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Optimization of Baking Temperature, Time and Thickness for Production of Gluten Free Biscuits from Keyetena Teff (*Eragrostis tef*) Variety

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Abstract

The demand for gluten-free foods is certainly increasing. Teff was becoming popular crop noticeably due to its very attractive nutritional profile and gluten-free nature. This study aims to develop Teff biscuits as an alternative food source for gluten intolerant people. Optimization of three independent variables, baking temperature (174,180 and 186°C), baking time (4, 8 and 12 min), and biscuit thickness (4.5, 5.5 and 6.5 ml) were taken as important factor to determine physical and nutritional quality of biscuits. There were twenty combinations created by Central Composite Design with the aid of Design-Expert software to get best quality Teff product. Moisture, protein, ether extract, fiber, ash, carbohydrate, and gross energy in biscuit samples were found in the range of 4.20-6.98%, 14.59-18.14%, 13.63-14.80%, 3.93-4.08%, 3.38 to 3.65%, 52.4-60.25%, and 414.22-422.15 Kcal/100 g respectively. Results showed that baking temperature and time were the most important factors that significantly affected (p <0.01) rehydration ratio, biscuit hardness, water activity, bulk density, protein, moisture content, carbohydrate and gross energy. Biscuit diameter, thickness, ash, fat, fiber, and mineral were not significantly affected by interaction of baking conditions. Based on all parameters with exception of sensory analysis the best treatment combination of temperature, time, and biscuit thickness were; 174°C, 9 min, and 4.5 mm. Biscuits sensory score shows variation in terms of colour and crispness. Biscuits baked at low temperature slighter thickness got better overall acceptability. Generally, result of this study confirmed that the possibility of production of gluten free Teff based biscuit as an alternative food source for gluten intolerant or tolerant consumers.

Keywords: Biscuit; Celiac disease; Gluten free diet; Teff; Sensory evaluation

Introduction

Teff (*Eragrostis tef* (Zucc.) Trotter) is a tropical cereal used throughout the world as grain for human consumption and as forage for livestock [1]. Teff grain flour is widely used in Ethiopia for making injera (staple spongy like bread made from fermented dough of Teff flour), sweet unleavened bread, local spirit, porridges, and soups [2]. Hopman et al. [3] and Dekking et al. [4] investigated the presence or absence of gluten in pepsin and trypsin digests of 14 Teff varieties. The digests were analyzed for the presence of T-cell-stimulatory epitopes. In contrast to known gluten containing cereals; no T-cell stimulatory epitopes were detected in the protein digests of all the Teff varieties assayed, thus confirming the absence of gluten in Teff. Because of this and nutritional merits, there is a growing interest on Teff grain utilizations like Quinoa. Due to this Teff has an increasingly important grain for individuals who suffer from gluten intolerance [5], and hence it is recommended as functional food for celiac patients.

Celiac Disease (CD) is a chronic entheropathy produced by gluten intolerance, more precisely to certain proteins called prolamines, which causes atrophy of intestinal villi, malabsorption and clinical symptoms that can appear in both childhood and adulthood [6]. Prolamins of wheat, barley, and rye are characterized by high proline content [7]. These proteins are the main constituents of gluten; contain toxic that can trigger celiac disease. Consequently, inadequate intakes of essential nutrients such as folate and vitamin B_{12} [8] calcium, iron, and fiber have been observed in those with CD [9] due to fear to eat products of those cereal crops. The recommended treatment for those with CD available to date is to follow a strict gluten-free diet [10].

Recent data indicated that the average worldwide prevalence of celiac patient is estimated as high as 1:266 (one out of 266 individuals). In Ethiopia, Celiac disease kills many children each year, mainly because it usually goes undiagnosed and untreated [11]. To alleviate the problem associated with gluten, value added gluten free product diversifications at national and international level are the only way to mitigate the problem. Biscuit produced from Teff could be used by celiac disease patients and those people who need to eat gluten free foods. In addition, there have been numerous studies on the effects of process conditions such as baking temperature, types of oven used and baking time to the final product qualities of biscuit made from wheat flour. However, so far there is no study conducted to investigate effect of baking conditions on physical and nutritional qualities of Teff biscuits. The study aimed to investigate and optimize certain baking processing conditions to produce better quality of Teff based biscuit.

Materials and Methods

Description of the study area

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Post-Harvest Management, Animal nutrition laboratory, and Ethiopian Public Health Institution (EPHI) laboratories for different parameters.

Experimental material collection and preparation

Teff variety used for preparation of biscuits was selected based upon market demand and colour. DZ-01-1681(Keyetena variety) was selected among red Teff varieties. Teff grain was brought from Debrezeit Agricultural Research Center (DZARC). Grain was manually cleaned, milled, and sieved using 0.5 mm sieve and stored in polyethylene bag till further use. Other ingredients like sugar, milk powder, mayonnaise,

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salt and baking powder was purchased from Jimma local market. Egg was obtained from JUCAVM Animal Science Department. Biscuits were prepared according to method (Method No.10.52) described in American Association of Cereal Chemists [12] with minor modification as indicated in Table 1.

Experimental design

The study was conducted in two separate phases. In first phase, optimization study was conducted to determine better baking temperature, time, and thickness of Teff biscuit. To accomplish this, treatment combinations were designed using statistical software package (Design-Expert®, version 6.02, Stat-Ease (Minneapolis USA). Response Surface Methodology Specifically Central Composie Design (CCD) was used to conduct experiment. Sensory analysis was conducted in subsequent phase.

Data collected

Determination of physical and functional properties: Bulk density was determined according to a method indicated in Adeleke and Odedeji [13]. Diameter and thickness were measured by two ways with digital Venire caliper (Fowler, US) and calibrated ruler as described in Ayo et al. [14] and AACC [1]. Rehydration ratio (RR) according to Yu et al. [15], Hardness of biscuit was determined with method of Nath and Chattopadhyaya [16], with stable micro system Texture Analyzer (TA-XT plus, 2012, UK). Water activity (aw) was measured with method of Hematian Sourki et al. [17] using Lab Master aw (Novasina AG, CH-8853 Lachen Sprint Switzerland) water activity meter.

Ingredients	Amount	Percentages (%)
Teff flour (g)	250	52.7
Mayonnaise (g)	45	9.5
Sugar (g)	60	12.7
Salt (g)	2.5	0.56
Baking powder (g)	5	1.08
Milk powder (g)	30	6.38
Whole egg (g)	50	10.6
Deionized Water (ml)	30	6.38

Table 1: The proportion of various ingredients for preparation of gluten free biscuit [56].

Proximate composition analysis: Proximate composition for flours and biscuits were done in duplicate and average value was taken. Moisture content of the samples were determined by air oven (Model: Leicester, LE67 5FT, England) method AOAC (Method 925.10) [18];, Ash (Method 923.03) [18], Crude protein content was determined by Kjeldahl method AOAC (Method 988.05) [18], Ether extract with Soxhlet method (Model: SZC-C fat determinate, China) (Method 45.06) [18], Crude fiber (AOAC method 962.09) [19], Total percentage carbohydrate was determined by the difference as reported in Ponka et al. [20] and gross energy according to Osborne and Voogt [21]. Mineral analyses were accomplished using Atomic Absorption Spectrophotometer (AAS) (AA 6800, Japan) method as per the AOAC method, 985.35 [19].

Sensory evaluation: Sensory analysis was conducted for biscuits baked at optimized baking conditions (baking temperature, time and biscuit thickness). Sensory attributes measured were colour, taste, aroma, crispness, and overall acceptability using a five-point Hedonic scale consisting of 1 (extremely dislike), 2 (dislike moderately), 3 (neither like nor dislike), 4 (like moderately) and 5 (extremely like) [22].

Data analysis

A statistical software package (Design-Expert®, version 6.02,

Run	A	B	C	D (mm)	DT (mm)	BD (g/ml)	Hardness (N)	*aw*	RR (%)	M.C (%)	Ash (%)	CP (%)	EE (%)	CF (%)	CHO (%)	Energy kcal/100g	Fe mg/ 100 g	Ca mg/ 100 g	Zn mg/ 100 g
1	186	6	6.5	53.4	0.3	0.7	85.4	0.6	12.9	6.4	3.6	17.2	14.3	4.0	54.5	415.7	46.6	229.9	6.2
2	186	12	6.5	52.7	0.0	0.7	95.3	0.4	15.4	5.0	3.5	15.6	13.9	4.0	58.2	420.2	45.9	229.1	5.6
3	180	9	3.8	53.0	0.2	0.7	93.1	0.5	15.8	5.3	3.4	15.8	13.8	4.0	57.7	418.4	45.8	229.4	5.9
4	180	9	5.5	53.6	0.4	0.7	92.7	0.5	15.1	5.7	3.5	16.8	14.2	4.1	55.7	418.0	46.2	229.3	5.8
5	190	9	5.5	52.6	-0.1	0.8	96.0	0.4	15.1	4.8	3.5	15.3	13.9	4.0	58.6	420.6	46.0	229.1	5.6
6	174	6	6.5	54.0	0.6	0.7	80.8	0.6	12.1	6.9	3.7	18.1	14.8	4.1	52.4	415.4	47.0	230.7	6.7
7	180	9	5.5	53.5	0.3	0.7	92.6	0.5	15.4	5.8	3.5	16.9	14.3	4.1	55.4	417.9	46.3	229.3	6.0
8	180	9	5.5	53.2	0.3	0.7	92.6	0.5	15.1	5.8	3.6	17.0	14.3	4.0	55.3	418.1	46.3	229.4	5.9
9	180	9	5.5	53.4	0.2	0.7	92.6	0.5	15.2	5.9	3.5	17.0	14.3	4.1	55.3	417.6	46.2	229.3	5.8
10	174	6	4.5	53.8	0.3	0.7	83.8	0.6	14.0	6.0	3.6	17.5	14.4	4.1	54.4	417.5	46.7	230.0	6.5
11	180	9	7.2	53.6	0.5	0.7	86.8	0.6	13.2	6.7	3.5	17.5	14.2	4.0	54.1	414.2	46.3	229.8	6.3
12	180	9	5.5	53.5	0.3	0.7	92.6	0.5	15.1	5.8	3.6	17.1	14.3	4.0	55.2	418.1	46.3	229.6	6.1
13	174	12	6.5	53.4	0.3	0.7	83.8	0.6	13.5	6.2	3.5	17.2	14.2	4.0	55.0	416.2	46.2	229.5	6.0
14	186	12	4.5	52.5	-0.1	0.8	100.2	0.4	16.2	4.2	3.4	14.6	13.6	3.9	60.3	422.2	45.7	228.9	5.5
15	180	14	5.5	52.8	0.0	0.8	92.7	0.4	16.0	4.7	3.4	15.2	13.8	4.0	58.9	420.8	45.8	229.0	5.5
16	174	12	4.5	53.2	0.2	0.7	88.9	0.5	15.6	5.1	3.5	15.9	14.0	4.0	57.6	419.7	46.2	229.4	5.9
17	186	6	4.5	53.4	0.3	0.8	87.9	0.6	13.6	6.0	3.5	16.7	14.2	4.0	55.5	417.0	46.3	229.5	6.0
18	180	9	5.5	53.5	0.2	0.7	92.7	0.5	15.2	5.7	3.6	16.9	14.3	4.0	55.4	418.2	46.3	229.4	5.9
19	180	4	5.5	53.8	0.5	0.7	79.9	0.6	12.3	7.0	3.6	18.0	14.6	4.1	52.7	414.3	46.8	230.0	6.6
20	170	9	5.5	53.8	0.3	0.7	82.8	0.5	13.8	6.0	3.6	17.2	14.4	4.1	54.7	417.6	46.5	229.9	6.4
Pure Teff flour										10.2	2.7	9.5	2.9	4.1	70.6	346.6	44.4	111.0	4.0
Mayonnaise (Herman Soybean oil based)										0	0	1	71.9	0	3.6	653.6	0.5	16.5	0.0
Milk powder (NIDO brand)												24.0	28.2	0	37.4	499.4	460.0	10	4.5
Where: A: Baking temperature; B: Baking time; C: Biscuit thickness before baking; D: Diameter; DT: Difference in thickness; BD: Bulk density; *aw* : Water activity; RR: Rehydration Ratio; MC: Moisture content; CP: Crude protein; EE: Ether extract; CF: Crude fiber; CHO: Carbohydrate																			

Table 2: Baking parameters and response variables for physical; functional and nutritional properties of Teff based biscuit.

(Minneapolis USA) was used to generate test of factor combination for better quality biscuit. Response surface methodology which involves design of experiments, fitting mathematical models and finally selecting levels of variables by optimizing the response [23] was employed in the study. The combinations were obtained based on a CCD. The statistical significance of terms in the regression equations were examined by analysis of variance (ANOVA) for each response and the significance test level was set at 5%. Data obtained from the sensory evaluation of biscuits were analyzed using CRD with three replicates by Minitab version 16.0 statistical software.

Results and Discussion

Effects of baking conditions on physical and functional properties

Diameter difference: Before baking biscuits had equal diameter but after baking their diameter varied from 52.5-54.0 mm with no significance difference. However, when average values are compared, bigger diameter (54 mm) was measured for biscuit baked at 174°C, 6 min, 6.5 mm thick and smaller one (52.5 mm) at 186°C, 12 min, 4.5 mm thick (Table 2). This implies that relatively higher temperature, longer baking time and slightly thick dough contributed less for expansion of the dough during baking. The bigger diameter from commercial point of view is the better quality as indicated Labuschagne et al. [24].

Thickness biscuit difference: Difference in thickness implies the difference in thickness of biscuit after and before baking. Since Teff flour is free from gluten, almost no significant variation was observed on thickness. The value ranged from-0.1 to 0.6 mm as shown on Table 2. The bigger (0.6 mm) difference obtained from biscuit baked at 174°C, 6 min, 6.5 mm thickness and the smaller in reduction (-0.1 mm) from biscuit at 190°C, 9 min, 5.5 mm and 186°C, 12 min, 4.5 mm thickness (Table 2). An increase in thickness of biscuits after baking is one of important features required by commercial producers for an increase in volume of production. However, like diameter of Teff based biscuits, a small increase in thickness of biscuits was observed at relatively lower baking temperature and time. In similar work, Biniyam [25] reported that, the average cookie thickness was not significantly reduced but a decrease in thickness of the cookies was observed as the baking temperature and time were increased. This might be associated with significant decrease in moisture content of biscuits which results in for shrinkage of the thickness.

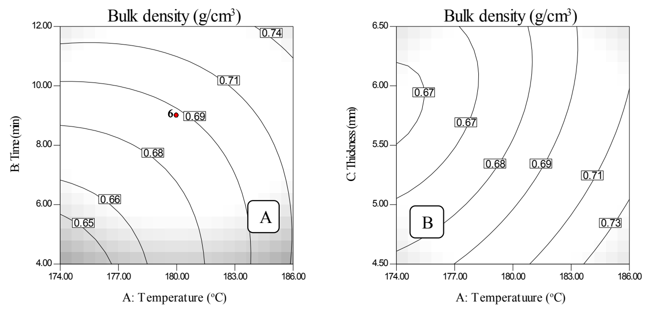

Figure 1: Effects of baking temperature and time (A) baking temperature and thickness (B) on bulk density of biscuits.

Bulk density of biscuit: Bulk density of Keyetena Teff flour was 0.62 g/ml and that of biscuits baked at different conditions showed significant (P<0.01) difference. The results ranged from 0.66 g/ml (186°C, 12 min, 4.5 mm) to 0.77 g/ml (174°C, 6 min, 6.5 mm) (Table 2). Bulk density shows significant (P<0.05) difference as temperature and time interacts as well as temperature and thickness interacts, but time and thickness showed non-significant effect. As indicated in Figure 1A as baking time and temperature increases there is an increment of bulk density on biscuit. Similarly, as indicated in Figure 1B with an increase in baking temperature and decrease in biscuit thickness an increment in bulk density is observed. This implies that a denser packaging material may be required for this type product [26] (Figure 1).

Hardness: Hardness showed highly significant difference in main, quadratic and interaction of model terms at (p<0.05) (Table 3). Result ranged between 80 N (180°C, 4 min, 5.5 mm) to 100 N (186°C, 12 min and 4.5 mm (Table 2). As expected, an increase in hardness with higher temperature, longer bakes time and thinner biscuit. The increase in baking time and higher temperature has shown a decrease in moisture loss of breads [27] and this might be true also for biscuits to have a harder texture due to higher temperature and longer baking time. Biscuits with relatively higher moisture content generally had a softer texture and therefore, there seems to be a good relation between the hardness of the biscuits and their moisture contents. Lower hardness in low temperature and shorter baking time indicates less brittle (but crunchy) biscuits with greater internal cohesiveness and springiness.

Source	D (mm)	D (mm)	Hardness (N)	*aw*	BD (g/cm³)	RR (%)	MC (%)	Ash (%)	CP (%)	EE (%)	CF (%)	CHO (%)	Energy K.cal / 100g	Fe mg /100g	Ca mg /100g	Zn mg /100g
Model	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Int	1.55	0.33	91.12	0.54	0.68	14.78	6	3.56	17.21	14.37	4.05	54.8	417.39	46.35	229.5	6.02
A	-0.31*	-0.103*	3.9***	-0.03**	0.03**	0.28**	-0.28**	-0.04**	-0.54*	-0.16*	-0.02**	1.03**	0.54**	-0.18*	-0.27**	-0.21**
B	-0.4**	-0.18**	3.79**	-0.09**	0.029*	1.74**	-0.87**	-0.07**	-0.99*	-0.3**	-0.04**	2.29**	2.38**	-0.45*	-0.524*	-0.45**
C	0.11*	0.078*	-1.9**	0.037*	-0.013*	-0.70*	0.376*	0.026*	0.40**	0.12**	0.017*	-0.94*	-1.08*	0.143*	0.179*	0.105*
A2	-0.08*	-0.063*	-1.2**	-0.012*	0.010*	-0.30*	-0.139*	-0.008*	-0.2**	-0.03^{ns}	-0.0 1*	0.41**	0.47**	0.014^{ns}	0.064^{ns}	0.027^{ns}
B2	-0.08^{ns}	-0.02^{ns}	-2.3**	0.006*	0.012*	-0.7**	0.020^{ns}	-0.006^{ns}	-0.17*	-0.03^{ns}	-0.02**	0.20*	-0.14^{ns}	0.071^{ns}	0.105^{ns}	0.082^{ns}
C2	-0.04^{ns}	0.025^{ns}	-1.0**	0.011*	0.010*	-0.3**	0.064*	-0.02**	-0.10*	-0.08*	-0.015*	0.15*	-0.53*	-0.04^{ns}	0.101*	0.066*
A*B	-0.07^{ns}	-0.05^{ns}	1.78**	-0.03**	-0.012*	0.36**	-0.262*	0.002^{ns}	-0.19*	0.01^{ns}	-0.02^{ns}	0.44**	1.10**	0.002^{ns}	0.087^{ns}	0.008^{ns}
A*C	-0.03^{ns}	-0.04^{ns}	0.10**	-0.013*	-0.006*	0.32**	-0.10**	-0.004^{ns}	-0.06^{ns}	-0.02^{ns}	0.001^{ns}	0.19*	0.31*	0.039^{ns}	-0.047^{ns}	-0.014^{ns}
B*C	0.03^{ns}	-0.02^{ns}	-0.6**	0.003^{ns}	0.002^{ns}	-0.06^{ns}	0.078*	0.005^{ns}	0.19*	0.003^{ns}	0.005^{ns}	-0.28*	-0.34^{ns}	-0.06^{ns}	-0.110^{ns}	-0.018^{ns}
LoF	0.6468	0.764	0.1816	0.222	0.1152	0.1614	0.2752	0.093	0.1995	0.0699	0.332	0.3406	0.0602	0.1176	0.3178	0.9424
Adj R^2	0.9098	0.879	0.9999	0.9877	0.971	0.9865	0.9922	0.9663	0.9857	0.9491	0.9534	0.9917	0.9756	0.9398	0.9045	0.9289

Where: A: baking temperature; B: baking time; C: biscuit thickness before baking; ns: non-significant difference; DT: Difference in thickness; BD: Bulk density; *aw* : water activity; RR: Rehydration ratio; MC: moisture content; CP: crude protein; EE: Ether Extract; CF: Crude fiber; CHO: Carbohydrate; P>0.05; *P<0.05; **P<0.01 and *** P< 0.001

Table 3: Estimated regression coefficients; degree of significance and lack of fit of parameters for physical; functional and nutritional parameters of biscuit model equations.

When the effects of temperature and time were compared, baking time was more effective for increment of biscuit hardness (Table 2).

But when compared to commercially available wheat based biscuits, Teff based biscuit was a bit harder to break. This might be due to lack of gluten in Teff flour to give the desired textural property related to hardness. In addition to absence of gluten, hardness level might be influenced by high iron and crude fiber contents of Keyetena Teff Umeta and Parker [28] as compared to commercially available wheat flour based biscuits. Similar increase in hardness of cookies was also reported in Singh et al. [29] on incorporation of sweet potato flour in wheat and concluded that an increase in fiber content in the formulation was the reason for variation (Figure 2) (Table 3).

Water activity: Correlating water activity with shelf-life is of critical importance in work with biocontrol formulations [30]. Typical of biscuits is not only low in moisture content but also has low value of aw. Foods with aw <0.60 are considered as microbiologically stable [31]. Water activity of biscuits in this study as indicated on Table 2 ranged from 0.39 to 0.64 with significant effects (P<0.05) in main, quadratic, and interaction of model terms (Table 3). From 20 treatment combinations, 3 of them exhibited a water activity above 0.6 (Table 2) which might not recommended for extended storage life of biscuit. Higher aw was obtained at 174°C for 6 min and 6.5 and lower at 186°C for 12 min and 4.5 mm thickness of biscuit (Figure 3).

The interaction of baking temperature and baking time was highly significant (P< 0.01) on water activity. As indicated in Figures 3A and 3B as baking time and temperature increases there is a decrement of water activity as expected. This is from the fact that application of heat results in the evaporation of the water molecules contributed for less aw value. Similar reports from Bojana [32], showed that increase of baking time and temperature on gluten free biscuits enriched with blueberry pomace with a decreased in aw from 0.6 to 0.326. Similar results also

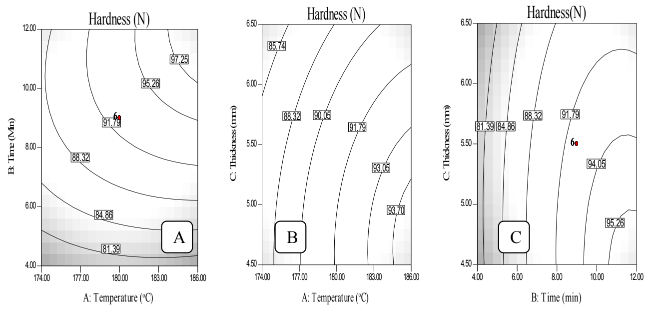

Figure 2:.Effects of baking temperature and time (A) baking temperature and thickness (B) baking time and thickness (C) on hardness of biscuits.

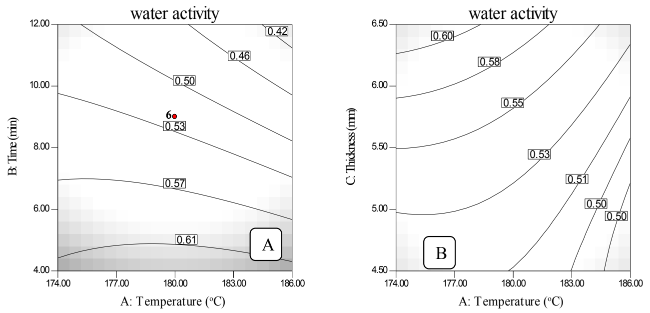

Figure 3: Effects of baking temperature and time (a) baking temperature and thickness (b) on water activity of biscuits.

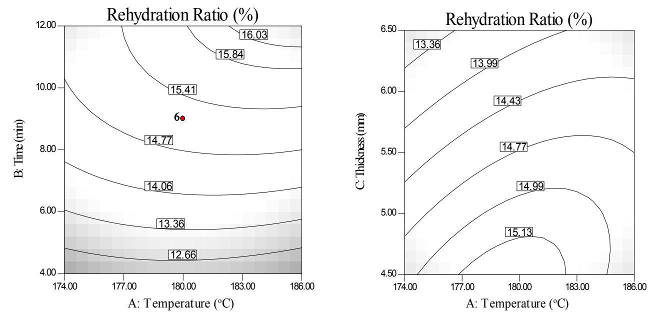

Figure 4: Effects of baking temperature and time (A) baking temperature and thickness (B) on rehydration ratio of biscuits.

reported in Manohar [33], indicated that sample of cereals and fruit-containing biscuits showed a decrease in aw with an increase in baking temperature.

Rehydration Ratio: Rehydration ratio as indicated on Table 2 ranged from 12.10% to 16.15% for biscuits. Higher result was recorded from biscuit baked at 186°C for 12 min at 4.5 mm thickness and lowest value from biscuit baked at 174°C, 6 min, 6.5 mm thickness. Rehydration ratio shows highly significant effect (P<0.01) in linear, quadratic and interaction of model terms. Effect of time and thickness had not a significant effect on rehydration ratio (Figure 4) .

The interaction of baking temperature and time was found to have highly significant (P<0.01) effect (Table 3) on rehydration ratio of biscuit. As indicated by contour line in Figure 4A as baking time and temperature increases there is an increase in rehydration ratio. This may be due to the more moisture removed from samples resulted in the more increase in water holding capacity. Similar result is reported in Mitra et al. [34] an increase in rehydration ratio with an increase in baking temperature and time. The interaction of baking temperature and thickness also exhibited highly significant (P<0.01) effect on rehydration ratio of biscuit (Table 3). As indicated in Figure 4B with decrease in thickness and baking temperature close to 180°C increases rehydration ratio. This might be due to the thinner biscuit with optimum baking temperature the change in microstructure of the biscuit might be in a position to absorb more water during rehydration process.

Nutritional composition of Teff flour based biscuit

Teff flour from different varieties contains almost similar proximate composition [35]. Nutritional compositions of pure Teff flour and other ingredients used for making of biscuits are indicated in Table 2. Proximate composition results obtained for Keyetena Teff variety is close to what is reported in Lovis [36] and Bultosa [37]. Also changes in nutritional composition of biscuits baked at different conditions are indicated in the same table.

Moisture: Moisture is an important parameter in baked foods that significantly affects shelf life and growth of microbial contaminants [38]. Moisture contents for biscuits baked at different conditions resulted in a range of 4.20 (186°C, 12 min, 4.5 mm) to 6.98% (180°C, 4 min and 5.5 mm) (Table 2). Moisture content showed significant effect (P<0.05) in main, quadratic, and interaction of model terms (Table 3). It is apparent that low moisture content is due to relatively high temperature, long time and thinner biscuit thickness as indicated in Figures 5A-5C.

As indicated by contour line in Figure 5A as baking time and temperature increases there is a reduction of moisture content. A similar result was reported in Piergiovanni and Farris [39] in which baking temperature and time was negatively affecting the amaranth

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cookie moisture content. Bojana [32] also showed that increase of baking time and temperature on gluten free biscuits enriched with blueberry pomace showed a decrease in moisture content from 10% to 5.2%. Similar result also reported in Patela et al. [40] and Kotoki and Deka [41], as bread baked at higher time-temperature combinations showed loss more water and the bread become harder and underweight. Figure 5B shows with an increase in baking temperature and a decrease in biscuit thickness, a decrease in moisture content. Baking time and thickness of biscuit have also a significant effect on moisture content as shown in Figure 5C. This might be associated with high rate of heat penetration on thinner biscuit than thicker one (Figure 5).

Ash: Ash content of Keyetena Teff flours was indicated on Table 2. This result is in agreement with Corke et al. [42] on review report indicated that, the ash level in Teff varieties varied from 2.66%-3.00% with typical value of 2.8%. Ciferri and Baldrati, [43] found that ash content of Teff flour ranged between 2.4% and 2.94% . The result for ash in this finding was comparable to the values indicated in Bultosa [13], which was reported in the range of 1.99% to 3.16%.

Results of ash for baked biscuits at different baking conditions are presented in Table 2 in range of 3.38 to 3.65. Ash content was not significantly affected by interaction of baking temperature, time, and thickness. According to Fenema [44] mineral elements, unlike vitamins and amino acids, cannot be destroyed by exposure to heat for a long time and this could be the reason that interaction of factors showed insignificant effects (P>0.05). However, increase in ash content of biscuits as compared to teff flour is associated with additional other biscuit ingredients which is in line with what is reported in Biniyam [25].

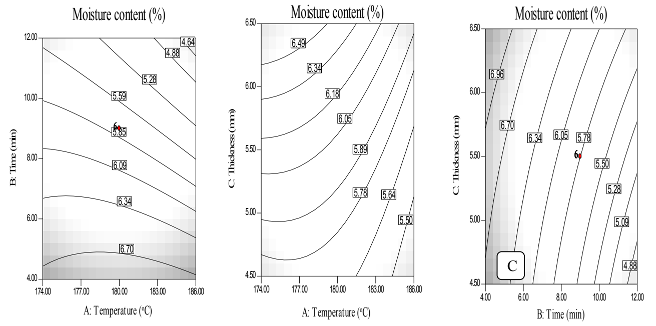

Figure 5: Effects of baking temperature and time (A) baking temperature and thickness (B) baking time and thickness (C) on moisture content of biscuits.

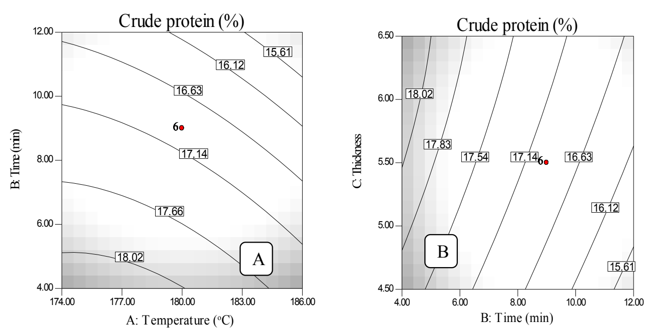

Figure 6: Effects of baking temperature and time (A) baking time and thickness (B) on moisture content of biscuits.

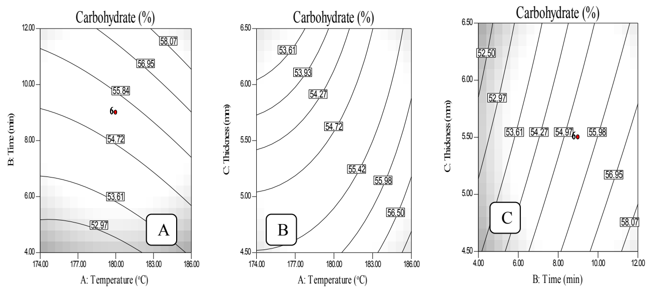

Figure 7: Effects of baking temperature and time (A) baking temperature and thickness (B) baking time and thickness (C) on carbohydrate content of biscuits.

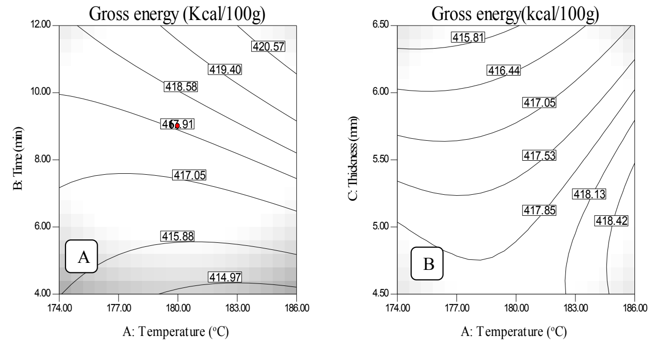

Figure 8: Effects of baking temperature and time (A) baking temperature and thickness (B) on gross energy of biscuits.

Crude protein: The statistical results regarding crude protein content of different biscuits baked at different conditions are presented in Table 2. The protein content of the biscuits significantly affected by main, quadratic and interaction of model terms at (P <0.05) as indicated on Table 3. The value varied from 14.59 to 18.14% this showed a significant increase as compared to Teff flour which might be due to effect of milk powder and mayonnaise added as an ingredient. Among the values, biscuits baked at 174°C, 6 min, and 6.5 mm thickness resulted in higher protein value than baked at 186°C, 12 min, and 4.5 mm thickness. This shows that, the higher the temperature, longer baking time and the thinner biscuit resulted in the reduction of crude protein content as indicated in Figures 6A and 6B.

Interaction effects of baking temperature and time as well as time and thickness have highly significant (P<0.01) effect on crude protein content. As indicated in Figure 7A as baking time and temperature increases crude protein content declines. This might be associated that, during baking, the physical and chemical properties of protein are altered due to the denaturation of protein where in the hydrogen bonds and non-polar hydrophobic interactions of the secondary and tertiary structures of proteins are disrupted by heat and the soluble amino acids leached out in the baking medium [45]. According to Hui et al. [46], the Maillard reaction, is also a responsible chemical reaction for the loss of protein due to reaction of free amino acids with reducing sugars (glucose and fructose) is favored at temperatures above 120°C. The extent of loss is increased by higher temperatures, longer baking time, and availability of larger amounts of reducing sugars [45].

Ether extract: Ether extract of flour is indicated in Table 3 with a value 2.93%. The ether extract value was comparable with those of Corke et al. [42], reported that the Ether extract content of Teff flour ranged from 2-3% with mean of 2.3%. Results presented in Table 2 indicated that the ether extract content of biscuits were not significantly (P<0.05) affected by interaction of model terms but linear terms had a significant effect (Table 3) and values ranged between 13.63 to 14.80 %. Usha [47] categorized biscuits in terms of their fat content as low

(7.5-15%), medium (15-27%), and high (more than 27%) fat biscuits. According to the author, Teff based biscuits baked under this study conditions are categorized in low fat biscuits. But when ingredient compositions are considered, similar to enhanced crude protein value of biscuits, addition of fat reach ingredient significantly enhances the ether extract of biscuits as compared to Teff flour as a major component. Higher ether extract measured for biscuits baked at relatively lower temperature and shorter time of baking, but when heating increased loss of ether extract observed. This finding was also in line with Fenema [44], high temperature heating trigger polymerization of fat molecules which contributing to its loss during baking, drying, and boiling. But when the effects of baking temperature and time were compared, baking time was found to be more effective on fat loss. This finding also in line with Biniyam [25] on fat content of cookies from wheat, quality protein maize and carrot composite flour which is ether extract was not significantly influenced by baking temperature.

Crude fiber: Fiber content of the Teff four is presented in Table 2. Relatively higher crude fiber content is reported for Keyetena Teff flour as compared to what are reported in Lovis [36] and Corke et al. [42], they reported that 3% and 3.8% respectively. However, the crude fiber of the final biscuit varied from 3.93 to 4.08 %. Higher fiber content was recorded from biscuit baked at 174°C for 6 min 6.5 mm thickness and lowest from 186°C for 12 min having 4.5 mm thickness. Fiber content significantly affected by baking temperature and biscuit thickness in both main and quadratic terms as indicated in Table 3. Lee et al. [46] and Bingham [48] reported that dietary fiber has a beneficial effect on bowel transit time, affects glucose and lipid metabolism, reduces the risk of colorectal cancer, and stimulates bacterial metabolic activity. Even though there is no report on incorporation of dietary fiber on gluten free biscuit, as indicated in this result, incorporation of Teff flour enhance fiber content of biscuits since other ingredients lack fiber.

Utilizable carbohydrate: Utilizable carbohydrates content of Keyetena Teff flours is presented in Table 2, 70.56%. This is lower than the values indicated in Lovis [35] (73.0%) and comparable with the value of 71.4% USDA [49]. Utilizable carbohydrate values of biscuits in this study varied from 52.4 to 60.25%. The decrease in carbohydrate could be associated with the addition of other biscuit ingredients high in protein and fat contents. Higher carbohydrate value obtained from biscuit baked at 186°C for 12 min having thickness of 4.5 and lowest value at 174°C for 6 min having 6.5 thicknesses (Figure 7).

Baking temperature and time significantly ($P<0.01$) affected carbohydrate content of biscuit Figure 7A with an increase in baking time and temperature carbohydrate content of biscuits increased. Since utilizable carbohydrate calculated by difference from other proximate compositions, the higher the other proximate compositions resulted in the lower carbohydrate value in similar baking condition. Furthermore, an increase in carbohydrate might also associate with starch degradation into dextrin and simple sugars [50]. Baking temperature and thickness has significant ($P<0.05$) effect on carbohydrate content of biscuit. As indicated in Figure 7B as baking temperature increased and biscuit thickness decreased there is an increase in carbohydrate content. Baking time and thickness effect was also significant ($P<0.05$) on carbohydrate content of biscuit as indicated in Figure 8.

Gross energy: Initially the gross energy was 346.61 kcal/100 g in Keyetena Teff flour. But an increase in gross energy was observed on biscuit in range of 414.22-422.15 kcal/100 g. The amount of calorie in the final product showed significant difference ($P<0.05$) in linear, quadratic and interaction model terms (Table 3). Higher calorie obtained from biscuit baked at 186°C, 12 min, and 4.5 mm thickness and lower value for biscuit baked at 180°C, 4 min, and 5.5 mm thick.

Interaction effect of baking temperature and time was highly significant ($P<0.01$) on calorie value of biscuit. As indicated in Figure 8A as baking time and temperature increases there is an increase in energy content. Since the calorie of biscuit was calculated from value of protein, ether extract, and carbohydrate with their conversion factor, higher carbohydrate value played the role for increment of energy content as baking time and temperature increases. Baking temperature and thickness was found to have highly significant ($P<0.01$) effect on gross energy content of biscuit. As indicated in Figure 8B as baking temperature increases and biscuit thickness decreases there is increment of energy content in keyetena Teff biscuit. This could be explained in line with justification provided in above points (Figure 8).

Minerals content

Iron, Calcium and Zinc content of Keyetena Teff flour are 44.4, 110.95 and 3.95 mg/100 g (Table 2). Due to addition of ingredients the minerals content of biscuits increased as compared to Teff flour. Increase in iron content was small as compared to calcium and zinc. However, increase in calcium content in biscuits is almost double as compared to Teff flour content (Table 2). This indicates that, in addition to get functional benefits of the ingredients, the mineral content of Teff based biscuit can be improved significantly. When effect of baking temperature, time and thickness are considered they showed significant effects mainly in the linear terms (Table 3) and with little or no quadratic and interaction effects. This implies that, baking parameters have additive effect on mineral contents. Result if this work is also in agreement with Fenema [44], mineral elements, unlike vitamins and amino acids cannot be destroyed by exposure to heat for a long time.

Optimized processing variables for better biscuit quality

Numerical optimization conducted to select baking conditions for better physical, functional and nutritional value of biscuit. Based up on desired target values of physical, functional and nutritional variables, biscuits having 4.5 mm thickness and baked at 174°C for 9 minutes resulted in best baking condition for best combination of biscuit qualities. Optimized values of Keyetena based biscuits for physical parameters like difference in diameter, hardness, water activity, bulk density, and water rehydration capacity are close to 1.65 mm, 86.89 N, 0.52, 0.7 g/m^3 and 19.6 ml respectively. Optimized proximate composition for moisture, ash, protein, ether extract, fiber, carbohydrate, and energy were; 5.81%, 3.65%, 17.8%, 14.3%, 4.04%, 55.15%, and 418.2 kcal/100 g respectively. For mineral elements iron 46.22, calcium 230.0, and zinc 6.18 mg/100 g were optimize results respectively.

Sensory evaluation

Sensory qualities are the main criteria that make the product to be liked or disliked by consumers [51]. Sensory evaluation was conducted after overall optimization of baking conditions for better physical, functional and nutritional quality parameters of biscuits (174°C, 9 min and 4.5 mm thickness). One of the major problems associated with production of gluten-free products is their inferior taste and/or structure. But the mean score value of biscuit samples for taste was 4.26 ± 0.09 (like moderately). This might be due to dominant effects of ingredients added (47.3%) in biscuit making. In agreement to this work, Gallagher et al. [52] also indicated that, Teff flour has a good taste than other gluten free cereals and hence allows an opportunity to produce gluten-free product with an attractive taste.

Color is one of the detrimental sensorial property significantly influences customers purchase decision. The color preference score ranged was 2.74 ± 0.19 which is in between dislike moderately to neither like nor dislike. The lower color value is might be due to dark color of biscuit because of red Teff color. This might be due to higher levels of pigmented material, such as tannins and polyphenols [28] in Keyetena Teff flour and Maillard reaction during high temperature baking. The aroma is one of the main sensorial properties for acceptability of biscuits. The average score value of panelist was 4.18 ± 0.02. This indicated that, baked biscuits were moderately liked by the panelist. This might be due to enhanced interaction and distinctive flavor of Teff flour together with ingredients interaction created during baking. Furthermore, aroma was mostly influenced by the reaction occurred between cross linking of Teff starch gelatinization and protein denaturation.

Biscuit lovers prefer to eat crispy biscuits since it is one of desirable textural properties. Average score value of crispness test of biscuit was 3.95 ± 0.10, which indicates that, panelist moderately liked the product. Hough and others [53] indicated that, dried or baked products loss their crispness when their water activity value greater than 0.5 ± 0.2. However, the water activity value for this study was above this value for better crispness. This might be associated with peculiar properties of Teff flour (high iron content and absence of gluten) for better crispness even at higher water activity value than wheat flour based biscuits [54-56].

The combination of the above sensorial parameters can be generalized or summarized by rating the overall acceptability of the product. Panelist's response to products is, of course, liking or acceptability to a certain degree. The overall acceptability of Teff based biscuits baked at optimized conditions was 3.98 ± 0.07. This implies that the biscuit was moderately liked by the panelists. Therefore, Teff based biscuit produced with the addition of required ingredients, not only good gluten free product in terms of other evaluated parameters, but also has good overall acceptability by the panelists.

Conclusion

The only effective treatment for celiac disease is the total lifelong avoidance of gluten ingestion or using a strict gluten-free but nutritious diet. Teff flour is an interesting gluten free raw material and considered as the major ingredient to produce gluten free biscuit. The study showed that, mainly higher baking temperature with longer baking time resulted in inferior biscuit quality. The sensory score lowered as baking temperature, time, and thickness increases. However, biscuits baked at relatively thickness of 4.5 mm, lower temperature of 174°C for 9 min, provided better quality product in terms of physical, functional, nutritional as well as sensorial properties. The out comes from this study would be used to generate baseline information for subsequent use and studies to produce nutrient enriched gluten free products from Teff flour. Many gluten-free products may not meet the recommended daily intake for fiber and minerals. However, gluten free biscuit made from Teff based flour under recommended baking condition will mitigate such limitations and provide alternative snack food source for gluten intolerant individuals.

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