Food and Nutrition Insights
https://journals.pagepal.org/index.php/fni
ISSN (Online), 3079-284X; (Print), 3079-3831
https://doi.org/10.54219/FNI.04.02.2026.614

Quality Evaluation of Cookies Prepared from Reduced Gluten Composite Flour

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Article Info

Keywords:
Reduced gluten; Composite flour; Proximate composition, Sensory evaluation

Date:
Received: 3 July 2026
Accepted: 26 September 2026
Published: 29 October 2026

Volume: 04 (02), 2026

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Abstract

The present study evaluated the physicochemical and sensory quality of cookies prepared by replacing wheat flour with maize (Zea mays L.) flour at six levels: WC_0 (100:0), WC_1 (80:20), WC_2 (60:40), WC_3 (40:60), WC_4 (20:80) and WC_5 (0:100; wheat: maize flour, w/w). Cookies were assessed initially and after 30, 60, 90 and 120 days of storage. Wet and dry gluten, proximate composition, physical characteristics (width and thickness) and sensory attributes were evaluated. Dry gluten decreased progressively from 7.05% in WC_0 to 0.00% in WC_5. Flour treatment significantly affected width, thickness, ash, protein, fibre, fat, carbohydrate and sensory attributes ($p<0.05$), whereas storage significantly affected moisture content, which increased from an overall mean of 3.63% initially to 3.78% after 120 days. The increase in moisture was likely related to moisture uptake during storage in polypropylene packaging; however, water activity was not measured and therefore cannot be inferred from moisture content alone. Among the reduced-gluten formulations, WC_1 (80:20 wheat: maize flour) had the highest overall sensory acceptability (7.92) and provided a favourable balance between gluten reduction and sensory quality. WC_5 had the highest protein (10.05%), ash (2.05%), fibre (6.09%), fat (19.96%) and carbohydrate (74.97%) values among the flour treatments, but its sensory scores were lower than those of wheat-containing formulations. The findings demonstrate that partial maize-flour substitution can reduce measured gluten while retaining acceptable cookie quality, although analytical gluten quantification and water-activity measurements should be included in future studies to strengthen product-safety and shelf-life conclusions.

1. Introduction

Low moisture content is used for the preparation of cookies. The cookies can be made with three main ingredients, sugar, fat and flour, and there are some other ingredients, such as chemical leavening agents, emulsifiers, syrups and salts, that they may contain, but may not always be used in the cookie formula and are typically used at a lower level (Akusu *et al*., 2016). The formulation baking properties and dough rheology sets cookies apart. Main ingredient in cookie dough formulae is flour, which has a major content of protein + starch, and a major content of water. Water binding ingredients in flour that contribute towards maximum dispersion of cookies seem to be starch, protein, arabinose and xylose. Low gluten wheat flour (Almeida *et al*., 2025) can also be used to develop gluten free snap cookies.

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The study was carried out in Food Science and Technology lab of The University of Agriculture Peshawar, Pakistan. Wheat flour and maize (Zea mays L.) flour were purchased from the local market and taken to the laboratory for preparing cookies. The prepared cookies were packaged in polypropylene bags and evaluated initially and after 30, 60, 90 and 120 days of storage.

Wheat is the largest grown cereal along with maize cereal, non-gluten containing and suitable for the health conscious, namely celiac consumer (Hera *et al*., 2014). The most important source of anthocyanins content is corn with pigments amongst all kinds of cereals. Pigmented maize kernels have a significant amount of anthocyanins, mainly cyanidin-3-glucoside (Cy-3-Glu) that give them the blue and red color. Due to the possibility of breakdown of anthocyanins and other phenolics during thermal processing, however, the use of colored maize in the baking and confectionery industries is difficult. The introduction of antioxidant (phenol) rich ingredients in cookies has caused interest due to their health properties, and potential effects against certain diseases. Given the significance in creating reduced gluten cookies with various flour composites, the aim of the present study was to assess cookies made from various flour combinations.

2. Materials and Methods

2.1 Procurement and preparation of raw materials

Experiments were carried out at Food Science and Technology (FST) Lab at University of Agriculture, Peshawar, Pakistan. Wheat flour and maize flour were purchased from the local market and delivered to the laboratory for further processing and preparation of cookies. The selected amounts of different kinds of flour were combined, and cookies were prepared and stored under the conditions described above to collect data and analyze later.

Analysis of the raw materials

Both the raw materials, i.e. maize flour and wheat flour were evaluated for proximate composition using standard AACC (2012) method.

2.2 Preparation of the supplemented flour

Wheat flour and maize flour were mixed as per treatment plan given in Table 1. to prepare wheat–maize composite flour along with the control. Resultant composite flour was stored in zip lock bags for further analysis and used to prepare cookies.

2.3 Analysis of the composite flour

Table 1. Treatment plan used in the study

Treatment	Wheat flour (g)	Maize flour (g)
WC_0	250	0
WC_1	200	50
WC_2	150	100
WC_3	100	150
WC_4	50	200
WC_5	0	250

WC = wheat–maize composite flour treatment
All treatments were prepared using 125g sugar, 125g vegetable ghee, 2 eggs and 2.5g baking powder.

All treatments were analyzed for the gluten content. The gluten content was measured with the dough-washing process. A rubber like mass was formed by thoroughly kneading and washing the dough under running water by hand. This mass was the gluten when it is wet and was weighed to obtain its wet weight. The wet gluten was then subsequently dried in the oven at 100°C overnight. The sample was then again dried, and its dry gluten content was determined.

2.4 Preparation of cookies

Cookies were prepared according to the AACC approved method for cookie preparation (AACC, 2010). Sugar and vegetable ghee were mixed, followed by addition of eggs. Wheat flour, maize flour and baking powder were then incorporated to form dough. The dough was sheeted and cut with a cookie cutter. The formulations were based on the flour treatments presented in Table 1. For each formulation, 250g flour, 125g sugar, 125g vegetable ghee, 2 eggs and 2.5g baking powder was used.

2.5 Physical analysis

Diameter and thickness were measured with the help of "Vernier Caliper" at different places in each cookie and the average was calculated for each.

2.6 Storage study

Cookies were stored in zip lock bags at room temperature and were evaluated at 0, 30, 60, 90 and 120 days for the following parameters.

2.6.1 Moisture content (%)

Moisture content was determined in triplicate using 2 g of sample dried at 100 °C to constant weight, following AACC (2012). The crucibles were cooled in a desiccator before weighing. Moisture content was calculated as:

$$Moisture\ (\%) = \frac{W1 - W2}{Sample\ Weight} \times 100$$

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Where W1 is the weight of crucible plus sample before drying and W2 is the weight after drying. The Sample were prepared and stored for four months prior to the investigation. Organoleptic evaluation and physiochemical of the sample was done at 1 month of storage interval.

2.6.2 Ash content (%)

Ash content was determined in triplicate by incinerating 2 g of sample in a muffle furnace at 550 °C for 3–4 h until a light-grey ash was obtained. Ash content was calculated as: following AACC (2010).

$$\text{Ash (\%)} = \frac{\text{W1} - \text{w2}}{\text{wt of sample}} \times 100$$

Where

W1 = weight of crucible + sample before drying

2.6.3 Crude fiber content (%)

Crude fiber was determined in triplicate by sequential acid and alkali digestion. 2 g sample was treated with 0.25 N H_2SO_4 and subsequently with 0.31 N NaOH, filtered, washed, dried and weighed. The residue was then ashed at 550–600 °C. For Kjeldahl analysis, 1 g of powdered sample was digested with the digestion mixture and concentrated H_2SO_4. The digest was diluted, distilled with NaOH, collected in boric acid containing indicator, and titrated against 0.1 N HCl. The recorded titre values were used to calculate nitrogen and protein content.

$$Crude\ Fiber\ (\%) = \frac{Dry\ wt - Ash\ wt}{wt\ of\ sample} \times 100$$

2.6.4 Crude protein content (%)

Protein was measured by using following reagents and with the aid of Kjeldhal instrument:

Concentrated H_2SO_4 (Sulfuric acid), 40% NaOH (sodium hydroxide solution), % Boric Solution, Methyl red (Indicator) and Digestion mixture K_2SO_4 and $SuSO_4$ (copper sulphate and potassium sulphate).

Procedure

Protein content was determined by taking 1g powdered sample in kjeldhal tube. The digestive mixture was prepared using about 7g of digestive mixture, 7ml of conc. H_2SO_4 was added and digestive tube was immersed in digestion mixture, the volume of digested mixture was increased to 100ml with distilled water when its digestion was completed. 10ml of digestive mixture was transferred to kjeldhal digestion tube and 10ml of 40% of NaOH was added which were distilled into the flask containing indicator (methyl red) and boric acid. Then the distillated was titrated with 0.1 N HCl until pinkish color was observed and then it was recorded as titration.

$$Protein\ (\%) = \frac{(S - B) \times N \times CF \times 100 \times 5.7}{Weight\ of\ sample \times V} \times 100$$

Where

N= Normality of the sulfuric acid used.

V = Sample volume taken for distillation.

B= Blank titration (in ml).

2.6.5 Crude fat content (%)

Soxhlet apparatus was used for the analysis of fat content. 2 g of the crushed cookies sample was taken, crushed and transferred to a thimble then transferred to an extraction tube. The extraction tube was attached to the round bottom flask which was sterilized and weighed, and fat extraction was carried out in 5 - 6 hours in petroleum ether at the boiling temperature range (60-80°C). Subsequently the fat was done, and thimble was taken from the extraction tube and the remaining petroleum ether was removed again for further use. This flask was then put into the oven and heated for an hour at 105°C and then transferred to a dedicator to get it room-temperature and reweighed.

2.7 Sensory evaluation

Prepared cookies were evaluated for colour, taste, texture and overall acceptability using a 9-point Hedonic scale, ranging from 9 (extremely like) to 1 (extremely dislike) (Larmond, 1977).

2.8 Statistical analysis

Data were analyzed using a two-factor completely randomized design (CRD), with flour treatment and storage period as the main factors. Analysis of variance (ANOVA) was followed by Fisher's least significant difference (LSD) test for mean separation at the 5% significance level.

3. Results and Discussion

3.1 Chemical composition of wheat and maize flour

The proximate composition of maize and wheat flour revealed variations in moisture, ash, protein, fiber, and fat contents (Table 2). Maize flour exhibited higher moisture (14.03%), ash (1.20%), fiber (2.70%), and fat (1.19%) contents than wheat flour, which contained 13.01% moisture, 0.60% ash, 0.59% fiber, and 0.60% fat. The protein content was also slightly higher in maize flour (11.22%) than in wheat flour (10.60%). Overall, maize flour showed comparatively higher values for all measured parameters, suggesting its potential to influence the nutritional composition of wheat–maize composite flour.

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Table 2. Composition of maize flour and wheat flour

Parameters	Maize flour (%)	Wheat flour (%)
Moisture %	14.03	13.01
Ash %	1.20	0.60
Protein %	11.22	10.60
Fiber %	2.70	0.59
Fat %	1.19	0.60

Table 3. Gluten content of wheat–maize composite flours

Treatments	Wet gluten (%)	Dry gluten (%)
WC_0	22.10^a	7.05^a
WC_1	20.05^b	6.12^b
WC_2	18.17^c	5.21^c
WC_3	15.12^d	4.05^d
WC_4	11.21^e	3.22^e
WC_5	0.00	0.00

WC_0 = 100% wheat flour (control); WC_1 = 80% wheat flour + 20% maize flour; WC_2 = 60% wheat flour + 40% maize flour; WC_3 = 40% wheat flour + 60% maize flour; WC_4 = 20% wheat flour + 80% maize flour; and WC_5 = 100% maize flour. Each formulation contained a total of 250 g of flour.

Similar results were noted by the previous studies (Hussain *et al*., 2006; Kamaljit *et al*., 2010).

3.2 Gluten content of wheat–maize **composite flour**

The wet and dry gluten contents of wheat–maize composite flour decreased progressively with increasing maize flour incorporation (Table 3). The control treatment (WC_0) exhibited the highest wet gluten content (22.10%) and dry gluten content (7.05%). In WC_1, wet and dry gluten contents decreased to 20.05% and 6.12%, respectively. A further reduction was observed in WC_2 and WC3, with wet gluten contents of 18.17% and 15.12% and dry gluten contents of 5.21% and 4.05%, respectively. WC_4 exhibited considerably lower wet and dry gluten contents of 11.21% and 3.22%, respectively. No gluten was detected in WC_5, as both wet and dry gluten contents were 0.00%.

The progressive decline in gluten content can be attributed to the dilution of wheat gluten-forming proteins by maize flour, which does not contain the gluten-forming protein network characteristic of wheat. As the proportion of maize flour increases, the concentration of gluten-forming proteins in the composite flour decreases, resulting in lower wet and dry gluten contents. The absence of detectable gluten in WC_5 suggests that this formulation contained no wheat flour or an insufficient amount of wheat-derived gluten to be detected under the applied analytical conditions. These findings indicate that increasing maize flour incorporation reduces the gluten-forming capacity of wheat–maize composite flour, which may affect dough elasticity, gas retention, and the structural characteristics of the final baked product.

3.3 Analysis of cookies prepared using wheat–maize composite flour

3.3.1 Physical analysis

The width of the samples gradually decreased from 45.72 ± 0.26 in WC_0 to 42.53 ± 0.08 in WC_5, whereas thickness increased slightly from 10.26 ± 0.03 to 10.38 ± 0.05 (Table 4). However, these variations were statistically non-significant ($p>0.05$), indicating that the different wheat–maize flour formulations did not significantly affect the width and thickness of the samples. These findings complement those of Sharif, *et al*. (2005) who found an increase in bakery product thickness with varying amounts of fibers incorporated. Moisture migration can affect crispness and texture in low-moisture baked products. However, water activity, which is a more direct indicator of the availability of water for physicochemical and microbial changes, was not measured in the present study. Therefore, the observed moisture increase should not be interpreted as direct evidence of changes in microbial stability or shelf life (Piga *et al*., 2005; Xu *et al*., 2020).

3.3.2 Proximate composition

During storage cookies were evaluated for the moisture content which increased as storage time increased (Table 5 & 6). Highest mean value was WC5 (3.77) and WC_4 (3.75) while lowest mean value was in WC_0 (3.64) and WC_1 (3.68). Initial day (3.63) was the lowest mean value and (3.78) was the highest mean value found at (120th) day. Cookies' Moisture level found significant ($p<0.05$) affected during storage and varies in combination of various ratios of maize flour.

The increased moisture content in the prepared cakes in combos of wheat and cocoyam flour was also noted by yetunde and chiemela (2015). Akusu *et al* (2016) claimed a high moisture content of cakes and chin-chin made from composite blend of wheat and cocoyam flour. Likewise, Miguel *et al*. (1999) reported that an increasing amount of peach fiber supplementation resulted in a higher moisture content of the muffin. The reason for this trend could be the water holding properties of dietary fiber which can hold

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Table 4. Effects of treatments on width and thickness of cookies

Parameters	WC_0	WC_1	WC_2	WC_3	WC_4	WC_5
Width	45.72±0.26	45.42±0.18	44.85±0.11	44.65±0.11	43.33±0.12	42.53±0.08
Thickness	10.26±0.03	10.29±0.04	10.30±0.04	10.33±0.04	10.35±0.04	10.38±0.05

WC_0 = 100% wheat flour (control); WC_1 = 80% wheat flour + 20% maize flour; WC_2 = 60% wheat flour + 40% maize flour; WC_3 = 40% wheat flour + 60% maize flour; WC_4 = 20% wheat flour + 80% maize flour; and WC_5 = 100% maize flour. Each formulation contained a total of 250 g of flour.

significant amounts of water within food products (Sudha *et al*., 2007). Similarly, the moisture holding capacity of wheat flour can be increased when rice bran is incorporated due to cellulose and hemicellulose present in its bran.

In fiber enriched products, moisture retention is related to the adsorption and absorption of water by dietary fiber, the water which is within the fiber matrix and free water that surrounds fiber matrix. Moisture content can also be affected by packaging and storage conditions. The biscuit moisture values could have been altered during storage due to moisture uptake from the stored environment which occurred if the biscuit were packaged in polypropylene bag (Piga *et al*., 2005). Uysal *et al*. (2007) also indicated that supplementing fiber significantly influenced moisture content of oven-baked biscuits, which is in agreement with these findings. The rise was attributed by the authors to the high-water holding capacity of fiber versus wheat flour.

Cookies ash content (AC) was measured at 0, 30, 60, 90, 120, 150 days intervals and the result showed that cookies in cuts WC_5 (2.05) has the highest value of mean followed by WC_4 (2.00) and cookies in cuts WC_0 (1.48) has the lowest value of mean followed by WC_1 (1.75). Level of ash content of cookies was not significantly affected during storage period of 120 days. The resulting differences, however, for ash content between the different treatments can be explained by the addition of fiber rich maize flour to the product containing extra mineral content. Also, measurers' Hussein *et al*. (2011) found a higher ash content after supplementation with fiber. However, baked products prepared from flour that come from grain mixtures containing more mineral content had higher mineral levels than those made from the pure flours (Pasha *et al*. 2011). As ovens continued to mount until 100% cocoyam flour was incorporated in cake with 50% in Chin-Chin, Ash content of all obanki cakes and Chin-Chin increased from 1.43% to 1.70% respectively (Akusu *et al*., 2016). The consequences were consistent with these findings as Kiin-Kabari and Banigo (2015) observed that cakes containing wheat and

Table 5. Effects of treatments on proximate analysis of cookies

Parameters	WC_0	WC_1	WC_2	WC_3	WC_4	WC_5
Moisture	$3.64±0.04^{f}$	$3.68±0.05^{e}$	$3.70±0.06^{d}$	$3.73±0.06^{c}$	$3.75±0.07^{b}$	$3.77±0.08^{a}$
Protein	$9.47±0.11^{f}$	$9.62±0.10^{e}$	$9.70±0.11^{d}$	$9.84±0.11^{c}$	$9.94±0.12^{b}$	$10.05±0.12^{a}$
Ash	$1.48±0.03^{e}$	$1.75±0.04^{d}$	$1.84±0.03^{c}$	$1.93±0.04^{bc}$	$2.00±0.07^{b}$	$2.05±0.07^{a}$
Fat	$18.74±0.38^{e}$	$19.15±0.40^{d}$	$19.59±0.41^{c}$	$19.71±0.45^{b}$	$19.84±0.49^{ab}$	$19.96±0.43^{a}$
Fiber	$3.01±0.07^{f}$	$3.57±0.08^{e}$	4.17±0.09d	$4.76±0.09^{c}$	$5.41±0.10^{b}$	$6.09±0.09^{a}$
Carbohydrates	$64.23±0.15^{f}$	$66.33±0.15^{e}$	$68.92±0.14^{d}$	$70.39±0.12^{c}$	$73.01±0.11^{b}$	$74.97±1.01^{a}$

WC_0 = 100% wheat flour (control); WC_1 = 80% wheat flour + 20% maize flour; WC_2 = 60% wheat flour + 40% maize flour; WC_3 = 40% wheat flour + 60% maize flour; WC_4 = 20% wheat flour + 80% maize flour; and WC_5 = 100% maize flour. Each formulation contained a total of 250 g of flour.

Table 6. Effect of storage on proximate analysis of cookies

Parameters	Initial	30 days	60 days	90 days	120 days
Moisture	$3.63±0.03^{e}$	$3.69±0.04^{d}$	$3.72±0.05^{c}$	$3.74±0.05^{b}$	$3.78±0.07^{a}$
Protein	$9.91±0.22^{a}$	$9.85±0.21^{b}$	$9.77±0.21^{c}$	$9.70±0.21^{d}$	$9.63±0.21^{e}$
Ash	$1.91±0.23^{a}$	$1.87±0.21^{a}$	$1.84±0.21^{c}$	$1.81±0.20^{e}$	$1.79±0.19^{e}$
Fat	$19.87±0.47^{a}$	$19.75±0.49^{b}$	$19.62±0.48^{b}$	$19.47±0.50^{bc}$	$18.79±0.41^{d}$
Fiber	$4.62±1.19^{a}$	$4.57±1.17^{b}$	$4.49±1.15^{c}$	$4.45±1.15^{d}$	$4.39±1.14^{e}$
Carbohydrates	$69.23±0.15^{b}$	$66.33±0.15^{bc}$	$69.58±3.91^{c}$	$69.65±3.91^{c}$	$70.07±4.48^{a}$

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plantain flour added with Bambara groundnut flour had a higher ash content. Protein content differed significantly among flour treatments ($p<0.05$), with means ranging from 9.47% in WC_0 to 10.05% in WC_5. Storage did not show a significant main effect. The treatment-related increase reflects the composition of the flour blends rather than an increase caused by storage.

Crude fiber content increased significantly with increasing maize flour substitution, from 3.01% in WC_0 to 6.09% in WC_5, whereas storage did not significantly affect fiber content. The higher fiber level is consistent with the greater fiber contribution of maize flour relative to the wheat flour used in the study.

The protein content of cookies increased progressively with increasing maize flour incorporation, rising from 9.47 ± 0.11% in WC_0 to 10.05 ± 0.12% in WC_5. This increase may be attributed to the comparatively higher protein content of maize flour than wheat flour used in the composite formulations. In contrast, during storage, protein content gradually decreased from 9.91 ± 0.22% initially to 9.63 ± 0.21% after 120 days, suggesting a slight reduction in measured protein content over the storage period.

Fat content differed significantly among flour treatments ($p<0.05$), ranging from 18.74% in WC_0 to 19.96% in WC_5, while the storage main effect was not significant. The treatment-related differences are consistent with differences in the composition of the two flour sources. The carbohydrate was calculated for corn flour supplemented cookies after 30 days interval. Results have shown that the quantity of carbohydrates increased in treatment and did not change during storage. Carbohydrate level in cookies was significantly ($p<0.05$) affected by the treatment, and the storage period had no effect. Within treatments the carbohydrate content was raised by the supplementation of wheat flour with maize flour. Dietetic value of cakes baked with the addition of whole flaxseed flour.

3.3.2 Sensory evaluation

Cookies samples were analyzed organoleptically for their color, flavor, texture, and overall acceptability. Taste scores differed significantly among flour treatments ($p<0.05$), ranging from 8.03 in WC_0 to 7.05 in WC_5 (Table 7 & 8). Storage did not show a significant main effect. Among reduced-gluten treatments, WC_1 (7.95) retained the highest mean taste score. Texture scores differed significantly among flour treatments ($p<0.05$), with means of 7.98 in WC_0 and 7.05 in WC_5. Storage did not show a significant main effect. WC1 recorded a mean texture score of 7.88 and therefore performed better than the higher-maize treatments on this sensory attribute. As per data, the quality of texture to some extent decreases its value within treatments and during storage period no changes were observed. Maximum mean was shown by WC_0 (7.98) chased by WC_1 (7.88), while minimum mean found in WC_5 (7.05) trailed by WC_4 (7.32). A significant difference ($p<0.05$) was obtained in the cookies while no difference was noticed at each interval of 30 days of the storage period of 120 days. Results were like those of Eneche (1999) who revealed maximum score for texture and overall acceptability for biscuits made from the

Table 7. Effect of treatment on sensory attributes of cookies

Parameters	WC_0	WC_1	WC_2	WC_3	WC_4	WC_5
Color	8.14 ± 0.09^a	8.07 ± 0.10^a	8.02 ± 0.10^a	7.92 ± 0.10^a	7.63 ± 0.23^a	7.35 ± 0.27^a
Taste	8.03 ± 0.10^c	7.95 ± 0.11^b	7.88 ± 0.10^b	7.82 ± 0.12^b	7.31 ± 0.24^c	7.05 ± 0.22^c
Texture	7.98 ± 0.10^d	7.88 ± 0.11^c	7.80 ± 0.12^d	7.73 ± 0.12^d	7.32 ± 0.13^d	7.05 ± 0.19^c
Overall acceptability	8.05 ± 0.09^b	8.07 ± 0.10^a	7.85 ± 0.11^c	7.76 ± 0.12^c	7.55 ± 0.12^b	7.26 ± 0.14^b

WC_0 = 100% wheat flour (control); WC_1 = 80% wheat flour + 20% maize flour; WC_2 = 60% wheat flour + 40% maize flour; WC_3 = 40% wheat flour + 60% maize flour; WC_4 = 20% wheat flour + 80% maize flour; and WC_5 = 100% maize flour. Each formulation contained a total of 250 g of flour.

Table 8. Effect of storage on sensory attributes of cookies

Parameters	Initial	30 days	60 days	90 days	120 days
Color	8.01 ± 0.26^a	7.94 ± 0.26^b	7.88 ± 0.27^c	7.81 ± 0.27^d	7.63 ± 0.45^e
Taste	$7.85\pm o.33^b$	7.78 ± 0.35^a	7.68 ± 0.40^c	7.59 ± 0.44^d	7.48 ± 0.48^e
Texture	7.79 ± 0.32^a	7.71 ± 0.34^b	7.63 ± 0.37^c	7.56 ± 0.38^d	7.46 ± 0.40^e
Overall acceptability	7.80 ± 0.28^a	7.74 ± 0.28^b	7.68 ± 0.29^c	7.87 ± 0.29^d	7.57 ± 0.32^e

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combination of 35% pigeon pea and 65% millet flour.

The acceptability of the cookies was analyzed at all 30 days of the storage period. The reduced acceptability during treatments, and no influence indicated during storage period were found in the results. The results show that there was a significant difference ($p<0.05$) as Adeyeye *et al* (2014) reported the attributes of biscuit produced using combination of sweet potato and maize flour. The results showed that Singh *et al*., (1993) obtained the highest sensory acceptability scores (30% soy flour) for their product with the highest acceptability scores for all sensory attributes.

The study demonstrated that increasing maize flour substitution progressively reduced measured wet and dry gluten in the flour treatments and significantly altered several physicochemical and sensory characteristics of cookies. Flour treatment affected width, thickness, ash, protein, crude fibre, fat, carbohydrate and sensory attributes, whereas storage mainly affected moisture. Mean moisture increased from 3.63% initially to 3.78% after 120 days. WC_5 (100% maize flour) produced the highest values for protein (10.05%), ash (2.05%), fibre (6.09%), fat (19.96%) and carbohydrate (74.97%), but it received lower sensory scores. WC1 (80:20 wheat: maize flour) had the highest overall acceptability among the reduced-gluten formulations, with a mean score of 7.92. Thus, WC_1 may be considered the most promising formulation within the tested reduced-gluten range based on the balance between measured gluten reduction and sensory acceptance, rather than being described as universally "best." Because water activity was not measured and the retained dataset did not contain independent replicate observations for estimating treatment × storage interaction, further work should include direct water-activity measurements, validated analytical gluten quantification and a fully replicated factorial design.

3.4 Integrated interpretation of treatment and storage effects

Overall, increasing maize substitution produced a clear formulation effect: measured wet and dry gluten decreased, while ash, protein, fiber, fat and NFE increased and cookie width and sensory scores generally decreased. Storage, in contrast, had its clearest effect on moisture, with relatively limited changes in the other reported main effects. These findings indicate a trade-off between greater reduction in measured gluten and sensory quality. WC_5 provided the greatest reduction in measured gluten and the highest values for several proximate constituents, but it had the lowest overall acceptability among the formulations. WC_1 therefore represents the most balanced reduced-gluten formulation in the present dataset from a sensory perspective.

Conclusion

Different ratios of wheat and maize flour were added to make reduced gluten cookies such as WC_0 (100% wheat flour) control, WC_1 (80:20), WC_2 (60:40), WC_3 (40:60), WC_5 (20:80) and WC_5 (100% maize flour). All the samples showed acceptability on behalf of physio-chemical and sensory properties. However, in all the samples, the best physio-chemical attributes were obtained by WC_5 (100: Maize flour) and WC_1 (80:20%) for the best sensory properties, respectively. Throughout storage, minimum mean value was indicated as (3.63) in beginning while maximum mean as (3.78) at final stage of storage. The only significant effect was observed for 120 days storage period in cookies, other than moisture content, no significant effect was observed on treatment. Overall, study revealed that all samples were in acceptable range. WC_1 (40:60) was confirmed as the best one based on sensory attributes. Although, the best results had been shown by physicochemical analysis were WC_5. The protein content was increased treatment wise with this formulation.

Funding

No external funding was reported for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Iftikhar Aziz and Muhammad Ayub: conceptualization, experimentation and manuscript preparation. Ali Muhammad and Irfan Ullah: data collection and manuscript review. Khitab Ullah and Muhammad Adnan Khan: statistical analysis and manuscript review.

Acknowledgments

The authors acknowledge the Food Science and Technology laboratory staff at The University of Agriculture Peshawar for assistance with laboratory analyses and cookie preparation.

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