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Tea Fungus Beverages from Torch Ginger (*Etlingera elatior*): Total microbial, Physicochemical, and Antioxidant Activity

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Abstract. Functional beverages are needed to maintain health and fitness as a part of the instant lifestyle and degenerative diseases. The scientific findings on torch ginger (*Etlingera elatior*) flower (TGF) as a tea fungus beverage (Kombucha) are still limited. This research evaluated total microbes, physicochemical properties, and antioxidant activity 7 and 14 days after fermentation (daf) of TGF kombuchas. In addition, TGF in variant 0% (TGF0), 5% (TGF5), 10% (TGF10), and 15% (TGF15) are fermented with 3% of SCOBY, 20% culture, 1% green tea, and 10% sucrose. The antioxidant activities of TGF kombuchas were evaluated by *2,2-diphenyl-1-picrylhydrazyl* (DPPH) and *2,2'-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid* (ABTS) assay—the phenolic and flavonoid content using Folin-Ciocalteu method-gallic acid equivalent, and quercetin acid equivalent, respectively. The result showed that the fermentation time ($p<0.05$) was affected by the properties of TGF kombuchas. Total microbes, physicochemical properties (pH, acidity, cellulose pellicle weight, phenolic, flavonoid content), and antioxidant activity were higher in 14 than in 7 days. Total soluble solid and total yeast count showed lower values in 14 days. The increase in total bacteria count, TGF15%, led to the highest increasing density from 8.08 ± 0.02 Log CFU/mL on seven daf to 13.34 ± 0.04 Log CFU/mL on 14 daf. The TGF 10% and 15% kombuchas in 14 dafs showed abundance in phenolic and flavonoid content, 121.45±1.07 mg GAE/mL and 1.70±0.04 mg QAE/mL, respectively. During 14 daf and TGF 10%, the Kombucha of torch ginger flower demonstrated high antioxidant activity at 85.92±0.07% DDPH and 63.05±0.97ABTS. It is expected to aid future research into developing functional kombucha beverages.

Keywords: antioxidant; Kombucha; microbial; physicochemical.

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INTRODUCTION

Increased public interest in health, functional drinks have become popular. A kombucha as functional drink is one type of available food. Functional beverages must fulfill two critical functions: nutritional intake and sensory satisfaction, such as flavor and texture. Functional drinks can support immune system development and give supplementary benefits such as probiotics (Devi et al., 2021). Kombucha is a sweet, partially sour beverage fermented with sugar by a *symbiotic culture of bacteria and yeast* (SCOBY) (Nyhan et al., 2022).

Many factors influence the chemical ingredients and antioxidant activities of kombucha beverages, including different types of teas, sugars as carbon sources, solution concentration, temperature, tea fungus, or acidic bacteria in kombucha broth during fermentation. Furthermore, the hardness of the water impacts the functionality of the kombucha beverage (Zubaidah et al., 2018 Can; Candra et al., 2023). According to the findings, herbal infusions have more potent antioxidant activity than black tea (Tapias et al., 2022). Furthermore, Kombucha's

phenolic components and radical scavenging capabilities increase with fermentation duration (Ahmed et al., 2020).

Many edible flowers are more than just a delicacy or a garnish; they are rich in nutritional properties such as protein and essential amino acids (Takahashi et al., 2020). Torch ginger (*E. elatior)* is an edible flower widely used as a salad green. Naufalin et al. (2021) state that torch ginger belongs to the Zingiberaceae family and is widely used in the community as a vegetable and medicine. Torch ginger is also utilized in medicine due to its antioxidant properties. The flower of torch ginger has the potential to be developed as a natural source of antioxidants for the prevention or treatment of diabetes (Muhamad et al., 2020).

Conversely, the nutritional drink made from torch ginger with tamarind and coconut sugar exhibits significant phenol and antioxidant activity (Naufalin et al., 2021). The addition of ingredients rich in antioxidants will enrich the resulting product. Torch ginger can be converted into syrup as a kombucha drink.

Expansion of torch ginger flower as a brewed drink began to be widely used as a tea brew. So far, the utilization of torch ginger flower in Kombucha functional drinks has yet to be studied for its functional properties. The torch ginger flower (TGF) kombucha can be a functional food component due to its health benefits (symbiotic beverage). In this context, this study aims to evaluate microbial load (bacteria count and yeast count), physicochemical properties, and antioxidant activity of TGF kombucha in various concentrations and fermentation times.

METHODS

Fermentation of torch ginger flower kombucha

Torch ginger flower (TGF) is sorted, washed, peeled, blanched, and dried in an oven dryer at 50°C for 12 h, then crushed into a powder using a food processor and, filtered with a 30 mesh, and transferred into the tea bag (3g). Torch ginger flower with different concentrations (w/v): 0% (TGF1), 5% (TGF2), 10% (TGF3), and 15% (TGF4) are fermented with 3% of *Symbiotic culture of bacteria and yeast* (SCOBY), 20% culture, 1% green tea (Dilmah) and 10% sucrose into the beaker glass and letting them ferment for 14 days in 37ºC temperature.

Determination of total bacteria and yeast count

Torch ginger flower (TGF) kombucha yeast and bacteria counts were performed using the standard plate count approach (PCA) described by Zubaidah et al. (2018), with an additional modification that entailed mixing 1 mL of the samples with 9 mL of sterile 0.1% peptone water (10^{-1}). Aliquots of the mixture and successive dilutions were diluted further with peptone water to achieve serial decimal dilutions ranging from 10^{-2} to 10^{-8}. One milliliter of each dilution was added to three duplicated petri dishes. Then 15 mL of sterile nutritional agar (which included cycloheximide to inhibit yeast growths) was added, mixed immediately, and allowed to solidify before the growths were counted after incubation at 37°C for 24 hours. The exact process was used for yeast counts, except potato dextrose agar (PDA) was used as the medium, and the incubation period was 48 hours at 30 °C instead of with the addition of cycloheximide. Growing colonies are counted. The number of cells is expressed in colony-forming units per ml (CFU/ml) as follows:

$$CFU\ s/mL = NCx\frac{1}{DF} \quad \text{.....(1)}$$

Wheres:
CFU s/ml = colony-forming units in 1 ml sample suspension (Log CFU/mL)
NC = number of colonies
DF = dilution factor

Cellulose pellicle weight

Cellulose pellicle production in the culture was monitored by gently removing it with a spatula. A paper towel was used to remove the moisture, and the fresh weight was measured on a digital scale in grams (g) after 7 and 14 days of fermentation.

Determination of acetic acid content (%)

Titratable acidity (TA) was evaluated by titrating the sample with a standard sodium hydroxide solution to a pH of 8.20 (Li et al., 2021). The TA was estimated as a percentage of acetic acid using the following equation:

$$\%\ \text{Acidity}\ (w/v) = N \times V1 \times \text{Eq wt} \times 100V2 \times 1000 \quad \text{.....(2)}$$

Wheres: N = Normality of NaOH (mEq/ml), V1= volume of titrant (mL), V2=Volume of the sample (mL), Eq wt = Equivalent weight of acetic acid (60.05mg/mEq).

Determination of pH and total soluble solid (TSS)

The pH value was determined using a pH meter, pre-calibrated to pH 4.0 and 7.0. The total soluble solid content was determined using a handheld analog refractometer (Pocket Refractometer Pal-H 3870 Atago Co., Ltd Japan).), and the results were expressed as brix. Changes in the clarity of the culture medium were observed in 0, 7, and 14-day fermentation.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu technique (Barbosa et al., 2022) with an additional modification using gallic acid (GA) as a standard. Weighing 1.3 mg of GA and adding 1.3 mL of methanol resulted in a 1000 ppm concentration GA solution. GA preparation solutions with concentrations of 240, 210, 180, 150, 120, 90, 60, 30, and 0 ppm were created to obtain a GA standard curve. Following that, a 96-well plate was prepared and filled with 20 uL of GA solution three times, then 100 uL of Folin-Ciocalteu reagent was added into the 96-well plate using a micropipette, then homogenized and incubated for 5 minutes. In the 96-well plate, 80 uL of Na_2CO_3 solution (20%) was added and incubated for 2 hours. Repeat the procedure with a 20-uL sample of TGF kombucha in each treatment. After 2 hours of incubation, the absorbance was measured at 750 nm using an Elisa Reader, and a calibration curve was built utilizing the connection between GA content and absorbance at each concentration. The x-axis shows the relationship between gallic acid concentration, and the y-axis shows the absorbance of the gallic acid reaction with the Folin Ciocalteu reagent. The total phenolic content was calculated as mg gallic acid equivalent (GAEq) per milliliter (mL) of Kombucha.

Determination of total flavonoid content

The total flavonoid levels were determined by producing quercetin standards. To create a quercetin solution with a concentration of 1000 ppm, weigh 1 milligram of quercetin and add 1 mL of methanol. Quercetin preparation solutions with concentrations of 160, 140, 120, 100, 80, 60, 40, 20, and 0 ppm were created to generate a quercetin standard curve. Following that, a 96-well plate was constructed and filled with 10 uL of quercetin solution three times, then 10 uL of 10% $AlCl_3$, 10 uL KCH_3COO 1 M, and 120 uL aquadest solutions were added to the 96-well plate using a micropipette and incubated for 30 minutes. Repeat the procedure with a 10 uL sample of TGF kombucha in each treatment. After incubation, absorbance was measured at 415 nm using an Elisa Reader, and a calibration curve was produced utilizing the relationship between quercetin content and absorbance at each concentration. The total flavonoid content was expressed as mg quercetin acid equivalents (QAEq) per mL.

Antioxidant activity of *2,2-Diphenyl-1-Picrylhydrazyl* (DPPH) assay in TGF kombucha

The first stage of antioxidant activity was creating a DPPH control solution using a modified technique (Barbosa et al., 2022). Dissolving 0.78 mg of DPPH powder in 10 mL of ethanol yielded a 0.2 mM DPPH solution. The 100% kombucha sample was then diluted with methanol to 10, 20, 30, 40, and 50%. A 40 uL sample was placed in a 96-well plate, and 160 uL of DPPH 0.2 mM was added. After 30 minutes in the dark, the absorbance was measured with an Elisa Reader at a wavelength of 517 nm. Weighing 1 mg of ascorbic acid and adding 1 mL of methanol prepared a standard curve for ascorbic acid with a concentration of 1000 ppm. Diluted ascorbic acid preparation solutions of 10, 20, 30, 40, and 50 ppm were used. Following that, a 96-well plate was constructed and filled with 40 uL of ascorbic acid solution three times, followed by the addition of 160 uL of 0.2 mM DPPH solution into the 96-well plate using a micropipette and incubation for 30 minutes. After incubation, the absorbance was measured with an Elisa Reader using a microplate reader (Multiskan® Go, Thermo Scientific, Vantaa, Finland) at 517 nm. The IC_{50} (the concentration of kombucha extract that causes a 50% loss of DPPH capability) was calculated using the absorbance versus concentration plot. The percentage of the antioxidant activity against DPPH radicals at each concentration of the sample solution was calculated by the formula:

$$\% \text{ Antioxidant activity} = (Ak - As) / Ak \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Wheres: (Ak)=the absorbance of DPPH, (As)= the absorbance of the sample.

Antioxidant capacity of 2,2′-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) in TGF kombucha

The radical-scavenging activity of extracts

was determined using a modified version of the Prasedya et al. (2021) method. An aqueous solution of 7 mM ABTS and a solution of 2.4 mM potassium persulfate were produced as stock solutions. The workable solution was made by combining the two stock solutions in equal parts (1:1) and allowing them to react for 16 hours in the dark at room temperature. The solution was then diluted by adding 250 uL ABTS with 12 mL ethanol to achieve an absorbance of around 1.1±0.02 units at 734 nm. Each test requires a new ABTS solution. After 2 hours of incubation at room temperature, 15 uL sample extracts of varied concentrations were mixed with 285 uL ABTS solution, and the absorbance was measured with an Elisa Reader at 734 nm using a microplate reader (Multiskan® et al.). The calibration curve was developed using Trolox as a standard. The ABTS scavenging activity of the extracts was determined using the same formula as above (3).

Sensory evaluation

A panel of 20 taste testers assessed the organoleptic qualities of the torch ginger flower kombucha. Analysis technique was used to analyze the sensory qualities of the kombucha samples, and a 6-point scale method was employed. Next, numbers and codes were added to each kombucha sample at different times (days 7 and 14) and concentrations (0%, 5%, 10%, and 15%). Color, taste, flavor, odor, and preference were assessed in triplicate. Results spanned from 1 to 6, as indicated by (1) strong distaste, (2) mild distaste, (3) neither distaste nor like, (4) mild inclination, (5) moderate inclination, and (6) strong liking.

Statistical analysis

IBM Statistics SPSS 23 with a one-way ANOVA was used to retrieve statistical data. Duncan's multiple range test ($p < 0.05$) assessed mean differences. The data were collected in at least three replicates and reported as the mean standard deviation (SD).

RESULTS AND DISCUSSION

Evaluation of the characteristics of torch ginger (*E. elatior*) flower kombucha

In this experiment, torch ginger (*E. elatior*) flower (TGF) kombucha in various concentrations (w/v)was cultivated in one substrate (green tea) and produced four different types of Kombucha: TGF1 (0%), TGF2 (5%), TGF3 (10%), and TGF4 (15%) respectively. The schematic representation of the fermentation process of TGF kombucha is illustrated in Figure 1. Figure 1 shows that the visual culture media (color and clarity) varied in final incubation (14 days after fermentation). Changes in the culture media of TGF kombucha were monitored during the fermentation process. At the end of the incubation, the TGF concentration (w/v) did not affect the visual (color and clarity) of the TGF kombucha. Finally, on day 14, the cultural media had turned a somewhat clear brown color. The TGF 15% has brightness, visual culture, color, and clarity. The physical and physicochemical properties (phenolic, flavonoid, TSS, and acidity) may be responsible for the medium's dark color changes during fermentation. TGF kombucha has vigorous antioxidant activity on day 14 of fermentation, which correlates with the high total phenolic and flavonoid concentrations. In addition, in a study by Yikmis & Tuggum (2019), the fermenting procedure has little effect on the color of fruit teas. Kombucha fermentation was better on color in herbal teas and coffee samples. One study found no statistical change in color values of all purple basil kombucha tea samples.

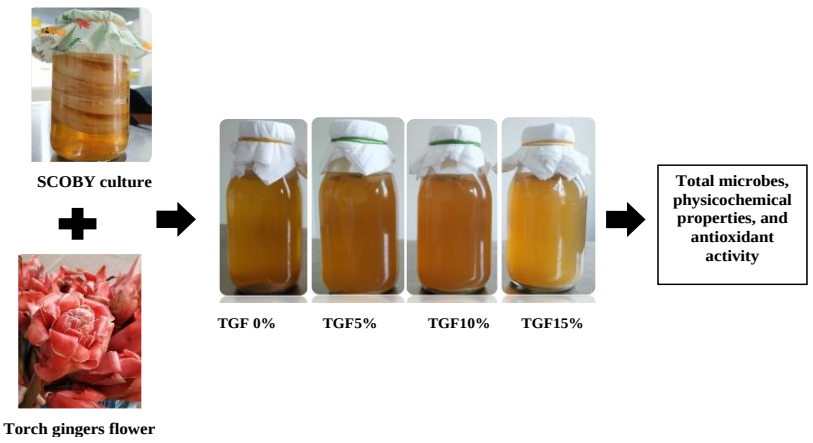

Figure 1. Kombucha tea of torch ginger (*E. elatior*) substrate in various concentration

Total bacterial and yeast count

The microbial load of TGF kombucha includes bacteria and yeast counts, shown in Figure 2. The fermentation time was significant ($p < 0.05$), increasing bacterial and decreasing yeast load in TGF kombucha. Total bacterial count was increased in all samples. The TGF15% showed the highest increasing density from 8.08 ± 0.02 Log CFU/mL on seven daf to 13.34 ± 0.04 Log CFU/mL on 14 daf—the fact demonstrating that the kombucha culture developed successfully in the torch ginger flower green tea medium enrichment. In contrast, the total yeast count demonstrated a decrease in cell density after 14 days of incubation compared to 7 days. The decrease in total yeast count is attributed to the increasing microbe-inhibiting chemicals in torch ginger due to modifications to raw materials, which provide varied nutrients such as sugar, vitamins, and minerals, which affect microbial growth. According to Zubaidah et al. (2018), sucrose is broken down by yeasts in the kombucha consortium into glucose and fructose, with the former being used and digested more to produce ethanol and carbon dioxide. Total microorganisms contributing to TGF kombucha fermentation increased in direct proportion to fermentation time. Acetobacter is one of the bacteria that can create microbial cellulose. The primary species in this genus is A. xylinum, which has been renamed *Gluconacetobacter xylinus* and, more recently, *Komagataeibacter xylinus* (Villarreal-Soto et al., 2019). So far, the only certainty is that kombucha cultures are simultaneously characterized by acetic acid bacteria (AAB) and yeast.

In contrast, lactic acid bacteria (LAB) are not always present (Diez-Ozaeta & Astiazaran, 2021). Kombucha (tea fungus) is a healthy beverage from sugared tea infusion fermented by a symbiotic consortium of yeast species and acetic acid bacteria. In Kombucha fermentation, many yeasts (including Pichia, Candida, Zygosaccharomyces, Brettanomyces, and Saccharomyces species) and Acetobacter xylinum have been found (Jayabalan et al., 2014). Indeed, using the benefits of probiotics may have various drawbacks. Live microorganisms must be delivered sufficiently (about 10^9 CFU/mL) to provide a health benefit and overcome various obstacles before reaching the gastrointestinal tract (Marco et al., 2021). Bueno et al. (2021) added that the *Lactobacillus rhamnosus* and *Lactobacillus casei* inoculated coffee kombucha. After 120 minutes of gastric and intestinal simulations and stored for 15 days at four °C, the concentrations remained more significant than the authorized dosage for food products (10^6 log CFU/mL). The addition of this isolate influenced changes in the abundance of bacteria in the intestinal simulations.

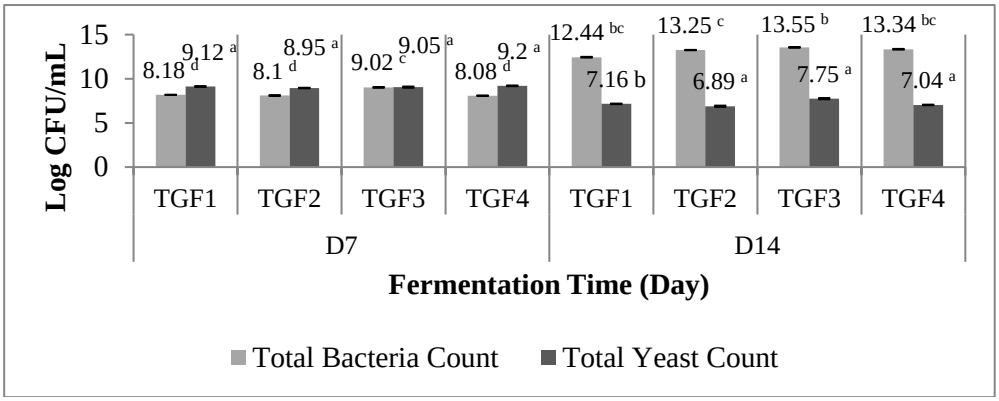

Figure 2. Total bacteria count and yeast count of TGF kombuchas during fermentation Notes: The bars followed by the same letters are not significantly different at $p < 0.05$. TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

The pellicle, pH, acidity, and TSS change in torch ginger (*E. elatior*) flower kombucha.

The fermentation time of TGF kombucha is affected by changes in pellicle weight, pH, total acidity, and TSS (Table 2, Figure 3). The pellicle weight of TGF kombucha samples increased significantly ($p<0.05$) with fermentation time (Figure 3.) The best combination to produce pellicle is TGF 5% on 14 days of fermentation (8.79±0.12g). Our finding indicated that 10% (w/w) of sucrose with green tea-torch ginger enrichment substrate is suitable for producing the new pellicles SCOBY layer. Trevino-Garza et al. 2020 reported that during the incubation of kombucha tea in various carbon sources, the turbidity and cellulose/pellicle production

increased in the distinct culture media.

The statistical analysis of the total acetic acid content (Table 1.) results suggests a significant ($p<0.05$) and positive relationship between the kombucha incubation period and acetic acid content ($R^2 = 0.9881$). The pH of TGF kombucha samples decreased significantly ($p <0.05$) with fermentation time. The original pH of the TGF0% kombucha was 4.39±0.01; however, it reduced to 3.37±0.03 after 14 days of fermentation (Table 1). Increased concentrations of torch ginger enhance the pH of the TGF kombucha and are consistent with the increasing total acidity. During fermentation, the bacteria found in a *symbiotic culture of bacteria and yeast* SCOBY utilized sucrose as a carbon source to make various metabolic products, including organic acids. The yeast invertase enzyme catalyzed the hydrolysis of sucrose into glucose and fructose, which were then used to create ethanol via the glycolysis pathway. Meanwhile, acetic acid bacteria, known as Acetobacter and Gluconobacter, use glucose and ethanol to make gluconic acid (Jayabalan et al., 2014). Previous research suggested that acetic acid bacteria identified in SCOBY use sugar as a carbon source, creating acetic acid as the major metabolite. Lactic acid bacteria, such as Lactobacillus sp. and Lactococcus sp., are present in kombucha culture in addition to acetic acid bacteria and may contribute to increased acidity. (Jayabalan et al., 2014 and Bishop et al., 2022). Furthermore, Zubaidah et al. (2022) discovered that increased concentrations of Javanese turmeric in the kombucha consortium enhance the pH of the Kombucha and are consistent with the increasing total acidity value.

In contrast to the pH, the TSS (ºbrix) of TGF kombucha was found to drop slightly in all treatments from day 7 (11.60±0.4°brix, 0%TGF) to day 14 (6.64±0.15 °brix, TGF15%). Increased concentrations of torch ginger are consistent with decreasing the total soluble solids of TGF kombucha. Jayabalan et al. (2014), because many nutrients were required for the bacteria' development and metabolism, the TSS may decrease with fermentation duration. The kombucha culture's use of sugars resulted in the creation of different organic acids, which eventually led to a pH decrease.

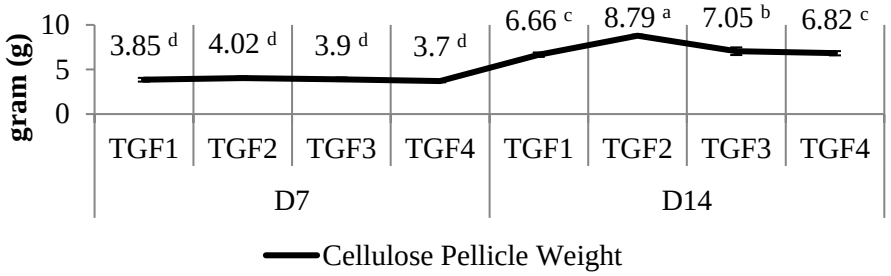

Figure 3. Total of cellulose pellicle of TGF kombuchas during fermentation
Notes: The bars followed by the same letters are not significantly different at $p < 0.05$. TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

Table 1. During fermentation, total soluble solids (TSS), pH, and acidity content (% acetic acid) of TGF kombuchas.

Fermentation time (day)	Kombuchas sample	Acidity (% acetic acid)	TSS (ºbrix)	pH
7	TGF1	5.05±0.15^{e}	11.60±0.48^{a}	4.39±0.01^{a}
	TGF2	5.35±0.08^{e}	10.38±0.17^{b}	4.27±0.02^{b}
	TGF3	6.88±0.10^{d}	10.60±0.37^{b}	4.25±0.01^{b}
	TGF4	5.90±0.27^{d}	10.55±0.14^{b}	4.07±0.01^{c}
14	TGF1	8.05±0.32^{c}	7.05±0.28^{c}	3.37±0.02^{de}
	TGF2	8.85±0.27^{b}	6.84±0.15^{d}	3.32±0.04^{e}
	TGF3	9.25±0.10^{a}	6.66±0.07^{d}	3.28±0.04^{f}
	TGF4	9.50±0.07^{a}	6.64±0.15^{d}	3.20±0.03^{g}

The means followed by the different letters in a column are significant (LSD test; $p < 0.05$). TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

Total phenolic and flavonoid content

During the fermentation, the total phenolic

and flavonoid content increased significantly ($p < 0.05$). Furthermore, varied torch ginger flower (TGF) concentrations (0%, 5%, 10%, and 15%) applied to the kombucha consortium, as well as time incubation (7 and 14 daf), affected the critical total phenolic and flavonoid content (Figure 4. and Figure 5.). Figure 4. shows the total phenolic content of the TGF kombucha; TGF10 % in 14 dafs have the highest and TGF15% in 7 dafs have the lowest content, 121.45±1.07 GAEq/mL and 28.55±0.15 GAEq/mL respectively (R^2=0.9815). Increased concentrations of torch ginger are consistent with the increasing total phenolic content. The total phenolic content of TGF kombucha was significantly higher than those identified in tea kombucha by Cardoso et al. (2020), who discovered 1.09 mg GAE/mL for black tea and 7.0 mg GAE/mL for green tea kombucha. In addition, according to Jakubczyk et al. (2020), the increase in polyphenolic compound content can be attributed to a variety of reactions that occur during tea fermentation, such as the oxidation of polyphenolic compounds by some enzymes, which results in the formation of catechins, flavonoids, and other compounds with beneficial properties, including antioxidant properties, as a result of a microbial hydrolysis reaction. The kind of biological material determines the abundance of phytochemicals (Samiyarsih et al., 2020).

Figure 5. showed the total flavonoid content of the TGF kombucha, TGF15% in 14 daf have the highest and TGF5% in 7 daf have the lowest content, 1.70±0.06 QAEq/mL and 1.47±0.04 QAEq/mL respectively (R^2=0.9876). Flavonoids and derivatives are known to contribute to the antioxidant content of the t flower. This study confirms the observations carried out by Chakravorty et al. (2016): During fermentation, polyphenols, especially flavonoids, rise, while thearubigin is converted into theaflavin, resulting in a shift in kombucha color from dark to light as fermentation progresses. Polyphenols and flavonoid levels in cascara kombucha were found to be significantly higher after 14 days of fermentation, confirming prior research that fermentation increases polyphenols and flavonoid fermentation catechins, flavonoids, and so on, which boost Kombucha's antioxidant capacity (Hur et al., 2014).

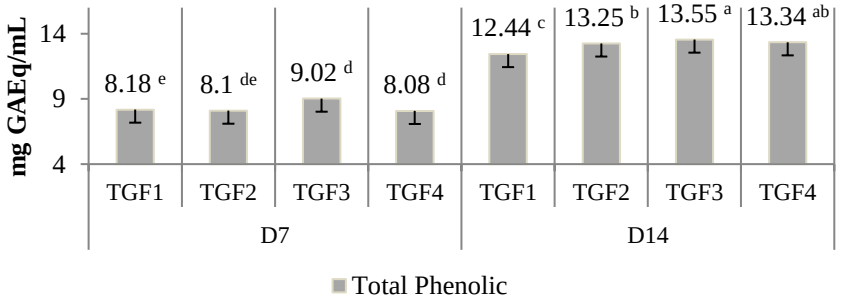

Figure 4. Total Phenolic Compounds of TGF Kombuchas During Fermentation
Notes: The bars followed by the same letters are not significantly different at $p < 0.05$. TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

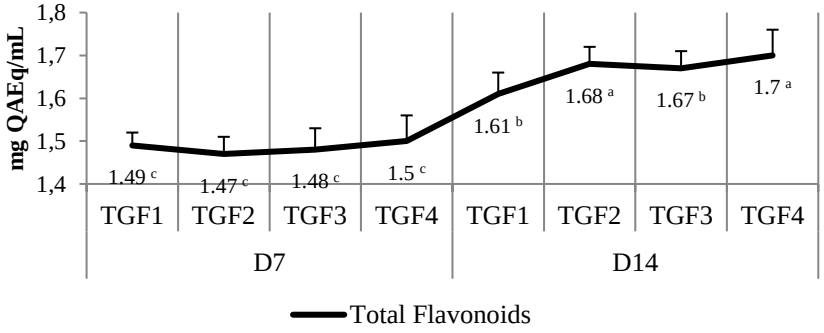

Figure 5. Total Flavonoids Compounds of TGF Kombuchas During Fermentation
Notes: The bars followed by the same letters are not significantly different at $p < 0.05$. TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

Antioxidant activity using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and 2,2′-Azino-bis 3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) assay

Table 2. shows the DPPH radical scavenging activity and ABTS activity obtained on 7 and 14 daf of torch ginger kombucha.

Table 2. Radical scavenging activity based on DPPH and ABTS values of TGF kombuchas during fermentation.

Fermentation time (day)	Kombuchas sample	Antioxidative activity DPPH (%)	IC_{50} DPPH (µg/mL)	Antioxidative activity ABTS (%)
7	TGF1	58.11±0.07		43.03±0.91
	TGF2	60.92±0.01		43.07±0.87
	TGF3	61.14±0.06		44.15±1.12
	TGF4	60.82±0.03		45.07±0.74
14	TGF1	81.70±0.07		60.02±0.84
	TGF2	83.12±0.01		61.10±0.59
	TGF3	85.92±0.07	148.95±11.30	63.05±0.97
	TGF4	84.30±0.03		60.09±0.52

The means followed by the different letters in a column are significant (LSD test; $p < 0.05$). TGF1 (0%), TGF2 (5%), TGF3 (10%), TGF4 (15%). D7 (7 days), D14 (14 days).

The fermenting duration of TGF kombucha affects the increase of antiradical capabilities. In contrast, the various TGF concentrations are unaffected. The antioxidative activity in 7 and 14 days ranged from 58.11% to 61.14% and 81.70% to 85.92% DPPH inhibition, respectively. The ABTS show activity in 7 and 14 daf, between 43.03% to 45.07% and 60.02% to 63.5% respectively. These antioxidant activities (Table 2.) correlated with the samples' phenolic and flavonoid contents (Figure 4-5). The IC_{50} concentration required to inhibit the free radical DPPH by 50%, 148.95 ug/mL. It belongs to the moderate antioxidant category. Furthermore, the results of the DPPH ABTS-antioxidant activity are consistent with previous work done by Chakravorty et al. (2016); they also found that The DPPH and ABTS radicals' scavenging activity increased by 39.7% and 38.36%, respectively, after 21 days. Hur et al. (2014) state that fermentation causes the structural breakdown of plant cell walls, releasing or producing numerous antioxidant chemicals. These antioxidant chemicals include free radical terminators, metal chelators, singlet oxygen quenchers, and hydrogen donors. Fermentation with metal ion chelation activity may influence the generation of protease, -amylase, and other enzymes. Kombucha's antioxidant efficacy varies depending on the kind and composition of the tea infusion prior to fermentation, as well as the SCOBY content, which defines the character of the forming metabolites and conditions the type of the forming products of polyphenol compound transformation (Jakubczyk et al., 2020).

Sensory Attributes of TGF Kombuchas

The results of Friedman's analysis showed that the treatment significantly affected the color, taste, flavor, odor, and preference (Figure 6). Finally, the optimal fermentation duration is crucial for the production of torch ginger kombucha. Our results indicated that 7–14 days of fermentation is suitable for achieving the desired sensory characteristics, such as color, taste, flavor, odor, and preference. Among all the evaluated sensory parameters, statistically significant differences were observed among torch ginger kombucha. The TGF10%-14 daf of TGF kombucha performs best in all senses (color, taste, flavor, odor, and preference). The flavor of kombucha tea changes from a delicious, fruity, acidic, and frothy flavor to a light vinegar-like flavor as fermentation develops, enhancing consumer tolerance of the flavor and other sensory elements of the drink (Marsh et al., 2014). Adding a different concentration substrate of torch ginger flower affects the sensory qualities of Kombucha, including its color, taste, flavor, odor, and preference, resulting in distinct color characteristics between samples.

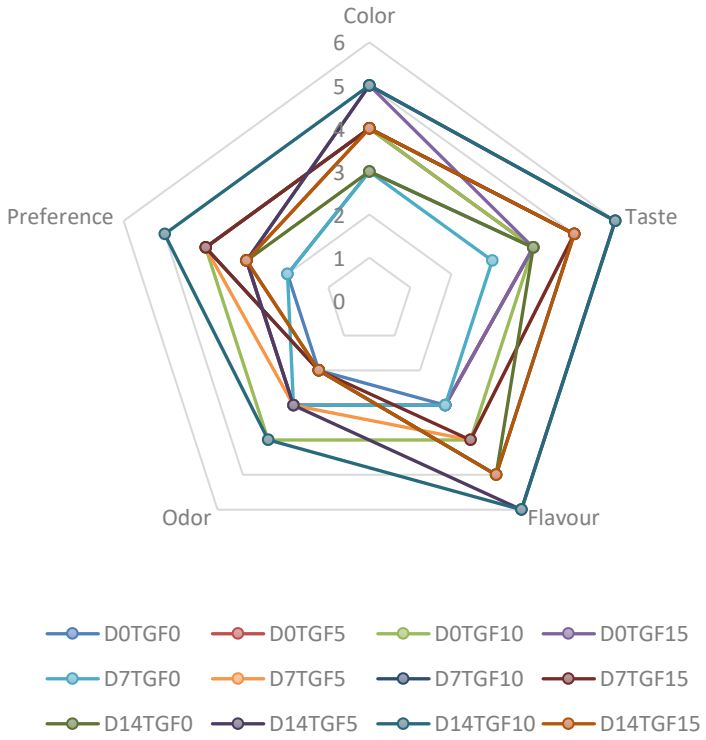

Figure 6. Spider web score of the average sensory steeping variable of torch ginger kombuchas in various combinations of concentration and incubation time.

Above all, it is essential to investigate the beverage's potential health benefits. As can be observed, most research studies have looked into the potential health benefits of Kombucha through in vitro studies. As a result, it is essential to emphasize that the results of in vivo and clinical research may differ significantly. Finally, in recent years, particular efforts have been made to research this attractive beverage. Kombucha beverages may be considered a model system for studying symbiotic interactions between microbes in the future, as well as symbiotic beverages for preventing degenerative diseases.

CONCLUSION

Reduced fermentation duration to 7 days results in lower antioxidant activity and phenolic and flavonoid content. As a result, increasing the fermentation time to 14 days is recommended to improve the overall quality and possible health benefits of torch ginger flower (*E. elatior*) kombucha. Our results showed that torch ginger flower kombucha might be a functional beverage for promoting gut health due to its symbiotic beverage. These findings suggested that consumption of torch ginger kombucha might confer benefits in preserving a healthy gut microbiota.

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