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Quality Analysis of Local Food Fermented Durian Paste (Tempoyak) in Pontianak Based on Nutrients and Bacterial Contaminants

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Tempoyak is a traditional food resulting from spontaneous fermentation of durian fruit. This fermentation involves the help of LAB which is present naturally. Tempoyak is often found in traditional markets and is a type of ready-to-eat food. The aim of this research is to analyze the feasibility of consuming Tempoyak from several traditional markets in Pontianak based on its nutritional (proximate), antioxidant and microbiological content. Tempoyak sampling was carried out in several traditional markets in Pontianak. The methods used to analyze nutrients are the Kjehdal method, Gravimetry and Solvent extraction, Antioxidants using DPPH, while analyzing microbial pollutants using Standard Plate Count, MPN and qualitative using a specific microbial growth medium by looking at the color indicators formed. The results of the analysis show that the tempoyak circulating in the Pontianak Traditional Market complies with SNI and the number of Coliforms and E. coli in the samples exceeds SNI, the samples do not contain S. aureus and Salmonella contaminants. The results of the analysis show that the tempoyak circulating in the Pontianak Traditional Market complies with SNI based on its nutritional and antioxidant content, and the number of Coliforms and E. coli in the samples exceeds the SNI so it is not suitable for consumption, even though the samples do not contain S. aureus and Salmonella contaminants.

Keywords: *Antioxidants; Coliforms; Escherichia coli; Nutrition; Staphylococcus aureus; Tempoyak*

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INTRODUCTION

High nutritional content in food, besides being essential for human needs, also serves as an excellent medium for microbial growth. Therefore, proper handling of such food is necessary to prevent contamination and ensure the food is safe for consumption (Molita et al., 2019; Susilawati et al., 2013). Improper handling, particularly poor hygiene and cleanliness, provides a pathway for microorganisms to contaminate food products.

Food safety, according to the World Health Organization (WHO), is the assurance that food or raw food materials will not adversely affect health or harm consumers when processed and/or consumed. In addition, the Indonesian government defines food safety, as stated in Government Regulation No. 86 of 2019, as the conditions and efforts necessary to prevent food from biological, chemical, and other contaminants that may disrupt, harm, or endanger human health. Furthermore, it must not contradict religious principles (halal) and societal culture, ensuring its safety for consumption.

Food that is suitable for consumption must not be repulsive (physical changes), free from biological hazards (microorganisms), and provide essential nutrients to the body. Spoiled food indicates a decline in quality and nutritional value, and if consumed, it may have adverse effects on health (Alang et al., 2022; Annisatity et al., 2023; Jimin et al., 2022). The characteristics used to measure food quality are based on chemical standards such as proximate analysis (carbohydrates, fats, proteins), physical characteristics assessed through organoleptic aspects like aroma, taste, texture, and color, and biological characteristics determined by the presence of microorganisms such as *Coliform, Escherichia coli, Staphylococcus aureus, and Salmonella spp.* (Molita et al., 2019; Mustika et al., 2019; Puspadewi et al., 2017; Sanjaya & Apriliana, 2013). Consuming food contaminated by pollutants can cause foodborne diseases (FBD), and several cases of FBD in Indonesia have been classified as extraordinary events (KLB) (Kusumaningsih, 2010; On & Rahayu, 2017).

Tempoyak is a type of Ready-to-Eat (RTE) food, which can be consumed directly without undergoing further processing (Tarmizi et al., 2020). High-quality RTE products are those that meet the Indonesian National Standard (SNI) in terms of their physical, chemical, and biological characteristics and are free from substances harmful to health (Alang et al., 2023). Tempoyak is a fermented food made from ripe or nearly overripe durian fruit (*Durio zibethinus*), commonly found on the islands of Sumatra and Kalimantan, and is typically served with shrimp or fish as a side dish (Anggraini & Widawati, 2015; Haruminori et al., 2018).

Numerous studies have been conducted on tempoyak, such as the isolation of lactic acid bacteria from tempoyak (Aisyah et al., 2014), the isolation of *Lactobacillus* bacteria producing exopolysaccharides from tempoyak (Khalil et al., 2018), and the isolation of lactic acid bacteria from tempoyak as an *anti-Escherichia coli* agent (Mursyida & Guspratiwi, 2019). However, research on the quality analysis of tempoyak has not yet been conducted. This study aims to analyze the consumption feasibility of tempoyak from several traditional markets in Pontianak, based on its nutritional content (proximate analysis), antioxidant properties, and microbiological characteristics. The results of this research are expected to provide scientific information and contribute to the body of knowledge, particularly for consumers, as well as for the city government, which holds the authority to enhance supervision from producers to consumers. This would help create traditional markets that meet the criteria for selling clean and healthy food, thereby reducing the risk of foodborne diseases.

METHOD

Time and Place

This research was conducted from February to April 2024. Sample collection was carried out at several traditional markets in Flamboyan, Pontianak City. The analysis of the nutritional content of tempoyak was performed in the Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, while the detection of contaminating microbes was conducted in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak.

Materials ad Tools

The equipment used in this study included an inoculation loop, test tubes (Pyrex), petri dishes (Pyrex), an incubator (Memmert), a laminar airflow (LAF) cabinet, an oven (Memmert), an autoclave (Memmert), and a colony counter. The materials used were alcohol, distilled water (aquadest), Plate Count Agar (PCA), Buffer Peptone Water (BPW), Lactose Broth (LB), Brilliant Green Lactose Bile Broth (BGLB), Eosin Methylene Blue Agar (EMBA), Selenite Cystine Broth (SCB), Bismuth Sulfite Agar (BSA), Trypticase Soy Agar (TSA), and Mannitol Salt Agar (MSA).

Procedure

The sampling of tempoyak was conducted at Flamboyan Market in Pontianak. The samples were then transported to the laboratory for quality analysis, which included chemical tests (nutritional content) and microbiological tests (Total Plate Count and contaminating microbes).

Analysis of Nutritional Content (Proximate Anaysis) and Antioxidant

The nutritional content analyzed in tempoyak includes protein, crude fiber, moisture, ash, fat, and carbohydrates. Protein content in the samples was analyzed using the Kjeldahl method. Crude fiber, moisture, and ash were analyzed using the gravimetric method. Fat analysis was performed through solvent extraction, while carbohydrate content was determined using the formula: fat content divided by protein content (Sholihah & Noer, 2014). The total antioxidant content was measured using the DPPH method, with a spectrophotometer set to a wavelength of 515 nm (Azis et al., 2015).

Dillution

A sample of 25 g of tempoyak was taken and mixed with 225 mL of Buffer Peptone Water (BPW) (for a 10^{-1} dilution). Subsequently, a series of dilutions was prepared up to 10^{-6}.

Total Plate Count (TPC)

The Total Plate Count (TPC) test was conducted using the Standard Plate Count (SPC) method in accordance with SNI 2009. Microbial inoculation was performed using the pour plate method, where 1 mL from each dilution was placed into a petri dish, followed by the addition of 20 mL of PCA medium, and then homogenized. The plates were incubated at 37°C for 24 hours. The bacterial colonies on the petri dish were then

counted using a colony counter. The TPC was calculated using the following formula (Surahmaida & Nurhatika, 2018; Wiratna et al., 2019).

$$\text{TPC of bacteria} : \frac{Number\ of\ microorganism\ colonies}{Volume\ counnted}$$

Contaminant Bacteria Testing

The enrichment media for Coliform used Lactose Broth (LB) and Brilliant Green Lactose Bile Broth (BGLB). For *E. coli*, Lactose Broth (LB) and Eosin Methylene Blue Agar (EMBA) were used. For *Salmonella sp.*, Selenite Cystine Broth (SCB) and Bismuth Sulfite Agar (BSA) were used. For *Staphylococcus aureus*, Trypticase Soy Agar (TSA) and Mannitol Salt Agar (MSA) were used.

Coliform testing was conducted by taking 1 mL from dilutions of 10^{-1} to 10^{-3} and transferring them into test tubes containing 5 mL of Lactose Broth (LB) and an inverted Durham tube. The samples were then incubated at 37°C for 48 hours. A positive result, indicated by gas formation in the Durham tube, was followed by transferring one inoculation loop (ose) of the sample into a test tube containing 5 mL of Brilliant Green Lactose Bile Broth (BGLB) and an inverted Durham tube. The tubes were again incubated at 37°C for 48 hours. A positive Coliform result was confirmed by the presence of gas in the Durham tube (Jimin et al., 2022).

The *E. coli* test was performed by taking one inoculation loop (ose) from the positive tube in the Coliform test and inoculating it onto Eosin Methylene Blue Agar (EMBA). The plates were then incubated at 37°C for 48 hours. A positive *E. coli* result in the sample was indicated by a metallic green sheen on the surface of the medium (Alang et al., 2023).

The *Salmonella sp.* test was conducted by taking one inoculation loop (ose) from the 10^{-1} dilution and streaking it onto Bismuth Sulfite Agar (BSA). A positive *Salmonella sp.* result in the sample was indicated by the formation of blackish-brown colonies with a metallic sheen on the surface of the medium (Alang et al., 2023).

The *Staphylococcus aureus* test was conducted by taking one inoculation loop (ose) from the 10^{-1} dilution and inoculating it into Trypticase Soy Agar (TSA) using the pour plate method. The colonies that grew were then inoculated onto Mannitol Salt Agar (MSA). A positive *S. aureus* result in the sample was indicated by a color change in the medium from red to yellow (Alang et al., 2022; Gahago et al., 2021; Jimin et al., 2022)

Data analysis

The data collected included the results of the nutritional analysis of tempoyak samples, observations of Total Plate Count (TPC) on petri dishes, results of the Most Probable Number (MPN) test, and color changes in selective media as indicators of the presence of test pathogens obtained from laboratory examinations. The values obtained were compared with the standards outlined in SNI No. 01-7388-2009. The data were then presented in the form of tables, figures, and descriptive analysis.

RESULTS AND DISCUSSION

Nutritional Content (Proximate) of Tempoyak

The results of the acidity test using a pH meter, antioxidant activity (DPPH), and the nutritional content or proximate analysis based on the research findings are presented in Table 1.

Table 1. Nutritional Characteristic

Characteristic	Sample		
	A	B	C
pH	4.0	4.0	3.8
Ash Content (%)	48.000	40.000	45.000
Moisture Content (%)	0.313	0.20	0.30
Fat Content (%)	0.68	0.5	0.62
Carbohydrates (%)	45	45.17	45
Protein (%)	23.12	23.05	20.23
DPPH (μg/mL)	10176.98	10175.10	10176.10

Proximate analysis is essential as it provides valuable information about the nutritional content of a food product. The proximate analysis conducted in this study included the assessment of ash, moisture, fat, protein, and carbohydrate content in Table 1. According to Muzaki et al. (2022), the proximate analysis aims to analyze or describe the nutritional composition of food, whether fresh or processed, and consists of protein, moisture, carbohydrate, fat, and ash content (Ambarsari et al., 2021; Sahirman, 2021)

Table 1 shows that the tempoyak samples obtained from markets A, B, and C have a low pH, ranging from 3.8 to 4.0, indicating an acidic condition. These results are consistent with the research conducted by (Aisyah et al., 2014), which reported that the fermentation of durian in the production of tempoyak leads to a decrease in pH, from 6.88 to 5.74, resulting in an acidic state. This acidity is attributed to the presence of lactic acid bacteria (LAB), which naturally occur during the fermentation process of tempoyak. This is in accordance with the findings of Erfisa et al., (2022); Fitri et al., (2023), who stated that the production of tempoyak, which takes 7-10 days, happens due to the presence of naturally occurring lactic acid bacteria (LAB) during the fermentation process. These results are further supported by Ardilla et al., (2022), who explained that the presence of LAB during the fermentation of durian into tempoyak causes the pH of the durian to decrease, resulting in a more acidic condition.

The ash content of samples A, B, and C was relatively high, at 48.000, 40.000, and 45.000, respectively, while the moisture content was relatively low, at 0.313, 0.20, and 0.30, respectively. This is consistent with the findings of Azis & Akolo (2019) who stated that the higher the ash content in a food product, the lower its moisture content. According to Azis & Akolo (2019); Palupi & Rahmatika (2022), ash content reflects the mineral or inorganic substance content in a food product. This indicates that tempoyak obtained from several traditional markets in Pontianak has a relatively high mineral or inorganic substance content. In addition, the low moisture content in tempoyak is due to

the production process, which involves the addition of salt (NaCl) to the durian fruit. According to Novia et al. (2011); Wulandari et al. (2014), chlorine (Cl) has absorbent properties, allowing it to absorb moisture from the food, thereby reducing its water content. The reduction of moisture in food naturally extends its shelf life, thus achieving the purpose of durian fermentation, which is to preserve the food for longer periods.

The fat content in tempoyak samples from several traditional markets in Pontianak was 0.68% (A), 0.5% (B), and 0.62% (C), whereas the study by Ardilla et al., (2022) reported a higher fat content of 1.4%. This is supported by Aisyah et al., (2014) who explained that the low-fat content in tempoyak is due to microbial activity, which degrades fat during the fermentation process.

Fat is one of the essential nutrients in food that functions as an energy source. However, according to Sartika (2008), consusming foods high in fat can trigger the occurrence of stroke and coronary heart disease. Several studies have mentioned that fermented foods are functional foods that have beneficial effects on health. This is further supported by St-Onge et al., (2000), who stated that consuming fermented foods can improve health, including reducing the risk of cholesterol-related diseases.

The carbohydrate content in tempoyak obtained from several traditional markets in Pontianak was 45% (A and C) and 45.17% (B), while the protein content was 23.13%, 23.05%, and 20.23%, respectively. A study by Aisyah et al., (2014) reported a carbohydrate content of 48.79% and a protein content of 6.37% in tempoyak. Typically, durian contains carbohydrates ranging from 60 to 70%. The lower carbohydrate and protein content in tempoyak, according to Aisyah et al., (2014) is due to the bacteria involved in the fermentation process, which use these components as carbon (C) and nitrogen (N) sources for their growth. Additionally, fermentation is a metabolic process that breaks down complex compounds into simpler ones, which leads to lower carbohydrate and protein content in tempoyak. This is further supported by Ardilla et al., (2022) who stated that the glycolysis process, which converts carbohydrates into pyruvic acid during tempoyak fermentation, results in a reduction of carbohydrate levels in the final product. Moreover, Aisyah et al., (2014) explained that during fermentation, bacteria hydrolyze proteins into amino acids as a source of energy.

The total antioxidant analysis of the tempoyak samples revealed that sample A had a total antioxidant content of 10,176.98 µg/mL, sample B had 10,175.10 µg/mL, and sample C had 10,176.10 µg/mL. According to Sayuti & Yenrina (2015), antioxidants are substances that can prevent or slow down cellular damage. These results indicate that tempoyak available in traditional markets has a high antioxidant content, which is beneficial for health. This is consistent with the findings of Fiter et al., (2017) who stated that tempoyak contains nutraceuticals and probiotics, giving it antioxidant properties, particularly in inhibiting the aging process.

Microbiological Characteristics of Tempoyak Samples

Regulations concerning microbial contamination standards in food in Indonesia are governed by POM No. HK.00.06.1.52.4011 and the Indonesian National Standard (BSN). The results of the microbiological testing of the tempoyak samples are presented in Table 2. The tests included Total Plate Count (TPC) values and contaminant bacteria, including *Coliform*, *E. coli*, *Salmonella sp.*, and *Staphylococcus aureus*.

Table 2. Microbiological Characteristics

Microbiology	Sample		
	A	B	C
TPC (CFU/g)	254 x 10^4	111 x 10^4	60 x 10^4
Coliform (MPN)	48,000	5,600	5,600
E. coli (Colonies)	740	560	400
S. aureus	-	-	-
Salmonella	-	-	-

Tempoyak, as a food product derived from durian, is undoubtedly rich in various nutrients. These nutrients are utilized by various organisms for growth, including pathogenic microbes. According to Hendriani & Budiarso (2020); Meilisnawaty et al., (2015), improper food handling can provide a medium for the growth of pathogenic microbes, making the food unfit for consumption and leading to potential health issues (Molita et al., 2019; Susilawati et al., 2013). Therefore, as Modibbo et al., (2014) stated, that it is crucial to determine the microbial load in a food product or pharmaceutical preparation to assess its safety status.

According to Rahayu & Nurwitri (2019), the Total Plate Count (TPC) or Angka Lempeng Total (ALT) is an analysis used to determine the microbial load in a product. The ALT value describes the number of bacteria present per milliliter or gram of the food being analyzed. The TPC results for tempoyak samples A, B, and C were 254 x 10^4, 111 x 10^4, and 60 x 10^4, respectively (Table 2). The Indonesian National Standard (SNI) specifies that the ALT value for lempok and processed fruits should be 1 x 10^4 CFU/g (SNI, 2009). According to Tama et al., (2023), while ALT is not directly related to food safety hazards, it serves to assess the quality, shelf life, contamination, and hygiene of the food. This indicates that the ALT values of tempoyak samples A, B, and C from several traditional markets in Pontianak do not meet SNI standards, suggesting that the tempoyak has poor quality and has been contaminated by bacteria.

The Coliform count in tempoyak sample A was 48,000 colonies, while samples B and C had 5,600 colonies each (Table 2). This indicates that the tempoyak is not suitable for consumption, as the Coliform levels in these samples do not comply with SNI 7388-2009, which sets a limit of < 20 MPN/mL or g (Molita et al., 2019). The analysis of *E. coli* in samples A, B, and C revealed counts of 740, 560, and 400, respectively. This indicates that the quality of the tempoyak does not meet the SNI 7388-2009 standard, which requires *E. coli* levels to be < 3 MPN/g. The presence of *E. coli* in food, according to Molita et al. (2019) the presence of *E. coli* in food indicates contamination with fecal matter. This contamination may originate from internal factors, such as raw materials, or external factors, such as workers, environmental sanitation, or improper food storage practices.

The analysis of *Staphylococcus aureus* and *Salmonella* in tempoyak samples using selective media revealed that all three samples were negative for these bacteria. This indicates that the samples met the criteria set by SNI 7388-2009. Tempoyak is made by adding salt to durian at a specific concentration. According to Yuliastuti et al., (2019), adding salt to food does not diminish its nutritional properties but instead preserves the food and enhances its flavor, making it more varied. Salt is a preservative, and its addition

to food extends the product's shelf life. This is because salt contains sodium (Na), which can lyse microbial cells (Rahayu & Nurwitri, 2019). This may explain the absence of Staphylococcus aureus and Salmonella in the tempoyak samples.

CONCLUSION

Based on the research results, it can be concluded that tempoyak sold in traditional markets in Pontianak meets the SNI standards in terms of nutritional content and antioxidant levels. However, the analysis indicates that the levels of *Coliform* and *E. coli* in the samples exceed SNI limits, making the tempoyak unsuitable for consumption, despite the absence of *Staphylococcus aureus* and *Salmonella* contamination.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Hasria Alang**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.