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Lima Bean Seed Powder as a Functional Ingredient for Improving the Antioxidant and Nutritional Properties of Pan Bread

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Abstract

This study assessed the nutritional, functional, rheological, physical, and sensory attributes of pan bread supplemented with lima bean seed powder (LBP) as a partial replacement for wheat flour (72% extraction rate). Phyto chemical selected analysis exposed that lima bean contained significantly higher protein (21.30%), fiber (7.60%), ash (3.20%), minerals (K, Ca, Mg, Fe, Zn, Mn), and antioxidant compounds compared to wheat flour, whereas wheat flour was richer in available carbohydrates and energy value. Amino acid profiling implied that LBP provided superior levels of essential amino acids, particularly lysine, valine, and threonine, with higher computed protein efficiency ratio (C-PER = 2.90) and biological value (BV = 80.41) than wheat flour. However, methionine and cysteine were limiting amino acids, emphasizing the importance of cereal-legume complementation. Dough rheology showed that increasing LBP levels increased water absorption and dough development time but decreased stability, extensibility, and elasticity due to gluten dilution. Bread quality analyses demonstrated that LBP supplementation enhanced protein, fiber, and mineral content, while slightly lowering available carbohydrates and energy values. Nevertheless, higher replacement levels (15–20%) negatively affected loaf volume, specific volume, crumb softness, and sensory acceptability. Optimal results were obtained at 5–10% LBP replacement, creating nutritionally enhanced bread with acceptable dough performance and consumer appeal. These findings support the potential of lima bean seed powder as a functional ingredient for improving the nutritional value of cereal-based foods.

Keywords: lamia beans- amino acids-antioxidants- rheology- bread

1. Introduction

Legumes rank second only to cereals as a global food source. [1] They are rich in essential nutrients, including minerals, carbohydrates, vitamins, and dietary fiber, and represent a cost-effective, sustainable alternative to animal-based proteins. [2] These crops are generally classified into major and minor groups. [3] While major legumes—such as soybean, cowpea, and black soybean—are extensively cultivated and well-studied, minor legumes—including lima bean, winged bean, marama bean, and Bambara groundnut—are often regarded as neglected and underutilized. Nevertheless, minor legumes possess significant potential to improve food and nutrition security, diversify farming systems, enhance dietary quality, open new market opportunities, and contribute to ecosystem resilience. [4, 5]

In addition, legumes present valuable options for cultivation under adverse environmental conditions. Lima bean (Phaseolus lunatus L.), native to Latin and Central America but grown across tropical and temperate zones, is considered an underutilized crop with notable agronomic and nutritional benefits. [6] In Egypt, its cultivation in the North Delta, particularly under the conditions of Kafr El-Sheikh Governorate, yielded about 2.5 tons/feddan with a protein content of roughly 24%. [4, 5, 7] Nutritionally, lima beans contain high levels of protein (8.61–26.02%), carbohydrates (50.44–77.39%), and dietary fiber, while being relatively low in fat (0.21–3.12%). [2] They are also good sources of vitamins such as thiamine, riboflavin, niacin, and vitamin B6, which act as coenzymes in energy and nutrient metabolism. [8] Beyond their nutritional profile, lima beans exhibit several bioactive functions, including gastroprotective, antimicrobial, antioxidant, antiviral, and antihypertensive activities. [9] Despite these advantages, their wider consumption is hindered by the presence of natural antinutritional factors (ANFs), including trypsin inhibitors, phytic acid, tannins, oxalates, and cyanogenic glycosides, which can impair protein digestibility, limit mineral absorption, and reduce overall food quality. [10]

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Compared with other legumes, the use of lima beans remains limited, largely due to low consumer awareness of their nutritional and health-promoting qualities as well as proper preparation techniques. Thanks to their mild flavor, however, they can be incorporated into a wide range of culinary preparations. [11] Traditionally, lima beans are cooked before consumption and may be consumed fresh, boiled, sautéed, baked, roasted, canned, dried, or frozen. [12] Beyond their versatility in the kitchen, lima beans are also a promising source of native and modified starches, offering potential for the food industry to address growing starch demands. In addition, they exhibit several medicinal properties. [13] A major limitation to their broader adoption is their hard-to-cook nature, though appropriate processing strategies can markedly enhance their functional attributes and consumer appeal. Commercializing underutilized crops such as lima beans could play a transformative role in boosting food security, improving dietary quality, diversifying cropping systems, increasing yields, and alleviating poverty in developing countries. [5]

Bread making is among the earliest food technologies discovered by humans. During baking, a mixture of wheat flour, water, yeast, and salt undergoes fermentation, during which yeast enzymes act on sugars to release carbon dioxide. The hydrated proteins of wheat flour form gluten, which provides the dough with its gas-retaining capacity—an attribute that distinguishes wheat flour from other cereals in bread production. [14] In many regions, legume seeds provide up to 80% of dietary protein, serving as the sole protein source for certain populations. [15, 16] Increasingly, legumes are being incorporated into wheat-based baked products, such as biscuits, for protein enrichment. When combined with cereals, legumes help create a complete protein profile. This makes them particularly valuable in developing countries, where research is focused on maximizing legume utilization to address protein-energy malnutrition and food insecurity. Lima beans, in particular, have been identified as a cost-effective supplement or even a replacement for conventional protein sources such as soybean and groundnut meal. [17] The current study aimed to assess the effect of partially replacing wheat flour with lima bean seed powder on the nutritional, phytochemical, amino acid, rheological, physical, and sensory possessions of pan bread, in order to explore its potential as a functional ingredient for improving the health value and quality of cereal-based foods.

2. Materials and Methods

2.1. Materials

Lamia beans (*Phaseolus lunatus* L.), wheat flour (72% extraction rate), and other basic ingredients for pan bread preparation - such as sugar (sucrose), table salt (sodium chloride), and butter - were obtained from the local market in Kafrelsheikh city, Egypt. All chemicals and solvents used in the study were procured from El-Gomhoria Company, Cairo, Egypt

2.2. Methods

2.2.1. Preparation of Lima bean seeds powder

Lima bean seeds were cleaned to remove broken, damaged and off-color seed, then ground using Quadrumat junior mill (Brabender- made in Germany) until they could pass through a 125 m mesh

2.3. Pan bread processing

Pan bread was prepared using the straight dough method according to El-Hadidy (2025). [18] The control formulation contained 100 g wheat flour, 1.5 g instant dry yeast, 2 g salt, 2 g sugar, 3 g shortening, and water adjusted based on farinograph absorption. In the experimental treatments (Control, B5, B10, B15, and B20), wheat flour was partially replaced with lima bean powder at 0, 5, 10, 15, and 20%, respectively. All ingredients were mixed in a bowl at 28 °C for 6 minutes, and the dough was manually folded 20 times to obtain a homogeneous mass, followed by a 10-minute resting period. The dough was then transferred into lightly oiled baking pans and proofed at 30 °C with 85% relative humidity for 60 minutes. Baking was performed in an electric oven at 250 °C for 20 minutes. The baked loaves were cooled at ambient temperature (25 °C) for 1 hour, packaged in polyethylene bags, and stored until analysis, as presented in Table 1.

Table 1: Pan bread formulations

Constituents (g)	Control	B 5	B 10	B15	B 20
Wheat flour (72%)	100	95	90	85	85
Lamia beans	0	5	10	15	20
Table Salt	2	2	2	2	2
Yeast	1.5	1.5	1.5	1.5	1.5
Sugar	2	2	2	2	2
Shortening	3	3	3	3	3

Control = Wheat flour 100% + 0 g Lamia beans powder
B5=95 g Wheat flour+5 g Lamia beans powder
B10=90 g Wheat flour+10 g Lamia beans powder
B15=85 g Wheat flour+15 g Lamia beans powder
B20=80 g Wheat flour+20 g Lamia beans powder

2.4. Chemical Analysis

2.4.1. Determination of Chemical Composition of Raw Materials and Blends

The proximate composition, including crude protein, crude fat, ash, crude fiber, and available carbohydrates, was determined for both the wheat flour and lamia beans powder and their blends according to the standard methods of AOAC (2019). [19] Mineral analysis was carried out on ash-digested solutions of the raw materials using an atomic absorption spectrophotometer (Perkin-Elmer 3300), following AOAC (2019) [19] procedures. Available carbohydrates content was estimated using the AOAC (2019) equation: [19]

Available carbohydrates (%) = 100 – (Protein% + Crude Fiber% + Fat% + Ash%)

The energy value (kcal/100 g) was calculated using the following formula:

Energy Value = (Carbohydrate% × 4) + (Protein% × 4) + (Fat% × 9)

All results were expressed on a dry weight basis (DWB) and presented as the means of three replicates.

Analyses were performed strictly in accordance with AOAC (2019) procedures. [19]

2.4.2. Assessment of Total Polyphenol Content

Total polyphenol content (TPC) was determined according to the procedure of Thaipong et al., (2006) [20] using the Folin–Ciocalteu reagent. Measurements were carried out with a UV spectrophotometer (Varian, Melbourne, VIC, Australia), and gallic acid was used as the calibration standard. Absorbance was read at 760 nm, and the results were expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

2.4.3. Evaluation of Total Flavonoid Content

Total flavonoid content was determined following the procedure described by Vuong et al. (2014). [21] Absorbance was recorded at 510 nm using a UV spectrophotometer (Varian, Melbourne, VIC, Australia). Quercetin served as the reference standard, and the results were expressed as milligrams of quercetin equivalents (mg QE) per gram of dry matter.

2.4.4. Antioxidant Capacity Determination

The antioxidant capacity of the samples was evaluated using the DPPH assay, following the method described by Sharma et al., (2022). [22]

2.4.5. Determination of amino acids composition

The amino acid composition of wheat flour (72%extraction rate) and lamia beans powder was analyzed at the National Research Center in Cairo, Egypt. The analysis was conducted using a Beckman amino acid analyzer (Model 119CL), following the method previously described by Duranti and AOAC (2019). [19]

2.4.6. Estimation of Tryptophan

The tryptophan content in the samples was determined using a colorimetric method, following the procedure previously outlined by Miller (1967). [23]

2.4.7. Computed protein efficiency ratio (C-PER) and biological value (BV)

Using the formula given by Alsmeyer et al. (1974) [24] the computed protein efficiency ratio (C-PER) was calculated as follows:

C-PER= -0.4687+0.454 (leucine)-0.105 (tyrosine).

The biological value (BV) was calculated using the formula BV=49.9+10.53C-PER, as explained by El-Shirbeeny et al., (1996). [25]

2.4.8. Chemical Score of Amino Acids

The chemical scores for essential amino acids were determined according to the FAO/WHO/UNU (1985) method, [26] using the following formula:

$$\text{Chemical score } = \frac{\text{Essential amino acid / 100 g protein in sample}}{\text{Essential amino acid / 100 g protein in FAO / WHO/UNU}} \times 100$$

* The amino acid with the lowest percentage value is referred to as the limiting amino acids, and the calculated ratio represents its chemical score.

2.5. Rheological Assessments

2.5.1. Farinograph assessment

Water absorption, arrival time, dough development time, dough stability, and degree of softening were measured using a Farinograph (Brabender, Duisburg, Type 810105001, No. 941026, West Ger-many), following the procedures described by AACC (2000). [27]

2.5.2. Extensograph Assessment

Dough extensibility, resistance to extension (elasticity), proportional number, and dough energy were determined using an Extensograph (Brabender, Duisburg, Type 860001, No. 946003, West Germany), according to the method specified by AACC (2000). [28]

2.5.3. Sensory properties of Pan bread

The pan bread blends were evaluated according to the method outlined by AACC. (2000) [27] A sensory panel consisting of twenty staff members from the Food Technology Research Department at the Agricultural Research Center in Sakha, Egypt, was assembled. Panelists were asked to assess the sensory attributes of the bread, including overall acceptability (out of 100), aroma (10 points), taste (20 points), crumb texture (20 points), crumb color (20 points), symmetry of shape (10 points), and crust color (20 points).

2.6. Color Parameters

As described by Brunton et al. (2006) [28] the color characteristics of the prepared pan bread-lightness (L^*), redness (a^*), and yellowness (b^*)-were measured using a Hunter Lab Scan Visible colorimeter.

2.7. Pan bread physical parameters

After allowing the loaves to cool for three hours, their average weight was recorded. Loaf volume was measured using the rapeseed displacement method, as specified by AACC, (2000). [27] The specific volume (cm^3/g) was calculated by dividing the loaf volume by its corresponding weight.

2.8. Texture Profile Analysis (TPA)

The hardness of the pan bread was evaluated following the procedures outlined by AACC, (2002). [29]

2.9. Statistical Analysis

The SPSS 26.0 program IBM Corp. (2019) [30] was used to analyze the data from the experiments, which were recorded as means ± standard deviations (SD) of triplicate measurements. Analysis of variance (ANOVA) was used to compare the means between groups, and Duncan's multiple range test was then performed to perform pairwise comparisons between the group means. The significance level was determined at a p-value of ≤ 0.05.

3. Results and Discussion

3.1. Phytochemical analysis of raw Lemia beans and wheat flour

As reported in **Table 2**, the phytochemical composition of raw lima beans and wheat flour exposed remarkable differences in their nutritional and functional possessions. Lima beans presented significantly lower moisture content (7.50%) compared with wheat flour (14.00%), suggesting improved storage stability. Crude protein in lima beans (21.30%) was almost double that of wheat flour (11.95%), confirming their role as a rich plant-based protein source. Moreover, ether extract, crude fiber, and ash values were noticeably higher in lima beans, [31] reflecting better nutritional density and mineral contribution than wheat flour. In contrast, wheat flour had higher available carbohydrate content (84.90%) and slightly greater energy value (413.01 kcal/100g) [32] compared with lima beans (64.90% and 380.72 kcal/100g, respectively), consistent with its function as a carbohydrate-dense staple. Mineral composition (Table 2) further highlighted the superiority of lima beans, which contained considerably greater amounts of potassium, calcium, phosphorus, magnesium, sodium, iron, zinc, and manganese. These higher mineral levels underline the potential of lima beans in combating micronutrient deficiencies when used in composite flour formulations. Furthermore, antioxidant analysis in **Table 2** shows that lima beans had much higher total phenolic content (275 mg GAE/100g), total flavonoid content (75 mg QE/100g), and antioxidant activity (70%) compared with wheat flour (70 mg, 20 mg, and 25%, respectively). This indicates a superior free radical scavenging potential and highlights their role as a functional ingredient with health-promoting benefits. [5, 7] In summary, **Table 2** demonstrates that raw lima beans provide significantly higher protein, fiber, minerals, and antioxidant compounds than wheat flour. Their incorporation into bakery formulations can therefore improve the nutritional and therapeutic quality of the final products. [7]

Table 2 shows the phytochemical analysis of raw lima beans and wheat flour (72% extraction) revealed marked differences in their nutritional and antioxidant profiles. Wheat flour exhibited significantly higher moisture content (14.00%), while lima beans contained lower levels (7.50%), suggesting better storage stability. Protein content in lima beans (21.30%) was nearly double that of wheat flour (11.95%), indicating its potential to improve the protein quality of composite flours. Similarly, ether extract (3.00%) and crude fiber (7.60%) were much higher in lima beans than in wheat flour (1.75% and 0.85%, respectively), reflecting their richness in lipids and dietary fiber. In contrast, wheat flour was superior in available carbohydrates (84.90% vs. 64.90%) and energy value (413.01 kcal vs. 380.72 kcal), confirming its role as a major energy source in baked products.

Lima beans also demonstrated a substantial advantage in mineral composition, being particularly rich in potassium (1450 mg/100 g), calcium (320.45 mg/100 g), phosphorus (380.20 mg/100 g), and magnesium (280.50 mg/100 g) compared to wheat flour. Micronutrients such as iron (15.50 mg/100 g), zinc (4.50 mg/100 g), and manganese (1.90 mg/100 g) were also significantly higher in lima beans, [2, 5, 31] emphasizing their potential contribution to addressing mineral deficiencies.

With respect to bioactive compounds, lima beans presented remarkably higher levels of total phenolic compounds (TPC, 275 mg GAE/100 g) and total flavonoid compounds (TFC, 75 mg QE/100 g) [5] compared with wheat flour (70 mg GAE/100 g and 20 mg QE/100 g, respectively). Thus, antioxidant activity was much greater in lima beans (70%) than in wheat flour (25%), emphasizing their functional food value. These findings suggest that partial substitution of wheat flour with lima bean flour could enhance the nutritional, mineral, and antioxidant properties of bakery products, making them more health-promoting and appropriate for functional food formulations.

Table 2: Phytochemical analysis of raw Lemia beans and wheat flour

Components (%)	Raw Lemia beans	Wheat flour (72% extraction)
Moisture	7.50±0.05^{b}	14.00±0.15^{a}
Crude protein	21.30±0.15^{a}	11.95±0.10^{b}
Ether extract	3.00±0.03^{a}	1.75±0.01^{b}
Crude fiber	7.60±0.01^{a}	0.85±0.01^{b}
Ash	3.20±0.02^{a}	0.55±0.01^{b}
Available carbohydrates	64.90±0.10^{b}	84.90±0.15^{a}
Energy value	380.72±0.12^{b}	413.01±0.13^{a}
Minerals (mg per 100g)		
K	1450.00±2.30^{a}	125.60±1.00^{b}
Ca	320.45±2.00^{a}	25.30±0.20^{b}
P	380.20±1.90^{a}	145.33±1.30^{b}
Mg	280.50±2.50^{a}	144.50±0.80^{b}
Na	45.00±0.50^{a}	8.40±0.05^{b}
Zn	4.50±0.02^{a}	3.50±0.01^{b}
Fe	15.50±0.03^{a}	1.80±0.02^{b}
Mn	1.90±0.02^{a}	1.10±0.01^{b}
Antioxidants status		
TPC (mg GAE/100g)	275±0.60^{a}	70±0.30^{b}
TFC (mg of QE/100g)	75±0.50^{a}	20±0.12^{b}
Antioxidant activity (%)	70±0.50^{a}	25±0.40^{b}

TPC= Total phenols compounds**.** TFC= Total flavonoids compounds

Amino acids profile of raw Lemia beans and wheat flour (g /100g of protein).

As shown in **Table 3**, the amino acid profile of raw lima beans was superior to that of wheat flour in terms of indispensable amino acids (EAA). Lima beans contained higher levels of lysine (7.80 g/100 g protein), isoleucine (4.60 g), leucine (8.20 g), phenylalanine (5.30 g), histidine (3.10 g), valine (5.60 g), and threonine (4.30 g) compared with wheat flour, and most values exceeded the FAO/WHO/UNU (1985) reference pattern, confirming their good protein value. However, sulfur-containing amino acids (methionine and cysteine) and tryptophan were relatively lower in lima beans, signaling the need for complementation with cereals to achieve a balanced amino acid profile. [2, 7] Regarding non-essential amino acids (NEAA), wheat flour was richer in glutamic acid (32.00 g/100 g protein) and proline (4.90 g), while lima beans were higher in aspartic acid (10.00 g), alanine (4.20 g), and arginine (6.40 g). The higher arginine content in lima beans is particularly noteworthy, given its role in immune modulation and nitric oxide synthesis. Overall, lima beans provided a greater proportion of EAAs (45.55 g/100 g protein) compared with wheat flour (35.45 g), which is reflected in their higher computed protein efficiency ratio (C-PER = 2.90 vs. 2.18) and biological value (BV = 80.41 vs. 72.83). These results demonstrate that lima bean proteins are nutritionally superior and could enhance the protein quality of wheat-based products when used in composite flour formulations. [2, 7]

Table 3: Amino acids profile of raw Lemia beans and wheat flour (g /100g of protein)

Amino acids	Wheat flour 72%	Lemia beans	FAO/WHO/UNU (1985) pattern
Lysine	3.00	7.80	5.80
Isoleucine	4.00	4.60	2.80
Leucine	6.70	8.20	6.60
Phenylalanine	4.10	5.30	
Tyrosine	3.90	3.40	6.30
Histidine	2.00	3.10	1.90
Valine	4.50	5.60	3.50
Threonine	2.60	4.30	3.40
Methionine	1.90	0.95	2.20
Tryptophan	1.15	1.00	1.00
Cysteine	1.60	1.30	
Total (EAA)	**35.45**	**45.55**	
Aspartic acid	7. 00	10.00	
Glutamic acid	32.00	17.0	
Serine	4.50	4.60	
Proline	4.90	4.20	
Glycine	4.70	4.50	
Alanine	3.40	4.2	
Arginine	3.95	6.40	
Total (NEAA)	**61.45**	**50.90**	
C-PER	2.18	2.90	
BV	72.83	80.41	

EAA: Essential amino acids. NEAA: Nonessential amino acids
C-PER = Computed protein efficiency ratio. BV = Biological value

The chemical scores of raw Lemia beans and wheat flour compared with the required pattern control recommended by FAO/WHO/UNU (1985). [26]

As illustrated in **Table 4**, the chemical scores of raw lima beans compared with wheat flour highlight the differences in amino acid adequacy relative to the FAO/WHO/UNU (1985) reference pattern. Wheat flour showed lysine (51.72%) as its first limiting amino acid, followed by threonine (76.47%) and methionine (80.36%), reflecting its poor balance of essential amino acids. By contrast, lima beans provided much higher chemical scores for most amino acids, with lysine (134.48%), histidine (163.16%), isoleucine (164.29%), leucine (124.24%), valine (160%), and threonine (126.47%) all exceeding the reference requirements. These results confirm the superior protein quality of lima beans, particularly in relation to lysine, which is deficient in most cereals. However, the first limiting amino acid in lima beans was methionine (43.18%)**,** followed by tryptophan (100%) and leucine (124.24%), which indicates that sulfur amino acids remain a constraint to their protein utilization. Despite this limitation, the overall amino acid balance of lima beans was nutritionally superior to wheat flour. Therefore, combining lima beans with cereal proteins, such as wheat, could provide mutual complementation and improve the amino acid profile of composite flours, leading to higher protein quality in bakery and other cereal-based products. [2, 7]

Table 4: The chemical scores of raw Lemia beans and wheat flour compared with the required pattern control recommended by FAO/WHO/UNU (1985).

Essential amino acids	FAO/WHO/UNU (1985) pattern	Chemical score wheat flour 72%	Chemical score Lemia beans
Lysine	5.80	51.72*	134.48
Isoleucine	2.80	142.85	164.29
Leucine	6.60	101.52	124.24***
Phenylalanine + Tyrosine	6.30	126.98	138. 10
Histidine	1.90	105.26	163.16
Valine	3.50	128.57	160
Threonine	3.40	76.47**	126.47
Methionine	2.20	80.36***	43.18*
Tryptophan	1.00	115	100**

Chemical score was calculated as a percentage of the FAO/WHO/UNU (1985) indispensable amino acid. * First limiting amino acid. ** Second limiting amino acid. ***Third limiting amino acid.

3.2. Farinograph characteristics of dough supplemented with lima bean seed powder

As shown in Table 5, incorporation of lima bean seed powder into wheat flour significantly affected the farinograph properties of the dough. Water absorption increased steadily with higher substitution levels, from 62.50% in the control (100% wheat flour) to 69.30% at 20% supplementation, which can be explained by the higher protein and fiber contents of lima beans that enhance water-binding capacity. Arrival time and dough development time were also prolonged with increasing substitution, suggesting slower hydration and a more gradual gluten network formation. In contrast, dough stability decreased from 10 minutes in the control to 6 minutes at 20% addition, indicating reduced resistance of the dough to mixing due to gluten dilution. Moreover, the degree of softening rose from 90 B.U in the control to 145 B.U at 20% replacement, further confirming weaker dough strength at higher levels of supplementation. Overall, Table 5 indicates that while lima bean seed powder improves water absorption and extends dough development, it compromises stability and increases softening when used at high levels. Therefore, moderate substitution levels (5–10%) appear most suitable for composite flour applications, maintaining acceptable dough properties while enhancing nutritional quality.

Table 5: Farinograph characteristics of dough supplemented with lima bean seed powder

Blends	Water absorption (%)	Arrival time (min)	Dough development (min)	Stability (min)	Degree of softening (B.U)
Control (100% Wheat flour)	62.50	1.5	2.5	10	90
5% lima bean seed powder	63.60	1.5	3.0	8.0	100
10% lima bean seed powder	65.20	2.0	3..5	7.0	110
15% lima bean seed powder	67.80	2.5	3.5	7.0	125
20% lima bean seed powder	69.30	3.0	4.0	6.0	145

Control = Wheat flour 100% + 0 g Lamia beans powder,
B5=95 g Wheat flour+5 g Lamia beans powder, B10=90 g Wheat flour+10 g Lamia beans powder,
B15=85 g Wheat flour+15 g Lamia beans powder, B20=80 g Wheat flour+20 g Lamia beans powder

3.3. Extensograph parameters dough supplemented with lima bean seed powder

As illustrated in Table 6, supplementation of wheat flour with lima bean seed powder caused noticeable changes in extensograph parameters. Dough elasticity decreased gradually from 380 B.U in the control to 270 B.U at 20% substitution, indicating a weakening of the gluten network. Extensibility also declined from 165 mm in the control to 95 mm at 20% replacement, reflecting reduced dough stretching capacity. Conversely, the proportional number (P.N) increased from 2.30 to 2.84 with rising levels of substitution, showing a trend toward stiffer and less extensible dough. In addition, dough energy, which represents the total work required to stretch the dough, dropped steadily from 80 cm^2 in the control to 50 cm^2 at 20% addition, confirming a reduction in overall dough strength. Overall, Table 6 demonstrates that increasing levels of lima bean seed powder impair dough viscoelasticity, leading to weaker and less extensible dough. Moderate levels (5–10%) may be tolerated in bakery formulations, but higher substitutions (15–20%) negatively affect dough performance and could compromise final product quality.

Table 6: Extensograph parameters dough supplemented with lima bean seed powder

Blends	Elasticity (B.U)	Extensibility (mm)	P. N	Energy (cm^2)
Control (100% Wheat Flour)	380	165	2.30	80
5% Lima Bean Seed Powder	340	140	2.43	75
10% Lima Bean Seed Powder	320	130	2.46	70
15% Lima Bean Seed Powder	290	115	2.52	60
20% Lima Bean Seed Powder	270	95	2.84	50

Control = Wheat flour 100% + 0 g Lamia beans powder,
B5=95 g Wheat flour+**0**5 g Lamia beans powder, B10=90 g Wheat flour+10 g Lamia beans powder,
B15=85 g Wheat flour+15 g Lamia beans powder, B20=80 g Wheat flour+20 g Lamia beans powder

3.4. Sensory attributes of pan bread supplemented with lima bean seed powder

The sensory assessment findings offered in **Figure 1** reveal that the partial replacement of wheat flour with lima bean seed powder significantly affected the overall quality characteristics of pan bread. The control sample consistently received the highest scores across all parameters, with crust color (19.20), crumb color (19.80), crumb grain texture (19.60), symmetry of shape (9.50), taste (19.00), aroma (9.40), and overall acceptability (96.50). As the level of replacement increased, a gradual decline in panelists' scores was detected. At 5% substitution (B5), the bread maintained relatively good sensory characteristics, with only slight reductions compared to the control, suggesting that low-level incorporation of lima bean powder was well accepted without major negative effects on bread appearance, texture, or flavor. However, higher substitution levels (10–20%) led to more pronounced decreases in all quality characteristics. At 10% (B10), noticeable reductions occurred in crumb texture, taste, and aroma, resulting in a lower overall acceptability score of 90.10. Further increases to 15% (B15) and 20% (B20) caused substantial deterioration, especially in crust color, crumb texture, taste, and shape symmetry, with overall acceptability scores dropping obviously to 86.10 and 80.00, respectively. These results imply that excessive addition of lima bean seed powder negatively impacts the sensory profile of pan bread, likely due to its influence on dough structure, flavor, and appearance. Therefore, incorporation up to 5% appears optimal for improving nutritional value while maintaining desirable sensory properties. [16]

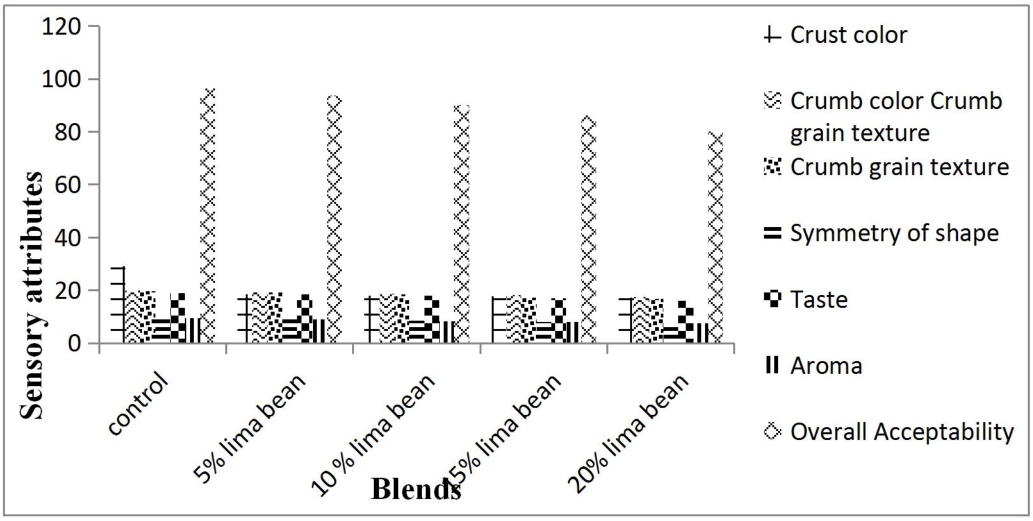

Figure 1: Flow chart for sensory attributes of pan bread supplemented with lima bean seed powder

3.5. Chemical compositions of pan bread supplemented with lima bean seed powder

The chemical composition of pan bread supplemented with lima bean seed powder is offered in **Table 7**. Progressive substitution of wheat flour with lima bean seeds powders significantly improved the nutritional value of the bread. Protein content increased progressively from 11.20% in the control to 13.00% at 20% replacement (B20), reflecting the higher protein content of lima beans compared to wheat flour. Also, fat, crude fiber, and ash contents all rose consistently with increasing supplementation, indicating improved lipid, dietary fiber, and mineral contributions from lima beans. In contrast, available carbohydrates decreased progressively from 83.06% in the control to 79.26% at B20, which can be attributed to the partial replacement of starchy wheat flour with nutrient-dense lima bean powder. The energy value also declined slightly with increasing substitution, from 426.87 kcal/100 g in the control to 420.76 kcal/100 g at B20, due to the reduction in carbohydrate content. These findings highlight that incorporation of lima bean seed powder enhances the protein, fiber, and mineral contents of pan bread, while slightly lowering its energy density. These results in agreement with Abdalla et al. [35] who reported that the addition of fermented chickpea flour in bread-making leads to an increase in nutritional and health values, as the proportions of protein, ash, fat, and dietary fiber were increased. Overall, **Table 7** reveals that moderate enrichment levels (10–15%) offer the best balance between nutritional enhancement and minimal reduction in available carbohydrates and energy value, thereby producing functionally superior bread with improved dietary quality. [33]

Table 7: Chemical compositions of pan bread supplemented with lima bean seed powder

Blends	Protein	Fat	Crude fiber	Ash	Available carbohydrates	Energy Value
Control	11.20^{e}±0.02	4.44^{e}±0.03	0.79^{e}±0.01	0.51^{e}±0.02	83.06^{a}±0.07	426.87^{a}±0.03
B5	11.65^{d}±0.03	4.50^{d}±0.01	1.11^{d}±0.02	0.64^{d}±0.03	82.10^{b}±0.06	425.33^{b}±0.06
B10	12.10^{c}±0.04	4.56^{c}±0.02	1.43^{c}±0.02	0.76^{c}±0.01	81.15^{c}±0.05	423.82^{c}±0.07
B15	12.55^{b}±0.02	4.61^{b}±0.01	1.74^{b}±0.03	0.89^{b}±0.04	80.21^{d}±0.04	422.27^{d}±0.05
B20	13.00^{a}±0.05	4.67^{a}±0.01	2.06^{a}±0.04	1.01^{a}±0.05	79.26^{e}±0.05	420.76^{e}±0.08

Values are expressed as mean ± standard deviation (n = 3).
Different superscript letters (a, b, c) in the same row indicate statistically significant differences among means at $P \leq 0.05$, according to one-way ANOVA followed by Dunkan test
Control Pan bread =100% wheat flour (72% extraction rate)
B5 Pan bread = 5% lima bean seed powder +95% wheat flour (72% extraction rate)
B10 Pant bread = 10% lima bean seed powder +90%wheat flour (72% extraction rate)
B15 Pan bread = 15% lima bean seed powder +85% wheat flour (72% extraction rate)
B20 Pan bread = 20% lima bean seed powder +80%wheat flour (72% extraction rate

3.6. Colors of obtained pan bread

The color parameters of pan bread crust and crumb were substantially influenced by the level of lima bean seed powder replacement (Table 8). The crust lightness (L*) decreased progressively from 63.88 in the control to 53.97 at 20% substitution, indicating darker crust formation with higher lima bean incorporation. This darkening trend may be attributed to the higher protein and reducing sugar content of lima beans, which enhances Maillard browning reactions during baking**.** [17] Conversely, both redness (a*) and yellowness (b*) values increased significantly with replacement level. The a* value rose from 14.60 in the control to 21.20 at 20% substitution, while b* increased from 23.20 to 33.75, reflecting a more intense reddish–yellow tone in the crust. A similar trend was observed in the crumb color: L* declined from 78.28 in control to 68.29 at 20% substitution, whereas a* and b* values increased from 5.62 and 16.14 to 11.00 and 24.43, respectively. These results suggest that the inclusion of lima bean powder not only darkened the crumb but also enriched it with more pronounced red and yellow hues. The observed changes are consistent with earlier reports on legume-enriched baked products, where intensified non-enzymatic browning and inherent legume pigments contributed to color modification. [17] From a sensory perspective, these alterations in color may enhance the visual appeal up to moderate substitution levels (5–10%), while higher levels (15–20%) could produce excessively dark tones that may reduce consumer acceptance.

Table 8: Colors obtained pan bread

Samples	Crust color			Crumb color		
Parameters	**L***	**a***	**b***	**L***	**a***	**b***
Control	63.88^a ±0.15	14.60^e ±0.10	23.20^e ±0.15	78.28^a ±0.15	5.62^e ±0.05	16.14^e ±0.10
B5	60.34^b ±0.20	17.70^d ±0.15	25.47^d ±0.20	76.41^b ±0.10	6.87^d ±0.09	18.38^d ±0.15
B10	57.88^c ±0.25	18.15^c ±0.12	27.05^c ±0.14	73.95^c ±0.19	8.68^c ±0.10	20.37^c ±0.20
B15	55.02^d ±0.30	19.10^b ±0.10	29.44^b ±0.12	70.73^d ±0.13	9.59^b ±0.05	22.47^b ±0.12
B20	53.97^e ±0.10	21.20^a ±0.12	33.75^a ±0.17	68.29^e ±0.24	11.00^a ±0.10	24.43^a ±0.20

Each value was an average of three determination ± standard deviation
Different letters indicate to significant differences between raw materials in the same column($p \leq 0.05$).
Control Pan bread =100% wheat flour (72% extraction rate)
B5 Pan bread = 5% lima bean seed powder +95% wheat flour (72% extraction rate)
B10 Pant bread = 10% lima bean seed powder +90%wheat flour (72% extraction rate)
B15 Pan bread = 15% lima bean seed powder +85% wheat flour (72% extraction rate)
B20 Pan bread = 20% lima bean seed powder +80%wheat flour (72% extraction rate)

3.7. Physical attributes of pan bread

The physical characteristics of pan bread were markedly affected by the partial replacement of wheat flour with lima bean seed powder (Table 9). Loaf volume decreased significantly with increasing substitution levels, from 1430 cm^3 in the control to 700 cm^3 at 20% substitution, while bread weight also declined slightly from 480 g to 410 g. Consequently, the specific volume, an important indicator of loaf quality, dropped from 2.98 cm^3/g in the control to 1.71 cm^3/g at 20% substitution, reflecting reduced loaf expansion and gas retention capacity. This reduction may be attributed to the dilution of gluten-forming proteins by non-gluten proteins and fibers in lima beans, which weaken dough structure and impair gas-holding ability. Bulk density showed the opposite trend, increasing gradually from 0.34 g/cm^3 in the control to 0.59 g/cm^3 at 20% substitution, confirming the formation of more compact loaves. Similarly, crumb hardness increased significantly from 1.40 Kgf/cm^3 in the control to 5.72 Kgf/cm^3 at 20% substitution, indicating firmer texture at higher replacement levels. The increase in hardness can be linked to reduced gluten development, higher fiber content, and lower loaf volume, all of which contribute to denser crumb structure. Overall, low substitution levels (5–10%) resulted in acceptable bread physical properties with moderate volume reduction, while higher inclusion levels (15–20%) produced smaller, denser, and harder loaves, which may negatively affect consumer acceptability. [16] This work confirms the great importance of applied science in bakery products. [34-48]

Table 9: Physical attributes of pan bread

Blends	Blends	Volume (cm^3)	Weight (g)	Specific volume (cm^3/g)	Bulk density (g / cm^3)	Hardness (Kg / cm^3)
WF: LBP (100:0)	**Control**	1430^a ±2.50	480^a ±0.90	2.98^a ±0.02	0.34^e ±0.01	1.40^e ±0.01
WF: LBP (95:5)	**B5**	1250^b ±1.50	460^b ±0.80	2.72^b ±0.03	0.37^d ±0.00	2.18^d ±0.02
WF: LBP (90:10)	**B10**	990^c ±1.20	450^c ±0.50	2.20^c ±0.02	0.45^c ±0.01	3.17^c ±0.01
WF: LBP (85:15)	**B15**	850^d ±1.30	440^d ±0.60	1.93^d ±0.01	0.52^b ±0.02	4.40^b ±0.02
WF: LBP (80:20)	**B20**	700^e ±1.60	410^e ±0.40	1.71^e ±0.02	0.59^a ±0.01	5.72^a ±0.03

Values are expressed as mean ± standard deviation (n = 3).
Different superscript letters (a, b, c) in the same row indicate statistically significant differences among means at $P \leq 0.05$, according to one-way ANOVA followed by Dunkan test
Control Pan bread =100% wheat flour (72% extraction rate)
B5 Pan bread = 5% lima bean seed powder +95% wheat flour (72% extraction rate)
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B15 Pan bread = 15% lima bean seed powder +85% wheat flour (72% extraction rate)
B20 Pan bread = 20% lima bean seed powder +80%wheat flour (72% extraction rate)

4. Conclusion

The incorporation of lima bean seed powder into wheat flour substantially enhanced the nutritional value of pan bread, particularly in terms of protein, fiber, minerals, antioxidant content and suggesting improved storage stability . Although higher replacement levels (15–20%) adversely affected dough rheology, loaf volume, texture, and sensory acceptability, moderate enrichment (5–10%) achieved an optimal balance between enhanced nutritional quality and desirable technological and sensory attributes. These findings highlight the potential of lima beans as a sustainable functional ingredient to enhance the dietary and therapeutic value of bakery products, while also contributing to better utilization of underexploited legumes in human nutrition. Through these results, it is possible to use lima bean seed powder in gluten-free products.

5. Consent for publication

All authors read and approved the final version of the manuscript.

6. Data Availability Statement

All data was provided in this paper.

7. Ethics approval

Not applicable.

8. Credit authorship contribution statement

All authors equally participated in the conceptualization, design of the methodology, validation procedures, data collection, statistical analysis, result interpretation, and the drafting and revision of the manuscript.

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11. Conflicts of Interest

No conflict of interest was reported by all authors.

12. References

[1]. Y. Maphosa, V. Jideani, The role of legumes in human nutrition, in: M.C. Hueda (Ed.), Functional food—improve health through adequate food, Intech Open, London, UK, 2017.
[2]. J.A. Adebo, A review on the potential food application of lima beans (phaseolus lunatus l.), an underutilized crop, Applied Sciences 13(3) (2023) 1996.
[3]. J. Popoola, O. Ojuederie, C. Omonhinmin, A. Adegbite, Neglected and underutilized legume crops: Improvement and future prospects, Recent advances in grain crops research, IntechOpen, London, UK, 2019.
[4]. M. Ogwu, M. Osawaru, R. Aiwansoba, R. Iroh, Status and prospects of vegetables in africa, University of Ilorin, Ilorin, Nigeria, 2016.
[5]. Y. Wu, W.-S. Shin, Germination-induced changes in the nutritional, bioactive, and digestive properties of lima bean (phaseolus lunatus l.), Foods 14(12) (2025) 2123.

[6]. L. Chel-Guerrero, V. Pérez-Flores, D. Betancur-Ancona, G. Dávila-Ortiz, Functional properties of flours and protein isolates from phaseolus lunatus and canavalia ensiformis seeds, Journal of Agricultural and Food Chemistry 50(3) (2002) 584-591.

[7]. S. El-Gohery, Effect of different treatments on nutritional value of lima bean (phaseolus lunatus) and its utilization in biscuit manufacture, Food and Nutrition Sciences 12 (2021) 372-391.

[8]. B. Holland, R.A. McCance, E.M. Widdowson, I.D. Unwin, D.H. Buss, Vegetables, herbs and spices: Fifth supplement to mccance and widdowson's - the composition of foods, 4th Edition ed., Royal Society of Chemistry, Cambridge, London, UK, 1991.

[9]. T. Agostini-Costa, A. Teodoro, R.B.N. Alves, L. Braga, I. Ribeiro, J. Silva, L. Quintana, M. Burle, Total phenolics, flavonoids, tannins and antioxidant activity of lima beans conserved in a brazilian genebank, Ciencia Rural 45 (2015) 335-341.

[10]. S.B. Yellavila, J.K. Agbenorhevi, J.Y. Asibuo, G.O. Sampson, Proximate composition, minerals content and functional properties of five lima bean accessions, Journal of Food Security 3(3) (2015) 69-74.

[11]. S. Sood, N. Gupta, Lima bean, in: M.K. Rana (Ed.), Vegetable crop science, CRC Press, Boca Raton, FL, USA, 2017, pp. 701-714.

[12]. A. Alves, A. Oliveira, A. Alves, C. Dornelas, E. Alves, E. Cardoso, A. Oliveira, I. Cruz, Lima beans production and economic revenue as function of organic and mineral fertilization, Horticultura Brasileira - HORTIC BRAS 26 (2008).

[13]. Z.M. Saleem, S. Ahmed, M.M. Hasan, Phaseolus lunatus linn: Botany, medicinal uses, phytochemistry and pharmacology, World Journal of Pharmacy and Pharmaceutical Sciences 5(11) (2016) 87-93.

[14]. Z. Gan, P. Ellis, J. Schofield, Mini review: Gas cell stabilization and gas retention in wheat bread dough, Journal of Cereal Science 21(3) (1995) 215-230.

[15]. K.E. Akande, U.D. Doma, H. Agu, H. Adamu, Major antinutrients found in plant protein sources: Their effect on nutrition, (2010).

[16]. S.S. Sobowale, O.H. Animashaun, O.M. Omosebi, Influence of traditional and back-slopping steeping methods on some quality attributes of lima bean-sorghum composite flour and its bread making potential, Journal of Food Processing and Preservation 45(1) (2021) e15030.

[17]. W.M. Mospah, E.G. El-Dreny, G.S. El-Hadidy, Preparation and evaluation of noodles from some legumes powder, Food Technology Research Journal 5(1) (2024) 1-14.

[18]. G.S. EL-Hadidy, M. Elabd, N.H. Hussien, Impact of planting date and tuber packaging on two jerusalem artichoke cultivars and its flour fortification on diabetic-bakery products, Egyptian Journal of Chemistry 68(9) (2025) 729-748.

[19]. AOAC international, 21st edition, official methods of analysis association of official analytical chemists, Association of Official Analytical Chemists,, Washington DC., 2019.

[20]. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D.H. Byrne, Comparison of abts, dpph, frap, and orac assays for estimating antioxidant activity from guava fruit extracts, Journal of food composition and analysis 19(6-7) (2006) 669-675.

[21]. Q.V. Vuong, S. Hirun, T.L.K. Chuen, C.D. Goldsmith, M.C. Bowyer, A.C. Chalmers, P.A. Phillips, C.J. Scarlett, Physicochemical composition, antioxidant and anti-proliferative capacity of a lilly pilly (syzygium paniculatum) extract, Journal of Herbal Medicine 4(3) (2014) 134-140.

[22]. S. Sharma, A. Ojha, C. Saravanan, B. Bhattacharya, Ultrasonic-assisted extraction of phytochemicals from eggplant peel: Physicochemical and antibacterial evaluation, Journal of Scientific Research 14 (2022) 569-581.

[23]. E. Miller, Determination of the tryptophan content of feedingstuffs with particular reference to cereals, Journal of the Science of Food and Agriculture 18(9) (1967) 381-386.

[24]. R.H. Alsmeyer, A. Cunningham, M. Happich, Equations predict per from amino acid analysis, (1974).

[25]. A. El-Shirbeeny, S.A. Farag, A.E. Nassef, Physicochemical studies for preparing quick-cooking rice by using gamma irradiation creators, Annals of agricultrural Sciences, Moshtohor 34(2) (1996) 641-652.

[26]. FAO/WHO/UNU Expert consultation, Technical report series: Energy and protein requirements, report of joint f.A.O/who/unu, world health organization, , WHO, Geneva, 1985.

[27]. American Association of Cereal Chemists (AACC), International methods approved of the american association of cereal chemists, 11th edition ed., Paul, Minnesota, USA, 2000.

[28]. N. Brunton, R.T. Gormley, F. Butler, E. Cummins, M. Danaher, M. O'Keeffe, Acrylamide formation in potato products, Teagasc, 2006.

[29]. American Association of Cereal Chemists (AACC), International methods approved of the american association of cereal chemists, 13th edition ed., Paul, Minnesota, USA, 2002.

[30]. Z. Čaplová, P. Švábová, Ibm spss statistics, Statistics and probability in forensic anthropology, Elsevier2020, pp. 343-352.

[31]. E.O. Farinde, O.T. Olanipekun, R.B. Olasupo, Nutritional composition and antinutrients content of raw and processed lima bean (phaseolus lunatus), Ann. Food Sci. Technol 19(2) (2018) 250-264.

[32]. W.M. Mospah, A. El-Sattar, A. Samir, G.S. El-Hadidy, Preparation of pan bread supplemented with amaranth cereal and soybean flour, Egyptian Journal of Food Science 51(1) (2023) 139-150.

[33]. S.R. Drago, H. Franco-Miranda, R.E. Cian, D. Betancur-Ancona, L. Chel-Guerrero, Bioactive properties of phaseolus lunatus (lima bean) and vigna unguiculata (cowpea) hydrolyzates incorporated into pasta. Residual activity after pasta cooking, Plant Foods for Human Nutrition 71(3) (2016) 339-345.

[34]. G. El-Hadidy, S. L Nassef, E. G El-Dreny, Chemical and biological evaluation of bakeries produced from golden berries, (2023).

[35]. M.E. Abdalla, M. Abou Raya, G.A. Ghoniem, G. EL-Hadidy, Evaluation anti-obesity activity of psyllium seeds powder on rats fed a high fat diet, Journal of Food and Dairy Sciences 12(11) (2021) 273-279.

[36]. H.H. Shaban, G.S. El-Hadidy, A.M. Hamouda, Evaluation of breadsticks prepared from chufa tubers as partial substitute of wheat flour, Asian J. Food Res. Nutri 2(2) (2023) 40-51.

[37]. G.S. El-Hadidy, W. Elmeshad, M. Abdelgaleel, M. Ali, Extraction, identification, and quantification of bioactive compounds from globe artichoke (cynara cardunculus var. Scolymus), Sains Malaysiana 51(9) (2022) 2843-2855.

[38]. G.S. El-Hadidy, H.H. Shaban, W.M. Mospah, Gluten-free crackers preparation, Journal of Food Research 11(3) (2022) 47-56.

[39]. A.E. Elbassiony, S.M. El-Elshehawy, F.Y. Ibrahim, G. El-Hadidy, Nutritional and sensory evaluation of prepared pan bread using amaranth, milk thistle, garden cress and wheat flour, Journal of Food and Dairy Sciences 16(5) (2025) 59-65.

[40]. G. El-Hadidy, Preparation and evaluation of pan bread made with wheat flour and psyllium seeds for obese patients, European Journal of Nutrition & Food Safety 12(8) (2020) 1-13.

[41]. G. Elhadidy, L. Shereen, A. Abd El-Sattar, Preparation of some functional bakeries for celiac patients, Current Chemistry Letters 11(4) (2022) 393-402.

[42]. H.H. Shaban, S.L. Nassef, G.S. Elhadidy, Utilization of garden cress seeds, flour, and tangerine peel powder to prepare a high-nutrient cake, Egyptian Journal of Agricultural Research 101(1) (2023) 131-142.

[43]. N.A. Abd Rahman, R.E. El-Gammal, E.I. Saafan, G. El-Hadidy, Utilization of germinated naked barley flour in the producing of balady bread, Journal of Food and Dairy Sciences 16(1) (2025) 1-6.

[44]. E. El-Dreny, M.A. Mahmoud, G. El-Hadidy, Effect of feeding iron deficiency anemia rats on red beetroots juices, Journal of Food & Dairy Sciences 10(8) (2019).

[45]. G. El-Hadidy, E.A. Yousef, A. Abd El-Sattar, Effect of fortification breadsticks with milk thistle seeds powder on chemical and nutritional properties, Asian Food Science Journal 17(2) (2020) 1-9.

[46]. G. Elhadidy, E. Rizk, E. El-Dreny, Improvement of nutritional value, physical and sensory properties of biscuits using quinoa, naked barley and carrot, Egyptian Journal of Food Science 48(1) (2020) 147-157.

[47]. G.S. El Hadidy, E. Rizk, Influence of coriander seeds on baking balady bread, Journal of Food and Dairy Sciences 9(2) (2018) 69-72.

[48]. E.-S.G. El-Dreny, G. El-Hadidy, Utilization of young green barley as a potential source of some nutrition substances, Zagazig Journal of Agricultural Research 45(4) (2018) 1333-1344.