Biosaintifika 18 (1) (2026): 33-44 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Microbiological and Nutritional Properties of Fermented *Artocarpus integer* Mesocarp (*Mandai*) from West Kalimantan, Indonesia

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Submitted: 2025-11-28. Revised: 2026-02-02. Accepted: 2026-04-05.

Abstract. *Mandai*—traditionally made from *Artocarpus integer* mesocarp—originates from Central, South, and East Kalimantan and has been adopted in West Kalimantan. However, data on the microbiological and nutritional properties of West Kalimantan *mandai* have yet to be revealed. Therefore, this study aims to analyze the microbiological and nutritional properties of *mandai*. *Mandai* samples were obtained from one traditional producer. The analysis of microbiological properties included bacterial isolation, followed by molecular identification through 16S rRNA gene sequencing and phylogenetic analysis. The analysis of nutritional properties includes the determination of proximate, crude fiber, amino acid, and essential mineral profiles. Microbiological properties showed that the *mandai* contains four *Enterobacter* species. However, no lactic acid bacteria (LAB) were detected in the samples. Meanwhile, the nutritional properties indicate a high concentration of moisture, ash, and essential minerals; low levels of total fat, protein, and carbohydrates; as well as the existence of L-serine and L-tyrosine. The total energy obtained is 15.50 kcal/100g. Therefore, it can be concluded that although *mandai* contains nutritional values, the presence of *Enterobacter* poses a food safety risk. As the first to analyze the microbiological and nutritional properties of *mandai* obtained from West Kalimantan, this study benefits science and society, as the unexpected finding of *Enterobacter* emphasizes the urgency of production process standardization. Moreover, this study correlates with Sustainable Development Goals (SDGs) 2 and 3 due to the main focus of this study, which reveals crucial information regarding the safety of consuming *mandai*, which can benefit health from the nutrients it contains.

Keywords: 16S rRNA; *Enterobacter*; *mandai*; proximate analysis; traditional fermented food

How to Cite: Yeni, L. F., Fajri, H., Faturrahman, M. A., Novahisa, P., Kumalasari, M., & Syakuran, L. A. (2026). Microbiological and Nutritional Properties of Fermented *Artocarpus integer* Mesocarp (*Mandai*) from West Kalimantan, Indonesia. *Biosaintifika: Journal of Biology & Biology Education, 18(1)*, 33-44.

DOI: http://dx.doi.org/10.15294/biosaintifika.v18i1.38812

INTRODUCTION

Traditional fermented foods are an essential part of Indonesian communities' local wisdom, serving as a source of nutrition with high economic and social values (Surya, 2024). *Mandai* is a traditional fermented food made from *cempedak* (*Artocarpus integer*) mesocarp and is one of the cultural heritages in the culinary aspect of the Banjar tribe, who inhabit the island of Kalimantan (Yudistira et al., 2025). A traditional fermented food cherished by various tribes and utilized as a condiment, *mandai* has long been known as one of the specialty products originating from Central, South, and East Kalimantan (Surono, 2016). The practice of fermenting *mandai* has spread and undergone modifications in various other regions, adapting to the local characteristics of each region. One of the regions that has adopted the *mandai* fermentation practice is West Kalimantan.

Variations in the microbiological and nutritional properties of traditional fermented foods can be prompted by geographical conditions (Fentie et al., 2025), the type of raw materials used (Tamang et al., 2016), the fermentation procedures carried out (Wang et al., 2023), and the dynamics of the dominant microbiota (Nemo & Bacha, 2020). These factors must be taken into consideration when analyzing the same traditional fermented food from different regions. No studies have been conducted on *mandai* from West

Kalimantan, even though this traditional fermented food has been extensively studied in South Kalimantan (e.g., Murwani et al., 2024) and East Kalimantan (e.g., Emmawati et al., 2016). The spontaneous fermentation required in the *mandai* production process involves a complex community of microorganisms that has not yet been fully studied and understood. In this regard, it is imperative to assess the safety of traditional fermented foods, which can be done through molecular identification of microorganisms (Basuki et al., 2024) and comprehensive proximate analysis (Helmi & Eni, 2024) to obtain accurate results.

Information regarding the microbial community, proximate composition, essential and non-essential amino acid profiles, crude fiber content, and mineral concentration of *mandai* from West Kalimantan is still unavailable. In general, the proliferation of lactic acid bacteria (LAB) such as *Lactobacillus casei* during the *mandai* fermentation process plays a role in increasing the phenolic components and antioxidant activity contained therein (Yudistira et al., 2025). Several studies have successfully identified the microbial community in *mandai* using molecular approaches, as demonstrated by Basuki et al. (2024), Juwana et al. (2020), and Murwani et al. (2024). However, these studies analyzed *mandai* originating from outside West Kalimantan, highlighting the urgency of conducting a comprehensive study to analyze the microbiological and nutritional properties of *mandai* originating from West Kalimantan, thereby explicitly offering novelty since existing studies have focused on products from Central, South, and East Kalimantan. Furthermore, the fact that *mandai* is made from *cempedak* peel as the raw material has the potential to show different results from previous studies, in line with the statement by Rejman et al. (2021) that the taste and appearance, as well as the health and nutritional properties contained in fruit, are highly dependent on the region where the fruit grows.

Based on the background described, this study has two main objectives. The first objective is to isolate and identify the microbial community in *mandai* through a molecular approach based on 16S ribosomal ribonucleic acid (rRNA) gene sequencing. The second objective is to analyze the nutritional properties of *mandai*, leading to the profiling of its moisture, protein, fat, ash, carbohydrate, amino acid, crude fiber, and mineral contents. This study contributes to the scientific understanding of the microbiological and nutritional properties of *mandai* from West Kalimantan. The results of this study are expected to serve as a step toward improving the quality of traditional fermented foods with an emphasis on food safety. In addition, the results of this study have the potential to support communities in Indonesia through the preservation of functional local wisdom based on ethnobiological resources.

METHODS

Study Design

This study uses a descriptive-analytical approach to analyze the microbiological and nutritional properties of *mandai* from West Kalimantan. The study was conducted from January to June 2025. Microbiological properties analyses were carried out at the Biology Education Laboratory and Integrated Laboratory at Universitas Tanjungpura, Indonesia. The 16S rRNA gene sequencing was performed at the commercial laboratory of Apical Scientific in Malaysia. Nutritional properties analyses were carried out at the Chemistry Laboratory at Universitas Tanjungpura and PT. Saraswanti Indo Genetech, both in Indonesia.

Samples Preparation and Collection

Mandai samples were obtained from one traditional producer in Pontianak, West Kalimantan, due to it being the sole producer of *mandai* in the province. Through a spontaneous fermentation method, *mandai* was produced by adding 15% salt (w/w) to fresh *cempedak* mesocarp that had been thoroughly cut and washed. The fermentation process was carried out in tightly sealed food-grade plastic containers at room temperature (28 ± 2°C) for 30 days. The *mandai* resulting from the process was sampled aseptically for further analysis. The *cempedak* used before fermentation and the finished *mandai* product are shown in Figure 1.

(a) (b)

Figure 1. (a) *Cempedak* mesocarp used prior to fermentation; (b) the resulting *mandai* product

Isolation and Molecular Identification of Microorganisms

The isolation of microorganisms began with grinding one gram of the *mandai* samples using a mortar and pestle. Nine milliliters of distilled water were then added to create an initial suspension. Microorganisms are then isolated using the spread plate method on two types of growth media, namely nutrient agar and de Man, Rogosa, and Sharpe (MRS) agar. Nutrient agar is a versatile medium that can support the growth of various non-fastidious bacteria, while MRS agar is a medium specifically used to promote the selective growth of LAB. Pure isolates are then obtained through the streak plate method on the same media.

Identification of microorganisms was based on the sequences of the 16S rRNA gene. Total genomic deoxyribonucleic acid (DNA) of each isolate was extracted using the Wizard® Genomic DNA Purification Kit by Promega, which was utilized according to the kit's protocol. The total genome was used for amplification of the 16S rRNA gene using polymerase chain reaction (PCR). The PCR was carried out using GoTaq® Green Master Mix by Promega, utilizing 27F forward primer (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R reverse primer (5'-GGT TAC CTT GTT ACG ACT T-3') (Murwani et al., 2024). The PCR conditions consist of pre-denaturation at 54°C for 2 minutes, denaturation at 53°C for 1 minute, annealing at 52°C for 30 seconds, polymerization at 72°C for 1 minute, and post-polymerization at 72°C for 5 minutes. The steps of denaturation, annealing, and polymerization are repeated for 35 cycles. The amplified results were confirmed by electrophoresis using 1% agarose gel.

The PCR products were purified and sequenced using Sanger DNA Sequencing with capillary electrophoresis at Apical Scientific Laboratory, Malaysia. Each sample was sequenced with the 27F forward primer and 1492R reverse primer. The 16S rRNA sequences from four isolates were aligned to the GenBank database using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). Alignment results were used to confirm the identity of isolates. Phylogenetic analysis was done based on the 16S rRNA sequences of *mandai* microorganisms and the reference data from GenBank. The ClustalW algorithm was used to align the 16S rNA sequences of the *mandai* microorganism and reference data. The phylogenetic tree was performed using the Neighbor-Joining method in the 12th version of Molecular Evolutionary Genetics Analysis (MEGA12) with 1,000 bootstrap replications (Juwana et al., 2020; Ngamnok et al., 2023).

Proximate Analysis

The proximate analysis performed includes the determination of moisture, protein, fat, ash, and carbohydrate content in accordance with Indonesian National Standards (*Standar Nasional Indonesia*, hereinafter abbreviated as SNI) and official methods. Moisture content was determined gravimetrically by drying the samples in an oven until a constant weight was obtained (SNI 01-2891-1992). Protein, fat, and ash content were analyzed using standard methods in accordance with laboratory technical instructions. Meanwhile, the total carbohydrate content was calculated using the difference method by subtracting moisture, protein, fat, ash, and alcohol (if any) from the total (Helmi & Eni, 2024).

Essential and Non-essential Amino Acid Analysis

The amino acid composition of the *mandai* samples was evaluated using ultra-

performance liquid chromatography (UPLC) with photodiode array (PDA) detection at the laboratory of PT. Saraswanti Indo Genetech (ISO/IEC 17025:2017-certified), following a standardized method (18-5-17/MU/SMM -SIG) to ensure precise analysis results. Based on the sample hydrolysis procedure involving HCl solution, filtration, and pre-column derivatization, the hydrolyzed samples were injected into the UPLC system with a C18 column and aqueous mobile phase eluent with a gradient pump at a column temperature of 49°C. Amino acid levels were calculated using the ratio of the analyte area to the internal standard (α-aminobutyric acid) with a standardized quantitative formula.

Determination of Crude Fiber

The crude fiber content was determined gravimetrically using the Association of Official Analytical Chemists (AOAC) method, which involved refluxing the samples with dilute sulfuric acid and dilute sodium hydroxide, filtering the mixture through Whatman® filter paper, washing the residue, drying it, and burning the residue at 550°C until a constant weight was achieved. The crude fiber content was calculated using a formula that takes the weight of the empty filter paper, the weight of the residue, the weight of the empty porcelain dish, and the weight of the residue ash into account (AOAC 945.18, 962.09, and 978.10; SNI 3836-2013).

Mineral Analysis

The mineral content was determined by the wet destruction method using microwave digestion. The samples were weighed, and concentrated nitric acid (and hydrochloric acid for specific analytes) was added before being destroyed using a microwave digester. After adding an internal standard solution of yttrium, the solution was diluted, filtered, and analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The mineral content was then quantified using a standard calibration curve with a linear equation of $Y = bx + a$.

Data Analysis

Data related to microbiological properties were analyzed through molecular identification involving gene sequencing and comparison of nucleotide composition, followed by phylogenetic tree construction to determine the relationship between *mandai* isolates and reference species. Meanwhile, data related to nutritional properties were analyzed statistically by determining the mean values and standard deviation (SD) of two replicates (mean ± SD).

RESULTS AND DISCUSSION

The agarose gel electrophoresis process indicated the success of amplifying the 16S rRNA gene from the analyzed bacterial isolates from *mandai*, marked by clear and singular bands. Fragments measuring approximately 1,500 bp were visible and are presented in Figure 2. The size of the fragments obtained was consistent with the expected size of the 16S rRNA gene. Parello (2020) explains that 16S rRNA is a component of RNA with a size of roughly 1,500 bp and is a ribosomal subunit in prokaryotic organisms, consisting of very conserved regions found in all prokaryotic organisms and species-specific hypervariable regions. Each isolate obtained (M1, M2, M3, and M4) showed consistency in amplification results and was suitable for proceeding to the sequencing and phylogenetic analysis stages.

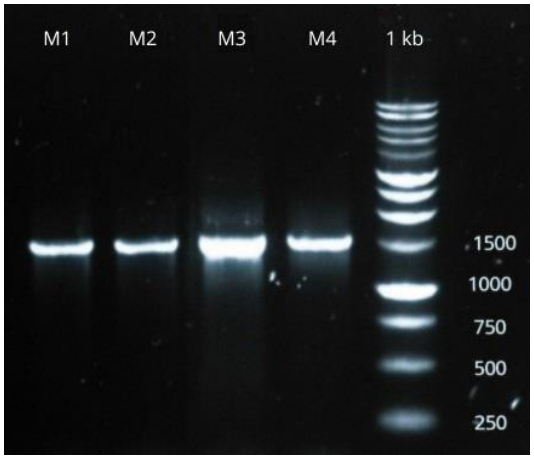

Figure 2. 16S rRNA gene fragments of four bacterial isolates from *mandai* (M1, M2, M3, and M4; M = 1 kb DNA ladder marker)

Molecular identification based on 16S rRNA gene sequencing and data matching with the NCBI's GenBank database revealed the diversity of bacterial species in the genus *Enterobacter* in *mandai* originating from West Kalimantan. The isolate codes and bacterial species identification results are presented in Table 1. Analysis of the 16S rRNA gene sequence of four bacterial isolates from *mandai* showed very high query coverage and percent identity as well as extremely low expect values (e-values), confirming the accuracy of the identification and indicating that the sequences obtained were consistent with the reference sequences in GenBank. This is consistent with the BLAST output, which shows accurate identification based on a query cover threshold >50–80% and a percent identity of more than 50%, ensuring that the BLAST match is truly authentic homologous, and e-values close to 0, meaning that the match is highly significant and very unlikely to occur by chance (Nestor et al., 2023).

The nucleotide composition of the 16S rRNA gene from the four *mandai* isolates reveals consistency with the nucleotide composition of the 16S rRNA of *Enterobacter* species data obtained from GenBank. The isolates obtained showed a balanced nucleotide base composition, with thymine (T) at 19.68–20.52%, cytosine (C) at 22.85–23.56%, adenine (A) at 25.02–25.31%, and guanine (G) at 31.33–31.84%. The total C+G content in the four *mandai* isolates ranged from 54.18% to 55.30%, with complete data on the nucleotide base composition of the four *mandai* isolates and reference strains from GenBank presented in Table 2. By comparing with reference strains from GenBank, the results obtained show a high similarity between *mandai* isolates and *Enterobacter* species. Isolate M1 showed similarity to *Enterobacter cloacae* strains 71241 (MH304298.1) and HSTU-ABk37 (MN795712.1). Isolate M2 showed similarity to *Enterobacter mori* strain 18.1 (PV441965.1). Isolate M3 indicates similarity with *Enterobacter hormaechei* strain 1.2.3 (CP134406.1). Isolate M4 implies similarity with *Enterobacter asburiae* strains gx-62 (FJ823004.1) and RG1 (CP129027.1). Meanwhile, a comparison of the nucleotide base composition with the LAB reference strain *Lactiplantibacillus plantarum* (LC880427.1) showed differences in the form of higher T+A content and lower C+G content compared to *Enterobacter*, confirming that there are differences in taxonomic classification between the *mandai* isolates and LAB. The length of the 16S rRNA gene sequence of the four *mandai* isolates that were successfully amplified varied between 814 and 1,235 bp, with sufficient sequence quality for further phylogenetic analysis. The consistency of nucleotide base composition between *mandai* isolates and *Enterobacter* reference strains reinforced the validity of the molecular identification performed in this study.

Table 1. Molecular identification of bacterial isolates from *mandai* based on 16S rRNA gene sequencing

Isolate	Species	Query Cover (%)	Percent Identity (%)	Expect Value	Accession Number
M1	*Enterobacter cloacae*	100	99.68	0.0	MN795712.1
M2	*Enterobacter mori*	100	99.59	0.0	PV441965.1
M3	*Enterobacter hormaechei*	100	99.92	0.0	CP134406.1
M4	*Enterobacter asburiae*	100	99.63	0.0	FJ823004.1

Table 2. Nucleotide composition of 16S rRNA genes from *mandai*-derived bacterial isolates and reference strains from GenBank

Isolate/Reference Strain	Nucleotide (%)						Nucleotide Length
	T	C	A	G	T+A	C+G	
Isolate M1	19.74	23.40	25.02	31.84	44.76	55.24	1.231
Isolate M2	20.16	23.09	25.04	31.71	45.20	54.80	1.230
Isolate M3	19.68	23.56	25.02	31.74	44.70	55.30	1.235
Isolate M4	20.52	22.85	25.31	31.33	45.82	54.18	814
Enterobacter asburiae strain gx-62 (FJ823004.1)	20.59	22.93	25.15	31.32	45.75	54.25	811
Enterobacter asburiae strain RG1 (CP129027.1)	20.59	22.93	25.03	31.44	45.62	54.38	811
Enterobacter hormaechei strain 1.2.3 (CP134406.1)	19.82	23.38	25.08	31.72	44.90	55.10	1.236
Enterobacter mori strain 18.1 (PV441965.1)	20.10	23.19	25.06	31.65	45.16	54.84	1.229
Enterobacter cloacae strain 71241 (MH304298.1)	19.81	23.38	25.00	31.82	44.81	55.19	1.232
Enterobacter cloacae strain HSTU-ABk37 (MN795712.1)	19.72	23.46	25.00	31.82	44.72	55.28	1.232
Lactiplantibacillus plantarum (LC880427.1)	24.02	21.99	25.45	28.54	49.47	50.53	1.328
Average	**20.45**	**23.12**	**25.10**	**31.33**	**45.55**	**54.45**	**1.126.27**

The phylogenetic tree constructed using the Neighbor-Joining method based on the 16S rRNA gene sequences of the bacterial isolates obtained and reference sequences from GenBank shows adequate relationships between isolates and reference species (Figure 3). This phylogenetic tree confirms the molecular classification obtained from BLAST analysis. The four *mandai* isolates analyzed in this study formed a clade adjacent to the corresponding reference species, with bootstrap values greater than 50% indicating the reliability of the phylogenetic tree. The distinct phylogenetic relationships were shown between the *mandai* isolates and the reference *Enterobacter* species. All four isolates from *mandai* (M1, M2, M3, and M4) are clustered within the genus of *Enterobacter.* Isolate M3 showed the closest relationship with *E. hormaechei* strain 1.2.3 (CP134406.1), with a bootstrap value 76%, while isolate M1 grouped with *E. cloacae*. Isolate M2 formed a clade with *E. mori* strain 18.1 (PV441965.1) with a bootstrap value 75%. Isolate M4 was positioned near the *E. asburiae* cluster, although with lower bootstrap support. These results indicate that the four isolates are phylogenetically related to members of the *E. cloacae* complex. Overall, the constructed phylogenetic tree confirms that isolates M1, M2, and M3 have been consistently grouped with bootstrap values ranging from 75–76%. Considering that branching with bootstrap values ≥70% is considered moderately supported (Rangkuti et al., 2022), this grouping is notable. Isolate M4 clustered with the reference strain *E. asburiae* with a relatively low bootstrap value, indicating weak phylogenetic support. Nevertheless, BLAST results showed a very high identity match, so that the designation of isolate M4 as *E. asburiae* could still be made tentatively. Therefore, identification based on phylogenetic trees provided consistent indications, although not strong enough to be the sole basis; consequently, the identification of species M4 required a greater reliance on BLAST sequence matching.

The findings contrast with previous studies that have found the presence of LAB in *mandai*, such as *Pediococcus pentosaceus* (Basuki et al., 2024) and the genus *Lactobacillus* (Juwana et al., 2020; Murwani et al., 2024), as well as *Aerococcus* and *Vagococcus* (Murwani et al., 2024). Although the salt concentration used in the *mandai* analyzed in this study was 15%, the finding of the absence of LAB contradicts the statement that the fermentation of *mandai* in brine with a concentration of 10% and 15% should facilitate the viability and growth of LAB (Surono, 2016). Murwani et al. (2024) reported the presence of unculturable LAB in *mandai*, which may require co-cultivation with other bacteria and additional components in the bacterial isolation medium, indicating the complexity of microbial interactions that are not yet fully understood. One of the interactions that may affect the abundance of *Enterobacter* in *mandai* is amensalism. Members of Enterobacteriaceae are known to produce antimicrobial peptides. For example, *E. cloacae* is known for producing microcin bacteriocin, which indicates ecological functions and affects the presence of other microorganisms (Baquero et al., 2019). Moreover, various unique interspecies interactions among microorganisms may alter their relative abundance over time, as demonstrated in fermented sour bamboo shoots (Zhang et al., 2025).

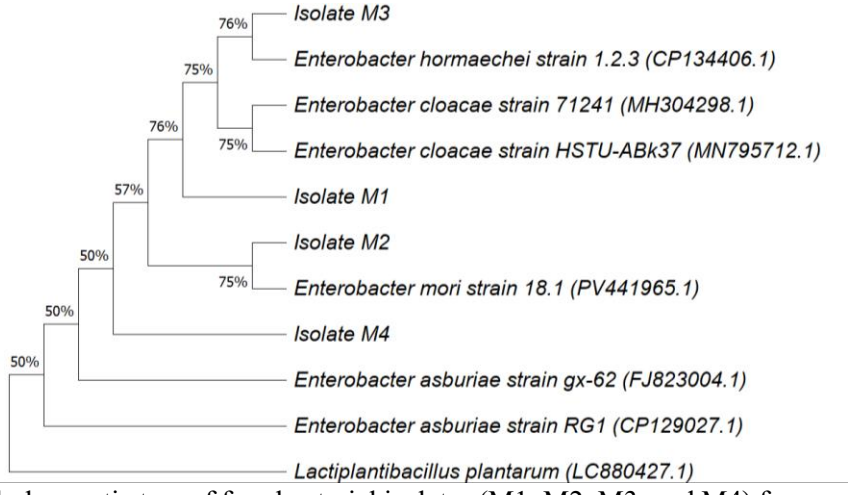

Figure 3. The phylogenetic tree of four bacterial isolates (M1, M2, M3, and M4) from *mandai* based on the Neighbor-Joining method (*L. plantarum* [LC880427.1] was used as an outgroup), with the number of bootstrap value of 1,000 replicates

The four identified *Enterobacter* species have food safety implications. *Enterobacter cloacae* is a pathogen that is resistant to various drugs, including amoxicillin, ampicillin, cefoxitin, and cephalosporins, regardless of whether it produces the AmpC-lactamase enzyme (Elbehiry et al., 2024). A case study in Austria identified the presence of *E. mori* isolates from the ear canal of a patient, which revealed the production of carbapenemase (Hartl et al., 2019). A specific strain of *E. hormaechei* was identified in Sichuan-style sausage as a producer of high levels of histamine (Pei et al., 2024). *Enterobacter asburiae* is commonly known as a cause of nosocomial infections, with the overproduction of AmpC β-lactamase triggering antibiotic resistance (Bo et al., 2017). Although members of the *Enterobacter* genus are generally considered pathogens, some *Enterobacter* species have been reported to play a positive role in specific fermentation systems (e.g., Zhong et al., 2023). However, it cannot be disputed that the dominance of *Enterobacter* species in the analyzed *mandai* is a matter that must be taken into serious consideration, especially from a food safety perspective. The conservation of traditional fermented foods based on local potential must be in line with quality and safety assurance principles so as not to cause health problems for consumers. This is supported by Oladeji & Ogidi (2025), who explains that inadequate storage methods and materials can increase the growth of unwanted microorganisms, compromising the quality and safety of traditionally fermented foods.

The results of microorganism species identification in *mandai* from West Kalimantan, which show the dominance of *Enterobacter*, do not necessarily indicate that LAB are completely absent from the product. Some LAB species may be in a viable but non-culturable or unculturable state. Traditional fermentation processes always employ microorganisms, especially LAB, which are capable of producing various organic acids and metabolites from the plant materials used, with these organic acids and metabolites playing a crucial role in enhancing flavor and nutritional value (Erem & Kilic-Akyilmaz, 2024; Shi et al., 2026). Therefore, an analysis of nutritional properties was also conducted, with the results presented in Table 3. The high moisture content in *mandai* contributes to its soft and moist texture. This finding is consistent with several previous studies (Gozali et al., 2024; Rohmah et al., 2022), which found that soaking *cempedak* mesocarp in water plays a role in softening the texture of the mesocarp without significantly altering it. The high moisture content in *mandai* plays two main roles: it affects the quality of *mandai* in terms of shelf life and spoilage resistance, and it supports the growth, development, and activity of microorganisms that play roles in the *mandai* fermentation process. In practice, *mandai* is commonly known to last up to one year in oxygen-free conditions. This serves as concrete evidence of the assertion by Wang et al. (2023) that the proper utilization of moisture content plays a crucial role in the successful production of fermented foods.

Table 3. Nutritional properties of *mandai*

No.	Parameter (Unit)	Value (Mean ± SD)[a]
1	Moisture content (%)	80.07 ± 0.08
2	Ash content (%)	16.06 ± 0.03
3	Total fat content (%)	<0.02[b]
4	Energy from fat (kcal/100 g)	0 ± 0
5	Total energy (kcal/100 g)	15.50 ± 0.42
6	Protein content (%)	0.65 ± 0.01
7	Carbohydrates (%)	3.23 ± 0.11
8	L-serine (mg/kg)	6,150.18 ± 0.01
9	L-tyrosine (mg/kg)	1,468.72 ± 0.71
10	Crude fiber (%)	0.35 ± 0.01
11	Sodium (mg/100 g)	5,395.18 ± 0.65
12	Calcium (mg/100 g)	49.83 ± 0.47
13	Potassium (mg/100 g)	176.98 ± 0.23
14	Magnesium (mg/100 g)	71.32 ± 0.52

Note: (a) The mean and standard deviation values were calculated from two measurements (duplicate analysis); (b) the value "<0.02" indicates results below the detection limit—therefore, the standard deviation was not calculated.

The ash content in the analyzed *mandai* indicates a relatively high mineral content. This may be due to the use of *cempedak* mesocarp as a raw material and the use of salt as a preservative in the fermentation process. The ash content value in the analyzed *mandai* is higher when compared to several other traditional Indonesian fermented foods, such as tempeh (Chalid et al., 2019) and red *oncom* (Firoh et al., 2024), which contain ash contents below 10%. Among the four minerals detected in *mandai*, sodium has the highest concentration, reflecting the *mandai* production process that involves soaking the mesocarp of *cempedak* in brine. Surono (2016) wrote that the addition of salt to *mandai* will trigger spontaneous fermentation and microbial selection, which will support microbial succession and prevent spoilage caused by a decrease in water activity. However, the extremely high sodium concentration is a concern for consumers because it can negatively affect health, especially for consumers at risk of hypertension and cardiovascular diseases. Meanwhile, the presence of calcium, potassium, and magnesium implies that *mandai* has the potential to support consumer health since the three minerals have various important roles in the body's physiology, as long as they are not consumed in excess (Baj et al., 2020). The presence of minerals in *mandai* is also assumed to influence the growth of microorganisms during the fermentation process. This is supported by the findings of Sun et al. (2025), who discovered that calcium and magnesium can increase the environmental adaptability of microorganisms through membrane structure stabilization and energy metabolism regulation.

The total fat content detected in *mandai* is very low, meaning that no energy from fat was detected. This indicates that the total energy value obtained from the *mandai* analyzed comes from carbohydrates and protein as the main macronutrients, even though they are relatively low in quantity. Therefore, the total energy of *mandai* is said to be insignificant, reflecting the negligible contribution of fat, making *mandai* more suitable as a low-calorie side dish than as a main food for energy. The carbohydrate content obtained in this study is low, accompanied by the presence of crude fiber, which was also found in low levels. Yudistira et al. (2025) stated that the salt concentration in *mandai* significantly affects its sensory attributes and fiber content, making it beneficial for consumers who prefer traditional fermented foods that are high in fiber and low in fat. Meanwhile, the low protein content of *mandai* (even lower than other Indonesian fermented foods, such as tempeh, as demonstrated by the results of a study by Chalid et al., 2019) is similar to previous studies that found that the protein content in *cempedak* peel and derived food products was below 10% (Rohmah et al., 2022; Sundarraj & Ranganathan, 2017; Yudistira et al., 2025). However, it should be emphasized that the protein content in *mandai* contributes to the presence of essential and non-essential amino acids, considering that amino acids act as the building blocks of protein. The analysis showed that the *mandai* samples analyzed contained significant amounts of L-serine and L-tyrosine. These two amino acids contribute to the nutritional value of *mandai*, as supported by Kawano et al. (2020), who explicitly wrote that L-serine and L-tyrosine play various important roles in the body's metabolism and biological functions. Nevertheless, these findings are not comparable to those of Rohmah et al. (2025), who successfully detected 15 types of amino acids from *mandai* powder, ranging from aspartic acid to arginine. The limited detection of amino acids in *mandai* from West Kalimantan may be due to several factors, such as very low concentrations (reflecting low protein content) or limitations in the sensitivity of the method utilized.

This study offers novelty as the first study to examine the microbiological and nutritional properties of *mandai* originating from West Kalimantan, considering that existing studies have only focused on *mandai* originating from other provinces (mainly South and East Kalimantan). Unlike previous studies that showed *mandai* contains LAB that contributes positively to human health, this study shows the dominance of four *Enterobacter* species, which raises concerns from a food safety perspective. Therefore, this study contributes to several implications for the development of science and society. This study fills the gap through an in-depth analysis of *mandai* in West Kalimantan and provides new information on the dominance of four *Enterobacter* species, opening up new opportunities to explore food safety in traditional fermented foods. The process of making traditional fermented foods is uncontrolled in nature, making it prone to reduced quality in terms of hygiene, which leads to inconsistencies in microbiological properties (Anyogu et al., 2021). In addition, this study emphasizes the need for measures to ensure the quality of traditional fermented foods in order to address concerns regarding food safety, such as through

standardization of the fermentation process, use of high-quality LAB starter cultures, routine monitoring and evaluation of microbiological properties, and use of more sensitive methods to identify non-culturable LAB. This study also contributes to the achievement of Sustainable Development Goals (SDGs) 2 and 3. Support for SDG 2 ("Zero Hunger") is obtained through a comprehensive assessment of *mandai*, playing a major role in promoting food diversification based on local potential and raising new avenues for improving the nutritional properties based on the information obtained. Meanwhile, support for SDG 3 ("Good Health and Well-being") is achieved through the detection of microorganisms contained in the analyzed *mandai*, which highlights the importance of food safety in traditional fermented foods and nutritional properties analysis that explicitly demonstrates the benefits of consuming *mandai*.

CONCLUSION

The microbiological properties revealed by molecular identification indicate the dominance of four *Enterobacter* species, with none of the LAB detected, contradicting previous studies and posing a potential risk to food safety. On the other hand, the analysis of *mandai* nutritional properties indicates significant moisture content, ash levels, and essential mineral concentrations; low levels of total fat, protein, carbohydrates, and crude fiber; a total energy value of 15.50 kcal/100g; as well as the presence of L-serine and L-tyrosine. These results highlight the urgency of standardizing production processes, implementing good manufacturing practices, and developing indigenous LAB starter cultures. This study has limitations in quantitative analysis of microbial load and in characterizing the pathogenic potential of isolates. Therefore, there is a need for further studies on quantitative microbial analysis, pathogenicity characterization, evaluation of toxin and biogenic amine production, and factors contributing to the absence of LAB to ensure consumer safety without neglecting the cultural value of traditional Indonesian fermented products.

ACKNOWLEDGEMENT

This study is funded by the Non-tax State Revenue of the Faculty of Teacher Training and Education, Universitas Tanjungpura in the 2025 fiscal year.

AUTHOR CONTRIBUTION STATEMENT

LFY contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, and writing – original draft. HF contributed to data curation, formal analysis, investigation, methodology, software, visualization, and writing – original draft. MAF contributed to data curation, formal analysis, methodology, investigation, visualization, and writing – original draft. PN contributed to conceptualization, formal analysis, investigation, visualization, and writing – original draft. MK contributed to formal analysis, investigation, visualization, and writing – original draft. LAS contributed to writing – review & editing.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the study, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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