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FORMULATION OF GLUTEN-FREE BREAD WITH DIFFERENT LEVELS OF HYDROXYPROPYL METHYLCELLULOSE, RAW BANANA FLOUR AND PROOF TIME: MODELLING, PROXIMATE AND SENSORY ANALYSES

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ABSTRACT

Semi-urbanization has increased the demand of bakery products triggering variousgluten-related disorders including Celiac Disease (CD), Non-celiac Gluten Sensitivity (NCGS), Gluten Ataxia and Wheat Allergy. Among these, CD accounts for nearly 1-2%global population. Gluten withdrawal- cornerstone treatment for CD is a bumpy road with nutritional deficiencies and poor quality of life. Additionally, there is a surge of people intentionally avoiding gluten for various reasons. The present work intends to optimize a formula for a gluten free (GF) bread and determine the effect of prooftime, hydroxypropyl methylcellulose (HPMC) and raw banana flour at different levels on moisture content, bake loss, specific volume, texture parameters andcrust-crumb brightness (L*) using response surface methodology (RSM) based on central composite rotatable design (CCRD).The results revealed moisture content, hardness, springiness, resilience and crust-crumb L*were significantly affected. Different proof time levels altered moisture content, bake loss and colour parameters with no influence on textural parameters. Increasing levels of HPMC impeded bake loss and crumb lightness but exacerbated hardness. Nonetheless, on its interaction with raw banana flour, there was antagonistic effect on hardness. Addition of raw banana flour darkened the bread. Thus, optimal conditions (HPMC 2%, raw banana flour 37.16% and proof time 40mins) were appliedto produce good quality bread. Furthermore, sensory and proximateanalyses of optimum GF bread were compared to whole wheat bread depicting higherprotein-fat content, lower carbohydrate content and overall acceptability score of 7.88for the former; the demand for which is steadily and incessantly expanding.

Keywords: bread, gluten-free, hydrocolloids, raw banana flour, response surface methodology

INTRODUCTION

The market of bakery products has sky-rocketed in the past few years owing to the change in societal habits, suburbanization, availability of disposable income and change in the household structure. The prevailing consumer marketshows preference towards products either nutritionally rich or/and promising reducedfood-related diseases. Unfortunately, a colossal amount of bakery products is gluten-based, the trigger for gluten-related disorder an umbrella term which encompasses CD, Non-celiac Gluten Sensitivity (NCGS), Gluten Ataxia and Wheat Allergy. Among these, CD is medically polymorphous and concomitantly less diagnosed. In this day and age, nearly 1-2% people are afflicted globally (Bascuñán, Vespa, and Araya, 2017; Koskinen *et al.,* 2020). It is an auto-immune inflammatory disorder of the small intestine in which ingestion of wheat (gliadin), rye (secalin) and barley (hordein) damage the villi on the mucosal surface leading to villous atrophy. The individuals are genetically predisposed carrying the gene alleles HLA DQ2 and/or HLA DQ8 (Padalino, Mastromatteo, Sepielli, and Nobile, 2011; Wolf *et al.,* 2018). The compound increases in CD awareness and prognosis, however, doesn't commensurate with increase in growth of GF market due to their cost and availability, inadequate information, poor labelling and inferior sensory properties; making adherence to GF lifestyle, the only efficacious treatment, difficult. Gliadin and Glutenin, gives gluten its characteristic elasticity and extensibility and its absence pose a technological challenge as the resultant products have reduced volume, crumbly and grainy structure with poor mouthfeel. Moreover, unlike their counterpart, starch, rice flour, corn flour forms the base of GF products and thus are a source of excessive carbohydrates but poor in protein, dietary fibre, macro- and micro nutrients (Lamacchia, Camarca, Picascia, Di Luccia, and Gianfrani, 2014).Of late, researchers (Camelo-Méndez, Tovar, and Bello-Pérez, 2018; Chompoorat, Kantanet, Hernández Estrada, and Rayas-Duarte, 2020; Masmoudi *et al.,* 2020; Rachman, A. Brennan, Morton, and Brennan, 2020)are working on millets, pseudo cereals and fruits and vegetables and their by-products to meet the health benefits and satiety.

In recent times, millets, pseudo cereals and unconventional flours have gained popularity due to their technical and nutritional properties. Buckwheat and Amaranth are grown globally, including different parts of India and are rich in proteins, dietary fibre, vitamins, polyphenols and flavonoids(Alvarez-Jubete, Arendt, and Gallagher, 2010; Gorinstein *et al.,* 2007)positively affecting the nutritional profile, total phenol content and antioxidant activity of GF bakery products. According to a clinical study by Bojñanská *et al.,* (2009), levels of iron, calcium and magnesium increased with introduction of buckwheat bread in diet. In a similar study, replacing wheat with amaranth in bread showed higher springiness and protein value(S. Liu, Chen, and Xu, 2019). Even though being an indigenous low-cost crop, pearl millet use is limited as compared to wheat in India. Pearl Millet in combination with different flours provide GF products with enhanced nutritional, sensory and functional qualities (Radhika *et al.,* 2019; Rai, Kaur, and Singh, 2014). Raw banana is another underutilized GF raw material of low commercial value which is a rich source of resistant starch, dietary fibre and flavonoids (Gomes, Ferreira, and Pimental, 2016; Martínez-Castaño, Lopera-Idarraga, Pazmiño-Arteaga, and Gallardo-Cabrera, 2020). Resistant starch intake seems to lower postprandial glycaemic and insulinemic responses, modulation of plasma cholesterol concentrations and increase satiety while providing functional properties to produce high-quality GF foods (Higgins, 2004; Korus, Witczak, Ziobro, and Juszczak, 2009). Several studies show that substitution with whole green banana flour increase total ash, dietary fibre, phosphorus, potassium, magnesium, sodium and calcium content and lowered starch digestion rates in GF products (Khoozani, Kebede, Birch, and Bekhit, 2020; Zheng, Stanley, Gidley, and Dhital, 2016).

Absence of gluten gives a cake-like batter which is difficult to handle resulting in need of hydrocolloids, enzymes, proteins and dietary fibre which mimic gluten. A recent study by our group has concluded that 3% HPM Cand 15% Whey Protein Concentrate improved the textural and nutritional properties of sorghum-based bread (Rustagi *et al.,* 2018). Similarly, Susanna and Prabhasankar (2013) demonstrated guar gum and HPMC amended the technological characteristics of GF pasta due to increased gelatinization temperature, higher protein content and less stickiness of pasta. Additionally, bread processing parameters which include kneading, proofing and baking have significant effect on its physical attributes and hence should be optimized. Several researchers have discussed how these process parameters positively influence the cell wall thickness and diameter thus producing high-quality end products (Bosmans, Lagrain, Fierens, and Delcour, 2013; Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson, 2014). The crust and crumb properties are influenced by baking time and temperature (Jusoh, 2008). During the proofing, the dough expands and entraps CO_2 in the protein matrix (Almeida and Chang, 2014); but in the absence of gluten this becomes difficult.

In this context, the aim of this work was to optimize a formula for a GF bread using RSM and determine the effect of proofing time, HPMC and raw banana flour at different levels. Further, the optimized bread was compared towhole wheat bread based on proximate and sensory analysis.

MATERIALS AND METHOD

Baking Ingredients

Whole buckwheat, pearl millet and amaranth flour were procured from Crop Connect (Delhi, India). Raw banana flour was obtained from Koko's Natural (Mumbai, India). HPMC (Methocel K4M, food grade, E464) was generously donated by Colorcon (Goa, India). Salt, sugar, compressed yeast, curd, egg, vegetable oil was purchased from the local market.

Experimental Design and Gluten-free Bread Manufacturing

RSM based on CCRD was used to investigate the effect of following factors at different levels: HPMC (X_1, 1-2%), raw banana flour (X_2, 30-60%) and proof time (X_3, 40-90 mins) on the physical properties of bread. The range (minimum and maximum) for these factors was determined based on previous literature (Karimi, 2012; Fabregat, 2015; Rustagi *et al.,* 2018; Martínez-Castaño *et al.,* 2020) and pre-trials. This design consisted of 20 random experiments (14 factorial experiments and 6 central point replicates). The base flour was a mixture of amaranthbuck wheat, pearl millet and raw banana flour in the ratio of 2:1:1: X_2. The sum of the flours was (100%) and inferred as the flour weight basis (fwb). The responses included moisture content, bake loss, specific volume, texture parameters (hardness, springiness, cohesiveness, resilience) and crust-crumb L*.

The bread was manufactured using straight dough method. For dough making, all ingredients were used either in fixed (Table 1a) or in variable quantity (Table 1b). Compressed yeast was used after being thawed in lukewarm water. All the ingredients were mixed usinga KitchenAid stand mixerwith a dough hook attachment at a speed of 4 for 10 mins until dough formation. Dough was then scraped and 800g was removed, rounded, placed in a pre-greased bake pan and proofed at 32°C at 85% RH. The proof time varied according to the experimental design (Table 1b). After proofing, the baking was done at 180°C for 45 mins in a deck oven. The breads were then depanned and allowed to cool for 2 hours. The loaves were stored in a polyethylene bags at 25°C until analysis. The analysis was performed within 24 hours in triplicates.

Design Expert version 11 software (State-Ease Inc., Minneapolis, MN, USA) was used for model generation, lack-of-fit, coefficient of variation, coefficient of determination and 3-D plot for statistical analysis.

Bread Quality Parameters

After cooling, the loaves were weighed and bake loss was calculated. The loaf volume was measured by rapeseed displacement according to AACC method 10-05.01 (AACC, 2010). The weight of the loaf was measured and the ratio of loaf volume and loaf weight was used to determine specific volume. The results were expressed in $cm^3\ g^{-1}$. The loaves moisture was determined according to AOAC method (AOAC, 2005).

The textural measurements- hardness, springiness, cohesiveness and resilience were performed on two bread slices that were taken from the centre of different loaves. Texture analysis was carried out using Texture Analyzer TA. HD *Plus* (Stable Micro Systems, Surrey, England) according to AACC method 74-09(AACC, 2010). Tests were conducted using flat probe of 75 mm diameter with

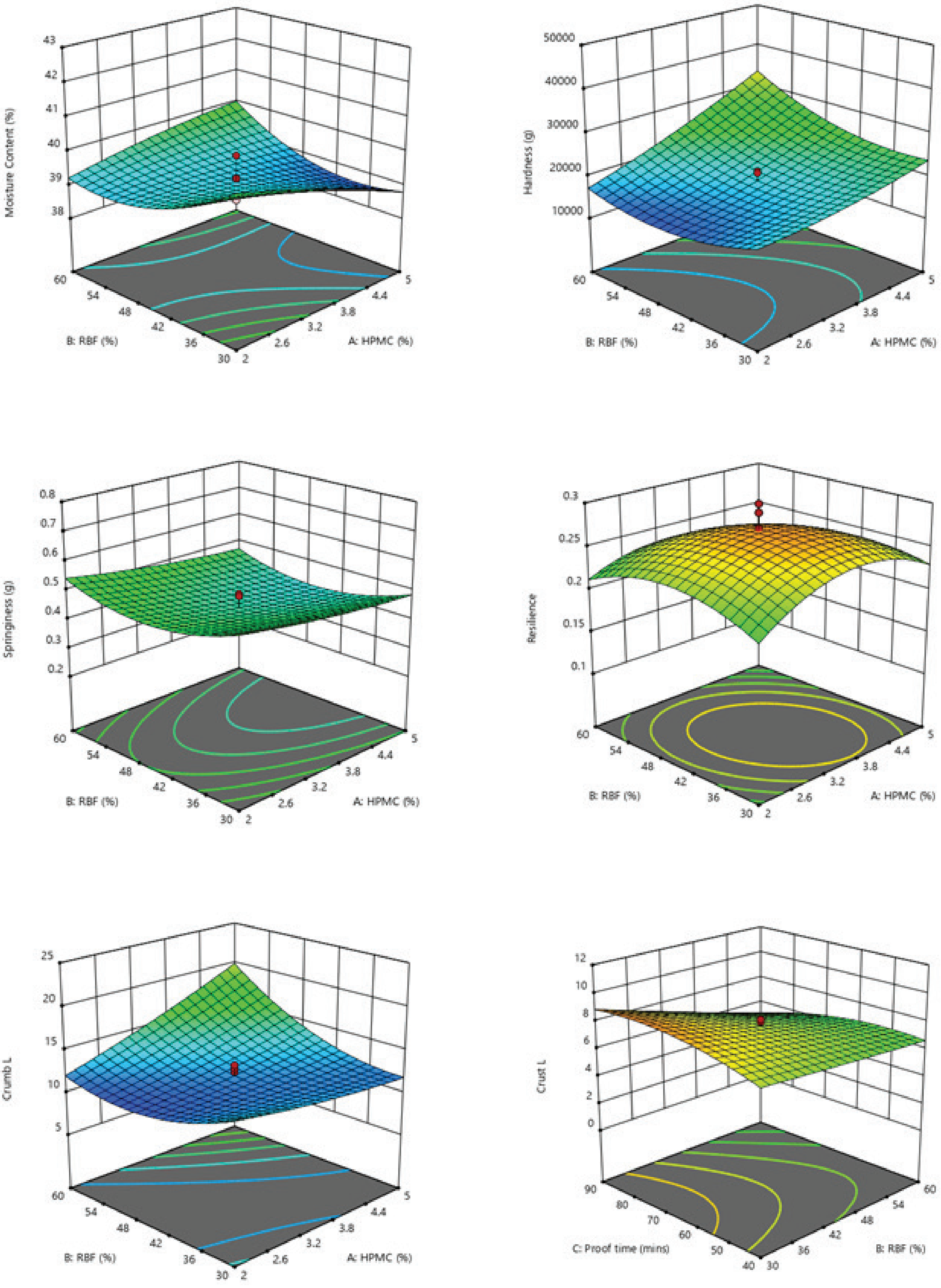

Figure 1: Response surfaces plots showing the effect of independent variables for responses a. moisture content; b. hardness; c. springiness; d. resilience; e. crust L* f. crumb L*. The changed variables were hydroxypropylmethyl cellulose, raw banana flour and proof time.

Table 1a: Gluten-free bread making formulation

Ingredients	**Remarks**
Base Flour (Amaranth: Buckwheat: Pearl Millet: Raw Banana Flour)	Blend of flours in ratio 2:1:1: X_2
Raw Banana Flour (X_2)	Variable, as per experimental design
Hydroxypropyl methylcellulose (X_1)	Variable, as per experimental design
Yeast	6%
Sugar	10%
Salt	3%
Curd	10%
Egg	40%
Water	40%
Vegetable Oil	5%

Table 1b: Plan of experiments and level of input variables

Coded Levels	**Actual Levels (%FWB)**		
	X_1 (%)	**X_2 (%)**	**X_3 (mins)**
-1.682	0.977311	19.7731	22.9552
-1.000	2	30	40
0.000	3.5	45	65
+1.000	5	60	90
+1.682	6.02269	70.2269	107.045

X_1 hydroxypropyl methylcellulose, X_2 raw banana flour, X_3 proof time
X_1 hydroxymethyl cellulose (%), X_2 raw banana flour (%), X_3 proof time (mins)

50% strain with a trigger force of 5.0 g. The probe speed parameters were: pre-test speed 1mm/s, test-speed 5 mm/s and post-speed 5 mm/s.

Bread crust and crumb samples were measured for L* system using 3nh NS810 portable spectrophotometer (Shenzhen Threenh Technology Co. Ltd., China)

Data Analysis and Optimization of Processing Parameters

Design Expert version 11 software (State-Ease Inc., Minneapolis, MN, USA) was used for CCRD regression analysis and analysis of variance (ANOVA) to study the effect of input variables (HPMC, raw banana flour and proof time) on output variables (moisture content, specific volume, bake loss, texture and colour parameters). The result for all models is depicted in Table 3. The following models were used to check the significance of the model terms.

$$Y= \beta° + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta 11X_1^2 + \beta 22X_2^2 + \beta_{33}X_3^2$$

Where Y= dependent variables; X_1, X_2 and X_3 are independent variables.

The competency of the models was determined using model F-value, lack-of-fit, coefficient of variance (*c.v.*) and coefficient of determination (R^2). The linear, quadratic and interactive effect on the variables was described using significance at 1% and 5% level of confidence. Based on the regression model significance, 3 D plots were then generated.

To obtain optimal parameters for the production of GF bread, an optimization method was used based on desirability function. The process parameters variables were subjected to certain constraints. The software predicted optimum values for responses. GF bread was baked under optimum conditions and the results were statistically compared to the predicted values from the fitted models. The accuracy of the models was established with two-tailed, one sample t-test using SPSS Statistics version 22 (IBM).

Sensory and Proximate Analyses

The sensory evaluation was carried out by 20 panellists using 9-point hedonic scale from 1 (dislike extremely) to 9 (like extremely). The samples were coded using random three-digit numbers. Sensory attributes included appearance, colour, texture, taste, aroma and overall acceptability. Sensory evaluation for optimized GF bread was assessed and compared with whole wheat reference bread. The bread samples were accepted if their overall acceptability score was above 5 (Gusmão *et al.,* 2019).

The proximate compositions of optimum GF bread formulation and whole wheat bread were determined as per AOAC methods (AOAC, 2005). The moisture content was based on weight loss when the sample was dried in an oven at 105°C. For ash content, the sample was incinerated in muffle furnace at 550°C. Fat was determined by the acid hydrolysis method. The total protein content was calculated using Kjeldahl method. Total carbohydrates were calculated by difference {100 - (moisture + ash + protein + fat)} (Khoozani *et al.,* 2020). Each value was calculated in triplicates.

Statistical Analysis

The analysis was carried out in triplicate and the results were expressed as mean ± SD (standard deviation). Independent samples t-test was performed using SPSS Statistics version 22 software (IBM). A value of $p< 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

According to the experimental design, Table 2 represents the results of response: moisture content, specific volume, bake loss, texture and colour parameters for bread formulated with HPMC, raw banana flour and proof time. The specific volume, bake loss and cohesiveness models were not significant ($p > 0.05$) and hence RSM was not suitable for predicting these responses. Values for moisture content, hardness, springiness, resilience and crust-crumb L* fitted the second order polynomial and high R^2 were observed. The regression models were used to generate the 3-D plots for the significant responses (Figure 1).

Effect of variables

Quantity of HPMC had significant effect on bake loss, hardness and crust-crumb L*. It had positive effect on all these parameters except bake loss and crumb L*. On increasing the HPMC levels, the GF system binds more water and become firmer and more inelastic. The hardened dough doesn't rise with the entrapped gas and shows increased hardness. Our findings were similar to those of (Crockett, i.e., and Vodovotz, 2011; Kim and Yokoyama, 2011; McCarthy, Gallagher, Gormley, Schober, and Arendt, 2005) HPMC had negative quadratic effect on cohesiveness and resilience textural properties and its interaction with raw banana flour had antagonistic effect on hardness as shown in Figure 1b. HPMC in combination with raw banana flour helps in strengthening the air micelles and provide mesh-like gluten structure. The hydrophobic groups of HPMC induce surface tension and thus improves cohesion in the dough system. Figure 1a shows that the HPMC- raw banana flour interaction had positive

Table 2: Experimental Design and result of response surface methodology

RUN	X_1	X_2	X_3	Moisture Content (%)	Specific Volume (%)	Bake Loss (%)	Texture Parameters				Colour Parameters	
							Hardness (g)	Springiness (g)	Cohesiveness(mm)	Resilience	Crust L*	Crumb L*
1	0.000	0.000	0.000	39.04 ± 0.69	95.369 ± 0.06	10.5 ± 0.33	17281.2 ± 2.45	0.49 ± 1.45	0.539 ± 3.66	0.289 ± 1.78	7.37 ± 0.02	12.9 ± 0.33
2	1.000	-1.000	1.000	39.69 ± 0.41	129.868 ± 0.08	9 ± 0.02	22507.6 ± 3.33	0.424 ± 2.21	0.588 ± 2.23	0.253 ± 1.34	10.21 ± 1.02	15.38 ± 0.78
3	0.000	0.000	-1.682	39.99 ± 0.15	128.24 ± 0.10	8.375 ± 0.15	34326 ± 1.45	0.713 ± 3.33	0.361 ± 4.56	0.127 ± 3.56	6.18 ± 2.04	10.44 ± 0.10
4	-1.682	0.000	0.000	38.23 ± 0.33	80.236 ± 0.09	11.12 ± 0.48	14345.5 ± 5.01	0.631 ± 1.78	0.422 ± 1.34	0.191 ± 3.21	3.73 ± 0.90	9.79 ±1.33
5	0.000	0.000	0.000	39.22 ± 0.56	94.321 ± 0.04	9.98 ± 0.61	16881.2 ± 4.67	0.378 ± 2.67	0.568 ± 2.22	0.299 ± 4.78	7.45 ± 0.33	11.2 ± 2.10
6	0.000	0.000	0.000	38.89 ± 0.89	91.348 ± 0.09	10 ± 0.87	18281.2 ± 3.78	0.421 ± 4.21	0.509 ± 1.67	0.258 ± 5.11	7.99 ± 1.45	11.23 ± 2.09
7	0.000	0.000	0.000	39.21 ± 0.44	99.899 ± 0.04	10.2 ± 0.45	21187.2 ± 2.12	0.488 ± 2.11	0.547 ± 4.67	0.271 ± 1.87	7.02 ± 1.04	11.56 ± 1.09
8	0.000	0.000	0.000	38.56 ± 0.61	93.123 ± 0.11	9.95 ± 0.22	18811.3 ± 3.12	0.395 ± 3.45	0.518 ± 5.22	0.262 ± 3.55	8.21 ± 2.05	12.5 ± 0.67
9	1.682	0.000	0.000	38.78 ± 0.23	88.354 ± 0.08	8.12 ± 0.56	32955.7 ± 1.78	0.299 ± 1.89	0.331 ± 1.56	0.12 ± 5.11	6.4 ± 1.90	14.69 ± 0.33
10	-1.000	-1.000	1.000	42.21 ± 0.15	140.112 ± 0.04	10 ± 0.78	26688.6 ± 1.45	0.587 ± 1.05	0.347 ± 4.78	0.134 ± 1.34	7.75 ± 0.56	12.71 ± 0.12
11	0.000	-1.682	0.000	40.78 ± 0.87	90.028 ± 0.03	9.75 ± 0.12	28662.9 ± 2.67	0.691 ± 2.56	0.399 ± 3.23	0.192 ± 2.87	8.46 ± 0.21	15.11 ± 1.04
12	1.000	-1.000	-1.000	38.89 ± 0.34	110.977 ± 0.09	11 ± 0.56	28978.2 ± 3.67	0.743 ± 3.45	0.41 ± 1.89	0.183 ± 3.56	6.39 ± 1.09	10.99 ± 1.33
13	1.000	1.000	-1.000	39.15 ± 0.56	120.657 ± 0.01	8.75 ± 0.49	41615.7 ± 5.55	0.661 ± 2.99	0.355 ± 1.78	0.159 ± 2.90	6.26 ± 1.56	18.39 ± 2.09
14	0.000	1.682	0.000	40.35 ± 0.55	89.164 ± 0.06	8.5 ± 0.42	32409.5 ± 3. 56	0.538 ± 1.56	0.542 ± 2.55	0.166 ± 1.56	6.42 ± 2.04	19.03 ± 1.22
15	-1.000	1.000	1.000	40.83 ± 0.68	135.053 ± 0.10	10.99 ± 0.19	29363.6 ± 1.34	0.624 ± 2.78	0.339 ± 3.56	0.148 ± 2.45	1.77 ± 1.99	13.48 ± 0.78
16	1.000	1.000	1.000	42.1 ± 0. 67	119.269 ± 0.04	9.75 ± 0.59	31613.3 ± 1.09	0.579 ± 3.67	0.388 ± 4.89	0.182 ± 3.33	7.07 ± 0.21	24.93 ± 0.89
17	-1.000	-1.000	-1.000	41.12 ± 0.22	139.969 ± 0.01	9.5 ± 0.41	15950.4 ± 2. 12	0.547 ± 4.78	0.472 ± 3.19	0.205 ± 2.43	7.42 ± 0.56	18.55 ± 1.03
18	0.000	0.000	1.682	40.97 ± 0.11	130.022 ± 0.11	11 ± 0.78	30657.2 ± 3.89	0.575 ± 4.02	0.374 ± 1.67	0.157 ± 1.78	4.62 ± 0.04	17.45 ± 2.04
19	0.000	0.000	0.000	39.89 ± 0.48	105.664 ± 0.09	9.25 ± 0.39	20781.2 ± 2.12	0.482 ± 2.56	0.58 ± 4.67	0.249 ± 2.87	8.17 ± 0.10	13.3 ± 1.56
20	-1.000	1.000	-1.000	39.99 ± 0.78	122.172 ± 0.05	9.12 ± 0.86	11982.8 ± 3.45	0.581 ± 1.32	0.395 ± 3.09	0.189 ± 2.56	4.49 ± 0.58	14.33 ± 0.67

effect on moisture content as it increases the water-binding capacity due to its hydrophilic nature (Ko, Kim, Baek, and Park, 2015; Sabanis, Lebesi, and Tzia, 2009).

Raw banana flour had significantly affected hardness and crust-crumb L*. All these parameters were negatively correlated to raw banana flour. This result is in accordance with Kaur *et al.,* (2017) who reported that use of whole grains and loss of characteristic white colour of wheat flour led to a darker colour product. The carbohydrate content of raw banana flour led to maillard reaction and thus producing darker bread (Khoozani *et al.,* 2020). Contrary to previous study of Loong and Wong, (2018), raw banana flour had significant negative effect on hardness linearly and a positive effect quadratically. The raw banana flour -HPMC interaction had significant effect on hardness with marked decrease in it. In their study, Korus *et al.,* (2009) showed that partial replacement in GF bread with resistant starch diminishes the crumb hardness. Raw banana flour is a rich source of resistant starch which only behave as a filler during gelatinization and does not help in structure formation. Increasing level of resistant starch decreases viscosity and thus lowers hardness. HPMC-raw banana flour improves water-binding capacity and thus reduces the hardness (Wang, Lu, Li, Zhao, and Han, 2017). However, quadratic effect of raw banana flour was opposite. As explained in a research conducted by Korus *et al.,* (2009) in GF system, excess of resistant starch leads to incomplete gelatinization, less expansion of gas cells and hence, dense and hard bread.

Proof time had negative

Table 3: Analysis of variance on the effect of experimental factors on the responses

Model	Moisture Content (%)	Specific Volume (%)	Bake Loss (%)	Texture Parameters				Colour Parameters	
				Hardness (g)	Springiness (g)	Cohesive-ness(mm)	Resilience	Crust L*	Crumb L*
X_1	-1.559	-12.050	-0.957*	+4401.81**	+0.048	+0.133	+0.086	+0.238**	-9.143**
X_2	-0.398	-1.586	-0.068	-1746.389*	-0.0310	+0.013	+0.012	-0.065**	-1.283**
X_3	-0.154*	-3.372	-0.009*	+127.977	-0.0149	+0.0090	+0.006	+0.153	-0.468*
X_1X_2	+0.029*	+0.122	-0.012	-148.640*	+0.000011	-0.0009	-0.0005	+0.031*	+0.113**
X_1X_3	+0.006	+0.015	-0.011	+1.037**	-0.002	+0.001	+0.0007*	+0.023*	+0.059**
X_2X_3	+0.0006	-0.002	+0.001*	+430.989	+0.00008	-0.000025*	-5.67E-06	-0.002*	+0.002
X_1^2	-0.042	+0.500	-	+15.130	+0.004	-0.025**	-0.015**	-0.362**	+0.164
X_2^2	+0.003**	+0.013	-	+6.553**	+0.0002*	-0.0001	-0.0001**	+0.00011	+0.009**
X_3^2	+0.001**	+0.027**	-	+127.976**	+0.0001**	+0.133**	-0.0063**	-0.0011*	+0.001*
ANOVA									
Type	Quadratic	Quadratic	2FI	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic
F-value	4.23	1.80	3.92	13.15	3.12	2.95	5.75	9.53	12.46
p-value	0.0171*	0.1866	0.0186*	0.0002**	0.0454*	0.0538	0.0058**	0.0008**	0.0002**
Lack of Fit	Ns	S	S	ns	ns	S	ns	ns	ns
C.V.	1.80	15.16	6.77	12.45	15.58	14.55	15.39	12.58	10.21
R2	0.7921	0.6183	0.6439	0.9221	0.7374	0.7261	0.8380	0.8956	0.9181
-1.000	41.12 ± 0.22	139.969 ± 0.01	9.5 ± 0.41	15950.4 ± 2. 12	0.547 ± 4.78	0.472 ± 3.19	0.205 ± 2.43	7.42 ± 0.56	18.55 ± 1.03
1.682	40.97 ± 0.11	130.022 ± 0.11	11 ± 0.78	30657.2 ± 3.89	0.575 ± 4.02	0.374 ± 1.67	0.157 ± 1.78	4.62 ± 0.04	17.45 ± 2.04
0.000	39.89 ± 0.48	105.664 ± 0.09	9.25 ± 0.39	20781.2 ± 2.12	0.482 ± 2.56	0.58 ± 4.67	0.249 ± 2.87	8.17 ± 0.10	13.3 ± 1.56
-1.000	39.99 ± 0.78	122.172 ± 0.05	9.12 ± 0.86	11982.8 ± 3.45	0.581 ± 1.32	0.395 ± 3.09	0.189 ± 2.56	4.49 ± 0.58	14.33 ± 0.67

*Significant at $p< 0.05$, **Significant at $p< 0.01$, R2coefficient of determination, c.v. coefficient of variation s significant, ns non-significant; X1 hydroxypropyl methylcellulose (%), X2 raw banana flour (%), X3proof time (mins)

Table 4: Predicted and measured values for responses of the optimized gluten-free bread formulation

Responses	Predicted Values	Measured Values ± SD
Moisture Content (%)	40.22a	40.18 ± 0.015a
Hardness (g)	14411.92a	14410 ± 1.63a
Springiness (g)	0.579a	0.575 ± 0.004a
Resilience	0.212a	0.211 ± 0.003a
Crust L*	6.704a	6.708 ± 0.002a
Crumb L*	14.65a	14.68 ± 0.052a

Means ± standard deviations; means followed by same superscript in each row are not significantly different (p>0.05)

Table 5: Proximate and Sensory Analysis of whole Wheat Bread v/s Gluten-free Optimized Bread

Sensory Acceptability Scores	Whole Wheat Bread	Gluten-free Optimized Bread
Appearance	8.24 ± 0.09a	6.78 ± 0.06b
Colour	8.43 ± 0.02a	8.12 ± 0.04b
Aroma	7.46 ± 0.015a	7.11 ± 0.08b
Texture	8.23 ± 0.019a	6.65 ± 0.016b
Taste	7. 85 ± 0.16a	7.32 ± 0.011b
Overall Acceptability	8.12 ± 0.07a	7.86 ± 0.09b
Proximate Composition (%)		
Moisture	38.72 ± 0.04a	40.18 ± 0.015b
Ash	1.605 ± 0.001a	1.606 ± 0.001a
Acid Insoluble Ash	0.187 ± 0.001a	0.153 ± 0.001b
Protein	6.78 ± 0.005a	8.47 ± 0.002b
Fat	10.34 ± 0.01a	12.35 ± 0.08b
Carbohydrate	42.37 ± 0.03a	37.24 ± 0.06b

Means ± standard deviations; means followed by same superscript in each row are not significantly different (p>0.05)

significant effect on moisture content, bake loss and crumb lightness. It showed no significant effect on textural parameters. At quadratic level, proof time significantly affected all the response variables. It showed positive correlation with moisture content, bake loss, specific volume and all textural parameters except resilience. Proof time- raw banana flour showed negative significant effect on cohesiveness and crust L* (Figure 1f). This could be due to raw banana flour starch producing stiff elastic mass yielding a greater cohesion as seen in the research conducted by Onyango *et al.,* (2011). In wheat-based bread, the CO_2 gas produced by yeast is entrapped by gluten matrix. But the GF batter is not able to hold the gas and therefore provide bread with poor textural properties. The darker colour of crumb can be attributed to the bread being over proofed. Over proofed bread develops larger cells which give access to more heat inside the loaf causing thick crust and darker bread with poor texture (*Bread Baking*, 1969). The antagonistic effect on moisture content and bake loss can be due to collapse of over proofed bread and escape of fermentation gas. As the centre of the loaf warms up, the activity of yeast decreases and with prolonged proof time, the bread physical properties are affected with loss of moisture and poor texture (Capriles and Arêas, 2014).

Optimal Parameters for Gluten-free Bread

The process variables were optimized under certain constraints by applying the desirability function of Design Expert Version 11 software. The optimal conditions were 2% for HPMC, 37.16% for raw banana flour and 40 mins for proof time, with a desirability function of 0.72. Bread was baked under the optimum parameters and the responses were recorded. The experimental values for moisture content, hardness, springiness, resilience, crust and crumb L* were 40.22%, 14411.92g, 0.579g, 0.212, 6.704, 14.65. The results of two tailed t-test are depicted in Table 4 and showed no significant difference between the predicted and measured values (p<0.05).

Sensory and Proximate Analysis

The sensory panellists evaluated the sensory scores based on appearance, color, texture, aroma, taste and overall acceptability of optimum GF bread and whole wheat bread (Table 5). The result of parameter showed significant difference at 5%. The color score for GF bread (8.12) was lower than whole wheat bread (8.43). The crust and crumb color of GF bread was darker compared to whole wheat bread. The crust lacked smoothness of regular white bread, was comparably dark and had cracks. Absence of gluten network is the element reason behind this. The use of GF flours greatly affects the aroma, texture and flavour of bread. Furthermore, the absence of characteristic aroma of wheat bread lowers the sensory score for GF bread. The overall acceptability of GF bread (7.88) was lower in comparison to whole wheat bread (8.12). Nevertheless, the overall acceptability score of 7.88 ≈ 8 (liked very much) of GF bread shows promising market and feasibility of further development. Similar studies, Gomes *et al.,* (2016) and X. Liu *et al.,* (2019), showed comparable results for sensory analysis for GF bread.

The results of proximate analysis between optimum GF bread and whole wheat bread are depicted in Table 5. All the responses except ash showed significant difference at 5%. It was observed that GF bread showed a protein content (8.47%) higher than that of whole wheat bread (6.77%). Use of millets and pseudo cereals based composite flour enhances the protein content in GF products (Taylor and Emmambux, 2008; Houben, Höchstötter, and Becker, 2012; Selimović *et al.,* 2014). This result was in contrast with that of Kulai and Rashid (2014). The fat content of GF bread (12.34%) was higher than that of whole wheat bread (10.34%). This is in accordance with the results of others research work (Allen and Orfila, 2018; Kulai and Rashid, 2014; Miranda *et al.,* 2018). In GF formulations, fat and oils are used in larger quantity to enhance the texture and mouthfeel (Roman, Belorio, and Gomez, 2019). Moisture, ash and acid insoluble ash of GF bread was comparable to those of whole wheat bread. In relation to whole wheat bread (42.37%) the value of carbohydrates of GF bread (37.24%) was lower. This can be attributed to moisture content as retention of water and carbohydrates share inverse relation as discussed by Roman *et al.,* (2019).

CONCLUSION

In the study, the effect of HPMC, raw banana flour and proof time was demonstrated on bread physical properties using CCRD. The results indicated that these variables had different effect on moisture content, specific volume, bake loss, texture parameters and colour parameters. HPMC showed positive correlation linearly but had negative effect on hardness on interaction with raw banana flour. An increase in the raw banana flour content significantly decreased the hardness and resilience. Proof time had no significant effect linearly on texture parameters and quadratically had antagonistic effect. Overall, the models for moisture content, hardness, springiness, resilience and colour parameters showed significant F-model with non-significant lack of fit. Thus, optimal conditions (HPMC 2%, raw banana flour 37.16% and proof time 40 mins) were applied to produce good-quality bread. The overall acceptability score of 7.88 ≈ 8 (liked very much) of GF bread showed promising results and its proximate and physical characteristics were studied. Use of GF flours showed higher protein-fat content and lower carbohydrate content when compared to whole wheat bread. This research highlights the use of amaranth, buckwheat, pearl millet and raw banana flour along with HPMC to produce bread comparable to whole wheat bread. With the increase in demand for nutritious GF food, this market is expected show growth trajectory and hence the need for such products.

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