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PROXIMATE, ANTIOXIDANT, AND COLOUR PROFILE OF WHOLE WHEAT-GERMINATED LIMA BEAN CHIPS

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ABSTRACT

Chips were produced from whole wheat and germinated lima bean flour in ratios 100: 0 (control), 95:5, 90:10, 85:15, and 80:20, representing samples AC, BC. CC, DC, and EC, respectively. The proximate, antioxidant properties, and colour profile were determined using standard protocols. Results indicated that incorporating germinated lima bean flour significantly increased the protein and ash contents of the chips, peaking at the 20% substitution level. Conversely, a proportional decrease was observed in crude fibre, crude fat, and carbohydrate levels. Furthermore, the substituted chips showed greater antioxidant activity, showing elevated 1,1-diphenyl-2-picryl hydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and total phenols (TP) levels compared to the 100% whole wheat (control). The colour also indicated higher lightness (L), whiteness (W*) values for the 100 % whole wheat chips while the substituted chips had higher values for the redness (a*) and yellowness (b*) values. Ultimately, the inclusion of germinated lima beans enhanced the nutritional contents and antioxidant properties of the chips, highlighting their potential as a healthy snacking alternative.*

Keywords: Antioxidants, Colour, Legumes, Proximate, Snacks

INTRODUCTION

Dietary habits, coupled with food insecurity, are major contributors to the rising global mortality rates associated with chronic diseases (WHO, 2018; Beyene, 2023). Consequently, there is also growing interest in the use of low-cost, adoptable technologies for the development of nutritious snacks. These snacks can provide macro and micronutrients, especially to vulnerable groups and across all ages (Chinma *et al*., 2021; Ojo *et al*., 2026). Whole-grain cereals are frequently combined with legumes to produce healthy snacks (Zungu *et al*., 2020). They are a good source of fibre and help reduce the risk of type 2 diabetes, cancer, and some cardiovascular diseases. Despite these advantages, consumption of whole wheat is reported to be extremely low compared with recommended levels, which stipulate that half of daily grain intake be whole grain (Li *et al*., 2019; Olamiti and Ramashia, 2024). Therefore, incorporation into diets has been widely advocated (Morey *et al*., 2025). Whole wheat retains the bran, germ, and endosperm, providing dietary fibres and other essential nutrients. It also offers other associated health benefits, such as the antioxidant properties that help improve digestive health and further reduce the risk of chronic conditions (Guo *et al*., 2022; Wei *et al*., 2022).

Legumes have transitioned from being traditional staple foods to key ingredients in innovative food formulations, particularly in snack foods, bakery products, meat analogues, and complementary foods (Melini *et al*., 2023). Recently, neglected and underutilised legumes (NULs) have gained significant attention as potential crops to alleviate malnutrition and boost food security in Africa (Popoola *et al*., 2023). This shift is supported by their excellent nutritional profile, functional properties, and potential health benefits, making them ideal for the development of functional food products (Wang *et al*., 2020). Legumes are valued for their high protein content (20-45 %), complex carbohydrates (60-65 %), and low-fat content (2-3 %), which significantly improve the nutritional value of composite food products (Ganesan and Xu, 2020; Multescu *et al*., 2024).

Germination is a natural process that reduces harmful components in crops while enhancing their nutrients and bioactive compounds (Chinma *et al*., 2020). Germination offers numerous health benefits to humans, primarily by boosting antioxidant activities. This increase is attributed mainly to their high polyphenolic content, which scavenges free radicals and reduces the oxidative stress linked to ageing and degenerative diseases (Singh *et al*., 2021). The use of whole grains, including whole wheat, in food formulations is common. However, reliance on staple grains such as whole wheat alone limits dietary diversity and increases vulnerability to disruptions in the food supply. Promoting legume-based composite flours can enhance local agricultural value chains and provide affordable, nutrient-dense food alternatives. This is particularly important in regions where protein-energy malnutrition is a major concern (Nwabueze *et al.,* 2023). This could serve the increasing number of people suffering from malnutrition in developing regions of the world, such as Nigeria.

Proximate composition provides a comprehensive view of a food's nutritional value (Maddaloni *et al*., 2025), which is crucial for evaluating novel foods and how they can address dietary needs or enhance a more sustainable diet. Therefore, this study investigates the proximate, antioxidant, and colour profiles of whole-wheat germinated lima bean chips, which can be utilised for healthy snacking.

MATERIALS AND METHODS

Whole wheat (*Triticum aestivum*) and the white variety of lima beans (*Phaseolus lunatus*) were procured from the local market in Minna, Niger State, Nigeria. These were authenticated at the Crop Production Department, and the experiment was carried out in the Food Processing Laboratory of the Federal University of Technology (FUT), Minna, Niger State.

Production of whole wheat flour

Whole wheat flour was prepared using the method described by Chinma *et al*. (2024), with slight modification. Five kilograms (5 kg) of whole wheat grains were sorted to remove foreign materials such as stones, chaff, and immature grains. After sorting, the grains were thoroughly washed with clean water and solar-dried to an appropriate moisture level. The dried grain was milled in an attrition mill and sieved through a 100 µm mesh to obtain fine whole wheat flour, which was then packaged in a plastic container, the lid tightly closed, and kept in a cool, dry place for further analyses.

Production of germinated white lima bean flour

Germinated white lima bean flour (GBF) was produced using the method described by Chinma *et al.* (2020). A total of two kilograms (2 kg) of white lima beans seeds were cleaned to remove broken seeds and foreign materials by sorting and were disinfect with 1200 mL of 1 % food-grade sodium hypochlorite solution for 30 min, to suppress mould growth, drained and soaked in distilled water in ratio 1:3 for 10 h at room temperature (32 ± 2^0 C) while shaking every 1 h to ensure uniform hydration. The water was drained, and hydrated beans were allowed to naturally germinate in the dark at ambient conditions (28±1 °C) for 72 h, the optimum period for high protein content reported by Amadeu *et al*. (2025) and Wu and Shin (2025). At the end of germination, the seeds were solar-dried, followed by milling in a high-speed blender (BLX750RD, Kenwood, Sheffield, UK) and sieving (100 μm mesh) to obtain 72h-germinated white lima bean flour (GBF). This was then packaged in an airtight Ziploc bag, stored in a plastic container with a lid, and kept in a cool, dry place for further analysis.

Flour blend formulations and chips production

The whole wheat and germinated white lima beans composite flour was formulated in the ratios 100:00 (AC), 95:5 (BC), 90:10 (CC), 85:15 (DC) and 80:20 (EC). Adeola *et al*. (2021) recommended substituting up to 20 % of legumes. After blending the flour samples to ensure uniformity, chips were produced from the whole wheat and germinated lima bean composite flours following the protocol of Chinma *et al*. (2020). Essentially, 100 g each of the various flours was mixed with 1 g of margarine, 3 g of baking powder, and 1.2 g of table salt, and then the flour was mixed with water (2:5: 1) in a Kenwood FP190 Series mixer (Shenzhen, China) for 8 min. The dough was rolled through a pasta maker, cut into uniform squares measuring 2.0 x 2.0 cm, and baked in a Gallenkamp oven (Widnes, Cheshire, UK) at 190°C for 10 min. After baking, the chips were cooled for 1 hour prior to packaging. Chips were produced from whole-wheat flour, which served as the control.

Determination of the proximate composition of chips

Basically, the AOAC (2012) technique was used for the proximate composition determination. The oven-dry method was employed for moisture content evaluation, while the micro-Kjeldahl technique with a nitrogen conversion factor of 6.25 was used to calculate crude protein content. The crude fat was determined by Soxhlet extraction with petroleum ether as the solvent. The crude fibre was determined by digesting the finely ground chip samples with diluted acid and alkali, and the ash content was analysed by incinerating (600 oC, 5 h) in a muffle furnace. The difference (100 – the total of moisture, ash, fat, and protein) was used to compute the value of carbohydrates, while the Atwater factor (Equation 1) was used in calculating the energy.

$$E = (\text{Carbohydrate} \times 4) + (\text{Fat} \times 9) + (\text{Protein} \times 4) \qquad (1)$$

E= Energy (Kcal)

Evaluation of antioxidant properties of chips

Preparation of extracts

The method described by Chinma *et al*. (2020) was used to prepare the methanolic extract. Briefly, each sample (0.2 g) was extracted with 4 mL of 80 % methanol in a shaking water bath (Genlab Ltd., Cheshire, UK) at 40 °C for 2 h, then twice centrifuged at 2000 × g for 10 min. The resultant methanolic extract was stored at refrigeration conditions (4 °C). The 1,1-diphenyl-2-picryl hydrazyl (DPPH) and total phenols of the extract were evaluated using the method described by Singleton *et al*. (1999), while the Ferric reducing antioxidant power (FRAP) was evaluated using the method of Pulido *et al*. (2000).

Analysis of Colour Profile

Colour analysis was conducted using a calibrated colourimeter (Konica Minolta CR-400/410) according to the method described by Chinma *et al*. (2020). Triplicate measurements of L (lightness), a (red-green axis), and b (yellow-blue axis) were taken on the chip surface, with the instrument calibrated against a white reference tile (L = 97.83, a = -0.43, b = 1.98). The whiteness value was calculated using equation (2):

$$\text{Whiteness}=\sqrt{(100-L)^2+a^2+b^2} \tag{2}$$

Statistical Analysis

Data were analysed using one-way Analysis of Variance (ANOVA) with a significance level of $p < 0.05$. Means were separated using Duncan's Multiple Range Test (DMRT). SPSS version 20 was used for the statistical analysis, and results were presented as mean ± standard deviation. All analyses were performed in triplicate except where stated

RESULTS AND DISCUSSION

The proximate composition of the chips is as illustrated in Table 1. Higher levels of GBF substitution increased the moisture content from 5.99% (AC) to 6.68% (DC, EC), ash ranged between 2.19% and 2.80% at AC and EC, and crude protein ranged from 11.37% (AC) to 16.26% (EC). Meanwhile, a decrease was observed in the crude fibre, crude fat and the carbohydrate contents at 3.48 % (EC)-3.52 % (AC), 2.22 % (EC)-3.47 % (AC) and 68.55-73.45 %, respectively, as the GBF substitution increases. The moisture content of the formulated chips at AC to CC was below the optimal value of 7 % for snack product shelf stability and resistance to microbial spoilage (Gautam and Gupta, 2017). The slight increase in moisture associated with higher GBF substitution could be due to greater water affinity of the hydrophilic functional groups in germinated lima bean flour (Wu and Shin, 2025).

Table 1. Proximate Properties of Whole Wheat-Germinated-Lima Beans Based Chips

Parameters (%)	AC	BC	CC	DC	EC
Moisture	5.99^{d}±0.00	6.21^{c}±0.02	6.40^{b}±0.01	6.68^{a}±0.10	6.68^{a}±0.10
Ash	2.19^{c}±0.00	2.53^{b}±0.06	2.60^{b}±0.00	2.71^{ab}±0.00	2.80^{a}±0.20
Crude fiber	3.52^{a}±0.00	3.25^{b}±0.00	3.19^{b}±0.09	3.25^{b}±0.00	3.48^{a}±0.00
Crude protein	11.37^{e}±0.03	13.30^{d}±0.08	15.42^{c}±0.00	15.82^{b}±0.02	16.26^{a}±0.00
Crude fat	3.47^{a}±0.05	3.40^{b}±0.01	2.38^{b}±0.00	2.28^{c}±0.01	2.22^{c}±0.04
Carbohydrate	73.45^{a}±0.00	71.29^{b}±0.13	70.01^{c}±0.02	69.25^{d}±0.03	68.55^{e}±0.02
Energy (KJ/100g)	370.51^{a}±0.31	368.96^{b}±0.04	363.14^{c}±0.03	361.20^{d}±0.02	359.22^{e}±0.10

Values are means ± Standard deviation of triplicate determinations. Values in the same row with different superscripts are significantly different ($p \leq 0.05$). AC=Whole wheat flour (control), BC=95 % whole wheat flour and 5 % germinated white lima beans flour, CC= 90 % whole wheat flour and 10 % germinated white lima beans flour, DC= 85 % whole wheat flour and 15 % germinated white lima beans flour, EC= 80 % whole wheat flour and 20 % germinated white lima beans flour.

Higher GBF substitution increased the ash (15.53–27.85%) and crude protein (16.97–43.01%) contents of the chips, probably due to the enhanced nutrient bioavailability in germinated legumes. Such elevated ash levels indicate a high mineral concentration, which supports metabolic growth and development (Elinge *et al*., 2012). Food with high ash content is expected to contain high concentrations of various mineral elements, which may accelerate and improve metabolic growth and development (Elinge *et al*., 2012). A similar increase in nutritional quality was observed by Liu *et al*. (2018) and Chinma *et al*. (2021) with the incorporation of nutrient-dense germinated mung bean into wheat-based noodles and germinated African yam bean (*Sphenostylis stenocarpa*) into chips snacks, respectively. This validates the suitability of germinated legumes as protein and mineral fortifiers.

The decrease in crude fibre, crude fat and carbohydrate content of the chips with increased substitution of GBF infers that these nutrients are low in the germinated lima bean flour. Fibre promotes the excretion of bile acids, fats and sterols, which have been implicated in the aetiology of certain diseases in humans (Lutter and Dewey, 2003). Crude fibre plays a crucial role in glycaemic control and helps reduce morbidity in diabetic patients. The fibre content of the formulated chips (3.19–3.52%) falls well within the recommended threshold of ≤5% for both children and adults (Hojsak *et al*., 2022).

Likewise, the carbohydrate content decreased slightly with GBF substitution. These findings are corroborated by Bello *et al*. (2022), who reported comparable trends when substituting carbohydrate-rich wheat flour with protein-dense legume flour. Furthermore, Dragomir *et al*. (2026) observed elevated protein and ash levels in bread formulated with germinated lentil flour. The superior protein profile of the germinated lima bean chips highlights the potential of germinated pulses as value-added ingredients in functional wheat-based foods, offering an accessible, health-focused alternative for the snack industry.

Antioxidant properties of germinated lima bean flour substituted chips

The antioxidant properties are shown in Table 2. The findings indicate that antioxidant properties in chips produced from the composite flour significantly ($p<0.05$) increased with the proportionate addition of GBF. The DPPH, FRAP and TP of the composite chips ranged from 70.31- 77.64 %, 19.16 -33.07 µmolFe2+/g and 42.03 -60.62 mgGAE/100g, respectively, against DPPH at 67.52 %, FRAP at 15.78 µmolFe2+/g and 40.62 mgGAE/100g for the control, respectively. Overall, the DPPH, FRAP and TP resulted in percentage increases ranging from 4.13 -14.99 %, 21.42 - 109.57 % and 3.47 -49.24 % when compared to the chips produced from 100 % whole wheat flour. Chips formulated with GBF showed significantly higher antioxidant activity than the control (100 % whole wheat) chips. This suggests a stronger antioxidant effects from the composite chips.

Table 2. Antioxidant Properties of Whole Wheat and Germinated Lima Beans Chips

Parameters	AC	BC	CC	DC	EC
DPPH (%)	67.52^e±0.00	70.31^d±0.00	71.63^c±0.00	74.51^b±0.00	77.64^a±0.00
FRAP (µmolFe^{2+}/g)	15.78^e±0.00	19.16^d±0.00	23.31^c±0.00	27.81^b±0.00	33.07^a±0.00
TPmg (GAE/100g)	40.62^e±0.00	42.03^d±0.00	53.15^c±0.00	57.62^b±0.00	60.62^a±0.43

Values are means ± Standard deviation of triplicate determinations. Values in the same row with different superscripts are significantly different ($p \leq 0.05$). DPPH= 1,1-diphenyl-2-picryl hydrazyl, FRAP= Ferric reducing antioxidant power, TP=Total phenolics. AC=Whole wheat flour (control), BC=95 % whole wheat flour and 5 % germinated white lima beans flour, CC= 90 % whole wheat flour and 10 % germinated white lima beans flour, DC= 85 % whole wheat flour and 15 % germinated white lima beans flour, EC= 80 % whole wheat flour and 20 % germinated white lima beans flour.

Research findings correlate high antioxidant activity with beneficial effects, including the elimination of naturally occurring free radicals in the body and the extension of product shelf life (Sattar *et al*., 2023). Consequently, higher antioxidant activity in GBF-substituted chips could help reduce the risk of developing non-communicable diseases while also preventing lipid oxidation by limiting carbonyl accumulation in the product (Jin *et al*., 2013; Ayoka *et al*., 2022). The regular consumption of these composite chips can contribute to the healthy bodily antioxidants, status that can stand as a dietary defence against the reactive oxygen species and free radicals formed during lipid peroxidation and normal cellular metabolism.

Colour characteristics of germinated lima bean substituted chips

Colour is a critical factor in determining consumer perception and acceptance of a food product, and it can be perceived positively or negatively depending on consumer/market preferences (Rathee and Rajain, 2019). The chip samples were significantly altered by the incorporation of germinated white lima beans, as presented in Table 3. The AC chips sample exhibited the highest lightness (L=53.25).

Table 3. Colour Profile of Whole Wheat Germinated Lima Beans Chips

Parameters	AC	BC	CC	DC	EC
L*	53.25^a±0.94	52.30^b±0.21	50.24^c±0.05	50.82^c±0.44	48.68^d±0.47
a*	1.66^d±0.06	2.22^c±0.23	2.57^b±0.17	2.79^a±0.21	2.79^a±0.09
b*	8.27^d±0.14	10.34^c±0.43	11.29^b±0.74	12.10^b±0.45	15.34^a±0.45
Whiteness	53.60^a±0.34	51.26^b±0.10	50.50^c±0.27	48.83^c±0.27	47.51^d±0.91

Values are means ± Standard deviation of triplicate determinations. Values in the same row with different superscripts are significantly different ($p \leq 0.05$). AC=Whole wheat flour (control), BC=95 % whole wheat flour and 5 % germinated white lima beans flour, CC= 90 % whole wheat flour and 10 % germinated white lima beans flour, DC= 85 % whole wheat flour and 15 % germinated white lima beans flour, EC= 80 % whole wheat flour and 20 % germinated white lima beans flour.

while EC was the darkest (L = 48.68). This darkening with increased GBF substitution is further supported by whiteness values at 53.60 and 47.51 for AC and EC, respectively. For the redness/greenness index (a*), samples DC and EC shared the highest values (2.79), showing a pronounced red component, whereas sample AC demonstrated the lowest redness (1.66).

In terms of yellowness/blueness (b*), sample EC exhibited the highest value of 15.34, and AC the lowest at 8.27. The addition of legume flours modifies colour parameters, and the shift in colour development could be due to non-enzymatic browning (Maillard reactions), which increased in the GBF-substituted chips. Other factors, such as the native pigments, could have been dominant at higher inclusion rates (Ohanenye *et al*., 2020). A similar darker colour result was reported in legume-enriched bread (chickpea, common bean, and broad bean) by Bojňanská *et al*. (2021). This characteristic increase in the a* (reddish shades) and b* (yellowish shades) values, can influence consumer acceptability (Cacak-Pietrzak *et al*., 2024).

CONCLUSION

The substitution of whole wheat with germinated lima beans (GBF) for chip production resulted in increased crude protein and ash, while crude fibre, crude fat, carbohydrate, and energy decreased. The chips' colour was deeper at higher GBF levels. Also, higher antioxidant capacities were observed in chips from composite flours than in those from 100 % whole wheat, suggesting that composite flours could serve as functional foods. Consumption of these chips could be nutritionally beneficial, increase the utilisation of lima beans, and, of course, add variety.

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