PHYSICOCHEMICAL AND MICROBIAL CHARACTERIZATION OF KOMBUCHA AND SCOBY-FORMULATED JAM FROM BLACK, OOLONG AND GREEN TEAS

By

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Chairman : Prof. Ir. Chin Nyuk Ling, PhD
Faculty : Engineering

This research was aimed at diversifying *kombucha* fermentation and leveraging its by-product *scoby* for jam formulation, followed by a detailed microbial analysis of the resulting products. The initial phase of the study showed that different pure and combinations of teas, *i.e*., black, oolong and green teas are potential alternatives for *kombucha* fermentation as all samples exhibited similar physicochemical trends to conventional black tea. Throughout fermentation, refractometric analysis of brix declined while acidity, alcohol and total polyphenol content increased, resulting in a pH drop to 2.70-2.79. After a 10-day fermentation, *scoby* yield ranged 64.89-109.93%. Pure oolong tea, with its highest total polyphenol content, presented the highest *scoby* yield and outperformed pure black and green teas with the highest acetic acid and *scoby* formation rates using modified Gompertz model. Other models, *i.e.*, Boltzmann, polynomial, rational and exponential effectively analysed substrate and pH changes (R^2 > 0.96). *Scoby* harvested from black, oolong and green tea *kombucha* fermentation was incorporated into mango jams at 2-10%, resulting in

enhanced quality by significantly ($P < 0.05$) reducing water activity (0.679-0.846), moisture content (19.92-29.48%), pH (3.19-3.38) and increasing brightness (31.82-40.83), with more pronounced effects at higher *scoby* concentrations. Jam texture properties increased with *scoby* concentrations, showing pseudoplasticity described by Power Law and Herschel-Bulkley models ($R^2 > 0.96$). *Scoby*-formulated jams had higher carbohydrates (up to 23.2 g/100g), total dietary fiber (up to 0.4 g/100g), protein (up to 0.3 g/100g) and lower fat (<0.1 g/100g) compared to commercial jam and received >70% for overall acceptability in the hedonic test. Microbiome analysis via Next-Generation Sequencing identified *Komagataeibacter* and *Zygosaccharomyces* as characteristic microorganisms of all *kombucha* and jams. The attempt to use *scoby* in jam formulation in this research for enhancing *kombucha* fermentation is novel. Both *kombucha* and *scoby*-formulated jams are clean-label foods with probiotic and bioactive functionality based on advanced microbial analysis.

Keywords: Jam, *Kombucha*, Microorganisms, *Scoby*, Tea

SDG: GOAL 3: Good Health and Well-being, GOAL 12: Responsible Consumption and Production, GOAL 15: Life on Land

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CIRI-CIRI FIZIKOKIMIA DAN MIKROB KOMBUCHA DAN JEM YANG DIFORMULASI SCOBY DARIPADA TEH HITAM, OOLONG DAN HIJAU

Oleh

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Penyelidikan ini bertujuan untuk mempelbagaikan penapaian *kombucha* dan memanfaatkan produk sampingannya, *scoby* untuk formulasi jem, diikuti dengan analisis mikrob terperinci untuk produk yang terhasil. Fasa awal kajian menunjukkan bahawa pelbagai jenis tulen dan gabungan teh, iaitu teh hitam, oolong dan hijau adalah alternatif yang berpotensi untuk penapaian *kombucha* kerana semua sampel mempamerkan trend fizikokimia yang serupa dengan teh hitam konvensional. Sepanjang penapaian, analisis refraktometri brix merosot manakala keasidan, alkohol dan jumlah kandungan polifenol meningkat, mengakibatkan penurunan pH kepada 2.70-2.79. Selepas penapaian 10 hari, hasil *scoby* berkisar antara 64.89-109.93%. Teh oolong tulen dengan kandungan polifenol keseluruhan yang tertinggi, menunjukkan hasil *scoby* tertinggi dan mengatasi prestasi teh hitam dan hijau tulen dengan kadar pembentukan asid asetik dan *scoby* tertinggi, menggunakan model Gompertz yang diubah suai. Model lain, iaitu Boltzmann, polinomial, rasional dan eksponen menganalisis perubahan substrat dan pH dengan berkesan (R^2 > 0.96).

Scoby yang diperoleh daripada penapaian *kombucha* teh hitam, oolong dan hijau dimasukkan ke dalam jem mangga pada kadar 2-10% dapat meningkatkan kualiti dengan ketara ($P < 0.05$) dengan mengurangkan aktiviti air (0.679-0.846), kandungan lembapan (19.92-29.48%), pH (3.19-3.38) dan meningkatkan kecerahan (31.82-40.83), dengan kesan yang lebih ketara pada kepekatan *scoby* yang lebih tinggi. Sifat tekstur jem meningkat dengan kepekatan *scoby*, menunjukkan pseudoplastisitas dengan model Power Law dan Herschel-Bulkley ($R^2 > 0.96$). Jem yang diformulasikan *scoby* mempunyai karbohidrat yang lebih tinggi (sehingga 23.2 g/100g), jumlah serat pemakanan (sehingga 0.4 g/100g), protein (sehingga 0.3 g/100g) dan lemak yang lebih rendah (<0.1 g/100g) berbanding komersial jem dan penerimaan keseluruhan adalah >70% daripada ujian hedonik. Analisis mikrobiom melalui 'Next-Generation Sequencing' mengenal pasti *Komagataeibacter* dan *Zygosaccharomyces* sebagai mikroorganisma ciri bagi semua *kombucha* dan jem. Percubaan untuk menggunakan *scoby* dalam formulasi jem dalam penyelidikan ini untuk meningkatkan penapaian *kombucha* adalah novel. Kedua-dua *kombucha* dan jem yang dirumuskan *scoby* adalah makanan berlabel bersih dengan fungsi probiotik dan bioaktif berdasarkan analisis mikrob lanjutan.

Kata kunci: Jem, *Kombucha*, Mikroorganisma, *Scoby*, Teh

SDG: MATLAMAT 3: Kesihatan Baik dan Kesejahteraan, MATLAMAT 12: Penggunaan dan Pengeluaran yang Bertanggungjawab, MATLAMAT 15: Kehidupan di Darat

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CHAPTER 1

INTRODUCTION

1.1 Overview

Fermentation is one of the oldest methods of preserving perishable foods, which takes a long time with minimal preparation and processing; however, it perpetuates the growth of benign microbial community and increases the complexity of food taste, which pays off with more flavours. Since the Neolithic age, humanity has started the fermentation of foods, which was discovered by inadvertent accidents long before comprehending the underlying science (Anal, 2019; Rahman, 2007). This ancient skill has not only facilitated the preservation of food, but also encouraged the transformation of ingredients, such as fruits into wine and cabbage into sauerkraut, which are served as staples or adjuncts to staples.

Over the course of time, interest in fermentation has burgeoned, shifting from ‘‘because we have to’’ to ‘‘because we love to’’. The shift from necessity to passion in fermentation is fueled by advancements in understanding the scientific principles behind this process, empowering individuals to appreciate the art of fermentation and health benefits it offers. This transformation is evident in the surge in popularity of fermented foods, which reflects how people have expanded their definition of wellness (Dimidi et al., 2019; Parvez et al., 2006). Fermented foods are now appreaciated for their health benefits such as improving gastrointestinal health and enhancing the bioavailability of nutrients (Dimidi et al., 2019; Parvez et al., 2006). The presence of bioactive compounds and probiotic features of microorganisms synthesised during the fermentation process has made fermented foods a cornerstone

of excellent health (Kort et al., 2015). In fact, consuming fermented foods is an easy and common way to modulate gastrointestinal microbiota by introducing potentially beneficial microbes (Marco et al., 2017). This method is more convenient than consuming probiotics that contain live microbes, yet both approaches ultimately provide similar health benefits (Marco et al., 2017). This allows fermented foods to remain a practical vehicle for providing access to probiotic benefits, particularly in poor countries where these benefits can come at a hefty price (Kort et al., 2015; Mpofu et al., 2014).

Apart from that, the natural process of fermentation, which meets consumers' expectations of attaining ''clean labeling'' without the usage of synthetic preservatives, also brings about the mega-fad around the positive image of fermented foods. Based on Bizzorero (2017), a survey conducted by specialist PR group Ingredient Communications reveals that 73% of consumers are willing to pay more for products made with ''clean'' and simple ingredients they recognise. With a high reputation for being both natural and healthy, the consumption of fermented foods is expected to fuel the growth of the overall market as ferments are on the rise, with the worldwide market expected to reach USD 1.08 billion and record a robust expansion of growth at a Compound Annual Growth Rate (CAGR) of 4.3% by 2024 according to ReportLinker (2019). As a result of high market demand and affordable procurement, food processing manufacturers are showcasing a substantial uptake in adoption of fermented ingredients for production (Persistence Market, 2017). Not only are an increasing number of companies commercialising traditional fermented products, but they are also creating novel products based on traditional ones (Terefe, 2016). This trend highlights how fermentation has evolved beyond food preservation,

with much effort being devoted to the development of better fermentation processes, equipment and food products since a century ago (Terefe, 2016; Xiang et al., 2019).

Kombucha is not an exception to the ever-evolving development of fermented foods as its renown has soared to new heights in recent times, in part due to its purported health benefits that cater to consumer demands. *Kombucha*, an acetic acid ferment, is a member of the vinegar family and has a rich history of traditional fermentation (Crum & LaGory, 2016). The process involves infusing sweetened black tea with a bacterial cellulose pellicle, known as *scoby* (symbiotic culture of bacteria and yeast). The fermentation of *kombucha* involves a combination of acetic acid bacteria and diverse strains of yeast, which work synergistically to produce the characteristic components of this popular beverage (Steinkraus, 2002). Despite its traditional roots, *kombucha* has undergone massive transformations (Chakravorty et al., 2019). The versatility of *kombucha* has opened the door for experimentation and innovation, resulting in a wide range of flavour combinations and specialised brewing techniques. However, the utilisation of oolong and green tea in *kombucha* fermentation has been relatively understudied, despite their beneficial compounds. Tea, from *Camellia sinensis* plant, has a complex chemical composition, comprising over 2000 components which varies among different tea types, *i.e*., black, green and oolong (Yashin et al., 2015). While it is challenging to generalise the relationship between tea components and health benefits, studies have shown that oolong tea was more effective in suppressing body weight compared to black and green teas (Kuo et al., 2004; Sun et al., 2019) while green tea was more efficient in lowering cholesterol levels than oolong and black teas (Kuo et al., 2004) and demonstrated superior anti-hyperglycemic activity compared to black tea (Tang et al., 2013). In addition, the

combination of black and green teas has shown synergistic potential (Malongane et al., 2017) with enhanced anticancer (Kobalka et al., 2015) and antibacterial effects (Betts et al., 2011). The differences in tea compounds contribute differently to health benefits, where it is also believe to impact fermentation outcomes as tea serves as vital nutrients for microorganisms (Yim et al., 2017). For instance, green tea showed a higher *scoby* yield (Gaggìa et al., 2018) and total polyphenol content (Hsieh et al., 2021; Wang et al., 2022) than black tea in *kombucha* fermentation and Coton et al. (2017) noted different bacterial communities in different tea types, with green tea fermentation having higher abundance of lactic acid bacteria compared to black tea fermentation. Comparisons of black, green and oolong tea in fermentation outcomes are limited and the exploration of using combinations of teas in *kombucha* fermentation which is not explored, opens up possibilities for potential synergistic effects, diversifying and enhancing the overall fermentation process. The microbial makeup of *kombucha* is also influenced by geographical location, as proven by studies in regions such as Brazil (Barbosa et al., 2021), Canada (Marsh et al., 2014), Indonesia (Angela et al., 2020), France (Coton et al., 2017) and Singapore (Amarasinghe et al., 2018) while this research is based on a new location of study which is Malaysia. To better understand the fermentation processes, the incorporation of mathematical modelling offers insightful prespectives, improves control over fermentation conditions and facilitates subsequent processes utilising the valuable by-products.

Scoby is a fermentation by-product whereby only a portion is used for further fermentation while the remaining goes to waste (Jayabalan et al., 2010). This valuable by-product should not be overlooked as it is believed to possess

advantageous characteristics and significant potential in food industry. As a form of bacterial cellulose, it offers a sustainable option as ingredient incorporation, functioning naturally as a binder, thickener, texturiser and emulsion stabiliser (Goh et al., 2012a; Mohite & Patil, 2014) to enhance texture, stability and overall quality of various food products. Its purity and water holding capacity heighten its desirability as a versatile ingredient, attributed to its compact cellulose network structure with ultrafine microfibrils that form hydrogen bonds more easily than commercial cellulose (Goh et al., 2012b; Mohite & Patil, 2014). Bacterial cellulose has shown its water-holding and structural stabilising capabilities, contributing to melting resistance, enhanced texture profiles and suspension stability of ice cream (Guo et al., 2018). Likewise, the application of *scoby* is particularly useful in production of jam, a widely enjoyed food product, where its water holding capacity can enhance gel formation and texture stability. This is crucial for achieving consistency in jam, preventing undesirable syneresis and ensuring a higher quality end product, yet it has not been studied previously. Also, in order to meet consumer demand for healthier food options, jam products can be reformulated using ingredients rich in beneficial compounds. Compared to previously researched jam-making ingredients such as pectin (Basu et al., 2013; Basu & Shivhare, 2012; Javanmard et al., 2012), carboxymethyl cellulose and sago starch (Javanmard et al., 2012), *scoby* may also provide extra health benefits due to its diverse functional microbial composition. While fermented foods contain beneficial microbes, many people are unaware of the microbiome and their roles within these foods. The microorganisms present can be identified through Next-Generation Sequencing (NGS) technology, a high throughput method which enables rapid and accurate characterisation of complex microbial communities (Arıkan et al., 2020). Unlike traditional culture-based

methods which are time consuming, this method has the capability to sequence millions of DNA fragments simultaneously, including those that are difficult to culture (Arıkan et al., 2020). With NGS, it is possible to prospect the composition of microbial communities in the functional foods, assessing the diversity within and between these microbial communities. This knowledge helps uncover the roles and potential benefits of these microorganisms, providing insightful information for product development and considerations related to human health.

Herein, the aim is to study *kombucha* fermentation using pure and combinations of tea substrates with application of mathematical modelling. The resulting by-product of this process, *scoby*, was used as a valuable resource to formulate a jam product where its properties were measured and evaluated. Subsequently, this investigation entails a thorough screening and characterisation of the microorganisms present in both the products of *kombucha* fermentation and jam production utilising NGS technology. With the study of microbiota, the contribution of these microorganisms to the nutritional and healthy attributes of food can be understood, paving the way for the development of novel and functional food products that meet the growing demand for natural and healthy foods.

1.2 Problem statements

Fermented foods give body a dose of healthful intestinal microbiome, which plays a role in fine-tuning immune system and wards off damaging inflammation in the body that contributes to a wide range of illnesses, including but not limited to obesity, diabetes and neurodegenerative diseases. However, in Malaysia, fermented foods are often unnoticed and undervalued in comparison to countries like China, Japan and

Korea, which have long-standing culture of fermentation. This knowledge gap presents a significant opportunity for research and exploration, particularly in the context of fermented foods like *kombucha*. While black tea-based *kombucha* has been widely researched, there is a lack of research investigating the impact of utilising alternative substrates such as green tea, oolong tea and tea combinations on the fermentation process and resulting product, *scoby*. The dynamics of fermentation, along with the extensive exploration of the microbial community of both *kombucha* tea and *scoby*, are also very scarce. Microbial composition studies of *kombucha* in different countries have highlighted geographical variations in microbial profiles, yet research specific to Malaysia remains lacking, leaving uncertainty about local factors that may influence microbial composition. Following each fermentation, *scoby* by-product contributes to resource wastage as only a portion is utilised for subsequent fermentation while the remainder is thrown away. The potential of *scoby* which has high water holding capacity, structural stabilising capability and is chock-full of microorganisms that confer health benefits represents a significant gap in the application of food product development, particularly in jam production where these characteristics are advantageous. In addition, the market today predominantly offers traditional fermented foods that have been available for a long time, overlooking the dynamic nature of food market today that is influenced by changing trends, preferences, needs, behaviours and habits. To address these gaps, this research aims to explore the fermentation of *kombucha* with alternative substrates and the utilisation of its by-product in the subsequent process of jam production, highlighting its significance and potential for broader application. Studying the microbial composition of the products is important to understand their impacts, roles and potential benefits which were investigated in this research.

1.3 Objectives

This research was conducted to investigate fermentation of *kombucha* and formulation of jam product, with a focus on understanding the diverse microorganisms inhabiting these products. Through the study of these supplementary foods, this research seeks to contribute to the advancement of knowledge in this field and provide valuable insights into their potential benefits. By exploring their properties, this study has the potential to pave the way for the development of novel and functional food products, offering new possibilities for the food industry. To accomplish these, the specific objectives were outlined as follows:

1. to compare physicochemical properties of *kombucha* made using three types of tea infusions, *i.e*., black, green and oolong tea,
2. to formulate jam product utilising *scoby* from *kombucha* fermentation, and
3. to screen and characterise beneficial microbes for health in production of *kombucha* and jam.

1.4 Scope of study

Figure 1.1 shows an overall research flow diagram, presenting three primary parts of the research study. The study on *kombucha* fermentation process used both pure and combinations of black, green, and oolong teas to assess their individual and combined impacts on fermentation outcomes. *Kombucha* was prepared and subjected to physicochemical analysis of *kombucha* tea through a 10-day fermentation. Subsequently, modelling of *kombucha* fermentation was done to better understand the fermentation process and improve quality control over fermentation conditions. To promote sustainability, *scoby* by-product from *kombucha* fermentation was used

to formulate a jam product. The produced jam was evaluated for its application on physicochemical, textural, rheological properties alongside with proximate analysis and sensory evaluation. Beneficial microbes in fermented products are important, thus, microbial analyses were conducted using NGS on both the fermented *kombucha* and jam products, inclusive of the by-product *scoby*.

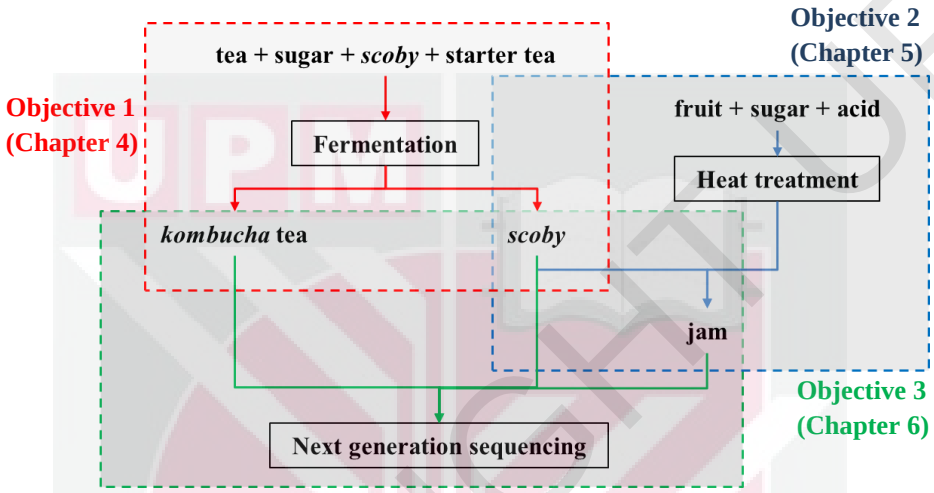


Figure 1.1: Overall research flow diagram

CHAPTER 2

LITERATURE REVIEW

2.1 Fermentation of food

Fermentation is defined as a metabolic phenomenon that induces chemical changes in organic substrates and energy-yielding from carbohydrates (Nout, 2014). In terms of fermentation of food, it refers to a dismutation of food substrates by microbes which results in desirable modifications of food ingredients (Nout, 2014). Fermented food can be known as palatable and wholesome foods prepared from various types of food ingredients which include primary agricultural raw materials, ranging from plant-based foods, *i.e.*, fruits, cereals and vegetables, to animal-based foods, *i.e.*, meats, eggs and fish (Nout, 2014). The main purpose of fermenting foods is to coax microorganisms into producing enzymes that may play a role in breaking down complex compounds to simple bio-molecules (Chettri & Tamang, 2014; Tamang et al., 2016; Tamang & Nikkuni, 1996). Microorganism, which is one of the several variables that give rise to different types of fermented foods, have to be in adequately large amount and metabolically active to exert their impact towards expected food characteristics such as taste, aroma, visual appearance, texture, shelf life and safety (Holzapfel, 2002). The use of microorganisms in food fermentation can be achieved either by using wild ferments or addition of starter culture (Figure 2.1).

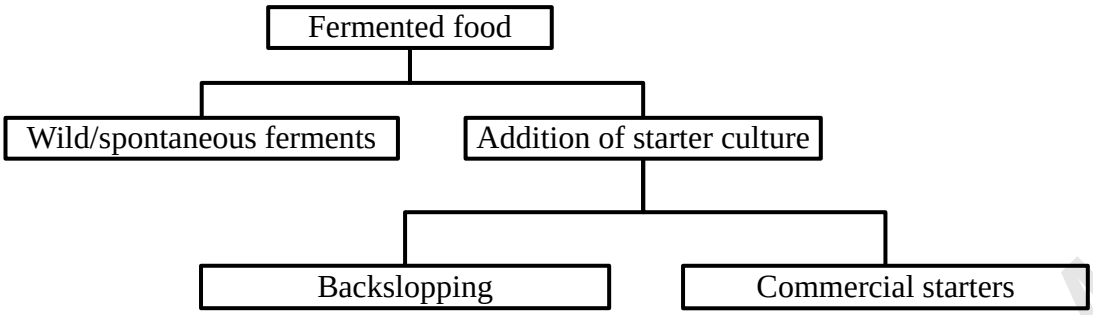


Figure 2.1: Approaches used in making fermented food

Principally, wild or spontaneous ferments need no added microorganisms into the ingredients whereby the microorganisms are present naturally and rely on process conditions that allow the survival and outgrowth of desirable microorganisms in the food (Dimidi et al., 2019). On the other hand, for foods fermented via addition of starter culture or starter-mediated, highly concentrated dose of desirable fermentation microorganisms are added to the ingredients which may have been previously freed of potential competitive microbiota and the fermentation process is carried out under favourable environment (Nout, 2014). This approach is achieved either by the ‘‘backslopping’’ process, *i.e.*, repeated cycles of adding a small amount of fermented products into fresh ingredient to obtain an active and matured starter or use of selected commercial starters for initiating the fermentation process. The latter approach is used to standardise organoleptic properties of final food in industrialisation of food production (Dimidi et al., 2019). The contribution of these ferments or starters is to convert carbohydrates to desired metabolites (Hansen, 2002), through diverse fermentation modes, such as lactic acid fermentation, alcohol fermentation, acetic acid fermentation and alkaline fermentation (Anal, 2019; Steinkraus, 1997).

2.2 *Kombucha*

Kombucha is a traditional beverage brought about by fermentation of tea and sugar source with symbiotic consortium of microbial in the category of acetic acid fermentation (Greenwalt et al., 2000; Kim & Adhikari, 2020; Steinkraus, 1997). The peculiarity of *kombucha* is that the microorganisms are embedded in a cellulose pellicle at the air-liquid interface of the *kombucha* tea (Gaggìa et al., 2018). The cellulose pellicle is commonly known as *scoby* (Figure 2.2), which will be used in this literature, referred to a ‘‘symbiotic culture of bacteria and yeast’’, added to initiate the fermentation process (Teoh et al., 2004; Tran et al., 2020). In some cases, acidic liquid or starter tea (ideally fermented *kombucha* tea from previous batch or distilled vinegar) will be added to acidify the mixture to help to inhibit growth of deleterious microbes and ensure safety of *kombucha* (Dutta & Paul, 2019; Goh et al., 2012a). *Kombucha* fermentation also requires the use of clean and sanitised utensils to avoid contamination (Nummer, 2013).

Figure 2.2: *Kombucha* tea and *scoby* (Susha & Jennifer, 2019)

Fermentation process of *kombucha* usually takes place for 7 to 10 days at a temperature range of 18 to 30^{o}C (Greenwalt et al., 2000; Kim & Adhikari, 2020). It is recommended to limit the fermentation to less than 10 days if *kombucha* is made

for human consumption, according to guidelines in the Food and Drug Administration Model Food Code for *kombucha* brewing (Nummer, 2013; Villarreal-Soto et al., 2018). Fermentation period beyond 10 days may lead to elevated acidity levels that could pose potential harm to consumers (Nummer, 2013). Along with each fermentation process, formation of a new layer of *scoby* can be used as a starter for subsequent brews through the practice of backslopping (Coton et al., 2017).

Sharing of *scoby* allowed the widespread of *kombucha* in the olden days (Kim & Adhikari, 2020). As its popularity grew in the east during historical periods, *kombucha* acquired various names (Table 2.1) (Kim & Adhikari, 2020). Eventually, it made its way to other regions including Russia, eastern and western European countries and North Africa, gaining traction and becoming a part of their cultural practices (Kim & Adhikari, 2020). It has gone through a significant transformation from its humble homemade origins. Now available in commercial products, it is promoted as a decent substitute for non-alcoholic beverages, gaining recognition as an appealing alternative to traditional soft drinks. Its invigorating benefits have been widely acknowledged, leading to its designation as ‘‘The Year of Fancy Water and *Kombucha*’’ in 2018 by the Wall Street Journal (Back, 2018).

Table 2.1: Names of *kombucha* in literatures

Other names of *kombucha*	References
Grib (mushroom), Tea Kvass, Kvass, Kombucha Fungus, Gout Jelly-fish Manchurian Tea, Russian Jelly-fish, Remedy of Immortality, Fungus Japonicus, Fungajapon Kombucha, Combucha, Kambotscha, Koucha Kinoko, Tea Mold, Indian Tea Fungus, Manchu Fungus, Kombucha Mushroom, Hongo Japanese Tea Fungus, Mother of vinegar, Manchurian Mushroom, Mushroom of Charity, The Divine Che, Miracle Fungus, Tea Mushroom, Mo-Gu, Russian Tea-Vinegar, Tea Cider, Volga-Spring, Teakwass, Tea Beer, Kargasok Tea	Chandrakala et al. (2019)
Khubdat Hamza	Alkhalifawi (2014)
Red tea fungus, Champignon de longue vie, Ling zhi, Kocha kinokko, Chainii grib, Chainii kvass	Malbaša et al. (2011)

The underlining causes that have been spurring *kombucha* boom encompass its multitude of potential health benefits, including improved intestinal microbiota, regulated nutrient absorption, reduced risk of chronic non-communicable diseases (Crowe & Francis, 2013; Manivasagan et al., 2020), body detoxification, enhanced immune system support, potential anti-cancer, anti-tumour and antimicrobial properties (Kapp & Sumner, 2019; Kim & Adhikari, 2020). These remarkable benefits are much dependent on the specific compositions and microorganisms in *kombucha* (Coelho et al., 2020; Fu et al., 2014; Jayabalan et al., 2007; Lee & Kim, 2000). However, the microbial consortium and compositions can vary based on fermentation conditions, inoculum source and ingredients used in *kombucha* preparation process (Bishop et al., 2022; de Miranda et al., 2022; Ferremi Leali et al., 2022; Jayabalan et al., 2008; Jayabalan et al., 2014).

2.3 *Kombucha* fermentation

There are various types of ingredients for *kombucha* brewing, nonetheless, choosing the right ingredients enables production of *kombucha* with highest content of healthy organic acids and generation of a healthy environment for *scoby* (Neffe-Skocińska et al., 2017; Nguyen et al., 2015). The medium required for *scoby* growth should necessarily include a carbohydrate element as a carbon source and tea extract as a nitrogen supplier, which in turn can be assimilated in the fermentation process to drive the growth and metabolism of microbes, making the overall yield of *scoby* (Laavanya et al., 2021).

2.3.1 *Scoby*

Scoby is the most conspicuous feature of *kombucha* and it plays a role in initiating the fermentation process (Harrison & Curtin, 2021). Generally, not all *scoby* contain the exact same strains of bacteria and yeasts but they contain all crucial microorganisms necessary to produce *kombucha.* Figure 2.3 shows *kombucha* metabolism. Yeast enzymatically cleaves sucrose into glucose and fructose, which they metabolise into ethanol and carbon dioxide (May et al., 2019). Concurrently, bacteria oxidise the ethanol, converting it into acetic acid while also produce cellulose which leads to biofilm formation (May et al., 2019). With increased acidity caused by production of acids, the slightly carbonated *kombucha* delivers vinegary notes and changes from sweet to fruity, sour taste (Dufresne & Farnworth, 2000; Jayabalan et al., 2008; Kim & Adhikari, 2020). The populations of microbes present in *scoby* comprise bacteria such as *Komagataeibacter xylinus*, *Acetobacter pasteurianus*, *Acetobacter aceti* and *Gluconobacter oxydans* and yeast species of the

genera *Saccharomyces*, *Brettanomyces*, *Candida* and *Zygosaccharomyces* (Gomes et al., 2018; Greenwalt et al., 2000; Treviño-Garza et al., 2020; Villarreal-Soto et al., 2018). Among all microorganisms, *Komagataeibacter xylinus* (formerly recognised as *Gluconacetobacter xylinus* (Yamada et al., 2012) and *Acetobacter xylinum* (Yamada et al., 1997) has been regarded to be the dominant species for the production of cellulose pellicle and has been known to be the core contributor for cellulose production in *kombucha* cultures (Dufresne & Farnworth, 2000; Marsh et al., 2014; Mikkelsen et al., 2009; Strap et al., 2011). As fermentation progresses, cellulose filaments produced by bacteria rise to the surface of the solution and aggregate together, generating a larger, thicker and stronger *scoby* (Dufresne & Farnworth, 2000; Villarreal-Soto et al., 2018). A new layer of *scoby* will be formed where the upper-most layer is always considered to be the newest. Development of *scoby* comes to a halt when it cultivates downward entrapping all bacteria and becomes inactive (Villarreal-Soto et al., 2018).

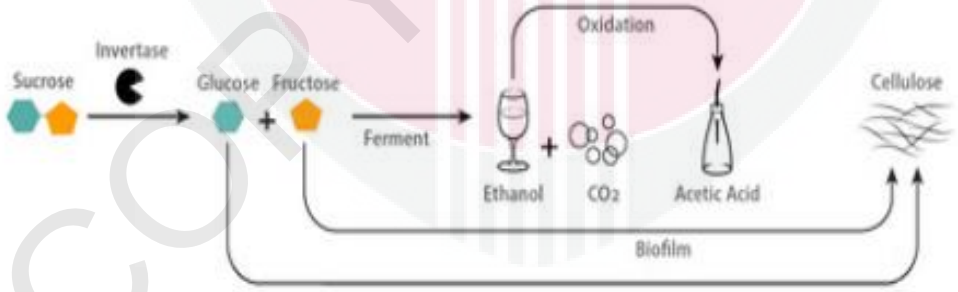


Figure 2.3: *Kombucha* metabolism (May et al., 2019)

The ability of *scoby* to regenerate with each batch of *kombucha* makes it a renewable source that can be continuously produced. It can be exploited as a sustainable alternative of raw material due to its structural characteristics such as high purity, high porosity and excellent water capacity (Treviño-Garza et al., 2020; Villarreal-

Soto et al., 2018). These characteristics suggest that *scoby* produced by *kombucha* fermentation has a wide assortment of applications in food and could be utilised in a variety of beneficial products (Goh et al., 2012a; Lin et al., 2016; Römling & Galperin, 2015; Treviño-Garza et al., 2020). In food application, it can be used as an emulsion stabiliser, thickener and source of dietary fiber (Emiljanowicz & Malinowska-Pańczyk, 2020). Compared to plant cellulose, it has higher potential in lowering triglycerides, low-density lipoprotein (LDL), total cholesterol, liver total lipids and liver total cholesterol (Emiljanowicz & Malinowska-Pańczyk, 2020). Hence, based on these properties, *scoby* can be a promising low-calorie food ingredient (Chau et al., 2008; Goh et al., 2012a). In maximising yield of *scoby*, aspects like volume of inoculated media, temperature, fermentation time, pH and other ingredients such as types of tea and sugar that contribute to the culture medium are to be given attention (Cacicedo et al., 2016; Laavanya et al., 2021; Treviño-Garza et al., 2020; Villarreal-Soto et al., 2018; Yim et al., 2017).

2.3.2 Tea

Picking the right tea for brewing is pivotal as tea is one of the foundational ingredients in *kombucha* fermentation that affects *scoby*'s health and taste of final brew. This is because tea provides essential nutrients and promotes the growth of microorganisms and cellular construction (Waisundara, 2019; Yim et al., 2017). Tea is a hardy evergreen shrub grown to produce beverage from its leaves and buds which originates from *Camellia sinensis* (L.) Kuntze in the flowering plant family *Theacea* (Dufresne & Farnworth, 2000; Oliveira et al., 2016). There are two major varieties, chiefly *Camellia sinensis var. sinensis* (China tea) and *Camellia sinensis var. assamica* (Assam tea) whereby the former has small leaves which is grown in

China, Japan and Taiwan while the latter has larger leaves that predominates from South and Southeast Asia including Malaysia (Oliveira et al., 2016). Teas obtained from *Camellia sinensis* plant can be classified as completely fermented (black tea), partially fermented (oolong tea) and non-fermented (green tea) with their appearance, organoleptic taste and compositions affected by different fermentation processes (Habtemariam, 2019; Oliveira et al., 2016; Sharangi, 2009). These true tea leaves are claimed to have cleanest flavour and most nutrients compared to scented, flavoured or herbal teas that contains chemical compounds such as natural oils which could have a negative effect on the growth of *scoby* (Teatulia, n.d.). It has also been observed that certain herbal teas cannot serve as alternative substrate due to their deficiency in purine derivatives (Hoffmann, 1998; Velićanski et al., 2014).

Of the total amount of tea produced, black tea is the most renowned and preferred type of tea, with an estimated consumption of 78% in the world (Oliveira et al., 2016). Black tea, displayed in Figure 2.4(a) undergoes a complex and lengthy processing among all teas. The leaves are plucked, withered, rolled and crushed which initiate fermentation or oxidation in order to crack the leaf surface so that oxygen will react with plant's enzyme and turns the leaves black (Oliveira et al., 2016). Fully oxidised black tea yields a dark, rich, reddish brown infusion with brisk, distinct flavour (Sharangi, 2009). *Kombucha* is most generally and traditionally made with black tea (Jakubczyk et al., 2020; Kumar & Joshi, 2016; Treviño-Garza et al., 2020). It is considered as the finest and most dominate substrate for the fermentation process (Amarasinghe et al., 2018; Chen & Liu, 2000). Black tea is claimed to be a good fermentation medium as it has high levels of nitrogen sources which are beneficial for the growth of *scoby* (Velićanski et al., 2014). Its infusion consists of

proteins, amino acids, volatile compounds lipids and polyphenols (Kumar & Joshi, 2016). As a result of extended fermentation, catechins' numeric structure in black tea is altered, resulting in dimeric and polymeric form of polyphenols, namely thearubigins and theaflavins (Coelho et al., 2020; Zaveri, 2006). The group of astringent polyphenolic compounds produced exhibit a higher radical scavenging property than non-fermented infusions and enrich flavour profile of fermented tea (Velićanski et al., 2014; Watawana et al., 2015). Hence, black tea is darker in colour and more aromatic attributed to the transformation of phenolic compounds into more substantial, dark coloured complexes and released aromatic compounds in enzymatic oxidation (Coelho et al., 2020).

(a)

(b)

(c)

Figure 2.4: (a) Black tea, (b) oolong tea and (c) green tea (Octavia tea, n.d.)

The cultivation and manufacture of oolong tea (Figure 2.4(b)) is similar to black tea. However, oolong tea is only allowed to undergo semi-oxidation (10-70%) with outer part of the leaf oxidised and center of the leaf kept green (Coelho et al., 2020).

Oolong tea leaves are often placed into a bamboo basket, where they are gently rubbed to induce interactions with oxygen. The tea leaves turns to a deep amber or medium green colour as they oxidise. While dark oolong teas reveal notes of chocolate and caramelised sugar, lighter oolong teas give a smoother and flowery flavour profile. The flavour, colour and caffeine are said to be in between green and black teas and express the characteristics of both green tea and black tea (Oliveira et al., 2016; Sharangi, 2009). Oolong tea contains a combination of the monomeric polyphenols and theaflavins with greater molecular weights (Zaveri, 2006). Presence of polyphenol contents in oolong tea claimed that it is significantly hypolipidemic and anti-obesogenic (He et al., 2013). It also has the strongest ability to lower serum level of total cholesterol compared to black tea and green tea (Preedy, 2012).

Green tea is becoming well known in recent years, however, still ranked second after black tea with 20% level of consumption in the world (Habtemariam, 2019; Oliveira et al., 2016). Green tea, shown in Figure 2.4(c) has either a very short oxidation period or no fermentation whereby the young leaves are usually steamed and rolled to halt the active leaf enzymes that would otherwise react with oxygen, thus retains its green colour (Oliveira et al., 2016). As a result, the unfermented delicate, light tea tends to be sweet after bitter. Green tea is also an excellent option for *kombucha*. The beneficial effect of green tea in *kombucha* production is due to the presence of polyphenolic compounds, specifically catechins, which constitute about 30% of the dried weight of green tea leaves. According to Zaveri (2006), the catechins are found at higher levels in green tea compared to black tea and oolong tea as green tea leaves are subjected to steaming and drying to inactivate the polyphenols oxidase enzyme, a process that is vital in retaining the polyphenols in their monomeric state. The

commonly found catechins are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (Coelho et al., 2020). The catechins imparts bitter and astringent flavours as well as sweet aftertaste of tea (Quiao-Won & Teves, 2018).

Table 2.2 shows the nutritional composition of different tea types. Generally, these teas have different nutrients that benefit microorganisms in *kombucha* (Coelho et al., 2020). Yim et al. (2017) pointed out that the presence of polyphenols, catechins, flavonols (theaflavins and thearubigins) and theophylline in tea could be a valuable source of nutrients for acetic acid bacteria. Coelho et al. (2020), Hoffmann (1999) and Kaewkod et al. (2019) stated that the presence of caffeine in teas is also an essential source of nutrients and suspected to have stimulating effect on the growth of microbes in *kombucha*. However, there have been instances where symbiotic culture of *kombucha* can thrive on substrate that does not contain caffeine, indicating the ability of microorganisms to utilise alternative nutrients (Coelho et al., 2020). In the preparation of *kombucha* fermentation process, it is vital to maintain an optimal concentration of tea. Laavanya et al. (2021) reported that the concentration of tea should not exceed 6 g/L. This precaution is necessary because an increase in tea concentration leads to elevated levels of polyphenols, which can inhibit the growth of acetic acid bacteria and thus decrease cellulose production of *scoby* (Kurtzman et al., 2001; Laavanya et al., 2021).

Table 2.2: Chemical components present in black, oolong and green teas

Compounds	Black tea	Oolong tea	Green tea	References
(a) Tea Catechin (mg/100ml)				He et al. (2009) & Henning et al. (2003)
Gallic acid	6.5	2.19	1.2	
Caffeine	36.2	23.51	33.1	
Epigallocatechin (EGC)	6.2	16.14	76.4	
Catechin (C)	2.7	1.65	5.8	
Epicatechin (EC)	5.3	5.08	11.9	
Epigallocatechin gallate (EGCG)	8.9	25.73	83.9	
Gallocatechin gallate (GCG)	4.2	6.68	1.1	
Epicatechin gallate (ECG)	4.5	5.73	13.7	
Catechin gallate (CG)	3.0	0.60	3.1	
(b) Amino acids				Wang et al. (2010)
Total (mg/g)	1.81-3.03	1.19-1.85	4.37–6.99	

2.3.3 Sugar sources

The carbon source has a profound influence on general growth and metabolism of nearly all microorganisms and this principle applies to the microbes found in *kombucha* as well. The sustenance of *kombucha* microbes greatly depends on the supply of a carbon source, with sugar, particularly sucrose, serving as their preferred choice (Goh et al., 2012a). Sugar, used to sweeten the brewed tea, is the fuel source for fermentation in *kombucha* as bypassing it could result in *scoby* starvation (Laavanya et al., 2021). As a result, sucrose will be hydrolysed into glucose and fructose which are used in cellulose production of *scoby*. The sugar concentration used in *kombucha* fermentation ranged from 60 g/L to 120 g/L, with an optimum sugar concentration of 90 g/L for the maximum yield of *scoby* (Goh et al., 2012a; Laavanya et al., 2021). When the concentration of sugar is too high, excess sugar will be converted to other metabolic products such as gluconic acid which will hinder the synthesis of *scoby* cellulose (Goh et al., 2012a; Laavanya et al., 2021). This is because excessive acid production results in lower pH values, creating an

unfavourable fermentation environment for microorganisms. A variety of sugar types, including white sugar, brown sugar and molasses, can be used as carbon sources in *kombucha* fermentation. The application of white or brown sugar resulted in intensive growth of *scoby* in the final products (Emiljanowicz & Malinowska-Pańczyk, 2020). In Muhialdin et al. (2019), research has been conducted to investigate the impact of various sugar sources *i.e.*, white refined sugar, coconut palm sugar and molasses sugar on the properties of *kombucha*. The results have shown that *kombucha* fermented with white refined sugar exhibited higher biomass formation compared to other sugar sources.

2.4 Physicochemical analysis

Physicochemical analysis plays a significant role in unravelling the characteristics of *kombucha*. Several key physicochemical analyses are performed on *kombucha*, including pH measurement, titratable acidity (TA), total soluble solids (^{o}Brix), alcohol content, total polyphenol content and yield of *scoby*. These analyses provide valuable insights into the composition and quality of *kombucha*, aiding in understanding its fermentation process and overall attributes.

2.4.1 pH

In *kombucha* fermentation, pH is one of the most essential environmental parameters as it controls the proper course of fermentation (Malbaša et al., 2008; Neffe-Skocińska et al., 2017). pH is a logarithmic measure of free hydrogen ions that is used to specify acidity or basicity of a solution (Wagner et al., 2013). It is vital as concentrations of hydrogen ions can activate or inhibit the development of each

microorganism in *kombucha* (Abuduaibifu & Tamer, 2019; Neffe-Skocińska et al., 2017). Each microorganism has a typical pH range, which facilitates its growth and development. Microorganisms in *kombucha* tea and *scoby* are tolerable to an acidic pH in the range of 2.5 to 4 (Laavanya et al., 2021). Acetic acid bacteria have the capability to tolerate pH of environment in the range of 3.6 to 6.3 (Abuduaibifu & Tamer, 2019). It is reported that the optimum pH required for the growth of *Acetobacter* spp. is between 4 and 6.3 (Goh et al., 2012a; Laavanya et al., 2021). While growth can potentially occur at a decreased pH due to presence of a symbiotic relationship among the microbes, it is worth noting that the extent of growth becomes significantly limited within the pH range of 7.0 to 8.0 (Goh et al., 2012a). An optimal pH for yeasts is influenced by species and strain, but on average it falls between 4.5 and 6.5 (Abuduaibifu & Tamer, 2019; Neffe-Skocińska et al., 2017). The growth of mould and other contaminants was found at pH 5.0 and mould was seen growing between the nanofibers at pH 6.0 (Laavanya et al., 2021). However, to ensure proper processing and uphold food safety standards, Pennsylvania Department of Agriculture (USA) has set a guideline stating that the pH in *kombucha* production should not be higher than 4.2 and lower than 2.5 to avoid microbial contamination and acidosis respectively (Coelho et al., 2020; Nummer, 2013).

Throughout the fermentation process, a decline in pH values within the culture medium serves as an indicator of the metabolic activity of *kombucha* microorganisms on the substrate (Treviño-Garza et al., 2020). According to Goh et al. (2012a), there is a significant drop in pH during fermentation when sugar is converted to acid. The concentration of sugar further influences the pH value. Research findings suggest that systems with a higher initial sugar concentration tend to exhibit a more

pronounced and drastic reduction in pH (Goh et al., 2012a). This decline in pH, caused by accumulation of organic acids as metabolic by-products, creates challenging conditions for bacterial growth and hinders the productivity of cellulose production, affecting the overall *scoby* yield. Hence, precise control of pH levels during *kombucha* fermentation is crucial for successful development of high-quality *kombucha*; likewise, pasteurisation is required to prevent further acidification (Coelho et al., 2020).

2.4.2 Titratable acidity (TA)

Titratable acidity (TA) is the concentration of acidic compounds in a solution and it provides valuable insights into the overall acidity (Sadler & Murphy, 2010). It provides a better prediction of the impact of acids on flavour compared to pH alone (Sadler & Murphy, 2010). This is crucial as organic acids are one of the end products of *kombucha* fermentation process and are primarily responsible for imparting the characteristic acidic flavour traits. Typically, a higher TA is associated with a stronger sour aroma and flavour profile. In the *kombucha* fermentation process, there is an interconnected relationship between TA and pH (Chakravorty et al., 2016; Kaewkod et al., 2019). As fermentation progresses, the production of various organic acids leads to an increase in TA. These organic acids include acetic, glucuronic, gluconic, lactic, malic, citric, tartaric, folic, malonic, oxalic, succinic, pyruvic and usnic acids (Neffe-Skocińska et al., 2017). Consequently, the rise in TA results in a decrease in pH, indicating a lower pH value (Chakravorty et al., 2016; Kaewkod et al., 2019). The combination of low pH and high TA can be associated with the metabolic conversion of sugars into organic acids by microbes that are capable of sustaining such niches (Treviño-Garza et al., 2020). This unique environment created

by the growth of these microbes not only facilitates the production of organic acids but also provides a level of protection against invasive contaminants (Treviño-Garza et al., 2020). The fermentation process should be terminated when TA reaches the value of 4 to 5 g/L in order to have a pleasant sour beverage with palatability, appealing and satisfactory sensory qualities (Markov et al., 2012; Villarreal-Soto et al., 2018; Waisundara, 2019). Hence, the extent of *kombucha* fermentation can be controlled more accurately by monitoring TA value. TA can be expressed as percent of acetic acid according to the formula below (Sadler & Murphy, 2010):

$$\% \text{ acid (wt/vol)} = \frac{(N)(V_1)(Eq\ wt)}{(V_2)(1000)} \times 100 \quad (2.1)$$

where:
N = normality of titrant, NaOH (mEq/ml)
V_1 = volume of titrant (ml)
$Eq\ wt$ = equivalent weight of predominant acid (mg/mEq)
V_2 = volume of sample (ml)
1000 = factor relating mg to g (mg/g)

2.4.3 Total soluble solids (^{o}Brix)

Brix reading expresses the amount of dissolved or soluble solids in a solution. Indicated in Brix degrees (^{o}Brix), it is a measure of sucrose concentration in a solution. One Brix degree can be defined as one gram of total soluble solids in a hundred grams of liquid solution. Measuring ^{o}Brix throughout fermentation of *kombucha* provides information into the availability of sucrose for yeast and the metabolic activity of glycolysis, enabling a comprehensive understanding of the sugar utilisation and fermentation process. The ^{o}Brix value in *kombucha* is dynamic and undergoes changes over time, reflecting its strong dependence on the various biochemical transformations occurring during fermentation. In Jakubczyk et al. (2020), analysis of sugar content presented that all tested tea types, including black,

green, white and red tea, exhibited the highest concentration of sucrose prior to the fermentation process. As the fermentation continued, total soluble solids decreased as yeast used sugar to produce ethanol. When it comes to *kombucha* prepared with black tea, total soluble solids decreased with progressing fermentation, achieving the lowest value on the 14th day of fermentation with 7.5 ^{o}Brix (Jakubczyk et al., 2020). In the case of *kombucha* made from green and red tea, the initial addition of *scoby* led to a decrease in total soluble solids, followed by a gradual increase over time, eventually approaching the initial ^{o}Brix value by the 7th day of fermentation (Jakubczyk et al., 2020). However, the results showed declination after day 7, with green tea *kombucha* reaching a value of 8.75 ^{o}Brix and red tea *kombucha* reaching 9.5 ^{o}Brix. In contrast, study by Kaewkod et al. (2019) shows that total soluble solids of *kombucha* prepared from black, oolong and green tea decreased significantly from 10 to 6 ^{o}Brix after 15 days of fermentation.

2.4.4 Alcohol content

Kombucha contains alcohol as a by-product of the fermentation process. The alcohol content of *kombucha* can exhibit variability due to several factors, such as the specific tea variety employed, the duration of fermentation process and the prevailing environmental conditions during fermentation (Jakubczyk et al., 2020; Yang et al., 2022). The presence of trace amounts of ethanol in *kombucha* has a few purposes, including acting as an antimicrobial agent, serving as a solvent to extract nutrients from the ingredients, facilitating the absorption of nutrients by the consumer and protecting the human body from cardiovascular diseases (*Kombucha Brewers International*, 2021; Leal et al., 2018). However, *kombucha* typically contains low levels of alcohol as the fermentation process is primarily driven by acetic acid

bacteria and yeast that produce organic acid rather than ethanol (Mellor et al., 2020). The yeast in *kombucha* produces ethanol as a by-product of its metabolism while the acetic acid bacteria in *kombucha* convert most of the ethanol into acetic acid and other organic acids (Leal et al., 2018). As a result, *kombucha* contains low levels of alcohol content. The small quantities of ethanol found in *kombucha* do not usually have an intoxicating effect as they are mixed with organic acids which counteract the harmful properties of alcohol (*Kombucha Brewers International*, 2021). However, for industrial production, it is necessary to monitor the alcohol content of *kombucha* to ensure compliance with regulations regarding the permissible level of alcohol in beverages. According to the Centre of Disease Control of British Columbia (BCCDC), Canada, alcohol levels should not exceed 1% or increase during the shelf life of the product (de Miranda et al., 2022). Based on *Kombucha* Brewers International 2021, *kombucha* should not contain more than 1.25% alcohol by volume (de Miranda et al., 2022; *Kombucha Brewers International*, 2021). The Department of Islamic Development Malaysia (JAKIM), which regulates the Malaysian Halal certification board, mandates a maximum ethanol content of 1% ABV in non-alcoholic beverages (Najiha & Nadiah, 2014; Sharifudin et al., 2021).

2.4.5 Total polyphenol content

Understanding the total polyphenol content of *kombucha* is crucial as the purported health-promoting properties of *kombucha* have been ascribed to the presence of phenolic compounds (Ayed et al., 2017; La Torre et al., 2021). Polyphenols are a class of plant-derived phytochemicals that are commonly found in various foods including tea (Jayabalan & Waisundara, 2019). The incorporation of tea as a primary ingredient in *kombucha* contributes to the presence of polyphenols. During the

fermentation process, some of the complex phenolic compounds might undergo degradation (Jayabalan et al., 2007; Watawana et al., 2015). In earlier research, the degradation of epicatechin isomers was noticed during fermentation of *kombucha* (Jayabalan et al., 2007). This degradation process is facilitated by the acidic environment of *kombucha* and the presence of enzymes such as phytase, α-galactosidase and tannase, released by microorganisms in *scoby* (Jafari et al., 2020; Kaewkod et al., 2019; Watawana et al., 2015). These enzymes facilitate the breakdown of complex polyphenolic compounds in tea, resulting in the formation of smaller and more bioavailable molecules (Jafari et al., 2020; Kaewkod et al., 2019; Watawana et al., 2015). As a result, *kombucha* tea contains a significant amount of total phenolic compounds, which provide a diverse range of health benefits. *Kombucha* tea exhibits the potential to modulate the immune system, reduce β-cell damage and effectively lower blood glucose levels, surpassing the effects of black tea itself due to its greater polyphenol concentrations (Aloulou et al., 2012; Bhattacharya et al., 2011). In addition, a number of studies have provided ample evidence for the strong candidacy of *kombucha* in alleviating oxidative stress and free radicals as well as enhancing enzymatic defenses (Aloulou et al., 2012; Gharib, 2009; Murugesan et al., 2009). Recent research also suggests that polyphenols have the potential to positively influence human health by modulating gut microbiota (Singh et al., 2019). Polyphenols have been demonstrated to help mitigate the risk of a wide range of diseases and conditions through various mechanisms, for instance, metabolisation by gut bacteria and promotion of beneficial bacterial growth while inhibiting harmful bacteria (Singh et al., 2019; Wang et al., 2022), highlighting their significant influence on overall well-being. However, the total polyphenol content of *kombucha* is influenced by several factors, including the type of ingredients and the

duration of fermentation process. The total polyphenol content in black tea *kombucha* has been reported as 234.1 mg GAE/L after 1 month of fermentation (La Torre et al., 2021), 412 mg GAE/L after 7 days of fermentation (Ivanišová et al., 2020), 420 mg GAE/L after 7 days of fermentation (Ivanišová et al., 2019) and a range of 201-320 mg GAE/L between 1 and 14 days of fermentation in black, green, white and red tea *kombucha* (Jakubczyk et al., 2020). Conducting total polyphenol content analysis enables comparisons between different samples and facilitates the exploration of the relationship between polyphenol contents and other properties, such as the yield of *scoby*, offering valuable information for achieving desired product characteristics.

2.4.6 Yield of *scoby*

The fermentation conditions (Table 2.3) play a vital role in shaping the formation of various metabolites and affecting the growth and metabolism of microbes, consequently influencing the yield of *scoby* (Laavanya et al., 2021). Therefore, the assessment of bacterial cellulose yield becomes indispensable as it offers insights into the multifaceted metabolic pathways triggered by different ingredients, ultimately enhancing *kombucha* production and maximisng *scoby* yield.

Table 2.3: Summary of *kombucha* fermentation requirement

Requirements	Descriptions	References
Ingredients	Tea, sugar, *scoby* (symbiotic culture of bacteria and yeast)	Kim & Adhikari (2020)
Additional ingredients	Starter liquid (previously fermented *kombucha*)	Dutta & Paul (2019) & Goh et al. (2012)
Fermentation time	Typically 7 to 10 days	Kim & Adhikari (2020)
Conditions	Temperature (18 to 30^{o}C)	Kim & Adhikari (2020)
pH level	2.5 to 4.2	Coelho et al. (2020) & Nummer (2013)
Equipment	Clean and sanitised utensils	Nummer (2013)

The bacterial cellulose growth and substrate utilisation are brought together using the concept of yield, which is the ratio of amount of *scoby* cellulose produced to the amount of substrate consumed, as described in Equation 2.2 (Goh et al., 2012a; Treviño-Garza et al., 2020). Table 2.4 shows *kombucha* fermentation with different parameters and corresponding weight and yield of *scoby*. In certain circumstances, the yield is in correlation with the carbon source in the system, in which the higher the sugar content, the higher the yield of *scoby*. However, when a surplus quantity of sugar is utilised, it results in the production of increased metabolic products which leads to product inhibition and hence hinders the synthesis of cellulose in *scoby* (Goh et al., 2012a). Goh et al. (2012a) conducted *kombucha* fermentation with sucrose concentrations ranging from 50 to 250 g/L, the maximum yield of 66.7% (wet weight: 60 g/L) was observed for the system with 90 g/L sucrose. The deliberate selection of tea varieties also exerts a significant influence on the intricate interplay of microbial growth and metabolism, thus impacting the overall yield. Yim et al. (2017) and Gargey et al. (2018) revealed a notable disparity in *scoby* production yield when using green tea, showcasing a remarkable superiority over the use of black tea.

$$\text{Yield (\%)} = \frac{\text{Wet weight of } scoby \text{ (g/L)}}{\text{Sucrose concentration (g/L)}} \times 100 \qquad (2.2)$$

Table 2.4: *Kombucha* fermentation with different parameters and corresponding weight and yield of *scoby*

No.	Tea substrate	Carbon source	Media components and concentration	Fermentation volume and vessel	pH	Temp. (°C)	Duration (days)	Weight and yield of *scoby*	References
1	Black	Sucrose	Tea: 18 g/L Sucrose: 70, 90, 110 g/L KT: 10% v/v *Scoby*: 3% w/v	1 L beaker	2.7-3.0	30 ± 3	8	Wet weight: 30.80 ± 0.57 g/L for 70 g/L sucrose 60.00 ± 0.28 g/L for 90 g/L sucrose 29.55 ± 0.92 g/L for 110 g/L sucrose Yield: 47.9% for 70 g/L sucrose 66.7% for 90 g/L sucrose 44.0% for 110 g/L sucrose	Goh et al. (2012a)
2	Black	Sugar	Tea: 5, 10, 15, 20% Sugar: 5% KT: 5-10% *Scoby*: 1% w/v	100 ml flask	3.0-7.5	25-45	7	Wet weight: 0.69-34.1 g/L	Ali & Shivanna (2017)
3	Black	Brown sugar	Tea: 10, 12, 24, 48 g Sugar: 9, 15 g *Scoby*: 20-37 g	0.6-1 L glass jar	5.6	-	56	Wet weight: 46-67 g for 10 g tea and 15 g sugar 21-34 g for 12 g tea and 9 g sugar 20-29 g for 24 g tea and 9 g sugar 18-21 g for 48 g tea and 9 g sugar	Amarasinghe et al. (2018)
4	Black	• White refined sugar • Coconut palm sugar • Molasses sugar	Tea: 1.2% w/v Sugar: 10% w/v KT: 10% v/v *Scoby*: 3% w/v	100 ml container	-	24 ± 3	14	Dry weight: 101.31 ± 0.09 g for white refined sugar 34.18 ± 0.00 g for coconut palm sugar 27.81 ± 0.18 g for molasses sugar	Muhialdin et al. (2019)

Table 2.4: Continued

5	Black	Glucose	Tea: 5 g/L Glucose: 20, 40, 60, 80, 100 g/L KT: 10% v/v *Scoby*: 2% w/v	1 L beaker	3.0	30 ± 1	20	Dry weight: 2.7-13.3 g/L for fresh tea leaves 2.0-13.8 g/L for used tea leaves Yield: 6.3-22.5% for fresh tea leaves 7.0-22.0% for used tea leaves	Sharma & Bhardwaj (2019)
6	Black and green	Sucrose	Tea: 6 g Sucrose: 50 g KT: 10 ml *Scoby*: 3 g	100 ml flask	-	20-22	18	Wet weight: 1-11 g for black tea 3-51 g for green tea 4-22 g for tea waste	Gargey et al. (2018)
7	Black and green	• Sucrose • Fructose • Honey • Corn syrup	Tea: 0.3% w/v Sugar: 5% w/v KT: 5% w/v	-	-	25-26	12	Yield: 9% for sucrose 3% for fructose 7% for honey 1% for corn syrup 240% for green tea 180% for black tea	Yim et al. (2017)
8	Green	High fructose corn syrup	Tea: 2.5 g/L Sugar: 12% w/v KT: 10% v/v	2L glass bottle	-	23	30	Wet weight: 461 g	Dima et al. (2017)
9	Green	• Glucose • Dextrose • Fructose • Saccharose	Tea: 3.6 g/L Sugar: 12.5 g/L KT: 10% v/v	500 mL glass container	2.2-2.7	25 ± 2	15	Dry weight: 4-48 g/L Wet weight: 160-350 g/L	Treviño-Garza et al. (2020)

KT: Previously fermented *kombucha* tea

2.5 Microbial analysis

The complexity of understanding *kombucha* fermentation is mainly due to the enormous diversity of microorganism present and symbiotic interplay between them. It is reported that the entire microbial spectrum of *kombucha* is dominated by acetic acid bacteria and yeast (Coelho et al., 2020). Microbial composition may vary depending on factors such as origin, climate, geographic location, substrates, medium used, production conditions and metabolites produced for fermentation process (Coelho et al., 2020; Laavanya et al., 2021). Nonetheless, the generally found microorganisms in *scoby* belong to the genera *Komagataeibacter*, *Gluconobacter*, *Acetobacter*, *Zygosaccharomyces*, *Saccharomyces*, *Schizosaccharomyces* (Laavanya et al., 2021; Nyhan et al., 2022) and *Candida* (Chakravorty et al., 2016). The genera *Komagataeibacter* and *Gluconobacter* dominated *kombucha* tea's microbial community (Chakravorty et al., 2016). Table 2.5 presents the diverse microorganisms commonly identified in *kombucha*. As a result of variations in inoculum sources and fermentation conditions across different studies, the precise composition of *kombucha*'s microbial community cannot be determined indefinitely, implying an inherent variability in the microbial composition of *kombucha*. The microbial types remain consistent in both *scoby* and *kombucha* tea throughout the fermentation process. For instance, *Gluconacetobacter* and *Zygosaccharomyces* were identified as the dominant microbes in *scoby* and *kombucha* tea on the 3rd day and 10th of fermentation (Marsh et al., 2014), *Komagataeibacter* and *Zygosaccharomyces* whose occurrence did not vary for 15 days (Barbosa et al., 2021), *Komagataeibacter* and *Dekkera* remained dominant up to 14 days of fermentation (Gaggìa et al., 2018).

Table 2.5: Comparison of microbial composition among different *kombucha scoby* sources

No	*Scoby* source	Tea substrate	Temp. (°C)	*Scoby*		Fermented *kombucha*		Methods used for identification	References
				Bacteria	Yeast	Bacteria	Yeast		
1.	Australia	Black	20-22		*Z. bailii* *Sz. pombe* *T. delbreuckii* *R. mucilaginosa* *Brett. bruxellensis* *C. stellata*		*Z. bailii* *Sz. pombe* *T. delbreuckii* *R. mucilaginosa* *Brett. bruxellensis* *C. stellata*	The Biolog Microstation system, physiology and morphological tests	Teoh et al. (2004)
2.	Brazil	Black	-	*Kb. hansenii* *Kb. europaeus* *Kb. xylinus* *Sg. melonis* *Kb. rhaeticus*	*Z. bailii* *R. mucilaginosa* *S. cerevisiae* *Malassezia* spp.	*Kb. hansenii* *Kb. europaeus* *Kb. xylinus* *Sg. melonis* *Kb. rhaeticus*	*Z. bailii* *R. mucilaginosa* *S. cerevisiae* *Malassezia* spp.	Sanger sequencing	Barbosa et al. (2021)
		Green	-	*Kb. hansenii* *Kb. europaeus* *Kb. xylinus* *Sg. melonis* *Kb. rhaeticus*	*Z. bailii* *R. mucilaginosa* *S. cerevisiae* *Malassezia* spp.	*Kb. hansenii* *Kb. europaeus* *Kb. xylinus* *Sg. melonis* *Kb. rhaeticus*	*Z. bailii* *R. mucilaginosa* *S. cerevisiae* *Malassezia* spp.		
3.	Canada	Black	23	*Gluconacetobacter* spp.	*Dekkera* spp. *Zygosaccharomyces* spp. *Davidiella* spp. *Pichia* spp. *Wallemia* spp. *Lachancea* spp. *Leucosporidiella* spp.	*Acetobacter* spp. *Gluconacetobacter* spp. *Lactobacillus* spp. *Lactococcus* spp. *Leuconostoc* spp. *Bifidobacterium* spp. *Thermus* spp.	*Dekkera* spp. *Zygosaccharomyces* spp.	DNA amplification and high-throughput sequencing	Marsh et al. (2014)
4.	India	Black	24-27	*A. aceti MTCC 2945.*	*Z. bailii* *Brett. claussenii*			-	Jayabalan et al. (2010)

Table 2.5: Continued

5.	Indonesia	Black	28-30			*Komagataeibacter* spp. *DS1MA.62A* *Kb. xylinus* *Kb. saccharivorans* *G. saccharivorans*	*Brett. bruxellensis*	DNA amplification and high-throughput sequencing	Angela et al. (2020)
6.	France	Black	-	*O. oeni* *Lq. nagelii* *A. okinawensis* *G. eurapaeus* *G. hansenii* *G. intermedius* *Gb. oxydans* *A. tropicalis*	*D. bruxellensis* *D. anomala* *H. valbyensis* *C. boidinii* *S. uvarum* *P. membranifaciens* *Z. bailii* *Zt. florentina*	*O. oeni* *Lq. satsumensis* *G. eurapaeus* *G. liquefaciens* *Gb. cerinus* *Gb. oxydans* *Gb. saccharivorans* *Gb. oboediens*	*D. bruxellensis* *D. anomala* *H. valbyensis* *Wm. anomalus* *Zt. florentina*	M13-PCR typing and bacteria species identifications; FTIR spectroscopy yeast dereplication and identification	Coton et al. (2017)
		Green	-	*O. oeni* *Lq. nagelii* *A. okinawensis* *A. tropicalis* *G. eurapaeus* *G. intermedius* *G. xylinus*	*D. anomala* *D. bruxellensis* *H. valbyensis* *S. cerevisiae* *Z. bailii* *Zt. florentina*	*O. oeni* *Lq. nagelii* *A. lovaniensis* *A. peroxydans* *A. syzygii* *A. tropicalis* *G. xylinus* *Tc. sakaeratensis* *Gb. oxydans*	*C. boidinii* *D. anomala* *D. bruxellensis* *H. valbyensis* *T. microellipsoides* *Zt. florentina*		
7.	Germany	Black	-	*Gb. oxydans*				Sanger sequencing	Hopfe et al. (2017)
8.	Mexico	Black	32	*Gb. oxydans* *A. aceti*	*S. cerevisiae* ATCC 18824 *Brett. bruxelensis* NRRL Y-1411 *Km. marxianus* NRRL Y-8281			Morphological and physiological tests	Belloso-Morales & Hernández-Sánchez (2003)

Table 2.5: Continued

9.	Singapore	Black	-	*A. xylinum* *A. xylinoides* *A. aceti* *A. pausterianus* *B. gluconicum*	*Kloeckera* spp. *Sz. pombe* *S. ludwigii* *S. cerevisiae* *Torulaspora* spp. *Z. bailii* *Pichia* spp.			-	Amarasinghe et al. (2018)
10.	Unknown	Black	25	*A. senegalensis* *A. tropicalis* *A. musti* *A. peroxydans* *Gb. oxydans* *G. europaeus* *Kb. xylinus* *Kb. rhaeticus* *Kb. intermedius* *Kb. saccharivorans* *O. oeni* *Pseudomonas* spp.	*Brett. bruxellensis* *Brett. anomalus* *P. fermentans* *C. sake* *Lh. fermentati* *Sz. pombe* *Z. bailii*	*A. senegalensis* *A. tropicalis* *A. musti* *A. peroxydans* *Gb. oxydans* *G. europaeus* *Kb. xylinus* *Kb. rhaeticus* *Kb. intermedius* *Kb. saccharivorans* *O. oeni* *Pseudomonas* spp.	*Brett. bruxellensis* *Brett. anomalus* *P. fermentans* *C. sake* *Lh. fermentati* *Sz. pombe* *Z. bailii*	DNA amplification and high-throughput sequencing	Huang et al. (2022)

Abbreviations: *A.*, *Acetobacter*; *B.*, *Bacterium*; *Brett.*, *Brettanomyces*; *C.*, *Candida*; *D.*, *Dekkera*; *Gb.*, *Gluconobacter*; *G.*, *Gluconacetobacter*; *Km.*, *Kluyveromyces*; *Kb.*, *Komagataeibacter*; *Lh.*, *Lachancea*; *Lq.*, *Liquorilactobacillus*; *O.*, *Oenococcus*; *P.*, *Pichia*; *R.*, *Rhodotorula*; *S.*, *Saccharomyces*; *Sd.*, *Saccharomycodes*; *Sz.*, *Schizosaccharomyces*; *Sg.*, *Sphigomonas*; *Tc.*, *Tanticharoemia*;; *T.*, *Torulospora*; *Wm.*, *Wickerhamomyces*; *Z.*, *Zygosaccharomyces*; *Zt.*, *Zygotorulaspora*.

There are various approaches in determination of *kombucha*'s microbial consortium, some of which are listed in Table 2.5 and one of the methods is the total viable count, which allows quantitative estimation of microorganism concentrations. For instance, studies have reported that the enumeration of acetic acid bacteria and yeast has higher microbial counts in *kombucha* tea compared to *scoby* (Chen & Liu, 2000; Laavanya et al., 2021; Marsh et al., 2014). As for the *scoby*, Laavanya et al. (2021) reported that the concentration of acetic acid bacteria was found to be higher on the upper part of the *scoby* which is more exposed to oxygen than *kombucha* tea beneath it. The microbial cells in *kombucha* increased for 6-10 days of fermentation in black tea (Goh et al., 2012a; Shi et al., 2023; Tran et al., 2020) and apple substrate (Zubaidah et al., 2018) and decreased after 14 days of fermentation (Tran et al., 2020; Zubaidah et al., 2018). The replication of microbial organisms comes to a halt after 14 days due to the production of various metabolite compounds that inhibit growth of microbial organisms (Zubaidah et al., 2018).

2.6 Next-Generation Sequencing (NGS)

In addition to the total viable count, a great number of culture-independent, molecular sequencing strategies have been developed to identify and quantify food-associated microbial groups and provide composition and diversity of complex microbial communities. The Next-Generation Sequencing technique is one of them. Next-Generation Sequencing (NGS) technique has revolutionised and advanced the metagenomics field through increased throughput (Arıkan et al., 2020). The sequencing allows production of thousands or even millions of sequences for precise identification of microbial taxa at the same time (Mayo et al., 2014). To date, numerous NGS-based microbiome studies investigating potential effects of different

parameters on *kombucha* have been conducted (Arıkan et al., 2020; De Filippis et al., 2018; Marsh et al., 2014).

Arıkan et al. (2020) conducted 16S rRNA and Internal Transcribed Spacer 1 (ITS1) sequencing using Illumina MiSeq platform to investigate the microbial communities of homemade *kombucha* fermentation from day 3 to day 15. Taxonomic analyses results from 16S rRNA revealed that 99% of the sequences assigned to the *Proteobacteria* phylum belong to *Komagateibacter* genus with relative abundance between 85.6% and 99.3% from the *Acetobacteraceae* family whereas ITS1 results revealed that genus *Zygosaccharomyces* dominated the fungal community (>99%) across all samples. De Filippis et al. (2018) explored effect of fermentation temperature on the bacterial community in *kombucha* using GS Junior platform. *Gluconacetobacter* was revealed as dominant genus in *kombucha* fermentation and was consistent with results reported in Marsh et al. (2014) and Coton et al. (2017). Elevated fermentation temperatures were found to have created an environment conducive to the growth of sub-dominant bacterial populations, including environmental contaminants such as *Acinetobacter* and *Propionibacterium*, and lactic acid bacteria such as *Lactobacillus* and *Streptococcus*. Coton et al. (2017) reported that presence of different bacterial communities from different tea types where lactic acid bacteria were mostly present during green tea fermentation while acetic acid bacteria dominated black tea fermentation. In summary, the application of NGS techniques is believed to have presented a significant advancement in microbial analysis, offering a powerful and effective means to explore the complex microbial composition and diversity in *kombucha*.

2.7 Mathematical modelling

In addition to the physicochemical and microbial analyses, the application of mathematical modelling offers a holistic approach to analyse the process of fermentation, thus allowing a deeper understanding of the dynamic interactions within the system. Examples of mathematical models used for various fermentation processes are listed in Table 2.6, each serving different purposes from describing substrate utilisation to predicting product formation and responses to environmental factors. The equations for the models and their corresponding parameters are stated in Table 2.7. The equations of sigmoidal models, such as Logistic, Gompertz, Richards and Schnute were originally developed to describe growth curves, however, they have found broader applications beyond their initial disciplines (Zwietering et al., 1990), such as those stated in Table 2.6. These models have been subsequently modified to provide parameters with biological meaning (Zwietering et al., 1990). The modified Gompertz model, a three-parameter model is the most commonly used and often provides a better fit to the data (Zwietering et al., 1990). It is preferred over the four-parameter model as it is simpler, more robust and provides more stable estimates with more degrees of freedom, which is particularly crucial when using a growth curve with a limited number of measured points (Erkmen & Alben, 2002; Zwietering et al., 1990). Most importantly, all three parameters in the three-parameter model can provide biological meaning compared to the four-parameter model as the fourth parameter in the four-parameter model is a shape parameter and it is difficult to explain biologically (Erkmen & Alben, 2002; Zwietering et al., 1990).

Table 2.6: Mathematical models for fermentation processes

	Description	Model	References
(a) *Kombucha*			
	Kinetics of saccharose fermentation in *kombucha* at two temperatures (22 and 30^{o}C) and in the samples inoculated with two inoculum quantities (10 or 15% (*V/V*))	Boltzmann	Loncar et al. (2014)
	Kinetics of lactose fermentation in milk with *kombucha* starter at two temperatures (37 and 42^{o}C)	Boltzmann	Kanurić et al. (2018)
	Prediction of experimental substrate consumption values and experimental total acidity values of *kombucha* fermentation	Logistic, Luedeking–Piret	Tarhan Kuzu et al. (2023)
(b) *Kefir*			
	Modelling of *kefir* grains growth and pH	Modified Gompertz, Exponential	Goršek & Tramšek (2008)
	Modelling of ethanol production by mixed *kefir* grains yeast as a function of temperature	Modified Gompertz	Zajšek & Goršek (2010)
	Modelling of *kefir* grain biomass growth with influence of various minerals and pH	Richards, Logistic, Gompertz, Multiple multiplicative factor, Quadratic, Polynomial, Exponential	Demirhan et al. (2011)
	Modelling of *kefir* grain biomass growth and pH as a function of propagation time	Richards, Logistic, Gompertz, Multiple multiplicative factor, Quadratic, Polynomial, Exponential, Rational	Apar et al. (2017)
(c) Others			
	Modelling of citric acid production and biomass formation by *Aspergillus niger* in undersize semolina	Gompertz, Logistic, Schnute, Boltzmann, Modified Gompertz	Erkmen & Alben (2002)
	Modelling pH profiles and apparent viscosity development with incubation time of yoghurt	Modified Gompertz	Soukoulis et al. (2007)
	Modelling of acidification kinetics of fermented vegetable-fruit beverages	Modified Gompertz	Yang et al. (2018)

Table 2.7: Mathematical models of fermentation, microbial growth and product formation

Models	Equations	Parameters definition	References
Logistic	$y = \frac{a}{[1 + \exp(b - cx)]}$	a, b, c: coefficients	Zwietering et al. (1990)
Modified Logistic	$y = \frac{A}{\{1 + \exp\left[\frac{4\mu}{A}(\lambda - t) + 2\right]\}}$	μ: specific growth rate, λ: lag time duration, A: asymptotic value	Zwietering et al. (1990)
Gompertz	$y = a\exp[-\exp(b - cx)]$	a, b, c: coefficients	Zwietering et al. (1990)
Modified Gompertz	$y = A\exp\{-\exp[\frac{\mu e}{A}(\lambda - t) + 1]\}$	μ: specific growth rate, λ: lag time duration, A: asymptotic value	Zwietering et al. (1990)
Richards	$y = a\{1 + \exp[b - cx]\}^{(-1/d)}$	a, b, c, d: coefficients	Zwietering et al. (1990)
Modified Richards	$y = A\{1 + v \cdot \exp(1 + v) \cdot \exp[\frac{\mu}{A} \cdot (1 + v)(1 + \frac{1}{v}) \cdot (\lambda - t)]\}^{(-1/v)}$	μ: specific growth rate, λ: lag time duration, A: asymptotic value, v: shape parameter	Zwietering et al. (1990)
Schnute	$y = \{y_1^a + (y_2^a - y_1^a) \cdot \frac{1-\exp[-b(x-c)]}{1-\exp[-b\,(d-c)]}\}^{(1/b)}$	a, b, c, y_1, y_2: coefficients	Zwietering et al. (1990)
Modified Schnute	$y = (\mu \cdot \frac{(1-b)}{a})[\frac{1-b\exp(a\lambda+1-b-at)}{1-b}]^{(1/b)}$	μ: specific growth rate, λ: lag time duration, v: shape parameter	Zwietering et al. (1990)
Polynomial	$y = a + bx + cx^2 + dx^3$	a, b, c, d: coefficients	Apar et al. (2017)
Rational	$y = \frac{a + bx}{1 + cx + dx^2}$	a, b, c, d coefficients	Apar et al. (2017)
Exponential	$y = a\exp(-bx) + c$	a, b, c: coefficients	Goršek & Tramšek (2008)
Boltzmann	$y = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{t-t_0}{\Delta t}}}$	A_1 and A_2: upper and lower asymptotes to the curve, t_0: t-coordinate of the point at which the slope has the highest value, Δt: width of step of an exponential decrease	Loncar et al. (2014)
Luedeking-Piret	$\frac{dP}{dt} = a\frac{dX}{dt} + bX$	P: metabolite secreted, X: biomass concentration, a, b: coefficients	Thierie (2013)
MMF	$y = \frac{ab + cx^d}{b + x^d}$	a, b, c, d: coefficients	Demirhan et al. (2011)

Polynomial, rational and exponential equations are commonly used basis functions for curve fitting which capture non-linear relationship between variables (Sit et al., 1994). Other models such as the Boltzmann model, were originally formulated based on sigmoidal logistic equation to describe behaviours observed when a certain factor

induces a transition between a steady state to another with markedly different magnitudes (Reséndiz-Muñoz et al., 2017). For instance, in some bacterial growth, ample food availability fosters exponential reproduction but eventually leads to a balance between stability and population size due to limited resources (Reséndiz-Muñoz et al., 2017). Luedeking-Piret model is used in cell cultures to elucidate the relationship between production of a metabolite, cell growth and biomass concentration (Luedeking & Piret, 1959; Thierie, 2013) while Multiple multiplicative factor (MMF) model is used for analysing datasets where multiple latent factors may exert influence on each data element (Marlin & Zemel, 2004).

2.8 Jam

Jam is an example of food product to which consumers pay increasing attention for better functionality and health benefits, *e.g.*, those with lower calories and lesser preservative ingredients (Abdullah & Cheng, 2001). Mordor Intelligence (n.d.) stated that the global market for jam, jelly, and preserves is forecasted to reach a CAGR of 4.9% from 2022 to 2027. In Malaysia, the market for spreads which include marmalade, jams, jellies, chocolate spreads, and peanut butter, is expected to grow annually by 7.43% from 2021 to 2025 (Statista, n.d.). Every spread's subcategory is rising with no exception for the jam. The use of jam has gone far beyond toast and sandwiches, benefiting from the increase in home baking and the more leisurely breakfasts that many started during the COVID-19 period. Jam products are not only preserving seasonal and perishable fruits but also enhancing the taste, nutritional value and year-round availability (Garg et al., 2019). The benefits of jam further include its positive impact on blood glucose regulation, as the presence of fiber and fructose content in jam helps to slow down digestion, stabilise blood sugar levels and

enhance satiety (Oo & Than, 2019), making it a valuable addition to a balanced diet. As it is naturally produced from fruits, some food processing industries prefer to use jam in place of caramel for flavouring and colouring of baked products (Ijioma et al., 2017).

Figure 2.5: Jam (Neha, 2020)

Figure 2.5 shows the semi-solid look of jam made by cooking fruits with sugar, with or without added pectin and acid to increase the total soluble solids content to more than 65% (Kaunsar Jabeen Shinwari & Rao, 2018). It can also be described as a blend of fruits and sweetening agent, processed to obtain a suitable gelled structure which may sometimes include other permitted ingredients (Ijioma et al., 2017). The fruits used to make jam can be sourced in a variety of forms, including fresh, dehydrated, frozen, concentrated or previously canned fruit (Featherstone, 2016). Fresh fruits, however, give the best jams (Hui, 2006). Sugar, pectin and acid are incorporated into jam to lower its pH value, bind available water and create an environment which restrains the growth of microorganisms (Garg et al., 2019). There are fruits that possess sufficient acidity and pectin content naturally in the jam making to desired jam texture (Kaunsar Jabeen Shinwari & Rao, 2018). The making of jam has to adhere to quality standards set by the Food Act (1983) and Food Regulations Malaysia (1985) in terms of the following:

- Jam shall be the product prepared by boiling one or more types of sound fruits, whether raw, processed or semi-processed, with a permitted sweetening substance with or without added pectin.
- For the purpose of these regulations, ginger shall be deemed to be fruits. Jam shall contain not less than 35% fruit except that passion fruit jam and ginger jam may contain not less than 6% and 5% of fruit respectively, and 65% of soluble solids determined by refractometer at 20°C, *i.e*., uncorrected for insoluble solids.
- Jam may contain permitted preservatives, permitted colouring substance, permitted flavouring substance and permitted food conditioner.
- There shall be written on the label on a package containing jam made up of more than one type of fruits the words 'mixed fruit jam'.

Figure 2.6 shows the steps and processes involved in its manufacturing process. In the preliminary phase of jam production, it should be noted that the fruit used should be fully mature, exhibit a rich flavour profile and possess the most desirable texture (Hui, 2006). After a thorough washing with water to eliminate any adhering dirt, the fruit is carefully peeled, cut and blended. Subsequently, the blended fruit undergoes a simmering and stirring process to draw out the pectin content of fruit. When it comes to the addition of sugar, the ratio of sugar to fruit may vary depending on factors such as the type of fruit, its ripeness level and the desired outcome, however, the most commonly used proportion of sugar to fruit is typically 1:1 (Hui, 2006). The boiling process holds paramount importance in jam making, as it serves to concentrate the product through evaporation of excess moisture, dissolves sugar and facilitates the union of all ingredients. Throughout the boiling process, the juice or

pulp should be skimmed, if necessary, to remove coagulated material and to diligently stir the mixture to ensure thorough mixing. The jam production is then ended with filling, packaging, cooling and storage.

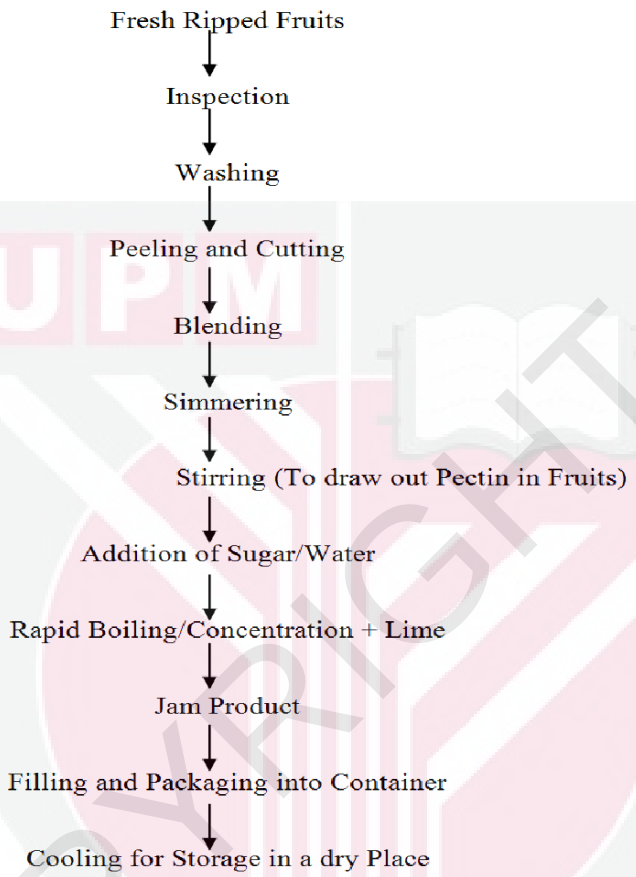


Figure 2.6: Flow chart for jam production (Ijioma et al., 2017)

2.8.1 Ingredients for making jam

The making of jam is essentially the formation of gel using the right amount of ingredients, namely the fruit, sweetening agent, gelling agent and acid (Bastin, 2004). Gelling agents and designated organic acids are incorporated during jam production to address any inadequacies of these substances in the fruits (Hui, 2006). The following paragraphs detail common and main ingredients used in jam-making.

Mango (*Mangifera indica* L.) (Figure 2.7) is one of the most cherished fruits because of its juiciness, sweet taste, exotic flavour and nutritional value (Lawson et al., 2019). It is a rich source of health promoting compounds such as vitamins (A and C), folate, minerals (potassium and calcium) (Kansci et al., 2008; Yadav et al., 2018), phenolic compounds and antioxidants, mainly b-carotene (Razak et al., 2018). The mango pulp is reported to be rich in pectin and could be used for jam preparation without additional pectin (Kansci et al., 2008).

The limited storage life of mangoes during their off-season restricts the commercialisation of mango products. Mangoes cannot be stored for long and can only be stored for a relatively short period of 4 to 8 days at room temperature (Razak et al., 2018; Yadav et al., 2018). The perishable nature of mangoes has encouraged efforts to explore the feasibility of processing mango fruits into a range of satisfactory products, including jams, mango nectar, pickles, mango juice, mango concentrates and puree sauces (Nour et al., 2011; Wongkaew et al., 2021). In making jam, it is essential to ensure a minimum fruit content of 350 grams to produce 1000 grams of finished product and not less than 450 grams per 1000 grams for the manufacture of ‘‘extra jam’’ (Javanmard & Endan, 2010).

Figure 2.7: Mango (*Mangifera indica* L.)

Hydrocolloids have a diverse range of functional properties in food applications. In jam production, they find specific use as gelling agents (Saha & Bhattacharya, 2010). The addition of a gelling agent to jam is recommended for fruits that have low pectin content, compensating for the deficiency in natural pectin or lack of uniformity in pectin levels (Featherstone, 2016) and ensuring that the resulting jam achieves desirable gel strength and palatable consistency (Ramaswamy et al., 1996). This is because gelling agents consist of a polymer network that effectively retains water, preventing it from flowing away (Hui, 2006).

A wide variety of gelling agents have become readily available such as traditional hydrocolloids that are plant–derived ingredients, *e.g.*, pectin, modified celluloses, modified starches, guar gum, konjac mannan, locus bean gum, and exudate gums; seaweed-derived ingredients, *e.g.*, agar, alginates, carrageenan and animal-derived ingredients, *e.g.*, collagen (Goff & Guo, 2019). Among all, pectin remains the universal choice for jam making (Hui, 2006; Ramaswamy et al., 1996). It is available in two types, *i.e.*, high methoxyl and low methoxyl pectin (Hui, 2006). Pectin is a polysaccharide found in plant cell walls and contains a linear chain comprised of D-galacturonic acid residues connected through α(1-4)-glycosidic bonds, where they function as a hydrating agent and cementing material for the cellulosic network (Khan et al., 2014; Vanitha & Khan, 2019). Besides the ability to form a mesh that traps liquid and holds suspended pieces of fruit, the two kinds of pectin are relatively stable at low pH levels, which makes them suitable to be used in fruit jams (Hui, 2006). Besides pectin, cellulose derivatives such as carboxymethyl cellulose (CMC) is also an acceptable gelling agent in food industry due to its thickening ability (Imeson, 2012; Javanmard et al., 2012). Alginate finds applications in bakery jams

due to its rapid setting behaviour and capability of forming gels without prior heating (Saha & Bhattacharya, 2010). Starch is also used as a gel ingredient in bakery jams for pie, cake and pastry fillings as it prevents flow and melt of the filling before biting (Javanmard et al., 2012). Other gelling agents such as carrageenan which provides only fiber with no nutritional value also find use in the food industry (Jancikova et al., 2019).

The introduction of innovative hydrocolloids, including gums derived from the fermentation process, including curdlan, dextran, xanthan and bacterial celluloses has emerged in recent years (Goff & Guo, 2019). Bacterial cellulose is a naturally occurring polymer that is produced by various bacterial species through a fermentation process (Gatenholm & Klemm, 2010; Guo et al., 2018), with *scoby* being one example. Bacterial cellulose, consisting of a fiber network structure, is formed by polymerisation of monosaccharides connected by β(1–4) glycosidic linkages in chain-like form (Knöller et al., 2020; Kruk et al., 2021). While bacterial cellulose and plant cellulose are chemically the same, bacterial cellulose exhibits unique physical and chemical properties, including high purity (free of lignin, hemicellulose and other biogenic compounds) and a high degree of polymerisation (Chau et al., 2008). Bacterial cellulose has a larger surface area of 24.709 m^2/g and pore volume of 0.089 cc/g compared to plant cellulose which measures 5.581 m^2/g and 0.019 cc/g, respectively (Ul-Islam et al., 2019). Its ultrafine network architecture renders it 100 times thinner while also exhibiting a remarkable water holding capacity that is 100 times greater compared to cellulose fibers derived from plants (Chau et al., 2008; Coelho et al., 2020). The notable high water holding capacity of 84.4% (Gayathry & Gopalaswamy, 2014) is advantageous in facilitating the

formation of gels and maintaining texture stability in products by preventing syneresis (Younis et al., 2015). Syneresis occurs when water is no longer bound within the food system and poses a potential risk to various food products, including jam (Younis et al., 2015). The bacterial cellulose has obtained ‘‘generally recognised as safe’’ (GRAS) status from the United States Food and Drug Administration (FDA), indicating that it is deemed safe for consumption as a food substance (Lin et al., 2020; Sharma & Bhardwaj, 2019). It has been explored as a food ingredient in ice cream for its potential in decreasing total calories, in maintaining its shape for at least 60 minutes after being removed from the freezer, in improving melting resistance and textural properties (Guo et al., 2018) and in Chinese-style meatballs which have helped in maintaining humidity of the meat and enhancing shelf stability properties without causing a detrimental effect on texture (Lin & Lin, 2004). Given its distinctive physicochemical features, bacterial cellulose of *scoby* is recognised for its potential as a functional food matrix, serving as a natural binder, thickener, texturiser and emulsion stabiliser (Goh et al., 2012a; Mohite & Patil, 2014). In addition, *scoby* is rich in crude fiber, crude protein and amino acid lysine (Jayabalan et al., 2014), making it a valuable source of dietary fiber and a promising low-calorie food ingredient with antioxidant and anti-microbial properties (Emiljanowicz & Malinowska-Pańczyk, 2020; Goh et al., 2012a). It contains 179.38 ± 0.04 g/kg dry matter of crude protein, 120.00 ± 0.03 g/kg dry matter of crude fiber, 44.14 ± 0.02 g/kg dry matter of crude lipid and 26.40 ± 0.04 g/kg dry matter of ash (Murugesan et al., 2005). In the context of mango jam, several studies have been conducted on exploring the use of different gelling agents, such as high methoxyl pectin, CMC, sago starch, xanthan gum, Arabic gum and guar gum (Javanmard et al., 2012; Razak et al., 2018). In light of the properties of *scoby*, such as its high water holding

capacity, superior purity, stability at low pH levels, and substantial degree of polymerisation, it presents itself as a viable substitute for conventional hydrocolloid in the production of jam.

Besides gelling agent, the addition of **sugar** to jam is vital as it contributes to soluble solids, which is crucial for achieving the desired physical, chemical, and microbial stability of jam (Basu & Shivhare, 2010). A deficiency in sugar content can lead to a decrease in total soluble solids, resulting in a diluted and low-consistency product. Sugar boosts gel-forming capability of the jam by drawing water to itself, thereby acting as a natural preservative and inhibiting the growth of detrimental microorganisms. Sugar also serves as a flavouring agent in jam, imparting its characteristic sweetness, adding body, controling viscosity which contributes to texture (Bastin, 2004; Hui, 2006) and improving appearance (colour and shine) (Basu & Shivhare, 2010). Hence, the incorporation of 40-75% sucrose in jam is essential as it imparts these various functionalities to the final product (Basu & Shivhare, 2010). The jam-making recipes call for a formulation of 45:55 fruit to sugar ratio (Featherstone, 2016). Since the percentage of sugar content for the production of jam is high, choosing a suitable type of sugar is vital due to the potential of sugar for recrystallisation. Sugars that have high tendency to crystallise like pure dextrose (glucose) are not used, in contrast, refined sucrose is recommended to be added to jam due to its low tendency to recrystallise (Javanmard & Endan, 2010).

2.8.2 Physicochemical properties of jam

Physicochemical analysis which includes the measurement of pH value, moisture content, water activity and colour, plays an essential role in assessing the quality and development of jam products. The pH value acts as an indicator of the quantity of organic acids present (Featherstone, 2016; Gindi et al., 2019), influencing both the gel formation and flavour of jam. Monitoring water activity and moisture content is important in determining whether a product supports the growth of a spoilage microorganism. Colour influences our judgement of food and acceptance of jam.

pH is a crucial factor in achieving ideal gel condition in jam production (Sutwal et al., 2019). Typically, the pH of jams falls within the range between 2.5 and 3.4, subject to the types of fruits used (Featherstone, 2016; Gindi et al., 2019). This specific pH range is crucial as it contributes to flavour, helps formation of gel-like consistency through the action of pectin and ensures proper setting of the jam (Featherstone, 2016; Gindi et al., 2019). Right amount of acid gives rise to gel formation, as too little acid will result in a failure to set while too much acid can lead to undesirable weeping in gel (Bekele et al., 2020). Acid aids in releasing pectin that is naturally contained within fruit cells during the heating process with sugar. Pectin is dissolved and undergoes a process of conversion to an unbound state, wherein the strands of pectin repel each other due to their negative electric charge (Bekele et al., 2020). When acid is added, it lowers the pH of the jam mixture, which also neutralises the negative charges on pectin strands so they can assemble into the network that will set the jam (Bekele et al., 2020). Maintaining the appropriate acidity level is important as it facilitates conversion of added sucrose during the cooking process, preventing crystallisation and giving an imperative effect on the

gelatinisation property of pectin (Bekele et al., 2020). The acidic pH range in jam also inhibits bacterial growth thus, ensuring better quality and a longer shelf life of the product (Emelike & Akusu, 2019; Gindi et al., 2019). pH values for mango jams have been reported at pH 2.5 (Kansci et al., 2008), pH 2.45 (Emelike & Akusu, 2019) and from 3.46 to 3.60 (Krishna et al., 2020). Notably, it was observed that jams with pH values of 2.55 and above exhibited a firmer texture compared to those with lower pH (Kansci et al., 2008).

Water activity (a_w) measurement is also an important basic element in foods including jam. By definition, it is the ratio of partial pressure of water in the substrate (ρ) and partial pressure of pure solution (ρ_0) under the same condition respectively (Sandulachi, 2012):

$$a_w = \frac{\rho}{\rho_0} \tag{2.3}$$

The water activity of a food characterises the energy property of water in the food and therefore the capability to serve as a solvent, take part in chemical processes and provide information regarding the possible growth of microorganisms (Sandulachi, 2012). Water activity ranges from 0 to 1. Zero water activity happens when water is tightly bound and does not have the propensity to escape from food in the form of vapour. Water activity is greater with products that are perishable as there is free water accessible for chemical reactions, facilitating microbial development and serving as a transportation medium for compounds (Sandulachi, 2012). Figure 2.8 displays the water activity thresholds for growth of various microbes.

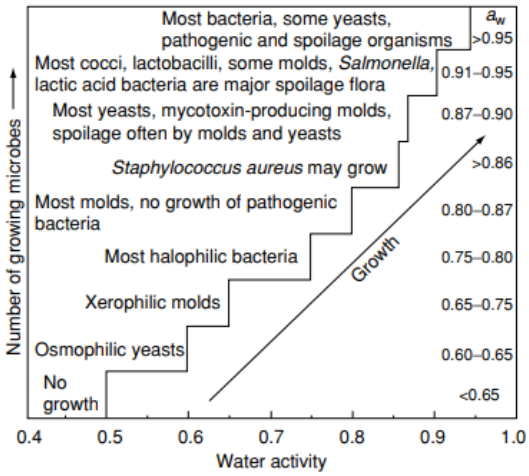


Figure 2.8: Growth of various microorganisms at different water activity conditions (Roos, 2003)

There will be an increasing likelihood of the growth of various spoilage microorganisms with increased water activity (Roos, 2003). Hence, control of water activity is crucial for ensuring safety, stability and food security of jam, with an expected range of 0.80 to 0.87 as reported by Roos (2003), where pathogenic bacteria are unable to grow. In some of the studies, water activity of mango jam samples were reported to be 0.81 (Tapia et al., 2020) and a range of 0.583-0.850 (Javanmard et al., 2012).

Water activity measures the free, unbound water of product while **moisture content** measures both, the free and bound water of product. The moisture content refers to the amount of water available relative to other components in jam. It is an intricate factor that influences the taste, texture and longevity of the product. High levels of moisture content can affect the viscosity of a product, resulting in an undesirable product. Additionally, excess moisture content can lead to microbial growth spoilage and shorten the shelf life of the product. The moisture content ranged from 22.02-29.87% (Bekele et al., 2020) and 23.07-51.89% (Javanmard et al., 2012) in mango

jam, 23.29-49.02% in guava and pineapple jam (Emelike & Akusu, 2019) and 31.23-33.36% in grape, apricot, blueberry and strawberry jams (Mohd Naeem et al., 2017).

Colour is one of the most vital characteristics of food from customers‘ point of view as it is closely associated with how the product is perceived and received (Banaś et al., 2018). When there is no additional colouring or flavouring agent in the production of jam, the appearance is highly dependent on ripeness and quality of fruits. However, colour of a food system may change depending on its physical state, chemical composition and structure (Basu & Shivhare, 2012). Discolouration may occur as a result of polymerisation during heating process of jam (Javanmard & Endan, 2010). The thermal treatment could be an initiation of several physicochemical reactions including Maillard browning and degradation of ascorbic acid, leading to alterations in the colour mix (Basu & Shivhare, 2010). Colour analysis is done to determine the colour values in terms of L^* (lightness/darkness), a^* (greenness/redness) and b^* (yellowness/blueness). The total colour change (ΔE) is calculated as the root-sum-square of the differences between the samples L^*, a^* and b^* values (denoted with subscript '$_s$') and L^*, a^* and b^* values of the control sample (denoted with subscript '$_c$') as the reference value (Robertson, 1977):

$$\Delta E = [(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2]^{\frac{1}{2}} \quad (2.4)$$

Hue is known to be the angular component of the polar representation and the hue angle (h^*) can be calculated as shown in (2.5) (Wrolstad & Smith, 2010).

$$h^* = \arctan(b^*/a^*) \quad (2.5)$$

2.8.3 Textural and rheological properties of jam

Jam quality is influenced directly by its textural and rheological attributes (objective) and indirectly by sensorial attributes (subjective). Texture of jam is pivotal as it indicates freshness and affects spreadability. The measurement of jam texture is normally achieved by instrumental measurement of texture profile, including hardness, work of shear, work of adhesion and stickiness. Hardness is the peak force on a product and presents the resistance to deformation whereas work of shear is related to spreadability, and is defined as the total force needed for the process of shearing (Javanmard et al., 2012). Stickiness refers to the force required to overcome the attractive forces between the product's surface and the test surface it interacts with while work of adhesion is the total force implied for the withdrawal of probe from food sample.

As foods like jams are complex systems and cannot be categorised exactly as solid or fluid, hence, food rheology is a significant tool to measure and interpret mechanical and flow behaviour of materials. This section places most of the emphasis on rheology, steady-state shear and rheology models on fruit jams. Food texture has been found to be closely related to food rheology as both involve the study of deformation, disintegration and flow of foods under well-defined conditions, in particular that of liquid and semi-solid foods (Mckenna & Lyng, 2013; Park, 2007).

In rheology, viscosity is an essential property that measures the ability of food materials to resist flow. Steady-state shear characterisation simulates a process shear rate and provides viscosity data of food materials. It generates information about response of materials to different flow rate regimes through viscosity measurement,

which is normally dependent on shear rate (Miri, 2011). Figure 2.9 shows a fluid confined between two parallel plates with an area *A*, separated by distance, *h*. The upper plate is set in motion at a constant velocity, *u* by applying a force, *F* in the X_1-direction while lower plate is kept stationary (Steffe, 1996). Initially, at $t = 0$, the velocity of fluid is uniformly zero everywhere except at the upper plate where the force is applied. As time progresses, the forces cause a linear velocity distribution to form, resulting in shear stress within the fluid. The shear stress, τ experienced by the fluid can be determined by Equation 2.6. Eventually, the system reaches a steady-state condition.

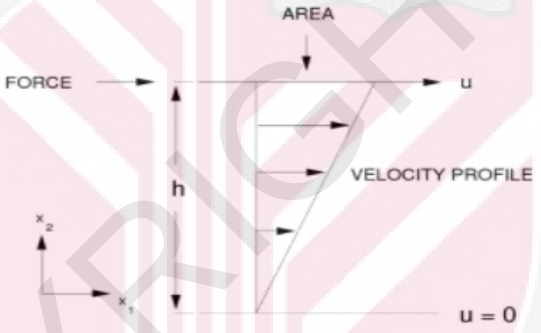


Figure 2.9: Velocity profile in steady flow between two parallel plates (Steffe, 1996)

$$\tau = \frac{F}{A}, \tag{2.6}$$

While considering the distance between two plates, *h*, the velocity gradient is *du/dh* and it is a measurement of shear rate or strain rate that is applied to the fluid. From Newton's law of viscosity (Sahin & Sumnu, 2005):

$$\tau = \mu\frac{du}{dh} = \mu\dot{\gamma} \tag{2.7}$$

where:

μ = viscosity (Pa.s)
$\dot{\gamma}$ = shear rate (1/s)

For a Newtonian liquid, viscosity is the slope of shear stress versus shear rate graph and it is independent of time (Miri, 2011). For non-Newtonian fluids which are shear thickening or shear thinning fluids, they obey the Ostwald-de Waele model, also known as Power Law model (Miri, 2011), displayed in Equation 2.8 below.

$$\tau = k(\dot{\gamma})^n \tag{2.8}$$

where:
k = consistency coefficient (Pa.s^n),
n = flow characteristics index (dimensionless)

The non-Newtonian fluids are classified by its n value where $n < 1$ describe shear thinning (pseudoplastic) fluids and $n > 1$ describes shear thickening fluids (Fischer et al., 2009). Different viscosities of non-Newtonian fluids will be obtained for different shear rates, hence, apparent viscosity is used (Sahin & Sumnu, 2005). Apparent viscosity, η is the ratio of shear stress to shear rate shown in Equation 2.9, Figures 2.10 and 2.11 for different types of fluids (Sahin & Sumnu, 2005). Apparent viscosity of shear thinning (pseudoplastic) fluids decreases when shear rate increases while apparent viscosity of shear thickening (dilatant) fluids increases with shear rate (Sahin & Sumnu, 2005). In the case of Bingham plastic fluids, shear stress exceeded the yield stress increases linearly with the shear rate. When the yield stress diminishes and reaches zero, the Bingham plastic fluid behaves like Newtonian fluid (Amoo & Layi Fagbenle, 2020).

$$\eta = f(\dot{\gamma}) = \frac{\tau}{\dot{\gamma}} \tag{2.9}$$

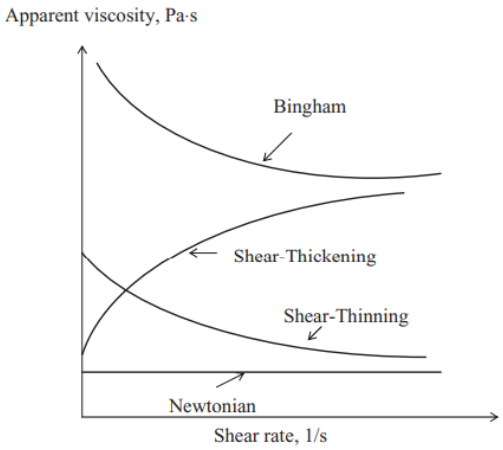


Figure 2.10: Apparent viscosities of Newtonian and non-Newtonian fluids (Sahin & Sumnu, 2005)

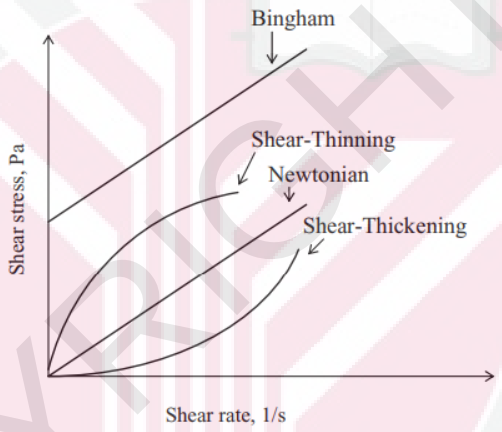


Figure 2.11: The slope of shear stress versus shear rate graph is not constant for non-Newtonian fluids (Sahin & Sumnu, 2005)

The description of fluid behaviour via relationship of shear stress to shear rate is known as rheological model. In addition to the Power Law, other models such as Power Law with yield stress (Herschel-Bulkley), Casson and Mizrahi-Berk have been introduced to describe flow characteristics of foods (Table 2.8). The Power Law model has been widely used for food processes that involve heating and cooling as measurements can be done with most types of rheological devices and additionally, it gives a good description of the fluid flow behaviour in a known shear rate range. The

Herschel-Bulkley model is used when non-Newtonian foods exhibit yield stress (Rao, 2010). Generally, most jams show non-Newtonian behaviour as jams contain particles of different sizes and shapes and pectin which behave as the non-Newtonian part (Javanmard & Endan, 2010). Different types of fruit jams or purees had been fitted to different rheological models as displayed in Table 2.9.

Table 2.8: Flow models describing shear rate versus shear stress data for time independent flow characteristics

Types of models	Equation
Newtonian	$\tau = k\dot{\gamma}$
Power Law	$\tau = k\dot{\gamma}^{n}$
Bingham	$\tau = k_o + k\dot{\gamma}$
Herschel-Bulkley	$\tau = k_{oHB} + k\dot{\gamma}^{n_s}$
Casson	$\tau^{0.5} = k_o^{0.5} + k\dot{\gamma}^{0.5}$
Mizrahi-Berk	$\tau^{0.5} = k_{oMZ} + k\dot{\gamma}^{n}$

k, k_o, and n are arbitrary constants and power indices, respectively determined from experimental data.

Table 2.9: Rheological models for different fruit jams or purees

Fruit Jams/Purees	Brix	pH	Model	References
Raspberry Fruits Strawberry Prune Peach Apricot	-	2.5-3.2	Power Law, Herschel-Bulkley, Cross, Carreau	Álvarez et al. (2007)
Raspberry Strawberry Peach Prune	-	2.56-3.38	Power Law, Herschel-Bulkley	Maceiras et al. (2007)
Mango	68.5	3.0-3.4	Herschel Bulkley	Basu & Shivhare (2010)
Mango	66-67	3	Power Law, Herschel Bulkley, Mizrahi-Berk	Javanmard & Endan (2010)
Physalis	50-65	3.09-3.36	Herschel-Bulkley	Pérez-Herrera et al. (2020)
Sapodilla	45-67	3.4	Power Law, Herschel-Bulkley	Shinwari & Rao (2020)
Sour cherry	46-66	-	Herschel-Bulkley	Nourmohammadi et al. (2021)

2.8.4 Sensory evaluation of jam

Texture influences the mouthfeel of a product, which is a sensory experience derived from the sensation in the mouth or on the tongue after mastication of a food (Basu & Shivhare, 2012). Hedonic test, widely used in food industry, evaluates customer preferences and purchase inclination by assessing their satsifcation and acceptance of product characteristics using a nine-point rating scale (Gámbaro & McSweeney, 2020). A food product is rated by the panellists according to how much it is liked or disliked, ranging from 1 = dislike extremely to 9 = like extremely including a neutral midpoint (neither like nor dislike). Hedonic scale had been used for sensory evaluation on colour, taste, odour, texture and overall acceptability of mango jam (Basu & Shivhare, 2010, 2012; Javanmard et al., 2012).

2.9 Summary

Fermented foods are food products that have been transformed by auspicious actions of beneficial bacteria and yeasts, resulting in a range of flavour, texture, and nutritional changes. One such food is *kombucha*, which is made from sweetened tea infused with a symbiotic culture of bacteria and yeast which is *scoby*. The process of fermentation affords the bacteria and yeast an opportunity to metabolise sugars present in the tea, resulting in the production of an array of organic acids, enzymes and other beneficial bioactive substances that can have wholesome effects on the body, including improved digestion, enhanced immune function and decreased inflammation. The conventional use of black tea as the primary substrate for *kombucha* has been longstanding. However, the reliance on black tea may limit the exploration of alternative substrates that could offer unique profiles and flavours.

Diversifying other potential pure and combinations of tea substrates such as green and oolong teas, which also originate from *Camellia sinensis* plant as black tea, have yet to be extensively explored. Different tea types could potentially influence the fermentation process through interactions with the microbial consortia. While transitioning from black tea to alternative substrates offers diverse possibilities, it is essential to study variations in key parameters affecting fermentation outcomes. As acetic acid is the major product of the fermentation process, maintaining proper pH levels is the most important factor in ensuring the successful creation of high-quality *kombucha*. By regulating acidity levels, the acidic conditions created by the bacteria and yeast during fermentation inhibit the growth of harmful microorganisms while promoting the production of beneficial compounds, thereby facilitating an ideal *scoby* yield. Given that *kombucha* is a fermented beverage containing a wide spectrum of microorganisms, the benefits can be further elucidated by identifying the presence and composition of these microorganisms using high-throughput sequencing techniques such as Next-Generation Sequencing (NGS). Microbial analysis is essential. The impact of different tea types on microbial composition of *kombucha* in the Malaysian region is not available. Further understanding of fermentation and its processes can be achieved through the use of mathematical modelling. In addition to the potable product, the by-product of *kombucha* fermentation, *scoby*, can be repurposed as a functional ingredient in food development, further amplifying the health-promoting potential of this unique fermented food. Given its exceptional properties including high purity, high degree of polymerisation and a remarkable capacity to hold water, *scoby* can be used as a hydrocolloid in jam, which is a spreadable food made from cooked fruits and sugar. Limited research exists on the application of *scoby*, particularly in jam production

and exploring its potential health benefits derived from microbial components. In Malaysia, jam production is subject to Food Act (1983) and Food Regulations Malaysia (1985) that must be adhered to, for instance, jam should not contain less than 65% of soluble solids determined by a refractometer at 20°C. Other qualities such as pH, water activity, moisture content, colour analysis, textural analysis and rheological analysis must be assessed to ensure the desired consistency, appearance as well as the overall quality of jam. Besides the analytical tests, sensory evaluation is also essential to understand the consumer's needs, preferences and expectations for the flavour, aroma, colour and texture of a new or modified product.

CHAPTER 3

METHODOLOGY

3.1 Overview of research

This chapter outlines methods used for each part of the research, including important materials and equipment. The statistical analysis used in this study is also discussed and illustrations are provided to aid in comprehension. Figure 3.1 shows the overall research framework, which has been structured into three main sections.

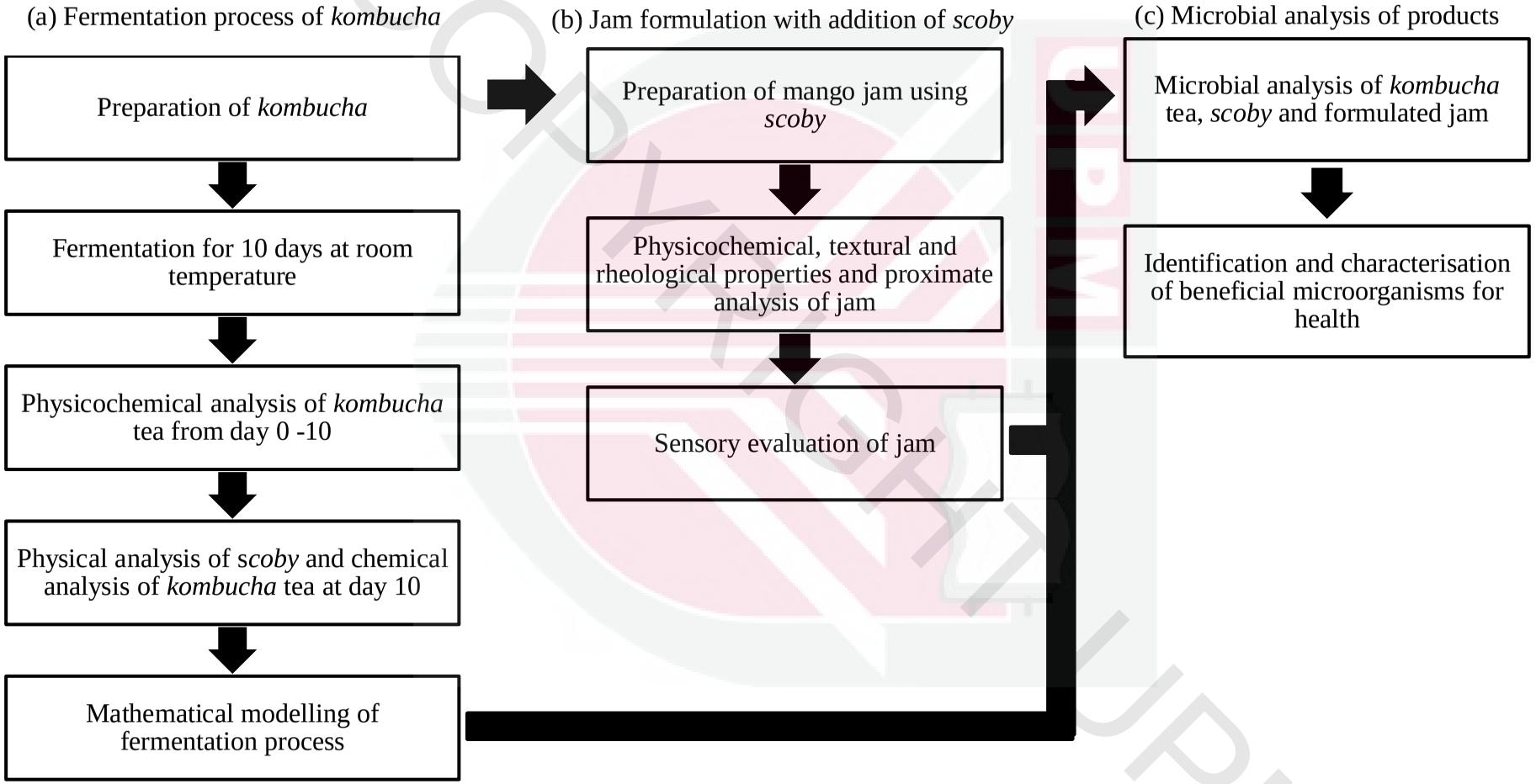


Figure 3.1: Overall research

3.2 *Kombucha* fermentation using three tea types

Figure 3.2 shows the process flow for *kombucha* preparation using tea, sugar, *scoby* and starter tea. *Kombucha* was prepared using pure teas and combinations of teas derived from black, oolong and green tea varieties. *Kombucha* fermentation produced tea and daughter *scoby*. *Scoby* used in the analyses and subsequent process refers to *scoby* produced from *kombucha* fermentation, in exact, the daughter *scoby*. The analyses performed on *kombucha* tea and daughter *scoby* are illustrated in Figure 3.3. In production of *kombucha* tea, part of the daughter *scoby* was used for producing jam, in Section 3.3.

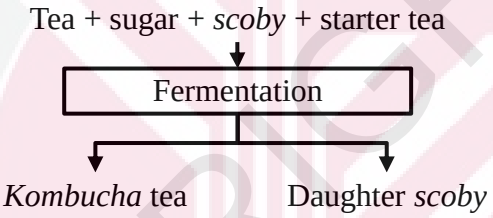


Figure 3.2: Process flow diagram of *kombucha* fermentation using *scoby* and producing *kombucha* tea and daugther *scoby*

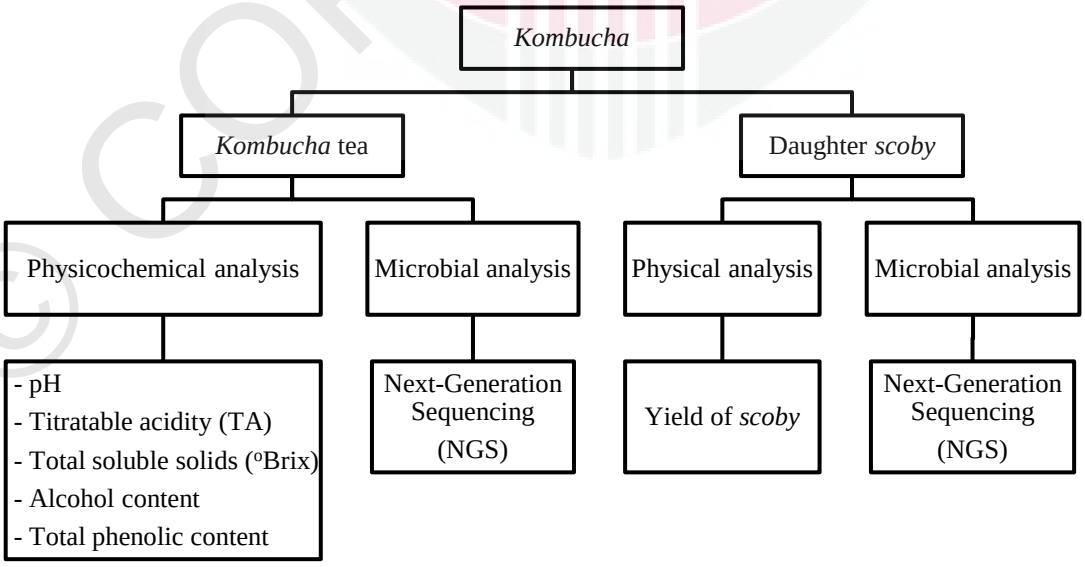


Figure 3.3: Analyses of *kombucha* tea and *scoby* samples

3.2.1 Preparation of *kombucha* tea

Figure 3.4 shows the main ingredients used in *kombucha* fermentation. The three different tea types, black tea (Yellow Label Tea, Lipton, Unilever, Indonesia), green tea (Pure Green Tea, Lipton, Unilever, China) and oolong tea (Legend of tea, Malaysia) were used. *Scoby* and starter tea were purchased from Herbal Remedies, Penang, Malaysia. Sugar used was *Gula Prai*, Malayan Sugar Manufacturing Company Berhad, Malaysia and Figure 3.5 shows the steps involved in *kombucha* fermentation. The steps used were referred from previous studies with some modifications, with the use of clean and sanitised utensils to prevent contamination (Ali & Shivanna, 2017; Chen & Liu, 2000; Kaewkod et al., 2019; Nummer, 2013; Sharma & Bhardwaj, 2019). Briefly, a known quantity of water (1 L) was heated and 90 g of sugar was added. The tea leaves (6 g) were then incorporated to steep in the solution. The solution was left standstill for 10 minutes to allow the components of the tea to mix in the water properly. Tea leaves were removed and the solution was allowed to cool at room temperature of 29^{o}C ± 0.3. Then, 30 g of *scoby* (3% w/v on wet weight basis) and 100 ml of starter tea (10% v/v) were added to the cooled tea. The tea was acidified by adding the starter tea (pH 2.7), a fermented *kombucha* tea to create an environment that favours the growth of *scoby* and prevents potential contaminants from developing (Dutta & Paul, 2019; Goh et al., 2012a). Then, the beaker was covered with a clean paper towel fitted with rubber band and left aside for fermentation for 10 days in a dark environment with room temperature of 29^{o}C ± 0.3. Fermentation period was set to 10 days to ensure the production of *kombucha* with good physicochemical and microbiological quality while also preventing the development of excessive acidity that could be harmful upon consumption (Neffe-Skocińska et al., 2017; Nummer, 2013). The experiments were carried out in

triplicates. Table 3.1 shows the proportions of ingredients used for the production of *kombucha* while Table 3.2 shows the proportions of three tea types used.

Figure 3.4: Types of tea comprising (a) black tea, (b) green tea, (c) oolong tea, (d) fine granulated sugar and (e) *scoby* used for fermentation of *kombucha*

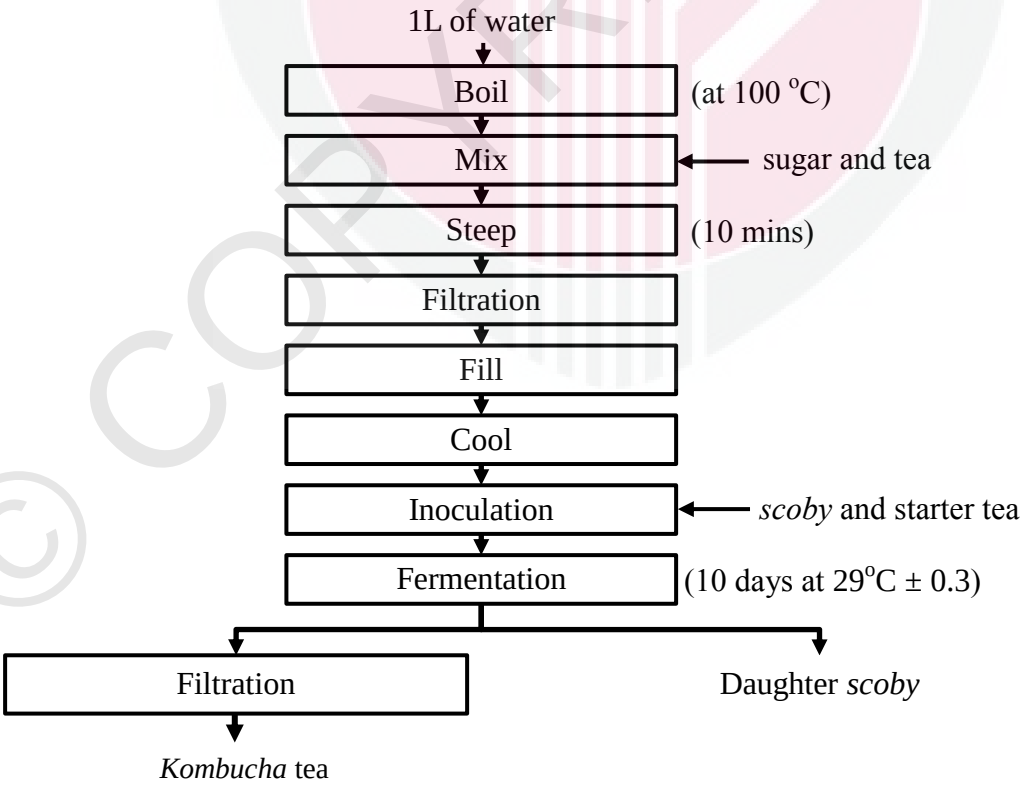


Figure 3.5: Steps used in preparing *kombucha*

Table 3.1: Quantity of ingredients used for production of *kombucha*

Ingredients	Quantity
Tea	6 g (0.6% w/v)
Sugar	90 g (9% w/v)
Water	1 L
Starter tea	100 ml (10% v/v)
Scoby	30 g (3% w/v)

Table 3.2: Proportions of three tea types at loading of 6 g for making *kombucha*

Samples	Percentage of tea (%)		
	Black tea	Oolong tea	Green tea
1	100	-	-
2	-	100	-
3	-	-	100
4	20	80	-
5	20	-	80
6	40	60	-
7	40	-	60
8	60	40	-
9	60	-	40
10	80	20	-
11	80	-	20

3.2.2 Physicochemical analysis

The *kombucha* tea was analysed for its physicochemical properties, including pH, titratable acidity (TA), total soluble solids and alcohol content throughout the 10 days fermentation. After 10 days of fermentation, total polyphenol content and yield of *scoby* were determined. The experiments were carried out thrice for all the samples. All chemicals used were of analytical grade. The pH was measured with a pH meter (Milwaukee MW-101, USA), shown in Figure 3.6. The pH meter was calibrated with buffer solutions of pH 4 and 7 respectively at room temperature. Then, the sensor tip was rinsed with distilled water prior to immersion into the aliquot. TA was measured using acid-base titration. 10 ml of fermented *kombucha* tea was titrated with 0.1 M NaOH using phenolphthalein as indicator (Kaewkod et al.,

2019; Treviño-Garza et al., 2020). The final TA of the fermented *kombucha* tea was expressed as percent acetic acid according to the formula (Sadler & Murphy, 2010):

$$\% \text{ acid (wt/vol)} = \frac{(N)(V_1)(Eq\ wt)}{(V_2)(1000)} \times 100 \quad (3.1)$$

where:
N = normality of titrant, NaOH (mEq/ml)
V_1 = volume of titrant (ml)
$Eq\ wt$ = equivalent weight of predominant acid (mg/mEq)
V_2 = volume of sample (ml)
1000 = factor relating mg to g (mg/g)

Figure 3.6: pH meter (Milwaukee MW-101, USA)

Content of total soluble solids was determined using a digital hand refractometer (Milwaukee MA871, USA), displayed in Figure 3.7, previously calibrated with distilled water (Kaewkod et al., 2019; Treviño-Garza et al., 2020). The results were expressed as °Brix. The volume of alcohol (%) was calculated from the specific gravity of *kombucha* tea (Appendix A) measured using a density bottle following AOAC (2004), with the equation below (Bustos et al., 2019; Ekumah et al., 2021; Feng et al., 2023; Heymann & Ebeler, 2017; Maskell et al., 2017):

$$\text{ABV } \% = (\text{SG}_{in} - \text{SG}) \times 131.25 \quad (3.2)$$

where:
ABV = alcohol by volume (%)
SG_{in} = the starting specific gravity of the *kombucha* tea before fermentation
SG = the current specific gravity of the *kombucha* tea

Figure 3.7: Digital hand refractometer (Milwaukee MA871, USA)

Total phenolic content was measured in accordance to Singleton & Rossi (1965) using the Folin-Ciocalteu spectrophotometric method. The sample of 0.1 ml was combined with 0.1 ml of the Folin-Ciocalteu reagent, 1 ml of 20% (w/v) sodium carbonate and 8.8 ml of distilled water and then placed in darkness for 30 minutes. The absorbance at 700 nm was measured with a spectrophotometer (Shimadzu UV-1800, Japan), shown in Figure 3.8. A standard calibration curve (Appendix B) was plotted using gallic acid with concentration range from 25 to 100 mg/L and the total phenolic content was expressed in mg/L of gallic acid equivalent.

Figure 3.8: Spectrophotometer (Shimadzu UV-1800, Japan)

After the fermentation process, the pellicle of *scoby* formed on the tea surface was removed carefully. An excess of moisture was removed with paper towels and the wet weight was recorded, expressed in g/L as mentioned in equation below (Treviño-Garza et al., 2020):

$$\text{Wet weight of } scoby \text{ (g/L)} = \frac{\text{Wet weight of } scoby \text{ (g)}}{\text{Volume of media (L)}} \quad (3.3)$$

Finally, percentage yield of *scoby* can be determined as follows (Goh et al., 2012a; Treviño-Garza et al., 2020):

$$\text{Yield (\%)} = \frac{\text{Wet weight of } scoby \text{ (g/L)}}{\text{Sucrose concentration (g/L)}} \times 100 \quad (3.4)$$

To evaluate the efficiency and effectiveness of the fermentation process in terms of *scoby* production, productivity of daughter *scoby* and conversion yield were calculated as follows (Sharma & Bhardwaj, 2019):

$$\text{Productivity of daughter } scoby \text{ (g/L} - \text{day)} = \frac{\text{Maximum } scoby \text{ weight (g/L)}}{\text{Fermentation time(day)}} \quad (3.5)$$

$$\text{Conversion yield (g/°Brix)} = \frac{scoby \text{ weight (g) at } t_{end}}{\text{Sugar (brix) at } t_0 - \text{Sugar (brix) at } t_{end}} \quad (3.6)$$

where t_0 is the starting time of fermentation, t_{end} represents the end of fermentation

3.2.3 Modelling of *kombucha* fermentation process

The fermentation process was modelled by analysing substrate utilisation and production formation over the course of 10 days fermentation process. Among the various *kombucha* samples, those with high productivity and conversion yield were specifically chosen for further analysis in modelling process. The Boltzmann's (sigmoidal) model was selected to represent the changes of sucrose concentration throughout the fermentation process (Kanurić et al., 2018; Loncar et al., 2014):

$$S(t) = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{t - t_0}{\Delta t}}} \quad (3.7)$$

where:

S = total soluble solids, which change in time t
A_1 and A_2 = positions of two asymptotes to the $S(t)$ curve (upper and lower)
t_0 = t-coordinate of the point at which the slope has the highest value
Δt = width of step of an exponential decrease

The focus of this study was on the production of desirable products from *kombucha* fermentation, which were acetic acid and *scoby*. Daily measurements of *scoby* and

acetic acid production were recorded, with sequential harvesting of *scoby*, enabling the quantification of cumulative growth over time. The data was then utilised in the modelling process to enhance the understanding of fermentation dynamics. The modelling approach for the formation of products was based on the modified Gompertz equation (3.8) (Erkmen & Alben, 2002; Zwietering et al., 1990). This equation was used to model the products formation which gives the specific production formation rate (μ), lag phase duration (λ) and the upper asymptote value (A). The average values of experimental results from fermentation were used for the calculation of mathematical parameters and fitting the mathematical models.

$$y = A\exp\{-\exp[\frac{\mu e}{A}(\lambda - t) + 1]\} \quad (3.8)$$

where:
y = amount of acetic acid (% acid (wt/vol)) or weight of *scoby* (g/L) at time t
A = upper asymptote value
μ = specific formation rate of acetic acid (% acid (wt/vol)/day) or *scoby* (g/L-day)
λ = lag phase duration (days)
t = time (days)

In addition to the modelling substrate utilisation and production formation, modelling of pH was made using polynomial (3.9) (Apar et al., 2017), rational (3.10) (Apar et al., 2017) and exponential models (3.11) (Goršek & Tramšek, 2008). These models were used to capture and understand the pH variations observed during the fermentation process, facilitating improved control of the fermentation process.

$$y = a + bt + ct^2 + dt^3 \quad (3.9)$$

$$y = \frac{a+bt}{1+ct+dt^2} \quad (3.10)$$

$$y = aexp(-bt) + c \quad (3.11)$$

where:
a, b, c, d = coefficients
t = time (days)

3.3 Effects of *scoby* addition in jam formulation

Figure 3.9 shows the preparation of jam using product of *kombucha* fermentation, daughter *scoby* with raw ingredients, mango, sugar and lemon juice. Different concentrations of daugther *scoby* from three tea types were mixed with heated mango fruit, sugar and acid. The analyses of mango *scoby* jam samples are detailed in Figure 3.10.

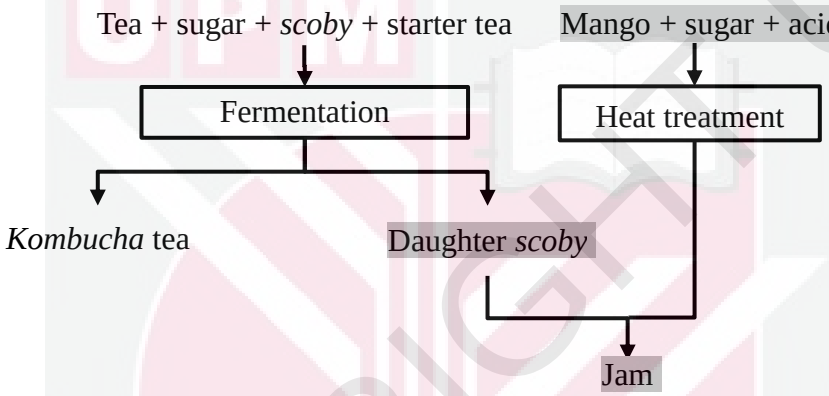


Figure 3.9: Production of jam using daughter *scoby* produced by fermentation process described in Figure 3.2

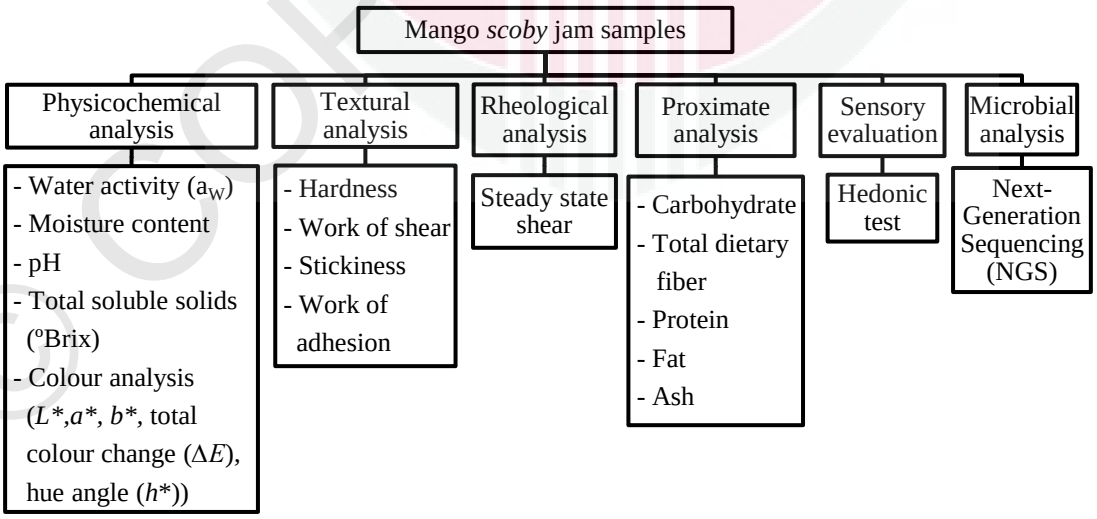


Figure 3.10: Analyses of mango jam formulated with *scoby*

3.3.1 Preparation of jam

Ripe mangoes (*Mangifera indica* L. cv. Susu) and lemons were obtained from local market. Commercial sugar was used in the form of coarse granulated sugar (*Gula Prai*, Malayan Sugar Manufacturing Company Berhad, Malaysia). The *scoby* used was daughter *scoby* obtained from the black, green and oolong tea *kombucha* fermentation in Section 3.2.1. Figure 3.11 shows the basic jam production procedures used referring to Javanmard et al. (2012) with slight modification. The mango pulp and sugar were premixed using a kitchen blender (Philips HR2059, Netherlands) for 10 seconds and heated to a temperature of 103-105^{o}C (Howard et al., 2010). Then lemon juice was added to reach desired pH level (Basu & Shivhare, 2010). Evaporation would be continued until a Brix value of 65-66^{o} was reached (Basu & Shivhare, 2010). After cooling to room temperature of 29^{o}C, the mango puree-sugar was pulse blended 3 times with daughter *scoby* using the same kitchen blender. To maintain a minimum fruit content of 35% (Javanmard & Endan, 2010), different concentration levels of daughter *scoby* were adjusted accordingly (Table 3.3). The concentrations were calculated based on total weight of jam (400g). The jams were carefully filled into individual airtight containers. Basic jam formulation composed of mango puree (base material), sugar (55% w/w) and lemon juice were prepared as control sample (without addition of *scoby*) (Javanmard et al., 2012).

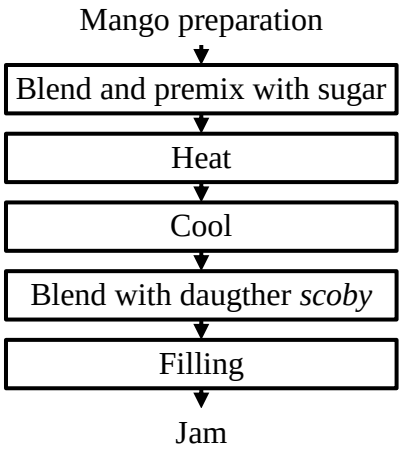


Figure 3.11: Steps used in preparation of jam

Table 3.3: Experimental plan for mango jam formulations at 5 levels of daughter *scoby*

Parameter	Level	Detail
Mango pulp	-	Base material
pH	-	3.4 ± 0.1
Sugar	1	55% (w/w)
Daugther *scoby*	5	2%, 4%, 6%, 8% and 10% (w/w)

3.3.2 Physicochemical properties

Physicochemical properties of jams included pH, total soluble solids, water activity, moisture content and colour determination. pH was measured with a pH meter (Milwaukee MW-101, USA), displayed in Figure 3.6. Content of total soluble solids was determined using a digital hand refractometer (Milwaukee MA871, USA), displayed in Figure 3.7, previously calibrated with distilled water (Basu & Shivhare, 2010). The results were expressed as °Brix. The water activity (a_w) was determined at 25^{o}C using a water activity meter (FA-st Lab, GBX, France) (Javanmard et al., 2012), illustrated in Figure 3.12. The moisture content was measured using a moisture analyser (MX-50 moisture analyser, A&D Co. Ltd, Japan) (Javanmard et al.,

2012), shown in Figure 3.13. The experiments were conducted thrice for each of the samples.

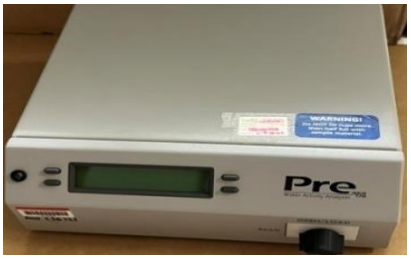


Figure 3.12: Water activity meter (FA-st Lab, GBX, France)

Figure 3.13: Moisture analyser (MX-50 moisture analyser, A&D Co. Ltd, Japan)

Colour properties of jam samples were measured with a handheld spectrophotometer (FRU WN700D, China), shown in Figure 3.14 using L^*, a^* and b^* colour scheme. The L^* , a^* and b^* values indicate lightness/darkness, greenness/redness and yellowness/blueness, respectively. The total colour change (ΔE) was calculated as the root-sum-square of the differences between the samples L^*, a^* and b^* values (denoted with subscript '$_s$') and L^* , a^* and b^* values of the control sample (denoted with subscript '$_c$') as the reference value (3.4) and the hue angle (h^*) was calculated as shown in (3.5) (Robertson, 1977).

$$\Delta E = [(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2]^{\frac{1}{2}} \quad (3.4)$$

$$h^* = \arctan(b^*/a^*) \quad (3.5)$$

Figure 3.14: Handheld spectrophotometer (FRU WN700D, China)

3.3.3 Textural and rheological properties

The textural parameters of jam samples including hardness, work of shear, work of adhesion and stickiness were investigated using a texture analyser (TA-XT*plus*; Stable Micro Systems, UK) (Figure 3.15) with a TTC Spreadability Rig (HDP/SR) attachment (Javanmard et al., 2012). The TTC work of shear rig contained a set of accurately matched male and female perspex cones with 90^{o} angle. Female cone (sample holder) was fixed on the heavy duty platform of the texture analyser. The penetration test commenced once the male cone proceeded to the approach then penetrated into the sample, and extended to a depth of 2 mm above the surfaces of sample holder (Javanmard et al., 2012). The textural data were recorded as force (N) versus time (s) and were further analysed.

Figure 3.15: Texture analyser (TA-XT*plus*; Stable Micro System, UK)

Rheological analysis was done using a controlled rate rheometer (ARG2, TA Instruments, USA), displayed in Figure 3.16 with computer control. The geometry of rheometer used for the analysis was stainless steel hatched parallel plate with radius of 40 mm and the gap between 2 plates was fixed at 1000 µm. The steady-state shear tests were carried out under steady-state flow condition, in a range of 1 to 500s^{-1} at 20^{o}C (Basu & Shivhare, 2010; Javanmard & Koohikamali, 2011). The rheometer was set to maintain equilibrium for 10 minutes so that the required temperature was reached and sheared in a continuous manner. Rheological measurements were calculated using TA Rheometer Data Analysis software (version V5.7.0).

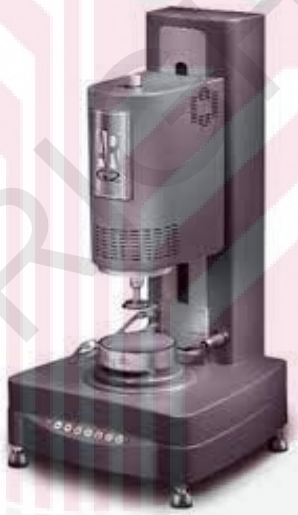


Figure 3.16: Rheometer (ARG2, TA Instruments, USA)

3.3.4 Proximate analysis

The proximate analysis of jam samples were measured following procedures stated in Table 3.4 (ALS Technichem (M) Sdn Bhd, Malaysia) and Appendix C.

Table 3.4: Methodology of proximate analysis

Analysis	Methodology	References
Total carbohydrate	Measurement by calculation using the following formula: Total carbohydrate (g/100g; % w/w) = 100% - (Total fat + protein + moisture + ash)	Sullivan & Carpenter (1993)
Energy calculated	Total energy was calculated as the sum of protein, fat, and carbohydrate measurements, multiplied by the respective factors: protein (16.7 kJ/g), fat (37.4 kJ/g), and carbohydrate (16.7 kJ/g)	
Total dietary fiber	Measurement of residual fiber after enzymatic digestion of defatted samples	
Ash	Measurement of inorganic residue after ignition of organic matter from 500 to 550°C	
Fat	Measurement of free lipids through direct solvent extraction using organic solvents	Kirk & Sawyer (1991); Sullivan & Carpenter (1993)
Protein	Measurement of organic nitrogen content by Kjeldahl procedure	

3.3.5 Sensory evaluation

The trained panel consists of fifteen members comprising male and female members from 25 to 35 years old. The descriptors were familiarised with texture terminology and the guidelines stated by Jowitt (1974). The mango jams were stored at domestic refrigerator and taken out for an hour before serving. Nine-point hedonic scale (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) was used in evaluation of texture (spreadability), colour, flavour (taste), odour (smell) and overall acceptability (Lawless & Heymann, 1998) (Appendix D and E). Mango jam samples were served at room temperature under normal light conditions within 50 mL cups coded with three-digit numbers randomly, one at a time. Panellists were provided with bread slices, spoons and drinking water (for palette cleansing after each testing). The mean values of the sensory scores were calculated followed by generation of sensory profile by plotting the average scores of the mango jam samples over the attributes (Javanmard et al., 2012).

3.3.6 Principal Component Analysis (PCA)

PCA was carried out using multivariate statistical procedures to study the correlations among a set of variables in order to determine the underlying structure of those variables (Shirali et al., 2016). The loading plots were used to show how much each variable contributes to the variation in data and to interpret variable relationships (Vilela et al., 2015). The analytical data obtained from the mango jam samples was analysed using PCA (Basu & Shivhare, 2012), generated using Minitab software (Version 16, Minitab Statistical Software, United States). The instrumental and sensory data obtained from the mango jam samples with *scoby* was analysed (Basu & Shivhare, 2012).

3.4 Microbial analysis

Next-Generation Sequencing (NGS) was used to determine microbial profile of the products, namely, *kombucha* tea, *scoby* and mango *scoby* jam, utilising 16S and ITS genetic regions for identification and classfication. 16S refers to the small subunit ribosomal RNA gene, widely used in bacterial studies while ITS (Internal Transcribed Spacer) identifies fungi. 16S rRNA gene enables the distinction between organisms at the genus level across various major bacterial phyla (Clarridge, 2004). The nuclear ribosomal RNA ITS region displayed the highest probability of correct identification (PCI) and well-defined barcode gap among numerous fungal lineages analysed, leading to its acceptance as the standard barcode marker for fungi (Badotti et al., 2017; Schoch et al., 2012). In overall, the major steps in carrying out NGS were DNA extraction, DNA amplification and amplicon sequencing as well as

bioinformatics data analysis adapted from Tung et al. (2016) (Apical Scientific Sdn. Bhd., Malaysia).

Genomic DNA was extracted from the samples following the manufacturer protocol using commercial kit by FastDNA™ Spin Soil Kit (MP Biomedicals). Subsequently, the quality of the purified DNA was initially examined using 1% TAE agarose gel in electrophoresis buffer. The concentration of DNA was determined with the use of a spectrophotometer (Implen NanoPhotometer® N60/N50) and fluorometric quantification using fluorescence microplate reader with iQuant™ Broad Range dsDNA Quantification Kit.

Next, PCR amplification and amplicon sequencing were done. The purified gDNA that passed DNA sample quality control was amplified using locus-specific sequence primers, bacterial 16S V3-V4 (5' to 3') with forward (CCTACGGGNGGCWGCAG) and reverse (GACTACHVGGGTATCTAATCC); Fungal ITS ITS2 (5' to 3') with forward (GCATCGATGAAGAACGCAGC) and reverse (TCCTCCGCTTATTGATATGC). All the PCR reactions were performed with REDiant 2X PCR Master Mix (1st BASE) which contains 0.06 U/µl *Taq* DNA polymerase, 400µM dNTPs, 3mM $MgCl_2$ and reaction buffer. Reaction volume was 25 µl, consisting 12.5 µl REDiant 2X PCR Master Mix, 1 µl of each primer, 1 µl DNA template and the remaining with nuclease-free water. The PCR conditions used were 95^{o}C initial denaturation for 1 min, 25 cycles at 95^{o}C for 30s (denaturation), 25 cycles at 55^{o}C for 30s (annealing), 25 cycles at 72^{o}C for 50s (extension) followed by a 5 min final extension at 72^{o}C.

The DNA samples that passed the amplicon PCR QC were proceeded to amplicon library preparation using 2-Step PCR, according to the Illumina's 16S metagenomic library preparation guideline (Amplicon et al., 2013). Bacterial 16S rRNA genes of the chosen regions (V3-V4) and Fungal ITS ITS2 were undergone amplification with the use of locus-specific sequence primers incorporating overhang adapters as below:

Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus- specific sequence]

Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus- specific sequence]

All the PCR reactions were carried out with KOD -Multi & Epi-® (Toyobo). Dual indices were attached to the amplicon PCR with the use of Illumina Nextera XT Index Kit v2 in accordance with the manufacturer's protocols. The library quality was determined with Agilent Bioanalyzer 2100 System using Agilent DNA 1000 Kit and fluorometric quantification using Helixyte Green™ Quantifying Reagent.

The libraries that passed the library QC were then processed to Next-Generation Sequencing. The libraries were standardised and pooled following the Illumina-recommended protocol and sequenced utilising MiSeq platform with 300 PE. The raw data was then processed and analysed based on the pipeline of bioinformatics.

Sequence data analysis of 16s and ITS was performed using QIIME 2. Quality assessment of raw reads was done with FastQC (available at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) after removal of primers and adapters with Cutadapt 3.5 (Martin, 2011). Paired-end reads were

processed and merged using Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline V1.18 and form an amplicon sequence variance (ASV) table (Callahan et al., 2016). Chimera screening and taxonomy assignment was done using the SILVA V138.1 databse or UNITE ITS database. ASVs were utilised to construct taxa abundance plot, rarefaction curves, alpha diversity and beta diversity in QIIME2. Alpha diversity analysis was employed to inspect the structure of ecological community of samples, utilising Shannon and Simpson metrics as indicators. Shannon and Simpson diversity indexes are quantitative indicators of species diversity by taking into account the richness and evenness of the community; nevertheless, Shannon diversity index (Shannon, 1948), emphasises species richness while Simpson diversity index (Simpson, 1949), considers species evenness more than species richness (Kim et al., 2017; Schloss et al., 2009; Schloss & Handelsman, 2006). On the other hand, beta diversity analysis, utilising Principal Coordinate Analysis (PCoA), was employed to compare microbial community composition and assess differences between samples.

3.5 Statistical analysis

In order to study different mathematical models in both *kombucha* fermentation and rheological analysis of jam, curve fitting was done with Microsoft Excel 2010 (Microsoft Corporation, USA) using solver function by applying generalised reduced gradient (GRG2) nonlinear optimisation algorithm to determine the parameters of the models (Chin et al., 2009). The coefficient of determination, R^2, was calculated as $R^2 = 1 - \frac{SSE}{SST}$ (SSE is the minimum sum of square errors and SST is the total correlated sum of squares) (Chin et al., 2009). Standard error was calculated as $SE = \frac{SD}{\sqrt{n}}$ where

SD represents the standard deviation and *n* denotes the number of experiment. Minitab software (Version 16, Minitab Statistical Software, USA) was utilised for conducting analysis of variance (ANOVA). One-way analysis of variance was used to investigate the effects of different tea types on physicochemical properties of *kombucha* fermentation and impacts of *scoby* on both the physicochemical and textural properties measured in jam (Javanmard et al., 2012). The *p*-value shows the individual significance probability of each independent variable. The term with $p < 0.05$ is considered to be statistically significant while $p > 0.05$ is considered non-significant on the response variable. Tukey's test was implemented to compare the differences among the mean values with a confidence level of 0.05.

3.6 Summary

This section provides a description of materials and methods used in the study. The methodology involves *kombucha* preparation, followed by physicochemical analysis using various techniques. The fermentation process was modelled using mathematical models to better understand the dynamics of the fermentation process. Subsequently, preparation of jam with ingredients, *i.e*., fruits, sugar and *scoby* were reported. The jams produced were subjected to several analyses, such as physicochemical, textural, rheological, and proximate analysis to ensure its quality. Sensory evaluation was also conducted to understand consumer preferences and needs. The third section involves microbial analysis of the products from *kombucha* fermentation and jam production using NGS. This analysis was used to identify the microorganisms present in the *kombucha* and jam samples, providing valuable insights into their microbial communities and potential health benefits.

CHAPTER 4

FERMENTATION PROCESS OF *KOMBUCHA* USING THREE TEA TYPES

This chapter discusses the results of *kombucha* fermentation process which yielded two products, *i.e.*, the *kombucha* tea and *scoby*. *Kombucha* was cultivated with pure teas (black, oolong and green) and tea blends (combinations of black tea with either oolong tea or green tea). Physicochemical analysis of *kombucha* tea and physical analysis of *scoby* were evaluated and compared.

4.1 Effects of tea types on physicochemical properties of *kombucha* fermentation

The use of black, green and oolong teas as a whole and in combinations for *kombucha* fermentation for 10 days was evaluated by measuring its pH, titratable acidity, total soluble solids and alcohol content.

4.1.1 pH

The pH levels of *kombucha* teas fermented with three types of teas, the black, oolong and green teas are shown in Table 4.1. Prior to the commencement of fermentation process, pH values after inoculation were about 3 while those found by Neffe-Skocińska et al. (2017) and Kallel et al. (2012) were 3.1 and 3.8 respectively. The use of different sources of acidic starter liquids is likely what caused the minor deviation. Figure 4.1 shows the pH trend over the fermentation period for different *kombucha* teas. A rapid decline in pH was seen in all *kombucha* teas on the first day of fermentation, with the largest drop found in *kombucha* prepared with a mixture of black tea and green tea (up to 0.31 units in the case of *kombucha* tea prepared with

40% black tea and 60% green tea). The drop in pH value could be due to the increased concentration of organic acids during fermentation process by microorganisms from *scoby* as they convert nutrients in the substrate (Amarasinghe et al., 2018). As fermentation progressed, the pH decreased as a result of the build-up of these organic acids. This decline was found to have significant differences ($P < 0.05$) from day 3 onwards, with variations corresponding to the types of tea utilised and the extent of organic acid production (Kaewkod et al., 2019). After the 5th day, a slower pH drop was observed which may be related to the buffer effect arising from the reactions between synthesised organic acids and minerals from the substrate (Malbaša et al., 2008). Figure 4.1(a) shows that the oolong tea had the lowest pH trend, followed by the green tea and lastly the black tea *kombucha*. A similar trend was shown in Coton et al. (2017) and Jakubczyk et al. (2020) whereby pH value of green tea was lower than that of black tea at the end of 8 days fermentation. Figure 4.1(b) shows that *kombucha* tea with a higher proportion of black tea (80%) had a slightly higher pH trend compared to the rest of the samples. Likewise, as shown in Figure 4.1(c), the highest pH can be found in a mixture of 80% black tea and 20% green tea *kombucha*. However, there was no marked difference for the rest of the samples.

Table 4.1: pH values of *kombucha* teas made with different tea types and combinations during a 10 days' fermentation process

Sample	Amount of tea (%)			Fermentation time (days)										
	Black tea	Oolong tea	Green tea	0	1	2	3	4	5	6	7	8	9	10
				pH values										
1	100	-	-	3.25 ± 0.05^{a}	3.10 ± 0.04^{a}	3.00 ± 0.00^{a}	2.96 ± 0.01^{a}	2.85 ± 0.01^{ab}	2.85 ± 0.01^{a}	2.81 ± 0.02^{a}	2.80 ± 0.00^{a}	2.79 ± 0.01^{a}	2.79 ± 0.01^{a}	2.79 ± 0.03^{a}
2	-	100	-	3.19 ± 0.01^{a}	3.00 ± 0.02^{a}	2.96 ± 0.04^{a}	2.87 ± 0.02^{b}	2.81 ± 0.01^{ab}	2.80 ± 0.01^{ab}	2.77 ± 0.03^{abc}	2.75 ± 0.05^{ab}	2.73 ± 0.00^{d}	2.73 ± 0.01^{bcd}	2.72 ± 0.00^{bc}
3	-	-	100	3.27 ± 0.02^{a}	3.04 ± 0.04^{a}	2.99 ± 0.03^{a}	2.93 ± 0.00^{ab}	2.85 ± 0.00^{ab}	2.84 ± 0.00^{a}	2.79 ± 0.00^{ab}	2.77 ± 0.00^{ab}	2.77 ± 0.00^{abc}	2.76 ± 0.01^{abc}	2.76 ± 0.01^{ab}
4	20	80	-	3.26 ± 0.04^{a}	3.04 ± 0.01^{a}	2.97 ± 0.01^{a}	2.87 ± 0.00^{b}	2.79 ± 0.00^{b}	2.77 ± 0.03^{b}	2.73 ± 0.00^{c}	2.71 ± 0.02^{b}	2.73 ± 0.00^{d}	2.70 ± 0.00^{d}	2.70 ± 0.00^{c}
5	40	60	-	3.22 ± 0.03^{a}	3.05 ± 0.05^{a}	2.95 ± 0.05^{a}	2.88 ± 0.02^{b}	2.81 ± 0.00^{ab}	2.79 ± 0.03^{ab}	2.75 ± 0.00^{bc}	2.73 ± 0.02^{ab}	2.74 ± 0.00^{cd}	2.73 ± 0.00^{bcd}	2.70 ± 0.00^{c}
6	60	40	-	3.21 ± 0.02^{a}	3.05 ± 0.03^{a}	3.00 ± 0.01^{a}	2.90 ± 0.00^{ab}	2.82 ± 0.00^{ab}	2.79 ± 0.00^{ab}	2.78 ± 0.00^{abc}	2.77 ± 0.03^{ab}	2.74 ± 0.00^{cd}	2.73 ± 0.00^{bcd}	2.72 ± 0.00^{bc}
7	80	20	-	3.17 ± 0.03^{a}	3.02 ± 0.06^{a}	2.99 ± 0.07^{a}	2.94 ± 0.07^{ab}	2.90 ± 0.08^{a}	2.80 ± 0.00^{ab}	2.79 ± 0.00^{ab}	2.77 ± 0.03^{ab}	2.76 ± 0.03^{abcd}	2.75 ± 0.03^{abc}	2.72 ± 0.02^{bc}
8	20	-	80	3.24 ± 0.03^{a}	2.97 ± 0.03^{a}	2.94 ± 0.03^{a}	2.90 ± 0.01^{ab}	2.82 ± 0.00^{ab}	2.81 ± 0.01^{ab}	2.80 ± 0.00^{ab}	2.74 ± 0.00^{ab}	2.74 ± 0.00^{cd}	2.73 ± 0.00^{bcd}	2.70 ± 0.02^{c}
9	40	-	60	3.28 ± 0.02^{a}	2.97 ± 0.04^{a}	2.95 ± 0.01^{a}	2.90 ± 0.01^{ab}	2.85 ± 0.00^{ab}	2.81 ± 0.03^{ab}	2.81 ± 0.00^{a}	2.74 ± 0.03^{ab}	2.74 ± 0.01^{cd}	2.73 ± 0.01^{cd}	2.71 ± 0.02^{c}
10	60	-	40	3.27 ± 0.05^{a}	3.00 ± 0.16^{a}	2.95 ± 0.09^{a}	2.92 ± 0.01^{ab}	2.84 ± 0.06^{ab}	2.83 ± 0.05^{ab}	2.81 ± 0.04^{a}	2.74 ± 0.03^{ab}	2.75 ± 0.03^{bcd}	2.73 ± 0.04^{bcd}	2.71 ± 0.01^{c}
11	80	-	20	3.28 ± 0.04^{a}	3.05 ± 0.00^{a}	3.02 ± 0.02^{a}	2.97 ± 0.06^{a}	2.87 ± 0.05^{ab}	2.83 ± 0.00^{ab}	2.81 ± 0.03^{a}	2.78 ± 0.03^{ab}	2.78 ± 0.00^{ab}	2.77 ± 0.01^{ab}	2.77 ± 0.01^{a}

Values are reported as mean ± SD.
Different small letters (a,b,c,d) within a column show significant difference ($P < 0.05$) based on Tukey's test.

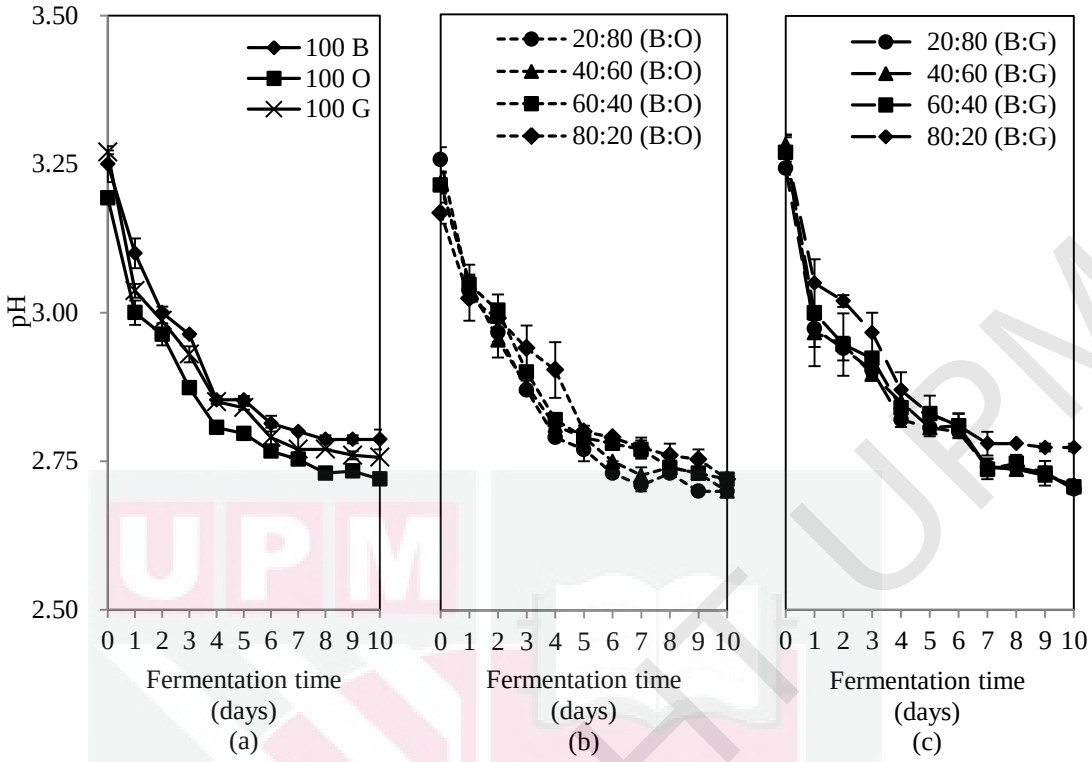


Figure 4.1: pH of *kombucha* teas made using (a) pure black, oolong and green teas, (b) combinations of black tea and oolong tea, and (c) combinations of black tea and green tea. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

pH drop during *kombucha* fermentation was reported by Goh et al. (2012) where pH of black tea *kombucha* dropped to a range of 2.53 to 2.78 after 8 fermentation days and by Jakubczyk et al. (2020) where pH of black and green tea *kombucha* dropped to a range of 2.38-2.62 and 2.32-2.53 within 7 and 14 days of fermentation respectively. The pH levels of *kombucha* teas at the end of fermentation ranging from 2.7-2.79 in the current study met the food regulations to guarantee the safety and quality of *kombucha* tea. In accordance with the Food and Drug Administration (FDA) Food Code model, Pennsylvania Department of Agriculture (USA), Normative Instruction (IN) in Brazil and the Centre of Disease Control of British Columbia (BCCDC), pH of *kombucha* teas should not be higher than 4.2 and lower than 2.5 to avoid microbial contamination and acidosis (Coelho et al., 2020; de

Miranda et al., 2022; Greenwalt et al., 2000; Kim & Adhikari, 2020; Nummer, 2013). Hence, it is important to have stringent monitoring and adjustment of parameters such as fermentation duration, sugar concentration and acid concentration as pH is primarily influenced by bacterial acid production and yeast metabolism, both of which facilitate the conversion of sugars into acids (Nummer, 2013; Villarreal-Soto et al., 2018). An example is to add fresh brewed tea if pH falls below the limit (Nummer, 2013).

4.1.2 Titratable acidity

Table 4.2 shows the titratable acidity (TA) of different *kombucha* teas. The TA of *kombucha* teas was in the range between 0.14 and 0.17% after inoculation with *scoby* from day 1 due to activation of acetic acid bacteria in *scoby* from the addition of starter liquid. Figure 4.2 illustrates the increase in TA of *kombucha* teas during the fermentation process. The microorganisms in the *scoby* metabolise the compounds found in *kombucha* tea, resulting in the production of different metabolites including acids. The production of acids, such as glucuronic, gluconic, tartaric, malic, citric, lactic, succinic and malonic acids continued to increase with fermentation time which resulted in an increase in TA. The TA however is expressed in terms of acetic acid, which is the dominant acid present in *kombucha* and is produced by acetic acid bacteria such as *Acetobacter*, *Gluconacetobacter* and *Komagateibacter* (Cardoso et al., 2020; de Miranda et al., 2022; Jakubczyk et al., 2020; Neffe-Skocińska et al., 2017; Wang et al., 2021).

Table 4.2: Titratable acidity of *kombucha* teas made with different tea types and combinations during a 10 days' fermentation process

Sample	Amount of tea (%)			Fermentation time (days)										
	Black tea	Oolong tea	Green tea	0	1	2	3	4	5	6	7	8	9	10
				% acid (wt/vol)										
1	100	-	-	0.12 ± 0.01^{a}	0.14 ± 0.01^{b}	0.16 ± 0.01^{a}	0.20 ± 0.01^{a}	0.26 ± 0.02^{a}	0.29 ± 0.00^{bc}	0.31 ± 0.01^{ab}	0.32 ± 0.02^{a}	0.33 ± 0.03^{ab}	0.34 ± 0.02^{bcd}	0.35 ± 0.02^{cde}
2	-	100	-	0.12 ± 0.01^{a}	0.14 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.20 ± 0.02^{a}	0.26 ± 0.02^{a}	0.31 ± 0.01^{ab}	0.32 ± 0.03^{ab}	0.32 ± 0.01^{a}	0.33 ± 0.02^{abc}	0.34 ± 0.02^{bcd}	0.36 ± 0.01^{bcde}
3	-	-	100	0.10 ± 0.02^{a}	0.14 ± 0.01^{b}	0.16 ± 0.01^{a}	0.18 ± 0.01^{ab}	0.24 ± 0.01^{a}	0.30 ± 0.00^{ab}	0.30 ± 0.01^{ab}	0.30 ± 0.01^{ab}	0.31 ± 0.01^{bcd}	0.32 ± 0.00^{cd}	0.33 ± 0.01^{ef}
4	20	80	-	0.12 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.17± 0.01^{a}	0.20 ± 0.00^{a}	0.22 ± 0.00^{b}	0.32 ± 0.02^{a}	0.33 ± 0.03^{a}	0.34 ± 0.02^{a}	0.35 ± 0.00^{a}	0.37 ± 0.00^{a}	0.41 ± 0.02^{a}
5	40	60	-	0.12 ± 0.00^{a}	0.15 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.19 ± 0.01^{ab}	0.21 ± 0.01^{b}	0.31 ± 0.02^{ab}	0.34 ± 0.01^{a}	0.34 ± 0.01^{a}	0.34 ± 0.01^{ab}	0.36 ± 0.00^{ab}	0.37 ± 0.00^{bcd}
6	60	40	-	0.12 ± 0.01^{a}	0.14 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.19 ± 0.02^{ab}	0.20 ± 0.00^{b}	0.29 ± 0.00^{bc}	0.25 ± 0.00^{c}	0.30 ± 0.00^{ab}	0.31 ± 0.02^{abcd}	0.34 ± 0.00^{bcd}	0.34 ± 0.02^{de}
7	80	20	-	0.13 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.16 ± 0.01^{a}	0.18 ± 0.00^{ab}	0.19 ± 0.00^{b}	0.25 ± 0.00^{cd}	0.25 ± 0.01^{c}	0.25 ± 0.02^{c}	0.28 ± 0.00^{d}	0.29 ± 0.00^{e}	0.30 ± 0.00^{f}
8	20	-	80	0.13 ± 0.01^{a}	0.17 ± 0.01^{a}	0.17 ± 0.01^{a}	0.20 ± 0.00^{a}	0.20 ± 0.00^{b}	0.26 ± 0.00^{cd}	0.30 ± 0.00^{ab}	0.31 ± 0.00^{ab}	0.35 ± 0.00^{a}	0.35 ± 0.00^{abc}	0.39 ± 0.01^{ab}
9	40	-	60	0.12 ± 0.01^{a}	0.17 ± 0.01^{a}	0.18 ± 0.01^{a}	0.20 ± 0.00^{a}	0.20 ± 0.00^{b}	0.24 ± 0.02^{cd}	0.30 ± 0.00^{ab}	0.30 ± 0.01^{ab}	0.33 ± 0.01^{abc}	0.34 ± 0.01^{bc}	0.38 ± 0.01^{abc}
10	60	-	40	0.12 ± 0.00^{a}	0.15 ± 0.01^{ab}	0.16 ± 0.02^{a}	0.17 ± 0.00^{b}	0.19 ± 0.00^{b}	0.25 ± 0.00^{cd}	0.28 ± 0.01^{bc}	0.28 ± 0.01^{bc}	0.29 ± 0.01^{cd}	0.33 ± 0.01^{bcd}	0.38 ± 0.01^{abc}
11	80	-	20	0.12 ± 0.00^{a}	0.15 ± 0.01^{ab}	0.16 ± 0.00^{a}	0.18 ± 0.01^{ab}	0.19 ± 0.00^{b}	0.24 ± 0.00^{cd}	0.28 ± 0.00^{bc}	0.28 ± 0.01^{bc}	0.29 ± 0.00^{d}	0.31 ± 0.00^{de}	0.34 ± 0.01^{de}

Values are reported as mean ± SD.
Different small letters (a,b,c,d,e,f) within a column show significant difference ($P < 0.05$) based on Tukey's test.

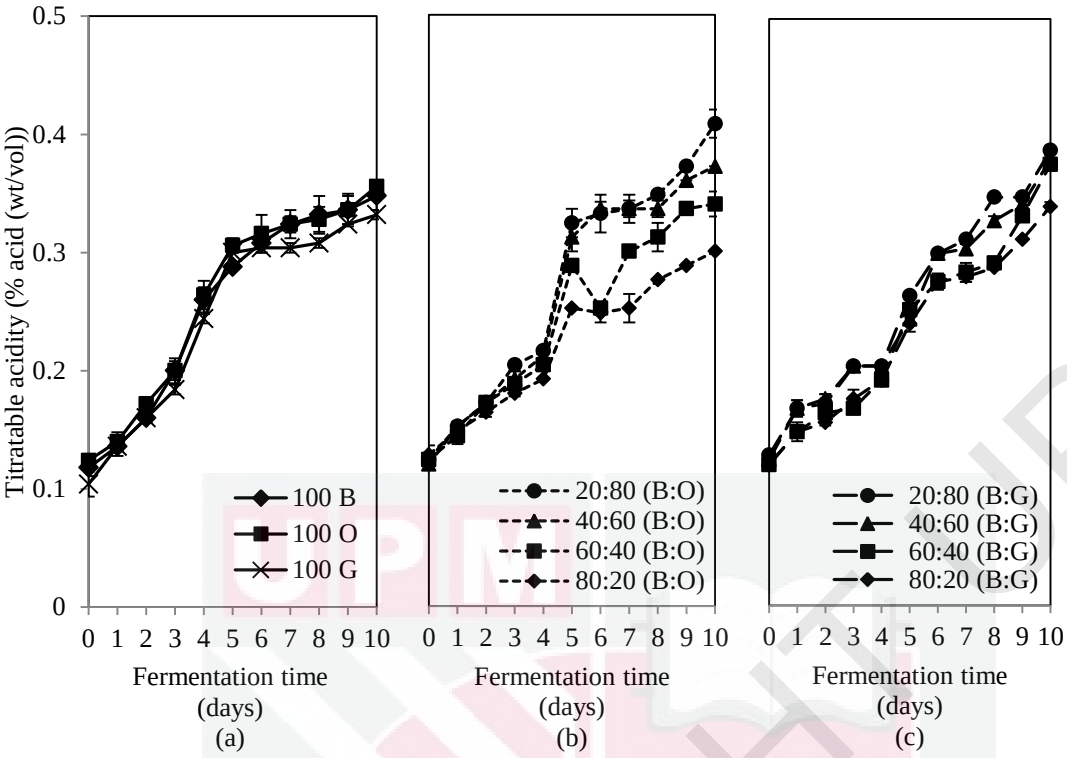


Figure 4.2: Titratable acidity of *kombucha* teas made with (a) pure black, oolong and green teas, (b) combinations of black tea and oolong tea, and (c) combinations of black tea and green tea. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

Significant differences ($P < 0.05$) were observed after day 1, indicating that different tea types influenced acid production during fermentation. However, while bacteria have well-defined roles in fermentation, such as producing acids from ethanol, the specific functions and interactions of many microorganisms involved in the process remain unknown (Coelho et al., 2020).

A sudden spike in elevation occurred on the fourth day of fermentation, a trend consistent with findings reported in other studies (Kaewkod et al., 2019; Loncar et al., 2014). The reason behind this could be attributed to the multiplication of acetic acid and acid-tolerant microorganisms which are responsible for the production of acids. This observation is supported by a remarkable surge in microbial counts for acetic

acid bacteria after the third day of fermentation in previous studies (Goh et al., 2012a; Neffe-Skocińska et al., 2017; Shi et al., 2023). At the end of fermentation, TA was between 0.3 and 0.41%, similar to the black and green tea *kombucha* with TA between 0.32 and 0.36% reported in Cardoso et al. (2020).

For pure tea *kombucha*, oolong tea showed the highest TA, compared to black tea and green tea (Figure 4.2(a)), consistent with previous findings indicating that oolong tea had the highest TA among green tea and black tea *kombucha* (Osiripun & Apisittiwong, 2021). A higher content of acetic acid bacteria was also detected in oolong tea, followed by green and black teas, potentially contributing to the greater TA (Osiripun & Apisittiwong, 2021). For *kombucha* made with combinations of teas, TA was the highest for *kombucha* made with 20% black tea and 80% oolong tea (0.41%) and 20% black tea and 80% green tea (0.39%), as shown in Figures 4.2(b) and 4.2(c) respectively.

4.1.3 Total soluble solids

Table 4.3 shows that the total soluble solids of *kombucha* teas decreased over the duration of fermentation process, with no significant difference ($P > 0.05$) observed in the effect of tea types on this decrease. The consistent initial sugar content across all tea types suggests uniformity in starting conditions, with the subsequent reduction resulted by the sucrose transformation process during *kombucha* fermentation (Randazzo et al., 2016).

Table 4.3: Total soluble solids of *kombucha* teas made with different tea types and combinations during a 10 days' fermentation process

Sample	Amount of tea (%)			Fermentation time (days)										
	Black tea	Oolong tea	Green tea	0	1	2	3	4	5	6	7	8	9	10
				°Brix										
1	100	-	-	9.6 ± 0.3[a]	9.4 ± 0.6[a]	9.4 ± 0.3[a]	9.3 ± 0.2[a]	9.2 ± 0.4[a]	9.2 ± 0.3[a]	9.1 ± 0.4[a]	8.9 ± 0.3[a]	8.9 ± 0.4[a]	8.9 ± 0.4[a]	8.7 ± 0.6[a]
2	-	100	-	9.4 ± 0.2[a]	9.4 ± 0.2[a]	9.4 ± 0.2[a]	9.3 ± 0.1[a]	9.3 ± 0.2[a]	9.3 ± 0.2[a]	9.2 ± 0.2[a]	9.0 ± 0.2[a]	8.9 ± 0.4[a]	8.9 ± 0.4[a]	8.7 ± 0.4[a]
3	-	-	100	9.6 ± 0.4[a]	9.6 ± 0.3[a]	9.5 ± 0.3[a]	9.4 ± 0.2[a]	9.4 ± 0.3[a]	9.3 ± 0.2[a]	9.2 ± 0.2[a]	9.1 ± 0.4[a]	9.0 ± 0.3[a]	8.9 ± 0.2[a]	8.9 ± 0.2[a]
4	20	80	-	9.8 ± 0.4[a]	9.8 ± 0.3[a]	9.7 ± 0.3[a]	9.7 ± 0.1[a]	9.6 ± 0.2[a]	9.6 ± 0.2[a]	9.6 ± 0.1[a]	9.5 ± 0.2[a]	9.4 ± 0.1[a]	9.2 ± 0.4[a]	9.0 ± 0.5[a]
5	40	60	-	9.7 ± 0.3[a]	9.7 ± 0.2[a]	9.7 ± 0.2[a]	9.7 ± 0.2[a]	9.6 ± 0.1[a]	9.6 ± 0.1[a]	9.6 ± 0.0[a]	9.3 ± 0.1[a]	9.1 ± 0.2[a]	9.0 ± 0.3[a]	8.9 ± 0.4[a]
6	60	40	-	9.7 ± 0.1[a]	9.6 ± 0.2[a]	9.6 ± 0.2[a]	9.4 ± 0.2[a]	9.5 ± 0.1[a]	9.5 ± 0.1[a]	9.5 ± 0.1[a]	9.3 ± 0.2[a]	9.1 ± 0.1[a]	9.0 ± 0.2[a]	8.9 ± 0.2[a]
7	80	20	-	9.8 ± 0.2[a]	9.7 ± 0.3[a]	9.6 ± 0.4[a]	9.5 ± 0.3[a]	9.5± 0.3[a]	9.4 ± 0.2[a]	9.3 ± 0.1[a]	9.3 ± 0.1[a]	9.1 ± 0.2[a]	9.0 ± 0.2[a]	8.9 ± 0.3[a]
8	20	-	80	9.4 ± 0.1[a]	9.4 ± 0.0[a]	9.4 ± 0.1[a]	9.4 ± 0.1[a]	9.5 ± 0.0[a]	9.4 ± 0.0[a]	9.3 ± 0.0[a]	9.0 ± 0.2[a]	8.9 ± 0.2[a]	8.8 ± 0.2[a]	8.7 ± 0.2[a]
9	40	-	60	9.6 ± 0.3[a]	9.3 ± 0.2[a]	9.4 ± 0.1[a]	9.3 ± 0.0[a]	9.3 ± 0.0[a]	9.4 ± 0.1[a]	9.1 ± 0.3[a]	9.0 ± 0.2[a]	8.8 ± 0.3[a]	8.8 ± 0.2[a]	8.7 ± 0.1[a]
10	60	-	40	9.6 ± 0.1[a]	9.5 ± 0.1[a]	9.4 ± 0.2[a]	9.3 ± 0.0[a]	9.2 ± 0.1[a]	9.2 ± 0.1[a]	9.2 ± 0.1[a]	9.0 ± 0.1[a]	8.8 ± 0.3[a]	8.8 ± 0.3[a]	8.6 ± 0.2[a]
11	80	-	20	9.4 ± 0.2[a]	9.3 ± 0.2[a]	9.3 ± 0.2[a]	9.3 ± 0.1[a]	9.2 ± 0.3[a]	9.2 ± 0.2[a]	9.1 ± 0.1[a]	8.8 ± 0.0[a]	8.7 ± 0.3[a]	8.5 ± 0.3[a]	8.3 ± 0.1[a]

Values are reported as mean ± SD.
Different small letters within a column show significant difference ($P < 0.05$) based on Tukey's test.

The refractometric analysis of brix values revealed that *kombucha* teas exhibited the highest sucrose concentration at the start of fermentation (Figure 4.3), corresponding well with other studies (Chen & Liu, 2000; Kallel et al., 2012; Malbaša et al., 2008). The drop in total soluble solids is caused by metabolism of sugar into glucose and fructose by yeast invertase (Randazzo et al., 2016). The total soluble solids reached the lowest value of 8.3-9 ^{o}Brix on the 10th day. Similarly, Kaewkod et al. (2019) revealed that the total soluble solids of *kombucha* tea steadily decreased from 10 ^{o}Brix at the beginning of the fermentation to a range between 8 and 9 ^{o}Brix on the 9th day of fermentation.

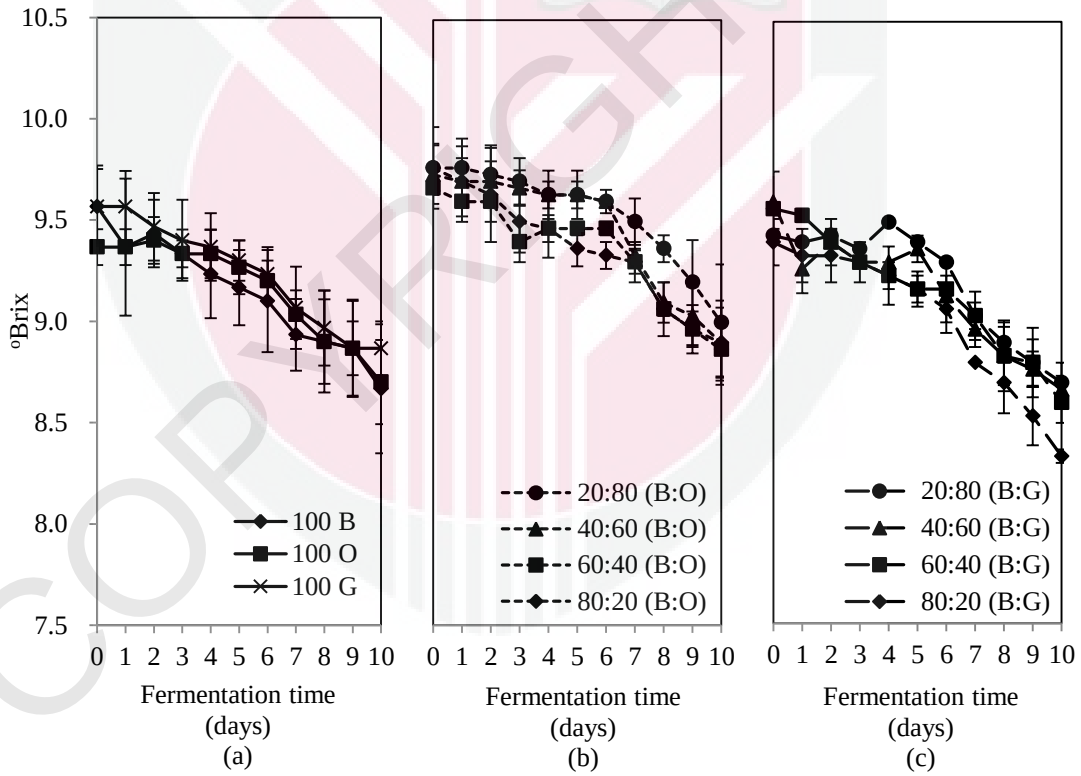


Figure 4.3: Total soluble solids of *kombucha* teas made with (a) pure black, oolong and green teas, (b) combinations of black tea and oolong tea, and (c) combinations of black tea and green tea. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

4.1.4 Alcohol content

Table 4.4 and Figure 4.4 show that alcohol content of *kombucha* teas made with different types and combinations increased to a maximum value on the 10^{th} day during fermentation. Similar trends have been found in other research, such as the work of Loncar et al. (2014) and Neffe-Skocińska et al. (2017), who found increasing levels of alcohol content along the fermentation period until day 10. The significant differences ($P < 0.05$) among fermentation days across different samples can be due to the dynamic processes involving yeast, such as *Saccharomyces* and *Zygosaccharomyces*, in ethanol production and subsequent utilisation of ethanol by acetic acid bacteria to produce acetic acid (Coelho et al., 2020; Jayabalan et al., 2014).

Table 4.4: Alcohol content of *kombucha* teas made with different tea types and combinations during a 10 days' fermentation process

Sample	Amount of tea (%)			Fermentation time (days)										
	Black tea	Oolong tea	Green tea	0	1	2	3	4	5	6	7	8	9	10
				Alcohol content (%ABV)										
1	100	-	-	0.00 ± 0.00^{a}	0.11± 0.02^{ab}	0.11± 0.01^{a}	0.16 ± 0.01^{a}	0.22 ± 0.01^{a}	0.22 ± 0.00^{a}	0.27 ± 0.01^{a}	0.38 ± 0.00^{a}	0.38 ± 0.00^{a}	0.38± 0.01^{a}	0.49 ± 0.02^{a}
2	-	100	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.11 ± 0.01^{a}	0.22 ± 0.01^{a}	0.27 ± 0.03^{a}	0.27 ± 0.02^{a}	0.38 ± 0.03^{a}
3	-	-	100	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.05 ± 0.02^{a}	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}	0.16 ± 0.02^{ab}	0.22 ± 0.02^{a}	0.27 ± 0.00^{a}	0.32 ± 0.01^{a}	0.38 ± 0.01^{a}	0.38 ± 0.01^{a}
4	20	80	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.11 ± 0.02^{a}	0.16 ± 0.01^{a}	0.22 ± 0.02^{a}	0.33 ± 0.01^{a}	0.43 ± 0.02^{a}
5	40	60	-	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{ab}	0.06 ± 0.02^{a}	0.22 ± 0.03^{a}	0.33 ± 0.01^{a}	0.38 ± 0.01^{a}	0.43 ± 0.01^{a}
6	60	40	-	0.00 ± 0.00^{a}	0.06 ± 0.01^{ab}	0.06 ± 0.01^{a}	0.16 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.11 ± 0.01^{a}	0.22 ± 0.01^{a}	0.33 ± 0.01^{a}	0.38 ± 0.02^{a}	0.43 ± 0.02^{a}
7	80	20	-	0.00 ± 0.00^{a}	0.05 ± 0.01^{ab}	0.11 ± 0.02^{a}	0.16 ± 0.02^{a}	0.16 ± 0.02^{a}	0.22 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}	0.38 ± 0.01^{a}	0.43 ± 0.03^{a}	0.49 ± 0.00^{a}
8	20	-	80	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.06 ± 0.00^{a}	0.22 ± 0.02^{a}	0.27 ± 0.01^{a}	0.32 ± 0.02^{a}	0.38 ± 0.01^{a}
9	40	-	60	0.00 ± 0.00^{a}	0.16 ± 0.01^{a}	0.11 ± 0.03^{a}	0.16 ± 0.02^{a}	0.16 ± 0.02^{a}	0.11 ± 0.01^{ab}	0.27 ± 0.01^{a}	0.32 ± 0.02^{a}	0.43 ± 0.01^{a}	0.43 ± 0.02^{a}	0.49 ± 0.01^{a}
10	60	-	40	0.00 ± 0.00^{a}	0.05 ± 0.00	0.11 ± 0.01^{a}	0.16 ± 0.00^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.32 ± 0.01^{a}	0.43 ± 0.03^{a}	0.43 ± 0.03^{a}	0.54 ± 0.02^{a}
11	80	-	20	0.00 ± 0.00^{a}	0.06 ± 0.00^{ab}	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{ab}	0.16 ± 0.01^{a}	0.32 ± 0.02^{a}	0.38 ± 0.00^{a}	0.49 ± 0.01^{a}	0.59 ± 0.01^{a}

Values are reported as mean ± SD.
Different small letters (a,b) within a column show significant difference ($P < 0.05$) based on Tukey's test.

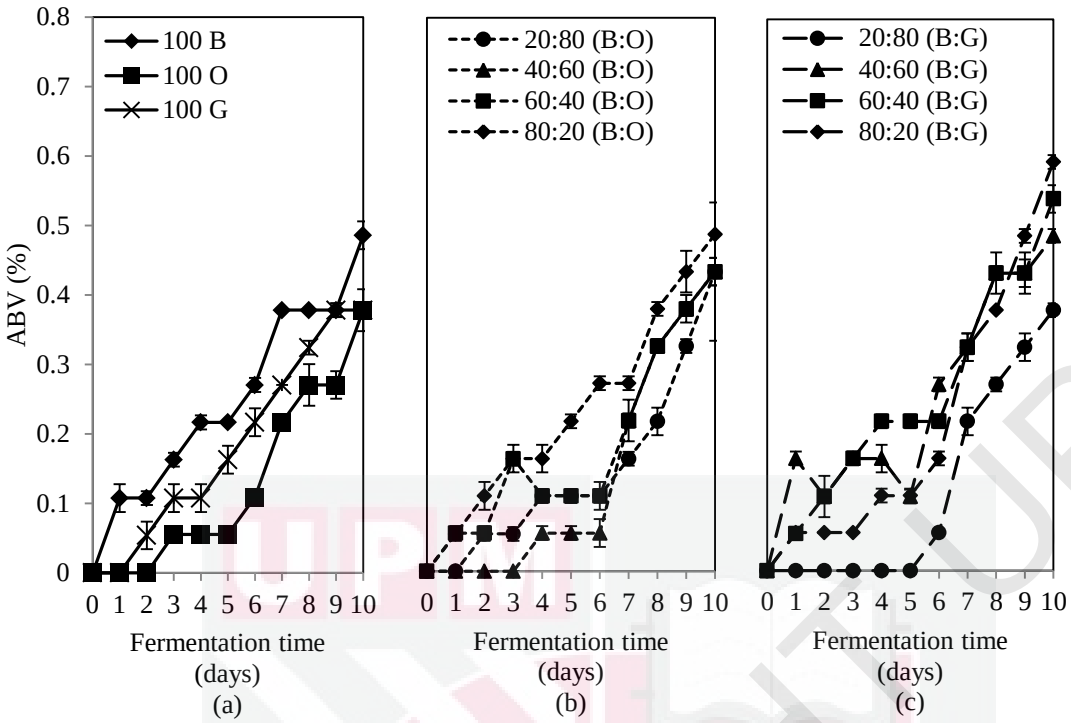


Figure 4.4: Alcohol content of *kombucha* teas made with (a) pure black, oolong and green teas, (b) combinations of black tea and oolong tea, and (c) combinations of black tea and green tea. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

Despite the highest alcohol content found in mixture of black and green tea *kombucha*, the *kombucha* in this study had low alcohol contents of about 0.5%, which is in compliance with Centre of Disease Control of British Columbia (BCCDC) plan, *Kombucha* Brewers International standards and The Department of Islamic Development Malaysia (JAKIM) (de Miranda et al., 2022; *Kombucha Brewers International*, 2021; Najiha & Nadiah, 2014; Sharifudin et al., 2021).

4.2 Effects of tea types on total phenolic content of *kombucha* fermentation

Table 4.5 and Figure 4.5 present the total phenolic content in *kombucha*. The total phenolic content showed no significant difference ($P > 0.05$) among all tea types before fermentation; however, it increased post-fermentation, with significant

differences observed as the proportion of black tea in tea blend increased. The elevation in total phenolic contents with the time of fermentation was confirmed by previous studies (Chakravorty et al., 2016; Jafari et al., 2020; Jakubczyk et al., 2020), attributed to enzymatic reactions catalysed by metabolic activity of microbes such as *Zygosaccharomyces*, *Schizosaccharomyces*, *Gluconobacter*, *Acetobacter*, and *Lactobacillus* spp. (Shi et al., 2023). These reactions are facilitated by enzymes such as glucosidase, pectinase, xylanase, cellulase and glucanase produced during fermentation (Kim et al., 2023). These enzymes degrade oligomeric and polymeric polyphenol compounds, resulting in the formation of smaller polyphenols like catechins with antioxidant properties, thereby contributing to the increase in total phenolic content (Jakubczyk et al., 2020; Kim et al., 2023).

The highest phenolic content was observed in oolong tea *kombucha*, followed by green tea and black tea, whereas higher proportions of black tea in tea mixtures resulted in lower total phenolic content of *kombucha*. The higher phenolic content of substrate may create a more favourable environment for microbial activity, potentially resulting in higher acid production and a lower pH (Piekarska-Radzik & Klewicka, 2021). This is because certain phenolic compounds such as catechin and gallic acid have been studied for their ability to stimulate bacterial growth (García-Ruiz et al., 2008; Plyuta et al., 2013; Rapeanu et al., 2014; Sabel et al., 2017). Osiripun & Apisittiwong (2021) demonstrated the highest total phenolic content in oolong tea compared to black and green tea *kombucha* while Kaewkod et al. (2019) revealed that green tea *kombucha* had the highest amount of total phenolic content, followed by oolong tea and lastly black tea. The deviation of phenolic content may be linked to numerous reactions taking place during *kombucha* fermentation, which

is the result of a microbial hydrolysis reaction (Jakubczyk et al., 2020), yet the exact reason remains unclear. Firstly, the phenolic profile of *kombucha* has not been fully elucidated and new phenolic compounds are still being identified (Cardoso et al., 2020; Shi et al., 2023). The second reason is that polyphenol biotransformation is extremely complex, resembling a metabolic labyrinth driven by microorganisms (Filannino et al., 2018; Shi et al., 2023).

Table 4.5: Total phenolic content of *kombucha* teas made with different tea types and combinations

Sample	Amount of tea (%)			Total phenolic content (mg GAE/L)	
	Black tea	Oolong tea	Green tea	Initial (day 0)	Final (day 10)
1	100	-	-	64 ± 1.41^{a}	116 ± 1.41^{a}
2	-	100	-	67 ± 2.83^{a}	119 ± 2.83^{a}
3	-	-	100	66 ± 4.24^{a}	116 ± 1.41^{a}
4	20	80	-	64 ± 1.41^{a}	115 ± 0.00^{a}
5	40	60	-	62 ± 1.41^{a}	113 ± 0.00^{ab}
6	60	40	-	61 ± 2.83^{a}	113 ± 0.00^{ab}
7	80	20	-	60 ± 1.41^{a}	111 ± 2.83^{ab}
8	20	-	80	62 ± 1.41^{a}	115 ± 0.00^{a}
9	40	-	60	61 ± 0.00^{a}	112 ± 1.41^{ab}
10	60	-	40	60 ± 1.41^{a}	110 ± 1.41^{ab}
11	80	-	20	60 ± 1.41^{a}	106 ± 1.41^{b}

Values are reported as mean ± SD.
Different small letters (a,b) within a column show significant difference ($P < 0.05$) based on Tukey's test.

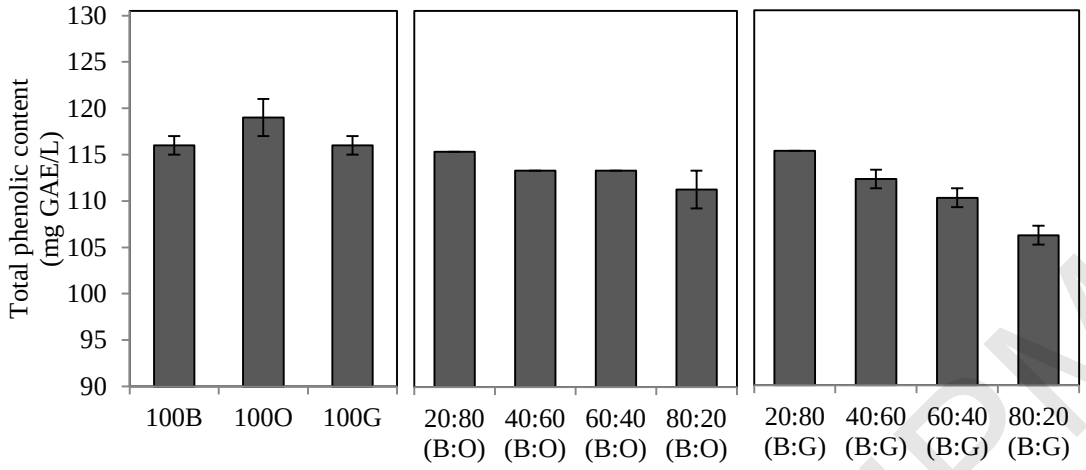


Figure 4.5: Total phenolic content of *kombucha* teas made with different tea types and combinations after a 10 days' fermentation. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

4.3 Effects of tea types on yield of *scoby* of *kombucha* fermentation

Table 4.6 shows that final weight of *scoby* increased after 10 days of fermentation, indicating that all teas can effectively sustain the livelihood of microorganisms in the production of bacterial cellulose. Tea extract stimulates bacterial cellulose production by activating cellulogenic complexes, making tea an essential nutrient supply for *kombucha* fermentation and cellulose synthesis as *scoby* cannot produce adequate cellulose independently (Laavanya et al., 2021; Sievers et al., 1995). Different tea types showed a significant influence ($P < 0.05$) on *scoby* yield, driven by the metabolic activities of acetic acid bacteria such as *Komagataeibacter*, *Acetobacter* and *Gluconacetobacter* during fermentation (Lahiri et al., 2021; Ramírez Tapias et al., 2022). These microorganisms convert various organic substances into cellulose, with pH alterations, secondary metabolites, nutrients and precursors availability playing key roles (Lahiri et al., 2021). Alongside the highest total phenolic content obtained, these factors could synergistically contribute to oolong tea exhibiting the highest yield of *scoby*, as presented in Figure 4.6. The

higher yield could also be attributed to the higher content of acetic acid bacteria, which was found in oolong tea, followed by green and black tea *kombucha* (Osiripun & Apisittiwong, 2021). The yield of *scoby* was higher in green tea *kombucha* fermentation than black tea, supported by studies showing its higher stimulating effect, resulting in faster production of fermentation products (Greenwalt et al., 2000; Jafari et al., 2020; Jayabalan et al., 2014). Lower black tea composition in tea blend *kombucha* with green and oolong tea resulted in a higher *scoby* yield.

Table 4.6: Initial weight, final weight and yield of *scoby* from *kombucha* made with different tea types and combinations

Sample	Amount of tea (%)			Weight of *scoby* (g/L)		Yield of *scoby* (%)
	Black tea	Oolong tea	Green tea	Initial (day 0)	Final (day 10)	
1	100	-	-	30.0	74.2 ± 5.17	82.48 ± 5.75^{bc}
2	-	100	-	30.0	98.9 ± 5.26	109.93 ± 5.85^{a}
3	-	-	100	30.0	84.2 ± 1.65	93.59 ± 1.83^{b}
4	20	80	-	30.0	85.6 ± 1.44	95.15 ± 1.60^{b}
5	40	60	-	30.0	83.1 ± 1.05	92.33 ± 1.17^{b}
6	60	40	-	30.0	83.0 ± 5.04	92.19 ± 5.60^{b}
7	80	20	-	30.0	68.3 ± 2.89	75.93 ± 3.21^{cd}
8	20	-	80	30.0	67.5 ± 5.12	75.04 ± 5.69^{cd}
9	40	-	60	30.0	65.1 ± 3.27	72.30 ± 3.63^{cd}
10	60	-	40	30.0	61.5 ± 4.29	68.37 ± 4.76^{d}
11	80	-	20	30.0	58.4 ± 4.41	64.89 ± 4.90^{d}

Values are reported as mean ± SD.
Different small letters (a,b,c,d) within a column show significant difference ($P < 0.05$) based on Tukey's test.

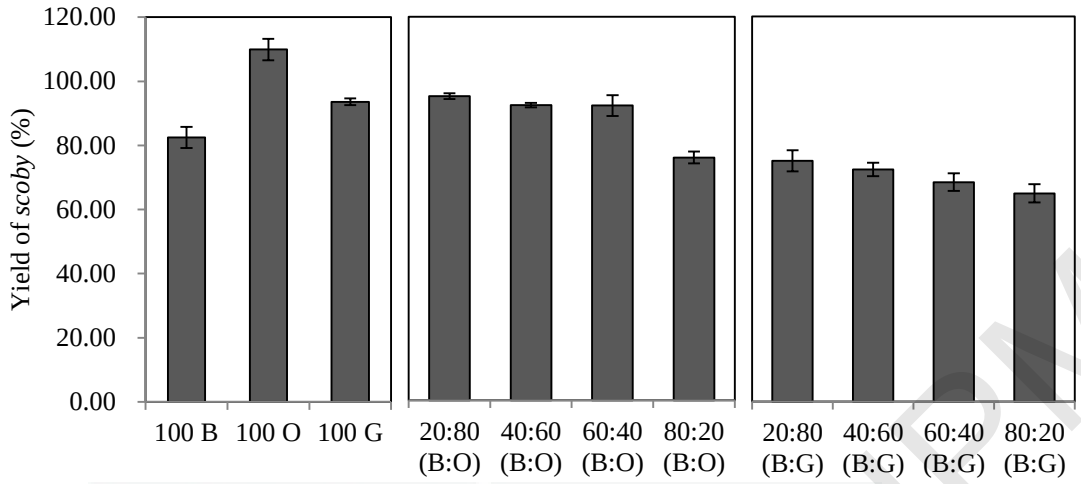


Figure 4.6: Yield of *scoby* of *kombucha* made with different tea types and combinations after a 10 days' fermentation. Abbreviations: B, Black tea; O, Oolong tea; G, Green tea

Most of the *scoby* showed higher yields than samples in earlier reports (21.5-66.7%) (Goh et al., 2012a; Lee & Kim, 2000). Such disparity of results can be mainly associated with a microbial variety of local *kombucha scoby* and the tea recipe itself, along with fermentation conditions (Chen & Liu, 2000; Neffe-Skocińska et al., 2017).

Table 4.7: Productivity and conversion yield of *scoby* from *kombucha* made with different tea types and combinations

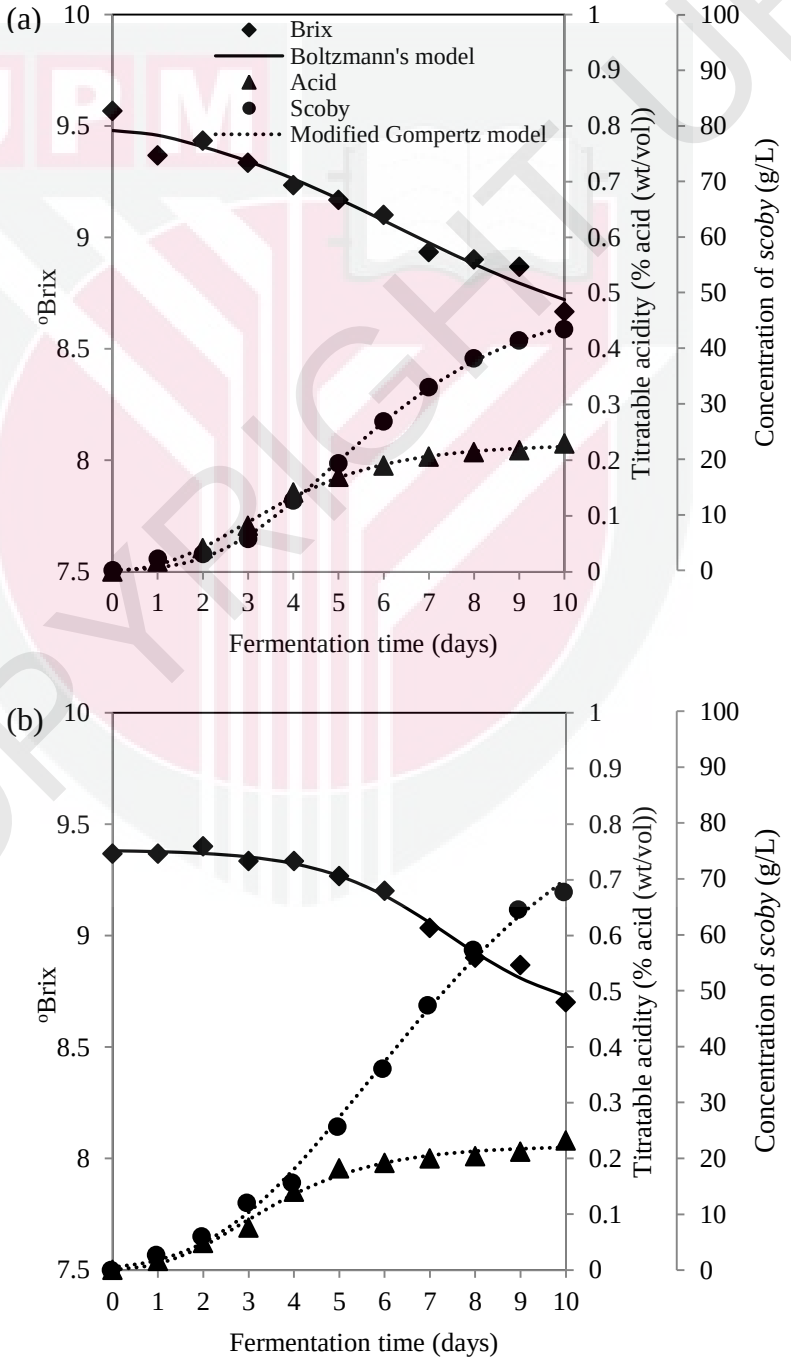
Sample	Amount of tea (%)			Productivity of *scoby* (g/L-day)	Conversion yield of *scoby* (g/^{o}Brix)
	Black tea	Oolong tea	Green tea		
1	100	-	-	7.42	82.48
2	-	100	-	9.89	148.40
3	-	-	100	8.42	120.33
4	20	80	-	8.56	111.69
5	40	60	-	8.31	99.72
6	60	40	-	8.30	103.71
7	80	20	-	6.83	78.85
8	20	-	80	6.75	92.09
9	40	-	60	6.51	69.71
10	60	-	40	6.15	63.66
11	80	-	20	5.84	54.75

Table 4.7 shows the productivity and conversion yield of *scoby* from *kombucha* made with different teas. With the same carbon source, oolong tea exhibited the highest results, followed by green tea and lastly black tea, suggesting oolong tea's potential for enhanced *kombucha* fermentation compared to black tea traditional. Mixed tea samples with presence of black tea gave lower yields as seen from the combinations with both green tea and oolong tea although the latter, oolong tea, managed to help boost the yield of *scoby* of the pure black tea *kombucha* (Figure 4.6).

4.4 Modelling of *kombucha* fermentation process

The variety of pure teas was chosen for *kombucha* fermentation modelling process using the Boltzmann model (Equation 3.7) to describe the change of substrate (sucrose) in terms of ^{o}Brix (Table 4.8). The reduction in sugar concentration was well described by the model, with R^2 values greater than 0.96, giving a reasonably good fit of data. Following the trends of sucrose changes (Figure 4.7), three stages were identified: (i) a minor decrease at the beginning of fermentation, (ii) a sharp decrease in the intermediate phase, and (iii) a gradual decline towards the end of fermentation (Loncar et al., 2014). This trend suggests that the fermentation process initially requires an adaptation period before the microorganisms start utilising the available sugars efficiently. Parameters A_1 and A_2 correspond to the positions of the upper and lower asymptotes of the curve respectively. Oolong tea exhibited the longest adaptation period, as evidenced by its highest t_0 value, representing the point at which the slope has the highest value, followed by green and black teas. Conversely, oolong tea had the smallest width on the step of exponential decrease (Δt), indicating a faster fermentation activity in comparison to green and black teas. The reduction of sucrose in *kombucha* fermentation is catalysed by enzymes

produced by diverse microflora, especially yeast presented in *scoby*. Sucrose molecules bind to the active sites of enzymes and are converted to products via a series of sequential and parallel processes (Loncar et al., 2014). As fermentation neared completion, sucrose conversion gradually decreased, attributed to yeast count which was found to stabilise after day 7 (Neffe-Skocińska et al., 2017).



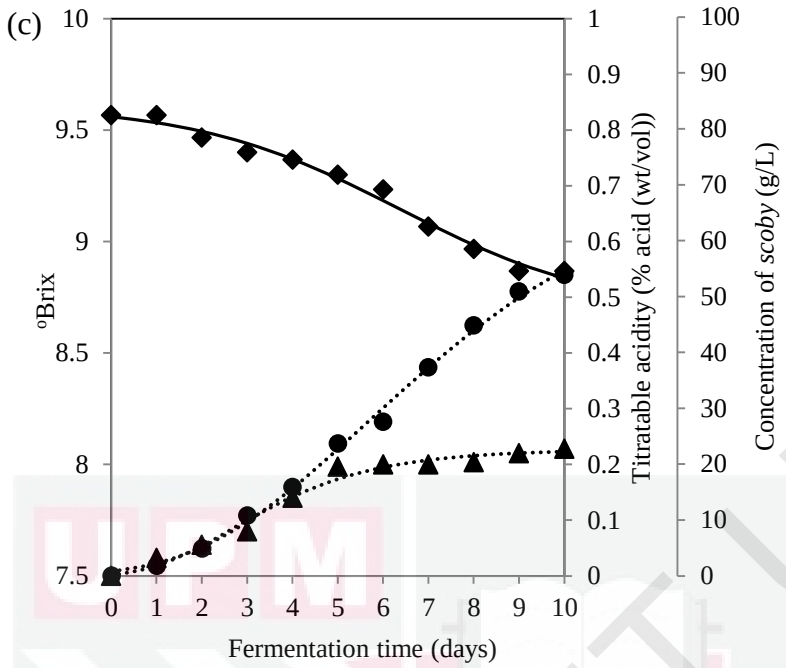


Figure 4.7: Modelling of *kombucha* fermentation in terms of sugar reduction in Brix using Boltzmann model and production of acid and *scoby* using modified Gompertz model for three different pure tea types: (a) Black tea, (b) oolong tea and (c) green tea

Table 4.8: Parameters of Boltzmann model

Parameters	Tea type in *kombucha* fermentation		
	Black tea	**Oolong tea**	**Green tea**
A_1	9.61	9.38	9.61
A_2	8.48	8.62	8.67
t_0	5.82	7.39	6.38
Δt	2.82	1.40	2.28
R^2	0.96	0.99	0.99

Contrasting to sugar reduction, the acid and *scoby* production during *kombucha* fermentation follows a different pattern, which was effectively described by the modified Gompertz model, as shown in Figure 4.7. The derived biological parameters of acid and *scoby* production are summarised in Table 4.9. The achieved coefficients of determination from the applied model were remarkably high (R^2 > 0.98), suggesting an excellent fit of data. The acetic acid specific formation rates (μ)

ranged from 0.047 to 0.049% acid/day and *scoby* specific formation rates ranged from 7.323 to 9.980 g/L-day, with the highest rates observed in pure oolong tea *kombucha*. The higher product formation rates not only serve as a key indicator of fermentation progress but also reflect a more conducive microbial environment for efficient metabolic processes. Matei et al. (2020) have shown that efficient metabolics during microbial fermentation can contribute to beneficial properties of *kombucha* such as enhancing protection against the growth of harmful bacteria, including *Bacillus pumilus* and *Salmonella typhimurium.*

Table 4.9: Parameters of modified Gompertz model: Acid and *scoby* production

Parameters	Tea type in *kombucha* fermentation		
	Black tea	**Oolong tea**	**Green tea**
Acid production			
A (% acid (wt/vol))	0.229	0.223	0.227
μ (% acid (wt/vol)/day)	0.048	0.049	0.047
λ (day)	1.126	1.164	0.871
R^2	0.99	0.99	0.98
***Scoby* production**			
A (g/L)	49.759	92.870	74.807
μ (g/L-day)	7.323	9.980	7.393
λ (day)	2.313	2.308	1.935
R^2	0.99	0.99	0.99

Mathematical models, *i.e.*, polynomial, rational and exponential models were used to capture the pH dynamics during *kombucha* fermentation, as shown in Figure 4.8. Table 4.10 presents the corresponding model parameters. The exponential decrease in pH during fermentation process can be described as a gradual and continuous decline that follows a diminishing pattern over time. Initially, the pH decline may be more pronounced, reflecting a rapid accumulation of acids during the active phase of fermentation. As fermentation proceeds, the pH change becomes less dramatic, showing a decreasing rate of acid production. The diminishing trend of pH

approaches a straight line, indicating a more stable pH level and suggesting the completion of the fermentation process. The mathematical models effectively describe the pH trend, with $R^2 > 0.98$. The rational model achieved the best fit among all models, providing a robust representation of pH trend and contributing to a higher level of efficacy in comprehending the dynamic changes of pH throughout the fermentation process.

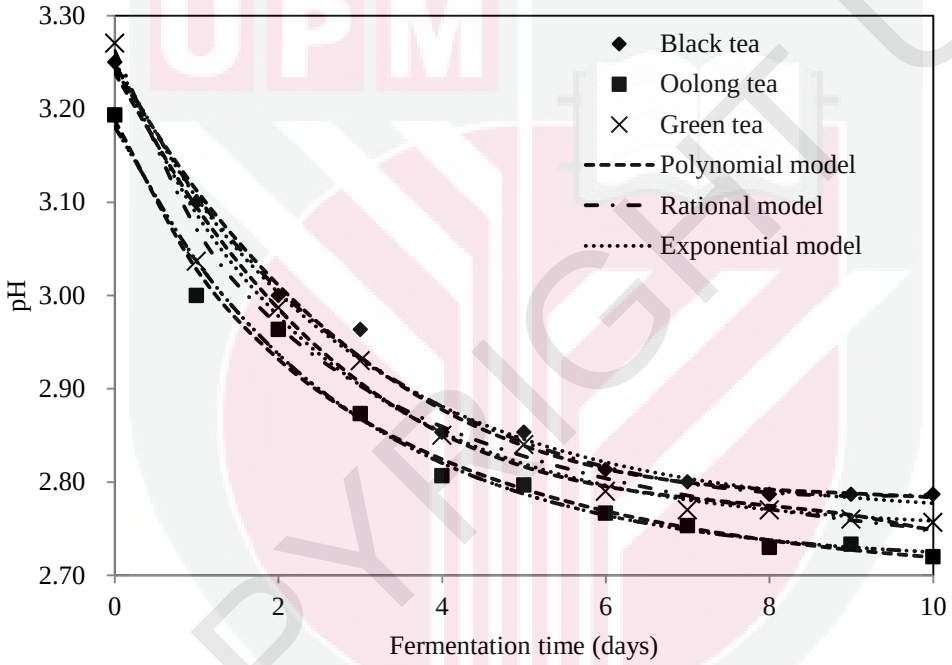


Figure 4.8: Modelling of pH on *kombucha* fermentation

Table 4.10: Parameters of pH modelling in *kombucha* fementation

Parameters	Tea type in *kombucha* fermentation		
	Black tea	**Oolong tea**	**Green tea**
(a) Polynomial model			
a	3.242	3.175	3.238
b	-0.145	-0.151	-0.163
c	0.016	0.019	0.020
d	-0.001	-0.001	-0.001
R^2	0.99	0.99	0.98
(b) Rational model			
a	3.248	3.187	3.262
b	0.298	0.778	1.096
c	0.143	0.310	0.419
d	-0.002	-0.001	-0.002
R^2	0.99	0.99	0.99
(c) Exponential model			
a	0.486	0.468	0.500
b	0.354	0.368	0.390
c	2.763	2.713	2.748
R^2	0.99	0.99	0.98

4.5 Summary

Kombucha fermentation using different tea types and their combinations has a significant impact on the fermentation outcomes. However, all samples consistently led to decreased pH and total soluble solids, along with increased TA, alcohol and total polyphenol content throughout the fermentation process. At the end of fermentation, a range of 106-119 mg GAE/L of total phenolic content and 64.89-109.93% of *scoby* yield were observed. Oolong tea demonstrated the highest total phenolic content, *scoby* productivity and conversion yield, emphasising its favourable characteristics in *kombucha* fermentation compared to other samples including the traditional black tea substrate. Mathematical modelling was conducted specifically on pure tea *kombucha*, with R^2 values of Boltzmann model for total soluble solids and modified Gompertz model for acetic acid and *scoby* production

greater than 0.96, giving reasonably good fit of data. The acetic acid specific formation rates (μ) ranged from 0.047 to 0.049% acid/day while *scoby* specific formation rates ranged from 7.323 to 9.980 g/L-day, with the highest results of both observed in pure oolong tea *kombucha*. Mathematical modelling of pH using polynomial, rational and exponential models showed an excellent fit of data (R^2 > 0.98). All in all, the results suggest that alternative substrates other than black tea can produce similar outcomes in *kombucha* fermentation, thereby demonstrating their potential as viable substitutes. By exploring different substrates in the *kombucha* fermentation process using modelling approach, the development of innovative products and generation of valuable by-products can be fostered as improvements in the food and beverage industry.

CHAPTER 5

EFFECTS OF *SCOBY* ADDITION IN JAM FORMULATION

This chapter presents the physicochemical, textural, rheological properties, proximate analysis and sensory evaluation of mango jams prepared using *scoby* obtained from the *kombucha* fermentation process using black, green and oolong tea.

5.1 Physicochemical properties of jam formulated with *scoby*

The physicochemical properties of mango jam produced with addition of *scoby*, which include water activity, moisture content, pH, total soluble solids and colour were measured due to their importance in determining the quality, stability and safety of the final product. The control sample and effects of *scoby* addition on water activity, moisture content, pH and total soluble solids are shown in Table 5.1. The baseline control for this study is represented by a mango jam sample without *scoby*, which serves as a benchmark for comparison with jam samples with added *scoby*.

Table 5.1: Water activity, moisture content, pH and total soluble solids of mango jams with different types of *scoby* added and a control sample

Scoby	Conc. (%)	Water activity (a_w)	Moisture Content	pH	°Brix
Control*	0	0.873 ± 0.003^{a}	31.65 ± 0.64^{a}	3.39 ± 0.02^{a}	67.8 ± 0.2^{a}
Black tea	2	0.846 ± 0.001^{b}	28.48 ± 0.50^{cd}	3.38 ± 0.01^{ab}	67.8 ± 0.1^{a}
	4	0.824 ± 0.000^{c}	26.49 ± 0.28^{f}	3.35 ± 0.01^{b}	65.6 ± 0.1^{cd}
	6	0.787 ± 0.008^{de}	22.58 ± 0.08^{hi}	3.29 ± 0.01^{cde}	65.6 ± 0.4^{cd}
	8	0.774 ± 0.002^{f}	20.04 ± 0.01^{j}	3.29 ± 0.01^{cde}	65.3 ± 0.1^{de}
	10	0.679 ± 0.001^{i}	19.92 ± 0.06^{j}	3.24 ± 0.01^{gh}	65.1 ± 0.1^{e}
Oolong tea	2	0.864 ± 0.002^{a}	27.96 ± 0.10^{de}	3.31 ± 0.01^{cd}	68.1 ± 0.1^{a}
	4	0.824 ± 0.001^{c}	26.31 ± 0.03^{f}	3.32 ± 0.01^{c}	67.9 ± 0.1^{a}
	6	0.781 ± 0.012^{ef}	24.27 ± 0.05^{g}	3.29 ± 0.01^{de}	66.5 ± 0.3^{b}
	8	0.738 ± 0.004^{g}	23.52 ± 0.70^{gh}	3.22 ± 0.01^{hi}	65.5 ± 0.1^{cde}
	10	0.701 ± 0.007^{h}	22.84 ± 0.20^{hi}	3.19 ± 0.01^{j}	65.5 ± 0.1^{cde}
Green tea	2	0.850 ± 0.001^{b}	29.48 ± 0.06^{bc}	3.35 ± 0.02^{b}	67.8 ± 0.1^{a}
	4	0.816 ± 0.002^{c}	29.81 ± 0.10^{b}	3.27 ± 0.01^{efg}	66.5 ± 0.2^{b}
	6	0.797 ± 0.002^{d}	27.11 ± 0.04^{ef}	3.27 ± 0.01^{ef}	65.8 ± 0.0^{cd}
	8	0.712 ± 0.004^{h}	26.50 ± 0.66^{f}	3.26 ± 0.01^{fg}	65.9 ± 0.0^{c}
	10	0.707 ± 0.005^{h}	22.14 ± 0.13^{i}	3.21 ± 0.01^{ij}	65.3 ± 0.1^{de}

*Control is sample without addition of *scoby*
Conc. refers to concentration
Values are reported as mean ± SD.
Different small letters (a,b,c,d,e,f,g,h,i,j) within a column show significant difference ($P < 0.05$) based on Tukey's test.

5.1.1 Water activity (a_w)

Table 5.1 shows that mango jams containing *scoby* had significantly lower ($P < 0.05$) water activity than control sample, with the exception of mango jam with 2% oolong tea *scoby*. This phenomenon can be attributed to the water retention ability and hydrophilic property of *scoby*, which enable them to extract and immobilise water molecules (Goh et al., 2012b; Laavanya et al., 2021). The interconnected molecular network with water reduces the amount of available water in the jam, lowering water activity.

Figure 5.1 presents a shared pattern in the relationship between *scoby* concentration and water activity across different types of jams, showing that higher *scoby* concentration levels correspond to lower water activity levels. The increase in the

propensity for binding water molecules corresponds to larger numbers of polymers in *scoby* (Saha & Bhattacharya, 2010), which can result in a competition for available water between sugar molecules in jam (Javanmard et al., 2012). Hence, increased concentrations of *scoby* caused the formation of a more compact network in jam, leading to an intensified attachment of water molecules and a reduction in water activity. It is imperative to reduce water activity levels in the preparation of jam with the intent of preventing conformational changes in enzymes and minimising the probability of deleterious microbiological growth (Bhagwat, 2019; Kanwal et al., 2017). The majority of the mango jams with *scoby* fell within the water activity limits of 0.6 to 0.85 for intermediate moisture foods (Erkmen & Bozoglu, 2016; Public Health England, 2020; Roos, 2003), showing a positive impact on enhancing the safety and quality of the product.

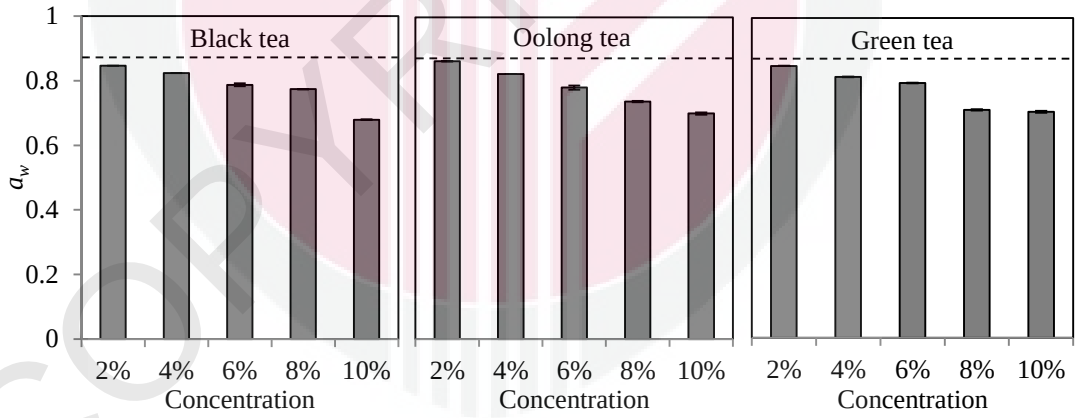


Figure 5.1: Water activity of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

5.1.2 Moisture content

Mango jams containing *scoby* had significantly lower ($P < 0.05$) moisture content than control sample, as presented in Table 5.1. Addition of *scoby* increased the solid

fraction, resulting in a decrease in moisture content. The magnitude of the decrease in moisture content was also influenced by the increase in concentration of *scoby* of all types, as shown in Figure 5.2. In Javanmard et al. (2012), similar findings were revealed with respect to hydrocolloids such as high methoxyl pectin and carboxymethyl cellulose. The increased hydrocolloid content in jam elevated the solid fraction and reduced moisture content (Javanmard et al., 2012) by enhancing water binding and retention within the jam matrix. This ability to absorb and immobilise moisture not only helps in structuring and thickening the jam but also has the added benefit of inhibiting the growth of detrimental microorganisms and prolonging the shelf life of jam. As intermediate moisture foods, jam should have a moisture content ranging from 10% to 50% (Roos, 2003) and mango jams with addition of *scoby* complied within this range (19.92 to 29.81%). For other hydrocolloids reported, the moisture content of mango jam ranged from 22.01 to 29.87% for pectin (Bekele et al., 2020) and 22.09 to 40.99% for high methoxyl pectin, carboxymethyl cellulose and sago starch (Javanmard et al., 2012).

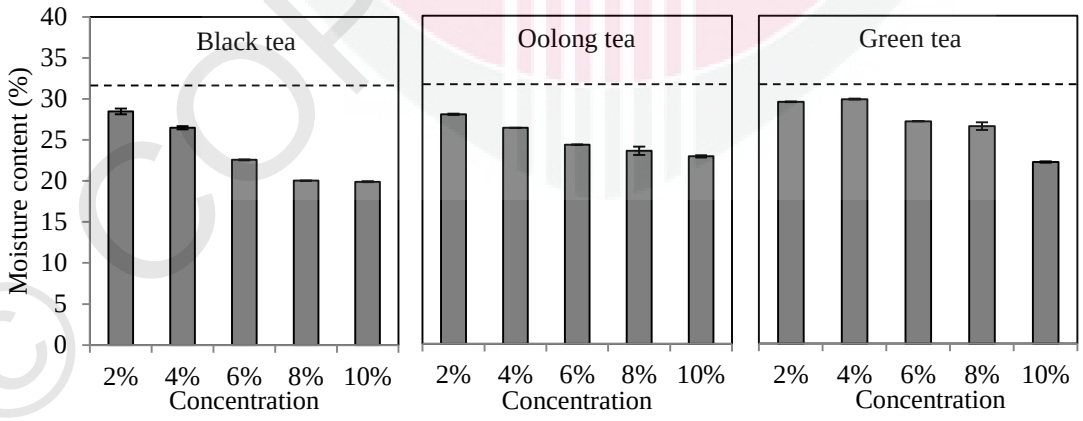


Figure 5.2: Moisture content of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

5.1.3 pH

Acidity, similar to water activity and moisture content, is a critical consideration in jam production. Based on Table 5.1, significant regression ($P < 0.05$) of pH values was observed, indicating that incorporation of *scoby* in jams had an impact on its pH. The mango jams with *scoby* resulted in a further decrease in pH values compared to the control sample without *scoby*. This is beneficial as higher acidity levels are preferred for jams (Bekele et al., 2020). The heightened level of acidity is desirable for facilitating gel formation (Bekele et al., 2020), ensuring the desired consistency in the aspect of flow properties (Razak et al., 2018), preventing the growth of spoilage microorganisms and ultimately enhancing the shelf life of jam (Emelike & Akusu, 2019; Gindi et al., 2019).

Figure 5.3 shows a decline in pH values of mango jams with an increase in concentration of *scoby*, suggesting an increase in acidity in jams. The outcome was consistent with the findings reported by Razak et al. (2018), who observed a similar correlation between increased concentrations of hydrocolloids and reduced pH values, regardless of the type of hydrocolloid used. Despite reduced pH levels, the pH of jam with *scoby* (3.19-3.38) remained within the commonly observed pH values of jams, ranging between 2.5 and 3.4 as reported in the literatures (Featherstone, 2016; Gindi et al., 2019).

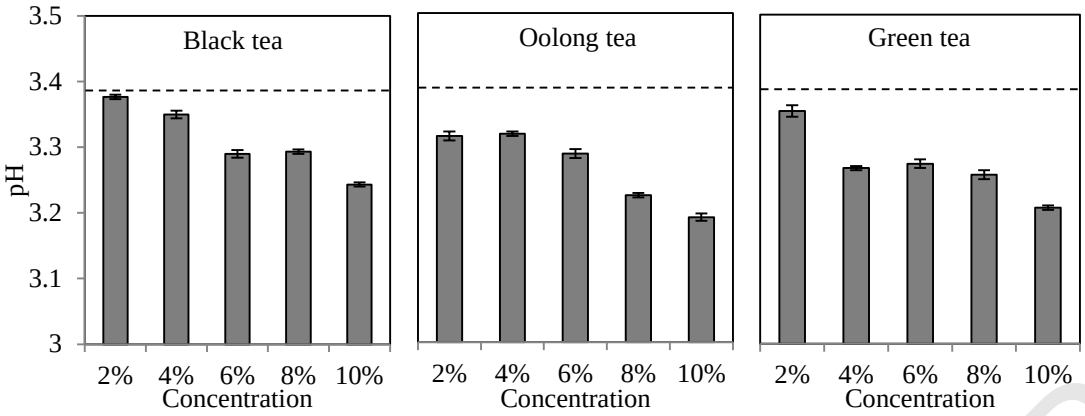


Figure 5.3: pH values of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

5.1.4 Total soluble solids (^{o}Brix)

The acidity and total soluble solids measured in °Brix in jam making are interdependent as higher total soluble solids can decrease acidity and both are vital parameters for achieving desired texture of jam. As expected from the results of increasing acidity, total soluble solids of mango jams decreased significantly ($P < 0.05$) with addition of *scoby*, as reported in Table 5.1.

Figure 5.4 shows that the ^{o}Brix values declined with increased concentrations of *scoby* in mango jam, irrespective of the type of *scoby* used. The increased concentrations of *scoby* corresponded to a lower percentage of mango fruit content in jam formulation, which presented lower total soluble solids because of the influence of high sugar content of mango fruit (Mgaya-Kilima et al., 2015). All samples, with different concentrations and types of *scoby*, complied with the requirements set forth by the Food Act (1983) and Food Regulations Malaysia (1985) which specify that the total soluble solids content in jam should not be less than 65%.

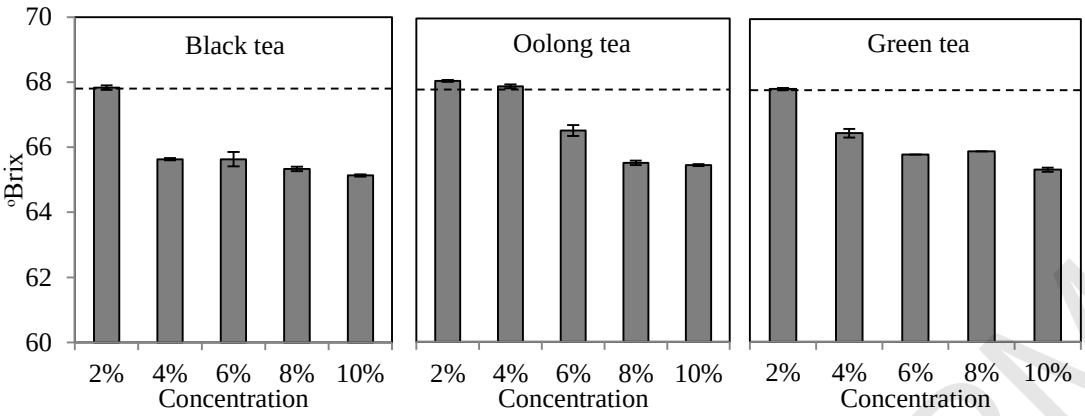


Figure 5.4: Total soluble solids of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample. All samples were above 65%.

5.1.5 Colour properties

The role of colour in indicating fitness and acceptability of a product for consumption is a paramount consideration that should not be overlooked. The colour properties, namely, L^* (lightness/darkness), a^* (greenness/redness), b^* (yellowness/blueness), ΔE (total colour change) and h^* (hue angle) to evaluate colour attributes of mango jams are shown in Table 5.2.

Table 5.2: Colour properties of mango jams with different types of *scoby* added and a control sample

Scoby	Conc. (%)	L^*	a^*	b^*	ΔE	h^* (^{o})
Control*	0	30.98 ± 0.76^{g}	8.91 ± 0.06^{de}	44.33 ± 0.74^{ab}	-	78.63
Black tea	2	34.85 ± 0.66^{e}	5.55 ± 0.21^{g}	40.16 ± 0.68^{defg}	6.73 ± 0.49^{c}	82.13
	4	37.55 ± 0.17^{d}	5.68 ± 0.48^{g}	41.29 ± 0.37^{cdef}	7.97 ± 0.47^{bc}	82.16
	6	38.18 ± 0.47^{bcd}	5.92 ± 0.48^{g}	41.52 ± 0.36^{cde}	8.30 ± 0.44^{abc}	81.88
	8	40.36 ± 0.43^{a}	6.16 ± 0.55^{g}	42.11 ± 0.09^{bcd}	10.06 ± 0.80^{a}	81.68
	10	40.83 ± 0.62^{a}	8.26 ± 0.53^{ef}	42.80 ± 1.12^{abc}	10.04 ± 0.45^{a}	79.08
Oolong tea	2	31.82 ± 0.56^{fg}	5.60 ± 0.55^{g}	36.34 ± 0.27^{i}	8.76 ± 0.65^{ab}	80.74
	4	33.41 ± 0.43^{ef}	9.43 ± 0.16^{cd}	36.36 ± 0.34^{i}	8.36 ± 1.03^{abc}	78.80
	6	37.61 ± 0.33^{d}	11.26 ± 0.16^{b}	38.95 ± 0.49^{fgh}	8.88 ± 1.22^{ab}	75.98
	8	37.87 ± 0.64^{cd}	13.28 ± 0.15^{a}	39.39 ± 0.58^{efgh}	9.57 ± 0.64^{ab}	76.75
	10	38.16 ± 0.54^{bcd}	13.43 ± 0.28^{a}	44.24 ± 2.32^{ab}	8.73 ± 0.30^{ab}	77.55
Green tea	2	34.89 ± 1.10^{e}	6.06 ± 0.23^{g}	37.20 ± 0.09^{hi}	8.73 ± 0.58^{ab}	81.24
	4	37.12 ± 0.21^{d}	7.60 ± 0.43^{f}	38.35 ± 0.32^{ghi}	8.69 ± 0.46^{ab}	75.46
	6	37.43 ± 0.24^{d}	9.92 ± 0.10^{cd}	39.75 ± 0.43^{defg}	7.99 ± 0.39^{bc}	73.88
	8	39.55 ± 0.45^{abc}	10.08 ± 0.18^{c}	42.78 ± 0.89^{abc}	8.88 ± 0.18^{ab}	71.37
	10	39.89 ± 0.83^{ab}	9.93 ± 0.09^{cd}	44.98 ± 0.38^{a}	9.03 ± 0.23^{ab}	73.11

*Control is sample without addition of *scoby*
Conc. refers to concentration
Values are reported as mean ± SD.
Different small letters (a,b,c,d,e,f,g,h,i) within a column show significant difference ($P < 0.05$) based on Tukey's test.

The presence of *scoby* in jam significantly ($P < 0.05$) increased L^* values, shown in Table 5.2, implying that it is likely to be more attractive and appealing as it had a brighter appearance. This is because brighter colours are often associated with freshness and desirable quality (Barrett et al., 2010; Pathare et al., 2013; Rababah et al., 2014). Figure 5.5 shows that increase in *scoby* concentrations of all types resulted in an elevation of L^* values. The contribution of increased lightness values could possibly be attributed to the presence of luminous gel in the final product, which was found to intensify with the escalating proportion of hydrocolloids in the jam (Javanmard et al., 2012). The present study showed a comparable pattern to the findings of Javanmard et al. (2012), where an analogous trend was observed with mango jams containing high methoxyl pectin, carboxymethyl cellulose and sago starch.

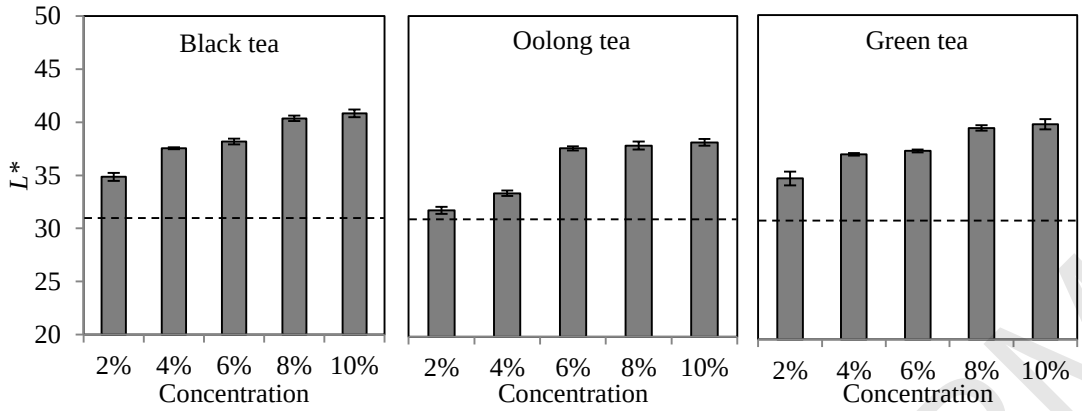


Figure 5.5: L^* values of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

The inclusion of *scoby* in mango jams exerted a significant ($P < 0.05$) effect on a^* values, as reported in Table 5.2. The increase in a^* indicates an increase in reddish hue. It was apparent that the reddish hue was not solely dependent on the anthocyanin content of mango fruit (Sudheeran et al., 2018) but was also affected by the presence of *scoby* which can cause colourant or pigment stability or destabilisation, resulting in changes to colour characteristics (Gao et al., 2017). Concomitant with such findings, Javanmard et al. (2012) and Phimpharian et al. (2011) reported similar results where an increase in hydrocolloid concentrations led to an increase in a^* value of fruit products including jam. The a^* values of mango jams with *scoby*, ranging from 5 to 13, are comparable to those reported by Basu & Shivhare (2012) in their study on mango jams made with pectin, whose study documented a^* values within the range of 8 to 11. This can be attributed to the influence of colour of mango as the main ingredient.

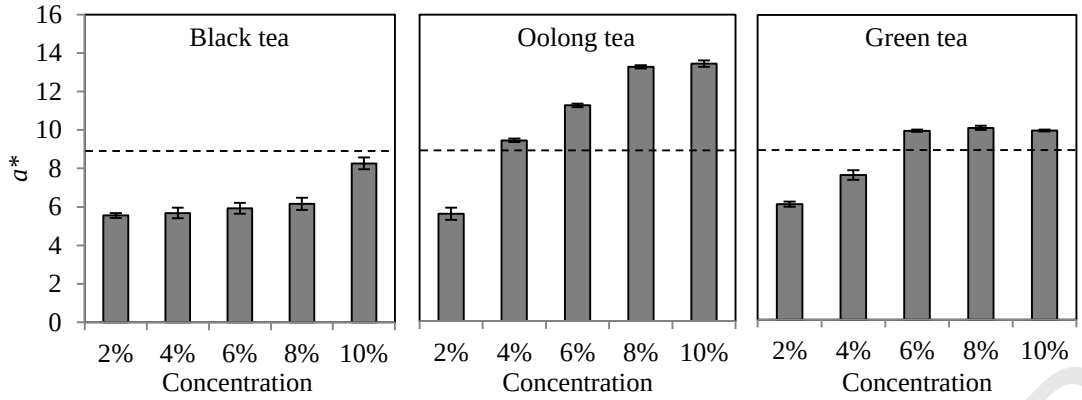


Figure 5.6: a^* values of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

Table 5.2 shows that presence of *scoby* in mango jam had a significant ($P < 0.05$) impact on b^* values, except for 10% concentration of oolong tea *scoby*. Figure 5.7 presents the b^* values of mango jams containing *scoby* from black, oolong and green *kombucha* tea at different concentrations, where an increase in *scoby* concentrations was observed to increase the b^* values of mango jams. The range of b^* values reported in Basu & Shivhare (2012) study was between 25 and 37, which is slightly lower than the b^* values found in current study. The minor difference in b^* values could be due to the use of distinct ingredients in the production of mango jam. The prior research added pectin while present study incorporated *scoby*. In addition to the pigments present in mango such as carotenoids and chlorophyll (Javanmard et al., 2012; Liu et al., 2013; Sudheeran et al., 2018), the yellow intensity of the jam may have also been influenced by the presence of *scoby*. The yellowish hue of *scoby* is believed to arise from Maillard reactions that occurred during fermentation and film conditioning between the sugars and amino acids, as well as from the presence of coloured phytochemicals (Ramírez Tapias et al., 2022). Comparing the mango jams prepared with different types of *scoby*, the differences in b^* values could be

attributed to the presence of melanoidins in *scoby*, which are nitrogen-containing compounds that are coloured and responsible for the colour of *scoby* (Ramírez Tapias et al., 2022). Despite the observed variations, all samples still maintained a yellow-orange colour of mango fruit. The hue angle (Table 5.2) of the samples ranged from 71^{o} to 82^{o}, providing further evidence of the yellow-orange colour of the mango jam samples. The hue angle (h^*) has a range between 0^{o} and 360^{o}, where 0^{o} represents purplish-red and 90^{o} represents yellow (Hutchings, 2011; Phimpharian et al., 2011).

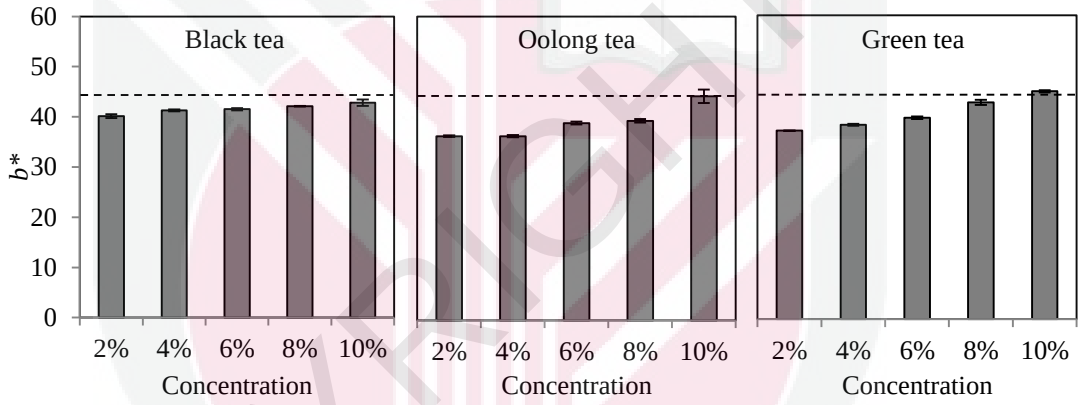


Figure 5.7: b^* values of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha*. Dotted line represents control sample.

The outcome of ΔE values is dependent on the difference of L^*, a^* and b^* data with the control sample. The presence of a total colour difference (Table 5.2) compared to the control can likely be attributed to the higher L^* and lower b^* values. In Figure 5.8, ΔE values of mango jams containing *scoby* from black, oolong and green *kombucha* tea at different concentrations are presented. The ΔE values of mango jams containing *scoby* from black tea *kombucha* increased with increasing concentrations while mango jams incorporating *scoby* from green and oolong tea *kombucha*

appeared to be relatively consistent with increasing concentrations, exhibiting minimal variation. This suggests that the *scoby* from black tea *kombucha* may contain compounds that are more sensitive to concentration changes and have a stronger impact on the colour properties of jam.

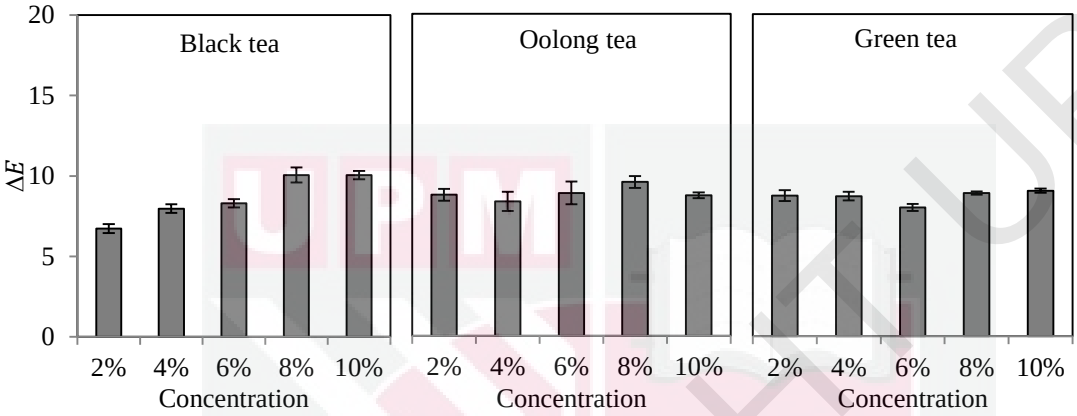


Figure 5.8: Δ*E* values of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha*

5.2 Textural and rheological properties of jam formulated with *scoby*

Textural properties, including hardness, work of shear, stickiness and work of adhesion of mango jam samples are displayed in Table 5.3.

Table 5.3: Textural properties of mango jams with different types of *scoby* added and a control sample

Scoby	Conc. (%)	Hardness (N)	Work of shear (N.s)	Stickiness (N)	Work of adhesion (N.s)
Control*	0	66.79 ± 0.40^{j}	23.26 ± 1.48^{h}	-71.03 ± 0.11^{b}	-13.99 ± 0.28^{a}
Black tea	2	83.02 ± 0.49^{f}	37.24 ± 0.37^{f}	-74.33 ± 0.04^{c}	-17.48 ± 0.20^{d}
	4	98.26 ± 0.78^{d}	39.18 ± 0.86^{ef}	-81.09 ± 0.79^{d}	-18.62 ± 0.49^{e}
	6	103.21 ± 0.15^{c}	39.66 ± 0.97^{ef}	-87.93 ± 0.66^{f}	-21.07 ± 0.48^{f}
	8	114.17 ± 0.65^{b}	48.28 ± 0.73^{b}	-92.45 ± 0.70^{g}	-23.40 ± 0.30^{g}
	10	119.14 ± 0.40^{a}	55.87 ± 1.29^{a}	-93.64 ± 1.40^{g}	-23.88 ± 0.15^{g}
Oolong tea	2	73.20 ± 0.73^{i}	29.61 ± 1.11^{g}	-64.78 ± 1.55^{a}	-15.19 ± 0.18^{b}
	4	75.38 ± 1.08^{hi}	32.06 ± 1.20^{g}	-71.01 ± 1.80^{b}	-16.49 ± 0.30^{cd}
	6	78.99 ± 2.78^{gh}	37.34 ± 0.76^{f}	-75.93 ± 1.95^{c}	-16.59 ± 0.15^{cd}
	8	87.11 ± 1.18^{e}	41.98 ± 1.50^{cde}	-79.96 ± 0.29^{d}	-17.43 ± 0.48^{d}
	10	96.74 ± 1.36^{d}	44.04 ± 0.16^{c}	-82.82 ± 0.50^{de}	-19.04 ± 0.55^{e}
Green tea	2	73.37 ± 2.96^{i}	29.95 ± 0.85^{g}	-70.62 ± 0.98^{b}	-15.73 ± 0.67^{bc}
	4	81.16 ± 1.64^{fg}	38.56 ± 0.97^{f}	-74.34 ± 0.39^{c}	-16.93 ± 0.18^{d}
	6	82.29 ± 0.59^{fg}	40.27 ± 1.00^{def}	-74.84 ± 0.44^{c}	-17.10 ± 0.20^{d}
	8	94.62 ± 0.43^{d}	43.40 ± 0.95^{cd}	-82.83 ± 1.42^{de}	-19.30 ± 0.25^{e}
	10	104.87 ± 0.52^{c}	48.55 ± 1.46^{b}	-85.52 ± 1.18^{ef}	-20.91 ± 0.20^{f}

*Control is sample without addition of *scoby*
Conc. refers to concentration
Values are reported as mean ± SD.
Different small letters (a,b,c,d,e,f,g,h,i,j) within a column show significant difference ($P < 0.05$) based on Tukey's test.

5.3.1 Hardness and work of shear

Based on Table 5.3, the incorporation of *scoby* into formulation of mango jam resulted in a significant increase ($P < 0.05$) in hardness and work of shear when compared with control sample. A more compact gel structure was obtained. This effect is consistent with previous studies that have shown the addition of hydrocolloids to similar food products produced greater firmness, such as addition of high methoxyl pectin, carboxymethyl cellulose and sago starch to mango jam (Javanmard et al., 2012) and addition of algae hydrocolloids (κ-carrageenan, ι-

carrageenan, furcellaran, and sodium alginate) to cream cheese (Vincová et al., 2023). The work of shear of mango jam with *scoby*, associated with spreadability, ranged from 29.61 to 55.87 N.s, closely aligns with the range observed for mango jams prepared with high methoxyl pectin, carboxymethyl cellulose and sago starch, which is 15.89 to 44.89 N.s (Javanmard et al., 2012) and falls within the limit of commercial spreads from 20 to 90 N.s (Glibowski et al., 2008).

Figures 5.9 and 5.10 present the hardness and work of shear of mango jams containing *scoby* from black, oolong and green tea *kombucha* at different concentrations, respectively. The results presented are in accordance with the findings of previous researchers who reported a similar trend where an increase in the concentrations of hydrocolloids resulted in an increase of hardness and work of shear of mango jams (Basu & Shivhare, 2010; Javanmard et al., 2012). The impact may be explained by the increased number of intermolecular interactions that contribute to the formation of junction zones (Goff & Guo, 2019). Consequently, the gel structure of jam has increased tensile force and turns out to be more rigid due to an increase in the number of elastically active polymeric chains within the gel network (Basu & Shivhare, 2010; Fu & Rao, 2001; Phimpharian et al., 2011). The mechanical behaviour of *scoby* is strongly reliant on the microfibrillar arrangement and the degree of interaction between the filaments by hydrogen bonds. It was observed that mango jam with black tea *scoby* had the highest hardness among all the samples tested. In Ramírez Tapias et al. (2022), mechanical strength including tensile strength and elastic module of black tea *kombucha scoby* was stated to be higher than that of green tea *kombucha scoby*. The difference in mechanical behaviour could be attributed to the presence of different quantities of low molecular weight substances

produced during the fermentation such as residual monosaccharides and organic acids which could plasticise the material (Ramírez Tapias et al., 2022). Water also acts as a plasticiser in the hydrophilic films of *scoby* by embedding itself between the polymer chains, hence, influencing the mechanical properties. Lower value of hydration content which corresponds to the water content of films, was presented in black tea *scoby* than in green tea *scoby* (Ramírez Tapias et al., 2022), contributing to a higher mechanical strength that affects the hardness of jam.

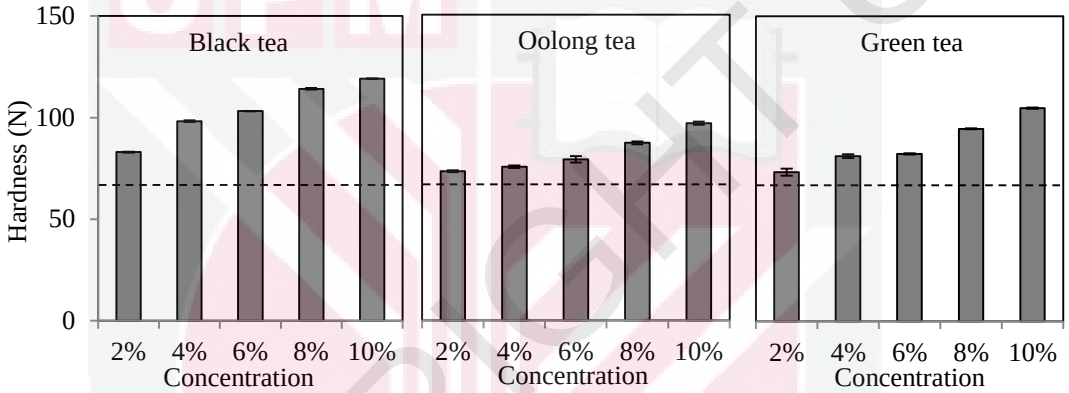


Figure 5.9: Hardness of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

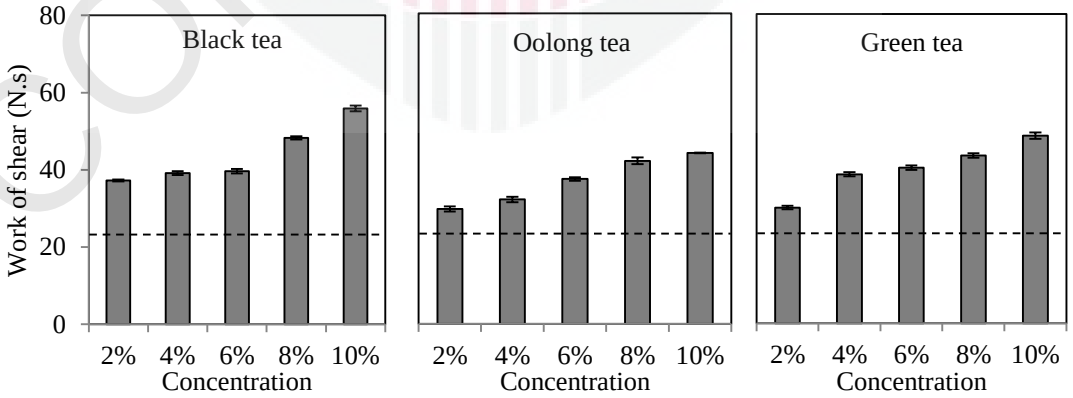


Figure 5.10: Work of shear of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

5.3.2 Stickiness and work of adhesion

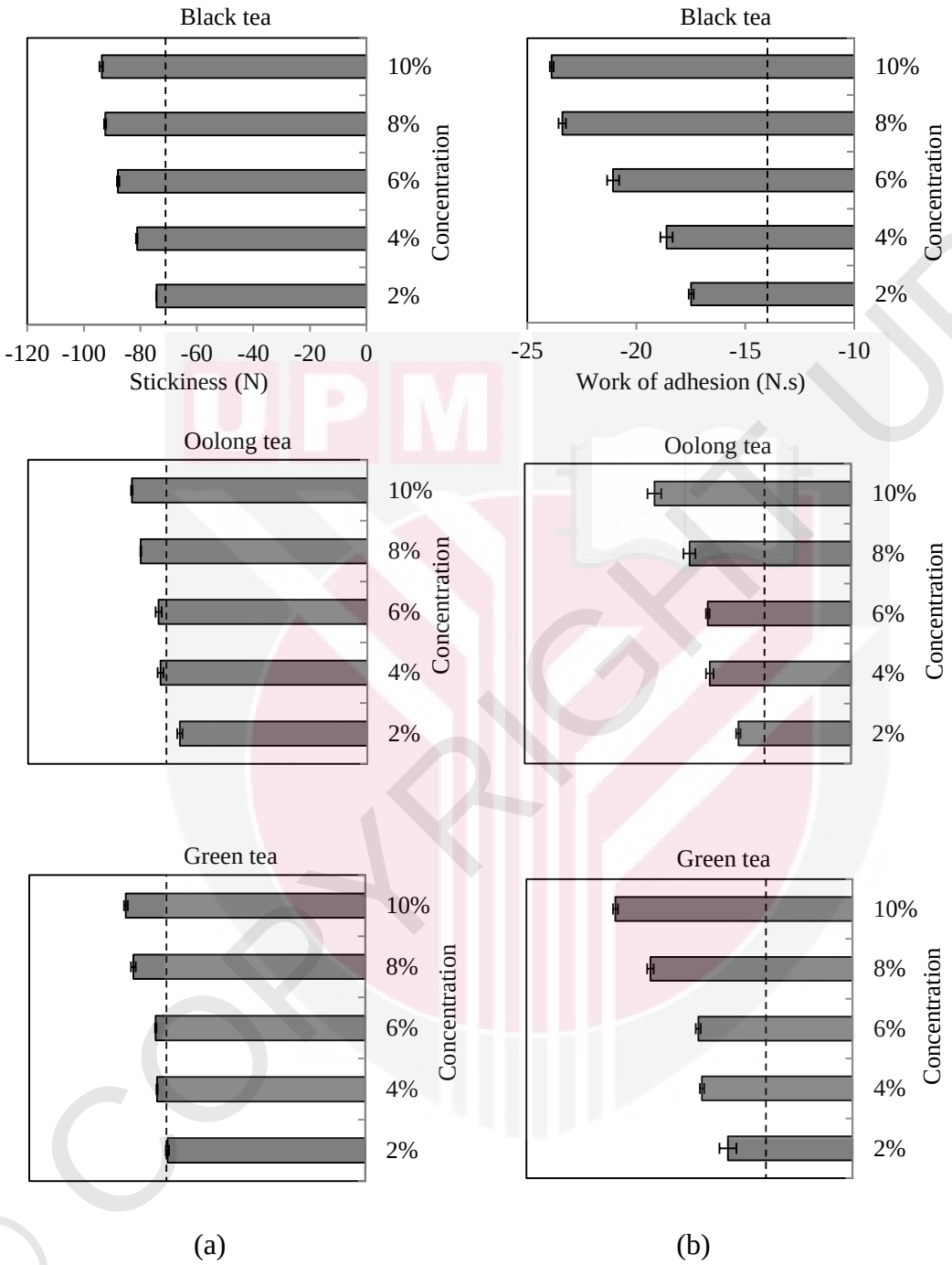


Figure 5.11: (a) Stickiness and (b) work of adhesion of mango jams containing *scoby* at different concentrations from the black, oolong and green tea *kombucha.* Dotted line represents control sample.

For stickiness, all jam samples displayed significant ($P < 0.05$) effect when *scoby* was added, except for mango jam with 2% green tea *scoby* and 4% oolong tea *scoby* compared to the control (Table 5.3). The insignificant effect could be attributed to the presence of low *scoby* concentrations in the jam matrix. For work of adhesion, the addition of *scoby* to mango jam formulation had a significant effect ($P < 0.05$) in contrast to the control sample.

Figure 5.11 depicts stickiness and work of adhesion of mango jams containing *scoby* from black, green and oolong tea *kombucha* at different concentrations. Having a similar effect with hardness and work of shear, stickiness and work of adhesion were also directly proportional to the concentrations of *scoby* added to the jam. Hydrocolloids thicken and impart stickiness to aqueous dispersion (Saha & Bhattacharya, 2010); similarly, higher concentrations of *scoby* resulted in a firmer and stickier sample.

5.3.3 Rheological properties

The rheological characteristics of mango jams were evaluated using parameters derived from rheological modelling. The two different models, namely Power Law and Herschel-Bulkley models, were selected to fit the obtained data as these models are most often used to describe flow behaviour of jams (Álvarez et al., 2007) and the parameters, *i.e.* flow index characteristics, *n* consistency coefficient, *k* and yield stress, k_{oHB} are depicted in Table 5.4. The rheograms of jam samples predicted by both models are shown in Figures 5.12 and 5.13, with an increase in shear stress as the rate of shear increased.

Both the Power Law and Herschel-Bulkley models showed high goodness of fit with R^2 > 0.9573. Similarly, these models have been used in describing the flow characteristics of jams and fruit purees, *i.e.*, mango jams (Basu & Shivhare, 2010; Javanmard & Koohikamali, 2011), blueberry puree (Kechinski et al., 2011), Murta berry pulps and purees (Ah-Hen et al., 2012), sapodilla jam (Shinwari & Rao, 2020), physalis jam (Pérez-Herrera et al., 2020) and sour cherry jam (Nourmohammadi et al., 2021).

Table 5.4: Parameters from Power Law and Herschel-Bulkley models of mango jams with different types and concentrations of *scoby* added and a control sample

Scoby	Conc. (%)	Power law			Herschel-Bulkley			
		n	k	R^2	n	k	k_{oHB}	R^2
Control	-	0.2882	31.4658	0.9965	0.6926	2.7091	51.7420	0.9676
Black tea	2	0.3061	31.7375	0.9872	0.7568	2.1181	51.4800	0.9829
	4	0.3102	35.5877	0.9980	0.6935	3.5516	61.9600	0.9687
	6	0.3019	40.3746	0.9979	0.6877	3.9306	69.4140	0.9703
	8	0.3469	41.9761	0.9969	0.7502	3.8648	74.8730	0.9757
	10	0.3632	51.2543	0.9923	0.8244	3.4363	84.0050	0.9795
Oolong tea	2	0.3120	29.7759	0.9929	0.6645	3.5758	53.4820	0.9573
	4	0.2996	33.9300	0.9962	0.6754	3.5036	58.4050	0.9633
	6	0.2943	38.8847	0.9937	0.7249	2.8631	63.1950	0.9781
	8	0.2991	41.8671	0.9689	0.7777	2.3389	66.0390	0.9833
	10	0.3742	33.2948	0.9823	0.7989	2.7412	66.2230	0.9903
Green tea	2	0.3376	28.1009	0.9862	0.7661	2.1990	53.7000	0.9859
	4	0.3314	33.6664	0.9832	0.7516	2.7352	58.9710	0.9882
	6	0.3390	34.657	0.9925	0.7578	2.8800	60.6060	0.9829
	8	0.3328	42.2625	0.9878	0.7631	3.2615	72.5340	0.9847
	10	0.3954	36.2123	0.9910	0.7942	3.5212	69.2150	0.9879

Conc. refers to concentration

In terms of rheological response, all mango jam samples showed similar features where the flow index characteristics, *n*, appeared to be less than 1. This phenomenon shows degree of pseudoplasticity and indicates a signature of shear thinning, non-Newtonian characteristics (Mohammadi Moghaddam et al., 2009). This shows

agreement with Javanmard & Endan (2010) that jams containing gels exhibit non-Newtonian behaviour. Similar behaviour of jams was also reported in Gao et al. (2011) with *n* values of 0.63, 0.57 and 0.71 for three commercial jams by brand names of St. Dalfour, Hero blueberry and Kewpie strawberry jams, respectively and Javanmard & Koohikamali (2011) with *n* values ranging from 0.6289-0.8308 for mango jam containing sago starch and 0.4864-0.5232 for commercial jam, using Herschel-Bulkley model. These results suggest that the flow index characteristics of mango jam made using *scoby* are comparable to the aforementioned jams, all of which are non-Newtonian. However, the *n* values did not show a definite trend with increased concentrations of *scoby* in jam. The consistency index, *k* showed no definite trend except an increasing trend for mango jams made with black tea *kombucha scoby* (Power law) and oolong tea *kombucha scoby* (Herschel-Bulkley model). The yield stress, k_{oHB} increased with increased concentrations of *scoby* of all types in mango jam. Yield stress is the minimal level of stress needed to initiate flow in jam. It is crucial as it plays a role in determining stability under low stress conditions, like spreadability upon usage and the perceived thickness or creaminess in the process of mastication (Schädle et al., 2022). Hydrocolloids have the ability to alter the structure of fruit solids and other components in the jam, resulting in the formation of complex network that prevents syneresis and increases the strength required to deform the jam. A similar effect was found in jam with *scoby* as observed by the increase in yield stress with the concentration of *scoby* in jam. In summary, while both Power Law and Herschel-Bulkley models have successfully characterised the flow behaviour of jam, a more appropriate fit was found with the latter model which shows a better alignment with yield stress.

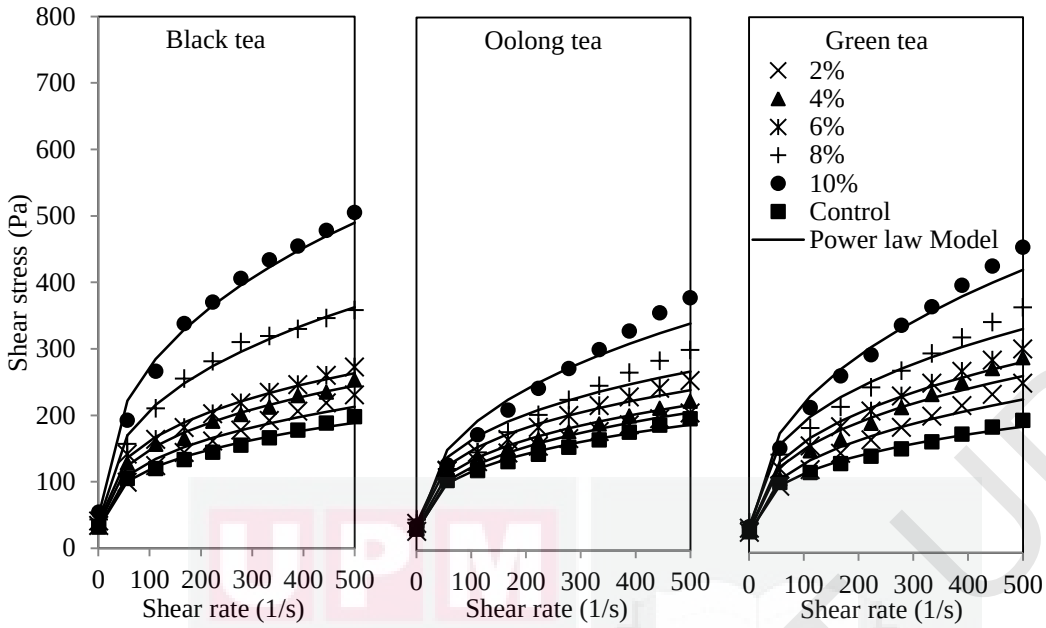


Figure 5.12: Rheogram of mango jams predicted by Power Law model

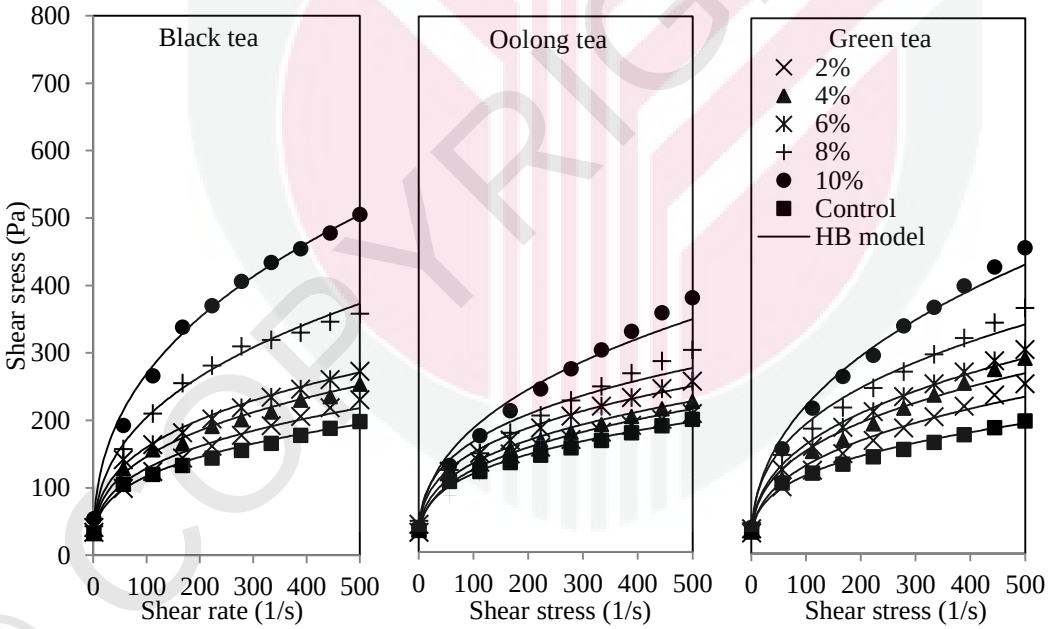


Figure 5.13: Rheogram of mango jams predicted by Herschel-Bulkley model

While Power law and Herschel-Bulkley models were used to characterise non-Newtonian fluids and their flow behaviour, the apparent viscosity (Equation 2.7) can be determined from the measured shear stress and shear rate data. Figure 5.14 shows

that apparent viscosity of all mango jam samples decreased with elevated shear rate. This is because when the shear rate is low, the disruption rate of entanglements in polymer chain of food is slower than or equivalent to the formation of new entanglements (Saha & Bhattacharya, 2010). However, as shear rate escalates, the disruption rate also elevates and surpasses that of formation; viscosity starts to decrease rapidly at this stage with respect to shear rate (Saha & Bhattacharya, 2010). Eventually, the progressive disentanglement of the long chain network throughout shearing results in lower intermolecular resistance to flow and lower apparent viscosity (Goff & Guo, 2019; Marcotte et al., 2001). This phenomenon also confirms the non-Newtonian behaviour of jam samples (Basu & Shivhare, 2012).

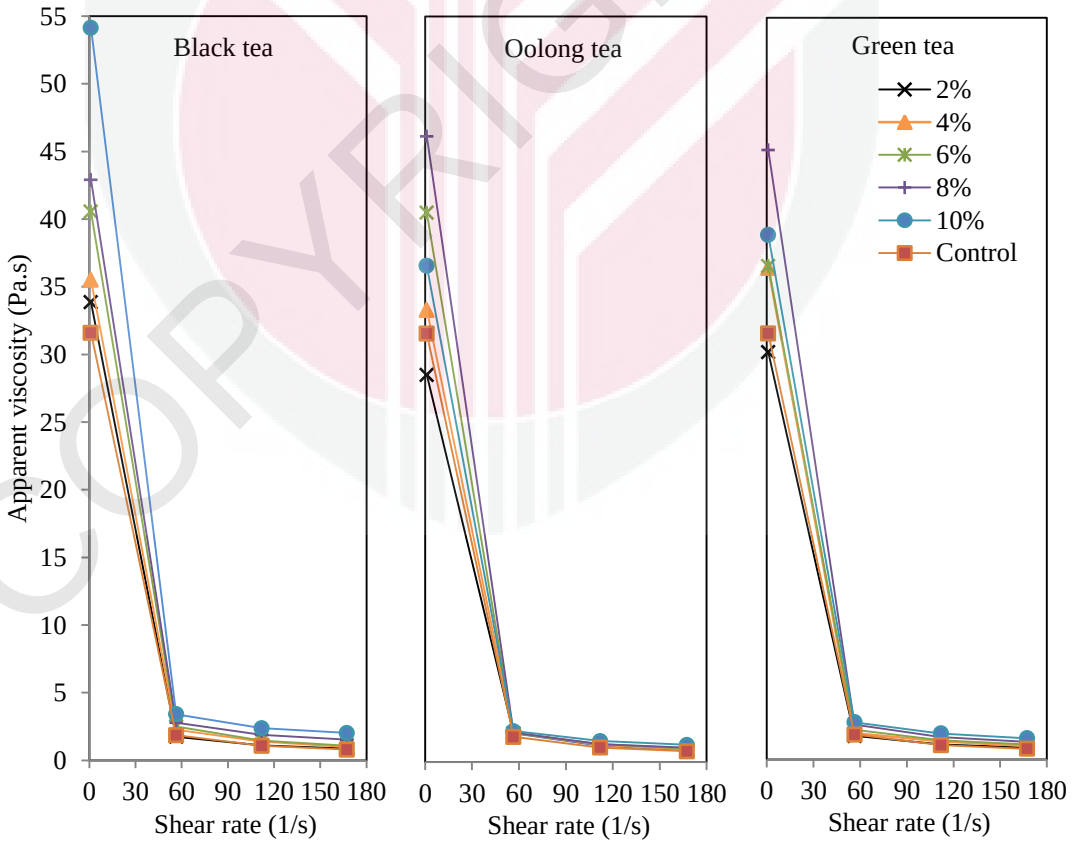


Figure 5.14: Apparent viscosity versus shear rate for mango jams with different types and concentrations of *scoby* added and control sample

The apparent viscosity of mango jam samples increased with higher *scoby* concentrations and these results were in agreement with previous studies by Javanmard et al. (2012) on the effect of sago starch in mango jam and Razak et al. (2018) on the effect of different hydrocolloids, *i.e.*, Arabic gum, locust bean gum and pectin in mango fillings. Gelation of polymer chains includes a series of structures and the most prevalent one is the aggregation of primary inter-chain connections into ‘‘junction zones’’, giving rise to the formation of basis for the three-dimensional network properties of jam (Saha & Bhattacharya, 2010). In a diluted dispersion, the individual molecules have higher mobility; on the other hand, the molecules have more restriction in their movement when these molecules interact and collide with each other in a concentrated system (Saha & Bhattacharya, 2010). The higher the amount of molecules in the junction zone, the higher the rigidity of the gel. Therefore, elevation of apparent viscosity with concentrations of *scoby* is probably due to the increased intermolecular interaction in jam.

5.3 Proximate analysis of jam formulated with *scoby*

Proximate analysis of jam provides crucial information about the composition and nutritional value of food, as shown in Table 5.5. Compared to the control sample, mango jam containing *scoby* from black and oolong tea *kombucha* had higher carbohydrate content while samples with black and green tea *scoby* had higher total dietary fiber content. The slightly higher total dietary fiber content could be due to variations in protein, ash and non-dietary fiber components considering that the total dietary fiber content is determined by subtracting the residual weight of these components from the initial sample (McCleary, 2023). The commercial jams have lower levels of both carbohydrate and total dietary fiber than jam formulated with

scoby, with 54.5 g/100g and 0.6 g/100g, respectively, as indicated in Table 5.5 and the other common fruit jams available in the Malaysian market, such as grape, apricot, blueberry and strawberry, have carbohydrate ranging from 65.99 to 67.65 g/100g and total dietary fiber ranging from 0.09 to 0.54 g/100g (Mohd Naeem et al., 2017).

Table 5.5: Proximate analysis of mango jams containing 10% *scoby* from black, oolong and green tea *kombucha* and a control sample

	Commercial jam[a]	Control[b]	*Scoby*		
			Black tea	Oolong tea	Green tea
Total carbohydrates (g/100g)	54.5	73.4 ± 14.7	77.7 ± 15.5	77.0 ± 15.4	70.7 ± 14.1
Energy (kcal/100g)	217	295 ± 59	312 ± 62	309 ± 62	284 ± 57
Energy (kJ/100g)	921	1240 ± 248	1310 ± 262	1300 ± 260	1190 ± 237
Total Dietary Fiber (g/100g)	0.6	0.8 ± 0.05	1.0 ± 0.06	0.5 ± 0.03	1.0 ± 0.07
Ash (g/100g)	-	< 0.1	< 0.1	< 0.1	< 0.1
Fat (g/100g)	0.3	< 0.1	< 0.1	< 0.1	< 0.1
Protein (g/100g)	0.0	0.4 ± 0.05	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.04

[a]Commercial jam is pineapple and mango jam
[b]Control is sample without addition of *scoby*

All mango jams with *scoby* exhibited a low ash and fat content below 0.1 g/100g. A low ash content in the samples suggests that there is a low concentration of minerals present in them (Gindi et al., 2019). The protein content of mango jam samples with *scoby* recorded a lower value than that of the control sample. This could be attributed to the ratio of composition of the fruit pulps. The commercial jam stated has a higher fat content and no protein content while the commercial jams of previous studies have reported that the fat content is typically below 0.1 g/100g, the ash content ranges from 0.12-0.25 g/100g and protein content is between 0.27 and 0.43 g/100g (Mohd Naeem et al., 2017). Taken together, the mango jam samples with *scoby* were

consistent with previous research that reported low-fat, ash and protein content in jams.

5.4 Sensory evaluation of jam formulated with *scoby*

The sensory analysis in terms of odour, flavour, colour, spreadability and overall acceptability of mango jams containing *scoby* from black, green and oolong tea at different concentrations are shown in Figure 5.15. All mango jams with *scoby* received scores >6.63 for spreadability, >6.5 for flavour, >7 for colour and >6.4 for odour. By comparing different sensory aspects, less desirable scores for odour could be due to the mild vinegary note from *kombucha scoby*. Despite the slightly lower scores on odour, the overall acceptability scores for samples had a rating of over 70%, ranging above 6.5 on a scale of 0-9. Notably, mango jam with 4 and 6% green tea *kombucha scoby* received the highest sum ranks across all five parameters. In particular, mango jam with 6% green tea *kombucha scoby* earned the highest score of 7.81 for overall acceptability.

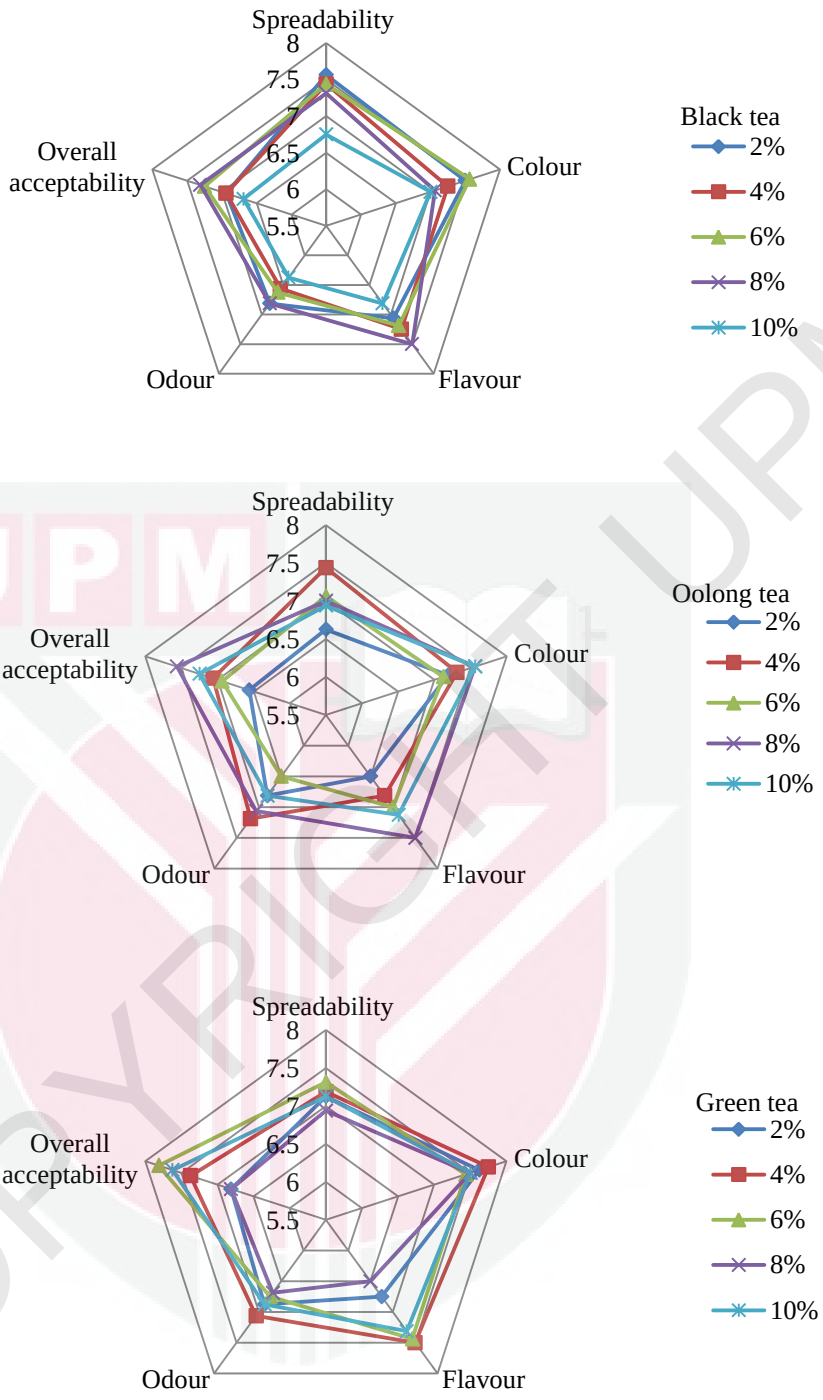


Figure 5.15: Sensory evaluation for mango jams containing *scoby* from black, oolong and green teas at different concentrations

5.5 Correlation analysis of properties of *scoby*-formulated jam using Principal Component Analysis (PCA)

PCA was performed on the average values of selected fourteen responses obtained from instrumental-based analysis including physicochemical, colour and textural properties and non-instrumental-based analysis, *i.e.*, via sensorial approaches, for all fifteen mango jam samples formulated with *scoby*, with the intention of identifying the relationships between responses from both approaches. Correlation loading of response variables is shown in Figure 5.16. The first and second principal components were plotted on two axes with total variation up to 72.3%, where PC1 explained 52.3% of the total variance and PC2 explained 20.0%. The first two generated PC from this analysis applied Kaiser's criterion with eigenvalues >1 (Massart et al., 1988). Variables with higher loadings located further away from the axis origin are significant for the considered PC and those with similar directions suggest a positive correlation while opposite directions indicate a negative correlation (Destefanis et al., 2000). Hence, textural parameters of stickiness, work of shear, hardness and work of adhesion with the highest loading on the right of PC1 and physicochemical parameters of moisture content, brix and water activity on the left are important attributes to be given highest consideration in developing this *scoby* formulated jam. This finding suggests that enhancing these desired texture parameters and reducing the undesired physicochemical properties will improve jam quality critically. Improvement in both textural and physicochemical properties can be done by modifying the formulation, *ie*., by a partial replacement of sucrose with xylitol which led to lower water activity and higher spreadability (Naknaen & Itthisoponkul, 2015) or by applying non-conventional techniques such as microwave

energy as an alternative to conventional heating which shows decreased water activity, moisture content, pH and increased consistency (Igual et al., 2010).

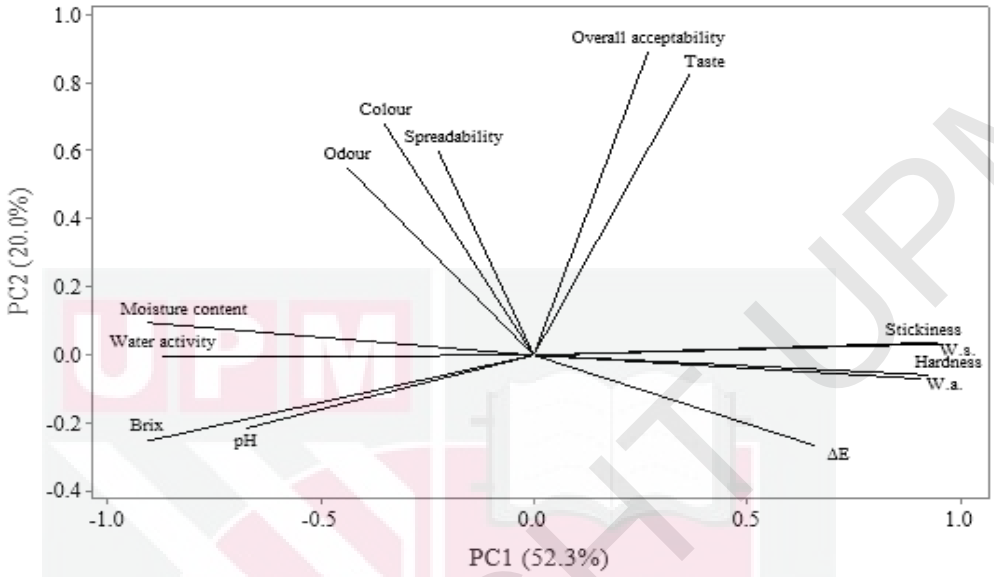


Figure 5.16: Principal component analysis for instrumental and sensory approaches. (Variables are represented by vectors)

5.6 Summary

This chapter has described the study of mango jams that were incorporated with 2-10% concentration of *scoby* from black, green and oolong tea *kombucha* fermentation. The inclusion of *scoby* into jam had significantly lower ($P < 0.05$) water activity and moisture content (except mango jam with 2% oolong tea *scoby*), pH and total soluble solids. All mango jams evaluated, with the exception of the 2% *scoby* concentration from *kombucha* fermentation using oolong tea and control, met the criteria of jam, exhibiting a moisture content below 50 percent and a water activity threshold ranging from 0.6 to 0.85 (Erkmen & Bozoglu, 2016). The results of jams formulated with *scoby* at total soluble solids contents of 65.5-67.8 ^{o}Brix demonstrated compliance with the requirements set forth by the Food Act (1983) and

Food Regulations Malaysia (1985) which specify a minimum of 65%. The colour analysis showed ΔE with *scoby* formulated jam had higher L^* and lower b^* values. All samples maintained a yellow-orange colour due to the colour of mango fruit, with hue angles ranging from 71^o to 82^o. Jams formulated with *scoby* are generally harder, requiring higher work of shear and and more sticky, requiring more work of adhesion, with the black tea *scoby* topping the list. *Scoby* formulated jams also exhibited a shear thinning, non-Newtonian characteristic with values of flow index characteristics, *n*, less than 1 from the Power Law and Herschel-Bulkey modelling exercises and trends of apparent viscosity reduction with shear rate increase. Jam formulated with *scoby* had higher levels of carbohydrates, total dietary fiber and protein and lower fat content compared to commercial jam while maintaining a similar nutritional composition to the control. Notably, sensory evaluation of *scoby* formulated jams scored above 6.5 on the scale of 0.9 for overall acceptability. Among all variations tested, jam with 6% green tea *scoby* is preferred due to its highest rating of overall acceptability in sensory evaluation while maintaining favourable physicochemical properties, such as water activity, pH and moisture content within acceptable quality and safety ranges. Based on PCA analysis, it was determined that textural and physicochemical attributes are the most important factors in *scoby* jam development. As a whole, jam formulated with *scoby* is a viable and satisfactory alternative as the results showed that it met the standards for quality and taste. It could be a sustainable approach to promoting eco-friendly practices as every time a batch of *kombucha* is made, a new film of *scoby* is formed which can be recycled for further use. Since *scoby* is typically discarded after *kombucha* production, using it to create a value-added product like jam can help businesses generate more revenue and reduce costs associated with waste management.

CHAPTER 6

MICROBIAL ANALYSIS ON FERMENTED PRODUCTS FROM *KOMBUCHA* FERMENTATION, TEA, *SCOBY* AND JAM

This chapter covers the microbial analysis conducted on the products of *kombucha* fermentation and jam production via Next-Generation Sequencing (NGS) method for bacterial identification using 16S ribosomal RNA and fungal identification using nuclear ribosomal RNA internal transcribed spacer (ITS). For the analysis, *kombucha* tea and *scoby* were prepared using pure and combinations of teas. The *kombucha* tea samples are abbreviated as KB (black tea), KG (green tea), KO (oolong tea), KBO (20% black tea and 80% oolong tea), and KBG (20% black tea and 80% green tea), while the *scoby* samples are abbreviated as SB (black tea), SG (green tea), SO (oolong tea), SBO (20% black tea and 80% oolong tea), and SBG (20% black tea and 80% green tea). The reference samples are *kombucha* tea KC and *scoby* SC from black tea which were obtained from an independent *kombucha* homemaker in Selangor, Malaysia for comparison. The microbial analysis for *scoby* formulated mango jams was done on jam samples using 10% *scoby* from black tea *kombucha* (abbreviated as JB), green tea *kombucha* (abbreviated as JG) and oolong tea *kombucha* (abbreviated as JO). A commercial probiotic butter (abbreviated as BR) was the most compatible comparison found in the range of probiotic spreads available in the commercial market.

6.1 Data filtering for microbial abundance and diversity of products

The NGS data generated for microbial analysis summed up to a total of 2,475,885 raw reads for bacterial 16S rRNA genes and 2,428,447 raw reads for fungal ITS

genes from all samples, which included *kombucha* tea, *scoby* and jam. After quality control measures of filtering and merging to assess microbial abundance, reads were reduced to 1,790,800 for 16S rRNA and 1,778,471 for ITS.

Subsequently, the raw reads of all samples were processed through a further filtering step to 88,504 (16S) and 88,485 (ITS) rarefied reads to be used for diversity analysis. The use of rarefied reads for diversity analysis ensured that the results were not biased by differences in sequencing depth among samples, with rarefaction curves shown in Figure 6.1. The curves reached a summit in each sample, which indicates that the number of unique genera identified is not expected to increase with additional sequencing. This suggests that the sequencing depth was sufficient to capture majority of the microbial diversity present in each sample. Overall, these results indicated that the sequencing approach used was appropriate for the detection and characterisation of the microbial communities in the samples.

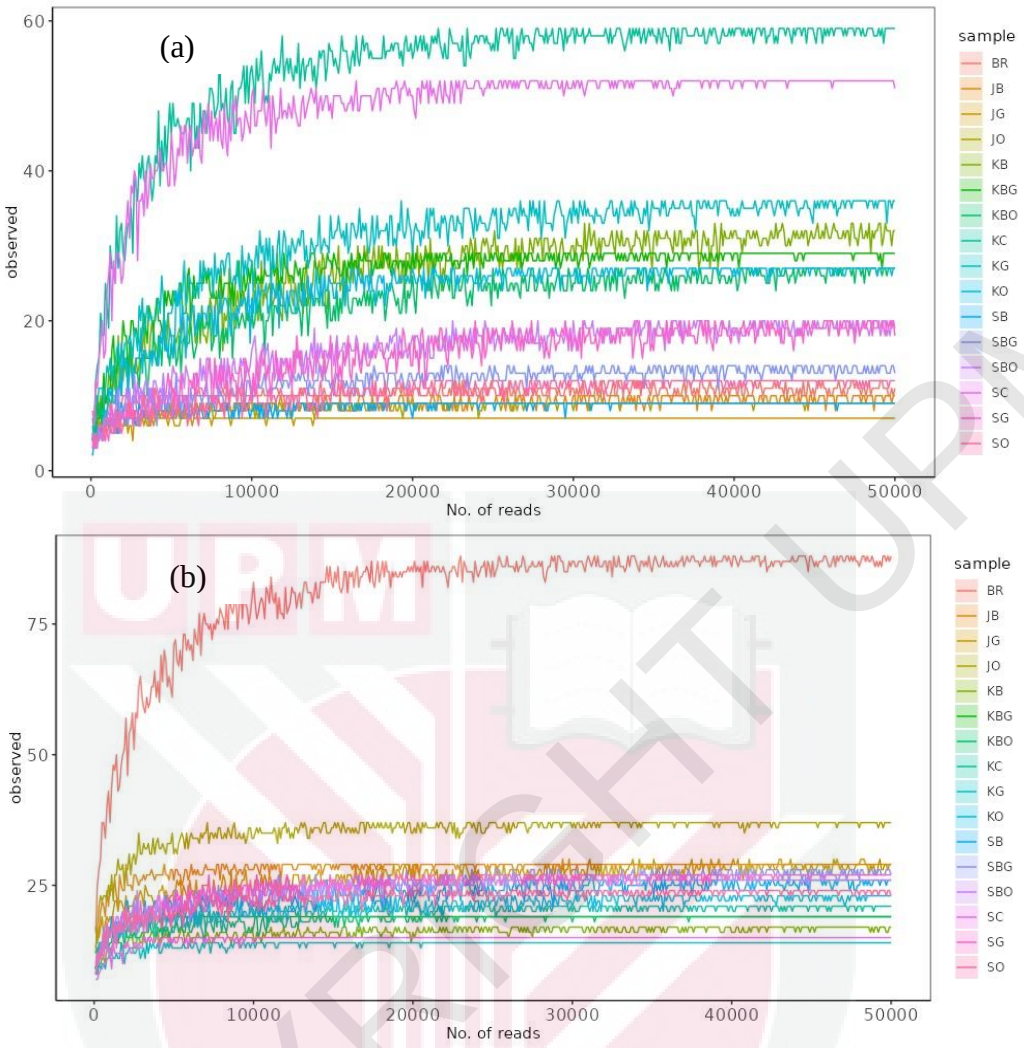


Figure 6.1: Rarefaction curves of (a) 16S and (b) ITS sequencing for all samples, inclusive of all *kombucha* teas, *scoby* and jams

6.2 Bacterial and fungal communities of *kombucha* tea and *scoby*

Figure 6.2 shows the main bacterial phyla (>0.1%) in the *kombucha* samples, with *Proteobacteria* being the most dominant in samples of *kombucha* tea (85-97%) and *scoby* (94-99%). The list continued with *Actinobacteriota* (2-11%), *Cyanobacteria* (0.1-3%), *Firmicutes* (0.2-1.2%) and *Bacteroidota* (0.1%) for *kombucha* tea and *Actinobacteriota* (1.1-5.6%) and *Firmicutes* (0.1%) for *scoby*. Several other *kombucha* studies have also reported *Proteobacteria* as the major phylum (Arıkan et al., 2020; Chakravorty et al., 2016; Fabricio et al., 2022; Gaggìa et al., 2018; Marsh

et al., 2014). In Lavefve et al. (2021), *Proteobacteria* is highlighted as one of the phyla involved in acidic fermentation processes. The comparison samples, KC and SC both have also shown phyla such as *Proteobacteria* (47-53%), *Actinobacteriota* (20-44%) and *Firmicutes* (2-31%).

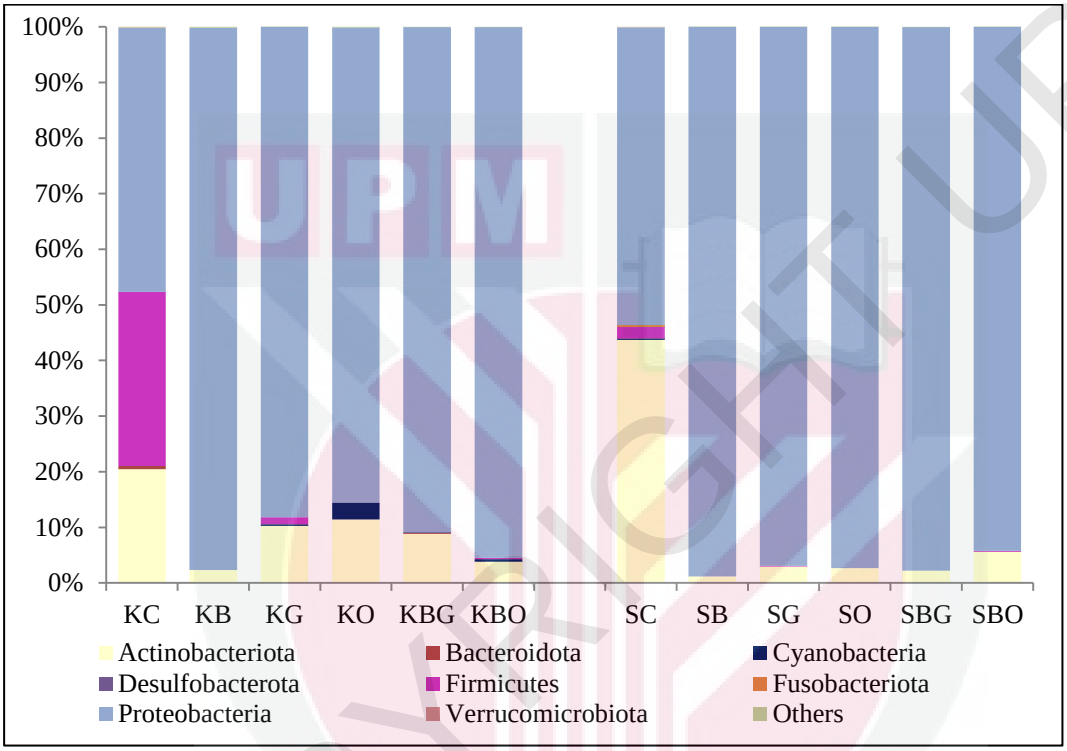


Figure 6.2: Bar plots representing relative abundances of bacterial phyla in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

At family level, 11 and 6 bacterial families (>0.1%) were identified for *kombucha* teas and *scoby*, respectively (Figure 6.3). *Acetobacteraceae* (78.4-93.2%) was the dominant family in all *kombucha* teas, followed by *Nocardiaceae* (2.3-11.4%). The other families that were presented in descending order were *Pseudomonadaceae* (2.2-10.8%), *Bacillaceae* (0.1%), *Burkholderiaceae* (0.1-0.9%), *Moraxellaceae* (0.1-0.8%), *Streptococcaceae* (0.1-0.7%), *Lactobacillaceae* (0.1-0.5%), *Enterobacteriaceae* (0.1%) and *Prevotellaceae* (0.1%). Similarly, for *scoby*, bacteria

families in descending order were *Acetobacteraceae* (89.4-97%), *Nocardiaceae* (1.2-5.6%), *Pseudomonadaceae* (1.5-4.7%), *Burkholderiaceae* (0.1-0.4%), *Lactobacillaceae* (0.1-0.2%) and *Streptococcaceae* (0.1%).

The samples showed a consistent trend with previous studies, which also identified *Acetobacteraceae* as the most abundant family in both *kombucha* tea and *scoby* (Chakravorty et al., 2016; Gaggìa et al., 2018). Notably, the prevalence of the *Acetobacteraceae* family was higher in *scoby* compared to *kombucha* tea, which is in agreement with findings in Coton et al. (2017). This is supported by their characterisation as aerobic chemoorganotrophic microorganisms that use oxygen to obtain energy by breaking down organic compounds (Hommel, 2014). The acetic acid bacteria under *Acetobacteraceae* are capable of oxidising ethanol as a substrate to acetate hydrate, which is in turn converted to acids (gluconic and acetic acid) by enzyme acetaldehyde dehydrogenase (Coelho et al., 2020; Laavanya et al., 2021). It is also responsible for cellulose *scoby* synthesis, which is produced in the form of fibrils attached to the bacterial cell (Fabricio et al., 2022; Villarreal-Soto et al., 2018). This process includes the synthesis of uridine diphosphate glucose (UDPG1c), which is the cellulose precursor (Coelho et al., 2020).

As the second dominant family in *kombucha*, certain genera within the *Nocardiaceae* family have the ability to utilise diverse carbon compounds like glucose (Goodfellow, 2014), which could have a role in the complex fermentation proceseses of *kombucha*. They are also known for synthesising bioactive compounds that might be of importance for the fermented beverage (Goodfellow, 2014).

Both control samples, *kombucha* tea (KC) and *scoby* (SC) showed dominance of the *Pseudomonodaceae*, which had not been documented previously. However, the *Bacillaceae* family was found to be present in certain *kombucha* (Arıkan et al., 2020; Harrison & Curtin, 2021). The variation might result from the notion that the *kombucha* culture was influenced by the origin of *scoby*, which was obtained from different geographical, climatic and cultural conditions as well as the types of bacteria that existed locally (Coelho et al., 2020; Kumar & Joshi, 2016). The *Nocardiaceae* family seems to be present consistently in both reference and experimental samples.

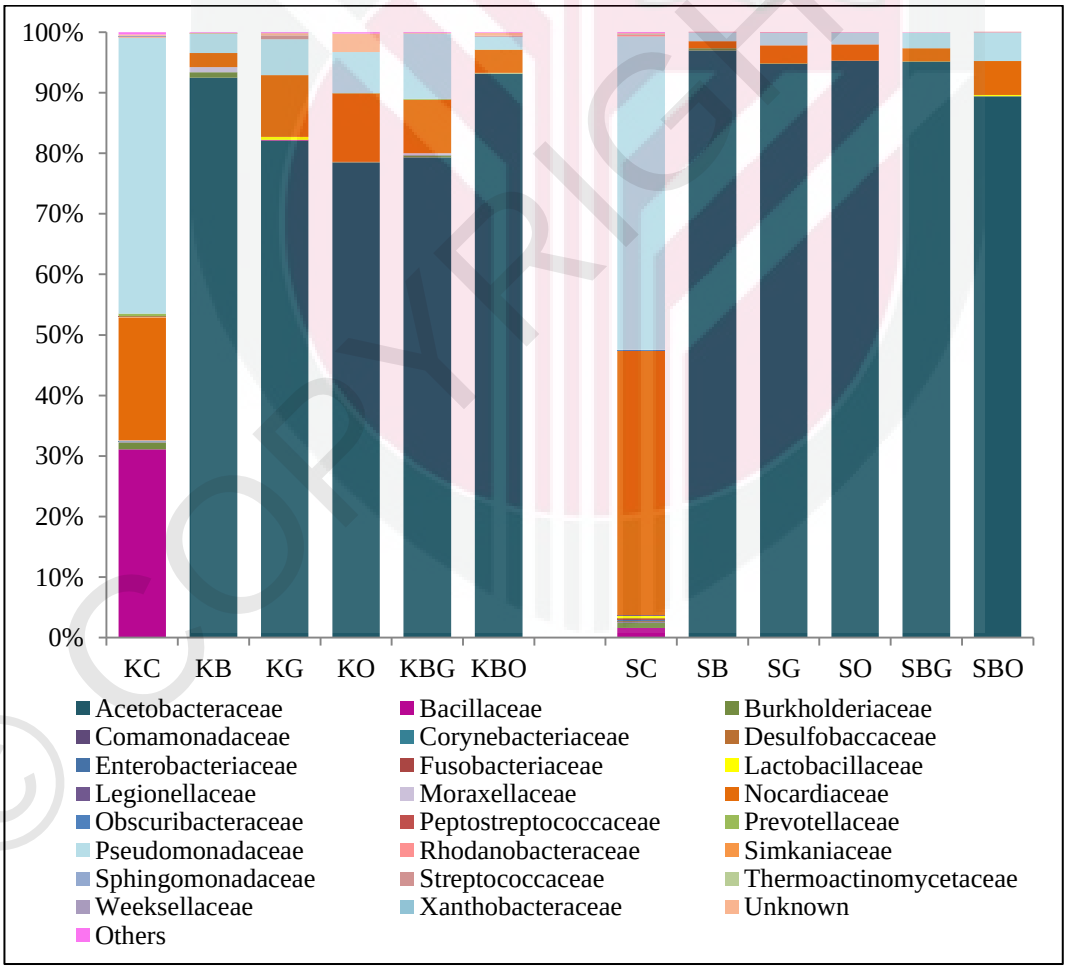


Figure 6.3: Bar plots representing relative abundances of bacterial families in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

For bacterial composition at genus level in Figure 6.4, there were a total of 14 and 6 genera detected in *kombucha* tea and *scoby* samples, respectively. The most abundant genus was *Komagataeibacter* (a revision of previous genus *Gluconobacter*) (Guillamón & Mas, 2017; Harrison & Curtin, 2021; Laavanya et al., 2021; Subbiahdoss et al., 2022; Yamada et al., 2012), which accounted for a range of 78.4-97.0% in all the *kombucha* samples of different tea substrates, followed by *Rhodococcus* (2.3-11.4%), *Pseudomonas* (2.2-10.8%), *Ralstonia* (0.1-0.9%), *Lactococcus* (0.2-0.7%), *Acinetobacter* (0.1-0.6%), *Enhydrobacter* (0.1-0.3%), *Weissella* (0.1-0.3%), *Burkholderia-Caballeronia-Paraburkholderia* (0.2%), *Lactobacillus* (0.1%), *Bacillus* (0.1%), *Limosilactobacillus* (0.1%), *Prevotella* (0.1%) and *Prevotella_9* (0.1%) in *kombucha* tea, and *Rhodococcus* (1.2-5.6%), *Pseudomonas* (1.5-4.7%), *Ralstonia* (0.1-0.4%), *Limosilactobacillus* (0.2%) and *Lactococcus* (0.1%) in *scoby*. The findings were in line with previous studies, which mainly found *Komagataeibacter* in both *kombucha* tea and *scoby* (Chakravorty et al., 2016; Gaggìa et al., 2018). Similarly, Harrison & Curtin (2021) reported *Komagataeibacter* as the most prevalent and abundant *scoby* genus throughout 103 commercial *kombucha* in North America. When comparing tea types, different dominant genera in black tea (*Komagataeibacter*) and green tea (*Oenococcus* and *Lactobacillus*) *kombucha* brewed in France were observed (Coton et al., 2017); however, the outcome was different in Barbosa et al. (2021), whereby tea types had no impact on bacterial species dominance, with *Komagataeibacter* still being the most abundant genus in both black and green tea *kombucha* brewed in Brazil. *Komagataeibacter* plays a vital role in *kombucha* fermentation. The formation of *scoby* relies upon the presence of this genus as it is the key microorganism for the production of cellulose from carbon sources (Pothakos et al., 2016; Subbiahdoss et

al., 2022). As a result of its ability to convert glucose, *Komagataeibacter* has been characterised with probiotic properties as it is capable of reducing glucose absorption and preventing weight gain by converting glucose into cellulose in the human gut (Kaashyap et al., 2021; Lavasani et al., 2019). In addition to *scoby* generation in *kombucha* fermentation, *Komagataeibacter* is responsible for nitrogen fixation and production of organic acids, which give *kombucha* its sweet and vinegary sour taste (Coelho et al., 2020; Harrison & Curtin, 2021; Reis & Teixeira, 2015; Villarreal-Soto et al., 2018). Organic acids produced include glucuronic acid, which acts as a precursor of vitamin C (Antolak et al., 2021) and is considered the main therapeutic and detoxification agent associated with the hepatoprotective effects of *kombucha* (Kumar & Joshi, 2016; Wang et al., 2022). Remarkably, gluconic acids released by *Komagataeibacter* have a putative role as mineral supplements to cure hypocalcaemia, hypomagnesaemia and anemia (Kaashyap et al., 2021). The organic residues increase acidity during the fermentation process and hence prevent contamination by inhibiting the growth of deleterious bacteria (Coelho et al., 2020). Findings from current study supported the absence of generally found pathogens such as *Penicillum* spp., *Salmonella* spp. and *Campylobacter* spp. (Neffe-Skocińska et al., 2017; Nguyen et al., 2015; Scallan et al., 2011) in the prepared *kombucha* samples. *Komagataeibacter* is also reported to relieve liver damage induced by alcohol through regulation of fatty acid metabolism and enhancement of diversity in intestinal microbiota (Lin et al., 2020). Its metabolic activity could lead to an increase in phenolic compounds (Shi et al., 2023), which are valuable antioxidants that confer antioxidant properties and may be effective in improving cognitive functions and preventing stratum corneum dryness (Jiang et al., 2019; Kozyrovska et al., 2021). In Jiang et al. (2019), it has demonstrated anti-inflammatory effects.

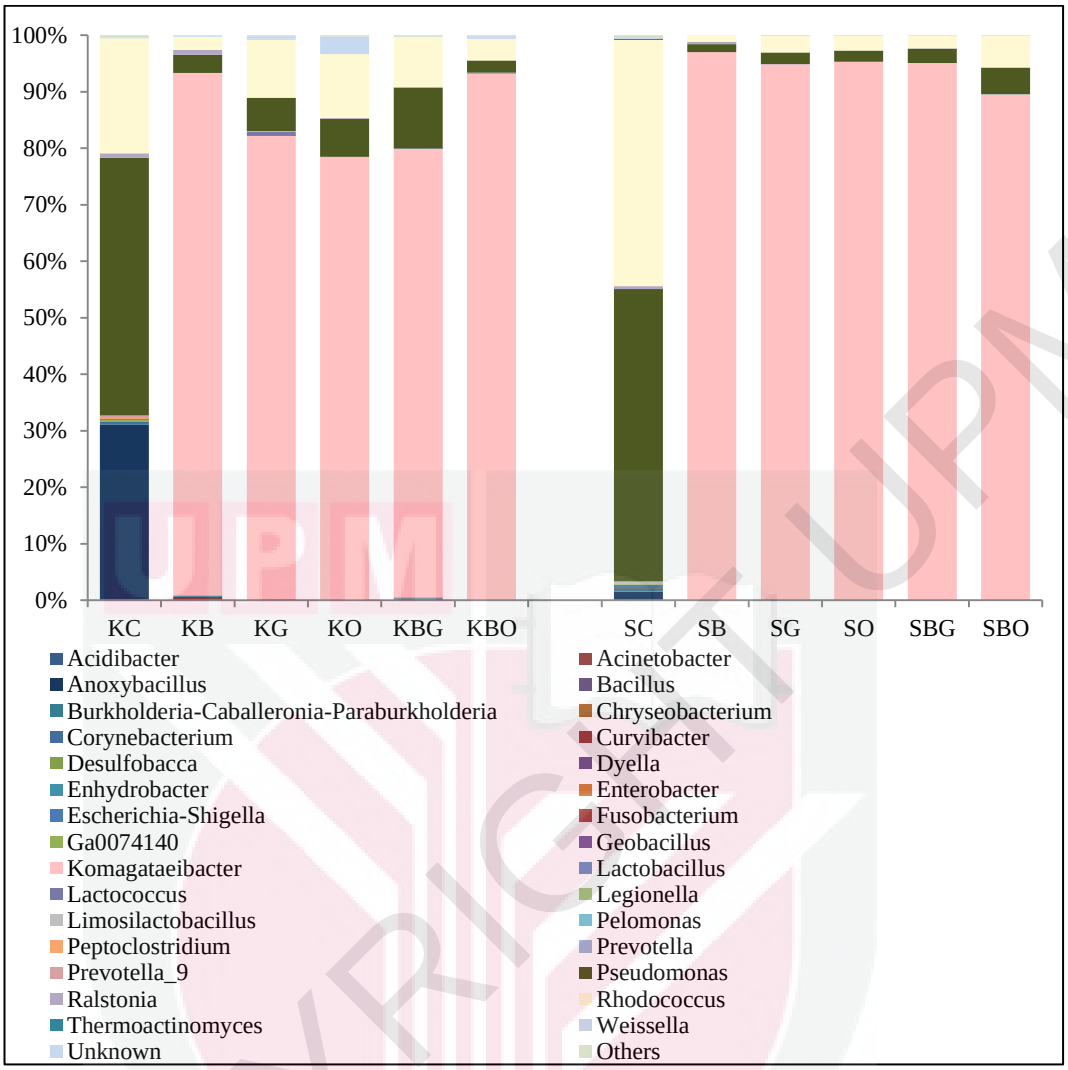


Figure 6.4: Bar plots representing relative abundances of bacterial genera in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Followed by the *Komagataeibacter*, *Rhodococcus* was the second dominant genus that harboured *kombucha* and it has also been reported to be present in this fermented beverage (Podolich et al., 2019). *Rhodococcus* is capable of producing cellulose through fermentative processes (Cacicedo et al., 2016; Iguchi et al., 2000; Jeremic et al., 2019) and cholesterol oxidases from *Rhodococcus* have also been found to possess cholesterol-degrading ability (Kim et al., 2018).

Here, the common bacterial pattern was similar among different tea types fermented with *scoby* from the same origin, even with different tea combinations. Nevertheless, some minor genera (relative abundance > 0.1%) were only presented in some of the samples; for instance, *Lactococcus* in KG, KBO and SG; *Lactobacillus* and *Weissella* in KG; *Limosilactobacillus* in KG and SBO and *Prevotella* in KO, KBG and KBO. Though these did not have as much occurrences as other genera, they still play a vital role in *kombucha*. Of the above-mentioned organisms, the presence of *Lactobacillus*, *Limosilactobacillus* and *Weissella* (members of the *Lactobacillaceae* family) and *Lactococcus* from the *Streptococcaceae* family is noteworthy due to their probiotic potential that may offer health benefits (Fijan, 2014; Piccioni et al., 2021; Teixeira et al., 2021). The health and nutritional benefits include protection against infectious agents, control of intestinal infections, reduction of serum cholesterol, prevention of certain cancers, improvement of bioavailability of vitamins and minerals, anti-allergenic effects, antioxidant effects and anti-obesity (Gilliland, 1990; Mathur et al., 2020). They are also responsible for producing d-saccharic acid-1,4-lactone (DSL), known for its hypocholesterolaemic, hepatoprotective and antihypergylcaemic effects (Emiljanowicz & Malinowska-Pańczyk, 2020; Içen et al., 2023). Additionally, they synthesise lactic acid and vitamin B and aid in the degradation of phenolic compounds (Antolak et al., 2021). However, the presence of beneficial lactic acid bacteria in *kombucha* varies according to different research studies (Bishop et al., 2022). In Chakravorty et al. (2016), De Filippis et al. (2018) and Marsh et al. (2014), lactic acid bacteria were only discovered in very small amounts while in Gaggìa et al. (2018), none were found. Alongside LAB, another significant bacterial genus, *Prevotella* has also been recognised for its potential benefits. *Prevotella* was found to improve glucose metabolism induced by prebiotic consumption (Kovatcheva-

Datchary et al., 2015). It is also related to the intestinal barrier, regulation of intestinal permeability and intervention in pathogen colonisation to prevent inflammation (Scher et al., 2013; Wang et al., 2021).

On the contrary, *Pseudomonas* from the *Pseudomonadaceae* family, exhibited the highest proportion in *kombucha* tea KC and *scoby* SC, highlighting how variations in sources can lead to distinct microbial communities. *Pseudomonas* is usually absent or found in relatively low abundance in *kombucha* (Huang et al., 2022; Reva et al., 2015). This genus is also present in other fermented foods such as kefir (Gao et al., 2013) and fermented soybean (Xie et al., 2020). *Pseudomonas* has high metabolic versatility and the capability to thrive in both aerobic and anaerobic processes (Wisplinghoff, 2017); hence, it can be inferred that *kombucha* is a favourable environment for the colonisation of this genus. *Pseudomonas* exhibits characteristics as a biofilm producer and it is assumed to play an exceptionally pivotal role in the carbon and nitrogen cycles in *kombucha* since it populated both the *kombucha* tea and *scoby* (Sah et al., 2021). However, *Anoxybacillus* found in KC and SC is not commonly associated with *kombucha* fermentation.

For the analysis of fungi, based on Figure 6.5, there were only two phyla of fungi found in *kombucha*: *Ascomycota* (99.8-100%) and *Basidiomycota* (0.2%), with the former phylum predominating. *Ascomycota* is commonly present and plays a significant role in the production of fermented foods (Cason et al., 2020), including *kombucha*, as evidenced by studies that have reported its dominance (Arıkan et al., 2020; Chakravorty et al., 2016).

Figure 6.5: Bar plots representing relative abundances of fungal phyla in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

At family level, two fungal families were observed in *kombucha* teas with *Pichiaceae* as the major family taxon, accounting for 63.9-93% followed by *Saccharomycetaceae* (7-36.1%) as shown in Figure 6.6. For *scoby*, 5 fungal families were detected, listed in descending order: *Pichiaceae* (68.7-90%), *Saccharomycetaceae* (17.9-31.3%), *Didymosphaeriaceae* (0.1%), *Phanerochaetaceae* (0.1%) and *Trichosporonaceae* (0.1%). The results showed congruence with a recent study that revealed *Pichiaceae* family as the most dominant family in both *kombucha* tea and *scoby* (Fabricio et al., 2022). The *Pichiaceae* family is also present in fermented products like Cupuassu (a chocolate-like product) (Ramos et al., 2020), wine (Benito, 2020), nuruk (a traditional Korean fermentation starter) and jang (a fermented soy product) (Jeong et al., 2023), with certain members of this family playing a role in producing important aromatic compounds and

enhancing flavours (Ramos et al., 2020). In comparison, SC showed a prevalence of *Pichiaceae*, while KC was dominated by *Schizosaccharomycetaceae* (Hu et al., 2011). The presence of *Schizosaccharomycetaceae* is not unusual, considering its occurrence in *kombucha* (Villarreal-Soto et al., 2020) and other fermented foods such as kefir (Ilıkkan & Bağdat, 2021).

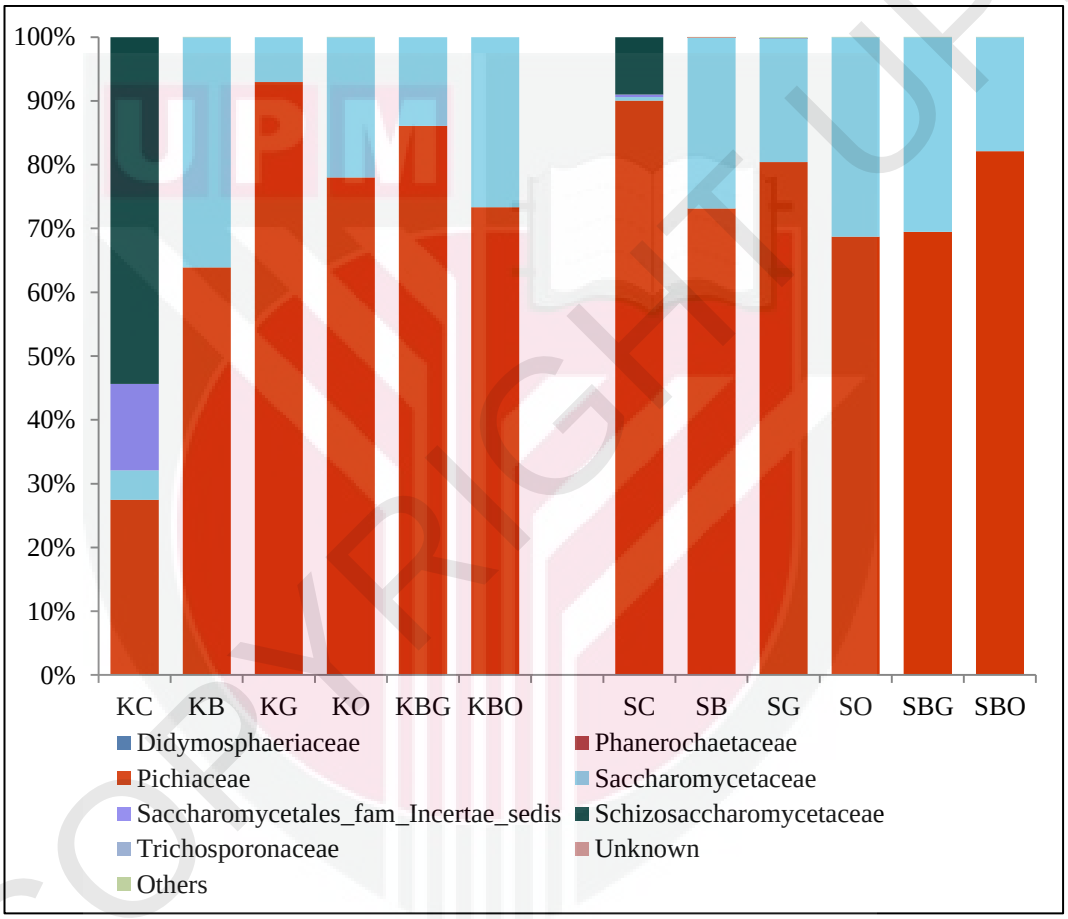


Figure 6.6: Bar plots representing relative abundances of fungal families in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Figure 6.7 illustrates the relative abundances of fungal genera in *kombucha* teas and *scoby*, with *Dekkera* (31.3-65.5%), *Zygosaccharomyces* (7-36.1%) and *Brettanomyces* (18.2-32.6%) identified across the *kombucha* teas. From the perspective of individual *kombucha* teas, differences were detected;

Zygosaccharomyces was found primarily in *kombucha* tea KB and *Dekkera* in the remaining *kombucha* teas. It appears that the selection of tea substrate had an effect on the fungal dominance. With the same origin of *scoby*, the findings call into question the substances impacting the fungal communities in *kombucha* teas. The major distinction between teas is how *Camellia sinensis* leaves are processed. As a result, differences in terms of types and concentrations of polyphenols were obtained (Coton et al., 2017), which may have an impact on fungal growth.

For *scoby*, there were 6 genera assigned, *Dekkera* (40.1-55.3%) and *Brettanomyces* (14.3-34.5%) which correspond to *Pichiaceae* family were found to harbour all the *scoby* at the highest percentage. The other genera in decreasing order were as follows: *Zygosaccharomyces* (17.9-31.3%), *Bjerkandera* (0.1%), *Pseudopithomyces* (0.1%) and *Trichosporon* (0.1%). Despite the fact that the genera mentioned typically populate the *kombucha* fungal community, studies have highlighted variability with different sampling and analysis approaches (Fabricio et al., 2022; Gaggìa et al., 2018; Marsh et al., 2014; Mayser et al., 1995; Teoh et al., 2004; Villarreal-Soto et al., 2018). For instance, *Zygosaccharomyces* was described as the most dominant in both *kombucha* tea and *scoby* with high-throughput sequencing (Marsh et al., 2014) and culture-dependent analyses (Teoh et al., 2004). *Brettanomyces* was identified to be dominant using a culture-dependent approach (Mayser et al., 1995), whereas other authors reported *Dekkera* as the major genera through culture-independent approach (Fabricio et al., 2022) or both culture-dependent and culture independent approaches (Coton et al., 2017). In contrast, differences in sources led to microbial deviation, with *Brettanomyces* dominating in *scoby* SC while *Schizosaccharomyces* was primarily found in *kombucha* tea KC which was also observed in Teoh et al. (2004).

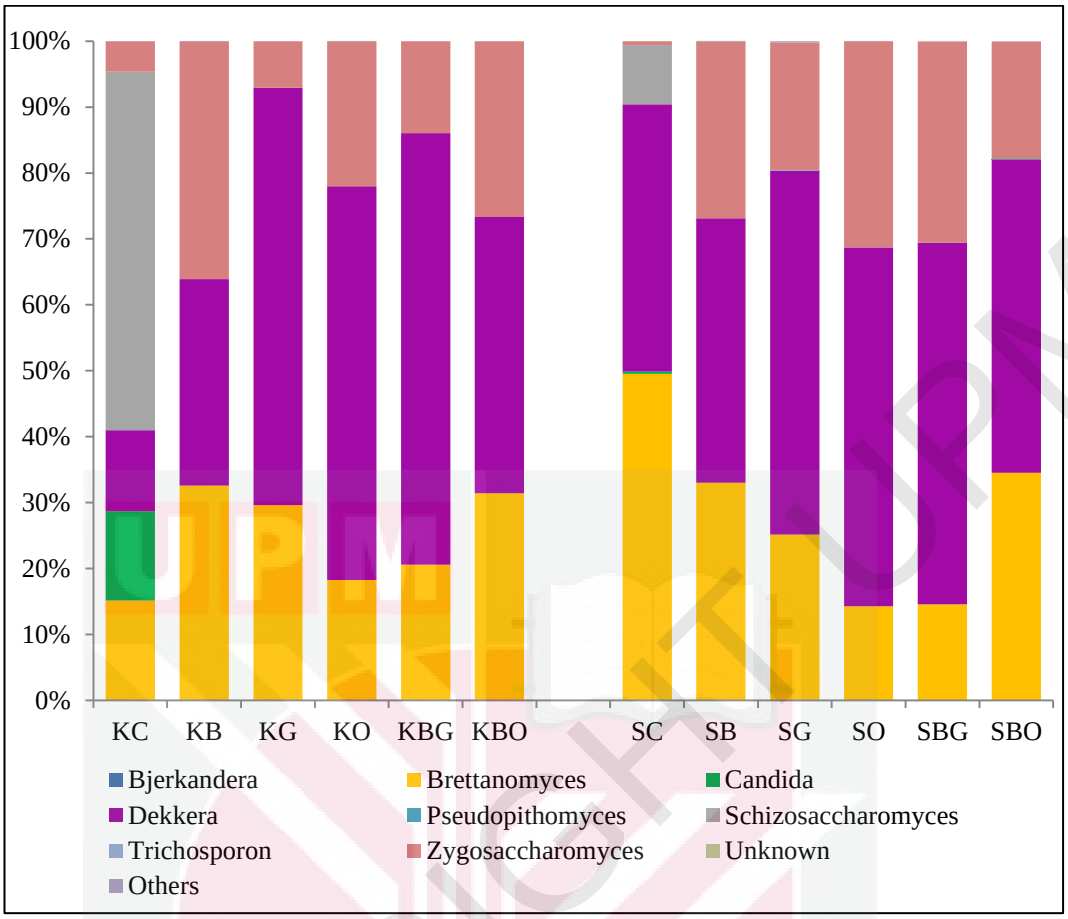


Figure 6.7: Bar plots representing relative abundances of fungal genera in *kombucha* teas and *scoby* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Studies indicate that osmotolerant yeasts such as *Zygosaccharomyces* and *Schizosaccharomyces* often initiate the fermentation process, while acid-producing yeast *Brettanomyces* acts subsequently (Coelho et al., 2020; Teoh et al., 2004). *Dekkera* and its anamorphic (asexual state) *Brettanomyces*, frequently found genera in fermented beverages like cider, wine and beer, are capable of adapting to harsh and limiting environmental conditions with a low pH value (Freer, 2002; Gaggìa et al., 2018; Schifferdecker et al., 2014). *Dekkera* presents high invertase activity, which is significant for *kombucha* fermentation. By releasing fructose and glucose through the action of invertase, *Dekkera* facilitates the metabolism of these sugars by

other microorganisms present in the *kombucha* (Coelho et al., 2020; Fabricio et al., 2022). Normally, *Brettanomyces* is also responsible for generating aromatic compounds that are of great importance in developing *kombucha*'s aroma (Harrison & Curtin, 2021). Its existence has the potential to alter the organoleptic characteristics of a product and contribute to exotic flavours (Serra Colomer et al., 2019). With respect to this, the impact of *Brettanomyces* upon flavour is crucial in beers like Belgian Lambic and Gueuze beers (Harrison & Curtin, 2021; Serra Colomer et al., 2019). As a result of growing craft beer industry, *Brettanomyces* applications for novel flavours have been expanded in beer styles yet to be explored (Serra Colomer et al., 2019); nonetheless, *Brettanomyces*-related flavours and tastes are still uncertain and unclear in *kombucha* (Harrison & Curtin, 2021). In terms of potential health benefits, *Zygosaccharomyces* produces phytases that boost mineral bioavailability and nutrient assimilation, mitigate phytic acid's adverse effects on health, improve functional properties and release health-promoting bioactive compounds (Joudaki et al., 2023) while *Schizosaccharomyces* exhibits promising probiotic potential (Staniszewski & Kordowska-Wiater, 2021).

6.3 Diversity metrics of microbial communities of *kombucha* tea and *scoby*

Alpha diversity indices, shown in Table 6.1, were used to summarise the structure of ecological community of all *kombucha* teas and *scoby*. The diversity of fungal communities outnumbers the bacterial communities in both *kombuch*a tea and *scoby*, supporting the observation from previous studies (Harrison & Curtin, 2021; Marsh et al., 2014). Environmental conditions have a significant impact on microbial growth and we can assume that *kombucha* provides a more favourable environment for fungal growth than bacterial growth. Yeast belonging to the fungi kingdom favours

low pH environment more than most bacteria (Bullerman, 2003) and *kombucha* fermentation which provides an acidic environment, accommodates yeast growth. The capability to ferment in both anaerobic and aerobic environments (Bishop et al., 2022), allows yeast to thrive in hypoxic conditions in *kombucha*. Although the entire microbial community contributes to *kombucha* fermentation, yeast still detains a predominant role since it initiates the fermentation process. The metabolic activity of yeast is higher than that of bacteria as bacteria's nutrient source must initially be utilised and produced by yeast (Goh et al., 2012a).

Table 6.1: Alpha diversity indices for bacterial and fungal communities of *kombucha* teas and *scoby*

	Bacterial community		**Fungal community**	
	Shannon	**Simpson**	**Shannon**	**Simpson**
(a) *kombucha* teas				
KC	1.51	0.70	1.78	0.74
KB	0.39	0.14	**2.07**	**0.85**
KG	0.70	0.32	1.67	0.76
KO	**0.77**	**0.37**	1.82	0.76
KBG	0.73	0.35	1.71	0.72
KBO	0.36	0.14	2.06	**0.85**
(b) *scoby*				
SC	1.00	0.54	1.52	0.73
SB	0.19	0.06	**2.10**	**0.86**
SG	0.27	0.11	1.90	0.80
SO	0.25	0.10	1.85	0.77
SBG	0.26	0.10	1.85	0.77
SBO	**0.45**	**0.20**	2.00	0.84

KO and SBO were inhabited by the most diverse bacterial community, as shown in Shannon and Simpson indices. On the other hand, KB, KBO and SB ranked highest in the diversity of fungal community. Despite the use of *scoby* from the same origin in *kombucha* fermentation, the shape of microbiome distribution was shown to be different with different substrates. However, by comparing with *kombucha* tea KC and *scoby* SC, it appears that these samples had higher diversity of bacterial

communities; nevertheless, lower diversity of fungal communities was observed, which could be elucidated by the presence of *Pseudomonas* genus in KC and SC that synthesis antifungal antibiotics (Reva et al., 2015).

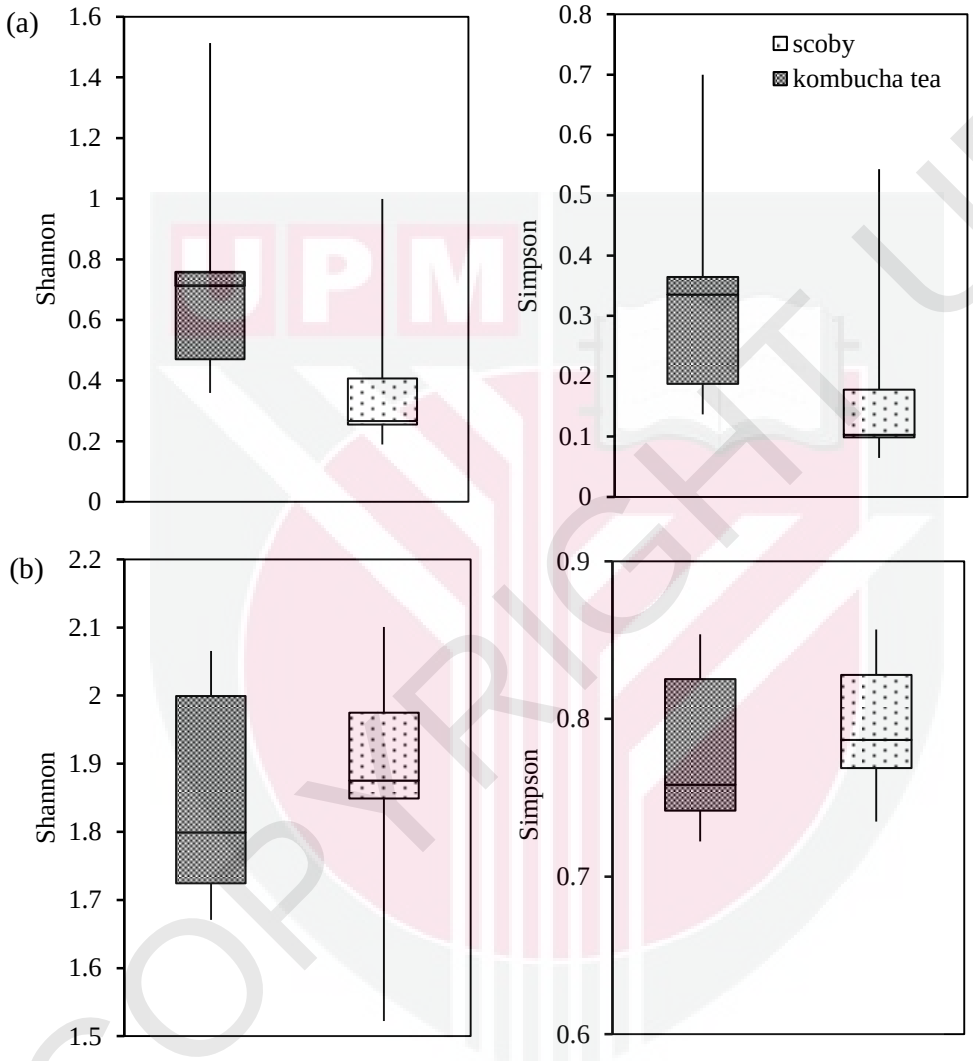


Figure 6.8: Alpha diversity analysis of overall *kombucha* teas and *scoby* based on Shannon index and Simpson index of (a) bacterial and (b) fungal communities

Given the compartmentalisation between *kombucha* tea and *scoby*, Figure 6.8 presents the comparison of alpha diversity analysis of overall *kombucha* tea and *scoby*. The bacterial community of *kombucha* teas was more diverse than *scoby*.

Both *kombucha* tea and *scoby*, however, had similar occurrences of fungal diversity. The environmental conditions prevailing in *kombucha* teas are most likely favourable for the growth of various bacteria. The nutrients are available in the tea for the culture while *scoby* could be undernourished and exhausted for higher diversity of bacterial growth. Meanwhile, fungi such as *Dekkera* and *Brettanomyces* are able to thrive in environments with limited availability of nutrients (Grassi et al., 2022).

Beta diversity was used to analyse the difference in bacterial and fungal communities among samples and was performed by generating Principal Coordinate Analysis (PCoA) plots, as visualised in Figure 6.9. In Figure 6.9(a), all the samples showed tight clusters, indicating a higher degree of similarity among samples. In contrast, the fungal communities displayed minimal separation from each other (Figure 6.9(b)), suggesting a subtle influence of the substrate. This finding highlights the impact of different substrates on fungal diversity, despite the samples originating from the same cultural source.

Obvious separations in both plots were observed for samples KC and SC compared to the rest of the samples, indicating a substantial variation in their microbial composition. Samples KC and KB from *kombucha* teas and samples SC and SB from *scoby* displayed distinct structures of symbiont composition despite being fermented with the same substrate, black tea. With *scoby* from different geographical origins, it is difficult to produce a microbial community, which is a hallmark of *kombucha* fermentation. This observation supports the explanation that variation in *kombucha* ecology is attributed to the origin of culture (Teoh et al., 2004). This situation can be explained by ‘‘terroir’’, an important concept in viticulture where microbial diversity

in wine production is associated with biogeographical patterns (Capozzi et al., 2015; Harrison & Curtin, 2021; Morrison-Whittle & Goddard, 2018).

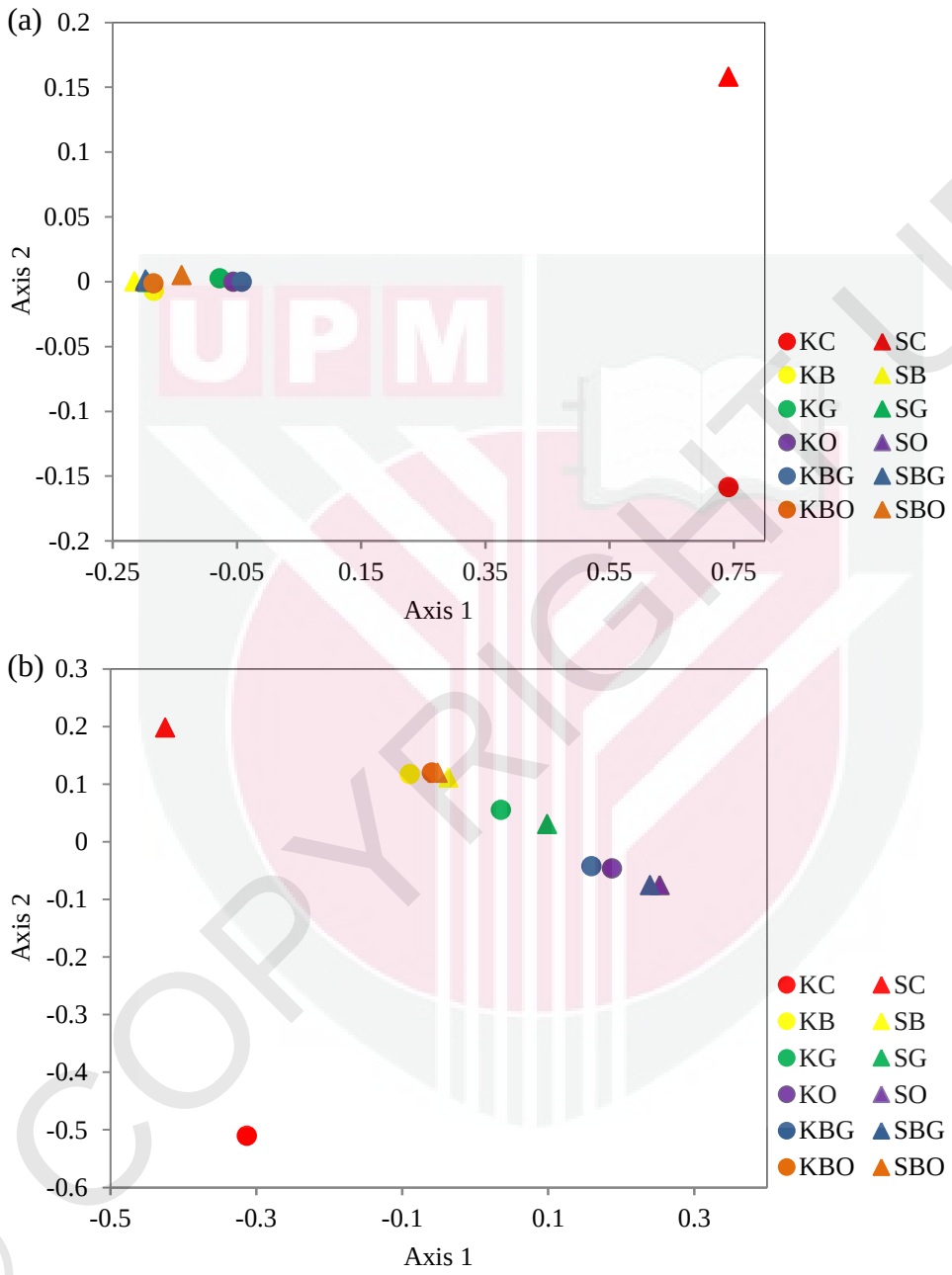


Figure 6.9: PCoA plots comparing (a) bacterial community and (b) fungal community of different *kombucha* teas and *scoby*

Another example is fermented meat products, of which many different variants are produced and characterised by diverse microbial compositions, believed to be

influenced by different starter cultures and processing technologies that are traditionally associated with local culture and origin (Van Reckem et al., 2019). In sourdough fermentation, such a pattern is apparent as well. It has been suggested that numerous regional variants of sourdough microbiome may be driven by geographical region as a result of probabilistic variations, for example, ingredients, processing conditions or local practices applied (De Vuyst et al., 2017; Palla et al., 2017; Van Reckem et al., 2019). Therefore, like other examples of fermented foods, the variation in microbial ecology observed in *kombucha* is influenced by the source of the culture.

6.4 Bacterial and fungal communities of jam formulated with *scoby*

The jam formulated with *scoby* in this research is rather a new concept; thus, bacterial and fungal analyses have been conducted in comparison to a commercial spread that has been enriched with probiotic cultures to evaluate the effectiveness of possible probiotic functions in jam formulated with *scoby*. Figure 6.10 shows relative abundances of bacterial phyla in jam formulated with *scoby* and commercial probiotic butter. Jams were primarily dominated by *Proteobacteria* (99.3-99.6%) while commercial probiotic butter showed a prevalence of *Firmicutes* (97.3%). However, all samples showed the presence of *Actinobacteriota* (0.4-1%). The presence of these three phyla may be attributed to their diverse metabolic functions (Lavefve et al., 2021), as they have also been prominently identified in other fermented foods, such as fermented soybean pastes (Sun et al., 2018), cheese (Falardeau et al., 2019), Zha-chili (Cai et al., 2021) and fermented plant-based products (Lavefve et al., 2021).

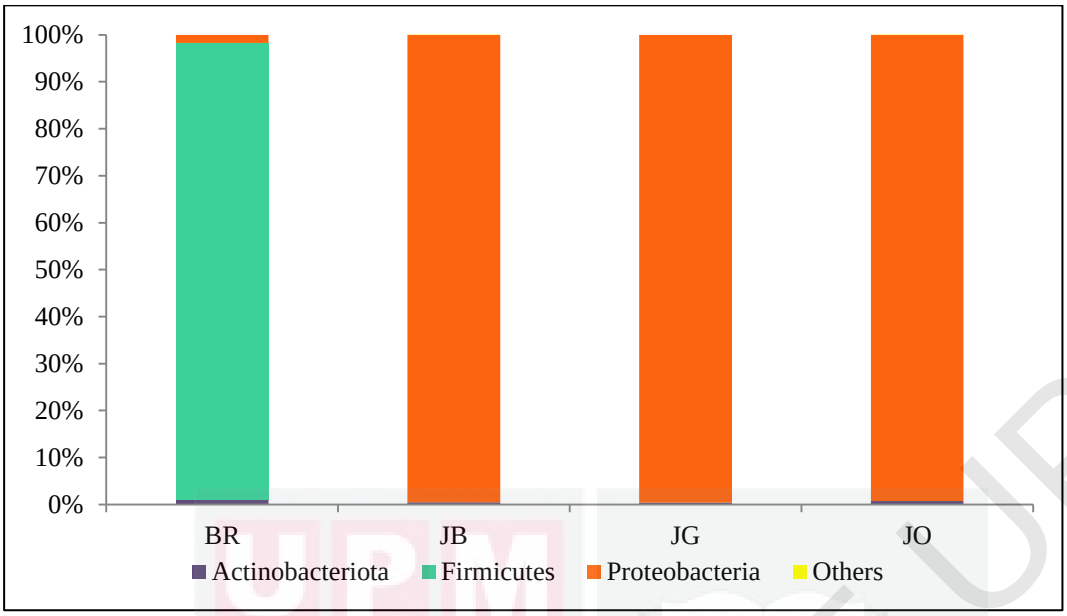


Figure 6.10: Bar plots representing relative abundances of bacterial phyla in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

The relative abundances of bacterial families in jam formulated with *scoby* from black, green and oolong tea *kombucha* and commercial probiotic butter are presented in Figure 6.11, showing that majority of the bacterial families of jam remained unknown. However, *Acetobacteraceae* (28.5-36.3%) family was identified as the second most abundant family in all the jams. On the other hand, the *Bacillaceae* (97.2%) family was found to be the most dominant family in commercial probiotic butter. While the dominant families of bacteria differed, the jams and commercial probiotic butter shared common families such as *Pseudomonadaceae* (0.1-1.7%) and *Nocardiaceae* (0.2-1%).

The variation in microbial groups was attributed to the types of fermentation used in each product. Jam formulated with *scoby* undergoes natural fermentation, relying on microorganisms naturally present in *scoby* while commercial probiotic jam involves intentional addition of specific probiotic cultures during production. The presence of

Acetobacteraceae in the jams was related to the presence of *scoby*, as this culture is typically dominated by bacteria from this family (Chakravorty et al., 2016; Marsh et al., 2014). The presence of *Bacillaceae* family in commercial probiotic butter is expected, given that it includes strains known for their probiotic properties (Brutscher et al., 2022).

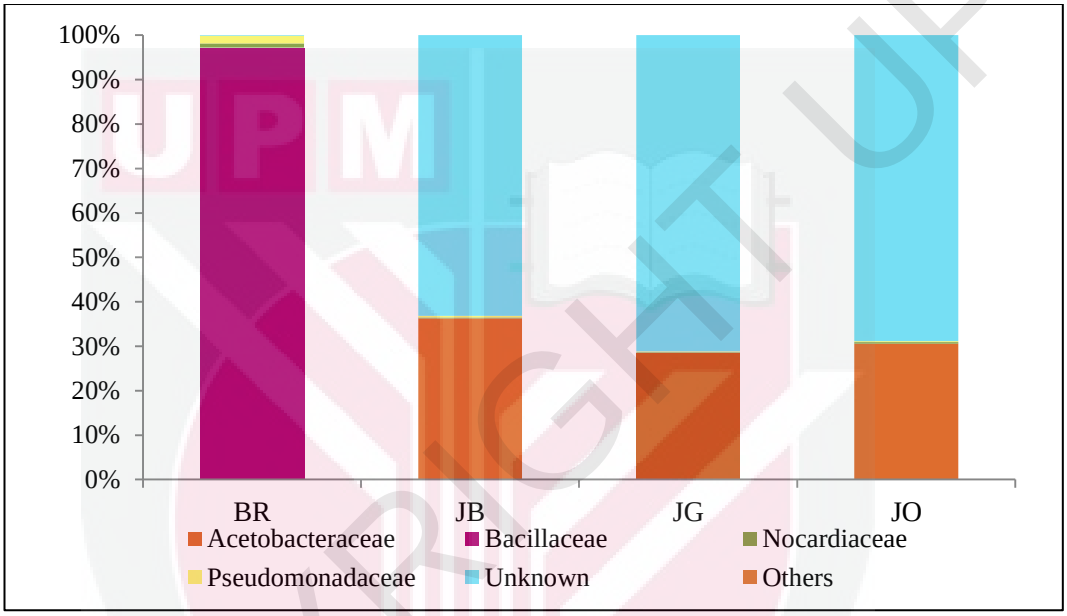


Figure 6.11: Bar plots representing relative abundances of bacterial families in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Figure 6.12 illustrates the relative abundances of bacterial genera in the samples. The findings revealed that *Komagataeibacter* (25-32.1%) from *Acetobacteraceae* family was the most prevalent in the jams while *Bacillus* (97.2%) from *Bacillaceae* family dominated the commercial probiotic butter. The other genera presented in both jams and butter were *Pseudomonas* (0.1-1.7%) and *Rhodococcus* (0.1-1%). The dominance of bacteria was unaffected by the different types of *scoby* in jam.

Komagataeibacter serves a significant function in the jam. It generates cellulose with excellent physical and mechanical properties (Laavanya et al., 2021), enhancing its suitability as a hydrocolloid. A recent report highlighted the results of a study that investigated the toxicological and dietary aspects of bacterial cellulose derived from *Komagataeibacter*, utilising an animal model (Dourado et al., 2017; Vigentini et al., 2019). The conclusion of the study affirmed the safety of bacterial cellulose from *Komagataeibacter*, concluding that it is safe to be used in food technology (Dourado et al., 2017; Vigentini et al., 2019), paving the path towards the acceptance of *scoby* in food production. In the delivery of food functional factors, presence of *Komagataeibacter* promotes probiotic properties with its ability to minimise glucose uptake and prevent weight gain in the human gastrointestinal tract by converting glucose to cellulose (Kaashyap et al., 2021; Lavasani et al., 2019). The functional mechanism of *Komagataeibacter* is involved in the synthesis of organic acids, which serve as the primary medicinal and detoxifying agents (Kumar & Joshi, 2016; Wang et al., 2022) and have the potential to treat hypocalcaemia, hypomagnesemia, and anaemia (Kaashyap et al., 2021). *Komagataeibacter* can also produce levan, a branched homopolymer with potential health benefits, for example, antioxidant and anti-inflammatory properties (Gomes et al., 2018). A study proposes valuable impacts of levan on the gut microbial community in farmed animals (Gomes et al., 2018). The use of levan in the food industry as a fat substitute and emulsifying agent may also have health benefits by reducing unhealthy fat intake and improving food product stability and texture (Gomes et al., 2018). In addition to the health benefits mentioned, levan can also have functional food properties due to its characteristics such as biocompatibility (Gomes et al., 2018). These findings further clarify that *scoby* in jam helps to improve quality and restrict calories.

On the other hand, *Bacillus* species are increasingly being studied for their potential health benefits in functional food research; hence, it is not unexpected to discover the presence of *Bacillus* in food. They have been used in functional food due to their ability to remain stable under various atmospheric conditions and survive the harsh conditions of the gastrointestinal tract (Elshaghabee et al., 2017; Lee et al., 2019). *Bacillus* probiotics have beneficial properties such as antimicrobial, anticancer, antioxidant, and vitamin production, but it is important to note that they can also produce toxins and biogenic amines, as well as potentially transfer antibiotic resistance genes (Lee et al., 2019). Despite the differences in nature, both products, jam formulated with *scoby* and commercial probiotic butter, offer beneficial microbes that could have positive implications for human health.

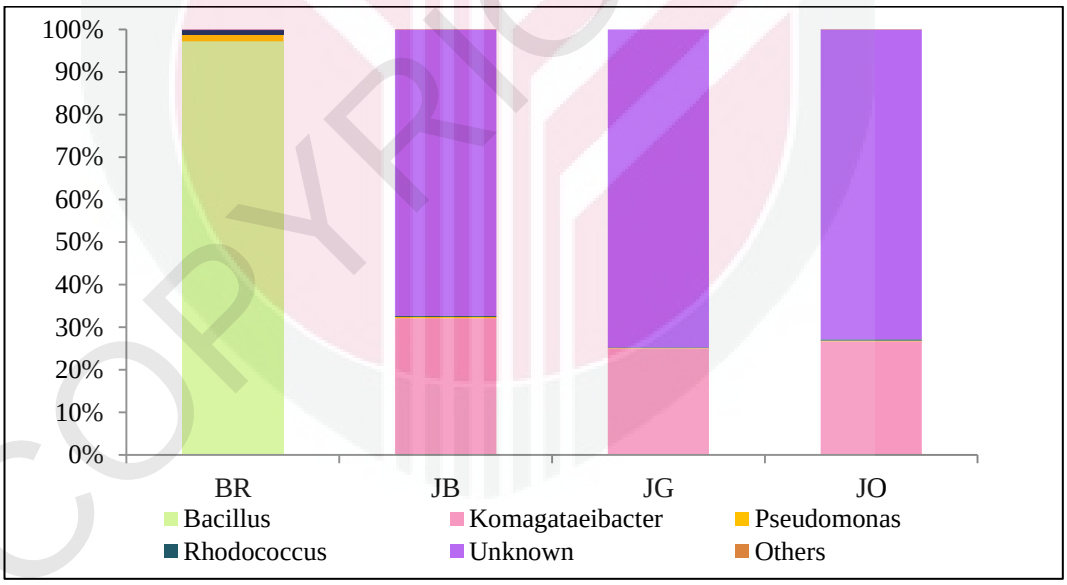


Figure 6.12: Bar plots representing relative abundances of bacterial genera in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Figure 6.13 shows the presence of two phyla: *Ascomycota* (60.6-75.6%) and *Basidiomycota* (0.34-0.59%) in commercial probiotic butter and jams formulated

with different *scoby*. *Ascomycota* is occasionally used in fermentation of foods such as bread, beer, wine and sake (Cousin, 2014), while *Basidiomycota* contributes to fermented food production as they secrete enzymes that facilitate the breakdown of substrates and yield valuable compounds such as antioxidants and flavour-enhancing compounds (Berger & Ersoy, 2022).

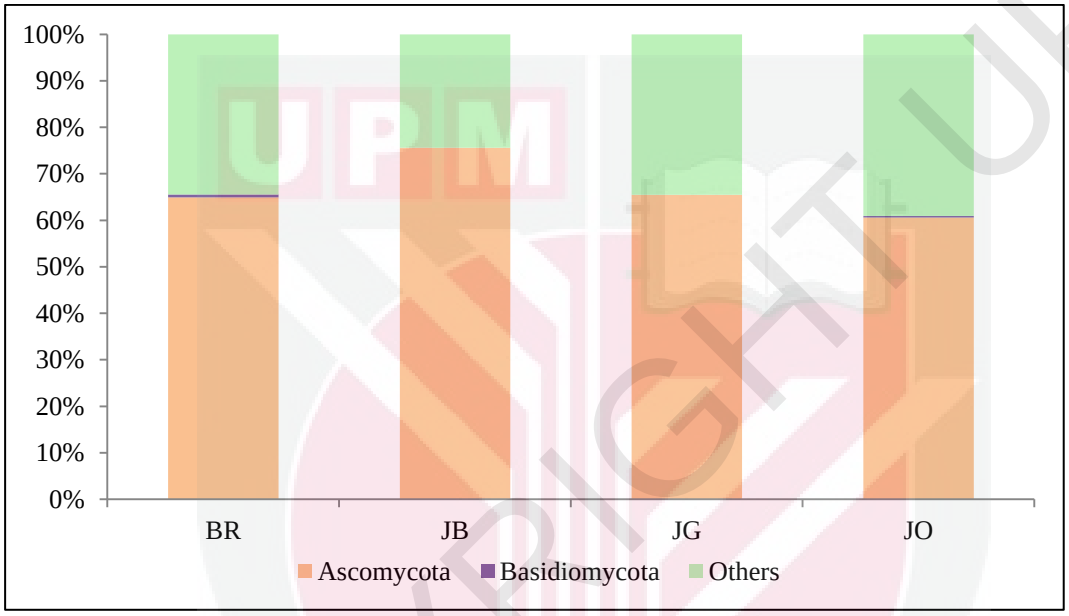


Figure 6.13: Bar plots representing relative abundances of fungal phyla in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

The dominant family, illustrated in Figure 6.14, was *Saccharomycetaceae* (32.29-73.18%), with *Pichiaceae* (1.68-29.97%) also detected in all samples, indicating a similarity between the two products. The other families were *Moniliellaceae* (0.3%) in jam and *Pleosporaceae* (0.7%), *Saccharomycetales_fam_Incertae_sedis* (0.5%), *Bulleribasidiaceae* (0.4%), *Mycosphaerellaceae* (0.4%), *Cladosporiaceae* (0.4%), *Nectriaceae* (0.2%), *Glomerellaceae* (0.1%) in commercial probiotic butter. *Saccharomycetaceae*, a family of food-fermenting yeasts, actively participate in fermentative metabolism (Gänzle, 2019) and contribute to flavours in fermented

foods (Hu et al., 2011). On the other hand, *Pichiaceae* is detected in various fermented foods (Jeong et al., 2023; Ramos et al., 2020), with specific members of this family recognised for their production of significant aromatic compounds, thereby enhancing the flavours of fermented foods (Ramos et al., 2020).

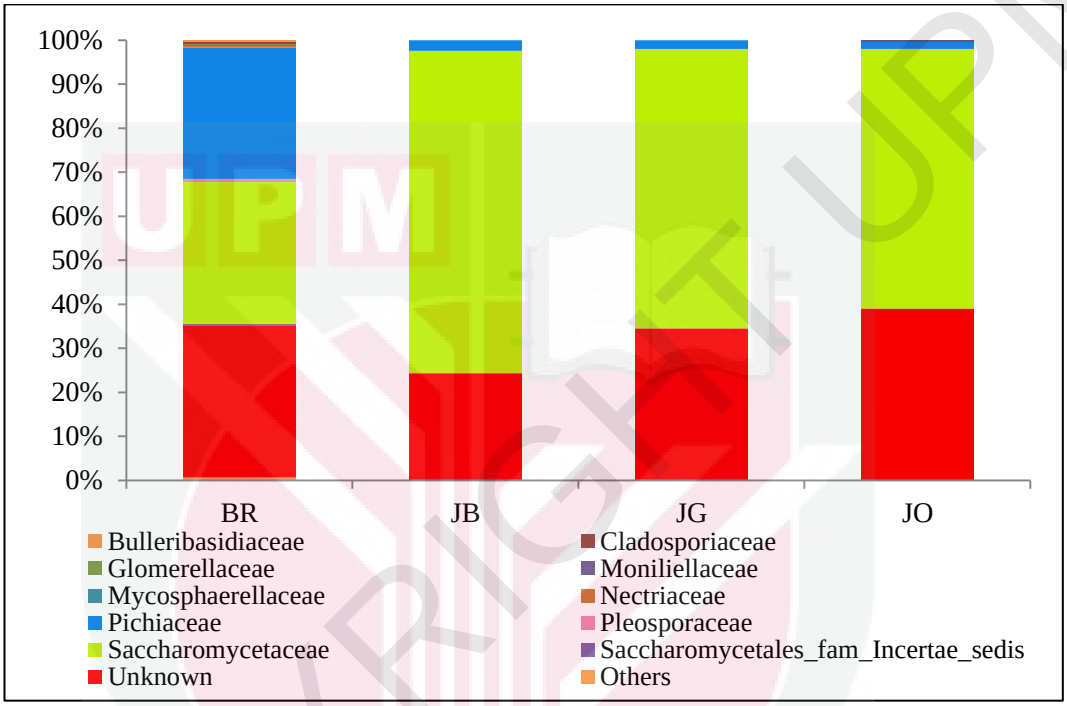


Figure 6.14: Bar plots representing relative abundances of fungal families in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ''others'')

Following that, the plots of fungal genera in the samples are shown in Figure 6.15. *Zygosaccharomyces* (58.9-73.2%) was the most encountered fungal representative in the jams, followed by *Dekkera* (1.4-1.6%) and *Brettanomyces* (0.2-1%) while the fungal communities in commercial probiotic butter were *Kluyveromyces* (25.8%), *Brettanomyces* (17.2%), *Dekkera* (12.7%), *Zygosaccharomyces* (6.1%), *Alternaria* (0.7%), *Candida* (0.5%), *Cladosporium* (0.4%), *Mycosphaerella* (0.4%), *Vishniacozyma* (0.4%), *Saccharomyces* (0.4%), *Fusarium* (0.2%) and *Colletotrichum*

(0.1%). *Zygosaccharomyces*, which populated the jam microflora, combined with *Dekkera* and *Brettanomyces* could provide taste modification (Serra Colomer et al., 2019) to the jams. Conversely, the species of the genus *Kluyveromyces,* present in commercial probiotic butter, shows potential for diverse industrial applications, including the production of aroma compounds, probiotic functionality and their stimulation through prebiotics (Karim et al., 2020).

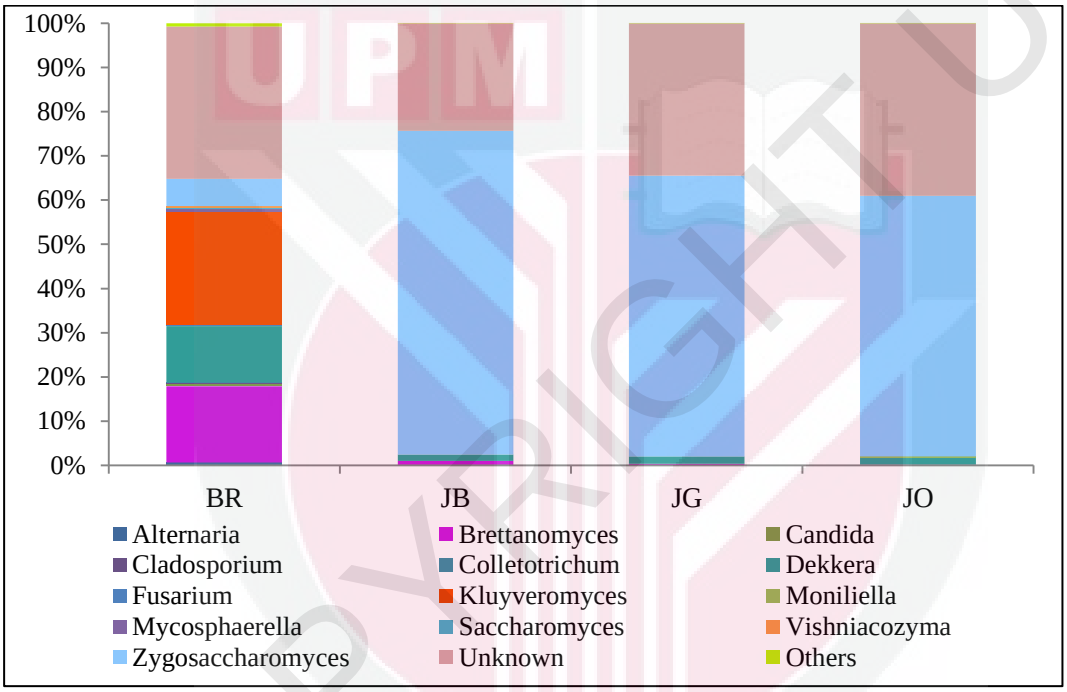


Figure 6.15: Bar plots representing relative abundances of fungal genera in commercial probiotic butter and jams with *scoby* from black, green and oolong tea *kombucha* (Taxa abundances < 0.1% have been combined under ‘‘others’’)

6.5 Diversity metrics of microbial communities of jam formulated with *scoby*

The bacterial and fungal communities of the samples were further investigated for their diversity using the Shannon and Simpson diversity indices, reported in Table 6.2. It appeared that all four samples had similar levels of bacterial diversity, as shown by the relatively consistent values for both indices. However, the commercial

probiotic butter appeared to have slightly higher diversity and evenness compared to the other three jam samples, with a Shannon diversity index of 1.32 and a Simpson diversity index of 0.61. Across all four samples, the Shannon diversity index ranged from 2.05 to 2.30 for fungal community. The Simpson index remained relatively consistent, ranging from 0.81 to 0.86, with the highest evenness found in jam formulated with green tea *scoby*.

Table 6.2: Alpha diversity indices for bacterial and fungal communities of commercial probiotic butter and jams with *scoby* from black, oolong and green tea *kombucha*

	Bacterial community		Fungal community	
	Shannon	**Simpson**	**Shannon**	**Simpson**
BR	1.32	0.61	2.24	0.81
JB	0.99	0.57	2.05	0.81
JO	0.93	0.53	2.19	0.85
JG	0.97	0.55	2.30	0.86

Principal Coordinate Analysis (PCoA), shown in Figure 6.16 was used to visualise and analyse similarities and differences among samples. Jams formulated with different *scoby* showed minimal separation in bacterial community while tight clusters were found in fungal community. The probiotic butter and jam samples were distinctly separated from each other, indicating a clear difference in the microbial and fungal composition of these samples. The distinct clustering of these samples was attributed to the different ingredients and nature of product affecting the microbiome.

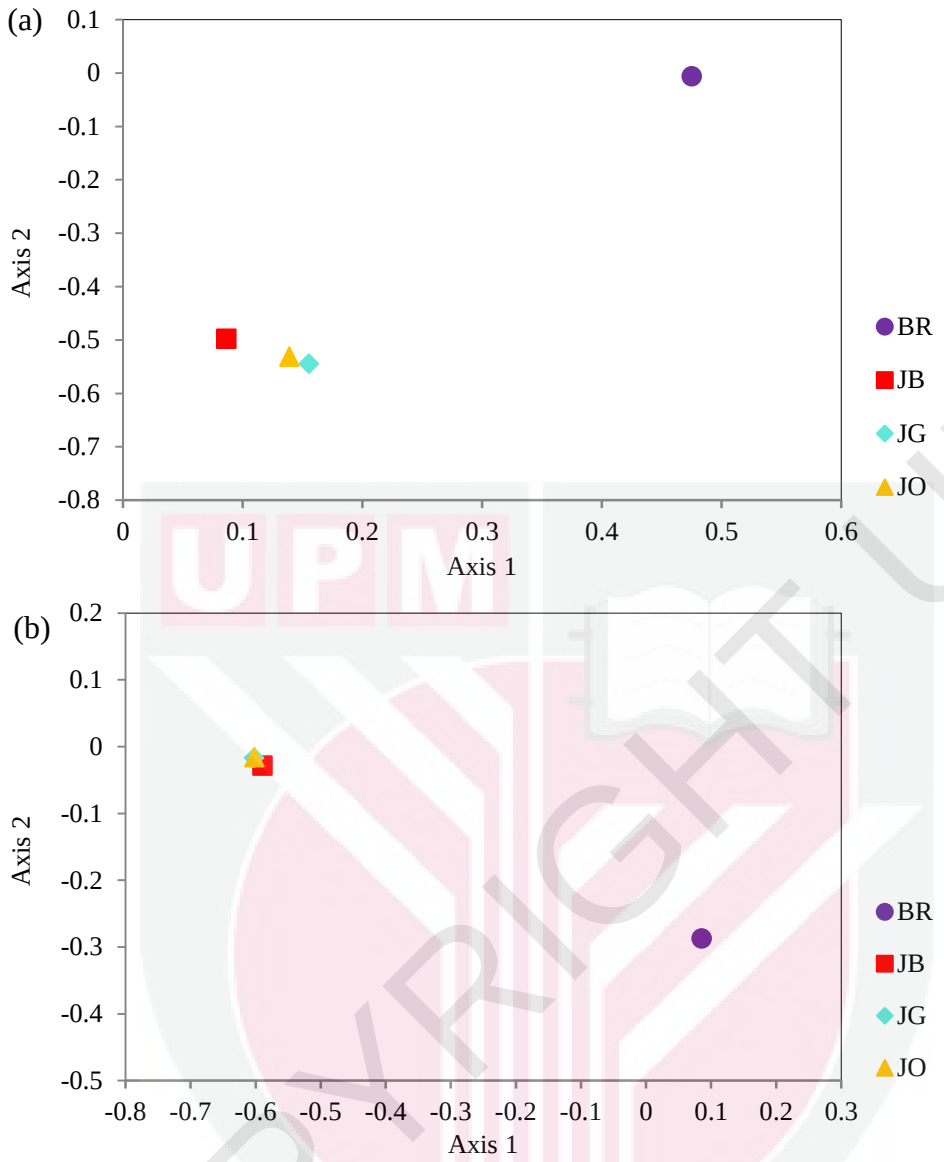


Figure 6.16: PCoA plots comparing (a) bacterial community and (b) fungal community of commercial probiotic butter and jams with *scoby* from black, oolong and green tea *kombucha*

6.6 Comparison on microbial changes in *scoby*, *kombucha* tea and jam formulated with *scoby*

In Figure 6.17, the changes in relative abundances of bacterial genera across *scoby*, *kombucha* tea and jam are shown, with *Komagataeibacter* being the major known genera in all the products. The dominance of *Komagataeibacter* in both *scoby* and *kombucha* tea of all three types of tea substrates indicated a strong correlation

between the two, suggesting a potential transfer of microorganisms from *scoby* to *kombucha* tea during the fermentation process. While most of the genera in the jam formulated with *scoby* remained unknown, the recurring dominance of *Komagataeibacter* showed a consistent microbial influence from the *scoby*. Given that mango is the major component in jam formulation, it could be the primary source of unknown microbes. This arises from the potential presence of plant materials like chloroplasts and mitochondria, which contain genetic sequences.

Pseudomonas and *Rhodococcus* persisted in all products, albeit at lower relative abundances, suggesting that these microorganisms are capable of adapting throughout the fermentation and jam-making processes. However, genera such as *Lactobacillus*, *Lactococcus*, *Limosilactobacillus*, *Prevotella_9* and *Weissella* were only found in *kombucha* and did not appear to be present in the jam samples.

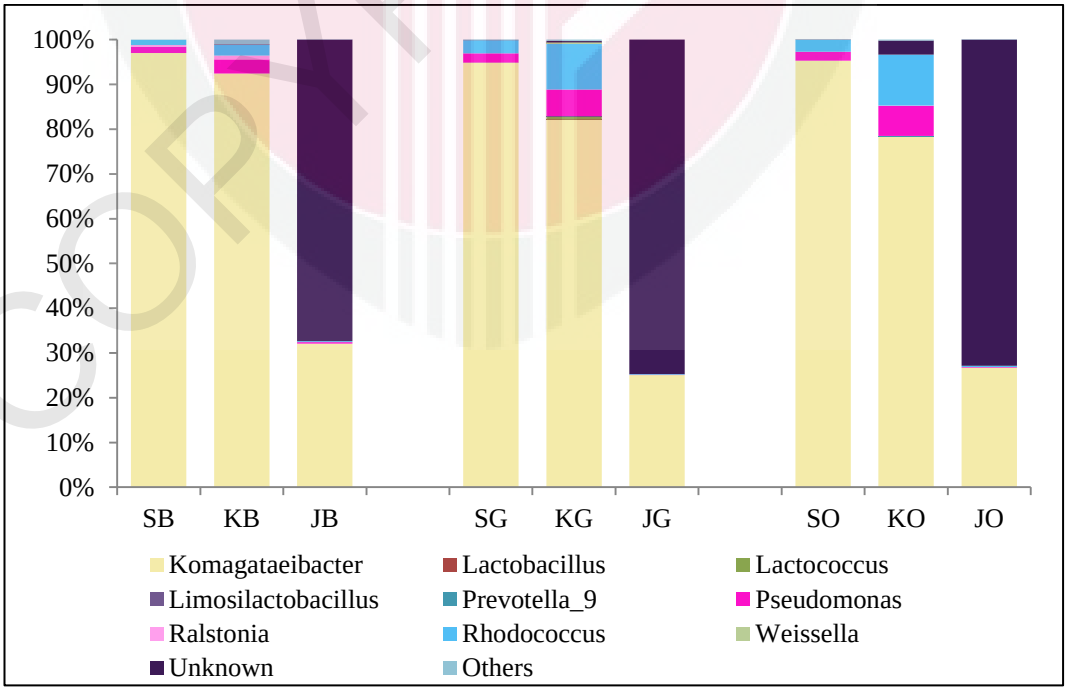


Figure 6.17: Bar plots representing relative abundances of bacterial genera in *scoby*, *kombucha* teas and jams (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Dekkera dominated all the *scoby* and *kombucha* samples except KB, while *Zygosacchromyces* was the dominant genus in the jams (Figure 6.18). The jams formulated with *scoby* did not follow the same dominance pattern, likely influenced by the jam-making process, yet they still contained *Dekkera* in low abundance. *Brettanomyces* was detected in all samples; however, its abundance was notably in a lesser fraction in jam compared to *kombucha* tea and *scoby*.

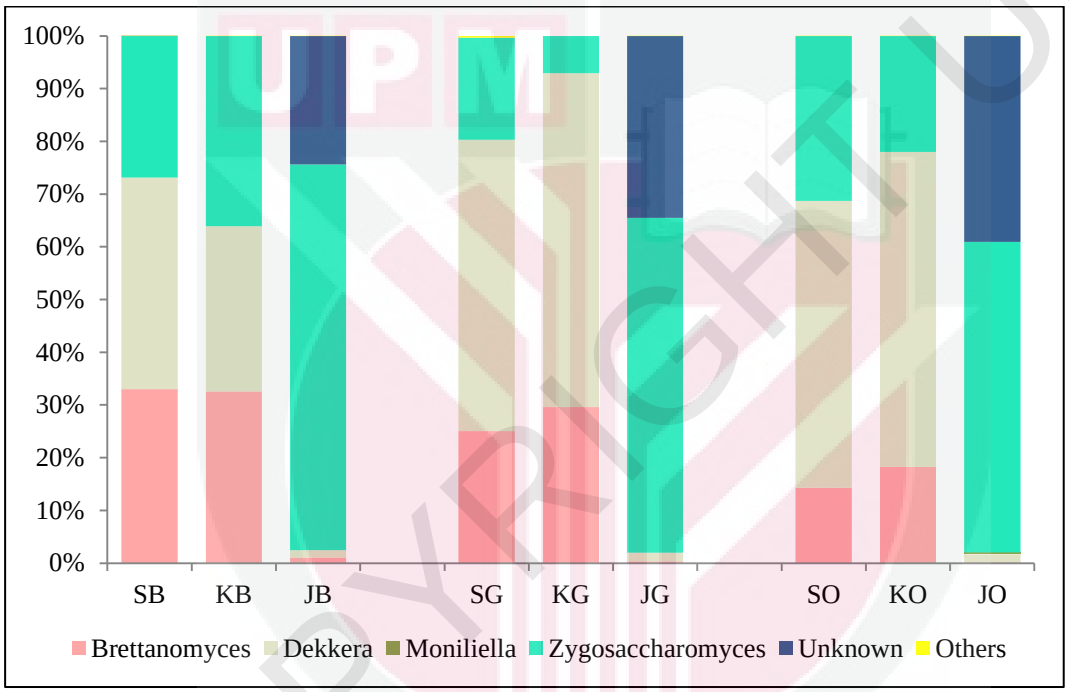


Figure 6.18: Bar plots representing relative abundances of fungal genera in *scoby*, *kombucha* teas and jams (Taxa abundances < 0.1% have been combined under ‘‘others’’)

Table 6.3 shows the summary of significant microorganisms and their functionalities in *kombucha* and jam products while Figure 6.19 shows the overall mechanism of microbial activity during fermentation which involves the symbiotic system of bacteria and fungi. Yeast converts sucrose into glucose and fructose, producing ethanol and carbon dioxide while acetic acid bacteria assimilates these monosaccharides to generate gluconic acid, glucuronic acid and ethanol for the

production of acetic acid (Neffe-Skocińska et al., 2017). Some yeast strains such as *Brettanomyces* and *Dekkera* have the ability to make acetic acid as well (Antolak et al., 2021; Laureys et al., 2020). Concurrently, acetic acid bacteria are responsible for creating new cellulose layers from substrates such as glucose and fructose and this anabolic process involves sucrose and ethanol (Laureys et al., 2020; May et al., 2019; Su et al., 2023; Valera et al., 2015). Meanwhile, lactic acid bacteria utilise glucose via Embden-Meyerhof-Pamas or pentose phosphate pathway to produce lactic acid (Antolak et al., 2021). While there is an accumulation of acids, the yeast strain demonstrate high acid-tolerance to maintain equilibrium of yeast and bacteria in their symbiosis (Nguyen et al., 2015).

On the other hand, the intricate phenolic compounds present in *kombucha* can undergo enzymatic degradation or conversion into smaller biological molecules facilitated by bacteria and yeast (Su et al., 2023). For example, epigallocatechin-3-gallate is hydrolysed into smaller molecules and transformed into epicatechin gallate, epigallocatechin and epicatechin (Jayabalan et al., 2008; Su et al., 2023). The microbial biotransformation ability of phenols in *kombucha* have been found in certain acetic acid bacteria, lactic acid bacteria and yeast like *Zygosaccharomyces* and *Brettanomyces*, contributing to the antioxidant properties of products (Antolak et al., 2021; Shi et al., 2023).

Table 6.3: Summary of significant microorganisms and their functionalities in *kombucha* and jam products

	Functionalities	References
(a) Bacteria		
(i) Acetic acid bacteria		
Komagataeibacter	• Production of cellulose • Production of organic acids, *i.e.*, acetic acid, glucuronic acid and gluconic acid for vinegary sour taste, hepatoprotective effects, mineral supplements, contamination prevention and synthesis of vitamin C • Conversion of phenolic compounds • Nitrogen fixation • Probiotic properties with capability of reducing glucose absorption and preventing weight gain • Mitigate alcohol-induced liver damage by regulating fatty acids • Demonstrate anti-inflammatory effects • Produce levan with antioxidant and anti-inflammatory benefits	Antolak et al. (2021), Coelho et al. (2020), Gomes et al. (2018), Harrison & Curtin, (2021) Jiang et al. (2019), Kaashyap et al. (2021), Kozyrovska et al. (2021), Kumar & Joshi (2016), Lavasani et al. (2019), Lin et al. (2020), Jiang et al. (2019), Pothakos et al. (2016), Reis & Teixeira (2015), Shi et al. (2023), Subbiahdoss et al. (2022), Villarreal-Soto et al. (2018), Wang et al. (2022)
(ii) Lactic acid bacteria		
Limosilactobacillus *Weissella* *Lactococcus* *Lactobacillus*	• Production of lactic acid • Conversion of phenolic compounds • Probiotic potential • Production of d-saccharic acid-1,4-lactone (DSL) that has hypocholesterolaemic, hepatoprotective and antihypergylcaemic effects • Synthesise B-complex vitamins	Antolak et al. (2021), Fijan (2014), Piccioni et al. (2021) & Teixeira et al. (2021)
(iii) Others		
Rhodococcus	• Production of cellulose • Possess cholesterol-degrading ability	Cacicedo et al. (2016), Iguchi et al. (2000), Jeremic et al. (2019) & Kim et al. (2018)
Pseudomonas	• Production of cellulose	Sah et al. (2021)

Table 6.3: Continued

Prevotella	• Improve glucose metabolism induced by prebiotic consumption • Regulation of intestinal permeability and intervention in pathogen colonisation to prevent inflammation	Kovatcheva-Datchary et al. (2015), Scher et al. (2013) & Wang et al. (2021)
(b) Fungi		
Dekkera	• Facilitate metabolism of sugars in production of ethanol • Production of acetic acid	Antolak et al. (2021), Coelho et al. (2020), Fabricio et al. (2022) & Laureys et al. (2020)
Zygosaccharomyces	• Initiate fermentation process • Production of ethanol • Conversion of phenolic compounds • Production of phytase that enhance mineral absorption, mitigate phytic acid's negative effects, improve functional qualities and release beneficial bioactive compounds	Antolak et al. (2021), Coelho et al. (2020), Joudaki et al. (2023), Shi et al. (2023) & Teoh et al. (2004)
Brettanomyces	• Facilitate metabolism of sugars in production of ethanol • Production of acetic acid • Generation of aromatic and flavour compounds	Antolak et al. (2021), Coelho et al. (2020), Harrison & Curtin, (2021), Serra Colomer et al. (2019), Teoh et al. (2004) & Villarreal-Soto et al. (2018)

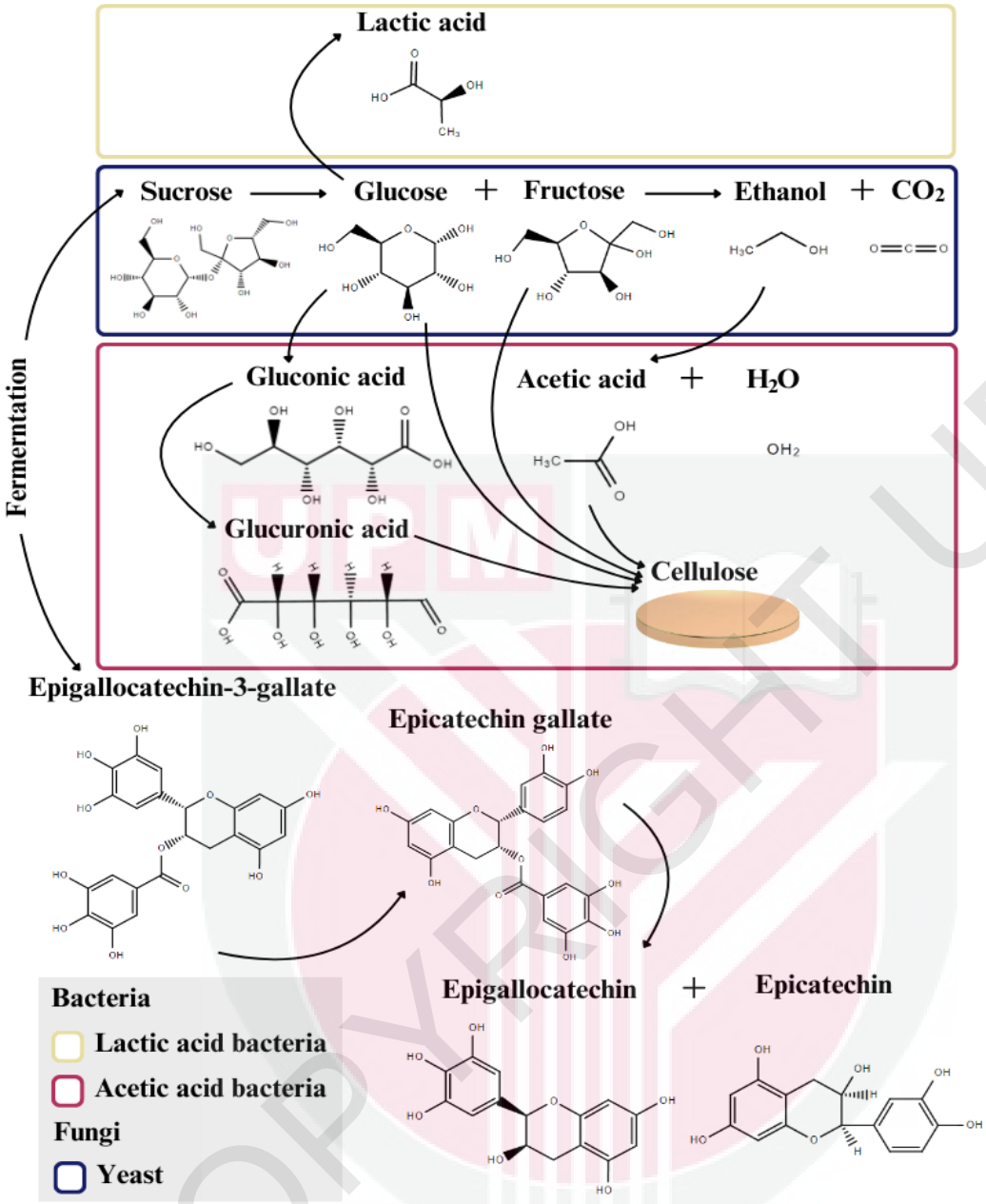


Figure 6.19: Overall mechanism of microbial activity during fermentation

The taxonomic assignment enabled the identification of microoganisms that contributed to the alpha diversity of bacterial and fungal communities of *scoby*, *kombucha* teas and jams, as reported in Table 6.4. Among the bacterial communities, jam formulated with *scoby* showed highest Shannon and Simpson indices, indicating greater species richness and evenness compared to *kombucha* teas and *scoby*.

Kombucha teas and *scoby* had a more dominant bacterial community, leading to lower diversity values whereas jam had a more diverse and balanced distribution of bacterial community, potentially attributed to the presence of a considerable number of unknown genera. In fungal community, the order of alpha diversity from highest to lowest was as follows: jam, *scoby* and *kombucha* tea, with the exception of black tea. Despite the order, the Shannon and Simpson indices showed similar values in all samples, indicating comparable richness and evenness among the products.

Table 6.4: Alpha diversity indices for bacterial and fungal communities of *scoby*, *kombucha* teas and jams

	Bacterial community		**Fungal community**	
	Shannon	**Simpson**	**Shannon**	**Simpson**
(a) Black tea				
SB	0.19	0.06	**2.10**	**0.86**
KB	0.39	0.14	2.07	0.85
JB	**0.99**	**0.57**	2.05	0.81
(b) Green tea				
SG	0.27	0.11	1.90	0.80
KG	0.70	0.32	1.67	0.76
JG	**0.97**	**0.55**	**2.30**	**0.86**
(c) Oolong tea				
SO	0.25	0.10	1.85	0.77
KO	0.77	0.37	1.82	0.76
JO	**0.93**	**0.53**	**2.19**	**0.85**

Figure 6.20 compares beta diversity of bacterial and fungal communities in *scoby*, *kombucha* teas and jam formulated with *scoby*. Bacterial community of black *kombucha* tea and *scoby* showed clustering while *scoby* of green and oolong tea remained distant from the corresponding *kombucha* teas. They were more consistent within each product type. In contrast, the fungal community showed a different trend, with similar substrates of *kombucha* teas and *scoby* displaying closer clustering, suggesting a more consistent microbial community for the same tea type used. In both communities, clear separation was observed between jams and *kombucha* teas

as well as *scoby*. Nevertheless, the bacterial and fungal communities of jams were not affected by different types of *scoby*.

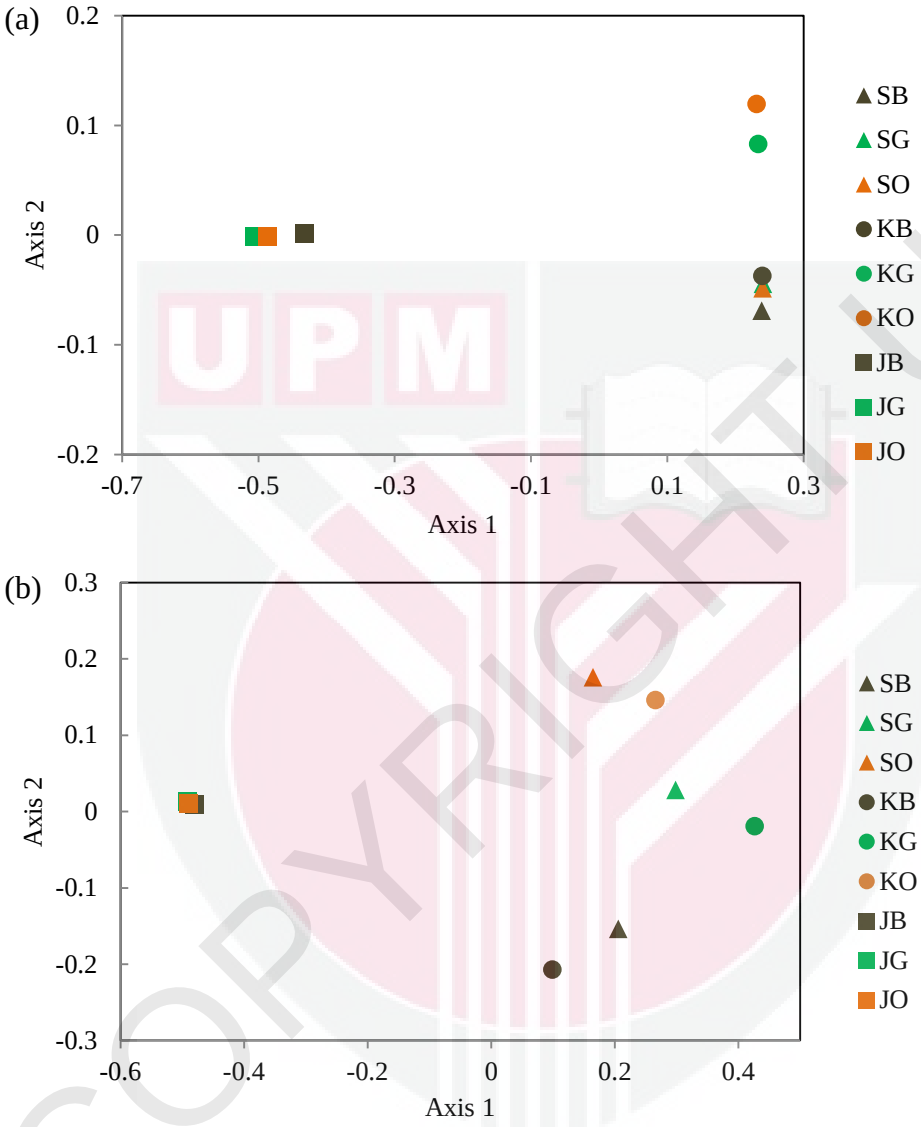


Figure 6.20: PCoA plots comparing (a) bacterial community and (b) fungal community of *scoby*, *kombucha* teas and jams formulated with *scoby*

6.7 Summary

This study utilised NGS to identify the microbiota in the products of *kombucha* fermentation and jam production. All samples reached plateau, indicating adequate sequencing depth to comprehensively capture the diversity of microbiota present in

the samples. The microbiome showed notable variations with starter cultures from different origins, while the impact of using various substrates was relatively minor. The dominant microorganisms identified in all *kombucha* tea and *scoby* samples were *Komagataeibacter*, *Zygosaccharomyces* and *Dekkera*, whereas the microbiota of samples KC and SC, which employed starter culture from a different location, were dominated by *Pseudomonas*, *Schizosaccharomyces* and *Brettanomyces*. The deviation of KC and SC from the rest of the samples was further validated through the examination of alpha and beta diversity in the study. The subsequent metagenomics sequencing showed that *Bacillus* and *Kluyveromyces* dominated commercial probiotic butter while *Komagataeibacter* and *Zygosaccharomyces* were the two most prevalent in jams made using *scoby*. Although there were different dominant microbes present, they still shared common bacteria and fungi, such as *Pseudomonas*, *Brettanomyces* and *Dekkera*. The dominance of *Komagataeibacter* and *Zygosaccharomyces* in both the *scoby* and jam suggests that the microbes that were present in *scoby* remain in jam. The identification of these microorganisms indicates that they could potentially offer multiple health benefits. For instance, *Komagataeibacter* displays probiotic properties, contributing to making the food functional. Overall, NGS has proven to be a powerful tool in supporting research on *kombucha* fermentation in greater depth through the identification and characterisation of microbes in detail. Its application holds promising prospects for subsequent fermentations and development of safer and healthier food products.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

7.1 Conclusion

In this study, *kombucha* fermentation expanded beyond the traditional use of black tea as substrate, incorporating green, oolong and various tea mixtures. The physicochemical analysis of the *kombucha* fermentation process revealed that different types of teas, whether in pure form or as a combined mixture, have presented comparable effects with a consistent trend. Brix values declined over the course of fermentation, while titratable acidity, alcohol and total polyphenol content increased. An increase in titratable acidity was associated with a corresponding decrease in pH values between 2.70 and 2.79. After 10 days of fermentation, the total polyphenol content in *kombucha* teas varied between 106 and 119 mg GAE/L while the yield of *scoby* ranged from 64.89 to 109.93%, with the highest results of both found in *kombucha* made with pure oolong tea. These findings suggest that type of tea used in the fermentation process plays an important role in determining the overall quality and yield of the final product. The use of pure oolong tea also resulted in a more efficient *kombucha* fermentation, achieving highest *scoby* productivity and conversion yield. This is further supported by modified Gompertz model analysis, showing higher acetic acid of 0.049% acid/day and *scoby* specific formation rate of 9.980 g/L-day compared to 0.048% acid/day and 7.323 g/L-day of black tea *kombucha* and 0.047% acid/day and 7.393 g/L-day of green tea *kombucha*. While pure oolong tea had the highest values, other tea types still fitted well in the modified Gompertz model, with $R^2 > 0.98$. The Boltzmann model, which was used to describe the change in sucrose substrate with respect to total soluble solids during the

fermentation period, also demonstrated a satisfactory goodness of fit of greater than 0.96 for all samples. Mathematical modelling of pH using polynomial, rational and exponential models showed an excellent fit of data with $R^2 > 0.98$. Incorporating a wider range of tea types, *e.g.*, the oolong tea instead of the traditional black tea in *kombucha* fermentation has paved the way for an innovative approach towards diversifying fermentation process. This advancement finds further clarity with the use of fermentation modelling. Manipulation of these substrates in *kombucha* has the potential to influence both the fermentation characteristics of *kombucha* and generation of *scoby* biofilms, offering alternatives for *kombucha* manufacturers, food and polymer engineers.

The application of by-product *scoby* from *kombucha* fermentation has advanced in the development of jam, with its properties being assessed. The physicochemical, textural, rheological, proximate analysis and sensory evaluation showed that different types and concentrations (2 to 10%) of *scoby* had an impact on jam. All samples showed significant regression ($P < 0.05$) with lower values of water activity, moisture, pH and total soluble solids in mango jams formulated with *scoby* and higher *scoby* concentrations. The jams complied with the requirements set by the Food Act (1983) and Food Regulations Malaysia (1985), with total soluble solids above 65%. For colour analysis, significant effect ($P < 0.05$) of L^*, a^* and b^* was found in comparison to the control sample. ΔE was observed in jam with *scoby* due to higher L^* and lower b^* values. The results also showed that addition of *scoby* to mango jam formulation had a significant effect ($P < 0.05$) on textural analysis when compared to the control sample such that hardness, work of shear, stickiness and work of adhesion were directly proportional to the concentration of *scoby*. On the

part of rheological analysis, the formulated jam showed degree of pseudoplasticity and indicated a non-Newtonian behaviour with shear thinning characteristic. The flow index, *n*, was less than 1 from the Power Law and Herschel-Bulkley (HB) models and apparent viscosity reduced with increased shear rate. *Scoby*-formulated jams had higher levels of carbohydrates (up to 23.2 g/100g), total dietary fiber (up to 0.4 g/100g) and protein (up to 0.3 g/100g) and lower fat content (less than 0.1 g/100g) compared to commercial jam while maintaining a similar nutritional composition to the control. The overall acceptability samples had scores greater than 6.5 on the score range of 0-9 with the highest score of 7.81 rated on mango jam with 6% green tea *scoby*. Principal Component Analysis (PCA) revealed that textural and physicochemical analyses are the most important among the set of variables studied for the quality of *scoby*-containing jam. Jam formulated with *scoby* showed enhanced properties while preserving a clean label composition. The favourable ratings further validated the quality of formulated jam as it received positive feedback and was well received by sensory panels, highlighting its appeal and sensory aspects. Repurposing the by-product of *kombucha*, *scoby*, in jam-making not only improves commercial value of *kombucha* and supports sustainable development goals but also offers environmental benefits by reducing waste and carbon footprint of food production.

In the analysis of microbes, the impact of pure and combined black, green and oolong teas on microbial composition of products, *kombucha* tea, *scoby*, and jam was examined, marking the first exploration of its kind. With Next-Generation Sequencing (NGS), sufficient sequencing depth was achieved for the test, as indicated by the plateauing of the rarefaction curves. The taxonomic analysis, which evaluated the overall abundance of bacterial and fungal communities, demonstrated

that the microbial composition of *kombucha* teas and *scoby* was influenced by the type of tea substrate and different culture sources in Malaysia. *Komagataeibacter*, *Zygosaccharomyces* and *Dekkera* were the characteristic microorganisms of all *kombucha* teas and *scoby* samples except the reference samples, *kombucha* tea KC and *scoby* SC from different culture source which were dominated by *Pseudomonas*, *Schizosaccharomyces* and *Brettanomyces*. The factor of tea varieties, including tea blends, which was first assessed in this study, revealed similarities in the bacterial and fungal taxa, however, the dominance of genera and diversity were slightly different. *Kombucha* tea from pure oolong tea and *scoby* from combinations of black and oolong teas exhibited the highest diversity in bacterial community based on Shannon and Simpson indices which reflect both richness and evenness, while black tea *kombucha* tea and *scoby* showed the highest in fungal community. Due to their different sources, KC and SC again showed a distinct separation from the main samples of this research as seen in the Principal Coordinate Analysis (PCoA) plots of both bacterial and fungal communities.

The metagenomic investigation showed that *Zygosaccharomyces* and *Komagataeibacter* were the two most prevalent in jams formulated with *scoby*, showing that the microbes that were present in the *scoby* remain in the jam while *Bacillus* and *Kluyveromyces* dominated commerical probiotic butter. Commercial probiotic butter exhibited the highest Shannon and Simpson diversity for the bacterial community, while jam with green tea *scoby* demonstrated the highest diversity for the fungal community. Probiotic butter and jam samples were distinctly separated from each other in PCoA plots, attributed to the different ingredients and nature of product. Despite having different diversities, both *kombucha* and jam

samples hold potential as functional foods, containing microorganisms that may contribute to their functional properties. In particular, the presence of *Komagataeibacter* as the dominant microorganism in the analysed samples is noteworthy as it brings along a host of health benefits owing to its probiotic properties. The presence of beneficial microorganisms in these products generates compounds that demonstrate potential as functional foods, catering to health-conscious consumers. As functional foods continue to gain popularity, these products can lead to increased consumer interest, satisfaction, and market expansion.

7.2 Recommendations for future research

Although diverse microorganisms' profiles were determined in the *kombucha* fermentation products, precise mechanism of the interactions between tea components and microbiota still remains unclear. Further studies investigating the impact of specific tea compounds on particular microbial populations are recommended to clarify this matter. Those that could lead to prebiotic, probiotic or paraprobiotic functions are also still not fully understood. This suggests research in the area of effects of the determined microorganism on the modulation of gut microbiota and its subsequent effects on host health. The use of *scoby* as an alternative ingredient can be expanded from an industrial point of view through a systematic product development course in order to achieve a novel food product with better health properties and sustainability. Research on applications of *scoby* for cellulose synthesis is also suggested for the relevant industries.

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APPENDICES

Appendix A

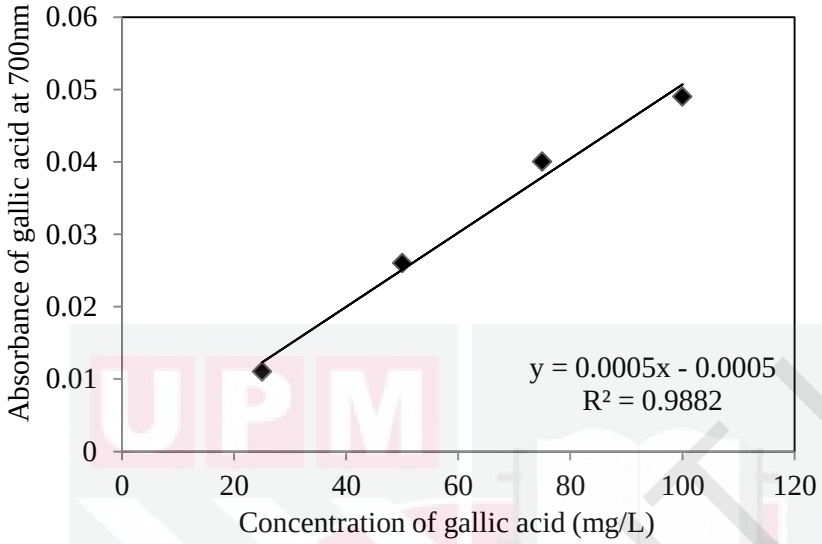
Specific gravity measurement

The specific gravity of the sample was calculated as follows:
Specific gravity = $\frac{B-C}{A-C}$
where:

A : Weight of distilled water and and density bottle
B : Weight of *kombucha* tea and density bottle
C : Weight of empty density bottle

Appendix B

Gallic acid standard curve for total polyphenol content



Appendix C

Methodology references for proximate analysis

Analysis	Methodology references	References
Total carbohydrate	Method of Analysis for Nutrition Labeling (1993), pg. 106	Sullivan & Carpenter (1993)
Energy calculated	Method of Analysis for Nutrition Labeling (1993), pg. 5 and 106	
Total dietary fiber	AOAC Official Method 985.29	
Ash	Method of Analysis for Nutrition Labelling, 1993, Chapter 10	
Fat	Method of Analysis for Nutrition Labeling, 1993, Chapter 18 & Pearson's, 1991, pg. 24	Kirk & Sawyer, (1991), Sullivan & Carpenter (1993)
Protein	Method of Analysis for Nutrition Labeling, 1993, Chapter 28 & Pearson's, 1991, pg. 17 and 20	

Appendix D

Hedonic rating scale

Name:

Age:

Gender:

Sample code:

Instruction: Infront of you is a coded sample. Taste the sample and tick how much you like or dislike it.

	Texture (Spreadability)	Colour	Flavour (Taste)	Odour (Smell)	Overall Acceptability
like extremely					
like very much					
like moderately					
like slightly					
neither like nor dislike					
dislike slightly					
dislike moderately					
dislike very much					
dislike extremely					

Appendix E

Definitions of the descriptors (sensory attributes) used for evaluation of jams

Attribute	Description
Texture (Spreadability)	Evaluate the smoothness of the texture by assessing its consistency and if it spreads evenly and effortlessly on a slice of bread
Colour	Assess the colour of jam by observing its hue, saturation and brightness, noting any variations or inconsistencies in the appearance of jam
Flavour (Taste)	Taste the flavour and rate based on the balance of sweetness and acidity
Odour (Smell)	Detect any aroma defects (*e.g.*, unclean, masked, unnatural, lacks freshness)
Overall acceptability	Rate the satisfactory of the sample by considering the combined sensory experience, including appearance, smell, taste and flavour profiles

LIST OF PUBLICATIONS

Chong, A. Q., Chin, N. L., Talib, R. A., & Basha, R. K. (2024). Modelling pH Dynamics, SCOBY Biomass Formation, and Acetic Acid Production of Kombucha Fermentation Using Black, Green, and Oolong Teas. *Processes*, *12*(7), 1301.

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