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Optimization of Spent Coffee Ground Extraction for Kombucha Production: Effect of Temperature on Fermentation Dynamics and Antioxidant Activity

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ABSTRACT

The valorization of agro-industrial waste into functional beverages offers a sustainable approach to food processing. This study aimed to optimize the extraction temperature of spent coffee grounds for kombucha production and to evaluate its impact on fermentation dynamics and antioxidant activity. Spent coffee grounds were extracted at three different temperatures (30°C, 60°C, and 90°C) and fermented for 14 days using a symbiotic culture of bacteria and yeast (SCOBY) and kombucha broth. Key parameters monitored included pH, total soluble solids (°Brix), reducing sugars, total phenolic content, and antioxidant activity. Each treatment was repeated three times, and the data was processed using simple statistical analysis. Results showed that all samples experienced a decrease in pH (from 3.95 to 3.3) and dissolved solids (by 3.23°Brix), alongside an increase in reducing sugars (from 0.02–0.05 mg/mL to 0.43–0.56 mg/mL), indicating active microbial fermentation. The 90°C extract exhibited the highest total phenolic content (823.82 µg GAE/mL) on day 2 and antioxidant activity (82.11%) on day 14, outperforming the 60°C and 30°C extracts. These results underscore the role of thermal extraction in enhancing the functional qualities of coffee ground kombucha and highlight its potential as a promising candidate for upcycled functional beverage development.

1. INTRODUCTION

Coffee is one of the world's most important commodities, grown in more than 80 countries on a total land area of 10.2 million hectares (Kim *et al*., 2025). As a widely consumed beverage around the world, coffee consumption generates substantial waste production. One significant by-product of the coffee industry is coffee grounds, which can be further utilized in various applications (Andrade *et al*., 2022). Coffee consumption in the global market reached 10.7 million tons in 2022 (International Coffee Organization, 2023). If 1 ton of coffee beans produces 650 kg of coffee powder and 0.91 grams of spent coffee grounds are generated for every 1 gram of coffee powder (Priena *et al*., 2023), then in 2022, over 6 million tons of spent coffee grounds were produced. Coffee grounds are rich in total phenolic content, antioxidant activity, and bioactive compounds. Numerous investigations have demonstrated the promise of coffee grounds as a source of bioactive compounds, reinforcing the application of coffee waste within a circular economy to enhance the value of coffee by-products (Andrade *et al*., 2022; Campos-Vega *et al*., 2015; Mitraka *et al*., 2021). Coffee grounds can also be used as an additive in chocolate products (Pezhman *et al*., 2022) or as a raw material for biofoam (Mialni *et al*., 2020). Coffee grounds can be used as a growing medium for kombucha through fermentation because of their high bioactive and phenolic content, as well as their potent antioxidant activity.

Kombucha is a functional beverage typically created by the fermentation of tea and sugar using a starter culture known as SCOBY. Functional beverages represent a category of food that delivers health benefits upon consumption due to their specific ingredients (Sulistiawaty & Solihat, 2022). The process of kombucha fermentation occurs at room

temperature over a period of 7 to 14 days. During the fermentation process, a variety of bioactive compounds are produced, including organic acids like gluconic acid, acetic acid, lactic acid, and amino acids. Additionally, water-soluble vitamins such as B1, B6, B12, and C are generated, along with catalase, β-carotene, carotenoids, and polyphenols including catechins, theaflavins, tannins, and flavonoids. Nonetheless, the primary constituents of kombucha include glucose, fructose, gluconic acid, lactic acid, enzymes, catechins, and flavonoids (Osiripun & Apisittiwong, 2021). Kombucha has been shown in various studies to have health benefits due to its antioxidant (Jakubczyk *et al*., 2020; Karyantina *et al*., 2024; Osiripun & Apisittiwong, 2021), antimicrobial (Cardoso *et al*., 2020; Kaewkod *et al*., 2019), antihyperglycemic (Srihari *et al*., 2013), and digestion-friendly (Dongoran *et al*., 2023) properties. In terms of taste, kombucha is a cool, slightly sweet, fizzy, and acidic functional tea that promotes health by means of symbiotic cultures of yeast and acetic acid bacteria (Leal *et al*., 2018).

According to studies by Kim *et al*. (2025) and Nizioł-Łukaszewska *et al*. (2020), the first brew of coffee is fermented, increasing the amount of phenolics, flavonoids, and compounds like caffeic acid, trigonelline, and chlorogenic acid. Significant changes in taste and aroma are also produced by fermentation, which also lowers pH, increases acidity, inhibits the enzyme α-glucosidase, and increases antioxidant activity (Ivanišová *et al*., 2020; Wang *et al*., 2022b; Zhao *et al*., 2018). Prior investigations have indicated that the rising popularity of kombucha and coffee presents avenues for the creation of kombucha utilizing coffee grounds (Błaszak *et al*., 2024).

Few studies have explored using spent coffee grounds to produce kombucha, despite extensive research on coffee-based kombucha. Moreover, very little research has been done on the effects of varying extraction temperatures on the fermentation profile and antioxidant content of coffee kombucha. Hence, this study sets out to optimize the extraction temperature of spent coffee grounds for kombucha production and to evaluate its impact on fermentation dynamics and antioxidant activity.

2. MATERIALS AND METHODS

2.1. Material

The spent coffee grounds are collected from a Salatiga coffee cafe. Both the kombucha culture and the chemicals that were utilized in this investigation were obtained from the Chemistry laboratory, Science and Math Faculty, Satya Wacana Christian University.

2.2. Preparation of Kombucha in Spent Coffee Grounds Extract Media

Preparation of Kombucha in Spent Coffee Grounds Extract Media with modifications was performed referred to Saito *et al*. (2024); Zubaidah *et al*. (2020). The spent coffee grounds are then dried in the oven at 50 degrees until dry. The sample is then stored in a jar. A total of 50 g of spent coffee grounds is brewed in 500 mL of water and extracted at 30, 60, and 90°C for 1 hour, then filtered. A total of 360 mL of extract is mixed with 36 g of sugar, sterilized (121°C, 15 minutes), then cooled. Spent coffee grounds extract was taken as much as 180 mL, added with 1 sheet of SCOBY diameter of 7cm and 10% coffee starter, incubated at 28°C. Samples were taken on days 0, 2, 4, 6, 8, and 14.

2.3. Characterization of Kombucha Fermentation

2.3.1. pH and Total Soluble Solids

The pH was measured using a pH meter (Hanna HI-9812-5), and for soluble solids using an ADOLF refractometer.

2.3.2. Reduced Sugar (Hidayat & Yunita, 2022)

Standard glucose solutions are prepared with a concentration of 100 to 1000 ppm. Each solution is taken 1 mL, 1 mL of DNS reagent is added, then vortexed and heated in boiling water for 10 minutes. After cooling, each solution is diluted 5 times. Absorbance was measured using a spectrophotometer UV-Vis at the maximum wavelength after scanning in the wavelength range of 300 to 800 nm. For sample measurement, as much as 1 mL of kombucha solution was taken, and 3 mL of DNS reagent was added. The next procedure follows the same steps as the standard solution. The absorbance value was then plotted on a standard curve to determine the reducing sugar content in the sample.

2.3.3. Total phenolic content (Aryal et al. (2019) with modifications)

Standard gallic acid solutions are prepared with a concentration of 10 to 100 µg/mL. Each solution is taken 1 mL, Folin-Ciocalteu 10% is added to 2.5 mL, then vortexed and incubated for 5 minutes. Furthermore, a 7.5% Na2CO3 solution was added as much as 2 mL and incubated in a water bath at a temperature of 50°C for 10 minutes. After the incubation process is complete, the mixture is cooled to room temperature. Absorbance was measured using a spectrophotometer UV-Vis at the maximum wavelength after scanning in the wavelength range of 400 to 900 nm. For sample measurement, as much as 0.1 mL of sample was added to 0.9 mL of aquadest and 2.5 mL of Folin-Ciocalteu 10% reagent. The next procedure follows the same steps as the standard solution. The absorbance values obtained are then plotted on a standard curve to determine the total phenolic content in the sample.

2.3.4. Antioxidant activity (Aryal et al. (2019) with modifications)

The sample is centrifuged to take the supernatant. A total of 0.2 mL of supernatant is taken, and 9.8 mL of methanol is added to the test tube. The diluted sample was taken as much as 1 mL, and 2 mL of 0.1 mM DPPH reagent was added to the test tube. The mixture is incubated for 30 minutes in a dark place at room temperature. Sample uptake was measured at a wavelength of 517 nm with methanol as a blank.

2.4. Data Analysis

The research data were analyzed using a two-way factorial design with analysis of variance (ANOVA). The factors were fermentation time (0, 2, 4, 6, 8, and 14 days) and extraction temperature (30°C, 60°C, and 90°C). The parameters that were examined were as follows: pH, total dissolved solids (TDS), absorbance, reducing sugar content (RSC), total phenol content (TPC), and antioxidant activity.

Fermentation duration, extraction temperature, and the interaction between the two were each subjected to analysis of variance testing to identify the primary impacts. Upon discovering significant differences, the study proceeded using Duncan's post-hoc test at a significance threshold of 5% ($\alpha = 0.05$) to delineate the distinct treatment groups.

3. RESULTS AND DISCUSSION

Kombucha fermentation begins when *Acetobacter xylinum* breaks down the sugar sucrose into glucose and fructose. Furthermore, glucose undergoes a series of biochemical changes through the pentose phosphate pathway. In this process, glucose is converted to glucose-6-phosphate, then glucose-1-phosphate, and finally to uridine diphosphate. This uridine diphosphate is then used to form cellulose with the help of the enzyme cellulose synthase. The cellulose that is formed is then seen as a thin layer or *pellicle* on the surface of the kombucha fermentation media (Zubaidah *et al*., 2018). In addition to producing cellulose, the metabolism *of Acetobacter xylinum* produces primary metabolites such as acetic acid, gluconic acid, glucuronic acid, malic acid, tartaric acid, citric acid, butyric acid, and lactic acid (Wang *et al*., 2022a; Zubaidah *et al*., 2018). Yeast (*Saccharomyces cerevisiae*) converts glucose and fructose into alcohol and CO_2 through glycolysis. Carbon dioxide reacts with water to form carbonic acid, and the alcohol is oxidized by *Acetobacter xylinum* into acetaldehyde and then into acetic acid (Wang *et al*., 2022a).

3.1. pH and Total Soluble Solids

Spent Coffee grounds extract has a neutral pH, which is between 7.4-7.5 (Narko *et al*., 2020). The addition of starters, both liquid and SCOBY, to the growing medium (the result of the extraction of coffee grounds and sucrose) caused a decrease in pH at the beginning of fermentation to 3.95. During the 14-day fermentation, the pH of kombucha drops from 3.95 to 3.25 (Figure 1). Statistical analysis confirmed kombucha's pH was not significantly affected by fermentation duration or extraction temperature (p-value = 0.716 and 0.978, respectively). This suggests that the pH drop was not induced by the treatments but rather happened naturally during fermentation. The decrease in the pH of fermented kombucha is caused by the production of acids, especially acetic acid, by acetogenic bacteria (Wang *et al*., 2022b).

The longer the kombucha ferments, the more organic acids are produced. Fermentation begins with the hydrolysis of sucrose into glucose and fructose. Yeast *Saccharomyces cerevisiae*, as part of the microbial community, metabolizes

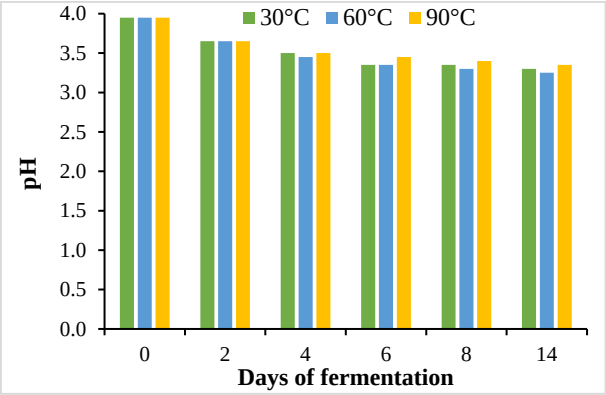

Figure 1. Kombucha pH during fermentation

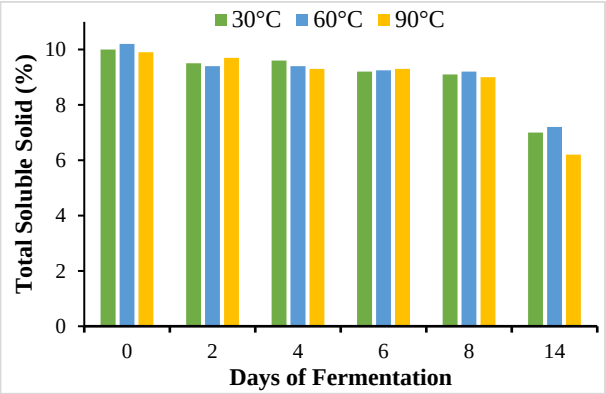

Figure 2. Total Soluble Solids during Fermentation

glucose into ethanol and carbon dioxide. Acetobacter bacteria oxidize ethanol into acetaldehyde, then into acetic acid, whose concentration increases during fermentation. Acetobacter also breaks down glucose into other organic acids such as gluconic acid, glucuronic acid, and lactate. Yeast has invertase enzyme to break down sucrose, whereas acetic acid bacteria lack this ability due to a lack of hydrolase and kinase enzymes (Zubaidah *et al*., 2018). The decrease in total soluble solids (TSS) during the fermentation process is shown in Figure 2; the percentage decrease in total soluble solids from the beginning of fermentation (day 0) to the end of fermentation (day 14) is about 6.2%. The results indicate that fermentation duration affects the decrease in total dissolved solids. The most distinct difference appears between prolonged fermentation (14 days) and the early stage (0–2 days). Meanwhile, in the intermediate stage (4–8 days), the mean values do not differ significantly, so they are categorized within the same homogeneous subset. In summary, the reduction in total dissolved solids occurs progressively and becomes more evident as the fermentation period increases. Fermentation time showed a marginal influence on TSS (p-value = 0.100), whereas extraction temperature had no significant effect (p-value = 0.796). The general downward trend of TSS reflect microbial utilization of sugars throughout fermentation.

According to Yuliana *et al*. (2023), the duration of fermentation of coffee grounds is positively correlated with an increase in the number of microbes that play a role in the fermentation process. One type of bacteria that can produce microbial cellulose is Acetobacter. The dominant species of this genus, which was originally known as *A. xylinum* and later reclassified as *Gluconacetobacter xylinus* and more recently as *Komagataeibacter xylinus*, undergoes a biochemical process involving the oxidation of glucose into gluconic acid. The biochemical process subsequently results in the synthesis of microbial cellulose, which forms a biofilm that persists on the surface of the liquid (Villarreal-Soto *et al*., 2019). The biomass yield (biocellulose) is greatly influenced by the availability of carbon sources in the system, where higher sugar content results in higher biomass yields.

3.2. Reducing Sugar

Reducing sugar levels at various extraction temperatures is shown in Figure 3. Neither fermentation time (p-value = 0.380) nor extraction temperature (p-value = 0.853) significantly affected reducing sugar content. The reducing sugar content in this study came from the basic ingredients of coffee grounds and sucrose that were added before fermentation. Assuming the same initial amount of sucrose, the reduction sugar increase is focused on coffee grounds that are extracted with water at different temperatures.

Spent coffee grounds contain lignocellulose, consisting of 30.2 % (w/w) cellulose, 25 % (w/w) hemicellulose, and 12 % (w/w) lignin (Zuluaga *et al*., 2024). In biomass, hemicellulose associates with cellulose fibers through non-covalent interactions, while lignin acts as a natural adhesive, occupying the spaces between them. These three components, cellulose, hemicellulose, and lignin—form a stable, cross-linked three-dimensional matrix (Haq *et al*., 2021). To enable efficient fermentation, it is essential to apply pretreatment methods that disrupt this complex structure, thereby increasing the accessibility of cellulose and hemicellulose for microbial enzymatic hydrolysis and subsequent conversion into fermentable sugars.

The results of the reducing sugar analysis (Figure 3) indicate that higher extraction temperatures enhance the hydrolysis of coffee grounds, leading to the breakdown of hemicellulose, cellulose, and lignin. Their breakdown (hydrolysis) is key in releasing bioactive compounds, sugars, and phenolics from the materials. While breaking down hemicellulose, several sugars such as mannose, xylose, arabinose, and galactose are released. Decomposition of cellulose results in the production of glucose and cellobiose. These sugars, which include glucose, galactose, xylose, and cellobiose, are examples of sugars that contain functional groups that are capable of performing the role of reducing agents. The results showed that 60°C was the most optimal extraction temperature to decompose the carbohydrate content in the spent coffee grounds to $C_6H_{12}O_6$ from day 0 to day 14 of kombucha fermentation.

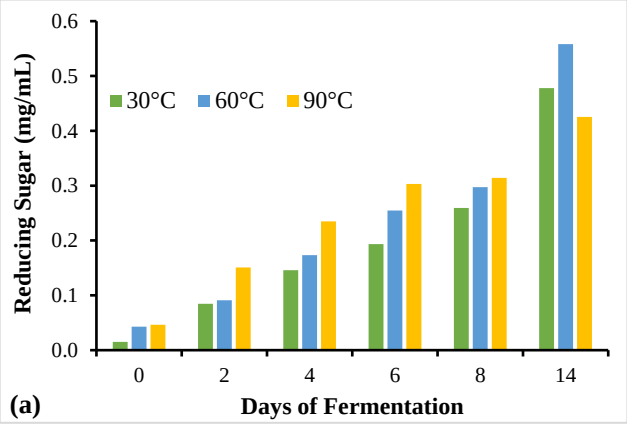

Figure 3. Reducing sugar dynamics during fermentation

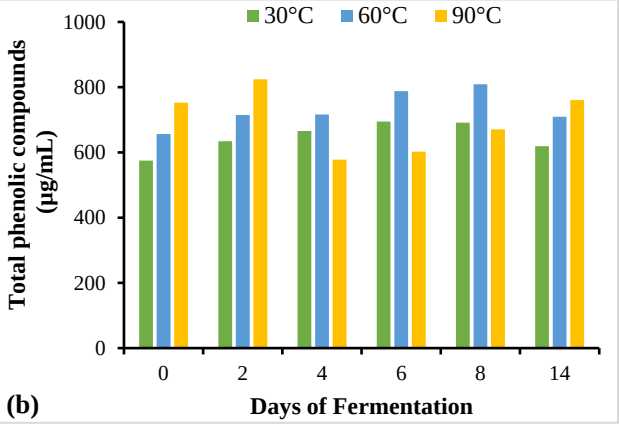

Figure 4. Total phenolics dynamics during fermentation

3.3. Total phenolic compounds

The total phenolic compounds (TPC) measured during the fermentation of spent coffee grounds kombucha can be seen in Figure 4. The extraction temperature has a significant effect on TPC (p-value = 0.039). The impact of temperature in promoting the release of bioactive compounds was demonstrated when the extraction temperature was increased from 30°C to 90°C, leading to higher phenolic levels. The duration of fermentation, however, exhibited no significant impact (p-value = 0.610). Increased phenolic production occurs, both in extraction at temperatures of 30°C, 60°C and 90°C. On the second day, an increase in total phenolic compounds was experienced both at all extraction temperatures with the highest phenol level for spent coffee grounds extraction at 90°C. Overall, extraction with a temperature of 60°C gives the highest phenolic product on day 8 of fermentation. During the fermentation process, the extraction of coffee grounds with a temperature of 60°C reaches the highest total phenolic compounds on day 6. Furthermore, the dynamics of phenolic levels occurred at all extraction temperatures, notably, the 60°C extraction resulted in the highest phenolics on day 8.

This study is in line with earlier research conducted by Hapsari *et al.* (2021), which obtained the highest total phenolic compounds for the fermentation of kombucha with *Alpinia purpurata* extract as substrate. The optimal total phenolic compounds produced in kombucha fermentation with a duration of 8 days was also obtained with a magnitude of 854.64±0.07 µgGAE/ml; this result is higher than spent coffee grounds extract kombucha, which reaches a total phenolic compound in the range of 670 to 809 µgGAE/ml. According to Coelho *et al.* (2020) during fermentation, enzymes from yeast and lactic acid bacteria hydrolyze glycosidic bonds in phenolic compounds, releasing simpler metabolites such as gallic acid and quercetin (Liang *et al.*, 2023); this enzymatic activity not only increases the bioavailability of phenolic compounds but also generates new metabolites with potentially enhanced health benefits (Gulsunoglu-Konuskan & Kilic-Akyilmaz, 2022). Meanwhile, the decrease in total phenolics and antioxidant activity is due to the acidic atmosphere, which causes phenolic compounds to become more stable and difficult to release protons (Villarreal-Soto *et al.*, 2019).

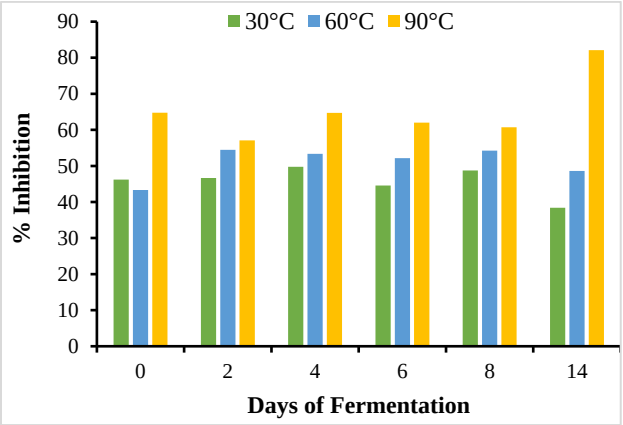

Figure 5. Inhibition dynamics during fermentation

3.4. Antioxidant Activity

Figure 5 illustrates the antioxidant activity seen during the fermentation of kombucha derived from wasted coffee grounds. Antioxidant activity was also significantly affected by extraction temperature ($p = 0.027$), showing a tendency to increase at higher temperatures. This pattern aligns with the rise in TPC, as phenolic compounds are known contributors to antioxidant potential. Fermentation time did not significantly influence antioxidant activity ($p = 0.939$). During the fermentation period from day 0 to day 14, the highest antioxidant activity, 82.11%, was achieved by extracts of spent coffee grounds obtained at 90°C on day 14, followed by 54.45% from the 60°C extract on day 2. In comparison, extraction at 30°C reached a maximum of 48.76% on day 8. Referring to the opinion of Villarreal-Soto *et al*. (2019) in the fermentation process, the decrease in antioxidant activity is caused by strong and stable proton bonds of active compounds, so that they cannot bind to DPPH. Based on the data obtained, throughout the fermentation carried out, there were fluctuations in DPPH inhibition, both at 30°C, 60°C, and 90°C extraction treatments. The phenolic activity of mainly polyphenols depends on their chemical structure, which is influenced by factors such as the number and position of phenolic hydroxyl groups, steric effects, and molecular properties (Santos *et al*., 2021).

The interaction between fermentation duration and extraction temperature was not significant for any studied parameters ($p > 0.05$). This finding indicates that the two elements functioned separately rather than synergistically in affecting kombucha properties. Changes in pH, total soluble solids, absorbance, phenolic content, and antioxidant activity were solely due to the individual impacts of fermentation time or extraction temperature, with no indication of combination or interacting effects.

4. CONCLUSION

Across all extraction temperatures (30°C, 60°C, and 90°C), fermentation from day 0 to day 14 showed a consistent pH decline, a reduction in dissolved solids, and an increase in reducing sugar concentrations. The highest total phenolic content was observed in the 90°C extract on day 2, followed by 60°C and 30°C. Antioxidant activity showed a clear temperature-dependent trend, reaching 47.76%, 54.45%, and 82.11% for the 30°C, 60°C, and 90°C extracts on days 8, 2, and 14, respectively. These findings suggest that higher extraction temperatures enhance phenolic release and antioxidant potential, supporting the use of thermal extraction as an effective approach in the valorization of coffee waste through kombucha fermentation.

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