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Research article

Effects of Jack Bean and Beetroot Flour Addition on the Amino Acid Composition, Nutritional Quality, and Physical Properties of Sorghum Crispbread

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Abstract

Foods enriched with bioactive compounds are increasingly valued for disease prevention. One example is sorghum which offer a number of functional advantages owing to its high fiber concentration. Due to its fiber-rich profile, crispbread made from sorghum is increasingly consumed as an alternative snacks. The addition of jack bean and beetroot flour serves as a protein, pigment and antioxidant source, enhancing nutritional, functional, and the appearance of sorghum crispbread. Although sorghum has been widely studied as a functional food ingredient, research on the development of sorghum-based crispbread added with jack bean and beet flour is very limited. Thus, the evaluation of nutritional and physical characteristics of the resulting product is needed. This study aimed to examine the amino acid, nutritional, and physical properties of sorghum-based crispbreads. A completely randomized design (CRD) was employed, incorporating with differences in the formulas of sorghum, jack bean, and beet flour. Chemical and physical analyses showed significant differences between the formulations. The sample with the highest jack bean flour (36%) had the highest protein (14.02%), soluble protein, red color intensity, and crispiness (117.39), while sample with the lowest jack bean flour (12%) had the highest total amino acid (7622.37 mg/100g) and dietary fiber contents. PCA showed that the addition of jack bean and beetroot flours significantly altered the nutritional and textural characteristics of the sorghum crispbread, shifting the product from a carbohydrate- and energy-dominated profile toward higher protein, amino acid, and fiber contents while modifying its physical properties. These results indicate that the addition of jack bean and beet flour has the potential of soghum-based crispbread as a high-fiber functional snack.

Keywords: amino acids; beetroot; crispbread; dietary fiber; jack bean

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1. Introduction

Healthy eating has become more important worldwide due to the rise in non-communicable diseases such as diabetes, obesity, and cardiovascular disease (Granato et al., 2023). Food is no longer just a source of energy and nutrients, but also a tool for illness prevention and quality of life. Thus, dietary products that meet nutritional needs and provide health advantages are becoming more important. Functional meals have bioactive components that benefit the body beyond nourishment. Functional food consumption improves nutrition and metabolism, and lower the risk of degenerative diseases (Iwatani & Yamamoto, 2019; Chen et al., 2024). Locally produced, nutritionally dense food innovation promotes healthy lifestyles and food security in a sustainable way (Aura & Fitriani, 2024).

Among the various functional food innovations, crispbread has gained popularity as a light, low-fat, and fiber-enriched bakery product that can serve as a healthy alternative to conventional snacks. Its simple composition and low moisture content allow for a long shelf life while maintaining desirable crispness and texture (Ameur et al., 2024). The physical, chemical, and functional characteristics of the resulting crispbread are influenced by the raw materials used. Crispbread is generally produced using cereals, legumes, and tubers. The use of chia seeds in carrot pomace crispbread can increase the antioxidant activity and total phenolic content of crispbread (Rutyna & Galus, 2025). This product can be manufactured using locally available cereals such as sorghum (*Sorghum bicolor L.*), which is naturally gluten-free and rich in dietary fiber, resistant starch, polyphenols, and antioxidants (de Sousa et al., 2018; von Schaumburg et al., 2021). Utilizing sorghum supports the government's ongoing initiative to promote national food self-sufficiency (Gunawan et al., 2017; Rohman et al., 2025).

Sorghum is a rich source of bioactive compounds, containing approximately 19% dietary fiber (Tanwar et al., 2023). The resistant starch content in sorghum reaches 60 g/100 g, depending on the genotype (Teixeira et al., 2016). Sorghum also contains phenolic compounds, flavonoids, and phytosterols that exhibit anti-obesity and anti-diabetic properties (Tanwar et al., 2023). Previous studies have demonstrated that regular sorghum consumption can help mitigate adiposity and inflammation (Arbex et al., 2018), decrease body mass index and liver weight (de Sousa et al., 2018). It can also reduce hepatic fat accumulation, lower fasting glucose levels, and enhance glucose tolerance and insulin sensitivity in obese mice (Moraes et al., 2018).

Sorghum-based crispbread can be enhanced through the incorporation of protein- and antioxidant-rich ingredients. Foods high in protein contribute to the prevention of non-communicable diseases such as obesity and type 2 diabetes mellitus (T2DM) by regulating metabolic processes (Leidy et al., 2015; Barber et al., 2024). High-protein crispbread formulations can be achieved by adding jack bean flour, which contains 30.8% protein and a well-balanced profile of essential amino acids (Anuntagool & Soonthonsun, 2023; Fitriani et al., 2024; Agustia et al., 2025a,b).

Additionally, beetroot flour can serve as a natural colorant with high antioxidant potential in sorghum crispbread. The dominant natural coloring and antioxidant compound in beetroot is betalain. Betalain include betacyanins and betaxanthins (Stoica et al., 2025). Beetroot provides approximately 2.075 g/100 g of betacyanin and 1.901 g/100 g of betaxanthin (Chen et al., 2021). These pigments exhibit both anti-inflammatory and antioxidant properties (Stoica et al., 2025). Moreover, beetroot concentrate contains 391 mg GAE/100 mL of total phenolics, 264 mg QE/100 mL of total flavonoids, and demonstrates 77.5% antioxidant inhibitory activity (Saleem et al., 2025). Therefore, the use

of beetroot can play a role in providing natural coloring for crispbread, with other benefits as an antioxidant.

Sorghum-based crispbread, characterized by its light texture and low moisture content, represents a potential healthy snack option that combines desirable sensory qualities with enhanced nutritional value. Therefore, evaluating the chemical and physical characteristics of sorghum crispbread is crucial for maximizing its formulation and supporting its development as a functional food product that aligns with current health and nutrition trends. This study aims to characterize the amino acid profile, nutritional, functional, and physical properties (texture and color) of sorghum-based crispbread. This research is expected to provide an alternative nutrient-dense snack with improved nutritional quality that can support healthier dietary patterns.

2. Materials and Methods

2.1 Materials

The components utilized in the preparation of sorghum-based crispbread consisted of white sorghum flour and beetroot flour purchased from local industries in the Yogyakarta city area. Jack bean flour was made in-house in the food chemistry laboratory, Universitas Ahmad Dahlan. All flour samples were standardized to 80 mesh particle size. Granulated sugar, butter, salt, and vegetable oil were purchased from local supermarkets. The materials utilized for chemical analysis were of analytical grade and were as follows: potassium hydrogen phosphate trihydrate (K2HPO4.3H2O), bovine serum albumin, Folin-Ciocalteu's phenol reagent (Merck #109001), ethyl alcohol, ethanol, acetone, distilled water, hydrochloric acid (HCl), potassium ferricyanide, sodium hydroxide (NaOH), D-glucose, α-amylase heat-stable (Merck #A3306), protease (Merck #P3910), amyloglucosidase (Merck #A9913). The various chemicals for liquid chromatography (LC) such as water, acetonitrile, formic acid, and ammonium formate, were all of HPLC grade.

2.2 Methods

2.2.1 Sorghum-based crispbread production

The method for producing sorghum-based crispbread pertains to the proportional formula of sorghum flour, jack bean flour, and beet flour as indicated in Table 1. The preparation of sorghum-based crispbread started with the blending of all ingredients in a mixing bowl using a silicone spatula till achieving homogeneity. The dough was allowed to rest for 15 min. Subsequently, the dough was shaped to a consistent dimension. The shaped dough was baked in an oven at 160°C for 25 min. The oven baking procedure consisted of two distinct phases: an initial baking for 18 min (160°C) followed by a 5-min resting period (room temperature) for the crispbread, and concluding with a second baking for 7 min (160°C). The dual oven baking strategy was implemented to avert the burning of the crispbread that would occur if baked in a single duration. The completed crispbread product was extracted from the oven and permitted to cool before being kept for subsequent analysis.

Table 1. Sorghum-based crispbread formulation

	F0	F1	F2	F3
Main Material				
Sorghum flour (%)	100	52	52	52
Jack bean flour (%)	0	36	24	12
Beetroot flour (%)	0	12	24	36
Total of Main Material	**100**	**100**	**100**	**100**
Additives Material				
Water (mL)	74	74	74	74
Butter (mL)	26	26	26	26
Salt (g)	0.8	0.8	0.8	0.8
Sugar (g)	11.2	11.2	11.2	11.2

2.2.2 Nutrition compound analysis

The nutritional components analyzed in this study included moisture content (gravimetric), ash content (dry ashing), lipid content (soxhlet), and total protein content (kjeldhal) using the AOAC (2005) method, as well as carbohydrate content done with different method of AOAC (2005) The soluble protein content was determined using the Lowry-Folin method (Fitriani et al., 2022), and total energy was calculated using the Atwater factors method (Merrill & Whatt, 1973).

2.2.3 Total amino acid analysis

The quantities of amino acids, except cystine, methionine, and tryptophan, were quantified utilizing the Acquity ultraperformance liquid chromatography (UPLC) H-Class amino acid analysis technique from Waters Corporation (2024). Simultaneously, the quantities of cystine, methionine, and tryptophan were quantified utilizing liquid chromatography tandem mass spectrometry (LC-MS/MS; LCMS-8060 NX, Shimadzu) due to their distinct chemical characteristics and the requirement for elevated sensitivity and specificity. The gradient elution protocol is presented in Table 2.

Table 2. Gradient elution utilized for evaluating amino acid content

Time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)	Eluent D (%)
0.00	10.0	-	90.0	-
0.29	9.9	-	90.1	-
5.49	9.0	80.0	11.0	-
7.30	8.0	15.6	57.9	18.5
7.69	7.8	-	70.9	21.3
7.99	4.0	-	36.3	59.7
8.59	4.0	-	36.3	59.7
8.68	10.0	-	90.0	-
10.20	10.0	-	90.0	-

Note: (A) 100% concentrated AccQ-Tag Ultra eluent A, (B) 10% AccQ-Tag Ultra eluent B, (C) double-distilled water, and (D) 100% AccQ-Tag Ultra eluent B

2.2.4 Starch determination

Titrimetric analysis was used to determine total starch content (Moorthy & Padmaja, 2002). The analysis was separated into three stages: sample preparation, titration, and quantification.

1) Sample preparation

Two grams of samples were measured into a 100 mL Erlenmeyer flask and immersed in 20 mL of 80% ethyl alcohol to extract the sugars. The residue was meticulously rinsed with two aliquots (10 mL each) of distilled water to eliminate any remaining sugar clinging to the sample fragments, and the filtrate was collected in the same flasks. The 2 N HCl (20 mL) was added. The substance was subsequently hydrolyzed on a hot plate (100°C; 30 min). The hydrolysate volume was augmented to 100 mL using distilled water, and the supernatant was utilized for titration. The alcoholic sugar filtrate from each sample was treated with 1 mL concentrated HCl and heated for 30 min on a hot plate to convert non-reducing sugars into reducing sugars and to evaporate the alcohol in the extract. The volume of the sugar extract was subsequently increased to 50 mL with distilled water.

2) Titrimetric assay

Ten milliliters of potassium ferricyanide reagent were pipetted into clean 100 mL Erlenmeyer flask. Subsequently, 5 mL of 2.5 N NaOH was added with thoroughly mixing by a swirling motion. Each flask was put above a gas flame to ease the boiling of chemicals. Titration had to be conducted while the sample was boiling. Five drops of diluted methylene blue indicator were introduced and each solution promptly transitioned to a blue-green color. The starch hydrolysate of the sample, measured at 2 mL using a blow pipette, was subsequently introduced to the boiling reagent incrementally, drop by drop. The approach to the endpoint was indicated by a color change from blue-green to bright violet.

3) Calibration and quantification

The calibration process was carried out using a standard D-glucose solution prepared for the same titration process as the sample as described previously. The starch content of the sample was calculated following equation 1.

$$\text{Starch (g/100 g fresh weight)} = \frac{10^a \times 100^b \times 0.9^c \times 100}{T \times 2.0^d \times 1000} \quad (1)$$

where *a* is the titer obtained for ferricyanide reagent; *b* is the total volume of the starch hydrolysate; *c* is 0.9 (the factor used for converting sugar to starch); *T* is titre obtained for the starch hydrolysate of the sample (mL); and *d* is weight of sample (g).

2.2.5 Dietary fiber analysis

Dietary fiber was measured using the gravimetric enzymatic approach (Garbelotti et al., 2003). The process involved enzymatic hydrolysis with α-amylase, protease, and amyloglucosidase in a MES-TRIS buffer solution. The previously dried, defatted, or carbohydrate-free sample was suspended (about 1 g) in 40 mL of MES-TRIS buffer. The

enzymatic hydrolysis was performed sequentially by adding 50 µL of thermostable α-amylase and incubating in a water bath for 35 min, followed by 100 µL of protease at 60°C for 30 min. After adjusting the pH to 4.0-4.7, 300 µL of amyloglucosidase was added and incubated at 60°C for 30 min. The soluble fiber was then precipitated (1 h; room temperature) with 95% (v/v) ethanol (60°C), with the ratio of ethanol to sample being 4:1 (v/v). The residue was filtered through fritted (sintered) glass crucibles (Gooch type) using glass wool as a filter aid. All residual particles were quantitatively transferred to the crucible and washed twice with 15 mL per wash of 78% ethanol, 95% ethanol, and acetone. Each crucible containing the residue was dried in an oven at 105°C, cooled in a desiccator, and weighed to a constant mass. The quantification of dietary fiber was accomplished using equation 2.

$$DF\ (\%) = \frac{M_2}{M_1} \times 100 \quad (2)$$

where M_2 is residue weights (mg); M_1 is the weights of the initial sample.

2.2.6 Texture profile analysis

Texture analysis was conducted utilizing an Ametek Brookfield CTX texture analyzer (Putri & Setiyoko, 2025). The texture analyzer was set with 0.05 mm/s test speed, 0.5 s wait time, 1 N preload/stress, 300 mm/min stress speed, and compression as the direction. Post-analysis of samples was conducted using the CTX software, evaluating the following attributes: hardness, cohesiveness, adhesiveness, fracture, crispness, and crunchiness.

2.2.7 Color determination

The color evaluation of the crispbread samples was conducted utilizing a Konika Minolta Optics Chroma Meter CR-400. The color evaluation was conducted with the Hunter L*, a*, and b* color classifications (Bimrew et al., 2025). The L value denotes the brightness level of the sample, +a* signifies a propensity for red, -a* denotes a propensity for green, +b* shows a propensity for yellow, and -b* signifies a propensity for blue.

2.2.8 Principal component analysis

Quantitative data of each result obtained were used to define a data set with 4 variables. The data were processed using Minitab 22 software by principal component analysis (PCA) in order to identify similarities among the samples.

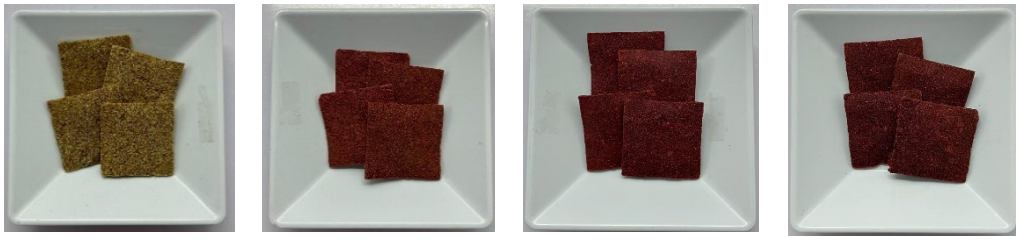
2.2.9 Experimental design and data analysis

This research employed a completely randomized design (CRD), in which four sorghum-based crispbread formulations served as the treatments. The data obtained were first analyzed for normality and homogeneity. All data were proven to be normal and homogeneous, so the data were classified as parametric data and were tested using the one-way ANOVA method at a significance level of 5%. If the test parameters showed a significant effect, the post-hoc test was continued with DMRT (Duncan's Multiple Range Test).

3. Results and Discussion

3.1 Sorghum-based crispbread products

Figure 1 illustrates a model of sorghum crispbread experiment. F0, produced with 100% sorghum flour, exhibited a distinct appearance compared to the other samples incorporating beetroot flour and jack bean flour. Despite F3 being derived from a formula with the maximum percentage of beetroot, its color appeared darker than that of F1, which was formulated with a smaller amount of beetroot. A comprehensive physical investigation of color and texture is detailed at the end of this Section.



F0 F1 F2 F3

Figure 1. Visual characteristics of the produced sorghum-based crispbread sample

3.2 Amino acids profile

Amino acids are crucial substances for providing dietary requirements, particularly essential amino acids. Amino acid evaluation is essential for assessing the protein quality in crispbread samples. According to Table 3, F0 has the lowest total amino acid concentration, measuring 3459.88 mg/100 g of material, in comparison to the other samples. F1, F2, and F3 had total amino acids of 7484.62 mg/100 g, 7608.70 mg/100 g, and 7622.37 mg/100 g, respectively. The variation likely resulted from the incorporation of jack bean flour in samples F1, F2, and F3, which significantly enhanced the overall amino acid content of the sorghum crispbread. The fortification of legume flour in cereal-based food items can enhance the limited amino acids present in each component. Lysine is classified as an essential amino acid, making its presence crucial in food products. The amino acid lysine is a limiting amino acid in cereal products (WHO/FAO/UNU, 2007). From Table 3, it was also noted that the incorporation of jack bean flour (F1, F2, F3) demonstrated the significant ability to double the concentration of the amino acid lysine, with the amount of 304.24±0.64, 276.68±5.58, 225.70±1.53 mg/100 g, respectively, when compared to F0 (118.82±0.30 mg/100g).

Cystine and methionine, which are sulfur-containing amino acids, are the limiting amino acids in jack bean flour. Among the total sulfur amino acids, methionine is nutritionally essential, but cysteine, a metabolic byproduct of methionine catabolism, relies on sufficient methionine availability to meet the requirements for both amino acids. They are nutritionally significant due to their limited concentrations in legume proteins, although being similarly prevalent in cereal and animal proteins. Despite their lower prevalence in proteins compared to other amino acids, they are metabolically significant, suggesting that their relative necessity for maintenance may exceed that for human growth (WHO/FAO/UNU, 2007). Despite sulfur amino acids being classified as limiting in legume products, the concentrations of methionine and cysteine in samples F1, F2, and F3 exceeded those in F0.

Table 3. Amino acid content of sorghum-based crispbread

Amino acids	Amino Acid Concentration (mg/100g)			
	F0	F1	F2	F3
Essential Amino Acid (EAA)				
L-Histidine	40.07±0.21^{a}	442.43±0.97^{d}	270.17±6.90^{b}	294.25±2.95^{c}
L-Isoleucine	80.53±0.36^{a}	185.52±0.76^{b}	317.46±8.38^{c}	332.50±1.37^{d}
L-Leucine	343.18±2.68^{a}	790.65±0.95^{c}	822.45±21.86^{d}	741.24±1.98^{b}
L-Lysine	118.82±0.30^{a}	304.24±0.64^{d}	276.68±5.58^{c}	225.70±1.53^{b}
L-Methionine	8.49±0.05^{b}	10.64±0.01^{d}	9.83±0.01^{c}	8.10±0.02^{a}
L-Tryptophan	67.19±0.03^{a}	104.66±0.08^{c}	130.26±1.24^{d}	79.13±0.37^{b}
L-Valine	172.46±0.05^{a}	283.99±1.15^{b}	404.66±9.66^{c}	426.93±3.00^{d}
L-Phenylalanine	159.65±0.91^{a}	357.21±0.14^{c}	365.99±10.22^{c}	325.37±3.29^{b}
L-Threonine	58.79±0.29^{a}	238.50±0.34^{d}	192.65±4.69^{b}	227.30±0.90^{c}
Total EAA	1049.17	2717.83	2790.15	2660.51
Non-Essential Amino Acid (NEAA)				
L-Alanine	381.43±2.37^{a}	684.58±1.83^{c}	673.76±16.62^{c}	549.64±0.54^{b}
L-Arginine	82.45±0.40^{a}	263.39±2.20^{b}	258.00±6.66^{b}	290.41±1.37^{c}
L-Aspartic acid	79.70±0.67^{a}	843.21±3.45^{d}	743.48±16.29^{c}	687.38±0.83^{b}
L-Glycine	109.06±0.87^{a}	397.56±2.11^{d}	353.17±8.94^{c}	255.38±0.05^{b}
L-Glutamic acid	1044.69±5.09^{a}	1436.08±4.63^{b}	1655.17±34.56^{c}	2094.39±1.62^{d}
L-Cystine	170.50±2.38^{a}	242.14±3.93^{d}	224.18±4.33^{c}	192.62±0.62^{b}
L-Proline	207.80±1.41^{a}	384.10±0.36^{c}	281.02±6.92^{b}	379.75±1.09^{c}
L-Serine	24.49±0.36^{a}	460.75±2.28^{d}	223.31±5.21^{b}	281.36±0.56^{c}
L-Tyrosine	310.60±1.98^{c}	174.10±1.03^{a}	406.46±7.56^{d}	230.93±3.93^{b}
Total NEAA	2410.71	4766.79	4818.54	4961.86
Total	3459.88	7484.62	7608.70	7622.37

The results indicate the mean±standard deviation. Variations in notation within the same row represent statistically significant differences ($P<0.05$).

The content of amino acids in sorghum crispbread was affected by the addition of beetroot. According to Table 3, the addition of beetroot influenced the elevation of the amino acid valine and glutamic acid. A greater concentration of beetroot resulted in an increased concentration of the amino acids valine and glutamic acid. F3 had the greatest significant content of valine (426.93 mg/100 g of material) in comparison to the other samples. Furthermore, the glutamic acid content in F3 (2094.39 mg/100 g of material) was nearly doubled in comparison to the other samples. Beetroot contains glutamic acid as its most abundant amino acid, with concentrations ranging from 900-1200 mg glutamic acid/100 g material, depending on the variety (Hu et al., 2019).

Numerous amino acids exhibit potential anti-obesity effects as they can enhance lipolysis, modulate appetite, and affect metabolic processes. This encompasses leucine, lysine, and amino acid derivatives such as β-aminoisobutyric acid (BAIBA) and L-carnitine. Leucine can suppress appetite and stimulate the secretion of appetite-regulating hormone.

Additionally, leucine has the potential to help prevent the effects of a high-fat diet without reducing food intake, thereby supporting increased energy expenditure

Lysine was recognized as a notably effective amino acid in animal research for mitigating obesity produced by high-fat diets and enhancing blood lipid profiles (Kobayashi et al., 2009). L-carnitine may facilitate weight loss by aiding the body in using fat for energy. BAIBA (β-aminoisobutyric acid) is an amino acid derivative that may enhance lipolysis (Zheng et al., 2021). Additional amino acids, such as isoleucine, methionine, phenylalanine, proline, threonine, and tryptophan, have shown promise in animal research regarding their impact on appetite management and body composition (Rigamonti et al., 2020; Simonson et al., 2020). The research findings indicated that the crispbread samples had elevated concentrations of the amino acid lysine and leucine. Consequently, the resulting sorghum-based crispbread has potential as a functional snack for preventing obesity. However, this study has not examined the in vivo anti-obesity and anti-diabetic benefits of crispbread, so anti-diabetic and anti-obesity claims cannot yet be attributed to sorghum crispbread products.

3.3 Proximate content and energy of sorghum-based crispbread

Table 4 shows the proximate and total energy values of the crispbread samples. In general, there were no significant differences among all samples in terms of moisture, ash, carbohydrate content, and total energy. Nevertheless, F0 exhibited the most substantial lipid concentration in comparison to the other samples. The lipid content of the raw materials used had an impact on the elevated lipid content of the crispbread samples. Sorghums contain 5% lipid (Tanwar et al., 2023), while jack beans contain 3% lipid (Purwandari et al., 2023). Consequently, the F0 sample, which contained a 100% sorghum flour ratio, yielded a product with the highest significant lipid content. Conversely, the F1 sample exhibited the highest crude protein content. The F1 sample was manufactured with the greatest proportion of jack bean flour, specifically 36%. Jack bean is recognized for its protein content, which ranges from 35 to 39%. Consequently, the F1 sample exhibited the highest crude protein content. Conversely, the F3 sample, which contained 12% jack bean flour, exhibited a low protein content of 10.78%. Nevertheless, this value was significantly higher than that of the control sample (F0), which was produced without the use of jack bean flour.

Table 4. Proximate content and total energy of sorghum-based crispbread

Sample	Moisture content (%)	Ash content (%)	Lipid content (%)	Crude protein content (%)	Carbohydrate content (%)	Energy (Kcal)
F0	5.19±0.07^{a}	3.83±2.85^{a}	18.63±0.43^{b}	7.41±0.09^{a}	69.92±2.52^{a}	477.01±13.54^{a}
F1	6.50±0.01^{a}	3.26±0.02^{a}	14.96±0.40^{a}	14.02±0.92^{d}	67.53±0.54^{a}	460.83±2.09^{a}
F2	7.86±1.96^{a}	3.96±0.00^{a}	15.07±0.69^{a}	12.35±0.41^{c}	68.28±1.19^{a}	458.17±3.06^{a}
F3	5.26±0.04^{a}	4.44±0.03^{a}	15.58±0.36^{a}	10.78±0.11^{b}	68.95±0.23^{a}	459.14±1.93^{a}

The results indicate the mean±standard deviation. Variations in notation within the same row represent statistically significant differences ($P<0.05$).

The moisture and ash content of this study were in accordance with comparable research on khakra products (crispbread) from India. The moisture and ash content of multigrain khakra products were 3.6% and 3.6%, respectively. In contrast, the lipid and protein contents documented in this investigation showed inversely proportional to that of multigrain khakra. The lipid and protein content of multigrain khakra were determined to be lower, at 1.6% and 10.1%, respectively (Chauhan et al., 2017). Consequently, the crispbread in this study can be considered to have a higher protein content than the multigrain khakra used in previous studies. Regrettably, the protein content of the F1 sample was lower than that of faba bean flour-supplemented crackers (22%) (Gangola et al., 2022).

3.4 Soluble protein

Analysis of soluble protein in crispbread is important because it reflects the degree of protein modification that occurs during processing, provides information on protein bioaccessibility and digestibility, and helps evaluate the influence of formulation and baking conditions on the nutritional quality and functional properties of the final product.

The soluble protein values in the crispbread samples exhibited a distinct trend from the total protein content, as illustrated in Figure 2. The total protein content of crispbread samples F2 (12.35%) and F3 (10.78%) was considerably higher than that of sample F0 (7.41%). Nevertheless, the soluble protein values in F2 (0.78%) and F3 (0.69%) were considerably lower than those in F0 (0.98%). Furthermore, the soluble protein in sample F0 did not exhibit any significant difference from that of sample F1 (0.91%).

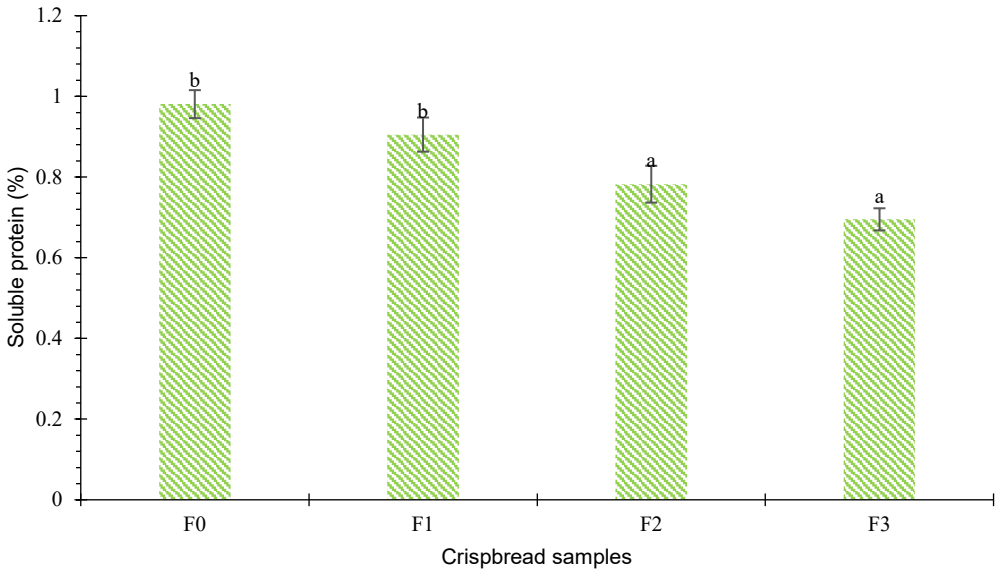


Figure 2. Soluble protein content of sorghum-based crispbread. Variations in notation represent a statistically significant difference ($P < 0.05$).

The elevated concentrations of soluble protein in samples F0 and F1 resulted from the soluble protein profile present in the raw materials. Sorghum and jack bean possess a greater proportion of soluble (polar) protein components, specifically globulin, in comparison to other protein fractions (Molina et al., 1974; Wahyuningsih et al., 2019). Consequently, F0 and F1 exhibited no discrepancies in soluble protein concentration. Conversely, samples F2 and F3, exhibiting a reduced jack bean ratio relative to F1, demonstrated diminished soluble protein levels. F2 exhibits no variations in soluble protein compared to F3.

Although the total protein content of crispbread formulations F2 (12.35%) and F3 (10.78%) were significantly higher than that of F0 (7.41%), the opposite trend was observed for soluble protein, where F2 (0.78%) and F3 (0.69%) showed lower values than F0 (0.98%). These results indicate that an increase in the amount of total protein is not always followed by an increase in the soluble protein fraction. The decrease in soluble protein in F2 and F3 was likely due to more intense interactions between protein and other food components, such as starch, dietary fiber, polyphenols, or antinutritional compounds, which can form insoluble protein complexes with protein during the mixing and baking processes (Ozdal et al., 2013; Shahidi & Dissanayaka, 2023). In addition, exposure to high temperatures during the baking process can cause protein denaturation and aggregation of protein molecules through the formation of hydrophobic bonds or disulfide bonds, thereby reducing protein solubility in the extraction medium (Batool et al., 2026; Günal-Köroğlu et al., 2023). This phenomenon indicates that some of the proteins contributing to the increase in total protein are in a denatured form or bound to the food matrix, and thus are not detected as soluble proteins (Cao et al., 2026). The absence of significant differences between F0 (0.98%) and F1 (0.91%) indicates that the level of substitution or addition of ingredients in F1 was not large enough to significantly change the protein structure or its interactions with the matrix components (Batool et al., 2026; Koh et al, 2026). Overall, these results indicate that formulations that increase total protein content can cause a decrease in protein solubility due to changes in protein structure and interactions during processing, which ultimately has the potential to affect the functional properties of the protein such as digestibility, emulsification ability, and water binding capacity, in the crispbread products.

3.5 Total starch and total dietary fiber

Figure 3 illustrates the total starch and dietary fiber composition of sorghum crispbread. Statistical analysis indicated that formula F3 had the greatest significant total starch concentration at 56.89% relative to the other samples. The quantitative examination of total starch revealed a continuous increase in total starch across samples, from F1 to F3, with F3 exhibiting the highest concentration of beetroot flour. F0 was determined to contain 44.87% total starch. F1 and F2 had total starch contents of 43.76% and 43.98%, respectively. The research results indicate that the higher the usage of sorghum flour and beet, the higher the total starch content in the sorghum crispbread. F0 exhibited the highest total starch content compared to F1 and F2 because F0 utilized 100% sorghum flour, whereas F1 and F2 used lower proportions of sorghum flour. On the other hand, the overall starch content in F3 exhibited a considerable increase. This outcome may be attributed to the impact of utilizing beetroot flour at the maximum proportion (36%).

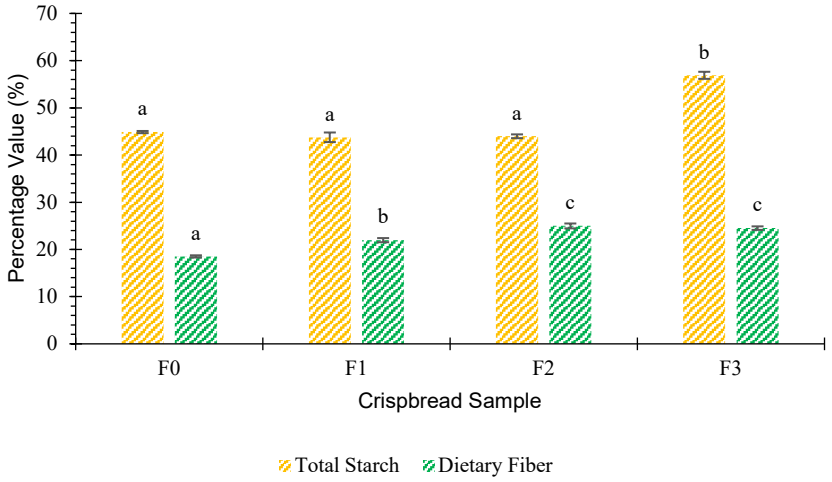


Figure 3. Total starch and dietary fiber content of sorghum-based crispbread. Variations in notation represent a statistically significant difference (P<0.05).

Sorghum was determined to possess 66.4% starch (von Schaumburg et al., 2021). The elevated starch content in sorghum flour accounts for the overall starch value in F0. Moreover, beetroot was determined to comprise 37.92% of the total carbohydrates (derived from the sum of all sugar types contained) and 1.82% starch (Andersson et al., 2022). Based on the starch analysis performed in this study, the starch content value was derived from the carbohydrate hydrolysis process, which generates simple sugars, and determined by converting the glucose value from the hydrolysis into starch. Consequently, the elevated total starch in F3 may have been affected by the inclusion of starch and starch hydrolysate in beetroot flour.

Total dietary fiber exhibited a comparable trend to total starch (Figure 3). A greater content of beetroot flour resulted in an increased dietary fiber concentration. F0 exhibited the lowest significant dietary fiber level at 18.53%, succeeded by F1 at 21.97% and F2 at 24.99%. The sorghum crispbread F2 exhibited no significant difference compared to F3 (24.52%). This is believed to be affected by the dietary fiber constituents present in beetroot. Beetroot has been documented to comprise 27.52% β-glucan, a soluble dietary fiber, and 8.58% non-starch polysaccharides (Andersson et al., 2022).

3.6. Physical properties color of sorghum crispbread

Color significantly impacts consumer food selections and directly affects product marketing. The color of food goods is determined by the materials incorporated in their formulations. The color attributes of sorghum crispbread products are presented in Table 5. The L* value denotes the brightness level of the sample. F0 had the greatest significant L* value (36.13) in comparison to the others. These results are confirmed in Figure 1. The brightness level of the sample was inversely related to the rising concentration of beetroot flour employed. An increase in beetroot flour usage correlated with a decrease in the resultant L* value. F3 exhibited the lowest significant L* value of 27.73. The findings of this study aligned with prior research (Herawati et al., 2021), which indicated that including beetroot in the making of arrowroot noodles diminished the L* value of the noodle product.

Table 5. Color properties of sorghum-based crispbread

Sample	Color		
	L*	a*	b*
F0	36.13±1.46^{c}	9.54±1.51^{a}	14.11±1.21^{c}
F1	31.54±0.14^{b}	18.67±0.08^{b}	9.28±0.75^{b}
F2	28.18±0.99^{b}	17.53±0.13^{b}	3.83±0.16^{a}
F3	27.73±0.07^{a}	16.95±0.11^{b}	3.48±0.15^{a}

The results indicate the mean ± standard deviation. Variations in notation within the same row represent a statistically significant difference ($P<0.05$).

The elevated L* value in F0 was affected by the incorporation of white sorghum flour in the study presented. White sorghum flour lacks anthocyanin colors, in contrast to red sorghum variants (Brantsen et al., 2021). Despite F0 possessing the greatest L* value, it remained well below 100 (lightness), signifying the product's brightness level. This is due to the light brown hue of the pericarp and endosperm in white sorghum flour. Consequently, F0 exhibits a L* value that was nearer to a score of 0 (darkness) than to a score of 100 (Adzqia et al., 2023). The presence of tannin and polyphenol oxidase activity in sorghum can induce enzymatic browning, hence influencing the color of sorghum flour (Brantsen et al., 2021). The color of sorghum is frequently evaluated qualitatively to estimate the amount of tannin and anthocyanin chemicals in sorghum and its derivatives (Boren & Waniska, 1992).

The a* value denotes the intensity of the red hue in the tested product. In principle, augmenting the concentration of beetroot in the creation of sorghum crispbread enhances the a* value. In actuality, though, the outcomes were the opposite. Despite the absence of a statistically significant difference among F1, F2, and F3, the F3 sample exhibited the lowest a value. This phenomenon is believed to result from the Maillard reaction between the sugar constituents in beetroot and the proteins present in the other ingredients (Račkauskiene et al., 2015). Figure 1 corroborates this argument, indicating that F3 presented a darker color impression than F1. The b* number denotes the strength of the yellow hue. F0 possesses the most significant b* value. This may happen due to the F0 formulation being composed entirely of white sorghum. Certain researches have indicated that white sorghum possessed carotenoid pigments that may enhance the intensity of the yellow hue (b value) (Kean et al., 2011; McDowell et al., 2024).

3.7 Texture profile of sorghum crispbread

The textural characteristics of sorghum-based crispbread are presented in Table 6. Hardness is defined as the maximum resistance force during the initial compression cycle, serving as an indicator of sample strength (Gravelle et al., 2017). The maximum hardness value (30.81 N) was observed in the sorghum crispbread formulation F0. Conversely, sample F3 exhibited the lowest hardness value of 17.13 N. The elevated hardness value in F0 is attributable to the incorporation of 100% sorghum flour in the crispbread formulation. The findings of this study are aligned with those of research on gluten-free bread. The hardness value recorded in that study increased with percentage of sorghum flour (Adzqia et al., 2023). The hardness of food products is affected by various factors, including flour type, dough formulation, processing method, and storage conditions. White sorghum variants have been documented to possess a tougher endosperm in comparison

to red sorghum (de Oliveira et al., 2022; Omer et al., 2023). Sorghum is also a gluten-free grain. This feature enhances the toughness of items derived from sorghum flour (Chavan et al., 2013). Similar results were also found in a study of crispbread making with linseed and beetroot. As the concentration of beetroot flour increased, the hardness value decreased. This was thought to be due to increased product porosity at higher beetroot flour concentrations. Another possible explanation was the low gelling agent formation, resulting in a less compact product structure (Niemira & Galus, 2024).

Table 6. Texture properties of sorghum-based crispbread

Sample	Hardness (N)	Cohesiveness	Adhesiveness (Nmm)	Fracture (N)	Crispness	Crunchiness (Nmm)
F0	30.81±1.12^{c}	0.34±0.15^{a}	0.01±0.01^{a}	7.70±0.71^{b}	107.79±1.08^{c}	16.79±0.99^{c}
F1	23.04±2.77^{b}	0.25±0.10^{a}	0.25±0.11^{ab}	12.03±1.27^{c}	117.39±1.41^{d}	12.68±0.10^{ab}
F2	23.87±0.85^{b}	0.69±0.00^{b}	3.15±0.42^{c}	4.51±0.51^{a}	95.76±0.85^{b}	13.30±0.99^{b}
F3	17.13±1.49^{a}	0.34±0.09^{a}	0.73±0.02^{b}	8.21±0.82^{b}	79.51±0.11^{a}	10.97±0.54^{a}

The results indicate the mean±standard deviation. Variations in notation within the same row represent statistically significant differences ($P<0.05$).

All samples exhibited poor cohesiveness and adhesiveness levels. Gluten is essential for cohesion and adhesion. The gluten protein found in wheat imparts elasticity and density to bread dough. Foods that are non-gluten and high in starch influence poor cohesiveness and adhesiveness levels (Onyango et al., 2011). Gluten-free crispbread composed of sorghum flour, jack bean flour, and beetroot lacks of gluten, leading to low cohesiveness and adhesiveness values (Gebremedihin & Abera, 2024; Paula & Conti-Silva, 2014; Rutyna & Galus, 2025). The results of this study align with those of several previous studies on the use of gluten-free ingredients in bakery products. Crispbread made from carrot pomace and chia seeds showed low cohesiveness and adhesiveness, possibly due to the product's predominantly starch and fiber structure and lacked of a gluten network (Rutyna & Galus, 2025). Onyango et al. (2011) showed that sorghum bread using cassava starch had low adhesiveness values owing to the lack of gluten. Carrot pomace and beetroot-apple pomace pastes were also found to have low cohesiveness and adhesiveness values. Researchers explained that the low cohesiveness and adhesiveness values were due to the absence of gluten in both ingredients, resulting in a lack of cohesion and adhesion (Kultys & Moczkowska-Wyrwisz, 2022).

The fracture value is derived from the force (N) at the maximum peak necessary to rupture the sample when the texture analyzer probe is suddenly dropped (BahramParvar et al., 2014). The maximum value before fracture is believed to correlate with the porosity of a snack product, influencing its crispness and brittleness. Establishing a low fracture value is a preferred textural quality among consumers (Roopa et al., 2009). The sample F1 had the greatest fracture value (12.03 N) in this investigation. Jack bean flour is recognized for its higher starch content (30-40%) (Purwandari et al., 2023), hence enhancing the gelatinization characteristics of the product. The elevated gelatinization capability of the dough necessitates greater power to fracture or compress the final product (Adzqia et al., 2023). On the other hand, the greatest fracture value of F1 likely occurred because the presence of jack bean as a protein source facilitated the formation of a protein

network with sorghum starch. The other sample has low fracture value. This can be expected due to the use of gluten-free sources. Similar results were found in a study of sorghum bread. This study showed that gluten-free sorghum bread with the addition of various types of starch produced a crumb that was brittle and easily broken. The low fracture force was associated with weak protein-starch interactions due to the absence of gluten, which functions as a network builder (Onyango et al., 2011).

The incorporation of sugar-containing beetroot flour (Andersson et al., 2022) can induce a Maillard reaction (Račkauskiene et al., 2015) with sorghum and jack bean proteins during baking, hence inhibiting starch gelatinization. Gelatinization may also be inhibited by heightened interaction between starch and protein. This may yield a brittle product (Zhang et al., 2025). The dough's thickness during baking significantly affects the resultant fracture value. Sorghum-based crispbread is produced with consistent dimensions with a mold of 3 cm × 3 cm and a thickness of 1 mm. A thinner dough facilitates more thorough water evaporation during baking, leading to reduced water content in the final product. This results in a smooth, homogenous, and less dense inner cellular structure, yielding a product that is less susceptible to fracture under stress (Ibe et al., 2025).

Crispness is typically assessed by sensory evaluation. The auditory phenomenon produced by food when it deforms and fractures during consumption is regarded as a significant indicator of crispness perception (Sanz et al., 2007). While certain texture assessments are conducted visually and manually, the principal examination of crispness transpires in the oral cavity. The correlation between the mechanical attributes of food and the impression of crispness is the auditory signal generated during fracture: all crunchy foods produce sound (Varela et al., 2009). The quantity and amplitude of mechanical events in a force-deformation curve have been suggested as a suitable method for quantifying crispness. The frequency distribution obtained from these measurements is regarded as the mechanical hallmark of crispy foods (Sanz et al., 2007). Crispy foods are mechanically brittle, fracturing suddenly with minimal deformation when subjected to a minor force. This contrasts with non-crisp and hard meals, which necessitate higher power to fracture, bend, or deform prior to breaking. Crispness can be assessed by quantifying the number of peaks (fractures) within a specified timeframe. An increased frequency of fractures per second signifies greater crispness (Varela et al., 2009). The computed crispness value is exactly proportional to the crispness attribute.

Based on Table 6, the crispiness values of the sorghum-based crispbread samples ranged from 79.51 to 117.39, with all formulations showing significant differences ($P < 0.05$). Sample F1 had the highest crispiness value (117.39), followed by F0 (107.79), F2 (95.76), and F3 (79.51). The elevated crispness value was derived from the frequency of fracture peaks in the sample generated per second. Higher crispiness values indicated that the product generated more fracture events during chewing or instrumental testing, thus providing a stronger crunchy sensation. The higher crispiness values in F1 indicated that the formulation produced a more porous and brittle internal structure, making it susceptible to repeated fractures under pressure. Conversely, the decrease in crispiness values in F2 and especially F3 indicates that increasing the proportion of certain ingredients in the formulation resulted in a more compact and less brittle product structure, resulting in a decrease in the number of fractures during testing.

Differences in crispiness values between samples can be attributed to the composition of sorghum flour, jack bean flour, and beetroot, which influences the formation of the product's microstructure during the baking process. The higher protein and fiber content of legume and beetroot flours can increase the density of the product matrix and reduce the formation of air voids that contribute to crispness. Furthermore, dietary fiber has water-binding properties, which can influence starch gelatinization and structure formation

during baking. A denser, less porous structure generally results in lower crispiness values because the product is less prone to repeated fractures under mechanical stress (Pasqualone et al., 2020; Proserpio et al., 2020). Similar findings were reported by (Paula & Conti-Silva, 2014), who stated that the composition of raw materials in gluten-free bakery products significantly influences texture characteristics, including crispness and brittleness. Furthermore, changes in the flour-to-starch ratio in gluten-free sorghum-based products can affect crumb structure and the resulting crispness level (Onyango et al., 2011).

Compared to other texture parameters, crispiness values in this study showed a trend that was opposite to hardness values. Sample F3 had the lowest hardness (17.13 N) and the lowest crispiness (79.51), indicating that softer products are not necessarily crispier. This indicates that crispiness is more influenced by the number and distribution of fractures in the product structure than simply the level of hardness. According to (Varela & Fiszman, 2011), crispness in dried products is largely determined by the layered structure, porosity, low moisture content, and the structure's ability to produce numerous small cracks during deformation. Therefore, the differences in crispiness values in sorghum crispbread observed in this study stemmed from microstructural changes resulting from variations in the composition of sorghum, koro benguk, and beetroot flours, which affected pore formation, starch-protein distribution, and product breaking characteristics during texture testing.

Crunchiness is determined by multiplying the force necessary to crush the sample by the entire area (Varela et al., 2006). The crunchiness values of sorghum-based crispbreads in Table 6 ranged from 10.97 to 16.79 Nmm and showed significant differences between formulations ($P<0.05$). Sample F0 had the highest crunchiness value (16.79 Nmm), while F3 showed the lowest value (10.97 Nmm). The high crunchiness value in F0 indicated that the product required more energy to break during testing, reflecting a stiffer and crispier structure. Conversely, the decrease in crunchiness values in F1, F2, and especially F3 indicated that the addition of jack bean and beetroot flours resulted in a more brittle structure and less resistance to mechanical forces. This difference was likely due to changes in the interactions between starch, protein, and fiber during baking, which affected pore formation and the structural integrity of the product (Mohammed et al., 2012; Sabanis et al., 2009). The crunchiness characteristics of dry products are strongly influenced by the microstructure, porosity, and distribution of cracks formed during mechanical deformation (Posadzka-Siupik et al., 2025; Raczyk et al., 2022).

3.8 Principal component

To obtain a more comprehensive picture of the relationships between chemical, physical, and functional parameters in the experimental products, the raw data obtained were analyzed using Principal Component Analysis (PCA). This method was chosen because it can reduce the complexity of multivariate data into a number of more concise principal components without losing significant information. Through PCA, the relationships between variables and the grouping patterns of samples based on their characteristics can be visualized more clearly. Therefore, the PCA results are presented in the form of a PC1–PC2 biplot (Figure 4) to show the contribution of each variable to sample separation, while also serving as a basis for interpreting differences in nutritional profiles and textures between the tested formulations.

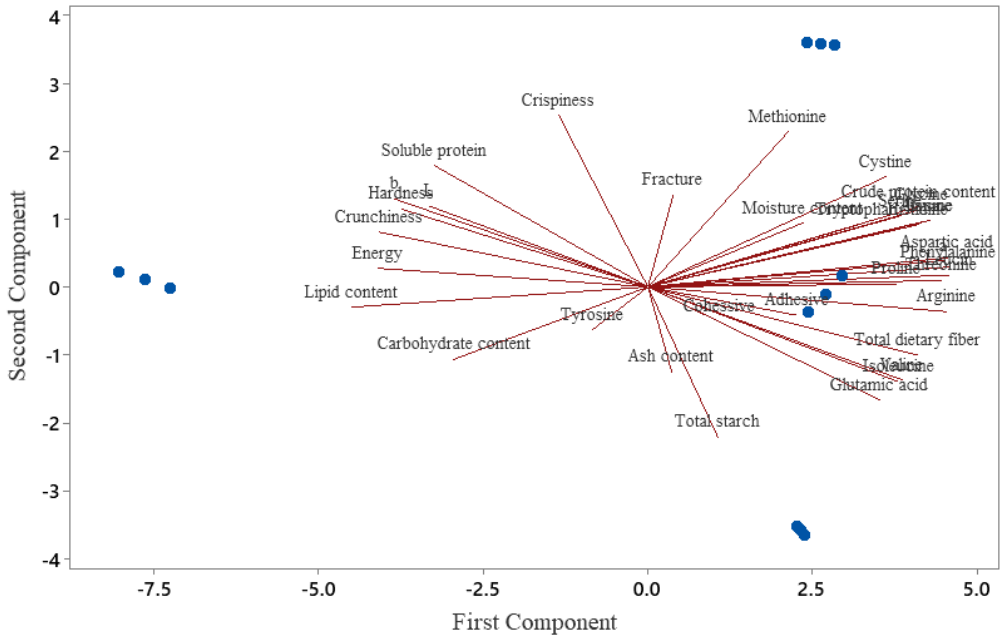


Figure 4. PCA biplot (PC1 vs PC2) of amino acid profile, nutrition, texture, and color properties of sorghum-based crispbread samples

The separation pattern in the biplot indicates that PC1 represents a nutritional gradient, depicting a shift from a high-carbohydrate and energy-based product toward one with higher protein, amino acid, and fiber contents. This was consistent with the raw data, which showed that formulations with added jack bean flours and beet flours had richer protein and amino acid profiles than sorghum-based controls. Conversely, samples with a higher proportion of sorghum tended to have higher carbohydrate and energy contents and a firmer texture. PC2 reflects differences in the physical-mechanical properties of the products, particularly related to friability and stickiness, which were influenced by the interactions between protein, starch, and water during the baking process. The angular relationships between the variable vectors in the biplot also indicated negative correlations between protein and carbohydrate, as well as between fiber and energy, confirming that increasing bioactive components led to a shift in overall product character. Thus, the PCA confirms that formulation modifications play a significant role in shaping the nutritional profile and texture of sorghum-based crispbreads.

4. Conclusions

The F1 sample (ratio formula of sorghum flour: jack bean flour: beetroot flour, 52:36:12) had the highest total protein (14.02%) and soluble protein levels (0.91%), accompanied by a low lipid content (14.96%). The highest total amino acid value (7622.37 mg/100g) was seen in the F3 sample (ratio formula of sorghum flour: jack bean flour: beetroot flour, 52:12:36). The highest concentration of essential amino acids (2790.15 mg/100g) was observed in F2 (ratio formula of sorghum flour: jack bean flour: beetroot flour, 52:24:24). The highest levels of dietary fiber (24.52%) and total starch (56.89%) were observed in F3.

F0 (100% sorghum) exhibited the highest brightness level (36.13). The highest intensity of red color was observed in F1 (18.67). F1 crispbread exhibited the maximum crispness value (117.39), accompanied by comparatively low hardness (23.04 N). PCA results confirmed that the composition of the ingredients plays a major role in determining the nutritional characteristics and texture of sorghum-based crispbread. The incorporation of jack bean flour and beetroot enhanced the chemical and physical qualities of sorghum-based crispbread. Moreover, the improved amino acid and dietary fiber composition suggests potential as a nutritionally enhanced snack.

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6. Authors' Contributions

All authors contributed equally to the main contributor to this paper. All authors read and approved the final paper. **Aprilia Fitriani**: Conceptualization; Methodology; Data curation; Writing - Original draft preparation; Funding acquisition. **Inasanti Pandan Wangi**: Visualization; Investigation. **Amalya Nurul Khairi**: Supervision; Data curation. **Helen Onyeaka**: Writing (review & editing), writing (original draft), investigation. **Putri Adelia Febriyanti**: Data curation; Formal analysis, Writing - Original draft preparation. **Chika Amelia Pratama**: Formal analysis; Data Curation; Project administration.

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7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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