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The Effect of Cooking Treatment on Antioxidant Activity in Soybean Tempeh

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Abstract

Indonesian people consume tempeh through various processing methods. Food processing by cooking has been reported to reduce the bioactive capacity contained in food ingredients. This study investigated the effects of food processing (frying, steaming, roasting, sautéing, and boiling) on antioxidants in soybean tempeh. The antioxidant activity of processed tempeh was measured using DPPH and reducing power methods. The results showed that the highest inhibition activity of the processed tempeh was achieved by roasting and boiling (40%). The roasting method yielded tempeh with the highest total phenolic and flavonoid content. Thus, the cooking method considerably influences the antioxidants contained in tempeh. The findings showed that tempeh processed through roasting and boiling has the highest antioxidant activity. The total phenolic and flavonoid profiles are not in line with antioxidant activity, which indicates that the contributors to antioxidant activity are not only phenolic compounds. The increase in antioxidant activity in soybean tempeh is suspected to be influenced by the non-phenolic bioactive peptide compounds found in tempeh.

1. Introduction

The traditional fermented food known as tempeh is well-known among Indonesians. According to Babu *et al.* [1], tempeh is a cheap food that is nutritious and suitable for consumption by people from various socioeconomic backgrounds. Extensive studies have also revealed high antioxidant activity in fermented soybeans [2]. Antioxidants are molecules that can slow or prevent the oxidation of other molecules. Antioxidants are abundant in phenolic compounds [3]. Piansti *et al.* [4] reported that the antioxidant activity of soybean tempeh extracted with 70% ethanol with a fermentation time of 2 days was 69.45±3.49. Chang *et al.* [5] reported that the IC_{50} value of soybean tempeh with two days of fermentation extracted using 95% ethanol was 21.11 ± 2.59 mg/mL.

Isoflavones are essential bioactive compounds that provide antioxidant activity in soybeans with health benefits for humans [6]. Soybean and tempeh are natural food ingredients that contain antioxidants in the form of isoflavones. Isoflavones can stimulate the expression of Cu-Zn SOD, which can protect against oxidative stress [7]. Cu-Zn SOD is an enzyme that alternately catalyzes the release of superoxide radicals into ordinary molecular oxygen and hydrogen peroxide [8]. A study by Nakajima *et al.* [9] reported the results of their research showing that the isoflavone content in tempeh fermented for 48 hours was 15%.

Indonesian people generally consume tempeh by processing tempeh into fried tempeh and chips which are the result of the cooking process using cooking oil. Frying conditions, such as temperature and duration, can affect changes in the nutritional value of fried foods [10]. Undesirable changes can occur together with desirable modifications, one of which is the loss of nutrients, especially vitamins, during the frying process [11]. These are general changes in the nutritional aspects of fried foods [12].

Sultana *et al.* [13] suggested that the cooking method can significantly influence the antioxidant properties of vegetables. In addition to the frying method, other methods such as sautéing, boiling, roasting with the

oven, and steaming cause a decrease in antioxidant activity in vegetables.

In a study by Hwang *et al.* [14], four different cooking process methods (boiling, steaming, sautéing, and roasting) changed the antioxidant activity of Cayenne pepper (*Capsicum annum* L). The antioxidant activity is lower in boiling and steaming than in other processes. The length of time in the cooking process also affects the decrease in antioxidant activity. Sauteing and roasting still retain nutrients and antioxidants compared to boiling and steaming. In comparison, the frying method [15] revealed that garlic was reduced by more than 50%, asparagus between 30% and 40%, and Swiss chard, cauliflower, and pepper between 5% and 30%.

In general, the cooking method of vegetables affects the antioxidant content. The effect of cooking on the antioxidant content of vegetables is mainly due to softening or denaturation of plant tissues or cellular disruption and separation of some phenolic compounds from cellular structures [16]. Natural antioxidants have different chemical structures and stability; for example, α-tocopherol is quite a heat resistant, and losses during processing are primarily due to oxidation processes. Ascorbic acid can be degraded irreversibly by heat, air, alkaline conditions, and enzyme activity to form oxalic and threonic acids. Since carotenoids are found in complex forms with proteins and have a lot of double bonds, they are moderately stable for cooking but highly susceptible to oxidation in plant cells [17].

In previous studies, many have examined antioxidants in uncooked tempeh, but this has never been done in soybean tempeh with cooking treatment. This study investigated the effect of cooking treatment on antioxidant activity in soybean tempeh using various cooking methods, namely steaming, sautéing, roasting in the oven, boiling, and frying.

2. Methods

2.1. Materials

Soybean tempeh was purchased from the traditional market. Methanol (Merck), 2,2- Diphenyl-1-picrylhydrazyl (Sigma Aldrich), quercetin (Merck), tamarind trichloroacetate (Merck), sodium dihydrogen phosphate monohydrate (Merck), potassium ferricyanide (Merck), ferric chloride hexahydrate (Merck) were ≥95% purity. Distilled water, 70% ethanol (Merck), disodium phosphate (Merck), sodium carbonate (Merck), Folin– Ciocalteu reagent, sodium nitrite (Merck), aluminum chloride (Merck), and sodium hydroxide (Merck).

2.2. Preparation of Tempeh Extract

Tempeh was cut into 2×2 cm sizes and subjected to different cooking processes (frying, steaming, sautéing, boiling, and roasting (190°C) for 3 to 5 minutes each. The cooked tempeh was dried at room temperature for 10 minutes and then dried in a freeze-dryer for 10 minutes. The dry samples were mashed and macerated in 50 mL of 70% ethanol and then put into a shaker at 100 rpm for 24 hours [18].

2.3. Determination of Total Phenolic Content

The total phenolic content was measured by spectrophotometric analysis using the Folin-Ciocâlteu method [19]. A 2.5 mL of tempeh extract was added with 0.5 mL of Folin–Ciocalteu reagent, shaken to make it homogeneous and allowed to stand for 8 minutes. The mixture was added 1.5 mL of 20% Na_2CO_3 and allowed to stand for 30 minutes at room temperature. The absorbance was measured at λ = 765 nm using a UV-Vis spectrophotometer.

2.4. Determination of Total Flavonoid Content

The total flavonoid content was determined following the method from [19]. A total of 1 mL of extract was added with 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$, allowed to stand for 5 to 6 minutes, then added 0.3 mL of 10% $AlCl_3$ and 4 mL of 4% NaOH. The mixture was added with distilled water up to the mark of the 10 mL volumetric flask and allowed to stand for 15 minutes. The absorbance was measured at λ = 510 nm using a UV-Vis spectrophotometer.

2.5. Antioxidant Activity Assay

2.5.1. DPPH Radical Scavenging Activity

The antioxidant activity of tempeh extract with various cooking treatments was determined using the DPPH method [20, 21]. Tempeh extract was added to 3.8 mL of DPPH solution (50 μM, in methanol), and the reaction was left for 30 minutes in the dark. The procedure was performed in triplicate. The absorbance of the mixed solutions was determined at 515 nm using a UV-Vis spectrophotometer (T60U Spectrometer).

2.5.2. Reducing Power Assay

The antioxidant activity of tempeh extract was measured using the reducing power method [22]. A 2.5 mL of the extract was mixed with 2.5 mL of phosphate buffer (2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) and incubated at 50°C for 20 minutes. The mixture was added to 2.5 mL of 10% trichloroacetic (TCA) solution and centrifuged at 5000 rpm for 10 minutes. A 2.5 mL supernatant was added with 0.5 mL of 0.1% $FeCl_3$ solution. The absorbance was measured at a wavelength of 700 nm using a UV-Vis spectrophotometer.

3. Results and Discussion

3.1. Preparation of Tempeh in Different Cooking Process

The preparation was conducted by preparing samples of tempeh cooked with various cooking methods. The cooking process was performed in five variations: sautéing, boiling, frying, steaming, and roasting with an oven. Cooking requires five minutes, except for frying, which requires 3 minutes because it cooks more quickly.

The results of tempeh from various cooking processes that have been freeze-dried can be seen in Table 1, which shows differences in color and texture after various cooking methods. The color of tempeh after

being given the frying and sautéing treatment turned brown, but the frying treatment gave a darker color. Boiled and steamed tempeh did not show a significant color difference; both were pale. The roasting process at 190°C produces brown tempeh and has a dry texture due to the influence of the heat from the oven. There was no significant difference in the characteristic appearance of the tempeh after freeze drying; only the texture of the tempeh became more easily crushed.

Table 1. Characteristics of tempeh in various cooking processes

Tempeh	Appearance	Description
Fried		The color is dark brown, and the texture is slightly dry and oily
Sauteed		The color is brown, and the texture is wet with oil
Boiled		The color is pale, and the texture is soft
Steamed		The color is pale, and the texture is soft
Roasted		The color is brown, and the texture is dry

3.2. Tempeh Extracts

Tempeh was extracted with 70% ethanol solvent. According to Suhendra *et al.* [23], ethanol is a solvent that may dissolve various substances, including phenolic compounds, that are less polar to polar. Ethanol can dissolve phenolic compounds because it can degrade cell walls, allowing bioactive compounds to be expelled from plant cells more easily. Ethanol has hydroxyl groups that can bind to hydrogen groups from the hydroxyl groups of phenolic compounds, which causes an increase in the solubility of phenolic compounds in ethanol). Vongsak *et al.* [24] reported that maceration with 70% ethanol was preferable to other methods in terms of simplicity, convenience, cost-effectiveness, and production of an extract with the highest concentrations of total phenolics and flavonoids and the highest antioxidant activity.

3.3. Total Phenolic Content

Phenolics or polyphenols are secondary plant metabolites most commonly found in plants with high medicinal value. Phenolic compounds contribute to the antioxidant potential of plants by neutralizing free radicals and preventing the decomposition of hydroperoxides into free radicals [25]. The total phenolic content of the extract can be evaluated by the spectrophotometer method using the Folin-Ciocalteu reagent. The principle of this method is the ability to reduce the functional group of phenol. The oxidation and reduction reactions of phenolic ions occur under basic conditions. Reduction of the Folin-Ciocalteu reagent by phenolic ions changes the color to blue. The color of the extract darkens, and the absorption level increases as the phenolic compound content increases [26]. The results of the total phenol analysis are shown in Figure 1.

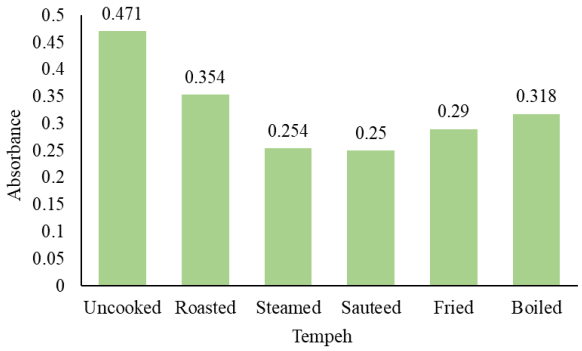

Figure 1. Total phenolic content of tempeh with various cooking methods

Based on the data shown in Figure 1, raw tempeh had the highest total phenol content but sauteed and steamed tempeh had the lowest content despite having around the same absorbance. Vegetables have phenolic compounds in water-soluble forms within their cell walls; however, contact of the tissue surface with water, high cooking temperatures, and long cooking durations may have disrupted the cell wall and damaged the phenolic compounds [27]. This causes the total phenol levels in tempeh after cooking to decrease. Loss of total phenol during cooking generally occurs due to heat and oxygen, so total phenolic compounds can be oxidized [28]. Mota *et al.* [29] added that phenolic compounds are soluble in water but insoluble in oil. The use of oil and the high temperature are likely to cause damage to the cell wall and plasma membrane in which phenolic compounds are abundant in the cell wall.

3.4. Total Flavonoids Content

Flavonoids are secondary metabolites with a polyphenolic structure found in many fruits and vegetables. Almost every flavonoid group can act as an antioxidant [30]. Total flavonoid content was measured based on spectrophotometric detection of the colored complex formed between Al(III) and the carbonyl and hydroxyl groups of flavonoids in alkaline conditions. The

results of the analysis of the total flavonoid are shown in Figure 2.

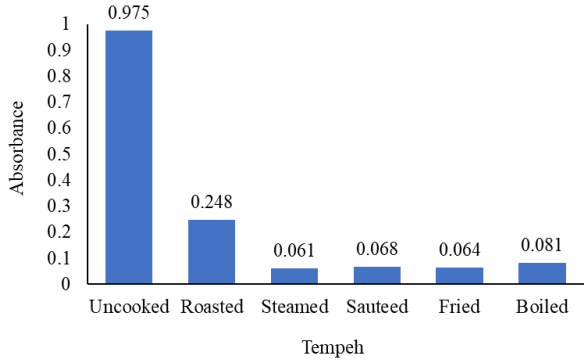

Figure 2. Total flavonoid content of tempeh with various cooking methods

Based on the data shown in Figure 2, the cooking process significantly influences the total flavonoid content of uncooked tempeh. Research by Surya and Romulo [31] also reported that the levels of flavonoids from uncooked tempeh and tempeh treated with cooking were not much different. The content of flavonoids will decrease with the cooking process due to damage to the structure of the flavonoids [32]. Research by Utari *et al.* [33] reported that the frying and boiling processes reduced the flavonoid content by 39.15% and 18.2%, respectively. Haron *et al.* [34] reported that the process of boiling and frying tempeh caused a decrease in flavonoid levels by 31.88% and 37.56%. In this study, the cooking processes such as steaming, sautéing, and frying showed no significant differences in total flavonoid content.

3.5. Antioxidant Activity

Antioxidant activity assay aims to determine the antioxidant capacity of tempeh extract that has undergone a cooking process. This research was carried out using the DPPH method and reducing power. The working principle of this approach is to combine DPPH free radicals with antioxidant compounds that can donate hydrogen in order to suppress free radicals. The results obtained are shown in Figure 3.

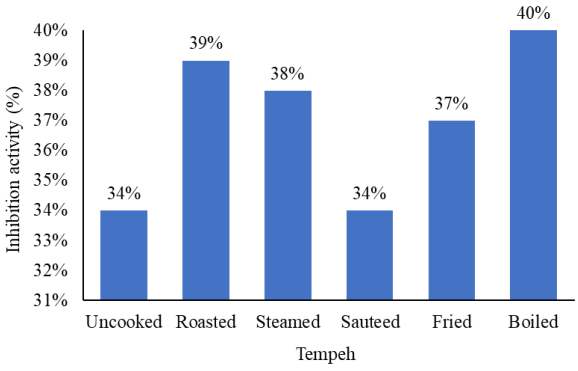

Figure 3. Inhibition activity of tempeh using the DPPH method

Based on Figure 3, the inhibition percentage for boiled tempeh had the highest inhibition activity of 39%, whereas sauteed dan raw tempeh had the lowest activity of 34%. In contrast, the steamed and fried tempeh did not show significant differences in inhibition activity. Many previous studies reported that antioxidant levels decreased after the cooking process depending on several factors, including cooking method, heating temperature, cooking time, enzymatic oxidation during preparation, and surface area exposed to water and oxygen [14]. However, several studies also reported that the same thing happened in this study: the antioxidant content increased after cooking. Sun *et al.* [35] reported that the antioxidant activity of sweet potato leaves increased after steaming and frying.

The DPPH method is based on the ability of antioxidants to inhibit free radicals by donating hydrogen atoms. DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution gives maximum absorption at 517 nm (purple color). The purple color of the DPPH fades when the antioxidants react with the DPPH solution because it is reduced due to the donation of hydrogen atoms. The DPPH purple color fades to yellow, causing the absorbance value to decrease and the inhibition percentage obtained to be higher [36]. The reaction between DPPH and antioxidant compounds with the donation of hydrogen atoms is shown in Figure 4.

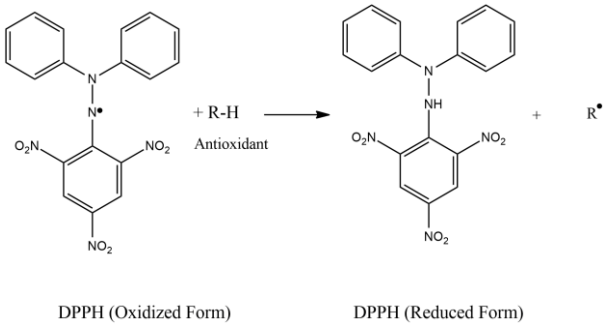

Figure 4. DPPH reaction with antioxidant compounds [37]

Reducing power is the following method for measuring antioxidant activity. This method is based on the reduction reaction of Fe^{3+} to Fe^{2+}. The reduction process of the iron (III) cyanide complex to Fe^{2+} can be measured using a spectrophotometer at a wavelength of 700 nm. The change can be seen in the solution's formation of a blue color. The higher the absorbance measured, the higher the reduction ability. The results of testing the antioxidant activity can be seen in Figure 5.

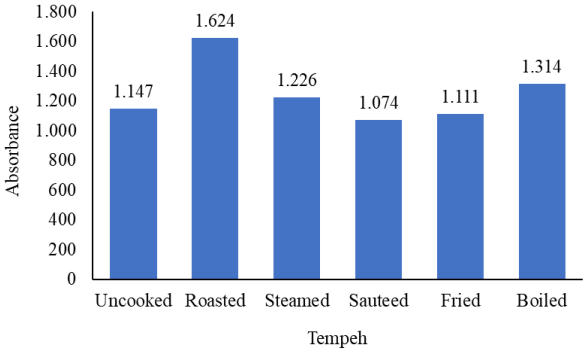

Figure 5. Antioxidant activity of tempeh using the reducing power method

Based on Figure 5, the tempeh sequence with the highest antioxidant activity starts from roasted, raw, boiled, steamed, and fried, while sauteed tempeh has the lowest antioxidant activity. The results are highly consistent with those obtained when assessing the antioxidant activity using the DPPH method. The tempeh that had been roasted and boiled received the highest activity from the two assays, whereas the sample of sautéed tempeh had the lowest activity.

Several studies show a correlation between antioxidant activity and phenolic content. However, this study did not show a correlation between the antioxidant activity of the DPPH method and the phenolic content of the extracts. Raw tempeh has a significantly higher total phenol content, which is a noticeable difference. This differs greatly from the capacity yield data on antioxidants using the DPPH method, which shows the lowest results. This finding is similar to research from Arbianti *et al.* [26], who reported no correlation between antioxidant activity and phenolic content for elephant apple leaf extract. This may be because fruits and vegetables contain many antioxidant components, including carotenoids, vitamins, phenolic compounds, and flavonoids, that can affect the measurement of antioxidant activity. Research that is more specifically in common with the results of this study is from Sun *et al.* [35]. His research on the effect of the cooking method on cassava leaves on antioxidants found that the sample extract without cooking treatment had a low antioxidant activity but a high total phenol content. This may be due to producing other redox-active secondary plant metabolites or breakdown products. However, it is likely related to changes in the structure of plant cell walls, the more efficient release of other antioxidants from intracellular proteins, and matrix modifications.

This study shows that the levels of antioxidants in the roasting and boiling processes increased while the levels of total phenols and flavonoids decreased. This is possible due to the activity of other compounds that act as antioxidants. The compound in question is an antioxidant peptide. Peptides have been reported to display significant antioxidant activity. Utari *et al.* [38] reported that the amino acids found in tempeh could be seen in Table 2.

A high proportion of hydrophobic amino acids has been reported in peptides with high antioxidant activity, compared to other hydrophilic amino acids, which is thought to be a critical factor in the ability of peptides to scavenge radicals. Hydrophobic amino acids frequently tested include histidine, phenylalanine, proline, glycine, lysine, isoleucine, and valine. Histidine residues exhibit strong radical scavenging activity in oxidative reactions, especially for enzyme-catalyzed reactions, due to the presence of the imidazole ring as an essential proton donor. Another study showed that the antioxidant capacity of peptides could be increased, especially in the presence of three aromatic amino acids (tryptophan, tyrosine, and phenylalanine) [39]. Previous research by Liu *et al.* [40] showed that samples cooked at 100°C showed higher antioxidant activity than those uncooked. This fact indicates that an increase in the potency of bioactive peptides after cooking is possible due to the formation of new antioxidant peptides with satisfactory heat stability. Therefore, the heat treatment used here can increase the antioxidant activity of some peptides by changing their secondary structure. In this study it is possible that tempe has increased antioxidant activity due to peptides with non-phenolic amino acids.

Table 2. Content of protein and amino acids per 100 grams of tempeh [38]

Parameter	Results %w/w net weight
Proteins	16.85
Amino acids	
Arginine	6.58
Glutamic acid	1.74
Aspartic acid	1.13
Serine	0.50
Histidine	0.31
Glycine	0.42
threonine	0.44
Alanine	0.47
Tyrosine	0.40
Methionine	0.15
Valine	0.58
Phenylalanine	0.53
I-leucine	0.51
Leucine	0.76
Lysine	0.95
Tryptophan	0.13

4. Conclusion

Raw tempeh has been successfully given different cooking treatments, such as roasting, steaming, boiling, sautéing, and frying. The ethanolic extracts of tempeh were obtained in the form of liquid. The total phenolic and flavonoid content of all processed tempeh decreased compared to raw tempeh. Among the various cooking methods, roasting had the highest total phenolic and flavonoid content, while the lowest was sautéing for total phenolic content and steaming for total flavonoid content. The results of the antioxidant activity assay were not in line with the total phenolic and flavonoid contents because the highest inhibition activity was obtained by boiling and roasting, while the lowest was sautéing.

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