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Effect of Steaming Time on The Protein Quality and Functional Properties of Soybean Paste

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ABSTRACT

Thermal processing is essential for enhancing the nutritional quality and protein digestibility of soybeans. Steaming, which uses hot vapor to minimize direct contact between the food and water, as in boiling, thereby better preserves nutrient content. However, excessive heating can decrease the quality and functional properties of the protein; therefore, the selected cooking time, specifically the steaming time, must be determined. This study investigated the effects of varying steaming times (25, 40, and 55 minutes) on the protein quality—specifically protein content and in-vitro protein digestibility—and functional properties, including emulsion stability and water holding capacity, of soybean paste. Results indicated that prolonged steaming significantly reduced both protein quality and functional properties. Extended thermal exposure resulted in excessive protein denaturation and aggregation, thereby disrupting the structural integrity required for effective water binding and emulsion stabilization. The selected steaming time was 25 minutes, yielding a protein content of 29.62 ± 1.10% and an in vitro protein digestibility of 44.40 ± 0.36%. Furthermore, at this selected steaming time, the soybean paste exhibited the highest emulsion stability and water holding capacity values of 92.79±1.76% and 71.25±1.77%, respectively. These findings support SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 12 (Responsible Consumption and Production) by establishing a resource-efficient method for producing high-quality, sustainable plant-based proteins. This 25-minute steaming duration establishes a standardized approach to enhancing the nutritional and functional profiles of soybean-based ingredients.

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1. Introduction

Soybeans are a type of legume known as a high-quality source of plant-based protein. Soybeans are considered one of the best food sources for fulfilling the body's protein needs compared to other types of nuts or legumes. In addition to their high protein content, which ranges from 35% to 40%, soybeans also contain other essential nutrients, including dietary fiber (9%), fat (20%), and water (8.5%). In general, the utilization of plant-based protein sources, such as soybeans, is highly relevant to efforts to achieve Sustainable Development Goal (SDG) 2: Zero Hunger, particularly the target of preventing malnutrition and ensuring access to nutritious and sustainable food. The amino acid composition of soybeans closely resembles milk and whey protein, especially when compared to other plant-based sources such as peas and barley. In terms of protein quality, the Digestible Indispensable Amino Acid Score (DIAAS) of soy protein is also comparable with animal protein sources such as milk and eggs. Typically, the DIAAS of milk and eggs ranges from 1.00 to 1.31, indicating their very high protein quality, while the digestibility of soybeans ranges from 0.84 to 0.92. This value is considerably higher than that of other plant-based materials and nearly approaches the DIAAS scores of milks and eggs, which are animal protein sources. Therefore, protein from soybeans can serve as an ideal alternative to animal protein [1,2].

The protein quality of soybeans can be further enhanced through various processing techniques, including cooking, soaking, germination (sprouting), extrusion, fermentation, and protein isolation or concentration. Among these thermal treatments, including heating, irradiation, and autoclaving, are commonly applied as standard methods in the processing of plant-based proteins. This is because they are effective at reducing the content of toxic and antinutritional compounds, such as protease inhibitors, which inhibit digestive enzymes, thereby increasing protein digestibility. The heating process also facilitates the decomposition of large protein molecules into smaller peptides and amino acids, thereby making them more readily absorbed by the body. However, prolonged heating can promote protein

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aggregation, thereby limiting amino acid release during digestion and reducing both bioavailability and nutritional value of the protein [2,3]

In addition to improving nutritional value, thermal processing of soybeans enhances sensory attributes, such as taste and aroma, thereby increasing consumer acceptability. Cooking can enhance flavor and aroma, inactivate pathogenic microorganisms, prolong shelf life, and improve nutrient bioavailability. Furthermore, cooking can enhance the nutritional value and physiological properties of food. Nevertheless, each cooking technique has a different impact on the physicochemical quality and nutritional content of the food material. This variation is influenced by factors such as temperature, heating time, and treatment intensity [2,4].

The conventional cooking methods commonly used nowadays are steaming, boiling, and frying. Steaming is a cooking technique that uses hot steam to achieve the desired degree of doneness. This method minimizes direct contact between food and water, thereby preserving nutrient content (e.g., protein, vitamin C, glucosinolates, and polyphenols) and complex structures within the food. Vegetables such as white cauliflower, broccoli, and Brussels sprouts exhibit preferable physicochemical, nutritional, and sensory characteristics when steamed rather than boiled [5]. Steaming also preserves the natural color of the food material, as it does not excessively trigger the Maillard reaction, which is common during frying. In addition, the resulting texture of the steamed ingredients is typically softer and moister, with higher moisture content. One factor influencing the steaming method is time. The steaming time requires investigation to ensure that the nutritional content of the cooked products is preserved or enhanced while minimizing energy consumption [6]. However, to date, no studies have investigated the variation of cooking time, specifically steaming time, on the characteristics of cooked soybeans.

The urgency of this study lies not only in food innovation but also in its direct contribution to the Sustainable Development Goals (SDGs), particularly SDG 2: Zero Hunger and SDG 3: Good Health and Well-being by enhancing nutritional utilization, and SDG 12 (Responsible Consumption and Production) by promoting resource-efficient processing. By refining the processing of a sustainable, locally sourced protein (soybean), this research aims to support Target 2.2 (ending malnutrition through high-quality food) and Target 3.4 (reducing premature mortality from non-communicable diseases by promoting healthier plant-based diets, as soybean is inherently cholesterol-free and naturally low in saturated fat—two primary dietary risk factors for cardiovascular diseases).

Soybeans are typically cooked by the boiling method, as demonstrated by Wijayanti et al. [7]. Moreover, cooked soybeans are typically milled into flour, whereas in this study, steamed soybeans of varying durations will be processed into a paste. This approach offers several benefits, including increased efficiency of soybean paste (SP) application in various plant-based products, as it eliminates the need for drying and milling, thereby enhancing process efficiency (SDG 12) and potentially retaining higher levels of soluble protein. The use of a paste form can retain higher levels of soluble protein, thereby enhancing the functionality and nutritional quality of soybean in more applicable formulations. Therefore, this research aims to determine the effect of steaming time on SP production and its impact on the quality and functional properties of soybean protein, thereby supporting the development of nutritious and sustainable food products.

2. Research Methodology

2.1 Materials and Equipment

The tools used in this study included analytical balance (Ohaus), table balance (Sartorius), blender (Phillips), chopper (Phillips), steamer (Phillips), stove, oven (Memmert UNB 500), water bath (Memmert), desiccator (Duran), glassware (Iwaki Pyrex), heater (Barnstead), Kjeldahl digestion dan distillation system (Buchi), centrifuge (Hettich Zentrifugen EBA 200), chromameter (Konica Minolta CR- 400). The materials needed in this research are soybeans, water, selenium (p.a, Smart Lab), K_2SO_4 (Smart Lab, Indonesia), H_2O_2 (Smart Lab, Indonesia), H_2SO_4 (Smart Lab, Indonesia), boric acid (Smart Lab, Indonesia), NaOH (Smart Lab, Indonesia), HCl (Smart Lab, Indonesia), hexane (Smart Lab, Indonesia), ethanol (Smart Lab, Indonesia), phosphate buffer, α-amylase enzyme, protease enzyme (Smart Lab, Indonesia), dan amyloglucosidase enzyme (Sigma Aldrich).

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2.2 Processing of SP

The SP production procedure was developed based on Apriliana et al. [8], Nazarena et al.[9] and Pradhananga [10] with modifications. Dehulled yellow soybeans were selected for this study and purchased from the online market in Jakarta. The process started with washing, draining and subsequent soaking the soybeans in 0.5% sodium bicarbonate solution (1:2, w/v ratio) for six hours; this crucial step serves to soften the cell wall, remove off-flavor compounds, and inactivate the lipoxygenase enzyme, although the time must be controlled to prevent the rupture of beans and subsequent leaching of protein reserves. Following soaking and draining, the soybeans are steamed for 25, 40, or 45 minutes [10]. Based on protein content in soybean paste at various steaming times during preliminary trials (20, 25, 30, 40, 50, and 55 minutes), it was observed that protein content increased from 20 to 25 minutes but declined at 30 minutes. Furthermore, a 15-minute steaming interval yielded a greater difference in protein content than 5- or 10-minute intervals. Consequently, the steaming time selected as a factor in this study begins at 25 minutes and proceeds with 15-minute intervals, specifically at 40 and 55 minutes. After steaming, the cooked beans are drained again and blended to yield the final SP. This paste is then analyzed for protein content (Kjeldahl method), In Vitro Protein Digestibility (IVPD) (enzymatic method), Water-Holding Capacity (WHC), and Emulsion Stability (ES), with the selected steaming time determined by protein content and IVPD.

The protein content measurement is conducted using the Kjeldahl method (AOAC 928.08) [11]. This analysis began with sample preparation, followed by digestion. After digestion, the analysis proceeds to the distillation stage. The resulting distillate is titrated with 0.2 N HCl until the solution turns pink. Following the titration step, the protein content of the sample can be calculated using the following formula (**Eq.1**). The production of soybean paste involves steaming, which triggers extensive protein denaturation and the formation of insoluble protein-carbohydrate complexes. Both colorimetric methods (Lowry and Bradford) require proteins to be in a solubilized, accessible state. In contrast, the Kjeldahl method uses high-temperature acid digestion (H_2SO_4) to completely mineralize the organic matrix, converting all organic nitrogen to ammonium sulfate ($[NH4]_2SO_4$). This ensures that protein quantification remains independent of changes in solubility or structural folding induced by steaming time.

$$Protein\ (\%) = \frac{V1-V2\ x\ N\ HCl x 14.008 x 6.25}{W x 100} \quad (1)$$

V1= volume sample (ml)
V2 = volume blank (ml)
W= weight of sample (g)

The protein digestibility analysis is conducted in vitro, a laboratory method that mimics the gastrointestinal tract [12], utilizing the enzyme pepsin to cleave proteins into amino acids. The procedure starts by preparing a 5 g sample and adding 20 mL of pH 2 Whaffole buffer. Afterwards, 2 mL of 1% pepsin solution is added, and the mixture is incubated for 1 hour at 40°C. To halt digestion and precipitate undigested protein, the sample is filtered and centrifuged, followed by the addition of 5 mL of 5% trichloroacetic acid (TCA). After settling for 1 hour, a 5 mL aliquot of the filtrate is taken for subsequent protein content determination, and the final protein digestibility is calculated by comparing the remaining (undigested) protein in the filtrate against the total initial protein, using the formula as stated in **Eq. 2**.

$$IVPD\ (\%) = \frac{protein\ content\ (enzyme\ treated)}{total\ protein\ content} \quad (2)$$

The WHC of the SP is analyzed using the centrifugal method [13], which begins by mixing a 2 g sample with 10mL of deionized water in a centrifugation tube. The tube is then subjected to centrifugation for 20 minutes at a speed of 3,000 rpm, leading to the separation of a supernatant from the solid pellet. The supernatant is carefully decanted, weighed, and its weight is recorded, allowing the WHC value—which represents the percentage of water retained by the sample—to be calculated using the formula (**Eq.3**) [14].

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$$WHC\ (\%) = \frac{W1 - W2}{W} x100\% \quad (3)$$

W1= weight of water added (g)
W2 = weight of supernatant (g)
W= weight of sample (g)

The ES analysis began by mixing 0.5 g of the sample with 5 mL of distilled water and 5 mL of soybean oil, then homogenizing for 1 hour using a vortex mixer. The mixture was initially centrifuged at 3000 rpm for 15 min. Subsequently, the sample was incubated in a water bath at 80°C for 30 min. After cooling at room temperature for 15 min, the sample underwent a second centrifugation cycle at 3000 rpm for 15 min until a visible separation layer formed. Phase separation was quantified by measuring the volume of the supernatant oil layer. The incorporation of additional water and oil was performed to evaluate the dough's emulsifying capacity under thermal stress. The lower percentage of ES indicates a stable protein-lipid interface capable of maintaining the fat fraction within the matrix during heat treatment. The percentage of ES is calculated using the following formula, as shown in **Eq. 4** [15].

$$ES\ (\%) = \frac{Ve}{Vt} x100\% \quad (4)$$

Ve: volume of emulsion layer
Vt: total volume of emulsion

2.3 Statistical Analysis

To determine the effect of steaming the soybean to produce SP, a completely randomized design with three levels of one factor, namely 25 minutes, 40 minutes, and 55 minutes. This research involved three replications, each with three repetitions. The data were analyzed using SPSS version 25, One-Way ANOVA, and Duncan post hoc tests.

3. Result and Discussion

3.1 Effect of Soybean Steaming Time on The Protein Quality of SP

Protein quality is assessed in this study using protein content and protein digestibility. Protein content measures the total amount of protein, while protein digestibility measures the extent to which the protein can be digested and absorbed by the body. Steaming is a heat-based processing method that uses hot vapor to cook the material until it reaches the desired level of doneness. Because the food has little to no direct contact with water, this technique helps retain nutrients such as protein, reduces anti-nutritional factors, and maintains physical attributes such as color [5]. The factors influencing the steaming method are temperature and time. Appropriate temperature and steaming time must be used to ensure that the cooked material achieves the optimal flavor profile and maintains or enhances its physicochemical characteristics [6]. However, during the steaming process, the steam temperature is generally relatively stable at 100°C. Therefore, the factor that most significantly affects the steaming process is the steaming time.

Table 1 shows the protein quality of SP in various steaming times and indicates a significant difference in protein content and protein digestibility of the SP across the varied steaming times. The 55-minute steaming time resulted in significantly lower protein content than both the 25-minute and 40-minute treatments. This observation suggests that prolonged steaming induces thermal degradation of soybean protein, reducing total protein content. This finding is consistent with Liao et al. [16], who reported a decrease in the protein content of black soybeans as the steaming cycle duration increased, with the value decreasing from 36.55% to 34.48% over time. Moreover, the results of this study are also supported by Samben and Puspaningrum [17]. The lowest protein content of pigeon pea was observed

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at 30 minutes of steaming (12.66%), whereas steaming for 10 minutes resulted in the highest protein content (12.98%).

Table 1 Protein Quality of SP in Various Steaming Time

Steaming time (minutes)	Protein content (%)	IVPD (%)
25	29.62±1.10[a]	44.40±0.36[c]
40	30.21±1.99[a]	33.20±0.09[b]
55	28.54±2.74[b]	27.67±0.52[a]

Notes: Value in the table shown by the average±SD. Value with different superscript in the same column has a significant difference at 5%

Furthermore, prolonged steaming time can lead to excessive Maillard browning, resulting in numerous amino acid amine groups reacting with sugars. This results in lower residual protein in the final product because a significant amount of protein is consumed during the Maillard reaction. The 25-minute and 40-minute steaming times did not differ significantly in protein content. However, both steaming durations yielded the highest protein content, whereas the 55-minute duration yielded the lowest. During the 25-minute and 40-minute time, the increase in protein is a function of mass balance; as heat facilitates the evaporation of water, the relative proportion of the remaining solid matter (including nitrogenous compounds) increases. This represents the optimal point of dehydration where the nutrient density is maximized. However, at 55 minutes, the intense heat causes amino acid amine groups to covalently bond with reducing sugars, forming melanoidins. This chemical transformation leads to a measurable decrease in residual total protein, as the nitrogen is incorporated into complex Maillard products that may alter the recovery during analysis. Thus, the 25–40 minute range serves as the peak equilibrium between physical moisture loss and chemical stability [18].

Table 1 also shows that as steaming time increases, the SP's IVPD decreases. During the steaming process, protein denaturation occurs, initially increasing protein availability. This protein denaturation is beneficial because the protein's structure unfolds, making it more accessible to enzymes and easier to hydrolyze. However, excessive or prolonged heating can trigger the Maillard reaction, in which the free amino groups of proteins (especially lysine) react with the carbonyl groups of carbohydrates. This reaction can form cross-links, thereby reducing protein solubility and increasing resistance to proteolytic enzymes. Consequently, this leads to a reduction in digestibility and the overall availability of amino acids (particularly lysine) [19,20]. Although initial denaturation unfolds the protein structure, prolonged heating can cause denatured protein molecules to aggregate, forming strong bonds (e.g., disulfide bonds) that subsequently lead to coagulation. Coagulation causes the protein matrix to become denser and more compact, thereby limiting the access of digestive enzymes to the matrix and its hydrolysis. As steaming time increases, Maillard reactions and protein aggregation become more pronounced, ultimately reducing protein digestibility [21,22].

According to Ohanenye et al. [23], the IVPD value for soybeans is approximately 56%. Although the IVPD value obtained in this study did not meet the reference value, the 25-minute steaming time showed the highest significant IVPD (44.40±0.36%) compared with the 40-minute (33.20±0.09%) and 55-minute (27.67±0.52%) treatments. The result indicates that a 25-minute steaming is sufficient to inactivate trypsin inhibitors and properly denature the protein without causing heat-related structural damage. This balance yields the highest digestibility. The significant decrease of protein and IVPD at 40- and 55-minutes shows that longer steaming time promotes further cross-linking within the protein matrix, which makes the protein harder for enzymatic hydrolysis, thus resulting in a decrease of IVPD [20,24,25]. This finding is also consistent with Adeleye et al. [26], who reported a decrease in protein digestibility of pigeon pea when cooked at 140°C (27.66%) compared to 100°C (64.95%).

3.2 Effect of Soybean Steaming Time on The Functional Properties of SP's Protein

ES is the ability of a protein to stabilize an emulsion (a mixture of oil and water). At the same time, WHC can bind protein to water. WHC is a functional property of a protein that measures the material's ability to bind and retain water molecules [14]. The WHC value will influence the product characteristics when the raw material, in this case, SP, is processed into a final product. A high WHC value will make the product chewier and less dry and will also increase its yield [13,15,27]. **Table 2** shows the functional properties of SP's protein in various steaming times. Based on **Table 2**, ES and WHC decreased

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significantly as steaming time increased, indicating structural damage to the protein caused by excessive heat, thereby reducing the SP's ability to stabilize emulsions and bind water.

Table 2 Functional Properties of SP's in Various Steaming Time

Steaming time (minutes)	ES (%)	WHC (%)
25	92.79±1.76^{c}	71.25±1.77^{c}
40	87.23±1.89^{b}	58.41±1.45^{b}
55	84.13±2.11^{a}	52.29±2.46^{a}

Notes: Value on the table shown by the average±SD. Value with different superscript in the same column has a significant difference at 5%

Before steaming, soybean proteins are mainly globular, with hydrophobic groups trapped inside the molecule and hydrophilic groups exposed on the surface. When heating begins—particularly during the first 25 minutes—the protein unfolds in a controlled manner, initiating denaturation without structural damage. Controlled denaturation creates conditions that allow the protein to function effectively in stabilizing emulsions and binding water. As the protein unfolds, previously buried hydrophobic groups become exposed to the surface, enabling these interactions. This exposed structure creates ideal conditions for coating the emulsion droplets formed within the soybean paste, resulting in a more stable emulsion and yielding the highest significant ES value of 92.79±1.76 %. The open protein structure also facilitates the formation of a more elastic gel matrix or a loose protein network. This elastic network condition creates larger interstitial spaces, allowing water to be bound both physically and chemically through hydrogen bonds, resulting in the highest WHC value of 71.25±1.77 % [28, 29, 30].

However, when the heating time is prolonged to 40 minutes and 55 minutes, the protein undergoes excessive denaturation and aggregation. This excessive aggregation causes proteins to bind permanently to one another via disulfide bonds, which leads to the formation of larger and insoluble aggregates, which in turn diminishes the protein's ability to stabilize emulsions and bind water [24,31, [32]. Once the proteins form these larger and more rigid aggregates, they can no longer effectively coat the emulsion system in the SP. This is demonstrated by the significantly lower ES value of 84.13±2.11% compared with the 25-minute steaming time. Furthermore, the increasingly rigid and dense matrix reduces the volume of the internal spaces, meaning it can no longer physically hold water. This causes water to be expelled from the matrix, and the protein loses its ability to bind or retain water, as evidenced by consistently lower WHC values (52.29±2.46%). The decreasing of WHC is also shown in field bean protein isolate that undergo steaming for 15, 30 and 45 minutes. The 45 minutes steaming resulting in a low WHC value (2.550±0.01%) compared to 15 minutes (2.785±0.01%) and 30 minutes (2.745±0.01%). The decreasing value of ES is also shown in brindle beans during steaming process using autoclaves. The ES of raw brindle bean is the highest (58%) compared to 5 minutes steaming (54%) and 20 minutes steaming (52%) [33,34].

Table 1 and **Table 2** demonstrate a linear relationship between the protein quality and the functional properties of SP. Steaming for 55 minutes resulted in a significant loss of protein quality, with IVPD falling to nearly half (27.67±0.52%) of the 25-minute value (44.40±0.36%). This suggests that prolonged heating induces protein-protein aggregation, making the protein less accessible to enzymes (Table 1) and less effective in stabilizing food systems (**Table 2**). The correlation between lower IVPD and decreased WHC and ES indicates that thermal damage to the protein structure is the primary cause of functional failure. This structural degradation is correlated with the results in Table 2, where (WHC) and ES show a downward trend. Specifically, the decrease in IVPD from 25 to 55 minutes corresponds to decreases in WHC from 71.25±1.77% to 52.29±2.46% and ES from 92.79±1.76% to 84.13±2.11%, as unfolded or aggregated proteins lose the surface-exposed hydrophilic and hydrophobic groups necessary for water binding and oil-water stabilization. These findings indicate that the thermal degradation of the protein primary/secondary structure (**Table 1**) is the underlying driver for the functional failures observed in the final food system (**Table 2**).

4. Conclusion

Steaming time significantly affects the protein quality and the functional properties of SP's protein. As steaming time increased, protein content and digestibility decreased, indicating a loss of protein quality.

Prolonged heating also reduced the ability of SP to stabilize emulsions and bind water, demonstrating a corresponding decline in its functionality. The selected steaming time for producing SP was 25 minutes. At the 25-minute steaming time, the SP exhibited protein content and protein digestibility of 29.62% and 44.40%, respectively. In addition, at this optimal steaming time, the SP showed ES and WHC values of 92.79±1.76% and 71.25±1.77%, respectively.

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