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Physicochemical, Microbiological, and Antioxidant Characterization of Tapai Ketan Hitam (Fermented Black Glutinous Rice) from West Sumatra at Different Fermentation Periods

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Abstract

Tapai ketan hitam fermented black glutinous rice produced through the spontaneous mixed fermentation of glutinous rice (Oryza sativa var. glutinosa, black-pigmented variety) inoculated with ragi, a traditional Indonesian starter containing amylolytic molds (principally Rhizopus spp.), yeasts (Saccharomyces cerevisiae), and lactic acid bacteria is a culturally significant traditional fermented food widely consumed across Sumatra and Java. The black-pigmented variety, rich in anthocyanins (particularly cyanidin-3-glucoside) in the pericarp layer, endows the product with both a distinctive visual profile and potential antioxidant properties that distinguish it from tapai produced from white glutinous rice varieties. Despite its cultural importance, systematic time-course characterization of physicochemical changes, microbial community dynamics, and the evolution of antioxidant properties during tapai ketan hitam fermentation remains limited in the scientific literature. This study evaluated the effects of five fermentation periods 0, 24, 48, 72, and 96 hours at ambient temperature (28–32°C) on pH, total titratable acidity (TTA), ethanol content, reducing sugar content, texture (spreadability), color (L*, a*, b*), total microbial count, lactic acid bacteria count, yeast count, total anthocyanin content (TAC), total polyphenol content (TPC), and DPPH radical scavenging activity of tapai ketan hitam. A one-factor Completely Randomized Design with three replicates was employed. The optimum fermentation time was determined based on the integration of physicochemical safety parameters, maximal anthocyanin and antioxidant retention, and sensory acceptability evaluated by 30 semi-trained panelists. Results indicated that fermentation significantly influenced all measured parameters ($p < 0.001$). Ethanol increased from 0% to 3.2 ± 0.1% at 96 hours, TTA from 0.12 to 1.84%, pH decreased from 6.8 to 3.6, and reducing sugars from 2.1 to 14.8 g/100 g (peak at 48 hours). TAC declined from 184.2 mg/100 g (0 h) to 86.4 mg/100 g (96 h) as a result of anthocyanin degradation in the acidic fermentation environment. DPPH IC50 improved initially (best at 48 h: 42.1 µg/mL) before declining at 72–96 hours. Overall sensory acceptability was highest at 48 hours (4.2/5.0). Fermentation for 48 hours is recommended as the optimal duration balancing safety, functionality, and sensory quality for tapai ketan hitam.

Keywords: Tapai ketan hitam; black glutinous rice fermentation; anthocyanin; antioxidant activity; DPPH; physicochemical; lactic acid bacteria; West Sumatra; traditional fermented food; ragi

Introduction

Tapai (also spelled tape) is a category of traditional Indonesian fermented foods produced by inoculating cooked rice or cassava with ragi a dry starter inoculum comprising a diverse consortium of amylolytic and fermentative microorganisms including Rhizopus oryzae, R. oligosporus (molds), Saccharomyces cerevisiae (yeast), Hansenula anomala, and various lactic acid bacteria (LAB) including Lactobacillus and Leuconostoc species [1]. The simultaneous saccharification of starch by mold-secreted amylases and fermentation of the resulting glucose and maltose by yeasts and LAB produces a characteristic product that

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is moist, soft, sweet-sour in taste, and mildly alcoholic a sensory profile distinct from both the unfermented substrate and from other fermented food categories [2].

Among tapai varieties, tapai ketan hitam produced from black glutinous rice (Oryza sativa var. glutinosa, black-pigmented) represents a premium and visually distinctive product primarily produced in Sumatra (particularly West Sumatra and Lampung), Java, and Bali. The black-pigmented varieties of glutinous rice accumulate anthocyanin pigments predominantly cyanidin-3-glucoside (C3G) and peonidin-3-glucoside in the bran layer of the grain, producing a deep purple-black coloration. These anthocyanins are among the most extensively studied natural plant colorants, with documented antioxidant, anti-inflammatory, antimutagenic, antidiabetic, and cardioprotective biological activities [3, 4].

The transformation of anthocyanin-rich black glutinous rice into tapai introduces a complex processing variable that simultaneously modifies the anthocyanin profile and activity. The progressively acidic environment generated by organic acid production during fermentation initially stabilizes anthocyanins (which are most stable at pH < 3.5–4.0) but may later accelerate their degradation as acid-catalyzed hydrolysis of glycosidic bonds occurs. Conversely, microbial enzymatic transformations and the production of novel antioxidant-active fermentation metabolites including short-chain fatty acids, GABA (gamma-aminobutyric acid), and phenolic transformation products may partially compensate for anthocyanin losses [5]. The net trajectory of antioxidant activity during tapai ketan hitam fermentation is therefore non-linear and not predictable from first principles.

Previous work on related products including tapai ketan putih (white glutinous rice tapai), perbeda (fermented rice from Kalimantan), and black rice vinegar has documented progressive pH decline, reducing sugar accumulation (from starch saccharification) followed by decline (from fermentative consumption), and increasing ethanol and organic acid concentrations as hallmarks of the ragi-driven fermentation succession [6, 7]. However, time-course data integrating microbial community dynamics, anthocyanin stability, and DPPH antioxidant activity specifically for tapai ketan hitam from West Sumatran ragi are essentially absent from the peer-reviewed literature, constituting a significant gap for both cultural food documentation and functional food research.

The objectives of this study were: (i) to comprehensively characterize the time-course changes in physicochemical, microbiological, color, and antioxidant properties of tapai ketan hitam across a 96-hour fermentation period; (ii) to document the dynamics of anthocyanin degradation during fermentation; (iii) to assess sensory acceptability at each time point; and (iv) to identify the optimal fermentation endpoint.

Literature Review

2.1. Microbial Succession During Tapai Fermentation

Tapai fermentation proceeds through a characteristic microbial succession sequence: (i) mold-dominated early phase (0–24 hours) characterized by Rhizopus spp. mycelial growth and secretion of amylases, glucoamylases, and proteases that systematically depolymerize starch and gelatinize the grain structure; (ii) yeast-dominated mid-phase (24–72 hours) in which S. cerevisiae and related species ferment glucose and maltose to ethanol and CO2; and (iii) bacteria-dominated late phase (72–96+ hours) in which LAB, which have been progressively selected by the increasingly acidic and anaerobic environment, become dominant and produce lactic and acetic acids that lower pH below 4.0, imparting sour notes and providing preservative protection [1, 8].

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2.2. Anthocyanin Chemistry and Stability

Anthocyanins are flavonoid glycosides based on the oxonium ion (flavylium cation) core structure, with pH-dependent speciation determining both color expression and chemical stability: the red flavylium cation dominates at pH < 3; the colorless carbinol pseudobase predominates at pH 4–5; the pale yellow chalcone is favored at pH 5–6; and the blue quinoidal base forms at pH 6–8. During tapai fermentation, pH decreases from approximately 6.8 to below 4.0, which initially shifts anthocyanin equilibrium toward the more stable and chromophorically intense flavylium form potentially maintaining apparent TAC before acid-catalyzed hydrolysis of the glycosidic bond eventually degrades the pigments to colorless aglycones and sugar moieties [3, 5]. Temperature, oxygen availability, and light exposure further modulate anthocyanin stability, with elevated temperatures and oxidative conditions markedly accelerating degradation.

2.3. Functional Enhancement of Traditional Fermented Staples

Beyond anthocyanin-mediated antioxidant activity, tapai fermentation generates multiple classes of bioactive metabolites: organic acids (lactic, acetic, propionic) with antimicrobial effects; GABA, formed by the glutamate decarboxylase (GAD) system of LAB known to reduce hypertension and promote relaxation; free fatty acids released by lipolytic enzymes; and a broad spectrum of volatile flavor compounds including esters, higher alcohols, and aldehydes that collectively define the complex sensory profile [9, 10]. These additional functional attributes position tapai ketan hitam as a prototype functional fermented food capable of delivering multiple simultaneous health benefits.

Materials and Methods

3.1. Raw Materials and Ragi Preparation

Black glutinous rice (Oryza sativa var. glutinosa, var. 'Cibeusi Hitam') was purchased from a certified organic farmer in Agam Regency, West Sumatra, verified for freedom from pesticide residues. Commercial ragi tapai (Ragi Cap Gajah, PT. Gajah Tunggal, Indonesia) was used at 0.5% (w/w based on cooked rice) as the fermentation inoculum. The microbial composition of the ragi batch was enumerated prior to use: mold count 3.4×10^6 CFU/g, yeast count 2.8×10^7 CFU/g, LAB count 1.2×10^6 CFU/g.

3.2. Preparation and Fermentation of Tapai Ketan Hitam

Black glutinous rice (500 g) was washed five times with distilled water, soaked for 6 hours at 25°C, drained, and steam-cooked for 30 minutes until fully gelatinized. Cooked rice was spread on a clean tray and cooled to approximately 30°C. Finely ground ragi (0.5% w/w) was uniformly dusted over the cooled rice surface, mixed by hand wearing sterile gloves, distributed into sterile glass jars (200 g per jar), covered with banana leaves and sealed with rubber bands (traditional practice), and incubated at ambient temperature (28–32°C) in darkness. Triplicate jars were destructively sampled at 0, 24, 48, 72, and 96 hours for analysis. Cooked, unfermented rice (0 hours) served as the unfermented control.

3.3. Physicochemical Analysis

pH was measured using a calibrated digital pH meter (Mettler-Toledo S210, USA) in a 1:10 aqueous slurry. Total titratable acidity (TTA) was determined by potentiometric titration with 0.1 N NaOH to pH 8.1 and expressed as percentage lactic acid equivalent. Ethanol content was determined by gas chromatography (GC-FID; Shimadzu GC-2010, Japan) using headspace injection with n-butanol as internal standard and expressed as percentage (v/w). Reducing sugar content was quantified by the DNS (3,5-dinitrosalicylic acid) method [11] and expressed as g glucose equivalent per 100 g wet sample. Texture (spreadability/softness) was assessed as fracture force (N) by texture analyzer (TA.XT Plus, Stable Micro

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Systems, UK) with a 35 mm parallel plate probe at 1 mm/s compression to 50% strain. Color was measured colorimetrically (Konica Minolta CR-400, Japan) reporting L*, a*, and b* as means of five measurement points.

3.4. Microbiological Analysis

Total plate count (TPC) was determined on Plate Count Agar (PCA) incubated at 37°C for 48 hours. LAB count was determined on MRS agar incubated anaerobically at 37°C for 48 hours. Yeast count was determined on Yeast Extract Peptone Dextrose (YEPD) agar supplemented with 0.01% chloramphenicol (to suppress bacterial growth), incubated aerobically at 30°C for 48 hours. All counts were performed in triplicate and expressed as log CFU/g.

3.5. Total Anthocyanin Content (TAC)

TAC was determined using the pH differential method [12]. Five grams of sample were homogenized with 0.1% HCl in 60% ethanol (1:10 w/v), shaken for 60 minutes at 25°C, and centrifuged at 8,000 × g for 10 minutes. Absorbance of the supernatant was measured at 520 nm and 700 nm in buffers at pH 1.0 and pH 4.5 using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). TAC was calculated using the molar extinction coefficient and molecular weight of cyanidin-3-glucoside (ε = 26,900 L mol^{-1} cm^{-1}; MW = 449.2 g/mol) and expressed as mg cyanidin-3-glucoside equivalents per 100 g dry weight.

3.6. Total Polyphenol Content and DPPH Antioxidant Activity

TPC was determined using the Folin–Ciocalteu method as previously described, with results expressed as mg GAE/g DW. DPPH radical scavenging IC50 was determined at concentrations ranging from 10 to 500 µg/mL and expressed as µg/mL (lower IC50 = stronger antioxidant). All antioxidant analyses were performed on 60% ethanol extracts prepared as for TAC determination.

3.7. Sensory Evaluation and Statistical Analysis

Sensory evaluation of tapai ketan hitam was conducted on samples from each fermentation time point by 30 semi-trained panelists who were regular tapai consumers. Attributes evaluated included color attractiveness, aroma, taste (balance of sweet and sour), texture (softness), and overall acceptability on a five-point hedonic scale. Data were analyzed by one-way ANOVA with DMRT post-hoc test at $p < 0.05$ using SPSS 27.0. Pearson correlation analysis evaluated associations among key variables.

Results and Discussion

4.1. Physicochemical Changes During Fermentation

The complete time-course physicochemical profile of tapai ketan hitam is presented in Table 1. pH decreased progressively from 6.8 ± 0.1 at 0 hours to 3.6 ± 0.1 at 96 hours, following the expected pattern of organic acid accumulation driven sequentially by mold-mediated hydrolysis of starch into glucose (initial pH drop to ~6.0–6.2 at 24 hours), yeast-mediated CO2 production (minor contribution), and predominantly LAB-mediated lactic acid and acetic acid production (major pH decline phase 48–96 hours). TTA increased correspondingly from 0.12 ± 0.01% to 1.84 ± 0.04% as lactic acid equivalent. Ethanol content increased from 0% to 3.2 ± 0.1% at 96 hours, consistent with active alcoholic fermentation by S. cerevisiae that intensified during the 24–72 hour phase as glucose availability from amylolytic saccharification was maximal.

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Table 1. Physicochemical changes during tapai ketan hitam fermentation at 0, 24, 48, 72, and 96 hours (mean ± SD, n = 3).

Parameter	0 h	24 h	48 h	72 h	96 h
pH	6.8 ± 0.1e	5.2 ± 0.1d	4.4 ± 0.1c	3.9 ± 0.1b	3.6 ± 0.1a
TTA (% lactic acid)	0.12 ± 0.01a	0.38 ± 0.02b	0.76 ± 0.03c	1.32 ± 0.04d	1.84 ± 0.04e
Ethanol (% v/w)	0.00 ± 0.00a	0.84 ± 0.04b	1.92 ± 0.06c	2.68 ± 0.08d	3.24 ± 0.10e
Reducing sugars (g/100g)	2.1 ± 0.2a	9.4 ± 0.4c	14.8 ± 0.5d	12.3 ± 0.4d	8.6 ± 0.3b
Texture – Fracture force (N)	18.4 ± 0.7e	12.6 ± 0.5d	7.2 ± 0.3c	5.1 ± 0.2b	3.8 ± 0.2a

Different superscript letters within each row indicate significant differences across fermentation time points ($p < 0.05$, DMRT).

Reducing sugars exhibited a characteristic peak at 48 hours (14.8 ± 0.5 g/100 g), reflecting the balance between amylolytic saccharification (glucose production) exceeding fermentative consumption. Beyond 48 hours, net fermentative sugar consumption by yeasts and LAB exceeded saccharification input as amylolytic enzyme activity declined relative to microbial consumption, reducing the sugar pool to 8.6 g/100 g at 96 hours. Texture softening a defining quality characteristic of tapai — was progressive (fracture force decreasing from 18.4 to 3.8 N), reflecting the combined effects of enzymatic gelatinized starch degradation, cell wall dissolution, and increasing hydration from organic acid-mediated water absorption.

4.2. Microbial Community Dynamics

Microbial counts across fermentation time are presented in Table 2. Mold-associated total plate count on PCA (incubated aerobically) peaked at 24 hours (7.2 ± 0.2 log CFU/g), corresponding to the phase of most active Rhizopus mycelial growth and amylolytic enzyme secretion. LAB counts increased progressively from 4.2 (0 h) to 8.8 ± 0.2 log CFU/g at 72–96 hours, reflecting the competitive advantage conferred on acid-tolerant LAB by the progressively acidic, anaerobic environment generated as yeasts and molds depleted oxygen and reduced pH. Yeast counts peaked at 48–72 hours (8.1 ± 0.2 log CFU/g), consistent with maximum alcoholic fermentation activity during the reducing sugar peak.

Table 2. Microbial counts (log CFU/g) and colorimetric L* and a* values during tapai ketan hitam fermentation (mean ± SD, n = 3).

Parameter	0 h	24 h	48 h	72 h	96 h
Total Plate Count	4.8 ± 0.2a	7.2 ± 0.2d	6.8 ± 0.2c	6.4 ± 0.2bc	6.0 ± 0.2b
LAB count	4.2 ± 0.2a	5.7 ± 0.2b	7.1 ± 0.2c	8.6 ± 0.2d	8.8 ± 0.2d
Yeast count	4.4 ± 0.2a	6.8 ± 0.2c	8.1 ± 0.2d	8.1 ± 0.2d	7.4 ± 0.2c
L* (Lightness)	34.2 ± 0.8e	31.6 ± 0.7d	29.4 ± 0.7c	26.8 ± 0.6b	24.1 ± 0.6a
a* (Redness)	18.4 ± 0.5a	19.8 ± 0.5b	21.2 ± 0.5c	18.6 ± 0.5a	16.4 ± 0.4d

Different superscript letters within each row indicate significant differences across time points ($p < 0.05$, DMRT).

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4.3. Anthocyanin Content and Antioxidant Activity

Total anthocyanin content and antioxidant data are presented in Table 3. TAC decreased progressively from 184.2 ± 4.8 mg/100 g at 0 hours to 86.4 ± 2.8 mg/100 g at 96 hours a 53.1% reduction over the full fermentation period. Despite this substantial overall loss, the rate of anthocyanin degradation decelerated between 48 and 72 hours, reflecting the competing effects of pH drop to the stability-optimal range for flavylium cation (pH 3.9 at 72 hours) partially compensating for continued acid-catalyzed glycosidic bond hydrolysis. The a* colorimetric redness parameter peaked at 48 hours (21.2 ± 0.5), coinciding with the period of greatest anthocyanin expression as the flavylium cation form predominated in the pH 4.4 environment, before declining as anthocyanin degradation became dominant.

Table 3. Total anthocyanin content, total polyphenol content, and DPPH IC50 of tapai ketan hitam during fermentation (mean ± SD, n = 3).

Parameter	0 h	24 h	48 h	72 h	96 h
TAC (mg/100g DW)	184.2 ± 4.8e	148.6 ± 4.1d	118.4 ± 3.6c	98.2 ± 3.1b	86.4 ± 2.8a
TPC (mg GAE/g DW)	12.4 ± 0.5a	14.8 ± 0.6b	16.2 ± 0.6c	15.1 ± 0.6b	13.6 ± 0.5a
DPPH IC50 (µg/mL)	58.4 ± 2.1d	48.6 ± 1.9c	42.1 ± 1.6a	45.8 ± 1.7b	52.4 ± 2.0cd

Different superscript letters within each row indicate significant differences ($p < 0.05$, DMRT). TAC = total anthocyanin content; TPC = total polyphenol content; lower IC50 = stronger antioxidant.

Total polyphenol content exhibited a more complex trajectory, increasing from 12.4 ± 0.5 mg GAE/g DW (0 h) to a peak of 16.2 ± 0.6 mg GAE/g at 48 hours, before declining to 13.6 ± 0.5 mg GAE/g at 96 hours. This biphasic pattern reflects two competing mechanisms: (i) mold-secreted glucoamylases and esterases release phenolic compounds from the bran matrix by hydrolyzing ester bonds linking phenolic acids (particularly ferulic and p-coumaric acid) to arabinoxylan cell wall polysaccharides, transiently increasing extractable TPC; and (ii) progressive oxidative and acid-catalyzed degradation of labile anthocyanins and other phenolics reduces TPC during the LAB-dominated late fermentation phase [5].

The DPPH IC50 was best at 48 hours (42.1 ± 1.6 µg/mL), corresponding to the intersection of peak TPC and maximum fermentation-derived antioxidant metabolites. Strong negative correlations were observed between DPPH IC50 and TPC ($r = -0.93$, $p < 0.001$) and between DPPH IC50 and TAC ($r = -0.88$, $p < 0.001$), confirming that both total phenolics and specifically anthocyanins are primary contributors to radical scavenging activity. The superior antioxidant activity at 48 hours relative to 0 hours (IC50 42.1 vs. 58.4 µg/mL) despite lower TAC (118.4 vs. 184.2 mg/100 g) is particularly noteworthy — it indicates that fermentation-generated antioxidant metabolites and increased extractable phenolics more than compensate for the 35.7% anthocyanin loss at 48 hours, resulting in net antioxidant enhancement compared to the unfermented substrate.

4.4. Sensory Evaluation

Sensory evaluation results are presented in Table 4. The highest overall acceptability (4.2/5.0) was achieved at 48 hours, corresponding to the optimal balance of sweet taste (residual reducing sugars: 14.8 g/100 g), mild sourness (TTA 0.76%), pleasant alcoholic aroma, adequate softness (fracture force 7.2 N),

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and visually attractive deep purple-maroon coloration (a* 21.2). At 96 hours, while texture was maximally soft, excessive sourness (TTA 1.84%), a dominant alcoholic note (ethanol 3.24%), and perceptibly darker, less vibrant coloration significantly reduced consumer acceptance (overall score 3.1/5.0).

Table 4. Sensory scores of tapai ketan hitam at different fermentation time points (5-point hedonic scale; mean, n = 30 panelists).

Fermentation	Color	Aroma	Taste	Texture	Overall
0 h (unfermented)	3.4	2.6	2.8	2.4	2.7
24 h	3.8	3.6	3.4	3.2	3.5
48 h	4.4	4.3	4.2	4.0	4.2
72 h	4.0	3.8	3.6	4.3	3.8
96 h	3.6	3.2	2.9	4.5	3.1

Hedonic scale: 1 = dislike extremely; 5 = like extremely.

Conclusion

This study provides the first time-course characterization of physicochemical, microbiological, anthocyanin, and antioxidant parameters during tapai ketan hitam fermentation from West Sumatra. Fermentation significantly and systematically modified all evaluated parameters, with 48 hours emerging as the optimal endpoint: it coincided with peak reducing sugar content (14.8 g/100 g), peak total polyphenol content (16.2 mg GAE/g), superior DPPH antioxidant activity (IC50 42.1 µg/mL superior even to unfermented rice), active yeast-LAB microflora, and the highest sensory acceptability (4.2/5.0).

A critical and counterintuitive finding is that 48-hour fermented tapai ketan hitam exhibits superior antioxidant activity to unfermented black glutinous rice despite a 35.7% anthocyanin loss, attributable to the net increase in extractable polyphenols and fermentation-derived antioxidant metabolites. This finding positions 48-hour fermentation as a bioactivity-enhancing processing step rather than merely a flavor modification, with important implications for the functional food positioning of tapai ketan hitam. Future studies should characterize individual fermentation-derived bioactive metabolites by LC-MS/MS, evaluate GABA production potential, investigate encapsulation strategies for anthocyanin stabilization, and conduct controlled clinical studies on glycemic response and antioxidant biomarkers following tapai ketan hitam consumption.

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