



Characterization of nutritional composition and bioactive compound of cowpea tempeh with moringa leaf powder as a functional food candidate for type 2 diabetes mellitus

Karakterisasi komposisi gizi dan senyawa bioaktif tempe kacang tunggak dengan bubuk daun kelor sebagai kandidat pangan fungsional untuk diabetes melitus tipe 2

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder that requires effective management, including dietary interventions. Functional foods, such as tempeh, enriched with bioactive ingredients, including cowpea and moringa leaf powder, may contribute to blood glucose regulation based on their fiber and antioxidant composition. This study aimed to evaluate the nutritional and bioactive properties of tempeh made from cowpea and moringa leaf powder and determine the optimal formulation for managing type 2 diabetes. A laboratory-based experimental design was conducted from September 2024 at the Diet and Culinary Laboratory, Universitas Brawijaya, using four formulations (T0, T1, T2, and T3) with varying proportions of cowpea and moringa leaf powders. The study sample consisted of cowpea and moringa leaf powders as raw materials for tempeh production. The analyses were limited to the proximate nutritional composition and selected bioactive compounds (vitamin C, antioxidant activity, and anthocyanin content). The results indicated that The best formulation was T2 (94% cowpea: 6% moringa leaf powder), which exhibited a moisture content of 58.93%, ash content of 1.50%, protein content of 14.90%, fat content of 0.39%, carbohydrate content of 24.27%, energy of 160.25 kcal, starch content of 8.86%, total sugar content of 12.73%, iron content of 15.3, vitamin C of 8.66, and antioxidant activity with an IC₅₀ value of 48.77. However, anthocyanin content was not detected in this study. Statistically significant differences were observed in the ash ($p = 0.027$), fat ($p = 0.014$), iron ($p = 0.016$), and antioxidant activity ($p < 0.000$) content. In conclusion, T2 shows promise as a functional food for type 2 diabetes management.

Keywords: Type 2 Diabetes, Cowpea, Moringa Oleifera, Functional Food, Antioxidant Activity

Abstrak

Diabetes mellitus (DM) adalah gangguan metabolik kronis yang memerlukan manajemen yang tepat, termasuk intervensi diet. Pangan fungsional seperti tempe yang diperkaya dengan bahan bioaktif, termasuk kacang tunggak dan bubuk daun kelor, berpotensi berkontribusi terhadap pengaturan kadar glukosa darah berdasarkan kandungan serat dan antioksidan. Penelitian ini bertujuan untuk mengevaluasi kandungan gizi dan bioaktif tempe yang dibuat dari bubuk daun kelor dan kacang tunggak, serta untuk menentukan formulasi terbaik dalam pengelolaan diabetes tipe 2. Penelitian eksperimental laboratorium dilakukan pada September 2024 di Laboratorium Diet dan

Kuliner, Universitas Brawijaya, dengan menggunakan empat formulasi (T0, T1, T2, T3) dengan proporsi bubuk daun kelor dan kacang tunggak yang berbeda. Subjek penelitian adalah kacang tunggak dan bubuk daun kelor yang digunakan sebagai bahan baku pembuatan tempe. Analisis dibatasi pada komposisi gizi proksimat dan beberapa senyawa bioaktif, meliputi vitamin C, aktivitas antioksidan, dan kandungan antosianin.. Hasil penelitian menunjukkan bahwa formulasi terbaik adalah T2 (94% kacang tunggak : 6% bubuk daun kelor), dengan kadar air 58,93%, kadar abu 1,50%, protein 14,90%, lemak 0,39%, karbohidrat 24,27%, energi 160,25 kkal, pati 8,86%, gula total 12,73%, zat besi 15,3, vitamin C 8,66, serta aktivitas antioksidan dengan nilai IC₅₀ sebesar 48,77, sementara kandungan antosianin tidak terdeteksi. Perbedaan yang signifikan secara statistik ditemukan pada kadar abu ($p=0,027$), kadar lemak ($p=0,014$), kadar zat besi ($p=0,016$), dan aktivitas antioksidan ($p<0,001$). Formulasi T2 menunjukkan potensi sebagai pangan fungsional untuk pengelolaan diabetes tipe 2.

Kata Kunci: Diabetes tipe 2, Bubuk daun kelor, Kacang tunggak, Pangan fungsional, Aktivitas antioksidan

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired insulin secretion or action, which leads to abnormal glucose regulation. The global burden of DM has been continuously increasing and poses a major public health challenge. According to the International Diabetes Federation (IDF), in 2024, 588.7 million adults (aged 20–79 years) lived with diabetes worldwide, and this number is projected to reach 852.5 million by 2030 (International Diabetes Federation (IDF), 2025). In Indonesia, the prevalence of diabetes mellitus increased from 10.9% in 2018 to 11.7% in 2023, based on the Indonesian National Health Survey or *Survei Kesehatan Indonesia (SKI) 2023*, a nationally representative cross-sectional survey conducted by the Ministry of Health of the Republic of Indonesia using standardized household interviews and health measurements (Ministry of Health of the Republic of Indonesia, 2023). This upward trend highlights the urgent need for effective management strategies, particularly through dietary interventions involving foods with a low glycemic index and are rich in fiber, antioxidants, vitamins, and minerals (Indonesian Society of Endocrinology, 2019). Functional foods, such as tempeh, are promising options for managing type 2 diabetes.

Tempeh is a traditional Indonesian fermented food commonly produced from soybeans (*Glycine max*) using molds such as *Rhizopus oryzae* and *Rhizopus oligosporus*. During fermentation, these microorganisms

produce enzymes such as proteases, lipases, and amylases, which enhance the digestibility and bioavailability of nutrients (Kristiadi & Lunggani, 2022). In addition to being a source of plant-based protein, tempeh contains essential nutrients, including iron, folic acid, and B vitamins, which support metabolic health (Agustin, 2024). However, domestic soybean production does not meet national demand, leading to a heavy reliance on imported soybeans. This highlights the need to develop non-soybean tempeh products based on local raw materials, particularly those with the potential to provide a lower glycemic index and higher antioxidant capacity for the dietary management of type 2 diabetes mellitus. Cowpea and moringa leaf powders are promising alternatives owing to their nutritional value and bioactive compound content.

Cowpea (*Vigna unguiculata* L.) is a legume that provides high protein and energy content, making it suitable as a raw material for functional foods. One hundred grams of cowpea contains 331 kcal, 24.4 g of protein, 56.6 g of carbohydrates, 1.9 g of fat, and minerals such as iron (13.9 mg), calcium (481 mg), phosphorus (399 mg), and zinc (5.9 mg) (Wiantini et al., 2019). Moreover, cowpeas are rich in anthocyanins (27.8 mg/100 g) and exhibit vigorous antioxidant activity, with an IC₅₀ value of 1.70 ± 0.02 mg/mL (Olabanji et al., 2018). From a mechanistic perspective, the high content of plant-based protein and dietary fiber in cowpeas may contribute to improved insulin sensitivity and glycemic response by slowing

down carbohydrate digestion and absorption. In addition, polyphenolic compounds, such as anthocyanins, have been associated with reduced oxidative stress and inflammation, which are closely linked to the development of insulin resistance in type 2 diabetes mellitus (Jayathilake et al., 2018).

Moringa leaves (*Moringa oleifera*) are known for their bioactive compounds, including flavonoids, polyphenols, vitamin C, and essential minerals. In its powdered form, moringa leaves contain 28 mg of iron, 17 mg of vitamin C, 368 mg of magnesium, and 1300 mg of potassium per 100 g (Zainuddin & Hajriani, 2021). Evidence from in vivo animal studies suggests that flavonoids and polyphenols in moringa leaves may exert antidiabetic effects by improving insulin sensitivity, enhancing glucose tolerance, and reducing oxidative stress and inflammation. In contrast, evidence from human studies remains limited; although some trials have reported the potential benefits of moringa leaf supplementation on glycemic parameters, the findings are inconsistent and require further clinical validation (Krawczyk et al., 2022). Therefore, the antidiabetic potential of Moringa leaves is primarily supported by preclinical evidence. In line with this, its antioxidant capacity, mainly demonstrated in experimental models, is reflected by an IC_{50} value of 0.118 mg/mL, with total phenolic and flavonoid contents ranging from 36.02–45.81 mg/g and 15–27 mg/g, respectively (Perveen et al., 2024).

Fermentation of cowpeas with the addition of moringa leaf powder may enhance the nutritional quality and antioxidant capacity of tempeh through the synergistic effects of microbial metabolism and bioactive components. Şanlıer et al. (2019) found that fermentation improves nutrient bioavailability, while Hestiyawati et al. (2021) reported that adding moringa leaf powder enhances antioxidant activity in food products. Furthermore, Ningrum et al. (2023) highlighted that moringa leaves contribute to the overall antioxidant properties of various food matrices. Despite this potential, research focusing on the nutritional and bioactive characterization of tempeh made from cowpea and moringa leaf powder remains limited. Specifically, no previous study has simultaneously analyzed proximate composition, iron, vitamin C, and antioxidant activity, and determined the optimal cowpea–moringa tempeh formulation using the

Zeleny method as a candidate functional food for type 2 diabetes management.

Therefore, this study aimed to evaluate the nutritional composition and bioactive compounds of tempeh produced from cowpea and moringa leaf powder and to identify the optimal formulation that can potentially be developed as a functional food to support type 2 diabetes management. It is important to note that the assessment in this study was limited to proximate composition and selected bioactive compounds. The findings are expected to provide evidence for developing local plant-based alternatives to soy-based tempeh and contribute to improving community nutrition and public health outcomes.

Methods

This study employed a laboratory experimental design using a Completely Randomized Design (CRD) with four treatments (T0, T1, T2, and T3) and three replicates, resulting in 12 experimental units (Rahmawati & Erina, 2020). Each treatment represented a different formulation ratio of cowpea (*Vigna unguiculata* L.) and moringa (*Moringa oleifera*) leaf powders.

The range of moringa leaf powder (0–30 g per 301.5 g total ingredients) was selected based on safety considerations, acceptable organoleptic properties, and previous research on functional food formulations. (Puyanda et al., 2023) reported that adding 3–6% moringa leaf powder to soybean tempeh maintained nutritional values and organoleptic quality, while (Suprihartini et al., 2021) found that higher moringa concentrations increased vitamin C and N-amino content. Considering these studies, this research explored a salt range of 0–30 g to evaluate its effect on the nutritional composition and bioactive compounds of cowpea tempeh.

Tempeh production was performed at the Diet and Culinary Laboratory, Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya. At the same time, nutritional and bioactive analyses were performed at the Food Quality and Safety Testing Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya. All experimental work was conducted in September 2024.

Materials and Equipment

The main food samples consisted of cowpea obtained from Toko Putra Perdana (Halal

certified), moringa leaf powder from *Bumi Daun Kelor Organik* (Halal certified), Raprima tempeh yeast, and distilled water. Analytical reagents included DPPH, HCl, NaOH, anthrone, H₂SO₄, chloroform, HNO₃, FeSO₄·7H₂O, ethanol, acetic acid, iodine solution, starch indicator, and buffer solutions (KCl and sodium acetate). The equipment used included analytical balances, ovens, UV-Vis spectrophotometers, atomic absorption spectrophotometers (AAS), vacuum ovens, vortex mixers, hotplates, blenders, and standard glassware (beakers, flasks, and pipettes).

Experimental Treatments

Tempeh fermentation was performed for 42 h at 28–30 °C using 0.5 % tempeh yeast based on the total ingredient weight. The treatment formulations were as follows.

Table 1. Composition for Each Treatment

Ingredient	T0 (g)	T1 (g)	T2 (g)	T3 (g)
Cowpea	300	291	282	270
Moringa leaf powder	0	9	18	30
Tempeh yeast	1.5	1.5	1.5	1.5
Total	301.5	301.5	301.5	301.5

Sample Criteria

The inclusion criteria consisted of mature cowpea seeds aged 54–75 days with a yellow-brown color and fine moringa leaf powder that was dark green and lump-free. The exclusion criteria were floating or burnt cowpea seeds during soaking, expired moringa powder, and contamination with foreign materials.

Tempeh Production Procedure

The ingredients for each treatment were weighed, washed, and boiled until soft. Cowpeas were soaked for 12 h to allow natural lactic acid fermentation and then re-boiled for 10 min to reduce microbial contamination.

After draining and cooling to room temperature, cowpeas were mixed with moringa leaf powder and tempeh yeast according to the formulation. The mixture was then packed in perforated plastic bags measuring approximately 20 × 15 cm, with a thickness of 2–3 cm, and fermented at 28–30 °C for 42 h. Temperature, humidity, and inoculum levels were monitored to ensure optimal *growth of Rhizopus* and uniform texture formation.

Nutrient and Bioactive Compound Analyses

The proximate composition of the sample was determined in accordance with SNI 01-2354.4-2006, which includes analyses of moisture, ash, protein (Kjeldahl method), fat (Soxhlet method), carbohydrates (by difference), and energy (Atwater method) content. All proximate results are reported as percentages of the sample weight.

Antioxidant activity was measured using the DPPH assay and UV-Vis spectrophotometry at 517 nm to determine the IC₅₀ value, expressed in µg/mL. Anthocyanin content was quantified using the differential pH method (Anggraini & Mirantana, 2022) and measured at the visible wavelengths.

The iron content was determined using Atomic Absorption Spectrophotometry (AAS) with standard calibration curves and expressed as mg/100 g of sample, calculated from mg/L results based on sample weight and extraction volume. Vitamin C content was analyzed using volumetric and spectrophotometric methods at 260.5 nm and reported as mg/100 g of sample. The starch content was assessed by acid hydrolysis and optical density measurement at 490 nm using a UV-Vis spectrophotometer (AOAC, 2005), and reported as g/100 g of sample. The total sugar content was determined using the anthrone method, with absorbance measured at 630 nm, using glucose as the standard, and expressed as g/100 g of sample. The limits of detection (LOD) for iron, vitamin C, and anthocyanin were 0.05 mg/100 g, 0.1 mg/100 g, and 0.1 mg/100 g, respectively. Concentrations below these limits were considered undetectable. For the DPPH assay, the LOD was 0.5 µg/mL, and values below this threshold were considered immeasurable.

Data Analysis

Data were analyzed using SPSS version 25.0. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Levene test, followed by one-way ANOVA. A significance level of 95% ($\alpha = 0.05$) was applied in this study. The Bonferroni post-hoc test was used when the data were normally distributed and homogeneous.

The optimal treatment was determined using the Zeleny method based on ideal scores of each nutritional and bioactive parameter, which were normalized against their ideal values, weighted according to the relative importance of

each parameter, and summed to produce a composite score. The parameters included in the analysis were moisture, ash, protein, fat, carbohydrate, energy, starch, total sugars, iron, vitamin C, and antioxidant activity. The treatment with the highest composite score was considered optimal.

Ethical Considerations

This study did not involve human or animal subjects; therefore, ethical clearance was not required. All experimental procedures were conducted in accordance with institutional laboratory safety protocols and adhered to the standard ethical principles for scientific research.

Result and Discussion

Table 2. Characteristics of Tempeh

Treatment	T0	T1	T2	T3
Product Image				
Color	Pale yellow	Light greenish-brown (+)	Dark greenish-brown (++)	Dark green like moss (+++)
Aroma	Dominant cowpea/tolo aroma; milder than T1-T3	Typical tempeh aroma with slight fresh aroma from moringa leaf powder (+); slightly stronger than T0, milder than T2-T3	Typical tempeh aroma with a slight fresh aroma from moringa leaf powder (++)	Typical tempeh aroma with stronger fresh aroma from moringa leaf powder (+++); strongest among all treatments
Texture	Outer texture firm, inner texture soft or firm (+++); firmer than T1-T3	Outer texture quite firm, inner texture soft or firm (++); softer than T0, firmer than T2	Outer texture slightly firm, inner texture firm (+); softer than T1, firmer than T3	Outer texture firm, inner texture soft, and prone to breaking or crumbling; softest and most fragile

Explanation: The more "+" signs, the higher the intensity of the color, aroma, and texture.

Characteristics of Cowpea and Moringa Leaf Powder Tempeh

The results of the physical characteristics of the tempeh are presented in Table 2. The tempeh in the control group (T0) exhibited a pale-yellow color and dominant cowpea aroma, with a firm outer and inner texture. The addition of 3 % moringa leaf powder (T1) produced a light greenish-brown color, a typical tempeh aroma with a mild moringa scent, and a softer inner texture.

Treatment T2 (6 %) resulted in a darker greenish-brown color with a moderate moringa aroma and a balanced outer and inner firmness. The highest concentration (T3, 10 %) produced an intense dark-green (moss-like) color, strong moringa aroma, and soft, fragile interior.

Nutritional and Bioactive Content

This study analyzed various nutritional parameters and bioactive compounds of tempeh formulated with moringa leaf powder as a substitute for cowpea. The parameters analyzed included starch, iron, vitamin C, protein, fat, carbohydrate, water, ash, total sugar, energy, and antioxidant activity.

Each formulation (T0, T1, T2, and T3) exhibited distinct characteristics based on the concentration of Moringa leaf powder. The average measurement values, standard deviations, and significance values (p-values) based on statistical tests are presented in Table 3.

Table 3. Nutritional and bioactive content

Parameter	Mean $\pm$ SD				Unit	P-value
	T0	T1	T2	T3		
Water Content	62,75 $\pm$ 2,27	61,34 $\pm$ 0,82	58,93 $\pm$ 2,57	58,00 $\pm$ 1,24	%	0,052
Ash Content	1,01 $\pm$ 0,46 ^a	1,31 $\pm$ 0,55 ^b	1,50 $\pm$ 0,73 ^b	1,86 $\pm$ 0,94 ^b	%	0,027
Protein Content	11,81 $\pm$ 0,30	14,62 $\pm$ 2,07	14,90 $\pm$ 1,66	12,70 $\pm$ 0,98	%	0,078
Fat Content	0,49 $\pm$ 0,07 ^{ab}	0,53 $\pm$ 0,06 ^{ab}	0,39 $\pm$ 0,04 ^a	0,61 $\pm$ 0,07 ^b	%	0,014
Carbohydrate	23,96 $\pm$ 2,01	22,20 $\pm$ 1,46	24,27 $\pm$ 4,13	26,82 $\pm$ 0,91	%	0,225
Energy Content	147,55 $\pm$ 8,59	152,04 $\pm$ 3,36	160,25 $\pm$ 1,23	160,60 $\pm$ 5,04	kcal/100 g	0,094
Starch Content	7,58 $\pm$ 0,66	10,20 $\pm$ 1,47	8,86 $\pm$ 1,14	7,72 $\pm$ 1,13	g/100 g	0,077
Total Sugars	12,00 $\pm$ 1,69	11,25 $\pm$ 0,31	12,73 $\pm$ 0,87	11,56 $\pm$ 1,29	g/100 g	0,464
Iron Content	9,85 $\pm$ 0,20 ^a	11,2 $\pm$ 0,21 ^b	15,3 $\pm$ 0,04 ^c	18,4 $\pm$ 0,14 ^d	mg/100 g	0,016
Vitamin C Content	0,00 $\pm$ 0,00	8,66 $\pm$ 0,72	8,66 $\pm$ 0,73	9,50 $\pm$ 0,06	mg/100 g	0,236
Antioxidant Activity	51,73 $\pm$ 0,25 ^b	51,41 $\pm$ 0,14 ^b	48,77 $\pm$ 0,16 ^a	48,54 $\pm$ 0,24 ^a	μ g/mL	0,000
Anthocyanin Content	Not Detected					

Moisture Content

The analysis of moisture content in cowpea-moringa tempeh showed no significant differences among the treatments ($p = 0,052$). Mean values ranged from 62.75 % in the control (T0) to 58.00 % in T3, all meeting the SNI 3144:2015 standard (≤ 65 %).

Although a decreasing trend in moisture content was observed with increasing levels of Moringa leaf powder, this trend was not statistically significant and should be interpreted cautiously. Fiber, especially in moringa, is known to have a high water-holding capacity, which can alter the moisture dynamics of food products (Gopalakrishnan et al., 2016). Similar findings were reported by Rizal et al. (2022), who noted that microbial metabolism during fermentation can modify the moisture content through the enzymatic degradation of macromolecules and water release. Lower moisture levels are desirable for a longer shelf life, as they limit microbial growth and spoilage, making the product more stable over time (Şanlıer et al., 2019). However, since the differences among the treatments were not statistically significant, the practical impact on shelf life is expected to be limited.

Additionally, the combined presence of cowpea and moringa leaf fibers may contribute to the development of functional foods relevant for type 2 diabetes management, as fiber intake has been shown to regulate blood glucose levels by slowing down carbohydrate digestion and absorption (Hanoum et al., 2024).

Ash Content

Ash content analysis showed significant differences between treatments ($p = 0,027$), with values increasing from 1,01 % in T0 to 1,86 % in T3. This increase corresponds to the mineral contribution of Moringa leaf powder, which is naturally rich in Ca, Mg, and Fe (Singh et al., 2015). The increase in ash content is consistent with the activation of phytase enzymes during soaking and fermentation, which degrade phytic acid and release bound minerals (Maldonado-Alvarado et al., 2023).

Phytic acid, a common anti-nutrient in legumes, can bind essential minerals such as calcium, zinc, and iron, thereby reducing their bioavailability. The activation of phytase enzymes, especially during fermentation, plays a crucial role in breaking down phytic acid and may improve mineral solubility (Rahmawati et al., 2023).

Additionally, microbial metabolism during fermentation may further potentially facilitate mineral release by altering the food matrix, facilitating the release of bioavailable minerals. These findings align with those of Perveen et al. (2024), who demonstrated that fermentation enhances the bioavailability of minerals in plant-based foods by reducing anti-nutritional factors. However, ash content represents the total mineral content and does not directly indicate mineral bioavailability or absorption.

Protein Content

The protein content of tempeh ranged from 11,81 % to 14,90 %, with the highest level in T2

(6 % moringa leaf powder) and the lowest in T0. Although one-way ANOVA showed no significant differences ($p = 0,078$), higher mean protein values were observed in treatments with moderate inclusion of Moringa. This observation may be related to the higher intrinsic protein content of Moringa leaves (29,4 g/100 g) compared with cowpea (24,4 g/100 g). The slight decline in T3 (12,70 %) may have resulted from protein hydrolysis by *Rhizopus oligosporus* during extended fermentation (Maldonado-Alvarado et al., 2023).

Comparable findings were reported by Suronoto et al. (2020), who observed improved protein quality in tempeh fortified with high-protein ingredients. Therefore, incorporating 3–6 % moringa leaf powder resulted in higher mean protein values while maintaining acceptable fermentation quality.

Fat Content

The fat content of cowpea-moringa tempeh differed significantly among the treatments ($p = 0,014$). Average values ranged from 0,30 % in T0 to 0,61 % in T3, with significant differences detected between T2 and T3. The increase in fat content with higher moringa concentration may be related to the greater lipid content of moringa leaves (5,2 g/100 g) compared with cowpea (0,2 g/100 g) (Singh et al., 2015; Ministry of Health, 2020).

Nevertheless, the overall fat content remained below the SNI 3144:2015 requirement for soybean tempeh (≥ 7 %). The lower mean fat value observed in T2 (0,39 %) may be associated with lipid hydrolysis during fermentation, as *Rhizopus oligosporus* produces lipase enzymes that convert fat into free fatty acids and glycerol, which are subsequently utilized for fungal metabolism (Hanafi et al., 2023). This enzymatic hydrolysis of fats during fermentation is a well-known process that may improve lipid digestibility and contribute to the formation of bioactive compounds (Asbur & Khairunnisyah, 2021). Similar reductions have been attributed to heat treatment and moisture loss during boiling, which accelerate lipid degradation and influence the final fat content (Syukri et al., 2022).

Furthermore, the presence of moringa may influence the lipid profile, as studies have shown that moringa leaf powder can improve the functional lipid profile of fermented products, suggesting its potential application as a functional food ingredient (Iqbal et al., 2023).

From a nutritional perspective, the relatively lowfat content observed across treatments is advantageous for the dietary management of type 2 diabetes, whereas the moderate fat level in T2 may represent a balance between nutritional quality and product stability.

Carbohydrate Content

Carbohydrate analysis showed no significant differences among the treatments ($p = 0,225$), with mean values ranging from 22,20 % in T1 to 26,82 % in T3. The high variability ($SD = 3,05$) may stem from the use of the by-difference method, which can amplify analytical error (Kawamura et al., 2015). Although no significant differences were observed, higher mean carbohydrate values were found in treatments with higher moringa leaf powder inclusion, which may be related to the carbohydrate and fiber content of moringa leaves (41,2 g/100 g) (Singh et al., 2015). Moringa leaves are particularly rich in soluble and insoluble fibers, which can contribute to both carbohydrate content and the ability to regulate blood sugar levels by slowing the absorption of glucose (Iqbal et al., 2023).

Comparable increases have been reported in soy-moringa beverages, where moringa addition elevated the carbohydrate content (Matabura & Rweyemamu, 2022). Conversely, *Rhizopus* fermentation produces amylases that degrade starch into simple sugars, partially explaining the lower carbohydrate content in T0 and T1 (Wang et al., 2022). The enzymatic breakdown of starch during fermentation by amylase enzymes plays a crucial role in reducing the carbohydrate content, as starch is converted into more readily digestible sugars. This finding aligns with studies on other legume-based tempehs, where fermentation significantly lowers carbohydrate levels (Syukri et al., 2022).

Thus, the carbohydrate composition of cowpea-moringa tempeh is influenced by both the substrate type and enzymatic conversion during fermentation, with moringa's fiber content potentially contributing to carbohydrate characteristics relevant for glycemic management.

Energy Content

Energy values ranged from 147,55 kcal in T0 to 160,60 kcal in T3, with no significant difference among treatments ($p = 0,094$). The increase in energy content corresponds to the higher fat proportion in formulations containing moringa

leaf powder, as lipids contribute 9 kcal per gram compared with 4 kcal from protein and carbohydrates. Moringa's relatively higher lipid content (5,2 g/100 g) compared with cowpea (0,2 g/100 g) explains the observed rise (Singh et al., 2015; Ministry of Health, 2020). Lipids are a highly concentrated source of energy, which may be beneficial for individuals requiring an increased caloric intake, such as those experiencing undernutrition (Hestiyawati et al., 2021). However, in the context of diabetes mellitus, a higher energy intake should be interpreted cautiously and aligned with the overall dietary energy requirements.

Additionally, the presence of moringa in cowpea tempeh not only increases its energy content but also enriches it with essential fatty acids that are beneficial for cardiovascular health (Hanafi et al., 2023). Thus, incorporating moringa leaf powder enhances the energy value of cowpea tempeh, providing a higher-density plant-based food, highlighting its potential role as a plant-based food option whose energy contribution should be considered within controlled dietary planning for individuals with diabetes.

Starch Content

The starch content of cowpea-moringa tempeh showed no significant differences among treatments ($p > 0,05$), with values ranging from $7,58 \pm 0,66$ % in T0 to $10,20 \pm 1,47$ % in T1. Although the differences were not statistically significant, higher mean starch values were observed in T1 than in the other treatments.. This observation may be related to the relative composition of the raw materials: cowpea contains more starch than moringa, whereas moringa leaf powder is richer in fiber and antioxidants (Zhang et al., 2020).

The presence of fiber in moringa could affect starch availability, as fiber-rich foods may slow down starch digestion and absorption, which may influence starch digestion characteristics relevant to the glycemic response (Singh et al., 2015). During fermentation, amylase enzymes from *Rhizopus oligosporus* hydrolyze starch into simpler sugars, thereby decreasing the total starch concentration (Martgrita et al., 2023).

Similar findings were reported by Rocchetti et al. (2020), who observed that Moringa fortification reduced the starch hydrolysis index

of food products. This suggests that Moringa may influence starch characteristics, including those related to digestibility. Antinutritional compounds, such as phytic acid, tannins, and saponins, can inhibit fermentation; however, soaking and boiling processes, as well as fungal phytase activity, can mitigate their effects (Azka et al., 2018; Lestari et al., 2021; Supriyanto et al., 2022). These findings highlight the importance of optimizing both raw material composition and fermentation conditions.

Total Sugars

The total sugar content determined by the Anthrone method ranged from $11,25 \pm 0,31$ % in T1 to $12,73 \pm 0,87$ % in T2, with no significant differences among the treatments ($p = 0,464$). Although no significant differences were observed, slightly higher mean total sugar values were found in T2 than in the other treatments, whereas higher moringa levels did not result in higher mean total sugar values. This trend is consistent with the findings of Shahidi & Ambigaipalan (2015), who demonstrated that during fermentation, *Rhizopus* species convert part of the released sugars into organic acids and alcohols, resulting in comparable total sugar content across treatments. Both cowpeas and moringa contain limited amounts of simple sugars and higher levels of starch and fiber, which are less readily digestible and contribute to lower glycemic indices (Singh et al., 2015).

Additionally, moringa polyphenols, including flavonoids, has been reported to influence glucose metabolism in previous studies (Iqbal et al., 2023; Devi et al. (2023)). These findings indicate that incorporating 6% Moringa leaf powder resulted in comparable total sugar levels while maintaining carbohydrate characteristics relevant for functional food development.

Iron Content

Iron analysis using Atomic Absorption Spectrophotometry (AAS) revealed significant differences among the treatments ($p = 0,016$). The highest iron concentration was found in T3 (18.40 mg/100 g), whereas T0 recorded the lowest value (9.85 mg/100 g). The observed increase in iron content with moringa supplementation may be related to the mineral-rich composition of moringa leaves, which contain approximately 28 mg of Fe per 100 g.

This trend is further explained by the fermentation-induced degradation of phytic acid, which potentially increases mineral availability (Sari & Setiyoko, 2023).

Cowpea provides non-heme iron with low absorption efficiency (2-20 %); however, soaking, boiling, and fermentation have been reported to reduce inhibitors such as phytate and tannins by 30–50 % (Pratiwi & Widari, 2018). These results are consistent with those of Ningrum et al. (2023), who found that adding 12 g of moringa powder to tempeh burgers increased the iron content from 2,41 mg to 2,98 mg per 100 g. Therefore, moringa leaf fortification resulted in a higher iron content in cowpea tempeh and may contribute to improving iron intake in plant-based diets.

Vitamin C Content

Vitamin C content analysis using UV-Vis spectrophotometry showed no significant differences among the treatments ($p = 0,236$). The average values ranged from $0,00 \pm 0,00$ mg/100 g in T0 to $9,50 \pm 0,06$ mg/100 g in T3. Although no significant differences were observed, slightly higher mean vitamin C values were found in T3, which may be related to the contribution of Moringa leaf powder, which contains 143,14–164,81 mg vitamin C per 100 g (Qadir et al., 2022). However, the overall levels remain low because of the thermolabile and oxygen-sensitive nature of vitamin C. Heating, boiling, and fermentation can cause up to 90 % loss, depending on the temperature and exposure duration (Wang et al., 2022; Knez et al., 2023).

The instability of vitamin C during food processing, especially under high-temperature conditions, leads to its degradation into less bioavailable forms. This degradation is influenced by oxidative reactions that occur during cooking and fermentation (Oktavia et al., 2023; Sari & Setiyoko, 2023). Despite these losses, moringa fortification resulted in slightly higher mean vitamin C values, consistent with previous reports on moringa-enriched fermented foods, in agreement with Aznury et al. (2021), who found similar increases in moringa-enriched fermented foods. The high polyphenol content of Moringa, particularly flavonoids, enhances its antioxidant capacity, which helps preserve other nutrients, such as vitamin C, by reducing oxidative degradation during processing (Devi et al., 2023).

These results suggest that incorporating Moringa leaves may enhance the functional characteristics of tempeh through antioxidant-related nutrients, particularly in relation to oxidative stability.

Antioxidant Activity

Antioxidant activity measured using the DPPH assay exhibited significant differences among the treatments ($p < 0,05$). The lowest IC_{50} value, indicating the highest antioxidant activity, was recorded in T3 ($48,54 \pm 0,24$ μ g/mL), whereas the control (T0) showed the weakest activity (>50 μ g/mL). The increase in antioxidant capacity with higher moringa inclusion is attributed to the abundance of phenolic and flavonoid compounds in the moringa leaves (Devi et al., 2023).

These bioactive compounds, particularly flavonoids and polyphenols, are known for their strong free radical-scavenging abilities, which help mitigate oxidative stress and inflammation (Iqbal et al., 2023). This result is consistent with the findings of Gopalakrishnan et al. (2016), who demonstrated a strong correlation between moringa supplementation and antioxidant enhancement in food matrices. The antioxidant potential of Moringa has been widely recognized, with studies showing that it significantly contributes to the health benefits of functional foods by protecting cells from oxidative damage, a key factor in the prevention of chronic diseases (Pareek et al., 2023).

Nonetheless, the antioxidant strength of the final tempeh was reduced compared with that of the raw ingredients owing to the degradation of phenolics and anthocyanins during heating, drying, and fermentation processes (Wijayanti & Dewi, 2021; Dewi et al., 2022). Although T3 (10 % moringa leaf powder) exhibited the highest antioxidant activity, as indicated by the lowest IC_{50} value, the optimal formulation was not selected solely based on antioxidant capacity. The best formulation was determined by a composite evaluation of the nutritional, bioactive, and physical attributes. Higher moringa inclusion in T3 was associated with less favorable physical characteristics, particularly a softer and more fragile texture, which may reduce product stability and consumer acceptability. In contrast, T2 (6 % moringa leaf powder) provided a more balanced profile, combining improved antioxidant properties with an acceptable texture and

compliance with tempeh quality standards. Therefore, T2 was selected as the optimal formulation based on its overall performance rather than the maximization of a single parameter.

Anthocyanin Content

Anthocyanin compounds were not detected in any of the treatments using the differential pH method at 500–700 nm. The absence of detectable anthocyanins is most likely due to thermal degradation during boiling and fermentation, where high temperatures and oxygen exposure break down anthocyanins into ketones and less-active derivatives (Sugiyanto et al., 2020). Microbial metabolism during fermentation can also convert anthocyanins into simpler phenolic compounds, thereby reducing their concentration (Mushollaeni & Tantalu, 2020).

Additionally, the relatively high moisture content of tempeh (58,00–62,75 %) promotes oxidative degradation (Tamaroh et al., 2018). Although cowpea and moringa contain 27,8

mg/100 g and 8,3 mg/100 g of anthocyanins, respectively, the processing stages in tempeh manufacture appear to diminish their stability below the detectable limits. These findings align with previous studies emphasizing that anthocyanins are highly unstable under heat and fermentation, suggesting that alternative drying or low-temperature fermentation methods may be necessary to preserve these compounds for functional food applications.

Best Formulation

The optimal formulation was determined using the Zeleny method, which evaluates the treatment performance by summing the L_1 , L_2 , and L_∞ values. In this approach, a smaller total value indicates superior quality, whereas higher values correspond to lower performance.

The ranking results are presented in Table 4, showing that treatment T2 (94 % cowpea: 6 % moringa leaf powder) achieved the lowest combined score (0,1249), followed by T3 (0,1597), T1 (0,1647), and T0 (0,3643). Thus, the best formulation was identified as T2.

Table 4. Best treatment calculation

Treatment	T0	T1	T2	T3
L1	0,2564	0,1262	0,0936	0,1149
L2	0,0169	0,0029	0,0016	0,0033
L_∞	0,0909	0,0356	0,0297	0,0415
Result	0,3643	0,1647	0,1249	0,1597
Ranking	4	3	1	2

The superiority of the T2 formulation can be attributed to the balanced composition of cowpea and moringa leaf powder, which produces optimal nutritional and functional characteristics. This formulation provided higher protein (14,90 %), moderate moisture (59,38 %), increased iron (16,24 mg/L), and the strongest antioxidant activity ($IC_{50} = 48,54$ mg/mL), indicating an enhanced nutritional profile suitable for functional food development.

Given the rising prevalence of type 2 diabetes mellitus 11,7 % in Indonesia in 2023 (Ministry of Health, 2023), the T2 formulation has potential as a functional food candidate for dietary planning related to glycemic management. Cowpea contributes plant-based protein and a low glycemic index, whereas moringa leaf powder enriches the product with fiber, minerals, vitamins, and bioactive compounds such as flavonoids and

isothiocyanates, which have been reported to be associated with improved insulin sensitivity and antioxidant activity (Pareek et al., 2023). Studies have shown that flavonoids and isothiocyanates in moringa may contribute, based on previous studies, to mechanisms related to blood glucose regulation by enhancing the body's response to insulin (Iqbal et al., 2023). Furthermore, the high fiber content of Moringa aids in slowing glucose absorption, helping to prevent rapid spikes in blood sugar (Devi et al., 2023).

Therefore, cowpea–moringa tempeh with the T2 formulation may be considered a potential nutrient-dense food option for dietary planning in individuals with type 2 diabetes mellitus, based on its nutritional composition and evidence from the literature. However, the potential metabolic and clinical benefits of this formulation require further confirmation through controlled interventional studies. It

should be noted that the present study was limited to compositional and in vitro analyses and therefore does not provide direct evidence of clinical efficacy.

Limitations of the Study

While this study provides valuable insights into the potential of cowpea-moringa tempeh as a functional food for diabetes management, several limitations should be considered. First, the study only evaluated the nutritional and bioactive properties of tempeh without assessing its sensory acceptability or consumer preferences, which are crucial for the practical implementation of functional foods. Second, the long-term effects of incorporating cowpea-moringa tempeh into the diet were not evaluated, and further research is needed to determine its impact on glycemic control under real-world conditions. In addition, this study did not assess the glycemic index, postprandial blood glucose response, insulin response, or other metabolic biomarkers. Therefore, any implication regarding the role of cowpea-moringa tempeh in the management of type 2 diabetes mellitus remains theoretical and based on compositional characteristics and supporting literature rather than direct physiological evidence.

Additionally, the study was conducted in a controlled laboratory setting, and the scalability of the production process and its cost-effectiveness for large-scale manufacturing remain unexplored. Finally, although this study used a well-established method for assessing the nutritional and bioactive content, the effects of other processing methods or storage conditions on the stability of bioactive compounds in the final product were not investigated.

Conclusion

This study demonstrated that tempeh made from cowpea and moringa leaf powder possesses promising nutritional and functional properties, indicating its potential as a functional food candidate for managing type 2 diabetes. The optimal formulation identified was T2 (94 % cowpea: 6 % moringa leaf powder), which provided the most balanced nutritional composition, higher antioxidant activity, and improved mineral profile without compromising texture or fermentation quality. Specifically, the T2 formulation was characterized by a high

protein content (14.90 %), increased iron level (15.30 mg/100 g), and strong antioxidant activity, as indicated by a low IC₅₀ value (48.77 µg/mL), while maintaining acceptable moisture and fat content. These characteristics support the selection of T2 as the optimal formulation based on a composite evaluation using Zeleny's method. The results confirmed that the partial substitution of cowpea with moringa leaf powder enhanced the nutritional value and bioactive potential of non-soy tempeh.

Regarding type 2 diabetes mellitus, the potential relevance of cowpea-moringa tempeh is based on its nutritional composition and supporting literature, rather than direct clinical evidence. Further research is recommended to assess the sensory acceptability, glycemic response, and long-term clinical benefits of this formulation in human subjects. At the practical level, promoting cowpea-moringa tempeh as a locally sourced, nutrient-rich food can support dietary diversification and contribute to national efforts to reduce the growing burden of type 2 diabetes mellitus in Indonesia.

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