Size Reduction Consequences on Anti-Nutritional and Chemical Properties of Tempe Made from Underutilized Legumes

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Abstract. Tempe is generally made from soybeans, but many underutilized legume species can potentially be tempe substrates, including jack bean and velvet bean from the Fabaceae family. This study evaluates the anti-nutritional and chemical properties of tempe made from underutilized legumes. Jack bean and velvet bean were used as tempe substrates. The whole-grain legume tempe was compared with the sliced-grain legume tempe to examine grain size reduction's impact on tempe properties. A completely randomized design was implemented in this study. This study exhibited that velvet bean tempe had higher levels of antinutrient compounds, protein, and carbohydrate content than jack bean tempe. It was also shown that grain legumes' size reduction significantly reduced the anti-nutritional features and carbohydrate content of tempe as a final product. Meanwhile, the protein and lipid content of tempe increased dramatically due to the size reduction of grain legumes. Thus, grain size reduction is recommended for a higher quality of underutilized legume tempe.

1 Introduction

Tempe (also known as tempeh) is an Indonesian traditional solid-based fermented food with excellent functional properties that benefit human health. As reported, bioactive compounds synthesized during tempe fermentation have some therapeutic effects such as anti-hypertensive, antibacterial, antioxidant, antidiabetic, and anticancer, depending on the specific amino acid sequences [1].

Although commonly made from soybeans, many other *Fabaceae* plants can also be used as tempe substrates. Preparing tempe from different raw materials would become a new alternative nutritional diet for vegans and vegetarians [2]. Moreover, it is crucial for developing countries like Indonesia, which face unavoidable population increases and fertile land scarcity, to explore other protein sources like neglected legumes to overcome malnutrition, especially regarding protein deficiency.

Jack bean (*Canavalia ensiformis* L.) and velvet bean (*Mucuna pruriens*) are underutilized legumes from the Fabaceae family that can be found in various regions in Indonesia. These legumes are regarded as reliable sources of dietary proteins and have comparable digestibility

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to other pulses such as soybean, lima bean, and rice bean [3,4]. Many studies also reported that underutilized legumes, such as canavalia and mucuna beans, exhibit various functional properties and pharmacological effects [4]. It has also been traditionally used as a food source, including as a tempe substrate, by certain ethnic groups in Indonesia.

However, due to their unique traits and grain sizes, processing tempe from canavalia and mucuna beans is trickier than soybean. These dimension legumes are about two to three times larger than soybeans. Besides, antinutrient existence frequently obstructs their utilization as food materials. All these issues required proper handling to obtain high-quality products.

An earlier study stated that reducing the size of grain legumes is necessary to obtain tempe with a solid texture [5]. However, there is little scientific evidence about the impacts of minimizing grain legumes on the nutritional content of tempe, especially for tempe made from unfamiliar materials. Thus, this study aims to compare the anti-nutritional and chemical properties of jack bean and velvet bean tempe by examining the impacts of grain size reduction. A better understanding of handling influences on underutilized legume tempe characteristics would help improve their quality.

2 Methods

Jack beans and velvet beans were bought from Temanggung, Central Java. Commercial tempe inoculum 'Raprima' (PT. Aneka Fermentasi Industri, Bandung, Indonesia) was obtained from a local market. This inoculum contained a mixture of cultures.

The tempe production process was adapted from the conventional Javanese method. Grain legumes were sorted, cleaned, and boiled in the water (100±5°C) at a ratio of 1:10 (b/v) for three hours. The boiling process attempts to soften the legume tissues and make separating the kernel epidermis from the grain easier. The dehulling process is essential since the kernel epidermis could interrupt the tempe fermentation process.

The boiled dehulled legumes were immersed in the water at a ratio of 1:4 (b/v) for 48 hours at room temperature, with water replacement every 12 hours. Subsequently, grain legumes were boiled for 15 minutes, drained, and cooled at room temperature. Before being inoculated, each legume was divided into two groups. The first group used whole grain legumes, and the second was sliced grain legumes (the grains were cut into four pieces with a thickness of about 0.5 cm). Then, about 100 g of legumes were inoculated with 0.2 g *Rhizopus oligosporus* starter, wrapped in banana leaves, and fermented at room temperature for 48 hours to ensure mycelium growth covered the legume surfaces entirely.

Analysis of anti-nutritional properties of legume tempe involved hydrogen cyanide (HCN) and phytate content. HCN measurement conformed to the previous method [6] using a Spectrophotometer UV-VIS at 510 nm absorbance and potassium cyanide (KCN) as a standard. Phytate content was measured by colorimetric procedure with slight modification [7]. Analysis of chemical properties (moisture, ash, protein, lipid, and carbohydrate) was determined using the American Association of Cereal Chemists method [8].

A completely randomized design was implemented as an experimental design. A one-way analysis of variance (ANOVA) was employed to evaluate the data at a significant difference of $p \leq 0.05$. The Duncan Multiple Range Test was applied in further analysis to compare various means. The statistical program SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used in this experiment.

3 Results and Discussion

3.1 Anti-nutritional properties

Despite being affordable protein sources in human and animal diets, jack beans and velvet beans are often restricted due to the presence of anti-nutritional components such as hydrogen cyanide and phytate. Table 1 presents the anti-nutritional properties of jack bean and velvet bean tempe. It was exhibited that using sliced grain allowed a better reduction in the antinutrient level of legume tempe ($p<0.05$).

This study found that tempe made from sliced legumes had lower cyanide content than tempe made from whole legumes, which indicated that grain size reduction helps the cyanide detoxification during processing tempe become more effective. The more effective cyanide detoxification would be more excellent since the presence of cyanide in foodstuffs could be detrimental to human health. It was reported that cyanide poisoning could be lethal to humans and animals, and the acute dose is around 1 mg/kg body weight [9]. However, another study claimed that the amount of hydrogen cyanide in velvet beans is well below the fatal level [4]. All of the tempe samples in this study indicated a safe amount of hydrogen cyanide.

Grain size reduction also showed effectiveness in decreasing phytate content. Phytate was categorized as an antinutrient since its presence decreases mineral bioavailability [10]. It was reported that phytate is the most potent antagonist of zinc absorption, producing insoluble complexes in the gastrointestinal system that cannot be digested or adsorbed due to the lack of intestinal phytase enzyme in humans [11]. Therefore, some studies recommended the threshold for phytate content in food products should be less than 25 mg/100 g to avoid micronutrient losses [12]. On the other hand, phytate also has several functional properties such as anticarcinogenic, antioxidant, and inhibitor of calcium kidney stone formation [10]. Although the human body can synthesize phytate, most urinary phytate comes from food rather than endogenous synthesis [10].

This study also showed that tempe from velvet beans had higher hydrogen cyanide and phytic acid than jack bean tempe ($p<0.05$). This finding indicated that antinutrient contents from the legume origin affect its content in the final product.

Table 1. Anti-nutritional properties of tempe made from underutilized grain legumes.

Substrate	HCN (mg/100g)	Phytate (mg/100g)
Whole jack beans	0.17 ± 0.03^{b}	0.24 ± 0.05^{a}
Sliced jack beans	0.09 ± 0.02^{a}	0.17 ± 0.02^{a}
Whole velvet beans	0.42 ± 0.01^{c}	0.70 ± 0.01^{c}
Sliced velvet beans	0.18 ± 0.04^{b}	0.44 ± 0.01^{b}
p-value	0.001	0.000

Different lowercase superscripts within the same columns indicate significant differences ($p \leq 0.05$)

3.2 Chemical properties

The results showed that the size reduction of legumes significantly improved ($p<0.05$) the protein and lipid content of tempe as a final product (Table 2). Previous studies associated the protein increase with the biomass increase [2], while the enhancement of lipid amount might relate to higher lipase activity [3]. Another study explained that reducing grain legume size would expand the contact area between substrate and microorganisms during fermentation, triggering several extracellular enzymes that lead to protein and lipid alteration [13].

Meanwhile, the carbohydrate content of tempe made from sliced grain legumes was lower than tempe made from whole grain legumes ($p<0.05$). This degradation might be attributed to the more significant hydrolysis of carbohydrate molecules in smaller legume sizes [13].

Table 2. Chemical properties of tempe made from underutilized grain legumes.

Substrate	Moisture (%)	Ash (% db)	Protein (%db)	Lipid (%db)	Carbohydrate (%db)
Whole jack beans	64.95±0.71 [a]	1.16±0.03 [a]	8.07±0.20 [a]	1.35±0.02 [ab]	20.54±0.13 [b]
Sliced jack beans	64.91±0.30 [a]	1.24±0.02 [a]	10.37±0.27 [b]	1.55±0.06 [c]	19.44±0.22 [a]
Whole velvet beans	64.93±0.25 [a]	1.35±0.04 [b]	12.01±0.02 [c]	1.29±0.01 [a]	24.66±0.14 [c]
Sliced velvet beans	64.07±0.18 [a]	1.39±0.05 [b]	13.68±0.23 [d]	1.41±0.00 [b]	20.17±0.04 [b]
p-value	0.239	0.011	0.000	0.005	0.000

Different lowercase superscripts within the same columns indicate significant differences ($p\leq 0.05$)

However, size reduction through slicing the grain legumes has no significant influence ($p>0.05$) on the moisture and ash content of the tempe. Another study also reported that tempe made from different legumes had no significant difference in moisture content [2].

Velvet bean tempe had lower lipids but higher protein and carbohydrates than jack bean tempe ($p<0.05$). This result might relate to the legume's original content. This occurrence was consistent with an earlier study [14], which stated that velvet tempe has a high amount of protein and carbohydrate and a low amount of lipid. Some literature reported that the protein content of velvet beans was about 23-35% [4], while the protein content of jack beans was around 23-27.6% [15].

4 Conclusion

The size reduction of under-utilized grain legumes effectively improves the quality of tempe as a final product. It was verified that slicing the grain legumes would help to reduce cyanide and phytate as antinutrients. Grain size reduction also contributes to increasing the protein and lipid content of legume tempe. In addition, tempe made from sliced legumes tends to have lower carbohydrates than tempe made from whole legumes. Different legume types would induce distinct chemical characteristics of tempe.

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