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APPLICATION OF D-OPTIMAL MIXTURE DESIGN TO OPTIMIZE THE WHEAT-PUMPKIN COMPOSITE FLOUR FOR BREAD PRODUCTION

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Abstract: *As compared to wheat flour, pumpkin flour is considered a high quality raw material in terms of nutrients and bioactive compounds essential in providing human health benefits. Due to its potential, pumpkin flour can be used as a functional ingredient and in bread making can be used in composite blends with wheat flour. In this study, the effect of wheat-pumpkin composite flour bread on some physical parameters of bread was investigated. For this purpose, three raw materials - pumpkin pulp flour, pumpkin seed flour, wheat flour were used and as statistical program the D-optimal mixture design (Design Expert 7.0) was used. As response variables, some physical parameters such as bread porosity, elasticity and H/D ratio were evaluated. The experimental data obtained were used to carry out the analyses of variance (ANOVA) and to develop the regression models. Special cubic model were used to explain all physical parameters considered, highlighting that the composite flour components interacted with each other and had effects on bread porosity, elasticity and H/D ratio. The best formulation of composite flour formula was chosen depending on the desirability function which showed a value of 0.94. The optimized formulation which showed desirable physical parameters contained 78.3% wheat flour, 15% pumpkin pulp flour and 6.70% pumpkin seed flour.*

Key words: *wheat flour, pumpkin flour, bread quality, mixture design approach, formula optimization*

1. Introduction

Bread is an important food product based on wheat flour which is consumed worldwide due to its competitiveness cost, ease of preparation, versatility, sensory and nutritional characteristics [1]. From the nutritional point of view, bread is a source of vitamins, minerals, dietary fiber and proteins [2 - 5]. However, by milling and processing, many of the nutrients from the wheat are destroyed, afectting from the nutritional point of view the quality of the wheat flour as raw material. Also, some vitamins such as A, C, D, and B_{12} lacked from the wheat flour, decreasing it nutritional quality [6]. The quality of raw materials used in bread formulation play an important role, influencing not only the nutritional value of bread but also functional, sensory and physicochemical quality of the final bread product [7]. Various ingredients can be added in bread products to improve wheat flour processing and/or nutritional value of bread. It is known that wheat flour proteins are of a lower nutritional quality than the milk, soy, pea and lupin proteins [8], e.g. because it is deficient in essential amino acids such as lysine, tryptophan and threonine [8 - 11]. Therefore, nowadays, the trend is to improved bread quality by incorporation of new types of raw materials to produce composite breads as functional foods [10], made from blend of wheat flour and non-wheat or pseudo-cereals flours [12] since they are rich in nutrients such as essential amino acids, dietary fiber, fatty acids, minerals, vitamins, phenolic compounds, e.g., with great potential for the human health. The blends between wheat flour and non-wheat flours can be successfully used to obtain a composite bread with a better overall balance of essential amino acids [13], especially the lysine content, significant in a balances diet [14], improving its nutritional characteristics. In this way, the used in wheat flour of not just one non-wheat flour but a mixture of legumes and seeds, can have as result from bread products an improved taste, aroma and nutritional quality. Some studies made in order to improve bread quality from the nutritional point of view by supplementation of wheat flour with non-wheat or pulses flours have been made by Mohammed et al. (2012) [13]; Harinder et al. (1999) [15]; See et al. (2007) [16]; Güemes-Vera et al. (2004) [17]. Also, some researches [18; 19] reported that the application of vegetable flours as functional ingredients in breads have in addition, many beneficial effects in controlling and preventing various metabolic diseases [20 - 22].

Pumpkin is an economically and agriculturally valuable vegetable cultivated around the word. Pumpkin fruits are suitable after processing for consumption, making a major contribution to human nutrition due to their composition which varies from one species or cultivar to another.

Pumpkin pulp provides a valuable source of vitamins such as vitamin A, vitamin B2, and vitamin C [23 - 25], carotenoids [23; 25; 26] that have significant roles in human nutrition. Also, pumpkin pulp is rich in bioactive compounds with high antioxidant activity such as phenolics and flavonoids [23; 27], minerals, pectin, dietary fiber [28] and other compounds which are beneficial to the human health [29; 30]. Among the dietary fiber, pumpkin flour contains cellulose, hemicelulose and lignin [31]. Therefore, by adding in composite flour, the pumpkin flour leads to a fiber enrichment of bread-making products, improving its nutritional quality [31]. Different levels of pumpkin pulp flour used for wheat flour substitution was used in preparation of various bakery products such as cakes and cookies [32; 33], biscuits [34], toast breads [35], having various effects on bakery products. It was showed that higher levels of pumpkin pulp caused an unpleasant aroma and taste. Noor and Komatchi (2009) [36] investigated the potential of peeled and unpeeled pumpkin pulp as a raw material for the production of flour for use in composite blend with wheat flour or as a functional ingredient in food products. Processed into flour which has a longer shelf-life, pumpkin can be use as functional ingredients in bakery products, supplementing cereal flours. The incorporate of pumpkin powder for preparation of cake lead to a increased of fiber content and β-carotene [32]. The β-carotene content increased with the increasing dose of pumpkin powder addition, increasing the level of vitamina A [33]. Same effects on pumpkin blend in biscuits were reported by Kulkarni and Joshi, 2012 [34]. In addition, they reported an increased of minerals content in biscuits as compared to the sample without pumpkin powder. Due to its highly-desirable flavor, sweetness and deep yellow-orange color, pumpkin flour can be use as a natural coloring agent in composite flour [35].

Pumpkin seeds are an interesting raw material for food application, highly nutritious than cereals due to its high protein content (39.25%), oil (27.83%), minerals (4.59%) and dietary fibers 16.84% [1; 37 - 39]. By the addition of pumpkin seed powder in bread the protein content of the product increases [1]. Pumpkin seed flour is recognized as a good source of lysine [40] improving nutritional value of products by incorporated them in bakery products, especially bread [41]. The addition of pumpkin seed flour to this product, conducted to an increased of the total essential amino acids, protein, fat and mineral content, showing that the pumpkin seed flour is a good source of protein and nutrients for bread fortification [40]. The level effects of pumpkin seed flour added in wheat flour on the bread quality were also studied [16], showing that the level of 5% is a feasible level to produce bread with good nutritional value and sensory characteristics.

Taking into account it nutritionally value, the pumpkin pulp and seeds flours can be a good supplement to wheat flour which can be used in formulation of the composite flours in order to improve quality of bread. By using in wheat flour not just one non-wheat flour but a mixture of vegetables and seeds oils we can obtain a better result regarding the quality of bread products. Although pumpkin pulp flour or pumpkin seed flour had been used in composite flour formulation, after our knowledge the addition of both pumpkin pulp and pumpkin seed flours in wheat flour in various ratio had not been studied before.

The used of statistically designed experiments in the bread-making processes to develop the new products or to improve the existing one is one of the priorities of the baking industry and not only that can lead to a lower general manufacturing costs. Mixture design is a statistical technique that can be used for evaluating the quality of final product based on the relative proportions of the components in the mixture. This technique uses an equation that describes how the independent variables in a mixture experiment affect the response variables [42]. D-optimal mixture design was applied to find the optimal response for any mixture of the components of composite flour, and to obtain the influence on the response of each component singly and in combination with other components.

The objective of this work was to investigate the effect of varying ratios of pumpkin pulp flour, pumpkin seed flour and wheat flour and their interaction on some physical parameters of bread by using a D-optimal mixture design approach and to determine the optimal mixture of wheat-pumpkin composite flour.

Also, the main objective of this study was to optimize the mixture of the three ingredients used, wheat flour, pumpkin pulp flour and pumpkin seed flour in the composite flour recipe, with the purpose of achieving the optimum physical parameters of produced composite breads. D-optimal criteria were applied to determine the optimal blend for the best physical parameters of composite bread obtained.

2. Materials and Methods

2.1. Materials

2.1.1. Wheat flour

The research study has been carried on 650 flour type (harvest 2015) obtained from S.C. MOPAN S.A. (Suceava, Romania). The flour used in this study presents the following characteristics: moisture content 14.5%, ash content 0.65%, protein content 12.6%, wet gluten content 34%, gluten deformation index 8 mm, falling number 380 s. The flour chemical composition was determined according to Romanian standard methods: moisture (SR EN ISO 712:2010), wet gluten content (SR 90:2007), gluten deformation index (SR 90:2007), ash content (SR EN ISO

2171:2010) and falling number index (SR EN ISO 3093:2010).

2.1.2. Pumpkin flour

Fresh pumpkins fruits (*Cucurbita moschata*) and commercial wheat flour were obtained from the local supermarket. In order to obtain the pumpkin pulp and seed flours, the unpeeled pulp and seeds of fresh pumpkins was manually separately and then washed under running tap water. The pumpkin pieces cut into slices of 2-3 mm thickness and the seeds were dried in hot air oven to a moisture content of 10 - 12% at 50°C for 24 hours. The shelled seeds and the dried pumpkin slices was grounded using a mechanical mill, and then sieved through a 150 µm mesh sieve in order to obtain fine flour.

2.2. Methods

2.2.1. Experimental design

D-optimal mixture design with three components was used to optimize the composite pumpkin - wheat flour bread formula. The wheat flour (WF or X1) as a bread composite flour base, pumpkin pulp flour (PPF or X2) used as a source of fiber, and pumpkin seed flour (PSF or X3) used as a source of proteins and minerals are the mixture components which was taken as independent variables.

The components proportions from composite flour formula, established from preliminary studies, are subject to multiple-components constraints [Cornell, 2002], as follows: $X_1 + X_2 + X_3 = 1$; $0.71 \leq X_1 \leq 0.92$; $0.05 \leq X_2 \leq 0.20$; $0.03 \leq X_3 \leq 0.09$, yielding a polyhedral constrained region. The dependent variables selected as the responses were some physical parameters of composite pumpkin - wheat flour bread such as porosity, elasticity and D/H ratio.

The Stat-Ease Design Expert 7.0.0 software package (trial version) was used to achieve as well as to analyze the experimental design.

The variations of wheat-pumpkin composite flour formulations and their replications including a total of 12 formulations are shown in Table 1 and for each treatment combination the sum of the component proportions was equal to one.

Table 1.
A D-optimal mixture design for the composite flour formulated with wheat flour (WF), pumpkin pulp flour (PPF) and pumpkin seed flour (PSF) in various proportion

Formulation number	Formulation variables		
	X_1 (WF)	X_2 (PPF)	X_3 (PSF)
1	0.920	0.050	0.030
2	0.855	0.055	0.090
3	0.831	0.108	0.061
4	0.766	0.200	0.034
5	0.842	0.128	0.030
6	0.710	0.200	0.090
7	0.766	0.144	0.090
8	0.876	0.083	0.041
9	0.799	0.138	0.063
10	0.855	0.055	0.090
11	0.766	0.200	0.034
12	0.710	0.200	0.090

According to the D-optimal approach effect of proportions of wheat flour (WF), pumpkin pulp flour (PPF) and pumpkin seed flour (PSF) on some physical parameters of composite pumpkin - wheat flour bread as response variables was evaluated and optimum combination was determined. The combination of independent variables in optimal proportions that lead to the best responses from bread physical parameters in terms of porosity, elasticity and height/diameter (D/H) ratio was determined.

2.2.3. Preparation of composite pumpkin-wheat flour bread

The pumpkin-wheat composite flour including pumpkin pulp flour, pumpkin seed flour and wheat flour at different proportions (Table 1) were used for the bread production. The 1.8 g compressed baker's yeast, 1% sodium chloride and 67% water (on 100% weight of mixed flour) were the other ingredients used for prepared the bread. All the ingredients were kneading in a mixer for 7 min at 28°C and then the modeled samples was proofed for 90 min at 30°C, 85% relative humidity and baked for approximately 30 min at 250°C. A 30 min after removal from the oven, the baked bread samples were evaluated.

2.2.4. Physical parameters of bread

The bread elasticity, porosity and H/D ratio are the physical parameters determined for assessed the effects of pumpkin-wheat composite flours formulation on bread quality. Loaf height, diameter, elasticity and porosity were determined according to SR 91:2007 and H/D ratio was calculated.

2.2.5. Statistical analysis and optimization

In this study, fitting response values was made using the linear, quadratic and cubic models (Eqs. (1) – (3)). Analysis of variance (ANOVA) at *p*-value of the model < 0.05, *p*-value of lack of fit > 0.05 and coefficient of determination, $R^2 > 0.85$ are the criteria's used for the selection of predictive model for each response.

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \varepsilon \quad (1)$$

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \varepsilon \quad (2)$$

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3 + \varepsilon \quad (3)$$

where Y is the predictive response (bread elasticity, porosity and H/D ratio); ε is the error of estimation; β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{123} are the constant coefficients for each linear and interaction term in the predicted models; and X_1, X_2, X_3 is proportion of pseudo-components.

Also, the regression coefficients of individual linear, interactive and cubic terms were determined. The significances of all terms in the regression models were assessed statistically by computing the *F*-value at a level of significance of 95% or 99%.

After evaluating the regression models that reveal the relationship between flours mixture components and response variables, the desirability function approach was used to simultaneously optimize bread porosity, elasticity and H/D ratio. The optimal levels of mixture components of the composite flour required to yield final optimized physical parameters of bread were obtained by numeric analysis based on criteria of maximizing porosity, elasticity and the value of 8 for H/D ratio.

The State- Ease Design Expert 7.0.0 software package (trial version) was used to statistical analysis, to generate the surface response of the fitted regression equations and to determine the optimum proportions of the components from composite pumpkin-wheat flour bread.

3. Results and discussion

3.1. Fitting for the best model

The best model for predicting physical parameters of composite flour bread is the regression model that has a low standard deviation, a low predicted sum of squares, and high R-squared [42]. Therefore, the results of ANOVA shown that special cubic model was found the best fitted for all response variables, bread porosity, elasticity and H/D ratio (Table 2).

The R^2 value obtained from special cubic model was found to be greater than 0.85, indicating a great fitting model. The mathematical response surface models were carried out using Design Expert software by applying pseudo components.

Table 2.

Regression coefficients of predicted special cubic models for physical parameters of composite flour bread formulation using data in pseudo components

Component	Physical parameters of bread sample		
	Porosity (%)	Elasticity (%)	H/D ratio
β_1 (WF)	80.33	82.38	0.96
β_2 (PPF)	91.02	85.06	0.96
β_3 (PSF)	-303.45	-146.73	2.99
β_{12} (WF x PPF)	-57.58	-21.44	-0.095
β_{13} (WF x PSF)	535.55*	311.73*	-3.15
β_{23} (PPF x PSF)	474.63*	297.52*	-3.39
β_{123} (WF x PPF x PSF)	280.19	162.14	-0.64
p-value	0.0270	0.0471	0.0133
Lack-of-fit	0.7765^{ns}	0.3072^{ns}	0.1473^{ns}
R^2	0.8897	0.8596	0.9184

β_1 – wheat flour (WF) value coefficient, β_2 – pumpkin pulp flour (PPF) value coefficient, β_3 – pumpkin seed flour (PSF) value coefficient;
^{ns} no significant effect at level < 0.05;
* Significant at the level of $p < 0.05$.

The physical parameters of bread sample were significantly predicted by independent variables pumpkin pulp flour, pumpkin seed flour and wheat flour. The values of linear and interaction terms show that pumpkin seed flour is the component which most affects the bread porosity, following the elasticity and H/D ratio. The pumpkin seed flour was again the most important variable in predicting all physical parameters of bread.

Residual analysis was also carried out in order to evaluate the adequacy of the prediction models. The residual values for porosity, elasticity and H/D ratio, explained through the difference between the observed and predicted values of these parameters, are normally distributed (Figure 1a), b) and c)). The plots showed that the special cubic model is adequate to describe the each response surface.

The predicted models were used to generate contour plots for response variables significantly predicted by independent variables. Figures 2-4 show the estimate response surface graphs for the porosity, elasticity and H/D ratio.

3.2. Effect of component from composite flour on quality measurements of bread

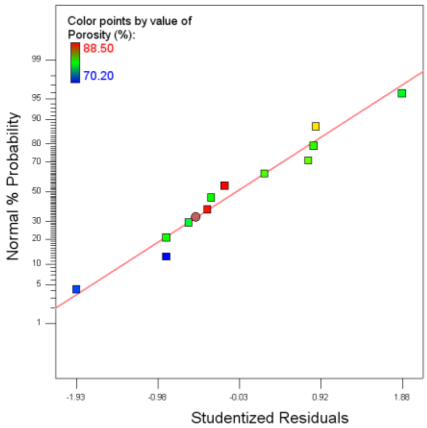
The bread samples produced with different proportion of wheat, pumpkin pulp and pumpkin seeds flours shows differences exist among the samples in porosity, elasticity and H/D ratio.

Bread porosity

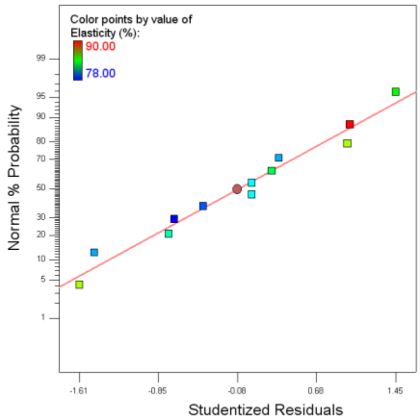
Bread porosity is an important parameter of bread quality which indicate it assimilability that can be higher or lower.

The influence of composite flour components on bread porosity has been shown in the response surface graph in Figure 2. Regarding to porosity, a high correlation (R^2 = 0.8897) was obtained. The formulation components, wheat flour (WF), pumpkin pulp flour (PPF) and pumpkin seed flour (PSF), independently not influenced significantly the porosity, however, the interactions between WF and

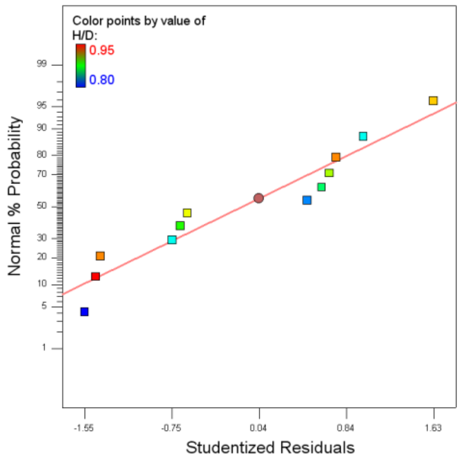
PSF, between PPF and PSF, significantly ($p < 0.05$) influenced bread porosity.



(a)



(b)



(c)

Fig. 1. Residual plots for porosity % (a), elasticity % (b) and H/D ratio (c)

The value of linear parameter PSF showed negative coefficient for porosity which indicated a decrease of this physical parameter.

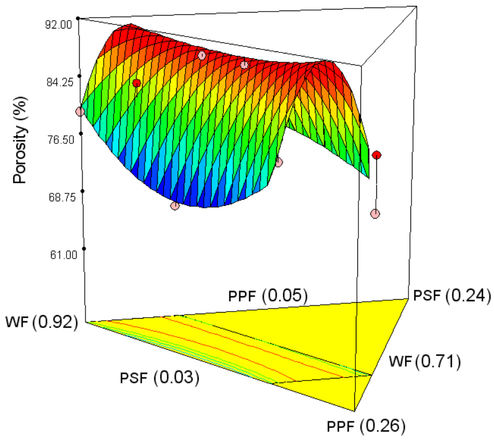


Fig. 2. The response surface plot of porosity

This result is in agreement with the results obtained by See et al. (2007) [16] who concluded that addition of 5% pumpkin seed flour in wheat flour resulted in bread with high loaf volume are most acceptable. After this level, the specific volume decreased. The increased level of PSF addition in bread composite flour up of 5% leaded to a decrease of bread porosity.

It was evident from this study that at lower PSF proportion the bread porosity increased. An increase in WF level in composite flours lead to an increase of bread porosity values. The increase in porosity value with increasing level of PPF in composite flour has also been reported previously [35], the bread obtained beeing acceptable at 10% level.

Porosity value is proportional to the increase level of PPF and WF in composite flour. Therefore, high proportions of PPF and WF in composite flour may increase the bread porosity, by improving gas-cell stability according to Ptitchkina et al. (1998) [31] which affirm that the enhanced stability comes from surface activity of pumpkin pectin. Pectin constitutes about 30% of the dry weight of pumpkin tissue. The increase in aeration is tentatively attributed to surface activity of the highly acetylated pectin present as a major component (about 30% of the total dry-matter content) of pumpkin tissue. Różyło et al. (2014) [27] showed that bread with pumpkin pulp had smaller and more compact pores.

Elasticity
The bread having high elasticity are considered to be having desirable features. Figure 3 shows response surface plot of elasticity as a function of wheat flour (WF) proportion, pumpkin pulp flour (PPF) proportion and pumpkin seed flour (PPS) proportion in composite flour.

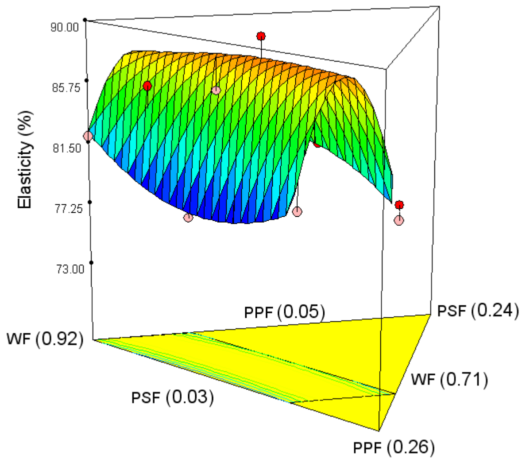


Fig. 3. The response surface plot of elasticity

Elasticity was maximum at intermediate to higher flours proportions. Addition of PPF to composite flour showed a positive coefficient indicated that PPF had a synergistic effect on porosity. The WF/PSF and PPF/PSF combinations showed a positive significant influence on porosity, while the three component combination is insignificant.
Also, in present study, interaction of WF/PPF showed a negative coefficient indicating an antagonistic effect on elasticity, while the interaction WF/PSF and PPF/PSF has a synergistic effect on this physical parameter.

H/D ratio
The predictive model for H/D ratio showed the three variables, WF, PPF and PSF hadn't significant effects, but which was positive. All the three flours showed not significant quadratic negative effects on H/D ratio. Comparatively with other components of composite flour, pumpkin seed flour has a high contribution on H/D ratio. Interactive effects between wheat flour and pumpkin pulp flour, pumpkin seeds flour and, triple interaction effect between wheat flour, pumpkin pulp flour and pumpkin seeds flour had a negative effect on H/D ratio of the bread. According to ANOVA, there was no significant effect of the regression model on H/D ratio (Table 2), but a high correlation (R^2 = 0.9184) was detected. In H/D ratio, the contour plot can be observed that the reduction of wheat flour content and increasing of pumpkin seed flour in composite flours formulation resulted in lower H/D ratio values (Figure 4).

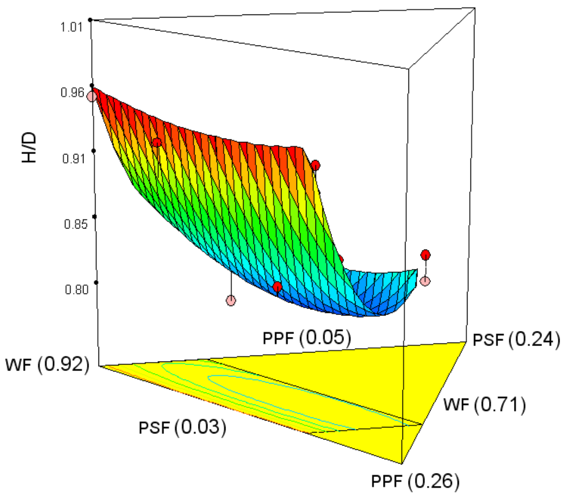


Fig. 4. Response surface plot showing effects of composite flour formulations on H/D ratio

3.3. Optimized composite flour formulation
Upon comprehensive evaluation of available Design Expert solutions, the optimum mixture ratio of flours with wheat flour, pumpkin pulp flour and pumpkin seed flour was determined on the basis of desirability function, using the

optimization process suggested by Derringer and Suich [43]. Simultaneous optimization was performed by imposing some constraints such as H/D ratio of 8, and maximum porosity and elasticity.

The best combinations between the proportion of wheat flour, pumpkin pulp and pumpkin seeds flours used in this study in order to obtain optimum values for some physical parameters of bread were extracted by State-Ease 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 94.1% was obtained for the optimal formulation of composite flour with wheat flour (WF), pumpkin pulp flour (PPF) and pumpkin seed flour (PSF) in proportion of 78.30%, 15.00% and 6.70% (w/w). The response-surface plot corresponding to D value is represented in Figure 5, and it was used to predict final mixture proportion.

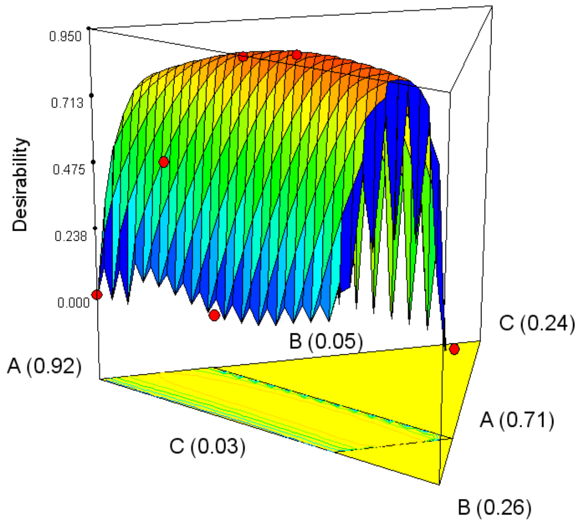


Fig. 5. Desirability function response surface

Under these mixture proportions, the predicted H/D ratio of 0.81, bread porosity of 89.80% and elasticity of 88.53% were obtained.

4. Conclusion

This study highlights the usefulness of D-optimal design in the assessment of the simultaneous influence of composite flour components and their interaction on bread physical parameters. The effects of composite flour components on response variables could be accurately predicted by special cubic type models. A high linearity was observed between predicted and actual value of response variables. By using numerical technique, the optimized pumpkin-wheat composite flour was obtained. The optimized composite flour formula chosen on the basis of desirability function revealed that the composite flour with 78.30% wheat flour, 15.00% pumpkin pulp flour and 6.70% pumpkin seed flour (w/w) is the best formulation which can be used to obtain pumpkin-wheat flour bread with the desirable physical parameters.

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