Optimization of Formulation and Process Conditions of Gluten-Free Bread from Sorghum using Response Surface Methodology

Shimelis Admassu Emire1* and Dawit Demelash Tiruneh2

1Food Engineering Graduate Program, Department of Chemical Engineering, Addis Ababa Institute of Technology, P.O.Box 385, Addis Ababa University, Ethiopia
2School of Agriculture, Department of Food Processing, Adama University, Ethiopia

Abstract

Grain and flour characterizations of two improved sorghum varieties (Gambella-1107 and 76T,#23) grown in Ethiopia was conducted from the perspective of their incorporation into Gluten-Free Bread (GFB). The sample varieties were evaluated for physicochemical and functional properties and their antinutrient compositions. In addition, GFB was produced from sorghum flour (50% flour weight basis), corn flour (20%), potato flour (10%) and chick pea flour (20%), Response Surface Methodology (RSM) was used to optimize the formulation and process conditions. The independent variables (factors) for the experiment were egg albumin level, milk powder level, baking temperature and time. The investigated responses were volume expansion, moisture loss, loaf height and bread hardness. Overall optimization, conducted by overlaying the 3-D plots under investigation, was able to identify an optimal range of the independent variables within which the four responses were simultaneously optimized. The point chosen as representative of this optimal area corresponded to egg albumin (2.5% flour-weight basis), milk powder (7.5%), temperature (220°C) and baking time (35 min), process conditions under which the model predicted volume expansion (597 cm³), moisture loss (4.02%), loaf height (3.43 cm) and bread hardness (3.88 N/g). The optimum GFB contain 78.9% dry matter, 12.3% crude protein, 3.9% crude fat, 2.4% crude fiber, 1.7% total ash and 58.6% total carbohydrates. Sensory evaluation indicated that the overall acceptability of the optimum GFB was much more similar to commercial GFB produced by local bakeries than the existing formulated GFB. Hence, the optimum formulation and process conditions can be used as starting point for bakers to produce GFB and to contribute to the eradication of the celiac problems in Ethiopia.

Keywords: Celiac disease; Gluten-free bread; RSM; Sorghum; Milk powder; Egg albumin

Introduction

Celiac disease is a disease of the digestive system that damages the small intestine and interferes with the absorption of nutrients from food. Celiac disease occurs when the body reacts abnormally to gluten, a protein found in wheat, rye, barley, and possibly oat. When someone with celiac disease eats foods containing gluten, that person’s immune system causes an inflammatory response in the small intestine, which damages the tissues and results in impaired ability to absorb nutrients from foods. The inflammation and mal-absorption create wide-ranging problems in many systems of the body [1].

Recent data indicated that the average worldwide prevalence of celiac patient is estimated as high as 1:266. In Ethiopia, Celiac disease kills many children each year, mainly because it usually goes undiagnosed and untreated. Unfortunately, knowledge /awareness of the disease in East Africa are almost non-existent. The acute lack of awareness and subtle ignorance about the disease leads the shocked increment of the disease in East Africa. Evidences show that there is a celiac disease problem in Ethiopia and because of this some bakery shops have started to prepare and sell gluten-free bread. A gluten-free diet is the only efficient treatment for celiac disease, and the results depend on rigorous discipline in its application, in many cases over the whole period of life. Food must be completely free of gluten, so all the products from wheat, rye, barley and oat must be replaced with corn, sorghum, teff, rice or millet equivalents or various types of starch or appropriate mixtures. The formulation of new, better recipes and technologies for gluten-free products is therefore, a priority.

Sorghum might therefore, provide a good basis for gluten free bread (GFB). However, the bulk of studies dealing with leavened breads containing sorghum have focused on composite breads from wheat and sorghum, in which a maximum of only 30% of low-tannin sorghum is regarded as acceptable. While such breads have been found acceptable by consumers, they are inappropriate for celiac patients [2].

Breads made from sorghum without added wheat, as are all GFB, require a different production technology. Gluten-free dough’s are more fluid than wheat dough’s and closer in viscosity to cake batters due to the lack of a gluten network. This batter-type dough has to be handled similarly to cake batters rather than typical wheat dough’s. Furthermore, gas holding is more difficult and the use of gums, stabilizers, and pregelatinized starch has been suggested as a means to provide gas occlusion and stabilizing mechanisms. Milk powder and egg albumin have also been described as having positive effects when used in GFBs and have been suggested for this application [3].

Only a limited number of studies have addressed wheat-sorghum breads, and most have used extra ingredients like methylcellulose, xanthan gum, carboxyl methyl cellulose and

*Corresponding author: Shimelis Admassu Emire, Food Engineering Graduate Program, Department of Chemical Engineering, Addis Ababa Institute of Technology, P.O.Box 385, Addis Ababa University, Ethiopia, E-mail: shimelisemire@yahoo.com

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yoghurt powder, egg, or rye pentosans [4]. Good quality GFBs were produced based simply on 70% sorghum and 30% cassava starch has been developed by Olatunji et al. [5]. Although previous research has been conducted on producing bread from sorghum flour, virtually no work has been done in Ethiopia using Response Surface Methodology (RSM) to determine the optimum formulation and process conditions for the production of GFB from sorghum flour. The factors (independent variables) included ingredients (milk powder, egg albumin) and processing conditions (temperature, time). The selection of independent variables was performed based on the standards set for evaluation of gluten-free bread [4]. The factors (skim milk powder and egg albumin) were chosen on the replacement of gluten with other protein sources which in turn improves appearance, sensory properties and overall quality of gluten-free bread. The other factors (baking time and temperature) were used for subsequent measurement of appropriate responses (volume expansion, moisture loss, loaf height and texture) in GFB production process.

Response surface methodology has been a popular and effective method for solving multivariate problems and optimizing several responses in many types of experiments, because it can simultaneously consider several factors at many different levels, and corresponding interactions among these factors using a small number of observations [6]. The aim of the study was to characterize flours from two sorghum varieties and optimize process conditions and some ingredients of gluten-free sorghum based bread using response surface methodology.

Materials and Methods

Material collection and sample preparation

The samples for the investigation were used two sorghum varieties (Gambella-1107 and 76T×23), which were grown under similar agronomic practices required for grain crops at Melkassa Agricultural Research Center, Nazareth, Ethiopia.

All the samples were cleaned manually to remove foreign matters, immature and damaged grains and then ground (Brabender break mill SM3, Germany) to different milling gaps. Grain and flours of the raw samples were evaluated for their physicochemical and, functional properties and their anti nutrient compositions. Additionally, the flours of the processed samples were used to study formulation and process conditions for the production of GFB. Samples were sealed and placed in plastic bags and stored at 21-23°C until used. Analytical grade reagents and chemicals were obtained from Ethiopian Health and Nutrition Research Institute and Addis Ababa University (Food Process Engineering Laboratory, Addis Ababa Institute of Technology), and used for the analyses.

Analytical methods

**Sorghum grain characterization:** Hundred seed mass was determined by counting one hundred seeds using an electronic seed counter and weighing. Hydration and swelling coefficients were determined by the method reported by Youssuf [7]. Hydration, swelling capacities and indices were determined by the Bishnoi and Khetarpaul [8] method.

**Sorghum flour characterization:** The proximate compositions of the sorghum flours were determined using AOAC [9] methods. Dispensibility of flour in water was determined by the method of Kuikarni [10].

Emulsion activity and stability was determined according to the method described by Yasumatsu et al. [11]. Flour particle size distribution was determined according to Whitby [12] using a sieving technique. Tannin contents of the samples were determined according to the modified Vanillin-HCl methanol method described by Price et al. [13]. The method of Haug and Lantzsch [14] was used for phytate estimation.

**Gluten-free bread formulation, processing and characterization**

The basic recipe of formulation for the breads was performed according to the method described by Schober et al [4]. Bread making experiments used the following basic formulation: Gambella-1107 sorghum flour (50%), corn flour (20%), potato flour (10%) and chick pea flour (20%). The sum of the flours was (100%) and interpreted as the flour weight basis (fwb). Salt, baking powder, sugar, active dry yeast, egg albumin, milk powder and water were used as ingredients. When comparing the bread making potentials of the sorghums, the amount of water to obtain constant consistency must be standardized; for the present study 70% water fwb was used. When testing additional ingredients using RSM, the amount of egg albumin and milk powder were varied and other ingredients remained unchanged in terms of proportion.

The bread was manufactured by the straight dough method. The processing stages GFB production includes: Sorghum flour sifting, blending/mixing, fermentation, dividing, rounding, proofing, baking, cooling and sealing. Baking was done in oven (Sheba, B-2371, Italy) at baking temperature between 190-230°C and baking time 20-40 min. After baking the loaves were depanned and cooled on cooling racks at room temperature, sliced and packed in polyethylene bags.

**Optimization of formulation and process conditions**

The central composite design of the RSM, consisting of a four-factor and five-level pattern, was used to conduct the experiment (Table 1). The design consisted of 30 runs in random order; all points in coded factor levels in the order of: egg albumin, milk powder, baking temperature and time. The center point (0, 0, 0, 0) was replicated six times, factorial points were not repeated and comprised all combinations of -1 and 1 levels of the factors; star points also were not repeated and comprised all combinations of -2.0, 0.0 and 2.0 levels of the factors.

The combination of the four factors (milk powder, egg albumin, baking temperature and time) studied in the response surface experiment and optimization was based on the +1 and -1 variable levels of the experimental design; their coded and actual values are shown in Table 1.

| Actual levels of factors | Ingredients (% fwb) | Process conditions |
|--------------------------|---------------------|--------------------|
| Coded levels | Egg albumin (%) | Milk powder (%) | Temperature (°C) | Time (min) |
| --- | --- | --- | --- | --- |
| -2.0 | 0.00 | 0.00 | 190 | 20 |
| -1.0 | 2.50 | 2.50 | 200 | 25 |
| 0.0 | 5.00 | 5.00 | 210 | 30 |
| 1.0 | 7.50 | 7.50 | 220 | 35 |
| 2.0 | 10.0 | 10.0 | 230 | 40 |

*Table 1: Coded and actual levels of the factors in the central composite design of the RSM.*

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Evaluation of gluten-free bread quality

Volume expansion and loaf height: Volume expansion was determined by the seed displacement method [9]. A rectangular box made from tin should be used. The box was filled with cleaned millet grain, leveled and poured out. The bread was placed in the same bowl and filled with the measured millet grain and leveled. The volume of the remaining grains from the same measured grains was taken as the volume of the loaf. The loaf height was determined by Varner caliper.

Moisture loss: Moisture loss of the bread was determined as follows:

\[
\text{Moisture Loss (\%)} = \frac{(W_1) - (W_2)}{(W_1)}
\]

Where \(W_1\): Weight of dough before baking
\(W_2\): Weight of product after baking

Texture profile test: The texture of GFB samples was determined using a texture analyzer (Model TA-PLUS, LLOYD instrument, England) equipped with cylindrical probe P/2. The individual samples of bread were cut into slices with 2.8 cm thickness; placed on the platform and the probe attach to the crosshead of the instrument. The texture analyzer setting keeps at: pre-test speed of 2 mm/s, test speed of 0.5 mm/s, and post-test speed of 10 mm/s. The initial significant peak force from the resulting curve was considered as the initial fracture force and the absolute peak force also consider as the hardness of the bread.

Sensory analysis: Sensory evaluation of GFB samples was carried out to predict consumer acceptance and preference using fifteen untrained sensory panelists, all diabetic’s patients who were primary victims of gluten intolerance, using a nine point Hedonic Scale (1 and 9, representing extremely dislike and extremely like, respectively). The qualities assessed included: color, taste, odor, texture and over all acceptance.

Statistical analysis

Statistical Analysis of Variance (ANOVA), the Lack-of-Fit test (LOF), Sequential Mean Sum of Squares (SMSS) and multiple regressions of the RSM were performed using Design-Expert version 8.0.1 software (Design-Expert, 2010).

Results and Discussion

Grain and flour characterization, bread production and characterization of two sorghum varieties obtained from the Melkassa Agricultural Research Center of Ethiopia were studied. The sorghum grains were produced in the same geographical location and all are harvested during the same season, which may lessen the influence of environmental factors on the analysis. Thus, the differences found can be attributed predominantly to genotypic characteristics of each variety.

Sorghum grain characterization

The physicochemical properties of the two sorghum varieties are presented in Table 2. The 100-kernel masses and kernel densities of the two varieties were not significantly different (p>0.05). The 100-kernel mass may be considered an indicator of the yield of products to be obtained from the sorghum grain. The hydration and swelling capacities of the two varieties were not significantly different (p<0.05). Gambella-1107 had a higher hydration index, and swelling coefficient than did 76T,#23 but Gambella-1107 had a lower swelling index and hydration coefficient. Alpaslan and Hayta [15] reported that hydration and swelling properties are important in terms of processing of cereal based foods.

Sorghum flour characterization

Moisture, crude protein, crude fat, total ash and, crude fiber content and pH values of the grain varieties are presented in Table 3. Components other than moisture contents are expressed on dry basis.

Protein content affects the rheological and end-use quality of wheat flours. Literature is scarce with respect to the effect of sorghum flour protein content on GFB quality. The protein contents of Gambella-1107 and 76T,#23 on a dry basis were comparable to those reported by Schober et al. [2] (9.5-12.9% db). Kim and De-Ruiter [16] reported that the color (lightness) of flour is affected by the ash content, and also indicated that ash content reflects the degree of bran contamination in the flour. Flour with low ash content is preferred for utilization in human food. The crude fat contents of the two varieties were within the range reported by Noha et al. [17] (2.4-4.2% db, dry basis). The crude fat content is related to the energy content of the flour.

### Table 2: Physicochemical properties of two sorghum varieties.

| Varieties         | Hundred seed mass (g/100seeds) | Seed density (g/ml) | Hydration capacity (g/seed) | Swelling capacity (ml/seed) | Hydration index | Swelling index | Hydration coefficient | Swelling coefficient |
|-------------------|-------------------------------|--------------------|-----------------------------|-----------------------------|-----------------|---------------|---------------------|----------------------|
| Gambella-1107     | 2.67±0.23a                    | 1.48±0.02a         | 0.01±0.01a                  | 0.02±0.01a                  | 0.45±0.02a      | 0.91±0.31a     | 1.04±0.06a          | 1.57±0.03a            |
| 76T,#23           | 2.63±0.15b                    | 1.47±0.07b         | 0.01±0.00b                  | 0.02±0.01e                  | 0.42±0.10a      | 1.00±0.00b     | 1.10±0.0b           | 1.41±0.01b            |

All values are means of triplicates ± standard deviation.

**Means with the same superscript letters within a column are not significantly different at P<0.05.

### Table 3: Proximate composition, antinutrient and pH values of sorghum flours.

| Varieties         | Proximate composition (g/100g) | Antinutrient (mg/100g) | pH |
|-------------------|-------------------------------|------------------------|----|
|                   | Moisture content              | Crude protein          | Crude fat | Ash | Crude fiber | Phytate | Tannins |                   |
| Gambella-1107     | 10.43±0.62a                   | 10.59±0.09a            | 3.08±0.19a | 1.64±0.11a | 3.38±0.07a | 276.23±1.79b | 11.178±0.61b | 6.89±0.03a |
| 76T,#23           | 10.41±0.59a                   | 11.36±0.25b            | 3.15±0.13a | 1.57±0.05a | 2.64±0.07a | 307.38±1.69b | 25.479±0.22a | 7.01±0.01a |

**Means with the same superscript letters within a column are not significantly different at P<0.05.
Gambella-1107 and 76T #23 varieties had higher crude fiber (3.38 and 2.64 g/100 g) contents than did the sorghum analyzed by Noha et al. [17]. Gambella-1107 sorghum had chosen for gluten-free bread manufacturing process due to its high crude fiber content compared to 76T #23. France [18] reported that high crude fiber is nutritionally appreciated because it traps less proteins and carbohydrates.

Flour moisture is typically an indication of flour quality and has an impact on functionality in specific products. The moisture contents of the two varieties were 10.43 and 10.41% for Gambella-1107 and 76T #23 respectively; which is also within the range reported by Emily [19] (8.5 - 12.2%). The pH values of flours from both Gambella-1107 (pH= 6.89) and 76T #23 (pH= 7.01) were similar to that of commercial sorghum flour (6.8), as reported by France [18].

Levels of phytate and tannins concentrations in Gambella-1107 and 76T #23 are shown in Table 3. The phytate content of 76T #23 was higher (p<0.05) than that of Gambella-1107. The levels of phytate were 276.23 and 307.38 mg/100 gm; respectively for Gambella-1107 and 76T #23. These values were lower than that of the sorghum flour (318 mg/100 g) analyzed by Noha et al. [17]. Variations in the phytate content may be due to factors such as variety, climatic conditions, location, and type of soil and length of the grown period. The tannin content of 76T #23 was higher (p<0.05) than that of Gambella-1107. The tannin contents of both sorghum varieties were much higher than the sorghum flour (0.18 mg/100 g) analyzed by Noha et al. [17]. According to Schober et al. [4], a maximum of only 30% of low-tannin sorghum is regarded as acceptable in a GFB formulation. By and large, Gambella-1107 variety had enhanced proximate and low anti nutrients composition. Thus, Gambella-1107 variety was selected to prepare GFB.

**Functional properties**

Functional properties are important characteristics of food products utilized in ground and canned meat formulations, baked goods, doughnuts, pancakes, soups, whipped cakes and desserts. Table 4 presents the functional properties of flours from the two sorghum varieties. The Water Absorption Capacity (WAC) of the two varieties were fell within the range of WAC values (2.7-3.6g/g) observed by Njintang et al. [20]. The difference in WAC between the two varieties may reflect differences in the amount and nature of hydrophobic constituents [5]. WAC is an important functional property required in food formulations especially those involving dough handling.

Fat absorption is an important property in food formulations because fats improve the flavor and mouth feel of foods. The result shows that oil absorption capacity of the two flour varieties did not have significant difference. Bulk density (BD) gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for greater ease of dispersibility and reduction of paste thickness [21]. The BD and dispersibility of the two sorghum flour varieties had comparable result.

Foaming properties are important parameters for the characterization of flour to be used in the preparation of various food products. Foam formation and stability are a function of the type of protein, pH, processing methods, viscosity and surface tension [11]. The difference in foaming capacity between the sorghum flour may reflect their protein contents (Table 4).

The Emulsion Activity (EA) values of the sorghum flours were higher than those reported for many other plant food flours, including 20% for fluted pumpkin [22]. Emulsion Stability (ES) of flour is one of the parameter for development of a food product. The 76T #23 sorghum variety showed greater ES than did Gambella-1107. The higher EA and ES suggest that it would form a better bread batter.

**Flour particle size distribution**

Flours milled in the laboratory provide information about their expected qualities and properties during the bread production. In the present study, sieve analyses were carried out with the described mesh size and milling gap adjustment [MGA] (Figure 1). Commercial flour was analyzed for comparison. The particle size distribution of the corresponding sorghum flour that was milled in the laboratory with 0.5 MGA is close to that of commercial flour.

Water absorption and dough stability are directly dependent on flour particle size. According to the study, fine flour particle size (MGA 0.5) was absorbing the added water more quickly than flour with a coarser particle size (MGA 1.0). Emily [19] concluded that the gluten matrix developed more quickly and the dough development time was correspondingly shorter for fine flour particle size, as the water needs more time to be absorbed completely by coarser flour particles.

If the particle size of flour is not known and differences occur, problems may arise during the production of baked goods. Therefore, the recommended flour particle size distribution were (0.5, <250 µm)
MGA and mesh size, respectively. Liman [23] concluded that gluten free bread made from flours produced with a MGA of 0.25 - 0.75 resulted in a product that exhibited better textural attributes than when a wider milling gap adjustment was employed.

**Gluten-free bread characterization**

The coded experimental conditions and responses (volume expansion, moisture loss, loaf height and bread hardness) are presented in Table 5. The minimum and maximum measured values of the responses for volume expansion, moisture loss, loaf height and hardness were 542.5-598.77 cm³, 4.03-5.48%, 3.1-3.52 cm and 3.25-4.9 N/g, respectively. All treatments resulted in dough’s that were moldable but that did not resemble dough form wheat flour, as they lacked viscoelastic characteristic. This is in agreement with the findings of Haque and Morris [24]. The replacement of gluten with other protein sources such as dairy proteins and egg proteins is an approach used to improve the quality of GFB. These proteins are capable of forming networks and strong cohesive viscoelastic structure [25].

The effect of formulation and process conditions on GFB quality

Response Surface Methodology (RSM) is an effective statistical technique which has been widely used to optimize processes or formulations with minimal experimental trials when many factors and their interactions are involved [6]. RSM was applied in studying the combined effects of egg albumin, milk powder, temperature and time on the physical properties of GFB produced in this study using a central composite design (Table 5).

For the important responses, Table 6 shows the fit summaries that were used to select the best models, and the significance of model coefficients. Figure 2 illustrates the results for volume expansion, loaf height, moisture loss and bread hardness, respectively, by using three-dimensional (3D) response surface plots. For each response, two plots are presented, each showing the effects of a combination of four factors, with the third and fourth kept constant at the medium level (corresponding to a coded factor level of 0.0).

Within the selected range of the factor levels, volume expansion was increased via raising the level of egg albumin and decreased by increasing the level of milk powder (Figure 2a). Both factors had significant (P < 0.001) quadratic effects. Similar increases in loaf volume with increased egg albumin addition levels have been reported by Gallagher et al. [26]. For higher milk powder level, the achievable volume expansion is eventually lower. In other words, increasing the level of milk powder can have a higher gas retarding property.

Strong significant effects of egg albumin and milk powder on the volume expansion were observed, due to large holes that impair crumb structure and with poor water-binding and stabilizing properties of egg albumin and milk powder. Beside this, an increase in the volume expansion was strongly associated with the in cooperation of factor...
variables milk proteins and egg albumin since they are highly functional ingredients.

A quick way to compare the effect of baking temperature and time on loaf size in this experiment is to determine the quadratic response of samples when the factors are varied. Generally, baking temperature had more significant effects than did baking time (Figure 2b). If the temperature and time levels are lower that approach the lowest levels, the bread achieves maximum volume expansion. The longest baking time resulted in the greatest volume expansion at the highest baking temperatures. It is believed that the higher baking temperature removed the moisture at the beginning of baking process.

Loaf height (Figure 2e) exhibited similar trends in that increased as the baking temperature and time decreased to minimum level (P < 0.001). The effects of egg albumin and milk powder in the present study were decrease between the coded level -0.5 and 0.5 (Figure 2e), at the same time increase at both coded level 1.0 in height were found. It is noteworthy that the treatments yielding the highest loaf heights gave poor quality breads with very large gas cells, thereby not truly reflecting loaf height. This agrees with the findings of Nishita et al. [27] and Haque and Morris [24] on rice bread, both of whom reported loaves with highest loaf height containing large air pockets. The overall findings reported in this section seem to indicate that there are complex interactions in GFB between formulation level and processing conditions.

Higher loaf height and volume have positive economic effect on bread at the retail end. Therefore, loaf height and volume reduction during baking is an undesirable economic quality to the bakers as consumers often get attracted to bread loaf with higher volume and height believing that it has more substance for the same price. The volume expansion and loaf height, have been generally adopted in the literature as a more reliable measure of loaf size. Loaf size is affected by the quantity and quality of protein in the flour as well as proofing time [28]. Beside this, loaf size is basically determined by the quantity of dough baked and the amount of moisture and carbon dioxide diffused out of the loaf during baking. In this study, higher level of milk powder and lower level of egg albumin and higher temperature and longer baking period caused reduction of loaf size (Figure 2 a,b,e,f) while it was found that volume expansion had a positive correlation with loaf height (P<0.01). Since the bread samples studied here have been produced from the same proofing time and dough size, the variation in loaf volume expansion and height could be attributed mainly to different rate of gas evolution and the extent of starch gelatinization due to differences in formulation, baking temperature and time.

The presence of high level of egg albumin and milk powder in gluten-free breads retarded the moisture loss during baking. The effects of egg albumin and milk powder in the present study were (P < 0.001), maximum moisture loss (Figure 2c) were observed at coded level of (-1.0, -1.0) egg albumin and milk powder, respectively. Milk powder network formed during baking could act as a barrier to the gas diffusion, decreasing the water vapor losses, and thus maintaining the final moisture content of the bread [29].

It was mentioned that baking temperature and time parameters
Figure 2: Three-dimensional response surface plots illustrating the effects of egg albumin, milk powder, baking temperature and time on responses (volume expansion, moisture loss, loaf height and hardness) for GFB.
Figure 3: Three-dimensional response surface plots illustrating the optimum response using numerical optimization.
affects moisture retention capacity of bread crumb [30]. The results showing the effect of baking temperature and time on moisture loss of GF bread are shown in (Figure 2d). The moisture loss (baking loss) of bread ranged from 4.03% to 5.48%. It was found that effect of temperature on the residual moisture loss in the loaves were not more significant than that of time (p<0.001), as the higher level of baking temperature and time, the lower moisture the bread attained. The amount of moisture loss in bread has some implication on the mechanical [28] and keeping qualities. It is also determined by the extent of gelatinization of starch in dough during baking. Higher moisture loss had a positive correlation with bread hardness (p< 0.05).

One of the most desirable characteristics of GF breads which lead to product acceptability is its textural profile. In the current study (Table 5), the bread hardness was ranged between 3.0-4.6N. The results showing the effect of egg albumin and milk powder on hardness of GF bread are shown in (Figure 2g). Results indicated that significant effect wasn’t observed on hardness of GF bread during low level incorporation of egg albumin and milk powder. As the level of incorporation increased consequently the hardness of the bread becomes lower. In (Figure 2h) it was revealed that bread hardness increases as baking temperature and time increase too.

Since gas filled cellular foods like bread are known to fail under mechanical force via brittle fracture, hardness test was therefore used to establish the amount of energy that may be required to fracture a given volume of the dried crumb. It was found that baking temperature and time had more significant positive effect on the hardness of dried bread crumb. Dried crumb hardness correlated positively with loaf volume expansion (p<0.01). The presence of plasticizing agent like water, known to affect mobility of the food polymers, has also been reported to be responsible for differing mechanical behavior of solid foods [31]. Also, higher moisture content is known to increase plasticization and reduce the tendency of solid foods to fail under brittle fracture, implying increased hardness.

**Optimization of formulation and processing conditions**

Although in bread making the perception of product quality is very personal, widely accepted quality criteria for gluten-free breads are large volume and height, soft crumb, and less baking loss. Conditions of optimum formulation and processing can be determined by both numerical optimization and graphical optimization techniques via response surface or, alternatively, contour plots. The two optimization techniques should arrive at similar results. Therefore, in a first case (numerical optimization), an attempt was made to maximize the responses for loaf volume expansion and loaf height, and to minimize baking loss and bread hardness.

Overall, the measured responses compared favorably to the predicted values (Table 7). These measured values were higher than those for GF bread described by Gallagher et al. [26]. Therefore, the experimental formulation yielded better results which were comparable to the predicted values from numerical optimization of predicted values (Figure 3a-h).

The high relative importance assigned to volume expansion, moisture loss, loaf height and hardness resulted in a calculated optimum that yielded breads with very good quality. In addition the calculated optimum desirability (0.910, Figure 4) reflected bread of notably better quality and was subsequently analyzed to compare predicted responses with measured values.

In case of graphical optimization, the most importance was assigned again to maximum responses for loaf volume and loaf height, and to minimizing baking loss and bread hardness. The counter plots (Figure 5) illustrate the graphical optimization of the optimum responses using overlay plot. The overlay plot indicates the optimum factor variable levels (-1, 1, 1, 1) of design points and responses. The optimum responses were 597 cm³, 4.02%, 3.43 cm and 3.88 N/g for volume expansion, moisture loss, loaf height and hardness; respectively. These graphical optimization values are analogous to those of treatment five from the experimental design of the coded experiment conditions and responses (Table 5).

From the results of both numerical optimization and graphical optimization, one can conclude that the optimum formulation and process conditions were the coded levels -1, 1, 1 and 1 or egg albumin (2.5%), milk powder (7.5%), temperature (220°C) and time (35 min), which are alike to those of treatment five from the experimental design/predicted value/ of the coded experiment (Table 1 and Table 5).

**Sensory analysis**

Descriptive sensory attributes (taste, texture, appearance, softness, flavor, overall acceptability) scores are given in Table 8. As seen from the results, there were significant differences (P < 0.001) among treatments. For this work, loaves of selected formulations of GFB which were lower and higher than the optimum, commercial GFB and the optimum formulation were evaluated by 15 untrained panelists (all diabetic patients) on the day of baking.

It must be noted that most panelists would be unfamiliar with breads based on sorghum or other gluten- free ingredients. Thus, their expectations of the characteristics of bread would be based entirely on products made from wheat flour. This may account partly for the low scores obtained. In the statistical analysis, there were no significant

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**Table 7:** Predicted and experimental values for selected responses of optimized bread.

| Parameter          | Predicted value¹ | Experimental value² |
|--------------------|------------------|---------------------|
| Volume expansion (cm³) | 597.00           | 563.51± 17.80       |
| Moisture loss (%)   | 4.02             | 4.63± 0.34          |
| Loaf height (cm)    | 3.43             | 3.31 ± 0.13         |
| Hardness (N/g)      | 3.88             | 3.90 ± 0.42         |

¹Optimum ingredients formulation and process conditions with coded levels (-1,1,1,1): egg albumin 2.5% wb, milk powder 7.5% wb, temperature 220°C and time 35min

²Experimental values

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**Figure 4:** Response prediction desirability of GFB using 3D plot.
Table 8: Sensory quality evaluation score of GFB.

| Hedonic parameters | Optimum GFB | Commercial GFB | GFB-1 | GFB-2 | Statistical effect of bread type |
|--------------------|-------------|----------------|-------|-------|---------------------------------|
| Taste              | 6.63±1.69   | 7.38±0.99      | 3.13±1.06 | 4.38±2.32 | *** 2                          |
| Texture            | 6.75±1.38   | 7.53±0.38      | 3.63±1.18 | 5.75±2.13 | ***                            |
| Appearance         | 8.38±0.64   | 7.25±1.40      | 4.13±1.96 | 6.25±2.21 | ***                            |
| Softness           | 8.25±0.17   | 8.50±0.71      | 3.13±1.60 | 5.03±2.70 | ***                            |
| Flavor             | 6.75±2.50   | 7.13±1.03      | 3.00±1.06 | 3.75±1.57 | ***                            |
| Over all acceptance| 7.35±1.82   | 7.56±1.14      | 3.40±0.87 | 5.03±2.01 | ***                            |

1 Hedonic parameters with scales: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like very much, 8 = like extremely

*** Means with the same superscript letters with in a row are not significantly different at P < 0.001.

The liking of softness of the optimum GFB had a significantly higher score than that of GFB-2 and GFB-1 bread. Similarly, the liking of flavor was significantly higher for the optimum GFB than that of GFB-2 bread and GFB-1 bread.

The improvement effect of egg albumin and milk powder on the sensory quality of bread could be due to its influence on the crumb structure, i.e., a softer crumb with small holes and thin cell walls. Significantly higher flavor scores for the optimum GFB compared to the GFB-1 and GFB-2 breads might be due to the heat stable gelation system of egg albumin, milk powder and starches created in gluten free composite flour. Overall, the optimum GFB was much more liked by the panelists than the existing formulated gluten free breads GFB-1 and GFB-2. The nutritional composition of optimum GFB consists of 21.1% moisture, 12.3% crude protein, 3.9% crude fat, 2.4% crude fiber, 1.7% total ash and 58.6% total carbohydrates.

Conclusions

This research output on grain and flour characterization demonstrated that Gambella-1107 sorghum flour was found enhanced proximate and low anti-nutrients composition; and thus GFB was prepared from this variety.

The RSM was successfully applied to the optimization of the gluten-free bread formulation and process conditions. The study showed that both formulation (egg albumin and milk powder) and process conditions (baking temperature and time) were important parameters affecting the physicochemical properties of gluten-free bread. Most polynomial models developed by RSM were highly effective in describing the relationships between the studied factors and the responses (volume expansion, moisture loss, loaf height and hardness).

RSM provided a simple and straightforward optimization analysis for GFB baking, and the results would help to develop a new value-added functional food containing gluten-free flours, egg albumin and milk powder, which are readily available and relatively inexpensive. The best treatment was recorded when the coded levels -1, 1, 1, 1 used, corresponding to egg albumin (2.5% flour weight basis), milk powder (7.5% flour weight basis), baking temperature (220°C) and time (35 min), which were very similar to those of treatment 5 from the experimental design.

In the sensory evaluations, there were no significant differences found in overall acceptability of the optimum GFB and commercial GFB. The ingredient formulation and process conditions should be viewed as a starting point for baking industry to produce gluten-free bread which in turn can contribute to eradicate celiac problems from Ethiopia and East Africa.

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