DEVELOPMENT OF KOMBUCHA FROM BLACK TEA AND DRIED ORANGE PEEL TEA

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Abstract.

Kombucha is a fermented tea beverage prepared by inoculating sweetened tea with symbiotic culture of bacteria and yeast (SCOBY). SCOBY is generally perceived as a cellulose-based thick biofilm floating in kombucha. Kombucha has been gaining increasing attention globally owing to its numerous health benefits. In this study, the first aim was to investigate the effect of SCOBY age on the quality of kombucha produced. Results showed that the color values of kombucha made from SCOBYs with 1, 3, 6 weeks of age were statistically different ($p \leq 0.05$).The 6-week-old SCOBY contributed to the overall darkest color (L*=41.07) as well as the lowest pH (3.13) and the highest acidity (0.39%) of kombucha. However, total soluble solids, ethanol content, antioxidant activity, and microbial counts were similar among kombucha produced from various ages of SCOBY ($p > 0.05$). The second aim was to evaluate the possibility to develop a new flavor of kombucha by incorporating dried orange peel tea (0, 15,30%) into the black tea base (15%). Kombucha including 30% orange peel tea displayed the darkest color (L*=35.44) compared with the one containing only black tea (L*=63.09). Orange peel tea addition significantly had an impact on the final kombucha regarding decreased ethanol content and microbial counts, and increased antioxidant capacity ($p \leq 0.05$). Kombucha with 15% orange peel tea received the highest acceptance by panelists in more sensory quality attributes ($p \leq 0.05$). Black tea in combination with dried orange peel tea was promising as the starting teas for developing a novel flavor of kombucha with enhancedhealth benefits.

Keywords: Kombucha, Scoby, Black tea, Dried orange peel tea, Antioxidant activities

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Introduction

Kombucha is a fermented tea beverage mainly produced from black, green, or oolong tea with added sugar. The exact origin of kombucha was uncertain. Historical records suggested that kombucha has been around since 220 B.C. during the Tsin Dynasty in Manchuria region, China (Jayabalan et al., 2014). It has then spread to other parts of theworld, but not been widely consumed until the past decade. Kombucha has recently gainedthe spotlight from its potential health benefits reported in many research studies. Positive effects associated with regular kombucha consumption include antioxidant, antimicrobial, anti-tumor properties, detoxification, immune stimulation, improvement of liver and gastrointestinal function, inhibition of cancer, cardiovascular disease, diabetes, and neurodegenerative diseases (Kapp & Sumner, 2019).

Kombucha fermentation is initiated by the starter culture known as symbiotic cultureof bacteria and yeast (SCOBY). The SCOBY itself has a jelly-like mat appearance made up ofcellulose produced by acetic acid bacteria (AAB). The most prevalent AAB genus in kombucha is *Komagataeibacter* (formerly *Gluconacetobacter*) including *K. xylinus*, *K. rhaeticus*, *K. saccharivorans*, *K. intermedius*, and *K. kombuchae* (also known as *K. hansenii*) (Harrison & Curtin, 2021). These species can accumulate up to 10-20% acetic acid in the medium (Gomes et al., 2018). Lactic acid bacteria (LAB) have also been isolated from kombucha. However, the concentration of LAB has been reported to be relatively low in most studies (Bishop et al., 2022). *Lactobacillus*, *Bifidobacterium*, *Lactococcus,* and *Oenococcus* are the genera of LAB found in kombucha (Coton et al., 2017). Working synergistically with AAB, yeast plays an important role in kombucha fermentation. Yeast hydrolyzes sucrose (the initial sugar) into glucose and fructose, then converts fructose to ethanol. AAB uses ethanol toproduce acetic acid, and simultaneously metabolizes glucose to yield gluconic acid (Kim & Adhikari, 2020). The dominant yeast strains present in kombucha include *Kloeckera* sp., *Schizosaccharomyces pombe*, *Pichia* sp., *Zygosaccharomyces bailii*, *Saccharomyces ludwigii*,
S. cerevisiae, and *Torulaspora* sp. (Goh et al., 2012).

A typical kombucha production process begins with preparing the sweetened tea base. Tea leaves are left to infuse in hot water for 5-10 min, followed by straining the tea to remove the leaves. Sugar is then mixed into the tea. Sucrose is the most common carbon source for kombucha microorganisms with dosage ranging from 5-15% (Jayabalan et al., 2014). Oncethe sugared tea cools down to room temperature, the starter culture is added comprising a small amount of previously fermented kombucha liquid and a SCOBY pellicle to initiate the fermentation (Wang et al., 2022). The proportions of the starter liquid and the SCOBY solid phase were reported to account for 3-10% and 2-5%, respectively, of the fermentation volume(de Miranda et al., 2022). The fermentation is usually carried out at ambient temperature (20- 30°C) in a duration of 7-14 days (Jayabalan et al., 2014). As the fermentation progresses, a new microbial biofilm develops on the liquid surface. This newly formed pellicle is often referred to as a daughter SCOBY (Wang et al., 2022b). The daughter SCOBY can be employed to continue making kombucha in a subsequent batch. In small-scale intermittent production of kombucha, the extra daughter SCOBY may be retained in some of the fermented tea or transferred to a SCOBY hotel. The SCOBY hotel is basically a temporary storage for excess SCOBYs until they are needed again for next fermentation. The hotel for SCOBYs can be created using a glass jar filled with sweetened tea and kombucha liquid similarly to preparing for a new batch of fermentation (Bond, 2023). Many research studies have mentioned several factors affecting kombucha quality, such as temperature, pH, oxygen, substrate, sugar, SCOBY origin, container shape, and fermentation time (Villarreal-Soto et al.,

2018). To our knowledge, the research area involving the age of SCOBY used to inoculate thetea to begin the fermentation has not yet been explored.

Traditional substrates for kombucha production are unflavored teas, mainly black, green and oolong teas (de Miranda et al., 2022). In recent years, various non-conventional substrates have been attempted for fermentation with SCOBY culture to produce novel kombucha-analog beverages. Examples of atypical raw materials comprise herbs, spices, medicinal flowers, coffee, oak leaves, fruit juices, soybean extract, and milk (Emiljanowicz &Malinowska-Pańczyk, 2019; Silva et al., 2021). Fermentation of herbal infusions from thyme, rosemary, fennel, and mint with kombucha SCOBY resulted in the beverage with improved antimicrobial activities compared to their unfermented version and the original black tea kombucha (Battikh et al., 2012). The fermented drink with kombucha culture from yarrow, a medicinal flower, exhibited enhanced antimicrobial and antioxidant properties as well as antiproliferative activity against tumor cells (Vitas et al., 2018). Kombucha made from green tea in combination with cinnamon was shown to exhibit higher antioxidant and antimicrobial activities (Shahbazi et al., 2018).

Orange peel, a by-product from orange juice processing, is a source of pectin, cellulose, limonene, vitamin C, flavonoids, iron, and copper (Czech et al., 2019). Several research studies have been successful in making use of this waste to create value-added products, such as bioethanol fermentation, pectin production, cellulose extraction, limonene recovery, and activated carbon generation (Deba-Rementeria et al., 2023). Zaki & Naeem (2021) incorporated orange peel powder into yogurt drink. Research revealed that orange peel itself possessed strong antioxidant capacity due to the presence of flavonoids and phenolic compounds, anti-cancer property against tumor cell lines, and antibacterial activities against major pathogens. Formulation of orange peel up to 2% in yogurt drink had no effect on sensory acceptance suggesting the promising application of orange peel in fermented beverage to boost the positive health effects. Ivanchenko et al. (2022) developed a fermented drink based on kvass wort supplemented with 1% dried orange peel. The resulting drink was found to contain 4 times more phenolic compounds and 1.5 times more vitamin C. Enriched with health-promoting constituents, orange peel could be considered as a functional ingredientto apply in novel food product development.

In the present study, kombucha production experiments were based on black teasubstrate. The first aim was to investigate the effect of SCOBY biofilm age on the physical, chemical and microbiological qualities of kombucha. Kombucha drinks made from SCOBYs with 1, 3, 6 weeks of age were compared to assess how different SCOBYs may contribute to the consistency of product quality. The second part of the study was aimed at developing a novel flavor of kombucha using dried orange peel tea as a co-substrate for fermentation. The level of orange peel tea integration at 15% and 30% was compared with the usage of black teaalone. The beverage quality in physical, chemical, microbiological and sensory aspects was considered to determine the suitability of the new recipe with partial orange peel tea addition.

Methods

Materials

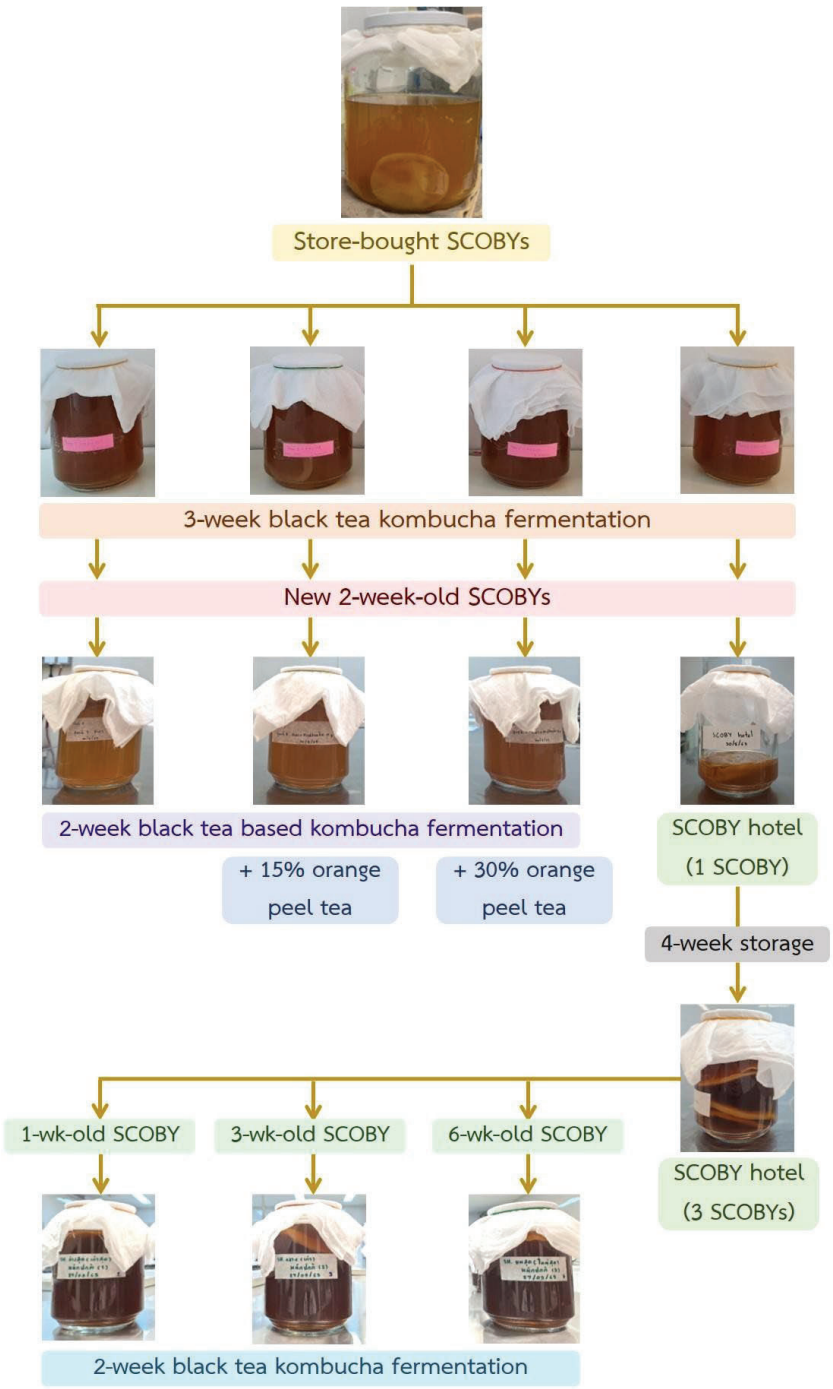
Kombucha starters (liquid and SCOBY) were bought from Down to Earth shop (Lamphun, Thailand). Organic black tea leaves (Suwirun tea shop), dried orange peels (Charmcha brand), organic cane sugar (Wangkanai brand), and mineral water (Purrá brand) were purchased locally in Tops supermarket, Thailand. Folin & Ciocalteu′s phenol reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, and Trolox were sourced from Sigma- Aldrich. Peptone, yeast extract, glucose, mannitol, agar, Potato Dextrose Agar (PDA), and *Lactobacillus* De Man, Rogosa and Sharpe (MRS) agar were acquired from HiMedia.

Preparation of kombucha from black tea and fermentation conditions

Black tea concentrate was made by steeping tea leaves (15 g) in boiled mineral water (300 ml) for 4 min. The leaves were then filtered out. Cane sugar (125 g) was dissolved in the tea concentrate in a glass jar. Mineral water (1.45 L) was added into the jar to dilute the tea. Store-bought kombucha liquid (125 ml) and one SCOBY were used to inoculate the sweetened black tea. The jar was covered with 8 layers of cheesecloth. A total of 4 fermentation jars was prepared identically as previously described and

placed away from direct sunlight in a well-ventilated room for 3 weeks. Afterwards, four new SCOBYs were generated in which three of them were subsequently used for the next round of kombucha fermentation from black tea and dried orange peel tea. The remaining new SCOBY was kept in a SCOBY hotel for the following SCOBY age experiment. Overview of experimental plan was illustrated in Figure 1.

Figure 1: Kombucha experimental diagram



Construction and management of SCOBY hotel

Black tea was prepared by steeping tea leaves (4.85 g) in boiled mineral water (485 ml) for 4 min. The leaves were then filtered out. Cane sugar (15 g) was dissolved in the tea in a glass jar. The jar containing sweetened black tea served as a hotel for extra SCOBYs to keep them alive and active before transferring them out to use as starter cultures for forthcoming fermentation. More tea (formulated the same way as previously described) was filled into the jar every 2 weeks of storage.

Preparation of kombucha from black tea and dried orange peel tea, and fermentation conditions

Black tea concentrate was made by immersing tea leaves (15 g) in boiled mineral water (300 ml) for 4 min. The leaves were then filtered out. Orange peel tea concentrate was prepared similarly by soaking dried peels (15 g) in boiled mineral water (300 ml) for 4 min. The peels were strained out. A total of 3 fermentation jars was prepared with 15% black tea- based substrates consisting of black tea only, black tea added with 15% orange peel tea, and black tea added with 30% orange peel tea. Cane sugar (125 g) was solubilized in the tea concentrate in each glass jar. Mineral water was added to dilute the tea and adjust the mediumvolume to 2 L for each fermentation jar. Kombucha liquid (125 ml) from preceding fermentation (Method 2.2) and one corresponding SCOBY (2-week-old) were used to inoculate the sugared tea. The jars were covered with 8 layers of cheesecloth, and placed away from direct sunlight in a well-ventilated room for 2 weeks.

Effect of SCOBY age on the quality of black tea kombucha experiment

Sugared tea broth was prepared in the same way as explained in Method 2.2 for 3 fermentation jars. All jars were inoculated with liquid (125 ml) and one SCOBY fromSCOBY hotel (1, 3 or 6 weeks of age). The jars were covered with 8 layers of cheesecloth, and placed away from direct sunlight in a well-ventilated room for 2 weeks.

Physical analysis of kombucha

The color values of kombucha (L*, a*, b*) were measured using Hunter Lab spectrophotometer (ColorQuest® XE, Hunter Associates Laboratory, USA) by the method modified from Achayuthakan et al. (2018). Kombucha was poured into a clear sample bottle and evaluated in TTRAN mode using CIELAB system.

The total soluble solids (°Brix) of kombucha were measured using a hand refractometer (Atago N1, Japan).

Chemical analysis of kombucha

The pH of kombucha was determined using recently calibrated pH meter (Starter 3100pH Bench, Ohaus, China).

The titratable acidity of kombucha was assessed following the procedures by Zubaidahet al. (2019). 10 ml of kombucha was blended with 20 ml of distilled water in a flask, and shaken well. The sample flask was then titrated against 0.1 N NaOH standard solution using phenolphthalein as an indicator. The titratable acidity (%) was calculated based on acetic acid concentration in kombucha using the following equation.

Titratable acidity (%) = [volume of NaOH (ml) x NaOH concentration (N) x 0.06 x 100] / sample volume (ml)

The ethanol content (%ABV) in kombucha was determined using GasChromatography (GC) coupled with Flame Ionization Detector (FID) as detailed by Bursova et al. (2015) (Shimadzu GC-2010 Plus, GCsolution software version 2.41.00 SU1, Japan). The analysis was performed using a single injection with a sample volume of 0.2 µl. The machine was equipped with RTX-BAC1-fused silica column (30 m x 0.32 mm ID x 1.8 µm, Restek, USA), RTX-BAC2 Plus (30 m x 0.32 mm ID x 0.6 µm, Restek, USA) and two FIDs. Nitrogen was used as a carrier gas. The oven temperature was held at 50°C for 4 min, and then raised to 180°C. The inlet temperature was 215°C, and the detector temperature was 250°C. Ethanol standards were run in the same condition to create a standard curve for ethanol quantification.

The antioxidant activity of kombucha was assessed using the DPPH radical scavenging method modified from Zubaidah et al. (2019). Briefly, 2 ml of 0.2 mM DPPH wasmixed with 1 ml of sample (ethanol was used as control). The reaction mixture was incubated in the dark for 30 min. The absorbance (A) for the sample and the control was measured usingUV-Visible Spectrophotometer (Evolution 201, Thermo Scientific, USA) at 517 nm. The radical scavenging activity (%) was calculated using the below equation.

Radical scavenging activity (%) = [(A control - A sample) x 100] / A control

The total phenolic content of kombucha was determined using the Folin-Ciocalteu assay adapted from Zubaidah et al. (2019). The sample (1 ml) was diluted with distilled water (9 ml). The diluted sample (150 µl) and 10% Folin-Ciocalteu reagent (150 µl) were added into distilled water (2.4 ml), vortexed and let stand for 2 min. The mixture was then reacted with 7.5% Na_2CO_3 (300 µl) while incubating in the dark for 2 h. The absorbance wasmeasured using UV-Visible Spectrophotometer (Evolution 201, Thermo Scientific, USA) at 765 nm. Gallic acid standards were also analyzed to create a standard curve. Total phenolic content was expressed as µM gallic acid equivalent (GAE)/ml sample.

Microbiological analysis of kombucha

Acetic acid bacteria (AAB) count was determined using the protocols modified from Wang et al. (2022a). 25 ml of kombucha and 225 ml of 0.1% peptone were mixed thoroughly.The sample was serially diluted to 10^{-3} and 10^{-4}. Each dilution (0.1 ml) was then spread onto Glucose Yeast extract Peptone Mannitol (GYPM) agar plate containing 0.2% glucose, 0.5% yeast extract, 0.3% peptone and 2.5% mannitol. The plates were incubated at 30°C for 5 days.Colonies were enumerated and expressed in log Colony Forming Unit (CFU)/ml.

Yeast count was performed using the method adjusted from Wang et al. (2022a). Inthe same manner as AAB count, kombucha sample was diluted to 10^{-3} and 10^{-4} concentrations. Each dilution (0.1 ml) was spread onto PDA plate supplemented with 0.1% tartaric acid. The plates were incubated at 30°C for up to 5 days. Colonies were enumerated and expressed in log CFU/ml.

Lactic acid bacteria (LAB) count was determined based on the procedures modified from Wang et al. (2023). As explained above, kombucha sample was diluted to 10^{-3} and 10^{-4} concentrations. Each dilution (0.1 ml) was spread onto MRS agar plate. The plates were incubated anaerobically at 37°C for 72 h. Colonies were enumerated and expressed in log CFU/ml.

Sensory evaluation of kombucha

Organoleptic properties of kombucha were evaluated in aspects of appearance, color, sourness, taste, mouthfeel, and overall liking. The affective test was conducted for kombucha evaluation using a 9-point hedonic scale (scores of 1=dislike extremely and 9=like extremely). The panelists were untrained 40 people with one criterion of having consumed any kinds of kombucha before in the past.

Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean ±standard deviation. Completely Randomized Design (CRD) was applied to all analyses exceptsensory evaluation using Randomized Complete Block Design (RCBD). Duncan's New Multiple Range Test (DMRT) was employed to compare the means using IBM SPSS 26.0 software.

Results and Discussion

Production of a new flavor of kombucha from black tea and dried orange peel tea

Prior to orange peel flavored kombucha fermentation, new starter cultures (kombucha liquid and SCOBY) were prepared in our laboratory by production of traditional kombucha from black tea using store-bought starters to generate all new SCOBYs along with corresponding kombucha liquid. This was to control the variation and minimize the impact of SCOBY age on the quality of kombucha. Based on our observation, a thin biofilm of daughterSCOBY started to develop after almost a week of kombucha fermentation. The normal black tea kombucha fermentation was then intended to last for 3 weeks to obtain all 2-week-old SCOBYs for subsequent fermentation.

To devise a novel orange peel-infused kombucha, two levels of orange peel teaaddition (15 and 30%) were compared to find the suitable proportion of orange peel tea in kombucha. Black tea kombucha was made in an identical condition to serve as a control. All fermentation jars were inoculated with 2-week-old SCOBYs and relevant starter liquid. Table 1 displayed the color values and total soluble solids (TSS) of kombucha prepared from incorporating dried orange peel tea (0, 15, 30%) into the black tea base (15%). Kombucha including 30% orange peel tea exhibited the darkest color (L*=35.44) compared to the one containing only black tea (L*=63.09). Orange peel tea addition also influenced brightershades of red (a*=9.08, 9.83) and slightly darker shades of yellow (b*=38.66, 40.97) ($p \leq 0.05$). Dried orange peel had a dark orange to light brown color which impacted the overall color of the beverage. All tea composition began and ended the fermentation with similar TSS of initial 5.97-6.07°Brix and final 4.93-5.07°Brix ($p > 0.05$). The decline of TSS was caused by the utilization of sugar and nutrients in the fermentation medium by kombucha microbes for their growth and metabolism (Hasan et al., 2019).

Chemical quality of kombucha was illustrated in Table 2. Regardless of tea substrate type, pH of kombucha decreased significantly after fermentation ($p \leq 0.05$). Sugar in the tea was assimilated by kombucha culture and converted into organic acids, mainly acetic acid, which caused the pH reduction (Jayabalan et al., 2014). However, partial orange peel tea kombucha displayed higher pH and lower acidity than kombucha made with black tea alone ($p \leq 0.05$). Orange peel tea, comparable to aqueous extract of orange peel, was reported to contain tannin, phenolics, saponins, and flavonoids (Shetty et al., 2016). These compounds were proven in numerous studies to possess antimicrobial activity. Nevertheless, the findings suggested that the ethanolic extract of orange peel was more potent than the aqueous extract in bacterial inhibition. This could explain the lower acid concentrations in kombucha partly made with orange peel tea as the microbes were slightly suppressed by orange peel components contributing to less amount of acids being produced. The ethanol content was below 0.5% in all conditions which can be categorized as a non-alcoholic beverage (Ivory et al., 2021).

Table 1: The color and total soluble solids of kombucha derived from 15% black tea and varied amount of dried orange peel tea

Color values / Total soluble solids (°Brix)	⅓ Orangepeel tea	3% Orange peel tea	0% Orange peel tea
L*	63.09 ± 0.05^{a}	45.34 ± 0.13^{b}	35.44 ± 0.12^{c}
a*	5.92 ± 0.02^{c}	9.08 ± 0.02^{b}	9.83 ± 0.06^{a}
b*	41.27 ± 0.08^{a}	40.97 ± 0.05^{b}	38.66 ± 0.20^{c}
TSS before fermentation^{ns}	5.97 ± 0.06	6.07 ± 0.06	6.03 ± 0.06
TSS after fermentation^{ns}	4.93 ± 0.06	5.07 ± 0.06	5.03 ± 0.06

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$). Row headers with ns superscripts indicate that values within the same row are not significantly ($p > 0.05$).

Table 2: The pH, titratable acidity, and ethanol content of kombucha derived from 15% black tea and varied amount of dried orange peel tea

Chemical values	5% Orangepeel tea	15% Orange peel tea	30% Orange peel tea
pH (before fermentation)	3.37 ± 0.02^{c}	4.11 ± 0.03^{b}	4.23 ± 0.03^{a}
pH (after fermentation)	3.03 ± 0.02^{b}	3.15 ± 0.02^{a}	3.14 ± 0.02^{a}
% Acidity (after fermentation)	0.43 ± 0.01^{a}	0.39 ± 0.05^{b}	0.40 ± 0.00^{b}
% Ethanol (after fermentation)^{ns}	0.27 ± 0.08	0.20 ± 0.03	0.17 ± 0.05

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

The antioxidant capacity of kombucha was shown in Table 3. Orange peel tea inclusion both at 15 and 30% significantly had an impact on the final kombucha regarding the increased quantity of phenolic compounds ($p \leq 0.05$). The phenolic content is known to have a positive correlation with the antioxidant activity (Hasan et al., 2019). Citrus flavonoids such as hesperidin and naringin are largely responsible for the antioxidant property of citrus peel extract (Kanaze et al., 2009). In the present study, the antioxidant ability of orange peel flavored kombucha inferred from DPPH scavenging activity was only slightly better than black tea kombucha. It was possibly due to the low initial amount of DPPH in the experiment which made the discrepancy in antioxidant activity between conditions not obvious.

The microbial count of kombucha was detailed in Table 4. Acetic acid bacteria (AAB)growth was not affected by the presence of orange peel tea ($p > 0.05$). As discussed earlier related to the antimicrobial substances in orange peel extract, minimal inhibition of yeast and lactic acid bacteria (LAB) was observed in kombucha comprising 30% orange peel tea($p \leq 0.05$). Incorporation of up to 30% orange peel tea into the traditional tea base was seemingly plausible as kombucha substrates without greatly impacting starter culture growth.

Table 3: The antioxidant activity and total phenolic content of kombucha derived from 15% black teaand varied amount of dried orange peel tea

ntioxidant ability / Total phenolics	5% Orange peel tea	15% Orange peel tea	30% Orange peel tea
% Radical scavenging activity	88.69 ± 0.34^{c}	90.07 ± 0.17^{b}	91.76 ± 0.33^{a}
µM Gallic acid equivalent/ml	$12,256.20 \pm 741.93^{c}$	$22,456.19 \pm 1,542.35^{b}$	$26,099.03 \pm 1,653.12^{a}$

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Table 4: The microbial count of kombucha derived from 15% black tea andvaried amount of dried orange peel tea

Microbial count(log CFU/ml)	0% Orangepeel tea	15% Orange peel tea	30% Orange peel tea
Acetic acid bacteria^{ns}	7.24 ± 0.08	7.15 ± 0.04	7.13 ± 0.05
Yeast	7.05 ± 0.06^{a}	7.02 ± 0.13^{a}	6.76 ± 0.01^{b}
Lactic acid bacteria	6.88 ± 0.04^{a}	6.76 ± 0.18^{a}	6.32 ± 0.08^{b}

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

Results of sensory evaluation of kombucha were shown in Table 5. Kombucha added with orange peel tea both at 15 and 30% was preferred by panelists in more sensory quality attributes (sourness, taste, mouthfeel, and overall liking) ($p \leq 0.05$). Considering sensory scores, phenolic content, and starter culture growth, the suitable proportion of orange peel tea to create a new flavor of kombucha was 15% in combination with black tea base of 15%.

Effect of SCOBY age on the quality of kombucha

In order to study how different SCOBY pellicles influenced the quality of produced kombucha, the first 2-week-old daughter SCOBY was generated from black tea kombucha fermentation in our laboratory. It was then transferred to a SCOBY hotel and preserved there for 4 weeks. During the 4-week storage, the second daughter SCOBY was formed in the second week, and the third daughter SCOBY was developed in the fourth week. Altogether, three SCOBYs with 1, 3, 6 weeks of age were obtained from the SCOBY hotel. They were subsequently employed as starter cultures along with the storage liquid from the hotel in blacktea kombucha fermentation. Table 6 displayed the color values and TSS of kombucha made from SCOBYs with 1, 3, 6 weeks of age. The 6-week-old SCOBY contributed to the overall darkest color (L*=41.07) as well as the darkest shades of red (a*=15.20) and yellow (b*=52.39) ($p \leq 0.05$). It was our speculation that the oldest SCOBY might have gradually absorbed the tea color and undergone chemical reactions related to browning while submerging in the tea for a long period. The impact of the oldest SCOBY application on the color characteristics of kombucha was noticeable compared to using the younger SCOBYs (1,3 weeks old). TSS of kombucha made from various ages of SCOBY was not different.

Table 5: Sensory evaluation (9-point hedonic test) of kombucha derived from 15% black tea and varied amount of dried orange peel tea

Sensory attributes	٪ Orangepeel tea	٪ Orangepeel tea	٪ Orangepeel tea
Appearance[ns]	6.53 ± 1.41	6.60 ± 1.30	6.48 ± 1.47
Color[ns]	6.48 ± 1.34	6.45 ± 1.36	6.40 ± 1.50
Sourness	4.77 ± 2.08[b]	5.43 ± 2.01[a]	5.55 ± 1.99[a]
Taste	4.75 ± 1.95[b]	5.75 ± 2.11[a]	5.58 ± 2.07[a]
Mouthfeel	5.68 ± 2.00[b]	6.18 ± 1.82[a]	6.00 ± 1.97[a]
Overall liking	5.38 ± 1.73[b]	6.25 ± 1.89[a]	6.13 ± 1.90[a]

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

Table 6: The color and total soluble solids of kombucha produced from different ages of SCOBY

Color values / Total soluble solids (°Brix)	-week-old SCOBY	-week-old SCOBY	-week-old SCOBY
L*	45.84 ± 0.01[b]	46.19 ± 0.04[a]	41.07 ± 0.09[c]
a*	17.21 ± 0.01[a]	16.02 ± 0.03[b]	15.20 ± 0.04[c]
b*	58.71 ± 0.14[a]	57.04 ± 0.13[b]	52.39 ± 0.01[c]
TSS before fermentation[ns]	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
TSS after fermentation[ns]	4.53 ± 0.06	4.53 ± 0.06	4.53 ± 0.06

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

Chemical quality of kombucha made from SCOBYs with 1, 3, 6 weeks of age was shown in Table 7. Kombucha from the 6-week-old SCOBY had the lowest pH of 3.13 and contained the highest concentration of acids (0.39%) ($p \leq 0.05$). However, the discrepancy of ethanol content among kombucha from various ages of SCOBY was marginal ($p > 0.05$).

Table 7: The pH, titratable acidity, and ethanol content of kombucha produced from different ages of SCOBY

Chemical values	-week-old SCOBY	-week-old SCOBY	-week-old SCOBY
pH (before fermentation)	4.75 ± 0.02^{a}	4.75 ± 0.01^{a}	4.67 ± 0.02^{b}
pH (after fermentation)	3.45 ± 0.03^{a}	3.46 ± 0.02^{a}	3.13 ± 0.02^{b}
% Acidity (after fermentation)	0.32 ± 0.00^{c}	0.34 ± 0.01^{b}	0.39 ± 0.01^{a}
% Ethanol (after fermentation)^{ns}	0.12 ± 0.01	0.14 ± 0.01	0.22 ± 0.13

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

The antioxidant capacity and total phenolic content were displayed in Table 8. DPPH radical scavenging activity of kombucha from various ages of SCOBY was similar ($p > 0.05$). The phenolic content exhibited a small variation with kombucha from 3-week-old SCOBY having the highest amount of phenolics ($p \leq 0.05$). In general, the antioxidant property and phenolic quantity did not seem to be markedly affected by the age of SCOBY biofilm.

Table 8: The antioxidant activity and total phenolic content of kombucha produced from different ages of SCOBY

ntioxidant ability / Total phenolics	1-week-old SCOBY	3-week-old SCOBY	6-week-old SCOBY
% Radical scavenging activity^{ns}	90.51 ± 0.58	90.54 ± 0.98	90.84 ± 0.59
µM Gallic acid equivalent/ml	$11,608.57 \pm 1,058.59^{b}$	$13,713.33 \pm 611.18^{a}$	$11,851.43 \pm 113.64^{b}$

Values are expressed as mean ± SD.

Values with different alphabet superscripts within the same row are significantly different ($p \leq 0.05$).

Row headers with ns superscripts indicate that values within the same row are not significantly different ($p > 0.05$).

The microbial count of kombucha produced from various ages of SCOBY was detailed in Table 9. Regardless of the age of SCOBY used in kombucha fermentation, the number of AAB, yeast, and LAB was not statistically different ($p > 0.05$). In summary, the use of older SCOBY with age from 6 weeks old or more apparently altered the color characteristics and the acidity of produced kombucha. Other qualities including TSS, ethanol content, antioxidant ability, and kombucha culture growth were undisturbed by SCOBY agedistinction.

Table 9: The microbial count of kombucha produced from different ages of SCOBY

Microbial count (log CFU/ml)	1-week-old SCOBY	3-week-old SCOBY	6-week-old SCOBY
Acetic acid bacteria^{ns}	7.27 ± 0.22	7.02 ± 0.11	7.13 ± 0.01
Yeast^{ns}	7.09 ± 0.07	7.06 ± 0.65	7.11 ± 0.47
Lactic acid bacteria^{ns}	7.20 ± 0.07	7.11 ± 0.08	7.13 ± 0.13

Values are expressed as mean ± SD.

Row headers with ns superscripts indicate that values within the same row are not significantly different (p>0.05).

Conclusion

In this study, orange peels were selected for value-added application in manufacture ofkombucha. Dried orange peel tea was utilized as a co-substrate with black tea base for kombucha fermentation. The appropriate ratio of black tea (15%) and orange peel tea (15%) was promising as the starting teas for developing a novel flavor of kombucha with enhanced health benefits and consumer acceptability. Regarding the effect of SCOBY age on kombuchaquality, the older SCOBY of 6 weeks old or more clearly impacted the beverage color and acidity. Other ages of SCOBY such as 4, 5, and more than 6 weeks old should be investigated in future researches to determine the threshold age for maintaining the product quality.

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