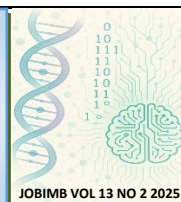


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## Valorization of Pineapple By-products (*Ananas comosus*) to Enhance Proximate and Antioxidant Properties of Green Tea Kombucha

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### Abstract

The shift towards health-conscious lifestyles has significantly influenced the functional foods market, leading to increased innovation in the development of fermented beverages. Pineapple (*Ananas comosus*), is recognized for its rich bioactive compounds such as bromelain, and dietary fiber, making their utilization not only nutritionally beneficial but also sustainable. Previous studies have shown that the fusion of pineapple with black tea increased the antioxidant properties of kombucha. However, the effects of pineapple infusion and its by-products on the proximate composition and antioxidant activities of green tea kombucha remain unclear. Therefore, this study aimed to investigate the proximate composition and antioxidant of green tea kombucha when infused with dried pineapple flesh (KPF) and its peels and cores (KPC). The green tea kombucha without any pineapple infusion was used as the control (KT). Proximate composition was analysed using Association of Official Analytical Chemists (AOAC) International standards meanwhile the antioxidant activity was measured using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP) assays. All analyses were conducted on day 13 of fermentation. Findings showed that the infusion with pineapple peels and cores (KPC) in green tea kombucha increased the crude fiber content, probably due to the release of fibrous microparticles during fermentation. Moreover, KPC had significantly the highest antioxidant activity with an IC<sub>50</sub> value of 443.9 µg/mL for ABTS and FRAP value of 77.82 µg TEAC/mL compared to other samples. The findings indicate that KPC has a strong potential as a functional ingredient. Future studies could focus on optimizing the formulation to enhance consumer acceptance of pineapple peel-infused green tea kombucha.

### INTRODUCTION

Functional foods have gained significant attention in recent years due to their potential health benefits beyond basic nutrition. Nowadays, people are looking for food products that not only provide quick preparation and convenience but also supply essential vitamins and minerals and support overall health [1]. Among these, fermented beverages such as kombucha have become increasingly popular [2]. Kombucha, a sweetened tea fermented by a symbiotic culture of bacteria and yeast (SCOBY), is known for its rich profile of bioactive compounds, including organic acids, vitamins, and polyphenols. Typically, the main component required to make kombucha is tea including black and green tea. Green tea's abundance of polyphenols (catechins), flavonoids (quercetin, kaempferol, and myricetin), proteins, and amino acids, among other components, renders it an excellent

choice as a fermentation medium [3]. According to Teixeira Oliveira et al. [4], the amount of catechins in green tea (70%) was higher than in black tea (30%) which exhibits a strong antioxidant capacity. The consumption of kombucha tea has been associated with several health benefits, such as its ability to treat digestive issues, reduce inflammation, and possess antibacterial, antioxidant, and anti-proliferative traits. These benefits are credited to the organic acids, vitamins, minerals, and phenolic compounds that are released during the fermentation process [5]. Each region or customer's preference may have an influence on the ratios of tea to sugar as well as the period of time and temperature at which the fermentation process takes place while making kombucha [6]. The market is replete with an array of commercially available kombucha brands, each offering an extensive selection of flavours to entice consumers.

Predominantly, these products feature a harmonious blend of at least two flavours, with the most prevalent flavourings encompassing fruits, herbs, or an intricate fusion of both. Incorporating fruit by-products, like peels, core, and flesh, into kombucha fermentation has emerged as a promising approach to enhance its antioxidant capacity. A recent report by Sornkayasit et al. [7] indicated that pineapple by-product infusion in black tea kombucha demonstrated higher Blautia and Lactobacillus levels and significantly reduced senescent CD4<sup>+</sup> T cells in older individuals, suggesting its potential as a health-promoting beverage. Pineapple (*Ananas comosus*), a tropical fruit rich in vitamin C, provitamin A (β-carotene), bromelain, and various phenolic compounds, is often underutilized beyond its edible parts. The flesh, peels, and core of pineapples are abundant in antioxidants, making them suitable for valorization in the production of functional beverages. Utilizing dried pineapple parts as substrates in green tea kombucha fermentation could lead to a beverage with enhanced antioxidant activity, providing an innovative and sustainable use of pineapple waste. For example, the fresh pineapple peels and cores have been traditionally used to make Tepache, a fermented Mexican drink with a pleasant tropical aroma. This beverage is not only sensorial appealing but also provides a boost in micronutrients and bromelain enzymes that improve digestive health.

In 2021, the Malaysian Pineapple Industry Board (MPIB) reported that Malaysia exported over 375,432 tonnes of pineapples, cultivated on 16,204 hectares. Johor, Sarawak, and Sabah were recognised as the primary contributors to Malaysia's pineapple production [8]. Approximately 80% of the pineapple plant, encompassing its peel, leaves, crown, and core, are abandoned during processing, shipping, and storage, significantly contributing to agricultural waste. This waste amounts to approximately 67,098 tonnes of leaf waste and 137,550 tonnes of peel waste [8]. Although the prices are cheap, these byproducts are useful raw materials that are utilised in making products with significant added value [9]. For long-term sustainability, utilising this agricultural waste as a functional food or value-added food item is crucial in compliance with Sustainable Development Goal (SDG) No. 12, which aims to achieve sustainable patterns of consumption and production [10]. Hence, the study aims to determine the proximate composition and antioxidant activities of green tea kombucha infused with dried pineapple flesh and its peels and cores.

## MATERIALS AND METHODS

### Sample preparation

The tea samples were produced following the methodology of Sornkayasit et al. [7] with minor modifications whereby the type of tea was changed from black tea to green tea and pineapple flesh was included in addition to pineapple peels as a substrate to align with the objectives of this investigation. In brief, three formulations were used in the present study as shown in **Table 1**.

**Table 1.** Infusions used for green tea kombucha.

Formulation	Infusion	Abbreviation
1.	Green tea kombucha (control)	KT
2.	Green tea kombucha + Pineapple flesh	KPF
3.	Green tea kombucha + Pineapple peels and cores	KPC

The present study used a pineapple (*Ananas comosus*) MD2 clone, which was purchased from the local market in Serdang, Selangor, supplied by Southern Valley. The ripe pineapples purchased were selected according to the colour of the peel (90%

yellowish colour with firm texture). The green tea used in the production of kombucha was purchased from the local market in Serdang, Selangor. The green tea was produced by BOH Cameron Highlands from the *Camellia sinensis* plant. The pineapples were cleaned, and the flesh was separated from the peels and cores. The pineapple flesh was diced into cubes while the peels and cores were cut into small pieces (2 cm x 2 cm).

The samples were dried at 50 °C in a hot air oven for three to five days until the moisture level attained 15%. The dried pineapple flesh, peels and cores were kept in an airtight container before being used in the green kombucha production. The tea bags were opened, and the tea leaves (weighing approximately 5 grams) were taken out to be used for brewing the green tea base for the kombucha. Prior to introducing the sample, distilled water was heated to a boiling point of 100 °C. Upon boiling the distilled water, the samples were introduced into the boiling liquid. After that, the hot tea was strained through muslin cloth to eliminate the tea residue and thereafter put into a stainless-steel bowl. Subsequently, granulated sugar (50 grams) was incorporated into the boiling tea and swirled until fully dissolved. The tea was cooled to room temperature (23 °C) before the introduction of the starting culture and SCOBY.

The cooled tea was thereafter put into a 1.5 L food-grade plastic container. The food-grade plastic containers (polypropylene) were chosen for their chemical resistance to the acidic fermentation environment, food-safe certification according to Malaysian standards, and practical advantages for small-scale experimentation. Subsequently, 50 ml of mature starter culture (a diameter of 10 cm) in a 1.5 L fermentation volume was introduced into the tea to ensure a consistent fermentation pace. Additionally, dried pineapple flesh, peels, and core were included in the cooled tea. The plastic jar mouth was covered in muslin cloth, and the samples underwent fermentation for thirteen days [7]. All formulations (KT, KPF, KPC) were prepared in triplicate. Following thirteen days of fermentation, the kombucha tea is complete for further analysis.

### Proximate analysis

The proximate composition of kombucha samples was conducted following AOAC international [11]. The details method was described as follows.

### Moisture content

Moisture content was determined using the oven-drying method of 3 g of liquid kombucha tea samples at 105 °C overnight. The clean, labelled crucibles and lids were pre-dried, cooled in a desiccator, and weighed before sample addition. After drying, the crucibles were cooled again in a desiccator for 15 minutes and reweighed. Moisture content was calculated based on weight loss and expressed as a percentage of wet weight. All measurements were performed in triplicate.

$$\% \text{ of wet weight} = \frac{a-b}{a} \times 100$$

Where ,

a = weight of kombucha tea sample use  
b = dry weight of kombucha tea sample

### Ash content

Ash content was determined by incinerating 3 g of kombucha tea sample in a muffle furnace. Pre-weighed crucibles and lids were dried at 105 °C, cooled in a desiccator, and weighed. Samples were added and placed in a muffle furnace at 550 °C for 8 hours

until no black residue remained. Crucibles were cooled overnight and reweighed. Ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{(\text{weight of ash} + \text{weight of crucible with lid}) - (\text{weight of crucible with lid})}{\text{Weight of kombucha tea sample}} \times 100$$

### Crude protein content

Crude protein content was determined using the Kjeldahl method. The kombucha tea sample (0.15 g) was digested in a micro Kjeldahl tube with 0.8 g of mixed catalyst and 2.5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (98%) until the solution became clear. After cooling, 5 mL of distilled water was added, and the digest was transferred to a distillation unit. Ammonia was collected and titrated with 0.05 N H<sub>2</sub>SO<sub>4</sub> until a purple endpoint. A reagent blank was processed in the same way. All analyses were performed in triplicate. The proportion of crude protein was determined based on the conversion factor of 6.25.

### Crude fat content

Crude fat content was determined by solvent extraction using petroleum ether. A 5 g kombucha tea sample was placed in a conical flask with 200 mL of petroleum ether and sealed with aluminium foil and parafilm. The mixture was stirred continuously on a magnetic stirrer hot plate for 8 hours. After extraction, the petroleum ether layer was separated using a separatory funnel and transferred to the pre-weighed round-bottom flask. The solvent was removed using a rotary evaporator. The flask was then dried again at 105 °C for 20 minutes, cooled for 15 minutes, and reweighed. The percentage of crude fat was calculated based on:

$$\% \text{ of crude fat} = \frac{D \times 100}{W}$$

Where,

D = weight of crude fat (g)

W = weight of the kombucha tea sample (g)

### Crude fibre content

Crude fibre content was determined through acid and alkaline digestion. A 2 g kombucha tea sample was placed into a 500 mL conical flask with 200 mL of sulphuric acid. The mixture was stirred and refluxed for 30 minutes, with the flask rotated every 10 minutes. The hydrolysed mixture was filtered through pre-weighed Whatman No. 541 ashless filter paper and rinsed with hot distilled water until acid-free. After filtration, the residue was rinsed with 5 mL of ethanol, drained, and transferred to a crucible. The crucible containing the filter paper and residue was dried at 105 °C overnight, cooled, and weighed. It was then ashed in a muffle furnace at 550 °C for 8 hours, cooled overnight, and reweighed. The percentage of crude fibre was calculated based on:

$$\% \text{ of crude fiber} = \frac{((C-B)-A) \times 100}{W}$$

Where,

W = Weight of kombucha tea sample

B = Weight of filter paper without ash

C = Weight of crucible + filter paper + dried residue

A = Weight of crucible + ash

### Carbohydrate content

The carbohydrate content was calculated using the carbohydrate by difference method. This method estimates the carbohydrate fraction by subtracting the measured percentages of moisture, ash, crude protein, crude fat, and crude fibre from 100 %. The formula used is as follows:

$$\% \text{ of carbohydrate} = 100 - (\% \text{ of moisture} - \% \text{ of ash} - \% \text{ of crude protein} - \% \text{ of crude fat} - \% \text{ of crude fibre})$$

### Antioxidant activities

#### Ferric Ion Reducing Antioxidant Power (FRAP) assay

For the FRAP analysis, the procedure outlined by Xiao et al. [12] was strictly followed accordingly. A 10 mM of TPTZ solution, 20 mM of FeCl<sub>3</sub>.6H<sub>2</sub>O, and 300 mM of acetate buffer were mixed together to produce the FRAP reagent. First, 3 mL of freshly made FRAP reagent was vortexed with 0.1 mL of diluted kombucha tea. After that, the combination was allowed to sit at room temperature for half an hour in the dark. A spectrophotometer was used to measure the absorbance at 593 nm, with distilled water serving as the blank. A standard curve was created using Trolox concentrations ranging from 0.2 to 1.2 mg/mL, which was used as the standard. The calibration curve equation that was often used was  $y = 1.2365x - 0.0412$  (R = 0.9951). The Trolox equivalent in microgrammes (µg TEAC/mL) was used to express the results. Three replicates of the analysis were performed for each kombucha tea sample.

#### 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS<sup>+</sup> scavenging capacity was determined using a slightly modified version of the Ramli et al. [13] method. Firstly, an amber bottle was incubated in the dark and at a low temperature for 16 hours to create the ABTS<sup>+</sup> radical cation. The solution contained 7 mM ABTS and 2.45 mM potassium persulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) in a 1:1 ratio. After dilution with distilled water to reach 0.70 ± 0.1, the absorbance of the ABTS<sup>+</sup> solution at 734 nm was then measured using a spectrophotometer. Next, 1.0 mL of ABTS<sup>+</sup> solution was mixed with 20 µL of diluted kombucha tea. Using distilled water, the material was further diluted to produce 20–80% inhibition of the blank. Three duplicates of the analysis were performed. Ascorbic acid was utilised as the positive control, and the following equation was used to determine the samples' and the positive control's percentage scavenging capacity based on the inhibition observed at 2 minutes:

$$\% \text{ scavenging capacity} = \left(1 - \frac{\text{Abs (sample)}}{\text{Abs (control)}}\right) \times 100$$

Subsequently, a plot was created to compare the half maximal inhibitory concentration (IC<sub>50</sub>) values of each sample with the concentrations of kombucha, based on the inhibition of ABTS scavenging activity. Three duplicates of the analysis were measured.

### Statistical analysis

The three replicates' means and standard deviations were used to illustrate the research's findings. Tukey's pairwise test with a level of significance of (p < 0.05) was used to examine the significant differences between mean values in both one-way and two-way analysis of variance (ANOVA). The statistical analyses were conducted using Minitab® 22.1 (Pennsylvania, USA).

## RESULT AND DISCUSSION

### Proximate analysis

The proximate analysis estimates the macro components constituents of food including moisture, crude protein, ash, crude fat, carbohydrate, and crude fibre. **Table 2** shows the proximate composition of three different kombucha tea samples (control (KT), kombucha tea infused with dried pineapple flesh (KPF), and kombucha tea infused with dried pineapple peels and core (KPC)) evaluated on 13 days of fermentation. There was a significant difference in moisture, crude protein, ash, crude fat, carbohydrate and crude fibre content between kombucha tea control and kombucha tea with added pineapple flavour.

**Table 2.** Proximate analysis of kombucha tea infused with pineapple flesh, and peel and core.

Composition	Sample		
	Kombucha control (KT)	Kombucha tea + dried pineapple flesh (KPF)	Kombucha tea + dried pineapple peels & core (KPC)
Moisture (%)	93.93 ± 0.02 <sup>b</sup>	96.27 ± 0.11 <sup>a</sup>	96.40 ± 0.02 <sup>a</sup>
Ash (%)	0.02 ± 0.01 <sup>b</sup>	0.06 ± 0.02 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>
Crude protein (%)	0.10 ± 0.17 <sup>a</sup>	0.19 ± 0.16 <sup>a</sup>	0.28 ± 0.00 <sup>a</sup>
Crude fat (%)	0.31 ± 0.08 <sup>c</sup>	1.13 ± 0.11 <sup>a</sup>	0.82 ± 0.03 <sup>b</sup>
Crude fiber (%)	0.05 ± 0.01 <sup>c</sup>	0.25 ± 0.04 <sup>b</sup>	0.46 ± 0.02 <sup>a</sup>
Carbohydrate (%)	5.61 ± 0.13 <sup>a</sup>	2.10 ± 0.18 <sup>b</sup>	1.98 ± 0.05 <sup>b</sup>

Note: KT – Kombucha tea control; KPF – Kombucha tea infused with dried pineapple flesh; KPC- Kombucha tea infused with dried pineapple peels and core. Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters indicate significant differences between the types of kombucha tea ( $p < 0.05$ ) using Tukey's pairwise test.

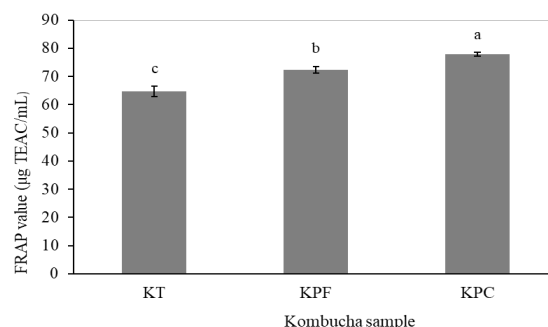
In terms of moisture content, kombucha tea control has the lowest moisture content (93.93%) followed by kombucha tea infused with dried pineapple flesh (96.27%) and kombucha tea infused with dried pineapple peels and core (96.40%). It can be observed that the addition of pineapple increases the moisture content, however, there was no significant difference between the incorporation of flesh and peels and core. The increase in moisture could be attributed to a more vigorous or complete fermentation by the SCOBY compared to the control kombucha resulting in the higher percentage of water in the infused kombucha tea increases relative to its total composition. In all three kombucha tea samples, moisture content was the predominant element in the proximate composition, mostly due to water being the primary component of kombucha tea, which exists in a liquid condition.

Carbohydrates were the second greatest proximate component in all three kombucha teas. This was mostly attributable to the incorporation of sugar in the production of kombucha tea. Sugar is a category of simple carbohydrates [14]. The use of sugar primarily served as a carbon source for the bacteria and yeast within the SCOBY. The infusion of pineapples flesh (KPF) and pineapple peels and cores (KPC) significantly reduced the carbohydrate content likely due to microbial and yeast-mediated sugar consumption. This reduction may also be partially attributed to the increased fiber content in KPF and KPC, as carbohydrate content is calculated using the by-difference method. Crude fat constitutes the third greatest proximate component in the kombucha tea evaluated in this study. Kombucha tea infused with dried pineapple flesh contains a higher amount of fat (1.13%) than kombucha tea infused with dried pineapple peels & core (0.82%). A possible explanation for this might be the types of lipids present in pineapple flesh are more readily extracted into the infusion and fermentation process compared to the lipids in the more complex and fibrous matrix of the peels and core [7].

The cell wall structures in the peel and core, rich in cellulose and other structural carbohydrates, might trap some of the lipids, making them less available for extraction into the kombucha tea. However, the amount of crude fibre in kombucha tea infused with dried pineapple peels & core is higher than in kombucha tea infused with dried pineapple flesh due to higher components of dietary fibre including cellulose and lignin. Pineapple peels and cores, which are structural parts, contain more insoluble fibres, whereas the flesh has more soluble components such as sugars and pectins [15]. During fermentation, soluble fibres like pectin may degrade, but lignin and cellulose remain, maintaining higher crude fibre levels in peels and core samples [16]. The composition of crude protein of all the samples was no significant difference and present in small amounts ranging from 0.10 - 0.28 %. Nordin et al. [17] stated that the protein found in the peel and core mostly derives from the hydroxyproline-rich glycoprotein located in the plant's primary cell wall. Glycoproteins are mostly attached to the cellulose of primary cell walls in the peel, creating a network of microfibrils. The small value of ash content indicates that only a small amount of minerals is present in the kombucha tea.

### Antioxidant activity

The antioxidant activities of kombucha tea samples (control (KT), kombucha tea infused with dried pineapple flesh (KPF), and kombucha tea infused with dried pineapple peels and core (KPC)) were evaluated using three distinct assays: FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging). The results for each assay are presented in **Fig. 1** and **Table 3**. The positive controls, ascorbic acid, were included as a references for antioxidant activity. All of the kombucha tea samples were evaluated for antioxidant assay on 13 days of fermentation. The FRAP assay evaluates antioxidant reducing potential ( $\mu\text{g TEAC/mL}$ ), while ABTS assays measure free radical scavenging activity, with  $\text{IC}_{50}$  values and percentage inhibition at 200  $\mu\text{g/mL}$ .



**Fig. 1.** Antioxidant activity of kombucha samples assayed using Ferric Ion Reducing Antioxidant Power (FRAP). KT – Kombucha tea control; KPF – Kombucha tea infused with dried pineapple flesh; KPC- Kombucha tea infused with dried pineapple peels and core. Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters indicate significant differences between the types of kombucha tea ( $p < 0.05$ ) using Tukey's pairwise test. NA indicates not available.

The Ferric Ion Reducing Antioxidant Power (FRAP) was quantified with Trolox as the standard reference. It was expressed as  $\mu\text{g Trolox equivalent (TEAC)}$  per mL of sample ( $\mu\text{g TEAC/mL}$ ) as illustrated in **Fig. 1**. The results show that there were significant differences in the FRAP value ( $p < 0.05$ ) between the kombucha tea of the same fermentation day.



**Table 3.** Antioxidant activity of kombucha samples assayed using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay.

Sample	ABTS	
	Concentration IC <sub>50</sub> (µg/mL)	% inhibition (at 200 µg/mL)
Kombucha tea control (KT)	901.98 ± 124.06 <sup>a</sup>	39.76 ± 0.67 <sup>c</sup>
Kombucha tea + dried pineapple flesh (KPF)	707.35 ± 71.64 <sup>b</sup>	41.58 ± 0.43 <sup>b</sup>
Kombucha tea + dried pineapple peels & core (KPC)	443.90 ± 40.80 <sup>c</sup>	44.13 ± 0.48 <sup>a</sup>
Positive control		
Quercetin	NA	NA
Ascorbic acid	60.46 ± 3.16	NA

Note: KT – Kombucha tea control; KPF – Kombucha tea infused with dried pineapple flesh; KPC– Kombucha tea infused with dried pineapple peels and core. Values are expressed as mean ± SD of triplicate measurement. Superscripts with different letters indicate significant differences between the types of kombucha tea (p<0.05) using Tukey's pairwise test. NA indicates not available.

As shown in **Fig. 1**, the KPC had the highest FRAP value of 77.82 µg TEAC/mL followed by KPF with FRAP values of 72.32 µg TEAC/mL compared to the control kombucha (64.72 µg TEAC/mL). The results were supported by findings from Mala et al. [18] who showed that pineapple peel extract had higher FRAP values (1,365.14 mg FeSO<sub>4</sub>/100 g DW), than pineapple pulp (544.00 ± 5.88 FeSO<sub>4</sub>/100 g DW). FRAP value was contributed by the presence of a high amount of ascorbic acid and polyphenol that fund the antioxidant activity [19].

According to Dzah et al. [20], pineapple peels possess a significant amount of natural antioxidants, including phenols, β-carotene, ascorbic acid, flavonoids, and additional bioactive compounds like catechin, ferulic acid, gallic acid, and epicatechin which collectively enhance antioxidant capacity through antagonistic and synergistic interactions. Ascorbic acid derived from fruits has been discovered to protect some flavonoids, including anthocyanins in green tea, against oxidative destruction [20]. Consequently, the incorporation of ascorbic acid enhances the antioxidative properties of green tea. In other words, polyphenols derived from both green tea combined with metabolites in pineapple might exhibit a synergistic antioxidant effect, therefore increasing the phenolic content in kombucha tea infused with pineapple. Similarly, Uslu et al. [21] reported that pineapple peels infused green tea poses a higher FRAP value (1207.66 µmol Fe (II)/g) than green tea alone (704.07 µmol Fe (II)/g).

The ABTS assay measures the ability of antioxidants in the sample to neutralize ABTS radicals, leading to a reduction in the blue-green colour as the radicals are stabilized. This study assessed the half maximum inhibitory concentration (IC<sub>50</sub>) and ABTS activity inhibition by adopting the method of Ramli et al. [13] using ascorbic acid as a positive control. IC<sub>50</sub> values have been determined as the concentrations of samples needed for the reduction of fifty percent of the ABTS radicals to their more stable molecular form compounds of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). The lower the IC<sub>50</sub> value indicates the higher antioxidant activity.

It can be observed in **Table 3**, that there were significant differences in the ABTS radical scavenging activity (p<0.05) between the types of kombucha tea samples. It was observed that the KPC exhibited the highest antioxidant activity, with the lowest IC<sub>50</sub> value (443.90 µg/mL) and the highest percentage of inhibition (44.13 % at 200 µg/mL) whereas the kombucha tea control had the lowest antioxidant activity of IC<sub>50</sub> (901.98 µg/mL). All of the kombucha tea had the ability to scavenge ABTS radical activity but kombucha tea with dried pineapple

peels and core was more effective compared to other kombucha tea. Research by Han et al. [22] reported that fermented foods naturally contain high antioxidant capacity due to interactions between phytochemicals and structure modifications of phenolic compounds in tea by microorganisms present. For example, the molecular structure of catechins in green tea enables it as a strong antioxidant due to free radical scavenging activity [23]. Catechins, one of the main flavonoids in green tea could contribute to the prevention of oxidative stress since it possess antioxidant activity [13].

Future work should therefore include phenolics and flavonoids measurements to complement the antioxidant inhibition data and to correlate phytochemical content with functional properties more precisely. Ascorbic acid, with an IC<sub>50</sub> value of 60.46 µg/mL, exhibits significantly higher antioxidant activity compared to all kombucha tea samples, emphasizing its role as a potent antioxidant. Furthermore, the pineapple by-products infusion may contribute to the distinctive aroma and flavour profile due to their phenolic acids and esters [24]. Hence, it is crucial to investigate consumer acceptance in future studies for successful commercial product development.

## CONCLUSION

In conclusion, the present research indicated the potential of infusing pineapple by-products (flesh, peels, and core) into green tea kombucha improved antioxidant activity compared to the control due to the release of bioactive compounds from pineapple components. The proximate analysis showed that the incorporation of pineapple by-products led to a modest improvement in the macronutrient profile, particularly in crude fiber and crude fat, while crude protein and ash levels remained relatively low. This research develops functional food innovation and promotes the sustainable use of agricultural by-products, in alignment with global sustainability goals.

## CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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