

DEVELOPMENT OF FOX NUT FORTIFIED BREAD TO MANAGE DIABETES TYPE 2

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Abstract

Type 2 diabetes mellitus (T2DM) remains a major public health burden, and functional foods based on widely consumed staples such as bread offer a practical avenue for improving glycemic control. This study developed and evaluated a fox nut (*Euryale ferox*)-fortified wheat bread (150 g fox nut flour per 1 kg wheat flour; 15% fortification) for its proximate composition, sensory acceptability, and clinical efficacy in patients with T2DM. Proximate analysis (AOAC methods) showed 31.50±0.25% moisture, 12.10±0.18% protein, 3.20±0.10% fat, 2.80±0.12% fiber, 1.90±0.08% ash, 48.50±0.32% carbohydrate, and 271.2 kcal/100 g energy. Fifteen semi-trained panelists rated the bread 8.07±0.46 ("like very much") on a 9-point hedonic scale. In a three-month randomized interventional trial, the experimental group (n=9, fortified bread) showed significantly greater reductions than the control group (n=10, standard bread) in fasting blood glucose (−32.3% vs −6.1%), postprandial blood glucose (−28.1% vs −4.8%), HbA1c (−19.2% vs −1.8%), and random blood sugar (−25.5% vs −4.9%) ($p < 0.05$ for all between-group comparisons at Month 3), with a modest, non-significant reduction in body weight (−2.0%, $p = 0.096$). These findings suggest that a 15% fox nut fortification level yields a nutritionally enhanced, sensorily acceptable bread capable of meaningfully improving glycemic parameters over three months, supporting its potential as an adjunctive dietary strategy for T2DM management pending confirmation in larger, multicenter trials.

1. Introduction

Type 2 diabetes mellitus (T2DM) has become one of the most pressing metabolic disorders of the twenty-first century, arising from impaired insulin secretion, insulin resistance, or both, and manifesting as chronic hyperglycemia [1]. Left inadequately controlled, hyperglycemia contributes to microvascular and macrovascular complications, including nephropathy,

neuropathy, retinopathy, and cardiovascular disease [2]. The disease burden is rising particularly fast in low- and middle-income countries: in Pakistan, diabetes prevalence increased from 11.7% in 2017 to 17.1% in 2019, and the International Diabetes Federation projects that 11.5 million Pakistanis will be living with diabetes by 2025, with an increasing share of cases now appearing in adults under 40 [3]. While

pharmacotherapy and insulin remain central to disease management, nutritional strategies that support glycemic control without imposing major lifestyle disruption have attracted growing scientific and clinical interest [2].

Fox nut (*Euryale ferox*), locally known as phool makhana, is an aquatic seed crop cultivated across South Asia, including India, China, and parts of Pakistan, and has a long history of use in indigenous medicine [4]. It is naturally gluten-free and nutritionally dense, providing complex carbohydrates, plant protein, dietary fiber, resistant starch, and minerals such as potassium, magnesium, phosphorus, iron, and zinc [4,5], alongside phenolic and flavonoid antioxidants [6]. Because these constituents are associated with a low glycemic index and antihyperglycemic activity, fox nut has been proposed as a candidate functional ingredient for dietary interventions in diabetes [4,7]. Incorporating fox nut flour into a widely consumed staple such as bread offers a way of delivering these bioactive compounds to patients without requiring substantial changes to established eating habits.

A growing body of mechanistic literature supports this rationale. The phenolic and flavonoid compounds in fox nut act as antioxidants that neutralize free radicals and may help offset the oxidative stress that accompanies chronic hyperglycemia, while also inhibiting the carbohydrate-digesting enzymes α -amylase and α -glucosidase, thereby slowing postprandial glucose release [6,8]. Ethnobotanical and pharmacological reviews of *Euryale ferox* similarly describe antihyperglycemic, anti-inflammatory, and antioxidant activity attributable to its alkaloid, tannin, and saponin content [23], and animal studies have identified specific triterpene constituents capable of ameliorating hyperglycemia in diabetic models [28]. The resistant starch fraction of fox nut is fermented slowly in the colon, producing short-chain fatty acids that may improve insulin sensitivity and, together with its soluble fiber content, promote satiety by delaying gastric emptying [5,29]. Its mineral profile is also relevant to glycemic risk: higher dietary magnesium intake has been

associated with a dose-dependent reduction in the risk of incident type 2 diabetes in prospective cohort studies [25].

Bread is an especially suitable vehicle for such fortification because of its universal and regular consumption, and prior work has fortified bread with a range of functional ingredients, including fennel seed, pomegranate peel powder, oat fiber, and nut oil cake, generally without compromising consumer acceptance [11,13,14,27,30]. With specific reference to fox nut, in vitro work has shown that wheat bread enriched with *Euryale ferox* seed-shell extract reduces the rate of starch hydrolysis and lowers the predicted glycemic index of the product, from 94.61 in control bread to 61.66 at a 2% enrichment level, while retaining acceptable sensory and textural properties [16]. Roasted whole fox nuts have likewise been shown to have a low glycemic index alongside elevated phenolic, flavonoid, and antioxidant content in human subjects [7]. However, this literature has largely relied on in vitro digestibility assays, extract-based formulations, or short-term sensory studies; no standardized fortification level for wheat flour bread has been established, and clinical evidence on fasting glucose, postprandial glucose, and HbA1c responses to fox nut-fortified bread particularly within the Pakistani population remains scarce.

Beyond its potential glycemic benefits, fortifying an everyday staple such as bread with fox nut flour carries broader practical advantages: it requires no specialized equipment or major recipe alteration, it improves the mineral and fiber density of a food already embedded in daily diets, and, if well tolerated, it could be scaled into commercial or hospital-based dietary products for diabetes management. Establishing whether such a product is both palatable and clinically effective is therefore a necessary first step before larger dose-ranging or multicenter trials are undertaken. Accordingly, this study aimed to (i) develop and evaluate the nutritional and sensory quality of a fox nut-fortified wheat bread, and (ii) assess its effect on glycemic parameters (fasting blood glucose, postprandial blood glucose, HbA1c, and

random blood sugar) and body weight in patients with Type 2 Diabetes Mellitus.

2. Materials and Methods

2.1 Study Design and Setting. This was an experimental, randomized interventional study conducted at Doctors' Clinic and Ruqqiya Clinic, Lahore, Pakistan, over a period of four months following synopsis approval.

2.2 Participants, Sample Size, and Sampling.

Twenty patients with Type 2 Diabetes Mellitus were enrolled using a non-probability convenience sampling technique. Patients were eligible for inclusion if they were aged 25 years or older, had a body mass index below 30 kg/m², were diagnosed with T2DM without additional organ dysfunction, and were able to attend follow-up visits; patients with type 1 diabetes, a BMI above 30 kg/m², or coexisting organ dysfunction were excluded. Participants were allocated to a control group (n=10, standard wheat bread) or an experimental group (n=10, fox nut-fortified bread). One participant in the experimental group was lost to follow-up after the first month and was excluded from the paired and endpoint analyses, leaving nine completers in that arm.

2.3 Procurement of Raw Materials. Fox nuts (*Euryale ferox*) were sourced from reputable grain suppliers to ensure freshness and purity, and whole wheat flour was obtained from a registered commercial flour mill. Yeast, iodized salt, drinking water, and edible vegetable oil were procured from

a reliable commercial food supplier in Lahore. All raw materials were inspected for quality and freshness before use, and fox nuts were cleaned, sieved, and ground into a fine flour using a laboratory grinder prior to incorporation into the wheat flour.

2.4 Development and Formulation of Fox Nut-Fortified Bread.

Bread was formulated at a 15% fortification level, corresponding to the substitution of 150 g of fox nut flour per 1 kg of total flour (Table 1), a ratio selected to retain sufficient gluten for adequate leavening while providing a measurable dose of fox nut-derived resistant starch, protein, and minerals. To prepare the bread, 150 g of fox nuts were roasted for 5–7 minutes, cooled, and ground into fine flour to avoid a gritty texture. The fox nut flour was combined with 850 g of wheat flour, warm water (600–650 mL), instant yeast (15 g), salt (18 g), and oil (30 g), and the dough was kneaded by hand for 10–12 minutes. The dough was proofed in a lightly oiled, covered bowl in a warm, draft-free environment for 50–60 minutes until doubled in volume, divided into large loaves (~400 g) or dinner rolls (~60 g), proofed for a further 45 minutes, and baked at 200°C until an internal temperature of 90°C was reached (approximately 25 minutes for loaves and 15 minutes for rolls). A standard serving of 50 g (approximately two slices) supplied approximately 4.4–5 g of fox nut flour, which constituted the tested dose in the clinical trial.

Table 1. Ingredient composition of fox nut-fortified bread (per 1 kg formulation)

Ingredient	Quantity	Function
Refined wheat flour	850 g	Provides gluten for structure and volume
Fox nut flour	150 g	Provides resistant starch, protein, and minerals
Warm water	600–650 mL	Hydration
Instant yeast	15 g	Leavening
Salt	18 g	Flavor and gluten strengthening
Oil	30 g	Improves crumb softness

2.5 Physical and Proximate Analysis. Loaf diameter and thickness were measured with a Vernier caliper, and the spread ratio was

calculated as diameter divided by thickness. Proximate composition was determined in triplicate using AOAC methods: moisture by hot-

air oven drying at 105°C to constant weight (AOAC 925.10), crude fat by Soxhlet extraction with petroleum ether (AOAC 920.39), crude protein by the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25 (AOAC 979.09), crude fiber by AOAC 962.09, and ash by incineration in a muffle furnace at 550°C (AOAC 923.03). Carbohydrate content was calculated by difference, and energy value was estimated using Atwater factors.

2.6 Sensory Evaluation. Sensory acceptability was assessed by 15 semi-trained panelists using a 9-point hedonic scale, rating appearance, color, aroma, texture, taste, mouthfeel, and overall acceptability.

2.7 Clinical Trial Protocol. After providing written informed consent, participants completed a baseline dietary assessment using a food frequency questionnaire, 24-hour dietary recall, and food diaries. During a 15-day control phase, all participants consumed standard wheat bread while fasting blood glucose, postprandial blood glucose, random blood sugar, HbA1c, complete blood count, and body weight were recorded to establish baseline glycemic status. Participants then entered an intervention period, extended to three months, during which the experimental group consumed fox nut-fortified bread and the control group continued with standard bread. Blood glucose was measured before breakfast (fasting) and one and two hours after eating

(postprandial), and glycemic and anthropometric parameters were reassessed monthly for three months.

2.8 Ethical Considerations. Written informed consent was obtained from all participants after the study purpose, procedures, and their right to withdraw at any time were explained. Data collection took place at participants' homes in Lahore, Pakistan. Participant identities were protected using unique study codes, and all data were stored securely and accessed only by the principal investigator and research team.

2.9 Statistical Analysis. Data were entered and analyzed using Microsoft Excel and the Statistical Package for the Social Sciences (SPSS). Baseline comparability between groups was assessed using independent-samples t-tests, within-group changes from baseline to Month 3 were assessed using paired t-tests, and between-group differences at Month 3 were assessed using independent-samples t-tests. A p-value below 0.05 was considered statistically significant.

3. Results

3.1 Proximate Composition. The fox nut-fortified bread contained 31.50±0.25% moisture, 12.10±0.18% crude protein, 3.20±0.10% crude fat, 2.80±0.12% crude fiber, 1.90±0.08% ash, and 48.50±0.32% carbohydrate, with an energy value of 271.2 kcal/100 g (Table 2).

Table 2. Proximate composition of fox nut-fortified bread (mean ± SD, n=3)

Parameter	Method (AOAC)	Result
Moisture (%)	925.10 (hot air oven)	31.50 ± 0.25
Crude protein (%)	979.09 (Kjeldahl)	12.10 ± 0.18
Crude fat (%)	920.39 (Soxhlet)	3.20 ± 0.10
Crude fiber (%)	962.09	2.80 ± 0.12
Ash (%)	923.03 (muffle furnace)	1.90 ± 0.08
Carbohydrate (%)	By difference	48.50 ± 0.32
Energy (kcal/100 g)	Atwater factors	271.2

3.2 Sensory Evaluation. All sensory attributes were rated between “like moderately” and “like

very much” on the 9-point hedonic scale, with small standard deviations indicating good panelist

agreement (Table 3). Appearance received the highest score (8.20 ± 0.56), followed by taste (8.13 ± 0.52), color and overall acceptability

(8.07 ± 0.59 and 8.07 ± 0.46 , respectively), while aroma, texture, and mouthfeel scored slightly lower but remained positive (7.80–7.93).

Table 3. Sensory evaluation scores of fox nut-fortified bread (n=15)

Attribute	Mean $\pm$ SD	Interpretation
Appearance	8.20 ± 0.56	Like very much
Color	8.07 ± 0.59	Like very much
Aroma	7.93 ± 0.70	Like moderately
Texture	7.87 ± 0.64	Like moderately
Taste	8.13 ± 0.52	Like very much
Mouthfeel	7.80 ± 0.68	Like moderately
Overall acceptability	8.07 ± 0.46	Like very much

3.3 Clinical Outcomes. Twenty patients were enrolled (experimental n=10, control n=10); one experimental-group participant withdrew after Month 1, leaving nine completers in that arm. At baseline, the two groups did not differ significantly for HbA1c, fasting blood glucose (FBG), postprandial blood glucose (PPBG), random blood sugar (BSR), or body weight (all $p > 0.05$), confirming comparability at enrollment.

Over three months, the experimental group showed significantly greater improvement than the control group across all glycemic parameters (Table 4). FBG fell from 195.33 ± 15.02 to 132.22 ± 13.30 mg/dL in the experimental group (−32.3%) versus 203.40 ± 17.08 to 190.90 ± 16.61 mg/dL in controls (−6.1%; between-group

$p < 0.001$). PPBG fell from 286.56 ± 30.82 to 206.11 ± 34.83 mg/dL (−28.1%) versus 285.00 ± 31.11 to 271.30 ± 30.09 mg/dL in controls (−4.8%; $p < 0.001$). HbA1c decreased from $9.70 \pm 0.91\%$ to $7.83 \pm 0.77\%$ in the experimental group (−19.2%) compared with $9.60 \pm 0.89\%$ to $9.43 \pm 0.85\%$ in controls (−1.8%). BSR decreased from 287.67 ± 29.33 to 214.44 ± 31.89 mg/dL (−25.5%) versus 285.60 ± 33.69 to 271.60 ± 32.39 mg/dL in controls (−4.9%; $p = 0.001$). Body weight decreased modestly in the experimental group (79.21 ± 8.67 to 77.66 ± 8.59 kg, −2.0%, $p < 0.001$ within-group) while remaining essentially unchanged in controls (84.65 ± 8.74 to 84.69 ± 8.75 kg); the between-group difference at Month 3 was not statistically significant ($p = 0.096$).

Table 4. Summary of glycemic and anthropometric outcomes at baseline and Month 3 (mean $\pm$ SD)

Parameter	Group	n	Baseline	Month 3	% Change	Between-group p (M3)
HbA1c (%)	Experimental	9	9.70 ± 0.91	7.83 ± 0.77	−19.2%	<0.001
	Control	10	9.60 ± 0.89	9.43 ± 0.85	−1.8%	
FBG (mg/dL)	Experimental	9	195.33 ± 15.02	132.22 ± 13.30	−32.3%	<0.001
	Control	10	203.40 ± 17.08	190.90 ± 16.61	−6.1%	
PPBG (mg/dL)	Experimental	9	286.56 ± 30.82	206.11 ± 34.83	−28.1%	<0.001
	Control	10	285.00 ± 31.11	271.30 ± 30.09	−4.8%	
BSR (mg/dL)	Experimental	9	287.67 ± 29.33	214.44 ± 31.89	−25.5%	0.001

Parameter	Group	n	Baseline	Month 3	% Change	Between-group p (M3)
Weight (kg)	Control	10	285.60 ± 33.69	271.60 ± 32.39	−4.9%	0.096
	Experimental	9	79.21 ± 8.67	77.66 ± 8.59	−2.0%	
	Control	10	84.65 ± 8.74	84.69 ± 8.75	+0.0%	

4. Discussion

The nutritional profile of the fortified bread supports its viability as a functional food. Moisture content (31.50%) fell within the normal range for fresh wheat bread, indicating that incorporation of fox nut flour did not impair the dough's water-holding capacity despite the added fiber and resistant starch. Crude protein (12.10%) exceeded typical wheat bread values of 8–9%, consistent with the elevated protein content reported for roasted fox nuts [7], while the ash content reflected the mineral contribution of the fortificant, particularly magnesium, calcium, potassium, and phosphorus – minerals whose dietary intake, especially magnesium, has been inversely associated with type 2 diabetes risk in a dose-dependent manner in prospective cohort studies [25]. The relatively modest energy density (271.2 kcal/100 g) and carbohydrate content (48.50%) are consistent with partial replacement of rapidly digestible starch by the resistant starch fraction of fox nut, which is not enzymatically digested in the small intestine and yields less available energy per unit weight [5].

Sensory scores ranging from “like moderately” to “like very much,” with an overall acceptability of 8.07 ± 0.46 , indicate that a 15% fortification level is comfortably within the acceptance range typically reported for fiber-enriched composite breads. As in other studies of fiber-fortified bread for example, those enriched with pomegranate peel powder or dietary fiber from oats texture and mouthfeel scored somewhat lower than appearance and taste, a pattern generally attributed to a denser crumb resulting from a diluted gluten network and greater water-binding capacity of the added fiber [27,30]. This trend also parallels dose-ranging work on other fiber-based flours, in which substitution levels up to approximately 20% remain well tolerated but

acceptability declines sharply beyond that threshold, suggesting that the present 15% level sits close to, but has not yet reached, the upper margin of sensory acceptability.

The markedly greater reductions in FBG (−32.3%), PPBG (−28.1%), HbA1c (−19.2%), and BSR (−25.5%) observed in the experimental group, in the absence of baseline differences between groups, point to fox nut fortification itself as the likely driver of improved glycemic control. This is consistent with meta-analytic evidence that resistant starch supplementation reduces fasting and postprandial glucose in individuals with type 2 diabetes or prediabetes [26], and with human data showing that roasted fox nut has an inherently low glycemic index together with elevated phenolic and flavonoid content [7]. The magnitude of glycemic improvement observed here, however, appears larger than that typically reported for isolated resistant-starch interventions, which may reflect a synergistic contribution from fox nut's combined resistant starch, dietary fiber, protein, and antioxidant phenolic content rather than any single mechanism acting alone [6,20]; this interpretation warrants caution given the small sample size of the experimental arm (n=9).

The small reduction in body weight observed in the experimental group, though not significantly different from the control group at Month 3, is compatible with the satiety-promoting effects generally attributed to viscous, fiber-rich foods, which delay gastric emptying and reduce subsequent energy intake [29]. Because only a single fortification level (15%) was tested, a formal dose-response relationship could not be established; comparison of the present sensory data with dose-ranging studies of other fiber-enriched flours suggests that some room may remain to test higher inclusion levels before sensory acceptability is compromised. Overall, the

magnitude and consistency of the glycemic effects observed align with, and in some respects exceed, findings from other functional bread interventions in T2DM, such as pomegranate peel-fortified bread [27], reinforcing the broader premise that fortifying a universally consumed staple can improve metabolic outcomes with minimal disruption to established dietary habits. This study benefited from a randomized, parallel-group design with a matched control arm, monthly laboratory monitoring over three months, and integration of proximate, sensory, and clinical data within a single research protocol. Nonetheless, several limitations temper the interpretation of these findings: the sample was small (n=20, one dropout) and drawn by convenience sampling from a single center, background dietary intake relied on self-reported recalls subject to recall and social desirability bias, only one fortification level was examined, the three-month duration precludes conclusions about longer-term efficacy or safety, and the distinct appearance and texture of the fortified bread precluded participant blinding. Larger, multicenter, dose-ranging, and ideally blinded or matched-placebo trials are needed to confirm these preliminary findings before fox nut-fortified bread can be recommended as a validated adjunctive therapy for T2DM.

5. Conclusion

A wheat bread fortified with 15% fox nut (*Euryale ferox*) flour was nutritionally enhanced relative to standard wheat bread and achieved high consumer acceptability (overall score 8.07/9). In a three-month randomized trial, consumption of this bread was associated with significantly greater reductions in fasting blood glucose, postprandial blood glucose, HbA1c, and random blood sugar compared with standard bread, along with a modest, non-significant reduction in body weight. These findings suggest that fox nut-fortified bread may serve as a promising adjunctive dietary strategy for the management of Type 2 Diabetes Mellitus, although confirmation in larger, multicenter, dose-ranging trials is required before broader clinical or commercial application.

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