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Effects of fermentation duration on α-glucosidase activity and vitamin C in temu mangga kombucha

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ABSTRAK

Latar Belakang: *Diabetes Mellitus Tipe 2 (DMT2) merupakan gangguan metabolik yang semakin meningkat di Indonesia, ditandai dengan tingginya kadar gula darah akibat gangguan fungsi sel β pankreas. Pangan fungsional yang memiliki sifat antioksidan dan kemampuan menghambat enzim pencernaan karbohidrat, seperti kombucha, berpotensi mendukung pengelolaan DMT2.*
Tujuan: *Menganalisis aktivitas inhibisi enzim α-glukosidase dan kadar vitamin C pada kombucha temu mangga.*
Metode: *Penelitian eksperimental dilakukan di UPT Laboratorium Terpadu, Universitas Diponegoro, Semarang, mulai Agustus sampai November 2024, menggunakan rancangan acak lengkap dengan empat lama fermentasi (5, 7, 10, dan 14 hari), masing-masing diulang tiga kali. Aktivitas penghambatan enzim α-glukosidase dan kadar vitamin C dianalisis menggunakan spektrofotometri UV-Vis pada panjang gelombang 540 nm dan 265 nm. Data aktivitas inhibisi α-glukosidase dan kadar vitamin C dianalisis dengan ANOVA, jika hasil uji menunjukkan perbedaan signifikan (p<0,05) dilanjutkan uji Post Hoc Tukey untuk menentukan kelompok yang berbeda dan Kruskal Wallis digunakan jika data tidak berdistribusi normal.*
Hasil: *Aktivitas penghambatan enzim α-glukosidase tertinggi diperoleh pada hari ke-14 fermentasi (75,84 ± 1,46%), menunjukkan penurunan signifikan dibandingkan kontrol (87,42 ± 0,96%). Kandungan vitamin C tertinggi ditemukan pada hari ke-5 fermentasi (2,06 ± 0,06 mg/100 mL), kemudian menurun seiring waktu fermentasi.*
Kesimpulan: *Kombucha temu mangga menunjukkan potensi sebagai minuman fungsional yang mampu menghambat enzim α-glukosidase secara in vitro dan mengandung antioksidan yang relevan. Penelitian lanjutan diperlukan untuk menguji efektivitas dan keamanannya secara klinis pada manusia.*

KATA KUNCI*: diabetes melitus; enzim α-glukosidase; kombucha; temu mangga (curcuma mangga); vitamin c*

ABSTRACT

Background: *Type 2 Diabetes Mellitus (T2DM) is a growing metabolic disorder in Indonesia, associated with impaired pancreatic β-cell function and elevated blood glucose levels. Functional foods with antioxidant and enzyme-inhibitory properties, like kombucha, may support T2DM management.*

Objectives: *To analyze the inhibition activity of the α-glucosidase enzyme and the vitamin C content in temu mangga kombucha.*

Methods: *An experimental study was conducted in UPT Laboratory, Diponegoro University, Semarang, from August to November 2024., using a completely randomized design with four fermentation durations (5, 7, 10, and 14 days), each in triplicate. α-Glucosidase inhibition and vitamin C levels were assessed using UV-Vis spectrophotometry at wavelengths of 540 nm and 265 nm. The inhibition activity data of α-glucosidase and vitamin C levels were analyzed using ANOVA. If the test results showed significant differences ($p<0.05$), a Tukey Post Hoc test was conducted to determine the different groups, and Kruskal-Wallis was used if the data were not normally distributed.*

Results: *The highest α-glucosidase inhibitory activity was observed on day 14 (75.84 ± 1.46%), showing a significant reduction compared to the control (87.42 ± 0.96%). The highest vitamin C content (2.06 ± 0.06 mg/100 mL) was detected on day 5, followed by a gradual decline with prolonged fermentation.*

Conclusions: *Temu mangga kombucha exhibits promising in vitro α-glucosidase inhibitory potential and relevant antioxidant content. These findings suggest its potential as a dietary adjunct for T2DM management. However, in vivo studies are warranted to confirm its efficacy and safety in human subjects.*

KEYWORDS*:* *α-glucosidase enzyme; diabetes mellitus; kombucha; temu mangga (curcuma mangga); vitamin c*

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INTRODUCTION

One of the health challenges that must be addressed today is the increasing prevalence of degenerative diseases year after year, such as diabetes mellitus which lasts for a long period (1). In recent decades, the prevalence of diabetes mellitus has shown a significant increase in almost all regions of the world. According to the World Health Organization (WHO), diabetes mellitus is one of the diseases that ranks fourth in priority for research on degenerative diseases in various countries around the world. WHO estimates that the number of diabetes sufferers increased from 200 million in 1990 to 830 million in 2022 (2).

Based on data from the Indonesian Health Survey (SKI) 2023, the prevalence of Diabetes Mellitus (DM) in Indonesia shows a significant figure, with variations based on demographic characteristics. Nationally, the prevalence of DM reaches 6.1%, with this figure indicating that the prevalence of DM reaches 8.3% in the age group of 55-64 years and 12.4% in those aged 65 years and older. Additionally, economic factors also influence prevalence, where households with higher economic status tend to have lower prevalence rates compared to those in the lower economic group. These findings illustrate the health challenges faced by Indonesian society and emphasize the need for effective interventions to address diabetes mellitus (DM) (3). Diabetes is a condition characterized by insufficient insulin synthesis by the pancreas to regulate the entry of glucose into cells due to damage to insulin-producing cells, which is categorized as type 1 diabetes (4). In another case, our body might produce enough insulin, but our body cannot use it well, which is known as insulin resistance and categorized as type 2 diabetes (5). The phenomenon of increasing incidence of this disease raises the need and interest of the community in functional food products, as their

health effects can reduce the risk of diabetes, especially type 2 diabetes (6).

One of the contributors to the control of diabetes mellitus is the activity of α-glucosidase enzyme inhibitors (7). The activity of the α-glucosidase enzyme is generally controlled through medications that have α-glucosidase inhibitory properties, such as acarbose, voglibose, miglitol, and emigrate, which are used to treat type 2 diabetes (8). This enzyme is usually found in the small intestine and plays a role in breaking down complex carbohydrates into simple sugars, thereby slowing the absorption of glucose from the digestive tract and helping to control blood sugar levels after meals (postprandial hyperglycemia). However, the use of this drug causes side effects such as bloating, stomach pain, and diarrhea, which can lead to decreased patient compliance (9).

Another important alternative in the management of diabetes mellitus is the consumption of functional foods (10). Functional foods like kombucha are known to have good physiological benefits and the potential to reduce various disease risk factors (6). Kombucha is a fermented drink made from sweet tea that is fermented with a Symbiotic Culture of Bacteria and Yeast (SCOBY) (6). Kombucha is rich in organic acids such as acetic acid, gluconic acid, citric acid, lactic acid, vitamins B1, B2, B6, B12, E, amino acids, and secondary metabolite compounds like phenols, flavonoids, and polyphenols that act as antidiabetic agents, particularly in inhibiting the activity of the enzyme α-glucosidase (11). Research conducted by Permatasari et al. (2024), which reported that kombucha from sea grapes can cause inhibition of the α-glucosidase enzyme, but the percentage of inhibition is not as high as acarbose (12).

The preclinical study conducted by Mulyani et al. (2022) mentioned that banana kombucha showed significant α-glucosidase inhibition (90.66%) after 12 days of fermentation, along with an increase in polyphenol content. The results of the study showed inhibition of the α-glucosidase enzyme, even exceeding that of acarbose (13). The research by Yunita et al. (2024) also reported that kombucha made from Rhizophora mucronata leaves showed inhibition of the α-glucosidase enzyme after 7 days of fermentation compared to non-fermented tea (14). These findings indicate that kombucha from various plant sources may provide potential benefits for blood sugar control due to its phenolic compounds and α-glucosidase inhibitory properties (12).

Vitamin C (ascorbic acid) is the main component in kombucha, synthesized by gluconobacter strains from glucose (15). In a study by Puspitsari et al., the analysis of vitamin C content in kombucha tea increased with the duration of fermentation and produced other bioactive components such as glucuronic acid, γ-aminobutyric acid, acetic acid, lactic acid, amino acids, proteins, hydrolytic enzymes, ethanol, polyphenols, and minerals that contribute to antioxidant activity and health benefits related to diabetes (15).

In addition, temu mangga (Curcuma mangga) is one of the native rhizomes of Indonesia that has great potential as a source of natural antioxidants (16). The addition of temu mangga in kombucha production can enrich the nutritional value of the product, as it contains bioactive compounds that can enhance health effects during the fermentation process, including as an antidiabetic (17). Temu mangga also contains bioactive compounds such as phenolics that have anti-inflammatory, antioxidant, and antimicrobial properties, as well as secondary metabolites that play a role in enhancing health, including as an antidiabetic (18).

This research presents an innovation in the form of the development of a functional kombucha drink fermented with the addition of temu mangga as a natural bioactive ingredient (19). Research on kombucha and turmeric has been extensively conducted, but the combination of making kombucha based on turmeric has never been done before. This research aims to explore the potential of temu mangga-enriched kombucha by analyzing its α-glucosidase enzyme inhibition activity and vitamin C content as a functional food alternative in the management of diabetes mellitus (20).

MATERIALS AND METHODS

Design of the study

This research uses an experimental design with a completely randomized design. The research was conducted at the UPT Terpadu

Laboratory of Diponegoro University, Semarang, with a research period lasting for four months starting from August to November 2024. This study involves four treatments of fermentation duration lasting 5, 7, 10, and 14 days, with each treatment repeated three times to obtain 12 samples for accurate results and the re-test treatment is conducted on the same product.

Table 1. Design research on *temu mangga* kombucha based on fermentation duration

Retest (U)	Duration of fermentation			
	5 days (T1)	7 days (T2)	10 days (T3)	14 days (T4)
1	T1U1	T2U1	T3U1	T4U1
2	T1U2	T2U2	T3U2	T4U2
3	T1U3	T2U3	T3U3	T4U3

(T1) Fermentation day 5, (T2) Fermentation day 7, (T3) Fermentation day 10, (T4) Fermentation day 14

Material and tools of the study

The research samples consist of temu mangga kombucha, made from ingredients such as temu mangga, water, sucrose, kombucha vinegar, and Symbiotic Culture Of Bacteria and Yeast (SCOBY), which were purchased online from Mambucha, while the temu mangga was obtained from a traditional market in Semarang. The tools used in the preparation of temu mangga kombucha include a grater, strainer, pot, stove, 3L jar, cloth cover, and raffia string. The tools used for testing include a pH meter, hand refractometer, and UV-Vis spectrophotometer (6).

Procedures

Sample preparation

The making of kombucha from temu mangga. As much as 0.7% of temu mangga is grated and cooked with 1 L of water, boiled at a temperature of 90 oC –100oC for 15 minutes, then add 100 grams of sugar. Then let it sit at room temperature until it reaches 30 degrees Celsius, then add kombucha vinegar and SCOBY (Figure 1). Cover the container with a clean cloth tied with a rubber band, so that air can enter but dust and insects cannot. The research was conducted with four fermentation duration treatments, namely 5, 7, 10, and 14 days, each repeated three times, resulting in a total of 12 samples. pH analysis is a modified method involving pouring 10 ml of temu mangga kombucha into a graduated cylinder, followed by measuring its pH using a pH meter. The measurement is performed by immersing the device in the test sample for a moment until a constant or stable pH value is obtained, and the results are recorded with three repetitions. This procedure aims to ensure the accuracy and consistency of pH measurements, which are important parameters in the physicochemical characterization of kombucha (21).

Total sugar testing

Testing the total sugar content in a solution is generally done using a brix refractometer, a device that operates based on the principle of light refraction. This method is very important in the food and beverage industry, especially for determining the sugar content in various products such as fruit juices, syrups, milk, and other beverages. The initial step that must be taken is the calibration of the refractometer, then the liquid sample to be tested is taken using a pipette and dropped onto the surface of the refractometer prism. The prism is then closed so that the sample spreads evenly and light refraction can occur optimally. Point the instrument towards the light source and observe the scale in the lens to read the measurement results, then repeat 3 times for each sample (22).

Testing Vitamin C concentration

The vitamin C content test was conducted using the UV-Vis spectrophotometry method. The determination of vitamin C content was carried out by first creating an ascorbic acid calibration curve, followed by the determination of vitamin C content in the temu mangga kombucha sample. First, the blank solution (distilled water) was measured at a wavelength of 265 nm. After that, ascorbic acid was prepared in concentrations of 2, 4, 8, 10, 12, and 16 ppm. Each ascorbic acid solution was measured for its absorbance at a wavelength of 265 nm, and this process was repeated three times. Next, the determination of the vitamin C

content in the sample was carried out. First, the absorbance of the blank (ethanol) was measured at a wavelength of 265 nm. Next, 100 mg of the sample was placed into a 100 mL volumetric flask, then ethanol was added up to the mark, resulting in a concentration of 1000 ppm. After that, 3.5 mL of the sample was placed into a cuvette to measure its absorbance at a wavelength of 265 nm, with three repetitions (23).

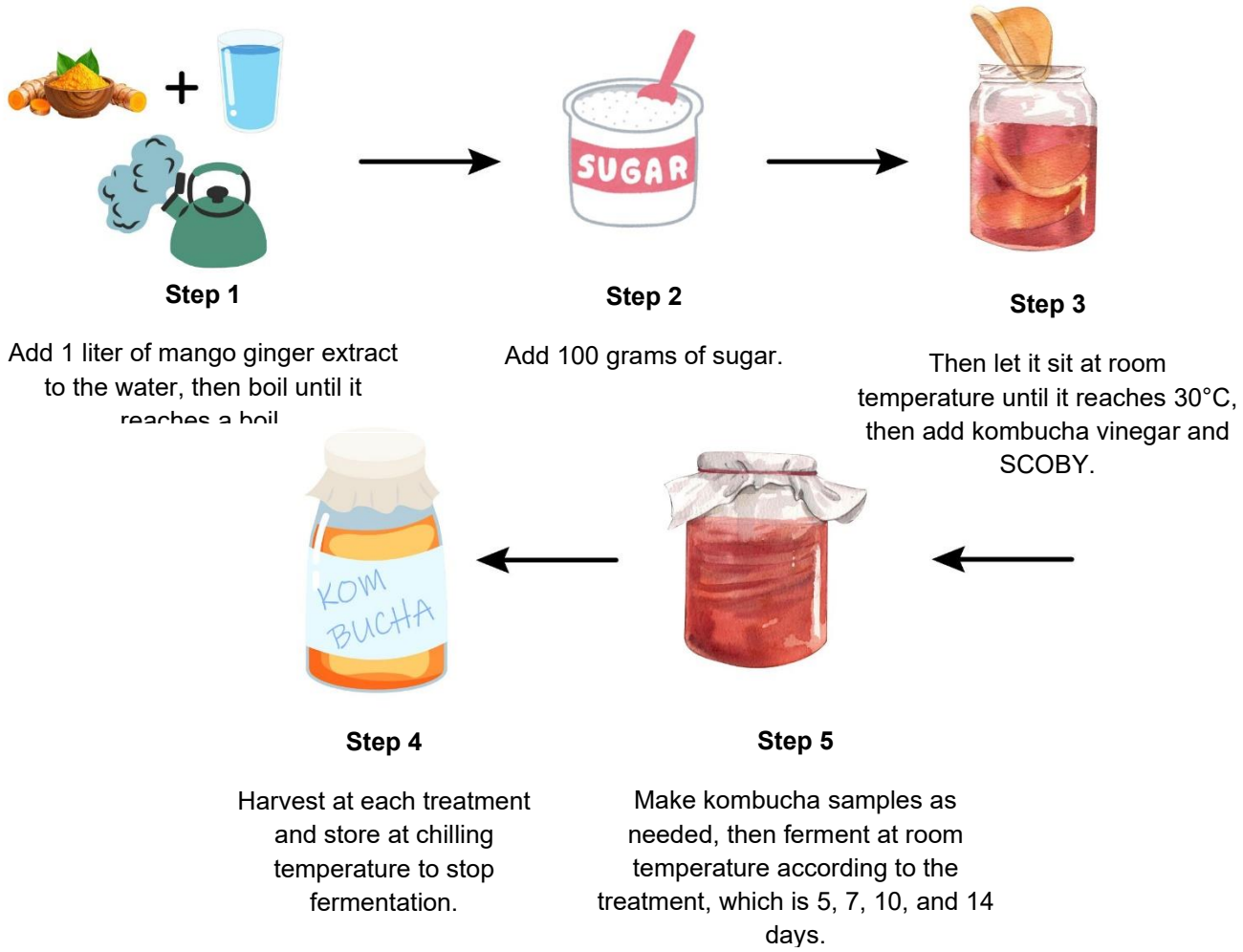

Figure 1. Preparation and fermentation of temu mangga kombucha pH analysis

Testing the inhibitory activity of the α-glucosidase enzyme

The α-glucosidase solution (1.52 UI/ml) was obtained by mixing 1 mg of powder (76 UI) with 50 mL of phosphate buffer (pH 6.9). The solution is then stored at -20°C, followed by mixing 0.1 mL of kombucha with temu mangga at a gradient concentration (3 mg/mL) with 0.35 mL of sucrose (65 mM) and maltose solution (65 mM). After being heated at 37°C for 5 minutes, 0.2 mL of α-glucosidase solution was added and then reacted at 37°C for 15 minutes. The reaction was carried out by heating the system in a 100°C water bath for 2 minutes. Acarbose is used in this experiment as a positive control (acarbose concentration 0.2 mg/ml, so 0.04 mg of acarbose is needed). The control treatment is the same as the kombucha with temu mangga treatment. The activity of α-glucosidase is expressed as the rate of glucose production in the experiment. 0.2 mL of the test solution was combined with the solution obtained from the α-glucosidase inhibition test, then 3 mL of dinitrosalicylic acid (DNS) reagent was added to the reactive system. The system was then heated

at 37°C for 5 minutes, and the absorbance of the solution was measured at a wavelength of 540 nm. The analysis of α-glucosidase enzyme inhibition was conducted by comparing the inhibition activity of the sample with acarbose (an α-glucosidase inhibitor) using the UV-Vis spectrophotometry method measured at a wavelength of 540 nm with the following formula (12).

$$\% \ Inhibition = \frac{(Blank\ Absorbance - Sample\ Absorbance)}{Blank\ Absorbance} \ x\ 100 \quad [1]$$

The % inhibition formula is used to calculate how much the sample can inhibit enzyme activity. The calculation works by comparing the absorbance of the blank (without sample) to the absorbance of the sample. The sample absorbance is subtracted from the blank absorbance to show how much inhibition occurs, then the result is divided by the blank absorbance to standardize the value. After that, it is multiplied by 100 to express the result as a percentage. The higher the % inhibition value, the stronger the sample is in inhibiting the activity of the α-glucosidase enzyme.

Data analysis

The obtained data were analyzed using SPSS 25.0 Windows with a confidence level of 95%. The activity of α-glucosidase enzyme inhibition and vitamin C levels are presented as numerical values in the form of mean and standard deviation, while the fermentation duration parameter is presented as a categorical value. The normality test of the data was conducted using the Shapiro-Wilk test. ANOVA (Analysis of Variance) was used to determine whether there were significant differences between groups, and if the results showed significant differences ($p<0.05$), the Post Hoc Tukey test was performed to identify the different groups. If the data is not normally distributed, then the non-parametric Kruskal-Wallis test is used (24)

RESULTS AND DISCUSSIONS

The research results are presented in the form of tables and graphs. The table illustrates the physicochemical characteristics, α-glucosidase enzyme inhibition activity, and vitamin C content of temu mangga kombucha. The presentation of data based on fermentation duration yields a comprehensive analysis of the impact of fermentation time on each parameter.

Physicochemical characteristics.

Table 2. Average results of ph and brix tests of *temu mangga* kombucha based on fermentation duration

	Duration of fermentation			
	5 days (T1)	**7 days (T2)**	**10 days (T3)**	**14 days (T4)**
pH (Asam, Netral, Basa)	4.2	3.7	3.7	3.5
Brix (%)	11.0	11.0	10.5	8.5

Data is presented as the mean of three repetitions (n=12) (4 treatments, 3 repetitions).

Table 2 shows that the total sugar and pH in the treatments of 5, 7, 10, and 14 days were 11% and 4.2; 11% and 3.7; 10.5% and 3.7; and 8.5% and 3.6, respectively. Total sugar was measured using a Brix refractometer, which indicates the percentage of sucrose and dissolved sugars present in temu mangga kombucha, while pH indicates the acidity level in the solution. The longer the fermentation time, the lower the Brix and pH values. Brix shows a decrease over time during the fermentation process due to the degradation of sucrose into simple sugars, glucose, and fructose by yeast (25).

As fermentation progresses, the pH of kombucha decreases due to the accumulation of organic acids, indicating increased acidity. It occurs as yeast degrades sucrose into glucose and fructose, which are subsequently metabolized by acetic acid bacteria into acetic, gluconic, and other organic acids (25). The extended fermentation period enhances microbial metabolic activity, contributing to a more pronounced pH reduction. This trend is

consistent with findings by Sulistiawaty & Solihat (2022), who reported a significant pH decrease from 4.28 on day 0 to 3.36 on day 12 of fermentation (26).

Similarly, the Brix value an indicator of soluble solids, including sugars declines over time due to the microbial consumption of sucrose and its conversion into metabolic byproducts (27). Although glucose and fructose temporarily increase during hydrolysis, the overall sugar content diminishes, which may be beneficial in the context of glycemic control (6). Kombucha typically exhibits Brix values ranging from 8.5% to 11%, aligning with prior reports on various tea-based kombucha types (28)**. Table 3** shows the average α-glucosidase inhibition activity for T1 (67.91 ± 0.36), T2 (46.49 ± 1.09), T3 (52.25 ± 1.81), T4 (75.84 ± 1.46), and the control at (87.42 ± 0.96). The results indicate that each treatment has significantly different inhibition percentages from one another, demonstrating that the fermentation duration of temu mangga kombucha can affect α-glucosidase inhibition activity (13).

Table 3. Average results of α-glucosidase enzyme inhibition activity test of *temu mangga* kombucha based on the duration of fermentation

	Duration of fermentation					
	5 days (T1)	**7 days (T2)**	**10 days (T3)**	**14 days (T4)**	**Control**	**P-value < 0.05**
α-Glucosidase Enzyme Inhibition Activity *(%)*	67.91 ± 0.36[a]	46.49 ± 1.09[b]	52.25 ± 1.81[c]	75.84 ± 1.46[d]	87.42±0.96[e]	0.000

The different superscripts within the same row showed significant difference (P<0.05), (statistical test using Post Hoc Tukey test).

The research results show that the fermentation of temu mangga kombucha for 14 days produces the highest α-glucosidase enzyme inhibition activity. Each treatment had significantly different inhibition percentages from one another, indicating that the duration of temu mangga kombucha fermentation can affect α-glucosidase inhibition activity (12).

The research by Mulyani et al. in vitro also mentioned that banana kombucha showed significant α-glucosidase inhibition (90.66%) after 12 days of fermentation, along with an increase in polyphenol content. The study indicated the inhibition of the α-glucosidase enzyme, even surpassing acarbose (13). Research by Yuningtyas et al. in vitro also mentioned that kombucha made from Syzygium polyanthum leaves showed strong inhibition (81.05-89.41%) of fermentation over 8 days (29). These findings indicate that kombucha from various plant sources may provide potential benefits for blood sugar control due to its phenolic compounds and α-glucosidase inhibitory properties (12). Based on a study of 60 male Kunming mice divided into five groups, a four-week kombucha intervention proved effective in reducing fasting blood glucose levels and enhancing β-cell function in a type 2 diabetes model (30). A double-blind, randomized prospective study by Chagai et al. found that consuming 240 ml of kombucha tea for 4 weeks reduced fasting blood sugar levels in 12 adults with T2DM (6). Through the scientific evidence presented above, kombucha generally has the potential as a therapy for managing diabetes mellitus, but further in-vivo studies at the human level with more specific subjects are still needed to confirm its validity.

Vitamin C

Vitamin C is a common product produced from the metabolism of kombucha in traditional kombucha drinks obtained from the fermentation of black and green tea (6)(28). The value of x is found from the linear regression equation y=0.0772x-0.0507, and R2=0.9953. The regression value of 0.9953 indicates that the accuracy level of ascorbic acid measurement approaches a value of 1, meaning that there is an influence between the concentration of vitamin C and its absorbance value as shown in the following **(Figure 1)** (31).

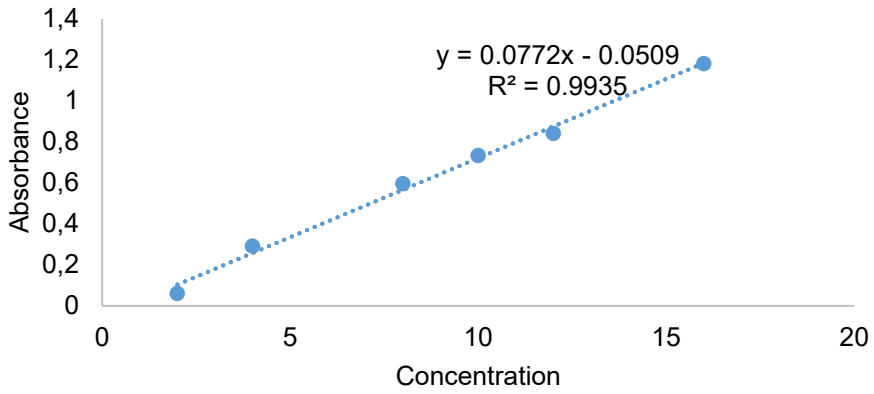

Figure 1. Vitamin C standard curve

Table 4. Results of vitamin C content testing in *temu mangga* kombucha based on fermentation duration

	Duration of fermentation				
	5 days (T1)	**7 days (T2)**	**10 days (T3)**	**14 days (T4)**	***P-value < 0.05***
Vitamin C (ml/L)	2.06 ± 0.06	1.93 ± 0.06	1.86 ± 0.06	1.86 ± 0.29	0.537

Statistical analysis used Non-parametric Kruskal Wallis test with a significance level of p<0.05. Data is presented as the mean of three repetitions ± standard deviation (SD).

Based on **Table 4**, it shows that the highest Vitamin C content of 2.06 ± 0.06 ml/L is found at the beginning of fermentation on the fifth day. At the beginning of fermentation, the amount of vitamin C remained stable until the 5-day fermentation treatment; the longer the fermentation, the more vitamin C decreased, as seen in the table above. This study is in line with the research by Winandari et al. (32) which shows that the highest vitamin C content in rosella kombucha is found on the 6th day of fermentation. However, on the 9th day, the vitamin C content decreased and continued to decline on the 12th day, indicating that a longer fermentation duration is associated with a decrease in vitamin C content in the product (15). This condition arises due to the degradation of vitamin C and the cessation of microorganism activity in producing vitamin C, caused by the reduced food supply, particularly sugar in kombucha. As a result, these microorganisms will produce other acidic compounds (33). Vitamin C is damaged due to the activity of bacteria that can produce the enzyme L-gulonolactone oxidase (15). This enzyme functions to convert compounds into 2-Keto-L-gulonolactone at the final stage of vitamin C synthesis, resulting in the production of various types of acids instead of ascorbic acid (vitamin C) (34). This is evidenced by data showing an increase in total acids. Total acid analysis in this study was conducted on the 5th, 7th, 10th, and 14th days of fermentation, respectively 0.99%, 0.90%, 1.05%, and 1.42% (15).

Total acidity is related to the pH of kombucha. The low pH is inversely proportional to the total acid produced. The increase in total acidity causes a decrease in pH value because the environment becomes more acidic (35). The decrease in pH occurs when the fermentation time is prolonged. The longer the fermentation lasts, the higher the acid concentration will be, causing the pH value of the kombucha to tend to decrease. With the decreasing pH obtained, it indicates that the acid content in the kombucha is increasing (35) (36).

LIMITATIONS

This study uses a limited number of samples, so the generalization of the results to a broader population may not be entirely accurate. Only four fermentation durations were tested, so the long-term effects of longer fermentation have not been thoroughly evaluated. This research was also conducted in vitro, which means the results obtained may not fully reflect the effectiveness of temu mangga kombucha in a real context within the human body, thus further in vivo research is needed

CONCLUSION AND RECOMMENDATION

This study demonstrates that fermentation duration significantly influences the physicochemical properties, vitamin C content, and α-glucosidase inhibitory activity of Curcuma mangga-based kombucha. The observed decrease in pH and sugar content and increased enzyme inhibition suggest potential antidiabetic functionality. These findings contribute to the growing body of evidence supporting the development of functional fermented beverages for the dietary management of type 2 diabetes. However, as the results are based on in vitro analysis, further in vivo and clinical studies are necessary to confirm the efficacy and safety of this product. Future research should also explore its potential application on an industrial scale.

This research is an initial study that is still in the in-vitro stage, thus having potential for further development. Future researchers are expected to continue the study in vivo on experimental animals and humans, as well as explore other phytochemicals in temu mangga kombucha and their impacts on other diabetes indicators. The consumption of functional foods like temu mangga kombucha plays an important role in improving public health. Kombucha combined with temu mangga not only offers a unique flavor but also contains various beneficial bioactive compounds, including antioxidants and probiotics. These compounds can help improve digestive health, boost the immune system, and potentially reduce the risk of metabolic diseases such as diabetes. With the increasing awareness of the importance of a healthy diet, integrating temu mangga kombucha as part of the daily diet can be an effective strategy to promote well-being and disease prevention among the community.

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