JURNAL KIMIA VALENSI
p-ISSN: 2460-6065; e-ISSN: 2548-3013
Journal homepage: https://journal.uinjkt.ac.id/index.php/valensi
Research Article

Exploration of Tempeh and Kidney Bean (*Phaseolus vulgaris*) Biscuits as Inhibitors of Angiotensin-Converting Enzyme (ACE-I)

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Article Info
Received: Sept 4, 2025
Revised: Sept 8, 2025
Accepted: Nov 30, 2025
Online: Dec 22, 2025

Citation:
Chalid, S. Y., Nurbayti, S., Ramadhani, S., Muawanah, A., Hermanto, S., & Rudiana, T. (2025). Exploration of Tempeh and Kidney Bean (*Phaseolus vulgaris*) Biscuits as Inhibitors of Angiotensin-Converting Enzyme (ACE-I) *Jurnal Kimia Valensi*, 11(2), 260-271.

Doi:
10.15408/jkv.v11i2.46424

Abstract
The study aims to improve the nutritional value of biscuits made from wheat flour and red kidney bean tempeh flour to functional antihypertensive biscuit as functional food by inhibiting angiotension-converting enzyme (ACE-I). Kidney beans were fermented for 32 hours at room temperature using 0.2% tempeh yeast. The tempeh was dried and ground using blender, filtered through a 100-mesh sieve. Kidney bean tempeh flour was then mixed with wheat flour in ratios of 40:60, 50:50, 60:40, 70:30, and 80:20.The dough was mixed with eggs, margarine, butter, and sugar, then baked in an oven. Organoleptic tests showed that panelists preferred biscuits 70:30 ratio of kidney bean tempeh flour to wheat flour, with texture, color, and aroma scores all above 3.The highest ACE-inhibitory activity 95.58% was observed in biscuits with an 80:20 ratio. Maillard reaction products in the biscuits contributed to increased ACE-I activity. Peptides of kidney bean tempeh are believed to play an active role in lowering blood pressure *in vitro*. Total phenolic and flavonoid contents were 0.025 mg GAE and 0.323 mg QE/g, respectively. These compounds also enhanced ACE-inhibitory properties. Based on the research, biscuits made from kidney bean tempeh flour show promising potential for development as functional antihypertensive food.

Keywords: ACE-inhibitor, biscuit, functional food, kidney bean, tempeh

1. INTRODUCTION

High blood pressure above 140/90 mmHg is a major cause of cardiovascular disorders, heart attacks, and strokes. This condition continues to rise, making it essential to pay attention to healthy food intake, particularly fiber-rich vegetables and specific, nutritious foods known as the Dietary Approaches to Stop Hypertension (DASH) [1]. Medications such as thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, and candesartan are recommended to help maintain stable blood pressure. In addition to medication, lifestyle changes are also advised to prevent hypertension [2]. These include weight loss, improving dietary habits by consuming high-fiber foods, avoiding alcoholic beverages, reducing intake of salty and fatty foods, and consuming fermented dairy products and grains. Flavonoids, phenolics, and peptides derived from soybeans have been shown to inhibit ACE, an enzyme that regulates blood pressure, making them promising candidates for development as functional antihypertensive foods. In *in vivo* studies, protein extracts from kidney beans hydrolyzed with alkalase, pepsin, and pancreatic enzymes were found to lower blood pressure in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY)[3]. Additionally, enzymatically hydrolyzed kidney bean peptides demonstrated antihypertensive properties, driven by hydrophobic amino acids and an 80% inhibition rate of ACE [4]. Hydrolysis of kidney bean using protease enzymes also successfully reduced blood pressure in hypertensive albino rats, indicating potential development for effective antihypertensive food ingredients [5].

Fermentation of kidney bean with *Cordyceps militaris (L.)* produces short-chain peptides with bioactive properties. This process increases protein levels and essential amino acids, which could inhibit the activity of angiotensin-converting enzyme (ACE), with an IC_{50} value of 0.63 mg protein [6]. Fermentation of kidney bean seeds with *Aspergillus awamori* significantly enhances the functional properties and mineral bioavailability of kidney bean. In addition, fermentation improves foaming and emulsifying properties, oil-holding capacity, thermal stability, and the bioavailability of iron and zinc in biscuits made from fermented kidney bean flour [7].

Substituting wheat flour with kidney bean flour in biscuit dough has been shown to increase levels of protein, crude fat, fiber, ash, and carbohydrates in the final biscuit product [8]. Biscuits made with kidney bean flour substitution could help prevent kwashiorkor, a protein-deficiency disease that commonly affects of children [9]. Hydrolysates of kidney bean globulin protein have demonstrated antihypertensive properties by inhibiting angiotensin-converting enzyme (ACE) activity by 28.4%. Meanwhile, hydrolysis of kidney bean albumin using pepsin has shown ACE-inhibitory (ACE-I) potential with an inhibition rate of 80% [10]. Biscuits produced from wheat flour fermented with *L. delbrueckii* subsp. *bulgaricus 151* exhibit ACE-I activity with an IC_{50} value of 43.63 mg mL^{-1}. In comparison, biscuits made from wheat flour fermented with *Lactobacillus casei* 2K show stronger ACE inhibition, with an IC_{50} value of 21.77 mg mL^{-1}. These findings indicate that fermenting wheat flour as a base for biscuit production could enhance ACE-I activity *in vitro* [11]. Substituting 30% of wheat flour with kidney bean flour in biscuit formulation has been proven to increase protein content, mineral levels, total ash, total phenolic compounds, and antioxidant capacity against DPPH radicals, it have value IC_{50} value of 0.0215 mg/L [12].

Chemically, the formation of Maillard reaction products occurs through complex and specific stages, and volatile compounds provide a distinctive aroma and an appealing brown color (**Figure 1**). The Maillard reaction happens between an amino acid and a reducing sugar. These products are responsible for the brown colour and appearance of processed food. However, this compound was able to provide positive effects on health, namely by exhibiting antioxidant activity [13].

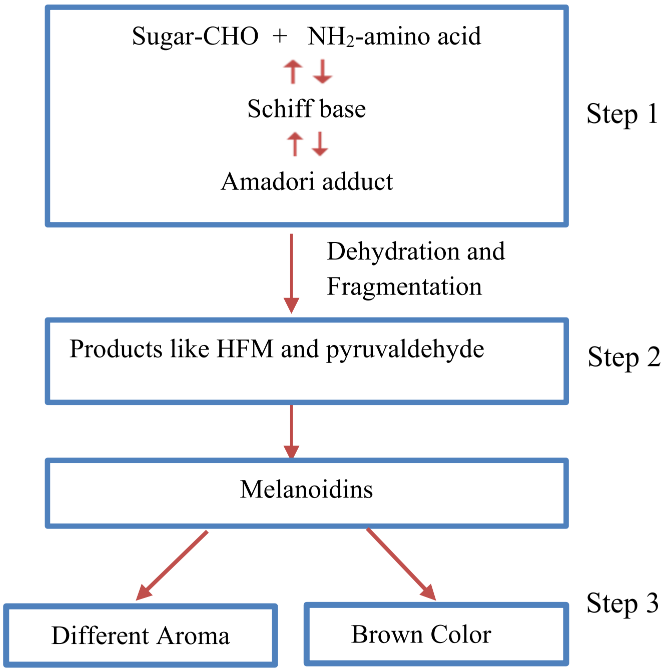

Figure 1. Schematic of the Maillard Reaction and aroma formation in food and beverage products [14]

Kidney bean contain the amino acids glycine, proline, and phenylalanine, while wheat flour also contains proline, phenylalanine, and glycine. The amino groups from lysine and arginine in peptides or proteins react with lactose or glucose as reducing sugars under heat, forming Maillard products with a brown hue. Treatment during the thermal processing of biscuit production causes nonenzymatic browning reaction. The reaction between the carbonyl group of sugars and the amino group of proteins under heat produces brown-colored compounds known as melanoidins [15].

2. RESEARCH METHODS

Materials and Instruments

Materials used for tempeh production included kidney bean obtained from a supermarket in South Tangerang, Indonesia; Raprima-brand tempeh starter containing *Rhizopus oligosporus* isolated from PT. Aneka Fermentasi Industri (AFI), and angiotensin-converting enzyme (EC 3.4.15.1, 0.5 U/mg) extracted from rabbit lungs. The substrate used was hippuryl-L-histidyl-L-leucine (Hip-His-Leu) sourced from Sigma-Aldrich (St. Louis, MO, USA). Additional reagents included sodium chloride (Merck), sodium tetraborate decahydrate (Merck), Folin–Ciocalteu reagent (Merck), aluminum chloride (Merck), gallic acid (Merck), quercetin (Merck), hexane (Merck), potassium sulfate (Merck), hydrochloric acid (Merck), sodium hydroxide (Merck), sulfuric acid (Merck), boric acid (Merck), and captopril (Farmoten).

Instruments used included a UV-Visible spectrophotometer (Lambda 25, Perkin-Elmer), centrifuge (Hettich EBA 20), and vortex mixer (Oregon). Other equipment included 10×20 cm plastic bags, glassware (Pyrex), micropipettes (Mettler Toledo), a gas stove (RI-3020S), water bath (Memmert), Whatman No.1 filter paper, 100-mesh sieve (Test Sieve), Soxhlet extraction apparatus (Barnsted Electrothermal), and additional glassware (Merck).

Statistical Analysis

The data in this study are presented as mean values with standard deviation (SD). Significant differences between treatments were determined by the Analysis of Variance (ANOVA) test at $p < 0.05$. The analysis was performed using the statistical tool IBM SPSS-25 [16] The ACE-I test was carried out twice.

Producing Kidney Bean Tempeh

Kidney beans were weighed at 800 g and soaked in 3.2 L of water for 16 hours, then boiled until fully cooked for 15 minutes. After cooling, the seed coats were removed, and the beans were dried by spreading them on a clean tray for 4-5 hours until dry. The cleaned beans were steamed for 30 minutes until tender, then dried by spreading them on a clean tray for 3-5 hours until dry. Then dried kidney beans were inoculated with 0.2% (w/w) Raprima-brand tempeh starter containing *Rhizopus oligosporus* and mixed thoroughly to ensure even distribution of the starter culture.

Kidney bean   Soaking   Boiling   Splitting   Yeast   Tempeh

Figure 2. Kidney bean tempeh production

A portion of 50 g of beans was placed into transparent plastic bags measuring 10×20 cm, which had been perforated with small holes to allow air circulation. Fermentation was carried out at room temperature for 32 hours or until a firm tempeh structure was formed [17]. Then fermentation was stopped once the surface of the tempeh was covered with white fungal hyphae from the starter mold, evenly growing across the entire surface (**Figure 2**).

Preparation of Kidney Bean Tempeh Flour

Kidney bean tempeh flour was produced by drying kidney bean tempeh in an oven at 50 °C for 6 hours [9]. The drying process aims to reduce the water content, making it easier to grind the tempeh into flour. The dried tempeh was ground using a blender to produce flour, then filtered through a 100-mesh sieve to obtain uniform-sized particles (**Fig. 3B**). The resulting red bean tempeh flour was stored in tightly sealed plastic bottles at room temperature.

Preparation Biscuit of Kidney Bean Tempeh Flour

The tempeh flour biscuit dough was prepared based on the method developed by Bedier et al. [8], with modifications involving the replacement of wheat flour with kidney bean tempeh flour. The biscuit doughs were labeled B1, B2, B3, B4, and B5, representing formulations combining kidney bean tempeh flour and wheat flour. Biscuit C1 served as a control, composed of 100% kidney bean tempeh flour without any wheat flour, while biscuit C2 was a control made with 100% wheat flour and no kidney bean tempeh flour. The biscuits were made with fat like butter, margarine, and egg yolk, and used stevia sugar as a sweetener (**Table 1**). Butter and margarine both play crucial roles in making biscuits, primarily affecting texture, flavor, and structure. The following procedure was pouring the flour mixture with wheat flour and tempeh flour while stirring evenly. Eggs and other ingredients were then added. All the ingredients for making biscuits have been mixed until they are homogeneous, and baking was carried out at 180 °C for 20 minutes [9]. After baking, the biscuits were cooled dan stored in polyethene bags for analysis. The supporting ingredients (butter, margarine, and milk powder) were mixed for 5 minutes and shaped using a cookie mold with a thickness of 2.5 cm. The biscuits were then baked at 130 °C for 30 minutes. The resulting biscuits underwent organoleptic testing, determination of total phenolic and flavonoid content, and analysis of ACE inhibition activity [11].

Table 1. Formulation of biscuits made from kidney bean tempeh flour

Ingredients	Biscuit formulation (g)						
	B1	B2	B3	B4	B5	C1	C2
Kidney bean tempeh flour	40	50	60	70	80	100	0
Wheat flour	60	50	40	30	20	0	100
Stevia sugar	30	30	30	30	30	30	30
Butter	80	80	80	80	80	80	80
Margarine	80	80	80	80	80	80	80
Egg yolk	50	50	50	50	50	50	50
Milk powder	58	58	58	58	58	28	28
Vanila	2	2	2	2	2	2	2

The addition of eggs serves to trap air or oxygen, allowing the dough to expand. Eggs also impart a yellow color, act as emulsifiers that bind fat and butter with liquid, and help improve the texture of the biscuits. Another function is to bind the dough so it does not easily crumble. Specifically, egg yolks contribute to creating biscuits with a soft and smooth texture, binding the flour, fat, and sugar, and serving as a source of antioxidants, folic acid, and vitamins [18].

Organoleptic Evaluation of Biscuits

Organoleptic or sensory evaluation was conducted to determine the panelists level of preference for biscuits made from kidney bean tempeh flour. Panelists were asked to fill out a provided form by assigning a score from 1 to 5. A score of 1 indicated strong dislike, 2 dislike, 3 moderate liking, 4 like, and 5 strong liking. The evaluated attributes included texture, color, aroma, and overall preference for the biscuits. This organoleptic test was carried out by 20 trained panelists [19].

Extraction of Kidney Bean Tempeh Biscuits

The method of biscuit extraction used in this study was the procedure developed by Wronkowska et al.[11], in which kidney bean tempeh biscuits were ground into biscuit flour. A total of 500 mg of this flour was extracted using 75% methanol solution. The extract was evaporated using an evaporator to produce a concentrated extract. The next step was to mix the concentrated extract with deionized water before subjecting the mixture to centrifugation at 6000 × g for 20 minutes at 4 °C. The resulting supernatant was used to test angiotensin-converting enzyme (ACE) inhibitory activity, expressed as a percentage of inhibition.

Angiotensin-Converting Enzyme (ACE)Inhibition Assay

The inhibitory activity of angiotensin-converting enzyme from kidney bean biscuit extract was expressed as a percentage, referring to the method developed by Wronkowska [11] and Chalid [20], using Hip-His-Leu (HHL), a synthetic peptide, as the substrate. A volume of 15 µL biscuit extract was mixed with 125 µL sodium borate buffer (pH 8.3) containing 7.6 mM HHL and 608 mM NaCl, followed by pre-incubation in a water bath for 5 minutes at 37 °C. The reaction was stopped by adding 125 µL of 1N HCl. The hippuric acid, formed as a result of the reaction between the sample and ACE (angiotensin-converting enzyme), was then mixed with 750 µL ethyl acetate solution and vortexed for 15 minutes. The resulting extract was centrifuged at 12,000 rpm for 10 minutes at a cold temperature. The upper supernatant was pipetted (500 µL) and dried in an oven at 90 °C for 30 minutes. The released hippuric acid was dissolved in 1 mL of distilled water, and its absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 228 nm. ACE inhibitory activity was determined using the following formula (1).

$$\text{ACE-Inhibitory} = \frac{C-A}{C-B} \, x\, 100\% \quad ......(1)$$

A = sample absorbance
B = blank absorbance
C = aquadest or distilled water

Proximate Properties Analysis

Proximate composition analysis was conducted on kidney bean tempeh flour, wheat flour, and kidney bean tempeh flour biscuits (B5). The proximate test included protein, fat, water, and carbohydrate content. Protein content was determined according to the Official Methods of Analysis [21], using a conversion factor of 6.25 to convert nitrogen to protein. Moisture and ash content were determined using the approved methods of the American Association of Cereal Chemists[8]. The difference method described by Ewunonu[9] was used to determine the total carbohydrate content.

Total Phenolic Content (TPC) Analysis

Tempeh flour and B5 biscuit flour were extracted using a mixture of methanol-water (70:30 v/v). The mixture was shaken at 300 rpm at room temperature for 10 minutes. The extract was centrifuged at 3000 rpm for 10 minutes. The total phenolic content (TPC) was measured using the Folin-Ciocalteu method. This method is based on the reduction of Folin-Ciocalteu reagent by phenolic compounds, which produces a blue color from molybdenum-tungsten complex.

A total of 0.5 mL of biscuit supernatant was added to the Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution. The mixture was then homogenized and incubated for 2 hours. The absorbance was measured using a UV-Vis spectrophotometer (Lambda 25, Perkin) at a wavelength of 725 nm after incubation for 40 minutes in a dark room. The total phenolic content in tempeh flour biscuit was expressed as gallic acid equivalents (GAE) per gram of extract [22].

Total Flavonoid Content (TFC) Analysis

Methanol extract of biscuit B5 was analyzed for total flavonoid content (TFC) by pipetting 0.25 mL of tempeh flour biscuit extract and mixing with 1.25 mL distilled water and 75 µL of 5% sodium nitrite solution. The reaction was allowed to proceed for 6 minutes, followed by the addition of 150 µL of 10% (w/v) aluminum chloride solution. After another 6 minutes, 0.5 mL of 1 M sodium hydroxide solution was added. The mixture was filtered and then adjusted with distilled water to a final volume of 2.5 mL. The solution was stirred until homogeneous, and absorbance was measured using a spectrophotometer at a wavelength of 510 nm. Flavonoid content was expressed as milligrams of catechin equivalent (CE) per gram of sample. The blank contained all the reagents without the sample [23].

3. RESULTS AND DISCUSSION

The Characteristics of Tempeh and Kidney Bean Tempeh Flour

Kidney bean tempeh was a fermented product made from kidney bean fermented for 32 hours using 0.2% (w/w) tempeh starter. Kidney bean as a fermentation medium represents an alternative fermentation technology that utilizes substrates other than soybeans. In this study, the resulting kidney bean tempeh met the Indonesian National Standard (SNI 3144-3:2015), characterized by a compact texture, evenly distributed white mycelium on the surface, and the distinctive aroma of tempeh without any ammonia odor, as required by SNI [24]. Alternative tempeh production using non-soybean legumes has been explored by combining two types of beans, soybeans with kidney bean, and soybeans with chickpeas. The results showed that such combinations can be developed into plant-based meat substitutes [25].The processing stages for kidney bean tempeh (soaking, boiling, and fermentation with mold) increased the protein content from 23.01% to 33.85% post-fermentation, while carbohydrate levels decreased. This process also led to an increase in protein and a reduction in isoflavone content.

During fermentation, macromolecules in the legumes are broken down into simpler molecules with lower molecular weight. The white mycelium of the tempeh starter evenly covered the surface of the tempeh (**Figure. 3A**) without producing ammonia odor. *Rhizopus oligosporus* produces enzymes such as protease, lipase, and alpha-amylase. These enzymes hydrolyze macromolecules into simpler compounds with functional properties that help regulate blood glucose and lipid levels [26]. Radiati and Sumarto[27] noted that bean size affects the ability of mold or tempeh starter to penetrate the legume substrate, thereby influencing the quality of the resulting tempeh. Several reasons support the use of kidney bean as a fermentation medium to replace soybeans, including the larger surface area of kidney bean compared to soybeans, which allows the mycelium to cover nearly the entire surface of the bean. Kidney bean flour fermented with *Aspergillus awamori* has been shown to enhance functional properties, improve mineral bioavailability, and act as a foaming agent, emulsifier, and oil and water retention agent. It also demonstrates thermal stability in a biscuit [7].

The fresh tempeh (**Figure 3A**) was manually crumbled by hand and then dried in an oven to be processed into tempeh flour by first drying the tempeh and then grinding it using a blender to produce kidney bean tempeh flour (**Figure 3B**). The red color of the flour originates from the metabolism of anthocyanins and flavonoids in the kidney bean skin, resulting in a red hue with black and gray specks on kidney bean skin [28]. In this study, kidney bean seed coat was included in the tempeh production process to ensure that the secondary metabolites in the skin could enhance the functional properties of the biscuit.

A. Kidney bean tempeh B. Kidney bean tempeh flour

Figure 3. Kidney bean tempeh and tempeh flour

The addition of 60% kidney bean seed coat in tempeh production aimed to increase the protein content of tempeh by 4.09%. achieved through the breakdown of complex proteins into amino acids via hydrolysis by protease enzymes from *Rhizopus oligosporus* [29]. Kidney bean contain anthocyanins, a class of flavonoid-derived compounds that serve as antioxidants, protecting the beans from photo-oxidative and oxidative stress [30]. Kidney bean tempeh flour also exhibits a reddish-brown color. According to Anggraini [31], theanthocyanin content in kidney bean tempeh reaches 40.37 mg per 100 g of tempeh extract, and this compound was responsible for the red coloration. Another functional property of red kidney bean is their potential as an alternative treatment for liver diseases caused by free radicals, viral and bacterial infections, and side effects from medications and alcohol. Kidney bean flour fermented with *Aspergillus awamori* has been shown to enhance functional properties, improve mineral bioavailability, and act as a foaming agent, emulsifier, and oil and water retention agent. It has also been shown to demonstrate thermal stability in a biscuit [7].

Characteristics of Biscuits of Kidney Bean Tempeh Flour

The intensity of the brown color in kidney bean tempeh flour biscuits varies depending on the composition of kidney bean tempeh flour and wheat flour. The higher the proportion of kidney bean tempeh flour used in the formulation, the darker the biscuit color becomes. Biscuits C1 and B5 exhibit similar brown color intensity, with biscuit C1 containing 100 g of kidney bean tempeh flour and biscuit B5 containing 80 g of tempeh flour (**Figure 4**). Biscuit C1 serves as the control sample, formulated exclusively with kidney bean tempeh flour and without wheat flour. The composition of kidney bean tempeh flour biscuits was presented in **Table 1**.

B1 B2 B3 B4

B5 C1 C2

Figure 4. Kidney bean-tempeh flour biscuits

The brown color of the biscuits is attributed to melanoidin compounds, which are brown pigments formed through the Maillard reaction or caramelization during baking or heating. The Maillard reaction between reducing sugars and amino acids produces a Schiff base, followed by an unstable glycosylamine-water interaction that leads to Amadori rearrangement. Melanoidin is the brown pigment resulting from the reaction between tempeh proteins and sugars present in the biscuit ingredients [32].

While the Maillard reaction contributes positively to the flavor and aroma of biscuits, making them enjoyable to consume, it also produces toxic compounds through non-enzymatic reactions involving lipids, proteins, and carbonyl compounds such as glucose and fructose. These reactions could lead to undesirable effects, including excessive browning and reduced protein [33] and nutritional value. Nevertheless, Maillard products are organoleptically favored by consumers. By-products such as acrylamide and hydroxymethylfurfural, which are known mutagenic agents [34].

During the baking process of bread or biscuits, the concentration of flavor and color-producing compounds increases, enhancing consumer appetite. Additionally, food flavor was influenced by the type of protein, hydrolysis conditions, polypeptide molecular weight, temperature, and pH. Foods and beverages containing proteins or peptides processed at high temperatures undergo Maillard reactions, where reducing sugars react with the NH_2 group of amino acids and the CHO group of sugars. This reaction is affected by sugar type, enzymatic hydrolysis, peptide molecular weight, processing temperature, and product acidity [35].

The brown color and distinctive aroma of biscuits are produced through a series of reactions between reducing sugars and amino groups of amino acids, beginning with the formation of a Schiff base, followed by Amadori products, and ultimately leading to advanced glycation end products (AGEs). Further reactions yield melanoidin compounds, which contribute to the biscuit's brown color and aromatic profile [36].

Angiotensin Converting Enzyme-Inhibitory

Biscuits developed from kidney bean tempeh flour represent one approach to creating functional snacks with ACE-inhibitory (ACE-I) potential, complementing earlier innovations such as soy tempeh protein explored by Chalid [20] and jack bean tempeh [37]. In this study, kidney bean tempeh flour was formulated with wheat flour to produce biscuits capable of inhibiting the activity of angiotensin-converting enzyme (ACE), an enzyme involved in regulating blood pressure. The inhibitory activity was measured as a percentage of ACE inhibition, indicating the extent to which biscuit extracts could suppress the conversion of angiotensin I into angiotensin II, a peptide known to constrict blood vessels and elevate blood pressure. Compounds or formulations that prevent this conversion are referred to as angiotensin-converting enzyme inhibitors (ACE-I).

The study aims to determine the effect of variations in kidney bean tempeh flour with wheat flour on the inhibition of angiotensin converting enzyme (ACE-I). The results of the study showed that variations in kidney bean tempeh flour were able to increase the inhibitory activity of ACE. The B5 biscuits, which were formulated with 80% kidney bean tempeh flour and 20% wheat flour had an ACE-I activity of 93.62 ± 0.07%. The value was higher than other kidney bean tempeh. Kidney bean tempeh flour biscuits of 100% (C1), was able to inhibit ACE by 93.90 ± 0.16. Biscuits of wheat flour or without kidney bean tempeh flour had an inhibitory activity against ACE of 44.83%. These results indicate that biscuits without kidney bean tempeh flour have a low ability to inhibit ACE-I (**Figure 5**). Captopril, as an antihypertensive drug, has a high inhibitory ability of 97.24 ± 0.06. The ACE inhibition value of biscuits made from wheat flour or without the addition of kidney bean tempeh flour was able to inhibit ACE by 44.83%. The value indicates that biscuits made by fortifying wheat flour with kidney bean tempeh flour are able to increase the ACE inhibition value.

These findings suggest that kidney bean tempeh flour biscuits hold strong potential as functional antihypertensive foods. The high ACE-inhibitory activity observed is likely attributed to Maillard reaction products, which may enhance bioactive compound formation during baking. Biscuits formulated with wheat flour, sunflower fatty acids, corn flour, skim milk, and sucrose have been shown to generate Maillard reaction products (MRPs) with functional properties, including antioxidant, antihypertensive, and prebiotic effects in Wistar rats [38]. Separately, black cricket protein hydrolysates were produced using protease enzymes. When concentrated black cricket protein was treated with Flavourzyme and Alcalase, combined with D-glucose, and heated at 100 °C for 60 minutes, it yielded Maillard products with ACE-inhibitory activity of 11.12%.

Protein hydrolysates obtained through Flavourzyme and Alcalase demonstrated a higher ACE inhibition rate of 30.83%, this significantly surpasses the level of untreated protein concentrates[39]. Biscuits made from fermented wheat flour using *Lactobacillus* bacteria exhibited ACE-inhibitory activity of 81.31 mg mL^{-1}, while non-fermented wheat flour showed a lower inhibition rate of 69.85 mgL [40]. These findings underscore the potential of fermentation, enzymatic hydrolysis, and Maillard reactions in enhancing the biofunctional properties of food products, particularly in the development of antihypertensive functional foods. Another study by Goulas et al. [41] showed that melanoidin compounds found in wine could reduce high blood pressure by blocking the action of an enzyme called angiotensin converting enzyme. The study found that a concentration of 2 mg/mL of melanoidin could reduce the activity enzyme by 58.2 to 75.3%

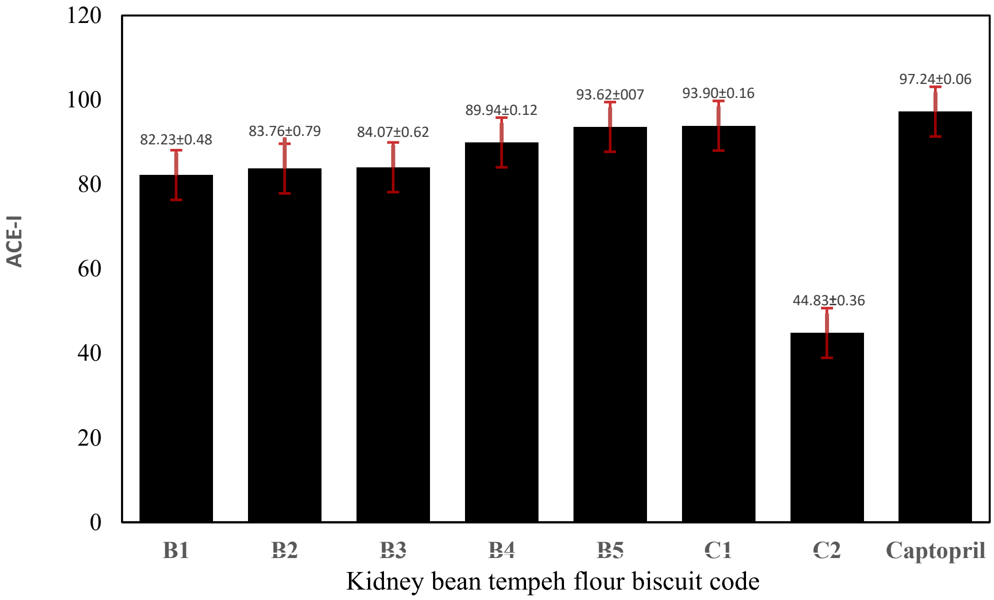

Figure 5. Angiotensin converting enzyme inhibition (%) of kidney bean flour biscuits (standard deviation (±) for two repetitions)

Table 2. Organoleptic evaluation of biscuits of kidney bean tempeh flour

Biscuit code	Texture	Color	Aroma	Taste	Overal likability
B1	2.87 ±1.04^{b}	3.55±0.80^{b}	3.89±0.92^{b}	3.11±1.03^{b}	3.03±0.89^{b}
B2	3.42±0.95^{c}	3.45±0.92^{b}	3.87±0.78^{b}	3.11±1.01^{b}	3.08±1.02^{b}
B3	3.34±0,97^{bc}	3.29±1.04^{b}	3.84±0.86^{b}	3.08±0.97^{b}	3.08±0.91^{b}
B4	3.21±1.12^{bc}	3.18±1.06^{b}	3.68±0.96^{ab}	2.76±1.10^{b}	2.87±0.99^{b}
B5	3.03±0.89^{bc}	3.13±0.94^{b}	3.79±0.91^{ab}	2.97±1.05^{b}	3.16±0.96^{b}
C1	4.11±0.84^{d}	4.05±1.14^{d}	4.13±0.94^{b}	4.03±0.89^{c}	4.03±0.92^{c}
C2	1.87±1.14^{a}	2.63±0,97^{a}	3.39±0.97^{a}	2.26±1.13^{a}	2.21±1.12^{a}

Results are expressed as mean ± SD. The same letter notation in the same column indicates no significant difference at the 5% confidence level according to Duncan's multiple range test. Conversely, different letters in the same column indicate a statistically significant difference at $p \leq 0.05$.

Organoleptic Evaluation Results

The development, monitoring, evaluation, and refinement of food product quality can be conducted through organoleptic testing[42]. In the study, the organoleptic test aimed to determine panelist preferences for kidney bean tempeh-based biscuits, focusing on texture, color, aroma, and taste. The substitution of kidney bean tempeh flour was intended to enhance the hedonic quality and organoleptic value of the biscuits. Data from the organoleptic evaluation are presented in Table 2. Statistically, panelists expressed a mild preference for biscuit formulations B4 and B5, which contained 70% and 80% kidney bean tempeh flour, respectively, combined with 30% and 20% wheat flour. These biscuits represent a novel innovation, and despite prior training, panelists still found them unfamiliar. However, they appreciated the texture and color of the biscuits. In contrast, the taste and overall likability were rated lower. The beany or slightly raw aroma of the kidney bean tempeh flour is suspected to be a contributing factor to the reduced taste preference.

Panelists favored the texture, color, aroma, and taste of the kidney bean tempeh flour biscuit labeled C1, which received a score above 4. This biscuit was produced using only kidney bean tempeh flour without any wheat flour. Panelists noted that biscuit C1 had a crisp texture, brown color, and a pleasant aroma and taste. The organoleptic test results indicated that biscuits made by substituting kidney bean tempeh flour were appreciated for their texture, color, aroma, and taste (**Table 2**).

Biscuits B4 and B5 were liked by panelists for their texture, color, and aroma, but were not favored in terms of taste and overall preference. Panelists gave high ratings to biscuits with a higher proportion of kidney bean tempeh flour, particularly for texture, color, and aroma. The brown color of the biscuits is a result of the Maillard reaction, which occurs between the amino acids (such as lysine, glycine, and phenylalanine) from kidney bean tempeh and wheat flour, and reducing sugars under heat (**Figure 4**). The Maillard reaction is influenced by protein hydrolysis conditions, protein type, peptide molecular weight, temperature, and acidity (pH) [35]. This evaluation is likely due to the fact that biscuits made from kidney bean tempeh flour are rarely found in markets or supermarkets, making the product unfamiliar to panelists. Despite this unfamiliarity, they recognized the biscuit as a newly developed product.

According to Giannou and Tzia [43], the presence of gluten from wheat flour improves texture, cutting strength, and structural retention during baking and storage. Therefore, the combination of gluten and kidney bean flour allows the cookies to have higher breaking strength, greater resistance to physical damage during packaging and distribution, and controlled fragility, resulting in a physically higher quality product compared to gluten-free kidney bean cookies.

Proximate Composition of Kidney Bean Tempeh Flour Biscuits

Proximate analysis was conducted on kidney bean flour, kidney bean tempeh flour, and biscuits made from kidney bean tempeh flour, specifically biscuit B5, which was formulated with 80% kidney bean tempeh flour and 20% wheat flour. The results revealed that the moisture content of biscuit B5 was 5.04%, the lowest among the three samples, indicating a drier and potentially more shelf-stable product compared to kidney bean flour and kidney bean tempeh flour. In contrast, the protein and ash contents were highest in kidney bean flour, exceeding those found in both kidney bean tempeh flour and the final biscuit formulation (**Table 3**). Similar findings were reported by Xiao et al [6], where fermentation of kidney bean with *Cordyceps militaris* increased protein content to 25.81%, compared to 23.61% in unfermented kidney bean. However, fat, ash, and carbohydrate levels were higher in the non-fermented kidney bean samples. These results suggest that fermentation and formulation processes influence the nutritional profile of kidney bean-based ingredients, with implications for both functional food development and ceremonial nourishment.

Kidney bean tempe flour contains carbohydrates and fat at 29.7 and 29.1% respectively, and kidney bean flour is a source of protein and carbohydrates with values of 22.4% and 57.7%. Kidney bean tempeh flour was a source of water, protein, and carbohydrate with 32.7, 12.8% and 50.1% respectively (**Table 3**). Ewunonu [9] stated that wheat flour and red bean flour in a ratio of 80:20 were used to make biscuits containing protein and carbohydrates of 13.6%, carbohydrates of 62.6% and fat of 15.8%.

In this study, kidney bean tempeh flour contained a moisture level of 32.71%, while the moisture content of kidney bean flour and its biscuit were found to be 14.17% and 5.04%, respectively, which were significantly lower than that of kidney bean tempeh. The elevated moisture content in tempeh is attributed to its processing, which involves soaking and boiling, as well as the steam produced during fermentation by tempeh yeast. A similar pattern was observed in the proximate analysis of biscuits fortified with kidney bean flour. The results showed an increase in moisture content from 5.23% to 6.93%, and in protein content from 10.77% to 13.98%. However, there was a decrease in fat and carbohydrate levels, from 33.31% to 17.37% and from 61.93% to 40.10%, respectively [19].

Table 3. Proximate composition of kidney bean, kidney bean tempeh, and biscuits (%)

Parameter	Kidney bean flour	Kidney bean tempeh flour	Biscuit
Moisture	14.17± 0.07	32.71 ± 0.31	5.04± 0.29
Ash	4.14 ± 0.06	2.78 ± 0.24	2.49± 0.01
Fat	2.89 ± 0.45	1.61 ± 0.03	29.14 ± 0.19
Protein	22.41± 0.58	12.79 ± 1.44	9.81 ± 0.26
Carbohydrate	57.72± 0.58	50.11 ± 1.40	29.7 ± 1.04

Standard Deviation (±), this represents the variation of sample values from the mean, based on two replications.

Total Phenolic and Total Flavonoid

Total phenolic and total flavonoid tests were conducted on B5 biscuits, which are biscuits formulated with 80 g of kidney bean tempeh flour and 20 g of wheat flour. Kidney bean tempeh flour mainly acts as a source of secondary metabolites, namely flavonoids, amounting to 0.442 mg QE/g, while biscuits are a source of phenolics (**Table 4**). According to Brewer [44], wheat flour contains a total of 0.29 mg FAE/g phenolic compounds that affect Maillard products. The baking process in biscuit production involves the Maillard reaction, which affects the colour, taste, nutrition, and safety of biscuits. Polyphenols(including flavonoids, flavan-3-ols, hydroxycinnamic acids, and tannins) play an important role in the taste, aroma, and colour of processed foods in the Maillard reaction.

The selection of B5 biscuits containing 20 g of wheat flour is a source of secondary metabolites in addition to kidney bean tempeh flour. The total phenolic content in 100 g of wheat flour has been found to be 56.87 mg [45]. The analysis of soluble and bound wheat flour revealed the presence of 0.29 mg of total phenolics per gram (FAF) and 1.95 mg of total phenolics per gram (FAF) in the samples, respectively. These values were expressed as ferulic acid equivalents (FAE). The total flavonoids obtained were 192.85 µg/g CE [44].

The total polyphenol and total flavonoid contents in fermented kidney bean were found to be higher than those in unfermented kidney bean [46]. The role of flavonoids as ACE inhibitors is attributed to compounds such as quercetin-3-glucuronide, flavan-3-ols, and anthocyanins, which have demonstrated efficacy both *in vitro* and *in vivo*. Additionally, catechins in polymerized form are effective ACE inhibitors. The antihypertensive properties of kidney bean tempeh biscuits are derived from bioactive peptides and secondary metabolites (including phenolic derivatives and flavonoid compounds such as luteolin, apigenin K, rutin, rhoifolin, quercetin, and kaempferol), all originating from kidney bean. Glycosylated quercetin has been shown to inhibit ACE activity 2–3 times more effectively than quercetin alone, through a competitive mechanism between quercetin and its glycoside form [47]. In this study, the total phenolic content in the biscuits was higher than that in kidney bean flour, whereas the flavonoid content was greater in the tempeh flour than in the biscuits.

Table 4. Total phenolic and flavonoid contents

Sample	Total Phenolic (mg GAE/g)	Total Flavonoid (mg QE/g)
Biscuit B5	0.025±0.10	0.313±0.02
Kidney bean tempeh flour	0.011±0.18	0.442±0.05

B5 = biscuits with a formulation of 80% kidney bean tempeh flour and 20% wheat flour, standard deviation (±), which represents the variation of sample values from the mean, based on two replications

Biscuits enriched with leaf extract flour from *Ficus asperifolia* (amplas) and administered to hypertensive rats were shown to significantly reduce. This effect is attributed to phenolic derivatives such as salicylic acid, naringenin, kaempferol, lutein, ellagic acid, quercetin, myricetin, and chlorogenic acid [48]. In the formulation of biscuits fortified with kidney bean flour, a total phenolic content of 12.50 mg GAE/g was obtained in biscuits containing 30% kidney bean flour [12]. The selection of B5 biscuits containing 20 g of wheat flour is a source of secondary metabolites in addition to kidney bean tempeh flour. The total phenolic content in 100 g of wheat flour has been found to be 56.87 mg [45].

4. CONCLUSIONS

The findings indicate that kidney bean tempeh flour biscuits possess the potential to function as antihypertensive foods, as evidenced by their ability to inhibit *in vitro* angiotensin-converting enzyme (ACE) activity. The ACE inhibitory properties of the biscuits are attributed to peptides resulting from the hydrolysis of kidney bean proteins by protease enzymes from the tempeh starter, as well as to phenolic and flavonoid compounds present in the tempeh flour used as the main ingredient. The optimal inhibition value was observed in biscuits composed of 100% kidney bean tempeh flour. Furthermore, increasing the proportion of tempeh flour resulted in enhanced biscuit properties, including an improved texture, colour, and flavour, rendering the biscuit more appealing. The Maillard reaction was indicated by the deepening of the biscuit's color, and this reaction also enhanced the biscuit's ACE-inhibitory capacity. The study successfully formulated a mixture of kidney bean tempeh flour and wheat flour for use in the production of biscuits, resulting in biscuits with functional angiotensin-converting enzyme (ACE) inhibitor properties. The investigation revealed that biscuits prepared with 4:1 ratio of kidney bean tempeh flour to wheat flour exhibited an inhibitory effect on angiotensin converting enzyme (ACE) with an efficiency of 93.62%.

5. ACKNOWLEDGMENTS

We extend our sincere gratitude to the Directorate General of Islamic Education, Ministry of Religious Affairs of the Republic of Indonesia, and to the Rector of Syarif Hidayatullah State Islamic University Jakarta for funding this research through the 2022 competitive grant program.

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