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Production and Evaluation of Gluten-Free Cookies for Celiac Patients Made from Rice Flour and Green Banana Flour

HANEEN HAMED MOUMINAH* and MAHA ALI ALTHAIBAN

Faculty of Human Sciences and Design, Departmen of Food and Nutrition, University of King Abdulaziz, Jeddah, Saudi Arabia.

Abstract

Demand for gluten-free food products is on the rise, largely due to heightened consumer awareness regarding celiac disease. This study was conducted to develop quality characteristics of gluten-free cookies produced by replacing rice flour with 0, 10, 20, 30 and 40% green banana flour. The methodology consisted of evaluating the cookie samples was evaluated in terms of chemical composition, physical properties (diameters, weights, thickness and spread ratio), textural properties (cookies hardness), color characteristics (L* (Lightness), a*(redness), b* (yellowness), hue angle and ΔE (Numerical total color difference), as well as microbiological analysis and sensory properties. The results indicated that the cookie samples showed a gradual reduction in moisture, protein, lipid, and total carbohydrate contents, while there was a gradual increase in both crude fiber and ash content with increasing levels of replacement with green banana flour. The physical properties showed a gradual increase in weight, thickness and hardness of cookie samples, while there was a gradual decline in both diameter and spread ratio with increasing percentage of replacement with green banana flour in comparison to the control sample. The color characteristics of the cookie samples showed that a gradual decline in the L* values and b* values, while the a* value of the gluten-free cookies was gradually increased with increasing levels of substitution with green banana flour compared to the control sample. Microbiological assessments showed that the cookie samples produced exhibited a low microbial count, confirming their microbiological safety. Neither Escherichia coli nor Salmonella spp. were detected in any sample throughout the storage periods of 2, 30, 60, and 90 days at ambient temperature (25 ± 2°C). Sensory evaluations revealed that cookie samples prepared by replacing rice flour with 20% green banana flour, received the highest ratings for color, flavor, texture, crispiness, and overall acceptability.

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CONTACT Haneen Hamed Mouminah hmouminah@kau.edu.sa Faculty of Human Sciences and Design, Departmen of Food and Nutrition, University of King Abdulaziz, Jeddah, Saudi Arabia.

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Introduction

Gluten-free products are specifically used for individuals diagnosed with celiac disease, i.e. people who are gluten intolerance resulting from the eating of wheat (gluten), rye, barley, and their hybrids. Celiac disease is an autoimmune disorder affecting the small intestine, primarily in genetically susceptible individuals across all age groups, and affecting between 0.5% and 5.6% of people worldwide.[1] This condition manifests through symptoms such as intense abdominal pain, bloating, diarrhea, and significant weight loss.[1,2] In addition, The celiac disease can only be treated by strict avoidance of gluten forming protein in diet throughout the patient's life. Wheat flour can be substituted with a variety of gluten-free flour and starches, including rice, green banana flour, maize, and sweet potato.[3] Various alternative ingredients have been tested to produce gluten-free food , such as cookies made from unripe banana flour.[4] bread from green banana flour and wheat flour,[5] cookies from rice and banana flour,[6] cookies from green banana flour, wheat flour,[7] pasta formulated with green matured banana flour and defatted soy flour,[8] biscuits with green banana flour,[9] gluten-free biscuit prepared with Acha-green banana Flour fortified with cowpea flour,[10] Gluten-free pasta production from banana and cassava flours.[11]

Cookies represent a significant category of baked goods that are highly sought after by individuals of all ages. This popularity can be attributed to their low production costs, extended shelf life, convenience, and palatable quality. Furthermore, cookies can serve as a valuable vehicle for providing essential nutrients to patients with celiac disease, who require an increase in their daily nutrient intake due to intestinal damage.[12] More than 65% of the world's population uses rice (*Oryza sativa* L.) as their staple food. Rice was the third most produced crop in 2020-2021, reaching approximately 509.87 million tons.[13] Broken rice is a by-product generated during the rice milling process, with approximately 15-25% of the rice being classified as broken during milling and polishing. This broken rice serves as a valuable ingredient in various industrial food applications.[14] Rice flour, derived from this broken rice, is abundant in essential minerals such as zinc, magnesium, selenium, and manganese, as well as antioxidants, fiber, and B vitamins. The consumption of rice flour products contributes to the remineralization of the body, lowers the risk of heart disease, and promotes a sense of fullness.[15,16] Additionally, rice flour is gluten-free, low in sodium, protein, and fat, while being high in easily digestible carbohydrates. It is frequently utilized as a wheat substitute in gluten-free food products, particularly for individuals with celiac disease.[17]

Green banana flour is rich in phenolic compounds, including prebiotic fibers, proteins, carotenoids, flavonoids, and a variety of vitamins such as A, B3, B6, B12, C, and E. Additionally, it contains essential mineral salts, including potassium, phosphorus, magnesium, and zinc.[18] Banana flour cookies are healthy, due to the nutritional components found in banana flour, including carbohydrates, minerals, and antioxidant properties. Additionally, utilizing banana flour in baked goods contributes to reduce postharvest losses of bananas.[19] Banana flour is currently used in the food industry for the preparation of various products, including bread, cakes, pasta, biscuits, cookies, and baby food.[5] Green banana flour has a mild raw bananas flavor and can be used as a healthy substitute to white wheat flours.[20] Banana flour is prepared, and it is used in bakery and confectionery industries. The bakery and confectionery industries.[18] Incorporating green banana flour into the diet can help in weight loss, control diabetes, improved bowel transit, and reduced cholesterol and triglyceride levels.[21] Additionally, using green banana flour in cookies can help control chronic conditions such as obesity and diabetes by lowering the glycemic index of products.[22,23] Rice flour combined with green banana flour presents a potentially nutritious and healthful option for consumers, attributed to the elevated levels of dietary fibers, minerals, and vitamins found in green banana flour. In light of these findings, the objective of this study was to produce gluten-free cookies suitable for individuals with celiac disease, utilizing rice flour and green banana flour, while also assessing the physicochemical, microbiological, and sensory characteristics of the resulting products.

Materials and Methods

The raw materials utilized in this study included second and third-grade rice, characterized by a significant proportion of broken grains, green bananas (*Musa paradisiaca* L.), sugar, fats, eggs, powdered milk flour, sodium bicarbonate, salt, and vanilla flavoring. These ingredients were procured from the local market in Jeddah, located in the Makkah region of Saudi Arabia.

In this study, all solvents, chemicals, reagents, and standards utilized for sample extraction and preparation were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of Green Banana Flour

The process of preparing green banana flour involves several steps. Initially, green bananas are detached from their bunches and thoroughly washed with water. They are then subjected to steam treatment at 82 °C for 15 min. Following this, the bananas are manually peeled, separating the pulp from the peel. The pulp is immersed in a 0.5% (w/v) citric acid solution for 10 minutes (To reduce the enzymatic browning), and 1% calcium chloride solution (for preservative and act as firming agent) for 15 mi. While submerged in the solution, the banana pulp is sliced into pieces measuring 0.5 cm in thickness. After that, the slices were placed on stainless steel trays and dried in an oven at 50 °C for a duration of 20 to 28 hours, or until the weight was constant. Once dried, the slices are milled using a laboratory mill (Brabender Automat Mill Quandrumat Senior, Germany) to produce uniform granules, which are then sieved through a 425 μm mesh to yield fine flour. The resulting flour is packaged in high-density polyethylene bags, heat sealed, and stored in a freezer until required.[4]

Preparation of Rice Flour

Rice flour was produced using the method described by.[24] Initially, the rice grains underwent a cleaning process to eliminate foreign materials such as stones, husks, and stalks. Subsequently, the grains were washed and soaked in water for one hour, followed by drying in a hot-air oven at a temperature range of 45 to 50 °C for six hours, until cracks appeared in the grains. The dried rice was milled with a laboratory mill to achieve uniform granules and then sieved through a 425 μm pore size to obtain fine flour. Finally, the flour was packaged in an airtight plastic container, labeled appropriately, and stored at room temperature (25 ± 2 °C) until required.

Table (A): Ingredients of the formulations used in prepared of gluten-free cookies

Ingredients (g)	formulations used in the manufacture of cookie samples				
	Control	F1	F2	F3	F4
Rice flour	100	90	80	70	60
Green banana flour	0	10	20	30	40
Sugar	30	30	30	30	30
Fat	45	45	45	45	45
Egg	30	30	30	30	30
Sodium Chloride	1	1	1	1	1
Baking powder	0.5	0.5	0.5	0.5	0.5
Powdered milk	6.7	6.7	6.7	6.7	6.7
Vanilla	1.0	1.0	1.0	1.0	1.0
Distilled water	25	25	25	25	25

Preparation of Gluten-Free Cookies

Various flour samples were prepared by partially replacing rice flour with 10%, 20%, 30%, and 40% green banana flour, which was utilized in the formulation of experimental gluten-free cookies, as presented in Table (A). The cookies were produced following the method outlined in reference.[25] Initially, sugar and fats were beaten together for a duration of five minutes, after which eggs and powdered milk were incorporated and the mixture was whipped until light and fluffy. Subsequently, flour, sodium bicarbonate, salt, and vanilla flavor were thoroughly combined and added to the creamed mixture, which was then mixed in a bowl mixer to form a dough. The dough was rolled out to a thickness of 1 cm and cut into circular shapes with a diameter of 5 cm using a round cutter. The cut portions were placed in a freezer for 30 minutes to rest before being baked in an oven at 170°C for 20 minutes. After baking, the cookies were allowed to cool, packaged in high-density polyethylene bags, and stored at room temperature for further analysis. The cookie samples made from 100% rice flour were used as the control.

Chemical Properties

Rice flour, green banana flour, and cookie samples were examined for their moisture, protein, ash, fat, and crude fiber content following the method described.[26] Total carbohydrate content was calculated by difference.

Physical Characteristics

The physical characteristics of cookie samples were assessed for the weight (g), thickness (mm), diameter (mm), and spread ratio as detailed.[27] The hardness of gluten-free cookie samples was measured using a Texture Analyzer (Comtech, B type, Taiwan) according to the procedure described.[28]

Color Characteristics

The color intensity of the top and down surfaces of gluten free cookie samples was measured according to the method.[29] by a Konica Minolta Color Measuring System (Chroma-meter model CR-400, Minolta, Japan), provided with a standard 2° observer and a D65 illuminator. Calibration of the device was performed using a white ceramic plate supplied by the manufacturer. This device recorded 3 parameters, L*, a*, and b*. L* (Lightness), a*(redness), b* (yellowness), and hue angle were calculated by arctan (b*/a*) to describe the overall color of the sample. While Numerical total color difference (ΔE) was computed using the formula:

$$\Delta E=\sqrt{(\Delta L^{*})^{2}+(\Delta a^{*})^{2}+(\Delta b^{*})^{2}}$$

Where

ΔL* = variation between lighter and darker (- = darker, + = lighter);
Δa* = variation between green and red (- = greener, + = redder),
Δb* = variation between blue and yellow (- = bluer, + = yellower)

Microbiological Analysis

The microbiological analysis of the gluten free cookies was carried out after 48 h of baking and then at 30, 60 and 90 days of storage. The total microbial count of gluten free cookies samples was determined using the standard microbiological method.[30] Samples of gluten free cookies from each formula were milled to powder under aseptic conditions. Five grams of the sample was dissolved in 45 ml of sterile water and homogenized samples at 3000 rpm /min for 5 min. The supernatant was decanted (basic dilution). Next, a 10 - fold dilution was performed for each homogenate sample. An aliquot of 1 ml from selected dilutions of each sample were aseptically inoculated onto media labeled agar plates in three replicates (agars: Nutrient Agar was used for total count of aerobic mesophyll bacteria, Endo agar for *Escherichia coli, Salmonella* and *Shigella agar* for *Salmonella*, and Dextrose Agar for mold counts).

Sensory Evaluation

The sensory attributes of cookie samples were assessed by a panel of fifteen members, chosen from various staff categories within the Department of Food and Nutrition at the Faculty of Human Science and Design, King Abdulaziz University, Saudi Arabia. All participants possessed a familiarity with the quality characteristics associated with cookies. The assessment utilized a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely).[31] Samples were coded with random three-digit numbers. Each sample was evaluated for color, flavor, texture, crispiness, and overall acceptability. Panelists were provided with tap water to clean their mouths before tasting the next cookie sample.

Statistical Analysis

All results were performed in triplicate except for sensory evaluation data (n = 15). The results were expressed as the mean ± standard deviation (SD) in this study. The collected data were statistically analyzed using SPSS software version 20. One-way analysis of variance (ANOVA) was used for further analyze the statistical significance of the resulting data. Differences were considered significant at $p < 0.05$.

Results

Tables 1-6 present the results for each of chemical composition of rice flour and green banana flour, chemical constituents of prepared cookie samples, physical properties of produced cookie samples, color attributes of produced cookie samples, total microbial counts (log cfu/g) of cookie samples during storage periods at room temperature,and sensory properties of prepared cookie samples.

Table 1: Chemical constituents of rice flour and green banana flour on a dry weight basis excluding moisture content

Components (%)	Rice flour	Green banana flour
Moisture	9.82± 0.16[a]	7.23 ± 0.12[b]
Crude protein	7.46± 0.14[a]	4.95 ± 0.07[b]
Total lipids	0.32± 0.02[b]	0.74 ± 0.04[a]
Total ash	0.61± 0.07[b]	2.23 ± 0.22[a]
Crude fibers	0.29± 0.08[b]	8.22 ± 0.25[a]
Total carbohydrate by difference	91.61± 0.22[b]	92.08 ± 0.17[a]

Values represent the averages of three replicates (mean ± SD). Means that are denoted by different letters between treatments indicate a significant difference at (P <0.05).

Table 2: Chemical constituents of prepared cookie samples on a dry weight basis excluding moisture content

Components (%)	Treatments (Formulations of gluten-free cookies)				
	Control	F1	F2	F3	F4
Moisture	5.95±0.12[a]	5.73± 0.19[b]	5.44±0.32[c]	5.17 ±0.23[d]	4.89±0.17[e]
Crude protein	13.12±0.53[a]	12.75±0.10[b]	12.24±0.08[c]	11.86±0.04[d]	11.42±0.02[e]
Total lipids	15.75±0.11[a]	15.49±0.09[a]	15.28±0.07[ab]	14.97±0.05[b]	14.76±0.03[b]
Total ash	1.27±0.04[c]	1.40±0.07[b]	1.56±0.10[b]	1.68±0.22[a]	1.81±0.19[a]
Crude fiber	2. 27±0.06[e]	2.88±0.11[d]	3.39±0.16[c]	3.90±0.19[b]	4.42±0.22[a]
Total carbohydrates	69.93±0.32[e]	70.36±0.25[d]	70.92±0.39[c]	71.49±0.62[b]	72.01±0.54[a]

Values represent the averages of three replicates (mean ± SD). Means that are denoted by different letters between treatments indicate a significant difference at (P <0.05).

Table 3: Physical properties of produced cookie samples

Parameter	Treatments (Formulations of gluten-free cookies)				
	Control	F1	F2	F3	F4
Weight (g)	6.95 ±0.26[a]	7.30 ±0.35[b]	7.73 ±0.22[c]	8.22 ±0.27[d]	8.63 ±0.18[e]
Diameter (mm)	42.69 ±0.33[a]	42.33±0.42[b]	41.92 ±0.30[c]	41.50 ±0.37[d]	41.17±0.29[e]
Thickness (mm)	5.24 ±0.16[e]	5.57 ±0.19[d]	5.92 ±0.22[c]	6.25 ±0.21[b]	6.58 ±0.33[a]
Spread ratio (D/T)	8.15 ±0.17[a]	8.08 ±0.15[ab]	7.08 ±0.29[b]	6.64 ±0.12[c]	6.26±0.22[d]
Hardness (N)	14.12± 0.30[e]	15.22±0.54[d]	16.30 ± 0.33[c]	17.43± 0.48[b]	18.53 ± 0.40[a]

Values represent the averages of three replicates (mean ± SD). Means that are denoted by different letters between treatments indicate a significant difference at (P <0.05).

Table 4: Color attributes of produced cookie samples

Parameter	Treatments (Formulations of gluten-free cookies)				
	Control	F1	F2	F3	F4
L*	63.92±0.32^{Ab}	61.33±0.82^{Bb}	59.65±2.52^{Cb}	56.93±3.22^{Db}	54.82±3.73^{Eb}
a*	4.22±0.41^{Ed}	4.86±0.21^{De}	5.62±0.35^{Ce}	6.27±0.27^{Be}	6.88±0.32^{Ae}
b*	25.43±12.13^{Ac}	22.75±2.63^{Bc}	20.82±0.37^{Cc}	19.10±0.30^{Dc}	17.24±0.56^{Ed}
Hue	80.58±0.39^{Aa}	77.94±0.16^{Ba}	74.89±0.22^{Ca}	71.82±0.50^{Da}	68.42±0.30^{Ea}
∆E	0.0	6.86Dd	11.12^{Cd}	16.37^{Bd}	21.96^{Ac}

Values represent the averages of three replicates (mean ± SD). Means that are denoted in the same row with different capital letters indicate a significant difference at (P <0.05). Means that are denoted in the same column with different lowercase letters indicate a significant difference at (P <0.05). L* (Lightness), a*(redness), b* (yellowness), Hue (hue angle), and ΔE (Numerical total color difference).

Table 5: The total microbial counts (log cfu/g) of cookie samples during different storage periods at room temperature (25 ± 2 °C)

Microbiological Parameters (Cfu/g)	Storage periods	Treatments (Formulations of gluten-free cookies)				
		Control	F1	F2	F3	F4
Total Bacterial	2 days	0	0	0	0	0
	30 days	3.11^{Bb} ± 0.21	3.15^{ABb} ± 0.24	3.20^{ABb} ± 0.22	3.24^{ABb} ± 0.25	3.62^{Aa} ± 0.26
	60 days	3.34^{ABa} ± 0.32	3.35^{ABa} ± 0.30	3.36^{ABa} ± 0.28	3.37^{Aa} ± 0.31	3.39^{Ab}± 0.29
	90 days	3.37^{Ba}± 0.37	3.39^{ABa}± 0.35	3.40^{Aa} ± 0.33	3.41^{Aa} ± 0.32	3.38^{Bb} ± 0.34
Total molds	2 days	0	0	0	0	0
	30 days	1.85^{ABb} ± 0.41	1.86^{ABb} ± 0. 40	1.87^{ABb} ± 0.37	1.91^{Ab} ± 0.35	1.93^{Ab} ± 0.33
	60 days	2.14^{ABa} ± 0.27	2.13^{ABb} ± 0.26	2.15^{ABa} ± 0.24	2.18^{Aa} ± 0.22	2.21^{Aa} ± 0.23
	90 days	2.19^{ABa} ± 0.29	2.22^{Aa} ± 0.25	2.21^{Aa} ± 0.26	2.22^{Aa} ± 0.27	2.24^{Aa} ± 0.28

Value represent the averages of fifteen participants (means ± standard deviation). Means that are denoted in the same row with different capital letters indicate a significant difference at (P <0.05). Means that are denoted in the same column with different lowercase letters indicate a significant difference at (P <0.05).

Table 6: Sensory properties of prepared cookie samples

Parameter	Treatments (Formulations of gluten-free cookies)				
	Control	F1	F2	F3	F4
Color	8.12±0.65^{ABa}	8.20±0.58^{Aa}	8.34±0.43^{Aa}	7.23±0.82^{Ba}	6.75±0.88^{Ca}
Flavors	7.20±0.98^{Cb}	7.65±0.84^{Bb}	8.37±0.69^{Aa}	7.11±0.56^{Cab}	6.60±0.42^{Db}
Crispiness	7.15±1.53^{Cb}	7.63±1.02^{Bb}	8.24±1.02^{Aa}	7.10±1.23^{Cb}	6.52±1.18^{Dc}
Texture	7.25±1.64^{Cb}	7.65±1.24^{Bb}	8.15±1.17^{Aa}	7.13±0.85^{Dab}	6.57±1.42^{Ec}
Overall acceptability	7.20±0.64^{Cb}	7.62±0.52^{Bb}	8.20±0.43^{Aa}	7.00±0.53^{Db}	6.60±1.02^{Eb}

Value represent the averages of fifteen panelists (means ± standard deviation). Means that are denoted in the same row with different capital letters indicate a significant difference at (P <0.05). Means that are denoted in the same column with different lowercase letters indicate a significant difference at (P <0.05)

Discussion

Chemical Constituents of Rice Flour and Green Banana Flour

The chemical composition of rice flour and green banana Flour is presented in Table (1). Moisture, crude protein, total lipids, total ash, crude fibers, and total carbohydrate contents of rice flour was 9.82, 7.46, 0.32, 0.61, 0.29, and 91.61 g/100g, respectively. These results align with the findings of,[32] which reported rice flour to contain 9.62% moisture, 7.78% protein, 0.21% crude fiber, 0.66% ash, 0.20% fat, and 91.36% total carbohydrates. Furthermore, these observations were corroborated by,[33] who indicated that ground rice flour had protein levels ranging from 6.60% to 9.30% and fat content between 0.18% and 0.50%. In contrast, the moisture, crude protein, total lipids, total ash, crude fibers, and total carbohydrates in green banana flour were found to be 7.23, 4.95, 0.74, 2.23, 8.22, and 92.08 g/100g, respectively. These findings are consistent with,[34] which reported that green banana flour contained 4.2% crude protein, 0.43% total lipids, 2.5% ash, 6.3% crude fibers, and 86.57% total carbohydrates. The same trend was obtained by[35] reported that green banana flour contains 4.41% protein, 0.45% fat, 1.08% ash, 8.49% fiber, and 94.06% carbohydrates.

Chemical Composition of Cookies

Chemical components of gluten-free cookies prepared by substituting rice flour with 0-40% green banana flour are given in Table 2. Chemical components of gluten-free cookies prepared by substituting rice flour with 0-40% green banana flour are given in Table 2. The results indicated that the cookie samples showed a gradual reduction in moisture, protein, lipid, and total carbohydrate contents, while there was a gradual increase in both crude fiber and ash content with increasing levels of replacement with green banana flour. The results showed that the moisture content of the cookie samples decreased with increasing levels of replacement with green banana flour. Specifically, the highest moisture content was observed in the cookie sample made entirely from rice flour (control), where the moisture content was 5.95%, then decreased to 5.73%, 5.44%, 5.17%, and 4.89% for formulations F1, F2, F3, and F4, respectively. The moisture content decreased gradually with the incremental addition of green banana flour. This phenomenon may be to the fact that banana flour contains a higher amount of solid matters and hydrophilic components compared to rice flour.[36] The moisture content of cookie samples exhibited a gradual decline with the increasing proportion of green banana flour, allowing for longer storage periods before microbiological degradation begins, and thus, had a prolonged shelf life compared to cookies prepared from100% rice flour (control).

Fat content of the cookie samples decreased with increasing levels of substitution with green banana flour compared with control sample , where the fat content of cookie samples made entirely from rice flour (control) was 15.75%, which decreased to 15.49%, 15.28%, 14.97%, and 14.76% for cookies prepared from formulations F1, F2, F3, and F4, respectively. The results in table (2), showed that the protein content of the cookie samples decreased with increasing levels of substitution with green banana flour, where the protein content of cookie samples made entirely from rice flour (control) was 13.12%, which subsequently decreased to 12.75%, 12.24%, 11.86%, and 11.42% for cookies prepared from formulations F1, F2, F3, and F4 respectively. These results were also confirmed by[36] who found that the protein percentage of cookie samples decreased with increasing level of banana flour. It may be due to the fact that green banana flour contained negligible amount of protein and as the banana flour replaces rice flour by increasing amount the protein content of rice flour was also replaced. Consequently, the addition of green banana flour into the cookie samples might lower the protein content of cookies. The results showed that the fiber content of the cookie samples increased with increasing levels of substitution with green banana flour compared with control sample.

The fiber content in cookie samples made entirely from rice flour (control) was 2.27%, rising to 2.88%, 3.39%, 3.90%, and 4.42% for cookie samples prepared from formulations F1, F2, F3, and F4, respectively. These results are consistent with,[37] who found that the fiber content of the cookie samples increased with the increasing percentage of green banana flour added.

The results showed that, the ash content of cookie samples increased with increasing levels of substitution with green banana flour where the ash content of cookie samples made entirely from rice flour (control) was 1.27%, and increased to 1.40%, 1.56%, 1.68%, and 1.81% for cookies samples prepared from the formulations F1, F2, F3, and F4, respectively. These results are consistent with those of,[37,38] who found that the ash content was higher in samples prepared with added green banana flour than in the control sample prepared without added green banana flour, indicating that these products possess high functional potential and serve as important sources of mineral substances.

The results showed that, the total carbohydrate contents of cookie samples increased with increasing levels of substitution with green banana flour,where the total carbohydrate contents of cookie samples prepared entirely from rice flour (control) was 69.93% and increased to 70.36, 70.92, 71.49 and 72.01% for cookie samples prepared with formulations F1, F2, F3, and F4 respectively.

Physical properties of produced gluten-free cookies
The results presented in Table 3. demonstrate that the cookie samples, prepared by substituting rice flour with different proportions of green banana flour (ranging from 0% to 40%), showed significant effects in their physical characteristics (diameter, thickness, weight, hardness, and spread ratio). The results indicate that, the weight of cookie samples showed a significant increased with increasing levels of substitution with green banana flour. The control sample (made entirely from rice flour) was 6.95 g while the weights of the cookie samples prepared from substituting rice flour with 10, 20, 30 and 40% green banana flour were increased to 7.30, 7.73, 8.22 and 8.63g respectively. The observed weight gain of cookies samples made with increasing percentage of green banana flour substitution may be attributed to linked to the dietary fiber present in banana flour, which has the ability to retain water molecules, thereby preventing moisture loss during baking. These results align with the findings of,[19] which suggest that the increase in weight of cookie samples may be attributed to the dietary fiber present in banana flour, which aids in water retention and minimizes moisture loss during baking.

The findings indicated that, the diameter of cookie samples was gradually decline with increasing proportions of replacement with green banana flour. The diameter of cookie samples made entirely from rice flour (control) was 42.69 mm, which subsequently decreased to 42.33 mm, 41.92 mm, 41.50 mm, and 41.17 mm for the cookie samples prepared from formulations F1, F2, F3, and F4, respectively. This reduction in diameter of cookie samples was more pronounced with increasing proportions of green banana flour addition, aligning with the results of previous studies conducted by,[37,38] which indicated that the diameter of biscuits decreased as the fiber content increased.

The results reveal that the thickness of cookie samples significantly increased progressively with increasing proportions of replacement with green banana flour. The thickness of the gluten-free cookies prepared entirely from rice flour (control) was 5.24 mm, which increased to 5.57 mm, 5.92 mm, 6.25 mm, and 6.58 mm for cookie samples prepared from formulations F1, F2, F3, and F4, respectively. These results are in agreement with,[23] who noted that the increase in cookies thickness could be attributed to the hydrophilic properties of the incorporated green banana flour.

The spread ratio of cookies is a critical quality attribute; a higher spread ratio correlates with greater product yield, as stated by.[34] The results indicate a gradual decline in the spread ratio of cookie samples with increasing proportions of substitution with green banana flour. The spread ratio for cookies prepared entirely from rice flour (control) was 8.15%, which decreased to 8.08%, 7.08%, 6.64%, and 6.26% for cookie samples which contained 10, 20, 30 and 40% of green banana flour respectively. The same trend was obtained by,[35] who reported a reduction in the spread ratio of cookies with higher proportions of added green banana flour.

The findings demonstrated that the hardness of cookie samples markedly increased with increasing proportions of replacement with green banana flour. Specifically, the hardness escalated from 14.12 N for cookies prepared entirely from rice flour (control) to 15.22 N, 16.30 N, 17.43 N, and 18.53 N for cookie samples prepared from formulated F1, F2, F3, and F4, respectively. These results were also confirmed by[37] who concluded that the hardness of cookies was significantly increased with increasing proportion of banana flour, which is characterized by a high fiber content. Previous studies[38] also found the positive relation of dietary fibers and hardness.

Color Attributes of Gluten-Free Cookies

The color characteristics of gluten-free cookie samples, which were prepared by substituting rice flour with varying proportions of green banana flour (ranging from 0% to 40%), with regards to L*, a*, b*, Hue, and ΔE values are presented in Table 4. The findings indicated that, the L* values of the cookie samples exhibited a gradual decline with increasing levels of substitution with green banana flour. The cookies control samples, which prepared entirely from rice flour, shoewd the highest L* (lightness) value, which was 63.92 and decreased to 61.33, 59.65, 56.93, and 54.82 for the cookie samples prepared from formulations F1, F2, F3, and F4, respectively. The reduction in L* values indicates that, the gluten-free cookies exhibited a darker color with increasing levels of substitution with green banana flour. The darker color of cookie samples may be attributed to the Maillard reaction. The Maillard reaction is depends on the levels of reducing sugars, amino acids, and proteins present on the surface of baked goods, as well as the temperature and duration of the baking process.[39]

The a* (redness) value of the cookie samples increased with increasing levels of substitution with green banana flour, where the lowest a* (redness) value was recorded in the gluten-free cookies prepared entirely from rice flour (control) sample, which was 4.22 and increased to 4.86, 5.62, 6.27, and 6.88 for cookie samples made from formulations (F1), (F2), (F3), and (F4), respectively. Additionally, the b* value, (which indicates the yellowness of cookie samples), significantly decreased with increasing levels of substitution with green banana flour. The highest b* value (indicating the yellowness) was recorded in cookies made entirely from rice flour (control samples), where b* value reached 25.43 and decreased to 22.75, 20.82, 19.10, and 17.24 for cookie samples made from formulations (F1), (F2), (F3), and (F4), respectively. These findings shown herein are consistent with those found by,[39,40] who indicated that the protein content may negatively impact the lightness of gluten-free cookies, thereby suggesting that non-enzymatic browning (Maillard reaction) significantly contribute in color formation. Furthermore, non-enzymatic browning and the caramelization of sugars contribute to the formation of brown pigments during the baking process.

The hue values of cookie samples exhibited a notable decline with increasing levels of substitution with green banana flour. The highest hue value was recorded in cookie sample made entirely with rice flour (control samples), where the hue value reached 80.58, and decreased to 77.94, 74.89, 71.82, and 68.42 for cookie samples prepared from formulations (F1), (F2), (F3), and (F4) respectively. Furthermore, the total color difference (ΔE) of cookie samples significantly increased with increasing levels of substitution with green banana flour. The total color difference (ΔE) values were 6.86, 11.12, 16.37, and 21.96 for cookie samples prepared from formulations (F1), (F2), (F3), and (F4) respectively. These findings coincide with,[29] who stated that, the increase in ΔE may be due to the rising proportions of green banana flour, as the darker coloration of the gluten-free cookies intensified with the increasing addition of green banana flour.

The Total Microbial Counts Of Cookie Samples

Table 5 shows the total microbial counts (log cfu/g) of gluten-free cookie samples, which were prepared by substituting rice flour with different proportions of green banana flour (ranging from 0% to 40%) during storage periods up to 90 days at ambient temperature (25 ± 2 °C). The findings indicate that, the increasing replacement proportions with green banana flour did not significantly effect on the total bacterial and mold counts across all cookie samples during the storage periods up to 90 days at ambient temperature (25 ± 2°C). After two days of storage at this temperature, the results showed no growth on both the nutrient agar plates for bacterial count and in the potato dextrose agar plates for molds count in all cookie samples. The lack of microbial presence can be attributed to the adherence to Good Manufacturing Practice (GMP) during the production of the cookie samples and the heat used in their production. These results were also confirmed by[41] who concluded that the cookies were prepared under good hygienic conditions and the integrity of the packaging material used was not compromised. Following a storage period of 60 days at ambient temperature (25 ± 2 °C), the findings revealed a rise in the microbial counts for both bacterial and molds. These results demonstrated that, the levels of bacteria and molds stayed within the permissible limits.[42] total counts of bacteria and molds did not change significantly during the storage periods of 30 to 90 days at ambient temperature (25 ± 3 °C). Following a storage period of 90 days, the bacterial count for cookie samples was observed to be low, being less than 104 cfu/g. Additionally, all cookie samples exhibited a mold count of less than 103 cfu/g. These findings indicated that, the cookie samples maintained a favorable stability when stored at ambient temperature for 90 days, as the microbial counts remained within acceptable limits. On the other hand, pathogenic bacteria such as Escherichia coli and Salmonella spp were not detected in any samples of cookie samples. These findings suggest that, the cookie samples were produced under good hygienic conditions, adhering to good manufacturing practices. It is important to note that improper preparation, handling, and storage can lead to an increase in microbial counts.

Sensory Properties of cookie Samples

The gluten-free cookies made by substituting rice flour with varying amounts of green banana flour, ranging from 0% to 40% assessed for color, flavor, crispiness, texture and overall acceptability Table 6. The findings of this study reveal that, cookie samples made by substituting rice flour with 20% of green banana flour (F2) received the highest ratings for sensory attributes followed by cookie samples made by substituting rice flour with 10% of green banana flour (F1), cookie samples made by 100% rice flour (C), cookie samples made by substituting rice flour with 30% of green banana flour (F3) and cookie samples made by substituting rice flour with 40% of green banana flour (F4) for color, flavor, texture, crispiness, and overall acceptability respectively. In contrast, cookie samples containing more than 30% green banana flour received the lowest scores for these sensory attributes. Notably, the cookies formulated with 40% green banana flour (F4) demonstrated the least favorable evaluations regarding color, flavor, crispiness, texture, and overall acceptability. These results are consistent with the results of,[39] which indicated a decrease in the sensory attributes of biscuits when rice flour was replaced with levels of more than 30% green banana flour.

Conclusion

Gluten-free cookies made from rice flour and green banana flour, obtained in this study, are suitable for people with certain conditions such as celiac disease, also referred to as gluten-sensitive enteropathy. The need for a gluten-free diet is clear for both children and adults diagnosed with celiac disease. Because they cannot properly digest gluten, gluten-free products, such as cookies, are essential to prevent adverse health effects in those with this condition. However, gluten-free diets may also provide advantages for individuals without celiac disease, as they are believed to enhance energy levels and promote a general sense of well-being. Gluten free cookies, which use easily digestible ingredients such as rice flour, green banana flour, sweet potato flour and tapioca dextrin, not only facilitate digestion but also provide children with the energy required to navigate their active days. Consequently, in an effort to formulate gluten-free food products with enhanced nutritional quality, gluten-free cookies were produced by substituting rice flour with green banana flour at rates 0, 10, 20, 30 and 40% . The results showed that, the chemical, physical properties, color attributes and sensory scores of the cookies samples showed significant differences when rice flour was replaced with increasing levels of green banana flour compared with control samples prepared with 100% rice flour. Notably, the formula consisting of replacing rice flour with 20% green banana flour was used to prepare gluten-free cookies for celiac disease with good nutritional value and received the highest scores for color, flavor, texture, crispiness, and overall acceptability. These findings suggest that, it can be concluded that banana flour substituted cookies may represent a good compromise between health benefits, technological quality and sensory attributes.

Future works, exploring the role of banana flour as a novel alternative ingredient in the development of various functional foods, are recommended.

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Author Contributions

- **Haneen Hamed Mouminah:** conceptualization, methodology, data curation, formal analysis, investigation, writing-original draft preparation (lead), writing-review and editing.
- **Maha Ali Althaiban:** data curation, formal analysis, investigation, writing-original draft preparation, writing-review and editing. .

References

1. Atlasy N, Bujko A, Baekkevold ES, Brazda P, Janssen-Megens E , Lundin KE, Stunnenberg HG. Single-cell transcriptomic analysis of the immune cell compartment in the human small intestine and in celiac disease. *Nature Commun.* 2022;13 (1): 4920.
2. Machado MV. New developments in celiac disease treatment. *International Journal Molec. Sci*. 2023;24 (2): 945.
3. Elzoghby AA, Atta MB, Salem MA, Sayed EE. Influence of sweet potato flour and okra mucilage on the rheological properties and water activity of rice bread. *Journal Sustainable Agric. and Environ. Sci.*2023; 2 (1):115-129.
4. Sawant BB, Relekr PP, Shirke GD and Kadam JH. Development of cookies from banana (Musa spp.) flour. *The Pharma Innovation Journal.* 2023;12(5): 4586-4593.
5. Júnior A, Machalela AA, Djedje FN, Nanelo R F. Physico-chemical and sensory characterization of bread based on green banana (Musa spp.) flour. *Universal Journal of Food Science and Technology*. 2023; 1(1): 56–70.
6. Stoin D, Jianu C, Velciov AB, Alexa E, Cocan I, Negrea M, Poiana MA, Moigrădean D. Evaluation of the nutritional and sensory profile of glutenfree cookies made from rice flour and green banana flour. *Journal of Agroalimentary Processes and Technologies.* 2021; 27(3): 348-356.
7. Norhidayah M, Noorlaila A, Izzati NFA. Textural and sensorial properties of cookies prepared by partial substitution of wheat flour with unripe banana (Musa x paradisiaca var. Tanduk and Musa acuminata var. *Emas) flour. International Food Research Journal.* 2014;21(6): 2133-2139.
8. Udachan I, Gatade A, Ranveer R, Lokhande S, Mote G, Sahoo A K. Quality evaluation of gluten-free brown rice pasta formulated with green matured banana flour and defatted soy flour. *Journal of Food Processing and Preservation*. 2022:46 (9):1-10. DOI:10.1111 /jfpp. 16448.2022.
9. Alvarenga N, Taipina M, Raposo N, Dias J, Carvalho MJ , Amaral O, Santos MT , Silva MM, Lidon FC. Development of biscuits with green banana flour irradiated by 60Co: Preservation in modified atmosphere packaging. *Emirates Journal of Food and Agriculture.* 2018. 30(6): 496-502.
10. Inyang UE, Nwabueze SO. Pasting Properties of Acha-Green Banana Composite Flour Fortified with Cowpea Flour and Quality

Evaluation of Gluten-Free Biscuit Made from the Blends. *EAS Journal of Nutrition and Food Sciences* 2020; 2: 61-72.

11. Rachman A, Brennan MA, Morton J, Brennan CS. Gluten-free pasta production from banana and cassava flours with egg white protein and soy protein addition. *International Journal of Food Science and Technology.* 2020; 55, 3053–3060.
12. Beers M, Berkow R. The Merck Manual of Diagnosis and Therapy. *Merck Research Laboratories,* USA. 2004.
13. Wafaa KG, Ali RG, Tawfik MM. Physicochemical and cooking properties of some rice varieties to produce salty and sweet puffed rice. *Alex. Journal Food Sci. and Technol.* 2019;16 (1): 21-30.
14. Liyanaarachchi GV, Mahanama KR, Somasiri HP, Punyasiri PA, Wijesena KA, Arachchi KJD. Profiling of amino acids in traditional and improved rice (Oryza sativa L.) varieties of Sri Lanka and their health-promoting aspects. Cereal Res. *Communications.* 2021; 49:441–448.
15. Rai S, Kaur A, Singh B. Quality characteristics of gluten-free cookies prepared from different flour combinations. *Journal Food Sci Technol.* 2014; 51(4): 785–789.
16. Prabha R, Chaudhari F, Nishesh T, Laxmi S, Ambika T, Deepak S. Rice Nutritional and Medicinal Properties A Review Article. *Journal of Pharmacognosy and Phytochemistry.* 2018; 7(2): 150-156.
17. Kouros J, Witczak M, Ziobro R, Juszczak L. The impact of resistant starch on characteristics of gluten-free dough and bread. *Food Hydrocolloids*. 2009; 23: 988-995.
18. Haslinda WH, Cheng LH, Chong LC, Aziah AN. Chemical composition and physicochemical properties of green banana (Musa acuminata balbisiana Colla cv. Awak) flour. *International Journal of Food Sciences and Nutrition*.2009; 60 (4): 232–239.
19. Adeola AA, Ohizua ER. Physical, chemical, and sensory properties of biscuits prepared from flour blends of unripe cooking banana, pigeon pea, and sweet potato. *Food Science and Nutrition.* 2018; 6(3): 532-540.
20. Sarawong C, Schoenlechner R, Sekiguchi K, Berghofer E. Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chemistry.* 2014; 143: 33–39
21. Singh B, Singh J P, Kaur A, Singh N. Bioactive compounds in banana and their associated health benefits – a review. *Food Chemistry.* 2016; 206: 1–11.
22. Fida R, Pramafisi G, Cahyana Y. Application of banana starch and banana flour in various food product: A review. International Conference on Food and Bio-Industry, *Earth and Environmental Science.* 2020; 443.
23. Inyang UE, Effiong CF, Edima-nyah AP. Physical properties, nutritional composition and sensory evaluation of cookies prepared from rice, unripe banana and sprouted soybean flour blends. *International Journal of Food Science and Biotechnology.* 2018; 3(2): 70–76.
24. Okpala LC, Egwu PN. Utilization of broken rice and cocoyam flour blends in the production of cookies. *Nig. Food J.* 2015; 33(1): 8-11.
25. Singh S, Riar CS, Saxena DC. Effect of incorporating sweet potato flour to wheat flour on the quality characteristics of cookies. *African Journal of Food Sci.* 2008; 2, 65-72.
26. AOAC. Official Methods of Analysis, 21st edition ed.; The Association, Washington DC, The USA, 2019.
27. Sai-Manohar R, Haridas RP. Effect of mixing period and additives on rheological characteristics of dough and quality of biscuits. *Journal of Cereal Sci.* 1997; 25: 197 -206.
28. Gaines CS. Instrumental measurement of the hardness of cookies and crackers. *Cereal Foods World.* 1991; 36: 989-996.
29. Mashau E, Rambau FD, Kgatla T. Influence of unripe banana flour Incorporation on the physical, antioxidant properties and consumer acceptability of biscuits. *Journal Microbiol Biotech Food Sci.* 2022; 12 (1): 1 – 7.
30. Giwa OE, Babalola RO, Kolawole SA. Microbial, physical, and sensory attributes of cookies produced from wheat flour fortified with Termitomyces robustus and spiced with curry leaves (Xylopia aethiopica). *Journal of Natural Sci. Res.* 2012; 2 (3): 40–46.
31. Meilgaard MC, Civille GV, Carr BT. Sensory

Evaluation Techniques. *Boca Raton: CRC Press*, 2016. Taylor & Francis Group.

32. Omran AA, Hussien HA. Production and evaluation of gluten-free cookies from broken rice flour and sweet potato. *Advances in Food Sci*. 2015; 37 (4): 184-192.
33. Cameron DK, Wang YJ. A better understanding of factors that affect the hardness and stickiness of long-grain rice. *Cereal Chem.* 2005; 82 (2): 113-119.
34. Khalil MM, Tabikha MM, Hosny MH, Kortam AA. Physiochemical and sensory evolution of some bakery products supplemented with unripe banana flour as a source of resistant starch. *Journal of Food and Dairy Sciences.* 2017; 8 (10), 411-417.
35. Bezerra CV, Amante ER, Oliveira DC, Rodrigues AMC, Da Silva LHM. Green banana (Musa calendrical) flour obtained in spouted bed – Effect of drying on physio-chemical, functional, and morphological characteristics of the starch. *Industrial Crops and Products.* 2013;41, 241–249.
36. Abdul Alim Md, Jui S, Rothy JNN, Tasnim J. Studies on the quality of biscuits incorporating green banana powder. *Journal of Agricultural Engineering,IEB.* 2022; 44: 1-7.
37. Amarasinghe NK, Wickramasinghe I, Wijesekara I, Thilakarathna G, Sathsara Deyalage ST. Functional, Physicochemical, and Antioxidant Properties of Flour and Cookies from Two Different Banana Varieties (Musa acuminata cv. Pisang awak and Musa acuminata cv. Red dacca). *International Journal of Food Science*. 2021; (4):1-9.
38. Parente MY, Bezerra FL, Costa RA, Aragão MF. Formulation and evaluation of gluten-free cookies from rice and green banana flour. *Revista Brasileira de Agrotecnologia.* 2021;11(2): 720-726.
39. Pereira D, Correia PM, Guine RP. Analysis of the physical-chemical and sensorial properties of Maria-type cookies. *Acta Chimica Slovaca.* 2013; 6 (2): 269–280.
40. Chevallier S, Colonna P, Della Valle G. Contribution of major ingredients during baking of biscuit dough systems. *Journal of Cereal Science*. 2000; 31(3): 241–25.
41. Yusufu PA, Netalaand J, Opega JL. Chemical, Sensory and Microbiological Properties of cookies produced from maize, African Yam Bean and Plantain Composite Flour. *Indian Journal of Nutrition.* 2016; 3 (1): 122.
42. ICMSF. International Commission on Microbiological Specifications for Foods. “Microorganisms in Foods 7”. Microbiological testing in food safety management. New York USA. *Kluwer Academic / Plenum Publishers.* 2002; 4 (1): 45-97.