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SENSORY EVALUATION OF MILLET-YELLOW FLAXSEED-HEMP COMPOSITE FLOUR GLUTEN-FREE COOKIES FOR OPTIMUM FORMULATION BY THE MIXTURE EXPERIMENTAL DESIGN

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Abstract: *The aim of the present investigation was to prepare and optimize the formulation of millet - yellow flaxseed- hemp composite flour gluten-free cookies by applying experimental design technique. Simplex-Lattice design and desirability function were used to evaluate and then to establish the optimal recipe for gluten-free cookies. Maximum sensorial quality and general acceptability were selected as dependent variables. Regression equations and contour plots were used to relate dependent variables, i.e. color, taste, smell, aroma, texture and general acceptability of cookies, with independent variables.The results of the experimental design revealed that the independent factors millet flour, yellow flax seed flour and hemp flour are significantly influenced by the general acceptability of the cookies. Based on the computed value of desirability function, it was concluded that the composite flour containing 21.50% millet flour, 20.00% yellow flaxseed flour and 58.50% hemp flour was in the optimum mixture proportions based on the maximum sensory characteristics of cookies samples.*

Key words: composite flour, gluten-free cookies, sensory evaluation, Simplex-Lattice design, desirability function

1. Introduction

In general, cookies are products favored by consumers being an important part of their daily diet. The main ingredient of cookies is wheat flour, but the health problems associated with celiac disease has limited the use of this type of flour in such products. Therefore, nowadays the requirement for gluten-free products is increasing worldwide. Considering this demand for obtaining cookies only from composite flours we used hemp, yellow flaxseed and millet flours which allowed us to improve the quality of cookies from the nutritional point of view due to the fact that these flours are nutrient-rich and also gluten-free.

In the food industry it is using only hemp (*Cannabis sativa L., Cannabaceae*) with a low level of δ-9-tetrahydrocannabinol [1]. It presents a great nutritional value generally composed of 25-35% lipids, 20-25% protein, 20-30% carbohydrates, and 10-15% fibers [2]. In addition to these components, hemp seeds also contain minerals and vitamins like phosphrous, potassium, magnesium, sulphur, calcium, iron, zinc, carotene and E, C, B_1, B_2, B_3, B_6 vitamins [3]. The lipid portion of hemp seeds is a healthy one due to it content up to 80% polyunsaturated fatty acids [4] notably essential linoleic acid (omega-3) and α-linolenic acid (omega-6) in the favorable ratio of 3:1 [2, 3, 5]. Moreover, hempseed oil contains γ-linolenic and stearidonic acid which can only be found in a few plant families [4]. Hemp seeds are also a good source of easily digestible proteins which contains all essential amino acids [2]. In the seeds contents substances such as methionine and cystine were also identified [6]. The hemp seed protein contains 65% globulin called edestin and 33% albumin [7].

The flaxseed (*Linum usitatissimum L.*) is rich in lipids, proteins, lignans and dietary fibers. The golden flaxseed contains a lower amount of fiber and a higher amount of soluble carbohydrates and fatty acids omega-3 and omega-6 than the brown variety [8]. In general, regarding the lipid content, flax seeds are rich in polyunsaturated fatty acid in especially omega-3 fatty acid with the highest amount of any seed oil. Omega-6 fatty acid is only about 16% from the polyunsaturated fatty acid content [9]. From the protein content point of view the flaxseed presents an amino acid composition similar to that of soybean protein, which is viewed as one of the most nutritious plant proteins [10]. The major proteins in flaxseed are albumins and globulins. Flax seeds contain also large amounts of minerals and vitamins such as vitamin E present as gamma-tocopherol, which function as an antioxidant [11], vitamin B (thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folate and cyanocobalamin (B12)) [12], potassium and phosphorus like major mineral elements and significant quantities of iron, zinc and manganese [13].

The term "millet" is used for several small seeded annual grases and can be cultivated in a wide range of soils and climates with a special importance in semiarid regions [14]. This food cereal is the forth tropical crop in the world [15]. Millet presents high levels of calcium, iron, zinc, lipids and high quality proteins [16]. Compared to other cereals millet has a better amino acid profile than maize and sorghum and a similar one to barley, rice and wheat [17]. The lysine and methionine content of pearl millet is with 40% and respectively 30% higher than the corn [18]. However, millet does not contain high level of lysine comparatively with other essential amino acids like methionine and tryptophan [19]. Regarding the lipid content, millet is rich in oil which contains linoleic acid and α-linolenic acid [20]. Linoleic acid is about 4% of the total fatty acids from this oil in a more high content than in maize [17].

Sensory properties of food have the highest influence on food choice in most situations. These sensory properties are perceived through the senses of smell, sight, touch, taste and hearing and are evaluated by different scientific methods. The evaluations of food preference from the sensorial point of view are based on foods accepted or rejected by using different preference sensorial tests.

Mixture experiment design is a statistical technique that can be used for evaluating the preference and acceptance of the final product based on the relative proportions of the components in the mixture. This technique describes through an equation how the response variables are affected by the independent variables in a mixture experiment [21]. Simplex-Lattice design can be applied as standard tool in order to find the optimal response for any mixture of the ingredients, and to obtain the influence on the response of each ingredient singly and in combination with other ingredients.

The main objective of this study was to optimize the mixture of the three ingredients, hemp, yellow flaxseed and millet flours from the gluten-free cookies recipe, with the purpose of achieving the maximum sensorial quality and overall acceptability of produced cookies. Simplex-Lattice design was applied as standard mixture designs in order to determine the optimal blend for high acceptance gluten-free cookies.

2. Materials and Methods

2.1. Materials

The millet seed, yellow flaxseed and hemp seed were purchased from the Romanian supermarket and used as raw materials for composite flour formulation. The gluten-free flours from these seeds were obtained through mechanical milling of the seeds, and then the mill was passed through a 150 μm mesh sieve in order to obtain fine flour.

2.2. Methods

2.2.1. Experimental design

In this study, the Simplex-Lattice design was used with a three components mixture, with lower and upper limit restrictions, for the determination of the optimum combinations of the gluten-free flours (components) in the recipe of cookies to produce acceptable product and to estimate the most acceptable product with the highest sensory score. The lower and upper limit restrictions of each component (gluten-free flour) were based on preliminary work. In this mixture design the following three factors: millet flour (X_1), yellow flaxseed flour (X_2) and hemp flour (X_3) were evaluated by changing their levels (proportions) simultaneously and keeping total level (proportions) constant ($X_1 + X_2 + X_3 = 1$) [22]. Blends of the formulations were the pure (X_1, X_2, X_3), two-component mixtures (X_1X_2, X_1X_3, X_2X_3) and the three-component mixtures ($X_1X_2X_3$) according to Montgomery (2000) [23]. The experimental design required 13 combinations of gluten-free flours, levels and experimental design in term of pseudo components and real levels which are presented in Table 1. The sensory characteristics such as: colour, taste, smell, aroma, texture and general acceptability were selected as the response variables. The value of response variable is a function of the level of ingredients present in a cookie recipe [24]. The goal of this experimental design is to model the mixture surface with a form of polynomial equation so that the responses (sensory characteristics) for any blend of ingredients from recipe of cookies can be predicted.

2.2.3. Preparation of cookies

The flour mixtures composed of hemp, yellow flaxseed and millet flours at different proportions (Table 1) were used for the cookie production. The other ingredients used were sodium chloride 1% and water 20% (on 100% weight basis of mixed flour). Dry flour mixture were homogenized using dough mixture for 3 min and sodium chloride dissolved in about 5 mL water were added into the mix. Further mixing was done with the rest of the water for 10 minutes. The dough was sheeted to thickness of 0.5 cm. The sheeted dough was made into circular shape samples by circular cookie cutter of 4 cm diameter. These circular samples were transferred in a baking oven and baked at 180-200^{0}C. The baking time varied between 10 and 13 minutes. After baking, cookies samples were cooled by resting on a paper napkin at room conditions for 30 minutes. Cookies were stored in HDPE bags in a refrigerator at 4^{0}C before analysis.

2.2.4. Sensory evaluation

Consumer affective testing was carried out using a nine point structured hedonic scale

(1 = disliked and 9 = liked extremely) for the evaluation of the color, taste, smell, aroma, texture and general acceptability. The test was conducted in the Sensorial Analysis Laboratory at the Ştefan cel Mare University, Faculty of Food Engineering. Samples (approximately 5 g) of each formulation coded with three-digit numbers were presented to 63 students on plastic plates. Unflavored, dry crackers and taste free water were provided for palate cleansing between samples. For minimize sensory fatigue there was a 3 minute break after each sample and a 15 minute break after the seventh sample.

2.2.5. Statistical analysis and optimization

Statistical analysis and modeling of response variables were carried out using Stat Ease Design Expert 7.0.0 software package (trial version). The multiple regression analysis with the intercept set to zero was used to generate polynomial models based on Scheffé's canonical model for each of the response variables. The adequacy of the regression model was verified by the sequential model sum of squares, lack of fit, multiple correlation coefficients (R^2) and other adequacy measures. In order to determine the significance of each term an analysis of variance (ANOVA) with a level of significance of 95% was performed, whereby the resulting model equations describe the sensory characteristics on the three components (millet flour, yellow flaxseed flour, and hemp flour) and their interactions.

The optimum levels of the factors (components used in recipe) were obtained by using the numerical optimization technique. This technique searches the design space, using the models created during analysis to find factor settings that meet the defined goals. To find out an effective solution, a multiple response method called desirability was applied [25]. The desired goal for each factor and response was selected so: the proportion of millet flour, yellow flaxseed flour and hemp flour were permitted to be at any level within range of the design, while all of the response variables were desired maximal. After applying these constraints, the optimal levels for ingredients used in cookies recipe and the predicted values for sensory quality and overall acceptability were obtained.

Table 1

Experimental design for ternary mixture of millet, yellow flaxseed and hemp flour in pseudo components and in real levels

Formulation	Component proportion in the composite flour					
	Pseudo components			Real levels (%)		
	Millet flour (X_1)	Yellow flaxseed flour (X_2)	Hemp flour (X_3)	Millet flour	Yellow flaxseed flour	Hemp flour
1	1.000	0.000	0.000	50.0	20.0	30.0
2	0.500	0.000	0.500	35.0	20.0	45.0
3	0.167	0.167	0.667	25.0	25.0	50.0
4	0.000	0.500	0.500	20.0	35.0	45.0
5	0.333	0.333	0.333	30.0	30.0	40.0
6	0.000	0.000	1.000	20.0	20.0	60.0
7	1.000	0.000	0.000	50.0	20.0	30.0
8	0.000	1.000	0.000	20.0	50.0	30.0
9	0.000	1.000	0.000	20.0	50.0	30.0
10	0.000	0.000	1.000	20.0	20.0	60.0
11	0.500	0.500	0.000	35.0	35.0	30.0
12	0.167	0.667	0.167	25.0	40.0	35.0
13	0.667	0.167	0.167	40.0	25.0	30.0

3. Results and discussion

3.1. Fitting for the best model

A Simplex-Lattice design was applied in this study to perform the experiments by changing the concentrations of component simultaneously and keeping total concentrations constant (Table 1). The quadratic model (Eq.1) was found the best fitted for all sensory characteristics of gluten-free cookies because has low standard deviation, low predicted sum of squares, and high predicted R-squared [21].

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 \quad (1)$$

where Y is the dependent variable; β_1, β_2, β_3, β_{12}, β_{13} and β_{23} are the constant coefficients for each linear and interaction term in the predicted models, and X_1, X_2, X_3 the proportions (levels) of each pseudo-components. Analysis of variance (ANOVA) showed that the sensory characteristics were significantly ($p < 0.01$) influenced from the linear terms of the predicted models despite the fact that the sensory characteristics were not significantly influenced from two component interactions (Table 2). Significance of model fitting was also assessed using ANOVA and *p* value was always less than 0.01. The R^2 value obtained through the analysis of predictive models was found to be always greater than 0.88, indicating a great fitting model.

Table 2

Regression coefficients and analysis of variance of the models for sensory characteristics

Coefficients[a]	Sensory characteristics					
	Colour	Taste	Smell	Aroma	Texture	General acceptability
β_1	6.83**	7.29**	7.37**	7.54**	7.21**	6.84**
β_2	6.79**	5.96**	6.01**	6.00**	5.77**	6.26**
β_3	8.04**	8.19**	8.45**	8.41**	8.25**	8.29**
β_{12}	2.11*	4.20*	3.82*	5.29*	5.48*	2.97*
β_{13}	1.62*	0.40	1.68	-0.25	1.56	1.94
β_{23}	-0.58	1.20	-2.86	-0.65	-1.92	-0.74
Significance of the model (*p*)	0.0004	0.0036	0.0019	0.0006	0.0018	0.0007
Lack of fit of the model	0.2920 ns	0.1922 ns	0.0502 ns	0.2773 ns	0.2570 ns	0.0610 ns
R^2	0.9408	0.8835	0.9033	0.9300	0.9057	0.9271

[a] β_1, β_2, β_3 correspond to the effects of millet flour, yellow flaxseed flour and hemp flour, respectively, in the model;
ns no significant effect at level < 0.05;
* Significant at the level of $p < 0.05$;
** Significant at the level of $p < 0.001$.

3.2. Sensory evaluation

Figure 1 shows the regression model of sensory characteristics for the millet-yellow flaxseed-hemp composite flour gluten-free cookies in a triangular plot of the statistically analyzed experimental design. Each corner of the equilateral triangle shown in Figure 1 represents a concentration of 50% (w/w) of millet flour and yellow flaxseed flour, and 60% of hemp flour, respectively.

3.2.1. Color

According to Table 2, the color scores response was well fitted to the quadratic model with high determination coefficient (R^2 = 0.9408). Two-component interaction, millet flour-yellow flaxseed flour (X_1X_2) and millet flour-hemp flour (X_1X_3) had significant influence ($p < 0.05$) on the color score response of cookies samples (Table 2). In the contour plot of the color score response, the highest values were given in the X_3 vertex and to edge of X_3 (Figure 1a).

3.2.2. Taste

The predicted regression coefficients for taste showed that the quadratic model is appropriate for this sensory characteristic (Table 2). For taste response, the interaction between millet flour and yellow flaxseed flour (X_1X_2) had a more notable influence on taste scores as shown by the higher coefficient value (Table 2). Higher taste scores were observed in the X_3 edge, in the vertex of the X_3 and to some extend in X_3X_2 edge. Increased yellow flaxseed (X_2) concentration decreased sample taste while, increased millet flour (X_1) also increased sample taste (Figure 1b).

High millet flour levels in cookies gave products with more taste scores compared to products with low levels added millet flour. Similar results were reported in the literature in which the composition on gluten-free flour cookies was optimized [26].

3.2.3. Smell

As can be observed in Table 2, R^2 value of the predicted model for smell response were higher than 0.90, indicating that this were relatively adequate for the prediction purpose. Figure 1c) illustrates ternary contour plot that indicates the effects of processing components on the smell sensory characteristic of cookies samples. The smell contour values increased toward the hemp flour (X_3) edge where maximum smell contour could be seed; however, the lowest smell scores were at yellow flaxseed flour (X_2) vertex and to some extend in X_2 edge. From this point of view, it could be said that the use of hemp flour in recipe gluten-free cookies increased the smell of the final product, while use of the others decreased.

3.2.4. Aroma

The aroma response was well fitted to the quadratic model with high determination coefficient (R^2 = 0.9300). Generally, the linear terms had a significant effect on aroma scores of cookie samples; however, interaction between millet flour and yellow flaxseed flour had lower coefficient compared to the linear terms (Table 2). Contour plot (Figure 1d) shows that aroma score was higher in the vertex of the hemp flour (X_3) and edge of millet flour (X_1). The lowest aroma scores were at yellow flaxseed flour (X_2) vertex. Therefore, high yellow flaxseed flour levels gave gluten-free cookie products with no acceptable aroma compared to products with low levels of this flour.

3.2.5. Texture

The quadratic regression model for texture response was highly significant ($p < 0.01$). Two-component interaction, millet flour and yellow flaxseed flour (X_1X_2) had significant influence ($p < 0.05$) on the texture response of cookie samples (Table 2). Contour plot showed that texture score seemed to behave symmetrical when yellow flaxseed flour concentration was equal to the millet flour (Figure 1e). Also, the highest texture score to be at millet flour and hemp flour edges and hemp flour vertex. However, the lowest texture scores were at yellow flaxseed flour vertex.

Consequently, it could be said that the use of hemp flour and millet flour in recipe increased the texture of the cookie samples.

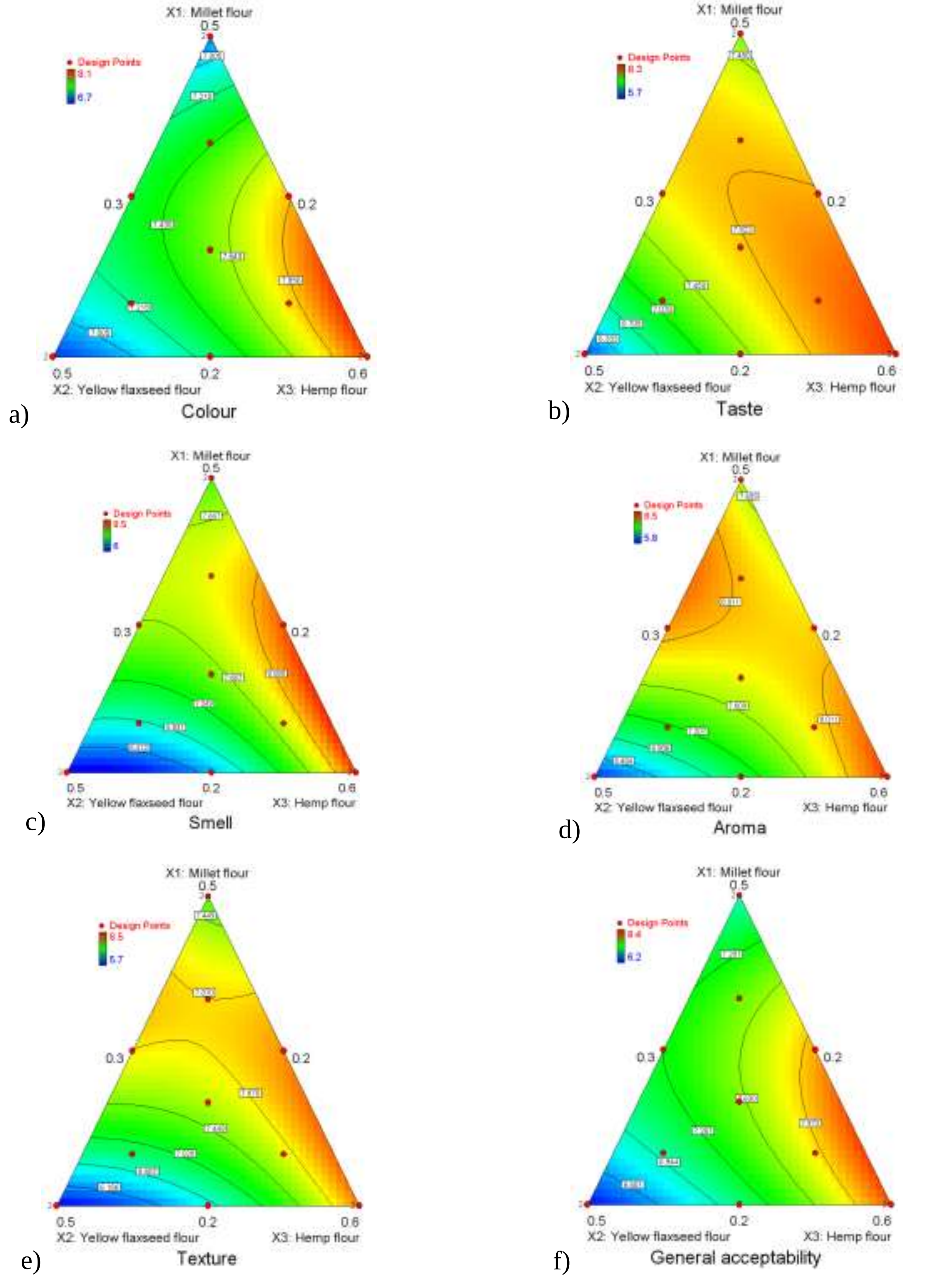

Fig. 1. Ternary contour plots of the effects of processing components on sensory characteristics of cookies: a) color, b) taste, c) smell, d) aroma, e) texture and f) general acceptability

3.2.6. General acceptability

General acceptability of millet-yellow flaxseed-hemp composite flour gluten-free cookies were significantly ($p < 0.01$) influenced from the linear terms of the predicted model as indicated by the adjusted regression model developed for general acceptability were not significantly ($p > 0.05$) influenced from two component interactions, millet flour-hemp flour (X_1X_3) and yellow flaxseed flour-hemp flour (X_2X_3). General acceptability score was higher at the points closest to hemp flour vertex and millet flour-hemp flour edge, indicating that the cookies samples were more preferred by the panelist when the millet flour and hemp flour was added in recipe at the higher levels comparatively with yellow flax seed flour (Figure 1f), probably because of the synergistic effects of the flours upon the sensory characteristic of the gluten-free cookie samples.

3.3. Mixture proportion optimization

Mixture proportion optimizations were performed to determine the optimum levels of gluten-free flours (millet flour, yellow flaxseed flour and hemp flour) used in the recipe of cookies to achieve the desired response goals. Finding maximum scores of sensory characteristics is the desired goal to obtain an acceptable product in terms of sensory characteristics.

The scores of following sensory characteristics: colour, taste, smell, aroma, texture and general acceptability were desired maximal. The best combinations between the proportions of these gluten-free flours from recipe of cookies in order to obtain maximum scores of sensory characteristics were extracted by Design Expert software that performs from the thousands of iterations and calculation the maximum desirability score and the conditions on which it was arrived. Using this approach, a set of combination of millet flour, yellow flaxseed flour and hemp flour was found but the best formulation (Table 3) to obtain an acceptable product in terms of sensory characteristics, at a total desirability value (D) of 0.955. Maximum colour (8.060), taste (8.169), smell (8.477), aroma (8.358), texture (8.268) and general acceptability score (8.305) could be obtained from this optimum mixture proportion (formulation of samples).

Table 3

Optimum mixture proportion

Components	Actual proportions (%)
Millet flour	21.50
Yellow flaxseed flour	20.00
Hemp flour	58.50

Overlay plots can also be produced by superimposing the contour for the sensory characteristics. By defining the limits of the color, taste, smell, aroma, texture and general acceptability score desired, the zone at the bottom right of the plot, as shown in Figure 2, defines the permissible scores of the response variables.

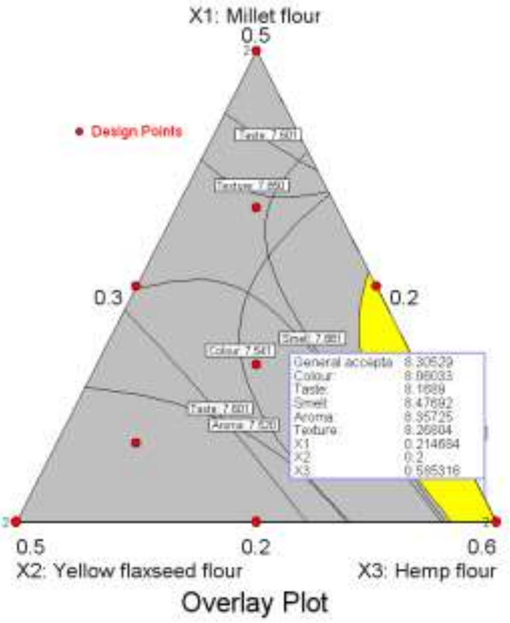

Fig. 2. Overlaid contour plot of sensory characteristics for millet-yellow flaxseed-hemp composite flour gluten-free cookies

According to these results, high proportion of hemp flour comparatively with millet flour and yellow flaxseed flour, are needed to obtain maximum sensorial quality and overall acceptability of produced cookies from millet-yellow flaxseed-hemp composite flour gluten-free.

4. Conclusion

The results of this study showed that Simplex-Lattice mixture design approach is a fitting method for optimization of millet-yellow flaxseed-hemp composite flour gluten-free cookies formulation. According to experimental results and contour plots were observed that hemp flour and millet flour significantly influenced sensory characteristics of cookies and increase levels of yellow flaxseed flour in recipe of cookies decreased sensory characteristics, especially smell characteristic. Therefore, millet-yellow flaxseed-hemp flour can be use as a mixture to produce gluten-free cookies when optimum combination proportions of these flours are taken account.

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