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Effects of Seed Germination on Physicochemical and Bioactive Compounds Characteristics of Velvet Bean Tempe

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Abstract

Indonesia, known as the largest tempe-producing country globally with approximately 160,000 producers, faces a challenge in meeting the demand for tempe due to insufficient local soybean supply. To address this issue, Indonesia has been compelled to import significant quantities of soybeans, reaching 1.68 million tons in 2020, which escalated by 47.78% to 2.49 million tons in 2021. This study aims to scientifically investigate the impact of velvet bean seed germination on the physicochemical characteristics and bioactive components of velvet bean tempe. The research methodology involved quantitative analysis to obtain reliable results. The preliminary analysis established that the optimal velvet bean tempe fermentation time was 48 hours. Following germination, the physical properties of velvet bean sprouts exhibited an increase in weight accompanied by a reduction in bulk density and absolute density. Furthermore, the germination treatment resulted in decreased brightness, saturation, whiteness, and pH values, while simultaneously increasing the hardness of the tempe. Chemical analysis demonstrated that germination of velvet bean seeds led to an elevation in moisture content and a decrease in carbohydrate content within the produced tempe. Additionally, bioactive component analysis revealed that the germination treatment contributed to a reduction in total phenol content from 52.46 to 36.30 mg AAE/100g, antioxidant capacity from 132.80 to 66.90 mg GAE/100g, and GABA content from 54.20 to 21.50 mg/100g in the tempe. These findings provide valuable scientific insights into alternative ingredient utilisation and production processes optimisation of velvet bean tempe.

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Introduction

Indonesia holds the distinction of being the largest tempe producer globally, representing a thriving soy-based market in Asia. With a substantial number of tempe producers reaching 160,000 units as of 2021, Indonesia's tempe industry plays a crucial role in the country's economy, particularly among micro, small, and medium enterprises (MSMEs).[1] However, the substantial tempe production in Indonesia necessitates a robust supply of local soybeans. Unfortunately, soybean productivity in the country remains relatively low, averaging only 1.5 tons per hectare.[2] Consequently, Indonesia has become heavily reliant on imports, with an annual requirement of 2.2 million tons of soybeans.[3] Notably, tempe producers exhibit a preference for imported soybeans due to guaranteed supply and more affordable prices when compared to local soybeans.

Amidst this scenario, exploring alternative raw materials for tempe production becomes imperative. One such option is the utilisation of velvet bean seeds (Mucuna pruriens (L)), locally known as koro benguk, as a substitute for soybeans. Although velvet bean seeds are relatively lesser known, they have already found applications in various regions of Java Island. In Indonesia, velvet beans demonstrate higher productivity, averaging around 3-4 tons per hectare, compared to soybeans' productivity of 1-2 tons per hectare. Additionally, velvet bean seeds contain approximately 24-37% protein content, which is comparable to soybeans' protein content of 43%.[4] Considering the local abundance and protein source potential, exploring velvet bean seeds as raw materials for tempe production is an attractive proposition. However, it is crucial to address challenges associated with high cyanide acid content and the seed's tough texture, which necessitates pre-treatment and specific processing conditions.

The germination process represents a simple method to enhance seed quality. Previous studies have highlighted the benefits of germination in improving digestibility and protein bio-accessibility in beans.[5] Enzymes become highly active during germination, facilitating the hydrolysis of macromolecules such as carbohydrates and proteins into simpler compounds, thereby enhancing digestibility and absorption.[6, 7]

This study aims to achieve three objectives: (1) analyse the physicochemical and bioactive component alterations resulting from velvet bean seed germination, (2) determine the optimal fermentation time for velvet bean tempe production, and (3) examine the physicochemical and bioactive compound characteristics of the resulting tempe. By addressing these objectives, this research contributes to a deeper understanding of the potential utilisation of velvet bean seeds in tempe production, paving the way for novel and sustainable approaches in the Indonesian tempe industry.

Materials and Methods

Materials

The materials used in this study were velvet bean seeds obtained from a supplier, a farmer from Situbondo, East Java. There was also tempe inoculum Raprima® as the source of *Rhizopus* spp. (tempe starter) mold in the fermentation process and perforated polypropylene plastic as tempe packaging. The materials used for analysis comprised filter paper, n-hexane, HCl, H_2SO_4, HgO, K_2SO_4, Na_2SO_3, boric acid (H_3BO_3), distilled water, phenolphthalein indicator (PP), NaOH, methanol, ascorbic acid, ethanol, DPPH, Folin-Ciocalteu reagent, sodium hypochlorite, phosphate buffer solution and Gamma-aminobutyric acid (GABA) standard solution 500 ppm.

The tools that were used in this study were tempe production equipment and analysis apparatus. The tools for the tempe production process consisted of a digital scale, basket, huller, stove, boiling pot, aluminium pan, fan, sealer, molder, fermentation shelf, and LPG gas holder. The analysis equipment was fluidised bed dryer (Indonesian local made), blender (Miyako BL-101 PL, Indonesia), oven (Memmert UF30, Germany), 80-mesh sieve (GB/T6003, China), desiccator, analytical scale (Fujitsu FS-AR210, Japan), Chromameter (CR-400 Minolta, Japan), magnetic stirrer, spectrophotometre UV-Vis (UVmini-1240. Shimadzu, Kyoto, Japan), pH metre, centrifuge with r = 18 cm (Eppendorf 5810, Germany), digital water bath (LABTECH, Korea), texture analyser TA1 (AMETEK Lloyd Instruments Ltd, USA) and various laboratory glassware.

Methods

This study was conducted in two phases, which were (1) determining the effects of germination on the physicochemical properties of velvet beans as tempe raw material, and (2) determining the optimum fermentation time of tempe produced from germinated and non-germinated velvet beans. Figure 1 shows the flowchart of the study. In the initial stage, the velvet gean germination was done for 24 hours, specifically until a 2-5 mm long radicle was produced. The germination process was done by watering the seeds every 6 hours and stored in dark conditions.

The second phase was the determination of the best time for tempe production, which needed to be based on the hyphae appearance of *Rhizopus* spp. on the tempe surface. The tempe production was done using Raprima® starter purchased from Rumah Tempe Azaki in Bogor, West Java, Indonesia. The non-germinated velvet bean seeds and germinated velvet bean seeds were processed into tempe simultaneously in different containers. The tempe production from germinated velvet bean seeds was done one day earlier than non-germinated velvet bean seeds so the harvesting could be done simultaneously.

Then, the analysis was done on four samples, namely: non-germinated velvet bean, germinated velvet bean, non-germinated velvet bean tempe, and germinated velvet bean tempe. The analyses which were done consisted of physical and chemical analyses. The physical analysis comprised brightness, saturation, hue, hardness, and pH. The chemical analysis consisted of proximate (water, ash, protein, fat, and carbohydrate contents) and bioactive components analysis (antioxidant capacity test, total phenolic content, and accumulation of Gamma-aminobutyric acid (GABA). The antioxidant capacity test and total phenol tests were done on each sample, why the GABA accumulation test was done only on tempe samples.

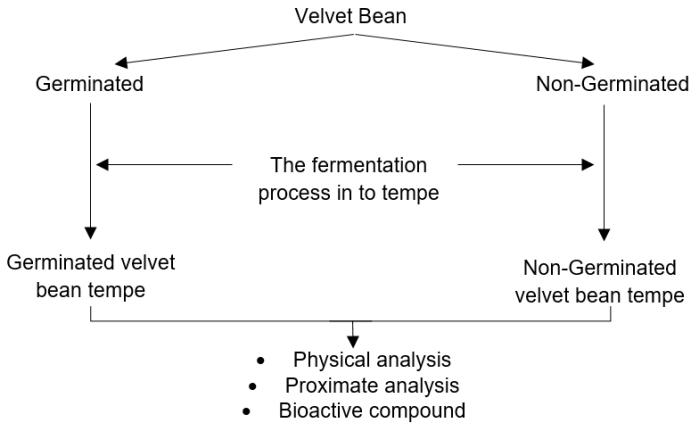

Fig. 1: Research flow chart

Physical Characteristics of Velvet Bean Seeds

The parameters used to determine the physical characteristics of non-germinated and germinated velvet bean seeds were weight per 100 seeds, bulk density, and absolute density. The measurement of 100 seeds was done using an analytical scale. One hundred velvet bean seeds were put inside a prepared dish and measured using an analytical scale. The measurement of bulk density was done using an analytical scale and beaker glass. Each sample was sorted into the beaker glass and adjusted at 100 mL. Then, the weighing of the 100 mL of the seeds sample was done using an analytical scale. The bulk density is defined as a gram/mL unit. The absolute bulk density was measured using an analytical scale and beaker glass. Unlike those of bulk density, absolute bulk density was measured based on added water volume. A sample of 20.00 ± 0.27 g was weighed and added into a 50 mL water-filled beaker glass. Then, the end volume of the water scale was read, and the increase (delta) of water was defined as the absolute volume of velvet bean seeds.

Production of Velvet Bean Tempe

The production of velvet bean tempe was conducted by referring to the flowchart shown in Figure 2. The sortation on velvet beans was done to sort the whole and dry beans. The sorted velvet bean seeds were soaked in clean water for 24 hours, then washed and placed in a basket layered with a damp cloth and covered. This germination treatment was performed on time variations of 0 hours (non-germinated as control) and 24 hours with periodic watering every 6 hours to maintain the environment humidity. The germination was done until a radicle of 2-5 mm sprouted.

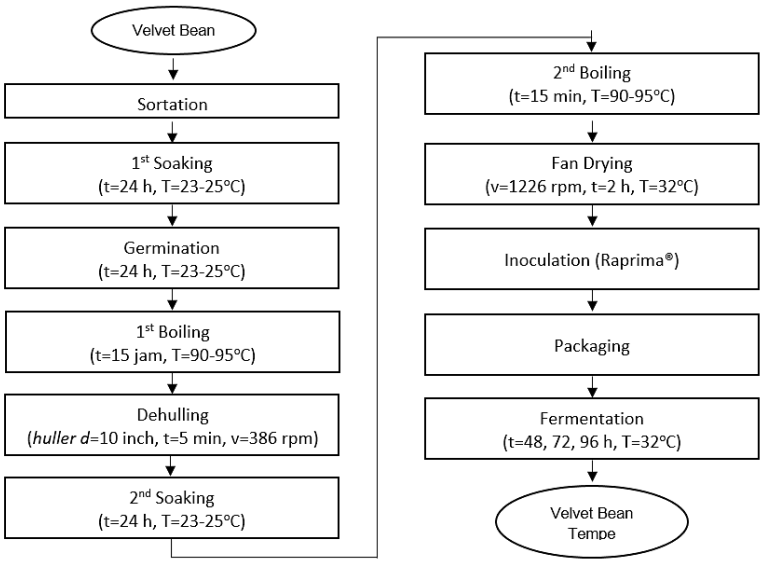

Fig. 2: Velvet bean tempe production flow chart

The velvet bean seeds were boiled at 90-95 oC for 1 hour with a ratio (w/v) of velvet bean: water of 1:10, then drained. Then, the velvet bean seeds were peeled using a huller to separate the skin from the seed. The second soaking was done on skinless velvet bean seeds, with a ratio (w/v) of 1:5 for 24 hours. This was a fermentation step by lactic acid bacteria to reduce the pH of the velvet bean to 4.5 – 5.0, which is the optimum pH range for the tempe starter to grow.

After the second soaking, the velvet bean seeds were boiled for 15 minutes, drained, and cooled at room temperature. The inoculation process was done by inoculating Raprima® starter onto the velvet bean seeds, with the starter dose of 2 g per 1 kg of velvet bean seeds was then mixed so the starter spread evenly on the seeds. Then, the mixture was packed into perforated polypropylene plastic bags. The production process of velvet bean tempe was performed for three alternative fermentation time, which was 48, 72, and 96 hours.

Determination of Optimum Fermentation Time

The optimum fermentation time is selected based on the appearance of mold hyphae on the surface and inside part of the tempe, as well as considering the protein content of the resulting tempe. To obtain the most optimum fermentation time, protein content analysis of tempe was done using Kjeldahl method on three different fermentation times; 48, 72, and 96 hours, with each treatment tested for eight repetitions. Then, the protein content of three samples was subjected to One Way ANOVA to determine the effects of fermentation time on tempe protein content. For a significantly different sample, it will proceed with the DMRT posthoc test to observe the significance of each treatment.

Physicochemical Analysis

The physicochemical analysis was done on four types of samples, namely non-germinated velvet bean seeds, germinated velvet bean seeds, non-germinated velvet bean tempe, and germinated velvet bean tempe. The tested parameters were texture,

pH, colour, proximate, and crude fibre analyses. The hardness measurement was carried out in the duplicate matter and defined as compression force in units of gf (gram force). The texture analyser instrument was used for the measurement. The used probe was the cylindrical model with a 2 cm diameter, 1.5 mm/second speed, and compression distance of 30 mm, and the sample was subjected to compression force to determine its maximum peak that presents the sample hardness level. The prepared non-germinated and germinated velvet bean seeds were skinless for this measurement. The specification of the tempe sample was cut into dice with a dimension of 1 x 1 x 1 cm3 and measured three times.

The colour measurement was carried out on four samples for two repetitions. The instrument used for the measurement was a Minolta chromameter CR-400. The outputs are L* and C* parameters. L* value is defined as a lightness parameter. C* value indicates chroma, which is the colour saturation of the sample. The higher the chroma value, the sample has more striking colour, and the lower the chroma value, the sample has more saturated/dull colour.

The proximate analysis[8] was done twice on each of the four samples. The analyses consisted of water (gravimetric method), ash (furnace method), fat (Soxhlet method), protein (Kjeldahl method), and carbohydrate (by difference method) content analyses. The analysis of crude fibre was performed using the gravimetric method.

Bioactive Compound Analysis

The samples were prepared by the flouring process. The sample was dried with a fluidised bed dryer for 2 hours, then milled with a blender at low speed for one minute, then sieved with 80-100 sieve. The flour samples were analysed for the following parameters; pH, antioxidant capacity, total phenolic content, and GABA accumulation.

One gram (dry sample) of flour sample was extracted with 10 mL of 95% methanol and vortexed for 2 minutes. The extraction process was followed with centrifugation at 3000 rpm, 4oC for 30 minutes. The resulted supernatant was taken and moved to a closed reaction tube to be analysed for its total phenolic content and antioxidant capacity.[9] The antioxidant capacity analysis referred to Barus *et al*.[10] Sample extract of 0.2 mL was taken and added with 0.8 mL of methanol and inserted into a closed reaction tube. DPPH reagent 170 ppm of 2.0 mL in methanol was dropped into the reaction tube using a micropipette. Then, the solution was homogenised using a vortex and set aside for 60 minutes in dark condition. Then, the sample was measured for its absorbance using UV-Vis spectrophotometre (UVmini-1240. Shimadzu, Kyoto, Japan) at 625 nm wavelength and calculated for its inhibition %. The result of antioxidant activity analysis was conducted two times and stated as the average mg equivalent of ascorbic acid/100g dry sample (mg AAE/100g).

The total phenolic content analysis referred to a study by Athaillah *et. al*.[11] Sample extract of 0.2 mL was mixed with 0.8 mL of 95% ethanol solution, then added with 0.8 mL of Folin-Ciocalteau reagent, which had been diluted ten times (freshly prepared) and 1.8 mL of Na_2CO_3 6%. After incubating for 90 minutes in dark storage, the sample was measured for its absorbance at 725 nm wavelength. The analysis was carried out two times and stated as mg equivalent of gallic acid per 100g dry sample (mg GAE/100g).

The GABA content analysis referred to Chalermchaiwat *et al*.[12] One gram of sample was inserted into a centrifuge tube and added with 10 mL of distilled water and then vortexed. The sample was centrifuged for 30 minutes at 3000 rpm at 4^{o}C. The supernatant was obtained, and the supernatant was drawn for 0.2 mL and inserted into a closed reaction tube, added with 0.2 mL of borate buffer (pH 9.0) and 1 mL of 6% phenol (b/v). The mixture was vortexed and cooled for 5 minutes before being heated in a water bath at 95oC for 10 minutes. Then, the tube was cooled in ice water for 20 minutes while shaken. Ethanol 60% (v/v) of 0.5 mL was added into the mixture and vortexed. Then, the sample was analysed using spectrophotometre at 645 nm wavelength. The standard curve was determined by determining the standard dilution of GABA with concentrations ranging from 0 to 500 ppm. The analysis was duplicated and stated as mg equivalent of GABA per 100g dry sample (mg GABA/100g).

Data Analysis

The data was processed by Microsoft Office Excel® and SPSS version 25. The analysis of variance and significance determination between the samples was

done using DMRT posthoc test at 5% significance level.

Results and Discussion

Physical Characteristics of Velvet Bean Seeds

The physical analysis of non-germinated and germinated velvet bean samples shows different characteristics, as stated in Table 1. The germination process resulted in a significant weight increase per 100 velvet bean seeds ($p<0.05$), from 70.40 to 108.38 g. The increased weight per 100 velvet bean seeds after germination was due to diffused water into the seeds, resulting in the increased weight. Bulk density and absolute density parameters results show a similar trend, and each sample experienced a significant decline ($p<0.05$) from 0.79 to 0.62 g/mL on the bulk density and from 1.35 to 1.14 g/mL on the absolute density. The decline of bulk density value was caused by the hydrolysis of complex compounds, such as carbohydrates and protein, due to the germination process.[13] The non-germinated velvet bean had higher bulk density than soybean, commonly around 0.81 g/mL.[14] Bulk density of a substance is contingent upon the solid density along with the specific geometrical dimensions, size, and surface attributes exhibited by each material.[15] The higher bulk density in the bean as tempe raw material will result in denser tempe, which eventually results in tempe that has greater weight in the same volume.

Table 1: Physical characteristics of non-germinated and germinated velvet bean seeds

Parameter	Velvet bean	
	Non-germinated	Germinated
Weight per 100 seeds (g)	70.59 ± 0.58^{a}	108.38 ± 0.26^{b}
Bulk density (g/mL)	0.79 ± 0.00^{b}	0.62 ± 0.00^{a}
Absolute density (g/mL)	1.35 ± 0.01^{b}	1.14 ± 0.01^{a}

Note: Means followed by different letters in the same row show significantly different at $\alpha = 0.05$.

Determining the Optimum Fermentation Time

The fermentation time variance for velvet bean tempe production comprised 48, 72, and 96 hours. The optimum fermentation time is chosen based on the best hyphae appearance on the surface and inside part of the tempe, as well the protein content. Figure 3 shows the hyphae appearance on three tempe samples fermented for 48, 72, and 96 hours. Tempe fermented for 48 hours had the most hyphae coverage on its surface compared to the 72- and 96-hours treatment. The proteolytic process by several species of yeast during prolonged fermentation is suspected in the decreased hyphae appearance on tempe.[16]

Visual and sensory-wise, tempe with longer fermentation (prolonged fermentation) had yellow-brownish colour, softer texture, and ammoniac-like odour.[17, 18] This was caused by Lactobacillus as the predominant yeast and *Lactococcus* as the predominant bacteria, contributing during the prolonged fermentation process.[19] The study by Abdurrasyid *et al*.[20] also showed a darker colour change in soybean tempe which were fermented for 72- and 96-hours. This was due to the mold experiencing the death phase and the appearance of oxidised double chain fatty acids (linoleic and linolenic acid). These fatty acids are vulnerable to oxidative damage, which has the potential to affect the colour of tempe. The process of oxidation not only damages the fatty acids, but also affects carotenoids, leading to the darkening of tempe's colour.[21]

Table 2 signifies the protein content of velvet bean tempe in various fermentation times. Velvet beans with 96 hours of fermentation time treatment had significantly higher protein content ($p<0.05$) than tempe fermented for 72 hours. However, the protein content in tempe that was fermented for 48 hours was not significantly different from the 72- and 96-hour treatments. Although the protein

content in 96-hours-fermented tempe was higher than 72 hours-fermented tempe, this does not always indicate the increase in overall protein content. On the contrary, prolonged hydrolysis from complex proteins into amino acids may occur during prolonged fermentation time. This can cause an increase in certain amino acid content, but it does not imply a significant increase in total protein content. This is supported by Utami *et al*., Abdurrasyid *et al*., Astawan *et al*., which show no significant difference in soybean tempe with prolonged fermentation treatment.[19,20,22] Therefore, it can be concluded that 48 hours was selected as the most optimum fermentation time.

48 h 72 h 96 h

Fig. 3: The presence of hyphae on the surface of velvet bean tempe after fermentation periods of 48, 72, and 96 hours

Table 2: Protein content of velvet bean tempe at various fermentation times

Fermentation Time (h)	Protein Content (%db)
48	39.94 ± 2.36ab
72	38.77 ± 1.84a
96	42.84 ± 4.70b

Note: Means followed by different letters in the same column show significantly different at α = 0.05.

Physical Properties of Velvet Bean Seeds and Tempe

The results of physical analysis on the seeds (non-germinated and germinated) and velvet bean tempe (non-germinated and germinated) are presented in Table 3. The germination treatment on velvet bean seeds resulted in a significant decrease in lightness value from 56.15 to 52.85. Non-germinated velvet bean tempe had the highest lightness value, which was 83.46, and it declined significantly ($p<0.05$) on germinated velvet bean tempe.

The chroma and colour intensity notation indicates the visibility of the sample ranging from the contrast to dull. The lower the chroma value, the sample looks duller in visual. Conversely, the higher the chroma value, the sample appearance will be contrasted. The germination treatment in velvet bean seeds reduced the chroma value significantly ($p<0.05$) from 7.84 to 6.33. The tempe also had a similar result, where the chroma value of the non-germinated tempe was significantly higher than the germinated tempe treatment. Whiteness intensity shows a significant difference in each sample. The highest whiteness intensity came from non-germinated velvet bean tempe (81.86), then followed by germinated velvet bean tempe (62.20), non-germinated velvet bean seeds (55.84), and germinated velvet bean seeds (52.42). The lower lightness and whiteness intensity in germinated tempe and seeds were caused by the high concentration of amino acids that increases the non-enzymatic browning reaction, such as the Maillard reaction.[23] The germination process also enables the increase of pigment quantity and activity, which results in lower brightness in germinated velvet bean seeds and tempe. Pigments that contribute to these changes include melanin, anthocyanin, and other phenolic compounds.[24]

Table 3: Physical properties of velvet bean seeds and tempe

Parameter	Non-germinated velvet bean	Germinated velvet bean	Non-Germinated velvet bean tempe	Germinated velvet bean tempe
Lightness	56.15 ± 0.08^{b}	52.85 ± 0.06^{a}	83.46 ± 0.88^{c}	53.84 ± 2,13^{ab}
Chroma	7.84 ± 0.48^{c}	6.33 ± 0.19^{a}	8.28 ± 0.07^{c}	7.00 ± 0.11^{b}
Whiteness	55.84 ± 0.01^{b}	52.42 ± 0.08^{a}	81.86 ± 1.33^{d}	62.20 ± 0.26^{c}
Hardness	88.63 ±0.02^{d}	16.47 ± 0.04^{c}	2.18 ± 0.06^{a}	5.03 ± 0.06^{b}
pH	3.25 ± 0.06^{a}	5.76 ± 0.06^{b}	6.52 ± 0.04^{d}	6.32 ± 0.02^{c}

Note: Means followed by different letters in the same row show significantly different at α = 0.05.

The hardness parameter shows significant differences ($p<0.05$) in all four samples. Non-germinated velvet bean seeds had the hardest texture of 88.63 kgf; then, it declined significantly after the germination process to 16.47 kgf. This was due to the shift in chemical composition during the germination treatment. This germination treatment can induce changes in chemical and structural properties in seeds which positively impact texture quality, resulting in softer texture.[25] The activity of hydrolysing enzymes during the seeds germination and fermentation process by the microbes results in the rupture of macromolecules that causes the seed cells to become softer.[26]

The tempe fermentation will significantly reduce the hardness value so that the tempe can meet consumer acceptance. The tempe texture profile, such as hardness, springiness, cohesiveness, gumminess, chewiness, and resilience, are also affected by substrate thickness, starter volume, type of beans, and the amount of mould.[27] The increase in hardness value in germinated velvet bean tempe was suspected due to the alteration of seed microstructure during the germination process, followed by hyphae formation during the fermentation process that covers and forms a network that contributes to tempe structure. It resulted in a higher hardness value in germinated velvet bean tempe.

Non-germinated velvet bean seeds had the lowest pH value because it contains phytic acid and cyanide acid. The germination process in velvet bean seeds enables in significant pH increase ($p<0.05$) from 3.25 to 5.76. This is caused by washing and rinsing velvet bean seeds during germination, removing the water-soluble acids. Tempe samples showed different results, where the germination process decreased the tempe pH from 6.52 to 6.32. This is suspected due to a metabolic process that occurs during germination that changes glucose to organic acids, resulting in tempe acidity.[28]

The processing of both germinated and non-germinated velvet bean seeds into tempe showed an increase in pH, which was caused by the release of ammoniac ions into the end product from the protein metabolism by the mould.[20] The fermentation process by *Rhizopus* mould in beans processing can reduce the phytic acid content due to the presence of phytase enzyme. The reduction of phytic acid becomes crucial to increase mineral absorption, thus resulting in tempe becoming one of the food products that have a decent source of minerals.[29]

Chemical Composition of Velvet Bean Seeds and Tempe

The proximate analysis of velvet bean seeds and tempe is presented in Table 4. The germination treatment causes a significant increase in water content ($p<0.05$) on velvet bean seeds from 14.05 to 45.86%. This also applies to germinated velvet bean tempe, which experienced a significant increase ($p<0.05$) from 60.72% to 61.43%. The increased water content was caused by absorbed water during the velvet bean germination. Moreover, the soaking and periodic watering during the germination process can also increase the water content of velvet bean seeds, so the tempe has a higher water content.[30] During the germination, the increase of simple hydrophilic compounds occurs, so more water is absorbed into the seed matrix.[31]

The germination process significantly reduces the ash content in velvet bean seeds ($p<0.05$) from 4.29 to 3.96%, but in velvet bean tempe, the decline occurs insignificantly ($p>0.05$). The higher the ash content in the sample, the higher the mineral content. This shows that the highest mineral content in the sample was found on non-germinated velvet bean seeds. The ash content in the non-germinated tempe treatment reduced significantly compared with its seed form. This was caused by the effects of various processes during the tempe production, such as washing, watering, and physical dehulling processes.[32]

Table 4: Chemical composition of velvet bean seeds and tempe

Parameter	Non-germinated velvet bean	Germinated velvet bean	Non-Germinated velvet bean tempe	Germinated velvet bean tempe
Moisture (%wb)	14.05 ± 0.06^{a}	45.86 ± 0.85^{b}	60.72 ± 0.46^{c}	61.43 ± 0.18^{d}
Ash (%db)	4.29 ± 0.17^{c}	3.96 ± 0.03^{b}	1.26 ± 0.05^{a}	1.02 ± 0.05^{a}
Fat (%db)	4.29 ± 0.22^{b}	1.29 ± 0.02^{a}	4.09 ± 0.15^{b}	4.87 ± 0.09^{b}
Protein (%db)	21.12 ± 0.57^{a}	30.39 ± 0.02^{b}	30.66 ± 0.41^{bc}	31.99 ± 0.15^{c}
Crude fiber (%db)	0.63 ± 0.21^{a}	0.95 ± 0.23^{a}	1.26 ± 0.16^{a}	1.31 ± 0.47^{a}
Carbohydrate (%db)	70.30 ± 0.57^{c}	64.36 ± 0.01^{b}	63.99 ± 0.60^{b}	62.12 ± 0.01^{a}

Note: Means followed by different letters in the same row show significantly different at $\alpha = 0.05$.

The germination process of velvet bean seeds could reduce the fat content from 4.29 to 1.29%. Meanwhile, germinated velvet bean fermentation into tempe increased the fat content significantly from 1.29 to 4.87%. This was caused by the decline of ash and carbohydrate contents in the seeds due to the germination treatment, so the % of fat content in tempe also increased. During fermentation, the mold releases lipase that triggers triacylglycerol hydrolysis into free fatty acids. Furthermore, Sharma *et al*. added that fatty acids are a substrate for the mould to grow, which results in decreased fat content during the fermentation process.[33]

The protein content of velvet beans experienced a significant increase ($p<0.05$) in non-germinated seeds from 21.12 to 30.39% in germinated seeds. The tempe production also contributed to a significant increase in protein content, both from non-germinated and germinated velvet bean tempe, sequentially 30.66 and 31.99%. The increased protein content in tempe was caused by the loss of water-soluble components, such as minerals and starch, from the velvet bean seeds.[34]

According to Ferreira *et al*., the protein content of tempe would increase by 21% compared to cotyledon.[35] Proteolytic activity from the mould mycelium also contributes to the increase of tempe protein content.[33] However, the protein content between germinated velvet bean seeds and tempe was not significantly different. This event was suspected due to the activity of the active protease enzyme after the germination and changes the complex protein into water-soluble peptides and amino acids with low molecular weight during the washing and soaking of velvet bean seeds before tempe processing. This is also supported by Yoshari *et al*., who mentioned that *Rhizopus* mould utilises amino acids as a nitrogen source for growth.[36]

The germination process significantly affects ($p<0.05$) the carbohydrate content of the resulting velvet bean seeds and tempe. Carbohydrate content in velvet bean seeds significantly decreased ($p<0.05$) from 70.29 in non-germinated seeds to 64.35% in germinated seeds. This was also applicable in tempe samples, where the carbohydrate content of non-germinated velvet bean tempe was decreased from 63.98 to 62.11% in germinated tempe. The decline of carbohydrates in germinated velvet beans is caused by carbohydrate hydrolysis into energy required for protein synthesis purposes.[37] Furthermore, radicle sprouting also requires energy obtained from the carbohydrate hydrolysis process. The germination of velvet bean seeds also reduced the carbohydrate

content in tempe. Abdurrasyid *et al*. mentioned that *Rhizopus* could turn into phenyl rings that increase free phenolic components.[38]

Antioxidant Capacity and Total Phenolic Content

The result of antioxidant capacity and total phenolic content of velvet bean seeds and tempe is shown in Table 5. Antioxidant capacity shows an insignificant decline ($p>0.05$) from 58.04 in non-germinated seeds to 57.17 mg AAE/100g after germinating. The antioxidant capacity decreased significantly ($p<0.05$) in tempe samples, both germinated and non-germinated tempe. The antioxidant capacity of tempe from germinated seeds was significantly lower than those of non-germinated tempe, respectively 36.30 and 52.46 mg AAE/100g.

The chemical composition, especially carbohydrate content, affects microbial metabolism during tempe fermentation. The fermentation process in beans utilise microbes that secrete primary and secondary metabolite, which can increase the antioxidant activity.[39–41] Based on the literature, the substrate positively correlates with antioxidant activity. The proximate analysis shows that the germination process can reduce the carbohydrate content since less carbohydrate acts as a substrate for microbial metabolism. This results in less antioxidants.

The germination treatment in velvet bean seeds caused a significant decrease in total phenolic content ($p<0.05$) from 233.80 in non-germinated seeds to 192.49 mg GAE/100g in germinated seeds. The total phenolic value plummeted even after processing into tempe. The total phenolic content in non-germinated tempe was 132.80, which decreased significantly to 66.09 mg GAE/100g in germinated tempe. The germination treatment affects changes in the phenolic compound of velvet bean seeds. The decreased total phenolic content was suspected due to the several soluble phenolic compounds being soluble in water during the soaking in the germination process.[22,42] The presence of soaking water continuously in contact with the surface of the seeds during germination causes several phenolic compounds to be washed away, resulting in decreased final total phenolic content.[9]

Table 5: Antioxidant capacity and total phenol of velvet bean seeds and tempe

Parameter	Non-germinated velvet bean	Germinated velvet bean	Non-Germinated velvet bean tempe	Germinated velvet bean tempe
Antioksidan capacity (mg AAE/100 g)	58.04 ± 1.09c	57.17 ± 0.75c	52.46 ± 0.78b	36.30 ± 1.38a
Total phenol (mg GAE/100 g)	233.80 ± 7.16d	192.49 ± 3.75c	132.80 ± 5.94b	66.09 ± 3.94a

Note: Means followed by different letters in the same row show significantly different at α = 0.05.

Table 6: GABA content in velvet bean tempe

Sample	GABA content (mg GABA/100g)
Non-germinated velvet bean tempe	54.01 ± 1.22b
Germinated velvet bean tempe	21.50 ± 0.39a

Note: Means followed by different letters in the same column show significantly different at α = 0.05.

GABA Content in Velvet Bean Tempe

The GABA content in non-germinated velvet bean tempe was significantly different from germinated velvet bean tempe ($p<0.05$), as presented in Table 6. The GABA accumulation in non-germinated velvet bean tempe (6.52) was higher, which was 54.02,

compared with germinated velvet bean tempe (pH 6.32), which decreased to 21.50 mg GABA/100g. According to Handoyo and Morita, the accumulation of GABA mainly occurs at pH 6.5.[26] The decrease of GABA was related to carbohydrate hydrolysis into glucose, as illustrated in Figure 4. Table 4 shows the carbohydrate content in germinated velvet bean tempe, which experienced a significant decline ($p<0.05$). This was positively correlated with the decline of GABA in germinated velvet bean tempe (Table 6).

Glucose is a substrate for GABA synthesis.[43] The decrease in GABA content was suspected to be related to a significant decrease in carbohydrate content in germinated tempe ($p<0.05$). During germination, carbohydrate is used as a energy source for radicle growth.[38] Figure 4 shows the GABA synthesis pathway through the RuMP pathway. The glycolysis changes the glucose into pyruvate to be followed by oxidative decarboxylation, which results in acetyl-CoA, which then enters the TCA (tricarboxylic acid) cycle. The GABA synthesis mechanism is related to three reactions catalysed by glutamate decarboxylase (GAD), GABA-Transaminase (GABA-T), and succinate semialdehyde dehydrogenase (SSADH). GAD will catalyse the α-decarboxylation reaction from glutamate to GABA through an irreversible reaction. GABA-T will then change GABA into succinate semialdehyde (SSA), and then SSADH will change SSA into succinate. The resulting succinate will enter the TCA cycle and takes part in electron transport inside the mitochondria as an electron donor.[44]

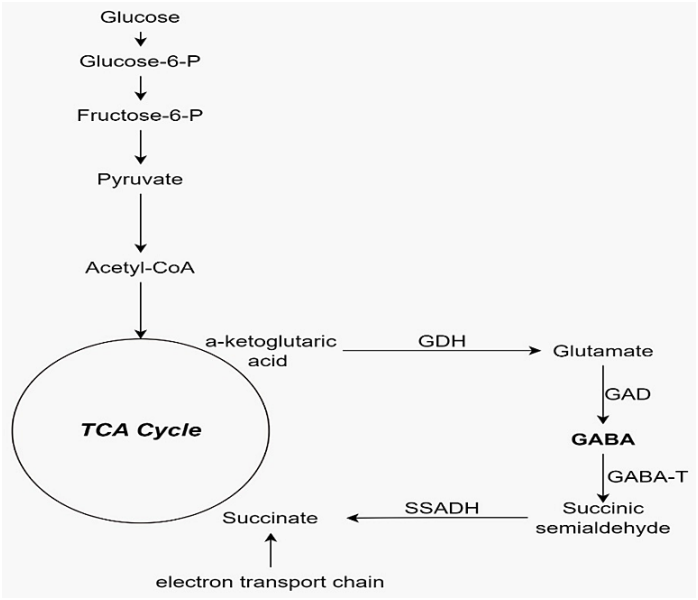

Fig. 4: GABA biosynthetic pathway through the RuMP pathway (GDH: *glutamate dehydrogenase*; GAD: *glutamate decarboxylase*; GABA-T: GABA *transaminase*; SSADH: *succinic semialdehyde dehydrogenase*)[44]

Conclusion

The optimum fermentation time to produce velvet bean tempe is 48 hours, shown by the best hyphae appearance. The germination process affects the physicochemical characteristics of the resulting velvet bean seeds and tempe: a decrease in lightness, saturation, whiteness degree, and pH; and an increase in tempe hardness. The germination treatment in velvet bean seeds increases the water content and decreases the carbohydrate content in the resulted tempe. Germination treatment in velvet bean seeds reduces the total phenolic content, antioxidant capacity, and GABA content in the resulted tempe. A correlation was found between the chemical composition in velvet bean seeds with the physical characteristics and bioactive components in the resulted tempe. The increased water content after germination reduces the hardness of the velvet

bean seeds. Meanwhile, the decline in glucose after the germination reduces GABA accumulation in the resulted velvet bean tempe.

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Conflict of Interest
The author(s) declares no conflict of interest.

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