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Polyphenol and antioxidant activities of Kombucha fermented from different teas and fruit juices

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Abstract

There are many inexpensive beverages with beneficial effects to the body such as preventing cancer, inhibiting heart disease, reducing cholesterol, and blood pressure, inhibiting diabetes, and developing the immune. In this study we assessed the polyphenol and antioxidant activities of Kombucha fermented from three sources: black tea, green tea, and oolong tea due to their high phenolic content and fruit juices such as pineapple, apple and pomegranate juices due to their strong antioxidant activity. The highest total phenolic content was found in oolong tea Kombucha fermented with pomegranate juice (994.42 mg GAE/L). While oolong tea Kombucha fermented with apple juice had the highest antioxidant activity (1,273.74±4.15 mg AAE/L). The main components found in these Kombucha are acetic acid, lactic acid, glucuronic acid, sucrose, fructose, and ethanol. A panel of 60 participants ranked the juices using a 9 point Hedonic scale, with pomegranate and pineapple juice receiving the highest scores. This clearly indicated that these Kombucha fermented from black tea, green tea and oolong tea with pineapple juice, apple juice and pomegranate juice not only contributed their enormous polyphenols and antioxidant activities to promote health and well-being, but also improved sensory properties.

Keywords: *antioxidant activities; black tea; fruit juice; green tea; Kombucha; polyphenol; oolong tea.*

1. Introduction

The ongoing coronavirus pandemic (2019-present) caused over 80 million deaths worldwide. Fear, anxiety and worry about COVID-19 not only impacted the mental and physical health of people but also disrupted economic, agricultural and food systems. During this situation, one methods to combat the coronavirus is strengthening the immune system with food and beverages. Kombucha is a healthy Chinese tea beverage fermented with symbiotic acetic acid bacteria (*Acetobacter aceti*, *Acetobacter pasteurianus* and *Gluconobacter oxydans*) and yeasts (*Saccharomyces* spp, *Torulopsis* spp, *Pichia* spp, *Brettanomyces* spp, etc.) called a *S*ymbiotic *C*ulture of *B*acteria and *Y*east or SCOBY. The respiration and oxidation of yeasts requires oxygen (O_2) (Alejandra, Beaufort, Bouajila, Souchard, & Taillandier, 2018). Yeasts hydrolyze sucrose in culture medium to fructose and glucose which are then metabolized to ethanol. Acetic acid bacteria further oxidize ethanol to acetaldehyde then to acetic acid, after that the acetic acid bacteria and yeast-derived glucose are synthesized to gluconic acid (Amarasinghe, Weerakkody, & Waisundara, 2018). Black or green tea has been used to ferment traditional Kombucha between 20-28°C for 7-10 days to obtain a slightly carbonated, sour, and refreshing beverage (Essawet et al., 2015). The most prominent polyphenols in tea leaves are pigallocatechin, epigallocatechin-3-gallate,

epicatechin-3-gallate, and epicatechin. Many bioactive compounds are released during the fermentation process such as, organic acids (gluconic acid, acetic acid, lactic acid and amino acid), water soluble vitamins (B_1, B_6, B_{12} and C), catalase, β-carotene, carotenoids and polyphenols (catechins, theaflavins, tannins and flavonoids) (Yikmis & Tuggum, 2019; Gamboa-Gomez et al., 2016). However, the main components of Kombucha are glucose, fructose, gluconic acid, lactic acid, enzymes, catechins, and flavanoids (Cvetković et al., 2019). Their high polyphenol and antioxidant levels have many beneficial activities: removal of free radicals that cause cancer, reduce cholesterol and blood pressure that causes cardiovascular diseases, stabilize diabetes related complications, detoxifies the liver, stimulate the immune system, and improve gastrointestinal functions (Jafaria, Naghavia, Daranib, Doudia, & Shahanipour, 2020). The gluconic acid and acetic acid can improve liver detoxification due to their antimicrobial activities (Martínez Leal, Valenzuela Suárez, Jayabalan, Huerta Oros, & Escalante-Aburto, 2018). The polyphenol composition and antioxidant properties vary according to the type of tea and parameters used for fermentation. Recently, the sensory properties of Kombucha can be developed from other substrates such as grape juice (Ayed, Abid, & Hamdi, 2017).

The pomegranate *(Punica granatum L.)* is a functional and healing food which has enormous health effects on cardiovascular disease, antipyretic, anthelmintic, vermifuge, hemorrhage, microbial infections, cure aphthae ulcers, diarrhea, and dysentery (Orgil et al., 2014; Manasathien, Indrapichate, & Intarapichet, 2012). Pomegranate juice has high total phenolic content and strong antioxidant activities which are associated with the age-related disorders, reduction of stress-related chronic such as, carcinogenesis, cardiovascular diseases, and neuro-degeneration (Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011; Aviram et al., 2008; Seeram, Zhang, Reed, Krueger, & Vaya, 2006). Pineapple, one of the most popular fruits not only exhibits taste and sensory acceptance, but also has high levels of natural antioxidants such as β-carotene (provitamin A), carotenoids, vitamin C, and fibers to scavenage free radicals, strengthens the immune system, protects membranes, inhibits hydrolytic and oxidative enzymes causing heart disease, exhibits anti-inflammatory functions and inhibits the development some types of cancers in human cells (Ferreira, Siqueira, Vilas Boas, Hermes, & Rios, 2016; Ramalho & Mascheroni 2012; Ramsaroop & Saulo, 2007; Naczk & Shahidi, 2004). Apple (*Malus* spp.) also has high phenolic compounds such as flavonoids and hydroxycinnamic acids which are used in food and pharmaceutical production due to their antioxidant and antimicrobial properties that promote health and well-being (Barreira, Arraibi, Isabel, & Ferreira, 2019). Apples have been validated in preventing cancer, diabetes, chronic heart, obesity, respiratory and pulmonary dysfunctions (Tu, Chen, & Ho, 2017). Besides the health benefits of Kombucha, in this study, black tea, green tea and oolong tea were fermented with pineapple juice, apple juice and pomegranate juice, to produce a functional beverage that has health-promoting properties, and also to investigate their contribution of polyphenols, antioxidants and improved sensory properties.

2. Materials and methods

2.1 Kombucha production

Green tea and oolong tea were purchased from the Royal Project located in Chiang Mai, Thailand. A 3-horse brand black tea was supplied by the Three horses Tea, Co. Ltd, Bangkok, Thailand. The local SCOBY, mixed culture as mentioned above was used and cultivated in Microbiology Laboratory, Faculty of Food Technology, Rangsit University. 100% UHT pomegranate juice (Tipco brand), pineapple juice and apple juice (Malee brand) were purchased from a supermarket located in Bangkok, Thailand. Natural cane sugar as sucrose was supplied by Mitr Phol., Co. Ltd, Bangkok. Black tea, green tea and oolong tea were prepared at a concentration of 12 g/L with water at 75°C for 2 min for green tea and at 95 °C for 3 min for black and oolong tea. Subsequently, the tea leaves were removed by stainless steel sieves. Then, 70 g/L sucrose and 3% (w/v) SCOBY were added as substrate in tea samples. Kombucha was fermented at 25°C for 7 days in sterile glass jars (2 L volume). Each individual sample broth was collected every 2 days and assessed for pH, total soluble solid total phenolic content, and total antioxidant activity. Black tea, green tea and oolong tea were fermented in 3 replications and the results are reported as the average±standard deviations. After the first

fermentation, 20% (v/v) of pineapple juice, apple juice and pomegranate juice were added and incubated at 25 °C for 3 days to determine pH, total soluble solid, total phenolic contents, and total antioxidant activities.

2.2 Materials and equipment

All standards and chemicals used for the analysis were supplied by Sigma-Aldrich and Merck. All solutions were freshly prepared for the identification and quantification. The equipment and glassware were supplied by the Microbiology and Chemistry Laboratory, Faculty of Food Technology, Rangsit University.

2.3 Determination of total acidity, sugar, organic acid, and alcohol contents

Individual Kombucha samples were collect once every 2 days. All samples were well mixed, then aliquots were taken to determine pH by electronic pH meter (Ohaus, starter 300, U.S.A.) calibrated at pH 4.0 and 7.0. Total acidity was determined by titration with 0.1 N NaOH using phenolphthalein as indicator (AOAC, 2005). Quantitative analysis of sugars (glucose, sucrose, fructose) and major organic acids (acetic acid, glucuronic acid and lactic acid) were measured by High Performance Liquid Chromatography (HPLC; Shimadzu, Japan) with a refractive index and UV-DAD detector, ion exclusion column (250 x 4.6 mm) compared to standard concentrations and expressed as mg/L. Samples were filtered through membrane (0.45 µm, Millipore) before injected. The analysis conditions were carried out with 170 µL/min sulfuric acid, pH 2.2 at 30 °C as mobile phase. The alcohol content was determined by spitless mode Gas Chromatography (GC; Shimadzu, Japan) at 100 °C injector and 260 °C FID detector in CBP column (250 x 0.33 mm) and expressed as % (v/v).

2.4 Determination of total phenolic content

The total phenolic content was determined corresponding to the absorbance of the standard gallic acid at 765 nm and expressed as mg of gallic acid equivalent (GAE) per liters of Kombucha solution (mg GAE/L) according to Folin-Ciocalteu colorimetric method with modifications (Siddiqui, Rauf, Latif, & Mahmood, 2017).

2.5 Determination of total antioxidant activity

DPPH (1,1-dipheyl-2-pierylhydrazyl) radical scavenging was used to determined total antioxidant activity. Diluted Kombucha samples (100µl) were mixed with the appropriate DPPH solution and kept at 20°C in the dark. The absorption rate was measured at 517 nm (Agilent 8453UV) as A_{sample}. A_{blank} was performed without the Kombucha samples. The total antioxidant activity was calculated as milligrams of ascorbic acid equivalents (AAE) per liters of Kombucha solution (mg AAE/L) according to the following equation (Marques et al., 2012).

$$\% \text{ inhibition} = 100 [A_{blank} - A_{sample}]/ A_{blank}$$

2.6 Sensory analysis

The sensory analysis of finished Kombucha fruit juice was performed with 60 panelists. A 9 points Hedonic scale (1-the lowest and 9-the highest) was used to evaluate appearance, color, taste, odor and general acceptance according to Malbasa et al. (Malbasa, Vitas, Loncar, Grahovac, & Milanovic, 2014).

2.7 Isolation of viable microbial cells

The most acceptable Kombucha fruit juice after 10 days fermentation from Section 2.6 was used to isolate the viable microbial cells. The serial dilutions samples from 10^{-2} to 10^{-8} were plated and incubated at 30 °C for 48 h on selective media such as Yeast Peptone Mannitol Agar (Difco) for acetic acid bacteria, Lactobacillus MRS Agar (Himedia) for lactic acid bacteria and Sabouraud-4% Maltose Agar (Merck) for yeast enumeration and expressed as colony–forming units per milliliter (cfu/mL) according to Kappeng and Pathom-aree (2009) with modifications.

2.8 Statistical analysis

All data in this study were determined statistically by Student's t-test at significant level of $P < 0.05$ with SPSS program (SPSS Inc., Chicago, U.S.A.) version 20.0. All studies were done with 3 replications and data are reported as mean±standard deviations.

3. Results and discussion

3.1 pH values, total acidity (TA), sugars and ethanol content of Kombucha fermented from different teas and fruit juices

The initial pH of black tea, green tea, and oolong tea Kombucha fermented with pineapple juice, apple juice and pomegranate juice were within the range of 3.2 to 3.3 to ensure that they were safe for human consumption (Nummer, 2013). After 10 days fermentation, the total acidity of these Kombucha beverages was significantly different ($p<0.05$) from each juice as shown in Table 1. These results are associated with the amounts of organic acids (mainly acetic acid and glucuronic acid) produced from symbiotic acetic acid bacteria and yeast using glucose as carbon sources during the fermentation process in accordance with the investigation of Jayabalan et al. (2015). The highest TA was oolong tea Kombucha fermented with pineapple juice (9.20 g/L), which was higher than pomegranate juice (8.60 g/L) and apple juice (7.60 g/L) when compared with the green tea Kombucha and black tea Kombucha, respectively. The black tea Kombucha fermented with different fruit juices had the lowest total acidity with no significant differences ($p<0.05$) compare to green tea Kombucha and oolong tea. Variations of TA had significant differences ($p<0.05$) due to the different metabolic pathways of bacterial diversity from teas and fruit juices during fermentation regarding the organic acid production (Jayabalan, Malbasa, Loncar, Vitas, & Sathishkumar, 2014).

The comparison of the final sugar concentration in oolong tea and green tea Kombucha showed significant difference ($p<0.05$) with the same pattern in accordance with the increase of organic acids. The results indicated that the metabolisms of yeasts that hydrolyzed sucrose to glucose and fructose, then metabolized these to ethanol were diverse across all fermentation treatments (Table 2). However, glucose could not be detected due to ease of metabolization by other microorganisms. Conversely, the ethanol production of different teas Kombucha after 10 days fermentation increased in the maximum value of 0.35±0.01 % (v/v). Nevertheless, the main components of these Kombucha are acetic acid, lactic acid, glucuronic acid, sucrose, fructose, and ethanol in agreement with the previous report by Cvetković et al., (2019).

Table 1 Total acidity of Kombucha beverages after 10 days fermentation

Tea	Total Acidity (g/L)		
	Apple juice	Pomegranate juice	Pineapple juice
Black tea	3.40±0.02[a]	3.60±0.01[a]	3.70±0.01[a]
Green tea	6.00±0.04[a]	5.20±0.04[b]	4.60±0.03[c]
Oolong tea	7.60±0.02[c]	8.60±0.00[b]	9.20±0.02[a]

Different letters in superscript within column indicate a significant difference at p<0.05 according to SPSS (SPSS Inc., Chicago, U.S.A.) version 20.0

Table 2 The amount of organic acids, sugars and ethanol after 10 days fermentation

Tea	Concentration						
	Acetic acid (mg/L)	Glucuronic acid (mg/L)	Lactic acid (mg/L)	Glucose (mg/L)	Fructose (mg/L)	Sucrose (mg/L)	Ethanol (%v/v)
Black tea	1488.73±11.90[a]	3389.03±170.83[a]	9.8±0.60[a]	ND	13414.32±92.21[b]	21507.28±23.05[b]	0.35±0.01[c]
Green tea	2382.12±34.46[b]	3945.41±107.42[b]	13.9±0.23[b]	ND	11609.46±57.71[a]	22253.83±137.51[c]	0.30±0.0[a]
Oolong tea	4306.59±3.29[c]	4816.92±155.74[c]	25.9±0.65[c]	ND	19298.82±14.40[c]	13167.71±26.79[a]	0.32±0.01[b]

ND in column indicate Not Detected
Different letters in superscript within column indicate a significant difference at p<0.05 according to SPSS (SPSS Inc., Chicago, U.S.A.) version 20.0

3.2 Determination of total phenolic content and total antioxidant activitiy

The total phenolic content as an antioxidant to scavenge the free radicals (Jayabalan et al., 2015) obtained from oolong tea Kombucha fermented with pomegranate juice gave the highest amount (994.42 mg GAE/L) with significant difference ($p<0.05$) compared to oolong tea Kombucha fermented with apple juice (660.36 mg GAE/L) and pineapple juice (526.30 mg GAE/L). This enhancement is related the higher ethanol content during fermentation with pomegranate juice which is higher than apple juice and pineapple juice (Alejandra, Beaufort, Bouajila, Souchard, & Taillandier, 2019). Comparison of the total phenolic content between black tea and

green tea Kombucha by different fruit juices found that higher total phenolic content was obtained from black tea fermented with pomegranate juice (348.77 mg GAE/L) as shown in Table 3. This may be due to the initial phenolic content in tea leaves, fruit juice and phenolic content which is released during the fermentation process (Jafaria et al., 2020), in accordance with the findings of Akbarirad et al. (2017).

Variations of total antioxidant activity of most Kombucha fruit juice significantly increased over time ($p<0.05$). The highest antioxidant activity was found in Kombucha fermented with apple juice (1,273.74±4.15 mg AAE/L) for 8 days compared with pomegranate juice (1,268.05±1.99 mg AAE/L) and pineapple juice (1,258.29±7.97 mg AAE/L), respectively. Pineapple juice exhibited the fastest change in antioxidant activity over the fermentation period, while apple and pomegranate juices were comparatively slower. The change of total antioxidant activity was higher than total phenolic content due to the microorganisms metabolizing vitamins, enzymes, organic acids, and polyphenols contributing to their total antioxidant activity during the fermentation time (Table 4) (Alejandra et al., 2019)

Table 3 Total phenolic content of Kombucha fermented with fruit juice for 10 days

Tea	Total phenolic content (mg GAE/L)		
	Apple juice	Pomegranate juice	Pineapple juice
Green tea	164.71±9.11^{b}	219.78±4.70^{a}	162.17±1.09^{b}
Black tea	310.36±1.02^{b}	348.77±10.25^{a}	325.58±7.17^{b}
Oolong tea	660.36±12.47^{b}	994.42±5.71^{a}	526.30±4.70^{c}

Different letters in superscript within column indicate a significant difference at p<0.05 according to SPSS (SPSS Inc., Chicago, U.S.A.) version 20.

Table 4 Antioxidant activity of Kombucha fruit juice during fermentation

Days	Antioxidant activity (mg AAE/L).		
	Apple juice	Pomegranate juice	Pineapple juice
0	1,235.53±3.04^{a}	1,253.41±3.45^{a}	1,042.85±3.04^{a}
2	1,261.54±4.15^{b}	1,253.41±8.68^{a}	1,225.77±5.75^{b}
4	1,263.17±3.45^{b}	1,263.17±1.99^{a}	1,254.23±1.15^{c}
6	1,273.74±3.04^{b}	1,267.24±10.97^{b}	1,257.48±2.30^{c}
8	1,273.74±4.15^{b}	1,268.05±1.99^{b}	1,258.29±7.97^{c}

Different letters in superscript within column indicate a significant difference at p<0.05 according to SPSS (SPSS Inc., Chicago, U.S.A.) version 20.0

3.4 Sensory analysis

A 9 points Hedonic scores of oolong tea Kombucha as sparkling, sour and vinegar-like taste with different fruit juices are shown in Table 5. Taste, color, odor, and overall acceptability of oolong tea Kombucha fermented with pomegranate juice were all acceptable for 60 panelists with the highest scores for pineapple juice and apple juice, respectively. According to this study, it can be concluded that pineapple juice, apple juice and pomegranate juice can improve the sensory and functional properties of Kombucha beverage as mentioned previously by Ayed et al. (2017).

Table 5 Sensory evaluation of oolong tea Kombucha fermented with different fruit juices

Appearance	Apple juice	Pomegranate juice	Pineapple juice
Color	5.90±1.54^{b}	6.53±1.53^{a}	6.18±1.35^{ab}
Odor	4.60±2.06^{b}	5.60±1.89^{a}	4.78±2.13^{b}
Taste	4.97±2.15^{b}	5.82±1.64^{a}	5.18±2.09^{ab}
Overall acceptability	5.10±1.98^{b}	5.88±1.70^{a}	5.25±1.87^{ab}

Different letters in superscript within column indicate a significant difference at p<0.05 according to SPSS (SPSS Inc., Chicago, U.S.A.) version 20.0

3.5 Isolation of microbial Kombucha

Comparison of viable microbial cells in black tea, green tea, and oolong tea Kombucha fermented after 10 days with pomegranate juice determined that the dominant lactic acid bacteria (3 . 7 9 log cfu/mL) in oolong tea Kombucha was higher than acetic acid bacteria and yeast in green tea (3.69 log cfu/mL) and black tea (3.19 log cfu/mL), respectively (Figure 1). The highest content of lactic acid bacteria was in accordance to the investigation of Amarasinghe et al. (2018) and Cvetković et al. (2019). The variation of lactic acid bacteria, acetic acid bacteria and yeast in Kombucha beverage depended on the local species or the cross contamination of bacteria and yeast in SCOBY and the condition for fermentation as mentioned previously by Malbasa et al., (2014).

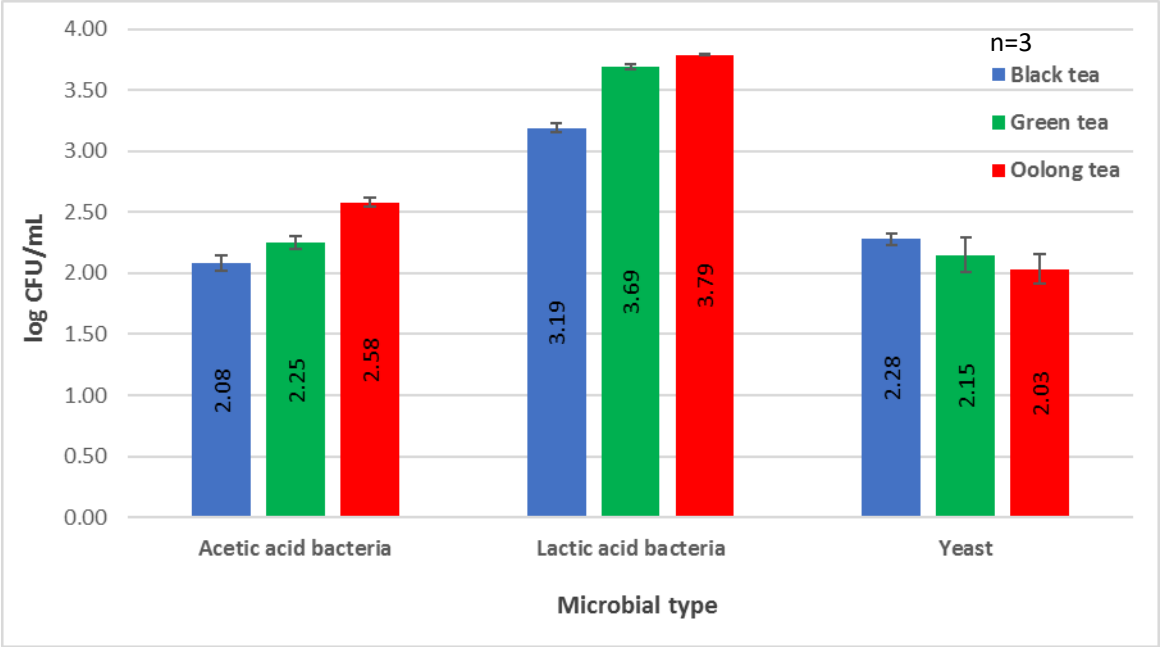

Figure 1 Microbial count from Kombucha beverage containing pomegranate juice

4. Conclusion

Results of this study clearly indicated that the polyphenols, antioxidant activities, and organic acids of black tea, green tea and oolong tea Kombucha fermented with pineapple juice, apple juice and pomegranate juice were greatly influenced by the type of teas, fruit juices, pH, microbial composition of tea fungus in SCOBY. These Kombucha fermented from different teas and fruit juices were enriched with the main metabolic compounds such as total phenolic content, total antioxidant activity and organic acids over time, which is beneficial to the body. The pineapple juice, apple juice and pomegranate juice can improve the sensory and functional properties of Kombucha beverages. Even though the variations of fermentation conditions may affect the different compositions of polyphenols, antioxidant activities, organic acid, and biological activities of Kombucha beverage, it still has potential benefits for inexpensive beverages to enhance immunity of the humans around the world. Furthermore, the commercial products of Kombucha fermented from different teas and fruit juices are a challenge to scale up for customization due to many cultivation factors which may affect the fermentation process. The optimum conditions, variation parameters to cultivate microorganisms without virulent bacterial and clinical investigation for the scale up process should be further studied.

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6. Conflict of interest

The authors declare that they have no conflict of interest.

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