RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 1 |22-28| January - March | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

UTILIZATION OF DURIAN SEEDS (*Durio zibethinus Murr*) AND SOYBEAN MIXTURES AS AN ALTERNATIVE SUBSTRATE FOR TEMPE PRODUCTION WITH *Rhizopus oligosporus*

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ABSTRACT

Durian seeds, a common waste product, pose environmental hazards when discarded improperly, necessitating innovative processing methods to repurpose them into value-added products. This study explores the use of durian seeds as an alternative substrate for tempe production employing the fungus *Rhizopus oligosporus*. Tempe was produced using varying mass ratios of durian seeds to soybeans (1:1, 1:2, and 2:1), with *Rhizopus sp*. and fermentation times ranging from 18 to 54 hours. The quality assessment involved organoleptic testing, protein content analysis, antioxidant activity measurement, and total isoflavone content evaluation. Results indicated that panelists favored tempe with a durian seed-to-soybean ratio of 1:2 and an inoculum mass of 3 grams, yielding an average organoleptic score of 3.7. The highest protein content (18.85%) was obtained in tempe with a 2:1 mass ratio and 5 grams of inoculum. Maximum antioxidant content (74.61%) was observed in the 2:1 mass ratio with 3 grams of inoculum. The optimal condition for isoflavone content (0.070%) was achieved with a fermentation time of 54 hours.

Keywords: Durian Seeds, Soybeans, Fermentation, *Rhizopus oligosporus*

RASĀYAN*J. Chem.*, Vol. 18, No.1, 2025

INTRODUCTION

Durian (*Durio zibethinus Murr*), a prominent tropical fruit in Indonesia, is widely cultivated but poses environmental challenges due to the substantial amounts of non-consumable seeds generated during the durian season. These seeds contribute to substantial waste and environmental pollution. Durian seed waste, like other lignocellulosic residues, can be innovatively transformed into value-added products.[1] Approximately 30% of the durian fruit is edible, while the remainder constitutes waste, with about 20–25% comprised of underutilized seeds.[2] The annual increase in durian seed waste, driven by rising durian demand, poses ecological risks and necessitates substantial management efforts.[3] In Indonesia, durian seeds are not commonly used in cooking and are consumed only by a small group of people after being roasted or boiled. Despite their potential for development into more appealing and flavorful dishes, durian seed by-products such as residue, chips, milk, and flour remain underutilized. Research indicates that durian seeds can be used as thickening agents due to their hydrocolloid content[4] and in the production of eco-friendly bioplastics, bioethanol, plywood fillers[5], and edible coatings.[6] Thus, further innovation in utilizing durian seeds for economically and nutritionally valuable products is necessary. Previous studies have shown that steamed durian seeds contain 0.25% fat, 4.13% crude fiber, 8.61% protein, 3.17% ash, 70.21% non-nitrogen extractable substances, and 3493 kcal/kg of metabolizable energy.[7] These components suggest that durian seeds could be an interesting alternative for tempe production. Tempe, a traditional Indonesian food product, has become an integral part of diets globally.

The fermentation process of tempe, primarily using soybeans, results in a product with distinctive texture, flavor, and nutritional value.[8] Tempe contains essential nutrients such as vitamin B12 and protein, making it attractive for vegans as vitamin B12 is typically found only in animal-derived products.[9] Additionally, tempe has antioxidant properties that benefit health[10] by reducing oxidative stress and scavenging free radicals.[11] Currently, soybeans are the primary ingredient in tempe production due to their high protein and fat content. However, Indonesia relies heavily on soybean imports, which impacts the national economy

http://doi.org/10.31788/RJC.2025.1819022

due to a consistent deficit in soybean sales each year. Local soybean supply is vulnerable to global market volatility, leading to potential soybean crises if international market disruptions occur.[12] This dependency on soybean imports can drive up prices, indicating a need for alternative sources for tempe production. Durian seeds, despite their high cellulose and fat content, remain an underutilized food source due to their relatively low nutritional quality. This study aims to evaluate the quality of tempe made from durian seeds and soybeans in various ratios using the *Rhizopus oligosporus* mushroom.

EXPERIMENTAL

Raw Material Preparation

Durian seeds were thoroughly washed and soaked for 24 hours, after which the seed hulls were removed. The de-hulled seeds were then boiled for 40 minutes and drained. After cooling for 40 minutes, the durian seeds were chopped, approximately 0.5 cm in size. Soybeans were ground to split into halves, de-hulled, and washed thoroughly. The soybeans were then boiled for 20 minutes, drained, and cooled for 40 minutes.

Preparation of Tempe and Testing Parameters

Tempe was prepared by mixing 150 grams of durian seeds with 150 grams of soybeans (1:1 ratio) from the pre-treated materials. *Rhizopus sp.* Tempe inoculum was added in varying amounts of 3 grams, 4 grams, and 5 grams. The mixture was then fermented with time variables of 18 hours, 36 hours, and 54 hours. Table-1 summarizes the composition of the Tempe prepared from the mixture of soybeans and durian seeds.[13] The mixture was compacted and subsequently wrapped in perforated clear plastic bags. The resulting Tempe was tested for organoleptic properties (texture, color, aroma, and taste), protein content, antioxidant content, and isoflavone content.

Table-1: Composition of Tempe from Soybeans and Durian Seeds

Treatment	Ratio	Mass (gram)		
		Soybean	Durian seeds	Tempe inoculum
Sample 1	1:1	150	150	3
Sample 2	1:2	150	200	3
Sample 3	2:1	200	150	3
Sample 4	1:1	150	150	4
Sample 5	1:2	150	200	4
Sample 6	2:1	200	150	4
Sample 7	1:1	150	150	5
Sample 8	1:2	150	200	5
Sample 9	2:1	200	150	5

RESULTS AND DISCUSSION

Organoleptic Test Results

Organoleptic testing is an evaluation method conducted by humans using their senses to assess the organoleptic qualities of a product, such as food or beverages. The objective is to gather information about the sensory attributes of a product and determine its acceptability to consumers in terms of taste, aroma, color, texture, and overall appearance.[14] Organoleptic testing is crucial in quality assessment, as it can provide indications of spoilage, deterioration, and other forms of product damage. In this study, a hedonic test with scoring was employed for sensory evaluation, involving 20 untrained panelists from among university students. The tempe was cut into 1 cm thick slices and fried over very low heat for 5 minutes without the addition of seasonings. Panelists assessed the tempe using a 1–5 scale (1 = Strongly Dislike, 2 = Dislike, 3 = Neutral, 4 = Like, 5 = Strongly Like). The scores given by the panelists were averaged using the following formula.[15]

$$\overline{X} = \frac{\sum X_i}{n}$$

The average sensory evaluation scores for the Tempe made from a mixture of durian seeds and soybeans are presented in Table-2.

Table-2: Organoleptic Test Results

Treatment	Organoleptic Result			
	Aroma	Color	Texture	Taste
Sample 1	2.15	3.7	3.8	3.3
Sample 2	3.65	3.7	3.65	3.85
Sample 3	3.7	3.6	3.7	3.6
Sample 4	2.05	4	4	4.2
Sample 5	2.55	4.1	4	4
Sample 6	2.35	3.8	4.05	4.3
Sample 7	2.7	3.6	3.9	4.1
Sample 8	2.9	3.55	3.45	3.5
Sample 9	2.5	3.65	3.8	3.5

Key: 1=Strongly Dislike, 2=Dislike, 3=Neutral, 4=Like, 5=Strongly Like).

Table-2 shows that the panelists provided diverse ratings for the aroma, color, texture, and taste of the durian seed and soybean Tempe mixture. For the aroma parameter, panelists generally expressed dislike for most tempe samples, except for samples 2 and 3, which were rated slightly favorable with values of 3.65 and 3.7 (between neutral and like). This suggests that Tempe with a lower inoculum mass was preferred by the panelists. Regarding color, panelists assigned similar scores ranging from 3.55 to 4.1 (between neutral and like), indicating that the color of the durian seed and soybean tempe was acceptable to them. The color of the Tempe is significantly influenced by the growth of the mycelium. However, the color assessed in this study was that of the fried tempe, and panelists' evaluations may also have been influenced by the frying time and temperature. Meanwhile, for texture and taste, the panelists provided favorable ratings. The best ratings were achieved for samples 4, 5, and 6, which received scores between like (4) and strongly like (5). From these results, it was found that panelists rated the Tempe with an inoculum mass of 4 grams most favorably. Based on evaluations across almost all parameters, the Tempe with the highest score was sample 2, which consisted of a durian seed and soybean mixture with a soybean-to-durian seed ratio of 1:2 and 3 grams of inoculum. This sample received an average score of 3.7 across all parameters. Panelists were less favorable towards the aroma of the Tempe, which was perceived as rancid due to excessive fermentation. Higher inoculum mass can lead to oxidation or excessive fermentation, resulting in a rancid odor.[16] Previous research on tempe made from cowpea (*Lablab purpureus (L.) Sweet*), fermented for 30 hours, also indicated that inoculum concentration and fermentation time significantly affected the taste, aroma, and texture of the Tempe, while color was not significantly impacted.[17]

Protein Content Analysis

Figure-1 illustrates the protein content in Tempe across different ratios of durian seeds to soybeans.

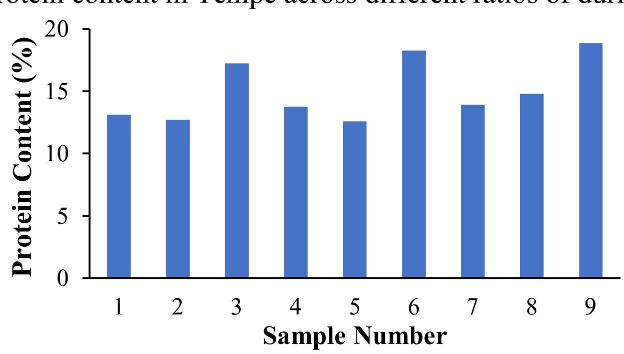

Fig.-1: Protein Content in Tempe Prepared from Soybean and Durian Seed Mixtures

As shown in Fig.-1, the ratio of soybeans to durian seeds and the variation in inoculum mass influence the protein content in Tempe. The highest protein content was observed in sample 3 (ratio 2:1 and 3 grams of inoculum), which can be attributed to the higher protein content in soybeans compared to durian seeds. Consequently, Tempe with a higher soybean-to-durian seed ratio yields a higher protein content.[18] The protein content in tempe with a 1:1 mass ratio and inoculum masses of 3, 4, and 5 grams was 13.11%, 13.76%, and 13.90%, respectively. For Tempe with a 1:2 mass ratio and the same inoculum masses, the protein contents were 12.70%, 12.58%, and 14.79%, respectively. In contrast, the 2:1 mass ratio sample had protein contents of 17.23%, 18.27%, and 18.85%. Based on these protein content results, the 2:1 mass ratio variation, with protein content below 16%, does not meet the protein standard for Tempe according to the Indonesian National Standards. Tempe produced from whole soybeans has a higher protein content compared to Tempe made from a mixture of durian seeds and soybeans, although both meet the SNI requirement of over 15%.[19] The lower protein content in Tempe made from a mixture of durian seeds and soybeans is likely due to the loss of soluble proteins, such as albumin, during the Tempe production process.[20] Additionally, the protein content in durian seeds is significantly lower than that in soybeans. Previous research by Kusumawati *et. al*[21] reported that Tempe made from 100% de-hulled soybeans contained 40.19% protein, which is more than twice as high as the maximum protein content of 18.85% observed in Tempe made from soybean and durian seed mixtures.

Antioxidant Content Analysis

Antioxidants are compounds with molecular structures that can donate electrons to free radical molecules without compromising their own function, thereby interrupting the chain reactions of free radicals.[22] The analysis of antioxidant content aims to demonstrate the nutritional quality and oxidative stability of tempe. The antioxidant content present in tempe is illustrated in Fig.-2.

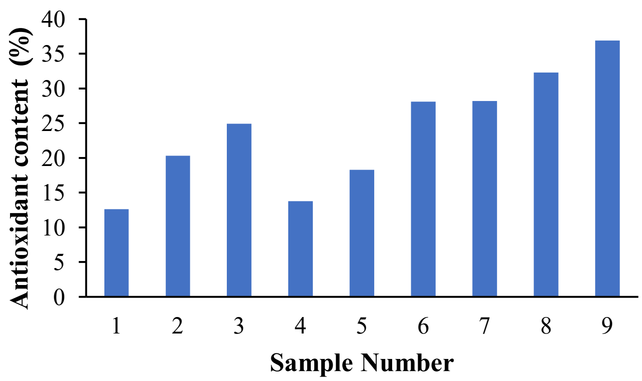

Fig.-2: Antioxidant Content in Tempe

Figure-2 shows that the nine samples exhibit varying levels of antioxidant content in Tempe. For samples 1, 2, and 3, the antioxidant contents were 12.61%, 20.3%, and 24.9%, respectively. Samples 4, 5, and 6 had antioxidant contents of 13.76%, 18.3%, and 28.1%, respectively. Samples 7, 8, and 9 showed antioxidant contents of 28.2%, 32.2%, and 36.9%, respectively. Among these samples, the lowest antioxidant content was observed in sample 1 at 12.61%, while the highest was in sample 9 at 36.9%. The increase in antioxidant activity can be influenced by several factors, including fermentation duration and the type of fungi used. Thus, it can be concluded that incorporating durian seeds into the tempe fermentation process enhances antioxidant activity.[23] Additionally, Tempe contains antioxidant compounds such as unsaturated fatty acids, amino acids, vitamin E, and beta-carotene, which may increase during fermentation.[24] Previous research on the antioxidant activity of tempe with moringa leaf powder indicated that higher amounts of moringa leaf powder resulted in increased antioxidant activity in Tempe. The antioxidant activity observed in this study with moringa leaf powder was higher.[25]

Isoflavone Content in Tempe

Isoflavones are a group of phytochemical compounds classified within the flavonoid class.[26] An analysis of isoflavone content in Tempe is necessary to evaluate the availability of isoflavone nutrients in Tempe. Generally, soybean Tempe contains beneficial nutrients and isoflavones that can enhance memory function.[27] For this reason, the Temple has significant potential to be processed as a functional food to improve cognitive function. As a functional food, tempe should meet the standard for total isoflavone content. The isoflavone content in Tempe can be observed in Fig.-3.

Figure-3 demonstrates that the nine samples contain varying levels of isoflavones in tempe. For samples 1, 2, and 3, the isoflavone contents were 0.029%, 0.031%, and 0.035%, respectively. Samples 4, 5, and 6 showed isoflavone contents of 0.039%, 0.041%, and 0.051%, respectively. Lastly, samples 7, 8, and 9 had isoflavone contents of 0.058%, 0.060%, and 0.070%, respectively. Among these samples, the lowest isoflavone content was found in sample 1 at 0.029%, while the highest was in sample 9 at 0.070%. The incorporation of durian seeds into tempe production with soybean mixtures contributes to an increased isoflavone content. This is attributed to the fact that longer fermentation can enhance the content of aglycone isoflavones in soybean Tempe.[28]

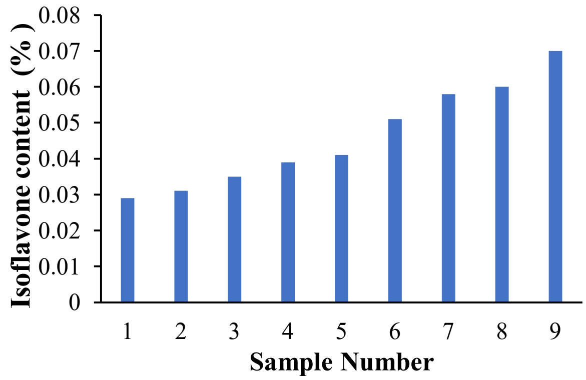

Fig.-3: Isoflavone Content in Tempe

In other studies, during fermentation, the enzyme glycosidase from *Rhizopus oligosporus* hydrolyzes isoflavone glycosides to isoflavone aglycones. This hydrolysis process has been described by several researchers.[29] Isoflavone glycosides can also be hydrolyzed in the digestive tract[30], though this process may be long and imperfect. In Tempe, most isoflavone glycosides have already been hydrolyzed into isoflavone aglycones.[31] Fermentation is thus a crucial final step, as isoflavone glycosides are hydrolyzed into more active isoflavone aglycones. Isoflavone glycosides are not absorbed by adults, whereas aglycones are.[32] Therefore, consuming Tempe ensures better absorption of isoflavones. The loss of isoflavone glycosides during tempe production is compensated by the formation of more active isoflavone aglycones in the human body. Furthermore, in Tempe, most macromolecules have also been hydrolyzed into simpler forms, thus enhancing their nutritional value compared to non-fermented soybeans.[33]

CONCLUSION

This study demonstrates the potential of utilizing durian seeds as a substrate for tempe production, revealing several key findings. The organoleptic tests revealed a moderate preference for the Tempe innovation combining durian seeds and soybeans, with a 1:2 ratio and 3 grams of inoculum. The tempe with a 2:1 soybean-to-durian seed ratio and 5 grams of inoculum exhibited the highest protein content of 18.85%. Antioxidant content varied significantly, with the highest observed in sample 9 at 36.9% in the 2:1 ratio with 5 grams of inoculum. Isoflavone content also increased with higher soybean ratios, peaking at 0.070% in the 2:1 ratio with 5 grams of inoculum. These results suggest that durian seeds can enhance the nutritional and functional properties of tempe, providing a sustainable alternative to traditional soybean-based tempe.

ACKNOWLEDGEMENTS

The authors would like to thank Universitas Syiah Kuala for supplying the facilities for this research.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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