## DEVELOPMENT OF KOMBUCHA TEA USING PECAH BELING TEA (Strobilanthes Crispus) AND POLY-HERBAL TEA (Strobilanthes Crispus and Orthosiphon Stamineus)

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#### ABSTRACT

The study focuses on the development of kombucha tea using Pecah Beling and Polyherbal tea leaves and evaluates its properties. Kombucha tea is a fermented tea made by combining tea leaves, sucrose, and SCOBY. The drink has been shown to possess various properties such as anticancer, antioxidants, antimicrobe, and antifungal. One of the challenges with kombucha tea is that it has an undesirable flavor that is quite like vinegar. To address this issue, the study explores using different types of tea leaves to make the drink more palatable. The researchers analyzed the physicochemical properties of the tea, including its nutritional properties, pH, sugar brix percentage (%), and alcohol content. They also evaluated its phytochemical properties, such as total phenolic content, total flavonoid content, and antioxidant activity using DPPH and ABTS analysis methods. In addition, the study assessed the antimicrobial properties of the tea using the agar well diffusion method and tested it against various bacteria and fungi. The results of the study showed that both types of tea leaves produced kombucha with acceptable phytochemical properties (13%-25%) and exceptional antimicrobial properties 2.71-7.52 mm inhibition area against pathogenic microbes. The analysis also found that the physicochemical properties of the kombucha changed gradually over the fermentation period, and the concentration of sucrose affected the tea's properties. However, the Poly-herbal tea showed an antagonistic reaction in TPC and TFC analysis when compared to black tea kombucha. Lastly, the study conducted an accelerated shelf-life assessment and found that kombucha tea containing 80g of sucrose had a shelf-life of approximately six months. In conclusion, this study found that kombucha tea production with different teas and sucrose concentrations affects sensory properties, nutritional composition, phytochemical and antioxidant properties, and antimicrobial activity. Kombucha tea with Pecah Beling tea had the most favorable sensory properties and antioxidant activity. The concentration of sucrose was found to promote nutritional composition and antimicrobial properties. The shelf-life of kombucha tea with 80g of sucrose was stable for 6 weeks.



#### ABSTRAK

Kajian ini memberi tumpuan kepada pembuatan teh kombucha menggunakan daun teh Pecah Beling dan Poly-herbal dan menilai sifat-sifatnya. Teh kombucha adalah teh yang difermentasikan dengan menggabungkan daun teh, sukrosa, dan SCOBY. Minuman ini mempunyai pelbagai kebaikan seperti anticancer, antioksidan, antimikrob, dan antifungal. Kajian ini menganalisis sifat-sifat fizikokimia teh, termasuk sifat-sifat nutrisi, pH, peratusan brix gula (%), dan kandungan alkohol. Kajian ini juga menilai sifat-sifat fitokimia teh, seperti kandungan fenolik keseluruhan, kandungan flavonoid keseluruhan, dan aktiviti antioksidan menggunakan kaedah analisis DPPH dan ABTS. Selain itu, kajian menilai sifat-sifat antimikrob teh menggunakan kaedah difusi sumuran agar dan mengujinya terhadap pelbagai jenis bakteria dan kulat. Hasil kajian menunjukkan kedua-dua jenis daun teh menghasilkan teh kombucha dengan sifat fitokimia yang diterima (13%-25%) dan sifat antimikrob yang cemerlang 2.71-7.52 mm kawasan larangan terhadap mikrob patogen. Sifat fizikokimia teh kombucha juga berubah secara beransur-ansur selama tempoh fermentasi, dan kepekatan sukrosa mempengaruhi sifat teh tersebut. Kesimpulannya, teh kombucha yang dihasilkan dengan daun teh yang berbeza dan kepekatan sukrosa mempengaruhi sifat-sifat sensory, komposisi nutrisi, sifat-sifat fitokimia dan antioksidan, dan aktiviti antimikrob. Teh kombucha dengan daun teh Pecah Beling mempunyai sifat sensory dan aktiviti antioksidan yang paling baik. Kepekatan sukrosa didapati dapat meningkatkan komposisi nutrisi dan sifat-sifat antimikrob. Kajian juga menjalankan penilaian jangka hayat produk dan mendapati bahawa teh kombucha yang mengandungi 80g sukrosa untuk setiap jenis teh mempunyai jangka hayat selama 6 bulan



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# LIST OF SYMBOLS AND ABBREVIATIONS

oC	<ul> <li>Degree celsius</li> </ul>
g	– Gram
μL	– Microliter
Kcal	– Kilocalorie
mL	– Milliliter
nm	– Nanometre
%	– Percentage
ABTS	– 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid
DPPH	– 2,2-diphenyl-1-picrylhydrazyl
AOAC	- Association of Official Analytical Chemists
ВТ	– Black tea
CFU	<ul> <li>Colony-forming unit</li> </ul>
GAE	– Gallic acid equivalents
GC-FID	- Gas Chromatography/Flame Ionization Detector
GC-MS	<ul> <li>Gas Chromatography-Mass Spectrometry</li> </ul>
MHA	– Mueller Hinton Agar
PBT	<ul> <li>Pecah Beling tea</li> </ul>
PHT	– Poly-herbal tea
RSDr	<ul> <li>Relative Standard Deviation for Reproducibility</li> </ul>
SDA	<ul> <li>Sabouraud Dextrose Agar</li> </ul>
TFC	- Total flavonoid content
TPC	- Total phenolic content
UV-Vis	– Ultraviolet-visible

#### LIST OF PUBLICATIONS

- Farid D. F., Abu Bakar M. F., (2022). Review about the benefit of Kombucha tea: Chemical component and Pharmacological activities. *Food Research.* 6, ICB2022-2938.
- Farid D. F., Abu Bakar M. F., Abdullah S. (2023) Comparative Analysis of Chemical properties, Nutritional, Phytochemical, and Antioxidant Properties of Kombucha Teas Black Tea, Pecah Beling Tea, and Poly-herbal Tea. *Letters in Applied NanoBioScience*. briac-2999.



## **CHAPTER 1**

#### **INTRODUCTION**

#### **1.1 Background of study**

Functional food is usually known as healthy food or natural food and although there is not a unique definition worldwide, foods can only be considered functional if, along with basic nutritional impact, they exhibit possible beneficial effects on specific functions in the human organism with the result of improving the physical condition and/or decreasing the risk of chronic diseases. The expansion of functional food industry can benefit to numerous factors including innovation in food science and technology, an aging population with growing health concern, an evolving regulatory environment allowing health claims on foods and increased marketing of functional food products which makes this industry has experienced rapid growth and development globally (Hasler, 2000). Among consumers, the awareness, and perceptions of older adults in relation to functional foods is of particular interest, as this population could greatly benefit from the incorporation of functional foods into their diets and due to that reason, the development of this kombucha tea will give benefits also opportunity towards the industry.

Tea product which undergoes the fermentation process is known as kombucha tea. This product is originally known in northeast China during Tsin Dynasty in Manchuria (Laureys *et al.*, 2020). Initially, kombucha tea was used as medicine due to its health-promoting properties, and studies are still being conducted to known its full potential full consumers health. Kombucha tea is a sweetened tea fermented in time by the presence of a symbiotic culture of bacteria and yeast (SCOBY), which creates a cellulose layer on top of the solution during the fermentation process (Laureys *et al.*, 2020). Kombucha beverage has been aggressively being known globally and show a



significant growth in its market throughout the years standing at USD 1.84 billion in 2019 and still growing and estimated reached a project growth of 23.2% by 2027 (Nyhan *et al.*, 2022). Kombucha tea usually consist of tea lea leaves, solute which is water, sucrose (5-20%) and SCOBY. Sucrose act as substrate for SCOBY which consist of acetic acid bacteria (AAB) and osmophilic yeast which trigger the natural fermentation process which lead to production of beneficial acid and alcohol (Gaggìa *et al.*, 2019). Tea is actually the second most known drank liquid after water and already been consumed since 3000 BC (Sharangi, 2009). The study about tea is still ongoing to show the optimum result for certain optimum point for potential health benefit and medicinal properties. The cultivation of tea in India in 1818 until 1834 can be considered as the origin of tea and it has been one of the most important agriculture product throughout the world (Harbowy *et al.*, 1997). All of the type of tea leaves such as green tea, black tea and oolong tea comes from the same species which is *Camellia sinensis L* but differ in their appearance, flavour and chemical component due to the exposure for fermentation and hydration process (Sharangi, 2009).



In the past 10 years, the market interest in kombucha tea has shown a positive increase towards the world and also, self-brewing for the production of kombucha tea has been quite common these days (Gaggia et al., 2019). Kombucha tea commonly being consumed due to the availability of beneficial chemical compounds and antioxidant value such organic acid, vitamins and tea polyphenols, and the low pH avoid bacterial contamination (Gaggia et al., 2019). Based on the quality of tea, the tea beverage usually based on species, season, age of tea leaves, position of plucking, climate, and horizontal practise which highly affected the composition of antioxidant and polyphenols which eventually affect the composition of fermented tea beverage (Lin et al., 1998). Kombucha can be claimed as one of probiotic product due to the present of beneficial microbe but also claimed as "unsafe medicinal tea" (Hartmann et al., 2000). There are many conceptions and misconception regarding the benefit of kombucha tea in term of chemical component due to very little clinical support and evidence. However, many studies have been done to improvise the production of kombucha tea focusing on the chemical component, microbial growth, alcohol production and even flavour. Only a few studies conducted regarding the insertion of other beneficial component such as commercialised juice product, fruits or foreign beneficial components to elevate the acceptance and the goodness of the product. In this work, it will assess regarding the benefit of kombucha tea with the insertion of black tea, Pecah Beling tea and Poly-herbal tea in term of physical properties, chemical components and antimicrobial properties.

#### **1.2 Problem statement**

Kombucha tea is well known as a fermented tea and contain probiotic microbes which contribute towards the beneficial components to the consumers. The acceptance of kombucha tea has been increasing in trend all over the world. However, the acceptance of this product is still considered as low in South East Asia and especially in Malaysia. This phenomenon may due to several reasons such as the lack of information about the product regarding quality, benefit, standard and efficacy of product (Batista *et al.*, 2022). Throughout the years, there are various research has been conducted with different formulation with additional processes to improve the quality of the product but only a few studies focusing on the acceptance of this product towards the consumer. As mentioned before, kombucha tea undergo fermentation process which reduce the pH value to acidic. Factors that play an important role in fermentation process. With different parameters for those factors creates different value of pH level thus different flavour that might affect the acceptance of customer.



The basic formulation for kombucha tea is sucrose, water, SCOBY and tea leaves which mostly black tea is the main choice. Nevertheless, the usage of black tea may result a pungent odour and strong vinegar-like flavour. This phenomenon largely affects the acceptancy of consumers towards the product. Moreover, there has been reports proving toxicity component is black kombucha if the fermentation process does not being controlled properly (Nyhan *et al.*, 2022). Some study has been conducted in overcoming this problem by inserting second fermentation process involving fruits or even combination of multiple tea leaves that might improve the palatability. A study done by Battikh, Bakhrouf and Ammar (2012) insert the infusion of multiple herbal component in kombucha tea formulation such as thyme, lemon verbena, rosemary and peppermint ((Battikh *et al.*, 2012). New formulation and additional of process does provide beneficial impact towards the product.

Hence, this new development of kombucha tea with Pecah Beling tea (Strobilanthes Crispus) and Poly-herbal tea (Strobilanthes Crispus and Orthosiphon

*Stamineus*) were focusing more on the first fermentation process involving a new herbal component which Pecah Beling tea and Poly-herbal tea act as an alternative for tea leaves.

## 1.3 Objectives of study

The objectives of the research are:

- i. To develop a kombucha tea using Pecah Beling tea (*Strobilanthes crispus*) and Poly-herbal tea (*Strobilanthes Crispus* and *Orthosiphon Stamineus*).
- ii. To analyse the phytochemical, physicochemical and antioxidant activity of each product
- iii. To analyse the antimicrobial and antifungal properties in each product
- iv. To determine the shelf-life duration of selected product from each type of kombucha tea based on physicochemical properties.

## 1.4 Scope of study

In order to achieve the objectives, scopes have been identified in this research. The scope of the research as stated as below:

- i. The study focuses on the formulation of kombucha tea using black tea (*Camellia sinensis*), Pecah Beling tea (*Strobilanthes Crispus*) and Poly-herbal tea (*Strobilanthes Crispus and Orthosiphon Stamineus*) involving 3 different sucrose concentration in each type of kombucha teas.
- ii. Sensory analysis was conducted involving 50 untrained panellists to analyse the preferability of consumers towards the new formulation with three different sucrose concentration.
- iii. Analysis of physicochemical were focused on pH analysis, alcohol analysis, sugar brix analysis and nutritional analysis (according to Malaysian food nutritional labelling)
- Phytochemical analysis was included total phenolic compound and total flavonoid content using Folin-Ciocaltue reagent and Aluminium chloride colorimetric

- v. Antioxidant assessment were conducted using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and ,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical assay.
- vi. Antimicrobial analysis was analysed using agar well diffusion method involving gram negative bacteria (*Escherichia coli*), gram positive bacteria (*Bacillus cereus*) and fungi (*Candida albicans*).
- vii. Shelf-life assessment was conducted on selected kombucha tea from each type to undergo accelerated shelf-life assessment.

### 1.5 Significance of study

Through earlier studies, it has proven that tea has the ability to act as an anti-cancer agent in various models of lung, pancreas, liver, breast, fore-stomach, oesophagus, duodenum, colon, and skin cancer. It also reported beneficial to reduce the risk of high blood pressure and cholesterol, atherosclerosis and coronary heart disease, and metabolism of xenobiotic compound (Sigurdsson H., 2000). Another beverage known as kombucha also uses tea and sugar as their main ingredient and a symbiotic culture of bacteria and fungi (SCOBY) and this product believe to be existed many years ago which may believe elevate the beneficial composition of tea. The composition and properties of tea are well documented, but scarce scientific information is available concerning the composition and the effects of Kombucha on health (Dufresne and Farnworth, 2000). Despite its long tradition of worldwide use as a health-supporting tea, kombucha is continuously being investigated for its intrinsic properties and many findings of common opinion are confirmed (St-Pierre, 2019). Due to this research, a new foundation may be discovered about the health benefit in Kombucha tea using multiple type of herbal tea and may consist synergistic effect towards the rate of consumption of kombucha beverages in Malaysia.

An increase in the price of pharmaceutical products may affect the population in general due to the fact that health is changing and technological development is exponential. The price of medicine would be the main concern in the factor of availability and affordability for low- and middle- income countries. From this phenomenon, people are starting to search for other options which are cheaper and easier to get to maintain their health. The availability of this studies give more



supporting information about the benefit of alternative products such as kombucha tea which has been claimed to contain beneficial properties.

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## **CHAPTER 2**

#### LITERATURE REVIEW

#### 2.1 Functional food

Functional food was first defined in the 1980s by the Ministry of Health and Welfare of Japan as foods that contain health benefits beyond nutritional effects to promote health conditions and reduce the risk of chronic disease. Functional food has been well known globally and already being a part of people's daily diet (Konstantinidi and Koutelidakis, 2019). Functional food is usually known as healthy or natural food with a basic nutritional impact which exhibits a beneficial effect on specific human functions, resulting in the risk of chronic diseases. Some food claimed to be functional food due to the presence of bioactive compound in the food. Epidemiological data supports that high intake of natural functional food can be associated with decreased risk of chronic disease such as cardiovascular diseases, cancer, metabolic syndrome, diabetes type II, and obesity (Karasawa and Mohan, 2018). There is a lot of studies have been conducted regarding natural bioactive compound presence in food such as resveratrol, epigallocatechin, curcumin, oleuropein, sulforaphane, quercetin, ellagic acid, anthocyanins, b-glucans, and other biomolecules which associated with the pathophysiology of cardiovascular diseases, diabetes, metabolic syndrome, and cancer which give a positive result for the people to consume in their daily diet.

The evolution of the functional food industry contributes benefits towards various factors including the progress in food science and technology, affecting additional claims and research information for food product, increasing life rate of human population which leads to expansion in marketing of functional food products (Hasler, 2000). Awareness particularly towards functional food product could benefit the consumers especially for older adult and could be inserted into their diet with the



appropriate dose of consumption. Due to that reason, the development of this kombucha tea will also include as functional food product which is competent in improving the health of the consumer and an opportunity towards the industry.

#### 2.2 Probiotic

Probiotic is used as denotation for "life" for bacterial association due to its beneficial effect on human and animal health (Kechagia *et al.*, 2013). But as knowledge and interest about the use of viable bacterial supplements have grown over time, along with advances in our comprehension of their mechanisms of action, the definition has also changed. This theory has its roots in Tissier's observation that the gut microbiota of healthy breastfed infants was dominated by rods with a bifid shape (bifidobacteria), whereas they were absent from formula-fed infants who had diarrhoea, suggesting that they played a role in preserving health. This observation was made more than a century ago (Kechagia *et al.*, 2013). todayly the definition officially specified as "probiotics are live microbial feed supplements which beneficially affect the host animal by improving microbial balance" and the definition was given approval by the Food and Agriculture Organization of the United Nations World Health Organization (Fuller, 1989).



Some of the most important representative microbial species which can be claimed as probiotic is Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus crispatus, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus, Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium breve, *Bifidobacterium* infantis, Bifidobacterium lactis, and Bifidobacterium longum (Holzapfel et al., 2018). Genera Lactococcus and Bifidobacterium also known as lactic acid bacteria due to their important properties which provide actual benefit towards the consumer. Lactic acid bacteria are Gram-positive bacteria which able to produce lactic acid as the end product from the fermentation of carbohydrate. This principle helps in most of the fermentation process which involve in making food and alcohol in the early ages. Figure 2.1 and 2.2 below shows an example for Lactobacillus sp and Bifidobacterial sp.



Figure 2.1: An example of Lan example of Lactobacillus sp. *actobacillus sp.* ((Holzapfel *et al.*, 2018)



Figure 2.2: An example of Bifidobacterial sp. ((Holzapfel et al., 2018)

Nevertheless, in the collaboration of probiotic in food there always have something to concerned about such as the probiotic dose which will be cultured into the formulation. The dose for probiotic involvement is vital and should be in the range of which will be proven effective to the consumer per gram. According to Food Regulation 1985, the probiotic cultures added must remain viable (live) and the viable count shall not be less than 106 cfu/ml or cfu/g of food during the shelf life of the food or beverage (George *et al.*, 1985).

## 2.3 Food fermentation

In order to preserve and change food materials, the process of fermentation starts the development and metabolic activity of microbes. The metabolites produced by the fermenting organisms during food fermentation limit the growth of spoilage and pathogenic organisms, increasing the shelf life of perishable goods. For instance, during the fermentation of lactic acid, lactic acid bacteria produce metabolites namely lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, bacteriocins, and antimicrobial peptides that work in concert to inhibit the survival and growth of pathogenic and spoilage microorganisms. (Di Cagno *et al.*, 2013). Even though this method involve microbe which may bring a bad preference to some people, fermentation process also give impact towards the characteristic of the food such as aroma, flavour, texture and nutritional profile (Terefe, 2016). Tempeh, natto and soy sauce are some of the example of fermented food that prove that fermentation can act as a antitoxin agent by removing antinutritional components and toxin namely phytic acid and trypsin inhibitors (Martin, 1974).

Traditional food fermentation can be classified in three different group which is lactic acid fermentation, fungal fermentation and alkaline fermentation. Lactic acid fermentation is one of most common process in producing fermented food involving lactic acid produced by lactic acid bacteria in yogurt, sausage, cheese, sauerkraut and kimchi. Fungal fermentation involves yeast species that cooperate with bacteria in producing organic acid that is beneficial to the consumers including kefir and kombucha. Most of the well-known soy-based fermented foods from Asia such as tempeh and soy sauce are produced by fungal fermentation, except natto, which is produced by alkaline fermentation (Terefe, 2016). For kombucha tea, it involves mixed process fermentation which is fungal fermentation converting glucose to alcohol by the present of yeast followed by bacterial fermentation which convert alcohol to lactic acid.

Fermentation is a process of breaking down sugar in alcohol or organic acid without the presence of oxygen in a favourable condition. This process catalysed by enzyme present in the microbe and the process is energy-generating in which organic compounds act as both donors and final acceptors of electrons. The biochemical pathways involve in this process is glycolysis with some additional reactions included



at the end. Figure 2.3 illustrate the pathway for alcohol fermentation and lactic acid fermentation process.

Figure 2.3: The flow for fermentation process (Some and Mandal, 2020).



Alcoholic fermentation is permitted by two steps which is the pyruvate is converted to two-carbon compound acetaldehyde through decarboxylation reaction followed by reduction to form ethanol. In the reaction, one molecule of glucose is converted into two molecules of carbon dioxide and ethanol each. Lactic acid fermentation is classified into two types which is homolactic and heterolactic. (Wang *et al.*, 2021). Homolactic fermentation is a process conversion of glucose into pyruvate and into lactic acid by the enzyme lactate dehydrogenase (Pyruvate reductase) while heterolactic fermentation also produce lactic acid as by product with an addition of carbon dioxide and ethanol (Eiteman and Ramalingam, 2015; Wang *et al.*, 2021).

### 2.4 Kombucha

Kombucha is the name of the beverage obtained from the fermentation of tea, mainly black tea and there are also other varieties that can be used as a base for its preparation, such as green and oolong tea, also known as blue tea, with added sugar as a substrate for fermentation. It is also possible to find variations which can be infused into the formulation such as mint, lemon balm or jasmine. Some metabolic products of Symbiotic Culture of Bacteria and Yeast (SCOBY), like acetic acid and other organic acids, possess antibacterial activity and prevents contamination of the drink by pathogenic bacteria (Ruiz et al., 2021). It is said that kombucha possibly made by accident like many other fermented foods. Kombucha also can be claimed as functional food due to its purported health benefit which include multiple functional properties such as anti-inflammatory potential and antioxidant activity, reduction of cholesterol level and blood pressure, reduction of cancer propagation, the improvement of liver, the improvement of immune system and gastrointestinal function (Kapp and Sumner, 2019). But kombucha is not yet universal and well known throughout the world because it is a live fermenting drink, required constant precaution and refrigeration making it more difficult to transport and distribute. Figure 2.4 shows UN AMINAT an example of kombucha tea during fermentation process.



Figure 2.4: An example of kombucha tea during fermentation process

Kombucha is prepared through fermentation which involve aerobic and static fermentation involving sucrose acting as substrate in black, green or blue (Oolong) with addition of symbiotic culture of bacteria and yeast (SCOBY) (Leal *et al.*, 2018).

The preparation usually starts with the dissolution of 50-100g of sucrose per liter of boiling water. Subsequently, tea leaves, either loose or within tea bags, are steeped for a short period, after which they are removed. Thereafter, the sweetened tea infusion is cooled to 20 °C and inoculated with a part of the SCOBY mat, which contain large amounts of indigenous yeast and bacteria. The addition of previously fermented tea lowers the starting pH, which may inhibit the growth of human pathogens such as *Clostridium perfringens, Bacillus cereus*, and *Clostridium botulinum*, as these microorganisms do not grow in an environment below pH 4.7. the fermentation process usually performed under aerobic condition and ambient temperature between 18-28°C for a period of 8-14 days (Pierre, 2019).

Fermentation process in the making of kombucha can be consider natural due to the addition of certain amount of a previous kombucha fermentation acting as the starter culture of the formulation. This practise (back slopping) also being used in other process in producing other food fermented products (Laureys and De Vuyst, 2014). The properties of sucrose play its role in changing the properties of kombucha tea through fermentation process which lasted for 8-14 days. In the fermentation process, the medium is aerobic which is high in sugar and sucrose and slightly acidic due to the addition of certain amount of a previous kombucha fermentation mixture. Due to the cellulose layer that forms on top of the fermenting beverage and the oxygen that the microorganisms in the SCOBY mat and the liquor use, the supply of oxygen of the beverages gradually diminishes. Due to microbial metabolism and cellulose layer formation, the substrate concentrations steadily decrease. A more anaerobic environment with low substrate concentrations and a high acidity may emerge at the conclusion of the fermentation process, which is characterised by a well-developed cellulose pellicle on top of the beverage and high quantities of yeasts and acetic acid bacteria.

#### 2.5 Fermentation in kombucha tea

The extensive study about the microbial diversity in kombucha fermentation process has been established and it shows that during fermentation process of kombucha tea yeast and acetic acid bacteria (AAB) are both present. The main component in the production of kombucha tea is tea leaves, water, sugar which act as a substrate and SCOBY as mention in the previous chapter. Sweetened black tea are one of the best

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VITA

Dzulkarnain Farhan bin Md Farid, born on September 11, 1997, in Kluang, Johor, is a driven individual with a passion for food technology and science. He commenced his academic journey at SK Senai Utama in Senai Utama, Johor, for primary school and later attended SMK Bandar Putra in Kulai for secondary school. Eager to expand his knowledge, he pursued his A-levels in Matriculation at Johor Matriculation College. Subsequently, he enrolled in Universiti Tun Hussein Onn Malaysia, where he earned his Bachelor's degree in Applied Science (Food Technology). During this time, he made significant contributions as the president of the Foodintech 4.0 program, showcasing his leadership abilities. Dzulkarnain Farhan's dedication to his studies and research also led him to participate in two exhibitions, where he received recognition for his outstanding work, securing a Gold award in the 2nd FAST Postgraduate Virtual Symposium 2020 and a Bronze award in the International Research and Symposium and Exposition (RISE2020). In pursuit of continuous growth, Dzulkarnain Farhan expanded his expertise, acquiring certificates in Food Handling, Food Quality Assurance, and Halal certification. His thirst for knowledge led him to pursue a Master's degree in Applied Science (Food Science) at Universiti Tun Hussein Onn Malaysia. Throughout his master's journey, he showcased his research prowess by competing in multiple exhibitions, garnering numerous accolades, including Gold awards in prestigious events such as the International Conference and Exposition on Inventions by Institutions of Higher Learning (PECIPTA2021), The National Innovation and Invention Competition (NIICE2022), Virtual Research and Innovation Exhibition (EREKA2022), and The National Innovation and Invention Competition (NIICE2021), as well as in the International Research and Innovation Symposium and Exposition (RISE2021). Through his remarkable journey, Dzulkarnain Farhan has demonstrated unwavering determination and a commitment to excellence in the field of food technology and science.

