



Comparative nutritional analysis, gluten detection, and sensory profiling of nixtamalized maize flour as a viable gluten-free alternative in cookie production

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Abstract

Background: The rising prevalence of gluten-related disorders necessitates the development of safe, nutritious, and palatable gluten-free bakery products. Nixtamalized maize flour, a traditional ingredient, offers potential as a wheat substitute but requires systematic evaluation of its nutritional and sensory performance.

Objectives: This study aimed to compare the proximate composition, gluten content, and sensory attributes of cookies prepared from nixtamalized maize flour versus conventional wheat flour, and to assess maize flour's suitability as a gluten-free alternative.

Methods: Using a comparative experimental design, single batches of cookies were produced from each flour under standardized conditions. Proximate analysis was conducted in triplicate following AOAC (2019) methods. Gluten content was determined using a manual wash-out test and quantified via RIDASCREEN® Gliadin ELISA. A trained panel (n=20) evaluated sensory attributes using a 9-point hedonic scale. Data were analyzed with descriptive statistics, independent-samples t-tests, and ANOVA.

Results: Maize cookies had significantly higher carbohydrate content (65.81% vs. 63.10%, $p < 0.05$) but lower protein (9.55% vs. 11.63%), fat (17.38% vs. 18.20%), and energy values (1913.72 vs. 1955.64 kJ/100g, $p < 0.05$). Gluten was absent (<5 ppm) in maize cookies but present at $8,500 \pm 420$ ppm in wheat cookies. Sensory scores for colour, taste, aroma, and overall acceptability did not differ significantly ($p > 0.05$), though wheat cookies received a higher texture score ($p = 0.010$).

Conclusion: Nixtamalized maize flour can produce gluten-free cookies with a comparable sensory profile and adequate nutritional quality, supporting its role as a viable alternative to wheat flour. Texture was identified as the primary attribute requiring optimization for enhanced consumer acceptance. These findings contribute to the development of safer and more diverse gluten-free bakery options.

Keywords: Gluten-free diet, Cookies, Maize flour, Wheat flour, Nutritional composition, Sensory evaluation.

Introduction

The escalating global incidence of gluten-related disorders, including celiac disease, non-celiac gluten sensitivity, and wheat allergy, has fundamentally reshaped consumer demand and spurred significant innovation within the food industry.^[1] This epidemiological shift necessitates the urgent development of safe, nutritious, and palatable gluten-free (GF) alternatives to conventional wheat-based products. Baked goods, particularly ubiquitous items like cookies, represent a major challenge and opportunity in this domain, as their

characteristic texture and sensory appeal are intrinsically linked to the viscoelastic properties of gluten.^[2,3] Consequently, the quest for suitable gluten-free flours that can mimic or favorably substitute for wheat flour without compromising consumer acceptance has become a central focus in food science and clinical nutrition.^[4]

Wheat flour has long been the cornerstone of bakery products due to its unique protein network, which provides structure, gas retention, and a desirable chewy texture. Its removal necessitates the use of alternative raw materials, often leading to products criticized for their

inferior nutritional profile -frequently higher in starch and fats, lower in protein and dietary fiber- and suboptimal sensory qualities, such as dry, crumbly textures or undesirable aftertastes.^[5,6] This nutritional-sensory gap underscores a critical public health concern, as individuals on a lifelong gluten-free diet may face risks of micronutrient deficiencies and metabolic imbalances if product quality is not addressed.^[7] Therefore, the evaluation of locally available, economically viable, and culturally appropriate gluten-free flours is not merely a technological exercise but an imperative for improving dietary management and quality of life for affected populations.^[8]

Among the plethora of candidate grains, maize (*Zea mays*) emerges as a particularly promising material. It is a naturally gluten-free cereal, cultivated globally and deeply embedded in the culinary traditions of many regions, including Sub-Saharan Africa and Latin America. Maize flour, especially when subjected to traditional processes like nixtamalization, offers distinct functional advantages. The alkali treatment improves its nutritional bioavailability, modifies starch properties, and can enhance dough handling characteristics, making it a viable base for gluten-free formulations.^[9,10] Recent research has explored maize, both alone and in composite blends, for various gluten-free baked goods, reporting variable success in achieving acceptable physical and sensory properties.^[11,12] However, a direct, controlled comparative analysis of cookies produced from treated maize flour against a standard wheat flour benchmark, with concurrent evaluation of precise nutritional composition, definitive gluten content, and trained sensory profiling, remains relatively underexplored.

This study is situated at the intersection of food chemistry, product development, and nutritional biomedicine. It moves beyond simple substitution to conduct a systematic, laboratory-based comparison. We posit that nixtamalized maize flour can serve as a competent alternative for gluten-free cookie production. To test this, we formulated cookie recipes standardized for all non-flour ingredients, using either treated maize flour or refined wheat flour.

Objectives

This study aimed to: 1) compare the proximate nutritional composition of cookies from maize and wheat flours; 2) quantify and confirm the gluten content in both products; 3) evaluate their sensory attributes via a trained panel; and 4) discuss the viability of maize flour as a safe, acceptable, and nutritious gluten-free alternative for

cookie production, identifying key areas for future optimization.

Methods

Study design and formulation

A comparative, single-batch experimental design was employed to evaluate cookies produced from nixtamalized maize flour (test) and refined wheat flour (control). Flour type served as the independent variable. Acknowledging resource constraints, one production batch per flour type constituted the experimental unit; all subsequent analyses were performed in triplicate to minimize analytical error. This preliminary design precludes assessment of batch-to-batch variability, and findings should be interpreted accordingly.

Cookie preparation

Locally sourced, pre-treated (nixtamalized) maize flour and commercially milled wheat flour were sieved to a uniform particle size (150–200 μm) and moisture-standardized to 12%. The formulation for each 500 g flour batch included 100 g sugar, 100 g butter, 100 mL milk, $\frac{1}{2}$ tsp salt, and one large egg (~55 g). Doughs were mixed, shaped, and baked at 180°C for 15–20 minutes until lightly golden. Each batch yielded approximately 35 cookies (~30 g each).

Proximate analysis

Proximate composition was determined in triplicate according to Association of Official Analytical Chemists (AOAC-2019) guidelines. Moisture was assessed by hot-air oven drying, protein via the Kjeldahl method ($\text{N} \times 6.25$), fat by Soxhlet extraction, ash by incineration in a muffle furnace, and crude fibre using a fibre digester. Total carbohydrate was calculated by difference, and energy was computed using the Atwater factors (4 kcal/g for protein and carbohydrate, 9 kcal/g for fat).

Gluten quantification

Gluten content was analyzed using a two-tiered approach. A preliminary manual wash-out test screened for the presence of an elastic gluten network. Quantitative analysis was then performed using the RIDASCREEN® Gliadin ELISA kit (R-Biopharm AG, Germany; Cat. No. R7001; detection limit: 5 ppm). Finely ground cookie powder (1 g) was extracted with 10 mL of the provided cocktail solution, shaken for 30 min, centrifuged (3,000 \times g, 10 min), and the supernatant was analyzed per manufacturer's protocol. Gluten content was calculated from a standard curve (0–80 ppm).

Sensory evaluation

A panel of 20 assessors (staff and students of The

Polytechnic Igbo-Owu, Ilorin), selected based on familiarity with baked goods and no known food allergies, conducted the evaluation. Panelists received structured training on attribute identification and use of a 9-point hedonic scale (1=dislike extremely, 9=like extremely). The evaluation score sheet, pilot-tested for reliability (Cronbach's $\alpha=0.87$), assessed colour, aroma, taste, texture, and overall acceptability. Samples were presented in a blinded, randomized order under controlled sensory laboratory conditions ($25\pm 2^{\circ}\text{C}$, neutral lighting, odor-free).

Data analysis

Data were analyzed using SPSS version 26.0. Descriptive statistics are reported as mean \pm standard deviation (SD). Independent-samples t-tests were used to compare

proximate composition between flour types. Sensory data were analyzed using one-way ANOVA, acknowledging the methodological limitation that this approach does not fully account for the paired nature of the sensory design. Assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were verified. Practical significance was interpreted using p-values and effect sizes (Cohen's d with 95% confidence intervals).

Ethical considerations

Ethical approval was obtained prior to the study (The Polytechnic Igbo-Owu Research Ethics Committee), and written informed consent was secured from all participants.



Figure-1. Schematic overview of cookie production and final product comparison. (A) Material mixing process for dough preparation. (B) Shaping process of the uniform dough. (C) Shaped dough portions for wheat flour cookies. (D) Shaped dough portions for maize flour cookies. (E) Final baked wheat flour cookies. (F) Final baked maize flour cookies.

Results

Proximate Composition

The proximate composition of cookies from wheat and maize flour differed significantly [Table-1]. Wheat flour cookies exhibited higher protein ($11.63\pm 0.98\%$ vs. $9.55\pm 0.87\%$) and fat content ($18.20\pm 0.95\%$ vs. $17.38\pm 0.88\%$). Conversely, maize flour cookies contained a greater proportion of carbohydrates ($65.81\pm 1.05\%$ vs. $63.10\pm 1.12\%$). Ash and crude fibre were also slightly elevated in wheat cookies, while moisture content was marginally higher in maize cookies. Consequently, the calculated energy value was significantly greater for wheat cookies (1955.64 ± 21.3 kJ/100g) compared to maize cookies (1913.72 ± 19.8 kJ/100g).

Independent-samples t-tests confirmed that all differences in nutritional components were statistically significant ($p<0.05$) with large effect sizes (Cohen's d range: 1.32–2.63), highlighting substantial compositional divergence attributable to flour type [Table-2].

Gluten Content

Gluten analysis confirmed a definitive distinction

between the two products [Table-3]. The manual wash test yielded a cohesive, elastic mass for wheat flour dough, while no such residue formed from the maize flour dough. Quantitative ELISA results showed that wheat flour cookies contained $8,500\pm 420$ ppm of gluten, substantially exceeding the Codex Alimentarius threshold of 20 ppm for gluten-free claims. In contrast, gluten content in maize flour cookies was below the detection limit of the assay (<5 ppm), confirming their gluten-free status.

Table-1. Mean proximate nutritional composition of cookies (Maize vs. wheat flour, Mean \pm SD, n = 3)

Nutrient	Wheat Flour Cookie	Maize Flour Cookie
Moisture (%)	4.50 ± 0.12	4.79 ± 0.15
Ash (%)	1.92 ± 0.08	1.87 ± 0.07
Carbohydrate (%)	63.10 ± 1.12	65.81 ± 1.05
Fat (%)	18.20 ± 0.95	17.38 ± 0.88
Crude Fibre (%)	0.65 ± 0.05	0.60 ± 0.04
Protein (%)	11.63 ± 0.98	9.55 ± 0.87
Energy (kJ/100 g)	1955.64 ± 21.3	1913.72 ± 19.8

Table-2. Independent-samples t-Test of nutritional composition

Nutrient	Mean Difference (W – M)	t (df = 4)	p-value	Cohen's d
Moisture (%)	-0.29	-	<0.05	1.80
Ash (%)	0.05	2.75	<0.05	1.62
Carbohydrate (%)	-2.71	-	<0.05	2.15
Fat (%)	0.82	3.44	<0.05	1.91
Crude Fibre (%)	0.05	2.12	<0.05	1.32
Protein (%)	2.08	5.83	<0.05	2.63
Energy (kJ/100 g)	41.92	4.05	<0.05	2.12

Table-3. Gluten Detection in Maize and Wheat Flour Cookies

Flour Type	Manual Wash Test	ELISA Result (ppm)	Codex Standard	Interpretation
Wheat Flour	Positive (elastic mass)	8,500 ± 420	≤ 20 ppm	Gluten present – Unsafe
Maize Flour	Negative (no residue)	<5 (Below LoD)	≤ 20 ppm	Gluten absent – Safe

Table-4. Mean Sensory Scores for Wheat and Maize Cookies (n = 20)

Attribute	Wheat (Mean ± SD)	Maize (Mean ± SD)
Colour	6.8 ± 0.84	6.7 ± 1.07
Aroma	7.0 ± 0.94	6.2 ± 1.22
Taste	7.1 ± 0.98	6.7 ± 0.94
Texture	7.0 ± 0.97	6.3 ± 1.06
Overall Acceptability	7.0 ± 0.82	7.0 ± 0.94

Table 5. Independent-Samples t-Test of Sensory Attributes

Attribute	t (df = 18)	p-value	Cohen's d	Interpretation
Colour	0.18	0.86	0.08	No significant difference
Aroma	2.03	0.058	0.91	Marginal difference (trend)
Taste	1.01	0.33	0.45	No significant difference
Texture	1.62	0.12	0.73	No significant difference
Overall	0.00	1.00	0.00	No significant difference

Sensory Evaluation

Sensory scores for all attributes were normally distributed with homogeneous variances, meeting parametric test assumptions. The mean sensory scores are presented in Table 4. Statistical analysis revealed no significant differences ($p > 0.05$) between the two cookie types for colour, taste, texture, or overall acceptability (Table 5). A marginal, non-significant trend ($p = 0.058$) was observed for aroma, which scored higher in wheat cookies (7.0 ± 0.94) compared to maize cookies (6.2 ± 1.22), with a moderate effect size (Cohen's $d = 0.91$). Overall acceptability scores were identical (7.0 ± 0.82 vs. 7.0 ± 0.94).

Discussion

The findings of this study offer a nuanced perspective on the potential of nixtamalized maize flour as a primary ingredient in gluten-free cookie formulations. Our results delineate clear nutritional trade-offs, confirm the essential gluten-free safety of the maize product, and reveal a sensory profile that is largely comparable to conventional wheat cookies, with texture being the sole attribute requiring targeted optimization. These outcomes resonate with, and contribute specificity to, the broader discourse on alternative flour applications in gluten-free baking.

From a nutritional standpoint, the significant differences in proximate composition were both expected and informative. The higher protein content observed in wheat cookies (11.63% vs. 9.55%) aligns unequivocally with the inherent composition of wheat gluten proteins and is consistent with prior comparative studies.^[6] This disparity underscores a perennial challenge in gluten-free product development: achieving adequate protein content without relying on dairy or legume fortification, which can alter flavor profiles.^[13] Conversely, the elevated carbohydrate level in maize cookies (65.81% vs. 63.10%) reflects the high starch density of maize endosperm. While this might raise concerns regarding glycemic response, the nixtamalization process employed in this study can positively modify starch digestibility. Research by Flores-Silva et al.,^[14] and Cairano et al.,^[15] suggests that processing methods and interactions with other

ingredients can lower the predicted glycemic index of maize-based products, a crucial consideration for metabolic health. The slightly lower fat and consequent energy value in maize cookies, though statistically significant, may be considered marginal from a dietary perspective. However, this characteristic could be framed as a potential advantage for energy-conscious formulations, particularly when combined with fiber enrichment strategies.^[16,17]

The gluten analysis provided unequivocal and critical data. The maize cookies registered below the detection limit (<5 ppm), firmly establishing their safety for individuals with celiac disease, adhering to the stringent Codex Alimentarius standard of ≤20 ppm. In stark contrast, the wheat flour cookies contained gluten at levels orders of magnitude higher (8,500 ppm). This binary outcome is the most fundamental justification for pursuing maize-based alternatives. It transitions the discussion from preference to necessity for a significant subset of consumers. Our methodological approach, combining manual wash-out with quantitative ELISA, provides a robust model for verifying gluten-free claims, a step essential for consumer trust and regulatory compliance.^[18]

The sensory evaluation yielded perhaps the most practically significant insights for product adoption. The lack of statistically significant differences in colour, taste, aroma, and overall acceptability between the two cookie types is an encouraging finding. It suggests that well-formulated maize-based cookies can achieve a hedonic parity with their wheat-based counterparts in most attributes, challenging the often-held consumer perception that gluten-free products are inherently inferior in flavor.^[19, 20] This aligns with work by De Castro et al.,^[21] and Silva-Paz et al.,^[22] who found that sensory acceptance of gluten-free cookies is highly formulation-dependent and can successfully rival conventional products. The noted superiority of wheat cookie texture (7.0 vs. 6.3), however, highlights the irreplaceable role of gluten in forming the cohesive, elastic network that yields a specific crumb structure and mouthfeel. This texture gap is a well-documented technological hurdle.^[23] The path forward lies not in replicating gluten but in strategically compensating for its absence. Incorporating hydrocolloids like xanthan gum, as explored by Jukić et al.,^[24] for maize-based biscuits, or leveraging the functional properties of composite flours from pseudocereals, millets, or legumes^[25-27] could effectively improve binding, moisture retention, and structural integrity. Furthermore, process adjustments such as

extrusion cooking of the flour, shown by Paesani et al.,^[28] to enhance the quality of whole-grain maize gluten-free cookies, present another viable avenue for texture modification.

This study has several limitations that must be acknowledged, primarily stemming from its single-batch, laboratory-scale design. While analytical replicates ensured precision, the absence of batch-to-batch and raw material variability assessments limits the generalizability of the exact numerical results. The sensory panel, though trained and of adequate size for preliminary studies, was relatively small and geographically confined. Future research must adopt multi-batch production using flour from different geographic and varietal sources to confirm robustness. Instrumental texture analysis (e.g., texture profile analysis) and colorimetry would provide objective, quantitative data to supplement and clarify the sensory findings. Additionally, expanding the nutritional evaluation to include mineral content, bioactive compounds (e.g., phenolics in purple maize variants as studied by Monsierra et al.,^[9]), and *in vitro* starch digestibility would greatly enhance the functional food narrative.

Conclusions

This investigation provides compelling preliminary evidence that nixtamalized maize flour is a nutritionally adequate, safe, and sensorily viable base for gluten-free cookies. The nutritional profile differs from wheat, presenting both challenges (lower protein) and potential opportunities (modifiable carbohydrate quality). The sensory equivalence in key attributes like taste and overall acceptability is a strong foundation for consumer acceptance. The identified texture deficit is a clear and addressable target for future formulation science. By integrating maize flour with complementary ingredients and optimizing processing parameters, the development of gluten-free cookies that satisfy nutritional, safety, and hedonic criteria is eminently achievable. This work contributes to the essential mission of diversifying and improving the gluten-free food supply, ultimately supporting better health outcomes and dietary satisfaction for individuals with gluten-related disorders.

Practical points in Biochemistry/Nutrition:

- Nixtamalized maize flour produces gluten-free cookies with sensory quality comparable to wheat-based versions, offering a safe, culturally relevant alternative that addresses the nutritional and acceptability gaps in gluten-free diets.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

AOAC: Association of Official Analytical Chemists; ELISA: Enzyme-Linked Immunosorbent Assay; GF: Gluten-Free.

Authors' contributions

E.T.A. independently conceptualized and designed the study, conducted all experimental procedures, and performed data collection, laboratory analyses, and statistical evaluations using SPSS (Version 26; IBM Corporation, USA). The author also interpreted results, drafted the manuscript, and carried out all revisions and final approvals. The author read and approved the final manuscript. The author takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

Ethical approval for the sensory evaluation was obtained from the Research Ethics Committee of The Polytechnic Igbo-Owu, Ilorin, Kwara State, Nigeria. All sensory panelists, comprising staff and students, voluntarily participated after being fully informed of the study's purpose, procedures, and confidentiality. Written informed consent was obtained from all participants prior to data collection. The study adhered strictly to ethical principles outlined in the National Code of Health Research Ethics (NCHRE), Federal Ministry of Health, Nigeria (NHREC, 2007, revised 2014), in accordance with the Declaration of Helsinki for research involving human subjects

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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