



Analysis of Primary Metabolites Content in Ketapang Seed Tempe (Terminalia Catappa)

***Siska D. Wulandari, Siti Nuryanti, Tahril & Sitti Aminah**

Chemistry Education Study Program/Faculty of Teacher Training and Education – Tadulako University, Palu – Indonesia 94119

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Abstract

Ketapang seed tempeh (Terminalia catappa) is made from ketapang seed waste through a fermentation process using Rhizopus sp., as with soybean tempeh. This study used a quantitative descriptive method to determine the content of primary metabolites, including fat, protein, and carbohydrates. Laboratory analysis was conducted at the Chemistry Education Laboratory, Tadulako University, using the Soxhlet method for fat, the Kjeldahl method for protein, and the phenol-sulfur method for carbohydrates, as well as organoleptic tests by 15 panellists. The results showed that ketapang seed tempeh had very low fat content (0.1714%), low protein (0.0189 g per 100 g sample), and moderate carbohydrate (10.06%). Fried ketapang tempeh was preferred by the panellists, although its acceptance level was still lower than that of soybean tempeh. The novelty of this study lies in the quantitative evaluation of primary metabolites in tempeh made entirely from ketapang seeds, which has not been previously reported. These findings indicate that ketapang seeds have the potential to serve as an alternative ingredient in the production of low-fat tempeh.

Keywords: Carbohydrates; fat; ketapang seed; protein; primary metabolites

Introduction

Indonesia possesses rich biodiversity, including various plant species that produce metabolites beneficial to human life (Nasrul & Chatri, 2024). One of these biological resources is the ketapang seed (*Terminalia catappa* L.), which grows naturally in coastal regions such as Ujumbou Village, Sirenja District, Donggala Regency. Although these seeds contain high levels of nutritional components, particularly lipids, their use as food ingredients remains limited (Jahurul et al., 2022).

The use of ketapang seeds as a fermentation substrate represents an innovative approach to increasing their added value and supporting the development of local functional foods (Nursyamsi et al., 2017; Irfan et al., 2025). Fermentation processes, such as those applied in soybean tempeh production, are known to modify the chemical composition of raw materials, especially primary metabolites including lipids, proteins, and carbohydrates (Halilu et al., 2023).

The presence of abundant unsaturated fatty acids, proteins, and carbohydrates in ketapang seeds indicates their potential as an alternative high-protein food source (Jahurul et al., 2022; Iyekowa et al., 2023). Furthermore, fermentation by *Rhizopus*

oligosporus has been widely reported to enhance nutrient bioavailability by breaking down complex molecules into simpler compounds (Ahnann-Winarno et al., 2021; Prativi et al., 2023).

Studies on soybean-based tempeh consistently show that fermentation alters primary metabolite levels, such as increasing amino acids and organic acids and reducing simple sugars (Prativi et al., 2023). Fermentation and soaking conditions have also been found to influence metabolite profiles, including amino and organic acid concentrations in tempeh soaked in acidic solutions (Dahlan et al., 2022).

Beyond soybeans, various non-soy materials, including mung beans, red beans, koro beans, and other local seeds, have been successfully utilised for tempeh production, yielding products with distinct metabolite characteristics while maintaining high nutritional value (Riyanto et al., 2024; Akanni & Adebo, 2024).

Primary metabolites such as carbohydrates, proteins, and lipids play essential physiological roles and constitute key components of fermented foods (Leeuwendaal et al., 2022; Fu et al., 2024; Fitsum et al., 2025). Lipids function as energy sources and influence sensory attributes; ketapang seeds are known to contain oleic, linoleic, and palmitic acids (Halaldeen et al., 2024). During fermentation,

*Correspondence:

Siska D. Wulandari

e-mail: dewideal2023@gmail.com

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proteins are degraded into peptides and amino acids, thereby improving digestibility (Prado et al., 2022). Carbohydrates serve as substrates for microorganisms and are converted into simple sugars and organic acids, affecting the pH, aroma, and texture of tempeh (Lo et al., 2022).

Primary metabolite analysis in raw and fermented materials is commonly conducted through proximate analysis, which measures moisture, ash, protein, lipid, and carbohydrate content (Santos et al., 2021; Hossain et al., 2025). Applying proximate analysis to ketapang seed tempeh provides quantitative insight into nutritional changes during fermentation, which is essential for assessing nutritional quality and chemical stability. The selection of Ujumbou Village as the sampling location is supported by its coastal ecology, which favours ketapang growth; seeds from such environments often contain higher lipid levels due to adaptation to saline conditions. Utilising ketapang seeds for tempeh production may also promote food diversification and reduce dependence on soybean imports (Ahnian-Winarno et al., 2021).

Although the chemical composition of ketapang seeds has been widely documented, a notable research gap lies in the limited studies examining changes in primary metabolites following fermentation—particularly regarding lipid, protein, and carbohydrate dynamics. Research on ketapang seed tempeh remains scarce and typically focuses on raw seed characteristics or mixed formulations rather than the fermented product itself.

Therefore, this study aims to analyse the primary metabolite content of traditionally fermented ketapang seed tempeh and assess consumer acceptance through organoleptic evaluation. The findings are expected to serve as a foundation for developing functional foods based on local materials and to support national food diversification efforts.

Methods

This research is an experimental study conducted in the Chemistry Education Laboratory of the Faculty of Teacher Training and Education, Tadulako University. The research sample was ketapang seeds from Ujumbou Village, Sirenja District, Donggala Regency.

Research tools and materials

The tools used in this study include a 10 ml measuring flask (Pyrex), oven (memmert), analytical balance (beadam), soxhlet apparatus, 50 mL measuring cylinder (pyrex), UV-Vis spectrophotometer (PG Instruments Ltd.), distillation apparatus, stand and clamp, burette, Erlenmeyer (schoot duran), 50 mL and 100 mL beakers (agc iwaki), funnel (pyrex), test tubes (iwaki pyrex) and test tube racks, hot plates, 50 mL and 100 mL measuring flasks, 5 mL measuring pipettes (iwaki), suction rubber (D&N), desiccator (iwaki).

The materials used in this study consisted of non-chemical and chemical materials, namely ketapang seeds, distilled water, tempeh yeast (Raprima 500gram), methylene red (MM), 30% NaOH, concentrated H₂SO₄, 1N NaOH, 0.1N HCl, glucose solution, concentrated HNO₃, 5% phenol, filter paper, cotton, N-hexane, kjedhal tablets, glucose, concentrated HCl, label paper (Basic), tissue (Paseo), and aluminum foil (Klinpak).

Research stages

Making Ketapang seed Tempeh

Ketapang seeds were washed thoroughly to remove dirt, then boiled for approximately 30 minutes until softened. After boiling, the seeds were drained, cooled, and then separated from the skin to obtain clean, white seeds. Next, the seeds were cut into small pieces, weighed to 100 g, and then 0.1 g of tempeh yeast was added. This mixture was wrapped in polypropylene plastic and fermented at room temperature for 48 hours. The fermentation process allowed the *Rhizopus* sp. fungus to grow and form a white mycelium that binds the ketapang seeds together into the tempeh (Sholeha, 2020).

Organoleptic testing

Sensory testing was conducted by 15 panellists using a hedonic scale (1–5) to assess four organoleptic quality parameters: colour, aroma, taste, and texture. Each panellist was given four samples: raw ketapang tempeh, fried ketapang tempeh, raw soybean tempeh, and fried soybean tempeh. Scores were assigned based on level of preference, ranging from 1 (dislike very much) to 5 (like very much). The average panellist data was analysed to determine consumer acceptance of ketapang seed tempeh compared to soybean tempeh. The organoleptic test results are calculated using the following mean equation:

$$\text{Average} = \frac{X_1 + X_2 + X_3 + \dots + X_{15}}{n} \quad (1)$$

(Safitry et al., 2021)

Fat content analysis (Soxhlet method)

$$\text{Fat content (\%)} = \frac{\text{final pumpkin weight} - \text{initial pumpkin weight}}{\text{dry sample weight}} \times 100\% \quad (2)$$

(Nurfadila, 2024)

Protein content analysis (Kjeldahl method)

Protein analysis begins with the digestion stage, which involves heating a 10 g tempeh sample with concentrated H₂SO₄ and a Kjeldahl catalyst tablet until a clear solution forms, indicating that organic nitrogen has been converted to ammonium ions. The resulting digestion solution is diluted and placed in a distillation apparatus. During the distillation stage, a 30% NaOH solution is added to release ammonia, which is then captured in 0.1 N HCl containing methyl red indicator. The distillate is then titrated with 0.1 N NaOH until the colour changes to pink. The nitrogen content is calculated and then multiplied by a conversion factor of 6.25

to obtain the total protein content. Protein is calculated using formulas 3 and 4.

$$\% N = \frac{(ts - tb) \times N_{HCl} \times 14,008}{Mg\ sample} \times 100\% \quad (3)$$

$$\% protein = \% N \times F \quad (4)$$

Note: F is the conversion value of the protein content.

(Nurfadila, 2024)

Carbohydrate content analysis (phenol-sulfate method)

A 1 g tempeh sample is extracted with distilled water and 0.1 M HCl, then filtered and transferred to a volumetric flask. The analysis was performed using the phenol-sulfate method, in which the sample solution was reacted with 5% phenol and concentrated H₂SO₄, yielding a yellow-orange complex. The colour intensity was measured using a UV-Vis spectrophotometer at 490 nm. To calculate the carbohydrate concentration, a standard glucose curve (20–100 ppm) was constructed, and the sample concentration was determined based on the linear regression equation of the resulting curve.

The formula for determining carbohydrates uses the following equation 5.

$$y = ax + b \quad (5)$$

Where:

y = Sample adsorbance on the calibration curve

a = Slope

b = Intercept

x = Carbohydrate concentration in the sample

Carbohydrate content can be calculated using formula 6.

$$\% Glucose = \frac{G \times FP}{W} \times 100\% \quad (6)$$

Where:

G = Sample glucose concentration

FP = Dilution factor

W = Sample weight

Converted to grams based on the percentage in formula 7.

$$Mass (g) = Total\ mass (g) \times \frac{Mass (\%)}{100} \quad (7)$$

(Purnama et al., 2022)

Analysis data

Organoleptic data, including colour, aroma, taste, and texture scores from 15 panellists, were averaged for each sample of ketapang seed tempeh and soybean tempeh. For primary metabolite content (fat, protein, and carbohydrates), each sample was analysed in triplicate, and the results were averaged across replicates. These average values were then used to compare the quality of ketapang seed tempeh and soybean tempeh, both in terms of nutrient content and consumer acceptance. The results are presented in simple tables and

graphs for ease of interpretation, and conclusions are drawn from differences in average values between the samples (Pratиви et al., 2023).

Results and Discussion

Organoleptic Testing

This study was conducted to evaluate consumer acceptance of tempeh products processed from two raw materials: ketapang seeds (*Terminalia catappa*) and soybean seeds (*Glycine max*). The evaluation was carried out using an organoleptic test method with a hedonic scale approach (preference scale). Organoleptic assessment was conducted on four parameters: colour, aroma, taste, and texture. The assessment was conducted by 15 untrained panellists using a hedonic scale of 1–5 (1 = very dislike, 5 = very like). presented in **Table 1**.

Table 1. Average Tempeh Organoleptic Scores Based on Assessment Parameters

Type of tempeh	Color	Aroma	Flavor	Texture
Raw Ketapang tempeh	4.2	3.0	3.6	4
Fried Ketapang tempeh	4.1	3.6	3.9	4.2
Raw Ketapang tempeh	4.5	3.7	3.6	4.4
Fried Ketapang tempeh	4.8	3.8	4.0	3.9

The results showed that fried soybean tempeh scored highest on all parameters, followed by fried ketapang tempeh, while raw ketapang tempeh scored lowest on aroma. This indicates that the frying process enhances the product's sensory characteristics by producing a distinctive aroma resulting from the Maillard reaction (Rizzo, 2024).

According to Akanni & Adebo (2024), the intensity of aroma and flavour in fermented tempeh is strongly influenced by the formation of amino acids and organic acids, which react to form volatile compounds. This was also observed by Riyanto et al. (2024), who found that the fermentation process during the soaking stage influences the volatile compound profile that contributes to tempeh aroma. The colour and texture of ketapang tempeh were highly acceptable (4.1–4.2), comparable to those of soybean tempeh. This is consistent with the results of Pratиви et al. (2023), who reported an increase in tempeh texture during fermentation due to the formation of a fungal mycelial network that strengthens the substrate matrix.

Fat content

Data Analysis: The fat content of tempeh made from ketapang seeds was analysed using the Soxhlet extraction technique. This study aimed to obtain free fat compounds from solid samples using a non-polar solvent. This method was chosen for its ability to extract fat through repeated heating and

solvent circulation optimally, thereby effectively separating fat compounds from the solid material. The results of the fat content calculations obtained from three repetitions are presented in **Table 2**.

Table 2. Results of fat content analysis of Ketapang seed Tempeh

Repetition I	Repetition II	Repetition III	Average
0.1428 %	0.19004 %	0.1816%	0.1714 %

The fat content obtained was 0.1714%, indicating a relatively low-fat content compared to soybean tempeh, which generally ranges from 18–22% (Ahnani-Winarno et al., 2021). This low value is due to the soaking and fermentation processes, which can cause lipid degradation through the lipase activity of *Rhizopus oligosporus* (Prativi et al., 2023).

Halilu et al. (2023) reported that *T. catappa* seeds contain high levels of oleic and linoleic acids, but most of these lipids are hydrolysed during fermentation. These results align with a metabolomics study by Riyanto et al. (2024), which found significant changes in the fatty acid profile during tempeh fermentation. The low-fat content of ketapang seed tempeh may also be influenced by the high water and crude fibre content of the raw material, as explained by Akanni & Adebo (2024) for non-soybean legumes.

To better understand the results of this analysis, the general structure of fat is shown in **Figure 1**. This structure depicts the basic form of a triglyceride, an ester compound formed from one glycerol molecule and three fatty acid molecules.

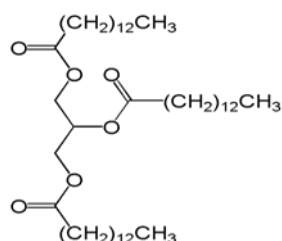


Figure 1. Fat structure

This structure indicates that fat contains an ester group, which plays a key role in determining its physical and chemical properties, such as solubility and melting point. This relationship explains that even though the fat content in ketapang seed tempeh was detected to be very low, the resulting fat compound still has the typical structure of fats.

Protein content data analysis

Protein content was determined using the Kjeldahl method, which begins by digesting the sample with concentrated sulfuric acid to convert organic nitrogen into ammonium ions. This is followed by neutralisation with a base (NaOH) and distillation to recover ammonia from the acidic solution. The amount of bound nitrogen was then

multiplied by a conversion factor (6.25) to obtain the protein content. The results of the protein content calculations from three replicates are presented in **Table 3**.

Table 3. Results of the protein content analysis of Ketapang seed Tempeh

Repetition I	Repetition II	Repetition III	Average
0.0162 gram	0.0225	0.0181	0.0189

The protein content of ketapang seed tempeh is 0.0189 g, lower than that of soybean tempeh, which generally contains 19–22% protein (Lo et al., 2022). This value indicates that fermentation of ketapang seeds increases nutritional value, but it is not yet comparable to that of soybeans. According to Iman et al. (2023), the increase in protein during fermentation is due to the proteolytic activity of *Rhizopus oligosporus*, which breaks down macromolecules into amino acids and simple peptides.

Halaldeen et al. (2024) found that the protein in *T. catappa* seeds is rich in essential amino acids, suggesting that fermentation can increase their bioavailability. A study by Prado et al. (2022) also confirmed that the fermentation process reduces antinutritional compounds such as tannins and phytates, thereby improving protein absorption. Thus, although the absolute protein content is low, its biological value is likely increased.

To clarify the results of this analysis, the general structure of the protein is shown in Figure 2. This structure shows that proteins are composed of chains of amino acids linked together through peptide bonds, forming complex polymers.

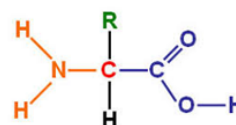


Figure 2. Protein structure

This structure illustrates that each protein molecule is composed of an amino group ($-NH_2$) and a carboxylic group ($-COOH$) linked together to form a long chain. This relationship indicates that the measured protein content originates from the nitrogen found in the constituent amino acids.

Carbohydrate content data analysis

The carbohydrate content of Ketapang seed tempeh was analysed using the Phenol-Sulfuric Acid method. The purpose of this study was to determine the carbohydrate content of Ketapang seed tempeh. The carbohydrate content in the samples was determined using a UV-Vis spectrophotometer at 490 nm. The results of the absorbance measurements of the standard glucose solution are presented in **Table 4**. Based on the data in **Table 4**, there is a linear relationship between glucose concentration and the resulting absorbance. The

following are the results of the carbohydrate content analysis presented in **Table 5**.

Table 4. Absorbance data for the standard glucose solution

Glucose concentration (ppm)	Absorbance 490 nm
20	0.218
40	0.252
60	0.295
80	0.353
100	0.403

Table 5. Results of the carbohydrate content analysis in Ketapang seed Tempeh

Repetition I	Repetition II	Repetition III	Average
9 %	9.34 %	11.84%	10.06 %

The average carbohydrate content was 10.06% by weight of the sample used. This indicates that ketapang seed tempeh has a relatively high carbohydrate content compared to previously obtained fat and protein levels.

Several metabolomics and fermentation studies on tempeh and non-soybean legumes have reported common phenomena in carbohydrate components during the fermentation process, which are relevant to explaining the 10.06% carbohydrate content in ketapang seed tempeh. Prativi et al. (2023), in a study of tempeh metabolites, showed that during fermentation, simple sugars (e.g., glucose, sucrose) are utilised by microorganisms, while organic acids accumulate as metabolic products. These changes in sugar transformation affect the carbohydrate profile measured by the total (phenol-sulfate) method, allowing total carbohydrate values to decrease or remain stable, depending on the initial substrate and fermentation duration.

Dahlan et al. (2022) demonstrated that soaking treatments and microbial conditions in the early stages of tempeh production can significantly alter the sugar and sugar alcohol composition; this influence explains the variation between replicate measurements in your samples (P1–P3). In other words, the small differences between 9.00%, 9.34%, and 11.84% could be due to variations in soaking conditions, fermentation time, or sample homogeneity, as also reported in tempeh metabolomic studies.

Fu et al. (2024) conducted a targeted metabolomic study of seed plants (e.g., peanuts). They confirmed that the composition of non-volatile carbohydrates (polysaccharides and oligosaccharides) is strongly influenced by seed type. Seeds with a high proportion of complex polysaccharides tend to maintain a high total carbohydrate fraction despite a decrease in reducing sugars, aligning with findings in ketapang seeds,

which contain complex fibre/starch, resulting in a relatively constant final total carbohydrate (despite some conversion to other metabolites).

In a review, Prado et al. (2022) and Akanni & Adebo (2024) stated that the final carbohydrate content of tempeh products is highly dependent on: (1) the initial substrate composition (starch/fiber ratio), (2) the activity of the starter microbe's amylolytic and proteolytic enzymes, and (3) process parameters (temperature, fermentation time, soaking conditions). Therefore, the 10.06% value in ketapang seed tempeh can be understood as resulting from limited starch degradation by the *Rhizopus* starter during the 48-hour fermentation period, combined with the presence of complex carbohydrates that are less hydrolysed.

The 10.06% carbohydrate content indicates a moderate level, comparable to that of soybean tempeh (9–11%) reported by Ahnan-Winarno et al. (2021). According to Fu et al. (2024), during fermentation, starch and oligosaccharides are broken down into simple sugars and organic acids, which are then used by microbes as an energy source.

Prativi et al. (2023) reported that fermentation causes a decrease in sucrose and an increase in organic acids (lactic and acetic), which lowers the total carbohydrate content. A similar effect is suspected in tempeh made from ketapang seeds. This carbohydrate content contributes energy and influences the texture of the tempeh due to the presence of water-soluble polysaccharides.

To provide a clearer picture of the components analysed, **Figure 3** shows the basic structure of a carbohydrate, namely glucose, one of the monosaccharides that make up polysaccharides.

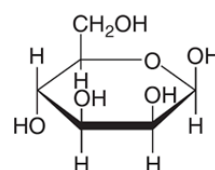


Figure 3. carbohydrates structure

This structure indicates that carbohydrates are composed of carbon (C), hydrogen (H), and oxygen (O) atoms with the general formula $C_6H_{12}O_6$. The hydroxyl group (-OH) on each carbon atom plays a crucial role in forming glycosidic bonds that make up polysaccharides such as starch and cellulose. This relationship explains that the carbohydrate analysis results reflect the polysaccharide content of ketapang seed tempeh.

Inter-replicate variability

In the analysis of fat, protein, and carbohydrates in Ketapang tempeh, there was variation between replicates (e.g., fat: 0.1428%, 0.19004%, 0.1816%), resulting in an average of 0.1714%. This variability can be caused by several factors, including sample homogeneity, extraction efficiency (especially for fat), variations in the

fermentation process, and sample handling conditions (drying/wetting, drying time, soaking, etc.).

This is common in fermentation research on non-soybean ingredients. In the literature on tempeh made from jackfruit seeds, for example, protein content varies with yeast concentration and fermentation conditions. Therefore, it is important to report deviations and result ranges, not just average values, to provide an overview of the data's variability and reliability.

Comparison with Soybean Tempeh and previous literature

According to general composition data for soybean tempeh, it contains approximately 20–21 g of protein, 8–9 g of fat, and 13–14 g of carbohydrates per 100 g of edible weight. These values are consistently much higher than those of Ketapang tempeh in your study—especially for protein and fat. This indicates that Ketapang tempeh cannot currently replace soybean tempeh in terms of meeting protein and fat requirements.

Literature on other non-soy tempeh also shows similar trends. For example, in a study substituting jackfruit seeds for soybeans, the addition of jackfruit seeds reduced the protein and fat content of the tempeh compared to a 100% soybean control (Irna et al., 2020; Jafarzadeh et al., 2024). However, there was significant improvement in sensory acceptability (colour, texture) of Ketapang tempeh, indicating the potential to optimise the process (fermentation, drying, leavening) and formulation (Nicole et al., 2021). Tempeh made from non-soy ingredients could become an alternative, especially when combined with other protein sources or enriched (Aaslyng & Højer, 2021; Teoh et al., 2024).

Conclusions

Tempeh made from ketapang seeds has very low fat and relatively low protein content. However, compared to other ingredients, it has a relatively high carbohydrate content. Taste and appearance tests indicate that people quite like fried tempeh made from ketapang seeds, although not as popular as tempeh made from soybeans. These findings suggest that ketapang seeds can be used as an alternative ingredient in tempeh production, particularly as a low-fat energy source. In practice, this potential can be used to expand the variety of foods by using local, more affordable ingredients. However, this study still has limitations, including the limited scope of taste and appearance testing and the lack of in-depth analysis of the impact of nutritional changes during fermentation. Therefore, future research should focus on developing improved fermentation methods, exploring the combination of ketapang seeds with other ingredients, and conducting larger-scale public acceptance tests to gain a more comprehensive understanding of the quality of ketapang seed tempeh.

Conflict of Interest

The author declares no conflict of interest.

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