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Optimization of Gluten-Free Bread Formulation Using Sorghum, Rice, and Millet Flour by D-Optimal Mixture Design Approach

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ABSTRACT

There is an increasing interest in Gluten-Free (GF) products as the prevalence of celiac disease. Sorghum, millet, and rice flours are the most suitable cereal flours for GF products. The objective of this study was to optimize mixtures of Sorghum Flour (SF), Rice Flour (RF), and Millet Flour (MF) for production of GF bread based on D-optimal mixture design approach. The characteristics of flours including moisture, proteins, fat, ash, fiber, and pH were measured. GF bread quality parameters such as specific volume, hardness, crumb structure, image characteristics and organoleptic evaluation were also analyzed. Our results revealed that three flour blends (SF, RF, and MF) had remarkable effect on physical and organoleptic properties of GF bread. Increasing MF and SF together with decreasing RF increased specific volume and mean cell area and produced GF breads with a softer texture. Color and taste improved with incorporation of RF, SF, and MF at high levels. The organoleptic evaluation of texture was correlated to instrumental texture analysis. The optimum formulation obtained according to organoleptic evaluation, specific volume, hardness, and crumb structure contained 67.18% SF, 17.82% RF and 15% MF with combined desirability equals to 0.791. In general, the results of the present study indicate that RF, SF, and MF can be used as a substitute for wheat flour in producing high quality GF bread. The data presented in this study could be useful in producing GF bread for celiac patients.

Keywords: Celiac, Desirability function, Optimum formulation, Organoleptic evaluation.

INTRODUCTION

The celiac disease is one of food intolerances diseases in which gluten in the diet causes inflammation of the small intestine. It affects the adsorption of nutrients as folic acid, fat soluble vitamins, iron or calcium (Iordăchescu *et al*., 2013). The only effective treatment for celiac disease is a strict adherence to a GF diet throughout the patient's lifetime (Gallagher *et al*., 2004; Dizlek and Ozer, 2016a). There is an increasing interest in GF products as the prevalence of celiac disease. The celiac prevalence was estimated about 1-2% of the world population (Reilly and Green, 2012).

RF is one of the most suitable cereal flour for GF products because it has low level of prolamine, low sodium content, mild flavor, desirable taste, white color, unique nutritional value, and hypoallergenic properties (Marco and Rosell, 2008; Sakač *et al*., 2011; Torbica *et al*., 2012; Nazni and Gracia, 2014). However, GF breads based on RF require polymeric substances that mimic the viscoelastic properties of gluten to provide structure and retain gas (Torbica *et al*., 2010). Hydrocolloid is such a compound that could improve volume and

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texture of rice-based GF breads in terms of gas retention and water absorbing characteristics (Phimolsiripol *et al*., 2012; Dizlek and Ozer, 2016b).

Regarding nutritional quality, rice-based GF formulations have, in particular, low contents of vitamins, minerals, proteins and dietary fiber (Phimolsiripol *et al*., 2012; Thompson *et al*., 2005). Hence, the enrichment of GF rice bread with other cereals seems to be necessary. The baking products made of RF have low specific volume and very compact crumb, because of the low content of prolamins fractions required for developing the specific dough's protein network.

Sorghum is an attractive raw material and a good source of protein for wheat-free products due to the neutral flavor, color of specific varieties, low allergenicity and its ability to grow in drought-like conditions. The use of SF in GF or composite bread can give us functional breads containing antioxidants and, therefore, helpful to relief celiac and tumor sufferers (Olatunji *et al*., 1992). Sorghum also has an advantage in composite flours because of its familiar bland taste which is similar to wheat (Kulp, 2000). The starches and sugars in sorghum are released more slowly than in other cereals and that could be beneficial to diabetic patients (Dahir *et al*., 2015).

Millets are highly tolerant of extreme weather conditions like drought and can be stored for a long time without insect damage (Obilana *et al*., 2002; Yang *et al*., 2012; Adekunle, 2012; Amadou *et al*., 2013). Millets are good sources of energy. They provide protein, fatty acids, minerals, vitamins, dietary fiber and polyphenols. Typical millet protein contains high amount of essential amino acids, especially the sulfur containing amino acids (methionine and cysteine) (Dykes *et al*., 2006; Amadou *et al*., 2013; Badiu *et al*., 2014). Millets can also be utilized in GF bread formulations. It possesses a low glycemic index and, therefore, helpful for diabetic patients (Chhavi and Sarita, 2012; Saleh *et al*., 2013).

Soy protein products are also known for their improved crust color, crumb, resilience and toasting characteristics in bread (Nilufer *et al*., 2008), also extending shelf-life of bakery products (Vittadini and Vodovotz, 2003). Proteins of legumes such as soy contain high amount of lysine, as an essential amino acid, and are also deficient in sulfur-containing amino acids that makes them a great complement to other cereal proteins which are deficient in lysine, but have good sulfur amino acid content (Eggum and Beame, 1983). Soy contains a high amount of minerals including phosphorus, calcium, magnesium, iron, and copper and is one of the richest sources of lecithin, essential for living cells, since it emulsifies cholesterol and helps in the assimilation of vitamins (Osella *et al*., 2014). Moreover, the consumption of soy protein causes reduction in total low-density lipoprotein cholesterol and also in triacylglycerols (Marco and Rosell, 2008).

Response Surface Methodology (RSM) is a statistical technique that has been successfully used in the development and optimization of cereal products. RSM consists of a group of mathematical and statistical procedures that can be used to study the relationships between one or more dependent variables and independent variables. In order to achieve optimization, RSM will reduce the number of trials and provide multiple regression approach (Dwivedi *et al*., 2013). The main objective of this study was to develop an optimized GF bread formulation in order to obtain bread containing optimal levels of SF, RF and MF using RSM.

MATERIALS AND METHODS

Material Collection and Sample Preparation

Rice (Hashemi varieties) flour, Sorghum (Red hybrid) flour and Millet flour (Miliaceum Panicum) were obtained from Agricultural and Natural Resources

Research and Education Center of Khorasan Razavi, Iran. Soy flour (inactivated natural enzymes) was obtained from Soyan Toos Co., Mashhad, Iran. Samples were sealed and placed in plastic bags and stored at 4-6°C.

Bread recipes also contained active wet (bread) yeast (Razavi Co., Mashhad, Iran), vegetable oil (Ladan Co., Behshahr, Iran), salt and sugar (Local market). Sodium Carboxy Methyl Cellulose (CMC) was obtained from AGC Industries Co., China.

GF Bread Preparation

The bread formula used for GF bread consisted of the following mixtures: (RF, SF, MF and soy flour), water (150 g 100 g^{-1} flour mixture), bread yeast (2 g 100 g^{-1} flour), salt (2 g 100 g^{-1} flour), oil (4 g 100 g^{-1} flour), CMC (2 g 100 g^{-1} flour) and white sugar (7 g 100 g^{-1} flour). In all tests, the water temperature was maintained between 20-22°C. Soy flour was added at a constant level of 10% to all flour mixtures, therefore, the combination of all three flours (RF, SF, MF and soy) were calculated from 90%. All ingredients were mixed for 15 minutes in a Mixer (Hobart Model Germany) and then 250 g of the batters were easily poured into rectangular mini toast pans with dimensions of 17×9×9 cm^3. Fermentation was performed at 37°C and 85% relative humidity for 60 minutes. After fermentation, batters were baked in an industrial oven (model Koenig, Germany) for 45 minutes at 200°C. After baking, samples were cooled at room temperature for 60 minutes. Finally, breads were packed in polyethylene bags and stored in an incubator at 20°C until use. Physical and textural analyses were carried out 8 hours after final baking.

Chemical Characteristics of Flours

The characteristics of flours including moisture, proteins, fat, ash, fiber and pH were measured according to AACCI methods (AACCI, 2000).

Flours Particle Size

The particle size distribution of flours were measured by Dynamic Light Scattering (DLS) using a Zetasizer nano-zs particle size analyzer (malvern instruments, model zen3600, UK) to determine fine and coarse fractions. The so-called fine flour had particle size lower than 125 µm, and the coarse fraction contained particles with sizes ranging between 125 and 180 µm.

Evaluation of GF Bread Quality

Physical parameters of GF bread were determined. Bread volume was determined by a rapeseed displacement method (AACCI method 10-05.01, [AACCI, 2000]). The specific volume of the loaf was calculated using the following formula:

Specific volume (cm^3 g^{-1})= Loaf volume/Loaf weight (Dizlek and Gul, 2009).

Image Processing

The crumb grain structural parameters such as mean cell area (mm^2) and total number of cells were evaluated. Briefly, digital pictures were taken by using Nikon cameras and at an angle of 90° (vertical). Lens focal length was 55 mm, Lens aperture: 18-55, ISO speed: ISO-800, Aperture range: F/56, resolution 4000×6016 pixels. The images were saved as JPG files format at a resolution of 300 dpi. All images were analyzed using ImageJ Software (1.48v).

Texture Evaluation

The peak force and the peak deformation point of GF bread were measured by compressing the GF bread samples twice at the surface with a 30 s interval between the two compression cycles. Texture Profile

Analysis (TPA) was carried out using a TA.XTplus machine (stable micro systems, UK) equipped with a 5 kg load cell and 10 mm aluminium cylindrical probe. A trigger force of 5 g was used to compress the middle of the bread crumb to 50% of its original height at a crosshead speed of 3 mm s^{-1} (Matos and Rosell, 2013) with some modification.

Organoleptic Evaluation

The organoleptic evaluation of the GF bread was done by 75 untrained panellists (Selection of the research faculty members of the center), 30 males and 45 females were asked to evaluate characteristics using a 9-points hedonic scale (1= Dislike extremely; 2= Dislike very much; 3= Slightly dislike; 4= Dislike; 5= Neither like nor dislike; 6= Like; 7= Slightly like; 8= Like very much; 9= Like extremely). The age of the panelists ranged from 18 to 50 years old. The panellists were presented with coded sample and water to rinse their mouths after tasting each sample. Each panellist evaluated samples for acceptability based on general appearance, crumb texture, crust texture, crust appearance, taste, aroma, crust color and crumb color.

Data Analyses and Validation of RSM Results

The Design-Expert (7.1.5) software was used to determine the optimum proportions of the GF bread formulation. Flour mixture component proportions are subject to constraints. Hence, a D-optimal mixture design was employed with some limitations. The design of this experiment was based on three components consisting of RF, SF, and MF with the sum of the component proportion of 100%. The component ranges were as follows:

$15 \leq RF \leq 100$, $0 \leq SF \leq 70$ and $0 \leq MF \leq 15$.

Design expert software designed 16 runs, of which 6 runs were different and 5 runs had two replicates. According to D-optimal approach, effect of these components on the properties of GF bread was evaluated and then the optimum combination was determined. Depending on the influence of each factor, the combination of factors that led to the best responses was determined. The best model was fitted according to high R-squared, low standard deviation and low predicted sum of squares (Nikzade *et al*., 2012). P-values of the acceptable models were lower than 0.05 and *P*-values of lack of fit were higher than 0.05.

RESULTS AND DISCUSSION

Flours Characterization

Moisture, crude protein, crude fat, total ash, crude fiber, pH values, and particle size analyses results of the MF, RF, SF, and soy flour are shown in Tables 1 and 2. Components other than moisture content are expressed on dry basis. Protein contents of soy, MF and SF were 30.06, 24.87, and 15.06%, respectively, which are higher than the RF with protein content of 9.25%. Several studies have shown that proteins of different sources could improve the quality of GF breads (Gujral *et al*., 2003; Gujral and Rosell, 2004a, 2004b; Moore *et al*., 2006; Storck *et al*., 2013). Therefore, SF, MF and soy proteins may improve GF bread quality (Gerrard, 2002; Taghdir *et al*., 2016). The crude fat content is related to the energy content of the flour (Emire and Tiruneh, 2012). The crude fat content of soy (24%) and SF (4.14%) are greater than RF (2.47%) and MF (1.19%). Therefore, they increase the energy content of GF bread. Ash content refers to the mineral content of flour. The ash content of soy (4.1%) and SF (2%) are greater than RF (1.34%) and MF (1.33%). Therefore, addition of soy and SF increases the mineral content of GF bread. Based on the nutrient composition, sorghum and pearl millet are considered highly nutritious cereals. Sorghum and pearl millet, blended with soy or protein-rich ingredients, such as legumes or groundnut (peanut) cake, give nutritionally balanced supplementary foods on extrusion (Rai *et al*., 2008; Taghdir *et al*., 2016; Malleshi *et al*., 1996).

Specific Volume and Crumb Textural Properties Measurement

Three flours were selected to study the effects of adding SF, RF, and MF with a constant level of 10% soy flour on GF bread quality. The values for the different responses are given in Table 3. According to Table 4, Special Cubic was the best model for specific volume. Each component (SF, MF and RF) and interaction of two components (SF/MF, SF/RF and MF/RF) had a positive coefficient, indicating increased specific volume. On the other hand, three component combinations (SF/RF/MF) showed a negative coefficient, indicating decreased specific volume (Table 5).

Specific volume was increased with raising the level of SF. It was also observed that increasing RF resulted in lower specific volume. Similar increase in specific volume was evident when using MF in combination with SF and RF. These results indicated that sample numbers 11 and 7 containing 17.8% RF, 67.2% SF, and 15% MF provided the highest specific volume. This observation may be related to the increased amount of protein. Similar increase in specific volume with increased amount of protein was reported by Andersson *et al*. (2011). The results showed that sample number 5 containing 100% RF provided the lowest specific volume. This could be due to the poor functional properties of its proteins and its inability to retain gas produced during the fermentation process, resulting in a product with low specific volume (Gujral and Rosell, 2004b; Capriles and Areas, 2014; Dizlek and Ozer 2016b, 2017).

According to Table 2 and our results, specific volume of GF bread increased with larger particle size. Therefore, addition of SF with coarse fractions (70% of fractions over 180 microns) increased specific volume. This is in agreement with De la Hera *et al*. (2012) who reported that coarse flour with large particles was best able to

Table 1. Chemical characteristics of flour samples.[a]

Flour	Crude protein	Crude fat	Moisture	Ash	Crude fiber	pH
Millet	24.87 ± 0.81[b]	1.19 ± 0.19[c]	4.77 ± 0.83[b]	1.33 ± 0.11[c]	0.7 ± 0.1[c]	5.81 ± 0.2[a]
Rice	9.25 ± 0.66[d]	2.47 ± 0.26[bc]	0.59 ± 0.09[d]	1.34 ± 0.16[c]	0.04 ± 0.05[d]	5.7 ± 0.26[a]
Sorghum	15.06 ± 1.09[c]	4.14 ± 0.12[b]	5.74 ± 0.4[a]	2 ± 0.1[b]	1.56 ± 0.2[b]	6.11 ± 0.35[b]
Soy	30.06 ± 1.09[a]	24 ± 0.5[a]	3.57 ± 0.16[c]	4.6 ± 0.26[a]	4.1 ± 0.26[a]	6.42 ± 0.37[b]

[a] Mean±*SD*. In each column, means (3 replication) with the same letter are not significantly different (P< 0.05). Comparison of means by Duncan's multiple range test was performed using SPSS software.

Table 2. Particle size distribution of flour samples.[a]

Flour	Flour 475 micron (%)	180 micron (%)	125 micron (%)	125 Sub-micron (%)
Millet	1±0.07[a]	51.4±0.98[b]	36.6±0.92[a]	11±0.49[b]
Rice	0.2±0.02[b]	20.8±0.8[c]	35.8±1.01[a]	43.2±1.7[a]
Sorghum	1.2±0.17[a]	70±2.7[a]	18.2±0.37[c]	10.6±0.4[b]
Soy	1±0.015[a]	72.4±2.03[a]	25±0.34[b]	1.6±0.1[c]

[a] Mean±*SD*. In each column, means (3 replication) with the same letter are not significantly different (P< 0.05). Comparison of means by Duncan's multiple range test was performed using SPSS software.

retain gas yielding bread with higher volumes and lower hardness. On the other hand, in samples containing 100% RF (samples 5 and 6), specific volume was lowest. This could be due to the smaller particle size of RF resulting in lower specific volume and increased hardness.

In a recent study, Różyło *et al*. (2015) showed that the bread volume is significantly dependent on the amount of water added in the recipe. For instance, Gallagher *et al*. (2003) suggested that increasing water level in the formulation by 10% and 20% increased the loaf volumes in bread. Based on the previous studies (De la Hera *et al*., 2014; Różyło *et al*., 2015) and our preliminary results (data not shown), we have concluded that addition of 150% of water (based on the flour weight) results in optimum loaf volume.

Experimental results obtained for hardness are shown in Table 3. According to Table 4, quadratic was the best model for hardness. Effect of each variable and interaction between them showed that RF and interaction of two components including MF/RF, MF/SF and RF/SF had positive coefficients, indicating increased hardness. MF showed negative coefficient, indicating that MF had a negative effect on hardness (Table 5). In samples containing blend of three components, it was evident that by adding 43.4% SF up to 70% and by decreasing the amount of RF, mean cell area and specific volume increased and hardness decreased. Our findings are in agreement with the results obtained by previous researchers who reported an inverse relationship between the specific volume and hardness (Gallagher *et al*., 2003; Sabanis *et al*., 2009; Dizlek, 2015; Dizlek and Ozer, 2016a). Olatunji *et al*. (1989) and Taylor *et al*. (2006) have also achieved good quality GF breads with the incorporation of 70% SF.

In addition, in samples 7 and 11 with similar formulations, particle size of flours was highest resulting in increased specific volume and decreased hardness. It is also important to note that excessive water causes overexpansion during baking resulting in large volume breads and big holes (De la Hera *et al*., 2014). This could be the explanation of the high specific volume of GF breads obtained from coarse flours (samples 7 and 11) and high water content (150%) allowing the maximum hydration of the coarse flour containing GF breads.

Digital Image Analysis

A Digital Image Analysis (DIA) system was applied to analyze the bread crumb structure at the surface. Parameters such as mean cell area (square millimeter) and number of cells were measured for all samples. Image analysis parameters are shown in Table 3. The best model for all image analysis parameters is presented in Table 4.

According to Table 5, each component (SF, MF and RF) and interaction of the three components (SF/RF/MF) had a positive coefficient, indicating increased mean cell area, however, interaction of two components (SF/MF, SF/RF, and MF/RF) showed a negative coefficient, indicating decreased mean cell area. The number of crumb cells showed the exact opposite trends to mean cell area.

Our results revealed that addition of SF and MF increased the mean cell area. However, with increasing the amount of RF, mean cell area deceased. The highest mean cell area of the crumb was found for GF bread with 67.183% SF, 17.817% RF, and 15% MF (sample number 11 and 7). The number of cells was significantly higher for GF bread with 85% RF and 15% MF. Sorghum has extremely hard endosperm and the pericarp is brittle (Zhao and Ambrose, 2016). In addition, Schober *et al*. (2005) reported that higher starch damage in sorghum-based GF bread is obtained if the kernel hardness is higher, and this high starch damage goes along with a large mean cell area, a small number of cells, and a soft crumb. They suggested that damaged starch is more easily degraded by amylases,

Table 3. Responses to different formulations of GF bread containing RF, SF, MF and soybean flour.

Run	Rice (%)	Sorghum (%)	Millet (%)	General appearance	Crust texture	Crumb texture	Crumb color	Crust appearance	Taste	Crust color	Aroma	Specific volume ($cm^3 g^{-1}$)	Hardness (gr)	Total number of cells	Mean cell area (mm^2)
1	85	0	15	7.2	6.9	7.2	7.2	7.4	7.2	7.2	6.3	2.11	805.711	1549	0.127
2	56.618	43.382	0	6.3	5.6	6.3	6.5	6.7	6.7	6.4	6.3	2.27	933.155	1101	0.191
3	24.244	70	5.756	7.2	7	6.9	6.9	7.2	7.2	6.9	8.3	2.4	842.815	874	0.274
4	69.739	15.261	15	6.9	4.8	5.1	5.7	6.4	5.1	6.1	5.9	2.04	850.524	1644	0.149
5	100	0	0	7.2	6.9	6.1	7.2	7.6	7.4	7.2	7.2	2.03	810.957	1050	0.147
6	100	0	0	7.4	6.1	6.7	7.2	7.7	7.2	7.7	6.7	2.04	790.809	992	0.151
7	17.817	67.183	15	7.2	7.2	7.2	7.2	7.2	7.2	7.2	6.9	2.4	664.237	810	0.39
8	81.122	18.878	0	6.1	5.6	6.1	6.3	6.5	6.1	6.1	6.4	2.19	1113.24	1637	0.189
9	45.271	54.729	0	6.9	6.5	6.3	6.9	6.9	6.7	6.7	6.3	2.32	758.977	961	0.268
10	35.71	64.29	0	7.2	6.9	6.3	7.2	6.9	7.7	6.7	6.7	2.42	739.565	681	0.344
11	17.817	67.183	15	7.2	7.2	7.2	7.2	7.5	7.2	7.2	6.4	2.56	574.06	805	0.403
12	60.709	30.863	8.428	6.6	6	6	6.4	6.6	6.3	6.3	6.3	2.12	1112.6	1467	0.169
13	60.709	30.863	8.428	6.9	6	5.9	6.6	6.6	6.3	6.6	5.7	2.19	1000.43	1332	0.19
14	31.863	53.137	15	7.2	6.9	6.3	6.7	6.3	6.9	6.6	6	2.32	863.435	1208	0.248
15	24.244	70	5.756	7.2	7.2	7.2	6.9	7.2	7.2	6.9	6.3	2.4	834.479	850	0.279
16	85	0	15	7.8	6.9	7.2	7.2	7.5	7.2	7.2	7.2	2.13	814.566	1764	0.125

Table 4. ANOVA for the evaluation of the best model.

Response	Standard deviation	*CV*	*R* square (Determination coefficient)			Lack of fit test		Best model	
			R square	Adjusted *R* square	Predicted R square	*P*-value	*F*-value	Model	*P*-value
Specific volume	0.049	2.17	0.946	0.91	0.823	0.752	0.48	Special Cubic	< 0.0001
General appearance	0.29	4.07	0.68	0.525	0.22	0.185	2.35	Quadratic	0.0235
Crumb texture	0.3	4.56	0.86	0.76	0.45	0.126	3.05	Special Cubic	0.0019
Crust appearance	0.16	2.27	0944	0.88	0.22	0.0534	5.57	Cubic	0.0010
Crust texture	0.37	5.72	0.87	0.73	-0.46	0.0743	4.57	Cubic	0.0137
Taste	0.48	7.08	0.62	0.43	0.157	< 0.0001	116.47	Quadratic	0.0535
Crust color	0.28	4.14	0.74	0.61	0.399	0.089	3.69	Quadratic	0.0090
Crumb color	0.11	1.6	0.97	0.94	0.58	0.0277	7.99	Cubic	0.0001
Aroma	0.55	8.32	0.49	0.24	-0.41	0.997	0.049	Quadratic	0.1719
Total number of cell	119.4	10.21	0.945	0.884	0.4246	0.0687	4.8	Cubic	0.0009
Mean cell area	0.00992	4.36	0.994	0.988	0.968	0.1553	2.77	Cubic	< 0.0001
Hardness	77.56	9.19	0.804	0.706	0.558	0.0583	4.66	Quadratic	0.0026

resulting in a larger amount of sugars for yeast fermentation and thus more gas production by yeast. This could be the explanation of large mean cell area, small number of cells, and soft crumb obtained from sorghum-based GF bread with high starch damage.

Organoleptic Evaluation

The results of organoleptic evaluation are presented in Table 3. The best model for all organoleptic parameters is presented in Table 4. It must be noted that panelists were unfamiliar with GF breads based on SF, MF and RF. Therefore, their evaluation is not error free.

Our results show that RF improved taste at high levels of incorporation. SF and MF exhibited similar trend and had positive effect on the taste of the GF breads. These findings are consistent with Lopez *et al*. (2004) and Mancebo *et al*. (2015) who reported that rice flour GF bread is generally better rated in terms of taste, appearance, and overall acceptability than maize-starch bread. Our results are also in line with Schober *et al*. (2005) who obtained desirable sorghum-based GF bread (70% SF and 30% corn starch) with regard to flavor and taste. Azarbad *et al*. (2015) reported that reduced-gluten Barbari bread containing 25% MF or more resulted in bread with a bitter taste. They concluded that high tannins content in millet could be responsible for bitter taste of GF bread. Therefore, in the present study we used MF at levels below 25%.

Results obtained for crumb texture by organoleptic evaluation are in line with results obtained by texture analyzer for hardness. Our results revealed that there was an inverse relationship between the crumb texture score and hardness, indicating that crumb texture score increased with decreasing hardness. According to the results of organoleptic evaluation, RF decreased crumb texture score, but SF and MF increased it.

Color together with texture and taste affects consumer satisfaction. GF breads are usually characterized by a light color, so, darkening of GF bread in general is desirable (Taylor *et al*., 2006). Schober *et al*. (2005) claimed that dark bread is common in various regions (e.g. Germany or Eastern Europe) as it is associated with “health”. They also reported that consumers accepted the appearance and color of a light-colored muffin as well as a dark brown one. This is in agreement with the results obtained in this study, as all the panelists rated light and dark GF breads similarly. Breads containing high amount of RF and SF achieved the highest score with regard to color. Addition of RF increased lightness of GF bread. On the other hand, SF increased darkness. This is due to relatively high ash content of SF. Similar to our results, Alhusaini (1985) showed that flour with higher ash content had a darker color, which would ultimately darken the bread. It is also important to note that red hybrid of SF was used in this study. Red sorghum contains high levels of tannins. This could also be the explanation for darker GF bread with high SF content. Our result is consistent with Schober *et al*. (2005) who reported that the use of red hybrid of SF resulted in pinkish-brown color GF bread. Millet breads were yellowish in color and the lightness decreased with increase in MF. This is in agreement with Mannuramath *et al*. (2015) who reported that millet breads tended to have a yellowish color. They also noted that crust and crumb color were highly dependent on the proportion of MF in the formulation. According to Table 3 and the panelists' rating for crust color and appearance as well as general appearance, it can be concluded that components that had positive effect on crust color and appearance also had positive effect on general appearance. Incorporation of SF, RF, and MF at high levels improved general appearance.

Table 5. Regression coefficients for models in D-optimal mixture design.[a]

Response [a]	β_1 (RF)	β_2(SF)	β_3(MF)	$\beta_1\beta_2$	$\beta_1\beta_3$	$\beta_2\beta_3$	$\beta_1\beta_2\beta_3$	$\beta_1\beta_2(\beta_1-\beta_2)$	$\beta_1\beta_3(\beta_1-\beta_3)$	$\beta_2\beta_3(\beta_2-\beta_3)$
Specific volume	0.02***	0.024***	0.02***	2.7 E-5^{ns}	3.7E-5^{ns}	2.9E-4^{ns}	-1.19E-5*	-	-	-
General appearance	0.07^{ns}	0.09^{ns}	0.056^{ns}	-6.09E-4**	4.7E-4^{ns}	-3.26 E-4^{ns}	-	-	-	-
Crumb texture	0.064^{ns}	0.068^{ns}	-0.85^{ns}	-1.5E-4^{ns}	0.011*	0.013*	-1.2E-4**	-	-	-
Crust appearance	7.61^{ns}	7.4^{ns}	6.7^{ns}	-3.7^{ns}	-41.6^{ns}	1.59^{ns}	31.76^{ns}	-2.57^{ns}	64.79^{ns}	-
Crust texture	6.59*	5.33*	-32.74*	-0.069^{ns}	9.35^{ns}	60.16^{ns}	3.88^{ns}	-11.76*	61.78^{ns}	-
Taste	0.073^{ns}	0.11^{ns}	0.23^{ns}	-9.28E-4**	--2.21E-3^{ns}	-3.09E-3^{ns}	-	-	-	-
Crust color	0.074^{ns}	0.089^{ns}	0.072^{ns}	-7.29E-4***	-1.98E-4^{ns}	-3.45E-4^{ns}	-	-	-	-
Crumb color	7.23^{ns}	6.46^{ns}	36.67^{ns}	-1.12^{ns}	-112*	-30.75^{ns}	74.14^{ns}	-7.27***	117.24**	-
Aroma	0.07^{ns}	0.098^{ns}	-0.27^{ns}	-8.63E-4*	3.81E-3^{ns}	2.92E-3^{ns}	-	-	-	-
Total number of cell	1048.5***	25.17***	-44169.7***	2915.52^{ns}	1.02E5^{ns}	58957.7^{ns}	-73960.8^{ns}	1791.35^{ns}	-66914.89^{ns}	-
Mean cell area	0.15***	0.73***	22.29***	-0.95***	-43.76***	-28.14***	27.32***	0.69***	25.82***	-
Hardness	8.39*	0.99*	-237.58*	0.168**	2.85**	3**	-	-	-	-

[a] *: Significant at $P< 0.05$; **: Significant at $P< 0.01$; ***: Significant at $P< 0.001$, ns: Not significant.

Table 6. Predicted and experimental values of the response variables at optimum formulation.

Response	Confidence intervals		Standard Error (SE)	Predicted values	Experimental values
	95% High prediction interval	95% Low prediction interval			
Specific volume	2.62	2.35	0.059	2.485	2.56
General appearance	8.04	6.54	0.34	7.294	7.2
Crumb texture	8.14	6.53	0.36	7.337	7.2
Crust texture	8.36	6.23	0.45	7.295	7.2
Crumb color	7.55	6.92	0.13	7.232	7.2
Crust appearance	7.76	6.85	0.19	7.305	7.3
Taste	8.48	5.94	0.57	7.206	7.2
Crust color	7.87	6.39	0.33	7.134	7.2
Aroma	8.07	5.22	0.64	6.64	6.4
Total number of cell	1180.26	492.25	145.48	836.255	805
Mean cell area	0.43	0.37	0.012	0.397	0.403
Hardness	848.76	442.35	91.20	645.55	574.06

Mixture Proportion Optimization and Desirability Function

Optimum formulation was obtained based on maximum score for all organoleptic parameters, minimum hardness, maximum specific volume, maximum mean cell area, and minimum number of cells.

The most frequently used general response is overall desirability function. The desirability function approach converts each estimated response value into a scale-free value (Harrington, 1965; Lazic, 2004; Sarteshnizi *et al*., 2015). In this approach, when the desirability value is between 0.8 and 1, product quality is considered to be acceptable and excellent. When this value is between 0.63 and 0.8, the product quality is considered to be acceptable and good, and if less than 0.37, the product quality is unacceptable (Lazic, 2004). In this study, total desirability was equal to 0.791, which is indicative of good quality bread. Desirability for each of the response variables and combined desirability are presented in Figure 1.

Taking into account the outcome of the highest and lowest degree of desirability presented in Figure 1 for physical and organoleptic characteristics of GF breads, sample 11 and 7 with similar formulations (17.8% RF, 67.2% SF, and 15% MF) received the highest score. This mixture was submitted to the same experimental procedures (Table 6). There was no significant difference between the estimated and observed values (P< 0.05), suggesting a good fit between the models and the experimental data.

CONCLUSIONS

The use of SF, RF, and MF combination in GF bread formulation improved the final bread quality greatly, with softer texture, higher specific volume, and better sensorial characteristics including taste, general appearance, and color. D-optimal mixture design approach was used to optimize the GF bread formulation. The optimum GF bread formulation contained SF 67.183%, RF 17.87%, and MF 15%. The optimum GF bread developed in this study is characterized by the minimum number of cells with larger size and a soft texture, unlike the soft wheat bread which is characterized by high number of cells with smaller size. This study provides insights that could promote the production of good quality GF bread for celiac patients.

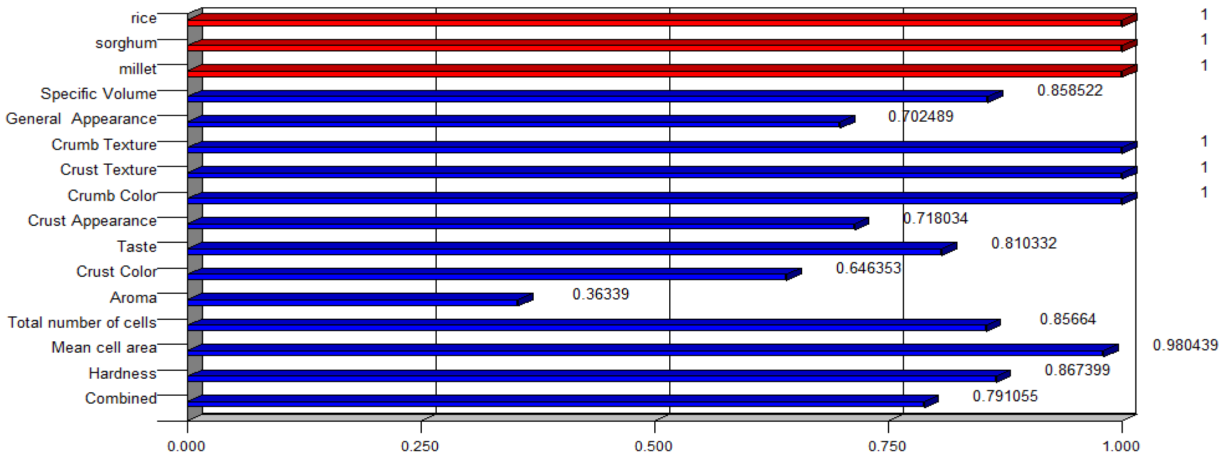

Figure 1. Desirability plot for optimum formulation.

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بهینه سازی فرمولاسیون نان بدون گلوتن شامل آردهای سورگوم، برنج و ارزن با استفاده از طرح مخلوط دی اپتیمال

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چکیده

گرایش به محصولات بدون گلوتن به دلیل شیوع بیماری سلیاک افزایش یافته است. آردهای سورگوم، برنج و ارزن برای تهیه محصولات بدون گلوتن بسیار مناسب می باشند. هدف از این تحقیق، بهینه سازی ترکیب آردهای سورگوم، ارزن، برنج برای تولید نان بدون گلوتن بر اساس طرح مخلوط دی اپتیمال می باشد. ویژگی آردها شامل رطوبت، پروتئین، چربی، خاکستر، فیبر و pH اندازه گیری شد. خصوصیات کیفی نان بدون گلوتن شامل حجم مخصوص، سفتی، ساختار سلولی مغز نان، ویژگی های تصویر و ارزیابی ارگانولپتیک نیز مورد تجزیه و تحلیل قرار گرفت. نتایج نشان داد که ترکیب آردهای سورگوم، برنج و ارزن تأثیر قابل توجهی بر روی خواص فیزیکی و ارگانولپتیک نان بدون گلوتن دارد. افزایش مقدار آرد ارزن و سورگوم همراه با کاهش مقدار آرد برنج موجب افزایش حجم مخصوص و اندازه متوسط سطح حفرات و نرمتر شدن بافت نان بدون گلوتن گردید. رنگ و طعم نان های بدون گلوتن در مقادیر بالای آردهای برنج، سورگوم و ارزن بهبود یافت. نتایج ارزیابی ارگانولپتیک بافت با داده های بافت سنجی دستگاهی مرتبط بود. فرمولاسیون بهینه بر اساس ارزیابی ارگانولپتیک، حجم مخصوص، سفتی و ساختار سلولی مغز نان بدون گلوتن شامل ۶۷/۱۸٪ آرد سورگوم، ۱۷/۸۲٪ آرد برنج و ۱۵٪ آرد ارزن با درجه مطلوبیت کل برابر با ۰/۷۹۱ می باشد. به طور کلی نتایج این تحقیق نشان می دهد که آردهای سورگوم، برنج و ارزن می توانند به جای آرد گندم برای تولید نان بدون گلوتن با کیفیت بالا مورد استفاده قرار گیرند. نتایج این تحقیق در تولید نان بدون گلوتن برای بیماران سلیاک قابل استفاده می باشد.