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ORIGINAL RESEARCH PAPER

QUALITY AND TEXTURAL PROPERTIES EVALUATION OF GLUTEN-FREE BISCUIT DEVELOPED FROM MAIZE, RICE, BUCKWHEAT, AND SOYBEAN

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Abstract: The research aims to develop a gluten-free biscuit from composite flour of maize, rice, buckwheat, and soybean by replacing wheat flour. Biscuits were prepared from four formulations of maize (40 parts), rice (20-35 parts), buckwheat (5-20 parts), and soybean (10 parts). The chemical, physical, textural, and sensory characteristics of the prepared biscuit were analyzed and compared with the control biscuit (wheat flour only). The fat (%), crude fiber (%), ash (%), and iron (mg%) of the formulated biscuits were significantly higher ($p < 0.05$) than the control biscuit, whereas phosphorous (mg/100 g) was found to be similar ($p < 0.05$). The spread ratio of all the formulated biscuits was significantly lower ($p < 0.05$) than the control biscuit. The hardness of formulated biscuits was found to be similar ($p < 0.05$) to the control biscuit, whereas the fracturability of the control biscuit was significantly higher ($p < 0.05$) than prepared gluten-free biscuits. The preference was high for the control biscuit than for gluten-free biscuits, but no formulations were found inferior based on sensory evaluation. The research concludes that other cereals and legumes excluding wheat have a good potential for the preparation of gluten-free biscuits.

Keywords: *fracturability, hardness, protein, spread-ratio*

INTRODUCTION

Biscuits are one of the fastest-growing industries due to their convenience to use and consume, yet customers are seeking both tastier and healthier alternatives [1]. There are many initiatives to make biscuits healthier by adding other cereals to increase protein, fiber, and minerals [1, 2]. Additionally, an attempt has also been undertaken to increase protein content with good amino acid balance, boost fiber content, and bioactive components by adding fruit or its by-product. The gluten content of wheat flour varies from 6 - 11 %. The rheological properties of dough are influenced by gluten content, so baked products, and noodles are prepared by using wheat flour [3, 4].

Celiac disease has affected 1 % of the total population, among which 83 % of patients are undiagnosed. The remedy for celiac disease is avoiding gluten-free diets and preventing cross-contamination with gluten-containing products [5]. The malabsorption of nutrients due to damage to intestinal villi by gluten is a major disorder associated with patients having celiac disease [6]. As reviewed by Di Cairano *et al.* [7], biscuits play an important role in the diet of people suffering from the celiac disease compared to bread.

Different researchers have tried different formulations to prepare the low glycemic index gluten-free biscuits Di Cairano *et al.* [7], however, we choose the ingredients based on the easy availability of ingredients. The goal of the study was to explore maize, buckwheat, rice, and soybean to prepare composite flour and to use in place of wheat flour for biscuit making. Buckwheat flour consists of natural antioxidants (rutin-a flavonoid), is good for health, and also inhibits lipid peroxidation. Soybean consists of 35-40 % protein and complements the limiting amino acid in the cereals. Besides that, it improves the rheological properties of dough by increasing water holding capacity. This improved the sheeting strength and gives a better finish [8]. Rice has a mild taste and is colorless, and is hypoallergic, so it is a suitable ingredient for the formulation [9]. Maize flour acts as a thickener and binder in bakery items [10].

Rai and Kaur [11] have investigated the preparation of gluten-free cookies from maize, sorghum, rice, and pearl millet. The research revealed that gluten-free cookies prepared from maize and millet have good pasting properties while combinations of rice and maize have a good spread ratio. However, a combination of sorghum and pearl millet was found to be acceptable based on sensory evaluation. Nedeljkovic *et al.* [12] found that 30 % of buckwheat flour incorporation has the highest antioxidant activities, confirming buckwheat as a good source of polyphenols that improves the nutritive value of biscuit. More *et al.* [13] also prepared gluten-free biscuits from a combination of white rice flour and brown rice flour with a good sensory score. Man *et al.* [14] reveals that the incorporation of soy flours up to 40 % improve the sensory and nutritive value of gluten-free biscuit. In major of the research was carried out to prepare gluten-free biscuits rice flour and maize flour were major ingredients.

There has been limited research in developing countries to prepare gluten-free baked products. The research will support the design of the product from composite flour, which is gluten-free and healthier due to increased fiber, minerals, and antioxidant properties. The combination of legumes and cereals will help to achieve a more balanced amino acid composition. The physical property (spread ratio) will help to identify the volume expansion of the baked products influenced by the raw materials.

The texture profile analysis will help to understand the effect of these ingredients on the texture profile of biscuits.

MATERIALS AND METHODS

Materials

Maize (*Rampur composite*), rice (*Khumal 4*), and soybean (*puja*) were collected from National Agronomy Research Centre, Nepal Agricultural Research Council. The buckwheat flour was procured from a local market. The formulation of multigrain flour is shown in Table 1. Other ingredients required for making biscuits as shown in Table 2 were procured from the local market of Kathmandu. Rice, buckwheat, soybean, and maize flour was sieved through mesh size (0.250 mm) after milling in a grinder.

Table 1. *Proportions of Wheat, maize, rice, soybean, and buckwheat flour*

Sample	Maize flour [g]	Rice flour [g]	Soybean flour [g]	Buckwheat flour [g]	Wheat flour [g]
T-1	746.64	746.64	746.64	746.64	-
T-2	653.32	559.96	466.64	373.32	-
T-3	186.68	186.68	186.68	186.68	-
T-4	93.36	186.72	280.00	373.36	-
Control	-	-	-	-	1680

Table 2. *Ingredients used in the preparation of biscuits*

Ingredients	T-1 [g]	T-2 [g]	T-3 [g]	T-4 [g]	Control [g]
Maize Flour	746.64	746.64	746.64	746.64	-
Rice Flour	653.32	559.96	466.64	373.32	-
Soybean Flour	186.68	186.68	186.68	186.68	-
Buckwheat Flour	93.36	186.72	280.00	373.36	-
Refined wheat flour	-	-	-	-	1680
Maize starch	120	120	120	120	120
Sugar	720	720	720	720	720
Butter	600	600	600	600	600
Guar Gum	30	30	30	30	30
Salt	12	12	12	12	12
Baking Powder	60	60	60	60	60

Methods

Biscuit making process

For biscuit making, all the required ingredients were mixed for 15 minutes manually and the prepared dough was rested for 15 minutes. A biscuit cutter was used to cut the dough sheet into a circular shape and was placed in a greased tray. The tray was placed in a preheated oven and baked at 180 °C for 30 minutes. The flowchart for the preparation of biscuits is shown in Figure 1.

Preparation of composite flour (maize, rice, soybean, and buckwheat flour)
↓
Mixing
↓
Resting (15 minutes)
↓
Molding
↓
Baking (at 180 ^{0}C, 30 min)
↓
Cooling at room temperature
↓
Packaging (LDPE)

Figure 1. *Flow chart for gluten-free biscuits preparation*

Chemical analysis of biscuits

Moisture, fat, protein, ash, and crude fiber were determined by the method as described by AOAC [15]. For the moisture content, biscuit samples were dried in a hot-air oven at 105° to constant weight as described by method AOAC, method number 930.15. The protein content of the biscuit was determined by calculating the nitrogen content by Kjeldahl's method as described by AOAC, method number 920.152, and multiplying the nitrogen by 6.25. A solvent (petroleum ether) extraction procedure was used to determine the fat content of the biscuit as described by AOAC, method number 991.36. The crude fiber f biscuit was determined by using a crude fibre analyzer and muffle furnace and ash content was determined by using a muffle furnace as described by AOAC, method number 934.01, and AOAC, method number 945.46 respectively.

The iron content and phosphorous content of biscuits were measured by the method described by Ranganna [16]. For iron content, the absorbance of red ferric thiocyanate was measured at 480 nm (Cary UV-Vis spectrophotometer, Agilent, USA). For phosphorus content, the absorbance of reduced phosphomolybdate was measured at 650 nm in a UV-vis spectrophotometer (Cary UV-Vis spectrophotometer, Agilent, USA).

Physical analysis of biscuits

The spread ratio of biscuits was calculated by dividing the average value of diameter (diameter of 6 biscuits was measured by vernier caliper) by the average value of thickness (thickness of 6 biscuits was measured by vernier caliper) [17].

Texture analysis of biscuits

Biscuit hardness and fracturability were measured by using a TA.XTPlus texture analyzer (Stable Micro Systems, Great Britain) equipped with a 2 mm cylinder Probe (P/2) (HDP/90) using a 5 kg load cell and heavy-duty platform. The test speed was set to 0.5 $mm \cdot s^{-1}$ and the trigger force was auto - 5 g. Peak positive force was recorded as the hardness value and Peak force was observed as the fracturability value.

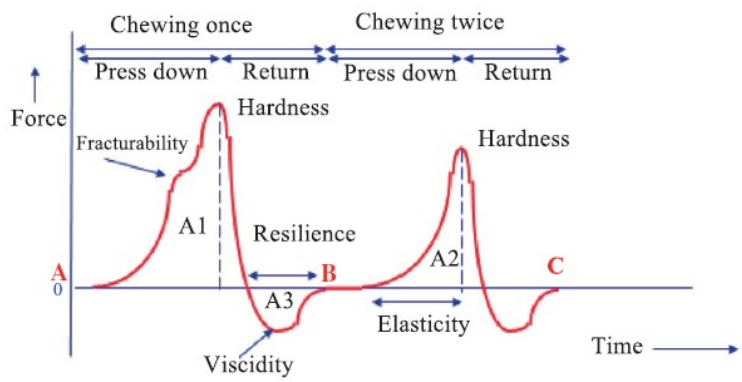

Figure 2. *The texturometer curve*

The hardness is represented by the peak value of force while the force value (fracture in the first compression curve) is fracturability [18].

Sensory evaluation of biscuit

The method described by Ranganna [16] was used to conduct a sensory evaluation. The parameters evaluated were color, taste, texture, and overall acceptance by 20 panelists.

Research design and data analysis

There were five treatments with a completely randomized design and a triplicate analysis was carried out for each parameter. Three lots (1074 g for each lot) of biscuits were prepared for each treatment. The data produced from chemical analysis, physical analysis, and textural analysis were analyzed by one-way Analysis of variance (ANOVA) using SPSS at a 5 % level of significance. The significant differences between them were studied by using Tukey HSD at a 5 % level of significance. The data of sensory evaluation were presented by spider plot and tabulated value reproduced from Kramer's rank-sum test (shown in Table 3) as described in Ranganna [16].

Table 3. *The tabulated value of upper pair and lower pair for 5 treatments and 20 panelists*

	At a 5 % level of significance
Upper pair	45-95
Lower pair	50-70

RESULTS AND DISCUSSION

Chemical composition of biscuits

Four formulations of biscuits and control biscuits (made only from wheat flour) were prepared and subjected to chemical, physical, texture, and sensory evaluation. The chemical analysis of biscuits is shown in Table 4.

There was no variation in moisture (%) of all the formulations and control biscuits. The fat (%), ash (%), and crude fiber (%) of all the formulations were significantly higher ($p < 0.05$) than the control biscuit. However, among the formulations, the protein content of formulations (T-3 and T-4) was significantly higher ($p < 0.05$) than formulations (T-2 and T-1). The formulation (T-4) has higher fat ($p < 0.05$) content compared to the formulation (T-1), whereas there was no significant difference ($p < 0.05$) among other formulations. There was no significant difference ($p < 0.05$) in ash and crude fiber among all the formulations. The phosphorous (mg/100 g) of all the formulations and control were not significantly different ($p < 0.05$). The iron content of formulations (T-2, T-3, and T-4) was significantly higher ($p < 0.05$) than control biscuits, whereas among the formulations the formulation (T-4) has significantly higher iron than formulations (T-1 and T-2), and the formulation (T-3) has significantly higher iron than formulation (T-1).

Table 4. *Chemical composite of prepared biscuits*

Biscuit	Control	T-1	T-2	T-1	T_4
Moisture [%]	1.1±0.02^{b}	1.15±0.05^{b}	1.27±0.01^{b}	1.47±0.22^{b}	1.34±0.1^{b}
Protein [%]	7.21±0.04^{a}	6.76±0.04^{c}	6.93±0.08b^{c}	7.30±0.152^{ab}	7.60±0.152^{a}
Fat [%]	16.13±0.06^{c}	19.10±0.07^{b}	19.60±0.51^{ab}	20.14±0.22^{ab}	20.97±0.42^{a}
Ash [%]	2.68±0.32^{b}	3.58±0.14^{a}	3.88±0.03^{a}	4.02±0.03^{a}	4.07±0.06^{a}
Crude fiber [%]	0.73±0.1^{b}	1.40±0.19^{a}	1.763±0.11^{a}	1.91±0.1^{a}	2.15±0.33^{a}
Phosphorous [mg/100 g]	100.33±7.08^{a}	98.30±8.80^{a}	99.81±3.73^{a}	93.26±4.07^{a}	94.84±5.95^{a}
Iron [mg/100 g]	2.00±0.286^{d}	2.69±0.03^{cd}	3.16±0.23^{bc}	4.04±0.20^{ab}	4.46±0.33^{a}

*Values followed by the same letter in columns are not significantly different at MSD at ($p \leq 0.05$)
**Each value was an average of three determinations ± standard error of the mean
***All parameters are on a dry basis except moisture.

Protein (%), fat (%), ash (%), crude fiber (%), and iron (mg/100 g) increase with an increasing ratio of buckwheat flour to rice flour. This might be due to the higher nutrient content in buckwheat compared to rice [19].

The moisture content of biscuits may range from 1 - 4.5 % [20], and as per the mandatory standard published by DFTQC [21], the maximum allowable moisture content in the biscuit is 6 %. As reviewed by Nogueira and Steel [22], the protein content of commercial biscuits ranges from 7-10 %. The higher protein content in biscuits is associated with greater hardness [23]. The fat percentage of commercial biscuits may range from 16-26 % depending upon the type [24]. The fat makes the biscuit crisper, less hard, and melts easily in the mouth, by insulating the gluten-forming protein. The crude fiber content in formulated biscuits is higher due to the high crude fiber in buckwheat, and soybean, compared to refined wheat flour [13]. Canalis *et al.* [25] reported that the quality of biscuits depends upon fiber percentage, composition, types, and sources. The protein (%), fat (%), and other compositions of biscuits depend upon the nutritional composition of raw materials [24].

Physical analysis of biscuit

Physical characteristics of biscuits formulated as treatment control, T-1, T-2, T-3, and T-4 as thickness, diameter, and spread ratio are presented in Table 5.

The spread ratio of the control biscuit was significantly greater ($p < 0.05$) than in other formulations. Among the treatment, increased buckwheat flour decreases the spread ratio of biscuits. The spread ratio of biscuits (formulation T-3 and T-4) was significantly lower ($p < 0.05$) than treatment T-1. The spread ratio of biscuits is affected by protein quality, crude fiber, and their water absorption characteristics [26, 27].

Table 5. *Physical analysis of biscuit*

Parameter	Control	T-1	T-2	T-3	T-4
Diameter [mm]	56.63±0.24^{a}	53.73±0.788^{b}	53.65±0.180^{b}	52.76±0.176^{b}	50.46±0.240^{c}
Thickness [mm]	9.91±0.297^{b}	10.44±0.159^{b}	11.24±0.0724^{a}	11.35±0.131^{a}	11.29±0.135^{a}
Spread ratio	5.73±0.186^{a}	5.15±0.121^{b}	4.77±0.021^{bc}	4.65±0.066^{c}	4.47±0.046^{c}

*Values followed by the same letter in columns are not significantly different at MSD at ($p < 0.05$)
**Each value was an average of three determinations ± standard error of the mean.

An increase in viscosity due to water absorption by a protein of other flours (composite flour) and increased hydrophilic sites decrease the spread ratio [28, 29]. Balijeet *et al.* [17] reported an increase in buckwheat flour and a decrease in rice flour in the formulation decreases the diameter of the biscuit. The spread ratio is an important parameter to access the quality of a biscuit, and good quality biscuit is characterized by a high value of spread ratio [30, 31].

Texture Profile of Biscuit

The texture profile of control biscuits and other formulated biscuits is shown in Table 6.

Table 6. *Texture profile of prepared biscuits*

Parameter	Control	T-1	T-2	T-3	T-4
Hardness [N]	33.91±5.39^{ab}	34.52±7.12^{ab}	22.05±2.98^{a}	32.29±5.79^{a}	47.54±3.8^{b}
Fracturability [N]	33.91±5.39^{a}	24.04±2.48^{b}	14.47±1.13^{c}	25.51±0.97^{b}	25.37±1.31^{b}

*Values followed by the same letter in columns are not significantly different at MSD at ($p < 0.05$)
**Each value was an average of three determinations ± standard error of the mean.

The hardness of the biscuit (T-4) was found to be significantly higher ($p < 0.05$) than biscuit (T-2 and T-3). The fracturability of the control biscuit was found to be significantly higher ($p < 0.05$) than all the formulated samples, while among the formulation, the biscuit (T-2) required significantly low force ($p < 0.05$) for fracture. The texture chart of control and formulated biscuits is shown in Figures 3a, 3b, 3c, 3d and 3e.

The hardness of a biscuit depends upon moisture, fat content, and fiber content [32 - 35]. The hardness of all the formulated biscuits was similar to the control biscuit. As reviewed by Chung *et al.* [36], changes in the structure of starch and degradation of macromolecules form a weaker matrix, which reduces the hardness. Consumers relate the hardness of biscuits with their freshness [37]. As reviewed by Kuchtova *et al.* [38], the hardness and fracturability of biscuits also depend upon the gluten content of the biscuit.

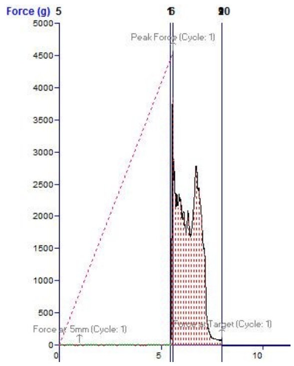

Figure 3a. *Texture chart of a control biscuit*

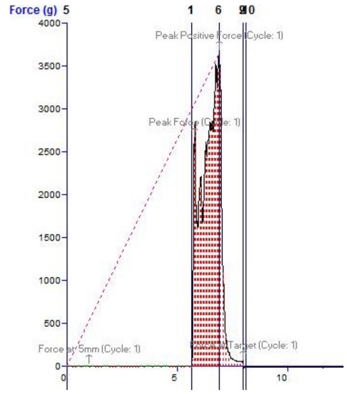

Figure 3b. *Texture chart of a formulation T-1 biscuit*

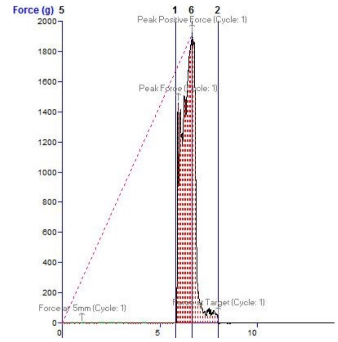

Figure 3c. *Texture chart of a formulation T-2 biscuit*

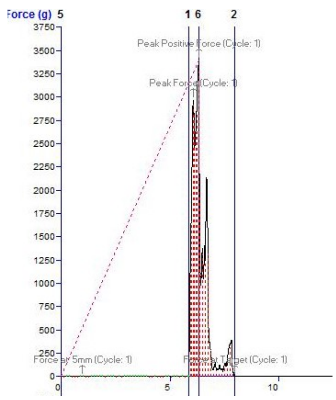

Figure 3d. *Texture chart of a formulation T-3 biscuit*

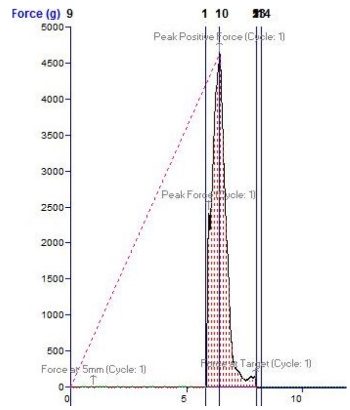

Figure 3e. *Texture chart of a formulation T-4 biscuit*

Sensory analysis of biscuit

The sensory evaluation of the biscuit was carried out to evaluate the organoleptic properties of the biscuit. The result of sensory evaluation is shown in figures 4a, 4b, 4c and 4d. The rank sum of taste falls within the upper pair and lower pair (table 3), so formulated samples were not significantly different ($p < 0.05$) than the control biscuit. For color, the rank sum of the control biscuit and formulations (T-1 and T-3) was below the lower limit of the upper pair, so they were significantly different ($p < 0.05$) from other formulations (T-2 and T-4). Similarly, for hardness and overall acceptability, the rank sum of the sensory score for control biscuits was lower than the lower limit of the upper pair, so was significantly different ($p < 0.05$) from other formulations (Table 3). Also, the control biscuit was significantly superior ($p < 0.05$) compared to other formulations based on hardness and overall acceptability when compared with a lower limit of lower pair (Table 3).

It was observed that an increase in buckwheat flour in the formulations decreased the perception of biscuits based on taste and overall acceptability. The control biscuit was liked by panelists and can be correlated to hardness and fracturability (Table 6). As reviewed by Cairano *et al*. [39], the sensory perception of biscuits depends upon the ingredients used, and the major difference was due to the legumes used in the formulation for gluten-free biscuits compared to wheat flour biscuits. Laguna *et al.* [40]

reported a decrease in sensory perception of biscuits due to the incorporation of maize flour.

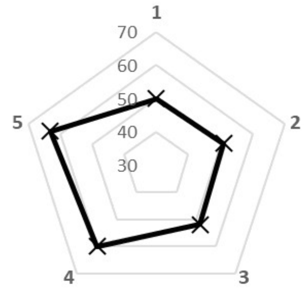

Figure 4a. *The sensory score for the taste of biscuits*

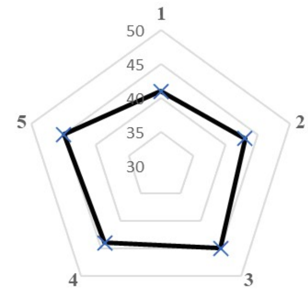

Figure 4b. *The sensory score for the color of biscuits*

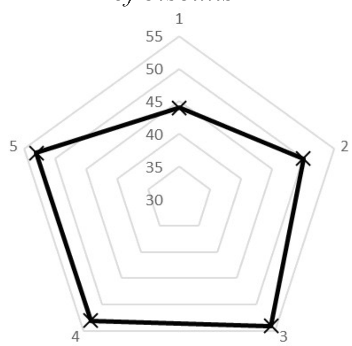

Figure 4c. *The sensory score for the hardness of biscuits*

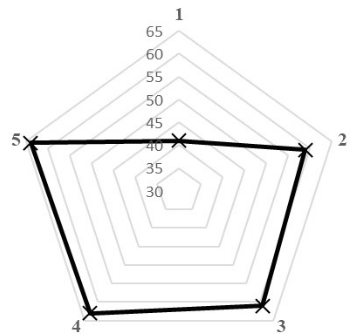

Figure 4d. *The sensory score for the overall acceptability of biscuits*

**1 = control biscuits, 2 = formulation T-1, 3 = formulation T-2, 4 = formulation T-3, 5 = formulation T-4*

CONCLUSIONS

The research attempt to highlight the potentiality of gluten-free biscuit formulated from maize, soybean, rice, and buckwheat flour. The research compares the nutritional quality of gluten-free biscuits with a biscuit made only from wheat flour. The result concludes that the gluten-free biscuit was better in terms of nutritional value (Fat, crude fiber, and minerals) than the control biscuit (wheat flour). However, the physical, textural, and sensory properties of the control biscuit were better than formulated biscuits. The inference can be drawn that there is a possibility for the development of gluten-free biscuits on a commercial scale, however, further research needs to be carried out to improve the physical, textural and sensory properties of gluten-free biscuits. Further underutilized crops like proso millet, foxtail millet, amaranth, etc. can be utilized for the preparation of gluten-free biscuits.

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