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Fermentation Time and Antioxidant Activity of Pineapple and Dragon Fruit Peel Kombucha

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ABSTRACT

Fruit-peel kombucha may provide antioxidant activity while valorizing agro-industrial waste; however, prolonged fermentation may also increase ethanol accumulation. This study evaluated kombucha prepared from pineapple peel juice, red dragon fruit peel juice, and their combination after 0, 8, and 14 days of fermentation. Descriptive sensory characteristics, pH, flavonoid screening, ethanol content, and DPPH radical-scavenging activity at 517 nm were assessed, with vitamin C as the positive control. The IC50 values on day 8 were 91.74, 78.34, and 72.01 microgram/mL for pineapple peel, dragon fruit peel, and combined kombucha, respectively, compared with 96.99, 91.43, and 86.77 microgram/mL on day 0. Antioxidant activity declined on day 14, with IC50 values of 393.56, 151.09, and 159.05 microgram/mL, respectively. Ethanol contents remained at 0.3-0.5% on day 8 but increased to 1-2% on day 14, while pH decreased to 2.70-2.90. Therefore, day 8 showed the most favorable observed balance between antioxidant activity and ethanol content in this study, particularly for the combined formulation. Confirmation of an optimal fermentation period requires complete post hoc analysis and further evaluation of microbiological safety, stability, and sensory acceptability.

Keywords:
Kombucha, Pineapple Peel, Dragon Fruit Peel, Fermentation Time, Antioxidant Activity

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1. Introduction

Kombucha is a fermented beverage from sweet tea solution fermented using SCOBY (Symbiotic Culture of Bacteria and Yeast) culture. Kombucha is known as a probiotic drink because it contains live microorganisms that are beneficial for health. This fermentation process produces bioactive compounds such as vitamins, enzymes, organic acids, and antioxidants that play a role in supporting the immune system, balancing intestinal flora, and fighting pathogenic microorganisms [1].

Initially, the main ingredients for making kombucha were black tea, green tea, or oolong tea. However, the development of research shows that various other ingredients rich in antioxidants can also be utilized. Some of these include mint leaves, bay flowers, rosella flowers, ginger, turmeric, and fruits such as pineapple and dragon fruit. These ingredients not only enrich the nutritional content of kombucha, but also increase its functional potential as a health drink [2]

Pineapple (*Ananas comosus*) is one of the tropical fruits that is easily found throughout the year in Indonesia and contains many bioactive compounds. Pineapple peels, which are generally discarded as waste, actually contain flavonoids, alkaloids, tannins, steroids, vitamin C, and natural sugars such as glucose and fructose. Flavonoid compounds and vitamin C in pineapple peels are known to have high antioxidant activity that can protect the body from oxidative damage [3].

Besides pineapple, red dragon fruit (*Hylocereus polyrhizus*) also has great potential in making kombucha. Both the flesh and skin contain functional compounds such as flavonoids, anthocyanins, phenolics, vitamins A, C, E, and carotenoids that are antioxidant, anti-inflammatory, and anticancer [4],[5]. In fact, dragon fruit skin, which has been considered waste, has potential as a natural colorant and value-added fermentation raw material [2].

Pineapple peel and red dragon fruit are agro-industrial wastes that are rich in bioactive compounds and environmentally friendly to be used as a base for kombucha. Previous research shows that phenol compounds, flavonoids, and vitamin C in fruit peels have high antioxidant activity. This activity is very important in neutralizing free radicals that can cause degenerative diseases such as cancer and heart disease [4].

One of the factors that can affect the antioxidant activity of kombucha is the length of fermentation. Fermentation affects the composition of active compounds, where at the beginning of fermentation there is an increase in phenol content and antioxidant activity, but after a certain time the activity decreases due to the degradation of bioactive compounds in acidic environmental conditions [6],[7]. Therefore, determining the right fermentation time is a crucial aspect in producing kombucha that is optimal in function and safety.

The method used in testing antioxidant activity is the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method, which is a fast, simple, and economical method to assess the ability of antioxidant compounds to neutralize free radicals. Measurements are made using a UV-Vis spectrophotometer at a wavelength of 517 nm, and the results are expressed in IC_{50} values, which is the concentration needed to reduce 50% of DPPH free radicals [8]. Based on this description, this study aims to analyze the effect of fermentation time on the antioxidant activity of kombucha made from pineapple peel juice, dragon fruit peel juice and a combination of pineapple peel and dragon fruit peel juice.

2. Methods

This laboratory experimental study used two independent factors: substrate type and fermentation time. The substrate treatments were pineapple peel juice, red dragon fruit peel juice, their combination, and a negative-control fermentation without fruit peel juice. Samples were evaluated on days 0, 8, and 14. Antioxidant activity was measured using the DPPH assay, and the effects of fermentation time, kombucha type, and their interaction were planned to be evaluated using two-way analysis of variance.

Material

The materials used in this study were ascorbic acid, distilled water, DPPH, ethanol p.a, sugar, concentrated HCL, filter cloth, label paper, cotton cloth, pineapple peel (*Ananas comosus*) and dragon fruit peel (*Hylocereus polyrhizus*), magnesium powder, SCOBY (*Symbiotic Culture Bacteria And Yeast*), and tissue.

Sample preparation of pineapple and dragon fruit peels

Pineapple peels and dragon fruit peels were obtained from fruit vendors. Both were washed with running water until clean, then drained and cut into pieces. Next, each skin was weighed and pulverized using a blender with a ratio of pineapple skin: water (1:3) and dragon fruit skin: water (1:5). Once smooth, the mixture was filtered using a filter cloth to separate the filtrate and residue (pulp). The filtrate of pineapple peel and dragon fruit peel was then pasteurized at 100°C for 10 minutes [1],[9].

Kombucha Preparation

The making of kumbucha is done by measuring 1 L of pineapple peel juice and dragon fruit peel juice which is put into a glass jar and then adding 200g of 10% (b/v) sugar, if the temperature of the solution has reached room temperature then 200 mL of 10% (b/v) kombucha and SCOBY starter is added and the top of the jar is covered with cotton cloth and tied and stored at a temperature of 25-30°C which is not exposed to sunlight. Fermentation was carried out on days 0, 8, After that, each treatment was collected and organoleptic testing was carried out, and antioxidant activity testing using a UV-Vis spectrophotometer [9].

Organoleptic Test

Organoleptic testing is carried out using the ability of the human senses to detect the color, smell and taste of kombucha. This organoleptic assessment was carried out on days 0, 8, and 14 of fermentation. The indicators observed were changes in color, aroma, sweetness and sourness in pineapple and dragon fruit peel juice kombucha [10].

pH test

In the pH test, standardization is previously carried out by rinsing the electrode using distilled water, then dried. The calibrated pH meter is then dipped into kombucha. The pH meter reading is obtained some time after the pH meter is dipped and the value is stable [11].

Flavonoid phytochemical screening test

Prepare and pipette as much as 3 mL of pineapple peel kombucha fermentation solution, dragon fruit peel kombucha, and a combination of both in each test tube. add 5 drops of concentrated HCl and 0.1 gram of Mg powder. If the solution shows yellow, red, and orange colors, it indicates the presence of flavonoids in Kombucha fermentation solution [12].

Alcohol content test

Alcohol content testing is done by taking each kombucha solution. Then, the distillation process is carried out. then 100 ml of distillation results are put into a measuring cup. Before measurement, the solution is calibrated first at 20°C. After that, the alcoholmeter is dipped into the distillate, and the alcohol content is read based on the scale visible on the liquid surface [13].

Antioxidant activity test

Antioxidant activity in this study was carried out using 0.1 mM DPPH (*2,2-diphenyl-1-picrylhydrazyl*) reagent made by dissolving 4 mg DPPH powder into 10 mL ethanol p.a, then diluted to a volume of 100 mL. To determine the maximum wavelength of DPPH, DPPH solution was mixed with ethanol p.a, homogenized, and the absorbance was measured in the wavelength range of 400-800 nm using a UV-Vis

spectrophotometer. As a comparison solution, vitamin C solution with a concentration of 20-100 ppm was prepared, each of which was reacted with DPPH solution, allowed to stand for 30 minutes in dark conditions, and then measured the absorbance at the maximum wavelength to calculate the percent inhibition.

Antioxidant activity test on the sample was conducted on pineapple peel kombucha, dragon fruit peel, a combination of both, and negative control, each with a variation of fermentation time on days 0, 8, and 14. Each sample was made 1000 ppm mother solution by mixing 10 mL of kombucha into 10 mL of ethanol p.a, then diluted to 20, 40, 60, 80, and 100 ppm. A total of 2 mL of each concentration was mixed with 2 mL of DPPH solution, covered with aluminum foil, homogenized, and allowed to stand for 30 minutes in dark conditions. After that, the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. UV-Vis spectrophotometer and calculated the percent inhibition to determine the antioxidant activity of each sample.

$$\% \text{ inhibition} = \frac{b - a}{b} \times 100\%$$

a= Sample uptake, DPPH given sample
b= blank uptake, DPPH without sample

The IC_{50} value is obtained from the intersection of the line between the inhibition and concentration axis, entered in the equation y = bx + a. The result is declared as an active antioxidant if the IC_{50} value is less than 200 ppm [14].

Data analysis

Data analysis was performed using Uv-Vis Spectrophotommeter with Two Way ANOVA statistical test. Two Way ANOVA has a function to see if there is a statistically significant difference in antioxidant activity between the averages of these three groups based on fermentation time days 0, 8 and 14 in pineapple peel kombucha, dragon fruit kombucha and combined kombucha.

3. Results and Discussion

Descriptive sensory observations

Descriptive observations of color, odor, and taste are summarized in **Table 1**. These observations indicate temporal changes during fermentation but should not be interpreted as formal consumer acceptability outcomes because no sensory panel or structured rating scale was used.

Table 1. Organoleptic Test Results on Kombucha

Sample	Time Fermentation	Color	Smell	Flavor
Pineapple Peel Kombucha	Day 0	Cloudy yellow dark	Typical neutral pineapple fruit	Sweet like fruit juice
	Day 8	Yellow cloudy slightly bright	Mild acid	Fresh sour, still sweet typical of pineapple & fizzy
	Day 14	Bright yellow clear	Sour pungent	Sour and fizzy

Dragon fruit peel kombucha	Day 0	Purplish red concentrated	Neutral	Sweet typical of dragon fruit
	Day 8	Lighter purplish red	Mild acid	Fresh sweet sour typical of dragon fruit and fizzy
	Day 14	Light red bright	Sour pungent	Sour and fizzy
Combination Kombucha	Day 0	Orange red dark	Neutral	Sweet blend pineapple and dragon fruit
	Day 8	Orange rosy bright	Medium sour	Sweet and sour fresh and fizzy
	Day 14	Light orange bright	Strong acid	Sour and fizzy
Negative Control (without sample)	Day 0	Clear	None Scent	Sweet
	Day 8	Clear	Slightly flavorful	Slightly sour sweet and slightly fizzy
	Day 14	Clear	Slightly flavorful	Sweet and sour and slightly fizzy

Table 1 shows that organoleptic characteristics with indicators including color, aroma, and taste of four types of pineapple kombucha samples, dragon fruit kombucha, combination kombucha and negative control at three fermentation times, namely day 0, 8, and 14. The observation results show that for the color aspect, there is a change in the color of kombucha due to pigment degradation by microbes. Where in Kombucha pineapple peel changes color from dark yellow to light yellow due to carotenoid degradation. Carotenoid pigments are lipophilic, soluble in the fermentation medium, and produce a yellow color that fades due to degradation by microbial activity [15].

Dragon fruit peel kombucha changes from intense purplish red to bright pink due to the degradation of betasianin, especially betanin. The purplish red color in dragon fruit peel juice kombucha comes from betacyanin which is soluble in the medium and degraded during fermentation into pale pigments such as neobetanin due to microbial activity, pH, and oxidation[16]. Kombucha the combination showed a shift from dark red-orange to light orange due to the mixing and degradation of the two types of pigments. In terms of aroma, pineapple peel kombucha, dragon fruit peel kombucha, and combined kombucha showed that the aroma of kombucha developed with fermentation, from neutral to sour on day 8 and became stronger until day 14. This change is caused by volatile compounds and alcohol produced by microbial activity, especially yeast [2], [17]. In terms of flavor aspects in pineapple peel kombucha, dragon fruit peel kombucha, and combined kombucha, the kombucha flavor changed from sweet to sour during fermentation. Mild sourness appeared on day 8 and became stronger on day 14. This change is influenced by the activity of microorganisms in fermenting sucrose, which increases the formation of total acid in kombucha [18]. While from these three aspects, the negative control showed no significant changes due to the slow or less than optimal fermentation process.

pH Test Results

The pH test was conducted to determine the level of acidity in the fermented kombucha made from pineapple fruit peel, dragon fruit peel, a combination of both, and negative control (without sample). Fermentation time was carried out on day 0, 8 and 14 at room temperature 25°C. The pH measurement was carried out at the three fermentation times for each sample using a pH meter for 3 repetitions.

Table 2. Kombucha pH results

Sample	Fermentation Time	pH
Pineapple Peel Kombucha	Day 0	4,22
	Day 8	3,39
	Day 14	2,70
Dragon fruit peel kombucha	Day 0	4,26
	Day 8	3,64
	Day 14	2,90
Combination Kombucha	Day 0	4,24
	Day 8	3,56
	Day 14	2,83
Negative Control (without sample)	Day 0	4,44
	Day 8	4,23
	Day 14	3,81

The test results (**Table 2**) showed that all samples experienced a decrease in pH as fermentation time progressed, but remained within the safe range for consumption. The pH value is still in accordance with the optimal standard for kombucha, which is between 4.6 to 2.5. The decrease in pH indicates that the longer the fermentation lasts, the acidity of kombucha increases [2]. On the other hand, in the negative control sample, the decrease in pH was not very significant (from 4.4 to 3.8) due to the absence of additional nutrient supply such as fruit juice for microorganisms, so that the fermentation rate becomes slower.

Flavonoid Phytochemical Screening Test Results

Flavonoid phytochemical screening test was conducted as an initial stage before the quantitative test of antioxidant activity. Flavonoid compound testing was carried out using the Shinoda reaction, which involves the addition of magnesium powder and concentrated HCl to the kombucha sample [19].

Table 3. Flavonoid Phytochemical Screening Results

Sample	Reagent	Positif result	Test result
Pineapple Peel Kombucha	Magnesium + concentrated HCl	the presence of yellow, red, and orange discoloration	(+) Turns into yellow color
Dragon fruit peel kombucha	Magnesium + concentrated HCl	the presence of yellow, red, and orange discoloration	(+) Changed to red color
Combination Kombucha	Magnesium + concentrated HCl	the presence of yellow, red, and orange discoloration	(+) Changed to red color

All kombucha samples showed positive results for the presence of flavonoid compounds, indicated by color changes. Pineapple kombucha showed a yellow color, while dragon fruit kombucha and the combination were red. The presence of flavonoids is indicated by color changes to yellow, orange, or red [20].

Alcohol Content Test Results

Alcohol content testing is conducted to evaluate the effect of fermentation duration on alcohol production and ensure the level remains within safe consumption limits. Where alcohol/ethanol levels in the final product of fermented beverages are tolerated a maximum of ≤ 0.5%. Alcohol measurement was carried out at the three fermentation times for each sample using an alcohol meter for 3 repetitions.

Table 4. Alcohol Content Test Results

Sample	Time Fermentation	Temperature Measurement	% Content Alcohol
Pineapple Peel Kombucha	0	20	0,0 %
	8		0,5 %
	14		1 %
Dragon fruit peel kombucha	0	20	0.0 %
	8		0,3 %
	14		2 %
Combination Kombucha	0	20	0,0 %
	8		0,3 %
	14		1 %
Negative Control (without sample)	0	20	0,0 %
	8		0,0 %
	14		0,0 %

Table 4 shows that on day 0, all types of kombucha (pineapple, dragon fruit, and combination) showed an alcohol content of 0.0%, indicating that the fermentation process had not started effectively [21]. After 8 days of fermentation, the alcohol content began to increase, with dragon fruit and combination kombucha reaching 0.3%, while pineapple 0.5%. All three samples have shown initial fermentative activity with alcohol levels that are still within safe limits. On day 14, the highest alcohol content was recorded in pineapple and combined kombucha (1%), while dragon fruit reached 2%. This increase reflects that fermentation duration affects ethanol accumulation.

Antioxidant Activity Test Results

Antioxidant activity testing was performed using UV-Vis spectrophotometry at a wavelength of 517 nm, which is the maximum wavelength for DPPH reagent (**Table 5**).

Table 5. Maximum wavelength and absorbance of blank solution (DPPH)

Material	Maximum wavelength	Absorbance
Blank (DPPH)	517 nm	0,574

From the measurement of the blank, the absorbance was obtained as 0.574. Blank solution is a solution that is treated the same as the sample and comparator, but does not contain the analyte. The purpose of its use is to measure the absorption derived from components other than the analyte [22]. Furthermore, the maximum wavelength was determined to ensure the highest absorption value. The largest absorption is obtained by determining which (λ) is used. Since the change in absorption is greatest at this wavelength for each unit concentration, sample measurements should be made at the maximum wavelength to maximize sensitivity and reduce errors. At a wavelength of 400-800 nm, DPPH free radicals show maximal absorption and have a complementary purple hue [23]. After determination of the wavelength of 0.1 mM DPPH, a max λ of 517 nm was found. Then incubation was carried out for 30 minutes with the aim of doing so because the reaction between the sample and free radicals runs slowly, so that sufficient time is needed so that the sample can react maximally with free radicals. The next process of the reaction is marked by a change in color to yellow. This color change indicates that each sample has the ability as an antioxidant.

Table 6 Antioxidant Activity Test Results of pineapple peel kombucha

Sample	Time Ferm	Concentration (PPM)	Average Abs ± SD	Inhibition (%)	Percent Liniear	IC_{50} (Mg/Ml)
KKN	Day 0	20	0.535 ± 0.001	6.79	y = 0.4643x	96.99
		40	0.498 ± 0.001	13.24	- 4.9652	
		60	0.453 ± 0.002	21.08	R^2 = 0.9348	
		80	0.419 ± 0.001	27.00		
		100	0.308 ± 0.001	46.34		
	Day 8	20	0.378 ± 0.001	34.15	y = 0.2239x	91.74
		40	0.356 ± 0.002	37.98	+ 29.46	
		60	0.324 ± 0.001	43.55	R^2 = 0.9922	

		80	0.307 ± 0.001	46.51		
		100	0.274 ± 0.000	52.26		
	Day 14	20	0.417 ± 0.001	27.35	y = 0.061x + 25.993 R^2 = 0.9231	393.557
		40	0.409 ± 0.001	28.74		
		60	0.406 ± 0.001	29.27		
		80	0.401 ± 0.002	30.14		
		100	0.386 ± 0.000	32.75		
KKBN	Day 0	20	0.547 ± 0.002	4.70	y = 0.4756x - 6.5157 R^2 = 0.9475	91.43
		40	0.497 ± 0.001	13.41		
		60	0.462 ± 0.001	19.51		
		80	0.419 ± 0.001	27.00		
		100	0.313 ± 0.000	45.47		
	Day 8	20	0.393 ± 0.001	31.53	y = 0.3458x + 22.909 R^2 = 0.9833	78.34
		40	0.373 ± 0.002	35.02		
		60	0.326 ± 0.001	43.21		
		80	0.288 ± 0.000	49.83		
		100	0.237 ± 0.000	58.71		
	Day 14	20	0.395 ± 0.001	31.18	y = 0.1446x + 28.153 R^2 = 0.9577	151.085
		40	0.374 ± 0.000	34.84		
		60	0.371 ± 0.001	35.37		
		80	0.348 ± 0.001	39.37		
		100	0.325 ± 0.001	43.38		
KK	Day 0	20	0.370 ± 0.001	35.54	y = 0.2082x + 31.934 R^2 = 0.9862	86.77
		40	0.340 ± 0.001	40.77		
		60	0.314 ± 0.001	45.29		
		80	0.301 ± 0.000	47.56		
		100	0.270 ± 0.000	52.96		
	Day 8	20	0.365 ± 0.001	36.41	y = 0.2666x + 30.801 R^2 = 0.9941	72.01
		40	0.341 ± 0.001	40.59		
		60	0.303 ± 0.001	47.21		
		80	0.271 ± 0.000	52.78		
		100	0.247 ± 0.001	56.96		
	Day 14	20	0.411 ± 0.001	28.39		159.052
		40	0.391 ± 0.000	31.88		

		60	0.376 ± 0.001	34.49	y = 0.1551x	
		80	0.361 ± 0.000	37.10	+ 25.331	
		100	0.337 ± 0.000	41.29	R^2 = 0.9924	
KN	Day 0	20	0.455 ± 0.002	20.73	y = 0.2622x	
		40	0.449 ± 0.001	21.78	+ 13.258	
		60	0.410 ± 0.001	28.57	R^2 = 0.9347	140.129
		80	0.390 ± 0.001	32.06		
		100	0.334 ± 0.001	41.81		
	Day 8	20	0.433 ± 0.001	24.56	y = 0.0923x	
		40	0.422 ± 0.001	26.48	+ 22.753	
		60	0.415 ± 0.001	27.70	R^2 = 0.9385	295.200
		80	0.394 ± 0.001	31.35		
		100	0.394 ± 0.001	31.35		
	Day 14	20	0.478 ± 0.002	16.72	y = 0.1185x	
		40	0.452 ± 0.001	21.25	+ 15.157	
		60	0.444 ± 0.002	22.65	R^2 = 0.9063	295.033
		80	0.442 ± 0.001	22.99		
		100	0.415 ± 0.001	27.70		
KP	Day 0	20	0.269 ± 0.001	53.13	y = 0.311x +	6.77
		40	0.226 ± 0.001	60.62	47.892	
		60	0.187 ± 0.001	67.42	R^2 = 0.9858	
		80	0.149 ± 0.001	74.04		
		100	0.129 ± 0.000	77.52		
	Day 8	20	0.260 ± 0.001	54.70	y = 0.3362x	5.49
		40	0.225 ± 0.001	60.80	+ 48.153	
		60	0.173 ± 0.000	69.86	R^2 = 0.9926	
		80	0.143 ± 0.001	75.09		
		100	0.108 ± 0.001	81.18		
	Day 14	20	0.279 ± 0.001	51.39	y = 0.3371x	
		40	0.211 ± 0.001	63.24	+ 47.3 R^2 = 0.9585	8.01
		60	0.174 ± 0.001	69.68		
		80	0.154 ± 0.000	73.17		
		100	0.114 ± 0.000	80.13		

Description :
KKN = Pineapple Peel Kombucha
KKBN = Dragon Fruit Peel Kombucha
KK = Combination Kombucha
KN = Negative Control (Kombucha without chili sauce)
KP = Positive Control (Vitamin C)

Based on the results of **Table 6**, the increase in sample concentration causes a decrease in the measured absorbance value. This indicates that the higher the sample concentration, the greater the percentage of free radical inhibition, characterized by low absorbance values [2]. The absorbance measurement results are used as the basis for calculating antioxidant activity, which is expressed in the form of percentage inhibition and IC_{50} value. Based on the calculation of percent inhibition, it is found that increasing the concentration of the sample affects the ability to neutralize free radicals. The higher the concentration, the greater the antioxidant activity as indicated by the increasing percent inhibition value. The ability of the sample to inhibit free radicals increases with increasing concentration. This is related to the content of secondary metabolites contained in the kombucha sample, one of which is flavonoid compounds [24]. Flavonoids are exogenous antioxidants that play an important role in protecting cells from oxidative stress damage. Flavonoids work by donating hydrogen ions to neutralize free radicals and reduce their toxic impact [25]

The results of antioxidant activity test on all samples showed differences in IC_{50} values during fermentation. A compound is categorized as having very strong antioxidant activity if its IC_{50} value is less than 50 µg/mL, strong if it is in the range of 50-100 µg/mL, moderate at 101-150 µg/mL, weak at 151-200 µg/mL, and not potential if it exceeds 200 µg/Ml [26]. Pineapple peel kombucha shows antioxidant activity that is classified as strong on day 0 and 8 with IC_{50} values. respectively 96.99 µg/mL and 91.74 µg/mL while on day 14 showed antioxidant activity classified as very weak with an IC_{50} value of 393.557 µg/mL. Dragon fruit peel kombucha showed strong antioxidant activity on day 0 and 8 with IC_{50} values of 91.43 µg/mL and 78.34 µg/mL, respectively, while on day 14 showed weak antioxidant activity with an IC_{50} value of 151.085 µg/mL. The combined kombucha showed strong antioxidant activity on days 0 and 8 with IC_{50} values of 86.77 µg/mL and 72.01 µg/mL, respectively, while on day 14 showed weak antioxidant activity with an IC_{50} value of 159.052 µg/mL. The three kombucha samples experienced an increase in percent inhibition until day 8 due to optimal production of bioactive compounds such as flavonoids and phenolics, but decreased on day 14 due to degradation of active compounds. This decrease was influenced by weak acidic conditions during continued fermentation, which limited the ability of active compounds to reduce free radicals [7]. Negative control kombucha showed low to very weak antioxidant activity at all fermentation times, with IC_{50} values of 140.129 µg/mL (day 0), 295.200 µg/mL (day 8), and 295.033 µg/mL (day 14), respectively, due to the absence of phenolic and flavonoid content of the plant material. The absence of bioactive compounds caused fermentation to not produce secondary metabolites that play a role in antioxidant activity, resulting in no significant changes in chemical or organoleptic parameters. In contrast, vitamin C showed very strong antioxidant activity at all three testing times with IC_{50} values below 10 µg/mL, as it is a single compound known to be effective as an antioxidant [27].

Statistical Analysis of Two Way ANOVA Test

The results of percent inhibition obtained from samples of pineapple peel kombucha, dragon fruit peel kombucha, and combined kobucha and vit C with concentrations of 20, 40, 60, 80 and 100 at variations in fermentation time on days 0, 8 and 14. Analysis of variance or ANOVA is one of the parametric tests that serves to distinguish the average value of more than two groups of data by comparing their variances, the use of the ANOVA test also provides statistical power so that the results can be considered more valid and reliable [28]. The Two Way ANOVA test is suitable for use in this test because it only has one independent variable and two dependent variables.Table 2. Kombucha pH results

Table 7. Two Way ANOVA Test Results

Group	Time Fermentation	Mean ± SD	*P- value*
Pineapple Peel Kombucha	0	22,89 ± 6,79	Fermentation Time = 0,001
	8	42,89 ± 3,18	
	14	29,65 ± 0,90	
Dragon Fruit Peel Kombucha	0	22,02 ± 6,91	Kombucha Type = 0.000
	8	43,66 ± 4,93	
	14	36,83 ± 2,09	
Combination Kombucha	0	42.42 ± 2,96	Interaction of both = 0.045
	8	46,79 ± 3,78	
	14	34,63 ± 2,20	
Vit C	0	66,55 ± 4,43	
	8	68,33 ± 4,77	
	14	67,52 ± 4,87	

Based on **Table 7**, the results of antioxidant activity measurements shown through the percent inhibition value are shown in the form of Mean± SD (Standard Deviation). Pineapple peel kombucha showed percent inhibition from 22.89 ± 6.79 on day 0 to the highest on day 8 which was 42.89 ± 3.18, then decreased on day 14 to 29.65 ± 0.90. This was also the same for dragon fruit peel kombucha, where the inhibition value started from 22.02± 6.91 on day 0 to 43.66± 4.93 on day 8, then decreased to 36.33± 2.09 on day 14. Combined kombucha showed a higher inhibition value than the two previous types, with the highest value on day 8 being 46.79± 3.78. As a comparison, the positive control (Vitamin C) had the highest and stable inhibition value at all fermentation times, with averages ranging from 66.55± 4.43 to 68.33 ± 4.77.

From the table above, the Two Way Anova test value was also obtained. Two Way Anova was conducted to determine the effect of fermentation time, type of kombucha, and the interaction between the two on antioxidant activity. There are three hypotheses tested, namely whether fermentation time affects antioxidant activity,

whether the type of kombucha affects antioxidant activity, and whether there is an interaction between fermentation time and type of kombucha in affecting antioxidant activity. The three hypotheses were declared significant if the p-value was <0.05. The analysis showed that fermentation time (p-value= 0.001), type of kombucha (p-value = 0.000), and the interaction between the two (p-value = 0.047) had significance values smaller than 0.05. This means that the three factors have a significant influence on the antioxidant activity of kombucha.

Study limitations and future directions

This study has several limitations. First, complete formulation details, including initial peel mass, juice yield, combination ratio, SCOBY amount, final batch volume, and independent batch replication, must be documented to ensure reproducibility. Second, the concentration basis for the liquid-sample DPPH assay and the use of sample blanks require clarification because intrinsic pigments may interfere at 517 nm. Third, antioxidant capacity was assessed only by DPPH, while total phenolic, total flavonoid, vitamin C, and betalain contents were not quantified. Fourth, ethanol was estimated using an alcoholmeter rather than a compound-specific chromatographic method, and descriptive sensory observations were not obtained from a formal panel. Finally, complete raw statistical output was unavailable in the submitted manuscript. Future studies should standardize the formulation, confirm ethanol by gas chromatography or another validated method, assess microbial safety and stability, use complementary antioxidant assays such as ABTS and FRAP, quantify major bioactive compounds, and conduct structured sensory acceptability testing.

4. Conclusion

Fermentation duration influenced the observed physicochemical and antioxidant characteristics of pineapple peel, red dragon fruit peel, and combined kombucha. Day 8 produced the strongest observed DPPH activity among the tested fermentation times, with the combined formulation showing the lowest fruit-sample IC50 value of 72.01 microgram/mL. At this time point, ethanol remained at 0.3-0.5%, whereas fermentation to day 14 was associated with weaker antioxidant activity, lower pH, and ethanol concentrations of 1-2%, which exceeded the stated reference level for a non-alcoholic beverage. Accordingly, day 8 appears to provide the most favorable observed balance in this experiment, but it should not be considered a confirmed optimum until formulation details, post hoc statistical results, microbiological safety, analytical stability, and product acceptability have been verified.

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Conflicts of Interest:

The authors declare no conflicts of interest related to this study or its publication.

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