Pharmaceutical and Biomedical Sciences Journal

Volume 8(1), June 2026, 157-170

Optimization of Fermentation Time Enhances the Antioxidant and Anti-inflammatory Activities of Tea Kombucha Cascara from Pagar Alam Robusta Coffee (*Coffea canephora*)

Eka Putri[1*], Lina Elfita, Ahmad Musir, Hendri Aldrat, Fathul Majidi, Fadhlika Putri Vihanda, Aulia Amira, Zulaila Hunafa

Department of Pharmacy, Faculty of Health Sciences, Universitas Islam Negeri Syarif Hidayatullah Jakarta, Jl. Kertamukti No. 5, Pisangan, Ciputat, South Tangerang 15419, Banten, Indonesia

**Corresponding author: eka.putri@uinjkt.ac.id*

Received: 28 May 2026; Accepted: 29 June 2026

Abstract: Coffee cascara, the dried pulp of coffee cherries, is a nutrient-rich agro-industrial by-product with considerable potential for developing sustainable functional beverages. Although kombucha fermentation has been reported to enhance the bioactivity of various plant substrates, the optimal fermentation period for maximizing the biological properties of robusta coffee cascara remains unclear. This study aimed to evaluate fermentation-dependent changes in the physicochemical characteristics, phytochemical profile, antioxidant activity, and in vitro anti-inflammatory potential of Tea Kombucha Cascara (TKC) prepared from red-ripe Coffea canephora cultivated in Pagar Alam, South Sumatra, Indonesia. Sweetened cascara infusion was fermented with a kombucha symbiotic culture of bacteria and yeast (SCOBY) for 15 days under controlled conditions (25–27 °C). Fermentation induced progressive physicochemical and organoleptic transformations, characterized by gradual acidification, pellicle formation, mild carbonation, and the development of a typical fermented beverage profile. The pH decreased from 3.783 to 3.390, while microbial contamination and ethanol remained undetectable under the applied analytical conditions throughout fermentation. Qualitative phytochemical screening consistently detected flavonoids, quinones, tannins, terpenoids, and saponins during the entire fermentation period. Biological activities exhibited a fermentation-dependent pattern, with the strongest simultaneous antioxidant and in vitro anti-inflammatory activities observed on day 5, where DPPH IC_{50} and protein-denaturation IC_{50} decreased from 2.028 to 1.137% (v/v) and from 8,697 to 5,640 % (v/v), respectively. Collectively, these findings demonstrate that five-day fermentation provides the optimal balance between bioactivity, physicochemical stability, and sensory acceptability, highlighting TKC as a promising cascara-based nutraceutical beverage and providing a scientific basis for fermentation endpoint optimization in coffee by-product valorization.

Keywords: antioxidant, anti-inflammatory, *Coffea canephora*, coffee cascara, kombucha fermentation, tea kombucha cascara.

DOI: https://doi.org/10.15408/pbsj.v8i1.51260

1. INTRODUCTION

Coffee processing generates substantial quantities of agro-industrial by-products, particularly coffee pulp, which accounts for nearly 40–50% of the fresh coffee fruit mass. In most coffee-producing regions, including Indonesia, this biomass is commonly discarded or used as low-value compost and animal feed, despite being rich in carbohydrates, dietary fiber, minerals, and diverse phytochemicals. The valorization of coffee by-products into functional foods has therefore become an important strategy to promote sustainability, reduce environmental burdens, and increase the economic value of the coffee industry. Among these by-products, coffee cascara, the dried pulp of coffee cherries, has attracted increasing attention as a promising raw material for nutraceutical and functional beverage development.

Coffee cascara contains numerous bioactive constituents, including chlorogenic acid, caffeic acid, flavonoids, tannins, caffeine, trigonelline, and other phenolic compounds that exhibit antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory activities (Braham and Bressani, 1979; Murthy and Naidu, 2012; Esquivel and Jiménez, 2012; Gemechu, 2020). These compounds contribute not only

to the nutritional value of cascara but also to its potential as a sustainable source of health-promoting ingredients. Accordingly, dried coffee cherry pulp from *Coffea arabica* and *Coffea canephora* has been authorized as a traditional food in the European Union, further supporting its application as a safe food ingredient when produced under appropriate quality standards [EFSA, 2021; Commission Implementing Regulation (EU), 2022].

Microbial fermentation has emerged as an effective bioprocessing approach to improve the functional properties of plant-derived materials. During fermentation, microbial enzymes hydrolyze complex macromolecules, release bound phenolic compounds, generate organic acids, and transform phytochemicals into metabolites with enhanced biological activities and bioavailability (Lemes et al., 2022). Among various fermentation technologies, kombucha fermentation has received considerable attention because the symbiotic culture of bacteria and yeast (SCOBY) simultaneously produces organic acids, microbial cellulose, vitamins, and other metabolites while preserving or enhancing the antioxidant capacity of plant substrates (Jayabalan et al., 2014; Coelho et al., 2020). Consequently, kombucha has become an attractive platform for developing functional beverages from agricultural by-products.

Previous studies have demonstrated that coffee cascara possesses considerable antioxidant potential and that kombucha fermentation may further enhance its biological properties. Fermented coffee pulp has also been reported to exhibit increased polyphenolic content following microbial biotransformation (Putri et al., 2023). More recently, kombucha beverages prepared from coffee cascara have shown promising antioxidant and anti-inflammatory activities (Sales et al., 2023; López-Parra et al., 2024). Nevertheless, previous investigations have primarily focused on changes in individual bioactivities or physicochemical properties. The optimal fermentation period that simultaneously maximizes physicochemical quality, phytochemical retention, antioxidant activity, and anti-inflammatory potential of Tea Kombucha Cascara (TKC), particularly those prepared from Pagar Alam Robusta coffee (*Coffea canephora*), has not yet been systematically investigated. This knowledge gap limits the establishment of scientifically supported fermentation conditions for developing standardized cascara-based functional beverages.

Therefore, this study aimed to evaluate fermentation-dependent changes in the physicochemical characteristics, phytochemical profile, antioxidant activity, and in vitro anti-inflammatory activity of Tea Kombucha Cascara (TKC) prepared from Pagar Alam Robusta coffee. Furthermore, by integrating these parameters throughout a 15-day fermentation period, this study sought to identify the optimal fermentation endpoint that provides the best balance between biological activity, physicochemical quality, and sensory acceptability, thereby providing a scientific basis for the development of cascara-based nutraceutical beverages.

2. MATERIAL AND METHODS

2.1 Raw material and cascara preparation

Fresh ripe Robusta coffee (*Coffea canephora*) cherries were obtained from Pagar Alam, South Sumatra, Indonesia. Cascara was prepared from freshly harvested red-ripe coffee cherries by manually separating the pulp from the beans, followed by drying under controlled conditions until constant weight was achieved. Commercial sucrose was used as the fermentation substrate. A kombucha symbiotic culture of bacteria and yeast (SCOBY) together with previously fermented kombucha broth was used as the starter culture. All analytical-grade chemicals and reagents used for physicochemical; phytochemical, antioxidant, and protein-denaturation assays were purchased from certified suppliers.

2.2 Preparation of tea kombucha cascara

Tea Kombucha Cascara (TKC) was prepared by infusing 65.6 g of dried cascara in 1 L of hot water at 90 °C for 6.5 min following the infusion procedure described by Apri et al. (2021). The infusion was

filtered through sterile muslin cloth to remove solid residues and allowed to cool to room temperature. Sucrose (200 g L^{-1}) was then added and completely dissolved to provide the primary carbon source for microbial fermentation. After cooling, the sweetened cascara infusion was aseptically inoculated with a kombucha starter culture consisting of 50 g of SCOBY pellicle and 60 mL of previously fermented acidic kombucha broth. Fermentation was carried out statically in sterile 1.5-L glass vessels at 25–27 °C for 15 days. The fermentation vessels were covered with sterile breathable cloth secured with rubber bands to maintain aerobic conditions while minimizing external contamination. All fermentation treatments were prepared in triplicate.

Fermentation progress was monitored daily through physicochemical and observational parameters, including pH, pellicle formation, carbonation, and changes in beverage color, aroma, and taste. These parameters were used as practical indicators of fermentation development throughout the incubation period.

The present study focused on evaluating fermentation-dependent changes in physicochemical characteristics, phytochemical profile, antioxidant activity, and in vitro anti-inflammatory screening activity. Quantitative analyses of total sugar, reducing sugar, glucose, fructose, or soluble solids (°Brix) were not performed. Therefore, microbial substrate utilization was not directly quantified, and the observed fermentation progression was interpreted based on physicochemical and sensory changes rather than sugar consumption.

2.3 Raw material, microbiological, and physicochemical characterization

Raw cascara was characterized for moisture, ash, and crude fat contents according to the official AOAC methods. Moisture content was determined by oven drying, ash content by dry ashing, and crude fat by Soxhlet extraction.

Microbiological quality of dried cascara was evaluated by total aerobic bacterial count and mold count using the plate count method. Briefly, 10 g of cascara was homogenized in 90 mL of sterile buffered peptone water and serially diluted prior to plating. Total aerobic bacteria were enumerated on Nutrient Agar (NA), whereas molds were enumerated on Potato Dextrose Agar (PDA). Plates were incubated under appropriate conditions according to standard microbiological procedures before colony enumeration. Microbial counts were expressed as colony-forming units per gram (CFU g^{-1}).

Fermentation progress was monitored daily by evaluating physicochemical and observational parameters, including pH, pellicle formation, carbonation, and changes in color, aroma, and taste. The pH was measured using a calibrated digital pH meter. Ethanol content was determined by distillation followed by pycnometric specific gravity determination.

2.4 Phytochemical screening

Qualitative phytochemical screening of raw cascara included alkaloids, flavonoids, saponins, tannins, quinones, volatile oils, steroids, triterpenoids, and coumarins. Fermented TKC was screened for flavonoids, quinones, tannins, terpenoids, and saponins from day 1 to day 15. Flavonoids were evaluated using the Shinoda test, quinones using alkaline reaction, tannins using ferric chloride reaction, terpenoids using Liebermann–Burchard reaction, and saponins using the foam test. Results were reported qualitatively as detected (+), not detected (-), or not tested (NT).

2.5 Antioxidant and anti-inflammatory assays

Antioxidant activity was determined using the DPPH radical-scavenging method. TKC samples were prepared in serial concentrations, mixed with 0.1 mM DPPH solution, vortexed, and incubated in the dark for 30 min. Absorbance was measured at 515.5 nm, and IC50 values were calculated from concentration–inhibition regression. Vitamin C was used as the positive control.

In vitro anti-inflammatory screening activity was evaluated using inhibition of bovine serum albumin (BSA) denaturation. TKC samples were mixed with BSA solution, incubated at 37 °C for 30 min, heated at 57 °C for 3 min to induce protein denaturation, cooled to room temperature, diluted with Tris-buffered saline pH 6.3, and measured at 660 nm. Sodium diclofenac was used as the positive control. Lower IC50 values indicate stronger activity in both antioxidant and protein-denaturation inhibitory assays.

2.6 Data treatment

Quantitative results were expressed as fermentation-day profiles. Percentage improvement in activity was calculated relative to day 0 using IC50 values. Organoleptic and phytochemical results were reported descriptively. Because the presented article focuses on endpoint screening and fermentation profiling, activity interpretation was made conservatively without overextending the in vitro findings into therapeutic claims.

3. RESULTS AND DISCUSSION

3.1 Raw-material quality of Pagar Alam robusta cascara

Red-ripe Robusta coffee cherries (*Coffea canephora* Pierre ex A. Froehner) harvested from Pagar Alam, South Sumatra, Indonesia, were processed into dried coffee cherry pulp (cascara) to serve as the substrate for Tea Kombucha Cascara (TKC) fermentation. The use of fully ripe cherries was intended to obtain a homogeneous raw material because fruit maturity influences sugar availability, organic acid composition, and phytochemical content, all of which may affect microbial fermentation and the quality of the final beverage. The preparation process, including fresh red-ripe cherries and the resulting dried cascara, is presented in Figure 1.

(A) (B)

Figure 1. Preparation of Pagar Alam Robusta cascara used as the substrate for Tea Kombucha Cascara (TKC). (A) Red-ripe Robusta coffee cherries; (B) dried cascara.

Processing 30,000 g of fresh coffee cherries produced 4,628 g of wet coffee pulp and 1,849.36 g of dried cascara, corresponding to a recovery yield of 6.16% based on fresh cherries and 39.96% based on wet pulp (Table 1). Although coffee pulp is generally regarded as an agricultural by-product, these results demonstrate that a substantial proportion can be recovered and converted into a value-added fermentation substrate, supporting more sustainable utilization of coffee-processing residues.

Proximate analysis indicated that the dried cascara contained 8.27% moisture, 8.10% ash, and 4.26% crude fat (Table 1). The relatively low moisture content suggests that the drying process effectively reduced water availability, thereby improving storage stability and minimizing the risk of microbial deterioration before fermentation. The ash content reflects the presence of naturally occurring mineral constituents derived from coffee pulp; however, the specific mineral composition was not determined in the present study. Meanwhile, the crude fat content is consistent with previous reports indicating that

coffee pulp contains only a modest lipid fraction, making carbohydrates and other soluble constituents the predominant components available during kombucha fermentation.

Table 1: Raw-material quality of Pagar Alam robusta cascara used for TKC production.

Parameter	Result
Fresh coffee cherries	30,000 g
Wet coffee pulp	4,628 g
Dried cascara	1,849.36 g
Cascara yield from fresh cherries	6.16%
Cascara yield from wet pulp	39.96%
Moisture content	8.27%
Ash content	8.10%
Fat content	4.26%
Mold plate count	0 CFU/g
Bacterial plate count	0 CFU/g

Microbiological quality assessment showed no detectable bacterial or mold colonies under the applied analytical conditions (Table 1). These findings indicate that the microbial load of the dried cascara was below the detection capability of the plate-count method employed and should not be interpreted as evidence of complete sterility. Instead, the results demonstrate that the drying and handling procedures were effective in reducing cultivable microbial contamination to levels suitable for subsequent controlled fermentation.

Overall, the combination of adequate drying, acceptable proximate composition, and low detectable microbial contamination supports the suitability of Pagar Alam Robusta cascara as a raw material for Tea Kombucha Cascara production. Establishing the quality of the starting material is essential because variations in substrate composition and microbiological status may influence fermentation performance and the physicochemical and bioactive characteristics of the final fermented beverage.

3.2 Phytochemical profile of raw cascara

Qualitative phytochemical screening was conducted to identify the major classes of secondary metabolites present in raw cascara. The dried cascara was positive for alkaloids, flavonoids, saponins, tannins, quinones, and triterpenoids, while volatile oils, steroids, and coumarins were not detected (Table 2). These findings indicate that Pagar Alam robusta cascara contains several secondary-metabolite classes that are relevant to antioxidant and anti-inflammatory screening.

Table 2: Qualitative phytochemical profile of raw cascara

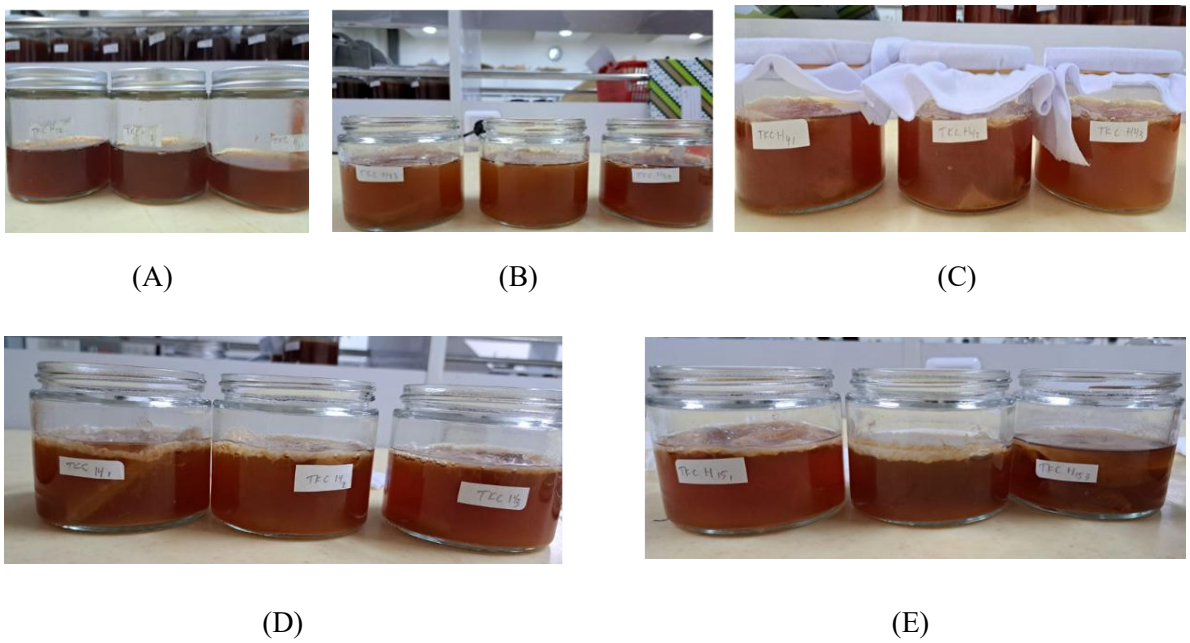
Phytochemical class	Raw cascara
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Quinones	+
Triterpenoids/Terpenoids	+
Volatile oils	-
Steroids	-
Coumarins	-

Note: + = detected; - = not detected

The presence of flavonoids and tannins is particularly important because these compounds are commonly associated with free-radical scavenging capacity and protein-stabilizing activity. Alkaloids detected in raw cascara may include caffeine or related methylxanthines, although qualitative screening cannot identify or quantify individual compounds. Similarly, the detection of saponins, quinones, and triterpenoids provides a broader phytochemical basis for the biological activity of the substrate. Future studies should quantify marker compounds such as caffeine, trigonelline, chlorogenic acid, caffeic acid, flavonoids, and tannins using validated chromatographic methods.

3.3 Fermentation characteristics and organoleptic transformation of TKC

Tea kombucha cascara was prepared by fermenting sweetened cascara infusion with kombucha SCOBY for 15 days. During fermentation, TKC underwent visible changes in color, surface pellicle formation, and appearance of fine bubbles. The beverage initially appeared as a dark reddish-brown infusion and gradually became brighter reddish-brown as fermentation progressed. The visual changes of TKC at selected fermentation days are shown in Figure 2.



(A) (B) (C)

(D) (E)

Figure 2: Visual changes of tea kombucha cascara during fermentation. (A) TKC day 1; (B) TKC day 4; (C) TKC day 6; (D) TKC day 13; (E) TKC day 15. Photographs were obtained from the research documentation.

The formation of a thin surface pellicle and fine bubbles during early fermentation indicates SCOBY adaptation and microbial activity in the cascara infusion. In kombucha fermentation, yeasts hydrolyze sucrose and produce ethanol and carbon dioxide, while acetic acid bacteria further oxidize ethanol and sugars into organic acids. The development of a new pellicle also suggests that cascara infusion provided nutrients sufficient to support SCOBY growth. This observation is important because it demonstrates that robusta cascara can function not only as a flavoring material but also as a fermentation substrate.

The organoleptic characteristics of TKC changed progressively throughout the 15-day fermentation period (Table 3). The initial preparation was characterized by a sweet, pleasant coffee-pulp aroma, a sweet and slightly astringent taste, and a dark reddish-brown color. During the first three days, the characteristic coffee-pulp aroma became less dominant, and a mild acidic note began to appear. From day 4 to day 6, sweetness decreased, astringency became weaker, and acidity increased.

After day 7, the beverage developed a more evident sour fermented aroma, refreshing carbonation, and a clearer reddish-brown appearance. In the late fermentation period, especially from day 13 to day 15, the sour acidic aroma became stronger, acidity dominated the taste profile, and the reddish-brown color became slightly brighter. This pattern indicates progressive acidification and metabolic transformation of the cascara infusion during kombucha fermentation.

Table 3: Organoleptic and visual characteristics of TKC during fermentation.

Fermentation period	Aroma	Taste	Color and visible characteristics
Day 0–1	Characteristic coffee-pulp aroma; sweet and pleasant	Sweet and slightly astringent	Dark reddish-brown
Day 2–3	Coffee-pulp aroma decreased; slight acidic note appeared	Sweet, slightly astringent, mildly acidic	Dark reddish-brown; early thin pellicle and fine bubbles appeared
Day 4–6	Mild acidic fermented aroma	Sweetness decreased; astringency became weaker; acidity increased	Dark reddish-brown with slightly brighter tone
Day 7–10	Sour fermented aroma became more evident	Acidic, slightly sweet, refreshing carbonation	Reddish-brown
Day 11–12	Sharper sour fermented aroma	Sour, slightly sweet, carbonated	Reddish-brown
Day 13–15	Strong sour and acidic fermented aroma	Dominant acidity, slight residual sweetness, carbonated	Brighter reddish-brown

The organoleptic profile is relevant for fermentation endpoint selection. Although prolonged fermentation may increase acidity and produce a stronger fermented character, excessive sourness may reduce consumer acceptability for a general nutraceutical beverage. Therefore, early-to-mid fermentation should be considered more favorable when biological activity and sensory feasibility are evaluated together.

3.4 Changes in pH and Ethanol During TKC Fermentation

The pH of Tea Kombucha Cascara (TKC) gradually decreased throughout the 15-day fermentation period, declining from 3.783 on day 0 to 3.390 on day 15 (Figure 3). The progressive decrease in pH indicates continuous acidification of the fermentation medium, which is consistent with the metabolic activity of the kombucha symbiotic culture of bacteria and yeast (SCOBY). During fermentation, yeasts hydrolyze sucrose into glucose and fructose and subsequently convert fermentable sugars into ethanol and carbon dioxide. The acetic acid bacteria then oxidize ethanol into organic acids, predominantly acetic acid, together with gluconic, glucuronic, lactic, and other organic acids, resulting in a gradual reduction of pH.

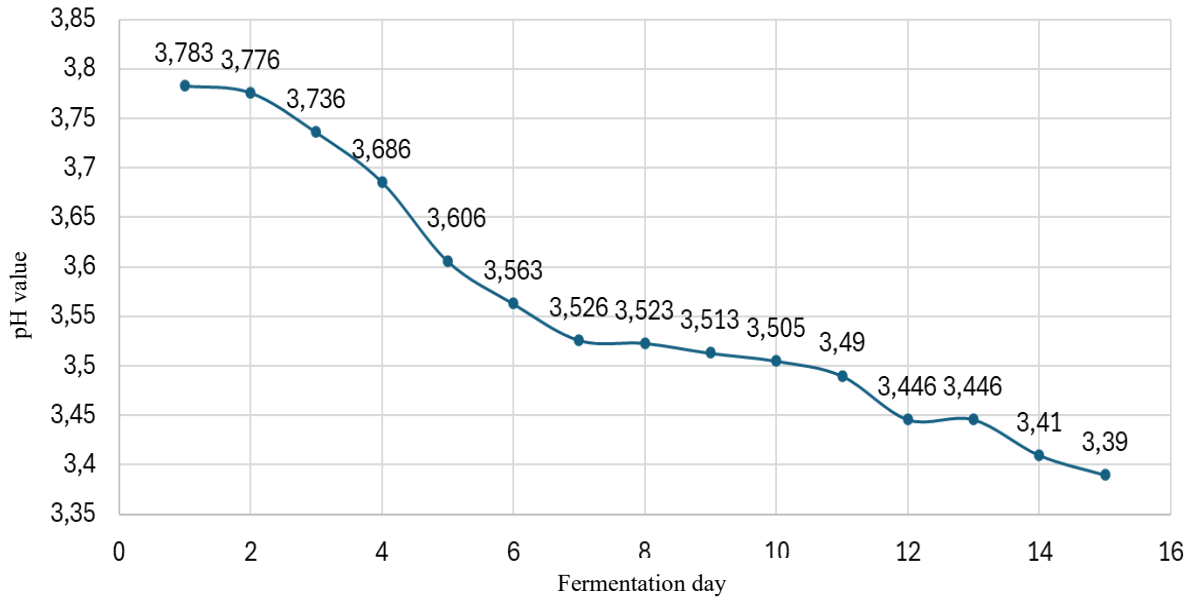


Figure 3. pH profile of TKC during 15 days of fermentation.

Although the decreasing pH is consistent with the expected biochemical changes occurring during kombucha fermentation, pH alone should not be considered definitive evidence of substrate utilization or fermentation efficiency. In the present study, fermentation progress was evaluated using physicochemical observations, including pH reduction, pellicle formation, carbonation, and changes in beverage color, aroma, and taste. However, quantitative measurements of total sugar, reducing sugar, glucose, fructose, or soluble solids (°Brix) were not performed. Consequently, substrate consumption by the microbial consortium could not be directly quantified and should be regarded as a limitation of the present study.

On day 5, the pH reached 3.563, representing a moderately acidic condition that remained within the typical pH range reported for kombucha beverages while avoiding the excessive acidification observed during prolonged fermentation. This intermediate acidity was considered together with antioxidant activity, in vitro protein-denaturation inhibitory activity, phytochemical profile, and sensory observations when determining the most suitable fermentation endpoint, rather than relying on pH as a single selection criterion.

Ethanol was not detected throughout the fermentation period using the applied distillation-based analytical method. These findings indicate that ethanol concentrations, if present, were below the detection capability of the analytical procedure employed under the experimental conditions. Therefore, the results should not be interpreted as the complete absence of ethanol.

The absence of detectable ethanol may be associated with the metabolic characteristics of the SCOBY consortium, in which ethanol produced by yeasts is subsequently oxidized by acetic acid bacteria into organic acids during aerobic fermentation. Nevertheless, because ethanol concentration was evaluated using a conventional distillation method, trace amounts of ethanol may not have been detected. More sensitive analytical techniques, such as gas chromatography coupled with flame ionization detection (GC-FID) or gas chromatography–mass spectrometry (GC-MS), are recommended for future studies, particularly when quantitative ethanol determination is required to support regulatory classification or non-alcoholic beverage claims.

The combination of gradual acidification and the absence of detectable ethanol under the applied analytical conditions suggests that the fermentation process proceeded under conditions favorable for organic acid production. However, because sugar consumption and ethanol formation were not quantified directly, these findings should be interpreted as indirect evidence of fermentation rather than definitive confirmation of microbial substrate utilization.

3.5 Phytochemical Profile of Fermented Tea Kombucha Cascara

Qualitative phytochemical screening demonstrated that flavonoids, quinones, tannins, terpenoids, and saponins remained detectable throughout the 15-day fermentation period. The consistent positive reactions obtained using the respective qualitative assays indicate that these major classes of secondary metabolites were still present in the fermented beverage under the applied analytical conditions. However, because the screening was qualitative in nature, these results indicate only the presence or absence of each phytochemical class and do not provide information regarding changes in their concentration during fermentation.

The persistence of positive reactions for flavonoids, tannins, quinones, terpenoids, and saponins throughout fermentation suggests that these metabolite groups remained chemically detectable despite microbial biotransformation. Kombucha fermentation is known to modify the chemical composition of plant-derived substrates through enzymatic reactions, hydrolysis, oxidation, and microbial metabolism. Nevertheless, qualitative phytochemical assays are not sufficiently sensitive to detect subtle quantitative changes or structural modifications of individual compounds. Therefore, the present results should not be interpreted as evidence that the concentrations of these metabolites remained unchanged during fermentation.

Although flavonoids, tannins, quinones, terpenoids, and saponins have been widely reported to possess antioxidant and anti-inflammatory properties, the present study does not establish a direct causal relationship between these phytochemical groups and the observed biological activities because

individual compounds or total phenolic content were not quantified. Consequently, any contribution of these metabolites to the antioxidant and in vitro protein-denaturation inhibitory activities of TKC should be regarded as a biologically plausible interpretation rather than definitive evidence.

An important observation was that alkaloids produced a positive reaction in the raw cascara but were not detected in fermented TKC using the Dragendorff qualitative assay. This finding should not be interpreted as complete degradation or elimination of alkaloids during fermentation. Coffee cascara naturally contains alkaloids, particularly caffeine and trigonelline, which are relatively stable compounds and are not expected to disappear completely during kombucha fermentation. The negative qualitative reaction observed after fermentation may instead reflect changes in sample acidity, dilution effects, matrix interference, or the analytical sensitivity of the colorimetric assay. Therefore, confirmation using quantitative analytical techniques, such as high-performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS), is required before concluding whether fermentation alters the alkaloid composition of Tea Kombucha Cascara.

Overall, the qualitative phytochemical profile indicates that fermentation did not eliminate the major classes of bioactive metabolites detectable by conventional phytochemical screening. However, future studies incorporating quantitative phytochemical analyses are necessary to elucidate the biochemical transformations occurring during kombucha fermentation and to better understand their relationship with the biological activities of the fermented beverage.

3.6 Antioxidant Activity of Tea Kombucha Cascara

The antioxidant activity of Tea Kombucha Cascara (TKC) was evaluated using the DPPH radical-scavenging assay and expressed as the IC_{50} value, where lower IC_{50} values indicate stronger antioxidant capacity. As shown in Figure 4, fermentation significantly affected the antioxidant activity of TKC (One-way ANOVA, $p < 0.05$), demonstrating that fermentation time influenced the radical-scavenging capacity of the beverage. The antioxidant profile exhibited a dynamic, non-linear pattern throughout the 15-day fermentation period.

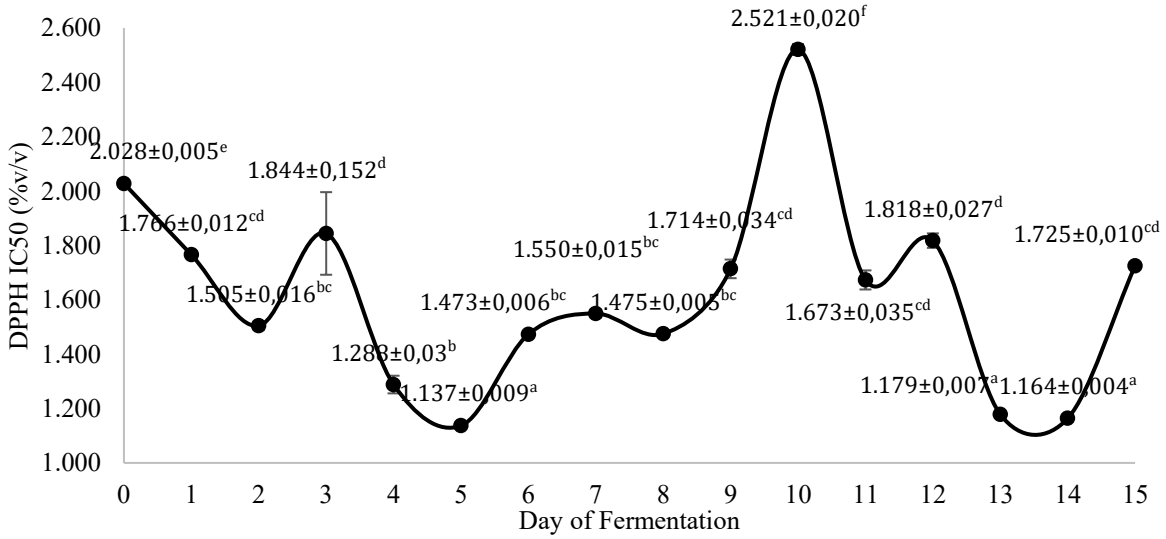


Figure 4. Changes in antioxidant activity (DPPH IC_{50}) of Tea Kombucha Cascara during 15 days of fermentation. Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences among fermentation times according to Tukey's HSD test ($p < 0.05$).

The unfermented sample (day 0) showed an IC_{50} value of 2.028 ± 0.005% (v/v). Antioxidant activity gradually increased during the early fermentation stage, reaching the lowest IC_{50} value on day 5 (1.137 ± 0.009% v/v), representing a 43.94% reduction relative to day 0. Antioxidant activity subsequently

declined during the middle fermentation period, with the highest IC_{50} value observed on day 10 (2.521 ± 0.020% v/v), indicating the weakest radical-scavenging activity. Following this transient decline, antioxidant activity increased again, producing another low IC_{50} value on day 14 (1.164 ± 0.004% v/v). These findings indicate that antioxidant activity fluctuated throughout fermentation rather than changing progressively with fermentation time.

Statistical analysis further demonstrated that fermentation time significantly affected antioxidant activity (One-way ANOVA, $p < 0.05$). Post hoc Tukey's HSD test revealed that the IC_{50} values obtained on days 5, 13, and 14 belonged to the same statistical group (group a), indicating that antioxidant activities during these fermentation periods were not significantly different ($p > 0.05$). In contrast, day 10 (group f) exhibited significantly weaker antioxidant activity than the optimal fermentation periods. These statistical results confirm that several fermentation stages produced similarly high antioxidant capacities despite differences in fermentation duration.

The dynamic antioxidant profile likely reflects continuous biochemical transformations occurring during kombucha fermentation. The symbiotic culture of bacteria and yeast (SCOBY) continuously modifies the chemical composition of the cascara substrate through enzymatic hydrolysis, oxidation–reduction reactions, and microbial metabolism. These transformations may alter the composition and reactivity of naturally occurring phytochemicals, thereby influencing their radical-scavenging capacity. However, because the present study only performed qualitative phytochemical screening without quantifying total phenolic content, total flavonoids, or individual antioxidant compounds, the underlying biochemical mechanisms cannot be confirmed directly. Therefore, the observed variation in antioxidant activity should be interpreted as evidence of fermentation-dependent biochemical transformation rather than as proof of increased phenolic concentration.

The presence of two antioxidant optima during fermentation (days 5 and 14) suggests that different stages of microbial metabolism may establish distinct biochemical equilibria capable of producing comparable antioxidant capacities. Similar non-linear antioxidant patterns have been reported in fermented plant-derived beverages, where simultaneous metabolite formation, transformation, and degradation occur throughout fermentation. Consequently, antioxidant activity may increase, decrease, or stabilize depending on the balance between microbial metabolism and phytochemical transformation.

Although antioxidant activities on days 5, 13, and 14 were statistically comparable, determination of the optimal fermentation endpoint should not rely solely on antioxidant capacity. In the present study, fermentation performance was evaluated using multiple complementary parameters, including pH, phytochemical profile, in vitro protein-denaturation inhibitory activity, and sensory characteristics. Considering these parameters collectively, day 5 was selected as the preferred fermentation endpoint because it achieved antioxidant activity statistically comparable to that observed during later fermentation while requiring a substantially shorter fermentation period and maintaining a moderately acidic profile. Therefore, the selection of day 5 represents the most practical fermentation condition within the scope of this study rather than implying universally superior antioxidant activity under all fermentation conditions.

3.7 In vitro anti-inflammatory screening activity of TKC

The anti-inflammatory activity of Tea Kombucha Cascara (TKC) was evaluated using the protein denaturation inhibition assay and expressed as the IC_{50} value, where lower IC_{50} values indicate stronger inhibitory activity against protein denaturation. As presented in Figure 5 and Table 7, fermentation time significantly affected the anti-inflammatory activity of TKC (One-way ANOVA, $p < 0.001$), indicating that microbial fermentation markedly influenced the biological activity of the beverage.

The unfermented TKC (day 0) exhibited an IC_{50} value of 8.697 ± 0.064% (v/v). During the early fermentation stage, the IC_{50} values progressively decreased from day 1 to day 5, reaching 5.640 ± 0.061% (v/v) on day 5. This reduction corresponds to an approximately 35.15% improvement in anti-inflammatory activity relative to the unfermented sample. Following day 5, the IC_{50} values temporarily increased during days 6–11 before declining again to 4.965 ± 0.019% (v/v) on day 12 and reaching the

lowest value of 4.533 ± 0.074% (v/v) on day 13, representing an approximately 47.88% improvement compared with day 0. These results demonstrate that the anti-inflammatory activity of TKC followed a dynamic non-linear pattern throughout fermentation rather than changing progressively with fermentation time.

Table 4. Anti-inflammatory activity (protein denaturation IC_{50}) of Tea Kombucha Cascara during 15 days of fermentation.

	(%v/v)
0	8,697±0,06
1	7,074±0,02
2	6,936±0,07
3	6,527±0,02
4	5,950±0,09
5	5,640±0,06
6	6,923±0,02
7	7,180±0,10
8	7,677±0,11
9	7,914±0,04
10	7,468±0,01
11	7,137±0,01
12	4,965±0,02
13	4,533±0,07
14	5,892±0,10
15	7,177±0,05

Statistical analysis further confirmed that fermentation significantly influenced the anti-inflammatory activity of TKC (One-way ANOVA, $p < 0.001$). Post hoc Tukey's HSD test demonstrated that the fermentation samples were distributed into several significantly different statistical groups. The strongest anti-inflammatory activity was observed on day 13 (group a), followed by day 12 (group b) and day 5 (group c). In contrast, the unfermented sample (day 0; group k) exhibited the weakest inhibitory activity. These findings indicate that microbial fermentation substantially enhanced the ability of TKC to inhibit protein denaturation compared with the non-fermented beverage.

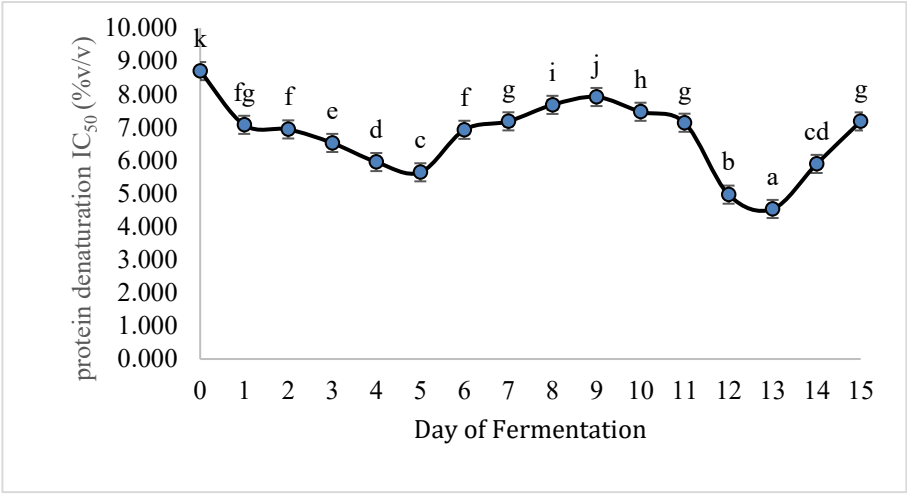


Figure 5. Changes in anti-inflammatory activity (denaturation protein IC_{50}) of Tea Kombucha Cascara during 15 days of fermentation. Values are expressed as mean ± SD (n = 3). Different letters indicate significant differences among fermentation times according to Tukey's HSD test ($p < 0.05$).

The observed fluctuations in anti-inflammatory activity are consistent with the complex metabolic processes occurring during kombucha fermentation. Throughout fermentation, the symbiotic culture of

bacteria and yeast (SCOBY) continuously modifies the chemical composition of cascara through enzymatic hydrolysis, oxidation–reduction reactions, and microbial biotransformation. These processes may alter the concentration and bioavailability of phytochemical constituents responsible for protein stabilization, including phenolic compounds, flavonoids, tannins, quinones, terpenoids, and other fermentation-derived metabolites. Consequently, the anti-inflammatory activity may increase or decrease depending on the dynamic balance between metabolite biosynthesis, transformation, and degradation during fermentation.

The phytochemical screening performed in the present study demonstrated that flavonoids, tannins, quinones, terpenoids, and saponins remained detectable throughout the fermentation period. Although qualitative screening cannot establish a direct quantitative relationship with biological activity, these metabolite groups have been widely reported to contribute to anti-inflammatory effects through multiple mechanisms, including stabilization of protein structure, inhibition of inflammatory mediator formation, reduction of oxidative stress, and modulation of inflammatory signaling pathways. Therefore, the enhanced protein-denaturation inhibitory activity observed after fermentation is likely associated with the collective action of these fermentation-derived phytochemicals rather than with changes in a single metabolite class.

Interestingly, the fermentation periods exhibiting the strongest anti-inflammatory activity closely corresponded to those producing the highest antioxidant activity. The antioxidant assay identified days 5, 13, and 14 as the periods with the greatest radical-scavenging capacity, whereas the protein denaturation assay demonstrated optimum anti-inflammatory activity on days 5, 12, and 13. This close temporal agreement suggests that microbial fermentation generated biochemical conditions favorable for the simultaneous enhancement of antioxidant and anti-inflammatory properties. Such concordance supports the hypothesis that fermentation-induced transformation of cascara phytochemicals contributes to multiple biological activities rather than affecting only a single functional property.

Although day 13 exhibited the lowest IC_{50} value for protein denaturation inhibition, determination of the optimal fermentation endpoint should not rely exclusively on anti-inflammatory activity. In the present study, fermentation performance was evaluated using multiple complementary parameters, including antioxidant activity, pH profile, phytochemical characteristics, and sensory observations. Considering these parameters collectively, day 5 was selected as the most practical fermentation endpoint because it simultaneously achieved high antioxidant activity, strong anti-inflammatory activity, a moderately acidic pH, and a substantially shorter fermentation time than later sampling days. Therefore, the selection of day 5 represents the most appropriate fermentation condition within the scope of the evaluated parameters rather than indicating that it possesses the highest activity for every individual biological assay.

4. CONCLUSION

This study demonstrates that Pagar Alam robusta cascara can be successfully valorized into TKC as a promising functional beverage through kombucha fermentation. The dried cascara exhibited suitable preliminary physicochemical and microbiological quality for fermentation, while the fermentation process promoted progressive acidification, stable retention of major phytochemical groups, and characteristic sensory changes associated with kombucha production. Ethanol was not detected throughout the fermentation period using the applied analytical method. Fermentation significantly influenced both antioxidant and anti-inflammatory activities, with dynamic changes observed throughout the 15-day fermentation period. Based on the integrated evaluation of antioxidant activity, anti-inflammatory activity, pH, phytochemical profile, sensory characteristics, and fermentation efficiency, day 5 was identified as the most practical fermentation endpoint. At this stage, TKC exhibited strong biological activities while maintaining a moderately acidic profile and requiring a shorter fermentation period than later sampling times, making it favorable for functional beverage development. The present findings highlight the potential of coffee cascara, an underutilized by-product of coffee processing, as a sustainable raw material for developing value-added fermented beverages with functional properties. Nevertheless, further studies are required to quantitatively characterize phenolic compounds and other bioactive metabolites, determine caffeine and trigonelline contents,

profile fermentation-derived organic acids, quantify trace ethanol using highly sensitive analytical techniques, characterize microbial community dynamics, and validate the observed biological activities through cell-based and in vivo studies. These investigations will provide a stronger scientific basis for the future development of TKC as a nutraceutical beverage.

5. ACKNOWLEDGEMENT

The authors acknowledge the 2024 BLU research funding from Pusat Penelitian dan Penerbitan (PUSLITPEN), Lembaga Penelitian dan Pengabdian kepada Masyarakat, Universitas Islam Negeri Syarif Hidayatullah Jakarta. The authors also thank the Pharmacy Study Program, Faculty of Health Sciences, UIN Syarif Hidayatullah Jakarta, laboratory staff and student assistants who supported sample preparation and laboratory work.

6. REFERENCES

Ameca, G.M., Ortega Cerrilla, M.E. and Cordoba, P.Z. (2018) 'Chemical composition and antioxidant capacity of coffee pulp', Ciencia e Agrotecnologia, 42(3), pp. 307-313. doi: 10.1590/1413-70542018423000818.

Arpi, N., Muzaifa, M., Sulaiman, M.I., Andini, R. and Kesuma, S.I. (2021) 'Chemical characteristics of cascara, coffee cherry tea, made of various coffee pulp treatments', IOP Conference Series: Earth and Environmental Science, 709, 012030. doi: 10.1088/1755-1315/709/1/012030.

Braham, J.A. and Bressani, R. (1979) Coffee Pulp: Composition, Technology, and Utilization. Ottawa: International Development Research Centre.

Chandra, S., Chatterjee, P., Dey, P. and Bhattacharya, S. (2012) 'Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein', Asian Pacific Journal of Tropical Biomedicine, 2(1 Suppl.), pp. S178-S180. doi: 10.1016/S2221-1691(12)60154-3.

Coelho, R.M.D., Almeida, A.L., Amaral, R.Q.G., Mota, R. and Sousa, P.H.M. (2020) 'Kombucha: Review', International Journal of Gastronomy and Food Science, 22, 100272. doi: 10.1016/j.ijgfs.2020.100272.

Commission Implementing Regulation (EU) (2022) 'Commission Implementing Regulation (EU) 2022/47 of 13 January 2022 authorising the placing on the market of Coffea arabica L. and/or Coffea canephora Pierre ex A. Froehner dried cherry pulp and its infusion as a traditional food from a third country', Official Journal of the European Union.

EFSA (2021) 'Technical report on the notification of dried cherry pulp from Coffea arabica L. and Coffea canephora Pierre ex A. Froehner as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283', EFSA Supporting Publications, 18, 6808E.

Esquivel, P. and Jimenez, V.M. (2012) 'Functional properties of coffee and coffee by-products', Food Research International, 46(2), pp. 488-495. doi: 10.1016/j.foodres.2011.05.028.

Gemechu, F.G. (2020) 'Embracing nutritional qualities, biological activities and technological properties of coffee by-products in functional food formulation', Trends in Food Science & Technology, 104, pp. 235-261. doi: 10.1016/j.tifs.2020.08.005.

Jayabalan, R., Malbasa, R., Loncar, E., Vitas, J. and Sathishkumar, M. (2014) 'A review on kombucha tea - microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus', Comprehensive Reviews in Food Science and Food Safety, 13(4), pp. 538-550. doi: 10.1111/1541-4337.12073.

Lemes, A.C., Egea, M.B., Oliveira Filho, J.G., Gauterio, G.V., Ribeiro, B.D. and Coelho, M.A.Z. (2022) 'Biological approaches for extraction of bioactive compounds from agro-industrial by-products: A review', Frontiers in Bioengineering and Biotechnology, 9, 802543. doi: 10.3389/fbioe.2021.802543.

Lopez-Parra, M.B., Gomez-Dominguez, I., Iriondo-DeHond, M., Villamediana Merino, E., Sanchez-Martin, V., Mendiola, J.A., Iriondo-DeHond, A. and del Castillo, M.D. (2024) 'The impact of the

drying process on the antioxidant and anti-inflammatory potential of dried ripe coffee cherry pulp soluble powder', Foods, 13, 1114. doi: 10.3390/foods13071114.

Murthy, P.S. and Naidu, M.M. (2012) 'Sustainable management of coffee industry by-products and value addition: A review', Resources, Conservation and Recycling, 66, pp. 45-58. doi: 10.1016/j.resconrec.2012.06.005.

Oktaviani, L., Astuti, D.I., Rosmiati, M. and Abduh, M.Y. (2020) 'Fermentation of coffee pulp using indigenous lactic acid bacteria with simultaneous aeration to produce cascara with a high antioxidant activity', Heliyon, 6, e04462. doi: 10.1016/j.heliyon.2020.e04462.

Plank, D.W. et al. (2012) 'Determination of antioxidant activity in foods and beverages by reaction with 2,2'-diphenyl-1-picrylhydrazyl (DPPH): Collaborative study first action 2012.04', Journal of AOAC International, 95(6), pp. 1562-1569. doi: 10.5740/jaoacint.CS2012_04.

Puspaningrum, D.H.D., Sumandewi and Sari (2022) 'Karakteristik kimia dan aktivitas antioksidan selama fermentasi kombucha cascara kopi arabika (Coffea arabika L.) Desa Catur Kabupaten Bangli', Jurnal Sains dan Edukasi Sains, 5(2), pp. 44-51. doi: 10.24246/juses.v5i2p44-51.

Putri, E., Rukayadi, Y., Sunarti, T.C. and Meryandini, A. (2023) 'Increase in polyphenolic substances from fermented robusta coffee pulp (Coffea canephora L.) by using indigenous actinomycetes', HAYATI Journal of Biosciences, 30(3), pp. 457-465.

Sales, A., Iriondo-DeHond, A., DePaula, J. et al. (2023) 'Intracellular antioxidant and anti-inflammatory effects and bioactive profiles of coffee cascara and black tea kombucha beverages', Foods, 12, 1905. doi: 10.3390/foods12091905.

Skocinska, K.N., Sionek, B., Scibisz, I. and Krajewska, D.K. (2017) 'Acid contents and the effect of fermentation condition of kombucha tea beverages on physicochemical, microbiological and sensory properties', CYTA - Journal of Food, 15(4), pp. 601-607. doi: 10.1080/19476337.2017.1321588.

Villarreal-Soto, S.A., Beaufort, S., Bouajila, J., Souchard, J.-P., Renard, T., Rollan, S. and Taillandier, P. (2019) 'Impact of fermentation conditions on the production of bioactive compounds with anticancer, anti-inflammatory and antioxidant properties in kombucha tea extracts', Process Biochemistry, 83, pp. 44-54.