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Effect of Steaming Time on the Bioactive Compound Content and Antioxidant Activity of Sprouted Cowpea (*Vigna unguiculata*) Tempeh Flour

Hamidatun [a*], Lukman Azis [b], Anisa Zahra [b], Nisa Alfilasari [c,d], Anik Nur Habyba [e,f]

[a] Department of Food Technology, Faculty of Food Technology and Health, Sahid University, Jakarta, Indonesia
[b] Department of Food Technology, Faculty of Science and Technology, University of Al-Azhar Indonesia, Jakarta, Indonesia
[c] Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
[d] Functional Food and Nutrition Program, Faculty of Agro-Industry, Prince of Songkhla University, Songkhla, Thailand
[e] Department of Industrial Engineering, Universitas Trisakti, Jakarta, Indonesia
[f] Department of Industrial Engineering and Management, Yuan Ze University, Taoyuan City, Taiwan

*Corresponding author, Email: hamidatun@usahid.ac.id

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ABSTRACT

Cowpea (*Vigna unguiculata*) is a local legume rich in protein, minerals, vitamins, and bioactive compounds such as GABA and antioxidants. One promising innovation is the use of cowpea sprouts as raw material for tempeh production. However, tempeh has a short shelf life, which can be extended by converting it into flour. Tempeh flour often exhibits a beany flavor, that may be reduced through steaming, although this process may also decrease its nutritional and bioactive components. This study contributes to evaluating the effect of steaming time on the proximate composition, GABA, total phenolic content (TPC), and antioxidant activity (IC_{50}) of sprouted cowpea tempeh flour. A completely randomized design with a single factor was employed, consisting of steaming times of 0, 15, 30, 45, and 60 minutes. Data were analyzed using one-way ANOVA, DMRT, correlation analysis, and determination of the optimal treatment using the De Garmo method. Results showed that steaming significantly decreased moisture, fat, and protein contents ($p<0.05$), while increasing ash and carbohydrate levels. The highest GABA content was observed at 15 minutes (8.95 mg/g), then declined due to thermal degradation. TPC reached its peak at 45 minutes (247.81 mg GAE/100 g). Antioxidant activity (IC_{50}) fluctuated with steaming time, with the highest activity in the control sample (IC_{50} 19.01 mg/mL). Correlation analysis indicated that antioxidant activity was more closely associated with total phenolic content than with GABA. Based on the De Garmo evaluation, considering GABA, TPC, and IC_{50}, 15 minutes was identified as the optimal treatment.

KEYWORDS
Antioxidant activity; Cowpea; GABA; Phenolic content; Steaming

1. INTRODUCTION

Cowpea (*Vigna unguiculata*) is a local legume that is still underutilized. One possible diversification is the production of cowpea tempeh. Cowpeas contain valuable nutrients, including protein, fat, fiber, various minerals, and vitamins, as well as bioactive compounds such as Gamma-Aminobutyric Acid (GABA), phenols, and antioxidants. Raw cowpeas contain 3.69 mg/g anthocyanins and 2.16 mg/g GABA, 5.75 mg GAE/g of total phenols and 5.72 mg QE/g of total flavonoids. In addition, cowpeas also exhibit antioxidant activity of 2.5 mg TE/g. Cowpeas have a higher protein content (19.75%) compared to mung beans (15.78%) [1], [2].

Cowpeas contain antinutritional compounds that may reduce the bioavailability of nutrients. However, processing methods such as germination and fermentation have been shown to enhance the bioactive compounds and antioxidant activity of legumes, as well as reduce the antinutrients. A previous study reported an increase in total phenols from 100.55 mg GAE/100g to 181.51 mg GAE/100g after 24

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hours of germination. Similarly, total flavonoids increased from 13.24 mg QE/100g to 20.46 mg QE/100g, while antioxidant activity increased from 22.24% to 33.78% in sprouted cowpea flour [3]. In addition, germination has been shown to enhance GABA levels in legumes. Previous studies reported an increase in GABA content in black beans from 1.47 mg/g to 4.78 mg/g after 24 h of germination, as well as a 10-fold increase in GABA levels in mung beans. This enhancement occurred due to an increase in enzymatic processes in legumes during the germination [4], [5].

Fermentation has also been reported to improve the bioactive properties of legumes. A previous study has shown that fermentation of tempeh made from jack bean sprouts can increase total phenols from 2.487 mg GAE/g to 7.0532 mg GAE/g after two days of fermentation [6]. Similarly, fermentation that occurred during soybean tempeh production can increase GABA content from 2.7 mg/100g to 7.1 mg/100g after 48 hours [7]. GABA is a non-protein amino acid produced by plants, animals, and microorganism [8]. In humans, GABA is the main inhibitory neurotransmitter in the central nervous system [9]. In addition, GABA also has several physiological benefits, including anti-anxiety effects, improvement of sleep disorders, antihypertensive, and antidiabetic potential [10], [11], [12]. Whereas phenolic compounds are a secondary metabolite in plants that has an antioxidant activity. Their antioxidant capacity is primarily attributed in their ability to scavenge free radicals, thereby neutralizing reactive species and interrupting the peroxidation chain reactions [13], [14]. Furthermore, previous study has reported that longer fermentation time led to a greater increase in antioxidant activity and total phenols in both soybean tempeh and cowpea tempeh [15].

Fresh tempeh is highly perishable. One way to extend its shelf life is flour processing. Tempeh flour has the advantage of lower moisture content, making it more durable for storage and suitable as a raw material for new products. However, the use of tempeh flour as a raw material for product development faces challenges such as the presence of bitterness and the typical beany flavor of tempeh [16]. One way to reduce these undesirable flavors is by heat treatment such as steaming. However, steaming has the potential to reduce the nutritional content and bioactive compounds of food materials, including tempeh and other legumes [17], [18].

Steaming was selected because it is more effective in retaining nutrients and bioactive compounds compared to other thermal processing methods. According to previous study, steaming caused a smaller reduction in GABA content in germinated soybean, decreasing from 0.498 mg/g to 0.407 mg/g, compared to roasting (0.498 mg/g to 0.306 mg/g) and boiling (0.498 mg/g to 0.204 mg/g) [18]. In addition, previous study reported that steaming for 10–15 minutes can reduce the beany flavor and improve sensory acceptance of tempeh [19]. Research done on tempeh made from soybean and corn demonstrated that steaming for 10 and 30 minutes did not significantly reduce antioxidant activity, with a notable decline only observed after 60 minutes of steaming. In terms of total phenolic and flavonoid content, steaming up to 60 minutes showed no significant effect [20]. However, no study has systematically evaluated the effect of different steaming durations on GABA retention and antioxidant activity in sprouted cowpea tempeh flour.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were cowpeas sourced from a local supplier in Bekasi, West Java, and commercial tempeh starter RAPRIMA. Chemicals used for proximate analysis included selenium, H_2SO_4, hexane, 40% NaOH, H_3BO_3, Brom Cresol Green-Methyl Red indicator, and HCl. Chemicals used for total phenolic, GABA, and antioxidant activity analyses included 2,2-diphenyl-1-picrylhydrazyl (DPPH) (SMART-LAB, Indonesia), distilled water, 6% phenol reagent (MERCK, Germany), 9% sodium hypochlorite, borate buffer solution pH 9 (MERCK, Germany), 7.5% Na_2CO_3 solution (MERCK, Germany), 10% Folin–Ciocalteu reagent (MERCK, Germany), gallic acid standard (MERCK, Germany), and 80% ethanol (MERCK, Germany). All reagents used in this study were of analytical grade (pro analysis).

2.2. Cowpea Germination

The germination process was performed according to the previous [21]. Cowpeas were first sorted. The sorted cowpeas were soaked in room temperature water for 12 hours, with the bean-to-water ratio of 1:3. After soaking, the beans were rinsed, drained, and weighed. The beans were then evenly spread on the germination basket and covered with clean cloth. Germination was carried out at room temperature (25–30 °C) for 24 hours, during which the beans were sprayed with water every 6 hours.

2.3. Tempeh Making

The tempeh-making procedure was adapted from a previous study [2]. Cowpea sprouts were boiled for 5 minutes at 100 °C, cooled, and peeled. The sprouts were then air dried for 30 minutes to remove excess moisture for 30 before inoculation with 0.02% (w/w) RAPRIMA starter. The inoculated sprouts were packed into perforated plastic bags (10×15 cm^2) to produce tempeh blocks with approximate dimensions of 10×15×3 cm. The packed sprouts were then incubated for 48 hours at room temperature (25–30 °C).

2.4. Sample Preparation

Tempeh was subjected to steaming treatments for 0, 15, 30, 45, and 60 minutes. Prior to steaming, tempeh was cut into 1 cm slices. Steaming was performed using a traditional household method at 95–98 °C. After steaming, the samples were drained, thinly sliced, and dried in a food dehydrator at 55 °C for 4 hours. The dried tempeh was ground using a mill, sieved through a 100-mesh screen, and stored in standing pouches with silica gel at -18 °C, until further analysis [20], [21], [22].

2.5. Proximate Analysis

Proximate analysis, including moisture, ash, protein, fat, and carbohydrate by difference, was conducted at the Biotechnology Research Center, IPB University, following AOAC (2023) methods [23].

2.6. Sample Extraction

Sample extraction was performed for all component analyses, including GABA, TPC, and antioxidant activity, based on a previous study [24]. One gram of tempeh flour was extracted with 10 mL of 80% ethanol using a single extraction method. The mixture was vortexed, allowed to stand at room temperature (25–30 °C) for 30 minutes, then centrifuged at 4000 rpm for 30 minutes. The supernatant was collected and stored at -18 °C until further analysis.

2.7. Determination of GABA

GABA analysis was conducted following previous study [24] with modifications. A total of 0.5 mL of sample extract was mixed with 0.2 mL borate buffer (pH 9), 1.0 mL 6% phenol reagent, and 0.4 mL sodium hypochlorite 9%. The mixture was heated in a water bath at 80 °C for 10 minutes, then cooled in an ice bath for 10 minutes. Standard GABA solutions were prepared at different concentrations (50, 100, 150, 200, and 250 ppm) to determine the GABA concentration of samples. The absorbance was measured at 645 nm using a UV-Vis spectrophotometer. A standard curve was constructed by plotting absorbance against standard concentration. From this curve, a linear regression of $y = ax + b$ was obtained, with a coefficient of determination (R^2) of 0.9857.

2.8. Total Phenolic Content

Total phenolic content (TPC) was determined following AOAC (2017) methods [25]. A total of 0.3 mL sample extract was mixed with 1.2 mL Folin-Ciocalteu reagent, vortexed, and allowed to stand for 3 minutes. Then, 1.5 mL of 7.5% Na_2CO_3 solution was added and mixed. The mixture was incubated at room temperature for 90 minutes. A reagent blank without the sample was also prepared using the same procedure. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid standard solutions were prepared at different concentrations (10, 20, 30, 40, 50, and 60 ppm) to construct the calibration curve. A linear regression of $y = ax + b$ was obtained, with a coefficient of determination (R^2) of 0.9974.

Table 1. Coefficients of determination (R^2) for each antioxidant activity sample.

Sample	R^2		
	1	2	3
0 minute (control)	0.9273	0.9201	0.9236
15 minutes	0.9970	0.9979	0.9984
30 minutes	0.8797	0.8887	0.8959
45 minutes	0.9241	0.9235	0.9223
60 minutes	0.9829	0.9465	0.9594

2.9. Antioxidant Activity (IC_{50})

Antioxidant activity was evaluated using the DPPH method, modified from previous study [24]. Different concentrations of extract (1.0 mL) were mixed with 3.0 mL of 140 µM DPPH solution and incubated in the dark for 60 minutes. Absorbance was measured at 515 nm. A blank solution containing 1.0 mL of 80% ethanol and 3.0 mL DPPH solution was used. Antioxidant activity was expressed as IC_{50} values (mg/mL). A smaller IC_{50} value indicates a higher antioxidant activity. The IC_{50} value is acquired from a linear equation between the percentage of inhibition to various sample concentrations (10,000; 20,000; 40,000; 60,000; 80,000; and 100,000 ppm). The coefficients of determination (R^2) for each sample were obtained from the linear equation (Table 1). The percentage of antioxidant activity of each sample was calculated as % inhibition using the equations (1).

$$\text{Inhibition (\%)} = \frac{\text{(blank absorbance - sample absorbance)}}{\text{blank absorbance}} \times 100 \quad (1)$$

2.10. Data Analysis

All measurements were performed in triplicate. Data were analyzed using one-way ANOVA to determine significant differences. If significant, Duncan's Multiple Range Test (DMRT) at a 5% significance level was applied to identify the specific differences among treatments. Correlations were tested using the Spearman's Rank correlation coefficient. This method was chosen because the normality test (Shapiro-Wilk) yielded a p-value of 0.011 ($p<0.05$), indicating that the data were not normally distributed.

Weighting analysis to identify the optimal treatment was carried out using the De Garmo method. Weighting analysis to identify the optimal treatment was carried out using the De Garmo method. Each parameter was assigned a variable weight (BV) ranging from 0 to 1. Based on the correlation analysis, TPC showed a stronger correlation with antioxidant activity compared to GABA, therefore higher weight was assigned to TPC. The assigned weights were antioxidant activity (1.0), TPC (0.9), and GABA content (0.8). The one-way ANOVA, DMRT, and correlation tests were conducted using SPSS, while the weighting analysis was performed in Microsoft Excel. This research methodology can be seen in Figure 1.

3. RESULTS AND DISCUSSION

3.1. Proximate Content

The results showed that the moisture content in sprouted cowpea tempeh flour was significantly affected ($p<0.05$) by steaming time, with values decreasing progressively as the steaming time increased (Table 2). The highest moisture content was observed in the control tempeh (9.80%), whereas the lowest was recorded after 60 minutes of steaming (7.50%). The decrease in moisture content in this study indicates that prolonged steaming time promotes greater water loss from the food matrix. Heat treatment can cause physicochemical and microstructural modifications in food materials. Heating has been reported to generate cracks or holes on the surface of rice grains [26]. This microstructural damage can generate water migration and accelerate moisture loss from the tissue matrix, which may explain the declining moisture content observed in the present study. Heat-treated pea protein isolate has also been reported to be less tightly bound to water [27], meaning that the free water in the protein matrix is more susceptible to migration during the drying process.

The ash content analysis presented in Table 1, showed no significant difference ($p>0.05$) between the control flour (3.38%) and the steaming treatments of 15 minutes (3.52%), 30 minutes (3.37%), and 45 minutes (3.35%). However, a significant rise was observed after 60 minutes of steaming, reaching 4.31%. This finding is consistent with previous findings for jack bean tempeh [18]. The increase in ash content may be attributed to water loss during steaming and drying in the food dehydrator, thereby concentrating the mineral fraction. The more water lost from the food matrix, the higher the ash content will be [28]. This theory is supported by the decrease in the water content of sprouted cowpea tempeh flour with increasing steaming time in the present study.

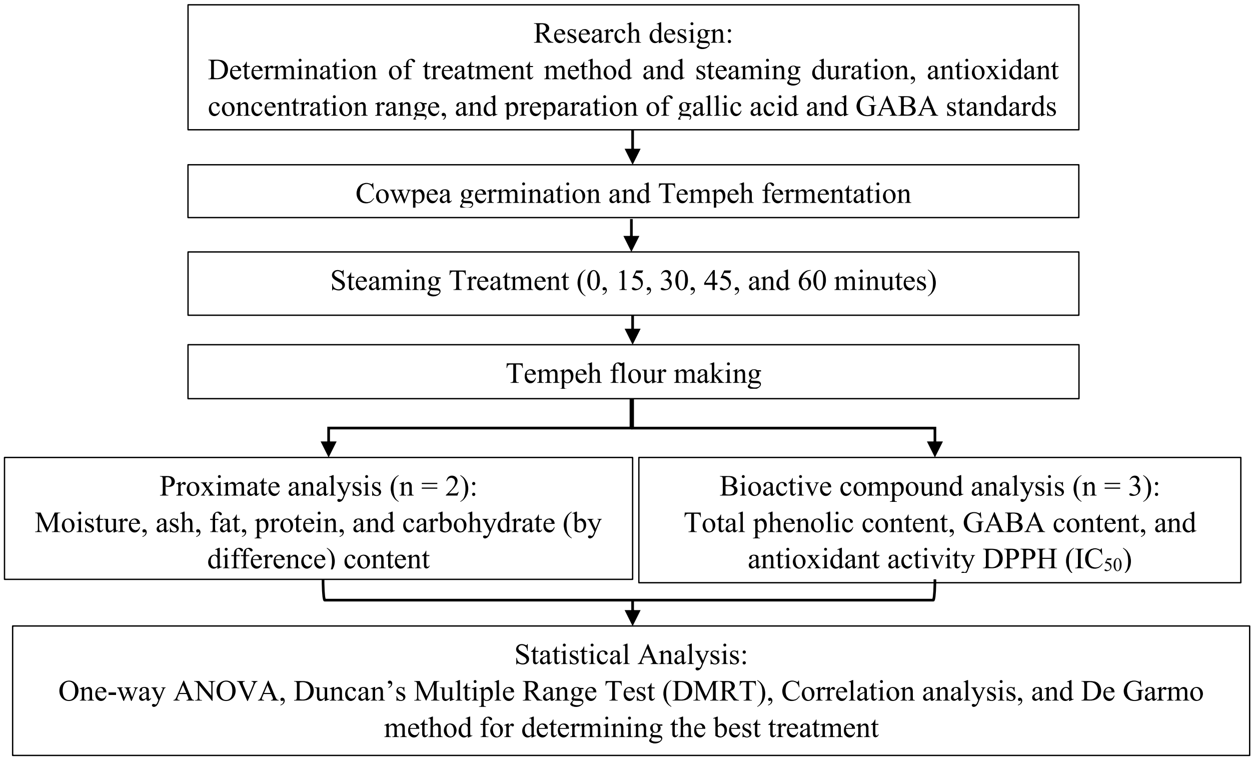

Figure 1. Research methodology.

The fat content of sprouted cowpea tempeh flour decreased significantly ($p<0.05$) with increasing steaming time (Table 2). The highest fat content was observed in the control sample at 2.21%, followed by the 15 minutes (1.90%), 45 minutes (1.62%), and 60 minutes (1.50%), while the lowest value was recorded at the 30 minutes of steaming (1.01%). A similar reduction in fat content following steaming has been reported for tempeh made from soybean and corn. The decrease in fat content is likely due to the structural changes in lipids during thermal processing. Heating can cause lipid hydrolysis, producing free fatty acid and glycerol, which may subsequently be lost during cooking processing [29].

In general, the steaming process significantly decreased ($p<0.05$) the protein content of sprouted cowpea tempeh flour (Table 2). The highest protein content was observed in the control sample at 35.59%, while the lowest was found in the 30 minutes steaming treatment, at 32.98%. A longer steaming time was associated with a progressive decline in protein content. This reduction may be attributed to the denaturation of protein during heating. Denatured proteins are more likely to migrate out of the food matrix and leach into the water released from the tempeh or condensed steam formed during steaming. A study on pea protein isolates demonstrated that heating could induce protein denaturation, thereby increasing solubility. Protein denaturation disrupts the protein structure and weakens non-covalent bonds, making some protein fractions more prone to redistribution within the food matrix [27].

The analysis revealed that steaming with different time had a significant effect ($p<0.05$) on the carbohydrate content of sprouted cowpea tempeh flour (Table 2). The control sample contained 48.54% carbohydrate. Overall, steaming treatments showed an apparent increase in carbohydrate content, with the highest value observed after 30 minutes of steaming (54.56%), followed by decreases at 45 minutes (51.96%) and 60 minutes (53.10%). It should be noted that carbohydrate content in this study was calculated by difference. Therefore, the observed increase does not necessarily indicate a true increase in carbohydrate. Instead, it reflects on the reduction of other proximate components, such as moisture, fat, and protein.

Table 2. Proximate content of steamed tempeh flour made from sprouted cowpea (%wet basis).

Steaming treatment (minutes)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate *by difference* (%)
0	9.80 ± 0.18^{d}	3.38 ± 0.32^{a}	2.21 ± 0.23^{d}	35.59 ± 0.26^{e}	48.54 ± 0.55^{a}
15	8.43 ± 0.06^{b}	3.52 ± 0.18^{a}	1.90 ± 0.00^{c}	35.13 ± 0.06^{d}	51.03 ± 0.19^{b}
30	8.20 ± 0.20^{b}	3.27 ± 0.18^{a}	1.01 ± 0.05^{a}	32.98 ± 0.08^{a}	54.56 ± 0.25^{e}
45	8.90 ± 0.21^{c}	3.35 ± 0.04^{a}	1.62 ± 0.07^{bc}	34.17 ± 0.01^{c}	51.96 ± 0.31^{c}
60	7.50 ± 0.02^{a}	4.31 ± 0.01^{b}	1.50 ± 0.04^{b}	33.60 ± 0.19^{b}	53.10 ± 0.27^{d}

Different superscripts indicate significant differences ($p<0.05$) at the same column as determined by one-way ANOVA followed by Duncan's MRT.

3.2. GABA

The GABA content of tempeh flour made from sprouted cowpea in this study ranged from 6.09 to 8.95 mg/g, which is higher than that of raw cowpea (2.16 mg/g) [1]. This increase may be attributed to the combined effects of germination and fermentation. Previous studies have reported that germination alone increases GABA content in mung bean [17], while fermentation enhances GABA levels during soybean tempeh production [7]. This increase can be explained by the proteolysis occurring during germination, which generates amino acids, including glutamic acid, the primary substrate for GABA synthesis. Moreover, germination enhances enzymatic activity, particularly that of glutamate decarboxylase (GAD), which is the enzyme responsible for GABA synthesis. Microorganisms involved in the fermentation of tempeh are also reported to have a GAD activity. *Rhizopus oligosporus*, the primary mold responsible for tempeh fermentation, has been shown to increase GABA levels during fermentation, suggesting it can produce GAD. In addition, lactic acid bacteria naturally associated with tempeh, were well known to synthesize GABA via GAD activity [7], [30], [31]. In other words, germination increases the pool of glutamate available, which can then be converted into GABA by GAD produced during fermentation. The GABA levels of tempeh flour in this study is displayed in Figure 2.

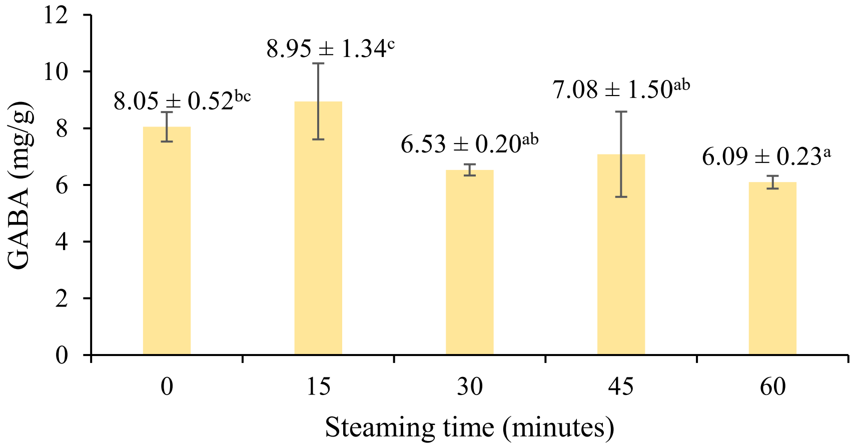

Figure 2. GABA content of sprouted cowpea tempeh flour at different steaming time.

Tempeh flour that was subjected to 15 minutes of steaming exhibited the highest GABA content (8.95 mg/g), although this was not significantly different from the control (8.05 mg/g). In legumes, including cowpea, GABA is predominantly synthesized in the cytosol, and may subsequently be transported to other organelles such as mitochondria or vacuoles [32]. This indicates that GABA is largely localized inside the cell, and under normal conditions, remains entrapped within intact cellular structures. Steaming may induce microstructural modifications by softening or disrupting the cell wall and food matrix, which enhances the release and extractability of bioactive compounds, including GABA. Consequently, the short steaming treatment (15 minutes) applied in this study likely facilitated the release of intracellular GABA accounting for higher measurable content compared to the unsteamed control. GABA levels decreased significantly after 30 minutes of steaming (6.53 mg/g) and 45 minutes of steaming (7.08 mg/g). The 60-minute steaming treatment had the lowest GABA level at 6.09 mg/g. A slight increase in 45 minutes of steaming compared to the 30 minutes of steaming may be associated with further disruption of the cellular structures, which enhance the release of GABA from the food matrix. However, prolonged steaming can lead to GABA degradation. A significant decrease in GABA levels has been reported in germinated red rice subjected to cooking treatments for 40 to 90 minutes, which was attributed to thermal decomposition as well as the consumption of amino acid compounds during the Maillard reaction that might occur during heat processing [33], indicating that heating time strongly influences GABA stability.

3.3. Total Phenolic Content

The total phenolic content (TPC) of sprouted cowpea tempeh flour in this study ranged from 155.49 to 247.81 mg GAE/100 g, exceeding the values reported for sprouted cowpea flour (100.55 mg GAE/100g–256.16 mg GAE/100 g) [3]. The higher values observed indicated that fermentation process during tempeh incubation increases total phenolic content. It has been reported that 48-hour fermentation of sprouted jack bean tempeh increased the total phenolic content from 2.49 mg GAE/g to 7.05 mg GAE/g [6]. The total phenolic contents of tempeh flour in this study are displayed in Figure 3.

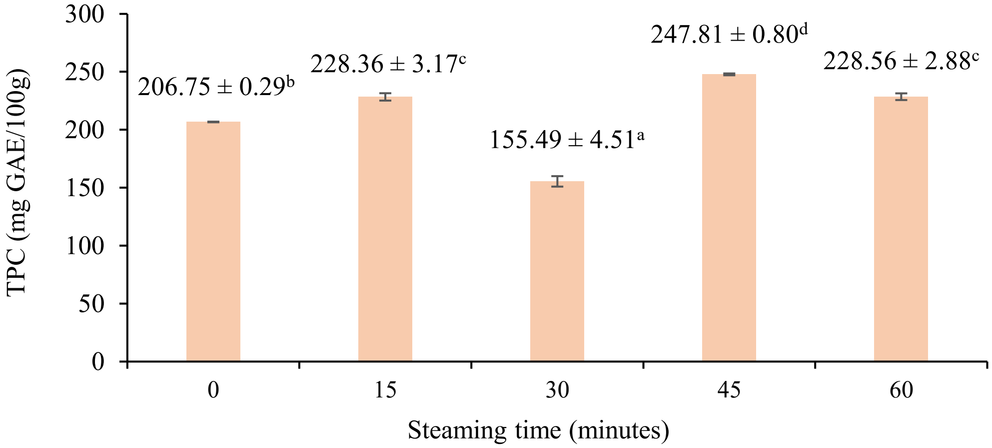

Figure 3. Total phenolic content (TPC) of sprouted cowpea tempeh flour at different steaming time.

The total phenolic content of steamed sprouted cowpea tempeh flour was significantly affected ($p<0.05$) by steaming time. The highest total phenolic content of sprouted cowpea tempeh flour was obtained after 45 minutes of steaming, reaching 247.81 mg GAE/100 g, followed by the 60 minutes (228.56 mg GAE/100 g), and the 15 minutes treatment (228.36 mg GAE/100 g). However, no significant difference was found between the 15 minutes and 60 minutes steaming treatments.

A possible mechanism underlying the increase of TPC is the disruption of cell walls by steam, which facilitates the release of phenolic compounds from the cell matrix. In addition, steam may liberate phenolic compounds from their complexes with other constituents, thereby enhancing their availability. Steaming may also inactivate polyphenol oxidase, an enzyme involved in the degradation of phenolic compounds [13].

In contrast, the 30 minutes steaming time resulted in the lowest phenolic content (155.49 mg GAE/100 g), likely due to a greater degradation of phenolic compounds. Although the exact mechanism for this sharp decrease cannot be fully confirmed in this study, it may indicate a phase in which oxidative degradation of phenolic compounds temporarily dominates over their release from the cellular matrix. A previous study has reported that heat processing can cause complex physical and chemical reactions that affect the stability of phenolic compounds. These reactions including degradation, oxidation, and the release of phenolics from their complex form [34]. the breakdown of phenolic components caused by heat may also produce unstable intermediates that are more susceptible to oxidation [35]. Previous studies have reported that phenolic retention during thermal processing is influenced by the balance between degradation, release from conjugates, and complex formation, which vary depending on heating conditions, such as temperature and time, and food type. Other factors that can influence the retention of bioactive compounds, including phenolic contents, and the cooking temperature and time [36], [37].

3.4. Antioxidant Activity (IC_{50})

The antioxidant activity of sprouted cowpea tempeh flour decreased significantly with steaming, as indicated by increased IC_{50} values compared to the control (Figure 4). The highest antioxidant activity was found in the control sample (IC_{50} 19.01 mg/mL), while the lowest was observed after 30 minutes of steaming (IC_{50} 46.09 mg/mL). Steaming for 15 minutes also reduced antioxidant activity (IC_{50} 33.74 mg/mL), while partial recovery occurred at 45 minutes (IC_{50} 28.89 mg/mL), which did not differ significantly ($p>0.05$) from the 60 minutes treatment (30.22 mg/mL), although both values remained higher than that of the control, indicating lower antioxidant activity. This trend is consistent with previous findings reported for jack bean tempeh, which showed a decrease in antioxidant activity following steaming, as indicated by a reduction in DPPH scavenging activity [18]. Similar reduction of antioxidant activity due to cooking processes have been reported for several types of cowpeas [38]. A possible mechanism for these decreases is that heating may degrade bioactive compounds, reduce their levels or form prooxidant components, and thereby reducing their antioxidant activity.

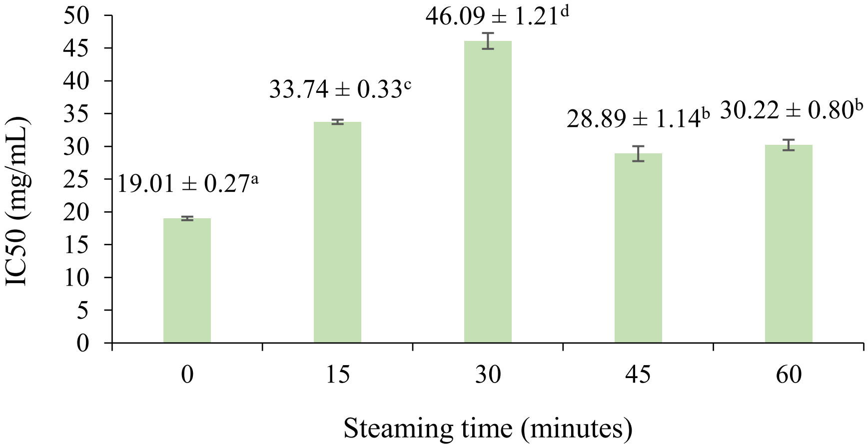

Figure 4. Antioxidant activity (IC_{50}) of sprouted cowpea tempeh flour at different steaming time.

3.5. Spearman's Rank Correlation

The correlation analysis in this study revealed a negative relationship between total phenolic content and antioxidant activity (IC_{50}) ($r = -0.336$; $p = 0.221$), indicating that total phenolics were not significantly correlated with IC_{50} values ($p>0.05$). Similarly, the relationship between GABA content and antioxidant activity also showed a negative correlation ($r = -0.175$; $p = 0.553$). The relatively high significance values, for both total phenolics and GABA ($p>0.05$), suggest that neither compound had a significant effect on antioxidant activity. This finding may indicate the involvement of other antioxidant compounds, apart from phenolics and GABA, which could have been degraded during heating. The antioxidant capacity of food materials is not attributed to a single compound but rather to the combined effects of multiple antioxidant constituents [39]. Although phenolics showed a stronger negative trend compared to GABA, neither variable significantly correlated with antioxidant activity. Nevertheless, a strong correlation has been reported between total phenolic compound and the antioxidant activity of cowpea, with total flavonoids being particularly prominent [38].

3.6. De Garmo Analysis

The determination of the best treatment in this study was carried out using the De Garmo method based on antioxidant activity, total phenols, and GABA content. The selection of these parameters was focused on bioactive compounds and antioxidant activity because these were the primary functional characteristics evaluated in this study. Variable weights were assigned based on the correlation analysis results. Antioxidant activity was given the highest weight (1.0), followed by TPC (0.9), and GABA content (0.8). the effectiveness index for each parameter was calculated and then multiplied by the parameter weight to obtain the product value. The product values of all parameters were then summed to obtain the total score for each treatment. The treatment with the highest total score was considered the optimal treatment.

Table 3. Total score and ranking of steaming treatments based on De Garmo methods.

Steaming time (Minutes)	Total score	Rank
15	0.8262	1 (best)
30	0.0462	4
45	0.8039	2
60	0.6026	3

Based on the calculation, the steaming treatment with the highest total score was 15 minutes (0.8262), followed by 45 minutes (0.8039), 60 minutes (0.6026), and 30 minutes (0.0462) (Table 3). The analysis results showed that the 15 minutes steaming treatment was the best in retaining the bioactive compounds and antioxidant activity, with antioxidant activity (IC_{50}) of 33.74 mg/mL, total phenols of 228.36 mg GAE/100 g, and GABA content of 8.95 mg/g.

4. CONCLUSION

Steaming time significantly influenced the proximate composition and bioactive compounds of sprouted cowpea tempeh flour ($p<0.05$). Prolonged steaming resulted in progressive decrease in moisture, protein, and fat contents, while carbohydrate content increased. Ash content experienced minimal alterations, except at 60 minutes of steaming where a significant increase was observed. Total phenolic content was significantly increased by steaming, with the highest level recorded at 45 minutes. GABA levels increased after 15 minutes of steaming but experienced a decrease after 30, 45, and 60 minutes, likely due to thermal degradation. Antioxidant activity (IC_{50}) fluctuated with increasing steaming duration, higher IC_{50} value observed at 15–30 minutes steaming indicated reduced antioxidant activity, reaching its lowest value at 30 minutes of steaming and partially recovered with 45 to 60 minutes of steaming. Correlation analysis indicated that neither TPC nor GABA showed a significant correlation with antioxidant activity. Based on evaluation using the De Garmo effectiveness index, 15 minutes of steaming was identified as the optimal treatment.

AUTHOR CONTRIBUTION

All author contributed equally to the main contributor to this paper. All authors read and approved the final paper. **Hamidatun**: Writing (original draft, review and editing), supervision, resources, methodology, and conceptualization. **Lukman Azis**: writing (review and editing), visualization and conceptualization. **Anisa Zahra**: Writing (original draft), data curation, visualization, and investigation. **Nisa Alfilasari**: Investigation, format analysis, writing (review), and supervision. **Anik Nur Habyba**: Data curation and visualization.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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