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บทความวิจัย

การพัฒนา kombucha จาก การ เบลน ด์ ชา สมุนไพร และ ชา ดอกไม้ และ ประโยชน์ ต่อ สุขภาพ

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kombucha

ชาเบลนด์

ฤทธิ์ต้านอนุมูลอิสระ

การประเมินทางประสาทสัมผัส

ฤทธิ์ยับยั้งเอนไซม์แอลฟาไกลูโคซิเดส

จุดประสงค์ของงานวิจัยครั้งนี้เพื่อพัฒนาเครื่องดื่ม kombucha จาก การ เบลน ด์ ชา สมุนไพร และ ชา ดอกไม้ สูตรต่าง ๆ โดยศึกษาปริมาณแบคทีเรียกรดอะซิติกและยีสต์ เปรียบเทียบฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์การยับยั้งเอนไซม์แอลฟาไกลูโคซิเดส และการประเมินคุณภาพทางประสาทสัมผัสของเครื่องดื่ม kombucha ที่ได้จากการหมักชาเบลนด์สมุนไพรและชาดอกไม้จำนวน 9 สูตร พบว่าปริมาณแบคทีเรียกรดอะซิติกและยีสต์ในแต่ละสูตรมีปริมาณแตกต่างกันอย่างมีนัยสำคัญ ($p < 0.05$) โดย kombucha จากชาเบลนด์สูตรที่ 3 (ชาอู่หลง เจียวกุหลานและคาโมมายด์) มีปริมาณแบคทีเรียกรดอะซิติกสูงที่สุดเท่ากับ $8.45 \pm 0.02 \log \text{CFU/ml}$ และ kombucha จากชาเบลนด์สูตรที่ 5 (ชาอู่หลง ใบชาหอมมะลิและกุหลาบ) มีปริมาณยีสต์สูงที่สุดเท่ากับ $6.19 \pm 0.02 \log \text{CFU/ml}$ ส่วน kombucha จากชาเบลนด์สูตรที่ 3 สูตรที่ 4 (ชาอู่หลง ใบชาหอมมะลิ และคาโมมายด์) และสูตรที่ 5 พบปริมาณกรดอะซิติกสูงที่สุดเท่ากับร้อยละ 1 และค่าพีเอชเท่ากับ 2.53 จากการทดสอบฤทธิ์ต้านอนุมูลอิสระของ kombucha จากการหมักชาเบลนด์ด้วยวิธี DPPH พบว่า kombucha จากชาเบลนด์สูตรที่ 8 (ชาอู่หลง ชาดาวอินคา และกุหลาบ) มีร้อยละการยับยั้งสารอนุมูลอิสระสูงที่สุดเท่ากับ 87.09 ± 0.006 ส่วน kombucha จากชาเบลนด์สูตรที่ 3 พบปริมาณสารประกอบฟีนอลิกสูงที่สุดเท่ากับ 0.398 ± 0.002 มิลลิกรัม เมื่อเทียบกับกรดแกลลิกต่อปริมาณตัวอย่าง ผลการทดสอบฤทธิ์ยับยั้งเอนไซม์แอลฟาไกลูโคซิเดสของ kombucha จากการหมักชาเบลนด์ พบว่า kombucha จากชาเบลนด์สูตรที่ 5 มีฤทธิ์ยับยั้งเอนไซม์แอลฟาไกลูโคซิเดสได้ดีที่สุด โดยมีค่าความเข้มข้นของ kombucha ที่สามารถยับยั้งปฏิกิริยาได้ร้อยละ 50 (IC_{50}) เท่ากับ 43.62 ± 1.28 ppm จากผลการประเมินคุณภาพทางประสาทสัมผัสของ kombucha จากชาเบลนด์สูตรต่าง ๆ พบว่า kombucha จากชาเบลนด์สูตรที่ 4 และ 7 มีคะแนนความชอบโดยรวมสูงที่สุดเท่ากับ 7.56 ± 1.89 และ 7.32 ± 1.75 ตามลำดับ ดังนั้นงานวิจัยนี้เป็นการพัฒนาสูตรเครื่องดื่ม kombucha จาก การ เบลน ด์ ชา ที่เหมาะสมจากการใช้สมุนไพรและชาดอกไม้เป็นวัตถุดิบทางเลือกอย่างชาอู่หลง ใบชาหอมมะลิ และคาโมมายด์ซึ่งได้รับการยอมรับจากผู้บริโภคสูงที่สุดและมีสมบัติเชิงหน้าที่อีกด้วย

Introduction

Kombucha is defined as a fermented tea beverage, made by adding a symbiotic culture of bacteria and yeast (SCOBY), resulting in a refreshing, bittersweet, sour taste and slightly carbonated drink. Kombucha is now considered a functional beverage because it contains both essential nutrients and bioactive compounds that promote human health benefits, including antioxidant, antimicrobial activity, immune system activation, hepatoprotective effects and antidiabetic (Chakravorty et al., 2016; Kapp & Sumner, 2019; Mahmoudi et al., 2016; Muhiyaldin et al., 2019; Coelho et al., 2020; Emiljanowicz & Malinowska-

Panczyk, 2020). During the fermentation of Kombucha, these microorganisms convert the sugar and generate metabolites such as organic acids (acetic, gluconic, glucuronic and citric), water-soluble vitamins (B₁, B₂, B₆, B₁₂, and C), ethanol, carbon dioxide, and cellulose. (Bauer-Petrovska & Petrushevska-Tozi, 2001; Jayabalan et al., 2007; Malbaša et al., 2011; Marsh et al., 2014; Neffe-Skocinska et al., 2017; Gaggia et al., 2018; Leonarski et al., 2021). Currently, Kombucha is one of the most popular world-fermented beverages with low alcohol (Kapp & Sumner, 2019), with a market value of USD 2.64 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of

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15.60 % from 2022 to 2030. Typically, Kombucha can be prepared with black or green tea. Recently, there has been a growing interest in Kombucha from alternative raw materials in the replacement of the traditional ingredients for its fermentation, such as herbal teas, fruit juices, milk, coffee and a wide range of agro-industrial materials, mainly by-products of fruit industries (Emiljanowicz & Malinowska-Panczyk, 2020; Kim & Adhikari, 2020). The use of alternative raw materials can promote the development of new beverages with novel functional properties due to their phytonutrient enrichment composition which provide Kombucha with diverse biological activities and differentiated sensory characteristics to attract consumer attention.

Blended tea is a final product that is produced by blending different teas or teas with other ingredients, such as added herbs, spices, flowers, dried fruits, and essential oils. In general, the blending tea formulation can use true teas (black, green, Oolong, yellow, or white) as tea base. Then, add a supporting ingredient for a complementary effect or flavor such as spices or herbs. The final ingredient is an accent which adds a pop of flavor (fruits and flowers) and can include a multitude of formulas to create a specific blend. In particular, appearance, liquor hue, flavoring and scenting are the most popular ways to create tea blends with well-balanced attractive flavors. Different types of tea are blended to produce a special taste, which helps in the achievement of different enhanced health benefits, including antioxidant, immunostimulatory, calming, and digestive stimulant. Oolong tea is referred to as semi-fermented tea results in an extremely wide variety of flavors, which is known for its distinct light and elegant floral aroma and very fruity flavor (Zeng et al., 2020). Because of the natural fruitiness of Oolong teas, they are often blended with other fruit flavors or a floral aroma that provides satisfying blend teas. Therefore, blended tea may be considered an interesting

substrate for Kombucha fermentation.

This study focused on the development of blended Kombucha from various herbal teas and flower teas by using Oolong tea as tea base. The populations of acetic acid bacteria and yeast during blended Kombucha fermentation were determined. A comparative study on the antioxidant activity, anti- α -glucosidase inhibitory profiles, and sensory evaluation of various blended Kombucha was performed.

Materials and methods

Preparation of blended herbal and flower teas

Three dried herbal teas including Jiaogulan, Jasmine rice leaves and Sacha Inchi and dried three flower teas including chamomile, roses and butterfly pea was purchased from local organic farms. Blend teas are created by combining Oolong tea leaves as tea base, dried herbs and dried flowers in a bowl using a 2:1:1 ratio. Then, mixing and put them into the tea bags. Keeping in ziplock bags and store at 4°C.

Blended Kombucha fermentation

Nine formulas of blended herbal and flower teas were set up as shown in Table 1. Preparation of each group infusion of blended Kombucha as described by using 4.8 g of blended dried herbs or flower teas allow it to steep for 15 minutes. Then, approximately 180 g of brown sugar was dissolved in 2 L of boiling water, added the commercial starter SCOBY (Kombuchadiyshop, Bangkok, Thailand) with 20% (v/v) of its liquid from a previous starter, and kept at room temperature in a glass container covered with clean cheesecloth and fastened with rubber bands for 10 days. After fermentation, SCOBY was removed from Kombucha liquids, and the remaining Kombucha samples were stored at 4°C. Kombucha samples were examined in terms of pH and total acidity, antioxidant and total phenolic compound, and α -Glucosidase inhibitory activity.

Table 1 Formulations of blended Kombucha sample

Blended teas formula	Tea base Oolong tea (g)	Herbal teas (g)			flower teas (g)		
		Jiaogulan	Jasmine rice leaves	Sacha Inchi	Chamomile	Roses	Butterfly pea
1	2.4	1.2	-	-	1.2	-	-
2	2.4	1.2	-	-	-	1.2	-
3	2.4	1.2	-	-	-	-	1.2
4	2.4	-	1.2	-	1.2	-	-
5	2.4	-	1.2	-	-	1.2	-
6	2.4	-	1.2	-	-	-	1.2
7	2.4	-	-	1.2	1.2	-	-
8	2.4	-	-	1.2	-	1.2	-
9	2.4	-	-	1.2	-	-	1.2

*** Control: Oolong tea.

Enumeration of acetic acid bacteria and yeasts during the blended Kombucha fermentation

The populations of acetic acid bacteria (AAB) during 10 days of fermentation were determined by plating on GYC agar and incubated at 30°C for 24-48 h. The viable cell numbers of yeasts were measured by plating on YEPD agar and incubated at 30°C for 24-48 h. The populations of both AAB and yeasts were expressed as log of colony-forming units per ml (log CFU/ml). The acidity of the Kombucha sample was determined and acetic acid content was analyzed by titration.

Determination of antioxidant activity of blended Kombucha

The antioxidant activity of samples was measured by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) was modified from Lu et al. (2011). Kombucha samples (50 µl) were mixed with 1.950 ml of 40 mg/l of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and incubated for 30 min in the dark. The absorbance of the sample was measured at 517 nm using a spectrophotometer (Genesys 10-S UV-Vis spectrophotometer, Thermo Fisher Scientific, USA). The results of the antioxidant capacity were calculated as trolox equivalent per gram of sample. % of radical scavenging activity was calculated according to the following equation:

$$\% \text{ of scavenging activity} = \frac{(A_{\text{control}} - A_{\text{sample}}) \times 100}{A_{\text{control}}}$$

wherein A_{control} is an absorbance at 400 nm in the control samples
 A_{sample} is an absorbance at 400 nm in the Kombucha samples

Determination of total phenolic compound of blended Kombucha

The total phenolic content was measured using the Folin-Ciocalteu assay modified from Matthäus (2002). Kombucha samples (50 µl) were added to 2 ml of 2 % (w/v) sodium carbonate, mixed with 10 % (v/v) Folin-Ciocalteu reagent (100 µl), and further incubated for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (Genesys 10-S UV-Vis spectrophotometer, Thermo Fisher Scientific, USA). The result was expressed as µg gallic acid equivalent per ml of Kombucha sample (µg GAE/ml).

Determination of α -glucosidase inhibition activity

The Inhibitory activity of α -glucosidase of blended Kombucha was determined according to the methods reported by Sugiwati et al. (2009). To analyze the inhibition activity of α -glucosidase, 50 µl of blended Kombucha samples fermented for 10 days of different concentrations (6.25, 12.5, 25 and 50 ppm) were added to 25 µl of α -glucosidase solution (0.5 U/ml), which was dissolved in 0.1 M phosphate

buffer (pH of 7), were mixed and incubated at 37°C for 15 min. After 5 µl of *p*-nitrophenyl- α -D-glucopyranoside (pNPG) (5 mM) was added to the mixture and incubated for 10 min at 37°C, 100 µl of Na₂CO₃ (200 mM) was added to stop the reaction. Acarbose was used in this experiment as the positive control. The reaction was monitored by a change of absorbance at 400 nm using a spectrophotometer (Genesys 10-S UV-Vis spectrophotometer, Thermo Fisher Scientific, USA). % of inhibition could be calculated with an equation.

$$\% \text{ of inhibition} = ((A_0 - A_1) / A_0) \times 100$$

wherein A₀ is an absorbance at 400 nm in control (DMSO) without sample and A₁ is sample absorbance with enzyme addition minus sample absorbance without enzyme addition.

The IC₅₀ for each sample was estimated using the fitted straight line (linear regression) plotted with the data derived (% of inhibition values) against µl of the sample. Acarbose was used as a positive control, and all treatment was conducted in triplicate (n=3). The concentration of the samples required to inhibit 50 % of α -glucosidase activity under the assay conditions was defined as the IC₅₀ value. A linear regression was made between % inhibition (Y) and sample concentration (X) to obtain a linear equation. IC₅₀ values of samples were calculated based on the equation obtained.

Sensory evaluation

Sensory evaluation of nine blend Kombucha samples was carried out by using 10 ml of the drink for an untrained sensory panel of 50 testers. Each blend Kombucha sample was subsequently coded with letters

and evaluated in triplicate for, color, clarity, odor, taste and overall acceptability were evaluated with the 9-point Hedonic scale method under natural light in a room by 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislikes slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = likes extremely.

Statistical analysis

All experiments were carried out in triplicate and the mean of replications with standard deviations were reported. The experimental data were subjected to analysis of variance (Duncan's test) at the significance level of 0.05 which were performed with SPSS software.

Results and Discussion

The appearance of blended Kombucha from herbal teas and flower

The appearance of blended Kombucha from herbal teas and flowers at the beginning of the fermentation and after 10 days of fermentation is shown in Figure 1. The appearance of blended teas broth formula 2, 4, 5, 7, 8 and 10 at the beginning of the fermentation was light yellow, while after fermentation, the color changed to transparent yellow. The color of blended Kombucha samples formula 3, 6 and 9 before fermentation was greenish blue then changed to purplish pink. In this study, the pH of blended Kombucha samples decreased during fermentation, thus the transformation in the color of blended Kombucha during fermentation was probably due to anthocyanins (Filipe-Ribeiro et al., 2019). The anthocyanin turns red-pink in acids (pH 1-6), reddish-purple in neutral solutions (pH 7) and green in alkaline or basic solutions (pH 8-14) (Fossen et al., 1998)

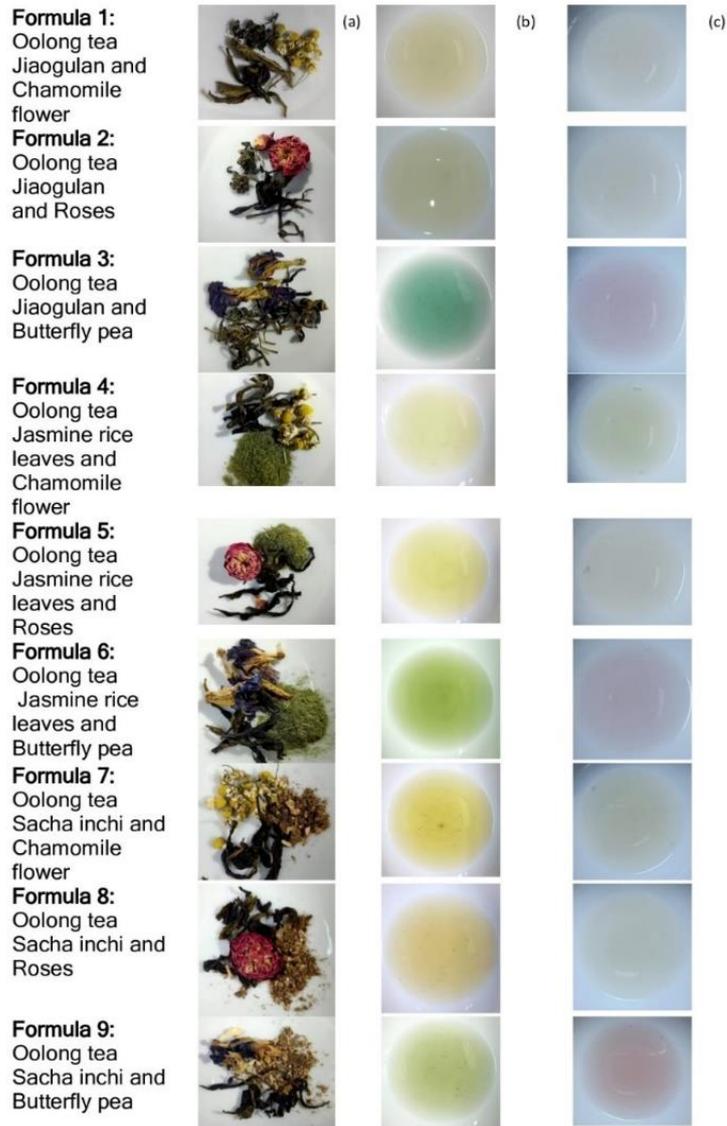


Figure 1 Appearance of blended Kombucha from herbal teas and flower; blended tea (a); blended tea at the beginning of fermentation (b); after 10 days of fermentation (c).

After 10 days of blended Kombucha fermentation, a thin layer of SCOBY grew across the top of the liquid from the fifth day, increasing its thickness with fermentation time as shown in Figure 2. During fermentation, acetic acid bacteria (AAB) utilize fructose and glucose to produce

bacterial cellulose, is an extracellular polysaccharide consisting of glucose molecules linked by β -1, 4-glycosidic bonds, and its production depends on the availability of carbon and nitrogen sources, ethanol, organic acids, and culture conditions (Leal et al., 2018).



Figure 2 Appearance of blended Kombucha from herbal teas and flower teas (from left to right) at the beginning of the fermentation process (a) 5 days of fermentation (b) and after 10 days of fermentation (c).

Enumeration of acetic acid bacteria and yeast during blended Kombucha fermentation

The growth characteristics of AAB and yeast of nine blended Kombucha samples during fermentation are shown in Figure 3. The microbial population of AAB and yeast in the blended Kombucha increased by nearly 2 log CFU/ml during fermentation for 10 days. Among the ten blended Kombucha samples, the population of AAB showed significant difference ($p < 0.05$) which the maximum concentration of AAB of formula 3 of 8.45 ± 0.02 log CFU/ml was observed. The results indicated that butterfly pea flower and Jiaogulan can promote the number of

acetic acid bacteria cells. According to Majid et al. (2023) showed that the development of butterfly pea flower Kombucha was studied on the 4 days of fermentation marked the peak of AAB growth at 6.29 log CFU/ml and yeast growth at 7.66 log CFU/ml. In addition, Jiaogulan presented the prebiotic properties which promote the growth of beneficial bacteria, particularly the short-chain fatty acid (SCFA) producers (Huang et al., 2022). On the contrary, formula 7 had the lowest of AAB population of 4.77 ± 0.10 log CFU/ml. Yeast population was detectable from the beginning of fermentation and increased until 10 days of fermentation, reaching

the maximum of 6.19 ± 0.02 log CFU/ml was found in formula 5, while formula 2 showed the lowest yeast count of 5.69 ± 0.03 log CFU/ml. The results indicated that Jasmine rice leaves provide a source of nitrogen and minerals for Kombucha microorganisms and have also valuable nutritional properties such as β - Glucan (Sudtasarn et al., 2008). In addition, roses are the alternative raw materials used in Kombucha production since it provides various nutrients for activating SCOBY. Zhang et al. (2020) who reported that rose and jujube kernel were proposed as an attractive alternative to Kombucha which showed the content of 6-feruloylspinosin and spinosin, as typical functional substances that exert sedative and hypnotic effects.

As depicted in Figure 4 the determination of acetic acid content of nine blended Kombucha samples during fermentation. It was found acetic acid increased dramatically at ten days of fermentation. Formula 3, 4 and 5 had the highest % of acetic acid content (1 %), and no significant ($p < 0.05$) differences were observed. The pH value declined quickly at 10 days of fermentation, meaning that the acetic acid bacteria produce the acetic acid during the fermentation of Kombucha by conversion of sucrose to ethanol and glucose and fructose (Spedding, 2015). Kombucha has less than 1 % acetic acid, with a typical

pH between 2.5 and 3.5 (Figure 5). Comparisons between the pH of blended Kombucha samples are shown in Figure 5. The pH of blended Kombucha samples decreased significantly ($p < 0.05$) during fermentation, mainly due to the increase in organic acid contents. However, at the beginning of fermentation, no significant ($p > 0.05$) differences were observed in the pH of blended Kombucha samples in comparison with Kombucha tea, whereas formula 3 exhibited the lowest pH (2.53) at the end of fermentation.

Determination of antioxidant activity of blended Kombucha

DPPH Assay

Comparisons between the antioxidant activity of blended Kombucha samples are shown in Figure 6. At the beginning of fermentation, the results revealed that formula 6 had the highest radical scavenging activity, which was 93.022 ± 0.004 % compared to the other blended Kombucha formulas. However, at the end of the fermentation, the highest antioxidant activity was found in formula 8 with scavenging activity of 87.090 ± 0.006 %. Lobo et al. (2017) determined that the metabolic conversion of tea components by microbial enzymes during fermentation may contribute to the increase in the antioxidant activity of Kombucha.

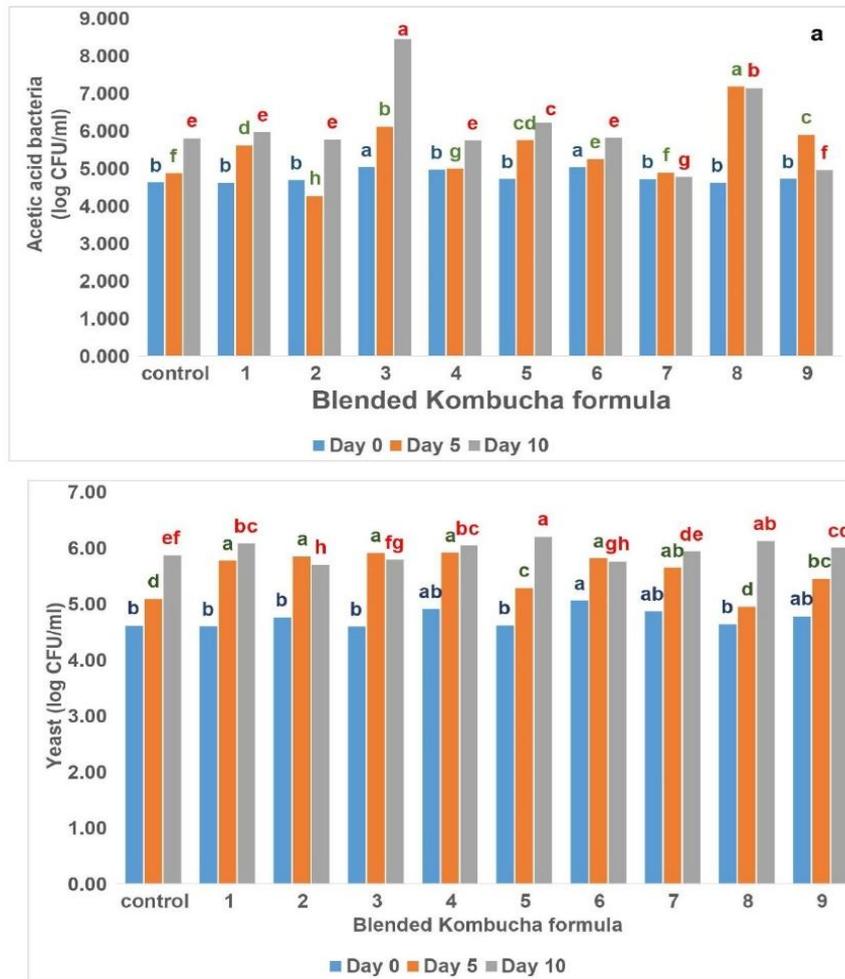


Figure 3 Growth patterns of acetic acid bacteria (a) and yeast (b) during blended Kombucha fermentation. Different letters show statistical significance ($p \leq 0.05$) between different Kombucha formulas at the same fermentation time. Different colors of letters represent different Kombucha fermentation with various blended Kombucha formulas.

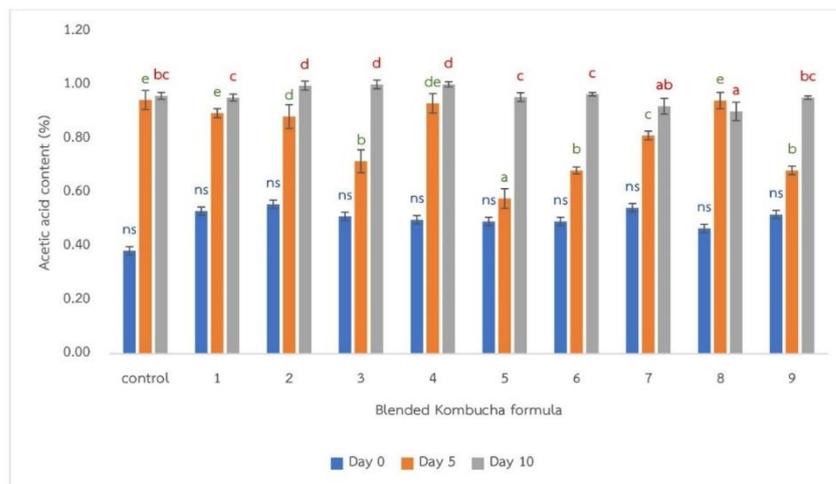


Figure 4 Comparisons between the acetic acid content (%) of various blended Kombucha during 10 days of fermentation. Different letters show statistical significance ($p \leq 0.05$) between different Kombucha formula at the same fermentation time. Different colors of letters represent different Kombucha fermentation with various blended Kombucha formula, whereas “ns” indicates not significant ($p > 0.05$).

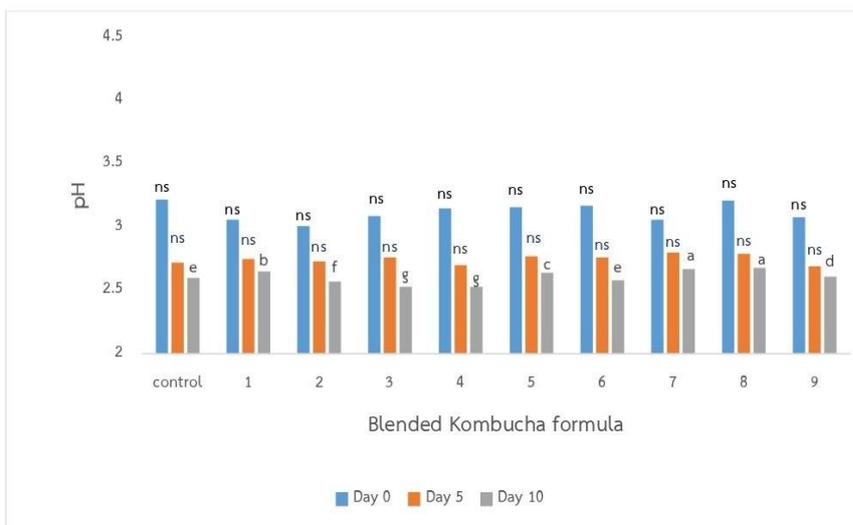


Figure 5 Comparisons between the pH of various blended Kombucha during 10 days of fermentation. Different letters show statistical significance ($p \leq 0.05$) between different Kombucha formula at the same fermentation time. Different colors of letters represent different Kombucha fermentation with various blended Kombucha formula, whereas “ns” indicates not significant ($p > 0.05$).

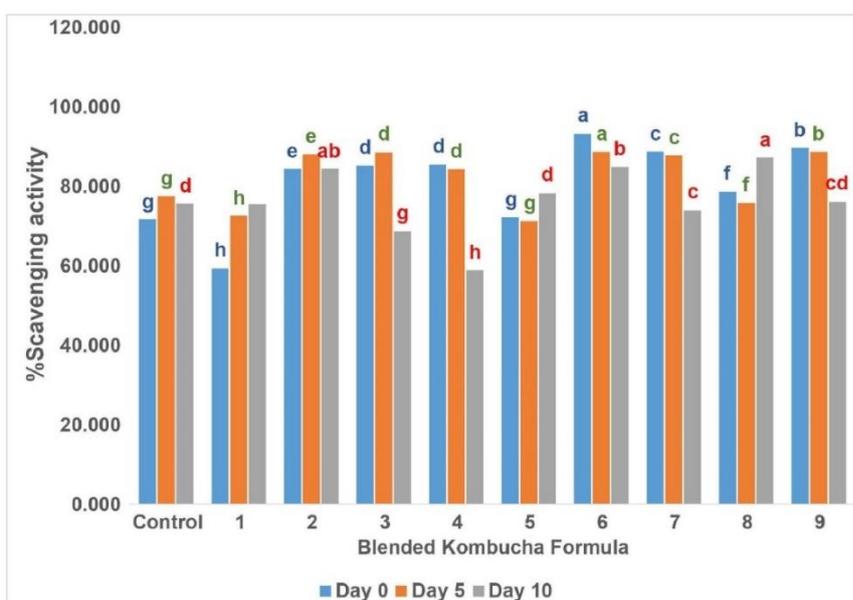


Figure 6 Antioxidant activities of various blended Kombucha during 10 days of fermentation. Different letters show statistical significance ($p \leq 0.05$) between different Kombucha formulas at the same fermentation time. Different colors of letters represent different Kombucha fermentation with various blended Kombucha formulas.

Total phenolic content

Following the total phenolic content assay, the results showed that the total phenolic content of all blended Kombucha formula increased during fermentation. Furthermore, the significantly highest total phenolic content is determined as 0.398 ± 0.002 mg/ml for formula 3 at 10 days of fermentation (Figure 7). According to Bhattacharya et al. (2013), an increase in phenolic content during fermentation may be

related to the enzymes of yeasts and bacteria such as glucosidase, esterase, dehydroxylase, and decarboxylase which convert polyphenolic complex into less complex phenolic components during fermentation. (Selma et al., 2009; Zubaidah et al., 2018). As well as Emiljanowicz & Malinowska-Pa (2020) reported that the phenolic components are more stable at acidic pH may cause differences in the total amount of phenolic substances during fermentation.

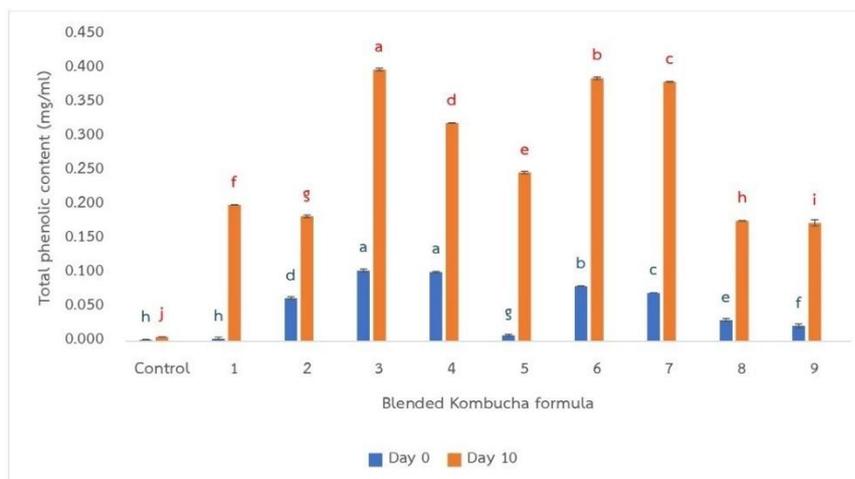


Figure 7 Total phenolic content of various blended Kombucha during fermentation. Different letters show statistical significance ($p \leq 0.05$) between different Kombucha formulas at the same fermentation time. Different colors of letters represent different Kombucha fermentation with various blended Kombucha formulas.

α -glucosidase inhibitory activity of blended Kombucha

α -amylase and α -glucosidase are essential in the breakdown of carbohydrates, which are present in the small intestinal brush border. Thus, inhibiting these enzymes' activity, thereby enhancing ability to prevent the breakdown of starch and reduce the glucose absorption rate in the blood (Koh et al., 2010; Liu et al., 2011). The results obtained for α -glucosidase inhibitory activity of blended Kombucha are shown in Figure 8. In all ten Kombucha samples had statistically significant difference in inhibitory effects on alpha-glucosidase activity ($p < 0.05$) was observed. Blended Kombucha formula 5 strongly inhibited α -glucosidase activity with IC_{50} value of 43.62 ± 1.28 ppm, followed by, blended Kombucha formula 1 showed α -glucosidase inhibitory activity with IC_{50} value of 50.54 ± 2.66 ppm, while the lowest activity of blended Kombucha formula 8 and 9 were observed. This finding indicates that the blended Kombucha from herbal teas and flower teas has the potential to be effective in controlling blood glucose levels functional drink. In agreement with the literature, Hardoko et al. (2020) who reported that Kombucha from *R. mucronata* herbal tea is more effective in

inhibiting α -glucosidase activity than the acarbose and the commercial Kombucha tea. Similar observations were also noted by Dechakhamphu et al. (2023) demonstrated that *C. rotundus* Kombucha showed alpha-glucosidase activity inhibition potential with IC_{50} values of 142.7 ± 5.2 μ l/ml. Inhibitory activity of the α -glucosidase at blended Kombucha may be due to the phytochemical compounds in plants have ability to inhibit the α -glucosidase, such as alkaloids, triterpenoids, flavonoids, and phenolic (Yin et al., 2014). In addition, the increased presence of the total phenolics content which can inhibit α -amylase produced during the fermentation process. In agreement with the literature, Mai et al. (2007) demonstrated a positive correlation between α -glucosidase inhibition and antioxidant activity. Like the previous study which revealed that phenolic compounds were the main contributors to α -glucosidase inhibitory activity of *Neptunia oleracea* (Lee et al., 2014) and *B. macrophylla* seed extract (Adam et al., 2016). Moreover, the glycoside content in herbal teas or flowers consists of sugars that may be structurally similar to carbohydrates which is a substrate of the α -glucosidase (Sugiwati et al., 2009).

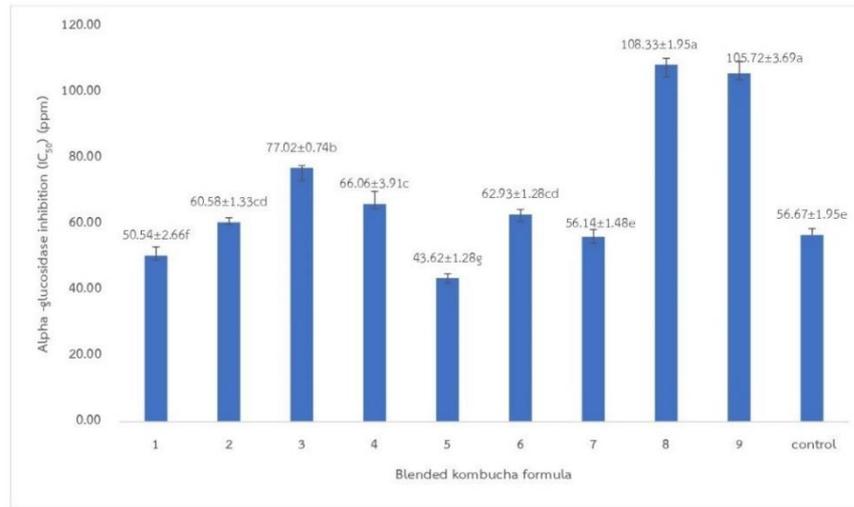


Figure 8 α- glucosidase inhibitory activity of blended Kombucha expressed in IC₅₀. Different letters show statistical significance (p ≤ 0.05) between different Kombucha formula at the same fermentation time.

Sensory evaluation

The results of the sensory evaluation of nine blended Kombucha formula samples are shown in Table 2. There was a significant difference in scores amongst the general acceptability of the blended Kombucha samples, whereas the clarity of blended Kombucha samples did not differ significantly. It can be seen that the highest score for color was assigned to formula 3 (7.80±1.25), whereas the lowest score was assigned to formula 2 (6.64±1.59). That may probably be

due to the attractive color of butterfly pea flower as a raw material for tea constituents which can enhance the product’s visual attractiveness to consumers. The highest score for odor of formula 3 was 6.30±2.03, which was higher than that of the other formulas. The score of taste of formula 3 and formula 4 was not statistically significant (p > 0.05), which was better than the other formula. Moreover, the most desirable overall acceptability was that of formula 4, while formula 2 had the least desirable overall acceptability.

Table 2 Sensory evaluation score of blended Kombucha samples

Blended Kombucha formula	Color	Clarity ^{ns}	Odor	Taste	Overall acceptability
1	6.88±1.38 ^{bc}	6.98±1.32	4.78±1.87 ^c	6.76±1.25 ^{ab}	6.54±1.27 ^{bc}
2	6.64±1.59 ^c	6.74±1.81	4.90±2.00 ^{bc}	5.22±1.93 ^e	5.60±1.82 ^d
3	7.80±1.25 ^a	7.42±1.32	5.28±2.18 ^{bc}	7.14±1.55 ^a	7.22±1.42 ^{ab}
4	7.46±1.49 ^{ab}	7.32±1.62	6.30±2.03 ^{abc}	7.50±2.05 ^a	7.56±1.89 ^a
5	7.06±1.53 ^{bc}	7.20±1.54	5.56±1.98 ^{abc}	6.3±1.85 ^{cd}	6.56±1.74 ^{bc}
6	7.42±1.49 ^{ab}	6.80±1.73	5.50±2.01 ^{abc}	5.72±1.90 ^{cde}	6.08±1.60 ^{cd}
7	7.16±1.43 ^{abc}	7.10±1.31	6.18±1.92 ^a	7.30±1.54 ^a	7.32±1.75 ^a
8	7.14±1.31 ^{abc}	7.06±1.36	5.78±1.93 ^{ab}	5.74±1.94 ^{cde}	6.34±1.35 ^c
9	7.06±1.71 ^{bc}	6.84±1.69	5.76±2.00 ^{ab}	6.12±2.21 ^{bcd}	6.50±1.89 ^c
Control	7.16±1.67 ^{abc}	7.16±1.65	5.66±1.73 ^{abc}	5.44±2.06 ^{de}	6.06±1.88 ^{cd}

Data from panelists are means ± standard deviations (n = 50); different letters show statistical significance (p ≤ 0.05) between different Kombucha formulas, “ns” indicates not significant (p < 0.05).

On the overall acceptability score, formula 4 showed the highest score for overall acceptability (7.56±1.89) among all blended Kombucha formulas. For the blended the Kombucha, taste, odor, and

overall acceptability of formula 4 were slightly better than formula 3, but the two samples were significantly higher than the control (p > 0.05) (Figure 9). It was evaluated that formulas 3 and 4 were most preferred by

participants which may probably be due to Jasmine rice leaves and Chamomile flower Kombucha being yellow a moderately amber and yellow color, while formula 3 had the attractive color of butterfly pea flower in acidic condition, pink-purple. The taste of blended Kombucha was evaluated as depending on the kind of herbal tea leaf and flower used for the Kombucha fermentation process. The blended Kombucha formulas 3 and 4 had acidic and pleasant taste and odor characteristics for the used extracts. Thus, the appearance of a sour taste due to the SCOBY metabolizes the sugars and ultimately produces acids during the fermentation process (Marsh et al., 2014; Kim & Adhikari, 2020). Acetic acid is the most prominent with a slightly sharp and hard sour aroma and taste. Moreover, gluconic acid is also found in Kombucha tea with a refreshing, soft, and light taste (Laureys et al., 2020). In addition, the flavors across fermentation can change from “fruity”, “sweet and sour”, “tea” and “sparkling” flavors to “vinegar” “sour”

“sparkling” and “apple cider-like” flavors (Marsh et al., 2014; Watawana et al., 2015; Amarasinghe et al., 2018). Besides, a beer taste is typical for Kombucha tea brews, since microbiota fermentation leads to the occurrence of a characteristic taste and aroma, similar to cider. Constituents such as tannins, catechins, amino acids contribute to the flavor of the tea. Catechins are predominantly known to contribute to 70-75 % of bitterness and astringency. On the other hand, the bitter taste is reduced by the Kombucha microbial activity during the fermentation process, since it produces amino acids reducing the bitterness of the tea alkaloids present (Gramza-Michałowska et al., 2016). Whereas caffeine and tannins are responsible for astringent or pungent taste. The brothy taste and sweet taste was due to amino acids (Nakagawa, 1975; Lee & Chambers, 2009; Zou et al., 2018). Therefore, blended Kombucha formula 4 is more suitable for the preparation of Kombucha beverage with delightful flavor.



Figure 9 Radar chart of sensory evaluation test of blended Kombucha; formula 3 (Oolong tea, Jiaogulan and butterfly pea), formula 4 (Oolong tea, Jasmine rice leaves and chamomile flower), control (Oolong tea).

Conclusion

In this work, we report the growth profile of acetic acid bacteria and yeast between the blended Kombucha beverages using different herbal tea and flowers during fermentation. The results showed that herbal teas and flowers are the good alternative raw materials used in Kombucha production that provide carbon and nitrogen sources for the

Kombucha cultures and alter its fermentation products to produce more beneficial and healthy bioactive beverages. The highest of acetic acid bacteria cell numbers was 8.45 ± 0.02 log CFU/ml for formula 3 (Oolong tea, Jiaogulan and chamomile flower), while formula 5 (Oolong tea, Jasmine rice leaves and roses) had the highest yeast cell at 6.19 ± 0.02 log CFU/ml. Additionally, the

total phenolic compounds and antioxidant activity increased during fermentation, the highest total phenolic compounds content and DPPH radical scavenging capacity values were evaluated in formula 3 and 6, respectively. To summarize the inhibitory effects on α -glucosidase of blended Kombucha, among of them, formula 5 was greatest inhibitory activity. In the sensory evaluation of overall liking, blended Kombucha formula 4 (Oolong tea, Jasmine rice leaves and chamomile flower) received the highest score with 7.56 ± 1.89 . Thus, the development of blended Kombucha from various of herbal teas and flower teas provide the profile of blended Kombucha of herbal teas and flower teas for promoting the growth of Kombucha starter culture and inhibition of α -glucosidase activity that provide a guideline for application to growth of functional beverage business, which could be beneficial for further research and industrial applications.

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Research article

Development of blended Kombucha from herbal teas and flower teas and their potential health benefits

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The purpose of this research was to develop blended Kombucha from various herbal teas and flower teas, to study the microbial population of acetic acid bacteria and yeast, to compare antioxidant activity, α -glucosidase inhibition activity, and to assess the sensory quality of the beverage. An experimental design with nine formulas of Kombucha herbal teas and flower teas was set up. The results showed that the population of acetic acid bacteria and yeast differed significantly in each blended Kombucha formula ($p < 0.05$). The highest acetic acid bacteria population was observed in formula 3 (Oolong tea, Jiaogulan, and Chamomile flower) at 8.45 ± 0.02 log CFU/ml. A maximum of yeast cells was found in formula 5 (Oolong tea, Jasmine rice leaves, and Roses) at 6.19 ± 0.02 log CFU/ml. Blended Kombucha formulas 3, 4 (Oolong tea, Jasmine rice leaves, and Chamomile flower), and 5 showed the highest acetic acid content at 1 % and a pH of 2.53. The antioxidant activity of blended Kombucha was determined using the DPPH method, with the highest antioxidant activity observed by inhibiting 87.09 ± 0.006 % of trolox equivalent with formula 8 (Oolong tea, Sacha inchi, and Roses). The high phenolic content was found in formula 3 blended Kombucha at 0.398 ± 0.002 mg gallic acid equivalents per milliliter. Evaluation of the α -glucosidase inhibition activity of blended Kombucha was performed, revealing that formula 5 obtained the best inhibition of α -glucosidase activity with an IC50 value of 43.62 ± 1.28 ppm. Sensory evaluation of blended Kombucha samples showed that formulas 4 and 7 had the highest overall liking scores, with scores of 7.56 ± 1.89 and 7.32 ± 1.75 , respectively. Therefore, this study highlights the best blended Kombucha formulation using herbal teas and flower teas as alternative raw materials (Oolong tea, Jasmine rice leaves, and Chamomile flower), which received the highest score in sensory acceptance and contains functional properties.

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