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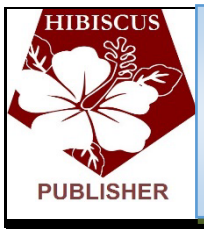

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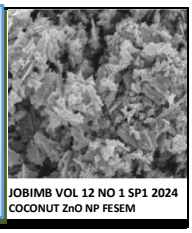

Physicochemical and Microbial Properties of Miracle Berry (*Synsepalum dulcificum*) Kombucha Tea Treated with Microwave Radiation

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ABSTRACT

Miracle berry (*Synsepalum dulcificum*) is a plant with unique taste-altering properties, which converts the sour taste of foods into sweetness. Therefore, it is highly interesting to incorporate it into kombucha, a well-known fermented product. This study aims to evaluate the effect of microwave radiation (MR) on the physicochemical and microbial properties of miracle berry kombucha tea (MBK) treated with MR. In this study, 75% miracle berry and 25% black tea kombucha were subjected to MR at varying times (5s and 10s). The total soluble solid (TSS) and pH, color, colony count of acetic acid bacteria (AAB) and lactic acid bacteria (LAB) were determined. Results showed that the pH and TSS values decreased over time, with microwave-treated samples exhibiting higher values than the control. The microwave-treated MBK at 5s significantly ($p<0.05$) promoted the growth of LAB (0.00 to 5.70 CFU/mL) from day 0 to 7 but inhibited the growth of AAB. In conclusion, the observed fermentation throughout 7 days of storage potentially shortened the fermentation period, and MBK under 5s treatment potentially enhanced the LAB growth. This study can provide fresh perspectives on the effects of MR on kombucha quality and properties.

INTRODUCTION

A symbiotic culture of bacteria and yeast known as a "SCOBY" is combined with sweetened tea to create kombucha, a fermented beverage [1]. Miracle berry (*Synsepalum dulcificum*) is a fruit that has a special taste-altering quality [3] due to the presence of the protein miraculin in this fruit. The miraculin transforms the sour taste into sweet and may be applicable in preparing less sour kombucha tea. Traditionally, kombucha is fermented for 7-21 days at temperatures between 27°C and 30°C in a warm and dark environment [1]. Microwave radiation is one alternative method that has been proposed to accelerate the fermentation process and control the microbial communities involved. However, limited research has been conducted on the effect of microwave radiation at low temperatures on microbial growth in kombucha preparation. This study focused on shortening the fermentation period while adding a new combination with MB to mask the unpleasant sour taste of kombucha. Thus, the objectives of this study are: to determine the effect of microwave radiation on the growth and viability of lactic acid bacteria (LAB) and acetic acid bacteria (AAB) while analysing the effect of microwave radiation on the physical and antioxidant properties of miracle berry kombucha tea.

MATERIALS AND METHODS

Raw Materials

The kombucha SCOBY, liquid starter and MB fruit were purchased from an online shopping platform. Black tea and sugar were purchased from a local market, Mydin in Gong Badak, Terengganu.

Preparation of Miracle Berry Kombucha Tea

Black tea incorporated with MB was prepared by mixing a total of 10 g MB fruits extract powder and black tea. Tea blends were made with 75% MB + 25% black tea. Then, sugar (20 g) is added to 300 mL of water. This whole procedure of kombucha preparation was followed by a method done by [6] with modifications. After that, the mixture of MB, tea, and sugar was steeped in 300 mL of hot water for 7 minutes, cooled to 20-30°C,

filtered into 3 glass jars, and cooled. Each jar was filled with 28.76 g to 33.77 g SCOBY and 0.5 mL of liquid starter for microwave treatment at 5s and 10s. Then, it was tightly wrapped with a clean, porous cloth. The kombucha was stored in a dust-free room for 7 days of fermentation. All steps were repeated for control using 10 g of dried black tea. After fermentation, both controlled and treated kombucha teas were kept at -18°C.

Microwave Treatment

A single-mode microwave oven with an internal capacity of 242 mm (H) x 412 mm (W) x 426 mm (D) was used to microwave treat the samples (National Microwave Oven NN-C988W, Japan). A magnetron oscillator with a maximum continuous-wave output power of 900 W at 50 Hz served as the source of the microwaves in this device, which was primarily made of regular rectangular waveguides. The sample in a 300 mL transparent glass jar was positioned in the center of the microwave and exposed to microwave radiation at 5s and 10s, respectively. After that, the temperature of the kombucha sample was immediately measured using a WT-1 digital food thermometer (China) to establish the standard temperature-time curve at various doses [7].

Determination of pH, TSS, and Color

The pH of the kombucha was measured using a pH meter (Orion Star A111, Indonesia, J11623). The total soluble solids (TSS) of the samples was determined using a portable digital refractometer Milwaukee MA871 (Romania, Europe) (0 to 85% °Brix). Colour analysis was performed using a Chroma meter CR-400 Konica Minolta (Japan).

Colony Count of AAB and LAB

Both analysis (AAB and LAB) was performed by doing a serial dilution in which 1 mL of treated kombucha sample was pipetted and placed into 9 mL of peptone water, respectively. This produced a sample with 10^{-1} diluted solution. The sample was shaken vigorously. Then, the procedure was repeated until 10^{-5} dilution. After that, 0.1 mL from the highest three dilutions were pipetted into Acetobacter Agar (AAB) and MRS agar (LAB), respectively. The inoculum was transferred in duplicate and was spread using an L-spreader aseptically left to dry for 10 minutes before being incubated aerobically at 30°C for 48 hours and the total plate count (CFU/mL) for each plate was determined [9]. All procedures were repeated with a controlled kombucha sample.

Statistical Analysis

Data were expressed as means ± standard deviations (SD) by one-way analysis of variance (ANOVA) using Tukey's tests, Minitab 21 statistical software. Each measurement was performed in triplicate (except for microbial analysis, which was carried out in duplicate).

RESULTS AND DISCUSSION

Determination of pH in Fermented Kombucha Tea

Table 1 represents the pH of fermented miracle berry kombucha teas with different time exposures under microwave treatment that were stored for 7 days. The pH values for all kombucha samples significantly differed between days 0, 1 and 7 *($p < 0.05$)*. The pH values of all samples were decreased from day 0 to day 7, respectively. According to a previous study [11], the pH value dropped over time, which caused by organic acids produced as a result of fermentation. Also, black tea typically has a lower pH compared to fruit-based teas, which can result in a lower pH in the final kombucha product [12]. Meanwhile, sample C recorded the lowest pH value (2.18 ± 0.00) on day 7 compared to other treatments, thus, suggesting that microwave treatment can accelerate fermentation by providing a favorable temperature for yeast growth, around 20-30°C [13].

Table 1. Results for pH of kombucha during fermentation. Values followed by different letters within the same column are significantly different *($p < 0.05$)* (n = 3 ± SD).

	pH			
Day	Control	A	B	C
0	4.18 ± 0.06^{a}	4.19 ± 0.01^{a}	4.19 ± 0.01^{a}	4.19 ± 0.01^{a}
1	2.59 ± 0.01^{b}	2.62 ± 0.03^{b}	2.62 ± 0.04^{b}	2.47 ± 0.02^{b}
7	1.92 ± 0.02^{c}	2.36 ± 0.04^{c}	2.30 ± 0.02^{c}	2.18 ± 0.00^{c}

Note:
where formulation = control (100% black tea); A (75% MB, 25% black tea for 0s); B (75% MB, 25% black tea for 5s); C (75% MB, 25% black tea for 10s).

Total Soluble Solids (TSS) of Fermented Kombucha Tea

Table 2 shows the TSS of fermented kombucha teas with different time exposures under microwave treatment that were stored for 7 days. It was found that all treatments showed a decrease in TSS values over time. This is because SCOBY in kombucha consumes the sugars in tea as a carbon source [2]. Meanwhile, samples A, B and C had higher TSS values than the control sample on day 0 and day 7. This could be related to the presence of natural sugar content in fruits, which leads to a higher TSS value. This was supported by the study [6,14], which concluded that the TSS is attributed to the sugar content and all soluble components in the solutions. The present study also found that there was a significant difference *($p < 0.05$)* in the TSS values between samples on day 0 and day 7. It can be highlighted that sample C recorded the highest value of TSS (8.10 ± 0.00 °Brix) at the end of fermentation. This could be related to the effect of longer exposure to microwaves compared to other samples. Thus, it affected the microbial activity in kombucha [15].

Table 3. Results for TSS (°Brix) of kombucha during fermentation. Values followed by different letters within the same column are significantly different *($p < 0.05$)* (n = 3 ± SD).

	TSS (°Brix)			
Day	Control	A	B	C
0	8.33 ± 0.49^{a}	9.37 ± 0.23^{a}	9.37 ± 0.23^{a}	9.37 ± 0.23^{a}
1	8.97 ± 0.75^{a}	8.03 ± 0.29^{b}	8.13 ± 0.06^{b}	8.30 ± 0.10^{b}
7	7.97 ± 0.06^{a}	8.03 ± 0.06^{b}	8.07 ± 0.06^{b}	8.10 ± 0.00^{b}

Note:
where formulation = control (100% black tea); A (75% MB, 25% black tea for 0s); B (75% MB, 25% black tea for 5s); C (75% MB, 25% black tea for 10s).

Colorimetric Analysis

Table 3 shows the values of L*, a* and b* that were used to determine the colour analysis of black tea kombucha and MB kombucha at different time exposure to microwave radiation for 7 days of fermentation storage. It was found that the control sample recorded the highest L* and b* values on day 0, 1 and 7, indicating that the black tea kombucha was lighter yellow compared to the samples. The addition of MB extract powder affected the yellow colour of kombucha tea because of its anthocyanin pigment, which was reported to be 11.04 mg/100 g FW, responsible for the fruit's red color [16]. Therefore, it produced a dark orange colour when added into black tea. Over time, the value of L* (lightness) and b* (blue-yellow) for each sample were increased. Meanwhile, the value of a* for sample A showed the highest value after 7 days of fermentation compared to samples B and C.

Table 3. Results for colorimetric analysis of kombucha during fermentation. Values followed by different letters within the same column are significantly different *($p < 0.05$)* (n = 3 ± SD).

Sample	Day	L*	a*	b*
Control	0	20.30 ± 0.08^{b}	8.24 ± 0.15^{a}	4.88 ± 0.04^{c}
	1	24.60 ± 0.22^{a}	8.61 ± 0.22^{a}	8.38 ± 0.26^{b}
	7	24.94 ± 0.04^{a}	7.63 ± 0.11^{b}	9.02 ± 0.01^{a}
A	0	19.49 ± 0.29^{b}	1.79 ± 0.05^{c}	1.14 ± 0.27^{b}
	1	20.20 ± 0.25^{a}	2.86 ± 0.11^{a}	2.38 ± 0.16^{a}
	7	20.19 ± 0.07^{a}	2.47 ± 0.11^{b}	2.79 ± 0.06^{a}
B	0	19.49 ± 0.29^{b}	1.79 ± 0.05^{b}	1.14 ± 0.27^{c}
	1	20.29 ± 0.03^{a}	2.31 ± 0.08^{a}	2.18 ± 0.07^{b}
	7	20.22 ± 0.20^{a}	2.26 ± 0.06^{a}	2.75 ± 0.13^{a}
C	0	19.49 ± 0.29^{b}	1.79 ± 0.05^{b}	1.14 ± 0.27^{c}
	1	20.32 ± 0.08^{a}	2.06 ± 0.07^{a}	1.93 ± 0.03^{b}
	7	20.35 ± 0.02^{a}	1.71 ± 0.06^{b}	2.78 ± 0.05^{a}

Note:
formulation = control (100% black tea); A (75% MB, 25% black tea for 0s); B (75% MB, 25% black tea for 5s); C (75% MB, 25% black tea for 10s). L*: darkness (0) to lightness (100); a*: greenness (-) to redness (+); b*: blueness (-) to yellowness (+).

Colony Count of Acetic Acid Bacteria (AAB)

Table 4 represents the acetic acid bacteria (AAB) count of fermented kombucha teas with different time exposure under microwave treatment during storage days. Throughout the fermentation process, the function of AAB is to impart a vinegary flavour to kombucha via the accumulation of acetic acid when converted from ethanol [13].

Table 4. Total plate count for AAB is present in miracle berry kombucha during fermentation. Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 3 ± SD).

Day	Acetic Acid Bacteria Count (CFU/mL)			
	Control	A	B	C
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
1	6.11 ± 0.47^{a}	4.64 ± 0.90^{a}	4.24 ± 0.34^{a}	4.78 ± 0.68^{a}
7	4.48 ± 0.00^{b}	4.95 ± 0.00^{a}	0.00 ± 0.00^{b}	2.45 ± 3.47^{a}

Note:
where formulation = Control (100% black tea); A (75% MB, 25% black tea for 0s); B (75% MB, 25% black tea for 5s); C (75% MB, 25% black tea for 10s); CFU: colony-forming unit.

The AAB count for control and sample A were significantly different between day 0 and day 7 ($p < 0.05$), and between day 0 and day 1 for sample B, with the highest AAB count at the end of fermentation recorded by sample A. On the other hand, the analysis showed no significant difference ($p > 0.05$) between fermentation days for sample C, with the lowest value recorded for AAB count after 7 days of fermentation along with sample B. It was found that the AAB count was relatively low throughout the fermentation process for all samples. This suggests that the miracle berry kombucha may not be an ideal environment for the growth of AAB. For control kombucha, it showed a slight increase in AAB count from day 0 to day 1, followed by a decrease by day 7. This pattern was consistent with research reported by [17], in which AAB initially increases to convert ethanol to acetic acid, but then reaches a stationary phase.

Colony Count of Lactic Acid Bacteria (LAB)

Table 5 shows the lactic acid bacteria (LAB) count of fermented kombucha teas with different time exposure under microwave treatment during storage days. The analysis revealed no significant difference *($p > 0.05$)* in LAB growth between days for control, sample A and C. This suggests that microwave treatment at 10s did not significantly impact the growth of LAB during the fermentation days. On the other hand, sample B treated for 5s showed a significant difference *($p < 0.05$)* in LAB growth between day 0 and day 7. This indicates that a 5s microwave exposure has positively influenced the proliferation of LAB in this study. The observed increase of LAB growth in sample B could be attributed to sublethal stress response. This can be explained by low-dose microwave exposure (5s) which might induce a mild stress response in LAB, stimulating their growth as a protective mechanism [19].

Table 5. Total plate count for LAB present in miracle berry kombucha during fermentation. Values followed by different letters within the same column are significantly different *($p < 0.05$)* (n = 3 ± SD).

Day	Lactic Acid Bacteria Count (CFU/mL)			
	Control	A	B	C
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
1	2.00 ± 2.83^{a}	2.00 ± 2.83^{a}	0.00 ± 0.00^{b}	2.00 ± 2.83^{a}
7	4.69 ± 0.30^{a}	5.79 ± 0.02^{a}	5.70 ± 0.02^{a}	6.24 ± 0.19^{a}

Note:
where formulation = Control (100% black tea); A (75% MB, 25% black tea for 0s); B (75% MB, 25% black tea for 5s); C (75% MB, 25% black tea for 10s); CFU: colony-forming unit.

CONCLUSION

The results of the study revealed that the optimal condition for fermentation of MB kombucha treated with microwave radiation was at 5s, which allowed the development of a functional beverage with enhanced beneficial microbes such as lactic acid bacteria (LAB). From the findings, the microbial analysis indicated low acetic acid bacteria (AAB) counts throughout fermentation influenced by microwave treatment. Meanwhile, LAB counts were not significantly impacted by microwave treatment, but the 5s treatment potentially enhanced LAB growth. Other than that, this study also found that the pH values decreased over time, with microwave-treated samples exhibiting higher pH compared to the control. In addition, the TSS also decreased over time, with microwave-treated samples showing higher values. It was also found that colorimetric analysis revealed changes in colour attributes influenced by the addition of MB.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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