UNIVERSITI PUTRA MALAYSIA

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF ENGINEERING (PROCESS AND FOOD)

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ABSTRACT

Kombucha tea is a fermented healthy beverage usually compose of black tea and sugar with symbiotic culture of bacteria and yeast (SCOBY). This work is focused on effect of temperature, pH value and alcohol content on the food safety of Kombucha tea to lengthen the storage period while maintaining its quality. The current study reveals that the storage temperature and preservative material used were greatly influences the pH value, alcohol content and biofilm formation of tea fungus on the Kombucha tea for a period during storage. Results showed there were no significant differences ($P<0.05$) when compared the trend of pH value between Kombucha tea without preservative with the Kombucha tea added with various type of preservative when all the samples were stored in chiller. The most ideal sample obtained when Kombucha tea sample added with pasteurized apple juice and stored in refrigerator temperature (<4°C) with safe consumption pH value (pH 3.28) and acceptable alcohol content (0.279±0.008 %w/v) after 28 days of storage. Kombucha tea should be stored in cold temperature (<4° C) to stabilize the pH, prevent high generation of alcohol and avoid formation of SCOBY. Kombucha tea manufacturing factory should be equipped with cold storage room to store the Kombucha tea product before distribution. A proposal was done for plant design of Kombucha tea manufacturing factory to provide guidelines and references for manufacturers of this industry.

ABSTRAK

Teh Kombucha adalah minuman sihat fermentasi yang biasanya dibuat daripada teh hitam, gula bersama dengan kultur simbiotik bakteria dan ragi (SCOBY). Karya ini menfokuskan pengaruh suhu, nilai pH dan kandungan alkohol terhadap keselamatan makanan teh Kombucha untuk memanjangkan tempoh penyimpanan sambil mengekalkan kualitinya. Kajian semasa menunjukkan bahawa suhu penyimpanan dan bahan pengawet yang digunakan telah mempengaruhi nilai pH, kandungan alkohol dan pembentukan biofilm jamur teh pada teh Kombucha untuk jangka waktu sepanjang masa penyimpanan. Hasil kajian menunjukkan tidak ada perbezaan yang signifikan ($P<0,05$) jika membandingkan trend nilai pH antara teh Kombucha tanpa bahan pengawet dengan teh Kombucha yang ditambahkan dengan berbagai jenis pengawet ketika semua sampel disimpan dalam peti sejuk. Hasil kajian juga menunjukkan sampel teh Kombucha yang ditambahkan dengan jus epal yang dipasteurisasi dan disimpan dalam suhu peti sejuk (<4°C) adalah sample yang paling baik dengan nilai pH yang selamat (pH 3.28) dan kandungan alkohol yang selamat (0.279±0,008% w/v) setelah menyimpanan selama 28 hari. Teh Kombucha harus disimpan pada suhu yang sejuk (<4° C) untuk menstabilkan nilai pH, mencegah penghasilan alkohol yang tinggi dan mengelakkan pembentukan SCOBY. Kilang pembuatan teh Kombucha harus dilengkapi dengan ruang simpanan sejuk untuk menyimpan produk teh Kombucha sebelum diedarkan. Satu cadangan ataupun pelan reka bentuk kilang pembuatan teh Kombucha telah diselesaikan untuk memberikan garis panduan dan rujukan kepada para pengeluar yang terlibat dalam industri ini.

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LIST OF ABBREVIATIONS

SCOBY - Symbiotic culture of bacteria and yeast

pH - Potential Hydrogen

SOP - Standard Operation Procedures

AAB - Acetobacter

HPLC - High Performance Liquid Chromatography

LAB - Latic Acid Bacteria

GC-FID - Gas Chromatography with Flame Ionization Detection

GMP - Good Manufacturing Practice

CHAPTER 1

INTRODUCTION

1.1 Background

Kombucha is a healthy tea fermentation drink which incorporates a symbiotic culture of bacteria and yeast (SCOBY) into a blend of green or black tea, sugar and other flavors (Ismaiel, 2015; Jayabalan et al, 2014). Several research and data have been portrayed that Kombucha has several health benefits particularly with its probiotic feature. Kombucha contains several species of bacteria with lactic acids that may have probiotic function (Marsh et al, 2013). Probiotics include a good bacterial to the human intestine that helps with digestion, inflammation and also helps reduce body weight. A rat analysis reveals that Kombucha is abundant in antioxidants and drinking Kombucha will routinely decrease the toxicity of the liver induced by harmful chemicals by at least 70 per cent (Murugesan et al, 2009). Kombucha is rich in tea polyphenols and acetic acid and has strong antibacterial properties , particularly against infectious bacteria that suppress the growth of unwanted bacteria and yeasts while not affecting beneficial, probiotic bacteria and yeasts involved in Kombucha fermentation (Houda Battikh, 2012). Additionally, a study in diabetic rats found that Kombucha helps manage type 2 diabetes by slowing down carbohydrate digestion and reducing blood sugar levels (Aloulou A, 2012). All these characteristics show that the Kombucha tea has the market appeal particularly in providing health benefits to humans. Hence, Kombucha tea should be promoted to the public and informed about the value of Kombucha tea. This research offers an incentive for the public to pre-introduce Kombucha tea via the Kombucha tea sampling and tastes studies.

It is important to note that Kombucha tea is only safe when correctly processed and preserved because the polluted or over-fermented Kombucha will trigger severe health and even death problems. Over-fermented Kombucha can also produce large levels of alcohol triggering signs or side effects such as allergic reaction, jaundice, diarrhea, vomiting, headache and neck pain (Srinivasan R, 1997). This research is therefore being performed to evaluate the composition in the manufacture of healthy Kombucha tea and to control the alcohol content of Kombucha during storage.

Kombucha tea can be produced by mixing the black tea, sugar and SCOBY followed by storing at room temperature (25°C) for 7 to 14 days of fermentation. The bacterium *Gluconacetobacter.xylinus* will transform the alcohols produced by the yeast into various types of acids such as acetic acid during the fermentation period (Jayabalan, R. et al. , 2008). Generation of such acids decreases the pH of the mixture before achieving the optimal degree of taste and acidity. Initially, this method may tend to be highly dangerous in terms of food health due to the decrease in pH in the range of pH 6.7- pH 4.7 which is the ideal optimal environment for bacterial development. However, if continuing fermentation, the pH that drops below 4.6 and is considered non-potentially hazardous because harmful bacteria would not be able to thrive or proliferate under such extreme acidic environment (BCCDC., 2015).

Kombucha has been a part of the food of many Malaysians for decades. However, the drinking Kombucha tea tradition, died out two decades back, and has been overlooked by many people. Kombucha tea is totally fresh to most youngsters. It is therefore important to implement and inform them about it and carry back to fashion Kombucha tea. Most of

the Kombucha available in Malaysia is imported from other nations, from Australia and America in particular. The Kombucha is normally pasteurized because the shipping of Kombucha took a long period of time. Pasteurization can cause the probiotic properties of the Kombucha tea to lose. The price of one bottle of Kombucha tea is high because of the shipping fee and other taxable costs. Perhaps, problem can be solved or reduced by the Malaysian-based Kombucha manufacturing factory. Kombucha tea is also believed to have the potential to expand and develop in Malaysia's market since there are fewer competitors or manufacturers producing this kind of beverage in this country. In other to produce large-scale Kombucha tea, there are many features that affect the quality of Kombucha tea to consider. This research is therefore being undertaken to provide recommendations and details on the risks involved with the Kombucha preparation. Since the Kombucha tea has a strong fermented acid smell, fruit juice is added to the Kombucha tea to enhance the taste and attract consumers or customers to purchase the Kombucha tea product. Thus, this research also conducts the analysis on the impact of pH on Kombucha tea, coupled with fruit juice.

Kombucha tea's pH is the key function for signaling Kombucha tea health. Therefore, in this analysis, the pH levels of Kombucha tea will be checked regularly during the brewing process to assess its safety in health. The influence of temperature on Kombucha tea can be measured and contrasted by measuring Kombucha's pH at both room temperature and cooler temperature during the storage process. To expand the study reach, the Kombucha tea that is stored at room temperature would be applied with preservative to decide the correct form and percentage of preservative needed to retain Kombucha tea if it is required to be preserved at room temperature.

1.2 Problem Statement

Ensuring the storage and safety consumption of Kombucha tea is not an easy task and have not satisfactorily addressed. The method proposed in this project follow several techniques for reducing several problems related to safety consumption of Kombucha tea. Follows are the list of problems that intend to tackle:

1. Kombucha tea dealing with a live and acidic probiotic culture. It should not involve any bad bacterial such as molds in the experiment.
2. The bad bacterial will affect the quality and efficiency of beneficial components produced by the Kombucha tea.
3. The problems of difficulty in transforming the homemade Kombucha tea into big industrial scale due to its problems of formation of high amount of bubbles during early fermentation, undefined tea to SCOBY ratio for preparation and difficulty in removing the big heavy SCOBY in fermentation tank after completed the fermentation.

1.3 Objectives

This research is proposed to:

1. To investigate the effect of preservative on pH value and alcohol content of Kombucha tea stored at both room temperature and refrigeration temperatures after the brewing process to determine its safety regarding health.

2. To study the effect of flavoring on pH value and alcohol content of Kombucha tea stored at both room and refrigeration temperatures by comparing the original Kombucha tea with Kombucha tea added unpasteurized fruit juice and Kombucha tea added pasteurized fruit juice.

3. To propose a plant design for manufacturing of Kombucha tea.

1.4 Scopes of Study

There are three major research scopes were carried out in order to achieve the objectives:

1. Standard Operating Procedures (SOP) of preparation of Kombucha tea

SOP of preparation of Kombucha tea include mixing of raw materials and fermentation until reaching the desired pH value. Kombucha's optimal pH fell in between pH 3.5-2.5. A group of Brazilian researchers reported that the antimicrobial activities of Kombucha worked against E.coli, Salmonellatyphi and M.canis and were considered to be the most effective after 28 days of fermentation (Rodrigo et.al, n.d). Their research also revealed the cyclical nature of the symbiosis of Kombucha tea. After 14 days of steady

pH decrease, the operation changed and the pH slowly increased and peaked again at 21 days, then moved again lower at 28 days as seen in figure 1.1.

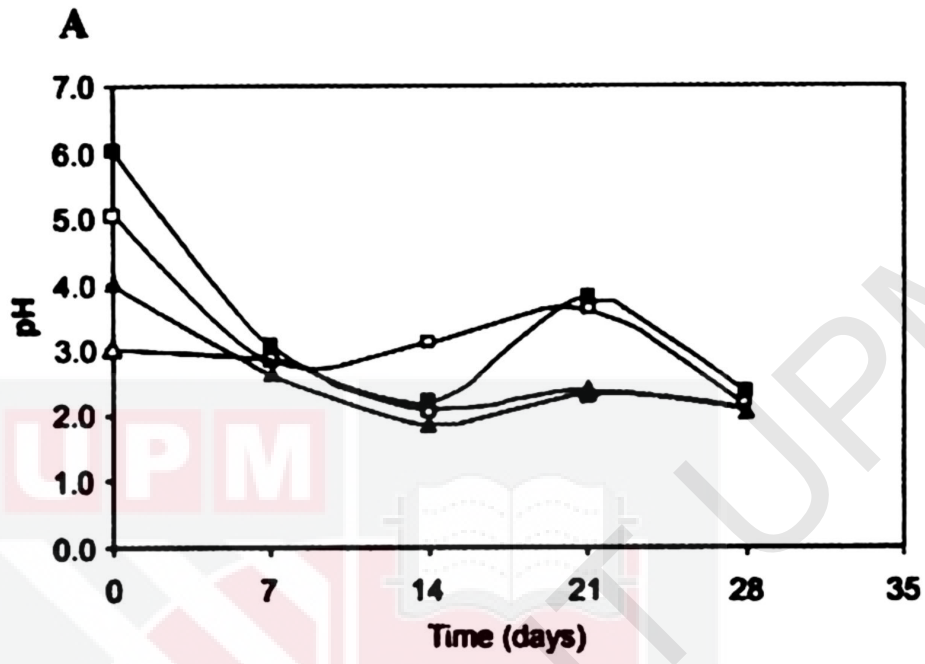


Figure 1.1: The graph of change of pH against time duration.

Temperature is essential control function because it can impact three specific aspects like fermentation duration, bacteria-yeast ratio and Kombucha tea taste. The optimal temperature for brewing Kombucha is between 24° C and 30° C. This temperature condition is just perfect for brewing of Kombucha tea with balanced yeast and bacterial populations as well as for the bacteria generating good organic acid levels. Chen and Liu (2000) reported that the concentration of ethanol in Kombucha rises with fermentation time, reaching an estimated maximum of 5.5 g/L on the 20th day of fermentation, followed by a gradual decrease. This is the reason make fermentation process is highly precise. The brew must stay within a range of temperature and should be fermented for a given time frame only. Long fermentation causes the flavor of Kombucha tea to become too vinegary, fizzy and contain too much alcohol to be legally marketed as a non-alcoholic drink. Conversely, if the Kombucha tea undergo fermentation for short duration, the beneficial bacteria may not develop well and the Kombucha may be less fizzy with high sweet taste.

Basically, Kombucha is a naturally fermented drink which can contain small amounts of alcohol, but never more than 0.5% if the handling and preparation follow Standard Operating Procedure (SOP). In Kombucha tea, the amount of alcohol should be small and negligible that it would have no effect on the person consuming it. Given the risk of manufacturing Kombucha tea with an alcohol level of more than 0.5 percent, it is necessary to carry out alcohol testing to ensure that the alcohol content do not exceed the limit and is safe to be consumed by all range of consumers.

2. Storage of Kombucha tea

In this study, the investigation of the effect of temperature on Kombucha tea will be carried out by storing the fermented Kombucha tea in both room temperature and refrigerator to observe the changes in pH, appearances, smell as well as the turbidity of the Kombucha tea. The pH of Kombucha tea is measured to see the pattern or how stable the tea is in regarding to the safety. Logically, the Kombucha tea in room temperature is not safe to be consumed due to the unstable pH condition arising from continuous fermentation. For expand the field of research relevant to health issues about the storage of Kombucha tea, tea stores in the room temperature is added certain preservative to achieve storage and healthy consumption requirements.

3. Production of Kombucha tea on industrial scale

Kombucha tea is usually homemade and prepare in small scale. It is simple to prepare homemade Kombucha tea as all the parameters that will affect the quality of Kombucha tea such as the amount of tea, sugar or SCOBY size can be easily controlled or monitored. However, there will be a lot of issues encounter when the Kombucha tea is produce in huge amount in the size of factory manufacturing scale. For example, longer

fermentation time will be required when the tea is fermented in a large tank. Besides, there might be extreme bubbles formation on the top layer of SCOBY due to the production of carbon dioxide during early stage of fermentation. Therefore, planning and plant design for production of Kombucha tea must be undertaken to build a viable Kombucha tea business. The plant design must include the process flow diagram, type of equipment as well as the plant layout for production of Kombucha tea. The cleaning method and plant hygienic issues should also be included in the plant design of Kombucha tea.

CHAPTER 2

LITERATURE REVIEW

2.1 Preparation of Kombucha Tea

Kombucha is one of the famous fermented beverages produced from tea, sugar and a starter culture named SCOBY. SCOBY is a sheet of yellowish membrane often referred to as ("mushroom") with a bacteria and yeast symbiotic population. This SCOBY is also an element which is used in Kombucha fermentation and processing. It is necessary to create a powerful symbiosis for the bacteria and yeasts present in the medium that can inhibit the growth of contaminating microorganisms (Vitas et al, 2013). The Kombucha consists of two phases: a floating biofilm (Figure 2.1) and a sour fluid phase (Figure 2.2).

Figure 2.1: SCOBY, a biofilm.

Figure 2.2: Kombucha tea.

Acetic acid, gluconic acid, and ethanol are the principal component in Kombucha. These components are also present in the SCOBY biofilm due to its great capacity to absorb water (Czaja et al, 2006). Over a span of 7 to 10 days, The symbiotic consortium of Kombucha is able to transform sugar and tea into a slightly carbonated, fizzy, sour and calming drink consisting of several acids, 14 amino acids, vitamins and other hydrolytic enzymes under aerobic conditions with optimal atmospheric temperature between 24°C to 26°C (Malbaˇsa et al, 2011). The typical process flow diagram of Kombucha tea is shown in figure 2.3.

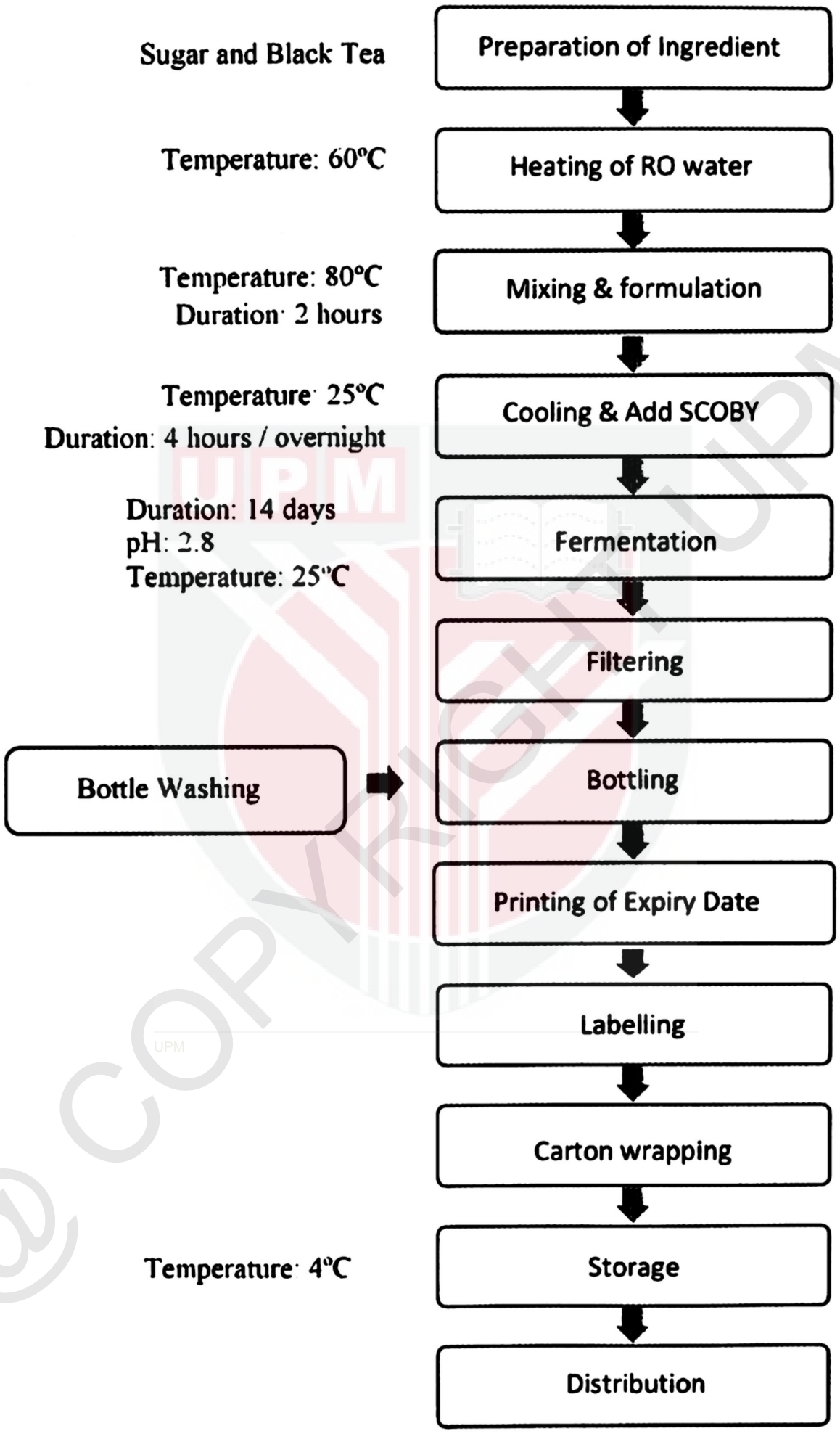


Figure 2.3: Process flow diagram of Kombucha Tea

2.1.1 Chemical composition

(Roussin, 1996) Fructose, acetic acid, and gluconic acid were the primary constituents of the tested ferments with fructose often the predominant component in Kombucha tea, using high-performance liquid chromatography (HPLC test) and mass spectrometry identification. This research also has shown that gluconic and acetic acid concentration in Kombucha differ, but generally considered to be occur in same quantities in the fermented product. There are also other ingredients commonly contained in the Roussin-tested Kombucha samples, generally in minimal quantities less than 1 g / liter, (Blanc, 1996) Specified Kombucha colony metabolites were formulated with various amounts of sucrose and observed that the number of metabolites present in the finished drink was closely linked to the volume of sugar used; lactic acid never exceeded amounts above 0.6 g / liter, and glucuronic acid was below 10 mg/liter. The metabolic process isn't always the same in Kombucha tea and this causes the final amount of sugar to vary from one fermentation (Chen and Liu, 2000). The general composition of Kombucha as seen in table 2.1.

Table 2.1: General chemical composition of Kombucha.

	Compound	Average composition	Initial sucrose	Fermentation time (days)	References
Organic acids	Acetic acid	5.6 g/L	70 g/L	15 d	Blanc (1996)
	Acetic acid	8.36 g/L	100 g/L	18 d	Jayabalan et al. (2007)
	Acetic acid	11 g/L	100 g/L	30 d	Chen and Liu (2000)
	Gluconic acid	39 g/L	100 g/L	60 d	Chen and Liu (2000)
	Glucuronic acid	0.0160 g/L	70 g/L	21 d	Lončar et al. (2006)
	Lactic acid	0.18 g/L	100 g/L	18 d	Jayabalan et al. (2007)
Vitamins	Vitamin B1	0.74 mg/mL	70 g/L	15 d	Bauer-Petrovska and Petrushevska-Tozi (2000)
	Vitamin B2	8 mg/100 mL	70 g/L	10 d	
	Vitamin B6	0.52 mg/mL	70 g/L	15 d	Malbaša et al. (2011)
	Vitamin B12	0.84 mg/mL	70 g/L	15 d	Bauer-Petrovska and Petrushevska-Tozi (2000)
	Vitamin C	25 mg/L	70 g/L	10 d	Bauer-Petrovska and Petrushevska-Tozi (2000) Malbaša et al. (2011)
General composites	Ethanol	5.5 g/L	100 g/L	20 d	Chen and Liu (2000)
	Proteins	3 mg/mL	100 g/L	12 d	Jayabalan et al. (2007)
	Tea polyphenols	7.8 Mm GAE	100 g/L	15 d	Chu and Chen (2006)
Minerals	Cu, Fe, Mn, Ni, Zn	0.1 to 0.4 μg/mL	70 g/L	15 d	Bauer-Petrovska and Petrushevska-Tozi (2000)
Anions	F^-, Cl^-, Br^-, I^-, NO_3^-, HPO_4^- SO_4^-	0.04 to 3.20 mg/g	100 g/L	7 d	Kumar, Narayan, and Hasanrajam (2008)

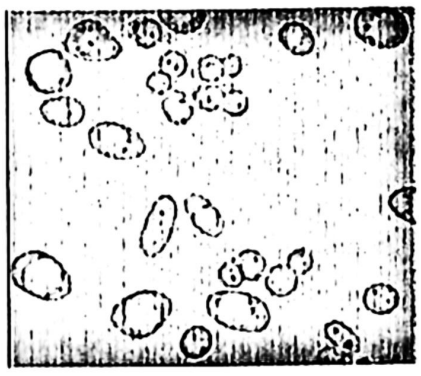
After 15 days, raising production of acetic acid output by fermentation would hit a maximum concentration of 9.5 g / L (Jayabalan et al, 2007). It reached a maximum concentration of 2.3 g / L on the 12th day for D-glucuronic acid generation and was detected in lesser quantity of 0.54 g / L of lactic acid on the 3rd day. In addition, as regards the anionic concentration, it persists at low values varying from 0.04 to 3.20 mg / g and it is observed that F− and Cl− are the most popular anions in Kombucha tea (Watawana et al, 2015). Also, the chemical composition and concentration of each metabolite produced in Kombucha will depend on the inoculum, initial concentration of sugar, and others.

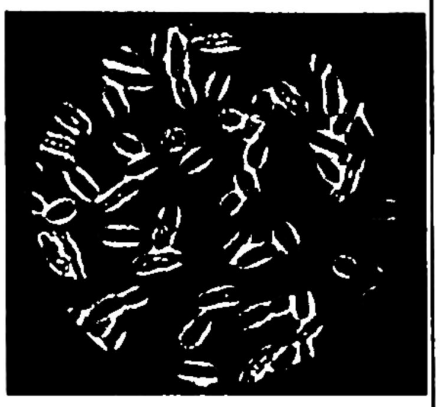
2.1.2 Yeasts

As described earlier, yeast is one of the most essential elements in Kombucha tea fermentation. Basically, most species of yeasts can ferment sugar to ethanol but this process takes time. Thus, many modern processes of alcoholic fermentation are initiated

by a single starting culture, usually by Saccharomyces cerevisiae because of its high fermentation performance. However in recent years, non-Saccharomyces yeasts have substituted the use of Saccharomyces yeasts in the industry for combined fermentations such as wine, tequila and others to enhance and enrich the aromatic profile, and to increase the complexity and kinetics of the final product (Lopez et al, 2014). Besides, the genus and species of yeasts in Kombucha culture are numerous.A wide range of yeast genus and species has been reported in the Kombucha culture including *Zygosaccharomyces, Candida, Kloeckera/Hanseniaspora, Torulaspora, Pichia, Brettanomyces/Dekkera, Saccharomyces, Lachancea, Saccharomycoides, Schizosaccharomyces,* and *Kluyveromyces* (Chakravorty et al, 2016). Furthermore, a research was undertaken on the identification of yeasts present in the Kombucha community (Watawana et al, 2015). Zygosaccharomyces are recorded as the prevalent yeast with the highest relative abundance percentage with 84.1% accompanied by Dekkera and Pichia species with 6% and 5% respectively.

Table 2.2: Some yeasts species present in Kombucha culture.

Species	Morphology	Characteristics
Zygosaccharomyces		•Highly osmo and halo-tolerant •Counteract better sugar and salt stress than *S. cerevisiae* (Dakal et al., 2014)

Brettanomyces		•High resistance to osmotic and ethanol stress. •Tendency to ferment sugars to ethanol and to develop high acetic acid concentrations under aerobic conditions (Steensels et al., 2015) produces large amounts of acetic acid.
Pichia		•Produces alcohol oxidase •More resistant to breaking efforts compared to bacteria.

2.1.3 Bacteria

Bacteria contribute to Kombucha tea in which Acetobacter (AAB) are dominant bacteria in Kombucha tea culture. AAB are aerobic bacteria which transform alcohol to acetic acid. The growth and activity of bacteria required large amount of oxygen. The metabolic cycle of bacteria relies on the conversion of acetaldehyde into ethanol and acetaldehyde hydrate into acetic acid by the enzyme acetaldehyde dehydrogenase. Several AAB are present in the tea fungus, including: *Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti, Acetobacter pasteurianus, and Gluconobacter oxydans* (Jayabalan et al, 2014).

2.1.4 Microorganisms Interactions

Interactions between the microorganisms during the Kombucha tea fermentation are known to have inhibitory effects on production of ethanol. Nonetheless, this is not an issue because the death and autolysis of yeast cells will release vitamins and other nutrients frequently that promote vital bacterial growth. Most microbial species create metabolic products which can either stimulate or inhibit the specific growth rate of the other species, producing social interactions that need to be analyzed extensively in order to understand this coexistence phenomenon. Several classes of bacteria like Latic Acid Bacteria (LAB) and AAB, as well as species of yeasts like Saccharomyces cerevisiae, have well-established functions in fermentation. Similar kinds of yeasts and types of bacteria in Kombucha act in parallel to create two separate final goods which are the fermented tea and the biofilm. In first stage of fermentation, yeast hydrolyzes sucrose into glucose and fructose, followed by ethanol development and finally AAB transforms ethanol into acetic acid, but gluconic and glucuronic acid production is also noteworthy, as shown in the figure below.

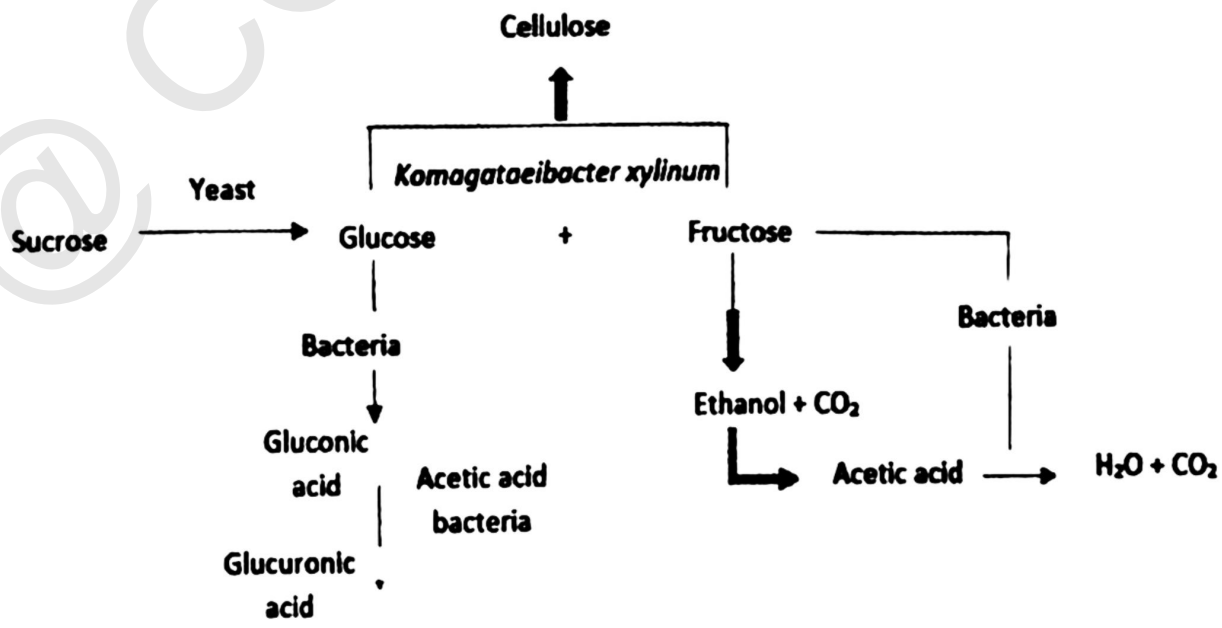


Figure 2.4: Main metabolic activity of Kombucha tea (Markov, 2003).

2.2 Factors Influencing Kombucha Fermentation

There are many factors that will affect the fermentation of Kombucha from the type of materials used to prepare Kombucha tea such as tea, sugar and starter culture, the amount of materials use, time, pH as well as the temperature for fermentation. Each of the features will affects the final product of Kombucha beverage in terms of appearance, taste, smell, probiotic value, antioxidant properties, pH and many others.

2.2.1 Substrate

Kombucha is produced by fermenting green or black sweetened teas. The Kombucha tea produced from black tea or green tea are varies. Black tea provides the highest concentration of purines which help in microorganism metabolism. The higher the level of purine, the more active culture it obtained. Therefore, black tea tends to be present in most Kombucha tea blends. Basically, black tea is *Camellia senensis* leaves that have been picked, withered, rolled, and allowed to completely oxidize. It is known to create a strong apple-cider flavor in Kombucha, with earthier tones, and a dark, golden color. A research showed that sweetened Echinacea (Echinacea purpurea L.) and winter savory (Satureja montana L.) can be used as alternate nitrogen sources, reducing fermentation time and generating similar characteristics to conventional drinks (Velicanski, 2013). A comparative study examining the microbiology, chemistry and antioxidant activity of Kombucha beverage from green, black and rooibos teas was conducted and the results showed that the yeast community in all tea substrates was very different (Gaggìa, 2018).

Furthermore, it is observed that Kombucha from rooibos has a small concentration of ethanol (1.1 mg / mL) and glucoronic acid compare to black tea.

2.2.2 Time effect

Kombucha tea fermentation usually ranges from 7 to 60 days and the biological activities may increase during this process. However, the best results were obtained in an average of 15 days (Chu, 2006). Since most antioxidant activities improve with the incubation period, but extended fermentation is not recommended due to aggregation of organic acids, which may exceed dangerous amounts for direct use. In addition, the carbon dioxide produced may begin to be accumulated at the interface between the biofilm and the broth and may block the transfer of nutrients creating a starved environment. Long fermentation also contributes to alcohol production and the level will go up to 5%.

2.2.3 Temperature effect

Regulation of the temperature during Kombucha fermentation is important for having a successful brew. Maintaining the optimum temperature during the fermentation results in a better microbial growth and enzyme activity in the Kombucha. Generally, the ideal Kombucha fermentation temperature usually varies from 24°C to 30°C. Fermentation temperature may affect Kombucha's final composition as research has shown that the amounts of acids, metabolites, and vitamin C produced in higher temperature samples were higher (Loncar et al, 2006).

2.2.4 pH effect

The pH is one of the most prominent factor that influence the Kombucha fermentation. Some of the acids generated during fermentation such as acetic acid and gluconic acid, may be accountable of the biological activities of the resulting beverages. The pH will impact the microbial growth and the structural modifications in the phytochemical compounds that may influence Kombucha's antioxidant function (Hur, 2014). The lowest acceptable pH value should not be lowered than 3 to prevent damage to the digestive tract when consumed (Loncar et al, 2006). pH monitoring is the key factor at early stages of first fermentation to ensure the process is going well. A main component in producing Kombucha is acidifying the sweet tea by incorporating starter tea from a previously brewing Kombucha batch. This starter tea causes the acidification process to continue, thus reducing the pH of the brew. An acidic pH prevents the growth of molds and the infection of harmful pathogens.

2.2.5 Alcohol content

Katarzyna et al (2020) There is a negative correlation between the acetic acid and the alcohol and sugar content during Kombucha tea fermentation. The content of alcohol and sugar is related to the production of organic acids and the use of substrates for their processing of Kombucha tea. The yeast from "SCOBY" is responsible for the breakdown of glucose into ethyl alcohol with presence of carbon dioxide during fermentation. The maximum alcohol concentration was reached on day 7 with as much as 3.5 per cent for kombucha prepared from white and red tea forms, 3.25 per cent for green tea and 3.0 per

cent for black tea, but the alcohol content marginally decreased on day 14 in the case of all the varieties studied to the level of 2–3 per cent. Villarreal-Soto (2018) In the second stage of fermentation, acetobacter bacteria use ethyl alcohol as a substratum to produce acetic acid. This causes decrease in alcohol content and further lead to an increase in acidity of Kombucha tea.

2.3 Health Benefits Associated with Consumption of Kombucha

Kombucha beverage is popular to offer many medicinal and therapeutic advantages. There are few researches reported that Kombucha drink can help with digestion, alleviate inflammation, act as a laxative, prevent microbial infections, battle stress and cancer, alleviate hemorrhoids, have a beneficial impact on cholesterol levels, and promote toxin excretion and blood cleaning (Jayabalan et al, 2014). Kombucha tea is also known as probiotic drink. This drink is associated with affecting the gastrointestinal microbial flora in humans and helping to stabilize the intestinal micro-biota, thereby promoting a degree of normalization of intestinal behavior (Silva et al, 2009). Furthermore, the existence of glucuronic acid produced during fermentation in Kombucha beverage imparts beneficial properties to this drink (Teoh, 2004). Glucuronic acid is naturally produced by a healthy human liver and is a carboxylic acid that is extremely water soluble. This acid can be transformed to collagen-associated glucosamine and chondroitin-sulfate and even the fluid that serves as a lubricant in the joints (Yavari, 2011). The generation of butyric acid in the fermentation process by the microbial consortium is considered to protect the cellular membranes of humans. In conjunction with glucuronic acid, this complex has the ability to reinforce the walls of gut and offer protection against

parasites (Dufresne et al, 2000). All the reviews and studies justify that Kombucha beverage having high value in term of providing human health benefit.

2.3.1 Probiotic Effects

As emphasized earlier, Kombucha is known to have probiotic effects. The bacterial portion of a probiotic mixture is essentially extracted from *Lactobacillus* or *Bifidobacterium* or a combination of these two strains. In support of these lines, there can be a few common yeast types such as *Saccharomyces boulardii* and *S. cerevisiae* in this mixture as well. Probiotic microbes are known to play a vital role in the wellness of human health. Probiotic microorganisms provide a balance intestinal micro-biota, standardize processes in the intestine and improve the immune system. They help enhance metabolism, combat toxic bacterial overgrowths, maintain conceptual balance and control of attitude as well as battle psychiatric disorders such as anxiety and depression. Several reports have shown that Kombucha tea is not just a probiotic drink but also a symbiotic, a mixture of prebiotics and probiotics (Dufresne et al, 2000). The function of probiotic is selectively helps the consortium's growth and development of beneficial microbes present in the human gut (Silva et al, 2009). The bacteria and yeast found in this drink function as probiotics and the micro cellulose allows the development of beneficial microbes in the intestine (Kozyrovska, 2012). All these studies have demonstrated the probiotic effect of the Kombucha beverage.

2.3.2 Detoxification

Kombucha beverage has a noteworthy function of detoxifying the human body. Detoxification is the complex process through which harmful compounds are eliminated from a living organism's body. The enzymes, bacterial acids and other secondary metabolites that the microbes produce during the fermentation process in Kombucha tea preparation have demonstrated the ability to detoxify the body (Dufresne et al, 2000). Many scientific studies have shown that Kombucha's detoxification activity is attributed directly due to the ability of glucuronic acid to attach with toxic molecules entering the body, and also to the ability to enhance the physiological excretion of such molecules with the help of the kidneys and intestines (Jayabalan et al, 2007). Research also found that people that frequently drink Kombucha can remove contaminants that have either been building up spontaneously as a consequence of disease or have been absorbed from the atmosphere (Teoh, 2004).

2.3.3 Antioxidant Activity

Many compounds with radical scavenging properties are extracted from the tea leaves themselves during the Kombucha fermentation (Malbaša, 2011). The primary group of compounds present in tea belonging to the flavanol group are polyphenols and catechins. Polyphenols are known to have high concentrations of strong antioxidant properties because they are capable of scavenging free radicals and reactive oxygen species (ROS) (Srihari, 2012). Degradation of large molecules to small molecules occurs when the complex phenolic compounds are present in an acidic environment and this

further contribute to an increase in the total phenolic compounds available in the Kombucha tea. Hence, as the fermentation begins, the overall phenolic content grows and the antioxidant level of Kombucha beverage rises,

2.3.4 Hepatoprotective Effects

Hepatoprotection is a popular therapeutic concept used to define the capacity to avoid harmful chemicals from causing harm to the liver. The environmental pollutants no matter from air, water or foods could induce hepatotoxicity and cause damage to the liver. Carbon tetrachloride (CCl_4) is a xenobiotic that cause peroxidation of the lipids. It will forms a free radical and accumulates lipid derived oxidants and cause injury to the liver (Jeon, 2003). Kombucha tea intake has been shown to inhibit CCl_4 activity and prevent hepatic injury in rats (Murugesan, 2009). Studies have also proposed that kombucha tea is capable of avoiding hepatotoxicity caused by paracetamol (Pauline, 2001). Also, histological analyzes of alloxane-induced diabetes rats have identified safe liver-kidney functions despite a diet containing Kombucha tea (Aloulou, 2012).

2.3.5 Other Therapeutic Effects

There are several reports of health advantages and uses relevant to beverage Kombucha. For example, there is research regarding the microbial mat produced in the fermentation had been used to create artificial skin (Vicente, 2001). This layer has been used by several experts to improve the healing mechanism and also as an antiseptic by

adhering to it for exposed injuries. Lactic acid produced during the fermentation of Kombucha tea can boost blood circulation and also avoid constipation (Dufresne et al, 2000). The yeast in Kombucha mat produce butyric acid that can be used to protect the human cellular membrane (Jayabalan et al, 2014). Moreover, healing of gastric ulcers in rats due to Kombucha tea was noticeable from histopathology and biochemical research (Banerjee, 2010). Kombucha beverage will also suppress gray hair development and improve the eyesight (Dufresne et al, 2000). There are many other studies and researches reported that Kombucha is a functional beverage with a lot of advantages.

2.4 Safety Issues and Controlling Potential Hazards of Kombucha

The preparation of Kombucha should be very cautious because pathogenic microorganisms may contaminate the tea during the preparation. This will increase the toxicity of the Kombucha and become harmful. There are issues of health disorders have been reported by some individuals with suspected dizziness and nausea, severe illness, allergic reactions, and head pain after consuming Kombucha. These problems leading to contraindication or restriction of Kombucha in pregnant and lactating women (Jayabalan et.al, 2014). The safety of Kombucha tea with possible liver injury, metabolic acidosis and skin anthrax infections is challenged by several case reports and case series (Ernst, 2003). Drinking Kombucha tea induces allergic symptoms and an uncomfortable stomach experienced by people with acid sensitivities and renal insufficiencies. Several studies have shown that Kombucha can induce nausea, shortness of breath, tightness of the throat, headache, dizziness and jaundice (Kole, 2008). However, whether such symptoms are the result of rare toxins produced in a specific batch of Kombucha tea was not clarified. There

is also a study claim that consumption of Kombucha might also be a health risk for HIV-positive patients (Jayabalan et.al, 2014). It was also discovered that acute renal failure may occur with lactic acidosis and hyperthermia due to intake of this beverage. As there are several concerns and health problems related to Kombucha, more research will therefore be performed to draw a comprehensive conclusion on the safety aspects of Kombucha tea consumption.

2.5 Production of Kombucha tea in industrial scale.

(Himjyoti Dutta and Sanjib K Paul, 2019) claimed that there are variety of home-scale Kombucha manufacturers have recently grown into bigger companies. GT's Living Foods began selling Kombucha bottled drinks in Los Angeles Health Food Store, Erewhon, in 1995. Kombucha's first commercial retailing in the modern world is considered to be this. Kombucha microbreweries recently introduced various flavored varieties for the manufacture of beverages. Denver, Colorado's Happy Leaf Kombucha Microbrewery opened in 2013, offering a variety of different-flavored Kombucha drinks. The fermentation is conducted in fermentation tanks of scale greater than 3500 L. In such tanks, the SCOBY weight increases by up to 90 kg. The liquor is moved to a stainless steel, glycol-lined brite tank as the pH level fall to approximate 2.7. Here, the Kombucha is kept under freezing temperature to deactivate the fermenting bacteria and yeast in the liquor. Besides, different flavors are added to it in this stage. The flavors take 2–5 days to be completely integrated into the product depending on the specific potency of the flavors compound. Transferring of Kombucha from fermenter tank to brite tank can result in loss of dissolved carbon dioxide (CO_2) due to agitation. Furthermore, consumers also prefer

higher and bubblier fizz in their Kombucha drink. Therefore, after stage of adding flavors, Happy Leaf Kombucha is filtered and pumped into kegs along with pressurized CO_2 dissolution. The carbonated Kombucha is then bottled, capped, labelled, and cartooned. The bottles and kegs are maintained at temperature of 2 – 4°C to ensure freshness of the drinks. A non-exhaustive list of different manufacturers and Kombucha products, brand name and product type are provided in Table 2.3.

Table 2.3: Examples of different manufacturers and Kombucha products, brand name and product type.

Company	Establishment Year	Brand	Company Websites	Products
The Kombucha Shop	2013	The Kombucha Shop	https://www.thekombuchashop.com/	Kombucha brewing kit, jars, bottles and accessories, organic blended kombucha premix, kombucha culture and starter.
Townshend's Tea Company	2008	Brew Dr.Kombucha	http://brewdrkombucha.com/	Organic herbal blended kombuch
Ithaca Kombucha Company	2015	IKC Lucky Brew	http://www.ithacakombuchacompany.com/	Kombucha sauce, ingredients, flavored kombucha drinks
WonderBrew	2018	WonderBrew	https://www.wonderbrew.co/	Lite Green Kombucha, Markisa Breeze Kombucha, Purple Pari Pari
Health-Ade kombuca	2012	HEALTH-ADE Kombucha	https://health-ade.com/	Blended kombucha beverages

CHAPTER 3

METHODOLOGY

3.1 Introduction

This study was concerned with investigating the effect of pH, temperature and alcohol content on the safety of Kombucha tea. The preparation of Kombucha tea was performed by referring to the Standard Operating Procedure (SOP) from a special report entitled Kombucha Brewing Under the Food and Drug Administration Model Food Code: Risk Analysis and Processing Guidance (Nummer B. , 2013). The pH of the Kombucha tea were monitor throughout the whole experiment. The samples with positive results (without molds, pleasant smell, and safe pH) were sent for analyzing of alcohol content. The works were conducted as shown in Figure 3.1.

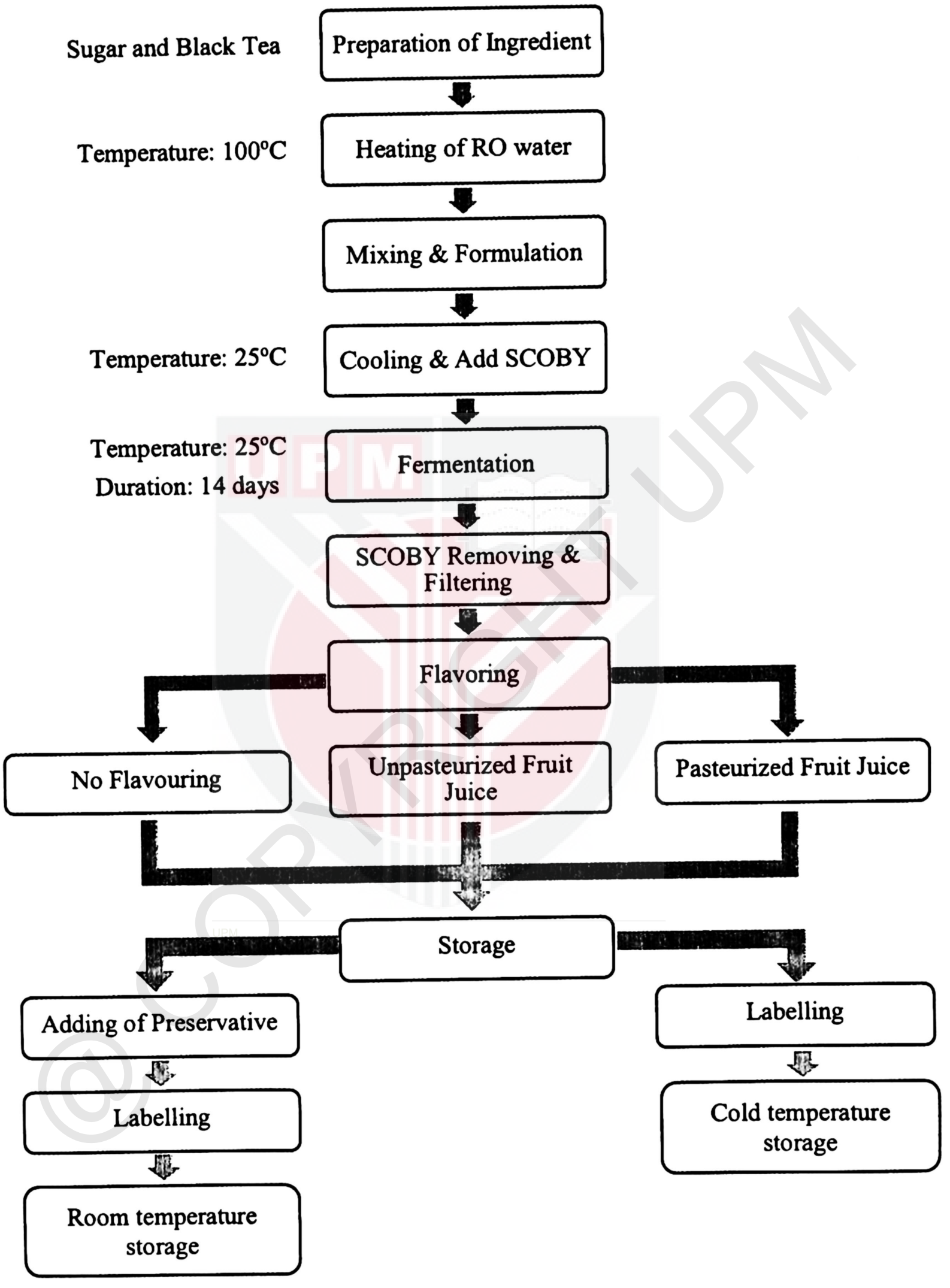


Figure 3.1: Work flow diagram.

3.2 Preparation of SCOBY

SCOBY is the key factor for fermentation of Kombucha tea. The SCOBY was bought from Home Remedies Online Seller. SCOBY is symbiotic culture of bacteria and yeast in form of membrane structure. The SCOBY obtained having diameter of ±8cm and a thickness ±1cm. The package of SCOBY included the previous tea that can be used to increase the fermentation rate of Kombucha tea.

3.3 Preparation of Kombucha Tea

7.5 liters of water was brought to a boil followed by adding of 0.413kg of sugar (1-liter tea: 0.055kg sugar) and 15 bags of black tea (1-liter water: 2 tea bags). BOH English Breakfast Black Tea Teabag was used for this purpose. The tea bags were removed after boiling for five minutes. Afterwards the sweet tea was left to cool down to room temperature. The tea was then filtered and divided into 3 separate glass jar (3L) containers equally. Each glass jar is then added with SCOBY and previous tea for fermentation, covered using cheese cloth and tied with an elastic band around the opening. The glass containers were left in room temperature condition for fermentation up to 14 days. The pH value of each jar was measured and recorded throughout the experiment. At the end of the fermentation, the pH of Kombucha tea must be less than pH 4.6 to prevent microbial growth.

3.3.1 Preparation of Kombucha tea sample (No Flavoring)

After 14 days of fermentation, the tea was filtered and poured into small glass bottles for sampling and test. The samples of Kombucha tea were then formulated and classified into few groups with labels O-R, O-RE, O-PS and O-SB. The type and percentage of preservative used in this research were referred to a report entitled Kombucha Brewing Under the Food and Drug Administration Model Food Code: Risk Analysis and Processing Guidance (Nummer B. , 2013). The summary of preparation of samples were depicted in Table 3.1. The descriptions of the samples were as below:

O-R: Original flavor Kombucha tea without preservative and stored at room temperature condition.

O-RE: Original flavor Kombucha tea without preservative and stored at refrigerator temperature condition.

O-PS: Original flavor Kombucha tea with 01% of potassium sorbate and stored at room temperature condition.

O-SB: Original flavor Kombucha tea with 01% of sodium benzoate and stored at room temperature condition.

3.3.2 Preparation of Kombucha tea sample (Unpasteurized Apple Juice)

After 14 days of fermentation, the tea was poured into small bottle for sampling and test. 375ml of apple juice were prepared (1-liter tea: 125ml of juice), filtered, poured

into a big container and mix well. The liquid is then poured into 4 glass bottles. The bottles of samples were prepared based on designed formulations and labelled as A-R, A-RE, A-PS and A-SB (Table 3.1). The descriptions of the samples were as below:

A-R: Unpasteurized apple juice Kombucha tea without preservative and stored at room temperature condition.

A-RE: Unpasteurized apple juice Kombucha tea without preservative and stored at refrigerator temperature condition.

A-PS: Unpasteurized apple juice Kombucha tea with 01% of potassium sorbate and stored at room temperature condition.

A-SB: Unpasteurized apple juice Kombucha tea with 01% of sodium benzoate and stored at room temperature condition.

3.3.3 Preparation of Kombucha tea samples (Pasteurized Apple Juice)

Another set of experiment was conducted by pasteurizing the fruit juice to kill the bacterial before adding into the Kombucha tea sample. Pasteurization was carried out by pouring the fruit juice into the pot on heater. Next, the heater was turned on high to heat the fruit juice. On-going monitoring of temperature was necessary during heating. A thermometer was used to check the temperature of fruit juice. The fruit juice was stirred evenly throughout the heating process. The fruit juice was heated until it reaches 70°C and held for 1 minute for complete pasteurization. The pasteurized juice was then added into bottles of Kombucha tea samples which were prepared based on designed formulations

and labelled as PA-R, PA-RE, PA-PS and PA-SB (Table 3.1). The descriptions of the samples were as below:

PA-R : Pasteurized apple juice Kombucha tea without preservative and stored at room temperature condition.

PA-RE: Pasteurized apple juice Kombucha tea without preservative and stored at refrigerator temperature condition.

PA-PS: Pasteurized apple juice Kombucha tea with 01% of potassium sorbate and stored at room temperature condition.

PA-SB: Pasteurized apple juice Kombucha tea with 01% of sodium benzoate and stored at room temperature condition.

Table 3.1: The summary of samples preparation for Original Flavor Kombucha tea, Unpasteurized Apple Juice Kombucha tea and Pasteurized Apple Juice Kombucha tea.

Description	Original Flavor Kombucha tea	Unpasteurized Apple Juice Kombucha tea	Pasteurized Apple Juice Kombucha tea
• No preservative • room temperature storage	O-R	A-R	PA-R
• No preservative • refrigerator temperature storage	O-RE	A-RE	PA-RE
• 0.1 % Potassium Sorbate • Room temperature storage	O-PS	A-PS	PA-PS

• 0.1 % Sodium Benzoate • Room temperature storage	O-SB	A-SB	PA-SB

3.4 pH Monitoring and Alcohol Analysis

The pH of the samples was measured using the Laboratory Digital Automatic Bench Table Top pH Meters, model pH 8601. The digital pH meter will be calibrated using buffer solution pH 7 and buffer solution pH 4 as well as washed using distilled water before dip into the samples of Kombucha tea to obtain the pH reading. The observation and changes of pH on each sample was taken every 7 days up to 28 days. The changes in pH during storage were recorded, compared, and analyzed.

The samples without molds, sediments, unpleasant smell and having stable pH reading throughout the one-month monitoring period were selected for alcohol test to identify the percentage of alcohol content. The samples were sent to UKM UNIPEQ to identify the alcohol content of the Kombucha tea. The type of analysis used to measure the ethanol content in Kombucha is headspace gas chromatography with flame ionization detection (HS-GC–FID). This method was conducted according to AOAC Official Method 2016.12 specified for determination of ethanol in kombucha (Konings, 2016).

All tests were carried out in parallel under the same conditions, whereas three times each quantity was measured. Averages of all measurements are the collected values used for further analysis and are described as mean ± standard deviation. Analysis of data was conducted using ANOVA under function in Data Analysis (Excel).

3.5 Plant Design for Production of Kombucha Tea

A visit to the Kombucha tea industry was made to clarify the state of the processing site. All the problems occur in the production were figured out and proposed with appropriate solution. A new plant layout for Kombucha tea production was designed by referring to the current plant layout, measurement and with considerations based on Good Manufacturing Practices (GMP).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, the results of the study are presented and discussed with reference to the aim of the study. which was to determine the effect of pH, temperature, and alcohol content on food safety of Kombucha tea. Changes in pH of Kombucha tea during 14 days of fermentation was first discussed and analyzed (section 4.2.1). The effect of preservative on Kombucha tea was described through comparison between the effect of preservative on pH of Original Kombucha tea, Kombucha tea with unpasteurized apple juice and Kombucha tea with pasteurized apple juice at room temperature storage upon 28 days of storage (section 4.2.2). The effect of storage temperature on Kombucha tea was determined by comparing the changes in pH during 28 days of storage for Kombucha tea samples stored at room temperature with the Kombucha tea samples stored at cold refrigerator temperature (section 4.2.3). The comparison between effect of preservative and effect of storage temperature on Kombucha tea was discussed and analyzed to determine the best method to ensure the safety of Kombucha tea (section 4.2.4). The effect of flavoring on Kombucha tea was also explained by comparing the changes in pH during storage of the Original Kombucha tea with the Kombucha tea added with Unpasteurized Apple Juice or Pasteurized Apple Juice (section 4.2.5). Lastly, the alcohol content of Kombucha tea before storage and after 28 days of storage was analyzed and discussed to figure out the sample with alcohol content within the alcohol acceptable limit (section 4.2.6).

The proposed plant design for production of Kombucha tea was presented at the second part of this chapter (section 4.3). The sub-topics that will be discussed under this section included the plant design overview (section 4.3.1), design objective (section 4.3.2), product description (section 4.3.3), productivity (section 4.3.4), process flow with mass balance (section 4.3.5), mechanical design (section 4.3.6) and plant layout for manufacturing of Kombucha tea (section 4.3.7).

4.2 Analysis of Kombucha tea

4.2.1 Changes in pH of Kombucha tea During Fermentation

Changes in pH of Kombucha tea during 14 days of fermentation was discussed and analyzed. A total of 4 packs of SCOBY were bought from the Home Remedies Online Seller. The condition of each SCOBY was checked by measuring the pH value. The pH value of the SCOBY was recorded as below.

Table 4.1: Original pH of the previous Kombucha tea with SCOBY.

Previous Kombucha tea with SCOBY	pH
A	2.60
B	2.49
C	2.70
D	2.71
Average	2.63 ± 0.09

The pH of previous Kombucha tea were in the range between pH 2.49 - 2.71. The average pH value is 2.63 with small deviation ±0.09. These showed that all the SCOBY were in high acidic condition before added into newly prepared Kombucha tea. The high acidic condition able to maintain the SCOBY in good condition by preventing growth of harmful microorganisms.

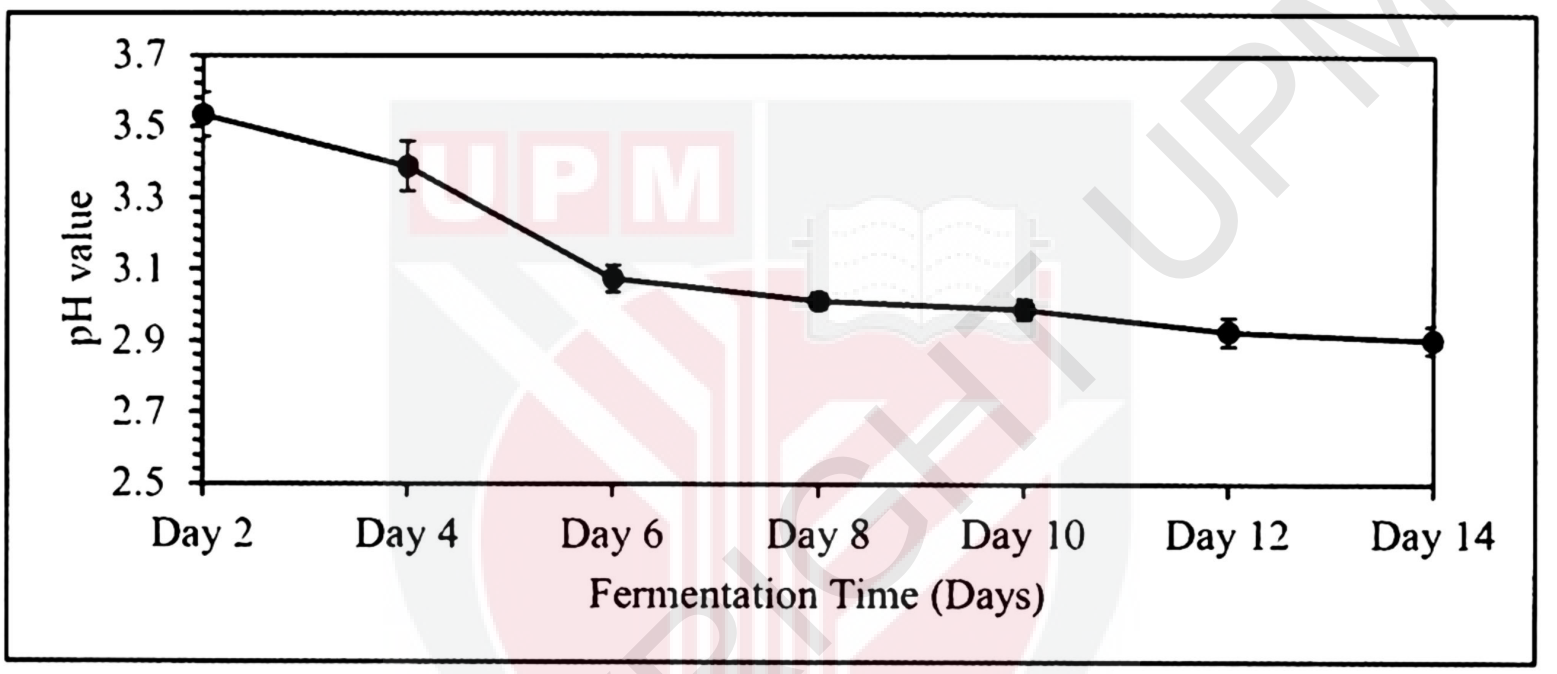


Figure 4.1: The change in pH of the Kombucha tea throughout the fermentation period.

The change in pH of the Kombucha tea throughout the fermentation period were observed and recorded. The purpose of this action was to monitor the change in pH of the Kombucha tea throughout the fermentation period. The pH value of the Kombucha tea were depend on the acid concentration level that generated by the good bacteria during fermentation of Kombucha tea. The type of acid includes acetic acid, glucuronic acid, gluconic acid, citric acid, oxalic acid and others. When the concentration of acid in the Kombucha tea is high, the pH value will be low. Based on the results (Figure 4.1), the pH of Kombucha tea drop significantly ($P<0.05$) from average pH 3.67 on the first day to average pH 2.91 on day 14. A previous research showed the pH dropped from pH 4.76 on

the first hours to pH 3.08 on hours 120 (Hammel et.al, 2016). The pH obtained were lower when compared with previous studies which have demonstrated that the pH can decrease from 5.44 to about 3.0 in roughly five to seven days (Jayabalan et.al, 2014). The drop in the pH value of Kombucha tea were more significant on the first 6 days in which the average pH drop form pH 3.67 on the first day to pH 3.53, pH 3.39 and to pH 3.01 on the day 2, day 4 and day 6 respectively. The difference of pH value between the first day and second day is 0.14 while difference between second day and third day is 0.15. The gap of pH value in between third day and day 4 is 0.31. There are just small changes in the pH value of Kombucha tea after day 6. The pH dropped from 3.01 on day 6 to pH 2.91 on day 14. Similar trend obtained from (Velićanski, 2013) which reported that the pH value decreased by 0.5-0.9 units per day on first two days, followed with decrease only 0.3 units and after, the pH value changed insignificantly and reached 2.82-2.95 at the end of the process. The result shows that the rate of fermentation was high at the early stage of fermentation in which at the beginning of the fermentation, yeast hydrolyze sucrose (sugar) into glucose and fructose, formerly the ethanol is produced and finally AAB transform ethanol into acetic acid, nonetheless the production of gluconic and glucuronic acids are also significant which lead to decrease in pH value. The rate of fermentation at after stage is lower because the Kombucha tea is now having low sugar concentration as it had been consumed by the yeast during early stage of fermentation. (Reiss, 1994) reported that the production of acetic acid is limited during prolonged tea fungus cultivation. (Velićanski, 2013) explained the fact that high medium acidity and lower amounts of sugar inhibit the fermentation activity of yeasts and the production of ethanol, and indirectly to the production of acetic acid.

4.2.2 The Effect of Preservative on Kombucha Tea

Comparison between the effect of preservative on pH of Original Kombucha tea, Kombucha tea with unpasteurized apple juice and Kombucha tea with pasteurized apple juice at room temperature storage will be discussed in this section. The changes in pH for samples added with 0.1% of potassium sorbate and 0.1% of sodium benzoate were higher than the samples without adding of preservative throughout 28 days of storage (Figure 4.2). The addition of potassium sorbate and sodium benzoate had successfully stabilized the changes in pH for Original Kombucha tea and Unpasteurized Apple Juice Kombucha tea (Figure 4.2 (a), (b)). Preservative can provide a higher pH condition to the samples and terminate the fermentation process Kombucha tea sample. Similar results reported by (Nummer, 2013) which reported that preservatives such as potassium sorbate and sodium benzoate can be used to inhibit further fermentation in Kombucha tea (Nummer, 2013). When the fermentation process stopped, the acetic acid or gluconic acid will not be produced and these lead to increase in pH value since the pH is highly depend on concentration of organic acid of the Kombucha tea. Others evidence taken from product nearly close to Kombucha tea, Ed Kraus, an experienced winemaker stated that Potassium Sorbate is able to alter the wine yeast from fermenting the newly added sugar (Kraus, 2020).

There was a significant drop ($P<0.05$) in the pH of Pasteurized Apple Juice Kombucha tea either for those samples added with preservative or without preservative (Figure 4.2 (c)). The pH of Pasteurized Apple Juice Kombucha tea without preservative decreased significantly ($P<0.05$) from pH 3.39 (day 1) to pH 2.52 (day 28th), same go to the pH of Pasteurized Apple Juice Kombucha tea with 0.1% Potassium Sorbate and 0.1%

of Sodium Benzoate in which the pH decrease from pH 4.25 to pH 2.97 and from pH 4.07 to pH 2.74 respectively. This condition indicated the yeasts in the Kombucha tea samples were behaved actively which caused the fermentation process to continue. This can be proofed by formation of SCOBY in Pasteurized Apple Juice Kombucha tea samples stored at room temperature storage as shown in APPENDICES (A.1.2). Based on (Kraus, 2020), Potassium Sorbate was able to stop fermentation but it does not kill the yeast. Therefore, the wine yeast still having ability to ferment sugar into alcohol and further converted into organic acid even though after adding preservative.

Addition of preservative into Kombucha tea was able to increase the overall pH of the samples and prevent from being too acidic. Preservative is able to stabilise the changes in pH of Kombucha tea during storage by preventing fermentation process and formation of new SCOBY, however, in some extent, fermentation process can continue, leading drop in pH as the yeast in the sample are not killed.

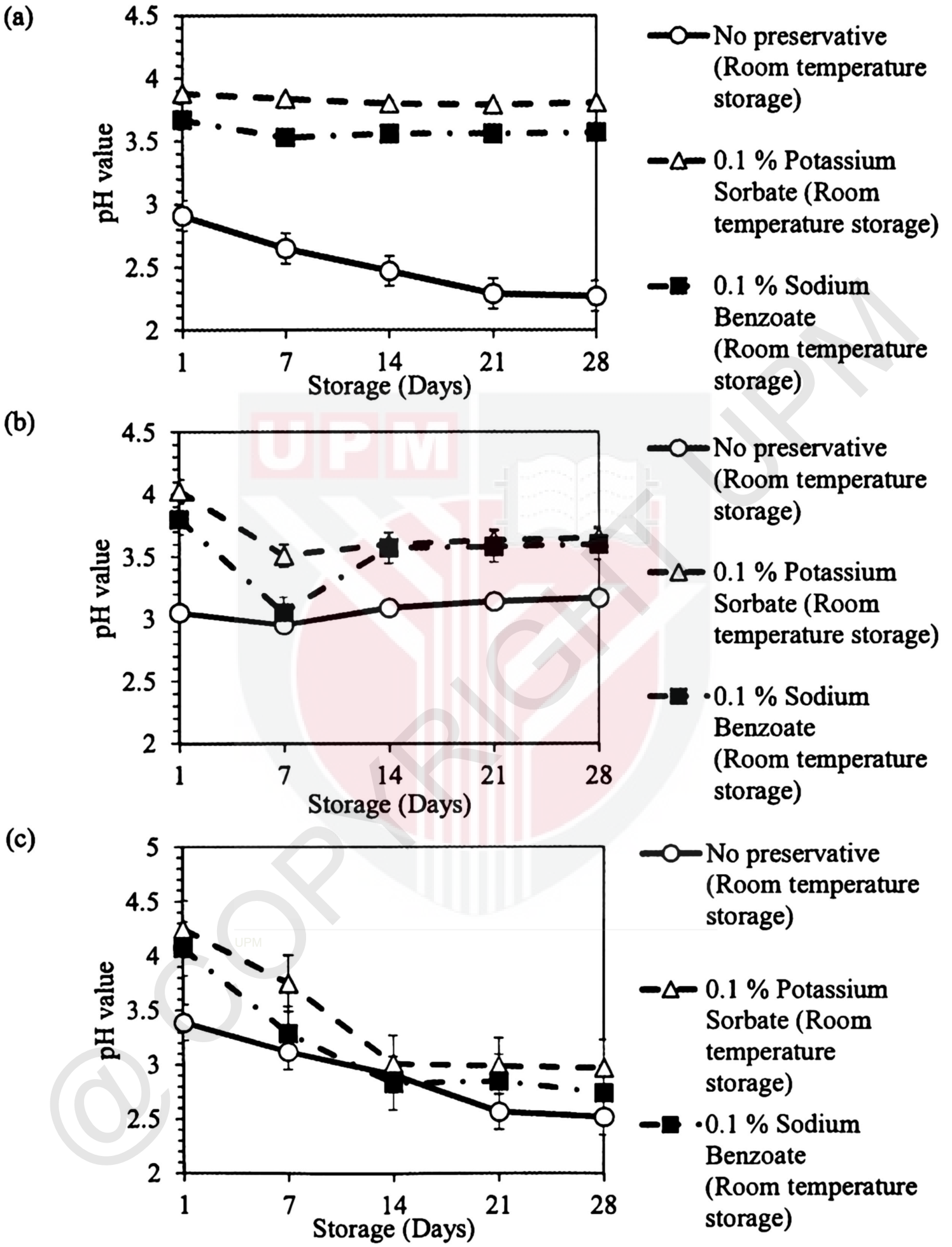


Figure 4.2: Changes in pH value of (a) Original Kombucha tea, (b) Unpasteurized Apple Juice Kombucha tea and (c) Pasteurized Apple Juice Kombucha tea during storage period of 28 days. Solid line represent sample without preservative and stored at room temperature, dashed line represent sample with 0.1% potassium sorbate stored at room temperature, dash-dotted line represent sample with 0.1% sodium benzoate stored at room temperature.

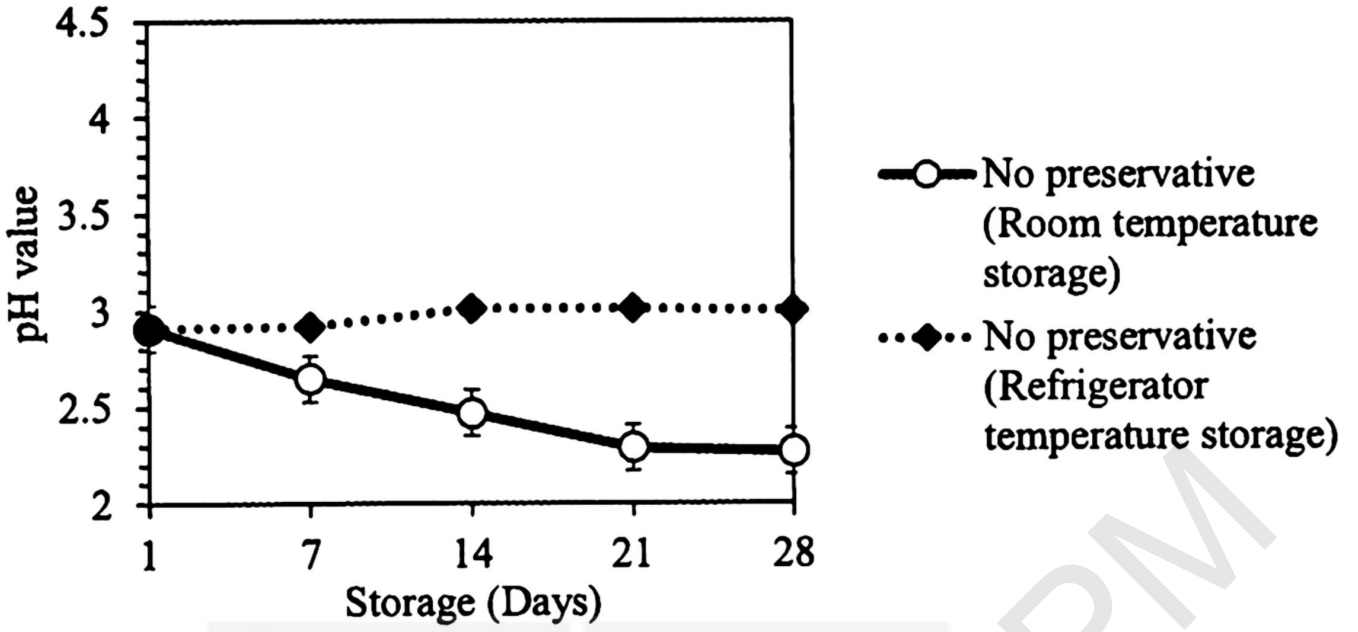
4.2.3 The Effect of Storage Temperature on Kombucha Tea.

Comparison between the effect of storage temperature on pH of original flavor Kombucha tea, Kombucha tea with unpasteurized apple juice and Kombucha tea with pasteurized apple juice without addition of preservative was analyzed. There were no significant changes ($P>0.05$) on the pH of Kombucha tea samples which stored at refrigerator temperature for upon 28 days of storage whether for Original Kombucha tea, Unpasteurized Apple Juice Kombucha tea or Pasteurized Apple Juice Kombucha tea. The pH of original flavor Kombucha tea without addition of preservative and stored at refrigerator temperature was well maintained throughout the storage periods from day 1, 7^{th} , 14^{th}, 21^{st} and 28^{th} with pH propagated at 2.91, 2.92, 3.01, 3.01 and pH 3.00 respectively. Similar trend obtained for both Unpasteurized Apple Juice Kombucha tea and Pasteurized Apple Juice Kombucha tea which stored at refrigerator temperature. The pH of Unpasteurized Apple Juice Kombucha tea stored at refrigerator temperature was controlled at pH 3.05 (day 1) to pH 3.20 (day 28^{th}), whereas the pH of Pasteurized Apple Juice Kombucha tea stored at refrigerator temperature was controlled at pH 3.39 (day 1) to pH 3.28 (day 28^{th}). A guidance provided by (Kombucha Brewers International , 2017) stated that cold storage ought to be maintained at temperature range of 1.1-4.4°C to slow the fermentation of the Kombucha throughout the supply chain. The growth rate of yeast for fermentation is highly depends on the temperature in which most yeast will survive at cold temperatures but the growth rate is very slow (Ed Kasper LAc Acupuncturist, n.d.). Finished product must be refrigerated at a temperature of 4°C to prevent the growth of foodborne pathogens (Control, 2015).

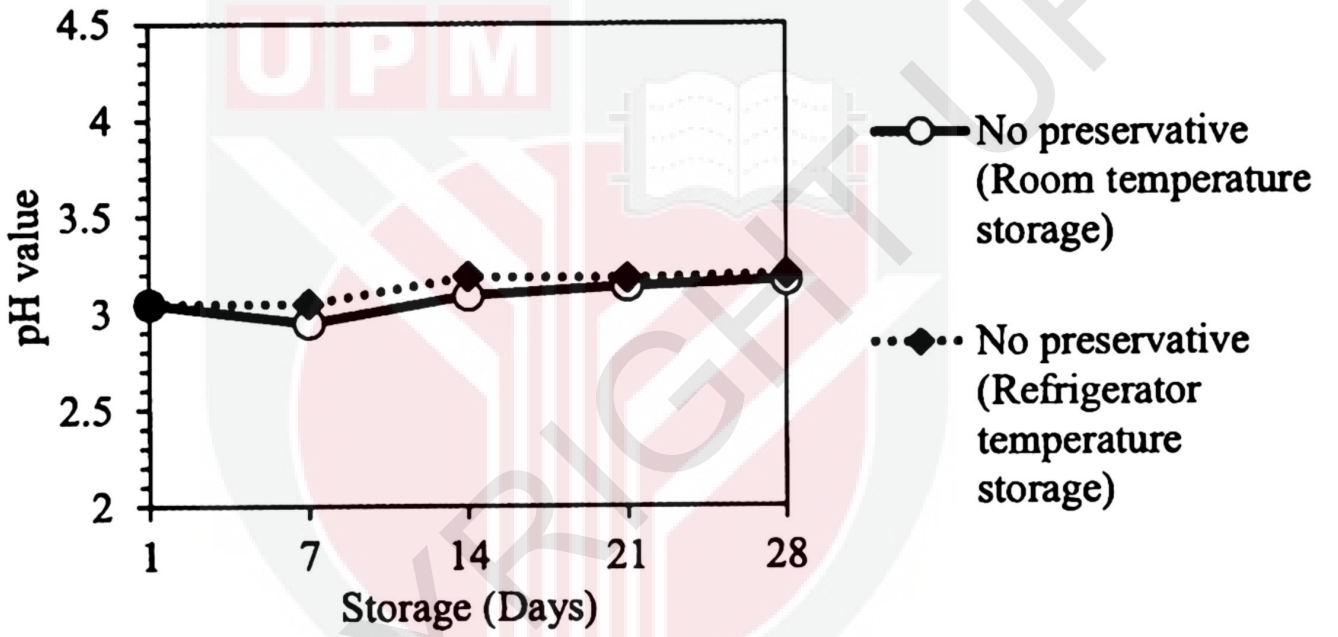
In contrast, there was a significant decrease ($P<0.05$) in the pH for both the Original Kombucha tea and Pasteurized Apple Juice Kombucha tea (without preservative and stored in room temperature) from pH 2.91 to pH 2.27 and from pH 3.39 to pH 2.52 throughout 28 days of storage. Again, the low pH indicated that fermentation process was carried in the Kombucha tea samples. The optimum yeast temperature range is 16°C – 30° C (Ed Kasper LAc Acupuncturist, n.d.). A report from (Nummer, 2013) stated that optimal temperature for the fermentation process ranges from 18°C to 26°C. The pH of both the Original Kombucha tea and Pasteurized Apple Juice Kombucha tea (without preservative and stored in room temperature) after 28 days of storage were close to the dangerous consumption pH level which is $pH<2.5$. Kombucha with a pH of below 2.5 is too acidic and should not be offered to consumers (Nummer B. , 2013). A guidance from (Agriculture, 2017) stated that a record of kombucha pH level monitoring log must be kept for each batch of Kombucha produced to verify that the pH fall within a safe level ($pH \leqslant 4.2$ and $pH \geqslant 2.5$).

Based on the results, Kombucha tea should be stored at refrigerator temperature (4°C) to minimize the changes in pH during storage by inactivate the yeast and slow down rate of fermentation. The cold refrigerator temperature storage is also important to prevent the pH from dropping below the safe consumption level ($pH<2.5$).

(a)



(b)



(c)

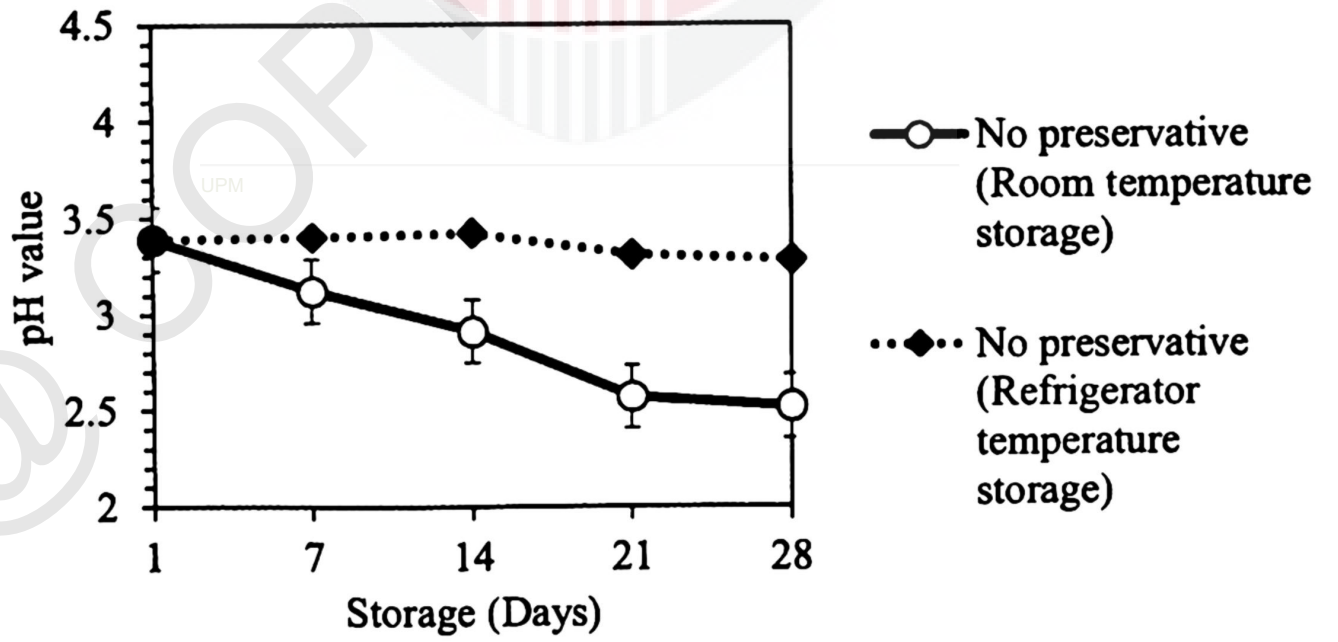


Figure 4.3: Changes in pH value of (a) Original Kombucha tea, (b) Unpasteurized Apple Juice Kombucha tea and (c) Pasteurized Apple Juice Kombucha tea during storage period of 28 days. Solid line represent sample without preservative stored at room temperature, dotted lines represent sample without preservative stored at refrigerator temperature.

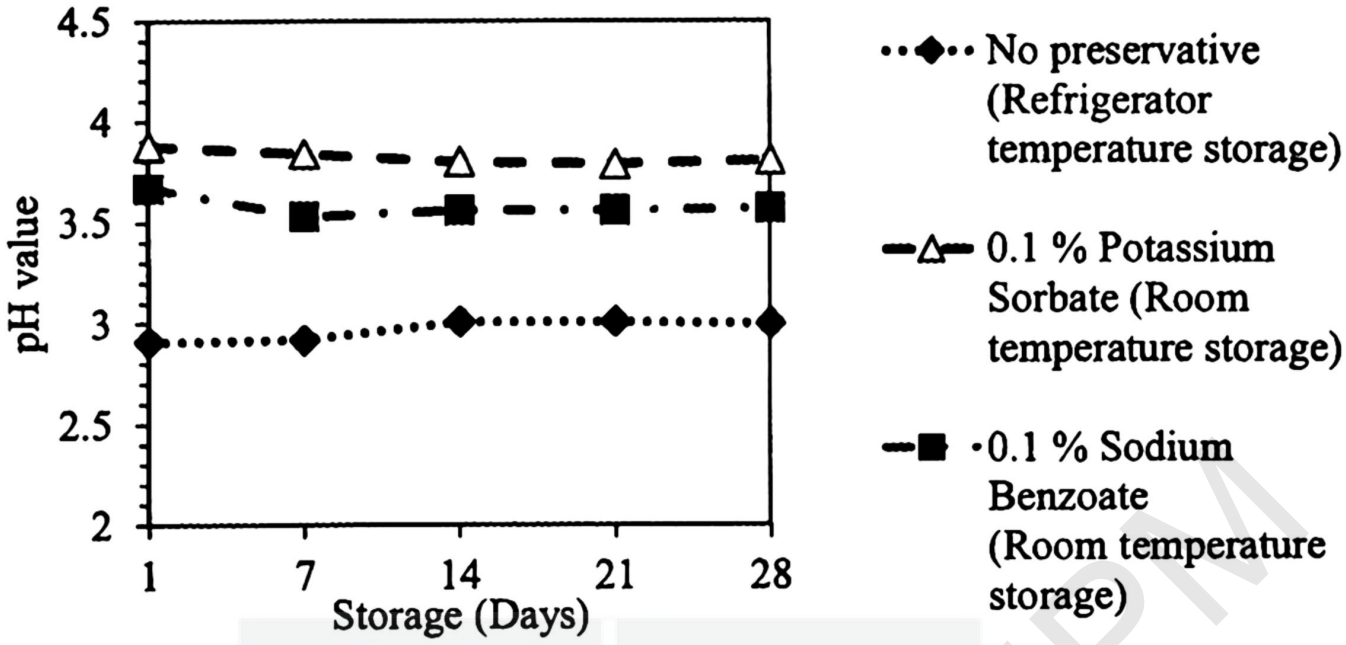
4.2.4 Comparison between Effect of Preservative and Effect of Storage Temperature on Kombucha Tea

The comparison between the changes in pH of samples (contain preservative and stored in room temperature) with the sample (without preservative and stored in refrigerator temperature) for original flavor Kombucha tea, Kombucha tea with unpasteurized apple juice and Kombucha tea with pasteurized apple juice can be observed as shown in Figure 4.4. Based on Figure 4.4, it was observed that additional of preservative to the Kombucha tea were able to maintain the pH value for both Original Kombucha tea and Unpasteurized Apple Juice Kombucha tea stored at room temperature, since the changes in pH value throughout the 28 days storage was not significant ($P>0.05$) except for Pasteurized Apple Juice Kombucha tea with preservative and stored at room temperature. In contrast, refrigerator storage temperature has successfully provided a constant result in which it has minimized the deviation or differences of pH value of all Kombucha tea samples during 28 days of storage. The cold temperature condition had stops or slows down the rate of fermentation in Kombucha tea sample. This can be further justified by the images as shown in APPENDICES A.1.1 and A.1.2, in which there were no formation of SCOBY in the Kombucha tea samples stored at refrigerator temperature,

Addition of preservative into Kombucha tea can provide an instant increase in the pH of Kombucha tea and inhibit further fermentation. However, the same condition can be achieved by storing the samples in chilled temperature condition. After storing in chilled temperature condition, the sample without preservative component having the similar trend of pH changes as the samples added with preservative and stored in room temperature condition. Based on the results, both methods either by adding of preservative

into the samples or by storing the sample in refrigerator temperature were able to control the pH value and rate of fermentation, but better to the refrigerator temperature storage. Kombucha tea should be stored in cold storage room to slow down fermentation as well as to ensure safety and stability of Kombucha tea. A report regarding Kombucha brewing and bottling guidelines (Agriculture, 2017) suggested that using refrigeration (≤41°F) in combination with antifungal preservatives such as adding 0.1% sodium benzoate and 0.1% potassium sorbate to kombucha with a pH≤4.2. were able to minimize hazards and spoilage.

(a)



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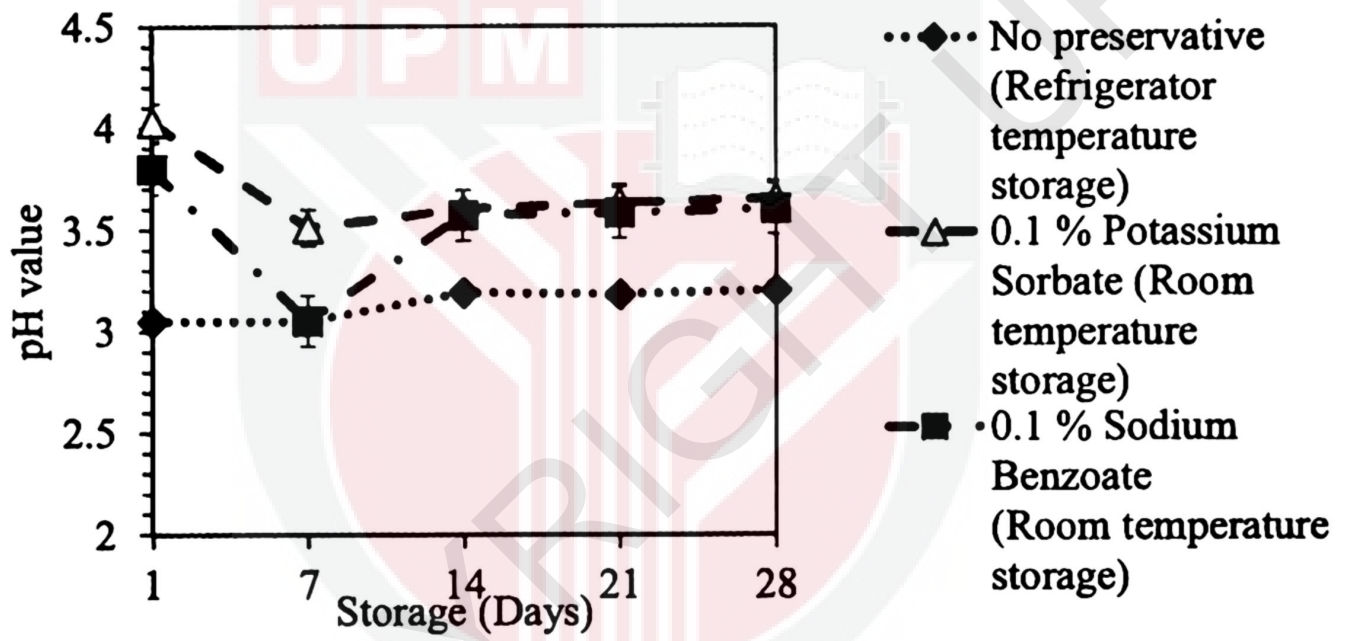

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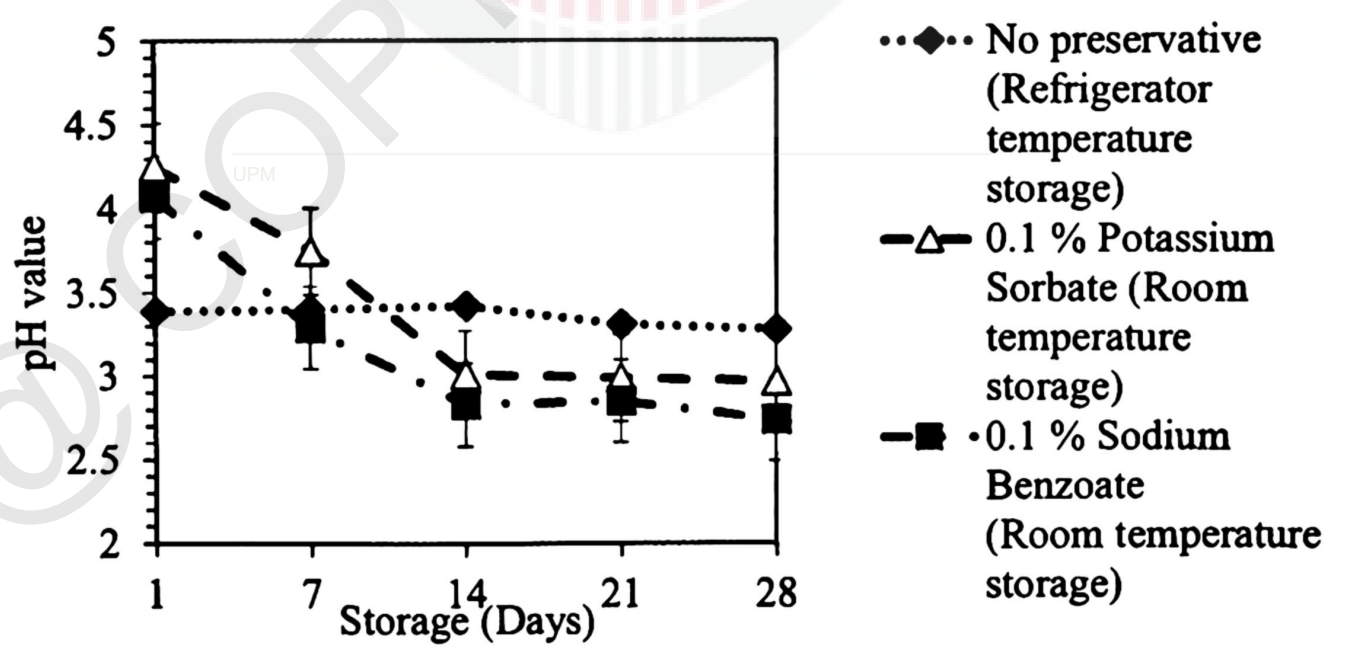


Figure 4.4: Changes in pH value of (a) Original Kombucha tea, (b) Unpasteurized Apple Juice Kombucha tea and (c) Pasteurized Apple Juice Kombucha tea during storage period of 28 days. Dotted lines represent sample without preservative stored at refrigerator temperature, dashed line represent sample with 0.1% potassium sorbate stored at room temperature, dash-dotted line represent sample with 0.1% sodium benzoate stored at room temperature.

4.2.5 The Effect of Addition of Flavoring on Kombucha Tea

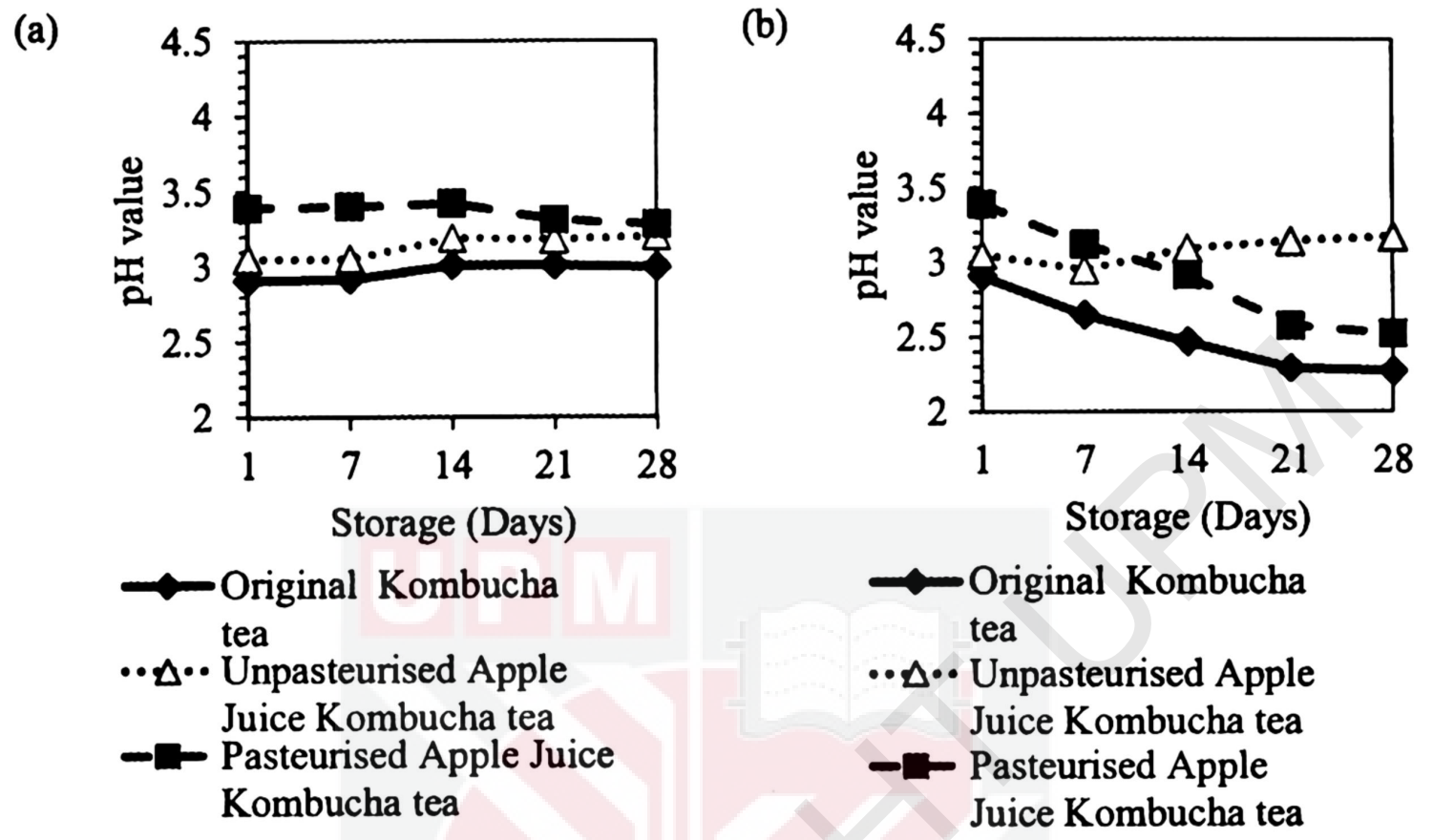


Figure 4.5: Changes of pH on Kombucha tea sample without preservative a) stored in refrigerator temperature and b) stored in room temperature. Solid line represents Original Kombucha Tea, dotted line represents Unpasteurized Apple Juice Kombucha tea, dashed line represents Pasteurized Apple Juice Kombucha tea.

The comparison between effect of addition of unpasteurized apple juice and pasteurized juice on pH of Kombucha tea sample without preservative at two different storage temperature was made. There were no significant changes ($P>0.05$) on the changes in pH of Unpasteurized Apple Juice Kombucha tea and Pasteurized Apple Juice Kombucha tea when compared to the Original Kombucha tea at refrigerator storage (Figure 4.5 (a)). However, there was a significant decrease ($P<0.05$) in the pH when the samples stored at room temperature (Figure 4.5(b)). These results proved changes on pH of Kombucha tea during storage were highly dependent on the storage temperature but not depend on type of flavoring added. Addition of Unpasteurized Apple Juice or

Pasteurized Apple Juice into Kombucha tea did not affect the stability of pH during 28 days of storage. The effect of addition of Unpasteurized Apple Juice or Pasteurized Apple Juice to the Kombucha tea is it will slightly increase the overall pH of the samples. It can be clearly seen on the Figure 4.5 in which the pH of samples added with flavoring were always slightly higher when compared to the Original Kombucha tea sample.

There was a special or unnormal case occurred in the changes in pH of Unpasteurized Apple Juice Kombucha tea in which the result was showing a stable trend of pH throughout 28 days of storage even stored under room temperature condition. There was no evidence to justify the condition, but it might be caused by formation of molds during storage (refer APPENDICES A.1.1, Label: A-R). Based on the observation during experiment, it was recorded thar there was presence of molds and undefined sediments at the bottom of the Kombucha tea sample which make it unclear with high turbidity. Besides, there was also formation of large bubbles on the surface of sample. This sample also consist of unpleasant smell, sour and acidic smell. There was an obvious difference when comparing the physical changes after 14 days of storage between Unpasteurized Apple Juice Kombucha tea with the Pasteurized Apple Fruit Juice at room temperature storage. A thick SCOBY was form on the surface of Pasteurized Apple Juice Kombucha tea without preservative and stored at room temperature condition after 14 days of storage (refer APPENDICES A.1.2, Label: PA-R). It was also recorded that there was no formation of molds and the liquid below is clear without present of any sediments.

The changes of Kombucha tea with unpasteurized apple juice after 14 days of storage were observed and recorded as shown in APPENDICES A.1.1. Few samples were added with different formulation of preservative to investigate whether the formulation

able to preserve the Kombucha tea with unpasteurized apple juice for long storage duration. Based on results, the change in pH of the Kombucha tea samples fall within the safety range and did not showed any significant changes within 28 days of storage. However, the diagram in APPENDICES A.1.1 showed that most of the samples grew molds and formed undesired sediments even though the samples were added with preservative or stored in chilled temperature. The results proved that unpasteurized fruit juice is not suitable to be added into Kombucha tea because it causes growth of mold or formation of undesired material which greatly increase the risk and hazard of Kombucha tea.

The visible changes of Kombucha tea added with pasteurized apple juice after storage of 14 days can be observed by referring APPENDICES A.1.2. Based on the observation, there were no molds formation in the Kombucha tea with pasteurized apple juice after 14 days of storage. However, it was observed that most of the samples grew SCOBY on the top of the liquid except the sample stored in chilled temperature. Formation of SCOBY explained that these formulations were failed to stop the fermentation of Kombucha tea. The pH of the samples of Kombucha tea with pasteurized apple juice stored in room temperature were significantly lower when compared to the sample stored in chilled temperature condition upon storage period due to the fermentation process. In conclusion, fruit juice can be added in Kombucha tea in order to enhance the taste, however, it is necessary to pasteurize the fruit juice before adding into Kombucha tea to prevent growth of molds as well as to extend the shelf-life. Kombucha tea products should be stored in chilled temperature condition to stop or slow down the fermentation and prevent it to become too acidic to reach pH<2.5.

4.2.6 Alcohol Content Analysis of Kombucha Tea

Table 4.2: Alcohol content (% w/v) of Kombucha tea before and after 28 days of storage.

Formulation	Before Storage	After 28 days Storage
Original Kombucha tea		
No preservative (Room temperature storage)	<0.5	0.979±0.049
No preservative (Refrigerator temperature storage)	<0.5	0.250±0.007
0.1 % Potassium Sorbate (Room temperature storage)	<0.5	Present of SCOBY
0.1 % Sodium Benzoate (Room temperature storage)	<0.5	Present of SCOBY
Unpasteurized Apple Juice Kombucha tea		
No preservative (Room temperature storage)	<0.5	Present of molds
No preservative (Refrigerator temperature storage)	<0.5	Present of sediments
0.1 % Potassium Sorbate (Room temperature storage)	<0.5	Present of molds
0.1 % Sodium Benzoate (Room temperature storage)	<0.5	Present of molds
Pasteurized Apple Juice Kombucha tea		
No preservative (Room temperature storage)	<0.5	Present of SCOBY
No preservative (Refrigerator temperature storage)	<0.5	0.279±0.008
0.1 % Potassium Sorbate (Room temperature storage)	<0.5	Present of SCOBY
0.1 % Sodium Benzoate (Room temperature storage)	<0.5	Present of SCOBY

The alcohol content in % w/v of the Kombucha tea before and after 28 days of storage was depicted in table 4.2. The alcohol content of Kombucha tea sample before storage were assume to be < 0.5 %w/v (safe alcohol limit) as all the samples were prepared under Standard Operating Procedures (SOP) for production of Kombucha tea with optimum fermentation temperature at 25°C for 14 days. The ethanol content obtained by (Sievers, 1995) was less than 5g/L (< 0.5 % w/v) after six days of fermentation, while (Reiss, 1994) obtained 3.3 g/L (0.33 % w/v) by using enzymatic tests. The alcohol content

obtained by gas chromatography was about 3 g/L (0.3 % w/v) after 10 days of fermentation (Chen and Liu , 2000).

The alcohol content for the samples of Original Kombucha tea (no preservative with room temperature storage), Original Kombucha tea (no preservative and refrigerator temperature storage) and Pasteurized Apple Juice Kombucha tea (no preservative with refrigerator temperature storage) after 28 days of storage were analyzed using headspace gas chromatography with flame ionization detection (HS-GC–FID). The alcohol content of Original Kombucha tea no preservative and stored at room temperature storage was 0.979±0.049 % w/v which was higher than the alcohol safety limit (0.5% w/v). The alcohol content can exceeded the alcohol limit because yeast continues to ferment sugars, producing alcohol and carbon dioxide in the Kombucha tea sample under room temperature (Nummer B. , 2013). The legal threshold for alcohol content of non-alcoholic drink is 0.5% by volume and any beverage with an alcohol content > 0.5% by volume cannot be legally manufactured or sold in the Commonwealth without a liquor license (Agriculture, 2017). The alleged functional beverage only contained traces of alcohol (<0.5% w/v alcohol) under normal conditions. (Watawana M. N., 2015). A report by (Severson, 2010) stated that there were cases where some of the commercial producers of kombucha were forced to recall products from shelves when the alcohol content exceeded 0.5%. The alcohol level must remain below 0.5% alcohol by volume (ABV) during production and after shipment for commercial sale as a non-alcoholic refrigerated beverage (Alcohol and Tobacco Tax and Trade Bureau U.S., 2015).

Both the alcohol contents of Original Kombucha tea (no preservative and refrigerator temperature storage) and Pasteurized Apple Juice Kombucha tea (no

preservative and refrigerator temperature storage) were within the alcohol safe limit (< 0.5% w/v) with 0.250±0.007 % w/v and 0.279±0.008 % w/v respectively after 28 days of storage. This proved that cold refrigerator temperature storage is important to prevent generation and accumulation of alcohol in the Kombucha tea to exceed the safe alcohol limit (<0.5% w/v). (Kombucha Brewers International , 2017) stated that fermentation process generates trace amounts of ethanol naturally, hence, kombucha should be keep cold to ensure the quality remains consistent and compliant.

4.3 Plant design for production of Kombucha Tea

4.3.1 Overview

The mission of Kombucha tea manufacturing company is to produce high quality probiotic Kombucha tea to enhance the quality of life and contribute to a healthier future starting from a healthier gut. Kombucha tea is well known with its probiotic function that provide many health benefits to the consumers. However, Kombucha tea is still new to almost all the Malaysian. Not many people familiar with this beverage as well as the health benefit of Kombucha tea. The vision of Kombucha tea manufacturing company is to enlarge the Kombucha tea market throughout whole Malaysia and to educate the Malaysian regarding the health benefit of Kombucha tea.

4.3.2 Design Objective

The design objectives of Kombucha tea manufacturing factory were as below:

1. To produce probiotic Kombucha tea beverage that are safe to consume and with a shelf life of 8 months.
2. To produce Kombucha tea beverage with alcohol contain less than 0.5%.
3. To improve the overall quality control of production of Kombucha tea.
4. To build Kombucha processing line with the space allocated which is about 2000 cm (length) x 500 cm (width).
5. To design three rooms for production of Kombucha tea included raw material and preparation room, processing room as well as chilled storage room.

6. To design the Kombucha production line based on the Good Manufacturing Practices (GMP) recommended clearance between machine, flooring and ceiling.
7. To design a suitable Kombucha tea fermentation tank with hygienic consideration.
8. To create the SOP and SSOP involved in Kombucha tea processing.

4.3.3 Product Description

Table 4.3: Product description.

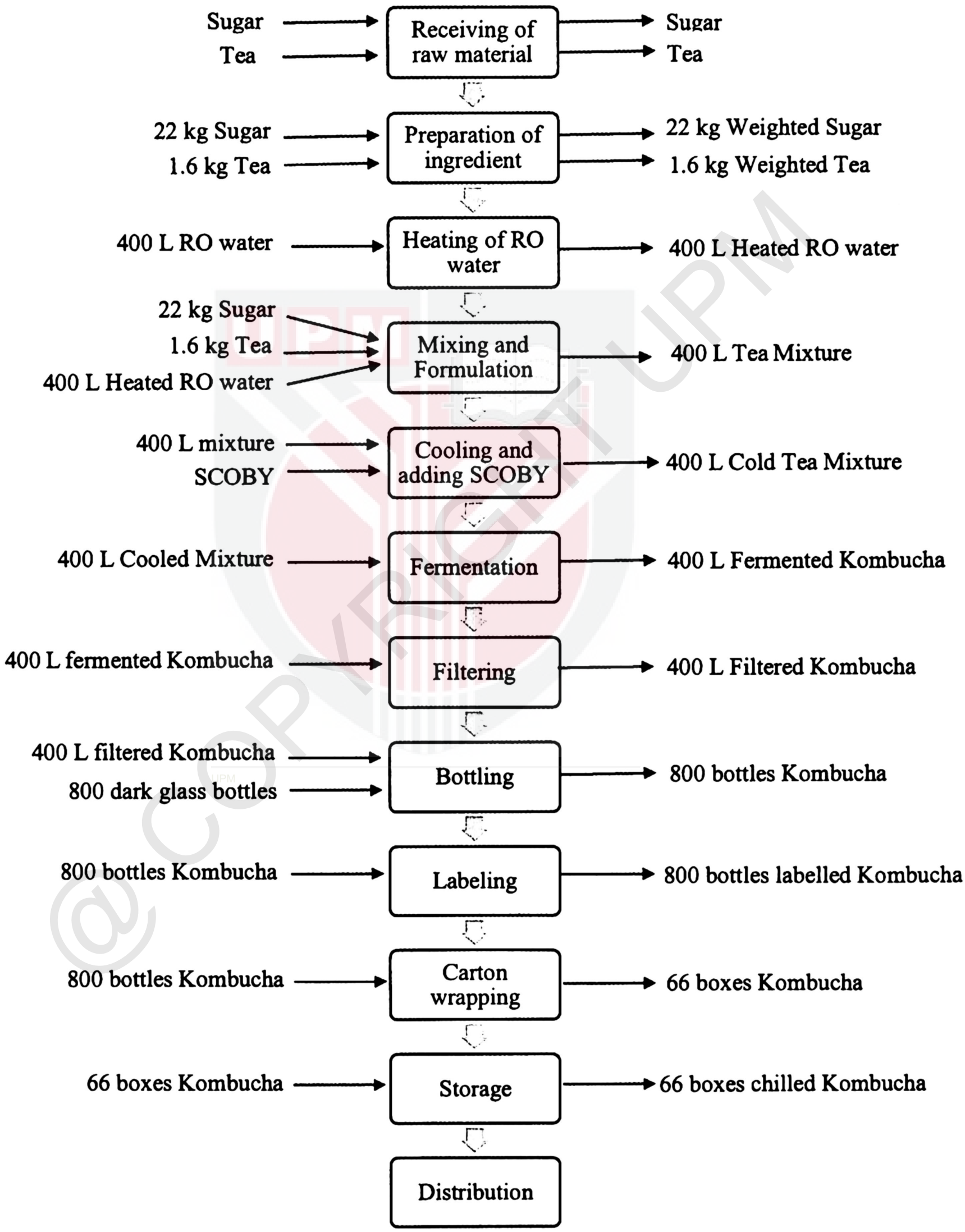
Product's name:	Kombucha tea
Product's volume:	500 ml
Product's shelves live:	8 months
Product's storage condition:	Fridge (temperature < 4°C)
Packaging:	500ml Dark Glass Bottle
Ingredient:	Boh Black Tea, CSR Sugar, SCOBY, RO water
Brief explanation:	Kombucha tea is a fermented tea that is naturally bubbly, fizzy with sweet taste. Kombucha is full with health benefits including detoxification, improve digestion, weight loss, cancer prevention, energy enhancement, cholesterol reduction and others.

	Kombucha is produced by mixing of sugar, tea and water with a symbiotic culture of bacteria and yeast which known as SCOBY. The mixture is then transferred into fermentation tank for fermentation process for a period of 14 days. The fermented tea is then filtered and filled into dark glass bottles. The finish goods are stored in chilled room with temperature condition less than 4°C before transported and distributed to the market.

4.3.4 Productivity

The volume of Kombucha produce per batch is 400 L. These 400L of Kombucha are filled into dark glass bottles. The quantity for each batch of production is 800 bottles/batch. The 800 bottles/batch of Kombucha will then undergo secondary packaging into 12 bottles/box. Therefore, there will be around 66 boxes of Kombucha tea produce per batch of production. Each batch of production will take 14 days to be completed if include fermentation. The factory will carries out one batch of production each day. The working hours per day is 8 hours and 5 working days per week.

4.3.5 Process Flow Diagram with Mass Balance



Process Steps Explanation

The process starts with receiving of raw materials which are the tea and sugar. The tea and sugar will be checked to ensure the goods received are in good condition and good quality. The raw materials will be labelled with batch number, quantity, date received and then stored in room temperature storage room according to first in first out (FIFO) arrangement. The tea and sugar are weighted based on the formulation before entering the main process step. The ratio of tea used in production of Kombucha tea is 1 L water to 0.004 kg of tea while the ratio of sugar used is 1 L tea to 0.055 kg sugar. Therefore, to produce 400 L of Kombucha tea, the weight of tea required should be 1.6 kg and sugar needed is 22 kg. At the same time when preparing tea and sugar, the reverse osmosis water is allowed to fill into the heating tank to be pre-heated to 60°C.There is a boiler that provide the heat to the heating tank to heat up the water through heat transfer. The water is further heated to 80°C when reach mixing and formulation step. This process will take around 2 hours to mix the tea and sugar with water evenly. After mixing and formulating process, the mixture is then transfer into a cooling tank to cool it to room temperature. When the mixture is cooled, it is then transferred to the fermentation tank through a pump. SCOBY is then added into the fermentation tank for 14 days of fermentation.

After 14 days of fermentation, the mixture is known as Kombucha. The pH of the Kombucha should be $\pm$ pH 2.8. Next, removes the SCOBY from the fermentation tank. The Kombucha is filtered to remove the sediments, residues or small solid particles. The Kombucha is then filled into dark glass bottles using a semi-automated fluid filling machine. The bottles are then label with important information such as the expiry date, ingredients used, nutrition facts, storage condition, company details and others before

moving to the carton wrapping process. The finished goods will be stored in chilled room with temperature <4°C before being transported or distributed to the retailers. A few samples will be selected from the finished goods to be sent for quality checking to ensure that the products produce reach the requirement or criteria of production. Quality checking of Kombucha may include identification of pH value, alcohol test as well as microbial test.

4.3.6 Mechanical Design

The main process in production of Kombucha tea is the fermentation process. Fermentation is a metabolic process takes place in the mixture of tea and sugar that produces chemical changes such as generating of acetic acid and gluconic acid through the action of enzymes from the previous tea and SCOBY. Fermentation of Kombucha tea normally takes place in the fermentation tank and hence, it is important to have an advance fermentation tank to aid the production of high quality Kombucha tea. There are a few features that must be considered in designing the fermentation tank of Kombucha tea as below:

1. Safety
2. Volume and temperature control
3. pH sensing and control
4. Sanitation
5. SCOBY removal

Safety

The material used to construct the fermentation tank for production of Kombucha should meet the food grade requirement. The material used should be inert and resist to corrosion since the fermentation process produces acid. Basically, stainless steel 304 is widely used in food production line to produce various types of food. Stainless steel 304 offers excellent resistance to corrosion by acidic liquid such as Kombucha tea. Direct contact of acid generated during fermentation causes the normal vessels to rust, giving the Kombucha tea a notable flavor and aroma. There are also others safety features that should be included in Kombucha tea fermentation tank design such as clean-in-place (CIP) facilities, modulating pump and transfer system, emergency shut-down systems and safe industrial control panel installation.

Volume and temperature control

Volume and temperature control during fermentation is the key factor to produce high consistency and repeatability Kombucha tea. The fermenter should process at optimal flow rates, volumes, and temperatures in order to make repeatable and consistent finished goods. There are sensors to detect the change in flow and temperature as well as controller to heat and cool the liquid precisely. The automation features that assure repeatability and precision in the fermentation process make work simple and easier.

pH sensing and control

The mixture of tea and sugar is fermented at optimum temperature until the desired pH obtained. There is a built in pH sensor to detect the change in pH of the Kombucha tea. The pH sensing and control system allow the operator to preset the pH band to either

notify the brewer automatically that the desired pH has been reached or to auto-cool the tank preventing over-fermentation.

Sanitation

Cleaning of fermentation tank involved removal of sugar, tea strain and yeast bacterial culture residues. It is time consuming and dangerous to enter a tank or vessel for cleaning works. The fermentation tank should be equipped with proprietary clean in place (CIP) technology to simplify the cleaning works. CIP is a method of cleaning the interior surfaces of pipes, vessels, process equipment, filters and associated fittings by simply pumping the sanitation solution to flow through the targeted area and generate impact to clean the features automatically without disassembly. The fermentation tank can be cleaned effectively and efficiently using the CIP cleaning method. Safety and sanitation are seamlessly managed in one simple operation.

SCOBY removal

The layer of SCOBY formed after 14 days of fermentation process in the fermentation tank is thick, heavy, and huge. The SCOBY should be removed once the fermentation is completed or when the Kombucha tea reached the desired pH value to prevent over fermentation. It will be very desired if the design of fermentation tank included a metal filter plate with automatic raising function to separate the SCOBY with the Kombucha tea mixture. The metal filter plate with automatic raising function is able to reduce the time and energy to remove the heavy SCOBY.

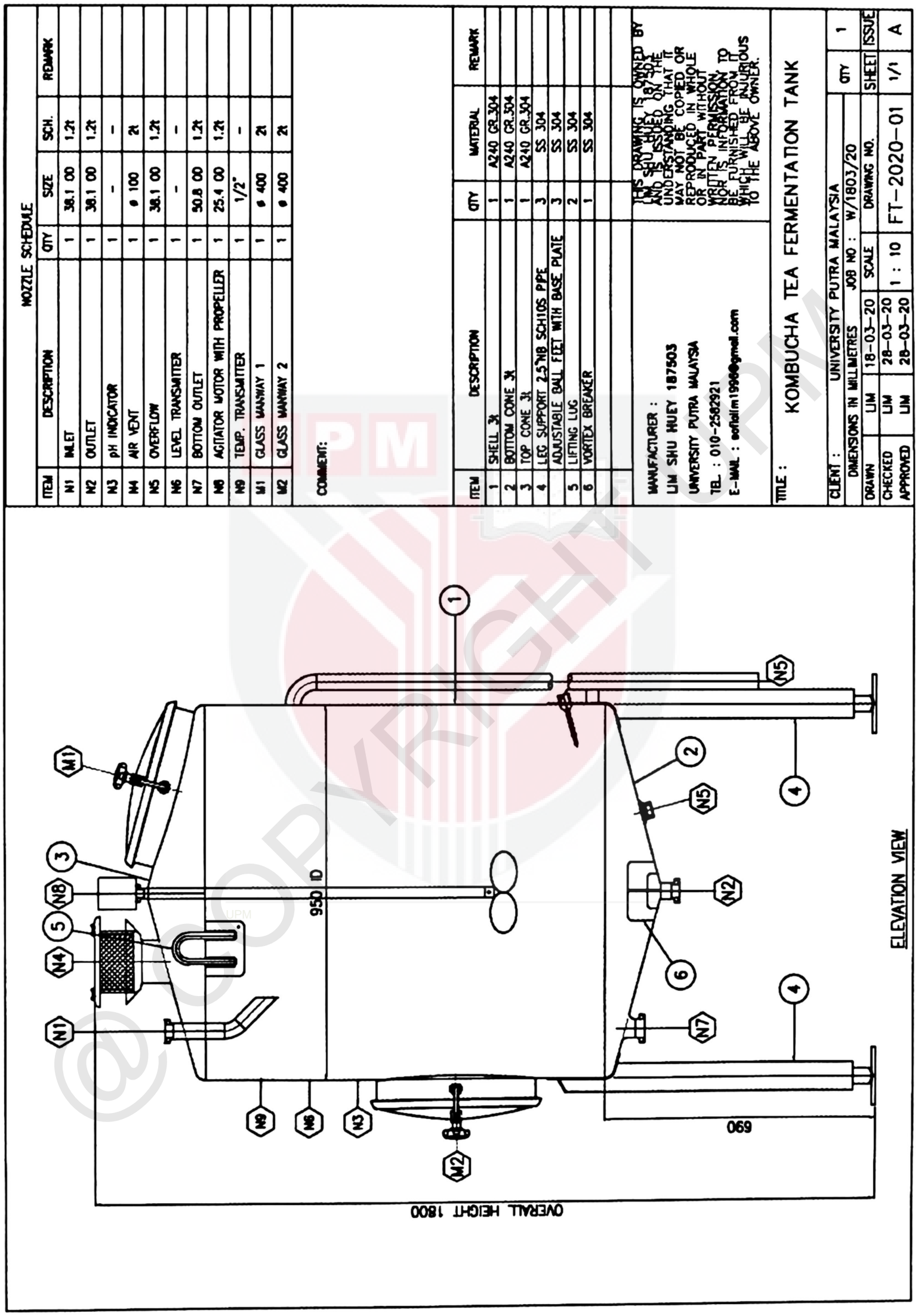


Figure 4.6: Mechanical drawing of fermentation tank.

Features of Fermentation tank and explanation:

Table 4.4: Feature of Fermentation tank and explanation.

Item	Description	Explanation
N1	Inlet	Inlet of Kombucha tea mixture.
N2	Outlet	Outlet of fermented Kombucha tea.
N3	pH Indicator	Detect the pH level of Kombucha tea during fermentation period and to prevent over fermentation.
N4	Air Vent	Allow exchange of gases between the inner tank with the external environment during fermentation period.
N5	Overflow	A hole which allow the overflow liquid to drain out. It is directly connected with drain out pipe.
N6	Level Transmitter	Detect the level of Kombucha tea in the fermentation tank.
N7	Bottom Outlet	Outlet for discharge of waste material.
N8	Agitator Motor with Propeller	A device to mix the fluid in the fermentation tank. The fluid need to be mix periodically to ensure balance fermentation.

N9	Temperature Transmitter	Detect the temperature inside the tank during fermentation period.
M1	Glass Manway 1	A protected glass hole to allow the operator to observe the growth of SCOBY during fermentation period.
M2	Glass Manway 2	A protected glass hole to allow the operator to observe the condition of Kombucha tea during fermentation. It is also important for tank maintenance such as periodic cleaning, inspection, and others servicing. The SCOBY formed during fermentation can be removed through this manway.
1	Shell	A cylindrical tank to keep the Kombucha tea mixtures.
2	Bottom Cone	The area where fermented Kombucha tea is being discharged.
3	Top Cone	A cover used to enclose the fermentation tank.
4	Leg Support	Provide support and hole the tank in a fixed position.
5	Lifting Lug	A plate with a hole in it where the hole is sized to fit a clevis pin for load transferring.
6	Vortex Breaker	A device used in tank to stop the formation of a vortex when fermented Kombucha tea fluid is discharged.

4.3.7 Plant layout

The spaces for production of Kombucha tea should be well planned and designed to create a safe and effective production site. In the design, it is assumed that the spaces allocated for Kombucha tea processing is 2000 cm (length) x 1150 cm (width). The plant layout should include raw material and preparation room, processing room and chilled storage room. The raw material and preparation room is the place where the raw material such as sugar and tea are checked, screened through foreign material detector and weighted based on the formulation before mixing process. The fermentation process, filtering, filling, labelling, and packaging process are carried out in processing room. On the others hand, it is essential to have a chiller storage room in Kombucha tea manufacturing industry. The chilled storage room is needed to store the finish goods (bottles of Kombucha Tea beverages) to prevent further fermentation that causes change in pH value, alcohol content as well as the taste and aroma of Kombucha tea. The suggested plant layout of Kombucha tea manufacturing industry based on measurement allocated is as shown in figure 4.10.

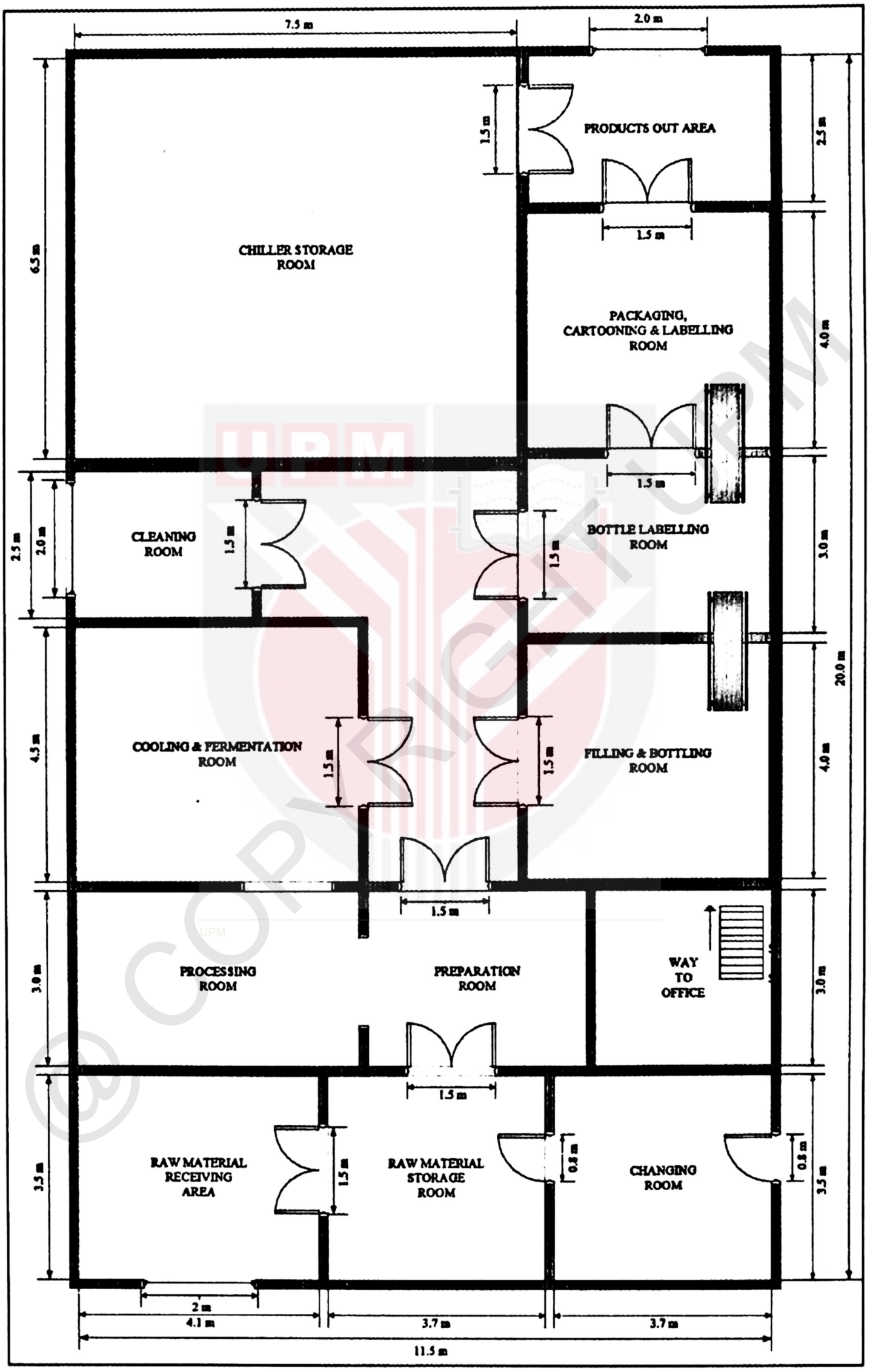


Figure 4.7: Plant layout of Kombucha tea manufacturing factory.

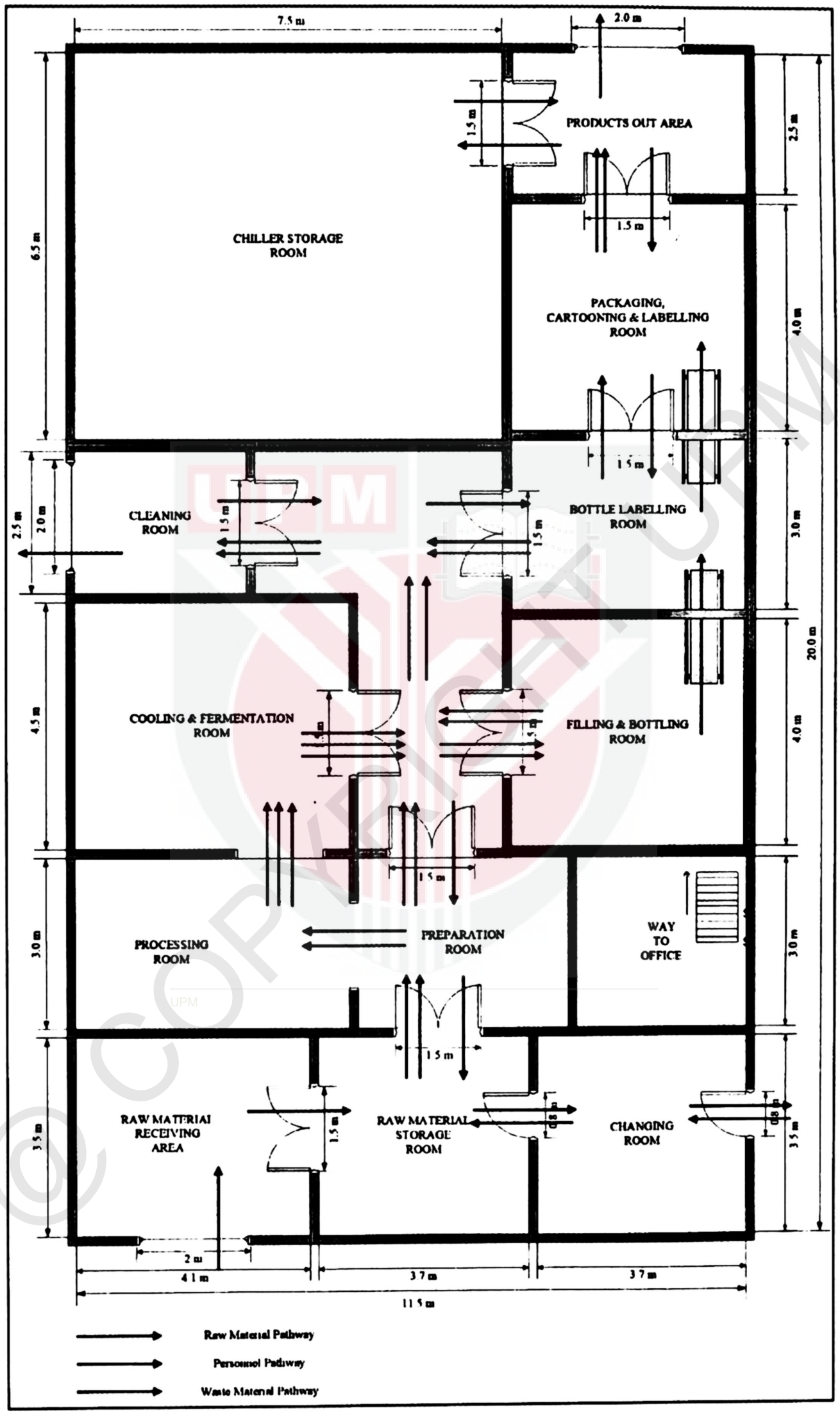


Figure 4.8: Plant layout with flow direction.

Plant layout flow description

The area is well divided into few rooms for Kombucha tea processing. There are raw material receiving area, raw material storage room, preparation room, processing room, cooling and fermentation room, filling and bottling room, bottle labelling room, packaging cartooning and labelling room, chiller storage room, products out area in the plant layout of Kombucha tea manufacturing industry. A changing room is prepared in the production area to allow the operators to change their attired and wash hand before enter production area. Besides, the plant layout also included a cleaning room for cleaning activities, placement of cleaning tools as well as to manage the waste products generated during Kombucha tea processing. Figure 4.11 shows the plant layout of Kombucha tea manufacturing industry with different pathways including raw material pathway (blue), personnel pathway (red) and waste material pathway (green).

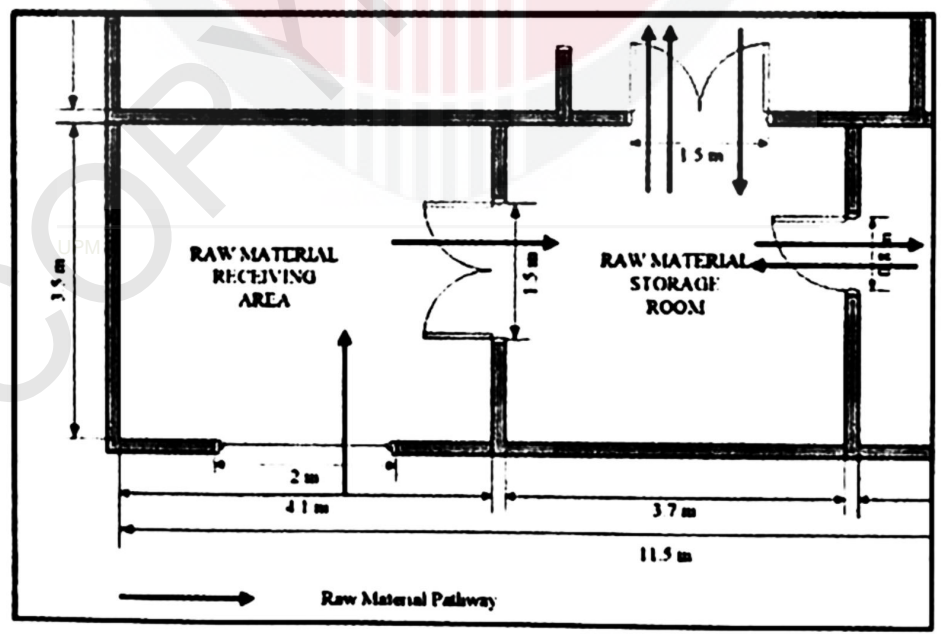


Figure 4.9: Raw materials enter pathway.

The raw materials will be received and enter the production area through the big front gate (Figure 4.12). All the materials such as tea, sugar and others packaging materials including the glass bottles, labelling stickers, corrugated boxes and plastic wrappers that

required in production of Kombucha tea will be unloaded in the raw material receiving area. These materials will be checked and labelled with batch number as well receiving date before stored in the raw material storage room and ready for further process. The Kombucha tea will be processed follow the production plan. The raw materials from the storage room will be transferred to preparation room follow first-in-first-out (FIFO) technique. In the preparation room, the tea and sugar are weight based on the Kombucha tea formulation. The tea and sugar prepared are transferred into the processing room for cooking and mixing process. The processing room is equipped with boiler (heat sauce) and multipurpose cooking tank to cook the tea. The sweet tea is then transferred to the fermentation tanks located in cooling and fermentation room. In cooling and fermentation room, the hot sweet tea is allowed to cool to room temperature. The room temperature sweet tea is then added with previous tea and SCOBY for fermentation process. The fermentation process of Kombucha tea is around 14 days.

After 14 days of fermentation, the fermented Kombucha tea is transferred to the filling and bottling room. The filling and bottling room should equipped with automatic filling capping machine as shown in figure 4.13 to increase the process efficiency. Automatic filling capping machine has multi functions in which it can carries out automatic bottle feeding, automatic liquid filling, automatic cap feeding, cap placing, cap screwing and automatic bottle out-feeding procedure. The filling station equipped with multiple filling nozzles and dual track conveying for fast and continuous production. After filling procedure, the bottles will be conveyed to the cap-placing machine, and start automatic cap placing, cap pressing and cap screwing process simultaneously. This

multipurpose machine design of cap-sorting, cap-pressing and cap-screwing process not only shorten the process time but also saving the space in production area.

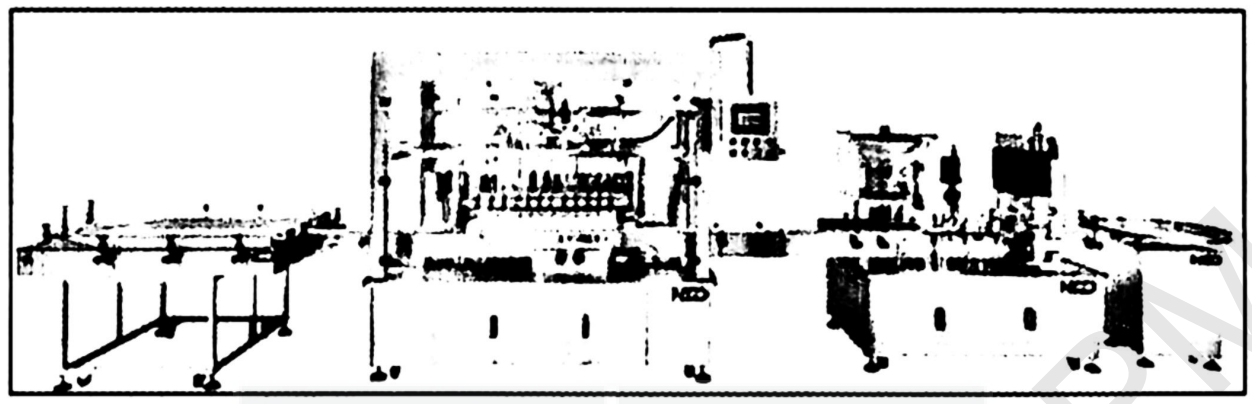


Figure 4.10: Automatic filling and capping machine.

The bottles of Kombucha tea are then conveyed to the bottle labelling room for labelling process. The bottle of Kombucha tea are labelled with sticker printed with necessary information such as nutritional fact, expiry date, ingredient and brand name. The bottle labelling process can be carried out effectively using fully automated round bottle labelling machine as shown in figure 4.14.

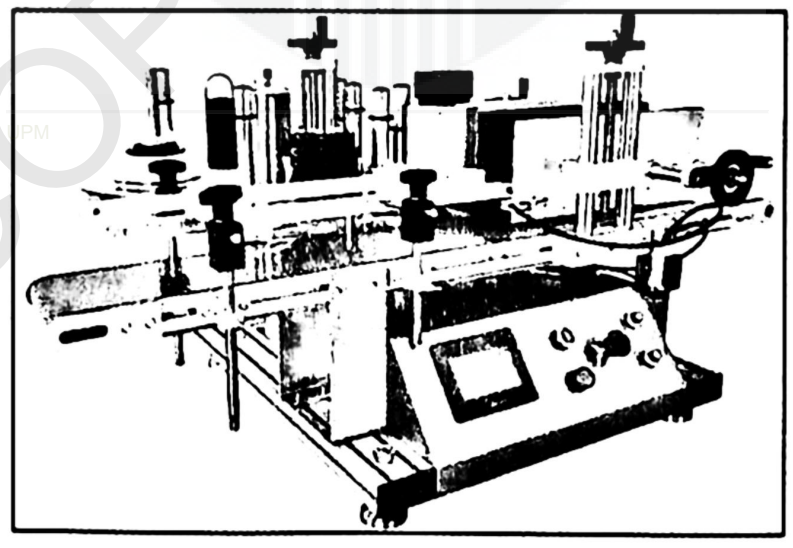


Figure 4.11: Fully automated round bottle labelling machine.

The bottle of Kombucha tea with label are then conveyed to the packaging, cartooning, and labelling room for final step of process. The finish goods are stored in the chiller storage room before transported and distributed to the market.

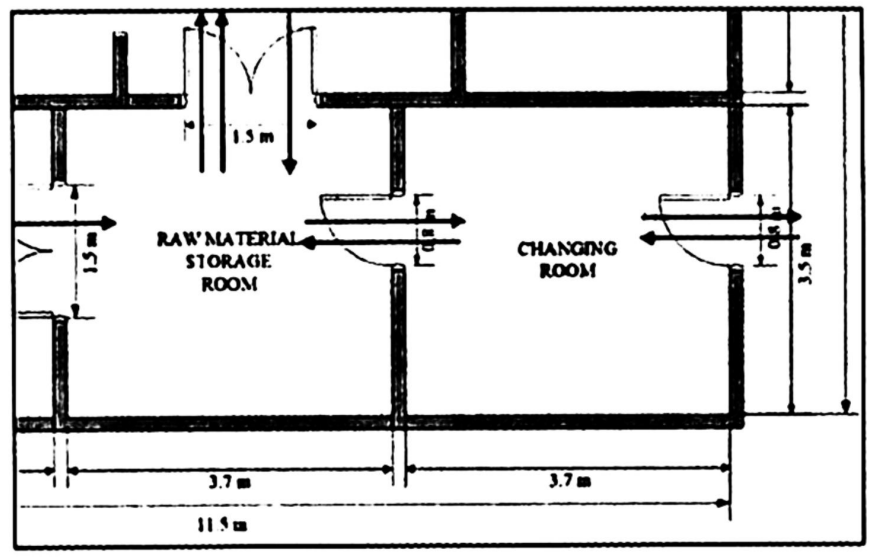


Figure 4.12: Personnel enter pathway.

The red direction arrow demonstrates personnel pathway. The operators can enter the production area through the changing room (figure 4.15). The changing room is an ideally single entrance to the food production area for all staff, visitors or contractors. The changing room should be facilitated with rack, hand washing and drying facilities. The rack is prepared for the staffs to keep their clothing and personnel belongings. All the stuffs must complete the personnel hygiene entry sequence includes changing of clothes and hand hygiene before enter the production area. It will be desired if facilities for cleaning and laundering factory clothing or footwear are included in production area.

The waste material flow is represented by the green arrow as shown in figure 4.11. Based on the international standard operation (ISO), the waste materials should flow from high risk zone to low risk zone. Basically, high risk zone in food production area is the place where the products are treated and filled in packaging material to prevent any contamination, while low risk zone is normally the processing room where the raw materials are washed, weighted, prepared and ready for further processes. In the Kombucha tea production area, the waste materials generated from different processing rooms are all collected in the cleaning room before transferred out for dispose purpose.

There is a back door in the cleaning room so that the waste materials can be directly transferred out from the production area without contaminate others processing room.

Chiller Storage Room

A chiller storage room is designed in Kombucha tea production area to store the finish goods. Kombucha tea must be stored in chiller temperature (<4°C) to slow down or prevent further fermentation that causes drop in pH and formation of alcohol inside the bottles of Kombucha tea.

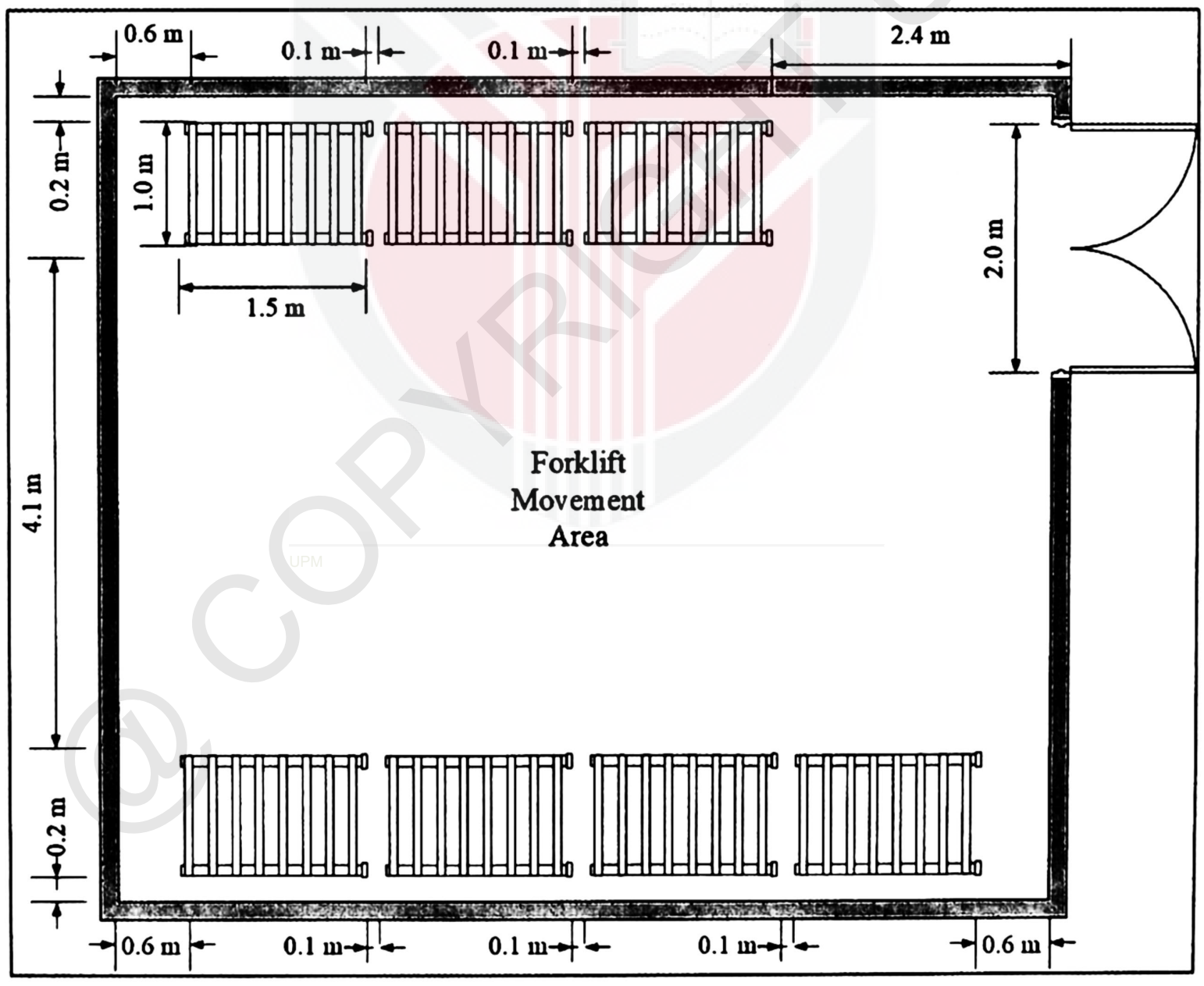


Figure 4.13: Chiller storage room of Kombucha tea.

The layout of storage room for an area of 6.5 m x 7.5 m is as shown in figure 4.16. The spaces allocated for the chiller storage room is small, hence planning and good arrangement system are essential to keep the finish goods of Kombucha tea. From the entrance of chiller storage room, there are racks arrange in parallel on the left and right. The rack near the entrance is smaller compared to the rack at opposite to enable enough spaces for movement of forklift at the entrance during transportation of heavy loads. The example of rack is as shown in figure 4.17. It is assumed that one pallet will occupied area of 1.0 m x 1.5 m and therefore this storage room is estimated available to keep 7 pallets of Kombucha tea in one layer in which 3 pallets on right while 4 others pallets on the left.

Figure 4.14: Pallet racking in chiller storage room.

The stack alignment must be perpendicular to the direction of air movement and the stacks placed close to the cooler in order maintain the circulation of air in a partly filled room. Stacking of pallet must be suitable for the layout prescribed, respecting loading limits and allowing space between the stacks and walls as well as below the pallets. (Krishnakumar, 2002) The palletization layout plan must take account of distances between cold store elements. They are in the range of 5 cm to 10 cm between

pallets, 15 cm to 20 cm along the walls and a stacking limit of 40cm to 60 cm below the ceiling. Based on the chiller storage room designed, stacking of pallets can be up to 3 layers with estimated height of 5 m to 6 m required. Safety concern is important in pallets stacking especially for those easy broken finished goods such as glass bottles Kombucha tea. The layer of stacking should not be too high in order to reduce the risk during handling or operation.

A chiller storage room is important in Kombucha tea manufacturing factory to keep the drinks. A good chiller storage room can keep the goods at constant temperature and maintain the quality of the products. The design of chiller storage room is depended on the area of production, type of products as well as storage loads capacity. When all the details are obtained, layout with suitable arrangement and stacking system should be constructed. Others important consideration that must be taken in designing of chiller storage room included the material of construction, insulation system, air circulation system, flooring, type of refrigerant, heat transfer, energy required as well as cost needed for construction of chiller storage room.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Finding

Fermentation of Kombucha tea take less than 14 days to drop to < pH 3.5. The rate of fermentation was high at the early stage with significant decrease ($P<0.05$) in pH value. The rate of fermentation at after stage is lower with minimum change in pH value. At the beginning of the fermentation, the concentration of sugar in Kombucha tea was high. The yeast hydrolyze sugar into glucose and fructose to produce ethanol and finally, AAB transform ethanol into acetic acid, nonetheless the production of gluconic and glucuronic acids are also significant which lead to decrease in pH value. However, the rate of fermentation decreases at later stage because the high medium acidity and lower amounts of sugar inhibit the fermentation activity of yeasts and production of ethanol, and indirectly reduce production of acetic acid.

Adding of preservative into Kombucha tea is not recommended to extent the shelf life of Kombucha tea. There were no significant differences ($P<0.05$) when compared the trend of pH value between Kombucha tea without preservative with the Kombucha tea added with various type of preservative when all the samples were stored in chiller. Kombucha tea should be stored in cold storage room to slow down fermentation as well as to ensure safety and stability of Kombucha tea.

To enhance the taste and flavor of Kombucha tea, fresh fruit juice can be added. Addition of flavoring into Kombucha tea does not perform significant effect on the pH value on ongoing storage. However, unpasteurized fruit juice is not suitable to be added

into Kombucha tea because it causes growth of molds and formation of undesired material which greatly reduced the shelf life and safety of Kombucha tea. Fruit juice can be added in Kombucha tea in order to enhance the taste, however, it is necessary to pasteurize the fruit juice in order to remove bacterial and pores before adding into Kombucha tea to prevent growth of molds and extend the shelf-life. Kombucha tea products should be stored in chilled temperature condition to stop fermentation as well as to prevent it to become too acidic.

The alcohol content of Kombucha tea increases when the storage time increases. The alcohol content of sample stored in room temperature condition had exceed the alcohol acceptable limit (<0.5% w/v) and classified as unsafe Kombucha tea. In contrast, the alcohol content of Kombucha tea samples that stored in chiller were still within the safety range after 28 days of storage. Therefore, Kombucha tea should be stored in the chilled temperature condition (4° C) to prevent generation of alcohol in Kombucha tea until exceeding the alcohol acceptable limit. Manufacturing factory of Kombucha tea must be equipped with chiller or chilled storage room in order to store the bottles of Kombucha tea before distribution.

Kombucha production line should be designed based on guidance and rules of food industry. Plant design of Kombucha tea manufacturing factory is important to create a sustainable business. The proposed plant design in this study can be used as references for Kombucha tea manufacturing factory. However, further research and experimental works should be carried out to implement the method in real practices.

5.2 Limitations of The Study

There are a few limitations have been identified throughout the process of conductivity in this study. First and foremost, the experiment was conducted on a small-scale preparation to replicate the large-scale process line. The results might not tally for all due to different in handling and preparation method to produce Kombucha tea. All the samples were prepared in small amount and therefore the results obtained may contain deviation compared to the samples produced in a large batch.

This study was done using the existing facilities and equipment in Food and Process Engineering Laboratory of University Putra Malaysia. The existing facilities and equipment are aging which then affect the accuracy of the results. The pH of Kombucha tea were calibrated using the digital pH meter with accuracy ± 0.01 pH. The results obtained may have higher accuracy if using a new and more advance device. Besides, the laboratory is not equipped with pasteurizer. Hence, the pasteurization of fresh fruit juice was carried out in a manual fashion and this condition might slightly affect the overall results.

For the alcohol test analysis, gas chromatography method was used to identify the ethanol content of Kombucha tea. This method required a gas chromatography instrument with specific columns and solution to analyze the alcohol content of the samples. This instrument induces high cost and not prepared in place. Therefore, all the samples required to send to UNIPEQ Sdn Bhd in University Kebangsaan Malaysia, Bangi for alcohol test. The fees for alcohol test are expensive (RM 180 /per sample) and these had limit the scope of research in this study due to limited financial resources (RM 500). Consequently, such limitations might be reflected in the results of the study.

5.3 Recommendations for Future Research

The research and study of pH on large batch of Kombucha tea should be carried out to identify whether similar results can be obtained as compared to the production of small batch Kombucha tea. Further study can be done in the processing method, storage method and stability of Kombucha tea which are crucial to improve the quality and safety of Kombucha tea to be consumed by the consumers. Also, simulation test of Kombucha tea production can be done using design software or simulator such as SuperPro Designer and Aspen BPS to determine the viability before implementing in real industry practices without wasting valuable resources. In conclusion, this study contains improvise space for further research.

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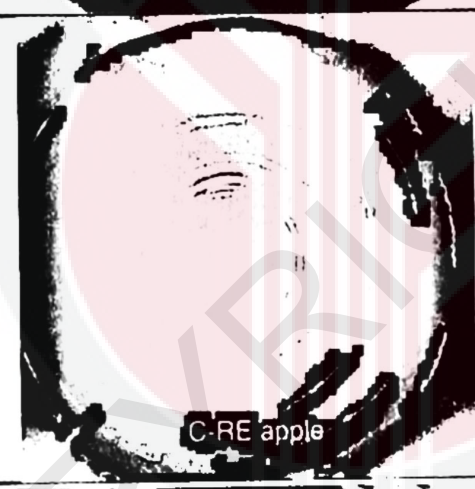
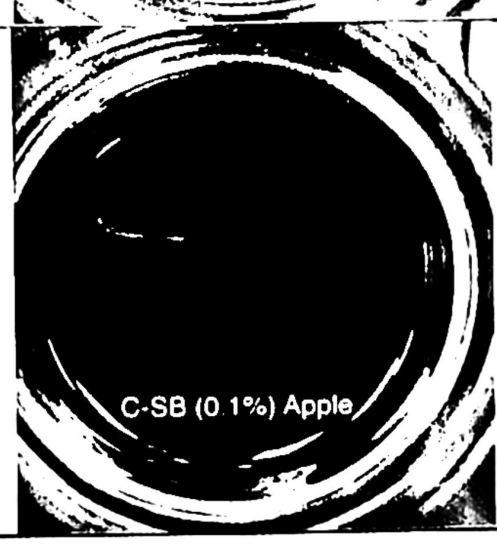
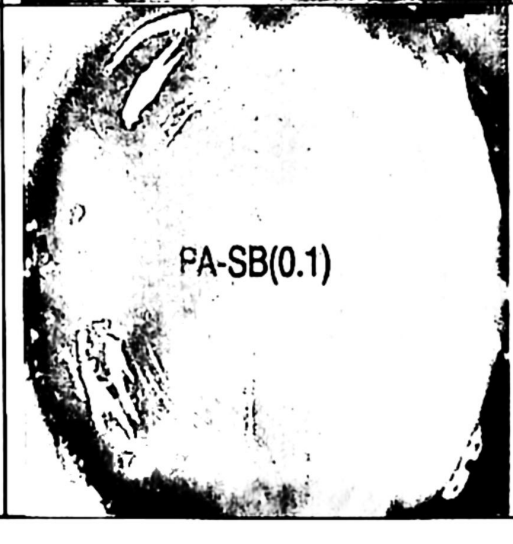
APPENDICES

A.1 Analysis of Kombucha tea

A.1.1 The changes of Kombucha tea with unpasteurized apple juice after 14 days of storage.

Label	pH	Observation	Justification
A-R	3.09		• Presence of molds. • Having unpleasant smell, sour and acidic smell. • The drink is unclear with high turbidity as well as present of sediments. • Formation of large bubbles. • The pH value is low.
A-RE	3.19		• Formation of mucus like textures and sediment at the bottom of the bottle sample. • Having good smell like apple cider beverage. • The pH value is at the safety range.
A-PS	3.60		• Presence of thin layer of membrane residues float on the top of the sample solution. • The drink is clear with less amount of residues • Having normal sour and acidic smell. • The pH value is at the safety range.
A-SB	3.57		• Little formation of molds with less amount of sediments at the bottom of the bottle sample. • Having good smell like apple cider beverage. • The pH value is at the safety range.

A.1.2 The changes of Kombucha tea with pasteurized apple juice after 14 days of storage.

Label	pH	Observation	Justification
PA-R	2.91		• Formation of thick SCOBY with thickness ± 1cm. • No formation of molds. The liquid below is clear without present of any sediments. • Strong fermentation, sour and acidic smell. • The pH value is low.
PA-RE	3.42		• No formation of SCOBY, • No formation of molds. • Having good smell like apple cider beverage. • The pH value is at the safety range.
PA-PS	3.01		• Formation of thin layer of SCOBY with thickness ± 0.1cm. • The liquid is clear without presence of molds. • Having normal sour and acidic smell. • The pH value is considered low.
PA-SB	2.83		• Formation of thin layer of SCOBY with thickness ± 0.2cm. • The liquid is clear without presence of molds. • No formation of bubbles. • Having normal sour and acidic smell. • The pH value is considered low.

A.1.3 Certificate of Analysis (Alcohol analysis using gas chromatography for Kombucha tea with pasteurize apple juice).

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Ref : ULUKM/1032/20
Date : 15/01/2020
Page : 1 of 1

CERTIFICATE OF ANALYSIS

Name of Customer : MS LIM SHU HUEY / DR NOR AMAIZA MOHD AMIN
Address : Department Of Engineering (Process And Food)
Faculty of Engineering, Universiti Putra Malaysia,
43600, UPM Serdang, Selangor
Tel. No : 010-2582921
Sample Description : One (1) samples describe as *Kombucha Tea*
Sample Ref. No : U0054/20
Date of Receipt : 13/01/2020
Test Performance Date : 14/01/2020

ANALYSIS RESULTS
(As per sample)

Sample Ref. No	Sample Description	Alcohol as Ethanol, %(w/v)	
U0054/20	*Kombucha Tea*	0.286	0.273

Standard Test Method: In-house Method, Ref. No STP/HA/02/Gas Chromatography (GC)

Remarks:

a) *B.D.L Below Detection Limit*
b) *Opened / balance samples will be discarded two weeks after issuance of Certificate of Analysis.*

SIRIM ISO/IEC 17025
TESTING
SAMM NO. 398

Bridge To A Better Product
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