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Unveiling the Proximate, Sensory, Microbial, And Shelf Life of Leavening Agent modified Gluten-Free Cookies Formulated with Indigenous Nepalese Hill Grains

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Abstract

The prevalence of gluten-related disorders has risen due to the increased consumption of bakery products made from refined flour in recent decades. This study focused on employing indigenous hill grain flours, such as Taichung 176 rice flour, maize flour, sweet buckwheat flour, and finger millet flour from Nepal, to create gluten-free cookies by incorporating fungal αamylase, baking powder, and baker's yeast as modified leavening agents, in contrast to the typical refined flour-based cookies. 16 different composite flours were made by combining millet, maize, buckwheat, and glutinous rice flours in a 75:25 percentage, then subjected to 30°C proofing for 1 hour for food grade fungal α-amylase with an improver and baker's yeast treated sample before blending with cream and baking for 20 minutes at 200°C.The ash content, moisture content, sensory qualities, microbial load, and shelf life of cookies were studied and compared with control cookies. Over 60-days storage period, most formulated cookies had moisture content below 5%, except BWBY cookies (6%to 8.37%). The ash percentage of cookies significantly increased from 0.81% to 2.59%. The highest ratings for taste (8.0), texture (7.6), and crunchiness (8.1) were achieved for cookies produced with 75:25% Millet: Taichung 176 rice flour, treated with fungal α-amylase, improver, and baker's yeast.Utilization of proofing techniques in that sample improved their color, taste and resolved the gritty texture issue Formulated gluten-free cookies (GFC) had higher ash and moisture but were still acceptable despite increased microbial load and moisture until the 60th day of storage. Overall acceptability (OAC) score slightly dropped but remained good (7 of 9) after 2 months of storage. Notably, gluten-free cookies exhibited higher nutritional value and retained their acceptability for up to 60 days when stored at ambient temperature (25 ± 2°C).

Keywords: Gluten free cookies, Millet-rice cookies, Maize-rice cookies, Buckwheat-rice Cookies

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Introduction

The current trend among consumers toward whole grains that are gluten-free, high in protein, and rich in dietary fiber has stimulated research into the use of composite flour as an alternative to refined wheat flour in baked goods such as cookies. Although challenges persist in achieving desired physical and sensory attributes in final products using mixed flours, researchers have discovered success by blending various grains such as millet, rice, maize, and buckwheat flours. This approach has led to the development of cookies that satisfy both physical and sensory characteristics [1].Wheat, rye, and barley typically comprise gluten, a viscoelastic protein that allows dough rise and imparts flavor. Due to these reasons, it is frequently utilized in a wide range of food products, which raises the overall amount of gluten absorbed in a conventional Western diet [2, 3].Celiac disease is an autoimmune condition linked to gluten, causing harm to the lining of the small intestine upon consumption of gluten-containing foods [4]. This damage to the intestine hinders the body's ability to effectively intake essential micronutrients and fat. The sole curative option for gluten-related disorders is to adopt a gluten-free diet for the remainder of one's life [5]. Demands for gluten-free products have increased as consciousness about gluten intolerances among individuals has grown [6]. Therefore, this research work is designed to formulate different hill grain-based cookies to improve the nutritional quality by developing low gluten cookies and increasing the utilization of locally available cheap grain flours in the hilly region of Nepal. As the prevalence of metabolic disorders, obesity, and gluten sensitivity rises, there is a growing desire to create fortified products that are rich in dietary fibers (DF) and possess antioxidant (AO) properties. Additionally, these products should have reduced gluten content while still maintaining a pleasant taste [7]. Gluten-free grains are those that are free of gluten or have a concentration below twenty parts per million of gluten [8]. In the current study, rice (*Oryza sativa*), maize (*Zea mays*), buckwheat (*Fagopyrum esculentum*) and pearl millet (*Pennisetum glaucum*) flours were used to replace traditional wheat

(*Triticum aestivum*) flour in the production of gluten-free cookies [9]. The main component of wheat flour, maize, millet, taichin rice and buckwheat flour is starch. Amylases are enzymes that break down starch into small dextrins and oligosaccharides, which yeast can use during dough fermentation, proofing, and early baking stages. Fungal α-amylases are a type of endoacting amylase that breaks down alpha – 1, 4 glycosidic bonds found in the inner part (endo) of amylose or amylopectin chains in starch polymer [10].

Conventional cookies made with refined wheat flour are deficient in dietary fiber, vitamins, minerals, and are high in carbohydrates and unsaturated fat. To address this issue, cookie recipes have been modified by substituting refined flour (maida) with whole grain millet flour, maize flour, buckwheat flour, wheat flour, and taichung176 rice flour. These flours are rich in protein, total dietary fiber, vitamins, minerals, and antioxidants. This substitution is believed to help prevent celiac disease, obesity, diabetes, and gastritis. Thus, there is a need for standard operating procedures to formulate, characterize, and study the shelf life of gluten-free cookies made from locally available and underutilized hill grains in Nepal.

Materials and Methods

Raw materials selection and processing

Maize, Finger Millet, Buckwheat, and Taichin rice (glutinous rice) were purchased, cleaned, graded, milled, and sieved to manufacture high-grade grain flours from Shiva Mill Center in Lokanthali, Bhaktapur, Nepal. Commercially available cookies made with refined flour were used as the control sample.

Leavening agents: Baker's yeast and baking powder were received from Nepal Dairy Pvt. Ltd (nd's), Dhapakhel, Lalitpur, Nepal. Food-grade fungal-amylase (FAA) enzyme with improver was obtained from St. Xavier's College's food microbiology lab in Maitighar, Kathmandu, Nepal.

Formulation of different gluten free cookies sample

By altering the AACC method 10-50.05 (American Association of Cereal Chemists, 2010) and employing the results from [11], 16 different gluten-free cookies were created. The given methodology (**Figure1**) and recipes (**Table 1, 2** and **3**) were used to develop sixteen distinct cookie samples.

Table 1. Formulation recipe for the preparation of Millet-taichin flour cookies (3:1)

Ingredients	MBK	MBY	MABK	MABY
Millet flour	375gm (75%)	375gm	375gm	375gm
Taichin flour	125gm (25%)	125gm	125gm	125gm
Icing sugar	200gm (40%)	200gm	200gm	200gm
Shortening	150gm (30%)	150gm	150gm	150gm
Butter	80gm (16%)	80gm	80gm	80gm
Sodium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Ammonium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Custard powder	10gm (2%)	10gm	10gm	10gm
Skim milk powder	20gm (4%)	20gm	20gm	20gm
Salt	5gm (1%)	5gm	5gm	5gm
Amylase enzyme	0gm	0gm	0.1gm	0.1gm
Baking powder	4gm (0.8%)	0gm	4gm	0gm
Baker's yeast	0gm	10.41gm(2.04%)	0gm	10.41gm
Butter flavor	4gm (0.8%)	4gm	4gm	4gm
Water	180gm	180gm	180gm	180gm

MBK=Millet cookies treated with baking powder, MBY=Millet cookies treated with baker's yeast,
MABK=Millet cookies treated with amylase and baking powder, MABY=Millet cookies treated with amylase enzyme and baker's yeast

Table 2. Formulation recipe for the preparation of whole grain buckwheat-taichin flour cookies (3:1)

Ingredients	BWBK	BWBY	BWABK	BWABY
Buck Wheat Flour	375gm (75%)	375gm	375gm	375gm
Taichin flour	125gm (25%)	125gm	125gm	125gm
Icing sugar	200gm (40%)	200gm	200gm	200gm
Shortening	150gm (30%)	150gm	150gm	150gm
Butter	80gm (16%)	80gm	80gm	80gm
Sodium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Ammoium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Custard powder	10gm (2%)	10gm	10gm	10gm
Skim milk powder	20gm (4%)	20gm	20gm	20gm
Salt	5gm (1%)	5gm	5gm	5gm

Amylase enzyme	0gm	0gm	0.11gm	0.11gm
Baking powder	4gm (0.8%)	0gm	4gm	0gm
Baker's yeast	0gm	10.41gm (2.04%)	0gm	10.41gm
Butter flavor	4gm (0.8%)	4gm	4gm	4gm
Water	190gm (38%)	220gm	203gm	203gm

BWBK=Buckwheat cookies treated with baking powder
BWBY= Buckwheat cookies treated with baker's yeast,
BWABK= Buckwheat cookies treated with amylase enzyme and baking powder,
BWABY= Buckwheat cookies treated with amylase enzyme and baker's yeast

Table 3. Formulation recipe for the preparation of whole grain maize-taichin flour cookies (3:1)

Ingredients	MZBK	MZBY	MZABK	MZABY
Maize flour	375gm (75%)	375gm	375gm	375gm
Taichin flour	125gm (25%)	125gm	125gm	125gm
Icing sugar	200gm (40%)	200gm	200gm	200gm
Shortening	150gm (30%)	150gm	150gm	150gm
Butter	80gm (16%)	80gm	80gm	80gm
Sodium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Ammonium Bicarbonate	2.5gm (0.5%)	2.5gm	2.5gm	2.5gm
Custard powder	10gm (2%)	10gm	10gm	10gm
Skim milk powder	20gm (4%)	20gm	20gm	20gm
Salt	5gm (1%)	5gm	5gm	5gm
Amylase enzyme	0gm	0gm	0.1gm	0.1gm
Baking powder	4gm (0.8%)	0gm	4gm	0gm
Baker's yeast	0gm	10.41gm	0gm	10.41gm
Butter flavor	4gm (0.8%)	4gm	4gm	4gm
Water	152gm (30.4%)	152gm	152gm	152gm

MzBK=Maize cookies treated with baking powder, MzBY=Maize cookies treated with baker's yeast, MzABK= Maize cookies treated with amylase enzyme and baking powder, MzABY= Maize cookies treated with amylase enzyme and baker's yeast

Preparation of composite flours

Composite flours were crafted by making minor alterations to the methodology outlined in the Chopra et al [12]. To create millet-taichin (glutinous) rice composite flour, 375 grams of well-cleaned and sifted millet flour were mixed with 125 grams of taichin rice flour, resulting in a total of 500 grams of composite flour. The same ratio was employed for all the samples. To prepare millet-taichin rice flour cookies, 125 grams of taichin rice flour were blended with lukewarm water until a sticky consistency was achieved, after which 375 grams of millet flour were added and thoroughly incorporated. When producing MBK, MABK, MBY, and MABY cookies, leavening agents were introduced as follows: baking powder, baking powder + amylase enzyme, baker's yeast, and baker's yeast + amylase enzyme, respectively. After the addition of leavening agents, except for the baking powder-only treated composite flour, all of the composite flours were subjected to a proofer at 30°C for 1 hour to stimulate yeast growth and enhance enzymatic activity. More detailed instructions for the preparation of composite flours are available (**Figure 1**).

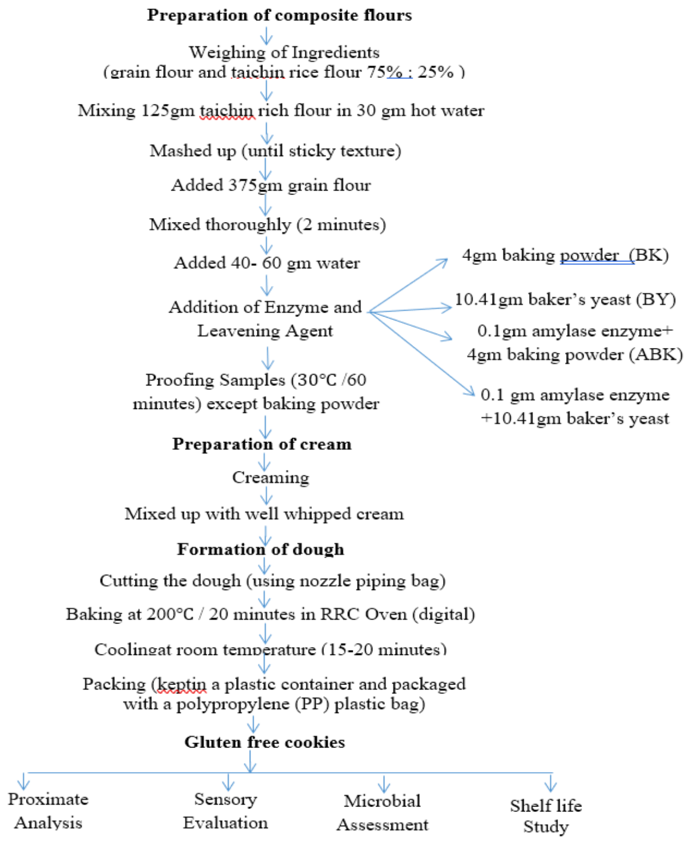


Figure 1. Flowchart showing the formulation process of gluten free cookies and its laboratory analysis

Making the whipped cream

The cream formation process reported by [13] was altered slightly to produce well-whipped cream. All of the ingredients were precisely weighed in accordance with the (**Table 4**). Subsequently, the pre-weighed ingredients were mixed thoroughly and kneaded manually until a dough-like consistency was achieved. The egg shaker was then used to mix the ingredients together, resulting in white well whipped cream. Finally, water was added to the mixture and blended by the egg shaker for a period of 2-5 minutes, resulting in a creamy white color. The formula for cream formation is provided in (**Table 4**).

Table 4: Recipe for the cream formation

S.N	Ingredients	Weight in gram
1.	Icing sugar	200 gm (40%)
2.	Shortening	150 gm (30%)
3.	Butter	80 gm (16%)
4.	Sodium Bicarbonate	2.5 gm (0.5%)
5.	Ammonium Bicarbonate	2.5 gm (0.5%)
6.	Custard powder	10 gm (2%)
7.	Skim milk powder	20 gm (4%)
8.	Salt	5 gm (1%)
9.	Butter Flavor	4 gm (0.8%)
10.	Water	120 gm (24%)

Table 5. Mean count (cfu/gm) on PCA and PDA of formulated cookies after 0 day, 30 days, and 60 days of storages

Sample	PCA 0day	PCA 30 days	PCA 60 days	PDA 0day	PDA 30 days	PDA 60 days
MBK	NG	5.3×10^2	3.01×10^3	NG	4.0×10^2	1.3×10^3
MBY	NG	2.37×10^3	4.16×10^3	NG	2.0×10^2	1.2×10^3
MABK	NG	1.8×10^2	2.6×10^2	NG	3.0×10^2	3.2×10^2
MABY	NG	1.8×10^2	2.1×10^2	NG	2.0×10^2	1.2×10^3
WBK	NG	6.0×10^1	2.6×10^2	NG	NG	2.0×10^2
WBY	NG	2.2×10^2	2.9×10^2	NG	NG	3.0×10^2
WABK	NG	1.6×10^2	1.8×10^2	NG	NG	3.0×10^2
WABY	NG	1.4×10^2	2.6×10^2	NG	NG	2.0×10^2
BWBK	NG	1.4×10^2	1.8×10^2	NG	NG	2.0×10^2
BWBY	NG	6.0×10^1	2.4×10^2	NG	NG	2.0×10^2
BWABK	NG	2.5×10^2	2.6×10^2	NG	NG	2.0×10^2
BWABY	NG	3.0×10^2	3.3×10^2	NG	NG	2.0×10^2
MzBK	NG	1.4×10^2	1.8×10^2	NG	NG	6.0×10^2
MzBY	NG	4.3×10^2	5.9×10^2	NG	NG	6.0×10^2
MzABK	NG	2.6×10^2	5.2×10^2	NG	NG	1.2×10^3
MzABY	NG	2.0×10^2	2.4×10^2	NG	NG	2.0×10^2
control	-	2.4×10^2	-	-	1.2×10^3	-

*NG indicates No growth of organisms

Preparation of the dough and production of cookies

The dough-kneading process was modified based on the technique stated in Singh et al [14] with certain deviations. The dough was created through a kneading process. It involved blending the composite flours with well-developed cream to form cohesive dough. Subsequently, this well-formed dough was loaded into a nozzle piping bag, and the cookies were shaped according to the desired specifications using the nozzle piping bag. Then it was baked in a preheated digital rotating rack convection oven set at 200°C for 20 minutes. After baking, the cookies were allowed to cool for 15 minutes at room temperature before being placed in an airtight plastic container for further examination.

Laboratory analysis

The prepared cookies were kept in a PET box and sealed in a polypropylene (PP) bag to be stored at room temperature, where samples were taken for analysis in the lab [15].

Microbiological analysis of cookies

Total plate count (TPC) and yeast and mold count (YMC) were used to identify the quality of 16 distinct gluten-free cookies. Cells per gram (cfu/g) were used to measure the numbers of the colonies on the dilution plates [16].

Proximate Analysis

The hot air oven method [17] was used to test the moisture content of the formulated cookies, while the ash content of gluten-free cookie samples was determined as described in [18].

Sensory evaluation of developed cookies:

A group of 16 panelists evaluated the formulated cookie samples based on their own sensory evaluations. They were instructed to evaluate the coded samples according to a 9-point hedonic scale, considering factors such as color, taste, texture, and acceptability. The measurement followed a 9-point hedonic rating test, ranging from "extremely like" to "extremely dislike," as described in [19].

Shelf life study

The shelf life studies were carried out for 60 days for 16 different gluten-free cookies. The cookies were kept in a PET box and packaged in a polypropylene (PP) plastic bag. Several factors, including the OAA (Overall Acceptability) score, moisture content, and microbial quality of the cookies, were considered during storage at ambient temperature to predict the shelf life. Evaluation and observation occurred at four specific intervals: within 24 hours of production, 30 days, and 60 days of post-production.

Statistical Analysis

The statistical analyses were performed using SPSS version 23 (Social Sciences software), SAS version 6.07 (Statistics Analysis System), and MS Excel. The outcomes of the lab experiment were computed and presented using triplicated findings and one-way (ANOVA) analysis of variance.

Results

Microbial evaluation of formulated cookies

Table 5 presents data on the average microbial counts in gluten-free cookies stored on PCA and PDA plates for 0, 0, and 60 days. Initially (0 days), no microbial growth was

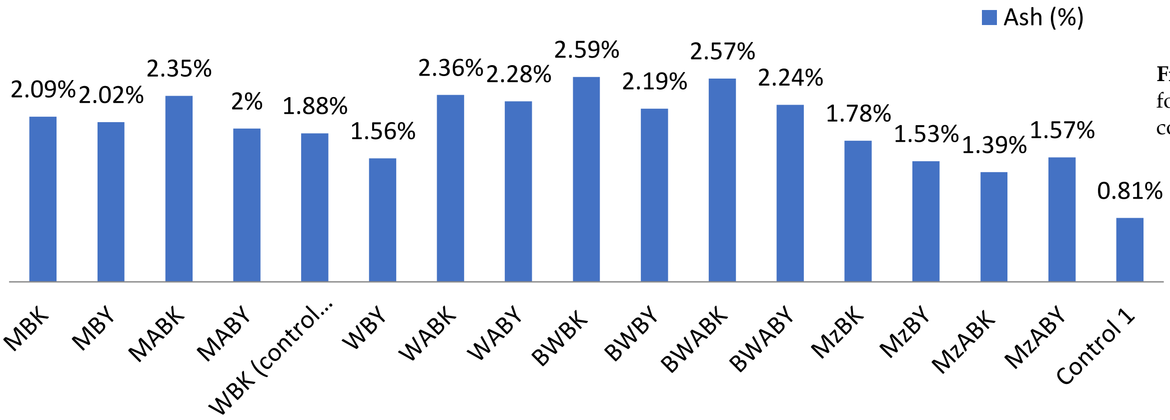


Figure 2. Ash content in formulated gluten free cookies and control

detected on any of the cookies. After 30 days, MBK and MBY cookies had the highest microbial counts, while WABY, BWABY, and MzABY had the lowest. Regarding yeast and mold counts (YMC) at 30 days, MBK and MABK cookies had the highest counts, while MBY and MABY had the lowest. None of the other samples showed growth. After 60 days, MBK, MBY, and MABY had the highest YMC, while MABK, WBK, WABY, BWBK, BWBY, BWABK, and MzABY had the lowest. In the majority of the cookie samples, the microbial load increased over time. However, the microbial counts remained within the permissible limit (below 1×10^4cfu/gm).

Table 6. Moisture content of cookies

Sample	Moisture% at 0 day	Moisture % at 30 days	Moisture% at 60 days
MBK	1.15%	1.20%	2%
MBY	3.80%	4%	5.16%
MABK	1.37%	2.80%	4%
MABY	2%	2%	2.97%
WBK	1.57%	1.68%	2.80%
WBY	1.80%	2.06%	3.10%
WABK	2.20%	2.21%	3.20%
WABY	3%	3.08%	3.27%
BWBK	4.16%	4.40%	4.63%
BWBY	6%	6.83%	8.67%
BWABK	2%	3.49%	4.06%
BWABY	3%	3.37%	3.81%
MzBK	2.09%	2.38%	3.33%
MzBY	2.02%	2.09%	2.32%
MzABK	2.35%	2.50%	2.62%
MzABY	1.05%	1.94%	2.39%
Control	-	2.10%	

Physio-chemical analysis

The moisture content, ash content, and shelf life of cookies were mentioned under physio-chemical analysis as the research was primarily focused more from microbial perspectives (limited within the scope of masters in food microbiology course).

Ash content of cookies

Among the 16 different cookie samples, BWBK had the highest ash content, with a percentage of 2.59%. Conversely, MzABK had the lowest ash content, measuring only 1.39%. When the test sample was contrasted with the control sample, the result revealed that the ash content increased from 0.81% to 2.59% (**Figure 2**).

Moisture content of cookies

The results of the moisture content of cookies are given in the (**Table 6**) and it is revealed that the moisture content of cookies increased from 1.05% to 5.16% over the 60 days of storage, except for BWBY-coded cookies. The entire test sample, BWBY only displayed the highest moisture content, ranging from 6% to 8.67%. Other buckwheat-based cookies, such as BWBK, BWABK, and BWABY, also showed the highest moisture contents, ranging from 2% to 4.16% in comparison with other samples. On the contrary, the lowest moisture content was found in a maize-based cookie coded MzABY ranging from 1.05% to 2.39%. However, it was discovered that the moisture content was less than 5% throughout the entire storage.

Sensory attributes of cookies

The provided **Tables 7, 8** and **9** compared the average sensory qualities of gluten-free cookies at different storage durations (0 days, 30 days, and 60 days) with a control sample. The findings revealed that incorporating amylase enzyme and bakery yeast led to positive feedback from the consumer panel. Cookies made with millet-taichung flour and maize-taichin flour were better received than other formulations. Sensory attributes of cookies made with bakery yeast, fungal amylase enzyme, and gluten-free composite flour received higher OAC scores compared to other formulations. For the majority of the samples, the average ratings on the hedonic scale

Table 7. Comparison of mean value on sensory attributes of gluten-free cookies after 0 day from packaging

SN	Sample	Color	Appearance	Taste	Texture	Flavor	Crunchiness	Overall Acceptability
1	MBK	7.750^{abcd}	7.687^{bcd}	7.750^{abcd}	7.562^{bcd}	7.562^{bcde}	7.562^{bcde}	7.687^{cd}
2	MBY	7.625^{bcde}	7.062^{e}	7.062^{e}	7.062^{d}	7.375^{cde}	8.063^{ab}	7.562^{de}
3	MABK	7.500^{cde}	7.875^{abc}	7.812^{abcd}	7.500^{bcd}	7.500^{cde}	8.063^{ab}	7.625^{cde}
4	MABY	7.625^{bcde}	7.875^{abc}	8.000^{abc}	7.625^{bc}	7.562^{bcde}	8.125^{a}	7.875^{abcd}
5	WBK	8.000^{ab}	8.000^{abc}	8.250^{a}	7.750^{ab}	8.00^{ab}	8.188^{a}	8.250^{a}
6	WBY	8.188^{a}	8.125^{ab}	8.188^{ab}	8.188^{a}	8.063^{a}	8.063^{ab}	8.125^{ab}
7	WABK	8.188^{a}	8.000^{abc}	8.063^{abc}	7.875^{ab}	7.625^{abcd}	8.000^{abc}	8.000^{abc}
8	WABY	8.063^{ab}	8.000^{abc}	7.562^{cde}	7.500^{bcd}	7.437^{cde}	7.687^{abcde}	7.750^{bcd}
9	BWBK	7.375^{de}	7.375^{ed}	7.625^{bcde}	7.750^{ab}	7.312^{cde}	7.500^{cde}	7.562^{de}
10	BWBY	6.312^{f}	5.750^{f}	5.062^{f}	5.000^{e}	7.187^{de}	7.187^{e}	6.625^{f}
11	BWABY	7.187^{e}	7.312^{ed}	7.500^{cde}	7.437^{bcd}	7.437^{cde}	8.000^{abc}	7.625^{cde}
12	BWABK	7.875^{abc}	7.875^{abc}	7.625^{bcde}	7.687^{abc}	7.75^{abc}	8.063^{ab}	7.625^{cde}
13	MzBK	8.125 ^{a}	8.188^{a}	7.750^{abcd}	7.750^{ab}	7.562^{bcde}	7.937^{abc}	7.937^{abcd}
14	MzBY	8.000^{ab}	7.875^{abc}	7.625^{bcde}	7.625^{bc}	7.500^{cde}	7.375^{de}	7.750^{bcd}
15	MzABK	7.875^{abc}	7.625^{cd}	7.312^{de}	7.500^{bcd}	7.687^{abc}	7.812^{abcd}	7.562^{de}
16	MzABY	7.187^{e}	7.125^{e}	7.125^{e}	7.187^{cd}	7.125^{e}	7.562^{bcde}	7.250^{e}

*Mean with the same superscript letters within each column are not significantly different from each other at the 5% level (P < 0.5). N=256; 1-9 point hedonic scoring scale."

Table 8. Comparison of mean value on sensory attributes of gluten-free cookies after 30days (1 month) from packaging

S.N	Sample	Color	Appearance	Taste	Texture	Flavor	Crunchiness	Overall Acceptability
1	MBK	7.5^{cdefg}	7.500^{abcd}	7.625^{abc}	7.437^{bc}	7.437^{ab}	7.437^{bcd}	7.437^{cde}
2	MBY	7.437^{defg}	6.937^{e}	6.937^{d}	6.937^{c}	7.187^{b}	7.937^{abc}	7.375^{de}
3	MABK	7.375^{efg}	7.750^{abc}	7.687^{abc}	7.375^{bc}	7.250^{b}	7.937^{abc}	7.500^{bcde}
4	MABY	7.437^{defg}	7.750^{abc}	7.875^{ab}	7.500^{bc}	7.437^{ab}	8.000^{ab}	7.750^{abcd}
5	WBK	7.9375^{abcd}	8.000^{ab}	8.188^{a}	7.625^{ab}	7.937^{a}	8.063^{a}	8.188^{a}
6	WBY	8.188^{a}	8.063a	8.188^{a}	8.188^{a}	7.937^{a}	7.937^{abc}	8.000^{ab}
7	WABK	8.188^{a}	7.750^{abc}	7.875^{ab}	7.625^{ab}	7.500^{ab}	8.000^{ab}	7.937^{abc}
8	WABY	8.000^{abc}	7.875^{ab}	7.437^{bcd}	7.375^{bc}	7.187^{b}	7.562^{abcd}	7.500^{bcde}
9	BWBK	7.250^{fg}	7.250^{bcde}	7.500^{bcd}	7.687^{ab}	7.187^{b}	7.375^{cd}	7.312^{de}
10	BWBY	6.312^{h}	5.750^{f}	5.062^{e}	5.000^{d}	7.187^{b}	7.187^{d}	6.312^{f}
11	BWABY	7.000^{g}	7.125^{de}	7.312^{bcd}	7.187^{bc}	7.375^{ab}	7.875^{abc}	7.375^{de}
12	BWABK	7.625^{bcdef}	7.562^{abcd}	7.437^{bcd}	7.375^{bc}	7.437^{ab}	7.937^{abc}	7.562^{bcde}
13	MzBK	8.063^{ab}	8.063^{a}	7.625^{abc}	7.625^{ab}	7.375^{ab}	7.750^{abcd}	7.750^{abcd}
14	MzBY	7.937^{abcd}	7.812^{abc}	7.500^{bcd}	7.562^{b}	7.375^{ab}	7.375^{cd}	7.687^{abcd}
15	MzABK	7.812^{abcde}	7.437^{bcde}	7.187^{cd}	7.375^{bc}	7.562^{ab}	7.750^{abcd}	7.375^{de}
16	MzABY	7.000^{g}	7.000^{de}	6.875^{d}	7.187^{bc}	7.125^{b}	7.500^{abcd}	7.192^{e}
17	Control	7.812^{abcde}	7.562^{abcd}	7.000^{de}	7.250^{bc}	7.000^{b}	7.375^{cd}	7.437^{cde}

*Mean with the same superscript letter within each column are not significantly different from each other at the 5% level (P < 0.5).N=256; 1-9 point hedonic scoring scale

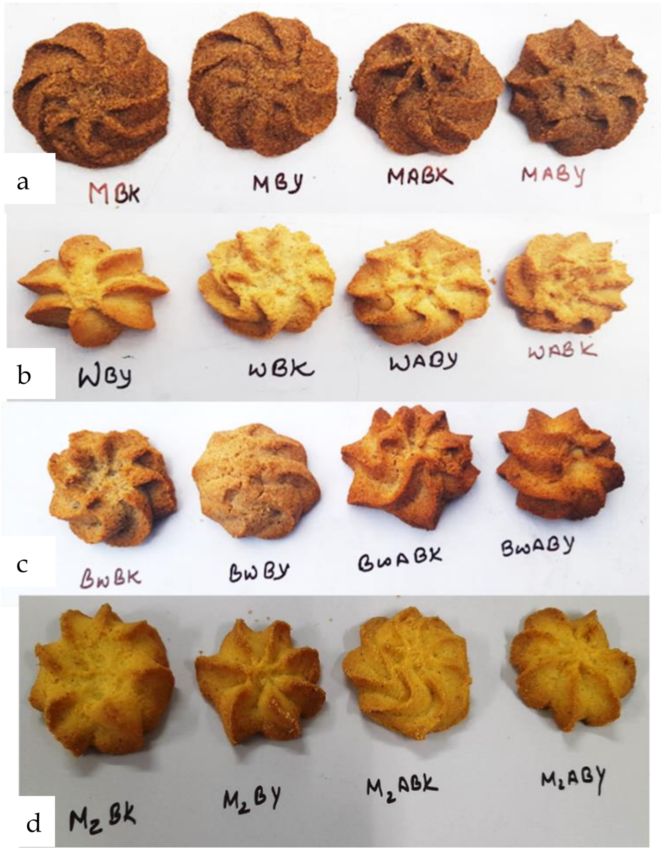
Table 9. Comparison of mean value on sensory attributes of gluten-free cookies after 60 days (2 months) from packaging

S.N	Sample	Color	Appearance	Taste	Texture	Flavor	Crunchiness	Overall Acceptability
1	MBK	7.000^{cde}	7.437^{abcd}	7.250^{ab}	7.187^{a}	7.250^{a}	7.687abc	7.187^{a}
2	MBY	5.812^{f}	6.312^{e}	6.125^{cd}	6.187^{bc}	6.125^{b}	6.312^{d}	6.187^{b}
3	MABK	7.125^{bcde}	7.187^{bcd}	7.250^{ab}	7.437^{a}	7.000^{a}	7.750^{ab}	7.437^{a}
4	MABY	7125^{bcde}	7.562^{abc}	7.125^{ab}	6.937^{a}	6.875^{a}	7.375^{abc}	7.125^{a}
5	WBK	7.562^{abc}	7.562^{abc}	7.625^{a}	7.250^{a}	7.312^{a}	7.250^{abc}	7.437^{a}
6	WBY	7.000^{cde}	7.125^{cd}	6.625^{bc}	6.875^{ab}	7.062^{a}	7.125^{bc}	7.187^{a}
7	WABK	7.437^{abcd}	7.375^{abcd}	6.875^{b}	7.000^{a}	7.000^{a}	7.500^{abc}	7.250 ^{a}
8	WABY	7.062^{cde}	7.062^{cd}	6.937^{ab}	6.875^{ab}	6.875^{a}	7.375^{abc}	7.250^{a}
9	BWBK	6.937^{de}	7.250^{abcd}	7.000^{ab}	6.875^{ab}	7.125^{a}	7.062^{c}	7.125^{a}
10	BWBY	6.562^{e}	6.312^{e}	5.875^{d}	5.937^{c}	5.625^{b}	5.500^{e}	5.937^{b}
11	BWABY	6.937^{de}	6.875^{de}	6.812^{bc}	7.125^{a}	6.875^{a}	7.562^{abc}	7.250^{a}
12	BWABK	7.312^{abcd}	7.375^{abcd}	7.312^{ab}	7.312^{a}	7.312^{a}	7.812^{a}	7.500^{a}
13	MzBK	7.750^{a}	7.562^{abc}	6.937^{ab}	7.000^{a}	6.937^{a}	7.437^{abc}	7.187^{a}
14	MzBY	7.687^{ab}	7.750^{ab}	7.062^{ab}	7.062^{a}	7.312^{a}	7.500^{abc}	7.375^{a}
15	MzABK	7.875^{a}	7.812^{a}	7.062^{ab}	7.125^{a}	7.125^{a}	7.500^{abc}	7.250^{a}
16	MzABY	7.375^{abcd}	7.500^{abc}	7.312^{ab}	7.062^{a}	6.937^{a}	7.125^{bc}	7.180^{a}

*Mean with the same superscript letter within each column are not significantly different from each other at the 5% level (P < 0.5).N=256; 1-9 point hedonic scoring scale consistently ranged from "moderately liked" to "liked very much" during the 60 days of ambient storage. This suggests that the panelists generally found the cookies to be satisfactory and enjoyable during this period. However, after 60 days from production, there were notable differences in the sensory characteristics of the cookies compared to 0 days and 30 days. Most samples received OAC scores ranging from 7 to 7.5, with the exception of the MBY and BWBY-coded samples, which scored 6.1 and 5.9, respectively. It's worth noting that all sensory aspects of the cookies received ratings higher than the minimum acceptable score, i.e., 5, indicating that all cookies were well-accepted by the panelists.

Shelflife studies

The shelf life of cookies was predicted using a number of tests over the course of 60 days, including microbiological counting on PCA and PDA, moisture content and sensory evaluation. The moisture percentage of the entire formulated cookies was roughly 5.04%, and their microbiological load and sensory characteristics were within acceptable limits. This finding showed that cookies could be easily preserved for up to two months at room temperature without the use of high-grade packaging materials or any artificial preservatives.



Photograph a. Final view of different millet-taichin rice flour cookies b. Final view of different whole grain wheat-taichin rice flour cookies c. Final view of different buckwheat-taichin rice flour cookies d. Final view of different maize-taichin rice flour cookies (from top to bottom).

Discussion

In this research, gluten-free cookies were created by substituting refined wheat flour with locally available, nutrient-rich hill grain flours like millet, maize, buckwheat, and taichin rice flour. Whole-grain wheat flour made up cookies and a store-bought cookie made with refined flour (Maida) served as control group. To develop gluten-free cookies, refined wheat flour was entirely replaced with a combination of hill grain flour and glutinous rice flour (taichin rice flour) in a 3:1 ratio. The inclusion of glutinous rice flour, which contains a higher concentration of the starch amylopectin, was aimed at enhancing the cookie's viscoelasticity. Even though starch does not dissolve in water, it absorbs moisture and swells, resulting in an increase in the cookies' volume. A study by Qin et al [20] demonstrated that dry-heat treatment had a more significant impact on glutinous rice flour (GRF), leading to increased viscoelasticity in the food industry. Gluten-related issues have gained significant attention due to their potential to trigger conditions such as celiac disease, gluten allergies, and non-celiac gluten sensitivity. These conditions can lead to damage in the lining of the small intestine, and a gluten-free diet is frequently the most successful approach for managing them [21].

The consumption of gluten-free cookies is on the rise in modern diets. Because of conventional cookies typically contain refined wheat flour (maida) as a primary ingredient, this flour undergoes chemical bleaching, a process that removes all dietary fibre, minerals, and vitamins, leaving behind a carbohydrate-rich product. These traditional cookies are deficient in fibre and essential amino acids while being high in fat and carbohydrates, which, as per earlier research by Goswami et al [22], is associated with an increased risk of diabetes. Consequently, by substituting wheat flour with millet flour, maize flour, buckwheat flour, glutinous rice flour, and whole grain wheat flour instead of refined wheat flour, the nutritional composition of these cookies was improved. This led to an increase in the ash content of the developed cookies (from 0.8% to 1.75%) and higher fibre content (up to 7.8%), as demonstrated in previous studies [23, 24].

In traditional cookie making, only baking powder is typically used as a leavening agent. However, in this study, a unique and innovative approach was taken by incorporating not only baking powder but also baker's yeast and fungal alpha-amylase enzyme. This recipe specifically utilized both baking soda (Sodium bicarbonate) and baking powder too. When baking soda alone proved insufficient in generating an adequate amount of carbon dioxide for the leavening process, this combination proved effective in providing additional lift during baking. The absence of gluten in the flour hindered its ability to form a structure capable of trapping gases during the fermentation phase of bread dough. To address this issue, enzymes were introduced into the gluten-free dough to enhance its viscoelastic properties, as demonstrated in studies [25, 26]. Consequently, gluten-free dough required additional treatment with fungal alpha-amylase to enhance its physical characteristics and make them comparable to those of dough containing gluten.

The gluten-free composite flours absorbed more moisture when the amylase enzyme and instant baker's yeast were added, together with a humid environment (proofer). With this adjustment, the issue of a gritty texture in gluten-free cookies was overcome, and fermentation and amylase processes improved the dough's quality. Using enzymes with baker's yeast has a significant impact on the texture and general quality of gluten-free baked goods [27].

The ash content of cookies increased noticeably from 0.81% to 2.59%. This increase was attributed to the substitution of refined wheat flour with high-ash-content wholegrain wheat flour, millet flour, maize flour, buckwheat flour, and glutinous rice flour. Buckwheat-based cookies exhibited the highest ash content, followed by millet-based cookies and maize-based cookies, respectively. This observation aligns with the findings of Bhavsar et al. [28]. The increased ash content in these cookies implies that they have higher mineral levels in comparison to conventional cookies. It was discovered that the ash content in cookies prepared with millet-taichin rice flour and maize-taichin rice flour was twice as high as that in conventional cookies made with solely refined wheat flour (C1). However, it was discovered that buckwheat-taichin rice flour (75:25%) cookies had nearly four times the ash amount of conventional cookies. .It was discovered that the ash content in cookies prepared with millet-taichin rice flour and maize-taichin rice flour was twice as high as that in conventional cookies made with solely refined wheat flour (C1). However, it was discovered that buckwheat-taichin rice flour (75:25%) cookies had nearly four times the ash amount of conventional cookies. This is in agreement with earlier researches [29, 30] that revealed equivalent ash content in buckwheat flour, maize flour, millet flour, and rice flour.

The study found that the majority of samples increased in moisture content over time. Because cookies are hygroscopic, they absorb moisture from the air during storage. The increase in moisture content has an effect on cookie quality and safety. The highest moisture content of the 16 formulated cookie samples was below 10% for up to 60 days at room temperature. This reduced the chance for microbial deterioration. The data correlates with previous studies (Aya et al. 2007), which found that low moisture content below 10% reduces the chance of microbial deterioration. Different studies[31, 32] revealed that the best moisture content for long-term storage is below 14%. Additionally, Adebowale et al [33] discovered that baked items with high moisture content, such as bread, cookies, and cakes, are vulnerable to bacterial, yeast, and mold growth.

When the cookies were freshly baked (0 days), there were no visible signs of bacterial or fungal growth on both PCA and PDA plates. This indicated that the cookies had undergone a high degree of dry heat treatment during baking, which made them free from microbial contamination. However, after 30 and 60 days of storage, there was a significant increase in the populations of viable bacteria and fungi. This increase could be post-contamination from the packaging materials used during storage. Most of the cookie samples showed an increase in microbial load gradually. However, the microbial counts were still below 1×10^4 cfu/gm and within acceptable limits [34]. The increase in viable microorganism counts in most of the test samples after 60 days of storage, compared to 30 days, may be due to factors such as the absence of preservatives, the cookies' hygroscopic nature, packaging quality, and the storage conditions that are conducive to microorganism growth. Interestingly, the control sample had a relatively lower count of viable microorganisms compared to most of the test samples after 60 days of storage. This could be attributed to the inclusion of the antioxidant tert-butyl hydroquinone (E319) in the control cookie, which helps protect against oxidative processes and inhibits the proliferation of various microbes during production. Similar findings were reported by Khezerlou et al [35], where tert-butyl hydroquinone (TBHQ) was used to avoid spoilage or rancidity in foods containing ingredients like lipids, edible oils, cereals, and shortening due to its antioxidant and antibacterial properties, with minimal impact on the sensory aspects of the food.

The overall acceptability scores for all of the formulated cookies decreased over time. After 30 days of storage, the Overall Acceptability Score (OAC) of the cookies made

with millet-taichin flour (coded as MBK) was comparable to that of the control sample. Similar results were noted in a study conducted by Swami et al [36]. The sensory evaluation findings revealed that cookies prepared with finger millet flour, which had a 30% oil content and were baked at 200°C for 20 minutes, received higher ratings (7 out of 9) for attributes such as texture, color, and overall acceptability. However, after 60 days of storage, there was a slight decrease in sensory attributes like taste, texture, flavor, crunchiness, and overall acceptability. This decrease may be attributed to factors such as the moisture-absorbing property, poor packaging, a lack of preservatives, and inadequate storage conditions.
Furthermore, using metalized polyester polyethylene (MET-PPE) pouches instead of polypropylene (PP) plastic pouches could have enhanced the shelf life of cookies and preserved their crisp texture. Jan et al [37] supported this finding, demonstrating that low-density polyethylene (LDPE) pouches had higher moisture absorption, increased levels of free fatty acids, peroxide value, and microbial growth compared to LP and MET-PPE pouches. Consequently, MET-PPE packaging can be employed to store cookies under ambient conditions (25 ± 2°C) for up to 4 months without compromising their crispness or other quality attributes. This study also revealed that cookies treated with food-grade fungal α-amylase in combination with dough improver, along with either baker's yeast or baking powder, were preferred by panelists in terms of crunchiness, taste, texture, color, and flavor compared to cookies treated with only baking powder or baker's yeast. The synergy between the food-grade fungal amylase enzyme and baker's yeast or baking powder helped address the issue of a gritty texture in gluten-free products and enhanced the color, flavor, and taste of the cookies [38]. The results of this research revealed that combinations of millet-taichin rice flour, maize-millet rice flour, buckwheat-rice flour, and whole grain wheat-rice flour (as a control) were greatly accepted in terms of sensory appeal and were microbiologically safe [39]. Therefore, this formulation recipe has the great potential to develop nutrient-rich gluten-free cookies that assist consumers in curing gluten-related diseases.

Conclusion

The bakery industry has struggled to develop gluten-free cookies with pleasing sensory characteristics. However, a groundbreaking solution has emerged through these innovative recipe formulations. The development of novel cookie recipes was accomplished by replacing entire refined flour with locally produced hill grains like maize, millet, buckwheat, and taichin rice flour from Nepal. This research concluded that incorporating these low-cost, highly nutritious and readily available composite flours into gluten-free cookie recipes boosted their nutritional value but also improved their texture and sensory appeal. The key finding of this research was that the addition of food-grade fungal alpha-amylase, in combination with either baking powder or baker's yeast during cookie production, played a crucial role in breaking down starch-rich flour into dextrin. This enzymatic process led to browning, enhanced flavor development, resolved the issue of gritty texture, and improved the even distribution of water in the dough. Furthermore, the research indicated that the shelf life of these formulated cookies could be extended by opting for packaging materials like metalized polyester polyethylene (MET-PPE) pouches or polyethylene (PE) plastic bags instead of polypropylene (PP) pouches. Additionally, the incorporation of preservatives could further increase the shelf life. As a result, these cookies hold the potential to serve as a dietary snack for individuals with gluten-related disorders.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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Ethical Approval and Consent

Not Applicable

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