EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION

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SEPTEMBER 2025

EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION

By

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A project report submitted to the Department of Agricultural and Food Science

Faculty of Science

Universiti Tunku Abdul Rahman

in partial fulfilment of the requirements for the degree of

Bachelor of Science (Honours) Food Science

September 2025

ABSTRACT

EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION

BRIAN CH'NG WEI SHEN

Kombucha is a functional fermented tea beverage that has been gaining consumer interest due to its rich bioactive compounds. While green tea kombucha and black tea kombucha are well studied, limited research has focused on kombucha fermented with oolong tea (semi-oxidised) and dark tea (post-fermented), which have different phytochemical and flavour profiles. The study found that the types of tea, fermentation time, and their interaction significantly affected the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH and FRAP) of kombucha. Green tea kombucha (GTK) consistently exhibited the highest TPC (up to 788.48 ± 5.84 mg GAE/L), TFC (up to 420.30 ± 2.92 mg QE/L), and antioxidant activities (up to 73.15 ± 0.38% in DPPH inhibition, 16.19 ± 0.07 mmol Fe^{2+}/L in FRAP), followed by oolong tea kombucha (OTK) and dark tea kombucha (DTK). All samples exhibited a significant increase in phytochemical content and antioxidant activities by Day 7, followed by a decrease by Day 14. From Day 0 to Day 7, GTK had the highest increase in TFC (+159.39 mg QE/L) and FRAP (+4.44 mmol Fe^{2+}/L), while the lowest increase was in DTK (+65.75 mg QE/L, +1.71 mmol Fe^{2+}/L). From Day 7 to Day 14, the highest decreases in phytochemical content (–85.45 mg GAE/L, –53.03 mg QE/L)

were observed in GTK, while the highest decreases in antioxidant activities (–4.24 %, –2.12 mmol Fe^{2+}/L) were observed in OTK. In the sensory evaluation, GTK and OTK received significantly higher ratings for aroma, sourness, and overall acceptability than DTK, with no significant difference in colour and sweetness among all types of kombucha. Overall, GTK was more recommended due to its higher phytochemical content, antioxidant activities, and consumer acceptability.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisor, Mr. Sim Kheng Yuen, for his invaluable guidance and continuous support throughout my Final Year Project. His insightful advice and feedback helped me greatly in accomplishing the oral presentations and thesis writing. I would also like to sincerely thank my ex-supervisor, Dr. Lye Huey Shi, for her approval of my project topic and provision of chemicals, as well as the bench.

I extend my thanks to kind laboratory officers, especially Ms. Nurul Farhanah and Ms. Hazlinda, for their technical assistance and willingness to lend me a complete set of micropipettes, which greatly facilitated the smooth progress of my experimental work. In addition, I am truly grateful to my friends, Tan Ee Ze and Aaron, for generously sharing information about antioxidant activity determinations and for helping me obtain a JMP software account. My heartfelt appreciation also goes to Mr. Looi Sin Yan and Dr. Ee Kah Yew for their valuable guidance in statistical analysis and helpful suggestions on thesis writing.

Lastly, I would like to thank my parents for their unwavering love, constant encouragement, provision of transportation, and financial support. The electronic devices they gifted me were the essential tools that supported my FYP experimental work, thesis writing, and the complete three-year journey at UTAR.

DECLARATION

I hereby declare that this final year project report entitled "**EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION**" is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Tunku Abdul Rahman or other institutions.

(BRIAN CH'NG WEI SHEN)

APPROVAL SHEET

This final year project report entitled "**EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION**" was prepared by BRIAN CH'NG WEI SHEN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

Date: 2 September 2025

(Mr. Sim Kheng Yuen)

Supervisor

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Date: 2 September 2025

PERMISSION SHEET

I, **BRIAN CH'NG WEI SHEN** (ID No: 22ADB07345), hereby certify that I have completed the final year project titled "**EFFECT OF DIFFERENT TEA TYPES (*Camellia sinensis*) ON ANTIOXIDANT AND SENSORY PROPERTIES OF KOMBUCHA FERMENTATION**" under the supervision of Mr Sim Kheng Yuen (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science.

I understand that the University may upload the softcopy of my final year project in PDF to the UTAR Institutional Repository, which may be made accessible to the UTAR community and the public.

Your truly,

_____

(BRIAN CH'NG WEI SHEN)

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LIST OF ABBREVIATIONS

β-glucuronidase	Beta-glucuronidase
β-glucosidase	Beta-glucosidase
SCOBY	Symbiotic culture of bacteria and yeast
AAB	Acetic acid bacteria
LAB	Lactic acid bacteria
ROS	Reactive oxygen species
–OH	Hydroxyl groups
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
DSL	D-saccharic acid-1,4-lactone
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
F-C	Folin-Ciocalteu
GAE	Gallic acid equivalent
$AlCl_3$	Aluminium chloride
Al^{3+}	Aluminium ion
QE	Quercetin equivalent
A	Absorbance
IC_{50}	Half maximal inhibitory concentration

Fe^{3+}	Ferric ions
Fe^{2+}	Ferrous ions
TPTZ	2,4,6-tripyridyl-s-triazine
HCl	Hydrochloric acid
w/v	Weight/volume
v/v	Volume/volume
GTK	Green tea kombucha
OTK	Oolong tea kombucha
DTK	Dark tea kombucha
ANOVA	Analysis of variance
Tukey's HSD	Tukey's Honest Significant Difference
p-value	Probability
SD	Standard deviation
β-glucuronidase	Beta-glucuronidase

CHAPTER 1

INTRODUCTION

1.1. Background of Study

Kombucha is one of the functional food options that has gained increasing interest due to its unique flavour profile and many potential health benefits (Fensterseifer Fabricio *et al*., 2023). Kombucha is believed to have originated from the Northern region of China and has been consumed for over 2,000 years (Vargas, Fabricio, and Ayub, 2021). It is produced by using a symbiotic culture of bacteria and yeast (SCOBY) to ferment tea with added sugar. During fermentation, the SCOBY converts tea components and sugar into various metabolites to develop kombucha's functional and characteristic fermented profile.

In Western nations like the United States, kombucha has become a popular functional beverage due to its many health benefits, including probiotics and antioxidants (Khaleil *et al*., 2020). Kombucha is not yet a commonly consumed probiotic drink in Asia countries due to its high price, limited production, and lack of awareness about its benefits (Ahuja, 2021). However, it has great potential in Asia because tea is deeply ingrained as a part in Asia culture and tradition (Gilbert, 2008). To be more successful in promoting kombucha as a health-functional beverage that aligns with both health trends and beverage

preferences, an understanding of the factors that affect its functional and sensory properties is necessary.

1.2. Problem Statement

Tea type and fermentation time are the two significant factors that affect the overall quality of kombucha, including the polyphenol content, antioxidant activity, and sensory properties (Andrade, 2025; Hsieh, Chiu, and Chou, 2021). Most existing studies focus on kombucha made with green and black teas. However, limited studies on oolong tea kombucha and dark tea kombucha were highlighted. Therefore, this gap needs to be filled because both the semi-oxidised oolong tea and the post-fermented dark tea have their unique flavour characteristics that may be favour for consumers and phytochemicals that may contribute to their potential antioxidative effects.

1.3. Significance of the Study

This study examined the effect of different tea types, including green tea, oolong tea, and dark tea, as well as fermentation times, on the phytochemical composition, antioxidant capacity, and sensory attributes of kombucha. The findings may contribute valuable insights for developing kombucha products that better meet both health expectations and regional flavour preferences of consumers. This may further expand the acceptance towards kombucha across various markets worldwide.

1.4. Objectives

The objectives of this study were:

1. To determine the phytochemical contents (TPC and TFC) and antioxidant activities (DPPH and FRAP) of kombucha prepared using different types of tea (*Camellia sinensis*) throughout fermentation of 0, 7, and 14 days.
2. To study the consumer acceptance of different tea-based kombuchas at their peak antioxidant activity using a sensory evaluation.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction to Kombucha

Kombucha is a traditional tea beverage fermented using a symbiotic culture of bacteria and yeast (SCOBY). Its history can be traced back to the Tsin Dynasty, where it was consumed for perceived health benefits in the Manchuria region (Troitino, 2017). Currently, kombucha is being promoted as a functional beverage and gaining more attention worldwide due to its unique flavour profile and various reported health benefits.

2.1.1. Kombucha Fermentation Process

Tea leaves (*Camellia sinensis*), sugar, water, a SCOBY, and starter tea are the five main ingredients to brew kombucha. The SCOBY is used as the starter culture. On the other hand, starter tea is an acidic liquid taken from previously brewed kombucha that is used to create an acidic environment for the SCOBY growth (Johnston, 2023). To make kombucha, tea leaves are first brewed in boiled water and sweetened with sugar, then cooled to room temperature. After cooling, a SCOBY and starter tea are added to start the fermentation. Kombucha fermentation is aerobic and usually carried out for one to two weeks at room temperature. In addition, it is preferably away from direct sunlight to avoid antimicrobial effects on the SCOBY (Azuma and Hayashi, 2021).

2.1.2. Symbiotic Culture of Bacteria and Yeast

The SCOBY is a jelly-like biofilm that is floating on the kombucha's surface **(Figure 2.1**). It offers both structural and functional benefits for the microbial community. It is typically composed of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and various yeast species like *Saccharomyces*, *Zygosaccharomyces*, and *Brettanomyces* (Harrison and Curtin, 2021). During fermentation, cellulose is synthesised by the AAB, leading to the progressive layer-by-layer formation and thickening of the SCOBY (Antolak, Piechota and Kucharska, 2021). This layered structure not only supports microbial colonisation but also allows the formation of a new SCOBY in each fermentation cycle.

Figure 2.1: A photograph of SCOBY culture in a kombucha (Johnston, 2023).

Each microbial group within the SCOBY works distinct but synergistic way to drive kombucha fermentation (**Figure 2.2**). The fermentation process usually starts with yeast. Yeast hydrolyses sucrose into glucose and fructose, then further metabolises into ethanol, glycerol, and carbon dioxide (Wang *et al.*,

2022). Subsequently, AAB ferments glucose into glucuronic acid and oxidises the ethanol produced by yeast to acetic acid. In addition, LAB produces various acids to contribute to the acidification. Moreover, *Gluconobacter* species convert D-sorbitol to L-ascorbic acid, also known as vitamin C (Su *et al.*, 2023). Importantly, the production of different enzymes by these microorganisms increases the overall bioavailability of polyphenols by breaking down complex ones into simpler forms (Onsun, Toprak, and Sanlier, 2025).

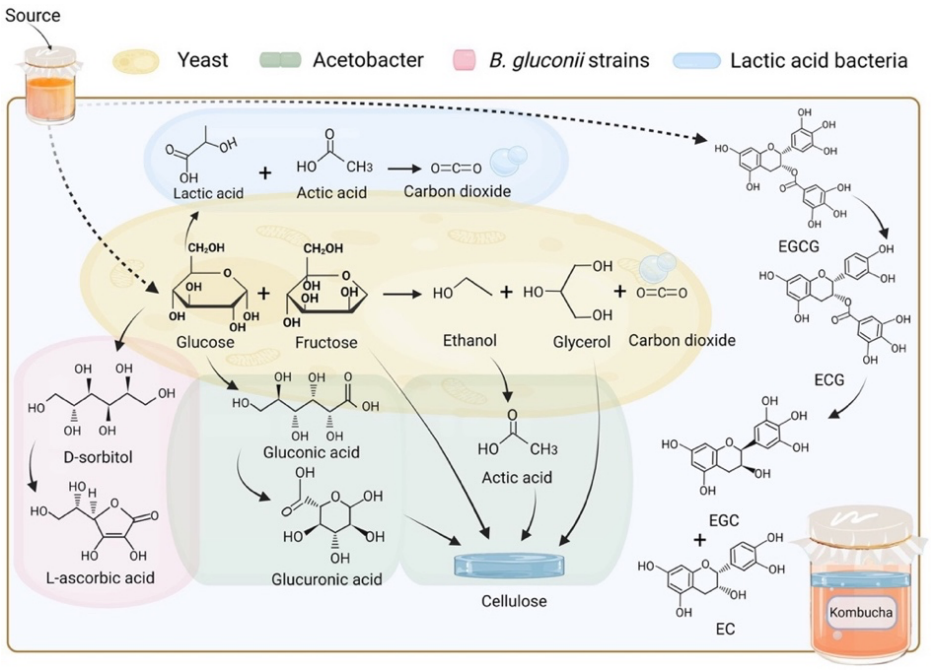


Figure 2.2: The role of various yeasts and bacteria within a SCOBY in kombucha fermentation (Su *et al*., 2023).

2.1.3. Key Metabolites of SCOBY

During fermentation, a variety of metabolites are produced by the SCOBY. These metabolites include phenolic derivatives, organic acids, vitamins, ethanol, and carbon dioxide. Phenolic derivatives are increased because of complex tea polyphenols being converted into simpler forms, which could contribute to the increased antioxidant capacity of kombucha compared to unfermented tea (Hu, Shi, and Ma, 2022). Furthermore, SCOBY produces various organic acids, including lactic acid, acetic acid, and glucuronic acid. These acids contribute to the kombucha's acidity and sourness. Moreover, *Gluconobacter* species, yeasts, and various lactic acid bacteria produce vitamin C and vitamin B-complex during kombucha fermentation (Sanwal *et al.*, 2023). Additionally, carbon dioxide and ethanol that yeast produces contribute to the fizziness and alcoholic taste of kombucha. In well-controlled fermentation, the alcohol content of kombucha usually falls below 0.5%, but it may reach up to 3% for homemade kombucha (Fonteles *et al*., 2024). The chemical composition of kombucha changes simultaneously across fermentation, with the increase in polyphenols and organic acids content and the decrease in sugar content.

2.1.4. Health Benefits of Kombucha

There are many reported health benefits of kombucha due to the various bioactive compounds produced during fermentation. These include antioxidant, antimicrobial, detoxification, gut health, and immune system boosting. Kombucha contain high amounts of polyphenols and certain organic acids,

which are antioxidants that can scavenge free radicals and active oxygen species to reduce oxidative stress (Ahmed, Hikal, and Abou-Taleb, 2020). In addition, acetic acid and catechin present in kombucha could exhibit antimicrobial properties to inhibit both bacteria (Al-Mohammadi *et al.*, 2021). Moreover, glucuronic acid produced by *Acetobacter* during fermentation can detoxify against drugs, pollutants, and excessive steroid hormones (Nguyen *et al.*, 2015). Furthermore, the presence of live probiotics like LAB and AAB may improve digestive health and the immune system by promoting gut microbiota balance (Sengun and Kirmizigul, 2020). Although these health claims have shown promising outcomes in many in vitro and in vivo studies, more human clinical tests are needed to substantiate many of these claims (O'Sullivan and O'Sullivan, 2024).

2.2. Factors Affecting Kombucha Quality

2.2.1. Tea Type

The phytochemical content, antioxidant activity, and sensory qualities of kombucha can be varied depending on the tea type used in preparation. Although green, oolong, and dark teas are derived from the same *Camellia sinensis* plant, their degrees of oxidation, catechin content, and sensory profile are different. This is due to the distinct manufacturing processes they undergo. Green tea is unoxidised and rich in catechins, particularly epigallocatechin gallate (EGCG), which contributes to its high antioxidant potential and its retained green colour (Jiang *et al.*, 2015). Oolong tea, on the other hand, is semi-oxidised by endogenous enzymes, resulting in partial transformation of catechins into

theaflavins and other oxidation products, giving it antioxidant properties that lie between green tea and fully oxidised black tea (Zhang, Qi, and Mine, 2019; Naveed *et al*., 2018). Dark tea, a special tea type that is post-fermented by microorganisms, contains a unique polyphenol profile with a high level of theabrownins and microbial metabolites (Aloo *et al*. 2024). These complex polyphenols are then biotransformed into smaller, more bioavailable compounds to enhance the antioxidant properties of kombucha. Moreover, these tea compounds also affect the flavour profile. For example, green tea gives a lighter flavour profile compared to the fruity to rich flavour profile of oolong tea (Bishop *et al*., 2022). Lastly, the enriched metabolites in dark tea may offer a mellow and sweet taste (Pan *et al*., 2022).

2.2.2. Fermentation Conditions

Fermentation conditions like temperature, time, and pH significantly influence the growth and metabolism of SCOBY, eventually affecting the overall quality of kombucha. The ideal temperature of kombucha fermentation is between 25°C–30°C, and the ideal pH is between 2.5–4.2 (Sadok *et al.*, 2025; Chong *et al*., 2024). The fermentation process may slow down when the temperature is below this range, while an excessive temperature can cause undesirable metabolic changes or the killing of the SCOBY. Kombucha fermented in a short period has a sweeter taste due to high sugar residual. As fermentation progresses, it turns acidic and tangy due to acid accumulation and lower sugar residual (Teoh, Heard, and Cox, 2004). This causes the pH of kombucha to gradually lower from approximately 5 to 2.5–3.5 by the end of fermentation (Nyhan *et al*., 2022).

However, prolonged fermentation may cause an excessive sourness that reduces consumer acceptability. According to Aung and Eun (2021), kombucha fermented at 30°C exhibited a faster pH drop, higher titratable acidity, and increased microbial activity, but showed a lower total bioactive content compared to fermentation at 25°C. A prolonged fermentation time also showed a decrease in bioactive compounds. This suggests that high temperature and prolonged fermentation can cause the degradation of beneficial compounds, lowering antioxidant potential.

2.3. Antioxidant Compounds in Kombucha

The antioxidant potential of kombucha is mainly attributed to various bioactive compounds produced via microbial fermentation of tea components. Polyphenols, L-ascorbic acid, and D-saccharic acid-1, 4-lactone are the most common and important antioxidants found in kombucha (Leal *et al*., 2018). These antioxidants could reduce oxidative stress via free radical scavenging, metal chelation, or reducing.

2.3.1. Polyphenols

Polyphenols that originate from the tea substrate are the main antioxidant components found in kombucha. They are characterised by having one or more phenol rings with one or more hydroxyl groups (–OH) and refer to a diverse family of plant-derived compounds (Belščak-Cvitanović *et al*., 2018). As shown

in **Figure 2.3**, the common polyphenols found in tea leaves include catechins, theaflavins, thearubigins, and their derivatives. Among these, catechins are the most abundant in tea leaves.

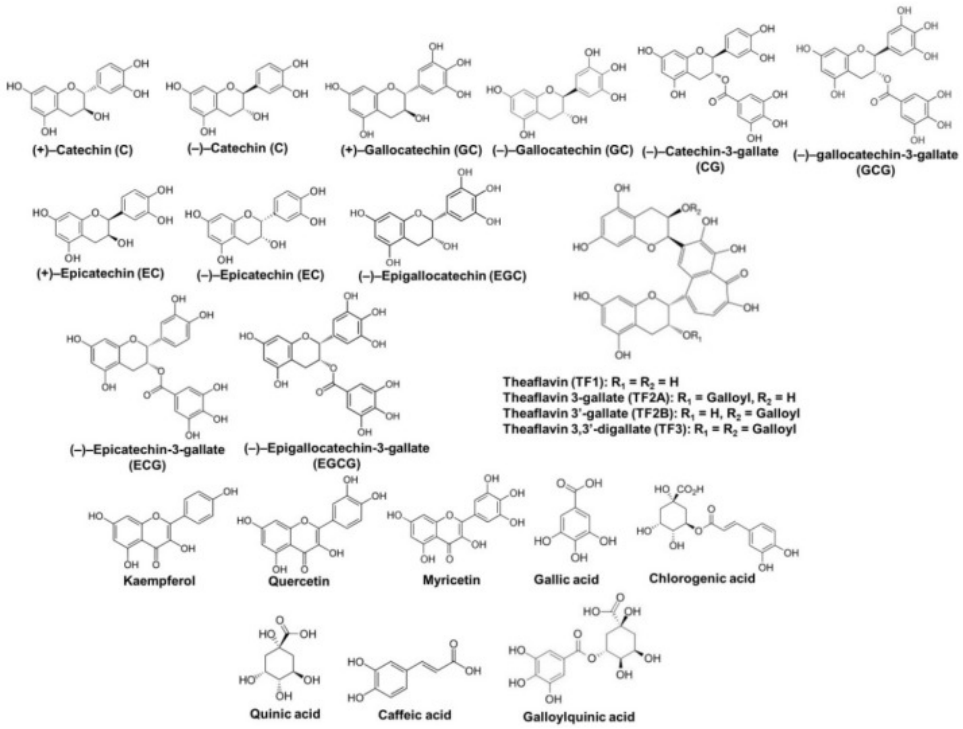


Figure 2.3: The chemical structure of various polyphenols present in tea (Truong and Jeong, 2021).

The hydroxyl groups on polyphenols' aromatic rings allow them to donate hydrogen atoms and electrons to scavenge reactive oxygen species (ROS) (Zhong, Ma, and Shahidi, 2011). The antioxidant efficiency of polyphenols highly depends on the number of hydroxyl groups in their structure (Truong and Jeong, 2021). The more hydroxyl groups present, the higher the hydrogen-donating ability to neutralise free radicals, thereby higher the antioxidant

potential. In addition, polyphenols can chelate to metal ions to prevent the formation of harmful metal-catalysed free radicals to exhibit antioxidant activities (Frei and Higdon, 2003). This process is known as metal chelation. Moreover, catechins can also act as indirect antioxidants to protect the body by upregulating antioxidant enzymes, inhibiting prooxidant enzymes, and modulating redox-sensitive transcription factors (Bernatoniene and Kopustinskiene, 2018).

During fermentation, various enzymes like tannase and phytase are produced by SCOBY to break down complex polyphenols into simpler polyphenol monomers like gallic acid and caffeic acid (Kaewkod, Bovonsombut, and Tragoolpua, 2019; Phung *et al.*, 2023). These phenolic acids may contribute to kombucha's increased antioxidant activity because they are typically more bioactive. However, the type and quantity of polyphenols are different in varied tea types. Therefore, varied tea types could undergo different biotransformation during kombucha fermentation, resulting in different antioxidant potential.

2.3.2. L-Ascorbic Acid

L-ascorbic acid, also known as vitamin C, is another potent antioxidant compound found in kombucha. It is produced by *Gluconobacter* species within the SCOBY from D-sorbitol during the glucose metabolism process (**Figure 2.4**). It can donate electrons to scavenge free radicals and effectively stop the oxidation from the chain reaction (Padayatty *et al.*, 2003). In addition, vitamin

C can support the action of antioxidants like polyphenols to exhibit a synergistic effect in reducing oxidative stress (Gęgotek and Skrzydlewska, 2022). Like polyphenols, vitamin C can act as a metal chelating agent to inhibit oxidation.

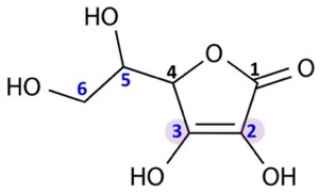


Figure 2.4: The chemical structure of vitamin C (Gęgotek and Skrzydlewska, 2022).

Many studies show that the vitamin C content increases during kombucha fermentation. Mousavi *et al.* (2020) and Sanwal *et al.* (2023) found that vitamin C content increased up to 25 mg/L and 29 mg/L after fermenting kombucha for 10 days, respectively. However, recent studies did not reveal the actual mechanism of L-ascorbic acid by *Gluconobacter* in kombucha fermentation. Instead of direct synthesis of vitamin C, some studies show that *Gluconobacter* species like *Gluconobacter oxydans* only produce L-sorbose by oxidising D-sorbitol. L-sorbose is just an intermediate form in industrial vitamin C production, and it requires other strains like *Ketogulonicigenium vulgare* and *Bacillus megaterium* that are absent within SCOBY to biotransform it to 2-keto-l-gulonic acid before forming L-ascorbic acid (Chen *et al.*, 2023; Gomes *et al.*, 2018; Zhang and Lyu, 2022). Therefore, vitamin C in kombucha is likely to be produced through minor alternative biosynthesis pathways.

2.3.3. D-Saccharic Acid-1, 4-Lactone

D-saccharic acid-1,4-lactone (DSL) is an important antioxidant found in kombucha (**Figure 2.5**). It is produced by *Gluconoacetobacter* species through the glucuronic acid pathway during kombucha fermentation (Jayabalan, Malbaša, and Sathishkumar, 2015). The production of DSL can be enhanced by cooperating with lactic acid bacteria (LAB) in SCOBY because LAB metabolites such as xylitol and acetic acid promote the growth of *Gluconoacetobacter* (Yang *et al.*, 2008). According to Leal *et al*. (2018), the DSL content reached a peak value of 132.72 µg/mL after fermenting kombucha for 8 days.

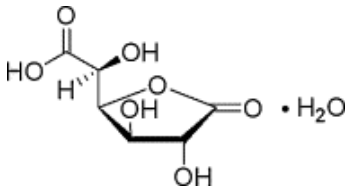


Figure 2.5: The chemical structure of DSL (Wang *et al*., 2010).

A vivo study by Bhattacharya, Gachhui, and Sil (2012) showed that taking DSL helped lower the harmful ROS in the liver of diabetic rats and restore intracellular antioxidant systems, preventing apoptosis in diabetic liver tissue. In addition, it can act as a protective detoxifier that indirectly contributes to the antioxidant activity by inhibiting β-glucuronidase that produces a pro-oxidant compound called aglycones (Wang *et al*., 2010).

2.4. Evaluation of Phytochemical Content and Antioxidant Activity

Antioxidant potential is one of the significant health-beneficial properties of kombucha. The main contributors of this antioxidant potential in kombucha are phenolic compounds and their subgroup catechins (Massoud *et al*., 2021). The microbial activity of SCOBY and various enzymatic activities may change the composition and concentration of these phytochemicals during fermentation, affecting the overall antioxidant potential. The total phenolic and total flavonoid contents are two common parameters for phytochemical content determination. On the other hand, DPPH radical scavenging activity and ferric reducing antioxidant power assays (FRAP) are commonly used to evaluate the antioxidant activity (Thennakoon *et al*., 2022).

2.4.1. Total Phenolic Content

The major antioxidants in kombucha are phenolic compounds, which are known for their ability to neutralise reactive oxygen species by donating electrons. The TPC content in foods is commonly assessed through the Folin-Ciocalteu (F-C) colourimetric assay. The principle behind this assay is the electron-donating ability of phenolic compounds. They can reduce the originally yellow oxidising F-C reagent to phosphomolybdenum blue that can be measured spectrophotometrically at 765 nm, as shown in **Figure 2.6** (Pérez, Dominguez-López, and Lamuela-Raventós, 2023). The colour intensity and absorbance reading are proportional to the TPC in the sample. However, the result of TPC in kombucha can be overestimated due to the presence of other reducing agents, like reducing sugars and ascorbic acids (Sánchez-Rangel *et al*., 2013).

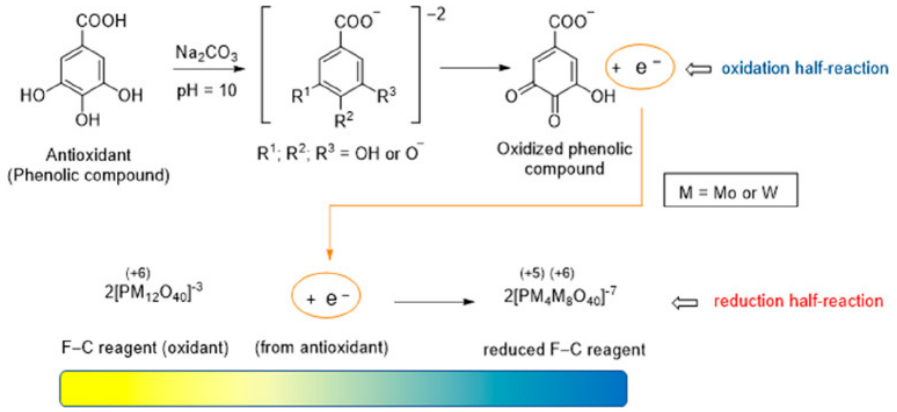


Figure 2.6: The working principle of Folin-Ciocalteu colourimetric assay (Pérez, Dominguez-López, and Lamuela-Raventós, 2023).

Sodium carbonate is often added to the sample mixture to create a condition where pH is around 10 for a successful reaction between phenolic compounds and F-C reagent (Munteanu and Apetrei, 2021). This may be due to the deprotonation of phenolic compounds in basic conditions, where their hydroxyl groups (–OH) lose a proton and become phenoxide ions, making them more reactive to free radicals (Priyadarsini *et al*., 1999). In addition, gallic acid is the most used reference in this assay to plot a standard curve for quantifying the TPC (Martins *et al*., 2021).

2.4.2. Total Flavonoid Content

Flavonoids, especially catechins, are a subclass of polyphenols that exhibit most of the antioxidant potential in kombucha. The TFC content in foods is commonly

assessed through the aluminium chloride ($AlCl_3$) colourimetric assay that is based on the metal chelation ability of flavonoids. The aluminium ion (Al^{3+}) from aluminium chloride can bind to specific functional groups on flavonoids. For instance, Al^{3+} can bind to the 3–4 site, 5–4 site, or 3′–4′ site of quercetin, forming stable yellow complexes that can be measured spectrophotometrically between 410 nm and 440 nm, as shown in **Figure 2.7** (Kukhtenko *et al*., 2024). The colour intensity and absorbance reading are proportional to the TFC in the sample.

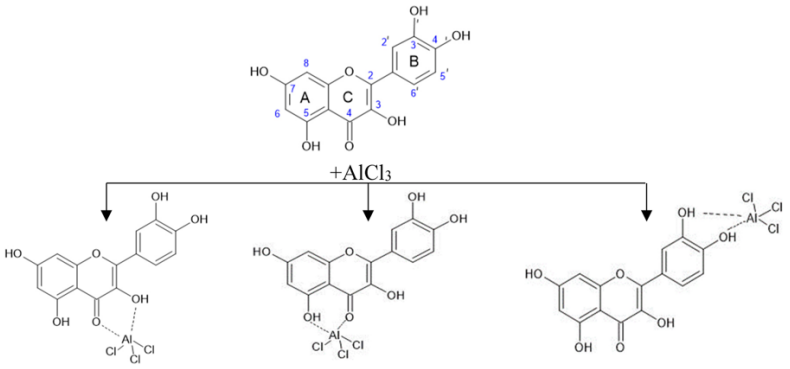


Figure 2.7: The possible binding sites on flavonoids by aluminium ions (Kukhtenko *et al*., 2024).

Acetate salt, such as sodium acetate or potassium acetate, has been added to the $AlCl_3$ in the classic TFC assay because it is believed to have a stabilising effect on the complex and help enhance the specificity of the assay for flavonoids (Shraim *et al*., 2021). However, recent studies show that acetate salt is not always necessary to be involved due to its minor effect on the formation of the complex (Nicolescu, Bunea, and Mocan, 2025). In addition, quercetin is a

flavonoid commonly used reference in this assay to plot a standard curve for quantifying the TFC.

2.4.3. DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a simple method that is commonly used to assess the radical scavenging ability of antioxidants. This assay is based on the hydrogen-donating ability of antioxidants to reduce the originally deep purple DPPH radicals to pale yellow 1,1-diphenyl-2-picrylhydrazine that can be measured spectrophotometrically at around 515 nm, as shown in **Figure 2.8** (Higgins *et al*., 2021). The more the discolouration of the DPPH solution and decrease in absorbance indicate the higher antioxidant potential for the sample used. The result can be expressed as DPPH inhibition percentage or IC_{50} if a fixed concentration of sample is used, which is the sample concentration that inhibits 50% of DPPH radicals (Xiao *et al*., 2020).

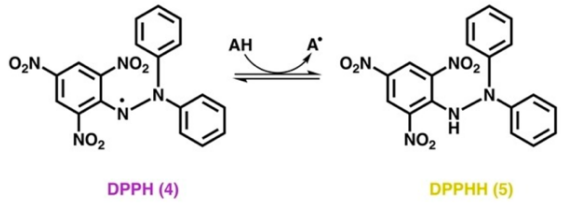


Figure 2.8: The working principle of DPPH assay (Higgins *et al*., 2021).

2.4.4. Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) assay measures the electron-donating ability of antioxidants. It is based on the conversion of the ferric ions (Fe^{3+}) within the 2,4,6-tripyridyl-s-triazine (TPTZ) complex to ferrous ions (Fe^{2+}) by antioxidants. The result is an intense navy blue complex that can be measured spectrophotometrically at 593 nm, as shown in **Figure 2.9** (Benzie and Choi, 2014). The colour intensity and absorbance reading are proportional to the reducing power of antioxidants in the sample.

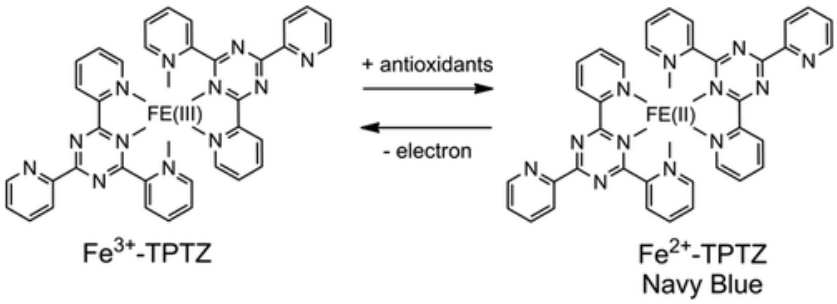


Figure 2.9: The working principle of the FRAP assay (Xiao *et al.*, 2020).

The FRAP solution needs to be freshly prepared by mixing TPTZ in hydrochloric acid, ferric chloride solution, and acetate buffer with a pH of 3.6, in a 1:1:10 ratio, and warmed before use (Proestos and Komaitis, 2009). The pH 3.6 acetate buffer provides an acidic environment to ensure iron solubility and potentially facilitate the electron transfer process. On the other hand, the TPTZ acts as a ligand to bind the iron ions released from the ferric chloride (Munteanu and Apetrei, 2021). In addition, ferrous sulphate can be used to plot a standard

curve for quantifying the reducing power (Wanna, 2019). The result is expressed as Fe^{2+} equivalents per mL or g of sample.

2.5. Sensory Evaluation

Sensory evaluation is a scientific method commonly used to analyse the sensory properties of a food product. It relies on human perception, primarily through smelling, observing, and tasting foods (Ruiz-Capillas and Herrero, 2021). Among all sensory evaluation methods, the 9-point hedonic scaling test is a popular method to determine consumer acceptance. It is simple and accurate without the need for trained panels or complex profiling methods (Lim, 2011). It involves asking panellists to score their levels of liking towards a sample or a specific attribute of that product, from "Dislike extremely" (1) to "Like extremely" (9). The collected data can be used to guide formulation adjustments that meet consumer satisfaction, which is important in functional products like kombucha (Wu *et al*., 2023). This ensures that the final product is both nutritionally beneficial and acceptable to target consumers.

CHAPTER 3

METHODOLOGY

3.1. Research Framework

The flow of the experiment for this study is shown in **Figure 3.1**, from kombucha starter and tea selection to data tabulation and statistical analysis.

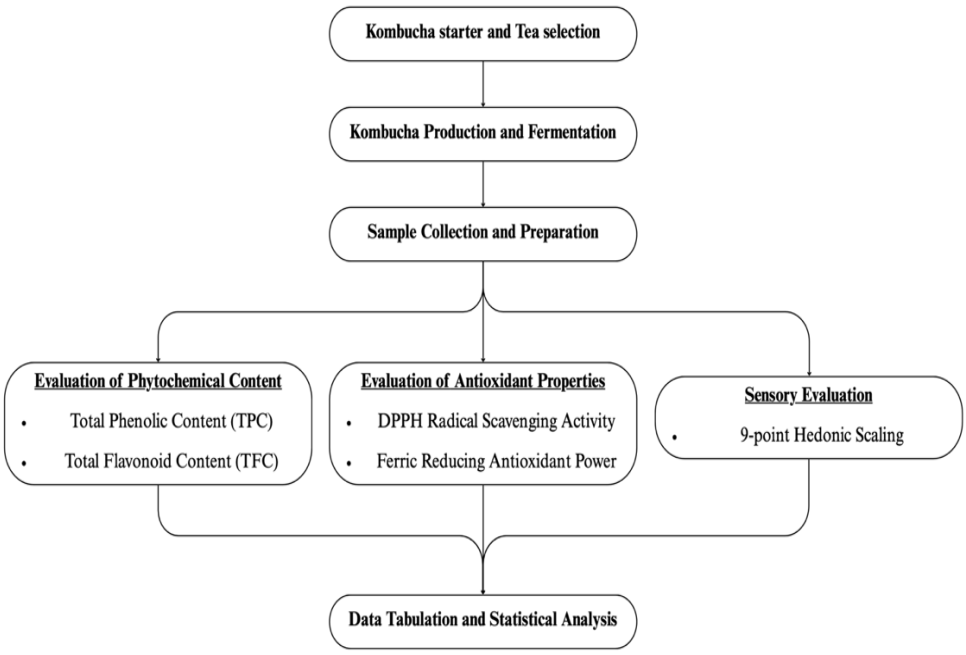


Figure 3.1: The experiment flow-chart of this study.

3.2. Chemicals and Equipment

The chemicals, equipment, consumables and glassware used to perform phytochemical and antioxidant determinations in this study are listed in **Appendices A** to **C**, including the manufacturer information and country of origin. Most of the materials were obtained from and used in Universiti Tunku Abdul Rahman, while some consumables like aluminium foil and disposable cups were purchased from the Lotus supermarket.

3.3. Kombucha Preparation

3.3.1. Kombucha Starter and Tea Selection

The SCOBY and starter tea used in this study were bought online via Shopee from a Penang, Malaysia seller named Herbal Remedies. As the supplier did not disclose the specific microbial composition of the SCOBY, it remained unidentified in this study. Three types of tea leaves were selected as the substrate of kombucha, including green, oolong, and dark teas. All teas were bought from the Billion Shopping Centre located at Sungai Petani, Kedah. Specifically, pure green tea from *Ahmad Tea London* was chosen as the green tea, Ti Kuan Yin, imported from China and packaged by *Super Tea Sdn. Bhd.* was used as the oolong tea, and ripened Yunnan Pu-erh tea, imported from China and packaged by *MGH Marketing*, was used as the dark tea. The packaging of selected teas is shown in **Figures 3.2-3.4**.

Figure 3.2: The packaging of pure green tea from *Ahmad Tea London*.

Figure 3.3: The packaging of Ti Kuan Yin, imported from China and packaged by *Super Tea Sdn. Bhd.*

Figure 3.4: The packaging of Yunnan Pu-erh tea, imported from China and packaged by *MGH Marketing*.

3.3.2. Kombucha Production and Fermentation

The kombucha was produced according to the formulation described by Jakubczyk *et al*. (2020). First, the glass jars were sterilised and properly labelled. To prepare sweetened tea, 6 g of tea leaves (0.8% w/v) and 75 g of fine sugar (10% w/v) were added to 750 mL of hot water (80–90°C) dispensed from a water dispenser into a sterilised 1.0 L glass jar. All tea leaves were brewed for 10 minutes. After tea preparation, the tea leaves were removed from the glass jar using a filter. The sweetened tea was then cooled to room temperature, around 25ºC. Once cooled, 75 mL of starter tea (10% v/v) and approximately 40 g of SCOBY were added to each jar containing sweetened tea. A clean cheesecloth was used to cover each glass jar and secured with a rubber band. All kombucha samples were fermented indoors at room temperature (~25°C) for 0, 7, and 14 days. The same procedure was repeated for each tea type (green, oolong, and dark tea).

3.3.3. Sample Collection and Preparation

During the sampling process, a sterile pipette was used to sample 10 mL of each kombucha sample into 15 mL centrifuge tubes. This step was repeated at the specified fermentation times of Days 0, 7, and 14. Before analysis, a centrifuge machine was used to centrifuge the samples under the settings of 15,000 rpm and 10 minutes. After centrifugation, a micropipette was used to collect the supernatant. Distilled water was used to dilute the supernatant into the required ratios. Specifically, for the phytochemical content determination and the FRAP assay, the supernatant was diluted at ratios of 1:20, 1:25, and 1:40 (v/v),

respectively. On the other hand, a fixed dilution of 1:100 (v/v) was used for the radical scavenging activity assay. All dilutions were freshly prepared on the same day as the assay measurements.

3.4. Evaluation of Phytochemical Content and Antioxidant Properties

3.4.1. Determination of Total Phenolic Content

The method used to determine the TPC for kombucha was the Folin-Ciocalteu (F-C) colourimetric assay, as described by Zheng *et al*. (2024), with some modifications. A 10% (v/v) F-C solution was prepared using 1 mL of F-C stock reagent with 9 mL of distilled water. Separately, a 7.5% (w/v) sodium carbonate solution was prepared by diluting 3 g of anhydrous sodium carbonate in 20 mL of distilled water. A total of 500 µL of 10% (v/v) F-C solution and 400 µL of 7.5% (w/v) sodium carbonate solution were then added to each 1.5 mL centrifuge tube containing 100 µL of sample supernatant and incubated in the dark. After 60 minutes of incubation, a micropipette was used to transfer 200 µL of each mixture into a 96-well plate. A microplate reader was used to measure the absorbance at 765 nm, with distilled water as the blank. Gallic acid was used as the reference compound to plot a standard curve. A 100 mg/L gallic acid stock solution was prepared by dissolving 10 mg of gallic acid in 100 mL of distilled water. This was then used to prepare five different concentrations of gallic acid standard solutions, from 5 to 80 mg/L, through serial dilution. These standard solutions were being analysed using the same procedures as the samples. The TPC value of the kombucha samples was expressed as mg of gallic acid equivalents per L of sample (mg GAE/L).

3.4.2. Determination of Total Flavonoid Content

The method used to determine the TFC for kombucha was the aluminium chloride colourimetric assay, as described by Pękal, A. and Pyrzynska, K. (2014), with some modifications. A 10% (w/v) aluminium chloride solution was prepared by diluting 1 g of anhydrous aluminium chloride in 10 mL of distilled water. Separately, 1 mol/L sodium acetate was prepared by dissolving 0.8203 g of anhydrous sodium acetate in 10 mL of distilled water. A total of 250 μL of each solution was then added to each 1.5 mL centrifuge tube containing 500 μL of sample supernatant. The mixture was shaken and incubated in the dark. After 10 minutes of incubation, a micropipette was used to transfer 200 μL of each mixture into a 96-well plate. A microplate reader was used to measure the absorbance at 425 nm, with distilled water as the blank. Quercetin was used as the reference compound to prepare a plot standard curve. A 100 mg/L quercetin stock solution was prepared by dissolving 10 mg of quercetin in 100 mL of distilled water. This was then used to prepare five different concentrations of quercetin standard solutions, from 10 to 80 mg/L, through serial dilution. These standard solutions were being analysed using the same procedures as the samples. The FPC value of the kombucha samples was expressed as mg of quercetin equivalents per L of sample (mg QE/L).

3.4.3. Determination of Free Radical Scavenging Activity

The method used to determine the free radical scavenging activity was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described by Fu *et al*. (2014), with some modifications. A 0.2 mmol/L DPPH solution was prepared by diluting

7.88 g of DPPH in 100 mL of methanol. A total of 1000 µL of this solution was then added to each 1.5 mL centrifuge tube containing 500 µL sample supernatant. The mixture was then shaken and incubated in the dark. After 30 minutes of incubation, a micropipette was used to transfer 200 µL of each mixture into a 96-well plate. A microplate reader was used to measure the absorbance at 517 nm. Distilled water was used as a control and was analysed using the same procedures as the samples. The DPPH radical scavenging activity was calculated using:

$$\text{DPPH Inhibition (\%)} = [(A_C - A_S)/ A_C] \times 100\%$$

where the A_C is the control's absorbance and the A_S is the sample's absorbance.

3.4.4. Determination of Ferric Reducing Antioxidant Power

The method used to determine the reducing power of kombucha was the Ferric Reducing Antioxidant Power (FRAP) assay, as described by Saimaiti *et al*. (2022), with some modifications. First, a 300 mmol/L sodium acetate buffer solution was prepared by dissolving 2.36 g of sodium acetate anhydrous in 100 mL of distilled water. Its pH was adjusted to 3.6 using glacial acetic acid. Second, a 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution was prepared by dissolving 0.312 g of TPTZ in 50 mL of pre-prepared 40 mmol/L HCl. Third, a 20 mmol/L ferric chloride solution was prepared by dissolving 0.54 g of ferric chloride hexahydrate in 100 mL of distilled water. Finally, a FRAP solution was prepared by combining these solutions in a 10:1:1 volume ratio and incubating them in a 37 °C water bath for 60 minutes before use.

A total of 900 µL of FRAP solution was added to each 1.5 mL centrifuge tube containing 25 µL of sample supernatant and incubated in the dark. After 10 minutes of incubation, a micropipette was used to transfer 200 µL of each mixture into a 96-well plate. A microplate reader was used to measure the absorbance at 593 nm, with distilled water as the blank. Ferrous sulphate was used as a reference compound to prepare a standard curve. A 1.0 mmol/L ferrous sulphate stock solution was prepared by dissolving 27.8 mg of ferrous sulphate heptahydrate in 100 mL of distilled water. This was then used to prepare four different concentrations of ferrous sulphate standard solutions, from 0.2 to 0.8 mmol/L, through serial dilution. These standard solutions were being analysed using the same procedures as the samples. The FRAP value of the kombucha samples was expressed as µmol of ferrous ion (Fe^{2+}) per L of sample (mmol Fe^{2+}/L).

3.5. Sensory Evaluation

A 9-point hedonic scaling test was applied as a sensory evaluation method to evaluate the consumer preference for the three different tea-based kombuchas at their peak antioxidant activity. These include green tea kombucha (GTK), oolong tea kombucha (OTK), and dark tea kombucha (DTK). Approximately 30 mL of each sample was labelled with random three-digit codes and served to each panellist random arrangement. A cup of water, a pencil, and a questionnaire (**Appendix D**) were also provided to each panellist. Panellists were instructed to taste each sample and evaluate five sensory attributes. The five sensory attributes evaluated are aroma, colour, sourness, sweetness, and overall

acceptability. Each attributes were assessed by giving a score from 1 to 9, meaning " extremely dislike" to "extremely like." A total of 50 untrained panellists carried out the sensory evaluation at the sensory room of Universiti Tunku Abdul Rahman. All responses and sensory scores were collected and recorded for further analysis.

3.6. Statistical Analysis

Phytochemical content and antioxidant activity determinations were performed in triplicate, except for the sensory evaluation. The obtained data were expressed as mean ± standard deviation (SD). The main and interaction effects of the tea type (GTK, OTK, and DTK) and fermentation time (Day 0, Day 7, and Day 14). A two-way analysis of variance (ANOVA) was used to analyse the data from phytochemical content and antioxidant activity determinations, while a one-way ANOVA was used for sensory evaluation data. A Tukey's Honest Significant Difference (Tukey's HSD) test was used to compare all possible pairs of means when significant differences were found ($p < 0.05$). All statistical analyses were performed using JMP Student Edition 18.2.2.

CHAPTER 4

RESULTS

4.1. Total Phenolic Content

The TPC values of kombucha prepared with different types of tea at various fermentation times were calculated using the linear regression equation in **Figure 4.1** and presented in **Table 4.1**. A two-way ANOVA revealed that both the main effects of tea type and fermentation time, as well as their interaction effect, were statistically significant ($p < 0.05$). Among all tea types, Green tea kombucha (GTK) consistently showed the highest TPC at all time points, with the highest value of 788.48 ± 5.84 mg GAE/L observed on Day 7. Conversely, dark tea kombucha (DTK) consistently showed the significantly lowest TPC at all time points, with the lowest value of 246.67 ± 2.78 mg GAE/L observed on Day 0. For all tea types, TPC significantly increased by Day 7 and then decreased by Day 14. By Day 7, the highest increase was observed in oolong tea kombucha (OTK) (+261.22 mg GAE/L), while the lowest increase was in DTK (+123.63 mg GAE/L). From Day 7 to Day 14, the highest decrease was in GTK (–85.45 mg GAE/L), while the lowest decrease was in DTK (–17.57 mg GAE/L).

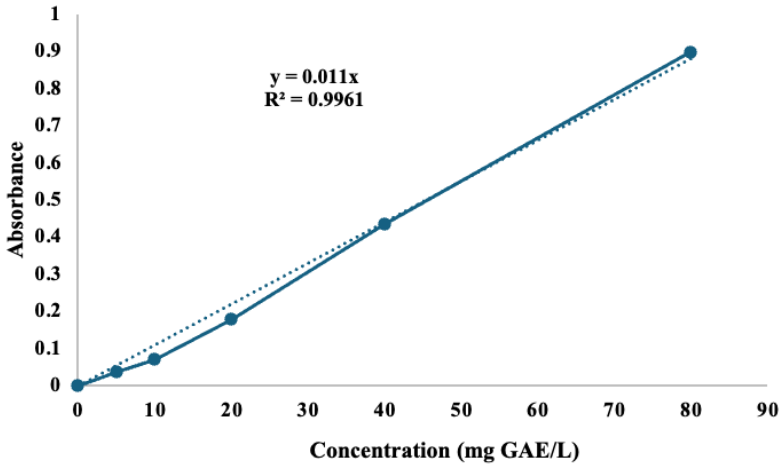


Figure 4.1: Standard curve prepared using various concentrations of gallic acid for TPC determination.

Table 4.1: TPC of kombucha fermented with different tea types at various fermentation times.

Tea Type	Total phenolic content (mg GAE/L)		
	Day-0	**Day-7**	**Day-14**
Green Tea	609.09 ± 7.27^{d}	788.48 ± 5.84^{a}	703.03 ± 2.78^{b}
Oolong Tea	365.45 ± 1.82^{fg}	626.67 ± 7.57^{c}	556.36 ± 3.64^{e}
Dark Tea	246.67 ± 2.78^{h}	370.30 ± 4.57^{f}	352.73 ± 3.64^{g}

Values are mean ± SD (n =3). Different superscript letters indicate a significant difference between means ($p < 0.05$) by Tukey's HSD test.

4.2. Total Flavonoid Content

The TFC values of kombucha prepared with different types of tea at various fermentation times were calculated using the linear regression equation in

Figure 4.2 and presented in **Table 4.2**. A two-way ANOVA revealed that both the main effects of tea type and fermentation time, as well as their interaction effect, were statistically significant ($p < 0.05$). Among all tea types, GTK consistently showed the highest TFC at all time points, with the highest value of 420.30 ± 2.92 mg QE/L observed on Day 7. Conversely, DTK consistently showed the significantly lowest TFC at all time points, with the lowest value of 102.73 ± 4.72 mg QE/L observed on Day 0. TFC significantly increased by Day 7 for all tea types. The highest increase was observed in GTK (+159.39 mg QE/L), while the lowest increase was in DTK (+65.75 mg QE/L). From Day 7 to Day 14, a significant decrease in TFC was only in GTK (–53.03 mg QE/L) and OTK (–51.82 mg QE/L).

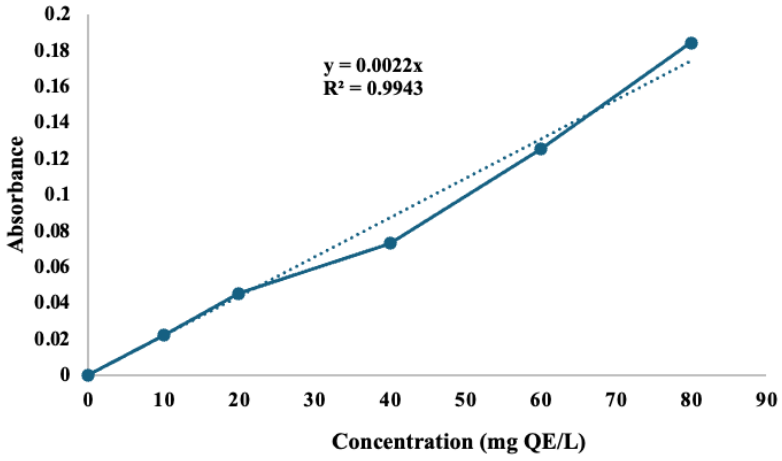


Figure 4.2: Standard curve prepared using various concentrations of quercetin for TFC determination.

Table 4.2: TFC of kombucha fermented with different tea types at various fermentation times.

Tea Type	Total flavonoid content (mg QE/L)		
	Day-0	**Day-7**	**Day-14**
Green Tea	260.91 ± 4.54^{c}	420.30 ± 2.92^{a}	367.27 ± 2.40^{b}
Oolong Tea	133.64 ± 2.73^{f}	270.00 ± 3.28^{c}	218.18 ± 4.54^{d}
Dark Tea	102.73 ± 4.72^{g}	168.48 ± 1.89^{e}	160.61 ± 1.39^{e}

Values are mean ± SD (n =3). Different superscript letters indicate a significant difference between means ($p < 0.05$) by Tukey's HSD test.

4.3. DPPH Free Radical Scavenging Activity

The DPPH radical scavenging activity (% inhibition) of kombucha prepared with different types of tea at various fermentation times is presented in **Table 4.3**. A two-way ANOVA revealed that both the main effects of tea type and fermentation time, as well as their interaction effect, were statistically significant ($p < 0.05$). Among all tea types, GTK consistently showed the highest DPPH inhibition at all time points, with the highest value of 73.15 ± 0.38% observed on Day 7. Conversely, DTK consistently showed the significantly lowest DPPH inhibition at all time points, with the lowest value of 28.21 ± 0.35% observed on Day 0. For all tea types, DPPH inhibition significantly increased by Day 7 and then decreased by Day 14. By Day 7, the highest increase was observed in DTK (+21.90%), while the lowest increase was in GTK (+13.90%). From Day 7 to Day 14, the highest decrease was in DTK (–6.80%), while the lowest decrease was in OTK (–4.24%).

Table 4.3: DPPH inhibition of kombucha fermented with different tea types at various fermentation times.

Tea Type	DPPH Inhibition (%)		
	Day-0	**Day-7**	**Day-14**
Green Tea	59.25 ± 0.55^{c}	73.15 ± 0.38^{a}	68.91 ± 0.06^{b}
Oolong Tea	39.38 ± 0.20^{g}	59.25 ± 0.26^{c}	55.25 ± 0.42^{d}
Dark Tea	28.21 ± 0.35^{h}	50.11 ± 0.45^{e}	43.31 ± 0.55^{f}

Values are mean ± SD (n =3). Different superscript letters indicate a significant difference between means ($p < 0.05$) by Tukey's HSD test.

4.4. Ferric Reducing Antioxidant Power

The FRAP values of kombucha prepared with different types of tea at various fermentation times were calculated using the linear regression equation in **Figure 4.3** and presented in **Table 4.4**. A two-way ANOVA revealed that both the main effects of tea type and fermentation time, as well as their interaction effect, were statistically significant ($p < 0.05$). Among all tea types, GTK consistently showed the highest FRAP at all time points, with the highest value of 16.19 ± 0.07 mmol Fe^{2+}/L observed on Day 7. Conversely, DTK consistently showed the significantly lowest FRAP at all time points, with the lowest value of 7.11 ± 0.09 mmol Fe^{2+}/L observed on Day 0. For all tea types, FRAP significantly increased by Day 7 and then decreased by Day 14. By Day 7, the highest increase was observed in GTK (+4.44 mmol Fe^{2+}/L), while the lowest increase was in DTK (+1.71 mmol Fe^{2+}/L). From Day 7 to Day 14, the highest

decrease was in OTK (–2.12 mmol Fe^{2+}/L), while the lowest decrease was in DTK (–0.79 mmol Fe^{2+}/L).

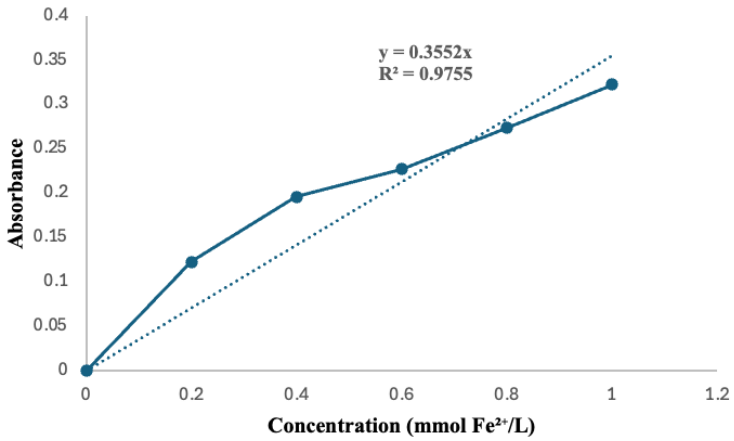


Figure 4.3: Standard curve prepared using various concentrations of ferrous ion (Fe^{2+}) for FRAP determination.

Table 4.4: FRAP values of kombucha fermented with different tea types at various fermentation times.

Tea Type	FRAP (mmol Fe^{2+}/L)		
	Day-0	**Day-7**	**Day-14**
Green Tea	11.75 ± 0.11^{c}	16.19 ± 0.07^{a}	14.40 ± 0.14^{b}
Oolong Tea	8.82 ± 0.11^{e}	11.05 ± 0.07^{d}	8.93 ± 0.18^{e}
Dark tea	7.11 ± 0.09^{g}	8.82 ± 0.18^{e}	8.03 ± 0.21^{f}

Values are mean ± SD (n =3). Different superscript letters indicate a significant difference between means ($p < 0.05$) by Tukey's HSD test.

4.5. Sensory Evaluation

The sensory attributes and acceptance of different types of kombucha at their highest antioxidant activity (Day 7) are presented in **Table 4.5**. There was no significant difference in the colour and sweetness among all three types of kombucha. In terms of aroma, sourness, and overall acceptability, DTK exhibited significantly lower values than the other kombuchas, while GTK and OTK did not differ from each other.

Table 4.5: Sensory attributes and acceptance of different types of kombucha evaluated using a 9-point hedonic scaling test.

Tea type	Green tea	Oolong tea	Dark tea
Aroma	7.18 ± 1.30^{a}	6.80 ± 1.24^{a}	5.36 ± 1.19^{b}
Colour	6.50 ± 1.58^{a}	7.10 ± 1.39^{a}	6.72 ± 1.23^{a}
Sourness	6.38 ± 1.41^{a}	6.38 ± 1.50^{a}	5.08 ± 1.48^{b}
Sweetness	6.36 ± 1.57^{a}	6.66 ± 1.66^{a}	6.42 ± 1.40^{a}
Overall Acceptability	6.66 ± 1.52^{a}	7.08 ± 1.26^{a}	5.74 ± 1.10^{b}

Values are mean ± SD (n =50). Different superscript letters within a same row indicate a significant difference between means ($p < 0.05$) by Tukey's HSD test.

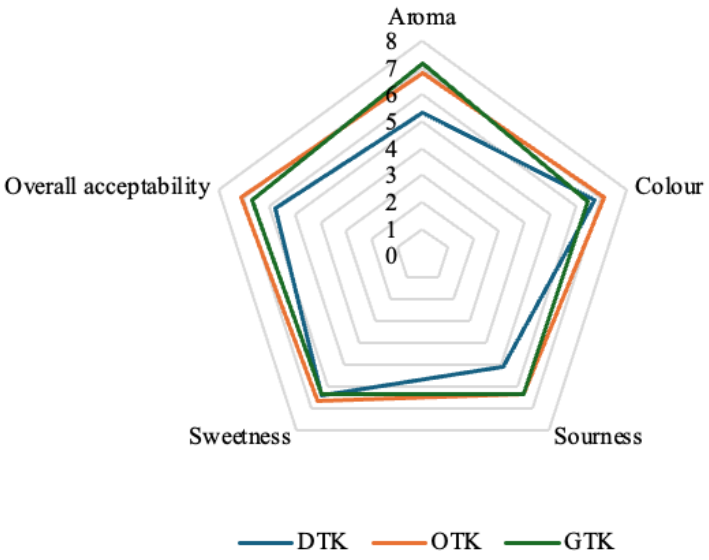


Figure 4.4: Radar chart of sensory attributes of different types of kombucha.

CHAPTER 5

DISCUSSION

5.1. Phytochemical Content

A two-way ANOVA revealed that the type of tea substrate and fermentation time, as well as their interaction, significantly affect the total phenolic content (TPC) and total flavonoid content (TFC) of kombucha.

5.1.1. Effect of Tea Type on TPC and TFC

Across all fermentation time points, green tea kombucha (GTK) consistently exhibited the highest TPC and TFC among all three types of kombucha. On the other hand, dark tea kombucha (DTK) exhibited the lowest in both phytochemical contents, while oolong tea kombucha (OTK) fell between GTK and DTK. The observations of Days 0 and 7 were aligned with Zheng *et al*. (2024), which show a trend of GTK > OTK > DTK across fermentation in both TPC and TFC. Wang *et al*. (2021) found that the total catechin content is ranked green tea (113 mg/g) > oolong tea (75.2 mg/g) > dark tea (7.22 mg/g). Therefore, the differences in TPC and TFC between kombucha samples can be attributed to the varied polyphenol content that is associated with the processing of tea.

According to Zhang *et al*. (2019), green tea is produced through fixation before rolling and drying, oolong tea through withering and rocking before rolling and drying, and dark tea through fixation and fermentation before shaping and drying. Green tea has undergone minimal processing compared to other tea types. In addition, green tea has undergone a fixation process, which involves heating to inactivate endogenous oxidative enzymes like polyphenol oxidase and peroxidase (Xue *et al*., 2023). This prevents green tea from oxidation and preserves the most tea polyphenols, especially catechins. As a result, more phenolic compounds are available to reduce the F-C reagent for TPC determination, and more flavonoids are available to chelate metal for TFC determination during kombucha fermentation.

The moderate TPC and TFC of OTK can be attributed to the partial oxidation during withering and rocking of oolong tea processing. The withering step involves exposing tea leaves to air or sunlight to reduce leaf moisture content and the semipermeability of the cells. This allows catechins in vacuoles to contact oxidative enzymes in the cytoplasm for oxidation (Lin *et al*., 2016). The next rocking step involves physical damage to the leaf edges, further promoting catechin oxidation until achieving the desired level. As a result, oolong tea retains only some catechins, but also produces oxidised polyphenols like theaflavins, thearubigins, and theasinensins (Sang, 2016). These oxidised polyphenols are present in smaller quantities and have more complex structures, which limits their contribution to the phytochemical contents.

The lowest phytochemical contents observed in DTK can be attributed to the formation of theabrownins during the post-fermentation of dark tea production. During this step, microorganisms like *Aspergillus* species convert tea polyphenols to larger, more complex theabrownins via oxidation, polymerisation, and condensation (Xiao *et al*., 2020; Wang *et al*., 2018). The total tea polyphenols are reduced with the production of theabrownins (Wang *et al.*, 2015). As a result, fewer phenolics and flavonoids are available in DTK to react with the F-C reagent for TPC measurement and chelate Al^{3+} for TFC determination. Moreover, theabrownin is a high molecular weight complex with unclear structure. It may contain polysaccharides and amino acids other than polyphenols, which may limit its reactivity compared with simpler tea polyphenols (Zhang *et al*., 2024).

5.1.2. Effect of Fermentation Time on TPC and TFC

Fermentation time also had a significant effect on TPC and TFC in kombucha. By Day 7, both TPC and TFC significantly increased in all types of kombucha. This observation aligns with Mihai, Cubi-Insuaste, and Catana (2024), in which both the TPC and TFC of 7-day fermented kombucha were higher than unfermented tea beverage. This increase can be explained by active microbial activity within kombucha during the early fermentation phase, when sugars and other nutrients are sufficient.

During fermentation, the SCOBY produces various enzymes like esterase, cellulase, and tannase to modify the structure of tea polyphenols or break down complex polyphenols to release smaller molecules (Jayabalan *et al.*, 2008; Neffe-Skocińska *et al*., 2023). A typical example is the hydrolysis of catechin gallate of epigallocatechin-3-gallate (EGCG) and epigallocatechin (ECG) by tannase and esterase to release smaller polyphenols, including epicatechin gallate (ECG), epicatechin (EC), and gallic acid (Macedo *et al*., 2012). In addition, depolymerisation of theaflavins and theobromines by the SCOBY also enhances the bioavailability of gallic acid, EC, and ECG to contribute both TPC and TFC amounts in kombucha (Su *et al*., 2023). Furthermore, β-glucosidase and other glycosidases can break down flavonoid glycosides like rutin into their aglycone forms like quercetin and kaempferol, increasing TFC values (Yang *et al*., 2023). These biotransformations enhance the bioavailability of phenolic compounds and flavonoids, thereby increasing the TPC and TFC in kombucha during active fermentation.

From Day 7 to Day 14, the TPC and TFC in all types of kombucha dropped significantly, except DTK showed an insignificant difference in TFC. A similar fermentation pattern was observed in kombucha prepared with green tea by Gaggìa *et al*. (2018), where TPC and TFC increased when measured at Day 7, followed by a significant decrease on Day 14. This suggests that the TPC and TFC of kombucha reached the peak amount after 7 days of fermentation, and prolonged fermentation to 14 days can lead to a decline in polyphenols. This may be attributed to the depletion of complex polyphenols for SCOBY

metabolism. The SCOBY may degrade the available phenolic compounds as an alternative energy and carbon source, especially when sugar is at a low level in the later fermentation. Another possible explanation could be that the prolonged fermentation causes excessive carbon dioxide accumulation between the SCOBY and the kombucha, thereby blocking the nutrient transfer and creating a starved condition (Villarreal-Soto *et al*., 2018). The insignificant difference of DTK in TFC from Day 7 to Day 14 suggests that its flavonoid profile, especially theabrownins, remained relatively stable during prolonged fermentation compared to other tea types.

Additionally, the decline in TPC and TFC from Day 7 to Day 14 may be partly due to the photooxidation of polyphenols. Polyphenols can be easily degraded when exposed to light and oxygen, as the reactive oxygen species (ROS) generated can oxidise phenolic hydroxyl groups into quinones and other oxidised compounds, reducing measurable content (Cao *et al*., 2021). Flavonoids, such as quercetins, are more susceptible to photooxidation, even under dark and low-oxygen conditions (Chaaban *et al*., 2017). Prolonged fermentation increases the exposure time of kombucha to these conditions, thereby contributing to the loss of polyphenols.

5.1.3. Interaction Effects of Tea Type and Fermentation Time on TPC and TFC

The interaction effect between tea type and fermentation time was also significant. Among three kombuchas, DTK exhibited the lowest increase in both TPC (+123.63 mg GAE/L) and TFC (+65.75 mg QE/L) from Day 0 to Day 7, as well as the lowest decrease (–17.57 mg GAE/L) in TPC and had no significant decline in the TFC from Day 7 to Day 14. These interaction effects may be linked to the presence of theabrownin that is unique to dark tea. It is a high-weight heterogeneous molecule that contains only 23.08–29.91% of polyphenols, while others are alkaloids, hydrocarbons, ketones, and nitrogen compounds (Liu *et al*., 2025). SCOBY may need a longer time to metabolise theabrownins, resulting in a slower fermentation. Moreover, the large amount of benzene rings in theabrownins may contribute to their stability against further oxidative degradation compared to monomeric catechins (Gong, Tang, and Peng, 2012). This suggests that the fermentation time for DTK can be extended for several days, but within a week.

After 7 days of fermentation, the highest increase in TPC was observed in OTK (+261.22 mg GAE/L). This may be attributed to the generally lower pH of OTK than GTK across fermentation, reaching a final pH of 2.72 on the tenth day of fermentation by Chong *et al*. (2024). This lower pH in OTK helps stabilise pH-sensitive tea polyphenols, especially for catechins with more hydroxyl groups, such as EGCG and EGC, preventing them from oxidative degradation (Zeng *et al*., 2016). Furthermore, the F-C assay used for TPC determination can respond

to non-phenolic reducing agents, giving an overestimation (Everette *et al*., 2010). For instance, glucose, fructose, and vitamin C are reducing agents produced during kombucha fermentation, may also contribute to the high TPC observed in OTK. However, for TFC, the highest increase was observed in GTK (+159.39 mg QE/L) because of its higher initial and unoxidised catechin content, which is more readily metabolised during fermentation.

5.2. Antioxidant Activity

A two-way ANOVA revealed that the type of tea substrate and fermentation times, as well as their interaction, significantly affect the antioxidant activities of kombucha, including 1,1-diphenyl-2-picrylhydrazine (DPPH) free radical scavenging activity and the ferric reducing antioxidant power (FRAP).

5.2.1. Effect of Tea Type on DPPH and FRAP

Across all fermentation time points, GTK consistently exhibited the highest antioxidant values in both assays, while DTK exhibited the lowest, and the OTK fell between them. This observation aligned with Zheng *et al*. (2024), which unoxidised green tea has a stronger antioxidant capability than oxidised oolong and dark teas. In addition, this observation showed a similar trend to phytochemicals discussed in **Section 5.1.1**. This suggests that the antioxidant potential of kombucha is closely linked to its phytochemical contents, which differ in different teas.

Previous studies reported that a significant correlation between catechins and antioxidant activities, as measured by both DPPH and FRAP assays, was observed in the study of Shafi *et al.* (2019). Therefore, the higher antioxidant capacity in GTK is mainly attributed to its higher catechin content, especially monomeric catechins like EGCG, around 60% of total catechins present in green tea (Naumovski *et al.*, 2019). According to Wang *et al*. (2023), EGCG and other unoxidised monomeric catechins had both significantly higher DPPH scavenging ability and ferrous reducing ability than dimeric and polymeric polyphenols in tea. The study identified that the sequence of antioxidant potential was monomeric catechins > theaflavins > thearubigins > theabrownins. The lowest antioxidant potential of theabrownin may be due to the presence of non-antioxidants in its structure, like polysaccharides. Therefore, the moderate antioxidant activities of OTK can be attributed to its higher amount of theaflavins and thearubigins, while the lowest antioxidant activities of DTK are due to the theabrownins from dark tea and its lower catechin content (Yashin *et al*., 2015).

5.2.2. Effect of Fermentation Time on DPPH and FRAP

All types of kombucha showed a significant increase in both antioxidant activities by Day 7, followed by a decrease by Day 14. These observations align with Gaggìa *et al*. (2018), in which DPPH inhibition and FRAP values significantly increased during kombucha fermentation and reached peak values

at Day 7, followed by a decline. In addition, these align with the trend observed in phytochemical contents discussed in 5.1.2, in which the antioxidant potential of kombucha increased with phenolic and flavonoid contents.

From Day 0 to Day 7, the increase in antioxidant potential of kombucha could be attributed to the increased amount of polyphenols released from the bound form in tea leaves (Onsun, Toprak, and Sanlier, 2025). During fermentation, β-glucosidase is produced by *Saccharomyces cerevisiae* within the SCOBY to hydrolyse the glycosidic bond of polyphenol glycosides, releasing their aglycone forms (Zubaidah *et al*., 2023). These aglycone forms often exhibit higher antioxidant activity and bioavailability (Antolak, Piechota, and Kucharska, 2021). In addition, a study found that the increased phenolic compounds and antioxidant activities of kombucha are closely related to the increased invertase produced by *Saccharomyces cerevisiae* (Jafari *et al*., 2020). Furthermore, L-ascorbic acid is the main vitamin produced by *Gluconobacter* species within the SCOBY, which can also donate electrons to scavenge DPPH free radicals and reduce ferric ions (Antolak, Piechota, and Kucharska, 2021). From Day 7 to Day 14, the decline in both antioxidant activities is likely due to the decrease in phytochemicals during prolonged fermentation. This may be a result of overfermentation by the SCOBY, during which polyphenols are used as nutrient sources (Phung *et al*., 2023).

5.2.3. Interaction Effects of Tea Type and Fermentation Time on DPPH and FRAP

The interaction effect of tea type and fermentation time was exhibited differently in the DPPH and FRAP assays. DTK exhibited the highest increase of 21.90% and decrease of 6.80% in the DPPH assay across fermentation. This was unexpected since theabrownins in dark tea are generally complex and require a longer time for SCOBY to metabolise. Therefore, the early increase might be due to the higher gallic acids that are more readily released to the kombucha during fermentation (Yashin *et al*., 2015). In contrast, the highest drop might be due to the low polyphenols available in dark tea, resulting in rapid depletion. GTK exhibited the lowest increase of 13.90% from Day 0 to Day 7. This was because of the high initial content of EGCG, which already high antioxidant potential due to the highly active trihydroxy groups, limiting the increase in antioxidant potential after breaking down into smaller polyphenols (Li, Cheng and Li, 2024). OTK exhibited the lowest decrease of 4.24% from Day 7 to Day 14, possibly due to the greater stability of complex theaflavins compared with monomeric catechins.

In the FRAP assay, GTK exhibited the highest increase of 4.44 mmol Fe^{2+}/L from Day 0 to Day 7. This finding aligns with its high catechin content, which possesses a strong electron-donating ability to reduce more ferric ions. DTK showed the lowest increase of 1.71 mmol Fe^{2+}/L and the lowest decrease of 0.79 mmol Fe^{2+}/L across fermentation. This reflects the limited ferric-reducing potential of theabrownins compared to catechins. A study showed that

theabrownins have a negative correlation with the tea's antioxidant activity (Wang *et al*., 2023). This may be attributed to the non-antioxidant compounds in its structure that limit its hydrogen-donating ability (Chen *et al*., 2022).

5.3. Sensory Evaluation

A one-way ANOVA revealed that DTK exhibited a significantly lower value for aroma, sourness, and overall acceptability than GTK and OTK, while GTK and OTK did not differ significantly in these sensory attributes. For colour and sweetness, there were no significant differences among all types of kombuchas.

The lower aroma ratings for DTK may be attributed to differences in volatile compound profiles associated with the degree of tea processing or fermentation. The more appealing aroma notes of GTK and OTK may be due to the higher amount and variety of volatile compounds (Zheng *et al*., 2024). GTK typically has chestnut-like and fresh aromas, while OTK has fruity fermented aromas due to its partial oxidation (Lin *et al*., 2023). In contrast, dark tea like ripe Pu-erh has undergone post-fermentation and extended microbial ageing, which transformed the initial fresh, fruity aromas of tea into heavier, stale, and woody notes (Ma *et al*., 2025). Furthermore, the less appealing aroma of DTK could also be related to differences in amino acid-derived volatiles. For instance, theanine is an amino acid that contributes to sweet and umami notes in tea aroma (Kaneko *et al*., 2006). However, these amino acids are lost significantly due to

the Maillard reaction or enzymatic conversion during the dark tea production (Zhu *et al*., 2015).

The lower rating in sourness and overall acceptability in DTK may be due to the theophylline formation and slower kombucha fermentation. During dark tea processing, caffeine was converted into theophylline by fungi like *Aspergillus pallidofulvus* and *Aspergillus sesamicola* (Zhou *et al*., 2019). Theophylline can enhance the sweet aftertaste to influence flavour, potentially reduce perceived sourness and alter the flavour balance, leading to lower overall acceptability (Yuan *et al*., 2025). Moreover, Hsieh, Chiu, and Chou (2021) found that the formation of zoogleal mat (cellulose-based film of SCOBY) was faster in green tea than in black and Pu-erh teas. This suggests that the tea with a less intensive oxidation or fermentation degree, like green tea and oolong tea, may ferment faster than dark tea. Therefore, the more intense characteristic sourness of kombucha fermented with green tea and oolong tea becomes more intense, which consumers generally prefer.

5.4. Limitations and Recommendations of the Study

A major limitation of this study was the undefined microbial composition of the SCOBY provided by the online seller. It was not feasible to confirm that all the SCOBY used for all tea types were consistent. Although the same percentage (w/v) of SCOBY was used, different microbial compositions may possess different fermentation dynamics, thereby influencing the antioxidant potential

of kombucha and offering a different sensory profile. To address this drawback, future studies should use laboratory-characterised SCOBY with a defined microbial composition to ensure consistency and reproducibility. In addition, physicochemical tests, such as total soluble solids and titratable acidity, were absent in this study. These physicochemical parameters could be used to explain the biochemical changes during fermentation and link to the findings from sensory evaluation. Future studies should include these comprehensive measurements to better correlate sensory evaluation data with underlying physicochemical changes.

CHAPTER 6

CONCLUSION

In conclusion, kombucha made with green tea consistently exhibited the highest values in TPC, TFC, DPPH free radical scavenging activity, and FRAP. This was followed by kombuchas made with oolong tea and dark tea kombucha. Across fermentation, all samples exhibited a significant increase in both phytochemical contents and antioxidant activities by Day 7, followed by a decline by Day 14. This reflects the release or transformation of polyphenols by the SCOBY, followed by subsequent degradation if prolonged fermentation. GTK showed the highest changes, particularly in TFC and FRAP, due to its naturally high catechin contents, while DTK showed the smallest changes due to the high stability of theabrownins.

Sensory evaluation revealed that GTK and OTK received significantly higher ratings in aroma, sourness, and overall acceptability than DTK. Colour and sweetness exhibited no significant differences between kombucha samples. The more appealing aroma of GTK and OTK was likely linked to higher levels of desirable volatile compounds, whereas the heavier, woody aroma of DTK was less appealing. The lower ratings of DTK in sourness and overall acceptability were likely linked to the theophylline formation and slower fermentation, which result in a less appealing flavour profile.

Overall, green tea remains the best option for fermenting kombucha due to its higher antioxidant and sensory qualities. For a more precise study, a SCOBY with a defined microbial composition should be used to ensure consistency. Physicochemical assays are also recommended to be included for a more complete study and understanding of the kombucha fermentation effect on both antioxidant activity and sensory properties.

REFERENCES

Ahmed, R.F., Hikal, M.S., and Abou-Taleb, K.A. (2020) 'Biological, chemical and antioxidant activities of different types of Kombucha', *Annals of Agricultural Sciences*, 65(1), pp. 35–41. Available at: https://doi.org/10.1016/j.aoas.2020.04.001

Ahuja, K. (2021) 'Kombucha industry expands in the Asia-Pacific region', *World Tea News*, 3 March. [online] Available at: https://www.worldteanews.com/products%20%26%20equipment/kombucha-industry-expands-asia-pacific-region (Accessed 3 July 2025).

Al-Mohammadi, A.-R. *et al.* (2021) 'Chemical constitution and antimicrobial activity of Kombucha fermented beverage', *Molecules*, 26(16), p. 5026. Available at: https://doi.org/10.3390/molecules26165026

Aloo, O.S. *et al*. (2024) 'Polyphenol constituents and impacts of fermented teas (*Camellia sinensis*) in human wellness', *Food Bioscience*, article number 60104389. Available at: https://doi.org/10.1016/j.fbio.2024.104389

Andrade, D.K.A. *et al*. (2025) 'Kombucha: An old tradition into a new concept of a beneficial, health-promoting beverage', *Foods*, 14(9), p. 1547. Available at: https://doi.org/10.3390/foods14091547

Antolak, H, Piechota, D., and Kucharska, A. (2021) 'Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY)', *Antioxidants*, 10(10), p. 1541. Available at: https://doi.org/10.3390/antiox10101541

Aung, T. and Eun, J-B. (2021) 'Impact of time and temperature on the physicochemical, microbiological, and nutraceutical properties of laver kombucha (Porphyra dentata) during fermentation', *LWT*, article number 154112643. Available at: https://doi.org/10.1016/j.lwt.2021.112643

Azuma, T. and Hayashi, T. (2021) 'Effects of natural sunlight on antimicrobial-resistant bacteria (AMRB) and antimicrobial-susceptible bacteria (AMSB) in wastewater and river water', *Science of the Total Environment*, 766, p. 142568. Available at: https://doi.org/10.1016/j.scitotenv.2020.142568

Baker, M.T. *et al*. (2022) 'Consumer acceptance toward functional foods: A scoping review', *International Journal of Environmental Research and Public Health*, 19(3), p. 1217. Available at: https://doi.org/10.3390/ijerph19031217

Belščak-Cvitanović, A. *et al.* (2018) 'Overview of polyphenols and their properties', in: C.M. Galanakis, ed. *Polyphenols: Properties, Recovery, and Applications*. Woodhead Publishing, pp. 3–44. Available at: https://doi.org/10.1016/B978-0-12-813572-3.00001-4 (Accessed: 3 July 2025).

Benzie, IFF. and Choi, S-W. (2014) ‘Antioxidants in food’, *Advances in Food and Nutrition Research*, pp. 1–53. Available at: https://doi.org/10.1016/b978-0-12-800270-4.00001-8

Bernatoniene, J. and Kopustinskiene, DM. (2018) ‘The role of catechins in cellular responses to oxidative stress’, *Molecules*, 23(4), p. 965. Available at: https://doi.org/10.3390/molecules23040965

Bhattacharya, S., Gachhui, R., and Sil, P. C. (2012) ‘The prophylactic role ofd-saccharic acid-1,4-lactone against hyperglycemia-induced hepatic apoptosis via inhibition of both extrinsic and intrinsic pathways in diabetic rats’, *Food & Function*, 4(2), pp. 283–296. Available at: https://doi.org/10.1039/c2fo30145h

Bishop, P. *et al.* (2022) ‘Chemical composition of kombucha’, *Beverages*, 8(3), p. 45. Available at: https://doi.org/10.3390/beverages8030045

Cao, H. *et al*. (2021) ‘Available technologies on improving the stability of polyphenols in food processing’, *Food Frontiers*, 2(2), pp. 109–139. Available at: https://doi.org/10.1002/fft2.65

Chaaban, H. et al. (2017) 'The photostability of flavanones, flavonols and flavones and evolution of their antioxidant activity,' *Journal of Photochemistry and Photobiology a Chemistry*, 336, pp. 131–139. https://doi.org/10.1016/j.jphotochem.2016.12.027

Chen, W. *et al*. (2023) ‘Symbiosis mechanism of associated bacteria on 2-keto-L-gulonic acid production via mixed fermentation: A review’, *Fermentation*, 9(12), p. 1000. Available at: https://doi.org/10.3390/fermentation9121000

Chen, X. *et al*. (2022) ‘Characterization of theabrownins prepared from tea polyphenols by enzymatic and chemical oxidation and their inhibitory effect on colon cancer cells’, *Frontiers in Nutrition*, 9. https://doi.org/10.3389/fnut.2022.849728

Chong, A.Q. *et al*. (2024) ‘Modelling pH dynamics, SCOBY biomass formation, and acetic acid production of kombucha fermentation using black, green, and oolong teas’, *Processes*, 12(7), p. 1301. Available at: https://doi.org/10.3390/pr12071301

Everette, J.D. *et al*. (2010) ‘Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu Reagent’, *Journal of Agricultural and Food Chemistry*, 58(14), pp. 8139–8144. Available at: https://doi.org/10.1021/jf1005935

Fensterseifer Fabricio, M. *et al*. (2023) ‘Revamping kombucha production: Achieving consistency and probiotic potential through a tailor-made microbial consortium’, *International Journal of Gastronomy and Food Science*, 34, p. 100844. Available at: https://doi.org/10.1016/j.ijgfs.2023.100844

Fonteles, T.V. *et al*. (2024) ‘Metabolic responses of kombucha consortium fermentation upon ultrasound-processing’, *Food Chemistry Advances*, 4, p. 100646. Available at: https://doi.org/10.1016/j.focha.2024.100646

Frei, B. and Higdon, J.V. (2003) ‘Antioxidant Activity of Tea Polyphenols In Vivo: Evidence from Animal Studies’, *Journal of Nutrition*, 133(10), pp. 3275–3284. Available at: https://doi.org/10.1093/jn/133.10.3275s

Fu, C. et al. (2014) ‘Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage’, *Food Science and Technology*, 34(1), pp. 123–126. Available at: https://doi.org/10.1590/s0101-20612014005000012

Gaggìa, F. *et al*. (2018) ‘Kombucha Beverage from Green, Black and Rooibos Teas: A Comparative Study Looking at Microbiology, Chemistry and Antioxidant Activity,’ *Nutrients*, 11(1), p. 1. https://doi.org/10.3390/nu11010001

Gęgotek, A. and Skrzydlewska, E. (2022) ‘Antioxidative and Anti-Inflammatory activity of ascorbic acid’, *Antioxidants*, 11(10), p. 1993. Available at: https://doi.org/10.3390/antiox11101993

Gilbert, M.J. (2008) ‘Chinese tea in world history’, *Education About Asia*, 13(2), pp. 25–29. [online] Available at: https://www.asianstudies.org/publications/eaa/archives/chinese-tea-in-world-history/ (Accessed 3 July 2025).

Gomes, R.J. *et al*. (2018) ‘Acetic acid Bacteria in the food industry: Systematics, Characteristics and applications’, *Food Technology and Biotechnology*, 56(2), Available at: https://doi.org/10.17113/ftb.56.02.18.5593

Gong, J-S, Tang, C., and Peng, C-X. (2012) ‘Characterization of the chemical differences between solvent extracts from Pu-erh tea and Dian Hong black tea by CP–Py–GC/MS’, *Journal of Analytical and Applied Pyrolysis*, 95, pp. 189–197. Available at: https://doi.org/10.1016/j.jaap.2012.02.006

Harrison, K. and Curtin, C. (2021) ‘Microbial composition of SCOBY starter cultures used by commercial Kombucha brewers in North America’, *Microorganisms*, 9(5), p. 1060. Available at: https://doi.org/10.3390/microorganisms9051060

Higgins, C.L. *et al*. (2021) ‘From Food to mobility: Investigating a screening assay for new automotive antioxidants using the stable radical DPPH’, *ChemistrySelect*, 6(34), pp. 9179–9184. Available at: https://doi.org/10.1002/slct.202102472

Hsieh, Y., Chiu, M-C., and Chou, J-Y. (2021). ‘Efficacy of the Kombucha Beverage Derived from Green, Black, and Pu’er Teas on Chemical Profile and Antioxidant Activity’, *Journal of Food Quality*, 2021, pp. 1–9, Available at: https://doi.org/10.1155/2021/1735959

Hu, T., Shi, S. and Ma, Q. (2022) 'Modulation effects of microorganisms on tea in fermentation', *Frontiers in Nutrition*, 9, p. 931790. Available at: https://doi.org/10.3389/fnut.2022.931790

Jafari, R. *et al.* (2020) 'Kombucha microbial starter with enhanced production of antioxidant compounds and invertase', *Biocatalysis and Agricultural Biotechnology*, article number 29101789. Available at: https://doi.org/10.1016/j.bcab.2020.101789

Jakubczyk, K. *et al*. (2020) 'Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea', *Antioxidants*, 9(5), p. 447. Available at: https://doi.org/10.3390/antiox9050447

Jayabalan, R. *et al.* (2008) 'Changes in free-radical scavenging ability of kombucha tea during fermentation', *Food Chemistry*, 109(1), pp. 227–234. Available at: https://doi.org/10.1016/j.foodchem.2007.12.037

Jayabalan, R., Malbaša, R.V., and Sathishkumar, M. (2015). 'Kombucha', in: *Reference Module in Food Science*. Elsevier. Available at: https://doi.org/10.1016/B978-0-08-100596-5.03032-8 (Accessed: 3 July 2025).

Jiang, H. *et al*. (2015) 'Determination of flavonol glycosides in green tea, oolong tea and black tea by UHPLC compared to HPLC', *Food Chemistry*, 183, pp. 30–35. Available at: https://doi.org/10.1016/j.foodchem.2015.03.024

Johnston, A. (2023) '*How to make kombucha',* [online] Available at: <https://www.bbcgoodfood.com/health/healthy-food-guides/how-make-kombucha> (Accessed 30 December 2024).

Kaewkod, T., Bovonsombut, S., and Tragoolpua, Y. (2019) 'Efficacy of kombucha obtained from green, oolong, and black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on colorectal cancer cell line', *Microorganisms*, 7(12), p. 700. Available at: https://doi.org/10.3390/microorganisms7120700

Kaneko, S. *et al*. (2006) 'Molecular and sensory studies on the umami taste of Japanese green tea', *Journal of Agricultural and Food Chemistry*, 54(7), pp. 2688–2694. Available at: https://doi.org/10.1021/jf0525232

Khaleil, M.M. *et al*. (2020) 'A bioprocess development study of polyphenol profile, antioxidant and antimicrobial activities of kombucha enriched with *Psidium guajava*', *Journal of Microbiology, Biotechnology and Food Sciences*, 9(6), pp. 1204–1210. Available at: https://doi.org/10.15414/jmbfs.2020.9.6.1204-1210

Kukhtenko, H. *et al*. (2024) 'Spectrophotometric and chromatographic assessment of total polyphenol and flavonoid content in *Rhododendron tomentosum* extracts and their antioxidant and antimicrobial activity', *Molecules*, 29(5), p. 1095. Available at: https://doi.org/10.3390/molecules29051095

Lau, T.-C. *et al*. (2013) 'Functional food: A growing trend among the health conscious', *Asian Social Science*, 9(1), pp. 198–208. Available at: https://doi.org/10.5539/ass.v9n1p198

Leal, J.M. *et al.* (2018) 'A review on health benefits of kombucha nutritional compounds and metabolites', *CyTA - Journal of Food*, 16(1), pp. 390–399. Available at: https://doi.org/10.1080/19476337.2017.1410499

Li, Y., Cheng, L., and Li, M. (2024) 'Effects of green tea extract epigallocatechin-3-gallate on oral diseases: A narrative review', *Pathogens*, 13(8), p. 634. Available at: https://doi.org/10.3390/pathogens13080634

Lim, J. (2011) 'Hedonic scaling: A review of methods and theory', *Food Quality and Preference*, Available at: https://doi.org/10.1016/j.foodqual.2011.05.008

Lin, J., and Lin-Shiau, S. (2006) 'Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols', *Molecular Nutrition & Food Research*, 50(2), pp. 211–217. Available at: https://doi.org/10.1002/mnfr.200500138

Lin, S-Y. *et al.* (2016) 'Effect of shaking process on correlations between catechins and volatiles in oolong tea', *Journal of Food and Drug Analysis*, 24(3), pp. 500–507. Available at: https://doi.org/10.1016/j.jfda.2016.01.011

Lin, Y. *et al.* (2023) 'Aroma identification and classification in 18 kinds of teas (*Camellia sinensis*) by sensory evaluation, HS-SPME-GC-IMS/GC × GC-MS, and chemometrics', *Foods*, 12(13), p. 2433. Available at: https://doi.org/10.3390/foods12132433

Liu, C. *et al*. (2025) 'Theabrownin: The 'rich hue' of Chinese dark tea, its extraction, and role in regulating inflammation and immune response', *Food Research International*, article number 209116185. Available at: https://doi.org/10.1016/j.foodres.2025.116185

Ma, B. *et al*. (2025) 'Revealing the formation of aged aroma in raw Pu-erh tea during the storage through comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry and molecular docking', *Current Research in Food Science*, article number 101038. Available at: https://doi.org/10.1016/j.crfs.2025.101038

Macedo, J.A. *et al*. (2012) 'Chemopreventive potential of the tannase-mediated biotransformation of green tea', *Food Chemistry*, 133(2), pp. 358–365. Available at: https://doi.org/10.1016/j.foodchem.2012.01.041

Martins, G.R. *et al*. (2021) 'A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açaí (Euterpe oleracea Mart.) seeds extract', *Journal of Food Science and Technology*, 58(12), pp. 4693–4702. Available at: https://doi.org/10.1007/s13197-020-04959-5

Massoud, R. *et al*. (2021) 'All aspects of antioxidant properties of kombucha drink,' *Biointerface Research in Applied Chemistry*, 12(3), pp. 4018–4027. https://doi.org/10.33263/briac123.40184027

Mihai, R.A., Cubi-Insuaste, N.S., and Catana, R.D. (2024) 'Biological activity and phenolic content of kombucha beverages under the influence of different tea extract substrates', *Fermentation*, 10(7), p. 338. Available at: https://doi.org/10.3390/fermentation10070338

Mousavi, S.M. *et al*. (2020) 'Recent progress in chemical composition, production, and pharmaceutical effects of kombucha beverage: a complementary and alternative medicine', *Evidence-based Complementary and Alternative Medicine*, 2020(1). Available at: https://doi.org/10.1155/2020/4397543

Munteanu, I.G. and Apetrei, C. (2021) 'Analytical methods used in determining antioxidant activity: a review', *International Journal of Molecular Sciences*, 22(7), p.3380, Available at: https://doi.org/10.3390/ijms22073380

Naumovski, N. *et al*. (2019) 'The association between green and black tea consumption on successful aging: a combined analysis of the ATTICA and MEDiterranean ISlands (MEDIS) epidemiological studies', *Molecules*, 24(10), p. 1862. Available at: https://doi.org/10.3390/molecules24101862

Naveed, M. *et al*. (2018) 'Pharmacological values and therapeutic properties of black tea (*Camellia sinensis*): A comprehensive overview', *Biomedicine & Pharmacotherapy*, 100, pp. 521–531. Available at: https://doi.org/10.1016/j.biopha.2018.02.048

Neffe-Skocińska, K. *et al*. (2023) 'Polyphenol and antioxidant properties of food obtained by the activity of acetic acid bacteria (AAB) – A systematic review', *Journal of Functional Foods*, article number 107105691. Available at: https://doi.org/10.1016/j.jff.2023.105691

Nguyen, N.K. *et al.* (2015) 'Screening the optimal ratio of symbiosis between isolated yeast and acetic acid bacteria strain from traditional kombucha for high-level production of glucuronic acid', *LWT - Food Science and Technology*, 64(2), pp. 1149–1155. Available at: https://doi.org/10.1016/j.lwt.2015.07.018

Nicolescu, A., Bunea, C.I., and Mocan, A. (2025) 'Total flavonoid content revised: an overview of past, present, and future determinations in phytochemical analysis', *Analytical Biochemistry*, article number 700115794. Available at: https://doi.org/10.1016/j.ab.2025.115794

Nyhan, L.M. *et al*. (2022) 'Advances in Kombucha Tea Fermentation: A review', *Applied Microbiology*, 2(1), pp. 73–103. Available at: https://doi.org/10.3390/applmicrobiol2010005.

O'Sullivan, E.N. and O'Sullivan, D.J. (2024) 'Viability and diversity of the microbial cultures available in retail kombucha beverages in the USA', *Foods*, 13(11), p. 1707. Available at: https://doi.org/10.3390/foods13111707.

Onsun, B., Toprak, K., and Sanlier, N. (2025) 'Kombucha tea: A functional beverage and all its aspects', *Current Nutrition Reports*, 14, p. 69. Available at: https://doi.org/10.1007/s13668-025-00658-9

Padayatty, S.J. *et al*. (2003) 'Vitamin C as an antioxidant: Evaluation of its role in Disease Prevention', *Journal of the American College of Nutrition*, 22(1), pp. 18–35. Available at: https://doi.org/10.1080/07315724.2003.10719272

Pan, H. *et al*. (2022) 'Dark tea: A popular beverage with possible medicinal application', *Chinese Herbal Medicines*, 15(1), pp. 33–36. Available at: https://doi.org/10.1016/j.chmed.2022.08.005

Pękal, A. and Pyrzynska, K. (2014) 'Evaluation of aluminium complexation reaction for flavonoid content assay', *Food Analytical Methods*, 7(9), pp. 1776–1782. Available at: https://doi.org/10.1007/s12161-014-9814-x

Pérez, M., Dominguez-López, I., and Lamuela-Raventós, R.M. (2023) 'The chemistry behind the Folin–Ciocalteu method for the estimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern', *Journal of Agricultural and Food Chemistry*, 71(46), pp. 17543–17553. Available at: https://doi.org/10.1021/acs.jafc.3c04022

Phung, L.T. *et al*. (2023) 'Changes in the chemical compositions and biological properties of kombucha beverages made from black teas and pineapple peels and cores', *Scientific Reports*, 13(1). Available at: https://doi.org/10.1038/s41598-023-34954-7

Priyadarsini, K.I. *et al*. (1999) 'Properties of phenoxyl radical of dehydrozingerone, a probable antioxidant', *Radiation Physics and Chemistry*, 54(6), pp. 551–558. Available at: https://doi.org/10.1016/s0969-806x(98)00298-9.

Proestos, C. and Komaitis, M. (2009) 'Antioxidant capacity of hops', in: V.R. Preedy, ed. *Beer in Health and Disease Prevention*. Academic Press. Available at: https://doi.org/10.1016/B978-0-12-373891-2.00045-6 (Accessed: 3 July 2025).

Rashidinejad, A. (2024) 'The road ahead for functional foods: Promising opportunities amidst industry challenges', *Future Postharvest and Food*, 1(2), pp. 266–273. Available at: https://doi.org/10.1002/fpf2.12022

Ruiz-Capillas, C. and Herrero, A.M. (2021) 'Sensory analysis and consumer research in new product development', *Foods*, 10(3), p. 582. Available at: https://doi.org/10.3390/foods10030582

Sadok, I. *et al*. (2025) 'Effect of temperature and time on mold growth, mycotoxin contamination, phytochemicals and microbiological characteristics of kombucha tea during fermentation', *Food Control*, article number 111296. Available at: https://doi.org/10.1016/j.foodcont.2025.111296

Saimaiti, A. *et al.* (2022) 'Antioxidant capacities and polyphenol contents of kombucha beverages based on vine tea and sweet tea', *Antioxidants*, 11(9), p. 1655. Available at: https://doi.org/10.3390/antiox11091655

Sánchez-Rangel, J.C. *et al*. (2013) 'The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination', *Analytical Methods*, 5(21), p. 5990. Available at: https://doi.org/10.1039/c3ay41125g

Sang, S. (2016) 'Tea: chemistry and processing', in: B. Caballero, P.M. Finglas and F. Toldrá, eds. *Encyclopedia of Food and Health*. Academic Press. Available at: https://doi.org/10.1016/B978-0-12-384947-2.00685-1 (Accessed: 3 July 2025).

Sanwal, N. *et al*. (2023) 'Kombucha fermentation: Recent trends in process dynamics, functional bioactivities, toxicity management, and potential applications', *Food Chemistry Advances*, article number 3100421. Available at: https://doi.org/10.1016/j.focha.2023.100421.

Sengun, I.Y. and Kirmizigul, A. (2020) 'Probiotic potential of kombucha', *Journal of Functional Foods*, article number 104284. Available at: https://doi.org/10.1016/j.jff.2020.104284

Shafi, W. *et al*. (2019) 'Variability in catechin and rutin contents and their antioxidant potential in diverse apple genotypes', *Molecules*, 24(5), p. 943. Available at: https://doi.org/10.3390/molecules24050943

Shi, J. *et al*. (2021) 'Impact of various Microbial-Fermented methods on the chemical profile of dark tea using a single raw tea material', *Journal of Agricultural and Food Chemistry*, 69(14), pp. 4210–4222. Available at: https://doi.org/10.1021/acs.jafc.1c00598

Shraim, A.M. *et al*. (2021) 'Determination of total flavonoid content by aluminum chloride assay: A critical evaluation', *LWT*, article number 150111932. Available at: https://doi.org/10.1016/j.lwt.2021.111932

Shraim, A.M. *et al*. (2021) 'Determination of total flavonoid content by aluminum chloride assay: A critical evaluation,' *LWT*, 150, p. 111932. https://doi.org/10.1016/j.lwt.2021.111932

Su, J. *et al*. (2023) 'Research progress on alternative kombucha substrate transformation and the resulting active components', *Frontiers in Microbiology*, 14. Available at: https://doi.org/10.3389/fmicb.2023.1254014

Teoh, A.L., Heard, G., and Cox, J. (2004) 'Yeast ecology of Kombucha fermentation', *International Journal of Food Microbiology*, 95(2), pp. 119–126. Available at: https://doi.org/10.1016/j.ijfoodmicro.2003.12.020

Thennakoon, T.M.E.S. *et al*. (2022) 'Total phenolic content, total flavonoid content and in vitro antioxidant activities measured by the FRAP, ABTS, DPPH and ORAC assays of Sri Lankan black and green tea (*Camellia sinensis*) infusions,' *Food Biology*, pp. 1–10. https://doi.org/10.25081/fb.2022.v11.7800

Troitino, C. (2017) '*Kombucha 101: Demystifying the past, present and future of the fermented tea drink*', [online] Available at: https://www.forbes.com/sites/christinatroitino/2017/02/01/kombucha-101-demystifying-the-past-present-and-future-of-the-fermented-tea-drink/ (Accessed 3 July 2025).

Truong, V-L. and Jeong, W-S. (2021) 'Cellular defensive mechanisms of tea polyphenols: Structure-activity relationship', *International Journal of Molecular Sciences*, 22(17), p. 9109. Available at: https://doi.org/10.3390/ijms22179109

Vargas, B.K., Fabricio, M.F., and Ayub, M.A.Z. (2021) 'Health effects and probiotic and prebiotic potential of kombucha: A bibliometric and systematic review', *Food Bioscience*, 44(Part A), p. 101332. Available at: https://doi.org/10.1016/j.fbio.2021.101332

Villarreal-Soto, S.A. *et al*. (2018) 'Understanding Kombucha tea Fermentation: A Review', *Journal of Food Science*, 83(3), pp. 580–588. Available at: https://doi.org/10.1111/1750-3841.14068

Wang, B. *et al*. (2022) 'Kombucha: Production and microbiological research', *Foods*, 11(21), p. 3456. Available at: https://doi.org/10.3390/foods11213456

Wang, K. *et al*. (2010) 'Determination of D-saccharic acid-1,4-lactone from brewed kombucha broth by high-performance capillary electrophoresis', *Journal of Chromatography B*, 878(3-4), pp. 371–374. Available at: https://doi.org/10.1016/j.jchromb.2009.12.003

Wang, Q. *et al*. (2015) 'Fungal isolates from a Pu-Erh type tea fermentation and their ability to convert tea polyphenols to theabrownins', *Journal of Food Science*, 80(4). Available at: https://doi.org/10.1111/1750-3841.12831

Wang, Q. *et al*. (2018) 'Physicochemical properties and biological activities of a high-theabrownins instant Pu-erh tea produced using *Aspergillus tubingensis*', *LWT*, 90, pp. 598–605. Available at: https://doi.org/10.1016/j.lwt.2018.01.021

Wang, S. *et al*. (2021) 'Chemical constituents and biological properties of Pu-erh tea', *Food Research International*, article number 154110899. Available at: https://doi.org/10.1016/j.foodres.2021.110899

Wang, Weiwei. *et al*. (2023) 'Effects of key components on the antioxidant activity of black tea', *Foods*, 12(16), p. 3134. Available at: https://doi.org/10.3390/foods12163134

Wanna, C. (2019) 'Free radical scavenging capacity and total phenolic contents in peel and fleshy crude extracts of selected vegetables', *Pharmacognosy Journal*, 11(6), pp. 1351–1358. Available at: https://doi.org/10.5530/pj.2019.11.209

Wu, T. *et al*. (2023) 'A review of natural plant extracts in beverages: Extraction process, nutritional function, and safety evaluation', *Food Research International*, article number 172113185. Available at: https://doi.org/10.1016/j.foodres.2023.113185

Xiao, F. *et al*. (2020) 'Guidelines for antioxidant assays for food components', *Food Frontiers*, 1(1), pp. 60–69. Available at: https://doi.org/10.1002/fft2.10

Xiao, Y. *et al*. (2020) 'Structural characteristics and hypolipidemic activity of theabrownins from dark tea fermented by single species *Eurotium cristatum* PW-1', *Biomolecules*, 10(2), p. 204. Available at: https://doi.org/10.3390/biom10020204

Xue, J. *et al*. (2023) 'Insights into the effects of fixation methods on the sensory quality of straight-shaped green tea and dynamic changes of key taste metabolites by widely targeted metabolomic analysis', *Food Chemistry X*, article number 20100943. Available at: https://doi.org/10.1016/j.fochx.2023.100943

Yang, Z. *et al*. (2008) 'Symbiosis between microorganisms from kombucha and kefir: Potential significance to the enhancement of kombucha function', *Applied Biochemistry and Biotechnology*, 160(2), pp. 446–455. Available at: https://doi.org/10.1007/s12010-008-8361-6

Yashin, A.Y. *et al*. (2015) 'Determination of the chemical composition of tea by chromatographic methods: a review', *Journal of Food Research*, 4(3), p. 56. Available at: https://doi.org/10.5539/jfr.v4n3p56

Yuan, X. *et al*. (2025) 'Identifying key contributors to the sweet aftertaste of raw Pu-erh tea through analytical and sensory methods', *Food Chemistry*, article number 144067. Available at: https://doi.org/10.1016/j.foodchem.2025.144067

Zeng, L. *et al*. (2016) 'Stability of tea polyphenols solution with different pH at different temperatures', *International Journal of Food Properties*, 20(1), pp. 1–18. Available at: https://doi.org/10.1080/10942912.2014.983605

Zhang, H., Qi, R., and Mine, Y. (2019) 'The impact of oolong and black tea polyphenols on human health', *Food Bioscience*, 29, pp. 55–61. Available at: https://doi.org/10.1016/j.fbio.2019.03.009

Zhang, L. *et al*. (2019) ‘Chemistry and biological activities of processed *camellia sinensis* teas: A comprehensive review’, *Comprehensive Reviews in Food Science and Food Safety*, 18(5), pp. 1474–1495. Available at: https://doi.org/10.1111/1541-4337.12479

Zhang, Q. and Lyu, S. (2022) ‘Microbial interactions in a vitamin C industrial fermentation System: Novel Insights and Perspectives’, *Applied and Environmental Microbiology*, 88(18). Available at: https://doi.org/10.1128/aem.01212-22

Zhang, Y, *et al*. (2024) ‘Physicochemical characterization of the fraction components of the theabrownin isolates from Pu’er tea’, *LWT*, article number 200116147. Available at: https://doi.org/10.1016/j.lwt.2024.116147

Zheng, Y, *et al*. (2024) ‘Comprehensive evaluation of quality and bioactivity of kombucha from six major tea types in China’, *International Journal of Gastronomy and Food Science*, article number 36100910. Available at: https://doi.org/10.1016/j.ijgfs.2024.100910

Zhong, Y., Ma, C.-M., and Shahidi, F. (2011) ‘Antioxidant and antiviral activities of lipophilic epigallocatechin gallate (EGCG) derivatives’, *Journal of Functional Foods*, 4(1), pp. 87–93. Available at: https://doi.org/10.1016/j.jff.2011.08.003

Zhou, B. *et al*. (2019) ‘Production of theophylline via aerobic fermentation of pu-erh tea using tea-derived fungi’, *BMC Microbiology*, 19(1). Available at: https://doi.org/10.1186/s12866-019-1640-2

Zhu, Y. *et al*. (2015) ‘Simultaneous determination of free amino acids in Pu-erh tea and their changes during fermentation’, *Food Chemistry*, 194, pp. 643–649. Available at: https://doi.org/10.1016/j.foodchem.2015.08.054

Zubaidah, E. *et al*. (2023) ‘Physicochemical and microbiological characteristics of fruit-based kombucha’, *Food Research*, 7(Supplementary 1), pp. 64–70. Available at: https://doi.org/10.26656/fr.2017.7s(1).8

APPENDICES

APPENDIX A

The manufacturer and origin of the chemicals used.

Table A.1: The manufacturer and origin of the chemicals used in this study.

Chemical	Manufacturer	Country of origin
2,4,6-tripyridyl-s-triazine (TPTZ)	Sigma-Aldrich	Germany
2,2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich	Germany
Acetic acid glacial	Bendosen	Malaysia
Aluminium chloride anhydrous	Sigma-Aldrich	Germany
Distilled water	Faculty of Science, UTAR Kampar	Malaysia
Ferric chloride hexahydrate	Nacalai Tesque Inc.	Japan
Ferrous sulphate heptahydrate	Bendosen	Malaysia
Folin-Ciocalteu reagent	Chemiz	Malaysia
Gallic acid anhydrous	Merck	Germany

Hydrochloric acid (0.1 mol/L)	Bendosen	Malaysia
Methanol	Merck	Germany
Quercetin	Sigma Life Science	Canada
Sodium acetate anhydrous	Bio Basic Inc.	Canada
Sodium carbonate anhydrous	Friendemann Schmidt	Malaysia

APPENDIX B

The manufacturer and origin of the equipment used.

Table B.1: The manufacturer and origin of the equipment used in this study.

Equipment	Manufacturer	Country of origin
Analytical balance	Mettler-Toledo	United States
Centrifuge	Newton Scientific	United Kingdom
Drying Oven	Binder	Germany
Micropipette (1-10 µL)	Thermo Fisher Scientific	United States
Micropipette (10-100 µL)	Thermo Fisher Scientific	United States
Micropipette (100-1000 µL)	Thermo Fisher Scientific	United States
Microplate reader	Thermo Fisher Scientific	United States
pH meter	Mettler-Toledo	United States
Precision balance	Mettler-Toledo	United States
Refrigerator (4 C)	KIM	Malaysia
Water bath	Memmert	Germany

APPENDIX C

The manufacturer and origin of the consumables and glassware used.

Table C.1: The manufacturer and origin of the consumables and classware used in this study.

Consumables/ Glassware	Manufacturer	Country of origin
96-well tissue culture plate	BioFil	China
Aluminium foil	Diamond	China
Beakers (50 mL, 100 mL)	Duran	Germany
Centrifuge tube (1.5 mL)	-	-
Centrifuge tube (15 mL)	Labchem	Malaysia
Cheese clothes	-	-
Disposable cups (75 mL)	Starfire	United States
Glass jar (1 L)	-	-
Glass rod (10 cm)	-	-
Gloves	Metex gloves	Malaysia
Measuring cup (500 mL)	-	-
Micropipette box	-	-

Micropipette tips (1–10 µL)	Gilson	France
Micropipette tips (10–10 µL)	Gilson	France
Micropipette tips (100–1000 µL)	Gilson	France
Microspatula	-	-
Rubber band	-	-
Schott bottles (100 mL)	Duran	Germany
Spatula	-	-
Test tube box	-	-
Transfer pipette	-	-
Weighing boat	-	-

APPENDIX D

9-Point Hedonic Scaling Test Questionnaire for Kombucha

Name:

Date:

Taste the three kombucha samples to assess your acceptance of each sensory attribute. Please rinse your mouth with water before tasting each sample.

Grade	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

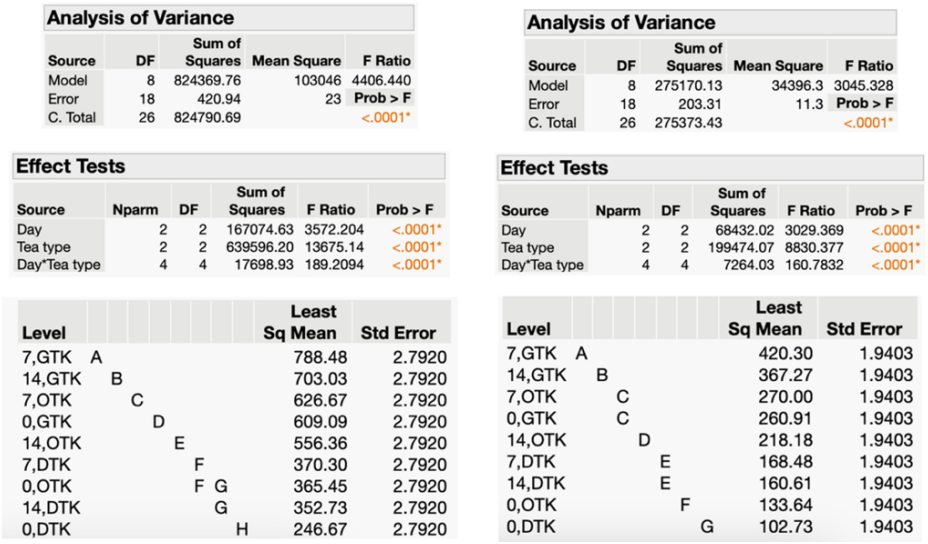
Sample Code			
Aroma			
Colour			
Sweetness			
Sourness			
Overall Acceptability			

Comments:

Thank you very much!

APPENDIX E

Statistical data of the two-way ANOVA and Tukey's HSD for phytochemical contents and antioxidant activities.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	824369.76	103046	4406.440
Error	18	420.94	23	Prob > F
C. Total	26	824790.69		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Day	2	2	167074.63	3572.204	<.0001*
Tea type	2	2	639596.20	13675.14	<.0001*
Day*Tea type	4	4	17698.93	189.2094	<.0001*

Level					Least Sq Mean	Std Error
7,GTK	A				788.48	2.7920
14,GTK		B			703.03	2.7920
7,OTK			C		626.67	2.7920
0,GTK				D	609.09	2.7920
14,OTK				E	556.36	2.7920
7,DTK				F	370.30	2.7920
0,OTK				F	G	365.45	2.7920
14,DTK					G	352.73	2.7920
0,DTK					H	246.67	2.7920

Analysis of Variance

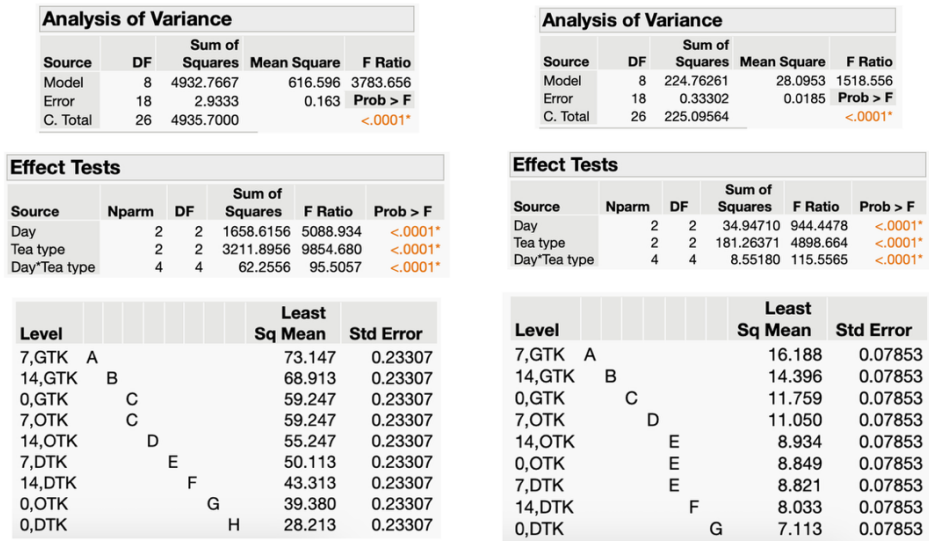
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	275170.13	34396.3	3045.328
Error	18	203.31	11.3	Prob > F
C. Total	26	275373.43		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Day	2	2	68432.02	3029.369	<.0001*
Tea type	2	2	199474.07	8830.377	<.0001*
Day*Tea type	4	4	7264.03	160.7832	<.0001*

Level					Least Sq Mean	Std Error
7,GTK	A				420.30	1.9403
14,GTK		B			367.27	1.9403
7,OTK			C		270.00	1.9403
0,GTK			C		260.91	1.9403
14,OTK				D	218.18	1.9403
7,DTK				E	168.48	1.9403
14,DTK				E		160.61	1.9403
0,OTK					F	133.64	1.9403
0,DTK					G	102.73	1.9403

Figure E.1: Statistical data of the two-way ANOVA and Tukey's HSD for TPC (left) and TFC (right).



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	4932.7667	616.596	3783.656
Error	18	2.9333	0.163	Prob > F
C. Total	26	4935.7000		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Day	2	2	1658.6156	5088.934	<.0001*
Tea type	2	2	3211.8956	9854.680	<.0001*
Day*Tea type	4	4	62.2556	95.5057	<.0001*

Level						Least Sq Mean	Std Error
7,GTK	A					73.147	0.23307
14,GTK		B				68.913	0.23307
0,GTK			C			59.247	0.23307
7,OTK			C			59.247	0.23307
14,OTK				D		55.247	0.23307
7,DTK					E	50.113	0.23307
14,DTK					F	43.313	0.23307
0,OTK					G	39.380	0.23307
0,DTK					H	28.213	0.23307

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	224.76261	28.0953	1518.556
Error	18	0.33302	0.0185	Prob > F
C. Total	26	225.09564		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Day	2	2	34.94710	944.4478	<.0001*
Tea type	2	2	181.26371	4898.664	<.0001*
Day*Tea type	4	4	8.55180	115.5565	<.0001*

Level						Least Sq Mean	Std Error
7,GTK	A					16.188	0.07853
14,GTK		B				14.396	0.07853
0,GTK			C			11.759	0.07853
7,OTK				D		11.050	0.07853
14,OTK					E	8.934	0.07853
0,OTK					E	8.849	0.07853
7,DTK					E	8.821	0.07853
14,DTK						F	8.033	0.07853
0,DTK						G	7.113	0.07853

Figure E.2: Statistical data of the two-way ANOVA and Tukey's HSD for DPPH assay (left) and FRAP assay (right).

APPENDIX F

Statistical data of the one-way ANOVA and Tukey's HSD for sensory evaluation.

AROMA

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	92.17333	46.0867	29.5969
Error	147	228.90000	1.5571	Prob > F
C. Total	149	321.07333		<.0001*

Level		Least Sq Mean	Std Error
GTK	A	7.1800	0.17647
OTK	A	6.8000	0.17647
DTK	B	5.3600	0.17647

Levels not connected by same letter are significantly different.

COLOUR

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	9.21333	4.60667	2.3264
Error	147	291.08000	1.98014	Prob > F
C. Total	149	300.29333		0.1012

Level		Least Sq Mean	Std Error
OTK	A	7.1000	0.19900
DTK	A	6.7200	0.19900
GTK	A	6.5000	0.19900

Levels not connected by same letter are significantly different.

SOURNESS

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	56.33333	28.1667	13.1344
Error	147	315.24000	2.1445	Prob > F
C. Total	149	371.57333		<.0001*

Level		Least Sq Mean	Std Error
GTK	A	6.3800	0.20710
OTK	A	6.3800	0.20710
DTK	B	5.0800	0.20710

SWEETNESS

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	2.52000	1.26000	0.5248
Error	147	352.92000	2.40082	Prob > F
C. Total	149	355.44000		0.5928

Level		Least Sq Mean	Std Error
OTK	A	6.6600	0.21913
DTK	A	6.4200	0.21913
GTK	A	6.3600	0.21913

OVERALL ACCEPTABILITY

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	46.97333	23.4867	13.7815
Error	147	250.52000	1.7042	Prob > F
C. Total	149	297.49333		<.0001*

Level		Least Sq Mean	Std Error
OTK	A	7.0800	0.18462
GTK	A	6.6600	0.18462
DTK	B	5.7400	0.18462

Figure F.1: Statistical data of the one-way ANOVA and Tukey's HSD for sensory evaluation using a 9-point Hedonic test.

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Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

UNIVERSITI TUNKU ABDUL RAHMAN

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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	BRIAN CH'NG WEI SHEN
ID Number(s)	22ADB07345
Programme / Course	Bachelor of Science (Honours) Food Science
Title of Final Year Project	Effect of Different Tea Types (*Camellia Sinensis*) on Antioxidant and Sensory Properties of Kombucha Fermentation

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 15 % **Similarity by source** Internet Sources: 10 % Publications: 6 % Student Papers: 12 %	
Number of individual sources listed of more than 3% similarity: 0	
Parameters of originality required and limits approved by UTAR are as follows: **(i) Overall similarity index is 20% and below, and** **(ii) Matching of individual sources listed must be less than 3% each, and** **(iii) Matching texts in continuous block must not exceed 8 words** ***Note: Parameters (i) – (ii) shall exclude quotes, bibliography and text matches which are less than 8 words.***	

Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor
Name: Mr Sim Kheng Yuen

Date: 7 October 2025