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Research Article

NOVEL TAMARILLO-RED GINGER KOMBUCHA: IMPACT OF CARBON SOURCE VARIATIONS ON PHYSICOCHEMICAL CHARACTERISTICS, ANTIOXIDANT POTENTIAL, AND SENSORY ACCEPTANCE

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Tamarillo and red ginger are rich in nutrients and high in antioxidants, so they have the potential to be used as raw materials for kombucha. This study aims to determine the effect of carbon source variation and substrate formulation of tamarillo-red ginger and to determine the treatment that produces the best tamarillo-red ginger kombucha drink. With carbon source variations in the form of coconut sugar and honey, the substrate formulation used consisted of three levels (tamarillo: red ginger) = F1 (87.5 : 12.5), F2 (75 : 25), F3 (50 : 50), and control sample (F0) with green tea substrate. During 12 days of fermentation, the variation of carbon source had a significant effect on kombucha characteristics. Compared to honey, coconut sugar excelled in various kombucha characteristics, especially in antioxidant activity and sensory parameters. Meanwhile, the tamarillo-red ginger substrate formulation generally did not have a significant effect on kombucha characteristics. Sample F1 of kombucha with coconut sugar carbon source was the best treatment of kombucha with tamarillo-red ginger substrate formulation.

1. Introduction

Tea (*Camelia sinensis* L.) is the main substrate for kombucha production. However, many studies have used other raw materials as substrates to produce kombucha. The effect of adding fruit juice is reported to increase the antioxidant potential of the fermented beverage (Ayed & Hamdi, 2015). Tamarillo (*Solanum betaceum*) is rich in vitamins A, B6, C and E, as well as minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, and zinc (Vasco *et al.*, 2009). The antioxidant activity of tamarillo is higher than antioxidant-rich fruits such as kiwi fruit, oranges, and grapes (Diep *et al.*, 2020). Tamarillo also contains anthocyanins, an antioxidant compound with 2-6 times greater antioxidant activity, compared to other common antioxidants such as ascorbic acid and glutathione (Prior *et al.*, 1998). Meanwhile, ginger can be utilized as a functional beverage because of its rich nutritional content, flavor, and health benefits. Ginger rhizomes contain very high amounts of total antioxidants (3.85 mmol/100 g). The antioxidant activity of red ginger is higher than that of elephant ginger and emprit ginger, with 88.26% inhibition of DPPH (Wahyuningsih *et al.*, 2018).

Kombucha with coconut sugar carbon source produced the highest antioxidant activity of 49%, compared to molasses (33%) and refined sugar (29%) (Muhialdin *et al.*, 2019). Meanwhile, the addition of honey will provide a better aroma, flavor, and composition of kombucha drinks (Putri *et al.*, 2023). In this study, fermentation was carried out for 12 days because it can produce more phenolics and increase the antioxidant activity of the drink (Muhialdin *et al.*, 2019). Based on this background, this study aims to evaluate the eff ect of variations in carbon sources and the potential of **tamarillo-red ginger (TRG)** in making kombucha drinks fermented for 12 days, especially through physicochemical and sensory examinations so that it is necessary to find the optimum formulation to produce kombucha drinks with the best quality characteristics.

2. Materials and methods

2.1. Materials

2.1.1. Honey and SCOBY Preparations

The ingredients used are mineral water, green tea bags "PRENDJAK", pure honey "Madu TJ", and coconut sugar “Gold Palm”. Tamarillo was obtained from Batu City, East Java, and red ginger were obtained from Pasar Gede Solo, Central Java. The starter culture used came from previous research, consisting of tea fungus (SCOBY) inoculated into tea broth and incubated at room temperature for 14 days.

2.2. Methods

2.2.1. Preparation of control kombucha tea

Control kombucha tea was made by combining green tea at 1% w/v was brewed in hot water. After reaching room temperature (±25 °C), 10% w/v coconut sugar or honey was added to the tea broth into sterile glass jars, and then pasteurized (85 °C, 15 minutes). After reaching room temperature, 10% v/v liquid kombucha starter and 3% w/v SCOBY were added. Then, the glass jar was covered with tissue and fermented for 12 days at room temperature.

2.2.2. Preparation of tamarillo-red ginger (TRG) substrate

The tamarillos were washed and cut into small pieces. Then, crushed with a blender, with a water:fruit ratio of 1:1. Next, filtered until the juice is obtained without pulp (Tamarillo juice preparation). Red ginger that has been washed and peeled clean of its skin, then sliced thinly and then blanching with hot water (±90 ˚C, 3 minutes). After that, the red ginger slices were baked (60 ˚C, 5 hours), then blended with red ginger:water of 1:2, and filtered. The result was red ginger juice without pulp (red ginger juice preparation).

2.2.3. Tamarillo-red ginger kombucha production

The tamarillo-red ginger kombucha was prepared by combining 10% v/v of tamarillo juice and red ginger juice with mineral water. Next, 10% w/v coconut sugar and honey were added to each sterile glass jar, and then pasteurized (85 °C, 15 minutes). After reaching room temperature (±25 °C), 10% v/v liquid kombucha starter and 3% w/v SCOBY were added. Then, the glass jar was covered with tissue and fermented for 12 days at room temperature (±25 °C).

2.2.4. Physical test

Pellicle Weight Growth (SCOBY): SCOBY weight growth = (M1 - M0)/M0 x 100%, where M1 = final mass, and M0 = initial mass (Muhialdin et al., 2019). Total Dissolved Solids (TDS): the sample is dripped onto the refractometer prism and the degree of Brix is measured (Wahyudi and Dewi, 2017).

2.2.5. Chemical test

Degree of Acidity (pH): before use, the pH meter is calibrated using pH 4.0 and pH 7.0 buffers. Every time you measure the pH of another sample, the pH meter must always be rinsed with distilled water (Fardiaz et al., 1992). Total Titratable Acid (TTA): Total titratable acid was performed using acid-base titration according to Rosada et al. (2023). In the initial stage, 1 ml of kombucha sample was taken and added with 10 ml of distilled water. Next, 1% PP indicator as much as 2-3 drops was added. Then, titrated with 0.1 N NaOH solution until the solution changes color to pink. Total Phenolic Compound (TPC): Polyphenols were determined by the Folin-Ciocalteu procedure. A total of 20 μL of the diluted extract was mixed with 20 μL of Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Then, 200 μL of 7% Na2CO3 was added, then vortexed. After incubation at room temperature for 1 hour in the dark, the absorbance was measured at 750 nm using a microplate reader, with gallic acid as the standard. Total phenolic compounds were expressed in mg GAE/mL (Gallic Acid Equivalent/mL) (Sari et al., 2023).

2.2.6. Antioxidant test

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Method: DPPH assay was performed according to Chu & Chen (2006) and (Frediansyah et al., 2023). Kombucha tea (0.025 mL) was mixed with 0.6 mL of 1.01 mM DPPH solution in a 96-well micro-titre plate. Afterwards, the samples were incubated for 30 min under dark conditions at room temperature. Next, the sample was vortexed and absorbed at 517 nm. The same treatment was also carried out for the blank solution (DPPH solution containing no test material).

The 2,2′-azino-bis-(3-ethylbenzothi-azoline-6-sulfonic)acid (ABTS) Method: kombucha samples (10 μL) were mixed with ABTS solution (90 μL). After 30 minutes of incubation at room temperature, absorbance was taken at 734 nm (Re *et al.*, 1999) and (Fitrianto et al., 2024).

Ferric Reducing Antioxidant Power (FRAP) Method: FRAP reagent (300 μL) and kombucha tea (10 μL) were mixed thoroughly and incubated under dark conditions for 30 min. Afterwards, the absorbance was measured at 593 nm using an ELISA reader (Benzie & Strezo, 1999).

2.2.7. Ogranoleptic test/ sensory test

Sensory analysis was conducted using the hedonic method with a 9-point hedonic scale. The number 1 represents extremely dislike and the number 9 represents extremely like. Thirty-five untrained panelists participated in the sensory evaluation. The kombucha samples were labeled with a three-digit code and presented randomly. Each panelist was given approximately 10 mL of kombucha drink and 220 mL of mineral water as a neutralizer. Each panelist scored the color, aroma, *acidity* and *sweetness*, *aftertaste*, and *overall* of the kombucha drink samples.

2.3. Experimental design and data analysis

This study used a completely randomized design (CRD) consisting of two factors, namely variations in carbon source and TRG substrate formulation. Observation data were statistically analyzed using the *Two Way Analysis of Variances* (ANOVA) method. If there was a significant difference in each treatment, *Duncan's Multiple Range Test* (DMRT) analysis was conducted at the 95% confidence level with α = 0.05. To determine the sample with the best formulation based on the results of the total phenol test, antioxidant activity, and sensory, an effectiveness index test was conducted using the method of de Garmo *et al.* (1984).

3. Results and discussions

3.1. Pellicle Weight Growth (SCOBY)

Based on the results in **Table 1**, the mean weight growth of SCOBY on coconut sugar carbon source is higher than that on honey. According to Jarrell *et al.* (2022), sucrose produces much heavier pellicles compared to other types of sugar. The high sucrose content and the presence of a number of amino acids that can be utilized as nitrogen sources cause coconut sugar to produce larger cellulose pellicles. The high fructose content in honey results in greater ethanol content, which can inhibit the growth of cellulose-producing bacteria (Leal *et al.*, 2018). The high cellulose production in tea substrate is due to the presence of caffeine in tea (Mamlouk *et al.*, 2013). Meanwhile, the low *SCOBY* weight on TRG substrate could be due to the more acidic final pH, resulting in *A. xylinum* metabolism not being optimum (Zahan *et al.*, 2015).

Table 1. Pellicle Weight Growth (SCOBY) after 12 Days of Fermentation

SCOBY Weight Growth (%)	Coconut Sugar	Honey	Mean
F0	203.37 ± 23.51	233.70 ± 21.25	218.54[b]
F1	188.96 ± 12.73	94.48 ± 24.62	141.72[a]
F2	207.81 ± 30.39	110.67 ± 22.76	159.24[a]
F3	192.19 ± 17.40	106.93 ± 9.46	149.56[a]
Mean	198.08[B]	136.44[A]	

3.2. Total Dissolved Solids (TDS)

Based on Table 2, the mean TDS of coconut sugar carbon source is higher than that of honey. TDS indicates the content of water-soluble materials such as reducing and non-reducing sugars, organic acids, pectins, proteins, and other soluble compounds (Nurhayati *et al.*, 2020). Maryani *et al.* (2021) reported that coconut sugar is high in sucrose content (86.86%). TDS in coconut sugar kombucha samples can be higher because metabolism during fermentation still leaves a lot of metabolic products in the form of sucrose, reducing sugars, and other minerals.

Table 2. Total Dissolved Solids (TDS) of Kombucha on Day 1 and Day 12 of Fermentation

TDS (°Brix)	Day 1			Day 12		
	Coconut Sugar	**Honey**	**Mean**	**Coconut Sugar**	**Honey**	**Mean**
F0	6.057 ± 0.82	5.443 ± 1.20	5.750[a]	9.833 ± 0.29	7.667 ± 0.33	8.750[b]
F1	9.833 ± 0.29	7.833 ± 0.76	8.833[bc]	8.833 ± 0.29	6.500 ± 0.17	7.667[a]
F2	10.000 ± 1.32	9.723 ± 1.57	9.862[c]	9.000 ± 0.00	6.167 ± 0.44	7.583[a]
F3	9.000 ± 1.32	7.333 ± 0.76	8.167[b]	8.667 ± 0.17	6.333 ± 0.93	7.500[a]
Mean	8.723[B]	7.583[A]		9.083[B]	6.667[A]	

The high TDS in green tea kombucha is because green tea brew contains soluble solids such as sugars, amino acids, caffeine, phenolic compounds, vitamins, and other minor compounds (Kim *et al.*, 2016). The longer the fermentation, the TDS value will decrease because the available nutrients are used for microbial growth and metabolism (Jayabalan *et al.*, 2014). Meanwhile, the increase in TDS during the fermentation process is because the hydrolysis of sucrose into glucose and fructose by *yeast* has not been used for microbial metabolism so that the measured TDS becomes greater (Jessica *et al.*, 2020).

3.3. Degree of Acidity (pH)

Honey is rich in various organic acids such as citric acid, lactic acid, malic acid, succinic acid, and fumaric acid. The high glucose content in honey (26.86-31.38%) will result in higher levels of gluconic acid, resulting in a decrease in pH (Mate *et al.*, 2018). During processing, the glucose oxidase enzyme in honey cannot be destroyed by hot or acidic conditions. As a result, the acidity of honey will increase (Bouhlali *et al.*, 2019). Based on Table 3, TRG substrate formulation does not significantly affect the pH value of kombucha because the composition of the formulation is not much different. The longer the fermentation, the pH of kombucha will decrease due to the conversion of sugar by bacteria and *yeast* into various organic acids (Jayabalan *et al.*, 2014).

Table 3. Degree of Acidity (pH) of Kombucha on Day 1 and Day 12 of Fermentation

pH	Day 1			Day 12		
	Coconut Sugar	**Honey**	**Mean**	**Coconut Sugar**	**Honey**	**Mean**
F0	3.833 ± 0.04	3.620 ± 0.00	3.726[a]	3.732 ± 0.01	3.293 ± 0.02	3.513 [a]
F1	4.043 ± 0.08	3.893 ± 0.02	3.968[c]	3.718 ± 0.03	3.159 ± 0.05	3.438[b]
F2	3.932 ± 0.01	3.856 ± 0.01	3.894[b]	3.715 ± 0.03	3.137 ± 0.00	3.426[b]
F3	3.929 ± 0.05	3.778 ± 0.01	3.853[b]	3.703 ± 0.01	3.112 ± 0.01	3.408[b]
Mean	3.934[B]	3.787[A]		3.717[B]	3.175[A]	

3.4. Total Titratable Acid (TTA)

Honey contains high levels of fructose and glucose that can increase the production of a number of organic acids, resulting in an increase in the total acid value of kombucha (Pebiningrum & Kusnadi, 2017). TRG substrate formulation produces a significantly different effect on the TTA value during 12 days of kombucha fermentation due to differences in the level of organic acid production (Velicanski *et al.*, 2014). Based on Table 4, the higher the concentration of red ginger extract, the pH value will increase and the total acid will decrease (Chasparinda *et al.*, 2014). The longer the fermentation, the acid content will increase because more organic acid compounds in kombucha are formed, especially acetic acid (Jayabalan *et al.*, 2014).

Table 4. Total Titratable Acid of Kombucha on Day 1 and Day 2 of Fermentation

TTA (%)	Day 1			Day 12		
	Coconut Sugar	**Honey**	**Mean**	**Coconut Sugar**	**Honey**	**Mean**
F0	1.667 ± 0.11	1.533 ± 0.30	1.601[a]	4.870 ± 1.68	4.400 ± 0.35	4.635[a]
F1	2.333 ± 0.11	2.133 ± 0.11	2.202[b]	7.410 ± 0.53	14.943 ± 0.42	11.177[c]
F2	2.200 ± 0.00	2.067 ± 0.23	2.168[b]	7.343 ± 0.11	14.610 ± 0.72	10.977[c]
F3	2.133 ± 0.23	2.000± 0.20	2.068[b]	7.010 ± 0.87	12.210 ± 0.40	9.610[b]
Mean	2.085[B]	1.935[A]		6.658[A]	11.541[B]	

3.5. Total Phenolic Compounds (TPC)

Based on Table 8. and Table 9., the mean total phenolics in coconut sugar carbon source is higher, compared to honey. During 12 days of fermentation, total phenolics in coconut sugar samples decreased due to polymerization of some phenolic compounds into molecules with higher molecular weight, leading to the detection of lower polyphenol content (Degirmen-cioglu *et al.*, 2020). Meanwhile, the addition of honey is thought to bind the phenolic components in the kombucha substrate, forming a glycoside bond between sugar and phenol (phenol glycoside), which has an effect on increasing the total phenol value of kombucha (Ferreira *et al.*, 2009). Green tea is rich in antioxidant activity. Oxidation of polyphenolic compounds by several enzymes results in the formation of catechins, flavonoids, and other simpler compounds (Samiyarsih *et al.*, 2020). Phenolic compounds bound to cell wall structural components such as cellulose, lignin, and protein through ester bonds will be released into free phenolic acids, resulting in an increase in total phenolic compounds (Nurhayati *et al.*, 2020).

Table 5. Total Phenolic Compounds of Kombucha on Day 1 and Day 12 of Fermentation

TPC (mg GAE/mL sample)	Day 1			Day 12		
	Coconut Sugar	**Honey**	**Mean**	**Coconut Sugar**	**Honey**	**Mean**
F0	18.595 ± 1.62	10.848 ± 0.61	14.722^{b}	33.684 ± 2.66	27.186 ± 2.30	30.435^{b}
F1	17.840 ± 1.07	9.620 ± 0.98	13.730^{ab}	15.966 ± 0.81	10.553 ± 1.65	13.259^{a}
F2	14.920 ± 0.51	10.662 ± 0.65	12.791^{a}	15.536 ± 0.58	11.219 ± 0.96	13.378^{a}
F3	17.422 ± 2.62	9.030 ± 0.32	13.226^{ab}	13.916 ± 1.99	12.249 ± 1.48	13.082^{a}
Mean	17.194^{B}	10.040^{A}		19.775^{B}	15.302^{A}	

3.6. Antioxidant Activity Test

Based on Figure 1 and Figure 2, the mean antioxidant activity of coconut sugar carbon source is higher than that of honey. Similar results were obtained by Muhialdin *et al.* (2019) in the form of the greatest antioxidant activity obtained in kombucha samples with coconut sugar (49.26%). The high antioxidant content can be due to coconut sugar contains a large number of phenolic compounds in the form of phenolic acids, as well as flavonoid compounds in the form of rutin, hesperetin, myricetin, quercetin, and apigenin. Coconut sugar also contains catechins amounting to 2.37 mg/100 g, which further plays a role in increasing its antioxidant activity (Hebbar *et al.*, 2020).

In TRG kombucha samples (F1, F2, and F3) there was no increase in antioxidant activity during fermentation. This can occur because acidic conditions in TRG kombucha can reduce antioxidant activity. In acidic conditions, phenolic compounds become more stable and difficult to release protons that can bind with DPPH so that antioxidant activity decreases (Ayu *et al.*, 2013). Meanwhile, the high antioxidant activity in green tea kombucha is because tea is rich in flavonoid compounds, with epigalocatechin gallate (EGCG) being the main catechin in tea (Fadhilah *et al.*, 2021). In another study, the Kombucha of torch ginger flower demonstrated high antioxidant activity using DPPH and ABTS at 85.92±0.07% and 63.05±0.97 respectively (Fitrianto et al., 2023).

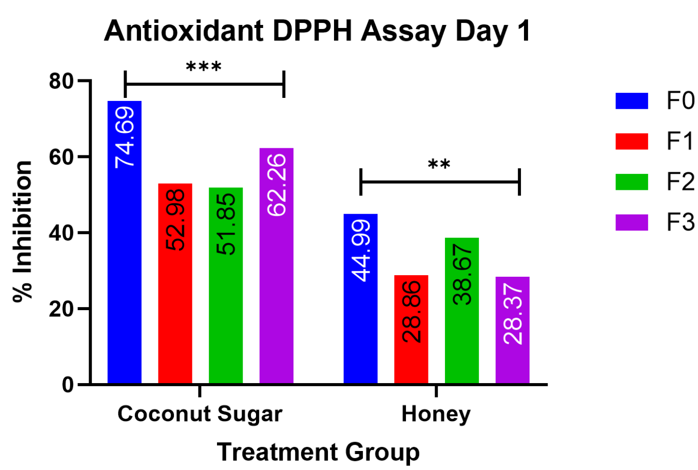

Figure 1. Antioxidant Activity of Kombucha on Day 1 of Fermentation (DPPH Test)

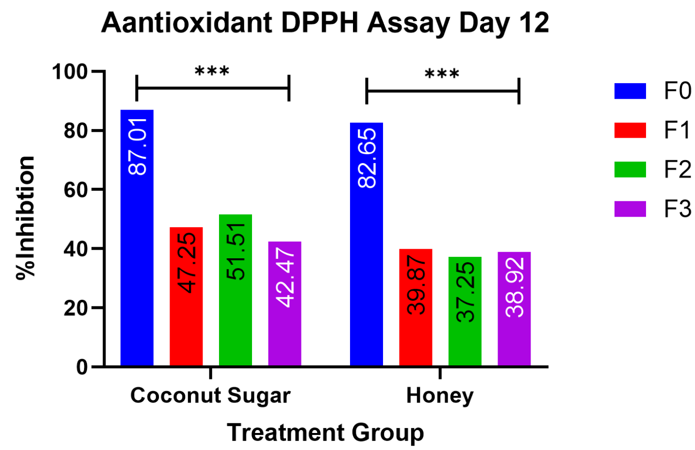

Figure 2. Antioxidant Activity of Kombucha after 12 Days of Fermentation (DPPH Test)

Figure 3 and Figure 4 show that in the ABTS method, the mean antioxidant activity of coconut sugar carbon source is higher than that of honey. The high antioxidant activity in green tea is due to the presence of polyphenols which are mostly represented by flavonol group, especially catechins (Wierzejska, 2014). Based on Table 14 and Table 15, the mean antioxidant activity of coconut sugar carbon source is higher than that of honey because coconut sugar is rich in antioxidant compounds such as vitamins C and E, vitamins K1 and K2; a number of essential amino acids and mineral substances, and phytonutrients such as antioxidants, flavonoids, anthocyanidins, and polyphenols. Coconut sugar also has a high *ferric-reducing antioxidant power* (mg ascorbic acid equivalent) value of 22.9/100 g coconut sugar (Hebbar *et al.*, 2022). Epigalocatechin gallate (EGCG) is the main catechin in tea and is the most powerful antioxidant (Min & Kwon, 2014), with its antioxidant power reaching up to 200 times stronger than other antioxidants such as vitamins C and E (Gramza *et al.*, 2005). Meanwhile, the results of FRAP testing in Figures 5 and 6, show that antioxidant activity in both groups with coconut sugar and honey treatment was highest in F0. Antioxidant activity is important to be able to protect the body from free radical attack *(*Ratnaningtyas *et al.,* 2024) which may cause inflammation reactions (Ratnaningtyas *et al.,* 2025).

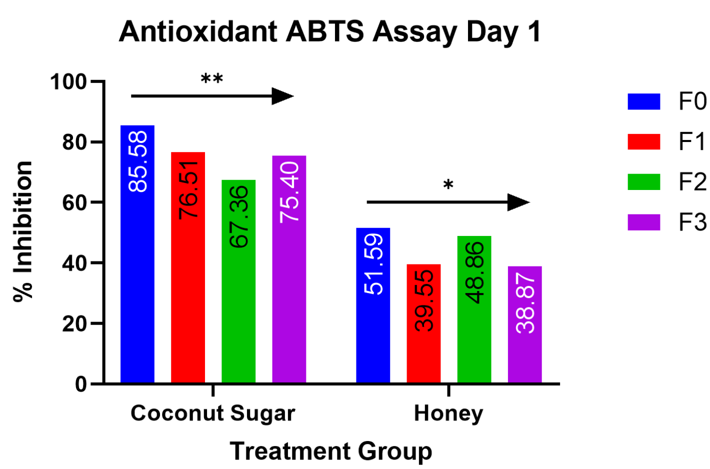

Figure 3. Antioxidant Activity of Kombucha on Day 1 of Fermentation (ABTS Test)

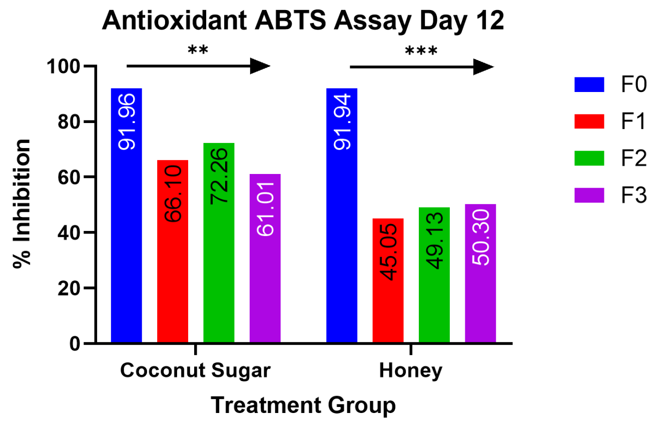

Figure 4. Antioxidant Activity of Kombucha after 12 Days of Fermentation (ABTS Test)

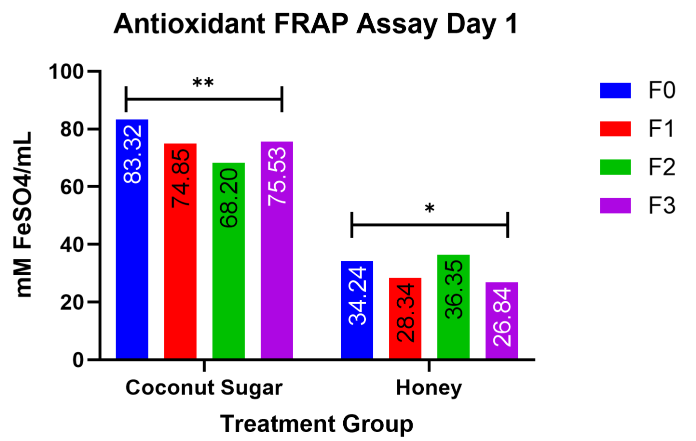

Table 5. Antioxidant Activity of Kombucha on Day 1 of Fermentation (FRAP Test)

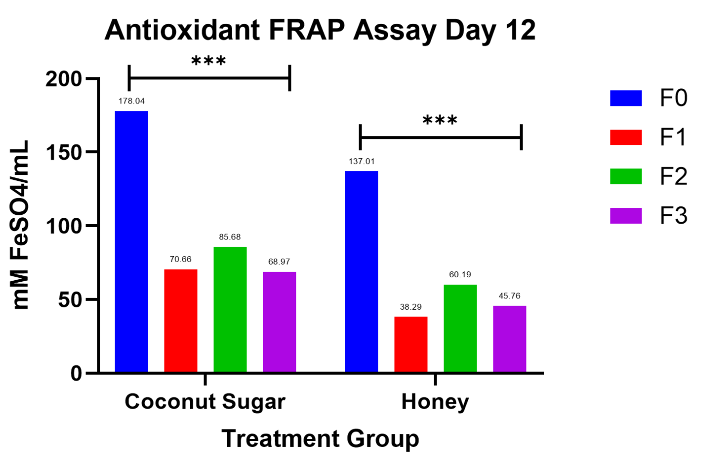

Figure 6. Antioxidant Activity of Kombucha after 12 Days of Fermentation (FRAP Test)

3.7. Organoleptic Test/ Sensory Test

Based on Table 6, the difference in kombucha color only occurs in carbon source variations, with higher color sensory values in the coconut sugar carbon source. The color of the substrate of origin affects the color of the product and changes in the color value of the beverage during fermentation (Ozyurt, 2020). The variation of carbon sources as shown in Table 6 has a significant effect on the color of the kombucha solution because from the initial raw materials, coconut sugar and honey are already seen to have quite a difference in color. The acidic atmosphere causes catechin degradation so that the color of the kombucha solution will be brighter during the fermentation process (Kallel *et al.*, 2012). Based on Table 6, there is no difference in the aroma of kombucha in each formulation and carbon source used. During fermentation, bacteria and *yeast* metabolize sugars resulting in the formation of volatile compounds that contribute to the aroma of kombucha such as aldehydes, alcohols, aldehydes, ketones, esters, benzene compounds, and organic acids (Zhao *et al.*, 2018).

Table 6. Sensory Test Results for Color and Aroma Parameters

Group	Color			Aroma		
	Coconut Sugar	Honey	Mean	Coconut Sugar	Honey	Mean
F0	6.34 ± 1.75	5.46 ± 2.03	5.900[a]	4.97 ± 1.82	5.63 ± 1.68	5.300[a]
F1	6.40 ± 1.61	4.77 ± 2.06	5.585[a]	5.77 ± 1.65	5.26 ± 1.79	5.515[a]
F2	6.14 ± 1.52	5.09 ± 1.54	5.615[a]	5.51 ± 1.85	5.23 ± 1.70	5.370[a]
F3	6.34 ± 1.68	4.97 ± 1.74	5.655[a]	5.91 ± 1.77	5.57 ± 1.77	5.740[a]
Mean	6.305[B]	5.073[A]		5.540[A]	5.423[A]	

Table 7. Sensory Test Results for Acidity and Sweetness Parameters

Group	Acidity			Sweetness		
	Coconut Sugar	Honey	Mean	Coconut Sugar	Honey	Mean
F0	5.80 ± 2.07	4.51 ± 1.82	5.155[c]	5.86 ± 2.12	4.46 ± 1.85	5.160[c]
F1	6.34 ± 1.55	3.17 ± 1.77	4.755[bc]	6.40 ± 1.58	3.20 ± 1.69	4.800 [bc]
F2	4.49 ± 2.04	2.97 ± 1.71	3.730[a]	4.57 ± 1.99	3.46 ± 1.93	4.015[a]
F3	5.37 ± 1.82	3.54 ± 1.70	4.455[b]	4.83 ± 1.79	3.63 ± 1.59	4.230[ab]
Mean	5.500[B]	3.548[A]		5.415[B]	3.688[A]	

Table 8. Sensory Test Results for Aftertaste and Overall Parameters

Group	Aftertaste			Overall		
	Coconut Sugar	Honey	Mean	Coconut Sugar	Honey	Mean
F0	5.46 ± 2.23	4.91 ± 1.70	5.185[b]	5.89 ± 2.00	5.03 ± 1.56	5.460[c]
F1	6.40 ± 1.54	3.51 ± 1.58	4.955[b]	6.51 ± 1.60	3.34 ± 1.64	4.925[bc]
F2	4.11 ± 2.04	3.43 ± 1.77	3.770[a]	4.54 ± 1.92	3.60 ± 1.52	4.070[a]
F3	4.31 ± 2.00	3.69 ± 1.55	4.000[a]	4.97 ± 1.89	4.11 ± 1.47	4.540[ab]
Mean	5.070[B]	3.885[A]		5.478[B]	4.020[A]	

Based on Figure 7 (spiderweb), it shows that the kombucha sensory test results are in the value range of 2.5 - 7.0. The highest sensory value is mainly in F2GK, which is almost high on each test parameter, while the lowest is the F2M group. Table 7 show that there are significant differences in the *acidity* and *sweetness* parameters of kombucha samples in each formulation and carbon source used, with coconut sugar carbon source producing greater sensory value. The longer the fermentation, the more acidic the kombucha will be due to the metabolic process by bacteria and *yeast* on sucrose to produce a number of organic acids (Jayabalan *et al.*, 2014).

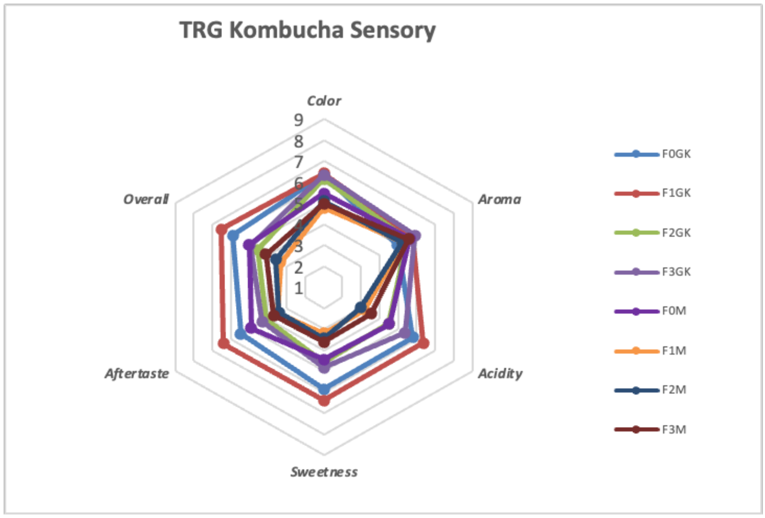

Figure 7. Kombucha Sensory Spiderweb Diagram

Notes: Scores: 1 = strongly dislike; 2 = strongly dislike; 3 = dislike; 4 = somewhat dislike; 5 = neutral; 6 = somewhat like; 7 = like; 8 = strongly like; and 9 = strongly like.

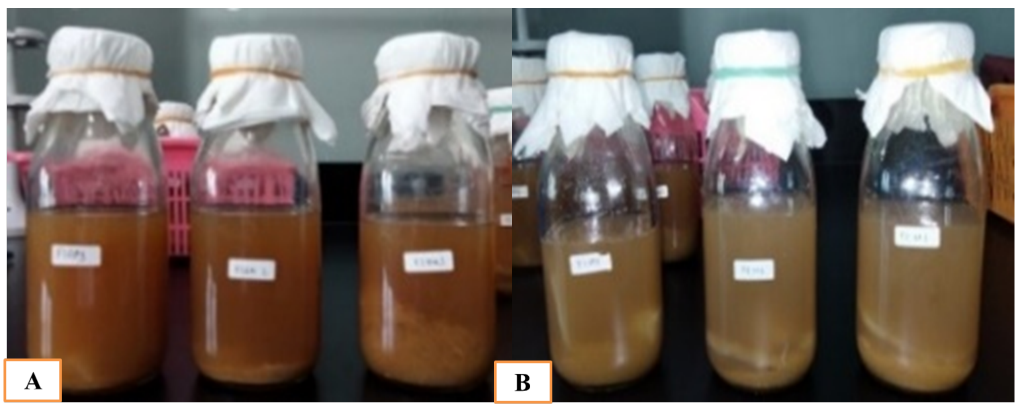

Figure 8. Kombucha Drink Samples of Coconut Sugar (A) and Honey (B) after 12 Days of Fermentation

Table 8 shows the sensory test value of the aftertaste and overall parameter, with the coconut sugar carbon source producing a greater sensory value. In Tetelepta's research (2024), the higher the concentration of red ginger extract will produce beverage products that are very red ginger flavored and reduce the level of panelist preference. *Aftertaste* that is too spicy and slightly bitter can reduce the sweetness and freshness of the beverage product (Kurniasari *et al.*, 2008). Based on Table 9, coconut sugar carbon source produces higher sensory value. Phenolic compounds also have good potential as antioxidants and kill pathogenic bacteria in the body, so kombucha containing high phenolics is very potential. Previous studies have shown that forzen granulation from P. cystidiosus mushroom can act as an antibacterial agent (Ratnaningtyas, Husen, Fitrianto, *et al.,* 2024).

The main objective of principal component analysis (PCA) biplot were generated to demonstrate distinctness between the tamarillo-red gingers kombucha formula based on physicochemical, characteristics, antioxidant potential, and sensory acceptance (Figure 9) The first and second components explained 83.87% and 11.10% of the variation, respectively. Thus, the studied principal components explained 94.08% of the total variation. A PCA biplot was used to identify the features responsible for the clustering patterns. Cluster F1 and F3 were characterized by aroma of sensory acceptance. The most different in variation is cluster F0 was characterized by physicochemical caharacters, antioxidant capacity, phenolic content, and sweetness, aftertaste and overall acceptance.

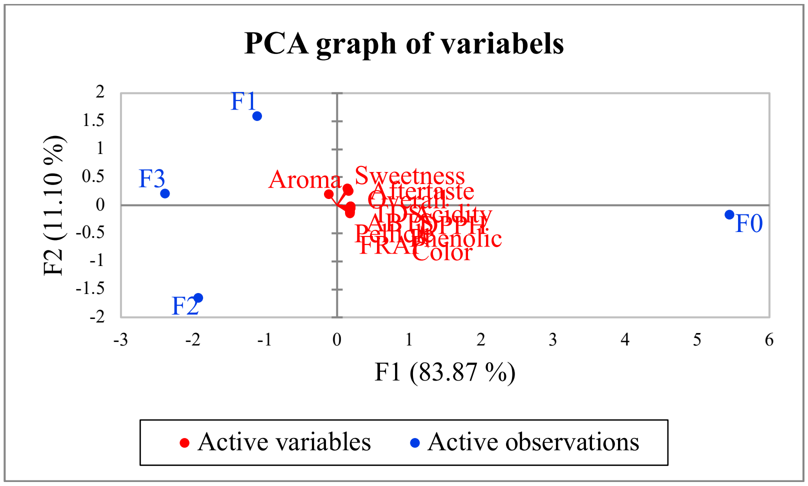

Figure 9. Biplot obtained from Principal component analysis (PCA) showing the correlation between the measured parameters and variation of tamarillo-red ginger kombucha formula. The total dissolved solid (TDS), 2,2-diphenyl-1-picryl hydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS).

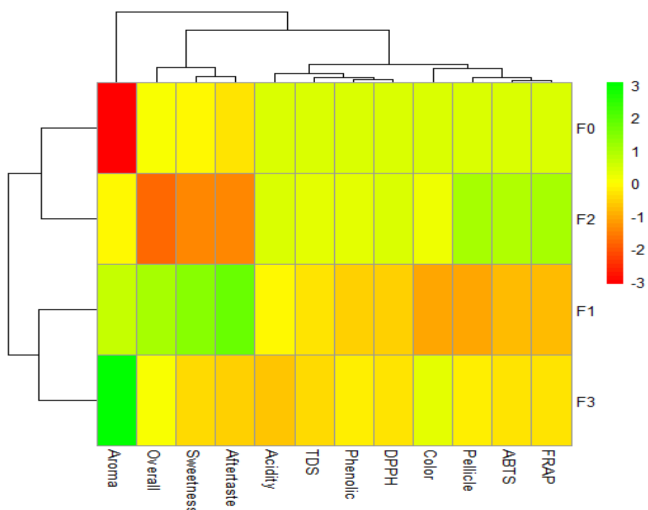

Figure 10. Heat map of the relationship between sample treatments and response variables of tamarillo-red ginger kombuchas formula, including their grouping cluster; color reflects the trend of the treatment effect from the lowest (red) to the highest (green).

The interrelationship between the tamarillo-red gingers kombuchas formula (physicochemical, characteristics, antioxidant potential, and sensory acceptance) based on the similarity of the parameters is visualized in Figure 2. Heat map of the relationship between sample treatments and response variables of tamarillo-red gingers kombucha including their grouping cluster. The formula of tamarillo red ginger F0 and F2 were categorized in the same clustering having the better antioxidant (DPPH, ABTS, FRAP) capacity than F1 and F3 formula. The second cluster generated was FI and F3 formula was shown relatively moderate antioxidant capacity but strong in sensory acceptance (aroma, sweetnes, aftertaste and overall parameter).

Table 9. Calculation of Determination of the Best Treatment of Kombucha Beverage

Parameters	Treatment Value						Best Value	Lowest Score	VW	NW
	F_1CS	**F_2CS**	**F_3CS**	**F_1H**	**F_2H**	**F_3H**				
Antioxidant (DPPH) (%)	47.25	51.509	42.661	39.868	37.246	38.921	51.509	37.246	1.000	0.101
Antioxidant (ABTS) (%)	66.103	72.262	61.013	45.052	49.125	50.303	72.262	45.052	1.000	0.101
Antioxidant (FRAP)	70.659	85.681	68.97	38.281	60.193	45.763	85.681	38.281	1.000	0.101
Total Phenol	15.966	15.536	13.916	10.553	11.219	12.249	15.966	10.553	1.000	0.101
Color	6.40	6.14	6.34	4.77	5.09	4.97	6.40	4.77	1.000	0.101
Aroma	5.77	5.51	5.91	5.26	5.23	5.57	5.91	5.23	0.900	0.091
Acidity	6.34	4.49	5.37	3.17	2.97	3.54	6.34	2.97	1.000	0.101
Sweetness	6.40	4.57	4.83	3.20	3.46	3.63	6.40	3.20	1.000	0.101
Aftertaste	6.40	4.11	4.31	3.51	3.43	3.69	6.40	3.43	1.000	0.101
Overall	6.51	4.54	4.97	3.34	3.60	4.11	6.51	3.34	1.000	0.101
∑	**152.8**	**225.01**	**117.6**	**133.77**	**157.8**	**147.26**	**225.45**	**131.155**	**9.900**	**1.000**

Table 10. Calculation of Determination of the Best Treatment of Kombucha Drink (Continued)

Parameters	F_1CS		F_2CS		F_3CS		F_1H		F_2H		F_3H	
	EV	**RV**	**EV**	**RV**	**EV**	**RV**	**EV**	**RV**	**EV**	**RV**	**EV**	**RV**
Antioxidant (DPPH) (%)	0.201	0.020	0.287	0.029	0.109	0.011	0.053	0.005	0.000	0.000	0.034	0.003
Antioxidant (ABTS) (%)	0.449	0.045	0.580	0.059	0.340	0.034	0.000	0.000	0.087	0.009	0.112	0.011
Antioxidant (FRAP)	0.232	0.023	0.339	0.034	0.220	0.022	0.000	0.000	0.157	0.016	0.054	0.005
Total Phenol	0.234	0.024	0.215	0.022	0.145	0.015	0.000	0.000	0.029	0.003	0.073	0.007
Color	1.000	0.101	0.840	0.085	0.963	0.097	0.000	0.000	0.196	0.020	0.123	0.012
Aroma	0.851	0.077	0.574	0.052	1.000	0.091	0.309	0.028	0.277	0.025	0.638	0.058
Acidity	1.000	0.101	0.451	0.046	0.712	0.072	0.059	0.006	0.000	0.000	0.169	0.017
Sweetness	1.000	0.101	0.428	0.043	0.509	0.051	0.000	0.000	0.081	0.008	0.134	0.014
Aftertaste	1.000	0.101	0.229	0.023	0.296	0.030	0.027	0.003	0.000	0.000	0.088	0.009
Overall	1.000	0.101	0.379	0.038	0.514	0.052	0.000	0.000	0.082	0.008	0.243	0.025
∑		**0.695**		**0.431**		**0.476**		**0.042**		**0.089**		**0.162**

The formulations favored by panelists in order started from formula F2, F3, F1, and formula F0 which was the most preferred sample formulation. Determination of the best treatment was carried out using the effectiveness index test by giving weight to each parameter used (de Garmo et al., 1984). The formula with the largest number of result values is the best formula. Based on Table 10, the effectiveness index test has been carried out on the six kombucha beverage samples by weighting the ten parameters that have been determined. The best TRG kombucha sample was obtained in sample F1GK, a kombucha sample with F1 substrate formulation (TB:JM = 87.5:12.5) with a carbon source of coconut sugar.

4. Conclusions

Kombucha fermentation for 12 days showed that the variation of carbon sources (coconut sugar and honey) had a significant effect on all test parameters, except for the sensory characteristics of kombucha aroma. Meanwhile, after 12 days of fermentation, the TRG substrate formulation only had a significant effect on physicochemical characteristics in the form of TTA, antioxidant activity (ABTS, and FRAP), and sensory characteristics in the form of color, *acidity*, *sweetness*, *aftertaste*, and *overall*. The best TRG kombucha drink treatment was obtained in sample F_1CS, kombucha drink with coconut sugar carbon source and substrate formulation of tamarillo: red ginger of 87.5: 12.5 parts.

5. References

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6. Ethical Approval

This study was conducted after obtaining ethical permission from the Health Research Ethics Commission of Dr. Moewardi Hospital, with ethical clearance number: 318/I/HREC /2024.