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DEVELOPMENT OF A FERMENTED BEVERAGE FROM WHITE MULBERRY JUICE USING THE KOMBUCHA CONSORTIUM

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ABSTRACT

White mulberry contains various nutrient elements and phytochemicals such as alkaloids, anthocyanins and flavonoids which possess a wide range of biological activities beneficial to human health. In this study, a novel fermented beverage from white mulberry juice was developed using Kombucha tea fungus. Effects of initial sugar concentration, fermentation temperature and recycling number of tea fungus biomass on quality of white mulberry Kombucha were investigated. Results showed that, at initial tea fungus biomass concentration of 100 g/L and initial sucrose concentration of 250 g/L, after 3 days of fermentation at 28 ^{o}C, the Mulberry Kombucha beverage showed desirable chemical characteristics with an acceptable total acidity content (9.45 g/L), as well as high total polyphenol and vitamin C contents (2099.9 μg/L and 0.51 mg/mL, respectively), giving the beverage a harmonious sweet and sour taste. In addition, the fermentation rate was increased when the tea fungus biomass was repeatedly reused; however, the viability of acetic acid bacteria during sequential tea fungus incubation was lost due to the out number of yeast cells, leading to a drop in total polyphenol and vitamin C contents.

Keywords: *Mulberry Kombucha; bacterial – yeast consortium; fermented beverage; tea fungus; total polyphenol; vitamin C.*

1. INTRODUCTION

Kombucha tea is a slightly sweet, sour sparkling beverage fermented from sweetened black tea infusion using a symbiotic consortium of bacteria and yeast called “tea fungus” [1,2]. The two portions of Kombucha tea are the fermented broth and the floating cellulose pellicle layer (tea fungus) [3-6]. This beverage is well-known for its therapeutic qualities including detoxification, antioxidation, energizing potency and immunity promotion [7,8]. According to many studies, these beneficial effects could have been due to the fact that not only many healthy compounds in tea infusions would be enriched after fermentation, but other organic substances can also be produced in the course of this process, especially glucuronic acid and vitamin C [1]. To diversify and expand the market for Kombucha, one approach is to replace traditional substrate (black or green tea) with other fruit juices, such as cherry juice [9], grape juice [10,11], pineapple juice [12], pomegranate juice [13], and cactus pear juice [14]. These studies indicated that the bioactive compounds in all fermented juices increased significantly after fermentation.

Mulberry fruits (*Morus*, Moraceae) are becoming increasingly trending among food and drink [15]. They are usually processed into jelly, juice, jam, dried fruit and wine, which are tasty and nutritious [16,17]. There are various studies depicting mulberry fruits as a natural source of alkaloids, anthocyanins and flavonoids [15]. Hence, mulberry juice may have potential effects on human health, mainly in cardiovascular disease prevention, anti-inflammation, metabolic diseases prevention, and maintaining a healthy liver [16,18]. Furthermore, it was reported that the polyphenol and vitamin C levels in mulberry juice were significantly higher than those in black tea, which were around 25.3 and 4 times higher, respectively [16,19-21]. Therefore, production of Kombucha beverages using mulberry juice could result in enhancement of existing healthy substances in mulberry juice. According to Mehdi et al. (2018), Kombucha tea with black mulberry syrup as an initial substrate showed a significant increase in the number of antioxidants in comparison with traditional Kombucha tea.

The white mulberry (*Morus alba*) is a fast-growing and widely cultivated plant in Vietnam, but its fruit is hardly commercially available due to its easily rotten characteristic and short storage period [23]. Besides, there are few studies involving Kombucha fermentation using white mulberry juice as a substrate. For these reasons, the aim of this study is to develop a fermented refreshing drink from white mulberry juice by using tea fungus. White mulberry juice was fermented under various conditions to find out the best approach for producing Mulberry Kombucha.

2. MATERIALS AND METHODS

2.1 Microorganisms and culture conditions

Kombucha tea fungus was purchased from Green House – Water Kefir, Milk Kefir, Kombucha (Binh Thanh District, HCMC). Tea fungus was maintained in sweetened black tea infusion. The seed culture was prepared by infusing 5 g black tea leaves into a glass bottle containing 1000 mL boiling water supplemented with 50 g/L sucrose. After 5 – minute brewing, the tea leaves were removed and the tea solution was cooled to ambient temperature. 24 g Kombucha tea fungus and 200 mL of previous fermented tea liquor were added to the container. The bottle was then covered with a clean cloth and the culture was kept at 28 °C. The broth was replaced with 1000 mL sugared black tea in alternate weeks to supply a nourishing medium for tea fungus. Before conducting experiments, the seed (tea fungus) was activated in mulberry juice for 48 hours at 2 °C [13].

2.2 Fermentation of mulberry juice

White mulberry fruits (*Morus alba*) were harvested from the fields in Da Lat city (Lam Dong province). The juice was obtained by pressing fresh fruits using household juicer and was filtered to remove the remaining pulp. Initial total sugar and reducing sugar concentrations in mulberry juice were 215.34 g/L and 151.2 g/L, respectively. Prior to fermentation, a certain amount of sucrose was dissolved into a glass bottle containing 1000 mL mulberry juice. In order to inhibit microbial contamination, 44.53 mg/L of sodium metabisulfite ($Na_2S_2O_5$) was added into the mixture. The mulberry juice was then inoculated with 100 g/L of tea fungus and incubated in darkness at constant temperature until the pH value was not less than 3.5.

2.3 Experimental design

2.3.1 Effects of initial sucrose concentration

The mulberry juice was supplemented with sucrose at different concentrations of 150 g/L (A1), 200 g/L (A2), 250 g/L (A3) and 300 g/L (A4). Initial tea fungus concentration was kept at 100 g/L and fermentation temperature was at 28 °C.

2.3.2 Effects of fermentation temperature

The mulberry juice was fermented at different temperature conditions of 10 °C (B1), 20 °C (B2), 28 °C (B3). Initial tea fungus concentration was kept at 100 g/L and sucrose concentration was at 250 g/L.

2.3.3 Effects of recycling number of tea fungus biomass on quality of mulberry kombucha

In this experiment, mulberry juice supplemented with 250 g/L sucrose and 100 g/L tea fungus was fermented at 28 °C. After every 4 days, the fermented broth was withdrawn and replaced with 1000 mL of fresh sugary mulberry juice (250 g/L sucrose). The used tea fungus served as inoculum for subsequent cycles. The tea fungus biomass was weighed at the end of each cycle.

In all experiments, broth culture was periodically collected to determine total sugar (g/L) and reducing sugar (g/L) concentrations, total acidity content (g/L), pH value, ethanol content (g/L), total polyphenol content (μg/L) and vitamin C content (mg/100 mL).

2.4 Analytical methods

Total sugar content was measured using polyphenol-sulfuric acid method [24], while reducing sugar content was measured using 3.5 – dinitrosalicylic acid method [25]. To determine total acidity, samples were titrated against 0.1N NaOH and polyphenolphthalein was used as an indicator [26]. pH was measured using pH meter (Mettler Toledo, US). Ethanol determination was conducted by titrating samples against nitrochromic reagent [27]. The total polyphenol in samples was measured by the Folin-Ciocalteu method [28]. Vitamin C determination was performed using spectrophotometric method with 2,4-dinitrophenyl hydrazine (DNPH) [29]. All the chemicals used for quantifying were of analytical grade.

3. RESULTS AND DISCUSSION

3.1 Effects of initial sucrose concentration on chemical composition of Mulberry Kombucha

Sucrose is an important substrate in both cellular growth and metabolite production [30]. The aim of this study is to find an appropriate sucrose concentration to not only control excessive biomass production but also improve the production of nutritious metabolites. Effects of four initial sucrose levels on chemical characteristics of Mulberry Kombucha were investigated.

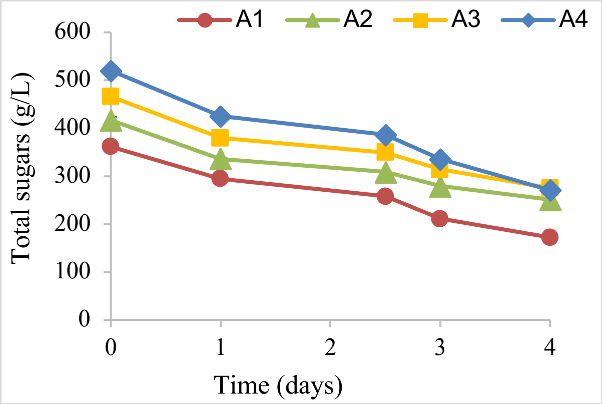
3.1.1 Total sugar and reducing sugar contents

Figure 1A shows the biodegradation of total sugar content during Mulberry Kombucha fermentation. Overall, the total sugar content decreased over the time due to the growth of tea fungus and the formation of metabolites [31]. At the beginning, sucrose was hydrolyzed by yeast invertase to release glucose and fructose, leading to the increase in reducing sugar levels on the first day (Figure 1B) [4,32-34]. At this stage, there was a slow growth rate of acetic acid bacteria as they could not assimilate sucrose [35]. However, yeast could develop rapidly through an aerobic respiration pathway using dissolved oxygen in the medium, producing H_2O and CO_2 [36]. The accumulation of CO_2 on the medium surface disrupted oxygen diffusion from the headspace, hence ethanol fermentation could take place due to the depletion of dissolved oxygen from the second day onwards. That was reflected by a rapid decline in total sugar concentrations of A1-A4. Ethanol then became a substrate for the growth of acetic acid bacteria. Acetic acid bacteria consumed glucose and fructose to produce gluconic acid, glucuronic acid, and extracellular cellulose [9,37]. Hence, the decrease in reducing sugars from day 1 to day 4 was also attributed to the acetic acid bacterial development [4].

Figure 1A also shows that the amount of total sugars consumed in A1, A2, A3 and A4 were significantly different after 4 days of fermentation. In particular, the highest total sugars consumption rate was 62.35 g/L-day (A4), while the other degradation rates were 47.68 g/L-day (A3), 44.02 g/L-day (A1) and 41.4 g/L-day (A2). This difference could be due to the difference in initial sucrose levels. According to Nguyen (2006), the solubility of oxygen decreases with the increase in sugar concentration. Due to oxygen deficiency, A3 and A4 could have approached the fermentation stage earlier, leading to a dramatic consumption of total sugars. In addition, the highest ethanol formation of A4 (Figure 3) could stimulate the growth of acetic acid bacteria, facilitating sugar consumption [4].

In the end, the remaining reducing sugar levels at four samples were 264.76 g/L (A4), 239.02 g/L (A3), 192.60 g/L (A2), and 129.06 g/L (A1). It can be concluded that the higher the initial sucrose level used, the more glucose and fructose hydrolyzed by yeast were produced. Similar trends were observed in the studies of Blanc (1996), Reiss (1994), Chen and Liu (2000). It should be noted that initial sucrose levels added in Mulberry Kombucha (150 g/L, 200 g/L, 250 g/L, 300 g/L) were higher than that in traditional Kombucha (70 g/L) [4]. With higher sucrose levels, the sour taste of mulberry juice could be well balanced, creating a sweet and sour brew because the total acidity in mulberry juice was much higher than that of black tea Kombucha (4.56 g/L versus 1.2 g/L) [35].

A)



B)

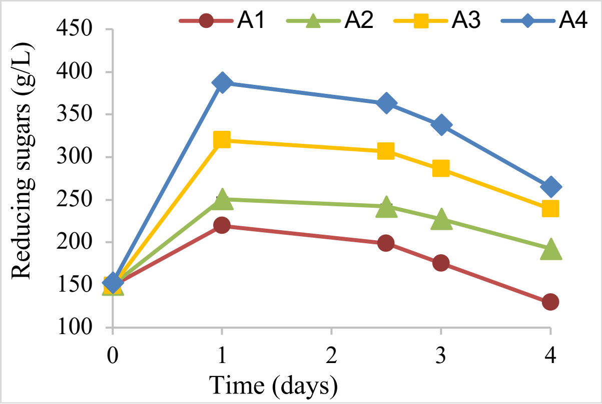


***Figure 1.** Effects of initial sucrose concentration on total sugars (A) and reducing sugars (B).*

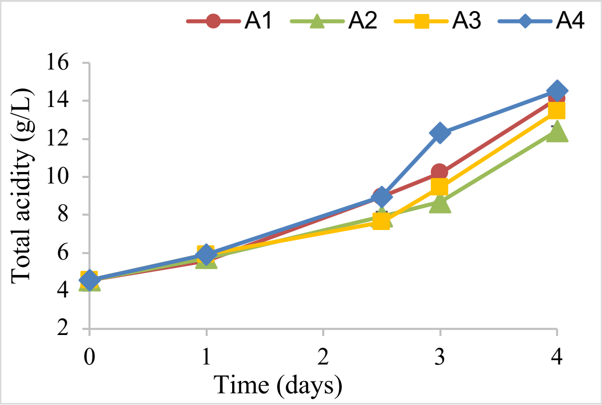
3.1.2 Total acidity and pH value

The organic acids in food could affect the odor, color, microbial development and the quality of final products [40]. pH changes and symbiotic relationship between yeast and bacteria are inextricably linked [41]. Hence, it is essential to determine total acidity and pH.

Figure 2 shows that the pH values of the four samples decreased gradually during 4 days of fermentation, corresponding with the increase in total acidity. After four days, the total acidity in four samples were 14.52 g/L (A4), 14.10 g/L (A1), 13.44 g/L (A3), 12.42 g/L (A2). Previous studies proved that acetic acid is one of the main metabolites produced during Kombucha fermentation [1, 4]. Hence, total sugars breakdown correlated closely with the total acidity and pH value. The final pH values in fermentation broths ranged from 3.62 (A4) to 3.71 (A2), all of which were in accordance with pH requirement of black/green tea Kombucha products, that is pH should not be less than 3.5 [4]. The pH value on the third day of A4 featured the highest drop compared to the others. It seems that the rapid assimilation of sugars (184.71 g/L from day 0 to day 3) had led to higher production of organic acids. Similar trends were observed by Reiss (1994) and Jayabalan et al. (2007).

The low pH value and high total acidity of Mulberry Kombucha could limit microbial contamination such as *Escherichia coli, Staphylococcus aureus, Agrobacterium tumefaciens* [11]. However, it is recommended that the total acidity in Kombucha brew should not excess 12 g/L [13]. Hence, day-3 fermented Mulberry Kombucha may be more acceptable than the day-4 one.

A)



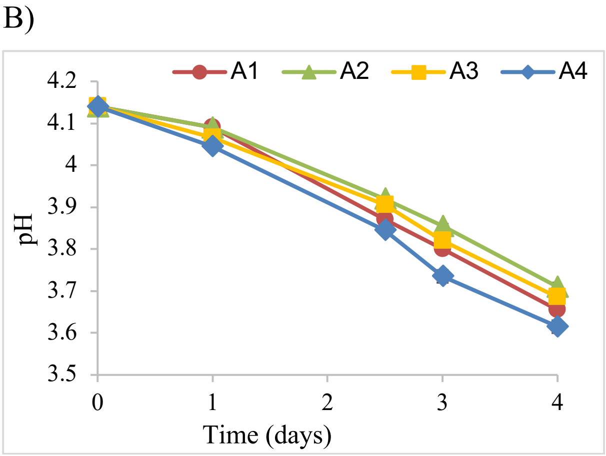


Figure 2. *Effects of initial sucrose concentration on total acidity (A) and pH (B).*

3.1.3 Ethanol content

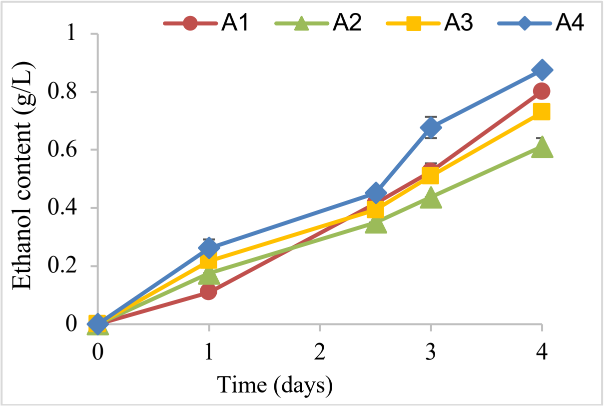


Figure 3. *Effects of initial sucrose concentration on ethanol content.*

As shown in Figure 3, the ethanol contents of all samples increased linearly and related to the biodegradation of total sugars (Figure 1A). Ethanol is formed as yeast cells strive to maintain their redox balance and make sufficient ATP for the continuing growth [32]. After the 4 – day fermentation, the ethanol contents in four media were 0.88 g/L (A4), 0.80 g/L(A1), 0.73 g/L (A3), and 0.61 g/L (A2). Ethanol contents on final days of all samples were quite low because part of ethanol content was oxidized by acetic acid bacteria to produce acetic acid (Figure 2) [33,42]. Moreover, the presence of acetic acid could stimulate the yeast to produce more ethanol [35]. As can be seen in Figure 3 and Figure 2A, ethanol formation was directly proportional to total acidity. This is an evidence of the interaction between yeast and acetic acid bacteria in tea fungus [43]. Similar results were also reported in previous studies [33,35,39].

According to Le (2011), Pham et al. (2014), the increased initial sucrose content resulted in higher final ethanol concentration because more substrates in media were available for use. With the highest initial sucrose content (300 g/L), final ethanol concentration in A4 was much higher than the other samples. However, the final ethanol content of A1 was higher than those of A2 and A3 (Figure 3). This could be because the higher density of growing yeast cells in A1 (due to the delay of fermentation stage) had accelerated the ethanol production rate [32].

3.1.4 Total polyphenol content

The phenolic compounds have been well known for their antioxidant activities. They may act as free radical scavengers to enhance human health [45]. As shown in Figure 4, total polyphenol levels of all samples increased significantly after one day of fermentation (1.61 ÷ 1.92 times higher than the initial quantity), then there was a slight increase in the following days. Previous studies elucidated that total polyphenol content increased due to the biodegradation of complex polyphenols to smaller monomers by enzymes excreted (excreting) from the Kombucha consortium [4,11], and by acid hydrolysis [11,42,46]. *Pichia manshurica* isolated from tea fungus could be able to break down polyphenols such as anthocyanins by β-glucosidase activity [47].

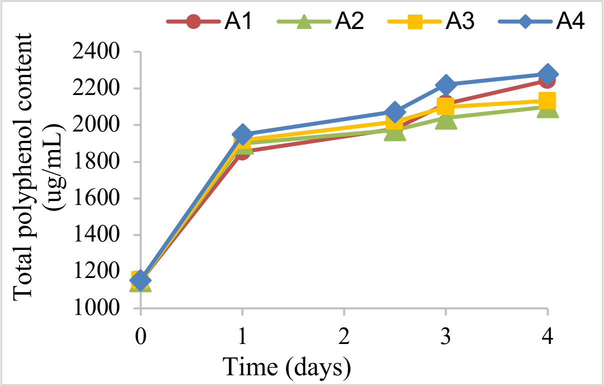


Figure 4. *Effects of initial sucrose concentration on total polyphenol content.*

In the first stage of fermentation, the increase in total polyphenol content (TPC) in all samples was similar (Figure 4). At day-4, the total polyphenol contents in fermentation media were 2278.99 µg/mL (A4), 2243.18 µg/mL (A1), 2132.48 µg/mL (A3), 2099,93 µg/mL (A2). Thus, the increase in total polyphenol content depends on activities of yeast – bacteria consortium [48]. The TPC in all samples increased slightly from day 3 to day 4. It was due to the combination of some of the polyphenolic compounds to form molecules of higher molecular weight [11,49]. Similar trends were also observed in other studies [11,34,49].

3.1.5 Vitamin C content

In addition to polyphenols, vitamin C also imparts antioxidant properties. The vitamin C contents were determined on the third day of fermentation when the total acidity was within the recommended level. Figure 5 depicts a sudden increase in vitamin C contents on day 3 of fermentation. The biosynthesis of vitamin C is a “side arm” of the glucuronic acid pathway, in which glucuronic acid is a precursor of vitamin C synthesis [34,48]. Acetic acid bacteria produced glucuronic acid from glucose [34,49], hence the increase in vitamin C content depends on the bacterial activity [48]. On the third day, the vitamin C contents were 54.57mg/100 mL (A4), 51.00mg/100 mL (A3), 50.29mg/100 mL (A1), 32.43mg/100 mL (A2), which were in agreement with the decrease in reducing sugars from the first day onwards (Figure 1B). A similar observation was also obtained by Malbaša et al. (2011).

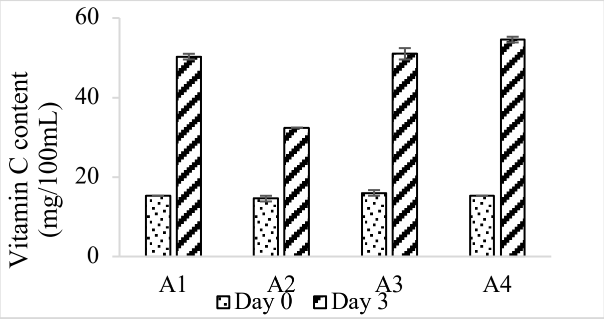


Figure 5. *Effects of initial sucrose concentration on vitamin C content.*

To sum up, mulberry juice containing 250 g/L initial sucrose concentration fermented at 28 ºC for three days could become a suitable drink regarding nutritious and sensory perspectives. Although A4 sample contained more biological active compounds, the total acidity content on day 3 of fermentation exceeded 12 g/L. A2 sample contained the lowest total polyphenol and vitamin C contents. In sample A1, the total acidity on the third day was relatively high, about 10.2 g/L, which could create a more sour taste due to the lowest remaining sugar content (Figure 1A).

3.2 Effects of fermentation temperature on chemical composition of Mulberry Kombucha

As was the case with many other fermentation processes, Kombucha fermentation also enriches antioxidant activity of food products through microbial growth and the excretion of their enzymes [50]. Moreover, environment temperature is one of the varied factors affecting this process, because extremely high or low temperature leads to the decrease in antioxidant activity [50]. Hence, it is imperative that appropriate temperature should be maintained to improve the Mulberry Kombucha benefits. In this experiment, mulberry juice with 250 g/L sucrose was fermented at the three different temperatures: 10 ± 1 °C (B1), 20 ± 1 °C (B2), and 28 ± 1 °C (B3).

3.2.1 Total sugar and reducing sugar contents

Figure 6A shows that total sugars in all samples decreased with different rates during fermentation. As the temperature increases, enzyme activity accelerates simultaneously, thus increasing sugar consumption rate and fermentation rate [30,37]. In other words, the closer to the optimal fermentation temperature of yeast and acetic acid bacteria it is, the more total sugars can be used. Since the optimum temperatures range from 20 to 30 °C for yeast [32] and from 25 to 30 °C for acetic acid bacteria [51], the total sugar consumption rate in B3 was highest (47.68

g/L-day (B3) versus 19.57 g/L-day (B2) and 10.61 g/L-day (B1)). More specifically, tea fungus in B3 consumed 190.67 g/L total sugars in 4 days, whereas the similar amount of total sugars (195.69 g/L) was completed in 10 days in B2 sample, and only 106.09 g/L total sugars were assimilated by tea fungus in B1 sample within 10 days.

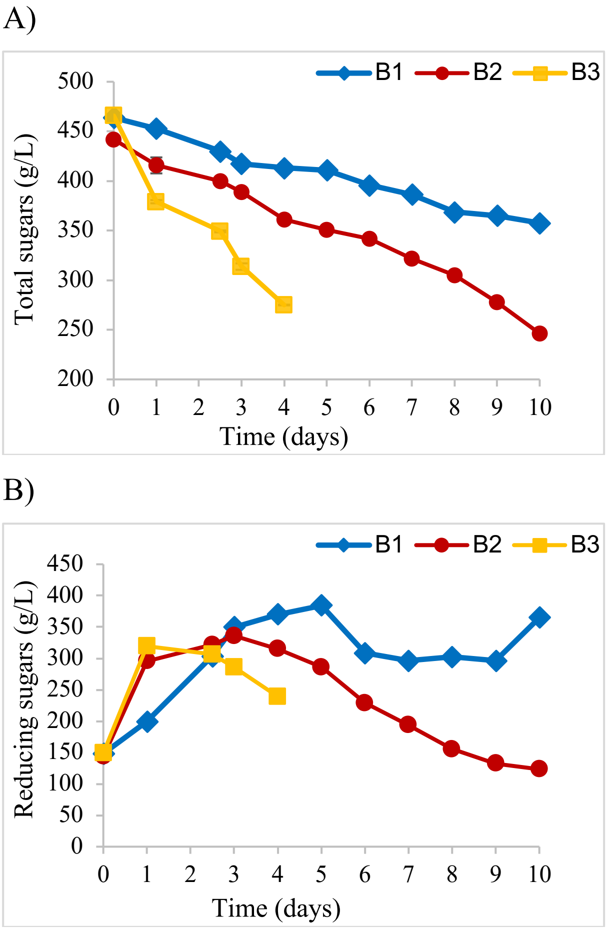


Figure 6. *Effects of fermentation temperature on total sugars (A) and reducing sugars (B).*

As mentioned above, the reducing sugar contents in all samples increased in the first stage of fermentation and decreased in the following days. However, the reducing sugar content in B1 samples fluctuated throughout the period, which decreased slightly from the 5th day but started to increase again on the 9th day. The first decrease in reducing sugar contents also started on different days, especially B3 illustrated the earliest decline. This could be inferred that acetic acid bacteria grew rapidly in B3 than the other samples. In contrast, the increase in reducing sugars on the final day of B1 could stem from the slow growth rate of acetic acid bacteria at low temperature (10 ± 1 °C). This observation in total sugar and reducing sugar contents was in agreement with the results reported by Lončar et al. (2014), Yavari et al. (2011, 2017).

3.2.2 Total acidity and pH value

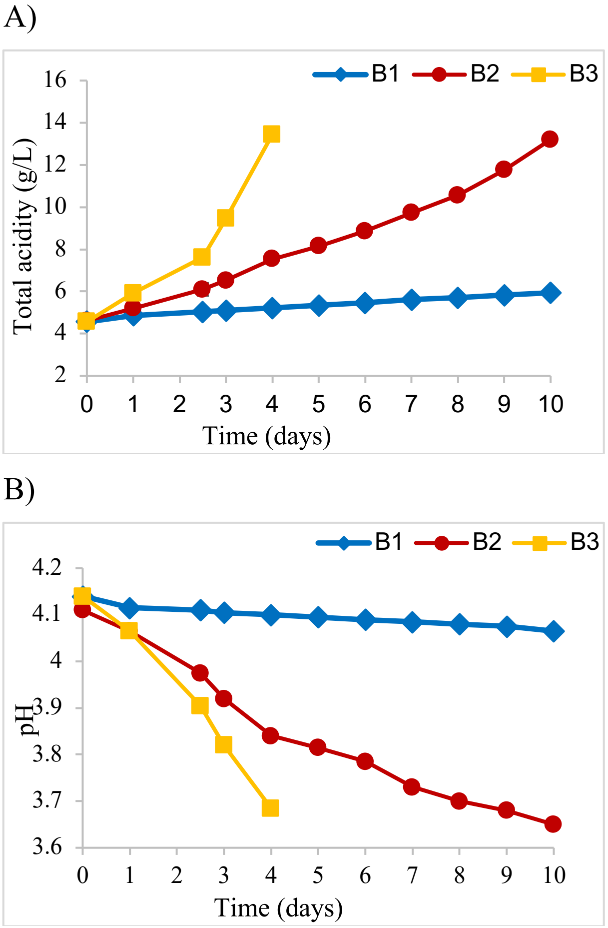


Figure 7. *Effects of fermentation temperature on total acidity (A) and pH (B).*

It can be seen in Figure 7 that the highest pH value was detected in sample B1, while the lowest pH value was obtained in B2 and B3. pH of sample B1 almost remained unchanged during fermentation. Therefore, the organic acid content in sample B1 was at lowest level. The results indicate that microbial growth was significantly slow at low temperature (10 °C). The total acidity of B2 on the 7th day (9.72 g/L) was approximately equal to that of B3 on the 3rd day (9.45 g/L), which may match organoleptic requirement (suitable to recommended total acidity). Hence, vitamin C content was determined on day 7 of B2 and day 3 of B3.

3.2.3 Ethanol content

Ethanol contents in all samples increased during fermentation (Figure 8). However, ethanol formation rate was dependent on environmental temperature. Higher fermentation temperature caused faster ethanol production rate. In particular, ethanol content in sample B1 was around 0.23 g/L on the final day (day 10), while B2 sample reached 0.78 g/L after the same period. It was noticeable that the B3 sample was able to create 0.73 g/L ethanol within 4 days.

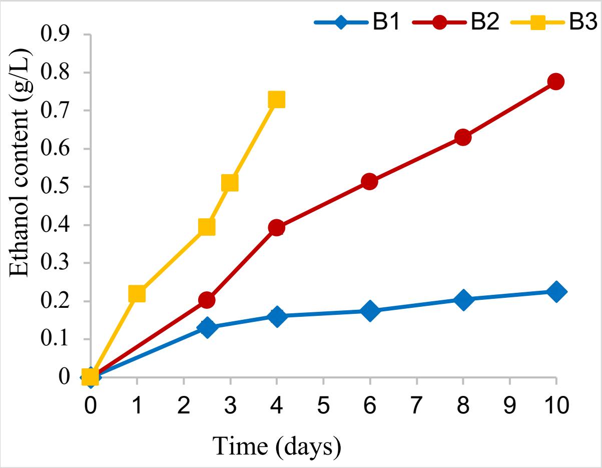


Figure 8. *Effects of fermentation temperature on ethanol content.*

3.2.4 Total polyphenol content

Apparently, the TPC increased significantly for sample B3 (reached 2099,93 µg/mL after 4 days of incubation), and increased slowly for samples B1, B2. After 6 days, total polyphenol content of sample B2 showed a decreased tendency. These results demonstrated that some of the polyphenolic compounds might be polymerized to more complicated molecules with higher molecular weight, leading to detection of lower total polyphenol contents [49]. Because of extended fermentation time, the total polyphenol content could suffer a decline over the whole process.

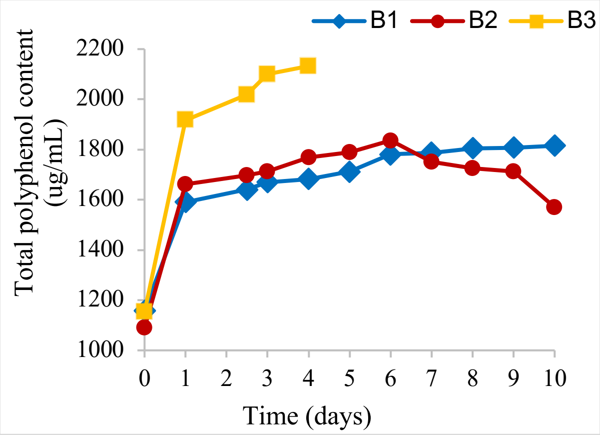


Figure 9. *Effects of fermentation temperature on total polyphenol content.*

3.2.5 Vitamin C content

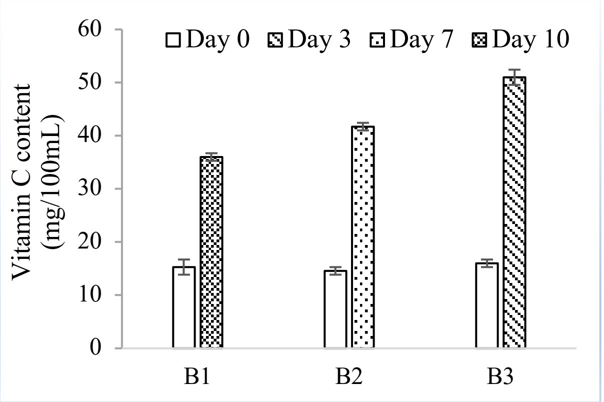


Figure 10. *Effects of fermentation temperature on vitamin C content.*

Figure 10 shows the difference in vitamin C content among samples B1-B3. The vitamin C formation productivities were 2.07 mg/100 mL-day (B1), 3.87 mg/100 mL-day (B2), and 11.67 mg/100mL-day (B3), significantly enhanced with the increased incubation temperature. These results indicated that the biotransformation from glucuronic acid to vitamin C strongly relies on fermentation temperature. As a result, the extension of fermentation time due to low temperature caused a steep decline on total polyphenol and vitamin C concentrations.

As can be seen from the above findings, the lower fermentation temperature could lead to the loss of polyphenol and vitamin C contents. Hence, Kombucha fermentation at 28 °C was more advantageous than the other temperatures.

3.3 Effects of recycling number of tea fungus biomass on chemical composition of Mulberry Kombucha

Sequential cultivation making use of tea fungus of previous batches proves effectiveness in saving inoculum development time. However, there were few studies addressing the impacts of reused tea fungus biomass on the quality of Mulberry Kombucha. Thus, four consecutive cycles of Kombucha fermentation were conducted at 28 ºC to examine the changes in chemical quantities.

3.3.1 Total sugar and reducing sugar contents

A)

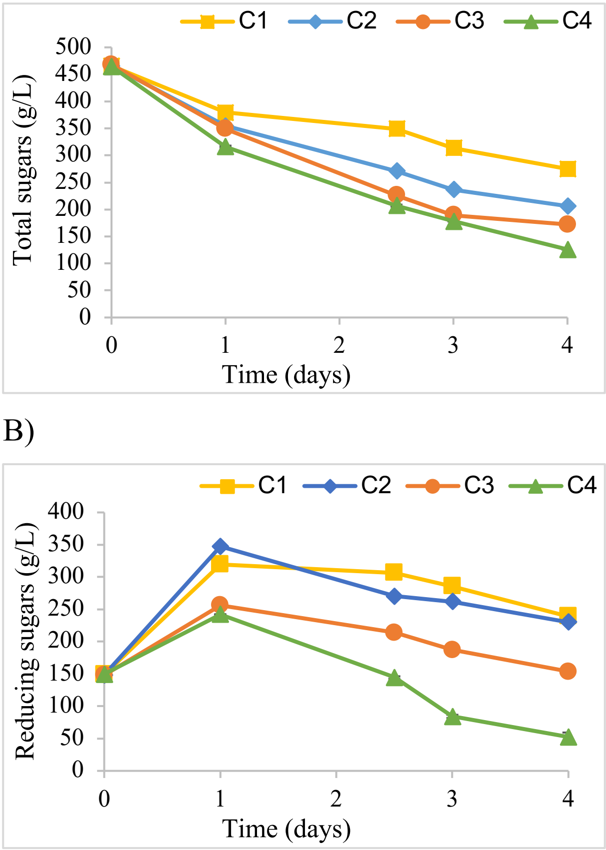


Figure 11. *Changes in total sugars (A) and reducing sugars (B) of C1 (cycle 1), C2 (cycle 2), C3 (cycle 3), and C4 (cycle 4).*

As shown in Figure 11A, total sugars in all cycles decreased during fermentation. It is also obvious that the total sugar biodegradation rate accelerated from cycle 1 to cycle 4. This could be due to the enhancement of yeast activity in tea fungus throughout the four cycles.

The changes in reducing sugar content had similar trends to those obtained in previous experiments. As shown in Figure 11B, from day 1 to day 4 of cycles 1, 2, 3 and 4, the amount of reducing sugars consumed gradually increased, from 80.4 g/L (C1) to 189.5 g/L (C4). This could be attributed to the increase in tea fungus amount on the last day of each cycle (Table 1). Especially, the amount of reducing sugar degraded in cycle 4 was the highest (189.5 g/L).

Table 1. *Changes in tea fungus weight after fermentation*

	First day	**Last day**
Cycle 1	100 g	117 g
Cycle 2	117 g	146 ± 1.00 g
Cycle 3	146 ± 1.00 g	137.5 ± 0.5 g
Cycle 4	137.5 ± 0.5 g	135.5 ± 0.5 g

3.3.2 Total acidity and pH value

A)

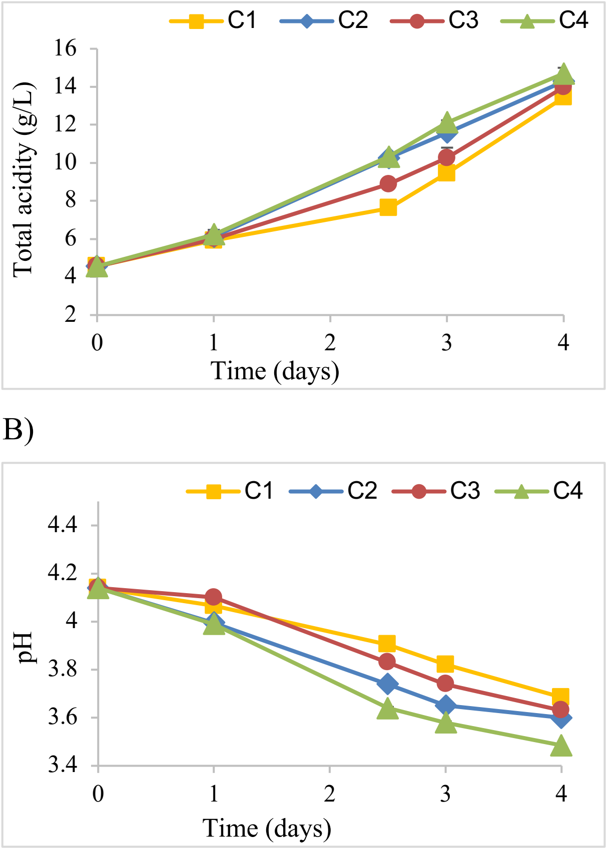


Figure 12. *Changes in total acidity (A) and pH (B) of C1 (cycle 1), C2 (cycle 2), C3 (cycle 3), and C4 (cycle 4).*

As can be seen in Figure 12, final pH values of the 4 cycles were quite close, ranging from 3.5 – 3.7. Because of the enhanced bacterial activities on cycle 1 and cycle 2, more acetic acid could be produced via the oxidation of ethanol [4]. Furthermore, excessive growth of yeast on cycle 3 and cycle 4 could also produce more organic acids, resulting in further reduction pH due to the solubility of CO_2 in water [32].

3.3.3 Ethanol content

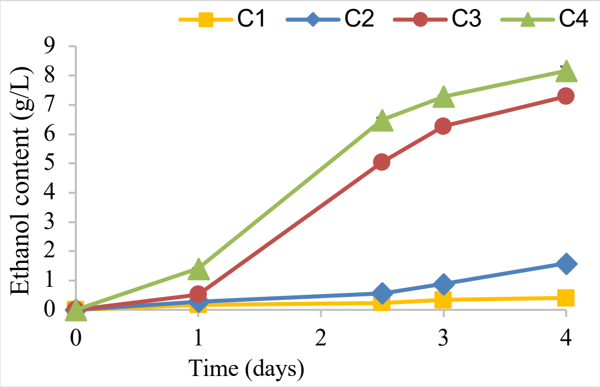


Figure 13. *Changes in ethanol content of C1 (cycle 1), C2 (cycle 2), C3 (cycle 3), and C4 (cycle 4).*

Results in Figure 13 shows that, after each cycle, the final ethanol concentration increased significantly, from 0.4 g/L in C1 to 8.1 g/L in C4. Especially, final ethanol concentrations of C3 and C4 were much higher than those of the majority of Kombucha products on the market, which are under 0.5% (v/v) alcohol.

3.3.4 Total polyphenol content

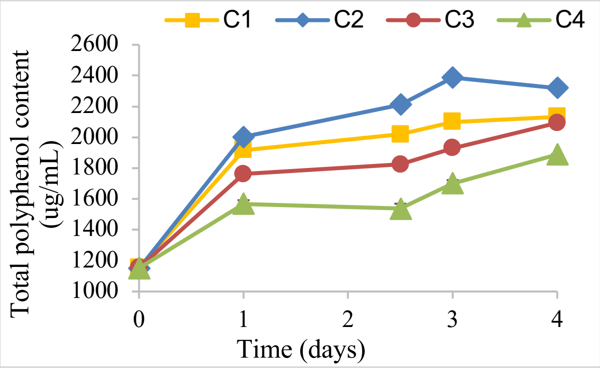


Figure 14. *Changes in total polyphenol contents of C1 (cycle 1), C2 (cycle 2), C3 (cycle 3), and C4 (cycle 4).*

It is shown in Figure 14 that the final TPC in C2 were significantly higher than those of C1, C3 and C4. As discussed above, the increase in TPC depends on Kombucha consortium activity [4,11]. In cycle 2, yeast and bacterial symbiotic relationship could be built up more strongly, resulting in an increase in TPC production (2318.87 µg/mL in C2 versus 2132.48 µg/mL in C1). As the tea fungus biomass accumulated over the course of C1 and C2, oxygen could become a limiting factor in C3 and C4. Hence, yeast cells could outgrowth acetic acid bacterial growth in C3 and C4, leading to a decrease in total polyphenol contents in these cycles.

3.3.5 Vitamin C content

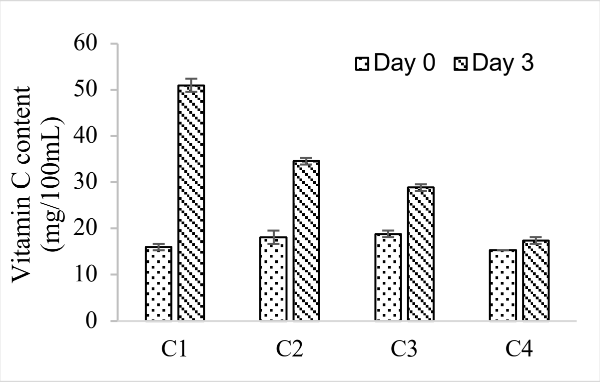


Figure 15. *The vitamin C contents on Day 0 and Day 3 of C1 (cycle 1), C2 (cycle 2), C3 (cycle 3), and C4 (cycle 4).*

As can be seen in Figure 15, in each cycle, the vitamin C contents all increased after 3 days of fermentation. However, the day-3 vitamin C contents gradually decreased, from 51 mg/100 mL (in C1) to 34.57 mg/100 mL (in C2), 28.86 (C3) and 17.43 mg/100 mL (C4). This means that the viability of acetic acid bacteria had an effect on the vitamin C content of mulberry kombucha. As discussed above, glucuronic acid, a precursor of vitamin C synthesis, is a secondary metabolite created through oxidation of glucose by acetic acid bacteria [34,48]. With the lower growth rates of acetic acid bacteria in later cycles, the vitamin C contents produced would be gradually reduced.

In conclusion, the total polyphenol and vitamin C contents all increased by the end of each fermentation cycle, but the significant increase occurred in C1 and C2. For C3 and C4, ethanol concentrations were relatively high compared to that of commercial Kombucha. Hence, the tea fungus biomass should not be reused more than 2 times at fermentation temperature of 28 °C.

4. CONCLUSION

The findings of this study confirmed that white mulberry juice can be used as a potential fermentation substrate for tea fungus to develop a novel beverage with enhanced nourishing properties. Total polyphenol and vitamin C contents in mulberry beverages were also markedly improved after Kombucha fermentation. It should be emphasized that initial sucrose concentration, fermentation temperature and recycling number of tea fungus biomass significantly affected chemical characteristics and sensory quality of Mulberry Kombucha.

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