



Efect of mocaf-soybean flour ratio on nutritional composition and antioxidant activity of gluten free snack bars

Pengaruh proporsi tepung mocaf dan tepung kedelai terhadap komposisi gizi dan aktivitas antioksidan pada snack bar bebas gluten

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Abstract

Innovations in gluten-free snacks based on local ingredients, such as fermented mokaf and soy flour, have the potential to produce healthier functional foods and support dietary diversity. This study aimed to assess the effects of varying mokaf-soy flour ratios on the nutritional profile and antioxidant capacity of snacks. Methods: This study used a completely randomized design with six formulations (100:0 to 0:100) and three replicates. The study was conducted at the Food Chemistry and Analysis Laboratory and Agricultural Product Processing Technology Laboratory, Muhammadiyah University Gresik, from November 2025 to January 2026. The parameters analyzed included moisture, ash, protein, fat, carbohydrate, calcium, iron, total phenolic contents, and antioxidant activity using the DPPH method. Data were analyzed using ANOVA and Tukey's post hoc test. The results showed that an increase in the proportion of soybean flour reduced the moisture (4.45% to 2.92%) and carbohydrate (78.45% to 69.80%) (p<0.05) contents. The protein content increased from 5.36% to 23.59%, calcium from 227 to 350 mg/100 g, and iron from 34.8 to 44.8 mg/100 g. The total phenolic content increased and the IC₅₀ value decreased, indicating stronger antioxidant activity. In conclusion, soy flour substitution improved the nutritional quality and antioxidant potential of the snacks. A mixed formulation is recommended to balance functional value and consumer acceptance, accompanied by further evaluation of its stability and sensory quality.

Keywords: Antioxidant activity, composite flour, functional food

Abstrak

Inovasi camilan berbasis bahan lokal bebas gluten seperti mocaf fermentasi dan tepung kedelai berpotensi menghasilkan pangan fungsional yang lebih sehat serta mendukung keragaman diet. Penelitian bertujuan untuk menilai pengaruh variasi rasio mocaf-tepung kedelai terhadap profil gizi dan kapasitas antioksidan camilan. Metode, penelitian menggunakan rancangan acak lengkap dengan enam formulasi (100:0 hingga 0:100) dan tiga ulangan. Studi dilaksanakan di Laboratorium Kimia dan Analisis Pangan serta Laboratorium Teknologi Pengolahan Produk Pertanian, Universitas Muhammadiyah Gresik, November 2025–Januari 2026. Parameter yang dianalisis meliputi kadar air, abu, protein, lemak, karbohidrat, kalsium, besi, total fenolik, dan aktivitas antioksidan metode DPPH. Data dianalisis menggunakan ANOVA dan uji lanjut Tukey. Hasil, peningkatan proporsi tepung kedelai menurunkan kadar air (4,45% menjadi 2,92%) dan karbohidrat (78,45% menjadi 69,80%) (p<0,05). Protein meningkat dari 5,36% menjadi 23,59%, kalsium dari 227 menjadi 350 mg/100 g, dan besi dari 34,8 menjadi 44,8 mg/100 g. Total fenolik meningkat dan nilai IC₅₀ menurun, menunjukkan aktivitas antioksidan lebih kuat. Kesimpulan, substitusi

tepung kedelai meningkatkan mutu gizi dan potensi antioksidan camilan. Formulasi campuran direkomendasikan untuk menyeimbangkan nilai fungsional dan penerimaan konsumen, disertai evaluasi stabilitas dan mutu sensori lanjutan.

Kata Kunci: Aktivitas antioksidan, tepung komposit, pangan fungsional

Introduction

Snack bars are becoming increasingly popular as a convenient and healthy solution for people living in cities who want both in one product (Kowalska et al., 2022). Using local raw materials to make new snack bars helps diversify the food supply, secure nutrition, and stabilize the economy (Wandeka et al., 2022). Fermented cassava flour (mocaf), extensively used in gluten-free applications, has enhanced texture, digestibility, and prebiotic components compared to native cassava flour (Monthe et al., 2018). Soybean flour is a significant source of plant-based protein, calcium, and iron, which are necessary for resolving protein-energy malnutrition, typically reported in underdeveloped nations (Messina, 2022). Combining mocaf and soybean flour in snack bars may result in an increased protein profile while maintaining desirable sensory qualities such as crispness and a neutral flavor (Zulaikha et al., 2021). This strategy satisfies the growing need for affordable functional meals that fulfill international safety and quality standards in the food industry.

Using indigenous gluten-free foods such as fermented mocaf and soybean flour to make new snacks is a good way to help with Indonesia's nutritional difficulties. In 2018, it was estimated that 30.8% of Indonesian children under five years of age were stunted. Anemia is still present in more than 20% of the population (Kementerian Kesehatan RI, 2019). Fermentation of cassava flour greatly eliminates anti-nutritional components, boosts resistant starch content, and promotes the growth of beneficial lactic acid bacteria (Dou et al., 2023). Such processing not only modifies the nutritional composition but also boosts the carbohydrate complexity and antioxidant profile of snacks (Galanakis, 2021). Soybean flour provides greater mineral concentrations and improved amino acid balance, making it suitable for school feeding and sports nutrition applications (Rizzo & Baroni, 2018). The adoption of mocaf in snack bar formulations also responds to the increasing prevalence of gluten-related intolerance among

Asian populations (Dharani & Sindhu, 2024). Snack bars produced using local components have exhibited promising sensory acceptance, notably in terms of color, flavor, and texture, as evaluated using standard hedonic tests (Haş et al., 2023).

Thermal processing, such as baking or roasting, can increase flavor development, regulate mouthfeel, control moisture content, and improve shelf-life stability (S. Liu et al., 2022). Several studies have demonstrated that optimal roasting settings can increase the phenolic content and promote antioxidant activity, hence supporting the preventive health benefits for frequent consumers (Liaquat et al., 2022). However, a crucial problem is the optimization of the quantities of mocaf and soybean flour to achieve a desirable texture while limiting the overwhelming soybean flavor (Hariadi et al., 2023). Moisture content is crucial for product stability, and ash and mineral tests are necessary to guarantee compliance with food safety regulations (Ajao et al., 2025).

Modern studies have utilized spectrophotometry, atomic absorption spectroscopy (AAS), and principal component analysis (PCA) to accurately and consistently evaluate the proximate composition, mineral content, and antioxidant characteristics of substances (Noh et al., 2020). Multivariate data analysis, including hierarchical cluster analysis (HCA), assists in identifying ingredient ratios that optimally meet the nutritional and sensory requirements (Kharbach et al., 2023). Snack bars made with mocaf and soybean flour might also be helpful in emergency nutrition programs because they do not spoil soon, provide a lot of energy, and use ingredients from the area (Rahmawati & Ismawanti, 2023). Over the next ten years, the demand for high-protein, gluten-free snack bars in the industrial sector is predicted to increase. This is because of changing consumer tastes and public health policy requirements (Boukid et al., 2022).

Therefore, to speed up the development of new snack bar items for both domestic and export markets, it is important to systematically study composite flour ratios, undertake thorough

chemical and sensory tests, and compare the results to international standards (Kumar et al., 2021). This report provides actionable recommendations for producers, dietitians, and legislators to foster healthy food environments and robust, sustainable supply chains. This study introduces an innovative approach to creating functional snack bars using local resources by combining fermented cassava flour (mocaf) and soybean flour through a systematic formulation ratio methodology. Unlike prior studies that typically investigate mocaf or soybean flour in isolation, this study thoroughly examined the effects of altering the proportions of both ingredients on the nutritional value, mineral content (specifically calcium and iron), total phenolic compounds, and antioxidant capacity of the resultant product.

This study also shows the collaborative effect between the mocaf fermentation process and soybean bioactive components that improve the functional properties of the snack bar after its baking. The main objectives were to assess how the ratio of mocaf and soybean flour affects the nutritional profile (ash, moisture, protein, fat, carbohydrate, iron, and calcium), total phenolics, and antioxidant activity of the snack bar, and to find the best formulation that can be used as a functional food, gluten-free, and utilizes local resources. The hypothesis of this study is that there is no difference in the proportion of mocaf and soybean flours in snack bar products in terms of moisture, ash, protein, carbohydrate, fat, calcium, iron, total phenolics, and antioxidant activities.

Table 1. Formulation of snack bar

Ingredient	F1	F2	F3	F4	F5	F6
Mocaf (g)	50	40	30	20	10	0
Soybean flour (g)	0	10	20	30	40	50
Sugar (g)	20	20	20	20	20	20
Margarine (g)	15	15	15	15	15	15
Milk powder (g)	10	10	10	10	10	10
Egg (g)	10	10	10	10	10	10
Baking powder (g)	1	1	1	1	1	1
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5

Information: F1(100:0), F2 (80:20), F3 (60:40), F4 (40:60), F5 (20:80) and F6 (0:100)

Table 1 presents the full formulas. The total batch weight of each formulation was approximately 106.5 g. We chose six different combinations of mocaf and soy flour (100:0 to 0:100) to determine the effect of replacing one with the other on the nutritional and functional

Methods

Research Etichs

This study adhered to the principles of research integrity and scientific ethics of the institution. This study did not require ethical approval as it did not involve human beings or experimental animals. All analytical methods used in the laboratory were standard and verified to ensure that the data were accurate, reproducible, and reliable.

Data collection, analysis, and sharing were performed honestly and openly, with no cheating, fabrication of information, or stealing of ideas. The authors declare no conflict of interest in this research, and all sources are properly mentioned.

Experimental Design

The research utilized a Completely Randomized Design (CRD) featuring six formulation treatments with different ratios of mocaf to soybean flour: 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. To ensure that the results were statistically valid, each treatment was performed and examined three times. These three repetitions were obtained based on the calculation formula for finding repetitions, namely $t(n-1) \geq 15$, where t is the number of treatments, which is 6. From the calculation results, the repetitions were obtained as many as three repetitions. Each formulation was manufactured three times in a row so that samples could be taken and analyzed based on the parameters mentioned above. The analysis showed that the right amount was met.

qualities of the snack bar. We utilized a 20% interval to observe changes in composition systematically and ensure that they were statistically significant with ANOVA. The extreme formulations (100% mocaf and 100% soy) were used as references to define the

carbohydrate-dominated and protein-mineral-dominated profiles. The mixed formulations helped identify transition patterns and possible synergies between the constituents. The goal of this research was to find the best mix that boosts nutritional content and antioxidant activity and is still useful for functional food products made from local resources.

Instruments and Equipment

The instruments utilized in this study comprised an analytical balance with 0.001 g precision, a temperature-controlled baking oven operating at 160 °C, a drying oven, a Kjeldahl apparatus, a Soxhlet extraction unit, an Atomic Absorption Spectrophotometer (AAS), a UV-Vis spectrophotometer, as well as a blender, mixer, beaker glass (Duran), Erlenmeyer flask (Iwaki), volumetric flask (Pyrex), and volume pipette (Pyrex).

Materials

The ingredients used were fermented cassava flour (Mocaf), soybean flour, sugar, margarine, milk powder, eggs, baking powder, and salt. For chemical tests, we used analytical-grade reagents such as Folin-Ciocalteu reagent, gallic acid standard, aluminum chloride (AlCl_3), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), and potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$).

Snack Bar Preparation

The dry ingredients (mocaf, soybean flour, sugar, milk powder, baking powder, and salt) were sifted and combined until they were homogeneous. Margarine and eggs were mixed separately until they were all the same, and then they were added to the dry mixture to make a dough that was all the same. The dough was rolled into bars of the same size (approximately $5 \times 2 \times 1$ cm). The bars were baked in a preheated oven for 20 min at 160 °C. The snack bars were placed in airtight containers and kept at room temperature until they were ready to be tested.

Analytical Parameters

The physicochemical, nutritional, and functional qualities of the snack bars were evaluated. Oven drying was used to determine the moisture content (Mauer, 2024). The Kjeldahl method was used to determine the protein content of the food, using a nitrogen-to-protein conversion

factor of 6.25 (Rizvi et al., 2022), and Soxhlet extraction was used to assess the fat content (Nisca & Tanase, 2025). The ash content was obtained by burning at 550 °C (Rahmawati & Ismawanti, 2023), and the amount of carbohydrates was determined by differences (Meyer et al., 2022).

Mineral analysis involved the quantification of calcium and iron concentrations by atomic absorption spectrophotometry with a wavelength of 248.3 nm for iron (with Fe standard) and 285.2 nm for magnesium (standard Mg) (Igbocheonwu et al., 2024).

We used the Folin-Ciocalteu technique to determine the total phenolic content and reported the results as mg gallic acid equivalents (GAE)/g sample. For phenolic analysis, the snack bar was ground and macerated for 8 h using methanol P.A and evaporated using a rotary evaporator, and then the thick extract was analyzed. The DPPH radical scavenging assay was used to measure the antioxidant activity, and the results are shown as IC_{50} values (Prayitno & Rahim, 2021).

Statistical Analysis

All data are presented as mean \pm standard deviation. One-way analysis of variance (ANOVA) was used to check for statistical significance, and Tukey's post hoc test was used at a significance level of $p < 0.05$. We used SPSS version 26.0 for statistical analysis.

Result and Discussion

The results of this study provide a comprehensive overview of the nutritional and functional qualities of snack bars made with different amounts of fermented cassava and soybean flours. We examined the proximate composition, mineral enrichment, total phenolic content (TPC), and antioxidant activity to determine how different amounts of substitution affect the quality of the product. Statistical analysis showed that the treatments were significantly different from each other ($p < 0.05$). This indicates that adding soybean flour not only alters the balance of macronutrients but also improves the functional qualities of the snack bars. These results highlight the importance of mixing mocaf with soybean flour to produce nutrient-rich foods that fulfill the needs of today's consumers.

Different treatments showed that mocaf and soybean flour work together in ways that go beyond nutrition. Mocaf is a gluten-free, carbohydrate-rich base that is easier to digest because it has been fermented. Soybean flour, on the other hand, delivers important proteins, minerals, and bioactive components. The way these nutrients work together creates different levels of hydration, proteins, minerals, and antioxidants. These changes will not only make cassava-based snacks healthier but also help address public health issues, including protein-energy malnutrition and vitamin deficiencies.

The results are compared with those of earlier investigations in the next sections to provide a scientific context and support. We examined each parameter, including moisture, ash, protein, carbohydrate, fat, calcium, iron, total phenolics, and antioxidant activity, to determine how changes in composition affect the functionality and quality aspects that matter to consumers, such as stability, texture, and bioactive potential. Overall, mocaf-soybean snack bars are marketed as functional foods that are both healthy and nutritious. They also stressed the need to determine the optimal flour ratios to obtain the best nutrition and taste. Table 2 presents a summary of the outcomes.

Moisture Content

The moisture content of snack bars is important because it affects their shelf life, texture, and stability against bacteria. The moisture content decreased dramatically as the fraction of soybean flour increased, dropping from 4.45%

in the 100% mocaf formulation to 2.92% in the 100% soybean formulation.

From a technological perspective, mocaf, which is rich in starch, can hold air and turns into a gel when baked, which means it holds more air. Soybean flour, on the other hand, is high in protein and fat but doesn't hold air as well, therefore the final product contains less water (Hapsari et al., 2022). However, if there is not much water, it can be hard and brittle. Therefore, changing the ratio of mocaf to soybeans is important for finding a balance between storage stability and taste.

This pattern is consistent with earlier research indicating a lower water-holding ability in protein-rich flours compared to carbohydrate-rich flours such as cassava (Ronie & Hasmadi Mamat, 2023). The decrease in moisture is due to the fact that soybean proteins and lipids are less hygroscopic, which means they hold less free water in the product matrix. For shelf stability, it is best to keep moisture levels under control. Excessive moisture can cause microbes to develop and lipids to oxidize (Mora & Pegg, 2025). However, insufficient moisture may render the textures excessively dry or brittle. This suggests that mixing mocaf and soybean flour may be the best way to achieve the right balance between shelf life and consumer acceptance.

Ash Content

The amount of ash in food indicates the mineral content and is a good way to determine its healthiness. The ash content increased from 2.03% in the 100% mocaf formulation to 2.90% in the 100% soybean formulation.

Table 2. Nutritional and antioxidant analysis of snack bar formulations (Mean \pm SD)

Parameter	F1 (100:0)	F2 (80:20)	F3 (60:40)	F4 (40:60)	F5 (20:80)	F6 (0:100)	p-value
Moisture (%)	4.45 \pm 0.12 ^a	3.84 \pm 0.09 ^b	3.61 \pm 0.08 ^c	3.44 \pm 0.10 ^d	3.22 \pm 0.07 ^e	2.92 \pm 0.06 ^f	<0.001
Ash (%)	2.03 \pm 0.04 ^f	2.25 \pm 0.05 ^e	2.41 \pm 0.06 ^d	2.60 \pm 0.07 ^c	2.75 \pm 0.05 ^b	2.90 \pm 0.05 ^a	<0.001
Protein (%)	5.36 \pm 0.11 ^f	7.85 \pm 0.12 ^e	10.45 \pm 0.14 ^d	13.29 \pm 0.18 ^c	17.29 \pm 0.20 ^b	23.59 \pm 0.25 ^a	<0.001
Carbohydrate (%)	78.45 \pm 0.96 ^a	80.12 \pm 0.88 ^a	75.30 \pm 0.74 ^b	73.20 \pm 0.79 ^b	71.10 \pm 0.85 ^c	69.80 \pm 0.67 ^c	<0.001
Fat (%)	5.00 \pm 0.15 ^f	5.20 \pm 0.12 ^e	5.50 \pm 0.14 ^d	5.70 \pm 0.11 ^c	6.10 \pm 0.10 ^b	6.40 \pm 0.09 ^a	<0.001
Calcium (mg/100 g)	227 \pm 8.5 ^f	250 \pm 9.2 ^e	270 \pm 8.4 ^d	289 \pm 7.8 ^c	305 \pm 8.3 ^b	350 \pm 7.6 ^a	<0.001
Iron (mg/100 g)	34.8 \pm 1.5 ^e	36.1 \pm 1.2 ^f	39.2 \pm 1.7 ^d	41.2 \pm 1.9 ^c	43.7 \pm 1.5 ^b	44.8 \pm 1.3 ^a	<0.001
Total Phenolics (mg GAE/g)	0.97 \pm 0.03 ^f	1.00 \pm 0.04 ^e	1.09 \pm 0.05 ^d	1.15 \pm 0.03 ^c	1.20 \pm 0.04 ^b	1.25 \pm 0.02 ^a	<0.001
Antioxidant Activity (IC ₅₀ , ppm)	313.73 \pm 0.20 ^a	248.66 \pm 1.00 ^b	247.45 \pm 1.10 ^c	211.70 \pm 0.20 ^d	211.10 \pm 0.10 ^e	192.70 \pm 0.05 ^f	<0.001

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

The ash content indicates the number of minerals in the product that affect its functional features, such as the stability of the matrix

structure, its reaction with protein and starch, and its browning without enzymes during baking (Mangabarani et al., 2023). The

product's color may darken slightly, and the flavor may change because of the inorganic substances present (Rosania et al., 2022). In addition, the mineral content of the dough might change its buffering capability and chemical stability during processing and storage.

This indicates that soybean flour has a higher mineral density. Soybean flour is recognized to contain many important macro- and micronutrients, such as calcium, magnesium, phosphorus, and iron (Bagale, 2021). Fermenting cassava flour may increase the bioavailability of minerals by breaking down antinutritional elements, such as phytates (Terefe et al., 2022). Higher ash content can make food more nutritious, but it can also change its appearance and taste, which shows how important it is to have balanced recipes.

Protein Content

As the amount of soybean flour increased, the amount of protein in the product increased significantly, from 5.36% in the 100% mocaf formulation to 23.59% in the 100% soybean formulation.

The increase in protein and soy flour content suggests that soy is a good source of vegetable protein that can improve the structure and quality of bread products (Amartia et al., 2025). During baking, proteins from soybeans interact with other proteins and starch to form a matrix network. This alters the texture, elasticity, and water-holding capacity of the product. In addition, increasing protein levels can help with emulsification and dough stability, which is very important for semi-dry foods, such as snack bars. However, excessive protein might change the color because of the Maillard reaction and make the texture thicker.

The high protein content and full set of essential amino acids in soybean flour caused this significant increase (Inyang et al., 2019). This type of enrichment is also useful for producing functional snack bars that help with protein-energy deficiency. The combination of mocaf and soybean flour not only increased the total protein content, but it may also make protein easier to digest by lowering the levels of antinutritional chemicals through fermentation. However, too much soybean protein may make the food taste and feel bad; therefore, it is better to use intermediate amounts of substitution to find a balance between nutrition and taste.

Carbohydrate Content

The more soybean flour was used instead of MOCAF, the lower the carbohydrate content. For example, pure mocaf bars contained 78.45% carbohydrates, whereas pure soybean bars contained 69.80%. This drop was due to the switch from cassava flour, which is high in starch, to soybean flour, which is high in protein and fat. Lower carbohydrate content may decrease the glycemic response and enhance metabolic compatibility, especially in patients with insulin resistance (Barazzoni et al., 2017).

The carbohydrate content decreased when mocaf starch was replaced with soybean protein and fat in the dough system. Starch is important for the gelatinization process that occurs during baking, as it creates the pore structure and crispy texture of snack bars. When the amount of starch decreases, the ability to make gelatin-based structures likewise decreases. This makes the soy protein network have a larger effect on the product matrix. This can change the density, cohesiveness, and crumbly nature of the result. Other studies (Puspaningtyas et al., 2022) have also shown that reduced carbohydrate levels can lower the glycemic index and change the product's energy profile. However, carbohydrates, especially starch, are very important for determining the texture of baked goods by gelatinizing them. Therefore, reducing carbohydrates too much may make things less crisp and less stable, which shows how important it is to get the mocaf-soybean ratio just right.

Although substituting soybean flour generally reduced the amount of carbohydrates, F2 (80:20) had a somewhat higher carbohydrate content (80.12%) than F1 (78.45%). This increase was not statistically significant ($p < 0.05$) and may be caused by the calculation method for carbohydrate-by-difference, which means that as the moisture content declines, the computed carbohydrate fraction increases. There was a clear downward trend at higher substitution levels ($\geq 40\%$), which showed that increasing the quantity of soybeans substantially decreased the carbohydrate content.

Fat Content

With more soybean flour added, the fat content increased from 5.00% to 6.40%, which is a small but important increase. This rise is because soybeans flour naturally have lipids that are high

in unsaturated fatty acids that are good for heart health (Petersen et al., 2024).

The fat content is probably due to the fact that some raw materials, such as soy flour or peanut parts, have a greater natural fat content. In these cases, the lipid fraction is primarily composed of unsaturated fatty acids. Fat in the snack bar matrix is vital for the structure because it affects the density, fragility, and mouthfeel of the food by interacting with carbohydrates and proteins and acting as a lubricant. A study Tanudjaja et al. (2026) indicated that the incorporation of fat into the snack bar matrix significantly influences texture formation via the lubrication effect mechanism and its interaction with starch and protein, thereby affecting the product's density, fragility, and mouthfeel.

Fat is important for flavor and texture, but excessive fat can shorten the shelf life of a product because it can oxidize. The fat levels observed were high enough to improve sensory quality without causing substantial stability problems.

Mineral Content: Calcium and Iron

With more soybean flour, the amounts of calcium and iron increased significantly. Calcium increased from 227 to 350 mg/100 g, whereas iron increased from 34.8 to 44.8 mg/100 g. Figure 6 demonstrates that the calcium content steadily increased. This indicates that the formulation composition significantly affects the mineral density of the product. This increase is probably due to the fact that some of the ingredients, such as soy flour or peanut-based binders, have a lot of calcium in them. Calcium is naturally present in protein and phytate matrices. Calcium can alter the stability and bioavailability of proteins and dietary fibers when they are mixed, heated, or fermented.

A review indicated that modest heat treatment typically does not harm minerals (Hartati et al., 2026). Calcium does not change significantly when heated, but anti-nutritional substances such as phytic acid can bind to Ca^{2+} ions, making it less available to the body (Ananta et al., 2025).

The iron content increased slowly over time. This indicates that changes to the recipe directly affected the micronutrient density of the snack bars. This increase is probably because there are more plant-based products that are high in iron, such as soy flour and legumes. In

these foods, iron is usually found in the non-heme form, which is attached to the protein matrix and fiber. This is confirmed by the fact that heating and mixing do not harm the minerals because Fe is stable at high temperatures. However, it is vital to consider how chemicals interact during processing and storage.

These minerals are important for maintaining bone health, facilitating oxygen transport, and ensuring proper brain function (Fekete et al., 2023). Plant-based minerals may not be as bioavailable because of phytates, although fermentation and heat processing can make minerals easier to obtain (Faizal et al., 2023). Therefore, mocaf-*soybean* snack bars could be quite useful as functional meals that are high in minerals.

The results of this study show that adding a lot of soy flour to these snack bars increased their calcium and iron content. However, an overall increase in mineral content does not necessarily correspond to an increase in the body's ability to absorb minerals. Soybeans and other plant-based foods contain substances that block nutrition, including phytic acid. This chemical binds to minerals with two charges, such as Ca^{2+} and $\text{Fe}^{2+}/\text{Fe}^{3+}$, and forms compounds that do not dissolve. These connections make it difficult for the body to absorb minerals in the small intestine. Therefore, even if calcium levels increased from 227 to 350 mg/100 g and iron levels increased from 34.8 to 44.8 mg/100 g, the body may not have absorbed as much as the chemical tests showed.

Some components of the snack bar in this study may change the bioavailability of minerals by mocaf fermentation, which uses microbial phytase enzymes to break down phytate and other antinutrients. In addition, roasting or heating can lower the action of several anti-nutrients, but not as much as fermentation. The Maillard reaction can also change the link between minerals and proteins, which can make them less available in some cases. In addition, interactions between the different foods in some formulations, especially F3-F5, may balance the high mineral content of soybeans and the lower phytate levels from mocaf fermentation. This could increase the bioavailability compared to formulations that only use soybeans.

Soybeans are a great source of protein and minerals, but they also contain many anti-nutrients, such as phytic acid, which can bind calcium, iron, and zinc. They also contain tannins, which can bind minerals and proteins, and trypsin inhibitors, which can stop enzymes that break down proteins. Saponins and other oligosaccharides can also alter the digestion of food. Heating and processing, especially prolonged roasting for a long time, lower the levels of these anti-nutrients.

Adding more soy flour can make the food more nutritious (more protein, calcium, iron, and phenolics), but using a lot of it (F6) can increase the likelihood of having antinutrient residues than other formulations. Therefore, intermediate formulations (such as F3–F5) would be the best choice because they can significantly increase protein and mineral levels, lower the chance of antinutrient dominance, and use the benefits of mocaf fermentation to make nutrients easier to obtain.

Total Phenolic Content (TPC)

The total phenolic content increased significantly with a higher proportion of soybean flour, from 0.97 mg GAE/g in 100% mocaf bars to 1.25 mg GAE/g in 100% soybean bars. Adding more ingredients to a formulation immediately increases the amount of bioactive compounds in the product. This increase is likely due to the use of phenolic-rich raw materials, such as soybeans and nuts. These materials contain isoflavones, phenolic acids, and flavonoids, which are natural antioxidants.

Heat treatment can break down phenolic compounds into smaller molecules. They also bind to proteins and polysaccharides through hydrogen interactions and covalent bonds (protein-phenol complexes), which can alter the values detected in tests. The increase in total phenols not only boosts antioxidant activity and the possibility of functional food claims but also helps the product remain stable by stopping lipid peroxidation.

This increase is due to the high levels of phenolic and isoflavone compounds in soybeans, such as genistein and daidzein, which are strong antioxidants (Liu et al., 2023). Fermentation of cassava may free up bound phenolics, but the total amount of phenolics is still lower than that in formulations that are high in soybeans. During baking, heat can break down phenolics, while

Maillard reaction products can add to the antioxidant activity of the bread.

Antioxidant Activity (IC₅₀)

As more soybean flour was added, the antioxidant activity, measured by IC₅₀ values, improved significantly. The IC₅₀ values decreased from 313.73 ppm in the 100% mocaf formulation to 192.70 ppm in the 100% soybean formulation.

This indicates that the antioxidant activity is stronger when there are more soybeans. A lower IC₅₀ value means that free radical scavenging is more effective. This improvement is strongly linked to the higher levels of phenolics and bioactive chemicals in soybean flour, especially isoflavones, which are good at donating hydrogen. Mocaf-based formulations demonstrated antioxidant activity through the release of phenolics during fermentation; however, soybean flour significantly enhanced the overall antioxidant capacity of the fermented product. Mixed formulations benefited from the synergistic interactions between fermented cassava components and soybean bioactive compounds, which led to gradually improved antioxidant profiles.

Soybean flour contains many bioactive chemicals, such as isoflavones (genistein and daidzein) and phenolics, that boost antioxidant activity (Grzymala & Amarowicz Ryszard, 2020). Other studies have shown that processing soybean flour (fermentation/tempeh) changes the isoflavone profile, boosting antioxidant and phenolic activity and making it better than starch flour (Chen et al., 2020). Other studies have shown that processing soybean flour (fermentation/tempeh) changes the isoflavone profile, boosting antioxidant and phenolic activity and making it better than starch flour (Schutte et al., 2024). Baked foods contain more phenolic and antioxidant compounds than raw foods. This is why snack bars offer more antioxidants, even though heat-sensitive phenolics break down (Mashau et al., 2020).

Fermentation is what makes mocaf work. It releases bound phenolics and strengthens the functional components (Khasanah et al., 2024). Mocaf has fewer antioxidant qualities than soybean flour because it is a main source of starch. This indicates that F1 (dominant mocaf) has the highest IC₅₀ (weakest antioxidant). Mixed

formulations (F3–F5) exhibited incremental antioxidant increase via synergy, utilizing mocaf as the fermented carbohydrate substrate and soybean flour as the phenolic/isoflavone source. The literature indicates that this augmentation of bioactive substances boosts DPPH antioxidant activity, correlating with a reduction in IC₅₀ and an elevation in total phenolic content (Casagrande et al., 2018).

Overall, these results show that replacing mocaf with soybean flour significantly improves the nutritional value and functional qualities of snack bars. Mixed formulations seem to offer the best compromise between health advantages and product quality.

Calorie calculation of snack bar products

The chemical energy obtained from essential nutrients, such as fat, protein, and carbohydrates, through metabolic processes is called calories, measured in kilocalories (kcal). This energy is required to support all movements and organ functions. The average daily energy requirement ranges from 2000 to 2500 kcal. Each gram of fat contributes 9 kcal/gram, whereas carbohydrates and proteins each contribute 4 kcal/gram. Based on the results of the snack bar product research, the calorie calculations are shown in Table 3.

Table 3. Calorie calculation of snack bar

Formulation	Calorie calculation			Total calorie (Kcal)
	4x Protein (Kcal)	4x Carbohydrate (Kcal)	9x Fat (Kcal)	
F1	21.44	313,80	45.00	380,24
F2	31.40	320,48	46,80	398,68
F3	41.80	301,20	49,50	392,50
F4	53.16	292,80	51.30	397,26
F5	69.16	284,40	54.90	408,46
F6	94.36	279,20	57.60	431,16

The energy from protein (4 kcal per gram) increased slowly, starting at 21.44 kcal in F1 and reaching a maximum of 94.36 kcal in F6. This means that the higher the protein content in the mixture, the more calories it adds to the total. This change had a significant impact on the total energy contribution of the F5 and F6 formulations. Simultaneously, the amount of energy provided by carbohydrates tended to decrease from 313.80 kcal in F1 to 279.20 kcal in F6. This drop suggests that some carbohydrate sources may have been replaced by foods with higher protein or fat content.

However, carbohydrates were the predominant energy source in each product design. The amount of energy from fat (9 kcal per gram) increased from 45.00 kcal in F1 to 57.60 kcal in F6. Fat has the most energy per gram; therefore, even a tiny increase in its amount can make a big difference in the total number of calories in a product. The total calorie increase from F1 to F6 illustrated that items with more protein and fat have higher energy density. F1 had the lowest total energy (380.24 kcal), whereas F6 had the highest (431.16 kcal). The difference is approximately 50–51 kcal, which is significant nutritionally, especially when it

comes to making functional meals or protein-enriched snack bars.

The table shows that changes in macronutrient composition directly affect the total number of calories in the snack bar. Carbohydrates provide the most energy, but adding more protein and fat also increases the total energy content. Therefore, the final formula needs to be suitable for the target customer, whether the product is strong in energy, high in protein, or requires a balanced mix of macronutrients.

This study had several limitations. First, the antioxidant evaluation was limited to the physicochemical composition, mineral content, total phenolic content, and antioxidant activity assessed using the DPPH method. Although DPPH is widely used, it reflects only one mechanism of antioxidant action and does not represent the biological activity *in vivo*. Therefore, additional assays such as ABTS, FRAP, or ORAC are recommended for a more comprehensive assessment. Second, sensory evaluation was not conducted, and increasing the proportion of soy flour may influence the taste, aroma, texture, and overall acceptability of the product. Third, shelf-life stability, including lipid oxidation and phenolic degradation during

storage, was not examined, despite the higher fat content of soy-based products, which may affect the oxidative stability. Finally, the mineral bioavailability was not directly measured. Although calcium and iron levels increased substantially, the presence of antinutritional factors, such as phytates, may influence their actual absorption and physiological utilization.

Conclusion

Modifying the ratio of mocaf to soybean flour significantly influenced the nutritional composition, mineral content, total phenolic content, and antioxidant activity of the developed gluten-free snack bars. Increasing the proportion of soybean flour enhanced the protein and mineral densities and strengthened the antioxidant capacity, reflecting the contribution of soybean-derived bioactive compounds. Mocaf also plays a complementary role, likely through fermentation-related mechanisms that support the release of functional components. Among the tested formulations, the 20:80 (mocaf:soybean) ratio provided the most balanced profile in terms of nutritional quality, antioxidant potential, and mineral bioavailability.

These findings support the potential use of soybean-enriched formulations for developing nutrient-dense, functional snack products based on locally available ingredients. From a practical perspective, food producers and small-scale industries should consider incorporating higher proportions of soybean flour to improve the functional and nutritional value of gluten-free products. Future research should evaluate sensory acceptability, storage stability, and mineral bioavailability, as well as optimize processing methods to reduce anti-nutritional factors while preserving antioxidant activity, thereby ensuring both health benefits and commercial feasibility.

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