9 show that if the 'bigger the better' is used and the amount of bran is selected to be 20% the targeted amount of phytic acid will be 460-500 (mg/100g).

DISCUSSION

Today, the importance of phytic acid has increased with higher consumption of whole flour bread or bran bread for a high intake of dietary fiber. Therefore, scientists have been increasingly interested in producing food with decreased contents of phytic acid.

In developed countries, nutrition with low-fiber foods has led to several diseases and medical disorders such as cardiovascular diseases, diabetes, colon cancer, diverticulosis, constipation and hemorrhoids. After the detection of a relationship between such medical conditions and daily intake of dietary fiber, the interest towards diet fiber has increased in communities conscious about nutrition (Egli et al., 2002).

The amount of phytic acid in bread increases with the amount of bran added to increase the amount of dietary fiber. Recently, scientists have been increasingly interested in reducing the phytic acid contents in bran breads. Previous studies have demonstrated that phytic acid can be partially hydrolyzed using phytase enzyme due to the fermentation process such as fermentation temperature, yeast amount, fermentation time and baking conditions (Hurrel, 2004).

Bread, the basic food for human, has no negative health effects when produced in appropriate conditions using appropriate materials. However, improper use of raw materials, such as nonoptimal amounts of bran, yeast or other additives in bread constitutes a threat for health.

Goals of response surface methods;

For accurate prediction of future values of the response variable, contribution expectations for the understanding of underlying mechanisms of a response system include the detection of an appropriate function (or model) that describes the relation between the response variable and input variables, investigation of the largest and smallest response values based on the type of problem and the detection of values of input variables $x_1, x_2, ..., x_k$ that can provide this value.

According to the results obtained using the Box-Behnken design of response surface methodology, it could be concluded that the amount of bran and the fermentation time have most effects on phytic acid, in other words, the amount of phytic acid is primarily influenced by the amount of bran according to the results of the Box-Behnken experimental design. It was also found that the amount of yeast either alone or in any interaction had no effect on the amount of phytic acid.

A three level BBD was used for the amount of bran and the fermentation time. The largest and the smallest response values were investigated and the values of input variables $x_1, x_2, ..., x_k$ that provide this value were detected. Based on the diversity of purpose, all possible results were presented.

Given the inverse proportion between the amount of phytic acid intake and the biological efficacy of important minerals such as Ca, Fe, Zn and Mn, the bread should be made by applying optimal fermentation time after selecting the wheat types with low phytic acid content and high bread baking quality, from which flours of lower extraction rather than those of high extraction should be made. Non-optimal fermentation time spoils dough quality, thus the resulting bread will cause various diseases in future.

In conclusion, the amount of phytic acid is primarily influenced by the amount of bran and the fermentation time, and increasing or decreasing the amount of bran directly increases or decreases the amount of phytic acid. The preliminary analysis revealed that the optimal fermentation time was 60 minutes. Thus, according to the results of the Box-Behnken experimental design, if the 'nominal the best' type is used and the amount of bran is selected to be10% with a fermentation time of 60 minutes the targeted amount of phytic acid will be 300-340 (mg/100g); if the 'smaller the better' type is used and the amount of bran is selected to be 0 % with a fermentation time of 60 minutes the targeted amount of phytic acid will be 120-150 (mg/100g); if the ' bigger the better' type is used and the amount of bran is selected to be 20% with a fermentation time of 60 minutes the targeted amount of phytic acid will be 460-500 (mg/100g). Additionally, an R^2 of 99,5% indicates that the regression equation explains 99,5% of the variance in the dependent variables.

In recent years, it has been debated to what extent the amount of phytic acid in bread is influenced by the wheat bran added to bread or other foods to enrich the diet in fiber and to what extent this is changed with the process conditions. The complete elucidation of these issues will significantly contribute to our understanding of the importance of bran bread for health.

This method, Minitab and Design Expert software packages for use in other applications available.

In this study, we investigated the changes in the amount of phytic acid in the breads made from flours containing wheat bran at different rates by adding yeast at different rates and applying different fermentation programs and different baking temperatures. Thus, the importance of process conditions for the nutritional quality of bran bread, the consumption of which has been rising in our country, was demonstrated.

This method provides savings in terms of time and the amount of material by limiting the amount of bran, fermentation time and the amount of phytic acid in certain levels of baking temperatures, thus allowing consumption of high quality bread, and eliminating possible health problems in future.

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