El-Hayah

JURNAL BIOLOGI

Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index

e-ISSN: 2460-7207, p-ISSN: 2086-0064

Original research article

Kombucha Based on Javanese Turmeric (*Cucurma zanthorrhiza* and *Cucurma zedoaria*): Effect of Various Concentrations and Antioxidant Activity

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DOI: 10.18860/elha.v10i2.35542

Article Info

Article history:

Received 16 December 2024

Received in revised form 24 February 2025

Accepted 24 March 2025

Key Word:

Antioxidant

Cucurma zanthorriza

Cucurma zedoaria

Kombucha

Abstract

Temulawak (C. zanthoriza) and white turmeric (C. zedoaria) are Indonesian herbal plants widely used to treat various diseases including anti-inflammatory properties, neuroprotective, nephroprotective, antitumor, and hepatoprotective. A long-standing tradition, Kombucha is a drink produced by fermenting tea using combine of lactic acid bacteria, acetic acid bacteria and mold. It's classified as a functional food due to its high antioxidant and antibacterial activity, suggesting potential benefits for disease prevention. This research aims to improve the functional value of kombucha by using temulawak and white turmeric. Kombucha is made from temulawak and white turmeric powder with various concentrations ranges 0.2 – 1% (w/v)) and a sugar concentration of 10% (v/v)). After 8 days of fermentation, temulawak and white turmeric kombucha were tested for pH, total acid, amount of phenol, and IC50. The effect of various concentrations temulawak and white turmeric as a substrate for kombucha demonstrated significant (p<0.05) in reduction of pH, increased total phenol content, IC50 value of antioxidant activity, also increased total acid. The best treatment of temulawak and white turmeric kombucha was obtained at a concentration of 1% with a pH of 2.77 for temulawak kombucha and 2.80 for white turmeric kombucha; total acid of 4.38% for temulawak kombucha and 3.60% for white turmeric kombucha; total phenols were 232.48 mgGAE/ml for temulawak kombucha and 113.00 mgGAE/ml for white turmeric kombucha; The IC50 value was 30.82 ppm for temulawak kombucha and 40.15 ppm for white turmeric kombucha.

1. INTRODUCTION

A beverage called kombucha is created by fermenting tea with a SCOBY, a complex culture of various bacteria and yeasts. This SCOBY contains different types of bacteria, such as lactic acid and acetic acid bacteria, along with yeasts. The global kombucha industry is experiencing rapid expansion, with forecasts predicting a roughly 20% growth from its 2020 valuation of approximately $2.2 billion. Antolak *et al.,* [1] suggests that kombucha will gain popularity in the future. According to Soares *et al.,* [2], This surge in kombucha's appeal aligns with the global trend toward prioritizing well-being. Kombucha is a source of various beneficial substances, including organic and amino acids, vitamins, and minerals [3]. Probiotics, antioxidants, diseases including cancer, cardiovascular, and other degenerative diseases prevention, as well as antimicrobials are some of these components' health-promoting properties [1].

According to numerous studies [4], kombucha cannot only be produced from black or green tea. Due to their abundance in beneficial bioactive components, kombucha rhizomes have the potential to be studied. The antibacterial characteristics of turmeric, as well as its immunomodulatory and hepatoprotective effects, have been studied in relation to kombucha rhizomes [5]. Temulawak has also been discovered to have hepatoprotective effects [6] and turmeric does as well.

The bioactive compounds present in rhizomes, most of which are bound to other components, are believed to enhance bioavailability during the fermentation process of rhizomes into kombucha, potentially increasing the anticancer activity of white turmeric and temulawak [7]. Given that Indonesia is the "Mother of Spices" and is abundant in many types of spices and rhizomes, the usage of white turmeric and temulawak is quite likely [8].

2. MATERIALS AND METHODS

Materials

Sliced white turmeric and temulawak from Oro-Oro Dowo Market in Malang, Indonesia dried for 5–6 hours at 60°C in a cabinet dryer. The 14-day-old starter utilised was produced by Presley. 10% (w/v) granulated sugar, distilled water, 7.5% Na_2CO_3, gallic acid, methanol, DPPH, Folin-Ciocalteu reagent, Gulaku brand, drinking water, and aluminium foil.

Research Design

The study utilized a factorial randomized block design (RAK) consisting of two factors: Factor I, which was the type of rhizome (Java ginger and white turmeric), and Factor II, which was the concentration of Java ginger and white turmeric (0.2%, 0.4%, 0.6%, 0.8%, and 1.0%), with three replications.

Statictic analysis

The data were evaluated using a Randomized Block Design (RBD), followed by analysis of variance (ANOVA), and further compared using Tukey's test at a 95% confidence level [9]. The optimal results were determined using the Zeleny method [10].

Kombucha Preparation

Temulawak and white turmeric are cleaned, peeled, and then thinly sliced about 3 mm and dried in a food dehydrator for 5-6 hours at 60°C. To prepare a solution with a concentration of 0.2%, 0.4%, 0.6%, 0.8%, and 1% in 500 ml of water, brew dried temulawak and white turmeric in boiling water according to the concentration (1 g, 2 g, 3 g, 4 g, and 5 g). The cooled temulawak and white turmeric liquid is mixed with up to 10% (v/v) kombucha starting. To protect it from dust, grime, and other impurities, place it in a sterile jar, cover it with a towel, and secure it with a rubber band. The fermentation process take place in the jar for 8 days at room temperature in an area that is not directly exposed to sunligt.

Analysis of Bioactive Compounds

Analysis of Total Phenols

The analysis of total phenols refers to the modification of Baek *et al.,* 2021.

Analysis of IC50

The analysis of IC50 refers to the modification of Chen *et al.,* 2006.

Total acidity and pH analysis

To determine total acidity, 10 mL aliquots of the samples were titrated with 0.1 M NaOH using phenolphthalein as an indicator. The titration continued until a permanent color change signaled the endpoint. Sample pH was measured with a pH meter.

3. RESULTS and DISCUSSION

Total Phenolic Compund

Table 1 shows the average rise in temulawak and white turmeric kombucha's total phenols.

Table 1. Total; phenols in temulawak kombucha and white turmeric kombucha have increased on average

Sampel	Day 0 (mg GAE /ml)	Day 8 (mg GAE/ml)	Enhancement
Temulawak 0.2%	42,33 ± 6,29	112,31 ± 32,22^{bcd}	69,98^{bc}
Temulawak 0.4%	39,13 ± 3,19	118,23 ± 33,04^{bcd}	79,10^{bc}
Temulawak 0.6%	46,02 ± 7,82	132,73 ± 34,03^{bc}	86,70^{bc}
Temulawak 0.8%	48,02 ± 6,47	176,79 ± 16,90^{ab}	128,77^{ab}
Temulawak 1%	50,58 ± 2,23	232,48 ± 40,62^{a}	181,90^{a}
White Turmeric 0.2%	36,68 ± 15,59	61,48 ± 6,62^{bcd}	24,81^{c}
White Turmeric 0.4%	39,09 ± 7,96	70,97 ± 3,09^{cd}	31,88^{c}
White Turmeric 0.6%	41,25 ± 3,83	75,49 ± 3,16^{cd}	34,23^{c}
White Turmeric 0.8%	44,78 ± 13,60	99,20 ± 7,18^{cd}	54,43^{bc}
White Turmeric 1%	48,86 ± 6,49	113,00 ± 11,45^{bcd}	64,14^{bc}
BNJ 38,8%			

Information:

Different figures show significant differences between the data on day 0 and day 8 for each of the observation parameters at $p < 0.05$.

On day 8 of fermentation, all concentrations in **Table 1** show an increase in total phenol. The findings of the analysis of variance demonstrated that the addition of temulawak and white turmeric concentrations throughout the fermentation process caused a significant difference (=0.05) in the rise in total phenols. This may be brought on by microbial metabolic processes that result in the production of phenolic chemicals through enzymatic processes. Bacteria and yeast create enzymes that break down complex polyphenol molecules into simpler ones [11]. Essawet *et al.,* [12] claimed that enzymes like invertase, cellulase, and amylase play important roles in the biotransformation of catechin compounds in kombucha tea during the fermentation process, which increases the total phenol content [12]. Because catechins and theaflavins are less stable in tea with a pH above 5 [13], pH has an impact on this biotransformation process as well.

The increased phenolic content observed during kombucha fermentation is attributed to the microbial community's biotransformation of phenolic compounds. These microbes possess enzymes like fructosidase and phosphoglucose isomerase, enabling them to break down complex phenolics into simpler forms, a finding supported by Chakravorty *et al.,* [14]. The production of hydrolytic enzymes such as invertase, cellulase, and amylase during fermentation, as noted by Kozyrovska *et al.,* [15], further contributes to this increase by breaking down the bonds between phenolic molecules and the kombucha matrix. Furthermore, the yeast *Candida tropicalis*, commonly found in kombucha, is known to transform various phenolic compounds into simpler derivatives [16].

Thearubigin depolymerization during the fermentation process, as well as structural alteration and microbial degradation, are suggested to be additional mechanisms for the rise in phenolic compounds in kombucha. The total phenolics rose as a result of modifications to the planar arubigin chromophore group's structure [17]. This is in line with studies [18] employing lemon and tea raw materials, which indicated that the total polyphenols of sweet tea on day 0 of fermentation were 347.1 g GAE /ml, and the amount grew to 574.61 GAE /ml after fermented for 7 days. The same phenomenon was demonstrated by the temulawak kombucha and white turmeric kombucha beverages, which saw an increase in phenol levels on day 8 compared to day 0 in both cases. Epigalocatechin gallate and epicatechin gallate can be metabolised by microorganisms in fermented tea [19]. Gallate and catechin are linked through an oxidation reaction that can be triggered by microorganism enzymes. By doing this, phenolic chemicals like gallate and catechin are added to kombucha at a rate that can reach 80%. According to Bhattacharya *et al.,* [11], the amount of polyphenol and flavonoid components rose linearly with the lengthening of the fermentation process for tea-based kombucha.

Activity of Antioxidants (IC50)

Table 2 shows how the temulawak kombucha and white turmeric kombucha had increased antioxidant activity.

Table 2. Enhanced Antioxidant Activity in White Turmeric and Temulawak Kombucha

Sample	Day 0 (ppm)	Day 8 (ppm)	Enhancement
Temulawak 0.2%	179,34 ± 24,18	95,45 ± 6,43^{a}	75,58
Temulawak 0.4%	174,75 ± 10,87	78,37 ± 20,20^{a}	96,37
Temulawak 0.6%	165,77 ± 29,47	73,01 ± 14,07^{ab}	92,77
Temulawak 0.8%	164,56 ± 21,84	44,79 ± 5,03^{bcd}	119,77
Temulawak 1%	172,23 ± 6,47	30,82 ± 1,41^{d}	141,40
White Turmeric 0.2%	196,71± 55,00	92,82 ± 10,19^{a}	76,07
White Turmeric 0.4%	192,71± 18,15	75,03 ± 4,01^{ab}	106,67
White Turmeric 0.6%	187,61 ± 8,30	68,63 ± 0,75^{abc}	118,98
White Turmeric 0.8%	160,05 ± 12,84	72,21 ± 4,93^{ab}	87,84
White Turmeric 1%	158,82 ± 40,32	40,15 ± 16,97^{cd}	118,66
BNJ 16,6%			

Note: Different figures show significant differences between data on day 0 and day 8 for each of the observational parameters at $p < 0.05$

The antioxidant potential of the kombucha samples was evaluated using the DPPH assay, which gauges the ability of the samples to neutralize DPPH radicals. The IC50 value, representing the concentration required to scavenge 50% of the free radicals, served as the measure of antioxidant capacity. A lower IC50 signifies a higher level of antioxidant activity. Based on IC50 values, antioxidants are classified as very strong (IC50 < 50 ppm), strong (50-100 ppm), moderate (100-150 ppm), or weak (151-200 ppm) [20]. Before fermentation, both the temulawak and white turmeric kombucha samples showed limited antioxidant activity (IC50 > 100 ppm). However, following an eight-day fermentation period, a substantial enhancement in antioxidant activity was noted, with both samples exhibiting strong antioxidant properties (IC50 < 50 ppm), as detailed in Table 2. This enhanced antioxidant capacity is linked to the increased phenolic content during fermentation. Phenolic compounds, plant-derived secondary metabolites, are known for their antioxidant potential [21]. They neutralize free radicals by donating hydrogen atoms from their hydroxyl groups, converting them into less reactive forms [22]. The number of hydroxyl groups on a phenolic compound directly correlates with its antioxidant strength, as these groups provide the hydrogen atoms necessary for scavenging free radicals [23].

During kombucha fermentation, microbial enzymes facilitate the breakdown of complex phenolics into simpler forms, according to Bhattacharya *et al.,* [11]. This process can enhance antioxidant activity by freeing the active sites of these compounds. Furthermore, the fermentation process can lead to increased gallic acid levels due to the deesterification of 3-galloyl catechin by microbial enzymes. *Abshenas et al.,* [24], Villarreal-Soto *et al.,* [19] and Zubaidah *et al.,* [25] suggest that the antioxidant mechanism of phenolics involves transfering of hydrogen atoms or electrons from their -OH groups to neutralize free radicals. Apart from changing phenolic compounds into simpler ones, microorganism enzymes can also change the structure of antioxidant compounds. Research by Huang *et al.,* [26] regarding turmeric fermentation said that lactic acid bacteria have the curcumin/dehydrocurcumin reductase enzyme. This enzyme is responsible for reducing curcumin into curcumin analogues in the form of tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin with the help of NADPH. These three compounds have a smaller number of double chains compared to curcumin. Apart from having fewer double chains, octahydrocurcumin also has more alcohol groups as a result of the reduction of the ketone group of curcumin.

The reduction of double chains to single chains and ketone groups to alcohol can increase antioxidant activity [27]. When free radicals oxidize these three compounds, the single chains of THC, HHC, and OHC will become double chains, while the alcohol group from OHC will become a ketone group. These three compounds will remain stable and will not turn into free radicals. Meanwhile, free radicals that are reduced by these three compounds are no longer dangerous radicals. In kombucha fermentation, there are also lactic acid bacteria which have enzymes to convert curcumin into tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin [28]. Temulawak, which is the fermentation medium for kombucha, also contains curcuminoids which can be converted into tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin during fermentation ([29]; [28]). This allows the conversion of curcumin into curcumin analogues during kombucha fermentation and increases the antioxidant activity of temulawak kombucha.

pH

The average pH reduction value for temulawak kombucha and white turmeric kombucha during the fermentation process can be seen in Table 3.

Table 3. Average value of pH decreases in Kombucha during the fermentation process

Sample	Day 0	Day 8	Decline
Temulawak 0.2%	3,67 ± 0,29	3,10 ± 0,06	0,57
Temulawak 0.4%	3,73 ± 0,25	3,03 ± 0,06	0,70
Temulawak 0.6%	3,77 ± 0,25	3,00 ± 0,10	0,77
Temulawak 0.8%	3,50 ± 0,06	2,87 ± 0,15	0,63
Temulawak 1%	3,53 ± 0,15	2,77 ± 0,10	0,77
White Turmeric 0.2%	3,67 ± 0,29	3,10 ± 0,06	0,57
White Turmeric 0.4%	3,47 ± 0,06	2,97 ± 0,06	0,50
White Turmeric 0.6%	3,50 ± 0,06	2,90 ± 0,20	0,60
White Turmeric 0.8%	3,67 ± 0,15	2,87 ± 0,23	0,80
White Turmeric 1%	3,60 ± 0,17	2,80 ± 0,06	0,80

Note: Different figures show significant differences between data on day 0 and day 8 for each of the observational parameters at $p < 0.05$.

According to Table 3, the fermentation of temulawak kombucha and white turmeric kombucha results in a process that lowers pH. According to the results of the analysis of variance, the treatment using various doses of temulawak and white turmeric could affect how quickly the pH dropped, but the difference was not statistically significant ($p > 0.05$). Due to the fermentation process's bacteria' conversion of the substrate into organic acids, the pH value drops. A reduction in pH will occur from an increase in the total amount of bacteria and total acid. Bacteria that create organic acids and release H+ ions are the reason of the pH being lowered. H+ ions can lower the pH level and create an acidic environment during fermentation [30].

With a 1% concentration, temulawak kombucha was the one that had the biggest pH drop. Curcumin is a substance found in temulawak. pH determines curcumin's stability, and a change in pH is followed by a change in colour. The enolate form of the hepta dienone chain will predominate as an electron donor in basic conditions, and the mechanism involved in phenolic activity occurs at alkaline pH. Therefore, curcumin will have a phenol group that forms a phenolic group and the curcumin solution will show a red colour. Curcumin, on the other hand, is in the yellow keto form in the acidic environment [31]. Curcumin is unstable under more acidic or alkaline pH circumstances, according to Suresh *et al.*, [32]. The findings indicated that alkaline environments caused significant curcumin degradation. In acidic environments, curcumin is highly stable. The amount of curcumin is more stable at acidic pH levels, particularly > 1 to 1.2, and the presence of protons released from the phenolic group, which causes the structure to break down, is indicative of this [32].

Although less so than with temulawak kombucha, white turmeric kombucha exhibited a pH drop. This is because white turmeric contains bioactive substances that have antimicrobial properties. Therefore, the pH does not significantly decrease and the total acid obtained is less as the concentration increases, which can have an impact on the activity of microorganisms during fermentation.

Total Acid

Table 4 displays the average rise in total acid of temulawak kombucha and white turmeric kombucha during the fermenting process.

Table 4. Average percentage of total acid in Kombucha that increases throughout fermentation

Sample	Day 0 (%)	Day 8 (%)	Enhancement
Temulawak 0.2%	0,32 ± 0,01	1,27 ± 0,05[f]	0,95[f]
Temulawak 0.4%	0,31 ± 0,01	1,38 ± 0,08[f]	1,06[f]
Temulawak 0.6%	0,31 ± 0,01	2,41 ± 0,26[de]	2,10[de]
Temulawak 0.8%	0,32 ± 0,01	3,26 ± 0,12[bc]	2,94[bc]
Temulawak 1%	0,32 ± 0,02	4,38 ± 0,17[a]	4,06[a]
White Turmeric 0.2%	0,32 ± 0,01	1,24 ± 0,03[f]	0,92[f]
White Turmeric 0.4%	0,33 ± 0,02	1,41 ± 0,10[f]	1,09[f]
White Turmeric 0.6%	0,33 ± 0,02	1,87 ± 0,19[ef]	1,55[ef]
White Turmeric 0.8%	0,32 ± 0,01	2,70 ± 0,32[cd]	2,38[cd]
White Turmeric 1%	0,33 ± 0,01	3,60 ± 0,52[b]	3,28[b]

BNJ 0,34%

Note:Different figures show significant differences between data on day 0 and day 8 for each of the observational parameters at $p < 0.05$

Based on **Table 4**, it can be seen that on fermentation day 8, the total turmeric kombucha acid increased with different turmeric concentrations. According to the findings of the analysis of variance, there was a significant difference between the amounts of white turmeric and temulawak in terms of the rise in total acid ($p > 0.05$). With a value of 0.31% to 0.33%, the increase in total acid on day 0 demonstrates that the acidity level is still modest. This demonstrates that the fermentation process had not started on the first day, when the microbes had not yet undergone the process of cell multiplication and breakdown and the total acid was still low. On day 8, as bacteria proliferate throughout the fermentation process, there is an increase in acidity, which results in an increase in total acid. Microbial metabolism, which generates organic acid molecules, may be to blame for the rise in total acid. As a result, more organic acid is created, increasing the overall acid content of kombucha [30].

Acetic acid, as well as other organic acids like gluconic acid, glucuronic acid, malic acid, tartaric acid, citric acid, butyric acid, and lactic acid, are produced by Acetobacter xylinum's metabolic process as primary metabolites ([33]; [25]). In the meantime, yeast (S. cerevisiae) uses the glycolysis process to turn glucose and fructose into alcohol and CO_2 during the fermentation process [34]. After that, CO_2 and water will combine to produce carbonic acid. Meanwhile, Acetobacter xylinum will convert the alcohol generated into acetaldehyde and then acetic acid [34]. The pH value will change as organic acids build up during the fermentation process. The acid in kombucha will release protons (H^+), which causes the pH value to decrease, so the higher the overall acid generated, the lower the pH value [30]. Acetic acid, which predominates in fermentation, has the ability to lower pH values as low as 2 [35].

Numerous reasons are suggested to be responsible for the increased acidity in temulawak and white turmeric kombucha. The source material is one thing that can affect how much acid is produced. Temulawak contains gum, which can be digested into simpler sugars and used as an energy source for the growth of microbes and an increase in total acid. The sugar level of the raw material will rise along with the temulawak concentration, increasing the total number of microorganisms and total acid in the kombucha as a result. The overall acid level is lower in white turmeric kombucha, though. The amount of the antibacterial component curcumin in turmeric can have an impact on this. Since curcumin is known to have strong antibacterial capabilities when the curcumin content is too high, it also kills fermenting bacteria in addition to pathogenic bacteria when this occurs.

4. Conclusion

In comparison to white turmeric, temulawak kombucha has the greatest total acid, lowest pH, most antioxidant activity, and highest total phenol levels.

5. ACKNOWLEDGEMENTS

This research was funded by Universitas Brawijaya through the Professor Grant (Hibah Profesor) of the Faculty of Agricultural Technology under contract number 3490.5/UN10.F10/PT.01.03/2023.

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