DEVELOPMENT OF KOMBUCHA BEVERAGE DERIVED FROM *Aquilaria malaccensis* TEA INFUSED WITH PINEAPPLE AND MINT

By

LOOI TZE SAN

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 (Hons) Food Science

September 2022

ABSTRACT

DEVELOPMENT OF KOMBUCHA BEVERAGE DERIVED FROM *Aquilaria malaccensis* TEA INFUSED WITH PINEAPPLE AND MINT

LOOI TZE SAN

Kombucha is a popular functional beverage known for its health benefits. The production of kombucha involves fermenting a tea with a symbiotic culture of bacteria and yeasts (SCOBY). The common types of tea that are used as the base of kombucha are black tea and green tea of *Camellia sinensis*. The research interests to explore the potential of utilizing locally available plants as the tea base for kombucha is expanding. In tropical countries, farmers are capitalising on the leaves of *Aquilaria* tree by making it into a tea drink and it is commonly known as agarwood tea. This tea is known to contain pharmacological and antioxidant properties. Up to date, there were limited studies utilizing agarwood tea as the base for kombucha beverages with the combination of pineapple and mint. This leads to the objective of this study to evaluate the difference in terms of the overall physicochemical, antioxidant properties, and consumer acceptance between agarwood tea base kombucha and green tea base kombucha. The kombucha was prepared by fermenting the sweetened tea with a SCOBY for 7 days, before the addition of flavouring and fermenting it again for another 3 days. The tests performed were the pH, Brix value, total titratable acidity, DPPH inhibition assay and total phenolic content. A sensory evaluation was also conducted with 30 panelists to determine consumer acceptance of the newly developed kombucha. It was found that the pH, total soluble solids, and DPPH inhibition between agarwood tea and green tea kombucha were similar by the end of fermentation. Although the total phenolic content of green tea kombucha was significantly higher ($p < 0.05$) than agarwood tea kombucha, the latter showed a higher taste acceptance by panelists. Hence, this study paves the way for the use of *Aquilaria* leaves in the development of kombucha.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my project supervisor Dr. Kwong Phek Jin for the continuous support, patience, and feedback during this project. Her guidance helped me in all the time of research and writing of this thesis. I would also like to thank my co-supervisor Dr. Ali Yassoralipour for his provided insight and knowledge on the subject matter.

I am also grateful to the lab officers, especially Ms. Hazlinda, for her assistance with using the laboratory equipment and providing the necessary materials for the experiments. Special thanks are also given to Adam for his patience and guidance during certain experimental procedures.

Finally, I would like to thank my family and friends for their continuous support and encouragement throughout the entire project.

DECLARATION

I hereby declare that this final year project report 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 UTAR or other institutions.

LOOI TZE SAN

APPROVAL SHEET

This final year project report entitled **"<u>DEVELOPMENT OF KOMBUCHA BEVERAGE DERIVED FROM *Aquilaria malaccensis* TEA INFUSED WITH PINEAPPLE AND MINT</u>"** was prepared by LOOI TZE SAN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

____

Dr. Kwong Phek Jin   Date: 23/09/2022

Supervisor

Department of Agricultural and Food Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 23rd September 2022

PERMISSION SHEET

It is hereby certified that **LOOI TZE SAN** (ID No: **18ADB07013**) has completed this final year project thesis entitled "DEVELOPMENT OF KOMBUCHA BEVERAGE DERIVED FROM *Aquilaria malaccensis* TEA INFUSED WITH PINEAPPLE AND MINT" under the supervision of Dr. Kwong Phek Jin (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science, and Dr. Ali Yassoralipour (Co-Supervisor) from the Department of Agricultural and Food Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project thesis in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(LOOI TZE SAN)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSL	D-saccharic acid-1,4-lactone
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigalocatechin
EGCG	Epigalocatechin gallate
FC	Folin-Ciocalteu
GAE	Gallic acid equivalents
IC	Inhibitory concentration
JMP	Jump
NaOH	Sodium hydroxide
ROS	Reactive oxygen species
SCOBY	Symbiotic culture of bacteria and yeast
SPSS	Statistical Package for the Social Sciences
TPC	Total phenolic content
TSS	Total soluble solids
TTA	Total titratable acidity

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Humans are constantly on the search for ways to improve their overall health and well-being, which can be seen from the development and use of technology in modern medicine. Despite all the recent medical advances, it can be said that a healthy body first comes from the foods that people consume. It provides nutrients for the body to function and keeps a person healthy depending on the type of food eaten. Functional foods can be defined in several ways, where Topolska, Florkiewicz and Filipiak-Florkiewicz (2021) describe them as those with the appearance of traditional food and are included in daily diet for reduced risk of non-communicable diseases. Meanwhile, the European Union classifies functional foods as products that have positive targeted functions in the body (Çakiroğlu and Uçar, 2018). Overall, functional foods are not necessarily needed by the body to perform its basic functions, but when consumed, can provide certain health benefits to improve one's well-being.

Kombucha can be considered a type of functional food. It is a type of probiotic beverage derived from the fermentation of sweetened tea by a tea fungus known as the symbiotic culture of bacteria and yeast (SCOBY). The slightly carbonated beverage has a tangy flavour, where its taste balances between sour and sweet. It has been purported to provide numerous health benefits upon consumption, where it is mainly known for improving digestion and is especially good for gut health. Other benefits of regularly consuming kombucha include boosting the immune system, lowering cholesterol, and providing anti-diabetic properties (Kapp and Sumner, 2018; Kayisoglu and Coskun, 2020). The kombucha industry has been growing in recent years as the younger generation is becoming more aware of their health and are taking the initiative as seen with health food trends. The common types of kombuchas found in the commercial market include those that are prepared from commonly grown tea leaves such as black and green teas, which come from the plant *Camellia sinensis* (Kim and Adhikari, 2020). There have been very few products that venture into other tea leaf varieties, which is why it can be interesting to study the potential use of other unique tea leaf varieties in the preparation of kombucha.

The consumption of kombucha in Malaysia has a prospective future as seen in a 2021 consumer survey conducted by GlobalData, with 59% of Malaysians having a positive perception towards probiotic beverages (Retail in Asia, 2022). Malaysia is known for its tea plantations located in Cameron Highlands where they mainly grow black tea leaves. However, there is another local tea plantation located in Gopeng, Perak that focuses on growing agarwood (*Aquilaria malaccensis* Lamk.) tea leaves. The *Aquilaria* tree is commonly used for its resin, but its plant material such as folium and bark have also been used by producers to market a product known as agarwood tea. This tea offers several pharmacological and antioxidant properties such as anti-inflammatory, cardioprotective, and anticancer properties (Hashim et al., 2016). As such, it is interesting to study the effects of agarwood tea that are incorporated into different types of functional foods. Currently, there are limited studies on the use of agarwood tea leaves as a tea base for the brewing of kombucha, and there is only one research paper by Nurmiati and Wijayanti (2018) that can be found with regards to this topic.

There are many different brands of commercially available kombuchas that can be found in the market which offer a variety of flavours for consumers to choose from. Most of these products contain at least two flavours rather than one, with the most common types of flavourings including fruits, herbs, or a combination of both (Kim and Adhikari, 2020; Nyhan et al., 2022). Pineapple is a tropical fruit that has also been found in several commercial flavoured kombucha products, thus showing the consumer demand for this flavouring. Its popularity was also ranked among 5 other flavours in countries including France, Spain, and the United Kingdom (MarketResearch.com, 2022b). In Malaysia, pineapples are one of the country's main exported produce which comes to show that the fruit is consumed by many worldwide. Commercially available pineapple juice products can also be found on market shelves, further showing demand for the fruit. Besides that, mint leaves are also another commonly used ingredient in the production of kombucha (Osiripun and Apisittiwong, 2021; Tanticharakunsiri et al., 2020). Many beverages are often added with mint on top with the purpose of providing refreshing sensations to elevate the overall enjoyability of the beverage. As both flavouring ingredients have proven to be widely used, the infusion of this combination into agarwood tea-based kombucha calls for an interesting study.

1.2 Problem Statement

There is still a large research gap in exploring the potential of using agarwood leaves as a tea base in the production of kombucha. Currently, there is only one paper that briefly explores this topic, but no sensory evaluation was involved to determine the consumer acceptability of the kombucha. There is also no research combining the infusion of agarwood tea kombucha with pineapple and mint for its antioxidant properties as well as consumer acceptance.

1.3 Objectives

The objectives of this study are as follows:

1. To assess the overall physicochemical, antioxidant properties, and consumer acceptance of agarwood tea kombucha and green tea kombucha infused with pineapple and mint.
2. To study the overall physicochemical, antioxidant properties, and consumer acceptance towards agarwood tea kombucha and green tea kombucha.

CHAPTER 2

LITERATURE REVIEW

2.1 Tea

Tea is a type of drink that is brewed by steeping tea leaves in hot water for several minutes for infusion before consumption. It originated from China and is now one of the most consumed beverages worldwide along with coffee (Marques, Alves and Coelhoso, 2017). There are many types of teas that are consumed globally depending on location. For instance, Chinese teas are more favoured in Asian countries while English teas are preferred in Western countries. Tea can be classified based on factors such as plant variety, grade, and market name (Adam, Lee and Mohamed, 2017). Some common examples of tea include black, green, white and oolong which they are categorized based on the type of processing that the leaves undergo. According to Khan and Mukhtar (2013), green tea leaves are non-fermented, black tea leaves are fermented and oolong tea leaves are semi-fermented, where the process of pan firing is done after fermentation to prevent further fermentation of the leaves. Generally, people consume tea for the health benefits it provides where green tea has higher antioxidant properties compared to black tea. The dry matter of teas has been found to have polyphenols, caffeine, sugars and fatty acids (Marques, Alves and Coelhoso, 2017).

Black tea and green tea are the most common types of tea consumed worldwide. They both belong to the plant species *Camellia sinensis*. Black tea contains compounds such as theaflavins, thearubigins and epigallocatechin gallate, while green tea contains catechins and flavonols (Prasanth et al., 2019; Rasheed, 2019). Catechins in green tea include epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Regular consumption of both teas provides multiple health benefits for the body. Black tea has antioxidants that can scavenge free radicals, thus decreasing the probability of the onset of chronic diseases (Rasheed, 2019). As for green tea, it can regulate cholesterol and reduce inflammatory responses in the body (Prasanth et al., 2019).

2.2 Kombucha

Kombucha is the beverage obtained as a result of fermenting sweetened tea with the use of a symbiotic culture of bacteria and yeast (SCOBY) for approximately one week (Alderson et al., 2021; Villarreal-Soto et al., 2018). Its taste is somewhat akin to that of apple cider vinegar, along with a strong sour smell. There is also a slightly noticeable effervescence present from the release of carbon dioxide by the microbes in the SCOBY (May et al., 2019). Due to this sensory profile, there will be people who enjoy drinking kombucha as well as those that do not, depending on preferences. The main purpose of kombucha is for consumption, where it is touted to provide several health benefits for the body. It possesses both antioxidant and antimicrobial properties which have been studied extensively *in vitro* and *in vivo* (Kapp and Sumner, 2018). Results from these studies have shown that kombucha is capable of treating ailments including indigestion, hypertension, and arthritis (Kayisoglu and Coskun, 2020). However, there may be certain doubts despite these studies. A systemic review conducted by Kapp and Sumner (2018) had come to the conclusion that there is currently a lack of empirical evidence for the health benefits of kombucha based on human subjects research. Despite this, it is still believed that the beverage does provide a certain extent of advantages for humans. In addition, it can be noted that the use of kombucha is not limited to consumption as it is now being used in skincare products (Pakravan et al., 2017; Ziemlewska et al., 2022). Companies market these products in a way that promotes the numerous benefits of kombucha for the skin due to its antioxidant content to scavenge free radicals.

Since kombucha is a type of fermented beverage, attention can also be brought to a similarly prepared drink. A lesser-known counterpart to kombucha is “Jun” (or Xun), which is another type of fermented beverage that also uses a SCOBY during brewing to obtain a similarly carbonated end product as kombucha. However, both beverages use different ingredients in which black tea and sugar are used for kombucha, while green tea and honey are used for Jun (Kulkarni, 2022a). Moreover, kombucha has to be fermented for at least one week while Jun can be fermented for between 3 – 7 days. This leads to a less acidic drink which may be more enjoyable for some consumers. At the time of writing, there is little to no scientific research on the comparison between kombucha and Jun despite the fact that both beverages are fermented drinks.

2.2.1 History of Kombucha

Kombucha had its roots in Manchuria, China during the Tsin Dynasty (220 B.C.) where it was famous for its detoxifying and energising properties (Jayabalan et al., 2014). It then started appearing in other countries as more people got to know about its health functions. Kombucha then had records of appearance in Russia, followed by other European countries as trade routes expanded beyond China (Jayabalan et al., 2014). However, there have been different versions for the etymology of kombucha. According to Amarasinghe et al. (2017), the name was derived from the Japanese words '*kombu*', meaning seaweed, and '*cha*', meaning tea. In other texts, it was said that a Korean physician named Kombu had used the drink to cure the Japanese Emperor Inkyo of his digestive issues (Dufresne and Farnworth, 2000). His name was thus used in combination with '*cha*' to name the functional beverage.

2.2.2 Kombucha Fermentation Process

The brewing of kombucha is relatively simple and does not require complicated procedures to obtain the final product. Therefore, many people choose to brew it in their own homes for personal consumption. Black tea leaves and white sugar are commonly used for the brewing of kombucha. However, other types of tea leaves and sugar sources can also be used. To prepare the beverage, the tea leaves are first added to hot water and allowed to steep for around 5 – 10 minutes (Kim and Adhikari, 2020). Following that, the tea leaves are removed and approximately 50 – 150 g/L of sugar is added to the hot tea (Greenwalt, Steinkraus and Ledford, 2000). The tea is allowed to cool until it reaches room temperature. Kombuchas are typically brewed in glass jars that have been sterilized to prevent contamination by spoilage microorganisms. The SCOBY and 10% (v/v) liquid starter are then added to the mixture to start the fermentation process. Liquid starter refers to the kombucha obtained from the previous batch of fermentation where the SCOBY was being propagated. The mouth of the glass jar is covered with cheesecloth and secured with rubber bands to prevent insects and other contaminants from entering. The inoculated tea is left to ferment at room temperature for a period ranging from 7 – 10 days for the first cycle of fermentation (Kim and Adhikari, 2020). During the fermentation period, sucrose acts as the carbon source to be hydrolysed by the invertase from yeasts, which is first converted into ethanol before being oxidized by acetic acid bacteria into acetic acid (Ahmed, Hikal and Abou-Taleb, 2020). At the same time, carbon dioxide will be released by the microbes in the SCOBY as they ferment on the available substrates. Once the fermentation period is over, the SCOBY is removed and the kombucha is ready to be consumed raw.

2.2.3 Factors Affecting Fermentation of Kombucha

The fermentation of kombucha can be affected by several factors including but not limited to fermentation time, temperature, and water. Firstly, the fermentation time can affect the activity of the SCOBY whereby an increased time allows for a higher antioxidant concentration. However, it should be noted that a prolonged fermentation period is not recommended as the SCOBY will start to produce too much organic acids that can be deemed unsafe for consumption. Hence, it is generally recommended to ferment kombucha for around 6 – 10 days. Secondly, a suitable fermentation temperature is also needed to ensure an optimal fermentation process by the microbes. A temperature ranging between 22 – 30°C was found to result in fermentation with higher concentrations of acids, metabolites, and vitamin C. Lastly, the type of water used to prepare the kombucha can also impact the final quality in terms of flavour based on its chemical composition. Since water is a source of medium for the enzymatic activity of the SCOBY to take place, it should not contain contaminants that can inadvertently affect the health of the microbes. Municipal tap water that has been filtered is the most suitable and commonly used source of water for the preparation of kombucha (Bishop et al., 2022).

2.2.4 Symbiotic Culture of Bacteria and Yeast (SCOBY)

A symbiotic culture of bacteria and yeast (SCOBY) is defined as a cellulosic film composed of bacteria and yeasts. It has a round mushroom cap-like appearance with a slippery touch and smooth surface. It is often produced by the biochemical activity of the inoculated microorganisms which results in a floating biofilm on the surface of the liquid. Not all SCOBYs contain the same types of microbes as it varies based on the origin of the inoculated microbial cultures (Harrison and Curtin, 2021). Hence, it can also be said that not all kombuchas will have the same microbial composition. Common types of bacteria found in a SCOBY include acetic acid bacteria (AAB) or lactic acid bacteria (LAB). These include *Acetobacter xylinoides*, *Bacterium gluconicum*, and *Acetobacter aceti* (Villarreal-Soto et al., 2018). Meanwhile, common yeasts include *Saccharomyces*, *Saccharomycodes*, and *Mycoderma* (Jayabalan et al., 2014). As mentioned earlier, a SCOBY is a cellulosic layer of beneficial microorganisms. According to Mohite and Patil (2014), bacteria such as *Aerobacter* and *Gluconacetobacter* are capable of producing microbial celluloses which contribute to the development of the SCOBY. The available glucose in the sweetened tea is oxidized into gluconic acid by the bacteria, where another metabolic process will slowly lead to the formation of microbial cellulose (Villarreal-Soto et al., 2018). The metabolic pathways involved can either be through exogenous hexose phosphorylation, or by the pentose phosphate pathway and gluconeogenesis processes (Lustri et al., 2015). When brewing kombucha, a mother SCOBY is first used to ferment the tea. As the microbes use up the available sugar in the sweet tea, they will form what is known as a daughter SCOBY. This process can take up to one week for the SCOBY layer to fully develop. By the end of the fermentation process, there will be two SCOBYs in the kombucha. According to Cacicedo et al. (2016), the yield of microbial cellulose is affected by the type of substrates used, such as the carbon source. The amount of growth a SCOBY undergoes is also influenced by the container it takes shape in as well as the fermentation period. If a small mother SCOBY is placed in a jar where the liquid has a larger surface area, the daughter SCOBY will end up taking the shape of the jar instead. The same can be said for the fermentation time, where a longer period allows the microbes to produce a thicker daughter SCOBY (Goh et al., 2012).

2.2.5 Antioxidant Properties of Kombucha

Reactive oxygen species (ROS) are metabolites created from oxygen that can be listed as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen. These metabolites can eventually form free radicals, which can take the form of lipids, proteins, and DNA radicals (Nakai and Tsuruta, 2021). Free radicals are generally known to negatively impact the body with effects such as aging. Hence, foods with antioxidant properties can help scavenge free radicals and prevent high oxidative stress on the body. Antioxidants can be divided into two types: enzymatic which converts the oxidative products into water, and non-enzymatic which disrupts the reaction of free radical chains (Nimse and Pal, 2015).

Studies have shown that kombucha exhibits antioxidant properties. The antioxidant activity of kombucha is mainly due to the polyphenols that are already present in tea. It is estimated that fresh tea leaves consist of around 30% polyphenols based on dry weight (Massoud et al., 2022). Polyphenols in tea mainly include catechins and flavonoids. The antioxidant mechanism of these polyphenols can be divided into four parts, first starting with the rise in antioxidant enzyme activity which is followed by lipid peroxidation inhibition. It then proceeds to free radicals scavenging and finally the chelation of metal ions (Yan et al., 2020). The overall result is that the polyphenols react with the ROS to scavenge free radicals into stable molecules.

Other chemical compounds that can be found in kombucha include acids (acetic, lactic, glucuronic, citric), vitamins (B and C) and enzymes (Massoud et al., 2022). These compounds also exhibit antioxidant properties, in particular, the vitamins and organic acids. Deghrigue et al. (2013) have stated that the compounds D-Saccharic acid 1,4-lactone (DSL), gluconic acid, glucuronic acid and lactic acid all contribute to the antioxidative properties of kombucha. At the same time, vitamin C is a well-known antioxidant that also acts by scavenging free radicals in the body. Vitamin C functions by stopping lipid peroxidation through the donation of an electron to the lipid radical (Nimse and Pal, 2015).

Furthermore, the type of tea leaves used can also affect the antioxidant activity of kombucha. According to Yan et al. (2020), green tea has been found to have a higher free radical scavenging activity compared to black tea. This can be attributed to the higher amounts of catechins and flavonoids in green tea which can interact with more ROS at a time. At the same time, the lower radical scavenging activity of black tea can be attributed to its lower concentration of flavonoids such as EC, EGC and EGCG compared to those of green tea (Peluso and Serafini, 2017). For that reason, it suggests that kombucha brewed with green tea leaves will have higher antioxidant activity compared to kombucha brewed with other types of tea leaves. In addition, the enzymes released from the SCOBY during fermentation directly influence the total phenolic content by breaking down the complex phenolic compounds in the tea such as epicatechin isomers (Massoud et al., 2022; Mojtaba Mousavi et al., 2020;

Srihari and Satyanarayana, 2012). For this reason, it is said that the total phenolic content of kombucha increases by the end of the fermentation process.

2.3 Commercial Kombucha Trends in Terms of Flavour Preferences

Commercial kombucha is becoming more accessible in the market as more brands are emerging with their own version of the beverage. The United States is one of the main kombucha manufacturers, but Malaysia is not short of any as well with well-known brands on the market such as ‘WonderBrew Kombucha’ and ‘Cha Cha Kombucha’. What often makes a product stand out from the rest can be partly attributed to its taste, which is influenced by the types of flavouring ingredients used. Based on a review paper by Kim and Adhikari (2020), majority of kombuchas in the market tend to be flavoured rather than unflavoured. It was also observed that many of these kombucha products contain at least one flavour, where the ingredients being used were either fruits, herbs, or a combination of both (Nyhan et al., 2022). This also applies to homebrewers, where adding flavouring to the kombucha can help enhance the overall taste and provide even more nutrients. Popular choices include fruits, flowers, and herbs such as berries, mint, etc. As for tea bases, rosella and rooibos are such examples of tisanes that are accepted in many other types of beverages.

2.4 Agarwood (*Aquilaria*)

Agarwood is the term used to refer to the wood obtained from the plant *Aquilaria* spp. (Thymelaeaceae), and it is commonly referred to as 'gaharu' in Malaysia. There are up to 19 species of *Aquilaria* that can be found in countries such as China, India, Malaysia, etc. (Liu et al., 2017). A healthy *Aquilaria* tree does not produce the agarwood that is usually sought after and will only do so when physiologically injured through wounding or fungal infections (Tan et al., 2019). The wood is often used for its fragrance in products such as incense and perfume, but the plant material has also been used in a wide range of items. HOGA tea valley is Malaysia's main tea valley for agarwood where they offer products that are incorporated with the plant material in the form of tea leaves, snacks, noodles, aromatherapy oils, and shower gels. The market demand for agarwood has been on the rise in recent years, but there is not enough supply to meet these demands. As a result, the price of agarwood becomes considerably high where wood chips can range between USD 20 – 6000 per kilogram (Tan et al., 2019). Since agarwood can only produce the desirable resin after a decade, young agarwood leaves are harvested for the production of tea in countries such as Thailand and Vietnam (Kamonwannasit et al., 2013). It is said that agarwood offers numerous health benefits when consumed regularly where it has been used for its medicinal properties for centuries in Ayurvedic, Chinese, and Southeast Asian medicine (Hashim et al., 2016).

2.5 Pharmacological Properties of Agarwood (*Aquilaria*) Tea Leaves

Consumption of agarwood plant material such as the leaves and bark can treat illnesses such as fever, rheumatism, coughs, and gastric problems as it exhibits anti-allergic, anti-inflammatory, and antioxidant activity (Hashim et al., 2016; Liu et al., 2017).

2.5.1 Antioxidant Activity

It has been studied by Kamonwannasit et al. (2013) that the extract of *Aquilaria* leaves exhibited antioxidant activity based on their reactions in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Additional compounds such as phenolics, anthocyanins, and flavonoids have also been found to be related to the antioxidant activity (Hendra, Moeljopawiro and Nuringtyas, 2016). An experiment conducted by Tay et al. (2014) found that ethanol concentration in solvent extraction was a factor in the final yield of total phenolic content (TPC), DPPH, and flavanol gallates of *Aquilaria*. An increasing ethanol concentration (0, 20, 40, 60, 80, 100% v/v) resulted in a higher percent scavenging activity in the DPPH test, whereas an ethanol concentration of up to around 60% resulted in the highest yields for total phenolic content.

The DPPH assay functions by using the free radical DPPH, a type of organic nitrogen radical with a dark purple appearance. When a DPPH solution is mixed with an antioxidant, the solution will turn yellow to indicate successful scavenging activity. The antioxidant activity of a substance will be determined based on its ability to reduce the DPPH radical at an absorbance ranging between 515 – 528 nm where the result can be expressed as either IC50 or percent scavenging of DPPH (Xiao et al., 2020). The Folin-Ciocalteu (FC) test determines the total phenolic content by using an FC reagent that is originally yellow in appearance. When mixed with an antioxidant along with sodium carbonate, the solution will turn blue. This test relies on electron transfer to measure the reducing capacity of an antioxidant (Lamuela-Raventós, 2017). Responses to this test depend on the number of phenolic groups available that scavenges free radicals via the donation of electrons or hydrogen (Parikh and Patel, 2018).

2.5.2 Anti-inflammatory Activity

The leaves of agarwood have also been found to contain alkaloids, terpenoids, saponins and tannins in which alkaloids can be used as an analgesic agent for pain relief (Adam, Lee and Mohamed, 2017; Batubara et al., 2021). The anti-inflammatory activity of agarwood is mainly due to the presence of flavonoids in the plant material. Inflammatory mediators have strong biological activity that activates other media systems to initiate further inflammation (Sun et al., 2018). Thus, the flavonoids act by reducing the release of inflammatory mediators while stabilizing cell membranes in the body. Other mechanisms such as the xylene-induced edema model and carrageenan-induced edema model have also been used to explain the anti-inflammatory activity of agarwood (Adam, Lee and Mohamed, 2017). Both models are commonly used for acute inflammation tests on live animals by determining the amount of inhibition by the substance of interest (Sun et al., 2018).

2.5.3 Gastrointestinal Regulation

Further studies have also discovered that agarwood leaves provide a certain extent of the gastrointestinal regulating effect. A study conducted by Wang et al. (2015) had shown that agarwood ethanol extract had advantageous effects including improving intestinal peristalsis and gastric emptying, as well as inhibiting gastric ulcers. Moreover, the ethanol extract of agarwood leaves had also shown laxative effects when tested on low-fiber diet-induced rats (Kakino et al., 2010). Hence, it can be noted that the consumption of agarwood plant material may be beneficial for gut health in humans by regulating the digestive system.

2.6 Pineapple (*Ananas comosus*) and Mint (*Mentha*)

Pineapple is a popular exotic fruit grown in several tropical countries, some of which include the Philippines, Thailand and Malaysia. The fruit is juicy and has a sweet taste that can either be eaten on its own or used in foods such as pineapple tarts and pina colada. In Malaysia, the main varieties of pineapple grown include ‘Moris’, ‘N36’, ‘Sarawak’, and ‘MD2’. The sweetness of a pineapple mainly depends on the stage of ripening, which can range between stages 1 – 5. A stage 1 pineapple has a mature green exterior, while a stage 5 pineapple is described to be fully yellow. On top of that, the pH of the fruit increases as the ripening stage advances which leads to a more palatable fruit (Ding and Syazwani, 2016).

Pineapple of MD2 variety was found to have the highest sweetness intensity based on a study of aroma qualities for different pineapple varieties conducted by Lasekan and Hussein (2018) with a total of 27 aroma-active compounds. Additionally, this variety has also been characterized to have a sweeter taste, higher fibre content, and more pleasant aroma compared to others (Mahmud, Abdullah and Yaacob, 2020). Much like any other fruit, pineapples are rich in antioxidants such as vitamin C, β-carotenes, and phenolic compounds that scavenge free radicals (Ferreira et al., 2016; Mahmud, Abdullah and Yaacob, 2020). At the same time, it has been recorded by Ding and Syazwani (2016) that the total phenolic content of MD2 pineapple had increased during ripening stages 1 – 3, but then decreased by the end of stage 5.

Mint (*Mentha*) belongs to the family 'Lamiaceae' that is widely distributed in regions throughout Europe, Asia, Africa, Australia, and North America (Salehi et al., 2018). The taxonomy of *Mentha* includes about 42 species and 15 hybrids with common types including *M. aquatica* L. (water mint) *M. piperita* L. (peppermint), and *M. spicata* L. (spearmint) (Tafrihi et al., 2021). The herb is known for its strong aromatic nature where it is commonly used as a garnish in foods and beverages to provide a cooling sensation. Mint is considered to be the third most common flavour in the world, placed behind vanilla and citrus (Arslan, Özcan and Mengeş, 2010). The main volatile compounds responsible for the fragrance of mint include menthol, menthone, and isomenthone (Straumite, Kruma and Galoburda, 2015). It can be noted that mint can provide certain health benefits as it contains polyphenols and phenolic compounds such as flavonoids. It has also been reported to exhibit antioxidant, antimicrobial, and anticancer activity based on preclinical studies (Tafrihi et al., 2021).

2.7 Response Surface Methodology (RSM) and Box-Behnken Design

Before conducting an experiment, it is important to consider the multiple factors that can affect the results. Response surface methodology (RSM) is a method used for optimizing processes by generating an experimental design that considers the various independent variables of an experiment (Mohamad Said and Mohamed Amin, 2015). Examples of optimized food processes include fermentation, baking and extrusion where the commonly used RSM methods for food product innovation are the 3n factorial, central composite design, Box-Behnken Design and D-optimal designs (Kidane, 2021). The Box-Behnken Design functions by providing a list of possible combinations that can be followed based on the number of parameters that influences the product. Using this experimental design for food, it can be understood how different ingredients and their interactions affect the quality of the food (Domingo et al., 2019). Moreover, this design is also favoured due to its simple and economical design (Kidane, 2021).

CHAPTER 3

MATERIALS AND METHODS

3.1 Agarwood Tea and Green Tea

The agarwood tea used in this study was obtained from HOGA Gaharu Tea Valley located in Gopeng, Perak (4.459986108314068, 101.1928984) as shown in Figure 3.1 below. One box contains 25 tea bags, where each 2 g tea bag contains 0.4 g folium and 1.6 g bark of *Aquilaria malaccensis*.

Figure 3.1: Agarwood tea bags from HOGA Gaharu Tea Valley in Gopeng.

The green tea was obtained from Lotus supermarket located in Ipoh, Perak (4.550132377263361, 101.11708585293755) as shown in Figure 3.2. One box contains 50 tea bags, where each 2 g tea bag contains 100% green tea leaves sourced from China.

Figure 3.2: Green tea bags from Lipton brand purchased at Lotus supermarket.

3.2 Preparation and Fermentation of Kombucha

The teas from both types of tea leaves were first prepared by emptying the contents of the sachet into separate beakers. The formulation used for both kombuchas was determined from the Box-Behnken Design using Jump (JMP) software as shown in Table 3.1 below. A randomized arrangement of 13 formulations (Appendix A) was first prepared for pilot testing performed by 25 untrained panelists recruited from the university campus, which included students and lecturers. From there, the most acceptable formulation was chosen to brew another batch for final sensory evaluation and antioxidant testing.

Table 3.1: Design of experiment for tea leaves, sugar, and liquid starter at three different levels.

Factor	Code	Level*			Unit
		-1	0	+1	
Tea leaves	A	10	12	14	g/L
Sugar	B	50	70	90	g/L
Liquid starter	C	8	10	12	mL/L

* -1 indicates lower level, 0 indicates middle level, and +1 indicates higher level.

The optimized formulation for the tea brewing started with 4.2 g of tea leaves being added to 300 mL of boiled filtered water. The leaves were allowed to steep for 10 minutes before it was sieved out, followed by the addition of 21 g of white sugar. The mixture was stirred and left to cool until room temperature in a fermenting jar that has been presterilized with boiled water. Following that is inoculation of the tea with 2.4 mL of liquid starter and a piece of SCOBY obtained from the local brand 'Herbal Remedies' (5.423145635613039, 100.32060671082814). The mouth of the jar was covered with cheesecloth, secured with rubber bands, and allowed to ferment for 7 days for the first cycle. Fermentation was carried out in the food processing lab in UTAR Kampar Faculty of Science (4.338508933572046, 101.14386585702515) at room temperature (23°C – 26°C). Both green and agarwood teas were prepared as shown in Figure 3.3 and Figure 3.4 below.

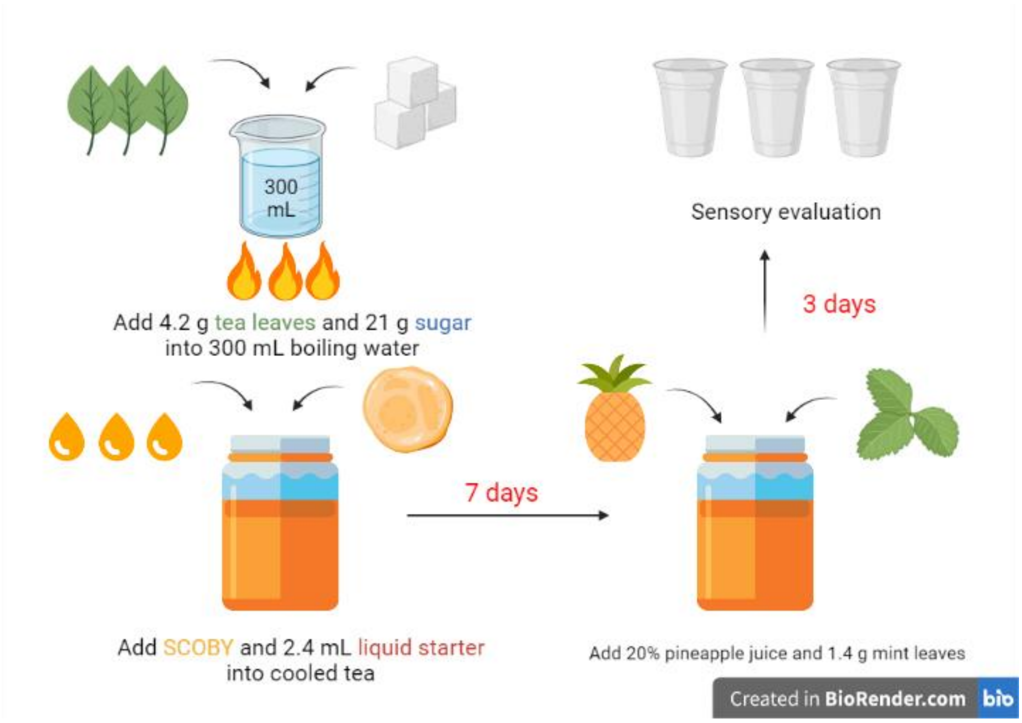


Figure 3.3: Experimental flow for preparation of agarwood tea kombucha.

Figure 3.4: Agarwood tea and green tea kombucha on Day 0 of fermentation.

After fermentation for 7 days, the SCOBY was removed from both jars and the kombucha was filtered. Fully ripened index 5 yellow pineapple of MD2 variety (Rompine) purchased from Jaya Grocer supermarket in Ipoh, Perak (4.596944878379712, 101.09070415344536) was blended and filtered before 20% (v/v) of pineapple juice was added to each jar. The amount used was adapted from a similar flavoured kombucha study conducted by Osiripun and Apisittiwong (2021), and the pineapple ripeness was chosen according to the index by AQINA Fruits (2015) in Figure 3.5. Mint leaves that were also purchased from Jaya Grocer mentioned above were rinsed with distilled water and 1.4 g was measured out before addition into the kombucha. The amount used was adapted with slight modifications from a study on kitchen mint kombucha by Tanticharakunsiri et al. (2020). The second cycle of fermentation lasted for 3 days. At the end of the fermentation period, the mint leaves were strained out and the kombuchas were filtered once more. The beverage was transferred to smaller sterile bottleneck jars as shown in Figure 3.6 and stored in the refrigerator before a sensory evaluation was conducted. The kombuchas were prepared in triplicates for both agarwood tea and green tea leaves respectively.

Figure 3.5: MD2 pineapple ripeness in reference to colour of exterior from index 1 (least ripe) until 5 (most ripe) (AQINA Fruits, 2015).

Figure 3.6: Agarwood tea kombucha infused with pineapple juice and mint leaves on Day 7 of fermentation.

3.3 Physicochemical Properties of Agarwood and Green Tea Kombucha

The samples tested included agarwood tea and green tea kombucha, as well as agarwood tea and green tea kombucha infused with pineapple and mint. All tests were performed on Days 0, 1, 3, 5, 7 and 10 of the study. Before the tests were conducted, the samples were first filtered through a filter paper.

3.3.1 Determination of pH Value

The pH of the kombuchas were determined with the use of a pH meter (FiveEasy Plus pH meter FP20-Std-Kit). The instrument was first calibrated at pH 4.0 and pH 7.0 before measuring the pH values of the agarwood tea and green tea kombucha. Approximately 10 mL of each sample was poured into a beaker and the pH meter electrode was immersed to determine the pH value. The electrode was rinsed with distilled water and wiped dry before each new measurement. The measurements for each sample were performed in triplicates.

3.3.2 Determination of Total Soluble Solids (TSS)

A pocket digital refractometer (ATAGO PAL-3) was used to determine the °Brix value for the samples. The instrument was first calibrated by dispensing a few drops distilled water onto the lens and wiping it dry using a paper towel before sample measurement was conducted. Approximately 5 drops of the sample were dispensed onto the lens for measurement and the results were recorded accordingly. The measurements were performed in triplicates and the lens were cleaned with distilled water each time before each new measurement.

3.3.3 Determination of Total Titratable Acidity (TTA)

The total titratable acidity of the kombucha samples were determined based on the amount of sodium hydroxide (NaOH) needed to titrate the sample in an acid-base titration. A concentration of 0.1 M of NaOH was first prepared by diluting 0.4 g of NaOH pellets with 100 mL distilled water. The kombucha sample was vortexed before 5 mL of it was poured into a conical flask. The sample was then titrated against the NaOH until the endpoint was reached with the use of phenolphthalein as the indicator. The TTA was calculated according to Formula 3.1 below by Jacobson (2006) where the results were expressed as grams of acetic acid per litre of sample.

Formula 3.1

$$\text{Total titratable acidity (g/L)} = 75 \times \text{Normality of NaOH} \times [\text{Titre volume (mL)} / \text{Sample volume (mL)}]$$

3.4 Evaluation of Antioxidant Properties of Agarwood and Green Tea Kombuchas

3.4.1 Determination of Free Radical Scavenging Activity

The method of determining the free radical scavenging activity for the kombuchas was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay adapted from Xiao et al. (2020) with minor modifications. A 1 mM DPPH stock solution was first prepared by mixing 3.94 mg of DPPH powder with 10 mL of methanol. The stock was then diluted to 0.1 mM by mixing 1 mL of the stock with 9 mL methanol. The freshly prepared solution was then wrapped with aluminium foil and stored at 4°C. Meanwhile, 100 µL of methanol was dispensed into each well on a 96-well plate excluding the first row. The kombucha samples of the same volume were then dispensed into the empty wells from the first row.

Ascorbic acid stock (1 mg/mL) was also prepared to be used as the standard for the assay, where 10 mg of ascorbic acid powder was mixed with 10 mL of methanol. The stock solution was further diluted to concentrations 0.04, 0.06, 0.08, 0.10 and 0.12 mg/mL in order to obtain a standard curve. A 2-fold serial dilution of the samples was then performed in the well plate by transferring 100 µL of the sample from the first well into the well directly below. The contents were then mixed 3 – 4 times before aliquoting the same volume and transferring it to the next well below. This was repeated until the last well of the column before discarding the remaining 100 µL. A micropipette was then used to aliquot 100 µL of the DPPH solution into each well before covering the well plate with an aluminium foil and leaving it to incubate for 30 minutes in the dark at room temperature. A microplate reader (FLUOstar Omega, BMG LABTECH) was used to measure the absorbance of the samples at 517 nm and the results were recorded. The inhibition ratio for the kombucha samples and the ascorbic acid standard was calculated based on Formula 3.2 below by Silva et al. (2021), where the results were expressed as percent inhibition.

Formula 3.2

$$\text{Percent inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 = absorbance of blank and A_1 = absorbance of sample

3.4.2 Determination of Total Phenolic Content (TPC)

The test used to determine the total phenolic content for the kombucha was the Folin-Ciocalteu (FC) method adapted from Silva et al. (2021) with minor modifications. Sodium carbonate stock solution of 20% (w/v) was first prepared by mixing 10 g of anhydrous sodium carbonate with 50 mL of distilled water. Following that, a gallic acid stock solution was prepared by adding 0.5 g of gallic acid powder with 10 mL of ethanol. The stock solution was then topped up with 100 mL of distilled water to create a 5 mg/mL concentration. The stock was then further diluted into the concentrations 0.1, 0.2, 0.3, 0.5, and 1.0 mg/mL in order to obtain a standard curve. For analysis of the kombucha samples, 20 µL of the sample was mixed with 1.58 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. The centrifuge tube containing the solution was wrapped with aluminium foil and left to incubate for 8 minutes. Following that, 300 µL of sodium carbonate stock was added to the solution before incubation in a 40°C shaking water bath for 30 minutes. The solution was then transferred into a 96-well plate and absorbance was measured at 765 nm. The total phenolic content was expressed as mg of gallic acid equivalent per L of sample (mg GAE/L).

3.5 Sensory Evaluation

A final sensory evaluation was conducted for the agarwood tea and green tea kombucha using the 9-point hedonic scaling test adapted from Gramza-Michałowska et al. (2016). For this scaling test, the rankings were as follows:

9 = like extremely	4 = dislike slightly
8 = like very much	3 = dislike moderately
7 = like moderately	2 = dislike very much
6 = like slightly	1 = dislike extremely
5 = neither like nor dislike	

A total of 5 samples were prepared which were unflavoured and flavoured kombuchas from both types of teas along with a commercially bought green tea kombucha from the local brand 'Wonderbrew Kombucha Malaysia' located in Subang Jaya, Selangor (3.074743771603687, 101.61799316137815). The commercial kombucha was bought from Jaya Grocer supermarket in Ipoh, Perak (4.596944878379712, 101.09070415344536), and the set-up of the sensory evaluation is shown in Figure 3.7.

Figure 3.7: Serving tray with 5 kombucha samples and rinsing water for sensory evaluation by panelists.

The hedonic test was prepared according to the general procedure for conducting sensory tests, where the samples were labelled with random 3-digit codes arranged in random order. On the provided scoresheet (Appendix B), panelists were asked to rate each sample in order according to the provided scale as ranging from a score of 9 (like extremely) to 1 (dislike extremely). The attributes that were assessed included appearance, aroma, taste, and overall acceptability. The sensory evaluation was performed in a sensory evaluation room where a total of 30 untrained panelists were recruited from the university campus. The responses were then received and recorded for further analysis.

3.6 Statistical Analysis

The results were expressed as mean ± standard error. All tests for the physicochemical properties and antioxidant properties in this study were conducted in triplicates, and the IBM SPSS Statistics 27 software was used to perform the statistical analysis. The mean comparison between agarwood tea and green tea kombucha added with pineapple and mint for physicochemical properties were compared using the independent sample t-test at a 95% confidence interval. Pearson *r* correlation between the physicochemical properties and the fermentation days were also analysed. Meanwhile, the mean comparison of the kombuchas between the different days of fermentation in terms of antioxidant activity and sensory evaluation were analysed using one-way ANOVA and Duncan's multiple ranged test at a 95% confidence interval where a p-value < 0.05 was statistically significant.

CHAPTER 4

RESULTS

The kombucha samples were analysed on Days 0, 1, 3, 5, 7, and 10 post-fermentation in triplicates. For the samples from Day 0, the one labelled as Day 0 (B) indicates before inoculation with the liquid starter, while Day 0 (A) indicates after inoculation with the liquid starter. The sample labelled Day 10 (P) indicates kombucha infused with pineapple juice and mint leaves.

4.1 Pilot Testing to Determine Optimum Kombucha Formulation

Based on the Box-Behnken Design, a total of 13 agarwood tea kombucha formulations were prepared for pilot testing accordingly (Appendix A). From the sensory evaluation analysis conducted, it was found that formulations 3 and 9 shared similar acceptance scores (Appendix C). A score of 1 indicated that the sample was disliked extremely, where an increasing score correlated to an increasing acceptability of the sample. A score of 5 indicated that the sample was neither liked nor disliked, while a score of 9 indicated that the sample was liked extremely. Formulation 9 had a mean score of 6.96 based on panelists' ratings. This formulation used 4.2 g of tea leaves, 27 g of sugar, and 3 mL of liquid starter. Meanwhile, formulation 3 was scored 6.72 by panelists. This formulation used 4.2 g of tea leaves, 21 g of sugar, and 2.4 mL of liquid starter.

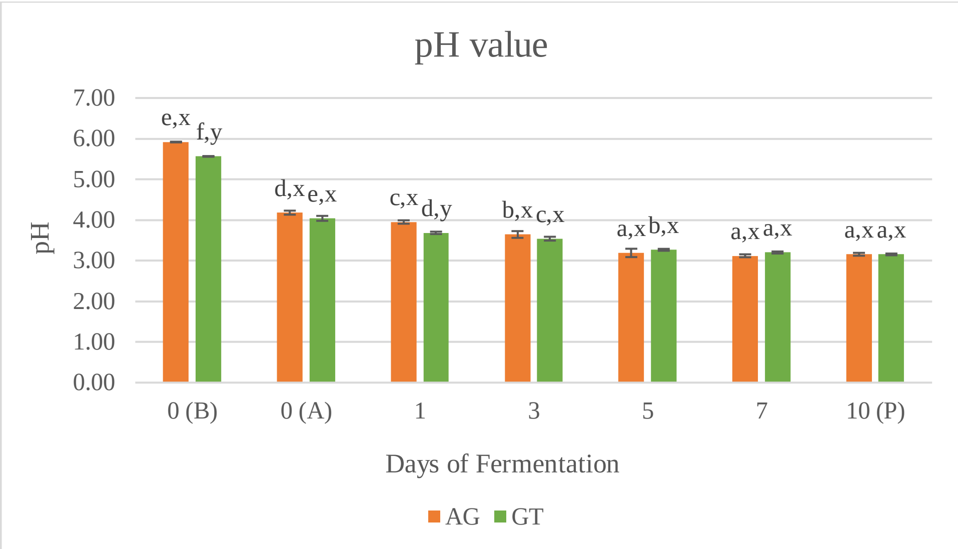
The kombuchas produced from both formulations underwent further analysis to narrow down the choice to one formulation.

4.2 Physicochemical and Antioxidant Properties Between Agarwood Tea Kombucha Formulations 3 and 9

The pH values and total soluble solids (TSS) were measured for the agarwood tea kombuchas. Formulation 3 kombucha had a pH of 2.82 ± 0.01, while formulation 9 kombucha had a pH of 2.84 ± 0.01 by Day 7. As for the total soluble solids, formulation 3 recorded a degree Brix (°Bx) value of 6.97 ± 0.19°Bx, while formulation 9 had a degree Brix value of 7.00 ± 0.12°Bx. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition ratio was also not significantly different ($p \geq 0.05$) between the two formulations, with formulation 3 recording 82.00 ± 0.12% inhibition and formulation 9 recording 82.55 ± 0.72% inhibition by Day 7 of fermentation. From the comparisons, formulation 3 for the agarwood tea kombucha was chosen for the subsequent experiment as it had similar antioxidant activity to formulation 9 but used less sugar. Lesser sugar content is not only better for overall health, but also reduces the cost of production. Thus, the final formulation adopted in the subsequent sub-experiment was as follows: 4.2 g tea leaves, 21 g sugar, and 2.4 mL liquid starter per 300 mL water.

4.3 Physicochemical Properties of Agarwood Tea and Green Tea Kombucha

The physicochemical properties of both kombuchas included pH values, total soluble solids (TSS), and total titratable acidity (TTA) were studied. Based on the results in Figure 4.1.1, the pH value for the agarwood tea kombucha observed a significantly decreasing trend ($p < 0.05$) from Day 0 (B) to Day 10, where the pH had decreased from a value of pH 5.92 ± 0.01 to pH 3.16 ± 0.04. The same decreasing trend was also observed for the green tea kombucha, with the pH values decreasing from pH 5.56 ± 0.01 on Day 0 (B) to 3.15 ± 0.02 by Day 10. The pH values between agarwood tea and green tea kombucha were not significantly different ($p \geq 0.05$) within the same day of fermentation, except for Days 0 (B) and 1.



AG refers to agarwood tea kombucha; GT refers to green tea kombucha. 0 (B) Means before inoculation; 0 (A) Means after inoculation; 10 (P) Means infusion with pineapple and mint

$^{a, b, c, d, e, f}$ Means different superscripts between different days of fermentation for agarwood tea and green tea kombucha are significantly different at ($p < 0.05$).

$^{x, y}$ Means comparison between agarwood tea and green tea kombucha within the same day of fermentation are significantly different at ($p < 0.05$).

Figure 4.1.1: Changes in pH values of agarwood tea and green tea kombucha throughout 10 days of fermentation.

A negative correlation was also observed between the pH values and the days of fermentation for both agarwood tea kombucha (Figure 4.1.2) and green tea kombucha (Figure 4.1.3), where the pH decreased as fermentation day increased.

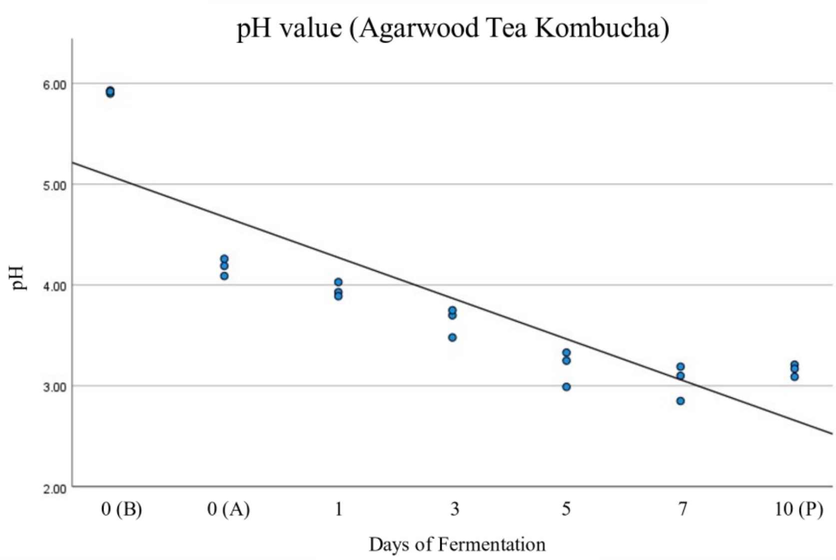


Figure 4.1.2: Correlation between the pH value of agarwood tea kombucha and the days of fermentation.

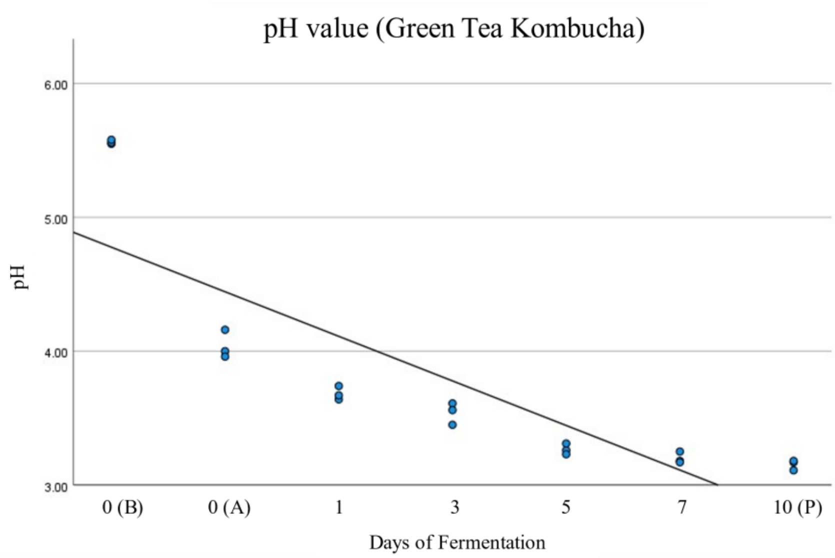


Figure 4.1.3: Correlation between the pH value of green tea kombucha and the days of fermentation.

Further analysis using the Pearson *r* correlation showed that there was a negative correlation between the pH values of the kombucha and the days of fermentation. For the agarwood tea kombucha in Table 4.1.1, there was a significantly high negative correlation between the days of fermentation and pH of the kombucha, with $r = -0.868$, $p < 0.001$. As for the green tea kombucha in Table 4.1.2, there was also a significantly high negative correlation between the days of fermentation and pH of the kombucha, with $r = -0.848$, $p < 0.001$.

Table 4.1.1: Correlation between days of fermentation and pH values of agarwood tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**pH**
Days of Fermentation	Pearson Correlation	1	-0.868**
	Sig. (2-tailed)		< 0.001
	N	21	21
pH	Pearson Correlation	-0.868**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

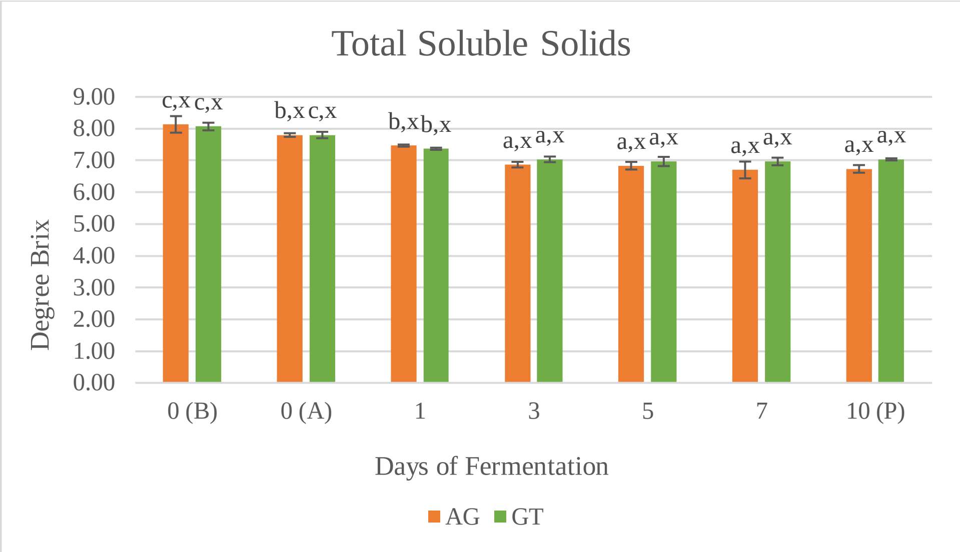
** Correlation is significant at the 0.01 level (2-tailed).

Table 4.1.2: Correlation between days of fermentation and pH values of green tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**pH**
Days of Fermentation	Pearson Correlation	1	-0.848**
	Sig. (2-tailed)		< 0.001
	N	21	21
pH	Pearson Correlation	-0.848**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

** Correlation is significant at the 0.01 level (2-tailed).

The total soluble solids (TSS) represented in Figure 4.2.1 showed that both agarwood tea and green tea kombuchas had recorded similar decreasing trends in the amount of TSS. For the agarwood tea kombucha, the TSS had significantly decreased ($p < 0.05$) from 8.13 ± 0.26 on Day 0 (B) to 6.73 ± 0.12 by Day 10 of fermentation. Similarly for the green tea kombucha, the TSS had also significantly decreased ($p < 0.05$) from 8.07 ± 0.12 on Day 0 (B) to 7.03 ± 0.03 by Day 10.



[a, b, c] Means different superscripts between different days of fermentation for agarwood tea and green tea kombucha are significantly different at ($p < 0.05$).

[x] Means comparison between agarwood tea and green tea kombucha within the same day of fermentation does not differ significantly at ($p \geq 0.05$).

Figure 4.2.1: Changes in total soluble solids of agarwood tea and green tea kombucha throughout 10 days of fermentation.

A negative correlation was also observed between the TSS and days of fermentation for both agarwood tea kombucha (Figure 4.2.2) and green tea kombucha (Figure 4.2.3), where the degree Brix value decreased as fermentation day increased.

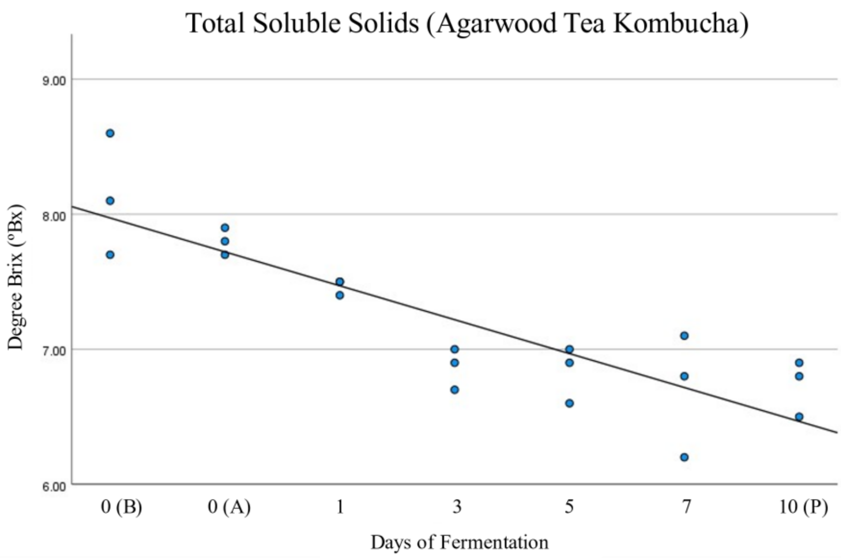


Figure 4.2.2: Correlation between the total soluble solids of agarwood tea kombucha and the days of fermentation.

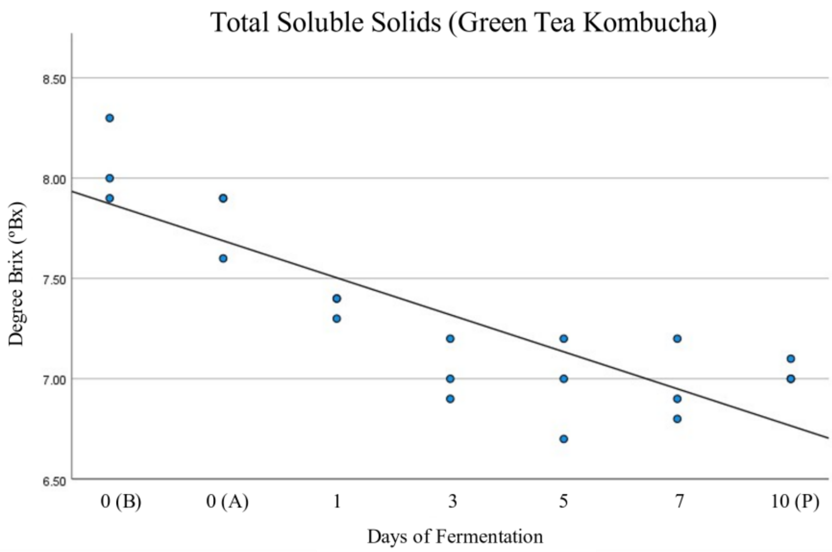


Figure 4.2.3: Correlation between the total soluble solids of green tea kombucha and the days of fermentation.

Further analysis using the Pearson *r* correlation showed that there was a negative correlation between the total soluble solids of the kombucha and the days of fermentation. For the agarwood tea kombucha in Table 4.2.1, there was a significantly high negative correlation between the days of fermentation and TSS of the kombucha, with $r = -0.863$, $p < 0.001$. As for the green tea kombucha in Table 4.2.2, there was also a significantly high negative correlation between the days of fermentation and TSS of the kombucha, with $r = -0.842$, $p < 0.001$.

Table 4.2.1: Correlation between days of fermentation and total soluble solids of agarwood tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**Degree Brix**
Days of Fermentation	Pearson Correlation	1	-0.863**
	Sig. (2-tailed)		< 0.001
	N	21	21
Degree Brix	Pearson Correlation	-0.863**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

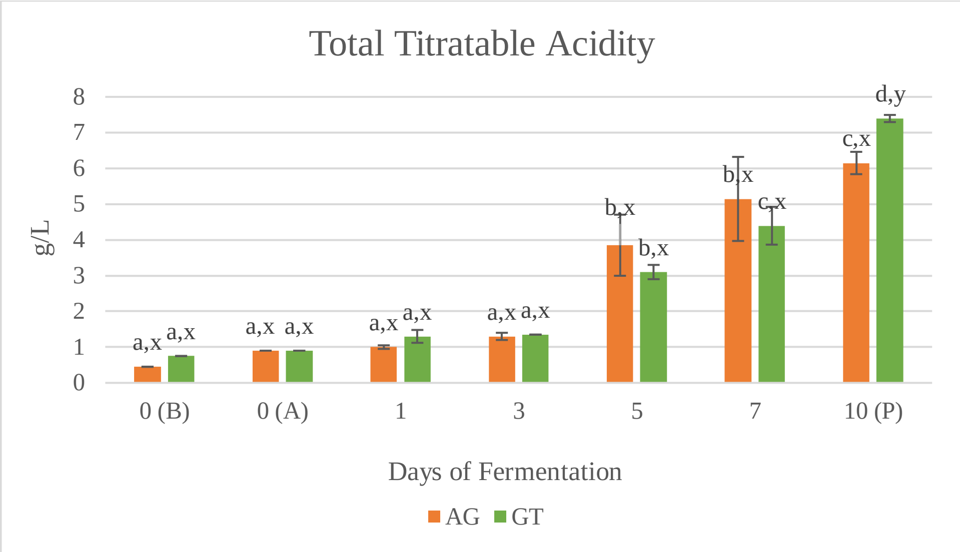
** Correlation is significant at the 0.01 level (2-tailed).

Table 4.2.2: Correlation between days of fermentation and total soluble solids of green tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**Degree Brix**
Days of Fermentation	Pearson Correlation	1	-0.842**
	Sig. (2-tailed)		< 0.001
	N	21	21
Degree Brix	Pearson Correlation	-0.842**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

** Correlation is significant at the 0.01 level (2-tailed).

From the chart in Figure 4.3.1 below, the total titratable acidity (TTA) between agarwood tea and green tea kombucha were not significantly different ($p \geq 0.05$) within the same day of fermentation, except for Day 10. The lowest amount was recorded on Day 0 (B) with values of 0.45 ± 0.00 g/L for agarwood tea kombucha and 0.75 ± 0.00 g/L for green tea kombucha. These values had increased by Day 10, with a TTA of 6.15 ± 0.31 g/L for agarwood tea kombucha and 7.40 ± 0.10 g/L for green tea kombucha.



[a, b, c, d] Means different superscripts between different days of fermentation for agarwood tea and green tea kombucha are significantly different at ($p < 0.05$).

[x, y] Means comparison between agarwood tea and green tea kombucha within the same day of fermentation are significantly different at ($p < 0.05$).

Figure 4.3.1: Changes in total titratable acidity of agarwood tea and green tea kombucha throughout 10 days of fermentation.

A positive correlation was observed between the total titratable acidity and the days of fermentation for both agarwood tea kombucha (Figure 4.3.2) and green tea kombucha (Figure 4.3.3), where the TTA increased as fermentation day increased.

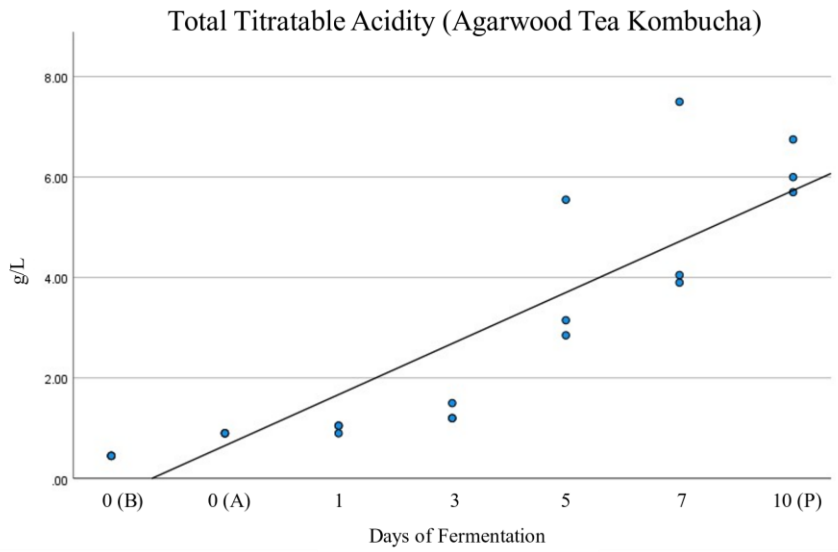


Figure 4.3.2: Correlation between the total titratable acidity of agarwood tea kombucha and the days of fermentation.

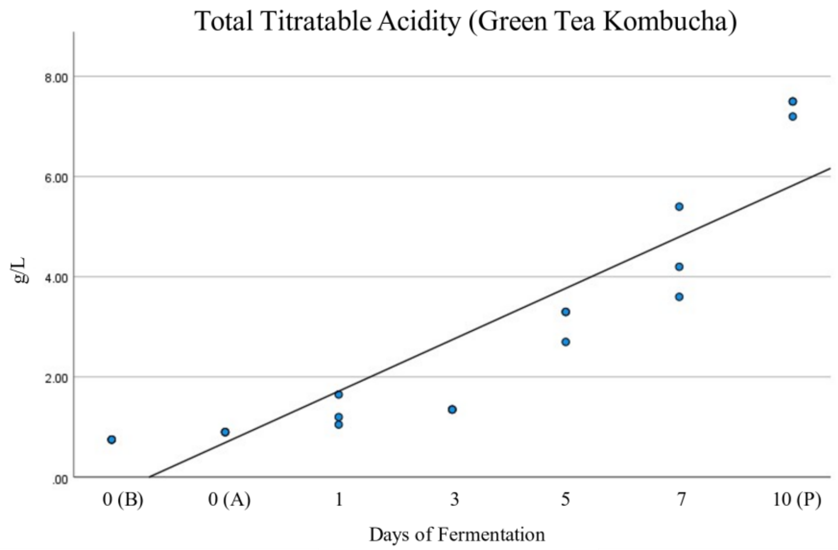


Figure 4.3.3: Correlation between the total titratable acidity of green tea kombucha and the days of fermentation.

Further analysis using the Pearson *r* correlation showed that there was a positive correlation between the total titratable acidity of the kombucha and the days of fermentation. For the agarwood tea kombucha in Table 4.3.1, there was a significantly high positive correlation between the days of fermentation and TTA of the kombucha, with $r = 0.886$, $p < 0.001$. As for the green tea kombucha in Table 4.3.2, there was also a significantly high positive correlation between the days of fermentation and TTA of the kombucha, with $r = 0.897$, $p < 0.001$.

Table 4.3.1: Correlation between days of fermentation and total titratable acidity of agarwood tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**TTA**
Days of Fermentation	Pearson Correlation	1	0.886**
	Sig. (2-tailed)		< 0.001
	N	21	21
TTA	Pearson Correlation	0.886**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.3.2: Correlation between days of fermentation and total titratable acidity of green tea kombucha throughout 10-day fermentation.

		Days of Fermentation	**TTA**
Days of Fermentation	Pearson Correlation	1	0.897**
	Sig. (2-tailed)		< 0.001
	N	21	21
TTA	Pearson Correlation	0.897**	1
	Sig. (2-tailed)	< 0.001	
	N	21	21

** Correlation is significant at the 0.01 level (2-tailed).

4.4 Determination on Antioxidant Properties of Agarwood Tea and Green Tea Kombucha

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition percentages had significantly increased ($p < 0.05$) from Days 0 – 7 for both the agarwood tea and green tea kombucha. As shown in Table 4.4, the highest observed percentage for both agarwood tea and green tea kombuchas were on Day 3, with a value of 87.45 ± 0.12% and 88.78 ± 0.21% respectively. Meanwhile, the inhibition values on Day 7 were the lowest with a value of 70.60 ± 0.74 % and 79.84 ± 0.32% accordingly. The inhibition percentages between the agarwood tea and green tea kombucha were also significantly different ($p < 0.05$) from each other on Days 0 – 7.

Table 4.4: DPPH inhibition percentage of agarwood tea and green tea kombucha over a fermentation period of 10 days.

Day	DPPH inhibition percentage (%)	
	Agarwood Tea Kombucha	**Green Tea Kombucha**
0 (B)	$81.50 \pm 0.24^{c,x}$	$85.28 \pm 0.24^{b,y}$
0 (A)	$82.53 \pm 0.14^{cd,x}$	$86.17 \pm 0.42^{b,y}$
1	$83.74 \pm 0.33^{d,x}$	$86.48 \pm 0.24^{b,y}$
3	$87.45 \pm 0.12^{e,x}$	$88.78 \pm 0.21^{c,y}$
5	$79.72 \pm 0.83^{b,x}$	$86.24 \pm 0.36^{b,y}$
7	$70.60 \pm 0.74^{a,x}$	$79.84 \pm 0.32^{a,y}$
10 (P)	$83.59 \pm 0.48^{d,x}$	$85.16 \pm 0.83^{b,x}$

$^{a, b, c, d, e}$ Means different superscripts within the same column are significantly different at ($p < 0.05$).

$^{x, y}$ Means different superscripts within the same row are significantly different at ($p < 0.05$).

The IC50 values determined from the DPPH results are shown in Figures 4.4 and 4.5. For the agarwood tea kombucha, the IC50 value on Day 0 (A) was 9.91 ± 0.34 µg/mL where this value had decreased to 6.02 ± 0.39 µg/mL by Day 7. For the green tea kombucha, the IC50 value on Day 0 (A) was 0.10 ± 0.02 µg/mL where it then decreased to 0.003 ± 0.001 µg/mL by Day 7.

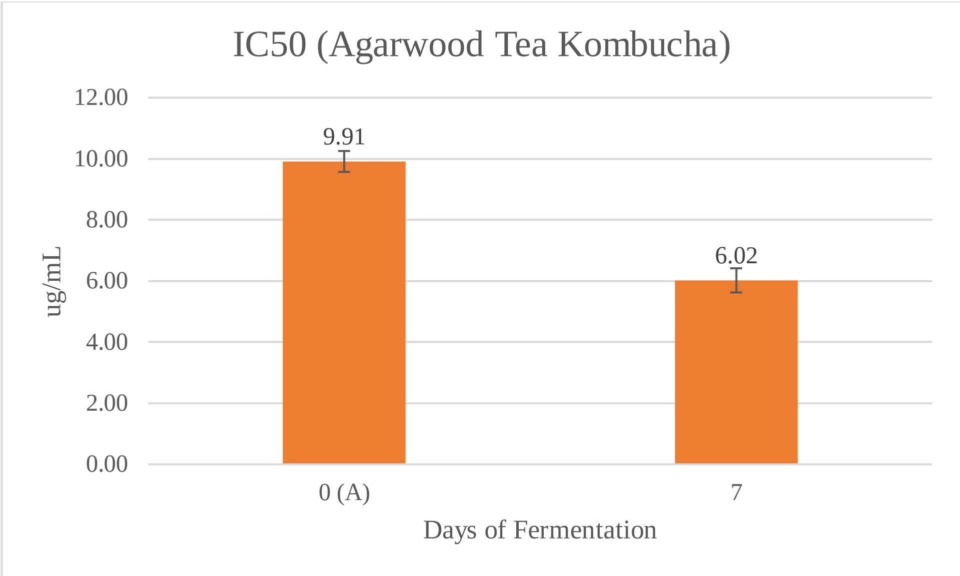


Figure 4.4: IC50 values of agarwood tea kombucha on Days 0 and 7 of fermentation.

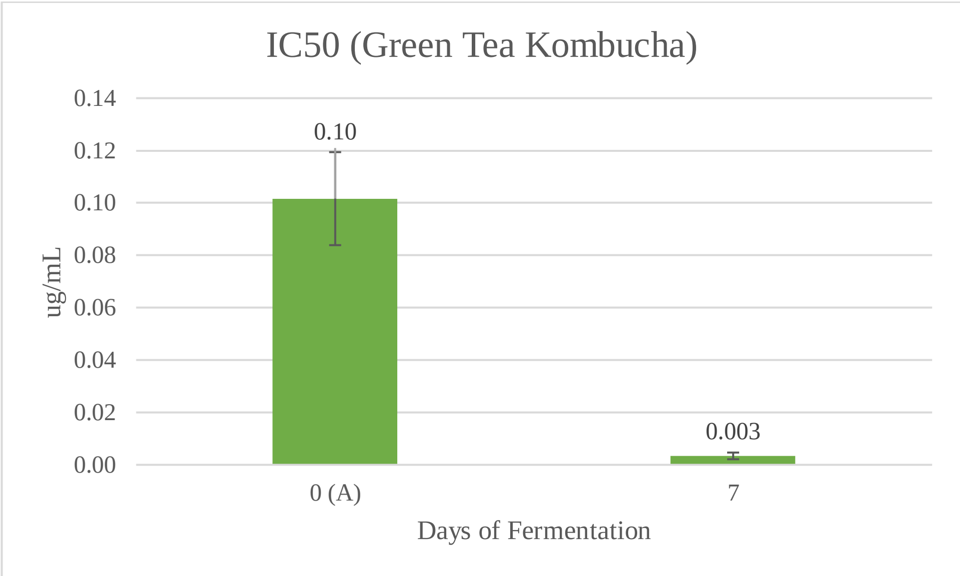


Figure 4.5: IC50 values of green tea kombucha on Days 0 and 7 of fermentation.

The total phenolic content (TPC) as tabulated in Table 4.5 indicates that there was a significant increase ($p < 0.05$) in both the agarwood tea and green tea kombucha from Days 0 – 7. The lowest values were observed on Day 0 (B), with a TPC of 142.42 ± 0.63 mg GAE/L for the agarwood tea kombucha and 833.97 ± 0.53 mg GAE/L for the green tea kombucha respectively. These values had then increased to 337.81 ± 1.00 mg GAE/L and 1344.53 ± 0.78 mg GAE/L respectively by Day 10. Throughout all 10 days of fermentation, the TPC of both kombuchas were also significantly different ($p < 0.05$) from each other.

Table 4.5: Total phenolic content of agarwood and green tea kombuchas over a fermentation period of 10 days.

Day	**Total phenolic content (mg GAE/L)**	
	Agarwood Tea Kombucha	**Green Tea Kombucha**
0 (B)	$142.42 \pm 0.63^{a,x}$	$833.97 \pm 0.53^{a,y}$
0 (A)	$151.86 \pm 1.04^{b,x}$	$946.70 \pm 1.21^{b,y}$
1	$187.53 \pm 0.28^{c,x}$	$1019.41 \pm 1.16^{c,y}$
3	$197.53 \pm 0.28^{d,x}$	$1108.81 \pm 0.68^{d,y}$
5	$231.97 \pm 0.74^{e,x}$	$1168.19 \pm 1.34^{e,y}$
7	$265.31 \pm 1.00^{f,x}$	$1236.14 \pm 1.00^{f,y}$
10 (P)	$337.81 \pm 1.00^{g,x}$	$1344.53 \pm 0.78^{g,y}$

$^{a, b, c, d, e, f, g}$ Means different superscripts within the same column are significantly different at ($p < 0.05$).

$^{x, y}$ Means different superscripts within the same row are significantly different at ($p < 0.05$).

4.5 Final Sensory Evaluation Results

The sensory evaluation ratings in Table 4.6 below show the mean scores for the 4 attributes by panelists. It was observed that the agarwood tea kombucha infused with pineapple and mint scored relatively higher with a value of 6.27 ± 0.23 in terms of overall acceptability, while unflavoured agarwood tea kombucha scored the lowest with 5.63 ± 0.31.

Table 4.6: Sensory evaluation of agarwood tea, green tea, and commercial kombucha.

Attribute	Kombucha				
	Agarwood Tea	**Agarwood Tea + Pineapple & Mint**	**Green Tea**	**Green Tea + Pineapple & Mint**	**Commercial**
Appearance	6.73 ± 0.21^{b}	6.30 ± 0.25^{ab}	6.60 ± 0.22^{ab}	5.90 ± 0.26^{a}	5.97 ± 0.32^{ab}
Aroma	5.90 ± 0.32^{a}	6.53 ± 0.28^{a}	5.67 ± 0.30^{a}	6.33 ± 0.25^{a}	5.77 ± 0.27^{a}
Taste	5.23 ± 0.34^{a}	5.73 ± 0.29^{a}	5.57 ± 0.36^{a}	5.27 ± 0.28^{a}	5.83 ± 0.37^{a}
Overall acceptability	5.63 ± 0.31^{a}	6.27 ± 0.23^{a}	5.77 ± 0.32^{a}	5.80 ± 0.22^{a}	5.87 ± 0.33^{a}

$^{a,\,b}$ Means different superscripts within the same row are significantly different at ($p < 0.05$). The sensory evaluation is based on a 9-point hedonic scale.

The bar chart shown in Figure 4.6 below represents the overall acceptability scores of the kombucha samples based on a 9-point hedonic scale. The agarwood tea kombucha infused with pineapple and mint, labelled as AG (P), was liked by 77% of the 30 panelists, which was determined based on ratings between 6 – 9. As for the green tea kombucha infused with pineapple and mint, labelled as GT (P), it was liked by 60% of panelists while 30% of them had a neutral response. The control in this sensory evaluation was the commercial kombucha which was liked by 53% of panelists, being the lowest out of all the samples. This sample also had equal scores for dislikes and neutral with a value of 23%. Meanwhile, the radar chart in Figure 4.7 illustrates the scores of the 5 kombucha samples relative to each other based on the attributes of appearance, aroma, taste, and overall acceptability. The scores were equally distributed, ranging between 5 to 7 for all attributes.

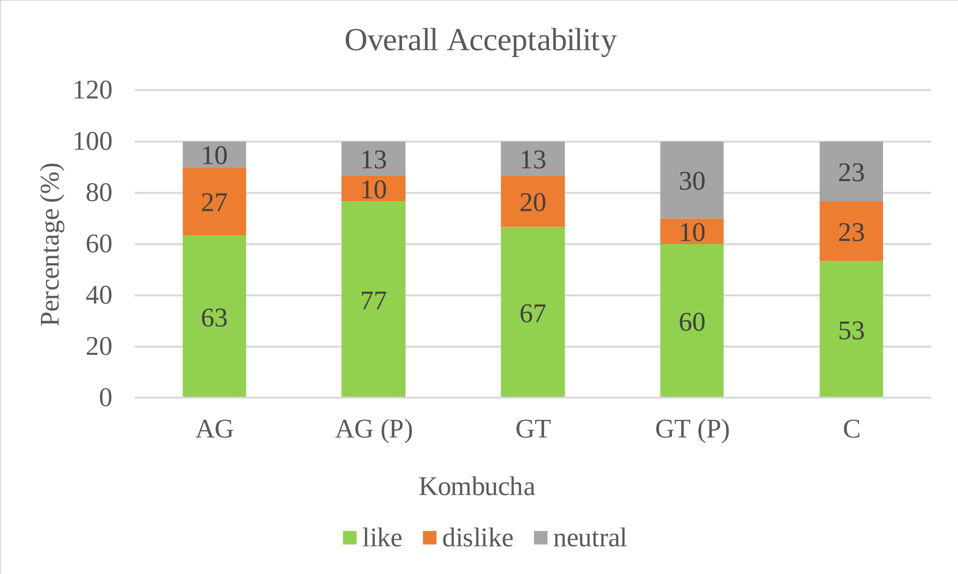


Figure 4.6: Panelists' ratings on the overall acceptability of the kombucha samples.

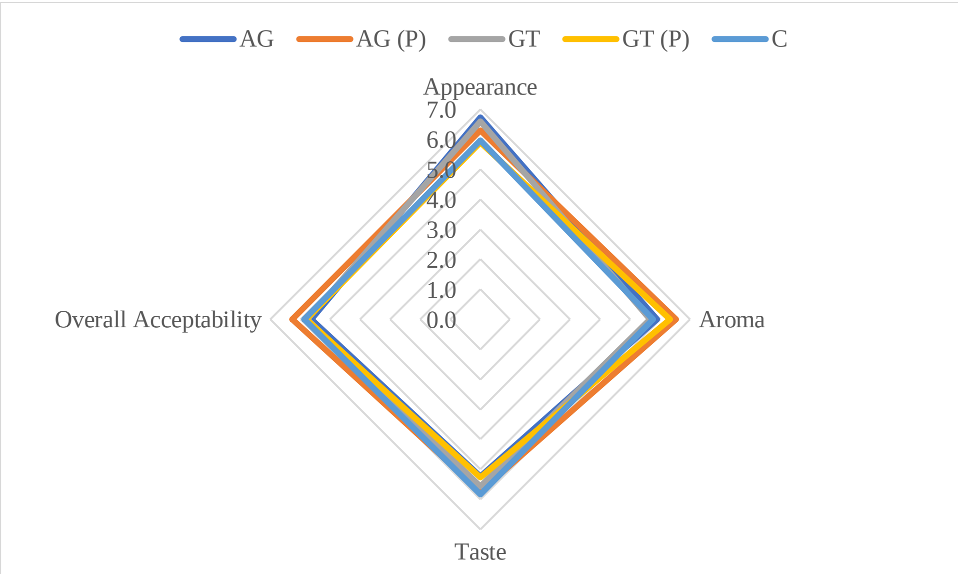


Figure 4.7: Appearance, aroma, taste, and overall acceptability evaluation of different kombucha samples.

CHAPTER 5

DISCUSSION

5.1 Physicochemical Properties of Kombucha

5.1.1 pH Values of Agarwood Tea and Green Tea Kombucha

From the results obtained in Figure 4.1.1, it can be deduced that the pH values between the agarwood tea and green tea kombucha were not significantly different ($p \geq 0.05$) within the same day of fermentation with exceptions to Days 0 (B) and 1. On Day 0 (B), the pH values were first measured for both tea types before inoculation with the liquid starter. The pH value of teas depends on the types of leaves, which includes the type and concentration of compounds contained in the leaves (Zhu et al., 2013). The tea leaf processing method as well as maturation period can also affect the pH of the tea (Nyhan et al., 2022). This explains why the pH of the agarwood tea was significantly higher ($p < 0.05$) than the pH of green tea on Day 0 before inoculation, as they are two different types of leaves with various compounds. In a study by Lunkes and Hashizume (2014), the pH values of brewed tea ranged between 6.75 – 7.89 which included green, chamomile, black, and lemongrass tea. This justifies that the types of tea leaves can impact the initial pH of the tea. However, upon inoculation with the liquid starter on Day 0, the pH of both agarwood tea and green tea kombucha were no longer significantly different ($p \geq 0.05$), which remained the same by the end of the 10-day fermentation period. This was supported by the results from Jakubczyk et al. (2020), where no significant differences in pH between kombuchas prepared from different tea types were found.

The high negative correlation between the pH and fermentation days was also proven to be significant ($p < 0.001$) for both the agarwood tea and green tea kombucha with negative Pearson correlation coefficients as shown in Table 4.1.1 and Table 4.1.2 respectively. The Pearson correlation coefficient determines how two variables are linearly associated, with values ranging between +1 to -1 (Laerd Statistics, 2022c). A positive value indicates a positive correlation and vice versa. The decrease in pH values for both kombuchas during the fermentation period was expected as the microbes in the symbiotic culture of bacteria and yeast (SCOBY) released organic acids while they feed on the carbon source in the tea (Bishop et al., 2022; Kayisoglu and Coskun, 2020). The pH values for both kombuchas had decreased from around pH 6.0 to pH 3.2 by Day 10 of fermentation. According to a similar study conducted by Quiao-Won and Teves (2018), the pH of their green tea kombucha had also undergone a decrease from pH 5.0 to around pH 3.0 by Day 10 of fermentation. Another study by Kayisoglu and Coskun (2020) determined that the pH of green tea kombucha had also experienced a similar decreasing trend from pH 6.94 to 2.75 after 14 days of fermentation. As for the pH value of agarwood tea kombucha, Nurmiati and Wijayanti (2018) found that it had decreased from a pH of 6.5 to 3.0 by Day 10 of fermentation. Therefore, the results agree with each other and proves that the pH values of kombucha should drop throughout fermentation.

One of the factors that affect the pH of kombucha is the amount of liquid starter inoculated into the tea at the start of fermentation, as it impacts the amount of organic acids released by the microbes from the SCOBY. When more liquid starter is added to the tea, it decreases the initial pH of the kombucha before the start of fermentation. As a result, the final pH of the kombucha will be lower compared to a kombucha initially inoculated with a lower amount of liquid starter. The amount of liquid starter added to the kombucha according to the formulation was 2.4 mL per 300 mL volume (0.8% v/v) which was lesser than the commonly used 10 – 20% v/v (Nyhan et al., 2022). Therefore, it was expected that the pH of the beverage in this study to be slightly higher than those from the kombuchas mentioned from other studies. It is also said that the pH of kombucha should range between pH 2.5 – 3.5 to ensure that the beverage has been optimally fermented after 7 – 14 days (Nyhan et al., 2022). Another factor of pH value is the level of carbon source added into the tea, which in this case is the white sugar. More sugar means that there is a higher carbon source for the SCOBY to act on, thus increasing their activity as well as the release of more organic acids (Muhialdin et al., 2019). The increased amount of organic acids will then further reduce the pH of the kombucha.

However, it was observed that the pH values of the experimental kombuchas by Day 10 were slightly higher than those on Day 7. This was because the ingredients pineapple juice and mint were added into the raw kombucha on Day 7 to produce flavoured kombucha. It was found by Ding and Syazwani (2016) that fully ripened stage 5 MD2 pineapple fruit had a pH value of 4.13, which was higher than the pH of the Day 7 unflavoured kombucha in this study (pH 3.05 ± 0.10 for agarwood tea kombucha and pH 3.20 ± 0.025 for green tea kombucha). Furthermore, the pineapple juice in this study was measured to be around pH 3.58 which was also higher than the pH of the unflavoured kombucha. Hence, it can be assumed that the addition of the pineapple juice as flavouring in the kombucha had increased the overall pH of the samples. The SCOBY had also been removed on the same day to allow for secondary fermentation to occur. Hence, the final pH of the flavoured agarwood and green tea kombucha did not show significant difference ($p \geq 0.05$).

5.1.2 Total Soluble Solids (TSS) of Agarwood Tea and Green Tea Kombucha

The total soluble solids determine how much sugar is contained in a sample during analysis. The soluble solids that can be found is mainly comprised of sugar but can also include soluble proteins and amino acids (Kusumiyati et al., 2020). In the brewing of kombucha, white sugar is commonly used as the carbon source as it can provide glucose and fructose for microbial metabolic pathways, where an optimal concentration ranges between 5 – 15% w/v (Kim and Adhikari, 2020; Nyhan et al., 2022). The type of sugar used can affect the properties of kombucha according to a study by Muhialdin et al. (2019), in which they used white refined sugar, coconut palm sugar and molasses sugar in different kombucha brews. It was discovered that the white refined sugar resulted in a higher sucrose content, thus having a higher content of total soluble solids.

For this experiment, the amount of white sugar used was 21 g per 300 mL of water (7% w/v) which was within the abovementioned optimal concentration. From the results in Figure 4.2.1, it can be found that there was a decreasing trend of a typical kombucha fermentation for the Brix values in both kombuchas. There was also a significant high negative correlation ($p < 0.001$) between the Brix values of the kombucha and the fermentation days based on the results in Table 4.2.1 and Table 4.2.2, where an increase in the number of fermentation days resulted in a decrease in the TSS in the kombucha. This is explained by the fact that the microbes have been utilizing the sugars during fermentation, thus reducing the amount of TSS in the kombucha. In particular, the reduction in Brix values is due to the sucrose in the tea being degraded by invertase that is produced by yeasts (Emiljanowicz and Malinowska-Pańczyk, 2019; Gramza-Michałowska et al., 2016).

The Brix value in this study for the agarwood tea and green tea kombucha had decreased from 7.80°Bx on Day 0 after inoculation to a range of 6.7°Bx – 6.97°Bx by Day 7. The Brix values between both kombuchas were not significantly different ($p \geq 0.05$) from each other as the amount of sugar added into the tea at the beginning of fermentation was the same, being 21 g sugar per 300 mL tea. Therefore, it was expected that the TSS would be similar throughout fermentation. In a study by Kayisoglu and Coskun (2020), the Brix value of their green tea kombucha was found to have experienced a similarly decreasing trend from 7.4°Bx to 6.75°Bx by the end of a 14-day fermentation period. In their study, 70 g of sucrose was added to 1 L of water which equates to the same amount of sugar used in this experiment. Since the final Brix value in their study falls between the range in this study, it can be said that the results agree with each other and a decrease in total soluble solids was part of the fermentation process. However, there was no studies reporting the change in Brix values for agarwood tea kombucha that can be compared with the results in this study. Despite that, it can be presumed that there will be a similarly observed decreasing trend for the agarwood tea kombucha as with other kombucha fermentation results. At the same time, it can also be noted that the TSS in this study on Day 10 were higher than those on Day 7. For both kombuchas in the experiment, pineapple juice and mint leaves were added on Day 7 for secondary fermentation until Day 10. This had led to an increase in the Brix values of the kombucha as the pineapple juice itself had a Brix value of 11°Bx. During secondary fermentation, more sugar is usually added into the kombucha to make it more carbonated (Kombucha To The People, 2022d). If more sugar is added to the kombucha, it will result in a higher amount of total soluble solids. In this case, fruit juice was added to replace sugar because not only can it be a source of carbon, but it also provides nutrients such as vitamins that can enhance the health properties of the kombucha. Once the juice was added, it acted as an additional carbon source for the microbes in the kombucha during secondary fermentation (Muzaifa et al., 2021). The microbial activity from the reinvigorated microbes thus caused the Brix values to further decrease from Day 7 to Day 10 for the agarwood tea kombucha (7.43°Bx to 6.73°Bx) and green tea kombucha (7.67°Bx to 7.03°Bx).

Unlike the pH values, the total soluble solids did not have a drastic change in values between Days 0 and 10 with the overall difference ranging between 1.0

– 1.40°Bx. This may be because up to 34.06% of sugar in kombucha usually stays unfermented even after 7 days of fermentation based on findings from several other studies (Jayabalan et al., 2014). Additionally, the TSS in kombucha can also be affected by other compounds such as proteins, pectin, pigments, and minerals (Zubaidah, Ifadah and Afgani, 2019). This includes any suspensions in the beverage samples, which was surmised to be part of the SCOBY. Due to the presence of these suspensions, it can also explain why the Brix values did not undergo a drastic decrease in values as fermentation progressed due to the cellulosic formation of the daughter SCOBY.

5.1.3 Total Titratable Acidity (TTA) of Agarwood Tea and Green Tea Kombucha

The total titratable acidity measures the total acid concentration of the kombucha and is often used to predict how the overall flavour of the product is affected (Tyl and Sadler, 2017). The TTA values followed an increasing trend of a typical kombucha fermentation, where it was significantly different ($p < 0.05$) between the infused agarwood tea and green tea kombucha by Day 10 of fermentation. The increase in TTA during fermentation was due to the release of organic acids during microbial activity by the SCOBY in the kombucha (Bishop et al., 2022; Kayisoglu and Coskun, 2020). The TTA is mainly affected by the type of tea leaves used due to different amounts of polyphenols and catechins that can impact the SCOBY activity to produce acids (Primiani, Mumtahanah and Ardhi, 2018). Liu et al. (2013) analysed the organic acid composition of tea leaves and found that they included citric, L-ascorbic, malic, oxalic, quinic, and tartaric acids at varying concentrations. Hence, a higher amount of tea leaves used will also result in a higher TTA as the concentration of organic acids has also increased. Osiripun and Apisittiwong (2021) recorded that the total acidity of kombuchas brewed with different tea bases (black tea, green tea, oolong tea) and infused with different fruit juices (apple, pomegranate, pineapple) were significantly different ($p < 0.05$) from each other after 10-day fermentation. Hence, their results are in accordance with the ones obtained in this study. In this case, the difference in TTA between the agarwood tea and green tea kombucha was due to the green tea's higher glucuronic acid concentration (Cardoso et al., 2020).

The Pearson correlation coefficients from Table 4.3.1 and Table 4.3.2 had also shown that there was a significantly high positive correlation ($p < 0.001$) between the TTA and the days of fermentation, where an increase in the fermentation days led to an increase in TTA. Based on the findings by Cardoso et al. (2020), the TTA of their green tea kombucha was around 4.32 g/L by the end of 10-day fermentation which comprised of acetic, glucuronic, and lactic acids. This value was lower than the obtained results of the agarwood tea kombucha (6.15 g/L) and green tea kombucha (7.40 g/L) in this study due to the following reasons. Firstly, the initial concentration of their kombucha was at 12 g/L and it was only infused for 2 minutes at a temperature of 75°C. A shorter infusion time prevents the compounds from the tea leaves to be fully extracted, causing a lower concentration of organic acids in the kombucha. This is supported by the results from a study by Sharif et al. (2013) in which the infusion time for tea leaves was directly proportional to caffeine concentration. In this experiment, the initial concentration of the kombucha was at 14 g/L and it was infused for a longer duration of 10 minutes. Thus, the kombucha in this experiment was expected to have a higher amount of TTA due to the higher tea concentration as well as infusion time. Secondly, the kombucha in this experiment was flavoured with pineapple juice, whereas the kombucha in the compared study was raw. Pineapple is known for its acidic content even when fully ripe, with the highest produced acid being citric acid by the end of ripening (Paull, Uruu and Chen, 2020). It is said that the TTA of pineapple can go up to 1.23% depending on genotype, with MD2 variety having a titratable acidity of 0.53% citric acid (Lu et al., 2014). As a result, this acid contributes to the total acidity of the final kombucha product.

5.2 Antioxidant Properties of Agarwood Tea and Green Tea Kombucha

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the free radical scavenging activity of the agarwood tea and green tea kombucha. The assay relies on free electron transfer between the antioxidant and the radical, with the limitation that it cannot determine hydrophilic antioxidant activity (Jakubczyk et al., 2020). Metabolites responsible for the antioxidant properties of kombucha include tea polyphenols, organic acids, D-Saccharic acid 1,4-lactone (DSL), and vitamins where the enzymes during fermentation can alter the structure of the polyphenols to increase antioxidant activity (Jayabalan et al., 2014).

The results in Table 4.4 showed a significant difference ($p < 0.05$) in DPPH inhibition values between the agarwood tea and green tea kombucha from Days 0 – 7, where green tea kombucha had significantly higher ($p < 0.05$) DPPH inhibition percentages than agarwood tea kombucha. This was because green tea had a higher amount of free radical scavenging catechins which include epigalocatechin gallate (EGCG), epicatechin (EC), epigalocatechin (EGC) and epicatechin gallate (ECG) (Jówko, 2015). On the other hand, it is said that agarwood tea shares a similar amount of ECG and EGCG as black tea (Leksono and Murtini, 2021; Tay et al., 2014). A higher catechin content is related to higher antioxidant activity, further supporting the results that green tea naturally has a higher antioxidant activity than agarwood tea.

Besides that, the DPPH inhibition activity was found to have fluctuated throughout the fermentation period, with the highest value observed on Day 3 and the lowest on Day 7 for both kombuchas. The inhibition percentage had increased by 2.09% for the agarwood tea kombucha (83.59%), while it decreased by 0.12% for the green tea kombucha (85.16%) on Day 10 of fermentation. The results from an experiment by Jakubczyk et al. (2020) showed that the highest inhibition values were also recorded before the 7^{th} day of fermentation and had even observed that there was a negative correlation between the antioxidant activity of kombucha along with the fermentation time. Their highest recorded inhibition value of green tea kombucha was on Day 1 at 94.61%, which then decreased to 91.40% by Day 7. Results from an experiment by Hsieh, Chiu and Chou (2021) showed that the effect of fermentation on the antioxidative properties of green tea kombucha in terms of increase in relative DPPH scavenging activity was comparatively lower than the those of black tea kombucha. Hence, it supports the obtained results in this study in which the fermentation of green tea kombucha had shown a lower increase in overall DPPH scavenging activity compared to that of agarwood tea kombucha. One possible explanation behind this result is the degradation of catechins, flavonoids, and caffeine in the kombucha as fermentation progressed (Jakubczyk et al., 2020; Zhao et al., 2018). Because green tea essentially contains more catechins than agarwood tea, it will thus experience a lower increase in scavenging activity due to the higher degradation of catechins during kombucha fermentation. Another possible factor that may have affected the fluctuating results was the fact that DPPH assay is sensitive to low pH, where a higher acidity of the beverage leads to a decrease in antioxidant activity (Kalinowska et al., 2020). This also explains why the inhibition value had increased by Day 10 of fermentation, as the addition of the pineapple juice on Day 7 had momentarily increased the pH of the kombucha thus increasing its antioxidant activity at the same time.

The IC50 values were also determined from the DPPH inhibition assay, which indicated the concentration of sample that is required to inhibit the radical by half. From the results in Figures 4.4, the agarwood tea kombucha recorded values of 9.91 µg/mL on Day 0 and 6.02 µg/mL on Day 7. Meanwhile, the IC50 values of the green tea kombucha in Figure 4.5 were lower with concentrations of 0.10 µg/mL and 0.0034 µg/mL on Days 0 and 7 respectively. The decline in values indicate that the fermentation process had increased the antioxidant activity, since the kombucha by Day 7 required a lower concentration to inhibit the DPPH radical. Moreover, the results suggested that green tea kombucha exhibited higher antioxidant properties than agarwood tea kombucha because of its lower IC50 concentration. There are currently limited studies to compare with the obtained results for the agarwood tea kombucha, so the IC50 values of agarwood extract was used instead for comparison. The results recorded by Halim et al. (2021) showed a value of 37.22 µg/mL based on ethanol extraction of agarwood leaves. With this data, it can be said that the agarwood tea kombucha exhibited higher antioxidant properties as it required a lower amount to inhibit 50% of the radical compared to pure agarwood leaf extracts. As for the IC50 values for green tea methanol extract, the values ranged between 20 – 30 µg/mL (Yadav et al., 2020). This range was lower than the value for the agarwood leaves extract, further proving that green tea itself is a stronger antioxidant than agarwood tea.

Besides the DPPH assay, the Folin-Ciocalteu (FC) assay was also conducted to determine the total phenolic content (TPC) of the agarwood tea and green tea kombucha. TPC values were measured based on the electron transfer from the phenolic compounds to the Folin-Ciocalteu reagent, therefore exhibiting antioxidant activity (Zhou et al., 2022). Phenolic content includes the compounds found in the tea as well as those contributed by microbes from the SCOBY that produce additional free phenolics using the available phenolic compounds in the tea (Kayisoglu and Coskun, 2020).

Based on the results in Table 4.5, the total phenolic content followed an increasing trend throughout the 10 days of fermentation, indicating that there was increasing antioxidant activity in the kombucha. There was a significant difference ($p < 0.05$) in TPC between agarwood tea and green tea kombucha, with green tea kombucha having a significantly higher TPC. For the agarwood tea kombucha, the TPC increased to a final value of 337.81 mg GAE/L after 10-day fermentation. Meanwhile, the TPC for green tea kombucha had increased to a final value of 1344.53 mg GAE/L. Zhou et al. (2022) found that the TPC of their green tea kombucha had also experienced an increasing trend, with a final value of around 1000 mg GAE/L by Day 9 of fermentation. This value was lower than the one in this study due to their use of lesser tea leaves along with a shorter infusion time. To further note, their study included only raw kombucha while this study included flavoured kombucha. Therefore, it was expected that the TPC value of the kombucha in this study would be higher due to the additional phenolic contribution from pineapple juice and mint leaves. To further support the results, the TPC of green tea kombucha in other experiments had increased to a final value ranging between 700 – 1200 mg GAE/L after 8 – 10 days of fermentation (Cardoso et al., 2020; Hsieh, Chiu and Chou, 2021). The concentration of the kombucha in these studies were lower (10 – 12 g/L) than the one in this study (14 g/L), along with the fact that the kombuchas were raw with no additional phenolic compound contribution from any flavouring ingredients. Thus, it can be deduced that these studies and this experiment shared similar results that the TPC of green tea kombucha should increase during fermentation. Meanwhile, the agarwood tea kombucha from this experiment recorded a value of 142.42 mg GAE/L on Day 0 and

337.81 mg GAE/L by Day 10. Currently, there are limited studies on the total phenolic content of agarwood tea kombucha. TPC values for agarwood tea and agarwood tea kombucha reported by Nurmiati and Wijayanti (2018) were 28.52 mg GAE/g and 62.86 mgGAE/g respectively. In the meantime, 60% ethanol extraction of *Aquilaria crassna* recorded a TPC of 260 mg/g (Tay et al., 2014). More findings are therefore needed to determine the TPC range of agarwood tea kombucha.

The TPC values of agarwood kombucha were significantly lower ($p < 0.05$) than that of green tea because it belongs to a different genus (*Aquilaria*) unlike green tea (*Camellia sinensis*). Antolak, Piechota and Kucharska (2021) stated that it is currently unclear as to how the type of tea leaves used in brewing kombucha can affect the fermentation by certain strains of SCOBY. For this case, the antioxidant properties between agarwood tea and green tea differ for various reasons. The first reason is that the phytochemicals found in each type of tea leaf differed. Batubara et al. (2021) performed a phytochemical screening on agarwood leaves hot water extract and found that they contained compounds such as flavonoids, tannins, triterpenoids, steroids, and glycosides. At the same time, alkaloids were absent in the sample. On the other hand, a phytochemical screening of green tea showed that it contained tannins, saponins, steroids, flavonoids, and alkaloids (Anita et al., 2014). Hence, agarwood tea leaves naturally contain less phytochemicals compared to green tea leaves. Moreover, it can be noted that the agarwood tea bag used in this experiment contained a mixture of *Aquilaria* leaf and bark instead of pure tea leaves. One 2.0 g tea bag contained 0.4 g leaf and 1.6 g bark of *Aquilaria* *malaccensis*. Meanwhile, the green tea bag used in the experiment was comprised of 100% green tea leaves. The amount of pure agarwood tea leaves used was therefore lesser than that of the green tea leaves, which may have also led to the obtained results. The age of the tea leaves used for brewing can also impact the antioxidant properties with supporting results from Hendra, Moeljopawiro and Nuringtyas (2016). It was found that young agarwood leaves had a lower antioxidant activity than old agarwood leaves due to the presence of more secondary metabolites in the latter. The age of the tea leaves used in this experiment cannot be determined, thus it was unclear as to how the antioxidant activity had been influenced. However, Adam, Lee and Mohamed (2017) mentioned that agarwood tea leaves prepared in China involved choosing young shoot, first and second leaflets. In addition, the leaves undergo fermentation and are roasted several times before packaging. With reference to this method, it can be assumed that the agarwood tea leaves used in this study were fermented young leaves which naturally have a lower antioxidant activity. Green tea leaves on the other hand, do not undergo fermentation so they are less processed and therefore possess higher antioxidant concentration. Additionally, agarwood tea has a lower catechin content compared to green tea. This was because the catechin concentration in fermented teas were found to be lower than that of green tea (Horie et al., 2017).

The antioxidant properties of kombucha can further be explained by the nature of the beverage. Release of peptides by the yeast *S. cerevisiae* during autolysis was found to increase antioxidant activity under the influence of an acidic environment (Ibrahim et al., 2019). Even though the DPPH inhibition values did not increase along with fermentation time, the total phenolic content did. It has been inferred that the phenolic compounds in the kombucha may have been degraded into phenolic compounds of lower molecular weight due to the acidic environment created during fermentation, therefore increasing the total phenolic content of the beverage (Srihari and Satyanarayana, 2012; La Torre et al., 2021).

5.3 Sensory Evaluation Between Agarwood Tea, Green Tea, and Commercial Kombucha

Sensory evaluation of the agarwood tea, infused agarwood tea, green tea, infused green tea, and commercial kombuchas was performed to determine which product was generally accepted or preferred by consumers. The parameters assessed included appearance, aroma, taste, and overall acceptability. The results in Table 4.6 showed that the 5 kombucha samples did not significantly differ ($p \geq 0.05$) in terms of aroma, taste, and overall acceptability. With additional results from Figure 4.6, it can be deduced that the agarwood tea kombucha infused with pineapple and mint was the most preferred product, followed by the green tea, agarwood tea, infused green tea, and commercial kombuchas. The infused agarwood tea kombucha also had the lowest dislikes by panelists compared to other products. Based on the 9-point hedonic scale, the ratings for all kombuchas ranged between scores 5 and 6, indicating that the beverage was generally accepted by panelists.

When assessing a product, appearance is one of the main factors in consumers' choices. It gives a first impression of the product before people are willing to take the next step to try it. Aspects of appearance can include colour, turbidity, and carbonation. Colour is known to influence consumers into the product's flavour intensity, where a darker colour is often attributed to a stronger flavour. The colour of kombucha is usually described to be brown because of the extracted polyphenols during the brewing of tea leaves (Bishop and Pitts, 2022; Tran et al., 2020). The colour of the agarwood tea kombucha was a dark yellow, but the infused agarwood tea kombucha was a slightly lighter yellow, due to the colour from the pineapple juice. The green tea kombucha and infused green tea kombucha also had a similar colour as the agarwood tea kombucha counterparts. On the other hand, the commercial kombucha had a light brown colour. Additionally, kombucha can either appear clear or turbid depending on if it has been filtered or not. The turbidity is caused by the suspended microbes and tea compounds. All kombuchas in this study were clear as they had been filtered beforehand to remove any present SCOBY strains that can influence panelist perception. It was discovered that the appearance ratings for the agarwood tea and green tea kombucha were higher than the flavoured and commercial kombucha. Thus, it can only be presumed that the addition of pineapple juice and mint to the kombucha had made the appearance less appealing.

Aroma and taste are closely related to each other when it comes to the flavour perception of a food product. The combination of these two can affect the intensity and overall quality of the food's flavour (Yin et al., 2017). Kombucha has a distinctive sour smell, akin to that of apple cider vinegar. Meanwhile, its taste can vary depending on ingredients used, but it is generally tart with a tinge of sweetness. Aroma and taste preferences can vary amongst individuals, so ratings will also vary. In this study, the agarwood tea and green tea kombucha infused with pineapple and mint had a detectable aroma of pineapple, which could have possibly influenced the ratings. This can be seen in the results, where the flavoured agarwood tea and green tea kombucha were scored higher than the other samples in terms of aroma. Agarwood kombucha scored higher than green tea kombucha for aroma as the agarwood tea originally had a sweeter aroma, while green tea had a more earthy aroma.

It was expected that the flavouring ingredients added may have also enhanced the taste of the product and led to a kombucha with higher scores. However, that was not the case as it was found that commercial kombucha was the highest rated, being only slightly higher than the agarwood tea kombucha infused with pineapple and mint. On the other hand, the flavoured green tea kombucha was scored second lowest for taste. The commercial kombucha was brewed with cane sugar and green tea, with no additional flavouring. Furthermore, carbonation was mentioned earlier to have an influence on trigeminal perception. The fizzy mouthfeel may have also contributed to overall enjoyability of the kombucha flavour for panelists. An optimum fermentation period should also be established to ensure that the kombucha product is not too sour for consumption. Though it was not known how long the commercial kombucha had undergone fermentation, it can be assumed that it followed the standard 7 days of primary fermentation. Several panelists' comments mentioned that the agarwood tea and green tea kombucha infused with pineapple and mint would cause choking upon consumption, most likely due to its high acidity from prolonged fermentation. The cooling effect provided by the mint leaves may have also contributed to this issue. As a result, this could have caused a lower rating for taste as it can be hard to consume in large amounts.

In terms of overall acceptability, the agarwood tea kombucha infused with pineapple and mint scored the highest, while the raw agarwood tea kombucha was the lowest rated. The other 3 samples were similarly rated. Thus, it can be deduced that the flavoured agarwood tea kombucha was the most accepted by panelists due its appealing appearance, aroma, and taste. It also showed that the flavoured agarwood tea kombucha was the most preferred, even surpassing the commercial kombucha.

5.4 Limitations and Recommendations

During the course of this study, there were certain limitations that hindered the progress of the project. Firstly, time constraints led to a lack of testing on the nutritional content of the kombucha which is usually what consumers look for when deciding to purchase a product. The experiment had also mostly involved antioxidant tests with no tests for understanding how the developed flavoured agarwood tea kombucha will perform during storage. Secondly, the number of respondents during the sensory evaluation could be increased to 50 instead of only 30. For the hedonic scale tests, around 100 responses are often needed to ensure that results are accurate. However, for small projects such as this study, around 50 responses will suffice. Unfortunately, only 30 responses were collected in the end due to limited manpower as well as the lack of respondents on campus.

With these taken into consideration, several recommendations can be implemented to improve future studies. For instance, shelf-life testing can be performed to determine how long it can last before it is deemed no longer safe for consumption. Alcohol testing can also be carried out to determine if the developed kombucha meets regulatory standards, and microbial count for the number and types of beneficial microbes in the kombucha can be determined. Secondly, the sensory evaluation can be performed earlier in the semester when there are many students on campus to recruit. Finally, a survey could also be conducted to better understand consumers' preferences and knowledge about kombucha. The information obtained can then be used to determine what flavours consumers prefer and whether they like or dislike consuming kombucha may have impacted the sensory evaluation.

CHAPTER 6

CONCLUSION

In conclusion, the development of kombucha beverage derived from *Aquilaria malaccensis* tea infused with pineapple and mint was performed to explore the potential of using locally available plants as the tea base for the brewing of kombucha. The objectives of this study had been accomplished, where the overall physicochemical, antioxidant properties, and consumer acceptance between infused agarwood tea kombucha was determined and compared with green tea kombucha. From the results, the infused agarwood tea and green tea kombucha did not differ significantly ($p \geq 0.05$) in terms of pH, total soluble solids, and DPPH inhibition values by the end of 10-day fermentation. This had shown that the agarwood tea kombucha has the same potential as green tea kombucha for scavenging free radicals. However, the infused green tea kombucha had a significantly higher ($p < 0.05$) total titratable acidity and total phenolic content than infused agarwood tea kombucha by the end of fermentation. This result was attributed to the green tea's naturally high phenolic content and antioxidant properties compared to agarwood tea. At the same time, a lower concentration of pure agarwood tea leaves was used compared to green tea leaves during the experiment, which further explained the difference in results. Meanwhile, sensory evaluation results had shown that most panelists preferred the infused agarwood tea kombucha over the green tea counterpart upon consumption. Hence, this study paves the way for the use of

Aquilaria leaves in the development of kombucha based on its antioxidant properties as well as sensory qualities.

REFERENCES

Adam, A. Z., Lee, S. Y. and Mohamed, R., 2017. Pharmacological properties of agarwood tea derived from *Aquilaria* (Thymelaeaceae) leaves: An emerging contemporary herbal drink. *Journal of Herbal Medicine*, [e-journal] 10, pp.37-44. https://doi.org/10.1016/j.hermed.2017.06.002

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

Alderson, H., Liu, C., Mehta, A., Gala, H.S., Mazive, N.R., Chen, Y., Zhang, Y., Wang, S. and Serventi, L., 2021. Sensory profile of kombucha brewed with New Zealand ingredients by focus group and word clouds. *Fermentation*, [e-journal] 7(3), pp.100 https://doi.org/10.3390/fermentation7030100.

Amarasinghe, H., Weerakkody, N. S. and Waisundara, V. Y., 2018. Evaluation of physicochemical properties and antioxidant activities of kombucha "Tea Fungus" during extended periods of fermentation. *Food Science & Nutrition*, [e-journal] 6(3), pp.659-665. https://doi.org/10.1002/fsn3.605

Anita, P., Balan, In., Ethiraj, S., Madan Kumar, P. and Sivasamy, S., 2015. In vitro antibacterial activity of Camellia sinensis extract against cariogenic microorganisms. *Journal of Basic and Clinical Pharmacy*, [e-journal] 6(1), pp.35. https://doi.org/10.4103/0976-0105.145777.

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*, [e-journal] 10(10), pp.1541. https://doi.org/10.3390/antiox10101541

AQINA Fruits, 2015. *Colour range of MD2 pineapple*. [online] Available at: <https://md2malaysia.com/2015/01/16/color-range/> [Accessed 22 September 2022].

Arslan, D., Özcan, M.M. and Mengeş, H.O., 2010. Evaluation of drying methods with respect to drying parameters, some nutritional and colour characteristics of peppermint (*Mentha* x *piperita* L.). *Energy Conversion and Management*, [e-journal] 51(12), pp.2769-2775. https://doi.org/10.1016/J.ENCONMAN.2010.06.013.

Batubara, R., Wirjosentono, B., Siregar, A. H., Harahap, U. and Tamrin, 2021. *Chemical compounds and antioxidant potential in hot water extract of cultivated agarwood (Aquilaria malaccensis) Lamk leaves*. In: IOP Science, International conference on science, infrastructure technology and regional development. South Lampung, Indonesia, 23-25 October 2020. Bristol: IOP Publishing Ltd.

Bishop, P., Pitts, E.R., Budner, D. and Thompson-Witrick, K.A., 2022. Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chemistry Advances*, [e-journal] 1, pp.100025. https://doi.org/10.1016/J.FOCHA.2022.100025.

Cacicedo, M.L., Castro, M.C., Servetas, I., Bosnea, L., Boura, K., Tsafrakidou, P., Dima, A., Terpou, A., Koutinas, A. and Castro, G.R., 2016. Progress in bacterial cellulose matrices for biotechnological applications. *Bioresource Technology*, [e-journal] 213, pp.172-180. https://doi.org/10.1016/J.BIORTECH.2016.02.071.

Cardoso, R.R., Neto, R.O., dos Santos D'Almeida, C.T., do Nascimento, T.P., Pressete, C.G., Azevedo, L., Martino, H.S.D., Cameron, L.C., Ferreira, M.S.L. and Barros, F.A.R., 2020. Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International*, [e-journal] 128, pp.108782. https://doi.org/10.1016/J.FOODRES.2019.108782.

Deghrigue, M., Chriaa, J., Battikh, H., Abid, K. and Bakhrouf, A., 2013. Antiproliferative and antimicrobial activities of kombucha tea. *African Journal of Microbiology Research*, [e-journal] 7(27), pp.3466–3470. https://doi.org/10.5897/AJMR12.1230

Ding, P. and Syazwani, S., 2016. Physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages. *International Food Research Journal*, 23(2), pp.549-555.

Domingo, C.J.A., De Vera, W.M., Pambid, R.C. and Austria, V.C., 2019. Playing with the senses: application of box-behnken design to optimize the *bukayo* formulation. *Food Research*, [e-journal] 3(6), pp.833-839. https://doi.org/10.26656/fr.2017.3(6).190.

Dufresne, C. and Farnworth, E., 2000. Tea, Kombucha, and health: a review. *Food Research International*, 33, pp.409-421.

Emiljanowicz, K.E. and Malinowska-Pańczyk, E., 2019. Kombucha from alternative raw materials – The review. *Critical Reviews in Food Science and Nutrition*, [e-journal] 60(19), pp.3185-3194 https://doi.org/10.1080/10408398.2019.1679714

Ferreira, E.A., Siqueira, H.E., Boas, E.V.V., Hermes, V.S. and Rios, A. de O., 2016. Bioactive compounds and antioxidant activity of pineapple fruit of different cultivars. *Revista Brasileira de Fruticultura*, [e-journal] 38(3). https://doi.org/10.1590/0100-29452016146.

Gaggìa, F., Baffoni, L., Galiano, M., Nielsen, D. S., Jakobsen, R. R., Castro-Mejía, J. L., Bosi, S., Truzzi, F., Musumeci, F., Dinelli, G. and Di Gioia, D., 2019. Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients*, [e-journal] 11(1). https://doi.org/10.3390/nu11010001

Goh, W.N., Rosma, A., Kaur, B., Fazilah, A., Karim, A.A. and Bhat, R., 2012. Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, 19(1), pp.109–117.

Gramza-Michalowska, A., Kulczynski, B., Xindi, Y. and Gumienna, M., 2016. Research on the effect of culture time on the kombucha tea beverage's antiradical capacity and sensory value. *Acta Scientiarum Polonorum, Technologia Alimentaria*, [e-journal] 15(4), pp.447–457. https://doi.org/10.17306/J.AFS.2016.4.43

Greenwalt, C.J., Steinkraus, K.H. and Ledford, R.A., 2000. Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects. *Journal of Food Protection*, 63(7), pp.976–981.

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

Hashim, Y. Z. H. Y., Kerr, P. G., Abbas, P. and Mohd Salleh, H., 2016. *Aquilaria* spp. (agarwood) as source of health beneficial compounds: A review of traditional use, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, [e-journal] 189, pp.331–360. https://doi.org/10.1016/J.JEP.2016.06.055

Hendra, H., Moeljopawiro, S. and Nuringtyas, T.R., 2016. Antioxidant and antibacterial activities of agarwood (*Aquilaria malaccensis* Lamk.) leaves. *AIP Conference Proceedings*, [e-journal] 1755. https://doi.org/10.1063/1.4958565.

Horie, M., Nara, K., Sugino, S., Umeno, A. and Yoshida, Y., 2016. Comparison of antioxidant activities among four kinds of Japanese traditional fermented tea. *Food Science & Nutrition*, [e-journal] 5(3), pp.639-645. https://doi.org/10.1002/fsn3.442

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, [e-journal] 2021. https://doi.org/10.1155/2021/1735959.

Huang, Y., Xiao, D., Burton-Freeman, B.M. and Edirisinghe, I., 2016. Chemical changes of bioactive phytochemicals during thermal processing. *Reference Module in Food Science*, 2016. https://doi.org/10.1016/B978-0-08-100596-5.03055-9.

Jacobson, J., 2006. *Introduction to wine laboratory practices and procedures*. New York: Springer.

Jakubczyk, K., Kałduńska, J., Kochman, J. and Janda, K., 2020. Chemical profile and antioxidant activity of the kombucha beverage derived from white, green, black and red tea. *Antioxidants*, [e-journal] 9(5), pp.447. https://doi.org/10.3390/antiox9050447

Jayabalan, R., Malbaša, R. v., Lončar, E. S., Vitas, J. S. and Sathishkumar, M., 2014. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, [e-journal] 13(4), pp.538–550. https://doi.org/10.1111/1541-4337.12073

Jówko, E., 2015. Green tea catechins and sport performance. In: M. Lamprecht, ed. 2015. *Antioxidants in Sport Nutrition*. Boca Raton: CRC Press. pp.123–140.

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*, [e-journal] 7(12), pp.700. https://doi.org/10.3390/microorganisms7120700

Kakino, M., Tazawa, S., Maruyama, H., Tsuruma, K., Araki, Y., Shimazawa, M. and Hara, H., 2010. *Laxative effects of agarwood on low-fiber diet-induced constipation in rats*. BMC Complementary and Alternative Medicine, [e-journal] 10(68). https://doi.org/10.1186/1472-6882-10-68.

Kapp, J.M. and Sumner, W., 2018. Kombucha: a systematic review of the empirical evidence of human health benefit. *Annals of Epidemiology*, [e-journal] 30, pp.66-70. https://doi.org/10.1016/j.annepidem.2018.11.001.

Kayisoglu, S. and Coskun, F., 2021. Determination of physical and chemical properties of kombucha teas prepared with different herbal teas. *Food Science and Technology (Campinas)*, [e-journal] 41, pp.393-397. https://doi.org/10.1590/fst.12720

Khan, N. and Mukhtar, H., 2013. Tea and health: Studies in humans. *Curr Pharm Des.*, [e-journal] 19(34), pp.6141-6147. https://doi.org/10.2174%2F1381612811319340008

Kidane, S., 2021. Application of response surface methodology in food process modeling and optimization. In: P. Kayaroga, ed. 2021. *Response Surface Methodology in Engineering Science*. London: IntechOpen.

Kim, J. and Adhikari, K., 2020. Current trends in kombucha: Marketing perspectives and the need for improved sensory research. *Beverages*, [e-journal] 66(1), pp.15. https://doi.org/10.3390/beverages6010015.

Kombucha To The People, 2022d. *Secondary Fermentation FAQ - KOMBUCHA TO THE PEOPLE*. [online] Available at: <https://www.kombuchatothepeople.com/secondary-fermentation-faq.html> [Accessed 25 August 2022].

Kulkarni, E., 2022a. *All you need to know about Jun, a probiotic alternative to kombucha*. [online] Available at: <https://www.lifestyleasia.com/bk/food-drink/drinks/jun-the-probiotic-alternative-to-kombucha/> [Accessed 25 August 2022].

Kusumiyati, Hadiwijaya, Y., Putri, I.E., Mubarok, S. and Hamdani, J.S., 2020. *Rapid and non-destructive prediction of total soluble solids of guava fruits at various storage periods using handheld near-infrared instrument*. In: IOP Science, Second international conference on sustainable agriculture. Yogyakarta, Indonesia, 30-31 July 2019. Bristol: IOP Publishing Ltd.

Laerd Statistics, 2022c. *Pearson Product-Moment Correlation - When you should run this test, the range of values the coefficient can take and how to measure strength of association.* [online] Available at: <https://statistics.laerd.com/statistical-guides/pearson-correlation-coefficient-statistical-guide.php> [Accessed 25 August 2022].

Lamuela-Raventós, R.M., 2017. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In: R. Apak, E. Capanoglu and F. Shahidi, eds. 2018. *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications*. Hoboken: John Wiley & Sons Ltd. pp.107–115.

Lasekan, O. and Khalifa Hussein, F., 2018. Classification of different pineapple varieties grown in Malaysia based on volatile fingerprinting and sensory analysis. *Chemistry Central Journal*, [e-journal] 12(140). https://doi.org/10.1186/s13065-018-0505-3.

La Torre, C., Fazio, A., Caputo, P., Plastina, P., Caroleo, M.C., Cannataro, R. and Cione, E., 2021. Effects of long-term storage on radical scavenging properties and phenolic content of kombucha from black tea. *Molecules*, [e-journal] 26(18), pp.5474. https://doi.org/10.3390%2Fmolecules26185474.

Leksono, A. and Murtini, E.S., 2021. Effects of fermentation duration on physicochemical and organoleptic characteristics of three agarwoods

(*Aquilaria*) leaves tea. *Jurnal Pangan dan Agroindustri*, [e-journal] 9(2), pp.73–82. https://doi.org/10.21776/UB.JPA.2021.009.02.1.

Liu, Y.Y., Wei, J.H., Gao, Z.H., Zhang, Z. and Lyu, J.C., 2017. A review of quality assessment and grading for agarwood. *Chinese Herbal Medicines*, [e-journal] 9(1), pp.22-30. https://doi.org/10.1016/S1674-6384(17)60072-8.

Lorenzo, J.M. and Munekata, P.E.S., 2016. Phenolic compounds of green tea: Health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine*, [e-journal] 6(8), pp.709–719. https://doi.org/10.1016/J.APJTB.2016.06.010.

Lunkes, L.B.F. and Hashizume, L.N., 2014. Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market. *RGO - Revista Gaúcha de Odontologia*, [e-journal] 62(1), pp.59–64. https://doi.org/10.1590/1981-86372014000100000092623.

Lustri, W. R., Barud, H. G. de O., Silva Barud, H. da, Peres, M. F. S., Gutierrez, J., Tercjak, A., Junior, O. B. de O., and Ribeiro, S. J. L., 2015. Microbial cellulose – biosynthesis mechanisms and medical applications. In: M. Poletto and H. Junior, eds. 2015. *Cellulose – fundamental aspects and current trends*. London: IntechOpen

Mahmud, M., Abdullah, R. and Yaacob, J.S., 2020. Effect of vermicompost on growth, plant nutrient uptake and bioactivity of ex vitro pineapple (*Ananas comosus* var. MD2). *Agronomy*, [e-journal] 10(9), pp.1333. https://doi.org/10.3390/agronomy10091333.

MarketResearch.com, 2022b. *Malaysia: Pineapple Juice Market and the Impact of COVID-19 on It in the Medium Term*. [online] Available at: <https://www.marketresearch.com/Williams-Marshall-Strategy-v4196/Malaysia-Pineapple-Juice-Impact-COVID-13624294/> [Accessed 25 August 2022].

Marques, M.P., Alves, V.D. and Coelhoso, I.M., 2017. Concentration of tea extracts by osmotic evaporation: Optimisation of process parameters and effect on antioxidant activity. *Membranes*, [e-journal] 7(1), pp.1. https://doi.org/10.3390/membranes7010001.

Massoud, R., Jafari-Dastjerdeh, R., Naghavi, N. and Khosravi-Darani, K., 2022. All aspects of antioxidant properties of kombucha drink. *Biointerface Research in Applied Chemistry*, [e-journal] 12(3), pp.4018–4027. https://doi.org/10.33263/BRIAC123.40184027.

May, A., Narayanan, S., Alcock, J., Varsani, A., Maley, C. and Aktipis, A., 2019. Kombucha: a novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *PeerJ*, [e-journal] 7(9). https://doi.org/10.7717/PEERJ.7565.

Mohamad Said, K.A. and Mohamed Amin, M.A., 2015. Overview on the response surface methodology (RSM) in extraction processes. *Journal of Applied Science & Process Engineering*, 2(1), pp.8-17.

Mohite, B.V. and Patil, S.V., 2014. A novel biomaterial: bacterial cellulose and its new era applications. *Biotechnology and Applied Biochemistry*, [e-journal] 61(2), pp.101–110. https://doi.org/10.1002/BAB.1148.

Mojtaba Mousavi, S., Alireza Hashemi, S., Zarei, M., Gholami, A., Wei Lai, C., Hung Chiang, W., Omidifar, N., Bahrani, S. and Mazraedoost, S., 2020. Recent progress in chemical composition, production, and pharmaceutical effects of kombucha beverage: A complementary and alternative medicine. *Evidence-Based Complementary and Alternative Medicine*, [e-journal] 2020. https://doi.org/10.1155/2020/4397543.

Muhialdin, B.J., Osman, F.A., Muhamad, R., Che Wan Sapawi, C.W.N.S., Anzian, A., Voon, W.W.Y. and Meor Hussin, A.S., 2019. Effects of sugar sources and fermentation time on the properties of tea fungus (kombucha) beverage. *International Food Research Journal*, 26(2), pp.481–487.

Muzaifa, M., Rohaya, S., Nilda, C., Muzaifa, M., Rohaya, S., Nilda, C. and Harahap, K.R., 2022. Kombucha fermentation from cascara with addition of red dragon fruit (*Hylocereus polyrhizus*): Analysis of alcohol content and total soluble solid. *Advances in Biological Sciences Research*, [e-journal] 17. https://dx.doi.org/10.2991/absr.k.220102.020

Nurmiati, E., and Dyah, W. (2018). Perbandingan kadar fenolik total antara seduhan daun gaharu dan kombucha daun gaharu (*Aquailaria malaccensis*). *Journal Cis-Trans (JC-T)*, [e-journal] 2(1). http://dx.doi.org/10.17977/um026v2i12018p006

Nyhan, L. M., Lynch, K. M., Sahin, A. W. and Arendt, E. K., 2022. Advances in kombucha tea fermentation: A review. *Applied Microbiology*, [e-journal] 2(1), pp.73–101. https://doi.org/10.3390/applmicrobiol2010005

Osiripun, V. and Apisittiwong, T., 2021. Polyphenol and antioxidant activities of kombucha fermented from different teas and fruit juices. *Journal of Current Science and Technology*, [e-journal] 11(2), pp.188–196. https://doi.org/10.14456/jcst.2021.20

Pakravan, N., Mahmoudi, E., Hashemi, S.A., Kamali, J., Hajiaghayi, R., Rahimzadeh, M. and Mahmoodi, V., 2018. Cosmeceutical effect of ethyl acetate fraction of Kombucha tea by intradermal administration in the skin of aged mice. *Journal of Cosmetic Dermatology*, [e-journal] 17(6), pp.1216–1224. https://doi.org/10.1111/JOCD.12453.

Parikh, B. and Patel, V.H., 2018. Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses. *Journal of Food Science* *and Technology*, [e-journal] 55, pp.1499–1507. https://doi.org/10.1007/s13197-018-3066-5.

Paull, R.E., Uruu, G. and Chen, N.J., 2020. Rapid Field Assay for Pineapple Fruit Acidity. *HortTechnology*, [e-journal] 30(5), pp.593–596. https://doi.org/10.21273/HORTTECH04664-20.

Peluso, I and Serafini, M., 2017. Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. *British Journal of Pharmacology*, [e-journal] 174(11), pp.1195-1208. https://doi.org/10.1111%2Fbph.13649.

Prasanth, M.I., Sundaram Sivamaruthi, B., Chaiyasut, C. and Tencomnao, T., 2019. A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients*, [e-journal] 11(2), pp.474. https://doi.org/10.3390/nu11020474.

Primiani, C. N., Pujiati, Mumtahanah, M. and Ardhi, W., 2018. Kombucha fermentation test used for various types of herbal teas. *Journal of Physics: Conference Series*, [e-journal] 1025(1). https://doi.org/10.1088/1742-6596/1025/1/012073

Quiao-Won, M. E. and Teves, F. G., 2018. Characteristics of kombucha fermentation from different substrates and cytotoxicity of tea broth. *Sustainable Food Production*, [e-journal] 4, pp.11–19. https://doi.org/10.18052/www.scipress.com/sfp.4.11

Rasheed, Z., 2019. Molecular evidences of health benefits of drinking black tea. *International Journal of Health Sciences*, 13(3), pp.1-3.

Ray, G., Leelamanit, W., Sithisarn, P. and Jiratchariyakul, W., 2014. Antioxidative compounds from *Aquilaria crassna* Leaf. *Mahidol University Journal of Pharmaceutical Sciences*, 41(4), pp.54-58.

Retail in Asia, 2022. *Looking at the kombucha drink market in Malaysia*. [online] Available at: <https://retailinasia.com/in-markets/looking-at-the-kombucha-drink-market-in-malaysia/> [Accessed 19 September 2022].

Salehi, B., Stojanović-Radić, Z., Matejić, J., Sharopov, F., Antolak, H., Kręgiel, D., Sen, S., Sharifi-Rad, M., Acharya, K., Sharifi-Rad, R., Martorell, M., Sureda, A., Martins, N. and Sharifi-Rad, J., 2018. Plants of genus *Mentha*: From farm to food factory. *Plants*, [e-journal] 7(3), pp.70. https://doi.org/10.3390/PLANTS7030070.

Sharif, R., Ahmad, S.W., Anjum, H., Ramzan, N. and Malik, S.R., 2014. Effect of infusion time and temperature on decaffeination of tea using liquid-liquid extraction technique. *Journal of Food Process Engineering*, [e-journal] 37(1), pp.46–52. https://doi.org/10.1111/JFPE.12058.

Silva, K.A., Uekane, T.M., de Miranda, J.F., Ruiz, L.F., da Motta, J.C.B., Silva, C.B., Pitangui, N.S., Gonzalez, A.G.M., Fernandes, F.F. and Lima, A.R., 2021. Kombucha beverage from non-conventional edible plant infusion and green tea: Characterization, toxicity, antioxidant activities and antimicrobial properties. *Biocatalysis and Agricultural Biotechnology*, [e-journal] 34. https://doi.org/10.1016/j.bcab.2021.102032.

Srihari, T. and Satyanarayana, U., 2012. Changes in free radical scavenging activity of kombucha during fermentation. *Journal of Pharmaceutical Sciences and Research*, 4(11), pp.1978-1981.

Straumite, E., Kruma, Z. and Galoburda, R., 2015. Pigments in mint leaves and stems. *Agronomy Research*, 13(4), pp.1104–1111.

Surjanto, Batubara, R., Hanum, T.I. and Pulungan, W., 2018. *Phytochemical and antioxidant activity of gaharu leaf tea (Aquilaria malaccensis Lamk) as raw material of tea from middle Tapanuli Regency, North Sumatera Province.* In: IOP Science, International conference on agriculture, environment, and food security 2018. Medan, Indonesia, 24–25 October 2018. Bristol: IOP Publishing Ltd.

Sun, K., Song, X., Jia, R., Yin, Z., Zou, Y., Li, L., Yin, L., He, C., Liang, X., Yue, G., Cui, Q. and Yang, Y., 2018. Evaluation of analgesic and anti-inflammatory activities of water extract of *Galla Chinensis In Vivo* models. *Evidence-Based Complementary and Alternative Medicine*, [e-journal] 2018. https://doi.org/10.1155/2018/6784032.

Tafrihi, M., Imran, M., Tufail, T., Gondal, T.A., Caruso, G., Sharma, S., Sharma, R., Atanassova, M., Atanassov, L., Valere, P., Fokou, T. and Pezzani, R., 2021. The wonderful activities of the Genus *Mentha*: Not only antioxidant properties. *Molecules*, [e-journal] 26(4), pp.1118. https://doi.org/10.3390/molecules26041118.

Tan, C.S., Zainal, Z., Md Isa, N. and Ismail, I., 2019. Agarwood induction: Current developments and future perspectives. *Frontiers in Plant Science*, [e-journal] https://doi.org/10.3389/fpls.2019.00122.

Tanticharakunsiri, W., Mangmool, S., Wongsariya, K. and Ochaikul, D., 2021. Characteristics and upregulation of antioxidant enzymes of kitchen mint and oolong tea kombucha beverages. *Journal of Food Biochemistry*, [e-journal] 45(1). https://doi.org/10.1111/JFBC.13574.

Tay, P.Y., Tan, C.P., Abas, F., Yim, H.S. and Ho, C.W., 2014. Assessment of extraction parameters on antioxidant capacity, polyphenol content, epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and iriflophenone 3-C-β-glucoside of agarwood (*Aquilaria crassna*) young leaves. *Molecules*, [e-journal] 19(8), pp.12304–12319. https://doi.org/10.3390/molecules190812304.

Topolska, K., Florkiewicz, A., Filipiak-Florkiewicz, A., Gł, D., Abska, ˛ and Tchounwou, P.B., 2021. Functional food – consumer motivations and expectations. *International Journal of Environmental Research and Public Health*, [e-journal] 18(10), pp.5327. https://doi.org/10.3390/ijerph18105327.

Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H. and Tourdot-Maréchal, 2020. Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. *Comprehensive Reviews in Food Science and Food Safety*, [e-journal] 19(4), pp.2050-2070. https://doi.org/10.1111/1541-4337.12574.

Tyl, C. and Sadler, G.D., 2017. pH and Titratable Acidity. In: S. Nielson, ed. 2017. *Food Analysis*. Cham: Springer, Cham. pp.389–406.

Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P. and Taillandier, P., 2018. Understanding kombucha tea fermentation: A review. *Journal of Food Science*, [e-journal] 83(8), pp. 580–588. https://doi.org/10.1111/1750-3841.14068

Wang, S., Yu, Z., Wang, C., Wu, C., Guo, P. and Wei, J., 2018. Chemical constituents and pharmacological activity of agarwood and *Aquilaria* plants. *Molecules*, [e-journal] 23(2), pp.342. https://doi.org/10.3390/molecules23020342.

Xiao, F., Xu, T., Lu, B. and Liu, R., 2020. Guidelines for antioxidant assays for food components. *Food Frontiers*, [e-journal] 1(1), pp.60–69. https://doi.org/10.1002/FFT2.10.

Yan, Z., Zhong, Y., Duan, Y., Chen, Q. and Li, F., 2020. Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Animal Nutrition*, [e-journal] 6(2), pp.115–123. https://doi.org/10.1016/J.ANINU.2020.01.001.

Yin, W., Hewson, L., Linforth, R., Taylor, M. and Fisk, I.D., 2017. Effects of aroma and taste, independently or in combination, on appetite sensation and subsequent food intake. *Appetite*, [e-journal] 114, pp.265–274. https://doi.org/10.1016/J.APPET.2017.04.005.

Zhao, Z.J., Sui, Y.C., Wu, H.W., Zhou, C.B., Hu, X.C. and Zhang, J., 2018. Flavour chemical dynamics during fermentation of kombucha tea. *Emirates Journal of Food and Agriculture*, [e-journal] 30(9), pp.732-741. https://doi.org/10.9755/ejfa.2018.v30.i9.1794.

Zhou, D.D., Saimaiti, A., Luo, M., Huang, S.Y., Xiong, R.G., Shang, A., Gan, R.Y. and Li, H.B., 2022. Fermentation with tea residues enhances antioxidant activities and polyphenol contents in kombucha beverages. *Antioxidants*, [e-journal] 11(1), pp.155. https://doi.org/10.3390/antiox11010155.

Zhu, J.J., Tang, A.T.H., Matinlinna, J.P., Tsoi, J.K.H. and Hägg, U., 2013. Potentiometric determination of fluoride release from three types of tea leaves. *International Journal of Electrochemical Science*, 8(9), pp.11142–11150.

Ziemlewska, A., Nizioł-łukaszewska, Z., Zagórska-Dziok, M., Bujak, T., Wójciak, M. and Sowa, I., 2022. Evaluation of cosmetic and dermatological properties of kombucha-fermented berry leaf extracts considered to be by-products. *Molecules*, [e-journal] 27(7). https://doi.org/10.3390/molecules27072345.

Zubaidah, E., Ifadah, R.A. and Afgani, C.A., 2019. *Changes in chemichal characteristics of kombucha from various cultivars of snake fruit during fermentation*. In: IOP Science, Second international conference on sustainable agriculture. Yogyakarta, Indonesia, 30-31 July 2019. Bristol: IOP Publishing Ltd.

APPENDICES

Appendix A

Agarwood Kombucha Formulations Based on Box-Behnken Design

	A (g)*	B (g)	C (mL)
1	4.2	15	3.0
2	4.2	21	3.0
3	4.2	21	2.4
4	3.6	21	2.4
5	3.6	21	3.0
6	3.6	27	2.4
7	3.0	27	3.0
8	3.6	15	2.4
9	4.2	27	3.0
10	3.6	27	3.6
11	3.0	21	2.4
12	3.6	15	3.6
13	3.0	15	3.0

*A Means amount of tea leaves, B Means amount of sugar, C Means amount of liquid starter

Appendix B

Questionnaire for Hedonic Scaling Test

Panelist No.: Date:

Product: Gaharu kombucha

Instruction:

Taste these samples in the following order and check how much you like or dislike each other. Please rinse your mouth with water before tasting each sample.

---

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

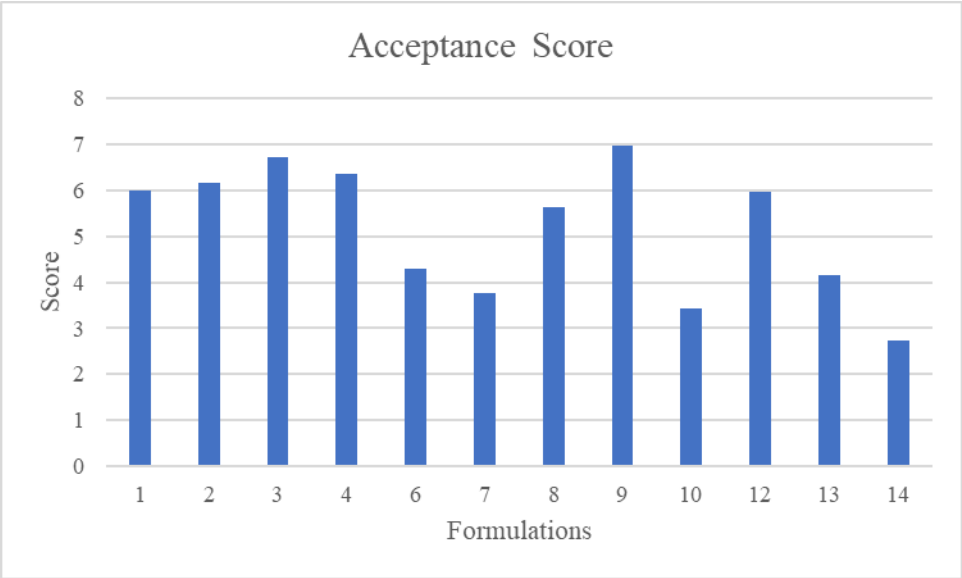
Attributes	**007**	**415**	**657**	**348**	**192**
Appearance					
Aroma					
Taste					
Overall acceptability					

Comment:

Thank you.

Appendix C

Preliminary Screening Results



Acceptance scores for agarwood tea kombucha formulations based on a 9-point hedonic scale rating system.

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 0 (B)

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood (B) Day 0	3	5.9167	.01528	.00882
	Green Tea (B) Day 0	3	5.5633	.01528	.00882

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.000	1.000	28.330	4	.000	.35333	.01247
	Equal variances not assumed			28.330	4.000	.000	.35333	.01247

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 0 (A)

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood (A) Day 0	3	4.1800	.08544	.04933
	Green Tea (A) Day 0	3	4.0400	.10583	.06110

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.343	.590	1.783	4	.149	.14000	.07853
	Equal variances not assumed			1.783	3.830	.152	.14000	.07853

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 1

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 1	3	3.9500	.07211	.04163
	Green Tea Day 1	3	3.6833	.05132	.02963

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.509	.515	5.219	4	.006	.26667	.05110
	Equal variances not assumed			5.219	3.612	.008	.26667	.05110

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 3

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 3	3	3.6433	.14364	.08293
	Green Tea Day 3	3	3.5400	.08185	.04726

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	1.729	.259	1.083	4	.340	.10333	.09545
	Equal variances not assumed			1.083	3.175	.354	.10333	.09545

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 5

Group Statistics

pH		N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 5	3	3.1900	.17776	.10263
	Green Tea Day 5	3	3.2667	.04041	.02333

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	6.157	.068	-.728	4	.507	-.07667	.10525
	Equal variances not assumed			-.728	2.206	.536	-.07667	.10525

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 7

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 7	3	3.0467	.17616	.10171
	Green Tea Day 7	3	3.2000	.04359	.02517

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	5.232	.084	-1.463	4	.217	-.15333	.10477
	Equal variances not assumed			-1.463	2.244	.268	-.15333	.10477

Appendix (Figure 4.1.1): Independent samples t test results for pH value between agarwood tea and green tea kombucha on Day 10 (P)

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 10	3	3.1567	.06110	.03528
	Green Tea Day 10	3	3.1533	.03786	.02186

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.763	.432	.080	4	.940	.00333	.04150
	Equal variances not assumed			.080	3.338	.941	.00333	.04150

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 0 (B)

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 0 (B)	3	8.1333	.45092	.26034
	Green Tea Day 0 (B)	3	8.0667	.20817	.12019

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	1.114	.351	.232	4	.828	.06667	.28674
	Equal variances not assumed			.232	2.815	.832	.06667	.28674

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 0 (A)

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 0 (A)	3	7.8000	.10000	.05774
	Green Tea Day 0 (A)	3	7.8000	.17321	.10000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	2.000	.230	.000	4	1.000	.00000	.11547
	Equal variances not assumed			.000	3.200	1.000	.00000	.11547

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 1

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 1	3	7.4667	.05774	.03333
	Green Tea Day 1	3	7.3667	.05774	.03333

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.000	1.000	2.121	4	.101	.10000	.04714
	Equal variances not assumed			2.121	4.000	.101	.10000	.04714

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 3

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 3	3	6.8667	.15275	.08819
	Green Tea Day 3	3	7.0333	.15275	.08819

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.000	1.000	-1.336	4	.252	-.16667	.12472
	Equal variances not assumed			-1.336	4.000	.252	-.16667	.12472

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 5

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 5	3	6.8333	.20817	.12019
	Green Tea Day 5	3	6.9667	.25166	.14530

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	.065	.812	-.707	4	.519	-.13333	.18856
	Equal variances not assumed			-.707	3.864	.520	-.13333	.18856

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 7

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 7	3	6.7000	.45826	.26458
	Green Tea Day 7	3	6.9667	.20817	.12019

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	1.882	.242	-.918	4	.411	-.26667	.29059
	Equal variances not assumed			-.918	2.792	.431	-.26667	.29059

Appendix (Figure 4.2.1): Independent samples t test results for Brix value between agarwood tea and green tea kombucha on Day 10 (P)

Group Statistics

	Brix	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 10	3	6.7333	.20817	.12019
	Green Tea Day 10	3	7.0333	.05774	.03333

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	5.000	.089	-2.405	4	.074	-.30000	.12472
	Equal variances not assumed			-2.405	2.306	.121	-.30000	.12472

Appendix (Figure 4.3.1): Independent samples t test results for total titratable acidity between agarwood tea and green tea kombucha on Day 1

Group Statistics

	TTA	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 1	3	1.0000	.08660	.05000
	Green Tea Day 1	3	1.3000	.31225	.18028

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	5.000	.089	-1.604	4	.184	-.30000	.18708
	Equal variances not assumed			-1.604	2.306	.233	-.30000	.18708

Appendix (Figure 4.3.1): Independent samples t test results for total titratable acidity between agarwood tea and green tea kombucha on Day 3

Group Statistics

	TTA	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 3	3	1.3000	.17321	.10000
	Green Tea Day 3	3	1.3500	.00000	.00000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	16.000	.016	-.500	4	.643	-.05000	.10000
	Equal variances not assumed			-.500	2.000	.667	-.05000	.10000

Appendix (Figure 4.3.1): Independent samples t test results for total titratable acidity between agarwood tea and green tea kombucha on Day 5

Group Statistics

	TTA	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 5	3	3.8500	1.47986	.85440
	Green Tea Day 5	3	3.1000	.34641	.20000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	8.145	.046	.855	4	.441	.75000	.87750
	Equal variances not assumed			.855	2.219	.475	.75000	.87750

Appendix (Figure 4.3.1): Independent samples t test results for total titratable acidity between agarwood tea and green tea kombucha on Day 7

Group Statistics

	TTA	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 7	3	5.1500	2.03654	1.17580
	Green Tea Day 7	3	4.4000	.91652	.52915

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	3.802	.123	.582	4	.592	.75000	1.28938
	Equal variances not assumed			.582	2.778	.605	.75000	1.28938

Appendix (Figure 4.3.1): Independent samples t test results for total titratable acidity between agarwood tea and green tea kombucha on Day 10 (P)

Group Statistics

	TTA	N	Mean	Std. Deviation	Std. Error Mean
VAR00001	Agarwood Day 10	3	6.1500	.54083	.31225
	Green Tea Day 10	3	7.4000	.17321	.10000

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
VAR00001	Equal variances assumed	3.821	.122	-3.812	4	.019	-1.25000	.32787
	Equal variances not assumed			-3.812	2.406	.046	-1.25000	.32787

Appendix (Table 4.1): One-Way ANOVA results for DPPH inhibition percentage for agarwood tea kombucha

One-Way ANOVA

Descriptive

DPPH inhibition percentage for agarwood tea kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood (B) Day 0	3	81.5033	.41621	.24030
Agarwood (A) Day 0	3	82.5333	.24194	.13968
Agarwood Day 1	3	83.7433	.56580	.32667
Agarwood Day 3	3	87.4500	.20785	.12000
Agarwood Day 5	3	79.7233	1.44500	.83427
Agarwood Day 7	3	70.5967	1.28337	.74095
Agarwood (P) Day 10	3	83.5867	.83716	.48333
Total	21	81.3052	5.06436	1.10513

ANOVA

DPPH inhibition percentage for agarwood tea kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	502.892	6	83.815	116.618	.000
Within Groups	10.062	14	.719		
Total	512.954	20			

Post Hoc Tests

Homogeneous Subsets

DPPH inhibition percentage for agarwood tea kombucha

Duncan[a]

DPPH	N	Subset for alpha = 0.05				
		1	2	3	4	5
Agarwood Day 7	3	70.5967				
Agarwood Day 5	3		79.7233			
Agarwood (B) Day 0	3			81.5033		
Agarwood (A) Day 0	3			82.5333	82.5333	
Agarwood (P) Day 10	3				83.5867	
Agarwood Day 1	3				83.7433	
Agarwood Day 3	3					87.4500
Sig.		1.000	1.000	.159	.118	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 4.1): One-Way ANOVA results for DPPH inhibition percentage for green tea kombucha

One-Way ANOVA

Descriptive

DPPH inhibition percentage for green tea kombucha

	N	Mean	Std. Deviation	Std. Error
Green Tea (B) Day 0	3	85.2767	.42147	.24333
Green Tea (A) Day 0	3	86.1733	.73555	.42467
Green Tea Day 1	3	86.4833	.42147	.24333
Green Tea Day 3	3	88.7767	.36501	.21074
Green Tea Day 5	3	86.2400	.62354	.36000
Green Tea Day 7	3	79.8433	.55537	.32064
Green Tea (P) Day 10	3	85.1567	1.44500	.83427
Total	21	85.4214	2.66562	.58169

ANOVA

DPPH inhibition percentage for green tea kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134.481	6	22.413	41.128	.000
Within Groups	7.630	14	.545		
Total	142.110	20			

Post Hoc Tests

Homogeneous Subsets

DPPH inhibition percentage for green tea kombucha

Duncan[a]

DPPH	N	Subset for alpha = 0.05 1	2	3
Green Tea Day 7	3	79.8433		
Green Tea (P) Day 10	3		85.1567	
Green Tea (B) Day 0	3		85.2767	
Green Tea (A) Day 0	3		86.1733	
Green Tea Day 5	3		86.2400	
Green Tea Day 1	3		86.4833	
Green Tea Day 3	3			88.7767
Sig.		1.000	.065	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 4.2): One-Way ANOVA results for total phenolic content (TPC) for agarwood tea kombucha

One-Way ANOVA

Descriptive

Total phenolic content for agarwood tea kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood (B) Day 0	3	142.4167	1.08960	.62908
Agarwood (A) Day 0	3	151.8600	1.80144	1.04006
Agarwood Day 1	3	187.5267	.47920	.27667
Agarwood Day 3	3	197.5267	.47920	.27667
Agarwood Day 5	3	231.9700	1.27330	.73514
Agarwood Day 7	3	265.3067	1.73339	1.00077
Agarwood Day 10	3	337.8067	1.73339	1.00077
Total	21	216.3448	65.05115	14.19532

ANOVA

Total phenolic content of agarwood tea kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	84607.985	6	14101.331	7882.704	.000
Within Groups	25.045	14	1.789		
Total	84633.029	20			

Post Hoc Tests

Homogeneous Subsets

Total phenolic content for agarwood tea kombucha

Duncan[a]

		Subset for alpha = 0.05						
TPC	N	1	2	3	4	5	6	7
Agarwood (B) Day 0	3	142.4167						
Agarwood (A) Day 0	3		151.8600					
Agarwood Day 1	3			187.5267				
Agarwood Day 3	3				197.5267			
Agarwood Day 5	3					231.9700		
Agarwood Day 7	3						265.3067	
Agarwood Day 10	3							337.8067
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 4.2): One-Way ANOVA results for total phenolic content (TPC) for green tea kombucha

One-Way ANOVA

Descriptive

Total phenolic content of green tea kombucha

	N	Mean	Std. Deviation	Std. Error
Green Tea (B) Day 0	3	833.9733	.92116	.53183
Green Tea (A) Day 0	3	946.6967	2.09872	1.21170
Green Tea Day 1	3	1019.4133	2.02073	1.16667
Green Tea Day 3	3	1108.8067	1.17411	.67787
Green Tea Day 5	3	1168.1933	2.33635	1.34889
Green Tea Day 7	3	1236.1367	1.73339	1.00077
Green Tea Day 10	3	1344.5267	1.34894	.77881
Total	21	1093.9638	165.94259	36.21164

ANOVA

Total phenolic content of green tea kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	550696.896	6	91782.816	30597.429	.000
Within Groups	41.996	14	3.000		
Total	550738.891	20			

Post Hoc Tests

Homogeneous Subsets

Total phenolic content of green tea kombucha

Duncan[a]

TPC	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
Green Tea (B) Day 0	3	833.9733						
Green Tea (A) Day 0	3		946.6967					
Green Tea Day 1	3			1019.4133				
Green Tea Day 3	3				1108.8067			
Green Tea Day 5	3					1168.1933		
Green Tea Day 7	3						1236.1367	
Green Tea Day 10	3							1344.5267
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix (Table 4.3): One-Way ANOVA results for sensory evaluation on appearance of kombucha

One-Way ANOVA

Descriptive

Sensory evaluation on appearance of kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood	30	6.7333	1.17248	.21406
Agarwood (P)	30	6.3000	1.34293	.24518
Green Tea	30	6.6000	1.22051	.22283
Green Tea (P)	30	5.9000	1.39827	.25529
Commercial	30	5.9667	1.77110	.32336
Total	150	6.3000	1.41777	.11576

*P Means infusion with pineapple and mint

ANOVA

Sensory evaluation on appearance of kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.467	4	4.117	2.109	.083
Within Groups	283.033	145	1.952		
Total	299.500	149			

Post Hoc Tests

Homogeneous Subsets

Sensory evaluation on appearance of kombucha

Duncan[a]

Appearance	N	Subset for alpha = 0.05	
		1	2
Green Tea (P)	30	5.9000	
Commercial	30	5.9667	5.9667
Agarwood (P)	30	6.3000	6.3000
Green Tea	30	6.6000	6.6000
Agarwood	30		6.7333
Sig.		.078	.053

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Appendix (Table 4.3): One-Way ANOVA results for sensory evaluation on aroma of kombucha

One-Way ANOVA

Descriptive

Sensory evaluation on aroma of kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood	30	5.9000	1.72906	.31568
Agarwood (P)	30	6.5333	1.54771	.28257
Green Tea	30	5.6667	1.66782	.30450
Green Tea (P)	30	6.3333	1.37297	.25067
Commercial	30	5.7667	1.50134	.27411
Total	150	6.0400	1.58381	.12932

ANOVA

Sensory evaluation on aroma of kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.893	4	4.223	1.716	.150
Within Groups	356.867	145	2.461		
Total	373.760	149			

Post Hoc Tests

Homogeneous Subsets

Sensory evaluation on aroma of kombucha

Duncan[a]

Aroma	N	Subset for alpha = 0.05
		1
Green Tea	30	5.6667
Commercial	30	5.7667
Agarwood	30	5.9000
Green Tea (P)	30	6.3333
Agarwood (P)	30	6.5333
Sig.		.057

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Appendix (Table 4.3): One-Way ANOVA results for sensory evaluation on taste of kombucha

One-Way ANOVA

Descriptive

Sensory evaluation on taste of kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood	30	5.2333	1.86960	.34134
Agarwood (P)	30	5.7333	1.59597	.29138
Green Tea	30	5.5667	1.95965	.35778
Green Tea (P)	30	5.2667	1.52978	.27930
Commercial	30	5.8333	2.01859	.36854
Total	150	5.5267	1.79745	.14676

ANOVA

Sensory evaluation on taste of kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.760	4	2.190	.672	.613
Within Groups	472.633	145	3.260		
Total	481.393	149			

Post Hoc Tests

Homogeneous Subsets

Sensory evaluation on taste of kombucha

Duncan[a]

Taste	N	Subset for alpha = 0.05
		1
Agarwood	30	5.2333
Green Tea (P)	30	5.2667
Green Tea	30	5.5667
Agarwood (P)	30	5.7333
Commercial	30	5.8333
Sig.		.260

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Appendix (Table 4.3): One-Way ANOVA results for sensory evaluation on overall acceptability of kombucha

One-Way ANOVA

Descriptive

Sensory evaluation on overall acceptability of kombucha

	N	Mean	Std. Deviation	Std. Error
Agarwood	30	5.6333	1.71169	.31251
Agarwood (P)	30	6.2667	1.28475	.23456
Green Tea	30	5.7667	1.73570	.31689
Green Tea (P)	30	5.8000	1.21485	.22180
Commercial	30	5.8667	1.83328	.33471
Total	150	5.8667	1.57014	.12820

ANOVA

Sensory evaluation on overall acceptability of kombucha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.867	4	1.717	.691	.600
Within Groups	360.467	145	2.486		
Total	367.333	149			

Post Hoc Tests

Homogeneous Subsets

Sensory evaluation on overall acceptability of kombucha

Duncan[a]

Acceptability	N	Subset for alpha = 0.05 1
Agarwood	30	5.6333
Green Tea	30	5.7667
Green Tea (P)	30	5.8000
Commercial	30	5.8667
Agarwood (P)	30	6.2667
Sig.		.171

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

UTAR
UNIVERSITI TUNKU ABDUL RAHMAN
Wholly owned by UTAR Education Foundation
(Co. No. 578227-M)

FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	Looi Tze San
ID Number(s)	18ADB07013
Programme / Course	Food Science (FD)
Title of Final Year Project	Development of kombucha beverage derived from *Aquilaria malaccensis* tea infused with pineapple and mint

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 10 % **Similarity by source** Internet Sources: 7 % Publications: 6 % Student Papers: - %	
Number of individual sources listed of more than 3% similarity: -	

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: Kwong Phek Jin

Date: 23/09/2022

Signature of Co-Supervisor
Name: Dr. Ali Yassoralipour

Date: 23 September 2022

ORIGINALITY REPORT

10%	7%	6%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Marieke M. ter Wee, Birgit I. Lissenberg-Witte. "A Quick Guide on How to Conduct Medical Research", Springer Nature, 2019 Publication	<1%
2	www.science.gov Internet Source	<1%
3	sigarra.up.pt Internet Source	<1%
4	www.ajfand.net Internet Source	<1%
5	Thinzar Aung, Jong-Bang Eun. "Production and characterization of a novel beverage from laver (Porphyra dentata) through fermentation with kombucha consortium", Food Chemistry, 2021 Publication	<1%
6	Ilkin Yucel Sengun, Aysegul Kirmizigul. "Probiotic potential of kombucha", Journal of Functional Foods, 2020 Publication	<1%

7	prj.co.in Internet Source	<1 %
8	Orapp.aut.ac.nz Internet Source	<1 %
9	link.springer.com Internet Source	<1 %
10	ir.jkuat.ac.ke Internet Source	<1 %
11	prr.hec.gov.pk Internet Source	<1 %
12	www.ifrj.upm.edu.my Internet Source	<1 %
13	www.jstage.jst.go.jp Internet Source	<1 %
14	www.pjbs.org Internet Source	<1 %
15	e-sciencecentral.org Internet Source	<1 %
16	repository.smuc.edu.et Internet Source	<1 %
17	worldwidescience.org Internet Source	<1 %
18	www.iobc-wprs.org Internet Source	<1 %

19	coek.info Internet Source	<1 %
20	openaccess.city.ac.uk Internet Source	<1 %
21	Acep Haryudin, Yana Yana, Efransyah Efransyah. "TEACHING SPEAKING USING COLLABORATIVE APPROACH AT SENIOR HIGH SCHOOL IN WEST BANDUNG", PROJECT (Professional Journal of English Education), 2020 Publication	<1 %
22	Nurten Abaci, Fatma Sezer Senol Deniz, Ilkay Erdogan Orhan. "Kombucha – An ancient fermented beverage with desired bioactivities: A narrowed review", Food Chemistry: X, 2022 Publication	<1 %
23	eprints.uthm.edu.my Internet Source	<1 %
24	Aimi Zafirah Adam, Shiou Yih Lee, Rozi Mohamed. "Pharmacological properties of agarwood tea derived from Aquilaria (Thymelaeaceae) leaves: An emerging contemporary herbal drink", Journal of Herbal Medicine, 2017 Publication	<1 %
25	porkinfo.osu.edu Internet Source	<1 %

26	www.iub.edu.pk Internet Source	<1%
27	Ana Karoline Nogueira Freitas, Paulo Henrique Machado de Sousa, Nédio Jair Wurlitzer. "Alternative raw materials in kombucha production", International Journal of Gastronomy and Food Science, 2022 Publication	<1%
28	etheses.whiterose.ac.uk Internet Source	<1%
29	hrcak.srce.hr Internet Source	<1%
30	psasir.upm.edu.my Internet Source	<1%
31	etd.uwc.ac.za Internet Source	<1%
32	Raquel Macedo Dantas Coelho, Aryelle Almeida, Rafael Queiroz Gurgel do Amaral, Robson Nascimento da Mota et al. "Kombucha: Review", International Journal of Gastronomy and Food Science, 2020 Publication	<1%
33	ir.unimas.my Internet Source	<1%
34	download.atlantis-press.com Internet Source	<1%

35	downloads.hindawi.com Internet Source	<1 %
36	lume.ufrgs.br Internet Source	<1 %
37	bio-protocol.org Internet Source	<1 %
38	www.diva-portal.org Internet Source	<1 %
39	Mario Coccia. "THE IMPACT OF LOCKDOWN ON PUBLIC HEALTH DURING THE FIRST WAVE OF COVID-19 PANDEMIC: LESSONS LEARNED FOR DESIGNING EFFECTIVE CONTAINMENT MEASURES TO COPE WITH SECOND WAVE", Cold Spring Harbor Laboratory, 2020 Publication	<1 %
40	core.ac.uk Internet Source	<1 %
41	www.thaiscience.info Internet Source	<1 %
42	Dita Kristanti, Woro Setiaboma, Lia ratnawati, Diang Sagita. "Robusta coffee cherry fermentation: Physicochemical and sensory evaluation of fermented cascara tea", Journal of Food Processing and Preservation, 2022 Publication	<1 %

digitalcommons.csbsju.edu

43 Internet Source <1 %

44 pdfs.semanticscholar.org
Internet Source <1 %

45 "Recent Advances in Intelligent Systems and Smart Applications", Springer Science and Business Media LLC, 2021
Publication <1 %

46 Elizabeth Varela-Santos, Araceli Ochoa-Martinez, Gipsy Tabilo-Munizaga, Juan E. Reyes et al. "Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice", Innovative Food Science & Emerging Technologies, 2012
Publication <1 %

47 Navindra P. Seeram, Susanne M. Henning, Yantao Niu, Rupo Lee, H. Samuel Scheuller, David Heber. "Catechin and Caffeine Content of Green Tea Dietary Supplements and Correlation with Antioxidant Capacity", Journal of Agricultural and Food Chemistry, 2006
Publication <1 %

48 www.biorxiv.org
Internet Source <1 %

49	Internet Source	<1%
50	www.rsu.ac.th Internet Source	<1%
51	Fan Xiao, Tao Xu, Baiyi Lu, Ruihai Liu. "Guidelines for antioxidant assays for food components", Food Frontiers, 2020 Publication	<1%
52	Shenoy K. Chandrakala, Reema Orison Lobo, Flora Olinda Dias. "Kombucha (Bio-Tea): An Elixir for Life?", Elsevier BV, 2019 Publication	<1%
53	www.buseco.monash.edu.au Internet Source	<1%
54	K Fibrianto, E Zubaidah, N A Muliandari, L Y Wahibah, S D Putri, A M Legowo, A N Al-Baarri. "Antioxidant activity optimisation of young Robusta coffee leaf kombucha by modifying fermentation time and withering pre-treatment", IOP Conference Series: Earth and Environmental Science, 2020 Publication	<1%
55	Kelly Alencar Silva, Thais Matsue Uekane, Jeniffer Ferreira de Miranda, Larissa Fernandes Ruiz et al. "Kombucha beverage from non-conventional edible plant infusion and green tea: characterization, toxicity,	<1%

antioxidant activities and antimicrobial properties", Biocatalysis and Agricultural Biotechnology, 2021
Publication

56	R. Jayabalan, Viduranga Y. Waisundara. "Kombucha as a Functional Beverage", Elsevier BV, 2019 Publication	<1 %
57	ethesisarchive.library.tu.ac.th Internet Source	<1 %
58	hal.univ-reunion.fr Internet Source	<1 %
59	www.jimmunol.org Internet Source	<1 %
60	Fahriha Nur-A Kabir, Tajnuba Sharmin, Neaj Ahmed, Md. Shohel Rana Palleb, Nilam Debi Bristi. "Effect of chemical preservatives on the shelf life of tomato juice", Acta Chemica Malaysia, 2019 Publication	<1 %
61	Ka - Wai Lai, Yu - Hsuan How, Liew - Phing Pui. " Storage stability of microencapsulated GG in hawthorn berry tea with flaxseed mucilage ", Journal of Food Processing and Preservation, 2020 Publication	<1 %

62	Mawiyah Mahmud, Rosazlin Abdullah, Jamilah Syafawati Yaacob. "Effect of Vermicompost on Growth, Plant Nutrient Uptake and Bioactivity of Ex Vitro Pineapple (Ananas comosus var. MD2)", Agronomy, 2020 Publication	<1%
63	Nathalie Barakat, Sandra Beaufort, Ziad Rizk, Jalloul Bouajila, Patricia Taillandier, Youssef El Rayess. "Kombucha analogues around the world: A review", Critical Reviews in Food Science and Nutrition, 2022 Publication	<1%
64	Palma, T.. "A review of postharvest events in cherimoya", Postharvest Biology and Technology, 199302 Publication	<1%
65	Katarzyna Ewa Emiljanowicz, Edyta Malinowska-Pańczyk. "Kombucha from alternative raw materials – The review", Critical Reviews in Food Science and Nutrition, 2019 Publication	<1%
66	Ruyi Li, Yongquan Xu, Jianxin Chen, Fang Wang, Chun Zou, Junfeng Yin. "Enhancing the proportion of gluconic acid with a microbial community reconstruction method to improve the taste quality of Kombucha", LWT, 2022	<1%

Publication

67	Shuai Wang, Zhangxin Yu, Canhong Wang, Chongming Wu, Peng Guo, Jianhe Wei. "Chemical Constituents and Pharmacological Activity of Agarwood and Aquilaria Plants", Molecules, 2018 Publication	<1 %
68	citeseerx.ist.psu.edu Internet Source	<1 %
69	ideas.dickinsonlaw.psu.edu Internet Source	<1 %
70	tudr.thapar.edu:8080 Internet Source	<1 %
71	www.old.spuvvn.edu Internet Source	<1 %
72	David Laureys, Scott J. Britton, Jessika De Clippeleer. "Kombucha Tea Fermentation: A Review", Journal of the American Society of Brewing Chemists, 2020 Publication	<1 %
73	S.C. Lazarini, C. Yamada, H.S. Barud, E. Trovatti, P.P. Corbi, W.R. Lustri. " Influence of chemical and physical conditions in selection of ATCC 23769 strains with high capacity to produce bacterial cellulose for application as sustained antimicrobial drug-release supports ", Journal of Applied Microbiology, 2018	<1 %

Publication

74	eprints.utm.my Internet Source	<1 %
75	ijp-online.com Internet Source	<1 %
76	llufb.llu.lv Internet Source	<1 %
77	skemman.is Internet Source	<1 %
78	text-id.123dok.com Internet Source	<1 %
79	www.scirp.org Internet Source	<1 %
80	E. Ivanišová, K. Meňhartová, M. Terentjeva, L. Godočíková, J. Árvay, M. Kačániová. "Kombucha tea beverage: Microbiological characteristic, antioxidant activity, and phytochemical composition", Acta Alimentaria, 2019 Publication	<1 %
81	Manisha Pathak, Shrikant Sharma, Prem Prakash Kushwaha, Shashank Kumar. "Functional lead compounds and targets for the development of drugs for the treatment of male infertility", Elsevier BV, 2020 Publication	<1 %

82	Rami Rahmani, Sandra Beaufort, Silvia Alejandra Villarreal-Soto, Patricia Taillandier, Jalloul Bouajila, Mohamed Debouba. "Kombucha fermentation of African mustard (Brassica tournefortii) leaves: Chemical composition and bioactivity", Food Bioscience, 2019 Publication	<1 %
83	Sakon Monggoot, Siam Popluechai, Eleni Gentekaki, Patcharee Pripdeevech. "Fungal Endophytes: an Alternative Source for Production of Volatile Compounds from Agarwood Oil of Aquilaria subintegra", Microbial Ecology, 2017 Publication	<1 %
84	Wilton R. Lustri, Hélida Gomes de Oliveira Barud, Hernane da Silva Barud, Maristela F.S. Peres et al. "Chapter 6 Microbial Cellulose — Biosynthesis Mechanisms and Medical Applications", IntechOpen, 2015 Publication	<1 %
85	Zhila Heydari Koochi, Kourosh Ghodrat Jahromi, Gholamreza Kavoosi, Sedigheh Babaei. "Citrus peel waste essential oil: Chemical composition along with anti ‐ amylase and anti ‐ glucosidase potential", International Journal of Food Science & Technology, 2022	<1 %

Publication

86	drs.nio.org Internet Source	<1 %
87	open.library.ubc.ca Internet Source	<1 %
88	pesquisa.bvsalud.org Internet Source	<1 %
89	repositorio.ufsc.br Internet Source	<1 %
90	repository-tnmgrmu.ac.in Internet Source	<1 %
91	umpir.ump.edu.my Internet Source	<1 %
92	www.southernbiological.com Internet Source	<1 %
93	Dileep Kumar, Syed Ibrahim Rizvi. "Black tea supplementation augments redox balance in rats: relevance to aging", Archives of Physiology and Biochemistry, 2017 Publication	<1 %
94	Iñaki Diez-Ozaeta, Oihana Juaristi Astiazaran. "Recent advances in Kombucha tea: Microbial consortium, chemical parameters, health implications and biocellulose production",	<1 %

International Journal of Food Microbiology, 2022

Publication

95 Kinga Topolska, Adam Florkiewicz, Agnieszka Filipiak-Florkiewicz. "Functional Food—Consumer Motivations and Expectations", International Journal of Environmental Research and Public Health, 2021 <1%

Publication

96 Min Yang, Qiong Wang, Wenjun Wu, Xinyan Cheng, Huixian Wu. "Effect of Salinity Change Resulting From Ballast Water Discharge on the Recipient Freshwater Eco-System – Inputs to Scientific Ballast Water Management of Inland Ports", Research Square Platform LLC, 2022 <1%

Publication

97 Shelly Ann Bartoo, >Neela Badrie. " Physicochemical, nutritional and sensory quality of stirred 'dwarf' golden apple (Sonn) yoghurts ", International Journal of Food Sciences and Nutrition, 2009 <1%

Publication

98 Teoh, A.L.. "Yeast ecology of Kombucha fermentation", International Journal of Food Microbiology, 20040901 <1%

Publication

99 dokumen.pub

	Internet Source	<1%
100	dspace.emu.ee Internet Source	<1%
101	erl.ucc.edu.gh:8080 Internet Source	<1%
102	fens.usv.ro Internet Source	<1%
103	ijhcs.com Internet Source	<1%
104	repositorio.animaeducacao.com.br Internet Source	<1%
105	researcharchive.lincoln.ac.nz Internet Source	<1%
106	www.djkn.kemenkeu.go.id Internet Source	<1%
107	www.fbscience.com Internet Source	<1%
108	www.madikombucha.com Internet Source	<1%
109	www.mjms.usm.my Internet Source	<1%
110	www.posnutricao.ufv.br Internet Source	<1%

111	www.utsakcongress.com Internet Source	<1 %
112	zaguan.unizar.es Internet Source	<1 %