Development and characteristics of green tea kombucha

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Abstract

Fermentation by microorganisms plays an important role in the development of manufactured food due to its ability to extend the shelf-life of the products as well as improving the nutritional value and sensory properties. Demand and interest in the consumption of fermented products, such as yoghurt, cheese, buttermilk and meat has continued to increase in the past few decades, due to their beneficial health effects which include prevention of chronic disease and enhancement of the immune system. However, because of the health challenges of these products such as lactose intolerance, high cholesterol and fat content, as well as protein allergies, consumer interest in the consumption of non-dairy and plant-based fermented products such as water kefir and kombucha is growing.

Kombucha is a traditional refreshing home-made beverage with a slightly acidic, sweet and alcoholic taste, which is thought to have originated in Germany, China and Russia, but is now consumed worldwide. Kombucha is usually produced by the fermentation of tea and sugar with a symbiotic consortium of acetic acid bacteria and yeasts, commonly known as the kombucha starter culture. The physico-chemical characteristics, microbial profiles and sensory properties of kombucha are significantly affected by fermentation conditions including sugar concentration, fermentation time and temperature. The majority of previous studies have focused on the comparison of different substrates for kombucha preparation and their effects on the final composition of kombucha. Therefore, there is scanty information in the development and characteristics of kombucha under different production conditions. The present study investigated the effects of sugar concentration, fermentation time and temperature on the fermentation of green tea kombucha aimed at optimising the process to develop a consistent high quality beverage.

The development and characteristics of green tea kombucha were investigated in four-integrated experimental phases. Phase 1 enumerated the microflora in the kombucha starter culture (acetic acid bacteria, lactic acid bacteria and yeasts). The effect of fermentation time (7, 10 and 14 days) on the development of kombucha was studied in Phase 2 in order to select the optimum fermentation time. In Phase 3, the effects of two sugar concentrations (7% and 10%) and two fermentation temperatures (22℃, 24℃) on the physico-chemical, microbial and sensory characteristics of green tea kombucha were studied, with the aim of selecting the optimum sugar concentration and fermentation temperature for the development of green tea kombucha. The antibacterial activity of the final optimised green tea kombucha were investigated and the stability of the beverage was monitored during storage (4℃) for 4 weeks in Phase 4. Various analyses of green tea kombucha samples were conducted during fermentation and storage in order to investigate the physico-chemical, microbial and sensory characteristics of the beverage: sugars, organic acids, ethanol, antioxidant, titratable acidity (TA) and viable cell counts (VCC) of kombucha microorganisms were analysed, pH, total soluble solids (TTS) and colour were also measured.

Acetic acid bacteria (6.08±0.06 log cfu/ml) and yeasts (7.13±0.07 log cfu/ml) were present in the kombucha starter culture used in this study, while no lactic acid bacteria were found. Results from Phase 2 showed that fermentation time contributed to the physico-chemical, microbial and sensory properties of green tea kombucha. In Phase 2, TA increased steadily from Day 7 (0.36±0.02 - 0.42±0.04) to Day 14 (0.88±0.04 - 1.01±0.06) ($p<0.05$), while pH, total soluble solids, VCC and overall consumer acceptability decreased ($p<0.05$). In Phase 3, kombucha samples containing 7% or 10% sugar and fermented at 24℃ for 7 days were characterised by higher levels ($p<0.05$) of organic acids with lower pH, TSS and VCC than kombucha fermented at 22℃. No significant differences ($p<0.05$) in colour, VCC and levels

of organic acids were observed between the samples containing 7% and 10% sugar during fermentation. Based on the physico-chemical, microbial and sensory characteristics of green tea kombucha beverage in Phases 2 and 3, the optimum fermentation conditions were kombucha containing 7% sugar and fermented at 22℃ for 7 days.

The results of the disc diffusion studies showed that the final optimised green tea kombucha had antibacterial activities against *Escherichia coli* 111, *Listeria monocytogenes* 15E03-1, *Salmonella typhimurium* ESR3479, *Staphylococcus aureus* MU-A57 and *Pseudomonas aeruginosa* MU-A26. High quantities of antioxidants (gallic acid = 5.7±0.04 µg/ml, EGC = 130.89±6.86 µg/ml, EGCG = 152.26±39.70 µg/ml and ECG = 41.11±16.23 µg/ml) were also present in this beverage. These observations suggested that consumption of green tea kombucha may exert beneficial health effects. During storage (4℃) for 4 weeks, the colour of the optimised green tea kombucha was stable and the consumer acceptability of green tea kombucha beverage remained high.

Green tea kombucha containing 7% sugar and fermented at 22℃ for 7 days was well-liked by consumer panellists (n=60) and this beverage contained 0.35±0.03% (w/v) gluconic acid, 0.31±0.00% (w/v) acetic acid and high levels of certain antioxidants which may confer beneficial effects on human health.

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List of Abbreviations

a*	redness-greeness
A. aceti	*Acetobactor aceti*
A. pasteuriaus	*Acetobactor pasteuriaus*
AAB	acetic acid bacteria
Acs	Acetyl-CoA synthetase
Adh	Alcohol dehydrogenase
ADH	Alcohol dehydrogenase
Ald	Aldehyde dehydrogenase
ALDH	Aldehyde dehydrogenase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
ATP	Adenosine triphosphate
b*	yellowness-blueness
C	Catechin
CFU	Colony forming per Unit
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSL	D-saccharic acid -1,4- lactone
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
ENO	Enolase
FBPA	Fructose bisphosphate aldolase
FSANZ	Food Standards Australia New Zealand
g	gramme
G. oxydans	*Gluconobacter oxydans*
GA	Gallic acid
Ga. Xylinum	*Gluconacetobacter xylinum*
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
GC	Gallocatechin
GC	Gas chromatography
HK	Hexokinase
HPLC	High performance liquid chromatography
L	Litre
L*	Lightness
LAB	Lactic acid bacteria
Min	Minute
mL	millilitre
mm	millimetre
MRS	de Man, Rogosa and Sharpe
NAD	Nicotinamide-adenine dinucleotide
NZ	New Zealand
OXPHOS	Oxidative phosphorylation
PDC	Pyruvate decarboxylase
Pdc	Pyruvate decarboxylase,

List of Abbreviations

Pdh	Pyruvate dehydrogenase
PFK	Phosphofructokinase
PGI	Phosphoglucoisomerase
PGK	Phosphoglycerate kinase
PGM	Phosphoglyceromutase
PQQ	Pyrroloquinoline quinone
PYK	Pyruvate kinase,
RI	Refractive index
SCOBY	Symbiotic colony of bacteria and yeast
SD	Standard deviation
spp.	species (plural)
TA	Titratable acidity
TCA	Tricarboxylic acid
TFA	Trifluoroacetic acid
TPI	Triose phosphate isomerase
TSS	Total soluble solids
UQ	Ubiquinone
UV	Ultra violet
VCC	Viable cell counts
YGC	Yeast extract glucose chloramphenicol
YPM	Yeast peptone mannitol

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1. Introduction

1.1 Background

It is well-documented that fermentation extends the shelf-life of products as well as improving the nutritional value and sensory characteristics of the food (Adams & Mitchell, 2002; Smid & Lacroix, 2013; Marsh et al., 2014). Fermentation of food is therefore described as an economical method for the processing and preservation of food. Fermentation by microorganisms is one of the oldest methods of manufacturing and preserving food, with the earliest records dating back to 6000 BC in the Middle East, describing the fermentation of vegetables, milks and meats (Caplice & Fitzgerald, 1999; Li et al., 2011).

There are four main roles of fermentation in food processing, comprising the formation of inhibitory metabolites, such as organic acids (acetic acid, lactic acid, propionic acid and formic acid), ethanol and bacteriocins (Bourdichon et al., 2012). Secondly, by inhibiting growth of pathogens, food safety can be improved, thus fermentation can extend shelf-life and remove certain toxic components (Bourdichon et al., 2012). Thirdly, through the synthesis of essential amino acids and vitamins, and improvement of fibre and protein digestibility, enhancing of micronutrient bioavailability and degradation of anti-nutritional factors during fermentation, there is a resultant increase in nutrient levels in the food (Giraffa, 2004; Smid & Lacroix, 2013). Finally, fermentation enriches the diversity of the diet by providing different textures, flavours and aromas (Valyasevi & Rolle, 2002; Wolfe & Dutton, 2015).

Manufacturing techniques for fermented foods vary world-wide, however the basic substrates remain the same; microorganisms and raw materials, such as fruit, vegetables, meat and milk. The different manufacturing techniques can be divided into four primary fermentation processes: lactic acid, acetic acid, alcoholic and alkali fermentation (Blandino et al., 2003). Lactic acid fermentation is mainly based on the use of lactic acid bacteria, primarily for milk and cereal products, with popular products including probiotic yoghurt, kefir beverages and sourdough bread. Acetic acid fermentation is mostly conducted by *Acetobacter* species and is usually used for the fermentation of cocoa beans, vinegar, acidic beers and a number of slightly acidic beverages such as water kefir, milk kefir and kombucha (Pothakos et al., 2016). Alcoholic fermentation is carried out by yeast and results in the production of ethanol, with

alcoholic drinks such as wine, beer and sake being common products (Flikweert, 1999). Fish and seeds are reported to undergo alkaline fermentation by mixed cultures dominated by *Bacillus subtilis.* Japanese natto from cooked soybeans, ugba from African oil beans and owoh from cotton seeds are examples of products of alkali fermentation (Wang & Fung, 2008).

The preparation of many traditional or indigenous fermented beverages and foods (water kefir, kombucha, yoghurt and Korean kimchi) remains popular, not only being carried out directly by consumers at home, but also by large-scale industry production (Aidoo, Nout & Sarkar, 2017). The popularity of fermented beverages is often attributed to their purported health benefits (e.g. prevention of heart and chronic diseases, enhancement of the immune system), improved nutritional attributes (enrichment of vitamins and essential amino acids) and improved sensory properties of these products. For these reasons, interest in the consumption of fermented foods such as yoghurt, cheeses, buttermilk, fermented sausages, kefir and kombucha beverages has increased worldwide (Blandino et al., 2003; Giraffa, 2004; Bourdichon et al., 2012). However, due to health challenges, such as lactose intolerance, high cholesterol and fat content, as well as milk protein allergies, there is growing consumer interest in non-dairy and plant-based fermented foods and beverages (Prado et al., 2008; Gawkowski & Chikindas, 2013; Kumar, Vijayendra & Reddy, 2015). Hence, kombucha, which is a natural, healthy and functional plant-based fermented beverage has recently gained attention (Dufresne & Farnworth, 2001; Malbaša et al., 2006; Jayabalan et al., 2008; Jayabalan et al., 2014).

Kombucha, also known as tea fungus, tea kvass, Manchurian mushroom, champignon de longue vie, and chainii grib (Steinkraus et al., 1996; Greenwalt, Steinkraus & Ledford, 2000; Malbaša, 2011; Aidoo, Nout & Sarkar, 2017) has a long history as a popular, healthy and functional traditional fermented beverage (Roussin, 1996; Velićanski, Cvetković & Markov, 2013; Jeszka-Skowron, Krawczyk & Zgola-Greskowiak, 2015; Chakravorty, 2016). The origin of kombucha can be traced back to 220 B.C. in northeast China (Manchuria), where it was noted for its healing benefits and detoxifying and energizing properties (Lončar et al., 2006). With the expansion of trade routes, kombucha spread throughout the world, and it is now popular in many western countries (Jayabalan et al., 2014).

Kombucha is a slightly sweet, acidic, sparkling and refreshing beverage, which is produced by the fermentation of sugar and tea with a symbiotic consortium of bacteria (acetic acid bacteria

and a small number of lactic acid bacteria) and yeasts named “tea fungus” (Dufresne & Farnworth, 2000; Jayabalan et al., 2006; Yang et al., 2010; Kallel et al., 2012). The composition of tea fungus varies with the climate and geographical conditions, and is also dependent on the origin of the mother culture (Bauer and Petrushevska, 2000; Malbaša et al., 2011). However, it primarily consists of strains of *Acetobactoe xylinum*, *Zygosaccharomyces, Saccharomyces, Schizosaccharomyces, Candida*, *Brettanomyces*, *Pichia* and *Torulopsis* (Chu & Chen, 2005; Cvetković & Markov, 2013; Vukiv et al., 2014; Sun, Li & Chen, 2015; Aidoo, Nout & Sarkar, 2017).

There are a large variety of possible substrates for the preparation of kombucha, such as red wine, fruit juices (grape juice, apple juice, etc.), dark beer, lemon balm, medicinal herbs, as well as lactose, molasses and whey (Malbaša, Lončar & Djuric, 2008; Četojević-Simin et al., 2012; Velićanski, Cvetković & Markov, 2013; Jayabalan et al., 2014; Vukiv et al., 2014; Ayed, Abid & Hamdi, 2017). However, black tea or green tea with organic sugars or materials containing sucrose are the most suitable and popular materials for the fermentation of kombucha beverages (Jayabalan, Marimuthu & Swaminathan, 2007; Iličić et al., 2012; Jayabalan et al., 2014). Sucrose is the carbon source for kombucha culture growth, it is hydrolysed by the yeasts into glucose and fructose, which are further utilized by acetic acid bacteria during fermentation (Chen & Liu, 2000). The main metabolic products from sucrose identified in kombucha are acetic acid, gluconic acid, glucuronic acid, ethanol, and glycerol (Greenwalt, Steinkraus & Ledford, 2000; Dufresne & Farnworth, 2000; Kallel et al., 2012).

Tea, as another important and suitable substrate for kombucha fermentation, provides essential nitrogen materials such as purine derivatives, caffeine and theophylline for the growth of the tea fungus (Velićanski, Cvetković & Markov, 2013) and it is the main source of polyphenols in the final product. Tea is the second most widely consumed beverage in the world after water (Yang & Landau, 2000; Yang, Baldermann & Watanabe, 2013). The consumption of tea is an ancient and traditional practice, originating from China and India about 5000 years ago (Dufresne & Farnworth, 2001; Cabrera, Artacho & Gimenez, 2006). Tea is made from the dried leaves of the *Camellia sinensis* plant, and approximately 3 million tons are produced and consumed annually (Yang and Landau, 2000). There are four major categories of tea based on the manufacturing process: unfermented green tea, post-fermented Pu-er tea, semi-fermented oolong tea and fully-fermented black tea (Cabrera, Artacho & Gimenez, 2006; Sang et al., 2011;

Bansal et al., 2013). The different types of tea are classified by the degree of fermentation and have different properties, including colour, appearance, taste and aroma (Senanayake, 2013).

Previous studies indicate that green tea exhibits stronger antioxidant activity than black tea and other fermented teas, primarily as a result of its higher catechin content (Manzocco et al., 1998; Cabrera, Artacho & Gimenez, 2006). Moreover, the caffeine content in green tea is higher than other teas, which is more favourable for the growth of microorganisms (Velićanski, Cvetković & Markov, 2013). Thus, green tea is more suitable for the preparation of kombucha tea beverages than other teas. As an abundant source of phenolic polyphenols, green tea is rich in various types of catechins, including (+)-catechin (C), (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC) and (+)-gallocatechin (GC). Together these phenolic compounds comprise 30% of the dry weight of green tea leaves (Balentine et al., 1997; Cabrera, Artacho & Gimenez, 2006, Chacko et al., 2010; Ananingsih, Sharma & Zhou, 2013). In addition, proteins, amino acids, lipids, alkaloids and certain minerals are also important constituents in green tea leaves (Harold & Graham, 1992; Zaveri, 2006; Reto et al., 2007; Senanayake, 2013). Previous reports have shown the presence of green tea polyphenols may help to decrease the risk and pathogenesis of certain chronic diseases, including cancer and cardiovascular disease (Cabrera, Artacho & Gimenez, 2006; Sang et al., 2011; Lorenzo & Munekata, 2016). In addition, green tea also assists with the control of body weight (Lorenzo & Munekata, 2016), bone and oral health (Cabrera, Artacho & Gimenez, 2006), physical functional performance (Jeszka-Skowron, Krawczyk & Zgola-Greskowiak, 2015), prevention of kidney stones (Cabrera, Artacho & Gimenez, 2006), and protects against ultraviolet radiation among other physiological effects (Dufresne & Farnworth, 2001; Reto et al., 2007; Chacko et al., 2010; Lorenzo & Munekata, 2016). These health benefits are mostly attributed to the polyphenols present in green tea, and these compounds remain in kombucha after fermentation and continue to elicit their beneficial health effects (Dufresne & Farnworth, 2000).

In the past few decades, with the increasing desire of people to live healthier life-styles, there has been growing interest in the development of healthy foods including kombucha (Dufresne & Farnworth, 2001). The composition of kombucha and the effects of different substrates (such as sucrose, molasses and lactose) on kombucha constituents have been studied (Greenwalt, Steinkraus & Ledford, 2000; Dufresne & Farnworth, 2000; Velićanski, Cvetković & Markov,

2013; Fu et al., 2014; Jayabalan et al., 2014; Liamkaew, Chattrawanit & Danvirutai, 2016). However the changes in the composition of kombucha during fermentation and the effects of fermentation conditions (sugar concentration, fermentation time and temperature, etc.) have rarely been mentioned. The absence of such information has impacted on the development of large scale commercial kombucha production. Therefore, understanding the fermentation process of kombucha is important for the production of a safe, wholesome beverage of consistently high quality.

1.2 Aim and objectives

Aim:

The aim of this study was to develop an optimised fermentation process for green tea kombucha with an acceptable sensory profile.

Objectives:

1. To determine the concentration of lactic acid bacteria, acetic acid bacteria and yeasts which may constitute the microbial community of the starter culture of green tea kombucha;
2. To select the optimum fermentation time by determining the effect of fermentation time on the physico-chemical, microbiological and sensory properties of green tea kombucha;
3. To select the optimum fermentation temperature and sugar concentration for preparation of green tea kombucha by:

   (a) Determining titratable acidity (TA), total soluble solids (TSS), organic acids (acetic acid and gluconic acid), sugars (sucrose, glucose and fructose), and ethanol content in green tea kombucha;

   (b) Measuring pH, colour, and analysing the microbial content (acetic acid bacteria and yeasts) during fermentation and storage for 2 weeks;
4. To determine the stability of the final formulation of fermented green tea kombucha during storage (4°C) for 4 weeks by analysing the physico-chemical (pH, TA, TSS), and microbiological content (acetic acid bacteria and yeasts) as well as evaluating of the products sensory characteristics;

5. To investigate the potential antibacterial activity of the final fermented green tea kombucha against selected common foodborne pathogens (*Escherichia.coli* 111, *Listeria monocytogenes* 15E03-1, *Salmonella typhimurium* ESR3479, *Staphylococcus aureus* MU-A57 and *Pseudomonas aeruginosa* MU-A26) using the disk diffusion method; and,
6. To analyse the concentration of antioxidants (gallic acid, ECG, EGC, EGCG, caffeine and theobromine) in the final fermented kombucha beverage.

2. Literature Review

2.1 Fermented foods and beverages

Food fermentation is a traditional, ancient and economical processing method for food preservation and production, which not only improves the nutritional composition of foods, but also enhances sensory attributes as well as inhibits growth of a large number of pathogens in the products (Sanni, 1993; Gadaga et al., 1999; Altay et al., 2013; Tamang et al., 2016). During fermentation, food substrates are utilised or metabolised by enzymes, particularly amylases, lipases and proteases which are produced by edible microorganisms (bacteria, yeast and moulds) existing in the products. The enzymes hydrolyse the polysaccharides, lipids and proteins to non-toxic substances with pleasant flavour, aroma and texture, these improving provide more favourable sensory attributes to the consumers (Steinkraus, 1997). The substances produced may include organic acids, ethanol, and carbon dioxide which may inhibit the growth of undesirable microorganisms (Caplice & Fitzgerald, 1999; Adams & Mitchell, 2002). Thus, fermented products have a relatively longer shelf life than the original products (Adams & Mitchell, 2002).

The origins of fermentation are not clear. However, available evidence shows that fermented honey, rice and fruit beverages date back to about 7000 B.C. in China. Whereas, health-promoting beverages fermented from milk, cereals, tea and other substrates, such as kombucha and Kefir originated around 220 B.C. (Alan et al., 2013). Many fermented beverages are classified as indigenous and are popular in many regions of Africa, Asia, Europe, South America and Middle East. Examples of traditional fermented beverages that are popular worldwide are shown in Figure 2.1. Today, fermented beverages and foods from milk, meat, soybean, cereal, vegetables and fruits are regarded as important in our daily diets, with more than 500 varieties of fermented beverages and foods being produced worldwide (Kabak & Dobson, 2011). Among the fermented beverages, kombucha is gaining popularity among consumers and researchers due to its health-promoting and nutritional properties (Lee, Baek & Lee, 2002; Altay et al., 2013; Watawana et al., 2015).

Figure 2.1 Typical fermented beverages from across the world (Hugenholtz, 2013)

2.2 Kombucha beverage

Kombucha is a slightly sweet, mildly acidic sparkling beverage consumed around the world (Malbaša et al., 2006; Jayabalan et al., 2014; Chakravprty et al., 2016; Mohammadshirazi & Kallor, 2016). The popularity of this fermented beverage is attributed to its energizing and detoxifying attributes, as well as for alleviating digestive problems (Loncar, 2006; Feng et al., 2009; Jayabalan et al., 2014; Vīna et al., 2014). Available information suggests that it originated from northeast China, and was then introduced to Japan by the physician, Kombu in 414 A.D. With the expansion of trade routes, kombucha appeared in Russia and other eastern European countries such as Germany, France and Italy around the turn of the 20th century (Aleksandra, 2014; Jayabalan et al., 2014; Essawet et al., 2015). Today, kombucha with different flavours is sold worldwide, not only in retail food stores, but also through online shopping websites (Jayabalan et al., 2014).

Kombucha beverage is traditionally fermented by a previously grown culture in a prepared tea infusion containing 5-10% sugar under aerobic conditions for 7-14 days at 20-28°C (Dufresne & Farnworth, 2000; Chen & Liu, 2000; Malbaša et al., 2006; Wu et al., 2012; Velićanski, Cvetković & Markov, 2013; Nummer, 2013; Mohsen, 2017). Green tea and black tea are the best media to provide the necessary nitrogen nutrients for the growth of the kombucha culture

(Jayabalan et al., 2014). Sucrose or materials such as red wine, vinegar, white wine, milk, fresh sweet whey, Echinacea, Mentha, and red grape juice which contain fermentable sugar (like molasses) are also essential substrates for kombucha fermentation, because they can be used as a carbon source for kombucha culture growth (Jayabalan et al., 2014; Ayed, Abid & Hamdi, 2017). At the end of fermentation, kombucha is composed of a sour liquid broth (beverage) and a floating cellulosic pellicle layer which is also called tea fungus (Figure 2.2). The fermenting microbial cultures are trapped in the tea fungus but are also present in the fermented broth (Resis, 1994; Chen & Liu, 2000). Therefore both the tea fungus and the broth can be used for subsequent fermentation of kombucha.

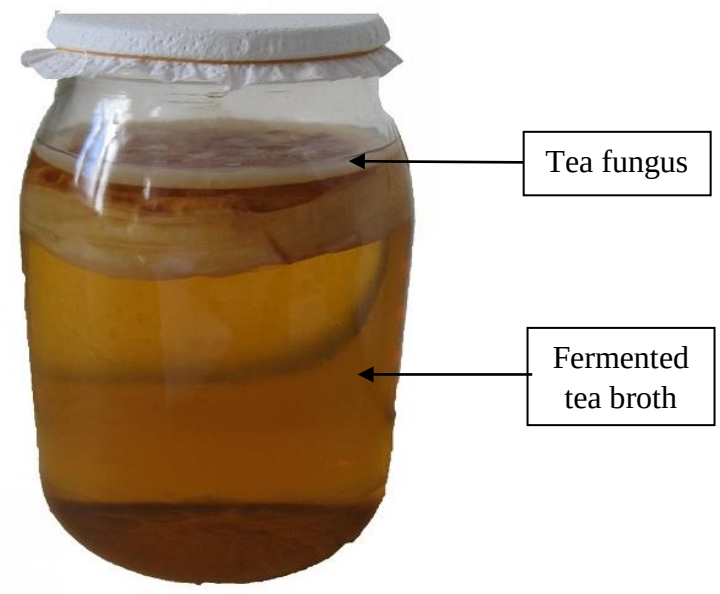

Figure 2.2 Kombucha tea fungus and liquid broth
(AHF, 2018)

2.3 Preparation of green tea kombucha

Fermented kombucha tea is prepared by inoculating the tea fungus and kombucha broth from a previous fermentation into a sweetened tea infusion, followed by fermentation at ambient temperature (Vijayaraghavan et al., 2000; Jayabalan et al., 2014; Jayabalan, Malbaša, & Sathishkumar, 2017). According to Jayabalan et al. (2014), typical preparation of kombucha involves adding tea leaves and sucrose into boiling water. The mixture is stirred and allowed to brew for about 5 min, the tea leaves are removed by filtration, and cooled at room temperature (20℃). Kombucha culture is then added into the sweet tea broth and the mixture is allowed to ferment for 7 to 14 days at about 21±1 °C (Jayabalan et al., 2014). However, the

concentration of black tea or green tea, sugar level, fermentation temperature and time, content and source of starter culture may vary (Dufresne & Farnworth, 2000; Jayabalan, Marimuthu & Swaminathan, 2007; Anna et al., 2016; Kumar & Joshi, 2016).

Alternative methods for producing kombucha include brewing 1.5 g/L of black tea and 7% (w/v) of sucrose in boiling water for 5 min, then adding 10% (v/v) fermented broth from a previous batch of kombucha and the mixture was fermented at 22±1℃ for 14 days (Malbaša, Lončar & Djurić ,2008). Yet another described method involves using 1.2% (w/v) green tea or black tea, 10% (w/v) sucrose, 10% (v/v) kombucha broth and 3 % (w/v) tea fungus to ferment kombucha at 24 ± 3℃ for 18 days. (Jayabalan, Marimuthu and Swaminathan, 2007).

Although there are various methods for the preparation of kombucha beverage, common materials are used with concentrations varying. For example, the concentrations of tea added to kombucha mixtures range from 0.1 to 0.6% (w/v), sucrose from 5 to 10% (w/v), tea fungus from 2% to 5% (w/v), kombucha broth from 10% to 20% (v/v). In addition, the fermentation temperatures vary (18℃-26℃) along with length of fermentation (7 to 14 days) (Dufresne & Farnworth, 2000; Teoh, Heard & Cox, 2004; Jayabalan, Marimuthu and Swaminathan, 2007; Battikh, Bakhrouf & Ammar, 2012; Markov, Cvetković & Velićanski, 2012; Jayabalan et al., 2014; Fu et al., 2014; Hrnjez et al., 2014; Chakravorty et al., 2016). A typical flow chart for the production of kombucha is shown in Figure 2.3.

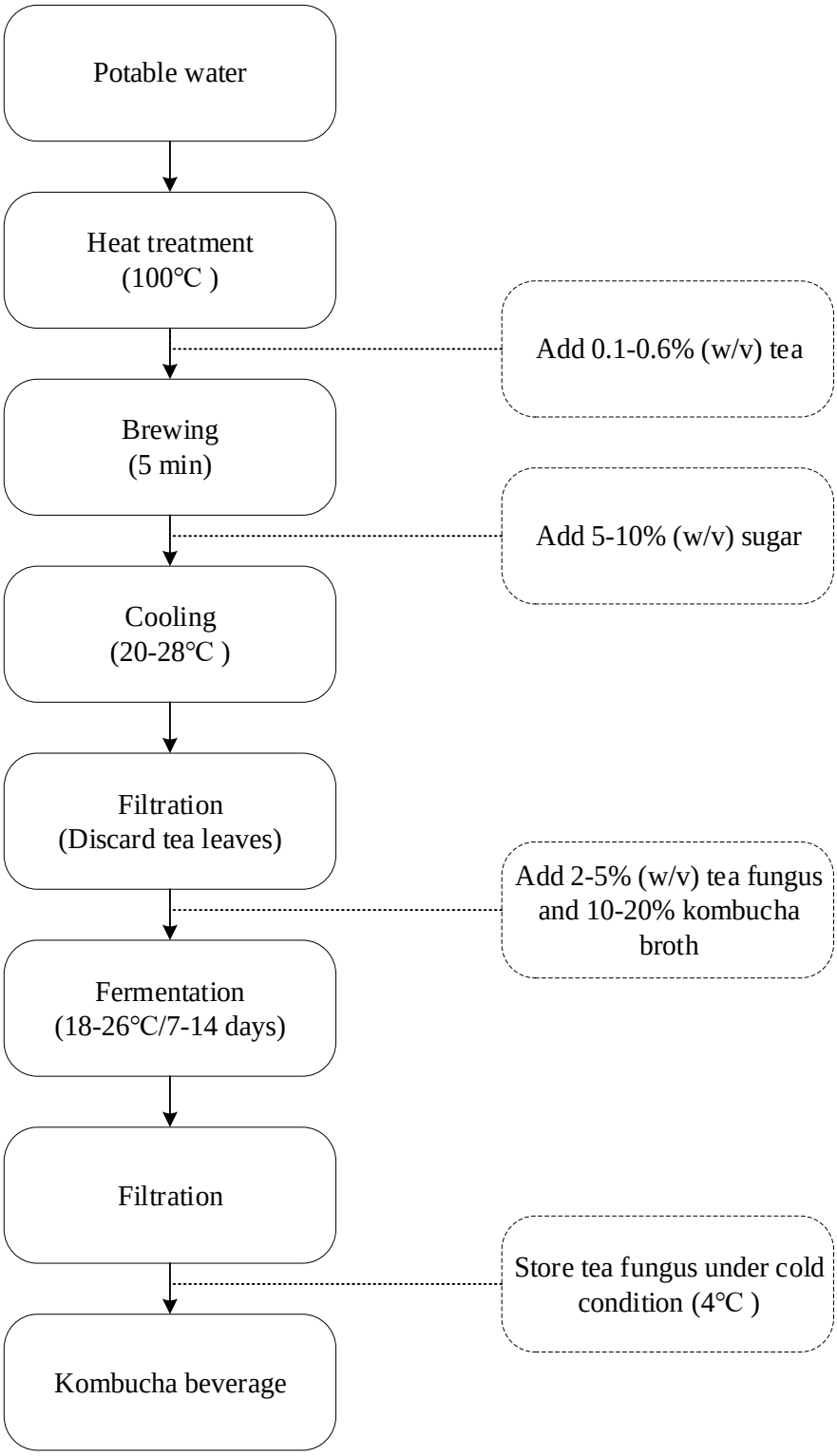

Figure 2.3 Overview of traditional procedure for preparation of kombucha tea

(Dufresne & Farnworth, 2000; Teoh, Heard & Cox, 2004; Jayabalan, Marimuthu and Swaminathan, 2007; Battikh, Bakhrouf & Ammar, 2012; Jayabalan et al., 2014; Fu et al., 2014; Hrnjez et al., 2014; Chakravorty et al., 2016)

Within a few days of the fermentation beginning, the beverage will start to produce a fermented odour and gas bubbles of carbon dioxide due to microbial activities, in particular yeast (Jayabalan et al., 2014). After 7-14 days, a newly formed membrane (tea fungus) becomes visible covering the entire surface of the liquid. The tea fungus is removed using a clean spoon and placed into a clean glass jar with a small volume of fermented kombucha, and is then stored in the fridge ready for use in the next fermentation. The remaining tea broth is filtered and stored at 4℃, but it may also be consumed immediately (Dufresne & Farnworth, 2000).

The taste of kombucha changes from a pleasant fruity, acidic sparkling flavour to a mild vinegary taste if the fermentation time is prolonged to more than 14 days. This is due to the acidity of the beverage increasing and it may increase to unacceptable levels (Adams & Hall, 1988). The concentration of sugar added, fermentation temperature and fermentation time are key factors that must be well-controlled in order to produce a healthy product with acceptable sensory properties (Jayabalan et al., 2014).

2.4 Kombucha starter culture

Kombucha starter culture is a symbiotic association of yeast and bacteria (Blanc, 1996; Sreeramulu, 2000; Malbaša, Lončar & Djurić, 2008; Nguyen et al., 2008; Velićanski, Cvetković & Markov, 2013; Velićanski et al., 2014; Aleksandra, 2014; Liamkaew, Chattrawanit & Danvirutai, 2016; Ayed, Abid & Hamdi, 2017). It is separated into two portions: kombucha broth and tea fungus (Figure 2.2). Kombucha tea fungus is a unique floating cellulose network that resembles a surface mould on the undisturbed medium, and it is produced by acetic acid bacteria (Jayabalan et al., 2010; Tan et al., 2012; Zhu et al., 2014; Hassan & AL-Kalifawi, 2014; Ramana & Batra, 2015). The composition of the cellulose network resembles the “mother of vinegar”, and the cell mass of yeast and bacteria are attached to this thin film (Sreeramulu, Zhu & Knol, 2000; Jayabalan et al., 2010; Yavari et al., 2010). During fermentation of the sugar in the tea, a new “daughter”, jelly-like cellulose membrane forms on the “mother” culture. At the end of fermentation, part of the newly formed tea fungus and liquid broth can be used as starter culture as it contains fermenting microbial cells (acetic acid bacteria and yeasts) (Blanc, 1996; Jayabalan et al., 2010). The thickness of the floating layer increases with fermentation time and ranges from a few millimeters to centimeters, with the shape changing according to the vessel used for incubation (Kalifawi & Hassan, 2013; Pothakos et al., 2016).

Several previous studies have investigated the diversity of microorganisms in kombucha, resulting in the identification of some of the bacteria and yeasts (Sievers et al., 1995; Steinkraus & Ledford, 2000; Dufresne & Farnworth, 2000; Teoh, Heard & Cox, 2004; Jayabalan et al., 2014; Marsh et al., 2014). However, the microbial composition of kombucha is highly variable as it depends on geographical, cultural and climatic conditions (Greenwalt, Steinkraus & Ledford, 2000; Teoh, Heard & Cox, 2004; Jayabalan et al., 2014). The most common species found in kombucha are listed in Table 2.1. The inoculum of kombucha mainly contains *Acetobacter* spp. and *Saccharomyces* spp. in kombucha matrix (Figure 2.4) (Greenwalt, Steinkraus & Ledford, 2000; Ram et al., 2000; Wu et al., 2004), although small concentrations of lactic acid bacteria have also been observed in some of kombucha cultures (Yang et al., 2010; Hrnjez et al., 2014).

Acetobacter spp. and *gluconobacter* spp. are the most abundant prokaryotes found in kombucha starter cultures, dominated by *Acetobactor xylinum* (Jarrell, Cal & Bennett, 2000) which was recently reclassified as *Gluconacetobacter xylinum* (*Ga. xylinum*). This type of acetic acid bacteria produces the cellulosic floating pellicle layer on the surface of the beverage, which is characteristic of kombucha cultures (Chu & Chen, 2006). The other dominant acetic acid bacteria (AAB) found in kombucha cultures are *A. pasteuriaus*, *A. aceti*, *Gluconobacter oxydans* (Liu et al., 1996; Sun, Li & Chen, 2015), *Gluconacetobacter saccharivorans* (Yang et al., 2010; Wang et al., 2014) and *Ga. Sacchari* (Marsh et al., 2014). *Gluconacebacter* sp. A4 which has a strong ability to generate D-saccharic acid -1,4- lactone (DSL) has also been isolated from preserved kombucha tea (Wang et al., 2007; Wang et al., 2010; Yang et al., 2010).

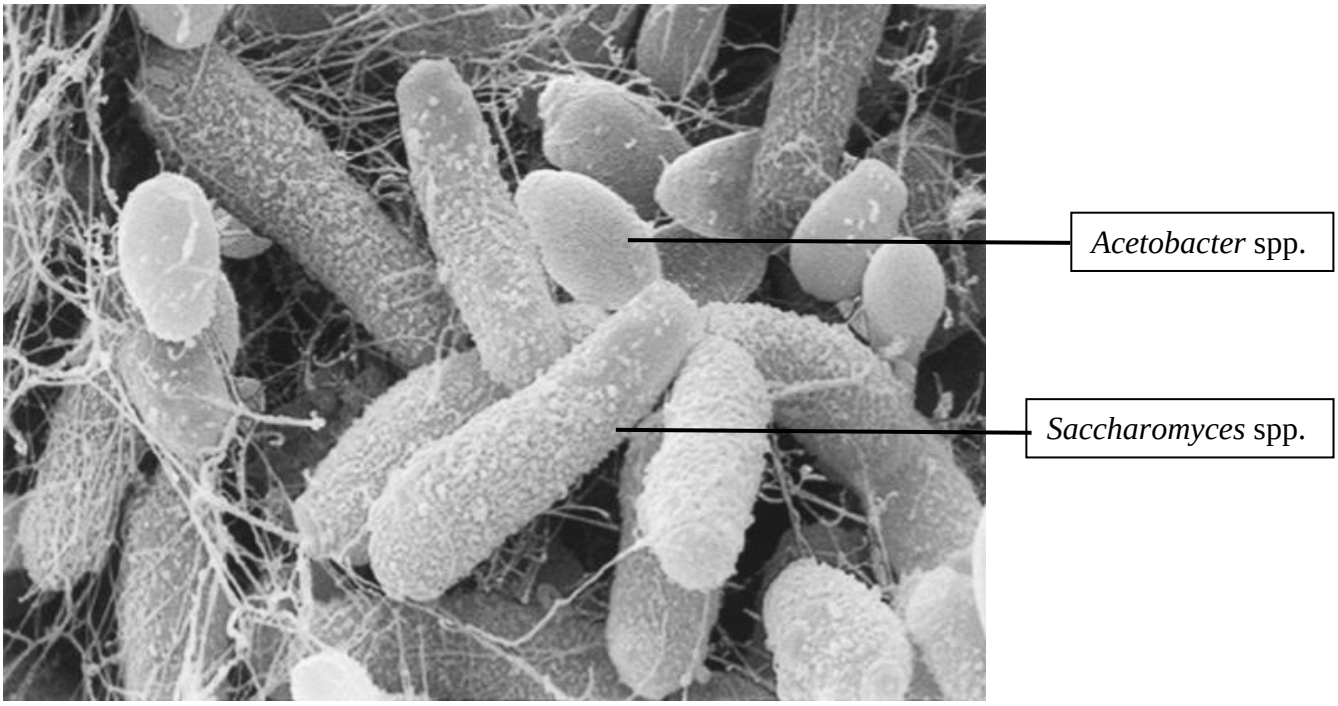

Figure 2.4 Electron micrograph of kombucha colony
(Greenwalt, Steinkraus & Ledford, 2000)

In addition to acetic acid bacteria, kombucha cultures contain several species of yeast. Teoh et al. (2004) isolated 163 yeast containing six dominant species from four kombucha cultures, which included *Schizosaccharomyces*, *Zygosaccharomyces*, *Torulospora*, *Candida*, *Brettanomyces/Dekkera* and *Rhodotorula* (Ai, Heard & Cox, 2004). Other species, such as *Saccharomyces*, *Saccharomycodes*, *Pichia*, *Mycotorula*, *Koleckera/Hansenisaspora* and *Mycoderma* have also been identified (Markov et al., 2001; Kurtzman et al., 2001; Kurtzman, Robnett & Basehoar-Powers, 2001; Ai, Heard & Cox, 2004; Markov, Cvetković & Bukvić, 2005; Jayabalan et al., 2008; Marsh et al., 2014).

The presence of lactic acid bacteria (LAB) in kombucha cultures have been reported (Fu et al., 2014; Hrnjez et al., 2014; Michatowska et al., 2016; Ayed, Abid & Hamdi, 2017). Marsh (2014) reported that *Lactobacillus* spp. were more common in kombucha than previously understood, with *Lactobacillus kefiranofaciens* subsp. *Kefirgranum* being the most abundant species (Hrnjez et al., 2014). The *Lactobacillus* spp. in kombucha can secrete bacteriocins and plantaricin; which is a small thermally stable peptide capable of inhibiting the growth of Gram-positive and Gram-negative pathogens under acidic conditions (Michatowska et al., 2016).

However, thorough investigations of lactic acid bacteria species in kombucha are still lacking (Pothakos et al., 2016).

Table 2.1 Isolated and identified acetic acid bacteria, yeast and lactic acid bacteria in kombucha

Type of bacteria	**Microorganism**	**Reference**
Acetic acid bacteria	*Acetobacter aceti*	Liu et al., 1996
	Acetobacter intermedius sp. nov.	Boesch et al., 1998
	Acetobacter nitrogenifigens sp. nov.	Dutta & Gachhui, 2006
	Acetobacter pasteurianus	Liu et al., 1996
	Acetobacter sp. A4	Yang et al., 2010
	Acetobacter xylinoides	Resis, 1994
	Bacterium gluconicum	Resis, 1994
	Gluconacetobacter xylinum	Jarrell, Cal & Bennett, 2000
	Gluconobacter oxydans	Liu et al., 1996
Yeast	*Brettanomyces*	Mayser et al., 1995
	Brettanomyces bruxellensis	Liu et al., 1996; Teoh et al., 2004
	Brettanomyces claussenii	Jayabalan et al., 2008
	Brettanomyces intermedius	Herrera & Calderon-Villagomez, 1989
	Candida famata	Herrera & Calderon-Villagomez, 1989; Kozaki et al., 1972
	Candida guilliermondii	Kozaki et al., 1972; Ramadani & Abulreesh, 2010
	Candida obusta	Kozaki et al., 1972
	Kloeckera apiculate	Safak et al., 2002; Kozaki et al., 1972
	Mycoderma	Jankovic & Stojanovic, 1994; Resis, 1987
	Pichia membranefaciens	Kozaki et al., 1972; Herrera & Calderon-Villagomez, 1989
	Saccharomyces	Kwanashie et al., 1990
	Saccharomyces bisporus	Markov et al., 2001
	Saccharomyces cervisiae	Liu et al., 1996; Markov et al., 2001
	Saccharomyces pombe	Resis, 1987; Teoh et al., 2004
	Saccharomycodes ludwigii	Resis, 1987; Ramadani & Abulreesh, 2010
	Torula	Resis, 1987; Jankovic & Stojanovic, 1994
	Torulaspora delbrueckii	Herrera & Calderon-Villagomez, 1989
	Torulopisis	Herrera & Calderon-Villagomez, 1989
	Zygosaccharomyces	Sievers et al., 1995; Marsh et al., 2014
	Zygosaccharomyces bailii	Liu et al., 1996; Jayabalan et al., 2008
	Zygosaccharomyces rouxii	Herrera & Calderon-Villagomez, 1989
Lactic acid bacteria	*Brevibacterium sp.*	Petrušic´ et al., 2011
	Lactobacilli kefiranofaciens subsp. kefirgranum	Hrnjez et al., 2014 Ayed, Abid & Hamdi, 2017
	Lactobacillus plantarum	Petrušic´ et al., 2011
	Streptococcus bovis	Petrušic´ et al., 2011
	Streptococcus lutetiensis	Petrušic´ et al., 2011
	Streptococcus thermophilus	

2.5 Symbiotic interactions between yeast and bacteria

The relationship between acetic acid bacteria and yeast during kombucha fermentation is known as symbiotic mutualism (Liu et al., 1996; Markov et al., 2001; Malbaša, Lončar & Djurić, 2008; Malbaša et al., 2011; Kallel et al., 2012; Ayed, Abid & Hamdi, 2017; Velićanski et al., 2014). During incubation, sucrose added into kombucha as a carbon source, is first hydrolysed into glucose and fructose by invertase from the yeast. The fructose is then utilised as a substrate to produce ethanol and carbon dioxide via glycolysis also by the yeast (Malbaša et al., 2006; Kallel et al., 2012; Jayabalan et al., 2014). Meanwhile, bacteria metabolise fructose and ethanol to acetic acid rather than gluconic acid (Resis, 1994; Chen & Liu, 2000; Lončar et al., 2006; Cvetković t al., 2008), while the glucose generated will be further metabolised by acetic acid bacteria to produce gluconic acid via the pentose phosphate pathway (Sreeramulu, Zhu & Knol, 2000; Soh & Lee, 2002; Marsh et al., 2014). Glucose is also metabolised by acetic acid bacteria to synthesise the cellulose, which is the main component of the tea fungus formed during fermentation (Greenwalt, Ledford & Steinkraus, 1998; Nguyen et al., 2000; Zhu et al., 2014). Chen and Liu (2000) analysed the concentrations of sucrose, glucose and fructose in black tea kombucha during 60 days of kombucha fermentation and found the metabolic fates of the three sugars to be different. Glucose was preferentially utilised by either yeast or acetic acid bacteria, while fructose was poorly metabolised, resulting in its accumulation in the broth. Liu et al. (1996) reported that during kombucha fermentation, production of ethanol by yeast assists in the generation of acetic acid by bacteria, while acetic acid production may further stimulate growth of yeast to produce ethanol.

2.6 Metabolism of microorganisms during fermentation

2.6.1 Metabolism of yeast

Yeasts are among the most common microorganisms present in the natural environment (Rodrigues, Ludovico & Leão, 2006; Hatoum, Labrie & Fliss, 2012). They are unicellular fungi widespread in aerial, terrestrial and aquatic environments; their high physiological adaptability to diverse conditions is responsible for their ubiquitous spread (Rodrigues, Ludovico & Leão, 2006). Like other heterogeneous microorganisms, there is a broad set of carbon sources utilisable by yeast, such as alcohols, amino acids, polyols and organic acids, although sugars are the favoured substrate for their growth. Sugar metabolism is similar between various yeast

species (Rodrigues, Ludovico & Leão, 2006), with two different pathways: respiration and fermentation, available for producing adenosine triphosphate (ATP) (Pfeiffer & Morley, 2014). High yields of ATP can be produced from the respiration pathway with approximately 18 moles of ATP being produced per glucose molecule by *Saccharomyces cerevisiae*. In contrast, the fermentation pathway results in only 2 moles of ATP per glucose molecule, however, no oxygen is required for this pathway (Pfeiffer & Morley, 2014). Yeast can generate ATP via independent respiration, fermentation or by concurrently using both pathways if high levels of oxygen and sugar are present. The two types of pathways are shown in Figure 2.5.

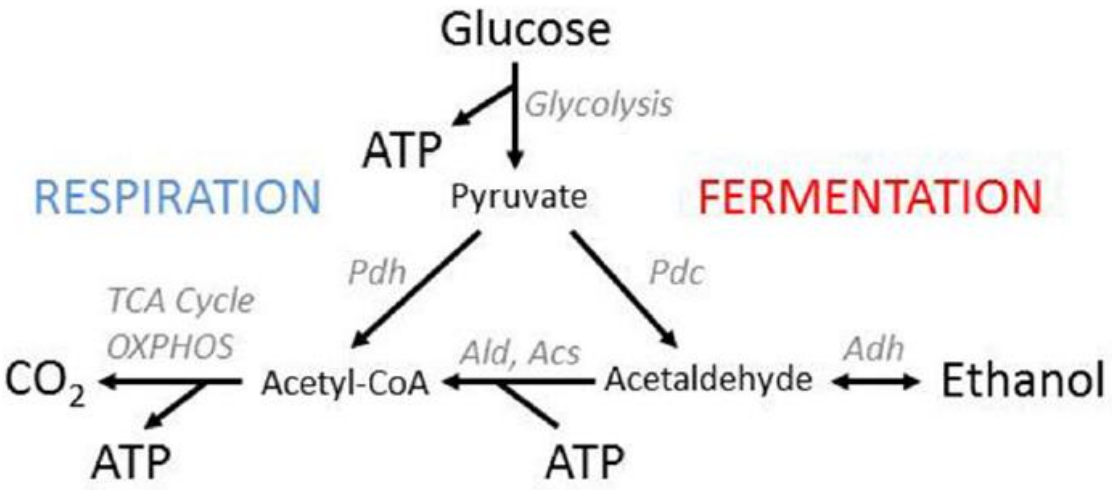

Figure 2.5 Metabolic pathway of glucose by yeast
(Pfeiffer & Morley, 2014)

Notes: Pdh = pyruvate dehydrogenase, Pdc = pyruvate decarboxylase, Ald = aldehyde dehydrogenase, Acs = acetyl-CoA synthetase, Adh = alcohol dehydrogenase OXPHOS = oxidative phosphorylation.

According to the energy generating processes involved in sugar metabolism, yeast can be physiologically classified into three types: non-fermentative, facultative-fermentative and obligately-fermentative (Rodrigues, Ludovico & Leão, 2006). Non-fermentative yeast, such as *Rhodotorula glutinis*, are not able to perform alcoholic fermentation because of their inability to synthesise key enzymes for fermentation. In contrast, *Candida slooffi*, an obligate-fermentative yeast can generate ATP through the fermentation pathway, since it is not capable of undergoing respiration (Dijken, Weusthuis & Pronk, 1993). There is a broad spectrum of yeast in kombucha, with typical genera being *Brettanomyces*, *Zygosaccharomyces* and *Saccharomyces* (Jayabalan et al., 2014). These fungi are facultative fermentative yeasts, which can metabolise sugar under either aerobic or anaerobic conditions. Depending on the conditions

of growth, the concentration and type of sugars present and the availability of oxygen, facultative-fermentative yeast may undergo either fermentation or concurrent respiration in a mixed respiration-fermentation metabolism (Dijken, Weusthuis & Pronk, 1993; Rodrigues, Ludovico & Leão, 2006).

A frequently observed effect associated with facultative-fermentative yeast is the Crabtree effect, which refers to the occurrence of alcoholic fermentation under aerobic conditions in the presence of excess sugar (Dijken, Weusthuis & Pronk, 1993; Johnston & Kim, 2005). In Crabtree-negative yeast, only respiration takes place, while in Crabtree-positive yeast, fermentation and respiration simultaneously occur (Pfeiffer & Morley, 2014). Most yeast present in kombucha starter cultures belong to the Crabtree-positive group, with *Saccharomyces cerevisiae* being a typical example (Flikweert, 1999). In Crabtree-positive yeast, at high glucose concentrations, respiration is usually suppressed. As the glucose content increases, pyruvate produced from glycolysis is diverted away from the citric acid cycle into ethanol synthesis where it is then converted to acetaldehyde and carbon oxide by pyruvate decarboxylase (Figure 2.5) (Deken, 1966). To reduce the Crabtree effect, sugar may be added to the fermentation vessel in small batches at several key stages during fermentation (Pfeiffer & Morley, 2014).

Figure 2.6 Metabolic pathway of yeast
(Bai, Anderson & Young, 2008)

Notes: HK = hexokinase, PGI = phosphoglucoisomerase, PFK = phosphofructokinase, FBPA = fructose bisphosphate aldolase, TPI = triose phosphate isomerase, GAPDH = glyceraldehydes-3-phosphate dehydrogenase, PGK = phosphoglycerate kinase, PGM = phosphoglyceromutase, ENO = enolase, PYK = pyruvate kinase, PDC = pyruvate decarboxylase, ADH = alcohol dehydrogenase.

Sucrose added during the preparation of kombucha beverage is first hydrolysed to monosacaccharides (glucose and fructose) by yeast invertase during fermentation (Greenwalt, Steinkraus & Ledford, 2000). Glucose is then metabolised via glycolysis (Figure 2.6), which involves transformation of one molecule of hexose into two molecules of pyruvate with the formation of two molecules of ATP (Lagunas, 1986). Pyruvate can be further degraded through either the respiration or fermentation pathways (Pfeiffer & Morley, 2014). During respiration, pyruvate is converted to acetyl-Coenzyme A by pyruvate dehydrogenase (Pdh), then oxidized to carbon dioxide through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) (Flores et al., 2000). Whereas in alcoholic fermentation, pyruvate is first converted into acetaldehyde by pyruvate decarboxylase (Pdc) and then to ethanol by alcohol dehydrogenase (Adh). In addition, acetaldehyde may be subsequently converted to acetyl-Coenzyme A as a substrate for the TCA cycle by acetaldehyde dehydrogenase (Aldh) and

acetyl-CoA synthetase (Acs) (Pfeiffer & Morley, 2014). In the absence of inhibition and presence of oxygen, pyruvate can be metabolised to ATP and CO_2 (Pfeiffer & Morley, 2014).

2.6.2 Metabolism of acetic acid bacteria

Due to their ability to grow and survive in both acidic and neutral media as well as the variety of substrates (alcohol, sugar and organic acids) they can metabolise, acetic acid bacteria are widespread in natural environments (González & Mas, 2011; Matsushita et al., 2016). Acetic acid bacteria can be isolated from various substrates and natural sources such as plants, fruits, flowers, herbs and fermented foods and beverages (Crotti et al., 2010). Ubiquitous acetic acid bacteria belong to the Acetobacteraceae family and are Gram-negative, catalase positive, oxidase negative, non-spore forming, obligate aerobic α-proteobacteria (Bartowsky & henschke, 2008; Ilabaca et al., 2008). They are rod-shaped to ellipsoidal cells varying in width between 0.4-1 µm and 0.8-4.5 µm long, and can occur singly, in pairs or chains. The optimum pH for their growth is 5-6.5, although they can exist at lower pHs of around 3 to 4 (Sengun & Karabiyikli, 2011). The optimum temperature for their growth ranges from 28℃ to 30℃ (Mamlouk & Gullo, 2013). Both peritrichously and polarly organisms are frequently found flagellated with acetic acid bacteria (Drysdale & Fleet, 1988).

Acetic acid is one of the most dominant by-products of the AAB oxidation of ethanol, which makes them significant in the vinegar industry. Early studies observed that the surface layer formed during vinegar production, commonly known as “mother of vinegar”, is a mass of living microorganisms including acetic acid bacteria (Mamlouk & Gullo, 2013). Other applications of AAB include the fermentation of certain foods, such as cocoa beans, *nata de coco* (fermented food from coconut), palm wine, *pulque* (beverage from agave) and kombucha (González & Mas, 2011; Mamlouk & Gullo, 2013). Acetic acid bacteria are also involved in the production of sorbose and cellulose, and the production of vitamin C (Sengun & Karabiyikli, 2011; González & Mas, 2011; Goh et al., 2012; Guillamón & Mars, 2017).

Acetic acid bacteria were originally classified into two main groups, *Acetobacter* and *Gluconobacter*, but more recently twelve genera have been recognised and included within the AAB taxonomy; these are: *Acetobacter*, *Gluconobacter*, *Acidomonas*, *Gluconacetobacter*, *Asaia*, *Kozakia*, *Swaminathania*, *Saccharibacter*, *Neoasaia*, *Granulibacter*, *Tanticharoenia*

and *Ameyamaea* (González & Mas, 2011). However, *Acidomonas*, *Kozakia*, *Swaminathania*, *Saccharibacter*, *Neoasaia*, *Granulibacter*, *Tanticharoenia* and *Ameyamaea* strains are rare found from common isolation sources, such as flowers, fruits, vinegar and wine (Sengun & Karabiyikli, 2011).

The most abundant prokaryotes in kombucha belong to the genera *Acetobacter*, *Gluconobacter* and *Glucoaetobacter*. The common bacteria are *Glucoacetobacter xylinum* (*Ga. xylinum*, formerly known as *Acetobacter xylinum*), *Acetobacter aceti* (*A. aceti*), *Acetobacter pasteurianus* (*A. pasteurianus*) and *Gluconobacter oxydans* (*G. oxydans*) (Greenwalt, Steinkraus & Ledford, 2000; Jayabalan et al., 2014). Ethanol and sugars are the favoured substrates for metabolism by these bacteria (Gullo & Giudici, 2008).

Ethanol present in the fermented broth, which is generated from the metabolism of sugars by yeast, is generally oxidised by AAB via two sequential catalytic reactions carried out by membrane-bound pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). These two enzymes are tightly linked to the respiratory chain, which transfers electrons to oxygen as a final electron acceptor via ubiquinone (UQ) and ubiquinol oxidase (Sakurai et al., 2012). During fermentation, ethanol is oxidised by ADH to acetaldehyde, which is further converted to acetic acid by ALDH (Equation 1 and 2). A temporary accumulation of acetate may be observed due to incomplete oxidation during the growth of *Acetobacter* and most *Gluconacetobacteria* species, but with the consumption of ethanol, the accumulated acetate is then completely metabolised. The acetic acid produced can be further utilised by acetyl CoA synthase (Figure 2.7) (Mamlouk & Gullo, 2013). The oxidation process usually takes place in *Acetobacter* rather than *Gluconobacter*. The reason being that since alcohol dehydrogenae activity in *Acetobacter* is more stable than *Gluconabacter* under acidic environments, which may explain the production of high levels of acetic acid by *Acetobacter* (Sengun & Karabiyikli, 2011).

$CH_3CH_2OH + PQQ \rightarrow CH_3CHO + PQQH_2$ (alcohol dehydrogenase)...……equation 1

$CH_3CHO + PQQ + H_2O \rightarrow CH_3COOH + POOH_2$ (aldehyde dehydrogenase) ...…equation 2

Figure 2.7 Ethanol oxidation by acetic acid bacteria in the cell
(Mamlouk & Gullo, 2013)

Notes: PQQ = pyrroloquinoline quinone, ADH = alcohol dehydrogenase, ALDH = aldehyde dehydrogenase, NAD = nicotinamide-adenine dinucleotide, UQ = ubiquinone.

Acetic acid bacteria have high oxidative capacity against sugars, particularly glucose but also for fructose, arabinose, sorbose, galactose and xylose (Mamlouk & Gullo, 2013). However, the pathways for sugar metabolism by the different bacteria: *Acetobacter*, *Gluconobacter* and *Gluconacetobacter* are different. In *Acetobacter* and *Gluconobacter* species, the initial phosphorylation of hexose and pentose is catalysed via the hexose monophosphate pathway (Figure 2.8) to acetic or lactic acid, which in *Acetobacter* species are further metabolised to water and carbon dioxide through the TCA cycle (Ribéreau-Gayon et al., 1998).

The metabolism of hexose and pentose is relatively weak in *A. aceti* and *A. pasteurianus*, possibly due to their inability to phosphorylate these substrates on entry into the cell (Drysdale & Fleet, 1988). In contrast, *Gluconobacter* species are capable of metabolising and utilising sugars more efficiently through the pentose phosphate pathway. The most characteristic metabolism of *Gluconobacter* is the direct oxidation of glucose to glucono-δ-lactone which is then converted to gluconic acid (Mamlouk & Gullo, 2013). This sequential reaction is highly active in *Gluconobacter* at high sugar levels.

Figure 2.8 Degradation of glucose by acetic acid bacteria (hexose monophosphate pathway) (Ribéreau-Gayon et al., 1998)

For cellulose synthesising *Gluconacetobacter*, such as *Ga. xylinum*, the Enter-Doudoroff pathway appears more active than the hexose monophosphate cycle or pentose-phosphate pathway (Mamlouk & Gullo, 2013). During cellulose synthesis for *Ga. xylinum* species, membrane bound cellulose synthase is the key enzyme which utilises UDP-glucose as a substrate. The pathway from glucose to cellulose comprise of four enzymatic steps shown in equation 3:

Glucose→glucose-6-phosphate→glucose-1-phosphate→UDP-glucose→ cellulose ……equation 3

2.7 Main kombucha preparation raw material - tea

Tea is the best medium for the preparation of kombucha, as it can provide the necessary nitrogen nutrients for the growth of the kombucha culture (Jayabalan et al., 2014). As one of the world's most popular beverages, tea (*Camellia sinensis* L., Family: *Theaceae*) is consumed by over two-thirds of the world's population and its consumption is second only to water (Cabrera, Artacho & Giménez, 2006; Khan & Mukhtar, 2013; Skowron, Krawczyk & Grześkowiak, 2015; Pastoriza et al., 2017). Originating from China and India, tea is produced from the leaves and buds of the plant *Camellia sinensis*, by steeping them in boiling water (Chopade et al., 2008; Sang et al., 2011; Hajiaghaalipour, Sanusi & Kanthimathi, 2016). Tea is cultivated in many regions of the world with high temperatures, high humidity and acidic soils (Dufresne & Farnworth, 2001). Over 3 million tons of tea are produced annually from the major producers comprising India (27.4%), China (24.7%) and Sri Lanka (9.8%) (Dufresne & Farnworth, 2001; Yang, Kumar, Narayan & Hassarajani, 2008; Baldermann & Watanabe, 2013).

Tea is an important source of polyphenols, which play a role in the maintenance of good health and reducing the risk of heart disease and cancer (Shen & Chen, 2008; Hayat, Lqbal & Malik, 2015; Lorenzo & Munekata, 2016; Cabrera, Artacho & Giménez, 2017). Previous studies (McKay & Blumberg, 2002; Khan & Mukhtar, 2007; Chopade et al., 2008; Ferrazzano, 2009; Lorenzo & Munekata, 2016) have revealed that tea can also aid in the control of body-weight, protect against ultraviolet radiation, and have positive effects on bone density, kidney stones, cognitive function and reduction of dental caries.

Based on the manufacturing process (Figure 2.9) and degree of oxidation, tea is categorised into four main types, non-fermented green tea, semi-fermented oolong tea, completely-fermented black tea and post-fermented Pu-erh tea (Cabrera, Artacho & Giménez, 2006; Rao & Ramalakshmi, 2011; Senanayake, 2013). About 78% of the tea produced around the world is black tea, which is mainly consumed in western countries (Bushman, 1998), whereas green tea, which consists of 20% of the world's tea production is primarily consumed in China and Japan (Sang et al., 2011). The remaining 2% is oolong tea and Pu-erh tea which are popular in Asian regions, such as Taiwan and Japan (Bansal et al., 2013). Due to its high levels of polyphenols, which are associated with the antioxidant activity, green tea has recently gained

attention among consumers and scientific communities (Zaveri, 2006; Rao & Ramalakshmi, 2011).

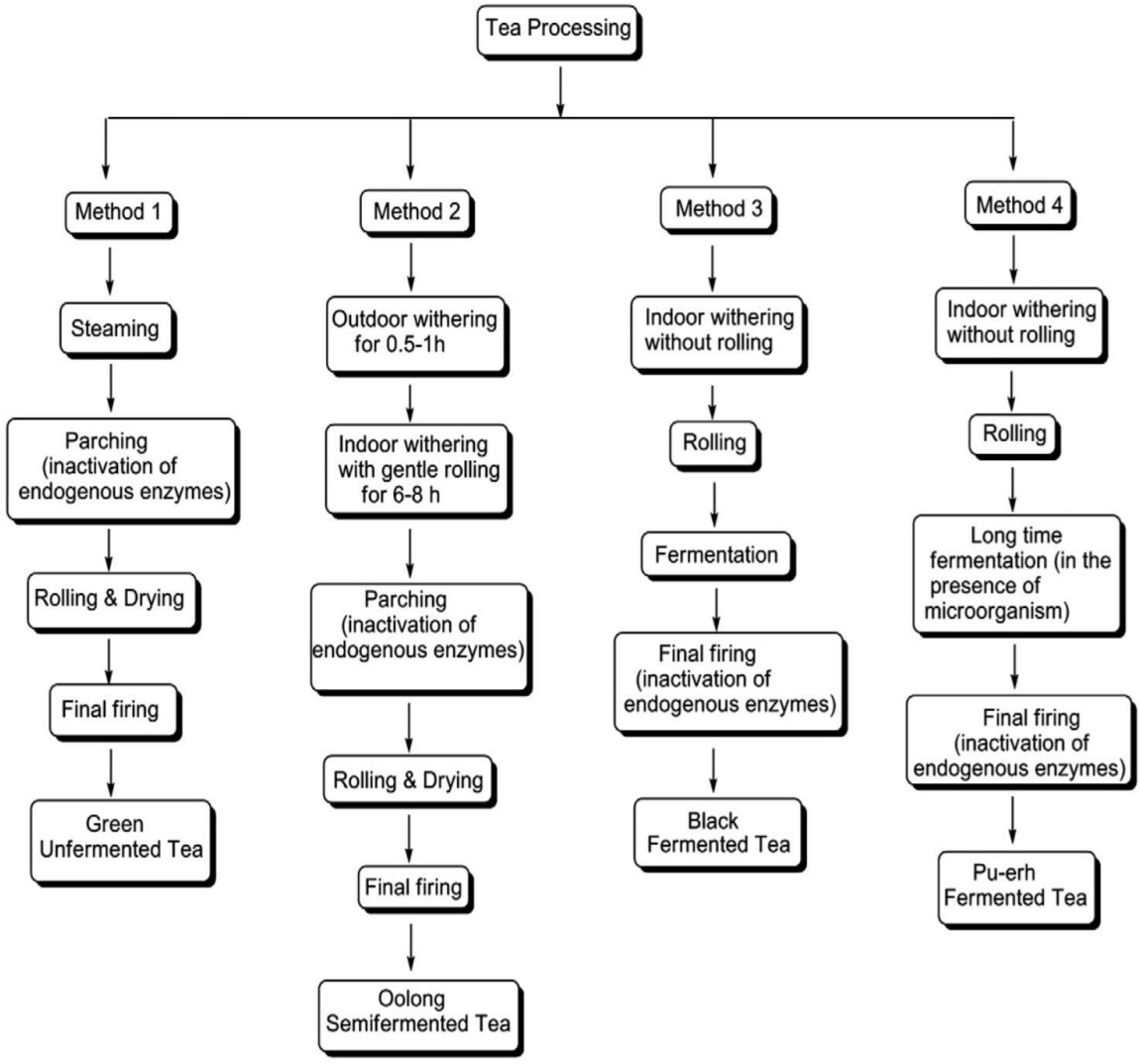

Figure 2.9 Conventional manufacturing process of green, oolong, black and pu-erh tea (Bansal et al., 2013)

2.7.1 Green tea

Green tea was first exported from India to Japan during the 17th century, and is now the preferred tea in China and Japan (Chako et al., 2010; Lin et al., 2010). The composition of the herb is associated with significant health effects, mostly attributed to the polyphenolic compounds, especially flavanols, also known as catechins (Zaveri, 2006; Alcazar et al., 2007). During manufacture, green tea undergoes heat or steam processing, and the heat-labile enzyme polyphenol oxidase in green tea leaves is inactivated to prevent oxidation of catechins (Hilal & Engelhardt. 2007). Thus, the polyphenols are present at higher levels in green tea than in

black tea, oolong tea or Pu-er tea which do not undergo heat processing (Zaveri, 2006; Bancirova, 2010).

2.7.2 Composition of green tea

The composition of green tea differs according to the climate, season, variety, age of leaves, and horticultural practices, as well as the position of the leaf on the harvested shoot (Harold & Graham, 1992; Cabrera, Artacho & Giménez, 2006; Bansal et al., 2013; Skowron, Krawczyk & Grześkowiak, 2015). The chemical content of green tea is complex, with polyphenols comprising 30% of dry weight and protein comprising 15-20% of the dry weight, principally from various enzymes. In addition to the contents shown in Table 2.2, sterols (stigmasterol), vitamins (B, C, E), xanthic base (caffeine, theophylline), as well as trace are also found in green tea leaves (Astill et al., 2001; Ferrara et al., 2001; Cabrera, Artacho & Giménez, 2006; Horžić et al., 2009; Pinto, 2013; Jiang et al., 2015). Of these chemical contents, flavonoids which belong to the polyphenol group, have important roles in conferring the biological activity of tea (Bansal et al., 2013). Besides of polyphenols, minerals and vitamins containing in tea leaves will continue to exist in the kombucha beverage after fermentation, and may contribute to human health following consumption (Bauer and Petrushevska, 2000; Rodrigo & Bosco, 2006; Jain et al., 2013).

Table 2.2 Composition (%) of green tea leaves

Compound	**(%, dry weight)**
Protein	15
Amino acids	4
Fibre	26
Other carbohydrates	7
Lipids	2
Pigments	2
Minerals	5
Phenolic compounds	30
Organic acids	1.5
Volatiles	<0.1

(Chacko et al., 2010)

2.7.2.1 Amino acids

Free amino acids are an important nutritive constituent of green tea, making up 1-4% (dry weight) of the total composition, which is the highest among all types of tea (Ding, Yu & Mou, 2002; Cabrera, Artacho & Giménez, 2006). Amino acids are the dominant metabolites from the nitrogen cycle of green tea trees as well as constituting the proteins in the tea leaves (Li et al., 2017). There are 26 kinds of amino acids recognized in tea leaves (Li et al., 2017), with over 50% the total free amino acids found in green tea being theanine, aspartic acid, glutamic acid, arginine and serine (Syu et al., 2008; Chako et al., 2010; Li et al., 2017). These amino acids provide an important nitrogen source for growth of yeasts and acetic acid bacteria in kombucha during fermentation. The amounts of some common amino acids found in green tea are shown in Table 2.3.

Table 2.3 Common amino acids levels in green tea

Amino acid	mg/g (dry weight)	Amino acid	mg/g (dry weight)
Alanine	0.19-0.68	Leucine	0.12-0.27
Arginine	0.12-1.31	Phenylalanine	0.20-0.50
Asparagine	0.30-1.37	Serine	0.36-0.77
Aspartic acid	1.12-2.33	Theanine	1.62-3.37
Glutamine	1.43-2.61	Threonine	0.15-1.25
Histidine	0.29-1.17	Tyrosine	0.16-0.50
Isoleucine	0.17-0.46		

(Alcázar et al., 2007)

As the predominant amino acid in green tea leaves, theanine (glutamic acid γ-ethyl amide; 5-*N*-ethyl glutamine) is reported to be responsible for the exotic and sweet umami taste of the green tea infusion (Syu et al., 2008). Theanine (Figure 2.10) is only present in the free (non-protein) form and it is the most important amino acid in green tea, not only because of its flavour attributes, but also because it is involved in the biosynthesis of polyphenols (Alcazar et al., 2007). The consumption of this bioactive has been reported to have anti-tumor effects and also decrease blood pressure (Ding, Yu & Mou, 2002), and increase dopamine, serotonin, and gamma-aminobutyric acid (γ-Aminobutyric acid) (GABA) levels in the brain, which imparts neuroprotective effects (Syu et al., 2008).

Figure 2.10 Chemical structure of theanine
(Juneja et al., 1999)

2.7.2.2 Phenolic polyphenols

Phenolic polyphenols, including flavanols and flavonoids, are the most abundant constituents in green tea leaves, accounting for 30% on a dry weight basis (Lin et al., 1998; Kome et al., 2010). Phenolic polyphenols are water-soluble and colourless; the compounds are associated with astringency and bitterness of the green tea flavour (Balentine, Wiseman & Bouwens, 1997). The classification of tea polyphenols is shown in Figure 2.11. These compounds are the most biologically active groups in green tea, and are responsible for the antibacterial, antioxidative, antiviral, antiallergenic, antiflammatory, anticarcinogenic and antimutagenic effects of its products including kombucha (Bushman, 1998; Bonoli et al., 2003; Rodrigo & Bosco, 2006; Reto et al., 2007; Mejia, Ramirez-Mares & Puangraphant, 2009; Jain et al., 2013; Lin et al., 2014; Filippis et al., 2018). Of these polyphenols, the catechin family, which is also a well-known member of a more general class of flavonoid, the flavan-3-ols, is the main contributor of the health-promoting benefits. The benefits are attributed to the free radical-scavenging capacity, enzyme modulation and metal chelating activities of catechins (Rodrigo & Bosco, 2006; Sang et al., 2011).

Figure 2.11 Classification of tea polyphenols
(Rao & Ramalakshmi, 2011)

There are six major types of catechins in green tea: (+)-catechin (C), (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC) and (+)-gallocatechin (GC) (Zhu et al., 1997; Yang & Landau, 2000; Leung et al., 2001; Gupta, Saha & Giri, 2002; Bonoli et al., 2003; Chu & Chen, 2005; Zaveri, 2006; Kome et al., 2010). The structure of these catechins are shown in Figure 2.12. EGCG is the most abundant catechin, making up 50-80% of the total catechins in tea leaves (Brannon, 2011; Ananingsih, Sharma & Zhou, 2013). One standard cup of green tea (2.5 g of green tea leaves/ 200 ml of water) is estimated to contain 90 mg of EGCG (Wu and Wei, 2002) followed by EGC, ECG and EC in decreasing order (Cabrera, Artacho & Giménez, 2006; Sang et al., 2011; Zaveri, 2006; Rao & Ramalakshmi, 2011; Jain et al., 2013). Fernández et al. (2002) analysed 45 green tea and black tea samples (from different geographical origins), and found green tea had higher levels of EGCG, ECG, EGC and EC than black tea. The highest amounts of ECGC and EGC from the 45 samples were 7.358% and 3.955% resepctively, while ECG ranged from 0.910% to 3.556%.

Figure 2.12 Structures of the major catechins found in green tea
(Zaveri, 2006)

Tea phenolic polyphenols in tea are released into the tea infusion during brewing in hot water (Velićanski et al., 2014). These compounds have been shown to increase from 40 to 120 μg/ml after 7 days fermentation with kombucha starter culture (Velićanski et al., 2014), with the main phenolic compound, catechin, reaching a level of 90.7 μg/ml after 7 days fermentation.

2.7.2.3 Alkaloids

Methylxanthines including caffeine (1, 3, 5-trimethylxanthine), and two minor isomeric dimethyxanthines (theobromine and theophylline) are the predominant alkaloids (Figure 2.13) in green tea leaves, which contribute to the mild stimulant effects of tea (Lin et al., 1998; Friedman et al., 2006; Seeram et al., 2006; Chopade et al., 2008; Komes et al., 2010). Caffeine, theobromine and theophylline not only stimulate the growth of bacteria in kombucha to produce cellulose (Greenwalt, Steinkraus & Ledford, 2000), but they are also essential nitrogen sources for kombucha culture growth (Velićanski, Cvetković & Markov, 2014).

In contrast to polyphenols, alkaloids make up only 2.5-5.5% of the total chemical composition of tea leaves (dry weight basis), with approximately 1.5-4% caffeine, 0.2-0.4% theobromine and ~0.02% theophylline (Lin et al., 1998; Schulz et al., 1999; Rao & Ramalakshmi, 2011). Although the amount of alkaloids change with tea-leaf size, brewing temperature, time and geographical origins (Fernández et al., 2002; Sang et al., 2011). The caffeine content of green tea and black tea are similar, a green tea infusion (2.0 g of green tea brewed in 200 ml water at 100 ℃ for 3 min) was found to contain 14.54 mg/L of theobromine, 6.34 mg/L of theophylline and 309.30 mg/L of caffeine (Horžić et al., 2009), and it is stable during the fermentation process (Sang et al., 2011). Caffeine remaining in kombucha acts on the central nervous system, and can stimulate alertness, and decrease the sensation of fatigue and facilitate association of ideas (Cabrera, Artacho & Giménez, 2006).

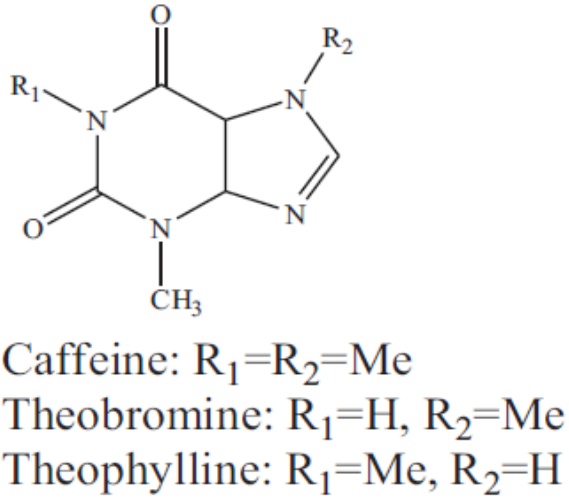

Figure 2.13 Structure of caffeine, theobromine and theophylline present in green tea (Sang et al., 2011)

2.7.2.4 Phenolic acids

Green tea contains several types of phenolic acids, such as gallic acid (GA), quinic, chlorogenic acid and caffeic acid, of which gallic acid (GA) (Figure 2.14) has the most important role (Dufresne & Farnworth, 2001; Cabrera, Artacho & Giménez, 2006). Besides the strong antioxidant activity that GA exhibits, astringent and styptic applications as well as some bioactivities, such as antineoplastic, bacteriostatic and antimelanogenic have also been reported (Yen, Duh & Tsai, 2002; Yizmaz & Toledo, 2003; Heleno et al., 2014). These healthy benefits may still derived from consumption of kombucha (Malbaša, Lončar, & Kolarov, 2004). The GA level is always higher in black tea than green tea and its quantity increases during fermentation, probably a result of its liberation from the catechins (Fernández et al., 2002). GA levels have been reported to range from 0.004 μg/ml to 0.168 μg/ml in 13 different types of green tea (Fernández et al., 2002).

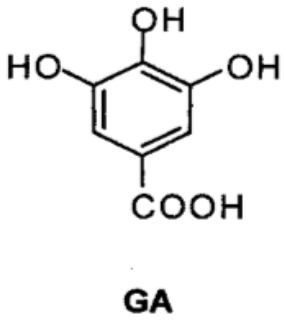

Figure 2.14 Structure of gallic acid in green tea
(Cabrera, Artacho & Giménez, 2006)

2.8 Chemical composition of kombucha

The chemical composition of kombucha beverages varies due to different fermentation conditions (e.g. fermentation temperature, fermentation time), type of substrates added (e.g. tea, sugars) and starter culture used (Chen & Liu, 2000; Petrovska & Tozi, 2000; Jayabalan et al., 2014). Organic acids are the major constituent in fermented kombucha, with the most common being acetic, gluconic, glucuronic acid, critic, L-lactic, malic, tartaric, malnonic, oxalic, succinic, pyruvic and usnic acid (Wu, Gai & Ji, 2004; Malbaša et al., 2011; Nguyen et al., 2014). With the exception of acetic acid and gluconic acid, the concentrations of the other organic acids are usually less than 1 g/L (Greenwalt, Steinkraus & Ledford, 2000). Several sugars, such as sucrose, glucose and fructose have also been reported in kombucha (Jayabalan

et al., 2014). In addition, vitamins B_1, B_2, B_6, B_{12} and C, amino acids, biogenic amines, purines, pigments, lipids, proteins, several hydrolytic enzymes, ethanol (0.5-1.5%), glycerol, carbon dioxide, phenols including tea polyphenols, minerals, anions, DSL, and other unidentified products of bacterial and yeast metabolites have also been detected (Dashti, Morshedi & Rafati, 2001; Chu & Chen, 2005; Malbaša, Lončar & Kolarov, 2006; Kozyrovska & Foing, 2010; Vitas et al., 2013; Jayabalan et al., 2014). Table 2.4 shows the main sugars and organic acids found in traditional kombucha beverages.

Table 2.4 Main sugars and acids detected in traditional kombucha beverages at the end of the fermentation

Component	Component content (g/L)	Initial sucrose (%)	Black tea	Fermentation temperature (℃)	Fermentation time (d)	Reference
Acetic acid	8	10	4 g/L	24±3	60	Chen and Liu (2000)
	4.69	10	12g/L	24±3	18	Jayabalan et al. (2007)
Glucuronic acid	0.0031	5	1.5g/L	28	21	Lončar et al. (2000)
	0.0026	7	1.5g/L	28	21	Lončar et al. (2000)
	0.0034	10	1.5g/L	28	21	Lončar et al. (2000)
	1.71	10	12g/L	24±3	18	Jayabalan et al. (2007)
Gluconic acid	39	10	4 g/L	24±3	60	Chen and Liu (2000)
Glucose	179.5	7	1.5g/L	28	21	Malbaša et al. (2002)
	24.59	7	1.5g/L	28	21	Lončar et al. (2000)
	12	10	2 bags	24±3	60	Chen and Liu (2000)
Fructose	76.9	7	1.5g/L	28	21	Malbaša et al. (2002)
	5.40	7	1.5g/L	28	21	Lončar et al. (2000)
	55	10	4 g/L	24±3	60	Chen and Liu (2000)
Remaining sucrose	192.8	7	1.5g/L	28	21	Malbaša et al. (2002)
	11	10	4 g/L	24±3	60	Chen and Liu (2000)
	2.09	7	1.5g/L	28	21	Lončar et al. (2000)

(Jayabalan et al., 2014)

2.9 Changes in the chemical and microbial composition of kombucha during fermentation

2.9.1 pH and organic acids

During fermentation, the pH of the kombucha beverage decreases due to the production of different organic acids (Chen and Liu, 2000; Kallel et al., 2012; Spasenija et al., 2012). In a study by Jayabalan et al. (2007), the pH in green tea kombucha decreased from pH 5 to pH 3 after fermentation for 12 days at 24 ± 3 ℃. A similar reduction in pH (from 3.8 to 2.6) was

observed by Kallel et al., (2012), after 15 days of fermentation at 24 ℃. With the pH decreasing, the titratable acidity (T.A.) increased concomitantly. An increase in T.A. from around 0.3 g/L pre fermentation, to 2.4 g/L post fermentation (10 days, 22 ℃), was observed in black tea kombucha, along with a decrease in pH from 6.4 to 3.6 (Lončar, 2014).

The major organic acids produced during fermentation of kombucha are acetic acid and gluconic acid (Malbaša et al., 2007; Jayabalan et al., 2014; Chakravorty et al., 2015). The concentrations of acetic acid and gluconic acid were measured by Chen and Liu (2000) using an L-6000 Hitachi High Performance Liquid Chromatography (HPLC) during fermentation. An increase of acetic acid from 2 g/L to 5 g/L and gluconic acid from 4 g/L to 8 g/L were detected during 14 days of fermentation of black tea kombucha at 24±3℃. Similar increases of the two acids have been reported by Chakravorty et al., (2015). The study by Chakravorty et al. (2015) reported acetic acid and gluconic acid in kombucha reached 5.5 g/L and 4/L respectively at the end of fermentation (14 days). However, quantities of organic acids in kombucha beverages vary according to the different substrates utilised (Chen and Liu, 2000; Malbaša, Lončar & Djurić, 2008; Chakravorty et al., 2015). When molasses was used instead of sucrose as the carbon source, acetic acid only increased to 0.28g/L by the end of fermentation (14 days), while acetic acid in kombucha fermented with sucrose under the same conditions was 0.53 g/L (Malbaša, Lončar & Djurić, 2008).

Fermentation conditions (e.g. fermentation time, fermentation temperature) can have a significant influence on the final concentration of metabolites in the kombucha product. Investigations into the effect of inoculum concentration and fermentation temperature on metabolite concentrations showed that about 4g/L of total acids were obtained from kombucha inoculated with 15% of starter culture broth and fermented at 30℃ for 10 days, while only 2.2g/L of total acids were detected in kombucha fermented at 22℃ with 10 % inoculum (Loncar, 2006). These results indicate that temperature and inoculum concentration affect the concentration of total acids produced during kombucha fermentation.

2.9.2 Sugar content and total soluble solids (TSS)

Sucrose, as the most common carbon source in kombucha decreases linearly during fermentation (Chen & Liu, 2000; Malbaša et al., 2006; Malbaša et al., 2008; Kallel et al., 2012). The decrease in sucrose is caused by the metabolism of the sugar by the yeast and correlates with a reduction in total soluble solids (TSS), which is indicative of the amount of remaining sugar in the beverage (Corona et al., 2016; Randazzo et al., 2016). In a study by Kallel et al. (2012), sucrose in green tea kombucha decreased rapidly at a rate (R^2>0.99) of 2.3 g. L^{-1}. d^{-1} until a concentration of 72 g/L was reached at the end of 15 days' fermentation. Meanwhile, glucose and fructose increased to 5.2 g/L and 12.2 g/L respectively, by the end of fermentation (15 days). These results are in agreement with the study of Chen and Liu (2000) who measured the concentration of sucrose, glucose and fructose and observed a steady decrease in sucrose from 94 g/L to 62 g/L after 14 days of fermentation, while glucose and fructose increased to 8 g/L and 18 g/L respectively (Chen & Liu, 2000). The degradation of sucrose by yeast invertase into glucose and fructose may be the reason for this result (Chen and Liu, 2000).

2.9.3 Ethanol

During fermentation, sucrose is first hydrolysed to two monosaccharides (glucose and fructose) by yeast invertase. Ethanol is then produced via glycolysis, with preference for fructose as a substrate (Blanc, 1996; Greenwalt, Steinkraus & Ledford, 2000; Malbaša et al., 2005; Lončar et al., 2006; Talebi et al., 2014; Michatowska et al., 2016). Several studies (Sievers et al., 1995; Chen and Liu, 2000; Malbaša et al., 2006) have reported the ethanol content in kombucha increases first and then decreases during prolonged fermentation. For example, Chen and Liu (2000) studied changes of ethanol content during prolonged fermentation of black tea kombucha for 60 days at 24±3°C by gas chromatography (GC) system (Hitachi G-3000, Tapan) equipped with a flame ionization detector. Ethanol content in this study increased linearly to a maximum of 5.5g/L at day 12, then slowly decreased to around 0.18g/L at the end of fermentation (60 days). A similar trend was reported by Sievers et al., (1995), in which ethanol content was determined enzymatically using combinations of test kits of Boehringer Mannheim (NO. 71626, NO. 428191, NO. 148261, NO. 176290), where the concentration of ethanol was around 3.6 g/L after 10 days of fermentation, and reached a maximum of 9.1g/L after 24 days of fermentation, then decreased to 0.7g/L after 62 days. The decrease in ethanol content during

prolonged fermentation may be attributed to the oxidation of ethanol to acetic acid by acetic acid bacteria. In a nutrient depleted environment, ethanol is used as a source of carbon by acetic bacteria (Sievers et al., 1995). According to the NZ Food standards on non-alcoholic beverages and brewed soft drinks (FSANZ Act 2.6.2, 2017), tea kombucha belongs to the brewed soft drink category and the alcohol content should be less than 1.15% by volume (< 9.0735 g/L). Previous studies (Sievers et al., 1995; Chen & Liu, 2000; Jayalaban et al., 2014) have shown that tea kombucha contains less than this amount of ethanol when fermented for less than 14 days.

A study by Guzel-Seydim et al. (2000) indicated ethanol increased from 0.4 µg/g to 0.8 µg/g after 21 days of storage (4°C) in a mixed-culture fermented beverage. Increasing ethanol levels in kombucha during storage were attributed to sugar metabolism by yeasts. Under anaerobic conditions, yeast hydrolysed glucose to pyruvate by glycolysis and pyruvate was then metabolised into acetaldehyde which was further hydrolysed to ethanol through the fermentation pathway (Bai et al., 2008; Pfeiffer & Morley, 2014).

2.9.4 Vitamins and minerals

Changes in the level of water-soluble vitamins and minerals, such as vitamins B_1, B_{12}, B_6, C and minerals (manganese, iron, nickel, copper, zinc, lead, cobalt, chromium, cadmium) in fermented kombucha drinks have been reported (Bauer and Petrushevska, 2000). Generally, most of the minerals, such as manganese, iron, nickel, copper and zinc increased after fermentation when compared to levels in the unfermented tea infusion. Manganese increased from 0.362 ± 0.015µg/ml in tea infusion to 0.462 ± 0.024 µg/ml, and iron increased from 0.257 ± 0.013µg/ml to 0.353 ± 0.018µg/ml. Cobalt however did not accumulate, probably due to its participation in the synthesis of vitamin B_{12} (Bauer and Petrushevska, 2000). A similar pattern has been reported by Jayabalan et al. (2010), with magnesium and iron content in the tea fungus increasing during fermentation; magnesium accumulated from 0.37 ± 0.01 to 0.45 ± 0.00 (g/100 g of dry matter) while iron increased from 0.05 ± 0.00 to 0.06 ± 0.00 (g/100g of dry matter). The increased levels of minerals has been attributed to the metabolic activity of kombucha starter cultures (Jayabalan et al., 2014)

In a study by Malbaša et al. (2011), vitamin B_2 in green tea kombucha increased from around 5 mg/100ml after three days of fermentation to a maximum of 9.6 mg/100 ml at the end of fermentation (10 days), with vitamin C increasing consistently from 1.4 mg/L to a peak of 8.50 mg/L over the same period. These results are in agreement with the study of Bauer and Petrushevska (2000), in which vitamins B_1, B_{12}, B_6 and C in black tea kombucha increased from 0.46 mg/ml, 0.36 mg/ml, 0.29 mg/ml, and 0.71 mg/ml to 0.74 mg/ml, 0.84 mg/ml, 0.52 mg/ml and 1.51 mg/ml respectively, after 8 days of fermentation. Biosynthesis of essential minerals and vitamins during fermentation is attributed to the metabolic activities of yeast in kombucha and the high content of these minerals and vitamins may contribute to the health promoting properties of kombucha.

2.9.5 Colour

The colour of kombucha beverage changes notably during fermentation, becoming lighter which means the lightness (L* value) generally increases as fermentation progresses (Jayabalan et al., 2007). Hrnjez et al. (2014) studied the colour of fermented kombucha dairy during storage for 14 days at -4°C and reported an increase of L* from 83.71 ± 0.17 to 84.22 ± 0.01, however the changes were insignificant ($p<0.05$). The redness and greenness (a*) of the beverage increased from -1.97±0.01 to 2.21±0.02 after 7 days of storage and decreased with prolonged storage until the 14^{th} day, reaching 2.06 ± 0.02. However, yellowness and blueness (b*) of fermented kombucha dairy initially decreased from 7.09±0.02 to 6.93±0.02 after one week of cold-storage and increased to 7.12 ± 0.01 at the end of storage (Hrnjez et al., 2014). The degradation or biotransformation of polyphenols by enzymes liberated by bacteria and yeast under the acidic environment, especially (degradation) of theaflavins and thearubigin may be responsible for the lighter colour (Jayabalan et al., 2014).

2.9.6 Viable cell counts of acetic acid bacteria and yeast during fermentation and storage of kombucha

Changes in viable cell counts of yeast and acetic acid bacteria have been reported in kombucha beverages during fermentation (Chen & Liu, 2000; Velićanski, Cvetković & Markov, 2013; Jayabalan et al., 2014; Lončar et al., 2014). Yeast cell counts in nine different samples of tea fungus initially increased with fermentation time, reaching a peak after 6 days of incubation,

with cell counts increasing from 0.21-2.74 $\times 10^6$ cfu ml^{-1} at day 0 to 12-78.5$\times 10^6$ cfu ml^{-1} at day 6 (Chen and Liu, 2000). However, with prolonged fermentation, yeast cells gradually decreased. In the same study (Chen and Liu, 2000), acetic acid bacteria increased during the first six days of fermentation, increasing from 1.50-18.8 $\times 10^3$ cfu ml^{-1} at day 0 to peak levels of 9.30-91.50 $\times 10^3$ cfu ml^{-1} at day 6, and then slowly decreased to 0-8.55 $\times 10^3$ cfu ml^{-1} after 30 days of fermentation.

Table 2.5 Yeast cell counts in nine kombucha samples inoculated with the same starter culture during prolonged fermentation

(Chen and Liu, 2000)

Changes in viable cell counts of yeast, and acetic acid bacteria in nine different kombucha tea samples during fermentation for 30 days are shown in Tables 2.5 and 2.6 (Chen and Liu, 2000). The reduction of microorganisms in the late phase of fermentation may be due to the increased acidic environment. In this study, the final pH of the beverage was around 2.5, which is much lower than the optimum pH for the growth of yeast (pH 5.4-6.3). Also, as the fermentation progresses, carbon dioxide produced by the yeast may accumulate at the interface between the liquid broth and the cellulose pellicle, thereby blocking the transfer of nutrients from the broth to the pellicle, as well as affecting the transfer of oxygen from the surface of pellicle to the broth. These two factors may have generated an anaerobic and nutrient-depleted environment, in which few genera of bacteria and yeasts could survive (Chen & Liu, 2000).

Table 2.6 Acetic acid bacteria cell counts in nine kombucha samples inoculated with same starter culture during prolonged fermentation

(Chen and Liu, 2000)

Fu et al., (2013) reported a gradual decrease in yeast and acetic acid bacteria cell counts in kombucha during storage at 4 ℃ for 14 days. In this study, the content of yeast and acetic acid bacteria were determined every two days. The cell counts of yeast decreased steadily from 1.45 x 10^7 cfu/ml to around 1.10 x 10^7 cfu/ml during first 10 days of storage and then followed by a rapid decrease to around 4.0 x 10^6 cfu/ml at the end of storage (14 days). Meanwhile, the number of acetic acid bacteria decreased steadily from 1.0 x 10^7 cfu/ml to 3.4 x 10^6 cfu/ml over the 14-day period. Decreases in viable yeast and acetic acid bacteria cell counts over the 14-day period were attributed to low pH and a nutrient-depleted environment (Fu et al., 2013).

2.10 Sensory attributes of kombucha beverage

The taste of kombucha is slightly acidic, sweet and sparkling which is significantly affected by fermentation temperature, time and materials used for the fermentation (Jayabalan et al., 2014; Grama- Michałowska et al., 2016). Ayed and Hamdi (2015) fermented cactus pear juice with kombucha culture for 15 days at 30℃ and compared the sensory properties of the fresh pear juice with juice fermented for 6 and 12 days. The taste varied significantly as the fermentation period increased with the juice fermented for 6 days being much sweeter than that fermented

for 12 days. Moreover, the juice fermented for 12 days was deemed too sour and acidic for consumption. The undesirable vinegary taste of the pear juice kombucha was attributed to the accumulating of organic acids (Ayed & Hamdi, 2015). According to Jayabalan et al. (2014), the taste of tea kombucha changes from a pleasant fruity sour-like refreshing flavour after fermentation for a few days to a mild vinegary-like taste after longer fermentation, even to unacceptable acidity levels which may pose potential risks when consumed (Sreeramulu et al., 2000). Thus, the concentration of added sugar, the fermentation temperature and fermentation time must be well-controlled to produce a healthy product with acceptable sensory properties (Jayabalan et al., 2014).

2.11 Beneficial effects of kombucha

2.11.1 Antioxidant activity

The use of naturally occurring phytochemicals in functional and antioxidant foods is an increasing global trend (Jayabalan et al., 2014). Polyphenols specifically catechins, which mainly belong to the flavanols group are proved to be the main antioxidants in kombucha (Cabrera, Gimenez & Lopez, 2003; Kilmartin & Hsu, 2003; Yang et al., 2009; Srihari & Satyanarayana, 2012). In addition, metabolites produced by kombucha's symbiotic consortium of bacteria and yeast, include vitamins B_2, B_6, C and catalase, have the ability to scavenge free-radicals (Malbaša et al., 2011). Health benefits of kombucha such as cancer prevention, alleviation of inflammation and arthritis, and enhancement of immunity may occur due to antioxidant properties (Dipti et al., 2003; Jayabalan et al., 2014; Vitas et al., 2018).

Jayabalan et al. (2008) investigated the antioxidant activity of kombucha by analysing 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability, inhibitory capacity on hydroxyl radical mediated linoleic acid oxidation and scavenging ability on superoxide anions. The results indicated the DPPH scavenging abilities of green tea kombucha increased as fermentation progressed, reaching around 84% at 12 days of fermentation, with the inhibitory capacity on hydroxyl radical-mediated linoleic acid oxidation being around 50% and a slight increase of superoxide radical scavenging ability. Fu et al. (2013) reported the DPPH scavenging ratio of green tea kombucha reached 95.30% after 90 hours of fermentation at 30℃ which was much higher than that of black tea kombucha (38.7%); this result was probably caused by the higher level of polyphenols in green tea than other teas (Fu et al., 2013). The increase in the antioxidant

activity of tea kombucha beverages after fermentation is probably due to the generation of low-molecular-weight substances (ascorbic acid and D-saccharic acid -1,4- lactone) and structural modifications of tea polyphenols by enzymes produced by yeast and bacteria during fermentation (Jayabalan et al., 2014). These studies indicate that tea kombucha has high antioxidant activity and may be consumed as a source of natural antioxidants with potential to promote health.

2.11.2 Antimicrobial activity

The antimicrobial activity of kombucha tea against a range of pathogenic microorganisms is well-documented (Mayser et al., 1995; Tu, Xia & Watanabe, 2005; Mo, Zhu & Chen, 2008; Santos, Batista & Rodrigues, 2009; Battikh, 2013). Kombucha can not only inhibit some Gram-positive bacteria, but also has antimicrobial potential against Gram-negative bacteria (Mo et al., 2008; Battikh et al., 2013). Previous studies have shown strains of *Aeromonas hydrophila*, *Enterobacter cloacae*, *Salmonella typhimurium*, *Helicobacter pylori*, *Staphylococcus aureus*, *Yersinia enterolitica*, *Escherichia coli*, *Agrobacterium tumefaciens*, *Campylobacter jejuni*, *Bacillus cereus*, *Shigella sonnei*, *Samonella enteritidis*, and *Candida albicans* can be also inhibited by kombucha (Dufresne & Farnworth, 2000; Tu, Xia & Watanabe, 2005; Mo, Zhu & Chen, 2008; Cetojevic-Simin et al., 2008; Battikh, Bakhrouf & Ammar, 2012; Jayabalan et al., 2014).

The antimicrobial efficacy of kombucha beverage is mainly due to the presence of organic acids, particularly acetic acid (Adams & Hall, 1988; Sreeramulu, Zhu & Knol, 2001; Battikh, Bakhrouf & Ammar, 2012; Ayed & Hamdi, 2015). Accumulation of dissociated acid anions to toxic levels and cytoplasmic acidification are the two main reasons for the antimicrobial activity of the organic acids (Velićanski et al., 2014). In addition, kombucha can however, still exert antimicrobial capacity against *E. coli*, *C. jejuni*, *S. typhimurium*, *S. sonnei* and *S. enteritidis* after thermal denaturation or at a neutral pH, suggesting the presence of antimicrobial compounds other than acetic acid (Sreeramulu, Zhu & Knol, 2000). Some studies (Mo, Zhu & Chen, 2008; An et al., 2008, Battikh, Bakhrouf & Ammar, 2012; Kallel et al., 2012; Bhattacharya et al., 2016; Lobanova et al., 2016) have attributed the antimicrobial potency of kombucha beverages after thermal treatment to large proteins and polyphenols derived from the tea infusion.

The antimicrobial effects of green tea kombucha against several pathogens, as represented by the presence of an inhibition halo zone are shown in Table 2.7. This study (Table 2.7) showed green tea kombucha had the strongest antimicrobial effects against *S. epidermidis*, *M. luteus*, *L. monocytogens* and *P. aeruginosa* (inhibition zone ≥ 18 mm) (Battikh et al., 2013).

Table 2.7 Antimicrobial effects of fermented green tea kombucha

Target microorganisms	**Inhibition zone Ø (mm)**
Staphylococcus epidermidis	22.0 ± 1.4
Staphylococcus aureus	12.0 ± 0.0
Micrococcus luteus	22.0 ± 2.8
Salmonella Typhimurium	14.0 ± 1.4
Escherichia coli	14.5 ± 0.7
Listeria monocytogenes	21.5 ± 2.1
Pseudomonas aeruginosa	18.0 ± 0.4
Candida parapsilosis	15.0 ± 1.4
Candida dubliniensis	13.5 ± 0.7

(Battikh et al., 2013)

2.12 Conclusion

In conclusion, fermentation as a food processing method prolongs the shelf-life of food as well as improving the nutritional content and sensory attributes. Kombucha as a traditional health-promoting fermented beverage, which is prepared with tea, sugar and symbiotic consortium of yeast and bacteria has generated interest recently. The fermented beverage contains a large number of nutrients from tea, such as polyphenols, minerals, amino acids and alkaloids, which may contribute to the health-promoting effects (antioxidant and antibacterial activities). Moreover, fermentation products, such as acetic acid, gluconic acid, ethanol, glycerol and glucose play important role in the sensory attributes of kombucha. However, the constituents of the final products of kombucha may be significantly affected by fermentation conditions such as fermentation time, fermentation temperature, and sugar added concentration, thereby compromising their health benefits. At present, kombucha on the New Zealand market is dominated by small scale and household artisan producers, resulting in the production of products with variable quality. Thus, it is essential to conduct a study to optimise the fermentation of kombucha in order to upscale for the production of safe, consistent high quality beverages.

3. Materials and Methods

3.1 Experimental design

Fermentation time, fermentation temperature and sugar concentration are the most important factors for producing green tea kombucha with optimum balance of chemical, physical, microbial and sensory characteristics (Jayabalan, 2014). In order to investigate the optimum fermentation conditions for green tea kombucha beverage, three factors were analysed in this study: fermentation time (7, 10 and 14 days), fermentation temperature (22 and 24℃) and sugar concentration (7 and 10%). The experimental design was carried out in four integrated phases as shown in Table 3.1.

Table 3.1 Experimental design of treatments for selecting the most promising formulation for fermentation of green tea kombucha beverage

Experiment number	Experimental code	% Sucrose (w/v)	Fermentation temperature (℃)
1	22/7	7	22
2	22/10	10	22
3	24/7	7	24
4	24/10	10	24

Phase 1

In Phase 1, the microbial composition (acetic acid bacteria, lactic acid bacteria and yeasts) of kombucha starter culture (SCOBY) supplied by K4 Kombucha Limited (Bay of Islands, NZ) was analysed. Yeast extract glucose chloramphenicol (YGC) agar was used to enumerate yeasts, while acetic acid bacteria were determined using yeast peptone mannitol (YPM) agar containing 4 mg/L of cycloheximide, and lactic acid bacteria were enumerated on de Man, Rogosa and Sharpe (MRS) agar.

Phase 2

In Phase 2, green tea kombucha beverages were prepared with two sugar concentrations (7% and 10%) and fermented at two fermentation temperatures (22℃ and 24℃) for 14 days (Table 3.1). Titratable acidity (TA), pH, total soluble solids (TSS), and colour were determined, and

enumeration of yeast and acetic acid bacteria were carried out over 14 days of fermentation. Consumer sensory evaluation was conducted on Day 7, 10 and 14 during the fermentation. The effect of time on fermentation of green tea kombucha beverage was analysed and the optimum fermentation time was selected according to the physico-chemical, microbial analysis and sensory evaluation.

Phase 3

In Phase 3, the effects of sugar concentration and fermentation temperature on the fermentation of green tea kombucha were investigated. In addition to the analyses and measurements (pH, TA, TSS, colour, cell counts) conducted in Phase 2, the concentrations of ethanol, organic acids and sugars were determined during fermentation for 7 days and storage (4℃) for 14 days. Sensory evaluation was conducted at the end of fermentation (Day 7), and during storage (4℃) after 1 and 2 weeks. According to the physico-chemical, microbial analysis and sensory evaluation, the most promising formulation was selected from this phase (3) and was used in Phase 4 to determine the storage stability of fermented kombucha at 4°C for 4 weeks.

Phase 4

The antibacterial activity and antioxidant content of the final kombucha products were determined after 7 days of fermentation in Phase 4 in order to investigate any potential beneficial effects of green tea kombucha. Microbiological and physico-chemical analyses of the final product selected from Phase 3 were analysed weekly during storage (4℃) for four weeks as previously described (Phases 2 and 3). The experiments were replicated twice and sample characteristics were either analysed in duplicate.

3.2 Description of key fermentation factors

3.2.1 Fermentation time

Previous studies showed that fermentation of black tea kombucha for 7 days at 28°C produced a product with acceptable sensory profile (Greenwalt, Steinkraus & Ledford, 2000; Loncar, 2006; Jayabalan et al., 2014). However, the acidity increases with increasing fermentation time, making the kombucha beverage sour and unpleasant (Chen and Liu, 2000). Therefore,

fermentation time plays an important role in the development of the sensory properties of kombucha beverage. Based on previous reports (Dufresne & Farnworth, 2000; Greenwalt, Steinkraus & Ledford, 2000; Jayabalan et al., 2014), fermentation times of 7, 10 and 14 days were chosen for use in this study. Microbiological, physico-chemical and sensory profiles of the fermented green tea kombucha were determined at Day 7, 10 and 14 during fermentation. Based on these results, the optimum fermentation time was selected for use in further experiments.

3.2.2 Temperature

The rate of fermentation and microbial species involved in green tea kombucha are affected by fermentation temperature (Pederson, 1971). Therefore, an appropriate fermentation temperature can create a favourable environment for microbial growth, which has a significant effect on the kinetics of substrate utilisation and hence composition of the final product (Loncar, 2006). In this study, 22 ℃ and 24℃ were used for the fermentation of kombucha (Chen and Liu, 2000; Lallel et al., 2012; Lončar et al., 2014).

3.2.3 Concentration of sucrose

The pattern of microbial growth in food fermentation is important, thus it is necessary to create a suitable growth environment in order to obtain the desired fermented product (Pederson, 1971; Amore & Faraco, 2013). One of the key fermentation conditions impacting on the sensory characteristics of the final product is the nutrient content. Sucrose, as the main carbon source for the fermentation of kombucha, has a significant influence on the production of sensory compounds such as acetic acid, gluconic acid, sucrose, fructose and glucose (Malbasa, 2008; Hamad, 2011). In previous studies (Malbaša et al., 2008; Jayabalan et al., 2014), 7% or 10% sugar added for kombucha fermentation produced a product with acceptable sensory properties. Therefore, in this study, two levels (7% and 10%) of sucrose were used for the preparation of green tea kombucha.

3.3 Materials

Kombucha tea fungus and kombucha vinegar used for preparation of kombucha starter culture and green tea kombucha beverage were supplied by K4 Kombucha Limited NZ (Bay of Islands, NZ) and were transported to Massey University, Albany Campus on ice (4°C). Upon delivery to Massey University, the materials were stored in a refrigerator (4°C). Organic green tea (Green Darjeeling, NZ) purchased from Keri Keri Organic Tea Company (Bay of Islands, NZ) was used to provide the liquid medium for kombucha beverage fermentation. Certified organic raw sugar (Chelsea Refinery, Auckland, NZ), purchased from Countdown Supermarket, (Sunnynook, Auckland, NZ) served as source of carbon for the fermentation.

3.4 Methods

3.4.1 Preparation of starter culture

Organic green tea, organic cane sugar, kombucha tea fungus and broth were used to prepare kombucha starter culture according to a modified method of Hrnjez et al., (2014), as shown in Figure 3.1. Potable water (1600 mL) was boiled (100 °C) using an electric kettle (Sunbeam, Australia) and 9 g (0.45%) of green tea leaves was added to the boiled water (100 °C), followed by 140 g (7%, w/v) of cane sugar. The mixture was stirred until completely dissolved, cooled to ambient temperature (22°C-28°C) and 2 mL (0.1%) of kombucha vinegar added to the mixture before being filtered through a coffee filter (Size 4, Fagg's coffee, NZ) into a 3 L glass jar. Thereafter, 50 g (2.5 %) kombucha tea fungus and 400 mL (20%, v/v) kombucha broth were added to the mixture (Figure 3.1). The jar was covered with a nylon cloth, secured with a rubber band and placed into an incubator (Labserv, Ireland) to ferment aerobically at 22°C for 7 days (Figure 3.1). At the end of fermentation, the mixture was filtered through a coffee filter to obtain a clear kombucha beverage. The tea fungus and broth (starter culture) were stored at 4°C until required for further fermentation procedures.

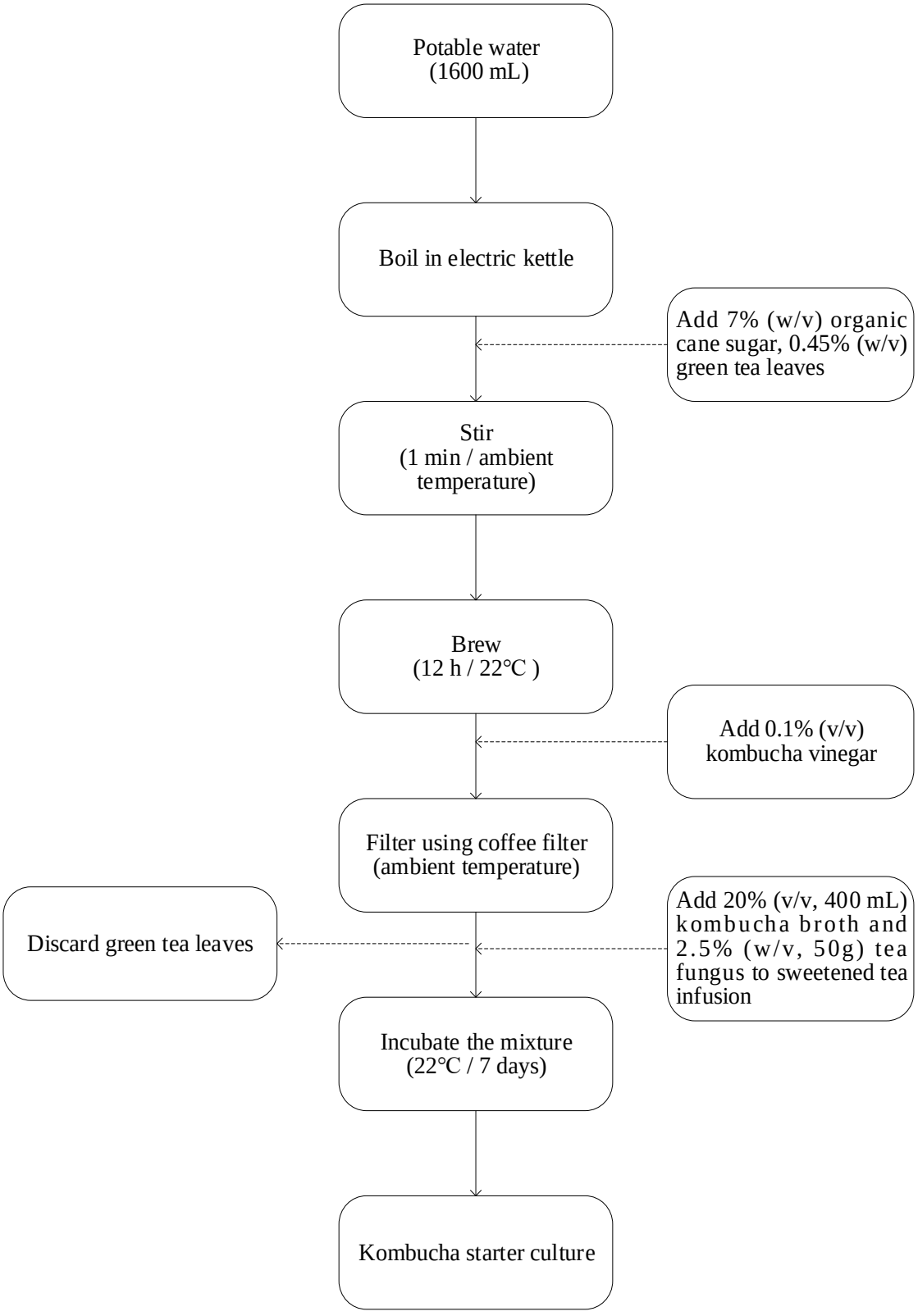

Figure 3.1 Preparation of green tea kombucha starter culture

3.4.2 Preparation of green tea kombucha beverage

General overview

Kombucha starter culture prepared as described in Section 3.4.1 was used to prepare green tea kombucha with green tea leaves, organic cane sugar and kombucha vinegar. Two litres (2 L) of the fermentation mixture contained 20% kombucha starter culture (400 mL of kombucha broth, 50 g of tea fungus) and 80% green tea medium (1600 mL) was fermented in a 3-L, sterile glass jar (Figure 3.2).

On Day 0, Kombucha starter culture (20% of kombucha broth and 2.5% of tea fungus) was transferred into a glass jar, followed by the addition of 15% sweetened green tea (240 mL). The jar was covered with a clean cloth and secured tightly using a rubber band (as shown in Figure 2.2, Section 2.2). The contents were allowed to ferment for 24 h at 22°C or 24°C (Figure 3.2).

On day 1, 30% sweetened green tea (480 mL) was added into the same glass jar which had been fermenting for 24 h at 22°C or 24°C. The glass jar was covered as previously described and the contents allowed to ferment for a further 24 h at either 22°C or 24°C (Figure 3.2).

On day 2, 55% sweetened green tea (880 mL) was added into the glass jar which had been fermenting for 48 h and covered. The contents were allowed to ferment for 5 or 12 days at 22°C or 24°C (Figure 3.2). The total fermentation times were either 7 days or 14 days.

Preparation of green tea medium

Potable water was boiled (100 °C) and then the following ingredients were added sequentially: green tea leaves (0.45%), organic cane sugar (7% or 10%). The tea infusion was stirred until the sugar was completely dissolved, and the tea infusion allowed to brew for 12 hours at 22°C, after which kombucha vinegar (0.1%) was added, mixed well and filtered through a coffee filter. The sweetened green tea infusion was then transferred into a 3-L glass jar.

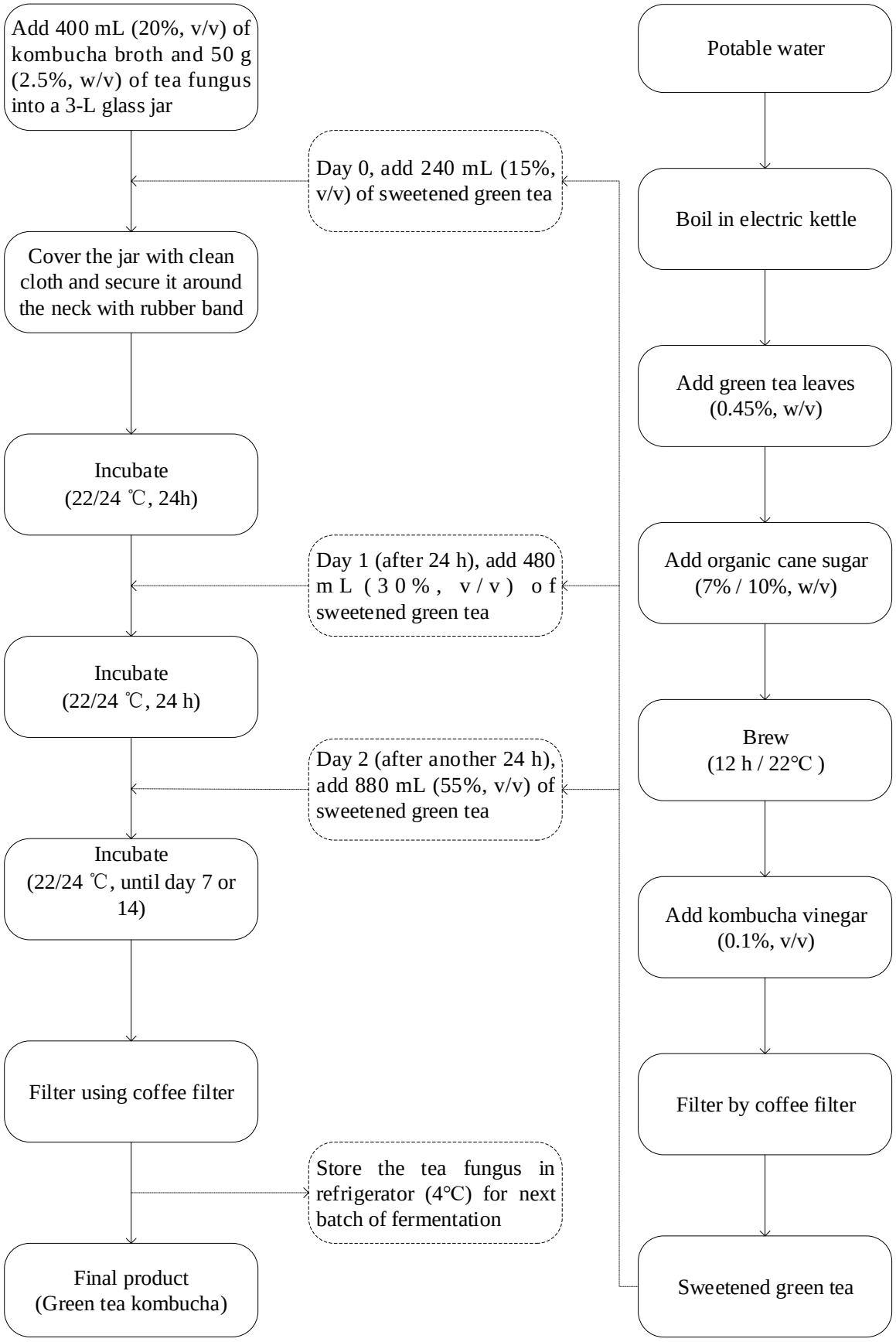

Figure 3.2a Preparation of green tea kombucha

Figure 3.2b Preparation of sweet green tea solution

3.5 Measurement of pH and chemical analysis

3.5.1 Measurement of pH

pH was measured in duplicate using a digital pH meter (Sartorius PB-20, USA) equipped with a glass electrode according to the AOAC method (AOAC 981.12, 2005). Before measurement, the pH meter was calibrated at pH 4.0 and 7.0 using standard buffer solutions (LabServ, Thermofisher, NZ). To measure pH, 10 g of kombucha were weighed into a 50 mL glass beaker using a top pan balance (AND FZ-500i, Korea), and 20 g of distilled water was added. The mixture was swirled and the pH was measured.

3.5.2 Determination of titratable acidity

Titratable acidity (TA) was determined by acid-base titration using phenolphthalein (0.1%) as the indicator according to the AOAC method (AOAC 947.05, 2005). Previously standardised 0.1 M NaOH (Univar, Ajax Finechem Pty Ltd, NZ) was used to titrate against 10 g of fermented green tea kombucha, which was pre-weighed using an analytical balance (Appliance Check, NZ) and mixed with 20 g of distilled water. A few drops (4-5 drops) of phenolphthalein solution (1%) were added to the mixture and swirled to mix. The mixture was titrated against standardised 0.1 M NaOH solution until the first persistent (30 s) pink colour was noted and the volume of titre used was recorded. Calculated titratable acidity was expressed in grams of acetic acid per litre of sample (equation 1). The titratable acidity was measured in duplicate and the experiment was conducted twice (AOAC 947.05, 2005).

$$\%\ Acetic\ acid = \frac{volume\ of\ NaOH\ \ used(ml) \times 0.0060}{sample\ weight\ (g)} \times 100 \quad \text{---------- (1)}$$

Molecular weight of acetic acid = 60 g/mol

1 mL 0.1 M NaOH = 0.0060 g acetic acid;

1 mL of test sample ≈ 1 g of sample

3.5.3 Determination of total soluble solids

Concentration of total soluble solids (TSS) was determined at ambient temperature (22-28°C) using a digital refractometer (Atago PR-101, Japan) previously calibrated with distilled water following the AOAC method 932.12 (AOAC, 1990); the results were expressed as °Brix (Note:

the °Brix is the unit of TSS; one degree Brix is defined as 1 gram of TSS in 100 grams of solution). The measurement of TSS was conducted in duplicate and the experiment performed twice.

3.5.4 Determination of colour

A Konica Minolta spectrophotometer (CM-5, Japan) was used to measure the colour of green tea kombucha following the method of Kurtmann et al., (2009) and the supplier's instructions (Konica Minolta, Japan). The L*, a*, b* colour system is a common system for the measurement of colour with L* representing whiteness (+) and blackness (-). An increase in L* means the colour becomes whiter. a* measures the redness (+) or greenness (-); a decrease of a* indicates the colour changes from red to green; while yellowness (+) and blueness (-) are shown by the b* value; a decrease of b* reflects a change in colour towards blue. Prior to measurement of colour, the spectrophotometer was allowed to warm up for 1 minute and then calibrated according to the manufacturer's instructions (Konica Minolta, Japan). The test sample (3 mL) was transferred into a 4-mL glass spectrophotometer cuvette (Sigma Aldrich, NZ) and illuminated with D65 artificial daylight (10° standard angle) under the conditions recommended by the manufacturer. Sample colour was measured directly; the measurements were performed in duplicate and the experiment was replicated.

3.5.5 Analysis of organic acids

The levels of acetic acid and gluconic acid in green tea kombucha beverage were determined by high performance liquid chromatography (HPLC) as described by Lin (2011) with minor modifications. The HPLC system consisted of: LC-10AT(Shimadzu Corp, Japan), column oven (CTO-10AS, Shimadzu Corp, Japan), auto-injector (SIL-10A, Shimadzu Corp, Japan) and system controller (SCL-10A, Shimadzu Corp, Japan) equipped with an ultra violet (UV) detector (SPD-10A, Shimadzu Corp, Japan) and a refractive index (RI) detector (RID-10A, Shimadzu Corp, Japan). Sulphuric acid (0.01 M), which was previously filtered through a 0.22 µm nylon membrane filter (Merck, Germany) and degassed in an ultrasonic bath (Bandelin Sonorex Super RK510, Germany) was used as the mobile phase at a flow rate of 0.4 mL/ min. A Rezex ROA-organic acid (8% cross-linked resin) column (300 × 7.8 mm) was used for the analysis of acetic acid and gluconic acid at 17 °C. Prior to analysis, a series of external

standards of acetic acid (≥99.5%, Fisher scientific, UK) and gluconic acid (49-53 %, w/v in water, Sigma Aldrich, USA) were prepared in distilled water, the concentrations of these two standards acids were 0.01%, 0.1%, 0.5%, 1.0%, 2.0% (w/v). All standards and test samples were filtered through 0.22-µm syringe filters (Merck, Germany) into 2-mL vials (Shimadzu Corp, Japan). Automatic injections (20 µL) of standards and test samples were conducted in duplicate. Acetic and gluconic acids present in each sample were identified and quantified by comparison with the retention times and calibration curves constructed using the peak areas of the standards obtained using Shimadzu LC solutions software (Shimadzu Prominence, Japan).

3.5.6 Analysis of sugars

Determination of sucrose, glucose and fructose in green tea kombucha beverage was performed by high performance liquid chromatography (HPLC) following the method of Stadie (2013) with minor modifications. The HPLC system used for the analysis of sugars is described in Section 3.5.4. The mobile phase used was distilled water, which was previously filtered through a 0.22 µm nylon membrane filter (Merk, Germany) and degassed in an ultrasonic bath (Bandelin Sonorex Super RK510, Germany), the flow rate was 0.6 mL/ min. A separation Rezex RCM- Monosaccharide, RCM $Ca2^{+}$ (8% cross-linked resin) column (300 × 7.8 mm) was used for the determination at 80 °C. Prior to analysis, a series of sucrose (≥99.5%, Sigma Aldrich, NZ), glucose (≥99.5%, Sigma Aldrich, NZ) and fructose (≥99%, Sigma Aldrich, NZ) standards were prepared and run as external standards, the concentration of sucrose standards were 0.01%, 0.1%, 2.5%, 5%, 10% (w/v); the concentrations of glucose and fructose standards were 0.1%, 1%, 3%, 5%, 10% (w/v). All sugar standards and test samples were filtered through 0.22-µm syringe filters (Merck, Germany) and stored in 2-mL vials (Shimadzu Corp, Japan) at -4℃ until required for use. Automatic injections (20 µL) were conducted in duplicate and each sample was identified and quantified by comparison with retention times and calibration curves based on peak areas of the (sugar) standards using Shimadzu LC solutions software (Shimadzu Prominence, Japan).

3.5.7 Analysis of ethanol

The concentration of ethanol in green tea kombucha was analysed by gas chromatography (GC), using a GC-17 A Shimadzu unit (Shimadzu Corporation, Japan). The alcohol was separated

using a Phenomex DBwax column (30m * 0.32 mm internal diameter) with 0.25 µm stationary phase and detection was achieved by using a flame ionisation detector (FID). Nitrogen (Oxygen free, BOC, NZ) at 76 mL/min was used as the carrier gas. The injector temperature was 150°C, and column temperature was programmed as shown in Figure 3.3:

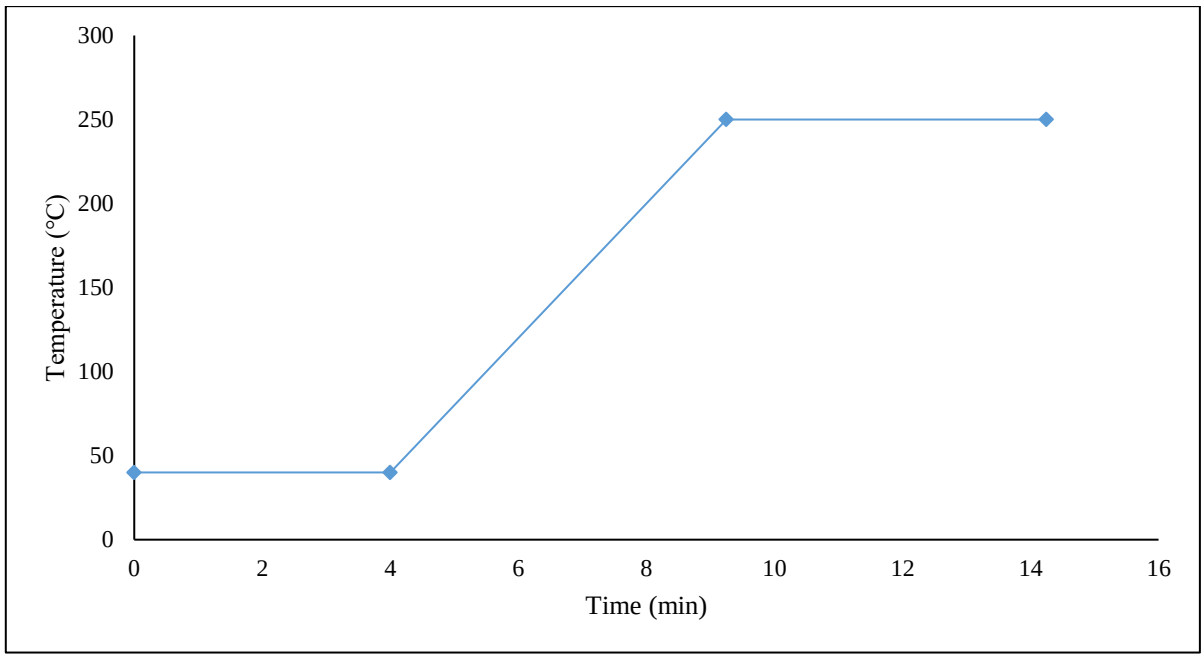

Figure 3.3 GC column temperature programme during analysis of ethanol

Notes: rate of temperature increase = 33.33 °C/ min; flow rate of carrier gas = 76 mL/min.; injection temperature = 150°C.

Prior to analysis, each sample was filtered through a 0.22-µm syringe filter (Merck, Germany) into 2-mL vials (Shimadzu, AUS). A sample (0.2 µL) was injected into the GC for the analysis which took 14.25 min. Ethanol (≥98%, Thermo Fisher Scientific, NZ) standards were prepared as follows: 0.01%, 0.1%, 0.2%, 0.5%, 1% and 1.5% (w/v) by diluting in distilled water and used to generate a standard curve, from which the concentration of the ethanol in green tea kombucha beverages was determined.

3.5.8 Analysis of antioxidants

Analysis of the phenolic compounds (gallic acid, EGCG, ECG and EGC) and alkaloids (caffeine and theobromine) in green tea kombucha beverages were conducted by HPLC using the method of Yao et al. (2004) with minor modifications. Phenolic and alkaloids compounds were determined by reversed phase HPLC (Shimadzu UFLC, Shimadzu Prominence, Japan)

with dual pumps (LC-20AD), an SPD-M20A photodiode array detector and an auto-sampler (SIL-20ACHT). Mobile phase A (0.1% Trifluoroacetic acid (TFA) in Milli-Q water) and mobile phase B (0.1% TFA in acetonitrile) were used for the analysis of antioxidants at 0.75 mL/min. A 5-µm Grace Smart RP18 column (250 × 4.6 mm) (Grace Davison Discovery Sciences, Deerfield, IL, USA) was used, and the analysis temperature was set at 18℃. Determination of the compounds was conducted at 270 nm, and the phenolic constituents and alkaloids were identified and quantified by comparison of retention times and peak areas with the standards. Peaks present on sample and standard chromatograms were integrated using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). Samples and standards were filtered through a 0.20 µm syringe filter (Merck, Germany) and stored in 2-mL vials at -4 ℃ (Shimadzu, AUS) prior to analysis. Samples and standards (20 µL) were auto-injected onto the column for analysis, which was conducted in duplicate.

3.6 Enumeration of yeast, acetic acid bacteria and lactic acid bacteria

3.6.1 Yeast

Yeast cells present in green tea kombucha starter culture and beverages were enumerated using YGC agar (Merck, Germany) as reported by Laureys & De Vuyst (2014). The agar was prepared according to the manufacturer's instructions (Astell Scientific, UK). Suitable dilutions of samples were prepared in 0.1% peptone water (Merk, Germany) using standard microbiological techniques (Laureys & De Vuyst, 2014). Diluted samples were plated by pour-plating in duplicate and solidified medium was incubated aerobically in a 25℃-incubator (Clayson IM1000R, Australia) for 5 days. Grown colonies were enumerated using a colony counter (Bibbyscientific, UK) and results were reported as log colony forming units per mL of sample.

3.6.2 Acetic acid bacteria

Enumeration of acetic acid bacteria (AAB) in green tea kombucha starter culture and beverages was determined by the pour-plating method using YPM agar (Aleksandra Velicanski, 2013). The medium was prepared following the manufacturers' instructions. Suitable dilutions of starter culture or kombucha were pour-plated and the solidified agar plates were incubated (Clayson IM1000R, Australia) aerobically at 28℃ for 7 days. Grown colonies were

enumerated using a colony counter (Bibbyscientific, UK) and results were reported as log colony forming units per mL of sample.

3.6.3 Lactic acid bacteria

Lactic acid bacteria in green tea kombucha starter culture were enumerated as described by Laureys & De Vuyst (2014), using MRS agar (Oxiod, UK). The medium was prepared according to the manufacturer's instructions and suitable dilutions were pour-plated. Solidified agar plates were incubated (Clayson IM1000R, Australia) at 37 ℃ for 72 hours under anaerobic conditions using an Anaerogen pack (AN0035A) (Mitsubishi Gas Chemical Company Inc., Japan). Grown colonies were enumerated using colony counter (Bibbyscientific, UK) and results were reported as log colony forming units per mL of sample.

3.7 Determination of antibacterial capability of fermented green tea kombucha

The antibacterial capability of green tea kombucha was determined by the agar diffusion method of Battikh (2013) with minor modifications. Gram-positive and gram-negative pathogens comprising *Escherichia coli* 111, *Listeria monocytogenes* 15E03-1, *Salmonella typhimurium* ESR3479, *Staphylococcus aureus* MU-A57 and *Pseudomonas aeruginosa* MU-A26 were used to determine the antibacterial ability of fermented green tea kombucha.

The frozen pathogenic bacteria were thawed in a refrigerator (4℃). Using a sterile automatic micropipette, one drop of each pathogen was transferred into 10 mL nutrient broth and then activated by incubation at 37℃ for 24 h. The grown culture was then purified by streaking on nutrient agar and incubating at 37℃ for 24 hours. A purified culture was then inoculated into nutrient broth using a sterilised loop and incubated for 24 h at 37℃. The absorbance of the overnight culture was measured using a spectrophotometer (Novaspec III, Biocherom Ltd, England) at 595 nm. The inoculated broth was then adjusted to an absorbance of 0.2±0.01 at 595 nm using sterile nutrient broth.

Nutrient agar was prepared according to the manufacturer's instructions. Molten nutrient agar was poured into sterile plates and allowed to solidify. Of each broth culture with adjusted absorbance, 0.2 mL were added into 7 mL of molten nutrient agar, followed by gentle mixing

to prevent the creation of air bubbles. The mixed liquid was then poured onto a previously solidified nutrient agar plate. The plate with added bacterium cells was gently tilted in order to completely cover the bottom solidified agar layer and allowed to solidify for 10-15 min at ambient temperature (20 °C). Six (6) mm paper discs (Sparks MD 21152, Becton, Dickinson and Company, USA) were immersed into the test sample (fermented kombucha) and then placed on the mixed agar with the bacterium; the agar plates with discs were incubated at 28℃ for 48 hours. The diameter of each visible growth inhibition zone was measured after 48 hours incubation. The experiment was conducted in duplicate.

3.8 Sensory evaluation

3.8.1 Sensory evaluation of green tea kombucha in Phases 2 and 3

Sensory evaluation of food aims to generate data in order to understand the acceptability and preferences of products, as well as provide insights into the formulation of commercial strategy and guide in the development of products (Syarief et al., 1985; Morten et al., 2007; Kemp, Hollowood & Hort, 2009). In Phases 2 and 3 of this study, informal focus groups of 5-7 experienced sensory panellists who were familiar with fermented kombucha evaluated the product at a round-table discussion. Informal focus groups are forums of small groups of people brought together to share their opinions and ideas on a given topic. It is an economic, quick and efficient way to collect data and obtain information (Beyea and Nicoll, 2000). Focus groups have been widely used in social science, market and health care research. Using informal focus groups in round table discussions strengthened the reliability of the results (Powell and Single, 1996).

During the sensory evaluation session, 20 mL of chilled (4℃) green tea kombucha beverage was served in a 25-mL transparent plastic cup covered with a lid to each of the sensory panellists. Each sample was evaluated for appearance, aroma, flavour, sweetness, sourness and overall acceptability followed by discussions by the informal focus group. Discussions were manually recorded as well as by 'Voice Memos' using iPhone 6s (Apple Inc., 2016). Prior to evaluation of each sample, panellists were required to rinse their pallate with potable water (22 °C). In Phase 2, sensory evaluation was conducted on products after 7, 10 and 14 days of fermentation (Table 3.2). While in Phase 3, sensory evaluation was conducted at Day 7 of fermentation, and thereafter during storage (4℃**)** after 1 and 2 weeks (Table 3.3).

Table 3.2 Samples used in sensory evaluation sessions of green tea kombucha in Phase 2 with 5 sensory panellists

Fermentation time (day)	Fermentation temperature (℃)	% Sucrose (w/v)	Sample code
7	22	7	7-22/7
7	24	7	7-24/7
7	22	10	7-22/10
7	24	10	7-24/10
10	22	7	10-22/7
10	24	7	10-24/7
10	22	10	10-22/10
10	24	10	10-24/10
14	22	7	14-22/7
14	24	7	14-24/7
14	22	10	14-22/10
14	24	10	14-24/10

Table 3.3 Samples used in sensory evaluation sessions of green tea kombucha in Phase 3 with 5 sensory panellists

Sampling time	Fermentation temperature (℃)	% Sucrose (w/v)	Sample code
Day 7	22	7	D7-22/7
Day 7	24	7	D7-24/7
Day 7	22	10	D7-22/10
Day 7	24	10	D7-24/10
Week 1	22	7	W1-22/7
Week 1	24	7	W1-24/7
Week 1	22	10	W1-22/10
Week 1	24	10	W1-24/10
Week 2	22	7	W2-22/7
Week 2	24	7	W2-24/7
Week 2	22	10	W2-22/10
Week 2	24	10	W2-24/10

3.8.2 Sensory evaluation of green tea kombucha during storage (4 °C)

Thirty (30) consumer sensory panellists were randomly recruited by email communications and signage posted at Massey University's Product Development Laboratory (Auckland). Before sensory evaluation, each participant was briefed about the project and then requested to complete an Ethics Form as approved by the Massey University Human Committee (Ethics Approval Number 40017016), regarding the aims of the study, composition of the beverages and privacy of the participants. Sensory panellists were required to evaluate each sample for appearance, aroma, sweetness, sourness and overall acceptability of the final (optimised) green tea kombucha using a 1-9-point hedonic scale (Meilgarrd, Civille, & Carr, 2006; Lamia Ayed, 2017). During sensory evaluation, 20 mL of chilled (4℃) green tea kombucha beverages contained in 25-mL transparent cups covered with lids were presented to the panellists. Potable water (22℃) in disposable cups was provided to the participants for rinsing their palate before tasting each sample. Sensory evaluations were conducted under white light and each panellist evaluated the sensory attributes of green tea kombucha beverages on a sensory score sheet (Appendix C). Sensory evaluation was conducted weekly during storage (4°C) of samples for 4weeks.

3.9 Analysis of data

For each sample, data for pH, TSS, TA, colour, viable cell counts, organic acid levels, sugar levels, ethanol content, antioxidant content, antibacterial capability, as well as sensory evaluation were collected in duplicate. All experiments were repeated twice. Minitab version 17 Statistical Software (Minitab Inc., State College, PA, USA) and Microsoft Excel 2016 (Microsoft Inc., Santa, CA, USA) were used for the analysis of data. All data are shown as mean ± standard deviation (SD) and MS Excel was used to calculate mean values and standard deviations, as well as generate graphs to show trends during fermentation and storage of kombucha. One-Way Analysis of Variance (ANOVA) was used to determine the effect of fermentation time and temperature, sugar concentration on physical (colour), chemical (pH, TSS, TA, organic acids, sugars, ethanol, antioxidants), microbiological (Acetic acid bacteria, yeast, antibacterial potential) and sensory properties of fermented green tea kombucha at $p<0.05$. Significant differences of the means between groups (95% C.I.) were compared using Tukey's test.

4. Results and discussion

4.1 Phase 1: Appearance and microbiological composition of kombucha starter culture

The kombucha tea fungus used for the preparation of starter culture in this study was provided by K4 Kombucha NZ, Bay of Island, New Zealand. The tea fungus was a white, slippery and elastic jelly membrane (zoogeal mat) comprising of several layers which floated on the top of the liquid (Figure 4.1). The same observations have been reported in previous studies of kombucha (Chen and Liu, 2000; Jayabalan et al., 2010; Jayabalan et al., 2014; Sun, Li & Chen, 2015; Ayed, Abid & Hamdi, 2017).

In this study, after fermentation for 7 days at 22°C, the kombucha contained high levels acetic acid bacteria (6.08±0.06 log cfu/ml) and yeasts (7.13±0.07 log cfu/ml). Lactic acid bacteria were not present. The viable cell counts reported here were higher than in previous work (Velićanski, Cvetković, & Markov, 2013), where the viable cell counts of acetic acid bacteria and yeasts were 5.57±0.12 log cfu/ml and 6.59±0.20 log cfu/ml after 7 days of fermentation at 28±1°C. This difference in cell counts may be attributed to differences in the type of strains used, geographical origin of the starter culture and fermentation conditions. When grown on cycloheximide-YPM agar, the acetic acid bacteria were characterised by the growth of small, round colonies on the surface and inside the medium. Yeast colonies were large, white and grew on the surface of YGC agar. The morphology of the yeasts and the acetic acid bacteria obtained in this study were similar to the previous reported mentioned here Velićanski, Cvetković, & Markov, 2013).

Microbiological composition of kombucha starter culture is highly dependent on the source of the tea fungus culture (Fu et al., 2014), intrinsic and extrinsic factors. The available nutrients and temperature play important roles in determining the composition of kombucha culture (Chen and Liu, 2000; Lončar, 2006; Malbaša, 2008).

Figure 4.1 Kombucha starter culture (broth and fungus) (a), and tea fungus (b) fermented for 7 days at 22℃

(Image was captured by iPhone 6S, Apple Inc., USA)

4.2 Phase 2: Effect of fermentation time on physico-chemical, microbial and sensory properties of green tea kombucha

4.2.1 pH and titratable acidity (TA)

The pH and TA of green tea kombucha beverages containing 7% and 10% sugar and fermented at 22℃ and 24℃ were determined on day 0, day 1 before addition of sweetened green tea (Day 1-BA), day 1 after addition of sweetened green tea (Day 1-AA), day 2 before addition of sweetened green tea (Day 2-BA), day 2 after addition of sweetened green tea (Day 2-AA), days 7, 10 and 14 (Figure 4.2 and Figure 4.3). During the initial fermentation period (Day 0 - Day 2-AA), pH decreased after one day of fermentation and then increased after the addition of sweetened green tea. Meanwhile, the titratable acidity of the kombucha beverages increased after one day of fermentation and decreased after the addition of sweetened green tea. The increase of pH and decrease of TA during this period was potentially caused by the addition of sweetened green tea which decreased the acidity of kombucha. After Day 2-AA, the pH decreased significantly ($p<0.05$), from 3.35±0.05 - 3.36±0.06 to 2.86±0.01 - 2.94±0.06 (Appendix C) at the end of the 14-day fermentation period. Similar trends have been reported in previous studies (Malbaša et al., 2011; Kallel et al., 2012, Chakravotry et al., 2016).

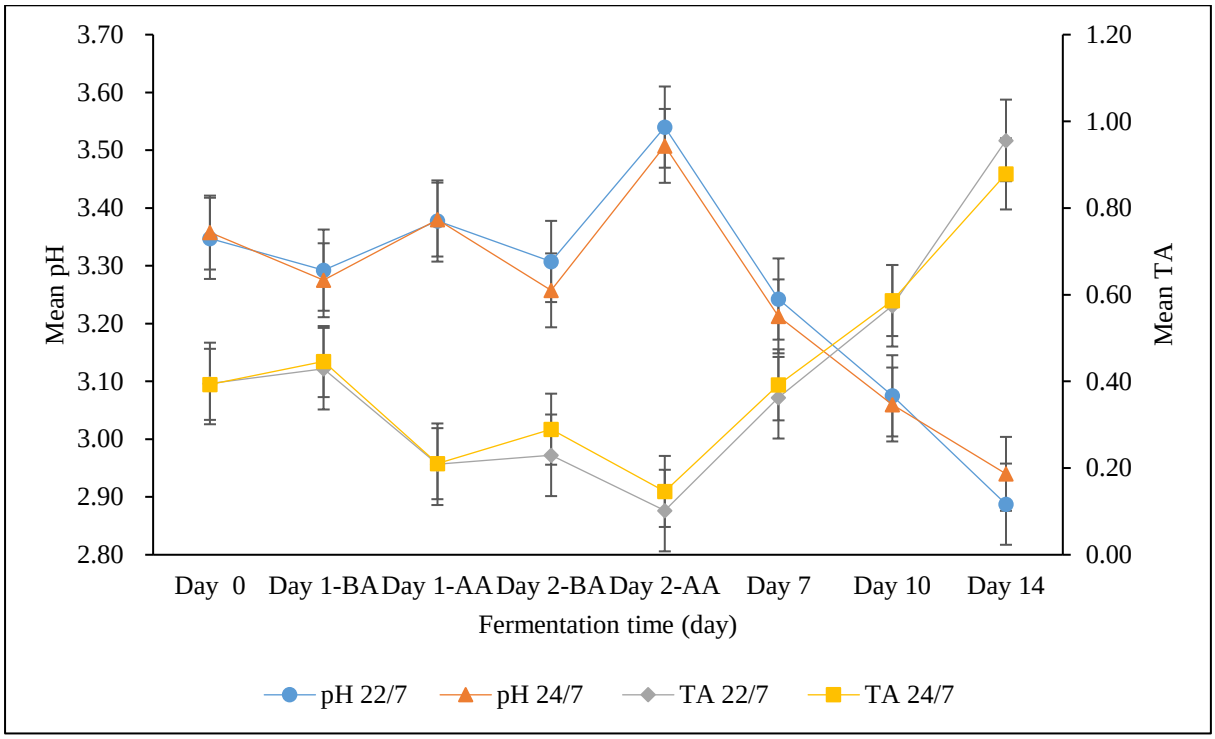

Figure 4.2 Mean pH and titratable acidity (%) of green tea kombucha beverages containing 7% sugar during fermentation for 14 days at 22°C and 24°C

Notes: TA = Titratable acidity; BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

Titratable acidity increased sharply from Day 7 (0.36±0.02 - 0.42±0.04) to Day 14 (0.88±0.04 - 1.01±0.06) ($p<0.05$), concomitantly with a decrease in pH (Figure 4.2 and 4.3). The significantly ($p<0.05$) increased acidity of the green tea kombucha beverages during fermentation coincides with the steady increase observed in the TA from day 6 to day 15 during black tea kombucha fermentation at 24±3°C (Chen & Liu, 2000). Similar changes in the levels of acids during fermentation have also been reported (Lončar et al., 2006) in black tea kombucha fermented at 22°C, during which the total acids increased from around 0.7 g/L at day 5 to 2.25 g/L at day 10. The increase in acid levels is caused by the activities of the kombucha culture which mainly comprise of yeast and acetic acid bacteria. During fermentation, sucrose added in the beverages is hydrolysed by yeast invertase into glucose and fructose, which is further metabolised by acetic acid bacteria into gluconic acid and acetic acid (Dufrene & Farnworth, 2000).

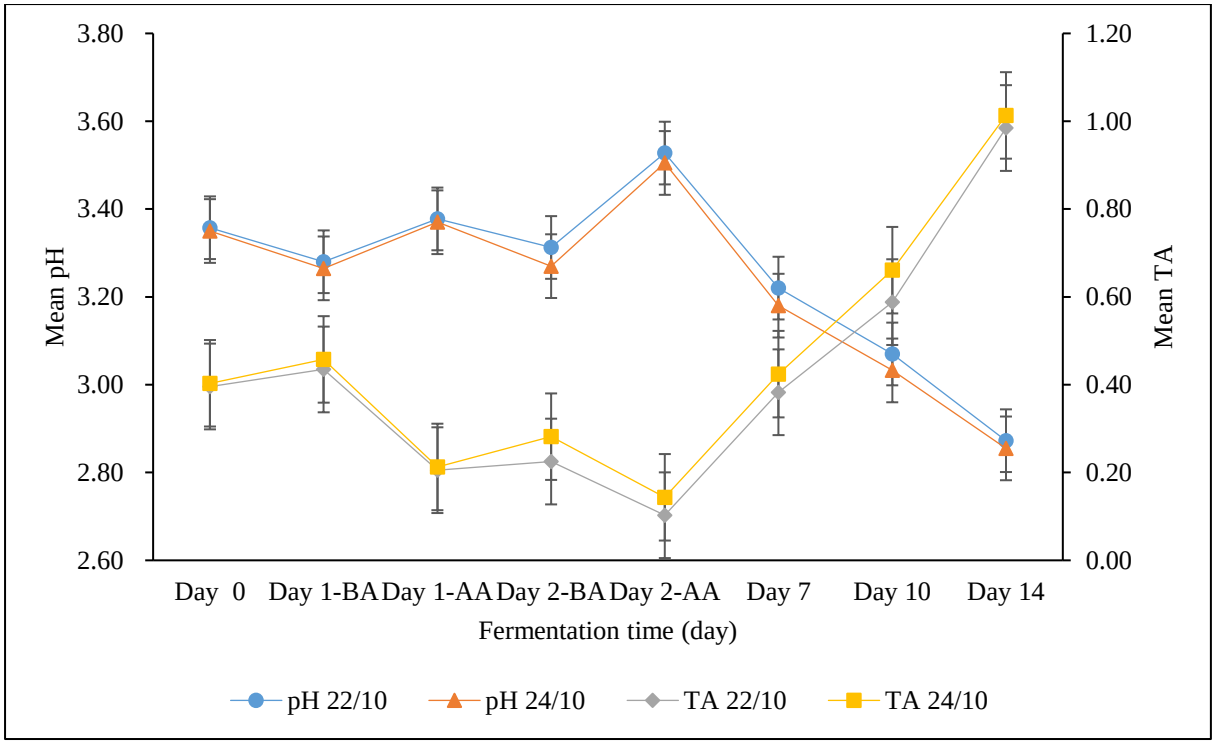

Figure 4.3 Mean pH and titratable acidity (%) of green tea kombucha beverages containing 10% sugar during fermentation for 14 days at 22℃ and 24℃

Notes: TA = Titratable acidity; BA = before addition of green tea; AA = after addition of green tea; Samples 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

4.2.2 Total soluble solids (TSS)

As the basic carbon source for the green tea kombucha (Section 3.2.3), sucrose plays an important role on the metabolism of acetic acid bacteria and yeast. The consumption of sucrose added in the green tea kombucha by the starter culture during fermentation influences the levels of total soluble solids (TSS) (Stadie et al., 2013). The changes of TSS in green tea kombucha during fermentation for 14 days at 22℃ and 24℃ are shown in Figure 4.4

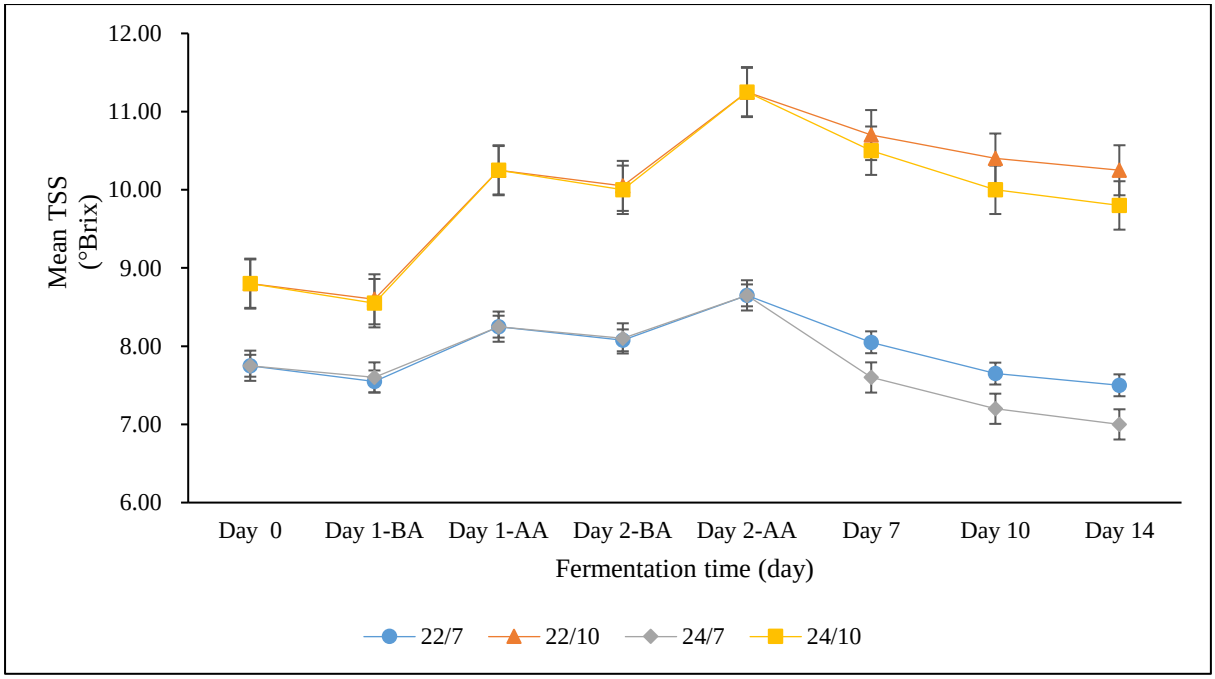

Figure 4.4 Mean total soluble solids (°Brix) of green tea kombucha beverages during fermentation for 14 days at 22℃ and 24℃

Notes: TSS = Total Soluble Solids; BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

During the initial fermentation period (Day 0 - Day 2-AA), total soluble solids decreased after one day of fermentation and increased after the addition of sweetened green tea, reaching the highest level at Day 2-AA. Variable concentrations of TSS were attributed to the addition of sweetened green tea. From Day 2-AA to the end of the fermentation (Day 14), the TTS in all samples decreased significantly ($p<0.05$). From Day 7 to Day 14, TSS in samples containing 7% sugar decreased from 7.60±0.00 - 8.05±0.05 to 7.00±0.00 - 7.50±0.00 ºBrix, and samples containing 10% sugar decreased from 10.50±0.50 - 10.70±0.40 to 9.80±0.40 - 10.25±0.15 ºBrix. The reduction in TSS during fermentation was likely caused by the metabolism of sucrose into fructose and glucose via the symbiotic consortium of yeasts and acetic acid bacteria in the kombucha culture, and further conversion of fructose and glucoese to organic acids (Chen & Liu, 2000; Feldmann, 2005; Dickinson & Kruckeberg, 2006).

4.2.3 Colour

Colour is a key attribute of green tea kombucha and has a significant influence on the appearance of the beverage, which directly affects the acceptability of the product by consumers (Chaturvedula & Prakash, 2011). The colour of kombucha was primarily influenced by the green tea infusion used for the fermentation, with both the infusion temperature and infusion time contributing to the colour of the green tea (Chatuvedula & Prakash, 2011; Kelebek, 2016). In this study, green tea used in the preparation of kombucha was infused in boiled (100 °C) water and allowed to brew at ambient temperature for 12 hours.

Colour measurements of green tea kombucha beverages were conducted during fermentation for 14 days (Figure 4.5, 4.6 and 4.7). During the initial fermentation period (Day 0 - Day 2-AA), the lightness (L*) of the beverages increased after one day of fermentation and decreased after addition of the sweetened green tea into the fermenting kombucha beverage. Following the addition, the lightness/ brightness of kombucha increased steadily as the fermentation time increased ($p<0.05$). From Day 2-AA to Day 14, the lightness of all samples increased from 85.93±0.58 - 87.18±0.58 to 89.41±0.61 - 91.90±0.09 which indicated the colour of the beverages became lighter during the fermentation. The observed increase in the colour of the kombucha was probably caused by the suppression of ionization and destruction of the polyphenols due to the enzyme activities of the kombucha cultures (Haslam, 2003; Jayabalan et al., 2014).

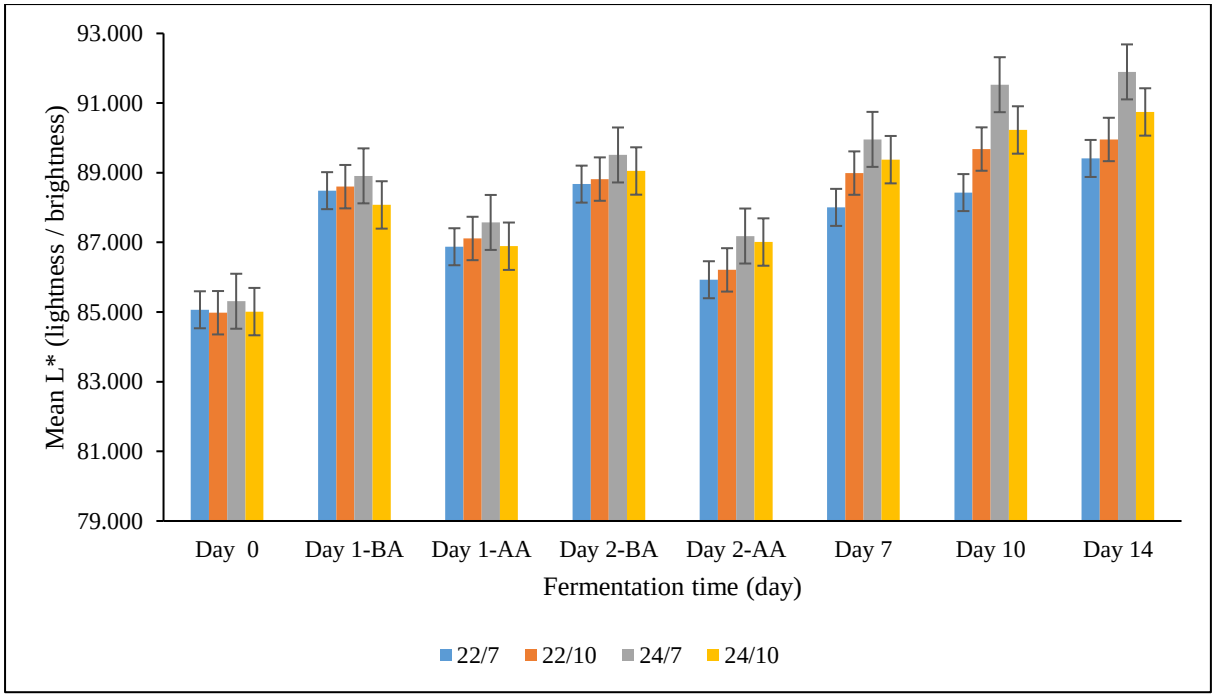

Figure 4.5 Mean L* (Lightness/brightness) of green tea kombucha during fermentation for 14 days at 22°C and 24°C

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

The redness-greenness of the green tea kombucha beverages is represented by a* value. The effect of fermentation time on redness-greenness is shown in Figure 4.6. The changes in redness-greenness (a*) were different from the lightness (L*), but similar to changes in the yellowness-blueness (b*) of the products. Both a* and b* of all samples decreased after fermentation and increased after the addition of the sweetened green tea. After Day 2-AA, a* and b*decreased during the remaining fermentation period.

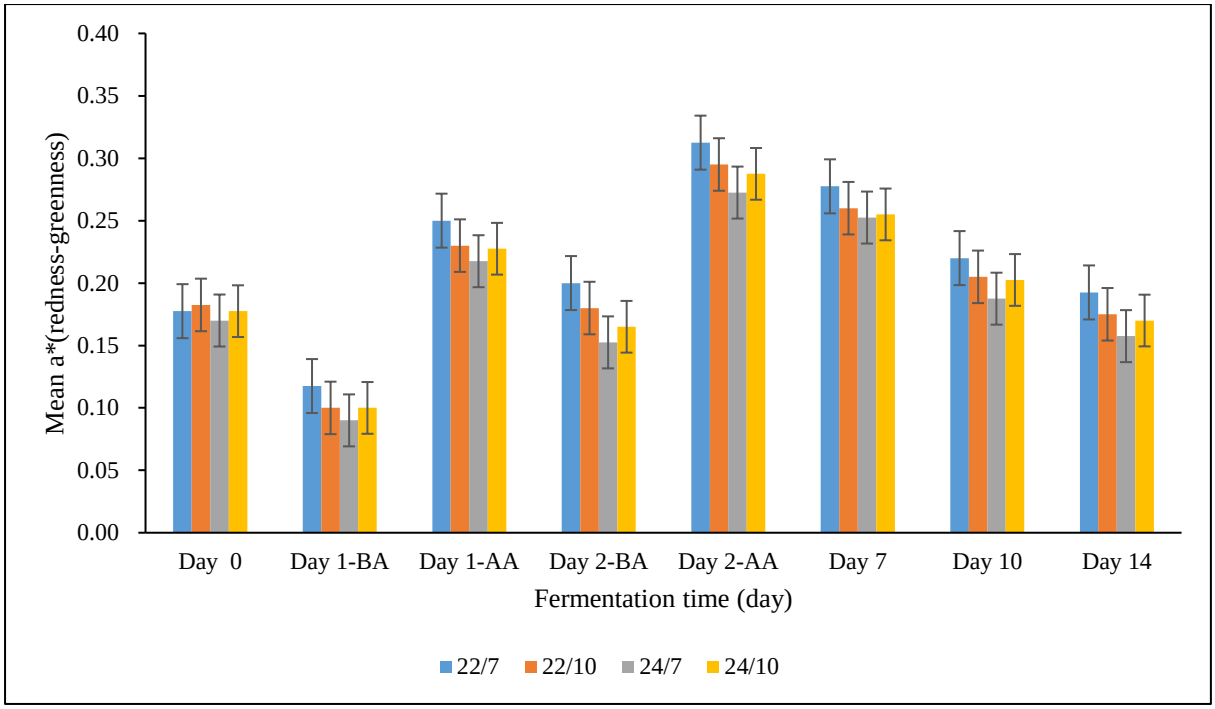

Figure 4.6 Mean a* (redness-greenness) of green tea kombucha during fermentation for 14 days at 22°C and 24°C

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

During Day 2-AA to Day 14, the redness-greenness (a*) of the four products decreased from 0.27±0.01 - 0.31±0.02 to 0.16±0.01 - 0.19±0.01 ($p<0.05$), and the yellowness-blueness reduced significantly ($p<0.05$) from 18.01±1.14 - 19.77±0.04 to 12.62±0.30 - 14.49±0.16. Based on these results, it appears that the fermentation time had a significant ($p<0.05$) effect on the colour of green tea kombucha, with the colour becoming lighter as fermentation progressed. The changes of colour may be caused by the transformation and degradation of the constituents in tea under the acidic environment during fermentation (Chu & Chen, 2006).

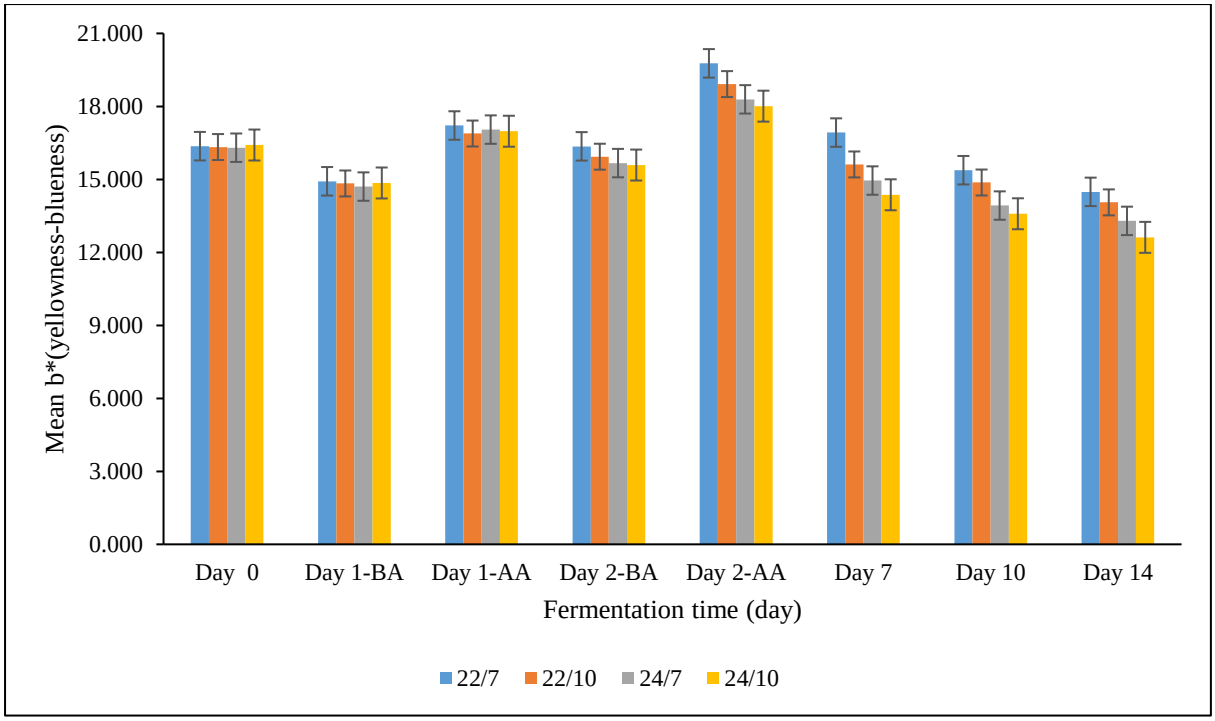

Figure 4.7 Mean b* (yellowness-blueness) of green tea kombucha during fermentation for 14 days at 22°C and 24°C

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea Kombucha fermented at 22°C; 22/10 = 10% sugared green tea Kombucha fermented at 22°C; 24/7 = 7% sugared green tea Kombucha fermented at 24°C; 24/10 = 10% sugared green tea Kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

4.2.4 Microbiology

The influence of fermentation time on the growth pattern of yeasts and acetic acid bacteria (AAB) in green tea kombucha beverages during fermentation is shown in Figure 4.8 and Figure 4.9. During the fermentation period, the yeast and acetic acid bacteria exhibited similar growth trends, suggesting the symbiotic metabolism of the two microorganisms. It is well-known that yeasts hydrolyse sucrose to glucose and fructose, which is then converted to gluconic acid and acetic acid by acetic acid bacteria. These two organic acids are the major compounds present in green tea kombucha. Liu et al. (1996) reported that acetic acid facilitates the yeasts to produce ethanol and the production of ethanol stimulates the acetic acid bacteria to grow. This growth pattern of yeast and acetic acid bacteria in kombucha is commonly called symbiotic mutualistic metabolism (Sreeramulu, Zhu & Knol, 2000; Ayed et al., 2017).

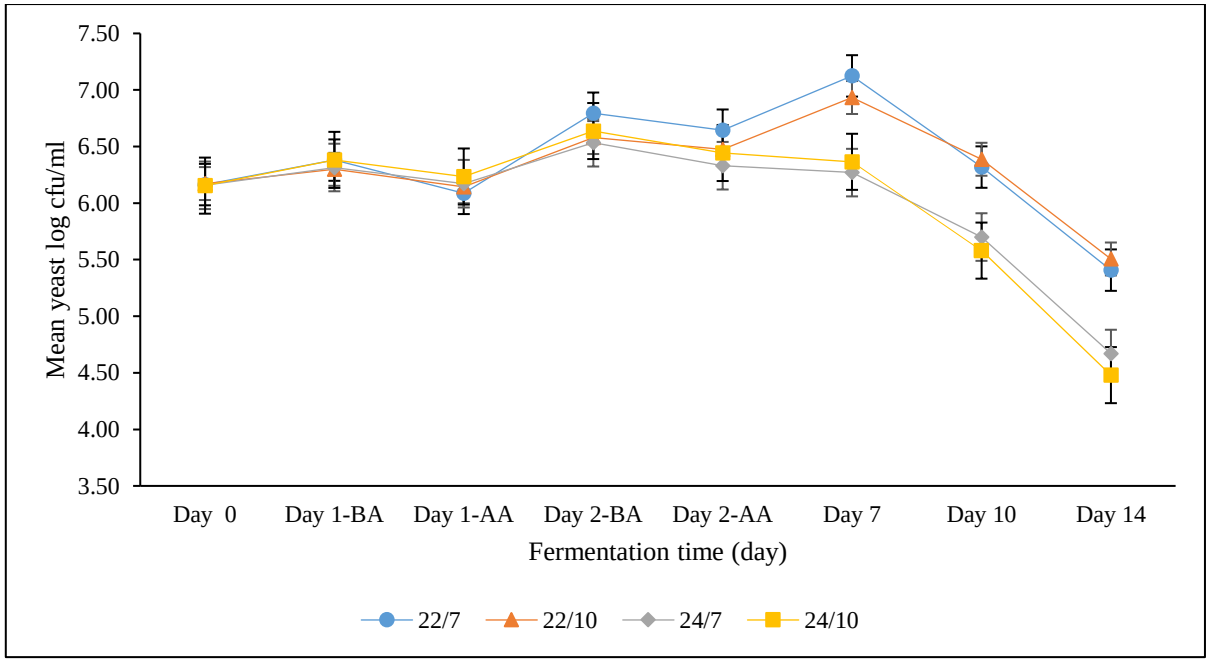

Figure 4.8 Mean log cfu/ml of yeast in green tea kombucha during fermentation for 14 days at 22℃ and 24℃

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

From Day 0 to Day 2-AA, both yeast and acetic acid bacteria (AAB) cell counts in the four different treatments increased after one-day incubation and decreased after the addition of the sweetened green tea, likely due to the dilution of the broth. From Day 2-AA to Day 14, the fermentation time had a significant effect ($p<0.05$) on the growth of yeast and AAB, with the yeast and AAB in the kombucha samples fermented at 22°C increasing firstly to 6.93±0.32 - 7.12±0.08 log cfu/mL and 6.00±0.23 - 6.13±0.08 log cfu/mL at Day 7, and thereafter decreasing to 5.41±0.18 - 5.51±0.16 log cfu/mL and 4.34±0.29 - 4.48±0.31 log cfu/mL respectively. In contrast, in samples fermented at 24°C, yeast and AAB numbers decreased from Day 2-AA, reaching their lowest values at Day 14. A similar decrease in yeast and AAB numbers was also observed by Chen and Liu (2000) during days 7 to 14 of black tea kombucha fermentation at 24 ± 3 ℃. The low pH might be one of the reasons for these results, because the optimum pH for the growth of yeasts is 5.4-6.3, while the pH in this study was 3.14 - 3.24 at Day 7 and 2.86 - 2.94 at Day 14 (Fleet & Heard, 1993). In addition, carbon dioxide produced during ethanol fermentation by yeasts separated the pellicle from the broth, resulting in an anaerobic and starved environment for the microorganisms (Chen and Liu, 2000). Very few genera of acetic acid bacteria and yeast can survive under such conditions (Chen and Liu, 2000).

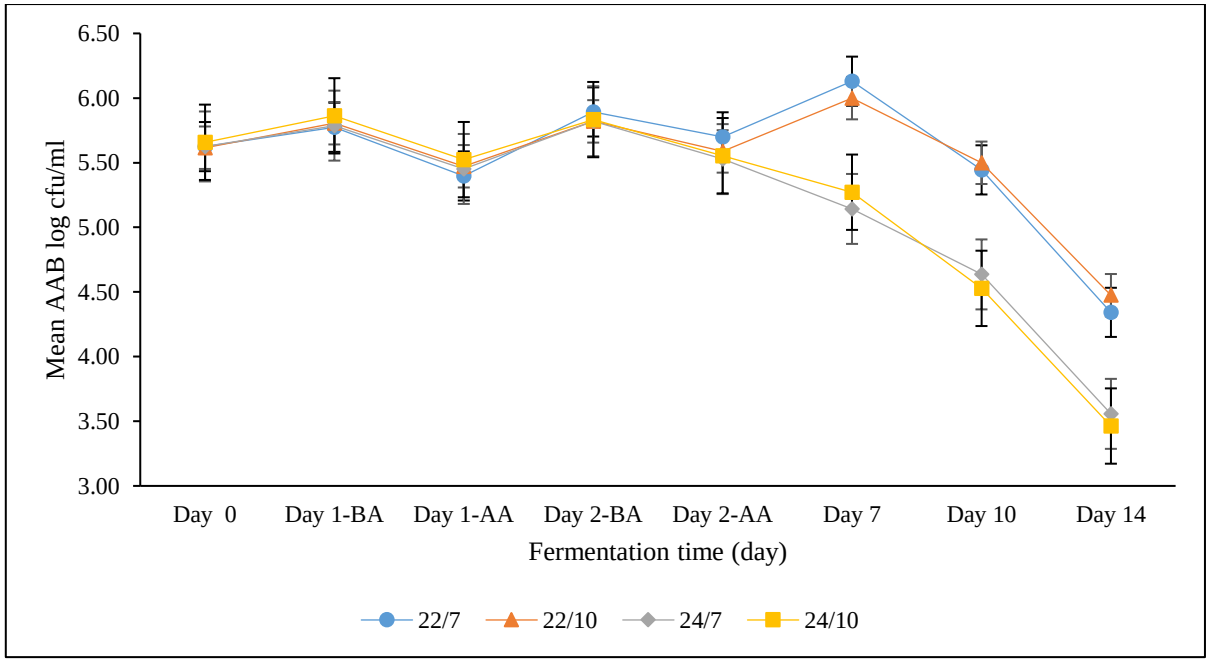

Figure 4.9 Mean log cfu/ml of acetic acid bacteria in green tea kombucha during fermentation for 14 days at 22℃ and 24℃

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; n=4; Error bars = ±SD; experiments were replicated twice.

Samples fermented for 7 days had the highest viable cell counts of yeast and acetic acid bacteria than that in samples fermented for 10 and 14 days, irrespective of the sugar concentration added and the fermentation temperature used (Figure 4.8 and 4.9). Similar results were reported by Sreeramulu, Zhu & Knol. (2000), in which the acetic acid bacteria and yeast cell counts reached peak at day 6 when fermented black tea kombucha at 25℃.

4.2.5 Sensory evaluation

Informal focus groups comprising of five panellists who were familiar with the products participated in this study for sensory evaluation. Fermented green tea kombucha was evaluated for appearance, aroma, flavour, sweetness, sourness and overall acceptability during fermentation (22℃ and 24℃) at Day 7, 10 and 14.

The appearance of the green tea kombucha beverages was described as a yellow, clear liquid similar to a natural green tea infusion, with visible small gas bubbles inside the liquid. The evolution of small gas bubbles was ascribed to fizziness, which is desirable for fermented

kombucha (Chen & Liu, 2000). Visually, there was no colour differences between samples containing different sugar concentrations and fermented at different temperatures for different periods. A vinegary smell could be detected from all the samples, irrespective of the fermentation conditions. The strongest vinegary smell was detected from the samples which had been fermented for 14 days, with the lowest intensity in the 7-day fermented beverage. The samples fermented for 7 days containing low vinegary smell and with some flowery aroma were the most favourable to the informal sensory panellists. Fizziness, which may be attributed to the produced carbon dioxide in the beverage by yeast (Chen & Liu, 2000), was noted in all samples, with the most in the 14-day fermented samples, most likely due to the extended fermentation period. Sourness and sweetness were perceived by the panellists in all samples. However, only the 7-day samples had a balanced flavour of the two attributes. The sourness in samples fermented for 10 days and 14 days were described as overpowering, particularly in the latter. From the overall acceptability, kombucha samples fermented for 7 days received the highest sensory scores and were well-liked by the panellists.

The increasing sourness and decreasing sweetness in the products fermented at longer periods (10 and 14 days) can be attributed to the production of organic acids and the depletion of sugar during fermentation by the kombucha culture. According to Chen and Liu (2000), the presence of acetic acid in kombucha is characterised by astringency and an acidic flavour, and gluconic acid is described as a mild sour taste, thus both of these two organic acids play important roles in the sourness of the kombucha beverage. During fermentation, accumulation of organic acids results in a sour product (Jayabalan et al., 2014), the titratable acidity (Section 4.2.1), in the 14-day samples ranged from 0.88±0.04 to 1.01±0.06, while in 7-day samples, the acidity ranged from 0.36±0.02 to 0.42±0.04, these results are in agreement with the sourness perceived in the products by the sensory panellists. Residual sugar is responsible for the sweetness, and the total soluble solids decreased (0.45 - 0.75 °Brix) between Day 7 to Day 14 (Section 4.2.2) suggesting the depletion of sugar during fermentation resulting in reduced sweetness in longer fermented kombucha products. These results are similar to those reported for a fruit-like kombucha drink which was fermented for 6-10 days, with prolonged fermentation resulting in a distinct vinegar-like flavoured beverage (Resis, 1994).

4.2.6 Conclusion

In Phase 2, fermentation time had significant effects on the physio-chemical, microbiological and sensory characteristics of green tea kombucha beverages. Irrespective of fermentation temperature, samples fermented for 10 and 14 days contained more acidity than samples fermented for 7 days. Low cell counts of yeast and acetic acid bacteria were reported in beverages fermented for a longer period than samples fermented for a shorter period. Samples fermented for 7 days received higher sensory scores compared with samples fermented for 10 days and 14 days. Therefore, these results show that the most suitable fermentation time for the fermentation of green tea kombucha was 7 days, which was selected for use in Phase 3 and 4 investigations.

4.3 Phase 3: Effect of fermentation temperature and sugar concentration on physico-chemical, microbial and sensory properties of green tea kombucha

4.3.1 pH and titratable acidity

In this phase, green tea kombucha beverages were fermented for 7 days, which was the fermentation time determined to yield beverages with the most favourable sensory characteristics in Phase 2. The pH and TA of the green tea kombucha samples made using either 7% or 10% sugar and fermented at either 22°C or 24°C were determined during fermentation (7 days) and also during storage at 4°C for 2 weeks. The pH and TA results of the products during this period are shown in Figures 4.10 (7% sugar samples) and 4.11 (10% sugar samples). During the 7-day fermentation period, the pH of the green tea kombucha beverages decreased after the initial fermentation and then increased after the addition of the sweetened green tea (Day 0 - Day 2-AA). The pH then steadily decreased until the end of the fermentation period. The pH in all four samples peaked on Day 2-AA, with values ranging from 3.42±0.03 - 3.51±0.04 and then decreased to 3.04±0.02 - 3.18±0.03 (Appendix C) at the end of fermentation (Day 7). Similar results have been reported for black tea kombucha, where the pH decreased to around 3.00 after fermentation for 7 days at 28°C (Velićanski et al., 2013).

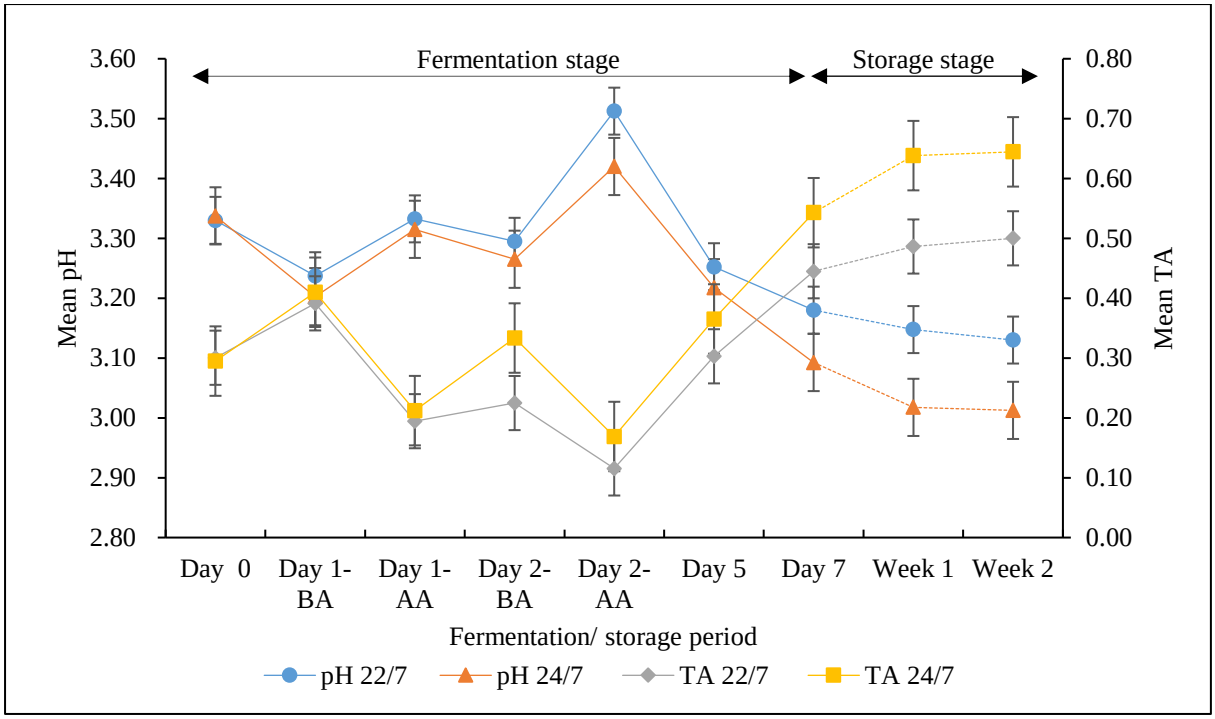

Figure 4.10 Mean pH and titratable acidity (%) of green tea kombucha beverages containing 7% sugar during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: TA = Titratable acidity; BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

Samples fermented at 24°C for 7 days (24/7, 24/10) resulted in lower pH values (3.09±0.02, 3.07±0.01) than samples fermented at 22°C (3.18±0.02 for 22/7 and 3.15±0.01 for 22/10) for the same period. The changes in TA were concomitant with the pH, with TA increasing from 0.12±0.01 - 0.17±0.01 at Day 2-AA to 0.45±0.02 - 0.58±0.01 at Day 7. Sample 24/10 had the highest TA (0.58±0.01) while sample 22/7 (0.45±0.02) had the lowest. These results indicated that the fermentation temperature had a significant effect ($p<0.05$) on the metabolic activities of the microorganisms, with more acids being generated in the products fermented at a higher temperature. Similar results have been reported for black tea kombucha beverages with samples fermented at 30°C for 10 days reaching higher TA levels than those fermented at 22°C for the same fermentation period (Lončar et al., 2006).

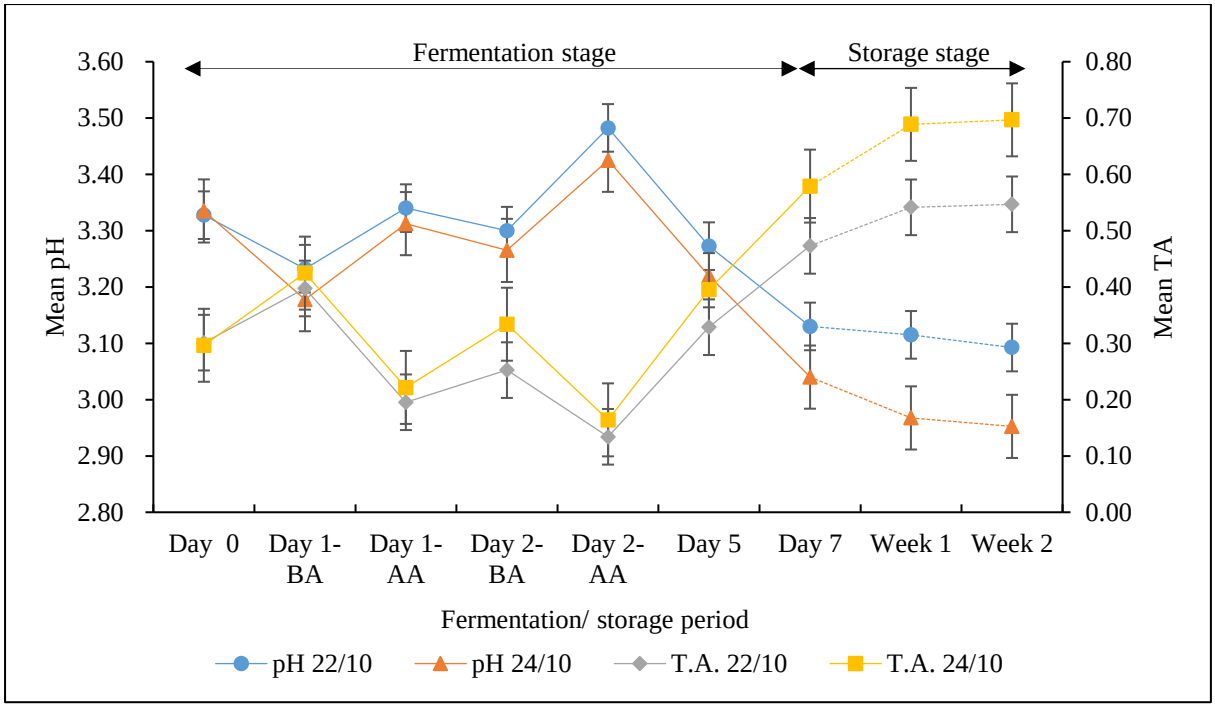

Figure 4.11 Mean pH and titratable acidity (%) of green tea kombucha beverages containing 10% sugar during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: TA = Titratable acidity; BA = before addition of green tea; AA = after addition of green tea; Samples 22/10 = 7% sugared green tea kombucha fermented at 22°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

The results also showed that the samples containing 10% sugar produced more acids than samples containing 7% sugar when fermented at the same temperature. As can be seen in Figure 4.10 and Figure 4.11, sample 22/10 (0.47±0.01) produced more acidity than sample 22/7 (0.45±0.02), while sample 24/10 (0.58±0.01) had higher TA than sample 24/7 (0.54±0.01). These results suggest the concentration of sugar may also have an effect on the microbial metabolic activities which may then influence acid production. These observations are in agreement with others, who reported that higher concentrations of sucrose (molasses) resulted in higher formation of organic acids during fermentation of black tea kombucha beverages (Malbaša et al., 2008). However, other studies reported no differences in the acidity of the final products fermented with variable concentrations of sucrose (Resis, 1994). The discrepancies in results obtained in this study compared to previous work may be attributed to the different origins of the starter culture, and differences in the fermentation conditions and substrate contents used.

During the 2-week storage of green tea kombucha beverages at 4℃, the pH decreased and the TA (Figure 4.10 and 4.11) increased as expected. However, the changes in acidity were not

significant ($p>0.05$). The changes observed for both pH and TA during storage were smaller in the second week than during the first week of storage. The reduction in pH and increase in TA during storage at 4°C were the result of further organic acid production, indicating the activities of the kombucha microorganisms continued during cold-storage, albeit at a lower rate, particularly during the second week of storage.

4.3.2 Total soluble solids

The trend of total soluble solids during the 7 days of fermentation was similar to that observed during the 14 days of fermentation in Phase 2 (Section 4.22). TSS ranged from 8.50±0.1 to 9.75±0.05 ºBrix at Day 0, decreased after one day of fermentation, then increased after the addition of sweetened green tea, reached the highest level (8.75±0.05 - 11.35±0.05 ºBrix) at Day 2-AA, and thereafter steadily decreased (Figure 4.12). The reduction in TSS during fermentation was probably due to the metabolism of sucrose into fructose and glucose by the enzyme invertase present in the yeasts in the kombucha starter culture (Feldmann, 2005; Dickinson & Kruckeberg, 2006), and also due to the conversion of monosaccharides to organic acids by acetic acid bacteria (Greenwalt et al., 1998; Velićanski et al., 2014)

Samples fermented at 24°C exhibited a greater reduction (1.60 ºBrix for 24/7, 1.25 ºBrix for 24/10) of TSS than samples (1.30 ºBrix for 22/7, 0.95 ºBrix for 22/10) fermented at 22°C ($p<0.05$) between Day 2-AA and Day 7. These results suggest that the higher fermentation temperature promoted the sugar metabolism by the microorganisms, which is in agreement with the study by Lončar (2006), who reported that higher temperatures resulted in higher metabolism of sugars in fermented black tea kombucha beverages.

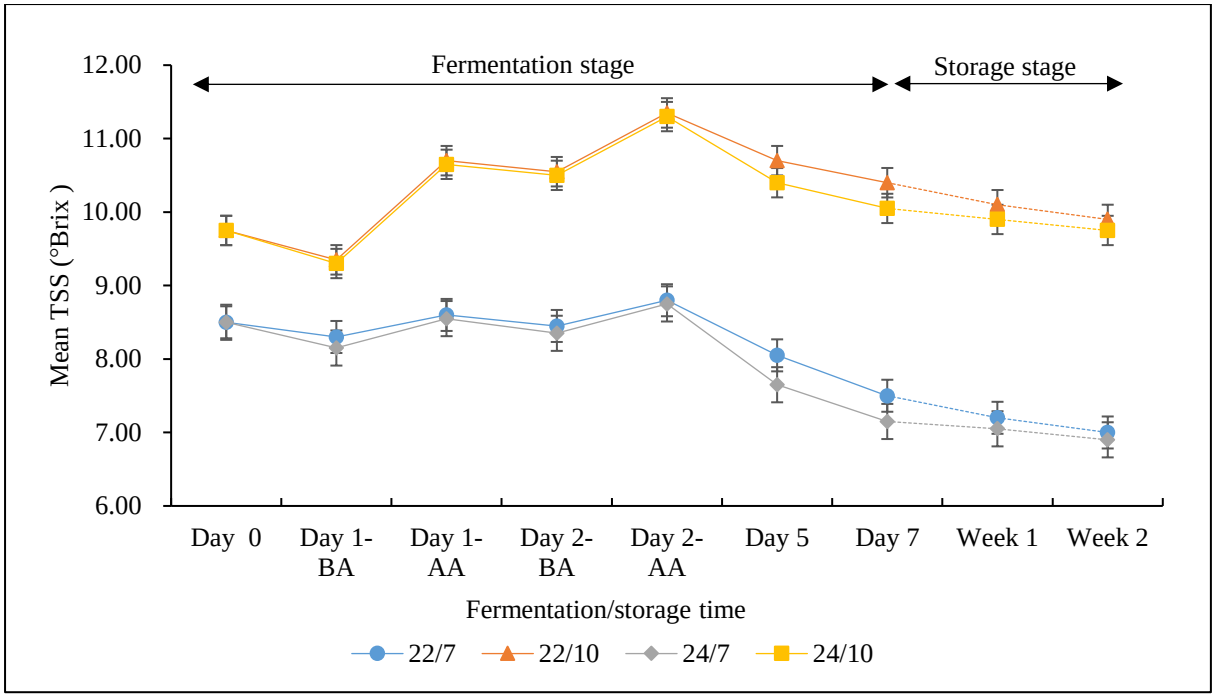

Figure 4.12 Mean total soluble solids (°Brix) of green tea kombucha beverages during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: TSS = Total Soluble Solids; BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

As expected, the reduction of total soluble solids continued during the 2 weeks of cold-storage (4°C), with the concentration at the end of storage ranging from 6.90±0.10 - 9.90±0.00 ºBrix. From Figure 4.12, it can be seen that rate of reduction in TSS during storage was slower than that observed during fermentation, and this trend was attributed to the low rate of sugar metabolism by kombucha culture at the low temperature.

4.3.3 Colour

The effect of fermentation temperature and sugar concentration on the lightness/brightness (L*) of four green tea kombucha samples observed during fermentation and storage is shown in Figure 4.13. The lightness/brightness of the samples ranged from 86.11±0.10 to 86.54±0.26 on Day 0, then increased after one day of incubation and then decreased after addition of the green tea infusion. After Day 2-AA, the L* increased steadily during fermentation and storage. At the end of fermentation, samples 24/7 (87.99±1.78) and 24/10 (87.62±0.44) had higher L* values than samples fermented at 22°C (86.27±0.11, 86.83±0.08), although the differences in

L* for the four samples were not significant (p>0.05). The changes in colour during fermentation were possibly caused by the suppression of ionization and destruction of the polyphenols due to the enzyme activities of the kombucha cultures (Haslam, 2003; Jayabalan et al., 2014).

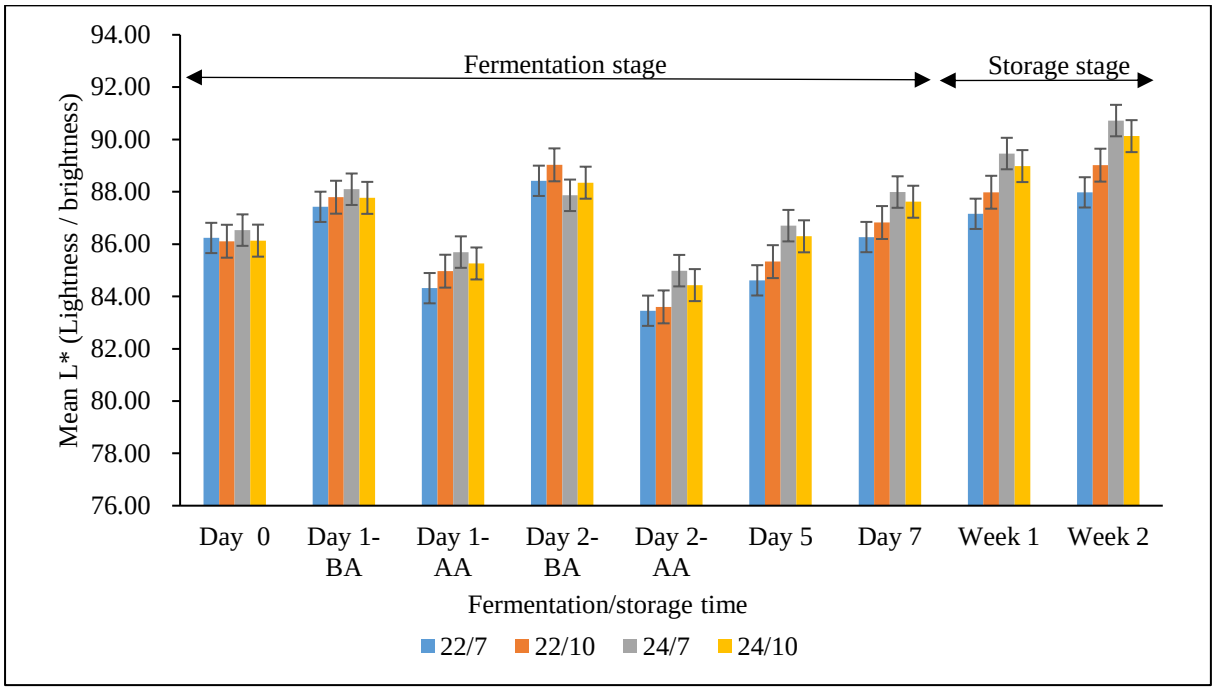

Figure 4.13 Mean L* (lightness/brightness) of green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

Changes in a* (redness/greenness) and b* (yellowness-blueness) values for the four samples are shown in Figures 4.14 and 4.15, respectively. Both a* and b* values of the kombucha beverages decreased after fermentation and increased after addition of the sweetened green tea. There was an increase in a* values at Day 2-AA where ranged from 0.40±0.01 to 0.46±0.02, followed by a decrease to 0.26±0.02 - 0.28±0.02 at Day 7. No significant differences (p>0.05) in a* were observed for the four products at the end of fermentation. The changes in b* values showed similar trends to that of a*, with the b* value also reaching a peak at Day 2-AA and steadily decreased to 15.59±0.40 - 18.00±0.09 at the completion of the fermentation period.

Samples fermented at 24°C had a greater reduction in yellowness-blueness than samples fermented at 22°C ($p<0.05$).

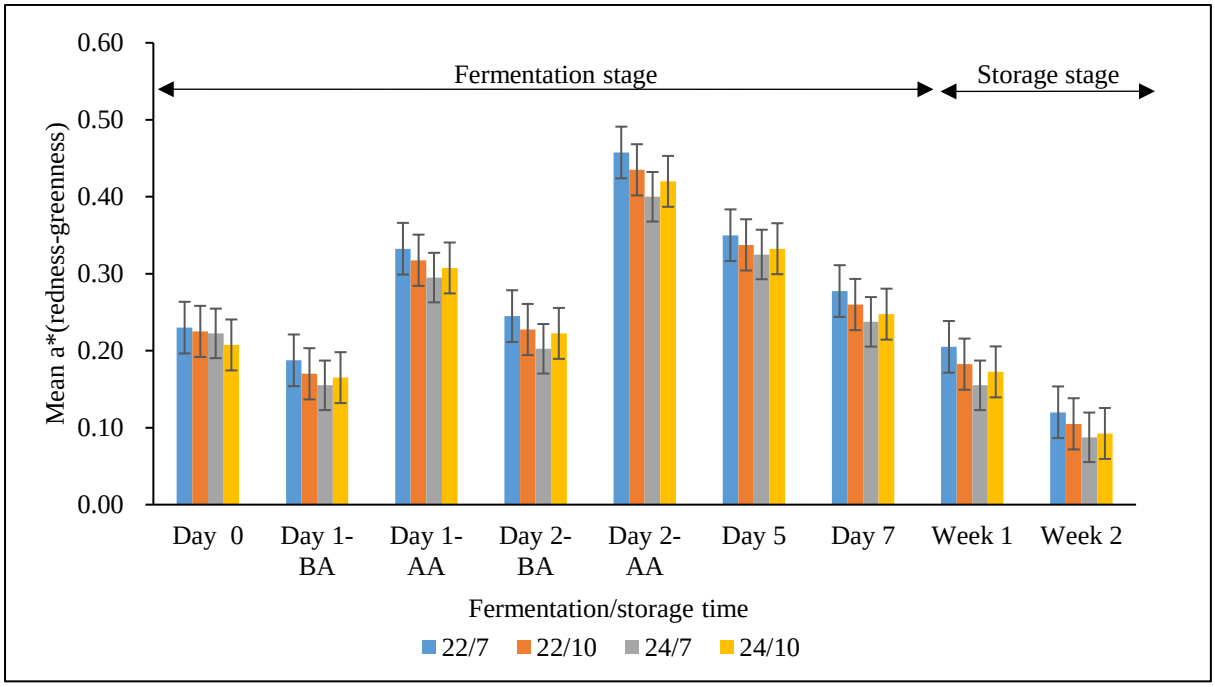

Figure 4.14 Mean a*(redness-greenness) of green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD.

During cold-storage, the L* increased, while a* and b* continued to decrease. At the end of storage, the lightness and brightness (L*) of the refrigerated kombucha ranged from 88.33±0.44 to 92.71±1.35, while a* of green tea kombucha beverages ranged from 0.07±0.02 to 0.12±0.05, and b* ranged from 13.15±0.69 to 15.81±0.30.

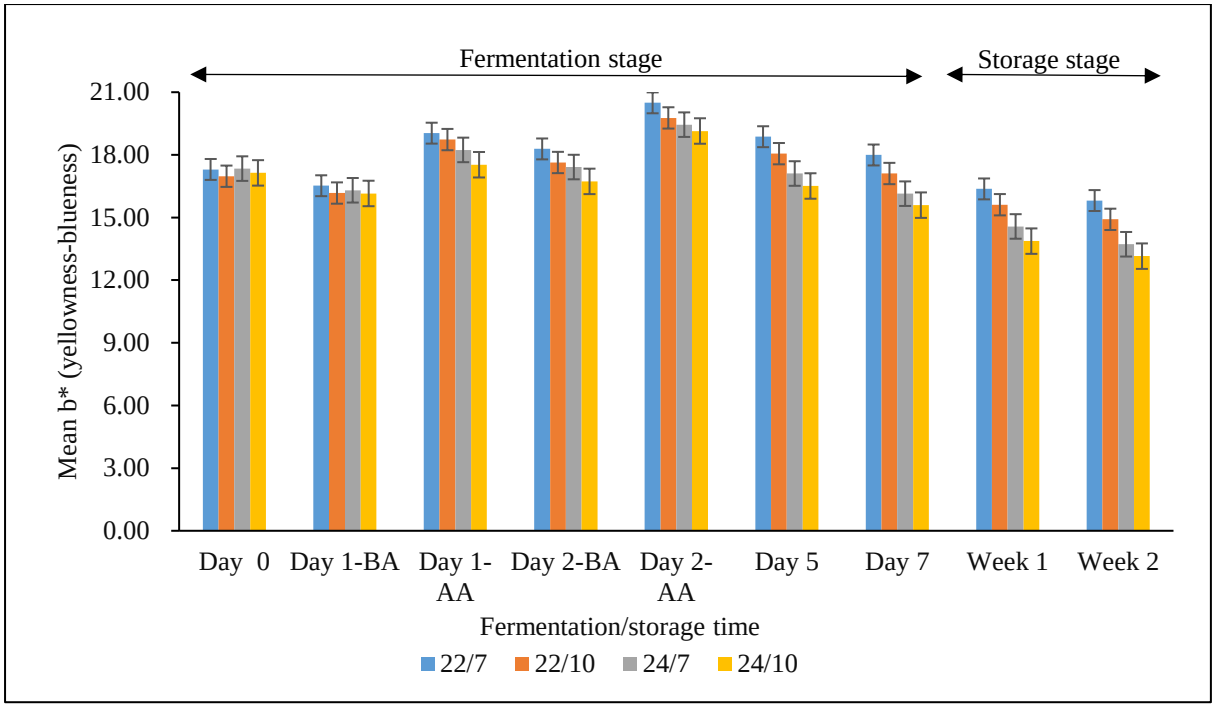

Figure 4.15 Mean b* (yellowness-blueness) of green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD.

4.3.4 Organic acids

Previous studies (Chen & Liu, 2000; Sreeramulu et al., 2000; Malbasa et al., 2008; Jayabalan et al., 2014; Chakravorty et al., 2016), have shown that acetic acid and gluconic acid are the major organic acids produced by the acetic acid bacteria and yeast during kombucha fermentation. Concentrations of gluconic acid and acetic acid in green tea kombucha during fermentation for 7 days and storage for 2 weeks at 4°C are shown in Figures 4.16 and 4.17 respectively.

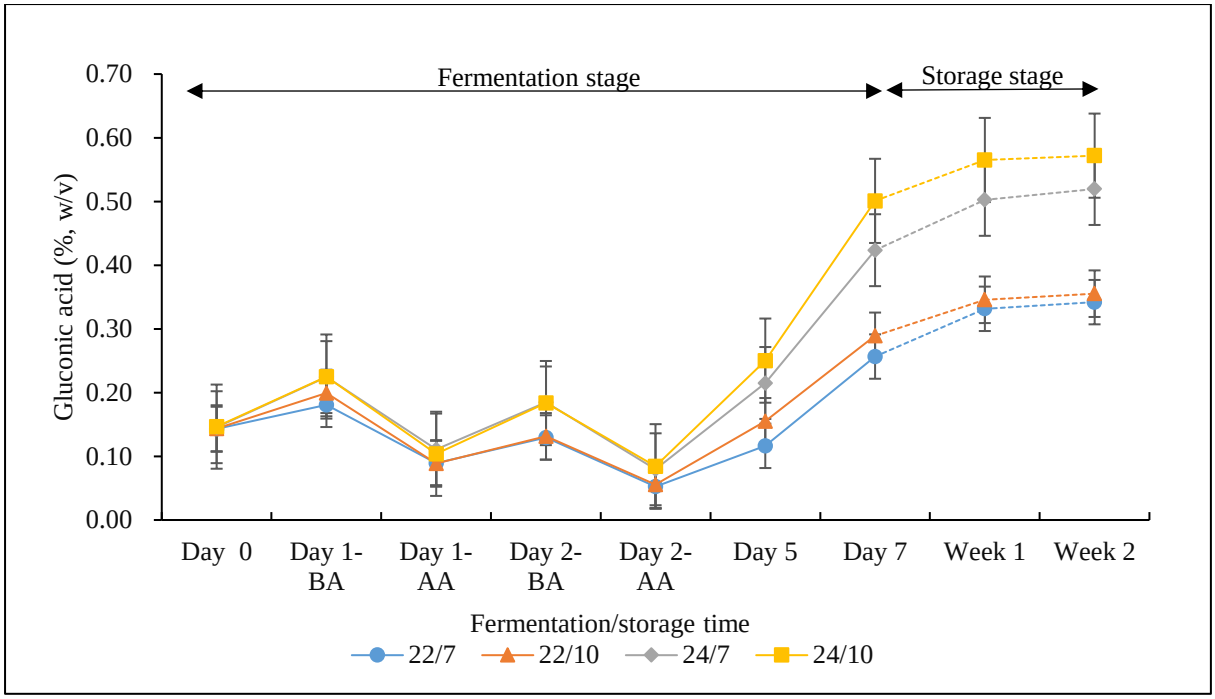

Figure 4.16 Concentration (%) of gluconic acid in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

The changes in gluconic acid and acetic acid levels during fermentation correspond with the observed changes in the titratable acidity (Figure 4.10 and Figure 4.11). During the initial fermentation period (Day 0 - Day 2-AA), both acetic acid and gluconic acid levels increased after fermentation and decreased after addition of the sweetened green tea. From Day 2-AA to Day 7, gluconic acid increased steadily from the lowest level (0.05±0.01 - 0.08±0.04) at Day 2-AA to 0.26±0.02 - 0.50±0.10% (w/v) at Day 7, and acetic acid increased from 0.06±0.01 - 0.10±0.02% (w/v) at Day 2-AA to 0.28±0.00 - 0.40±0.04% (w/v) when the fermentation finished. A similar trend was reported by Jayabalan et al. (2007), where acetic acid increased steadily to 0.3% and gluconic acid increased to 0.14% after 9 days of green tea kombucha fermentation at 24±3°C. However, the acetic acid and gluconic acid levels reported in the study by Jayabalan et al. (2007) are lower than those found in the present study. Chen and Liu (2000) also observed similar results to this study, with gluconic acid levels increasing to 0.5% and acetic acid levels increasing to 0.4% after 9 days of black tea kombucha fermentation at 24±3°C. The differences in acetic and gluconic acids found in the different studies may be due to

differences in the overall diversity of the microbial communities as well as the conditions used during fermentation (Vukic et al., 2014).

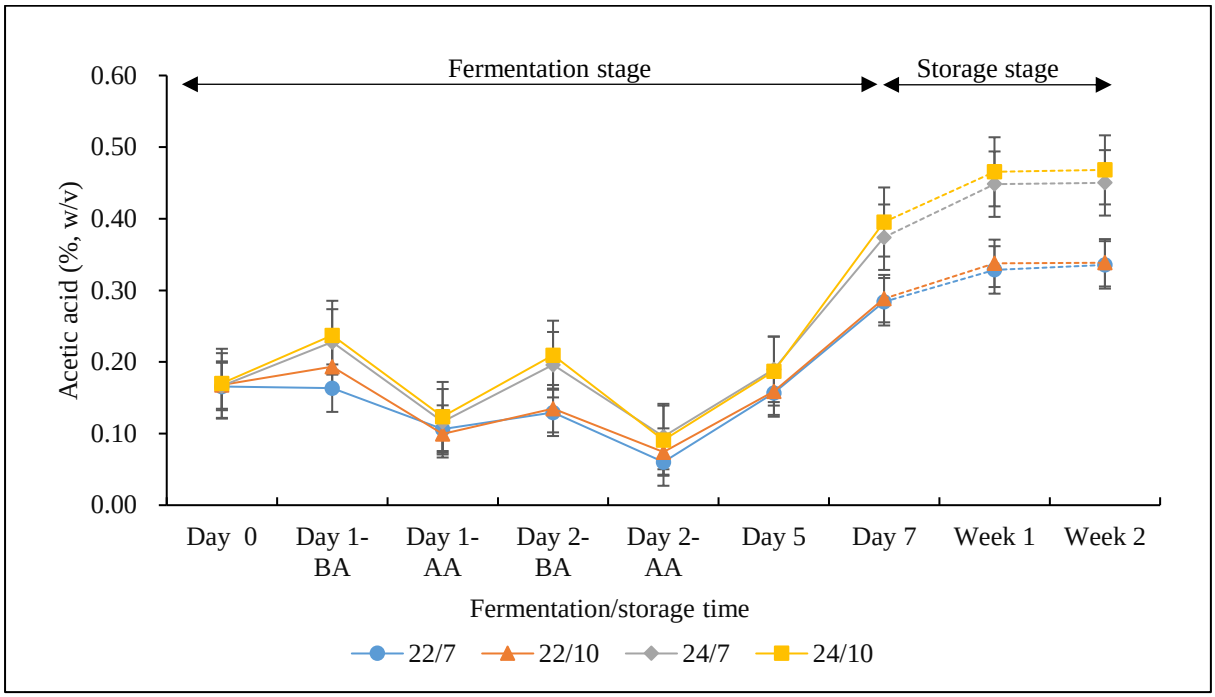

Figure 4.17 Concentration (%) of acetic acid in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

Samples containing 7% and 10% sugar fermented at different temperatures achieved different acetic acid and gluconic acid levels. As can be seen in Figures 4.16 and 4.17, samples fermented at 24°C generated higher levels of organic acids than those fermented at 22°C. From Day 2-AA to Day 7, gluconic acid levels in samples fermented at 24°C increased to 0.34 - 0.42% and acetic acid levels increased to 0.27 - 0.31%, whereas samples fermented at 22°C only produced 0.21 - 0.23% gluconic acid and 0.22% acetic acid. These results suggest temperature had a significant effect ($p<0.05$) on the yield of organic acids during fermentation. Similar results were reported by Lončar et al. (2006), with higher quantities of organic acids being generated in kombucha products fermented at higher temperatures. However, no significant differences ($p>0.05$) were observed between samples containing different sugar levels.

As expected, during the 2-week storage period, the rate at which acetic acid and gluconic acid increased was lower than during the fermentation period. During the second week of storage, almost no increase in organic acid levels occurred, which indicates that the metabolic activities of the acetic acid bacteria and yeasts remained during the cold-storage but were reduced as the storage time increased. These results are consistent with the pattern of increasing T.A during storage (Section 4.3.1).

4.3.5 Sugars

The concentrations of sucrose, glucose and fructose in green tea kombucha during fermentation for 7 days and storage (4°C) for 2 weeks are shown in Figures 4.18, 4.19 and 4.20, respectively. At the initial fermentation (Day 0 – Day 2-AA), sucrose decreased after one day of fermentation and increased after addition of sweetened green tea. In contrast, glucose and fructose concentrations changed in the opposite pattern to sucrose: they increased after fermentation and decreased after the sweetened green tea addition. During Day 2-AA to Day 7, sucrose decreased, while glucose and fructose steadily increasing. This phenomenon may be due to the yeast, which enzymatically hydrolyses extracellular sucrose into glucose and fructose (Lončar et al., 2014), thus as sucrose is hydrolysed, its levels will decrease, while the levels of products glucose and fructose will increase.

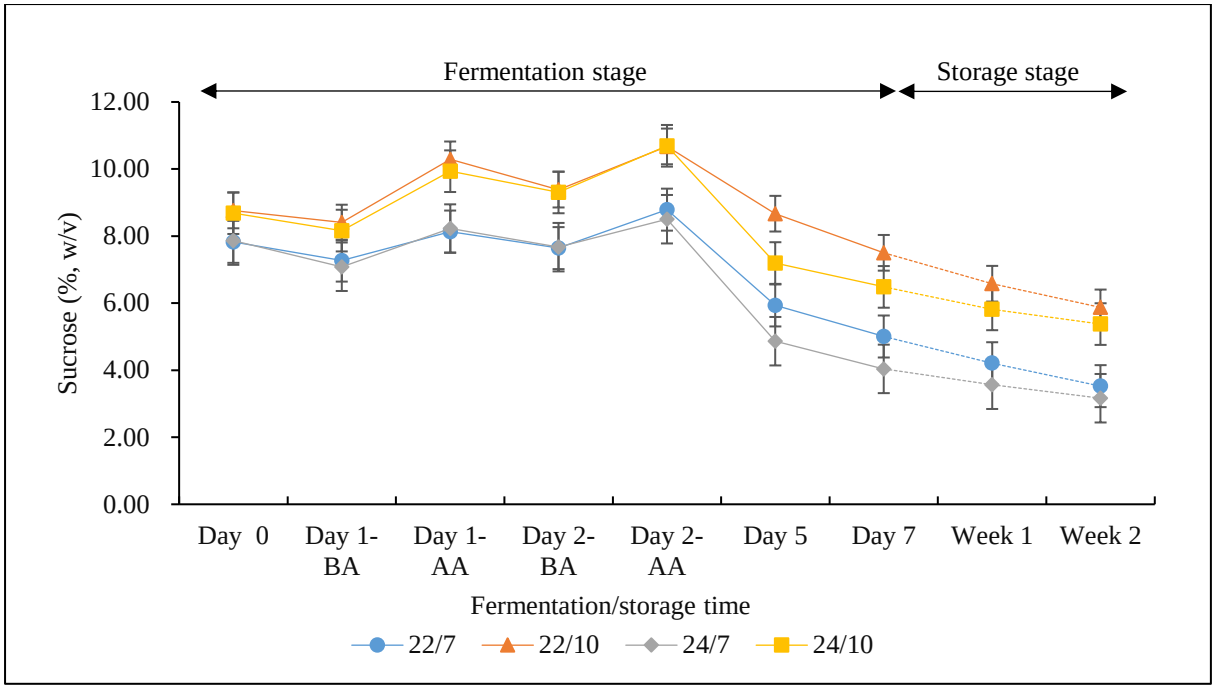

Figure 4.18 Concentration (%) of sucrose in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

As can be seen from Figures 4.18, 4.19 and 4.20, the sucrose decreased from the highest level of 8.50±0.39 - 10.69±0.21% (w/v) at Day 2-AA to 4.04±0.36 - 7.5±0.32% (w/v) at Day 7. In parallel, glucose and fructose levels increased from their lowest concentrations of 0.44±0.04 - 0.53±0.07% (w/v) and 0.16±0.04 - 0.25±0.04% (w/v) on Day 2-AA to 0.71±0.01-1.36±0.04% (w/v) and 0.44±0.04-1.46±0.09% (w/v) on Day 7, respectively. These trends are similar to those reported in several previous studies of kombucha (Chen & Liu, 2000; Malbaša et al., 2006; Malbaša et al., 2007; Lončar et al., 2014). For example, sucrose was reported to decrease from 94 g/L to 78 g/L after 9 days of fermentation of black tea kombucha at 24±3 °C, while glucose and fructose increased from 4.1 g/L and 5.0 g/L to 5 g/L and 10g/L, respectively (Chen & Liu, 2000).

In this study, the final concentration of glucose was higher than fructose, which suggests that the metabolic fates of the two sugars during fermentation were different (Chen & Liu, 2000). Here, fructose was preferentially utilised by the kombucha culture, a result in agreement with that of Sievers et al. (1995), who also reported that glucose levels were higher than fructose

levels after 8 days of fermentation at 20-22 ℃. However, others have reported different utilisation patterns for glucose and fructose, with glucose being used preferentially to fructose by kombucha microorganisms (Chen & Liu, 2000). Although both glucose and fructose can be used by yeast, the authors showed that fructose was poorly metabolized by *Gluconacetobacter xylinum* (*Ga. xylinum*) and therefore remained in the broth (Chen & Liu, 2000). The differences in sugar metabolism during kombucha fermentation is probably due to differences in the composition of the kombucha starter cultures.

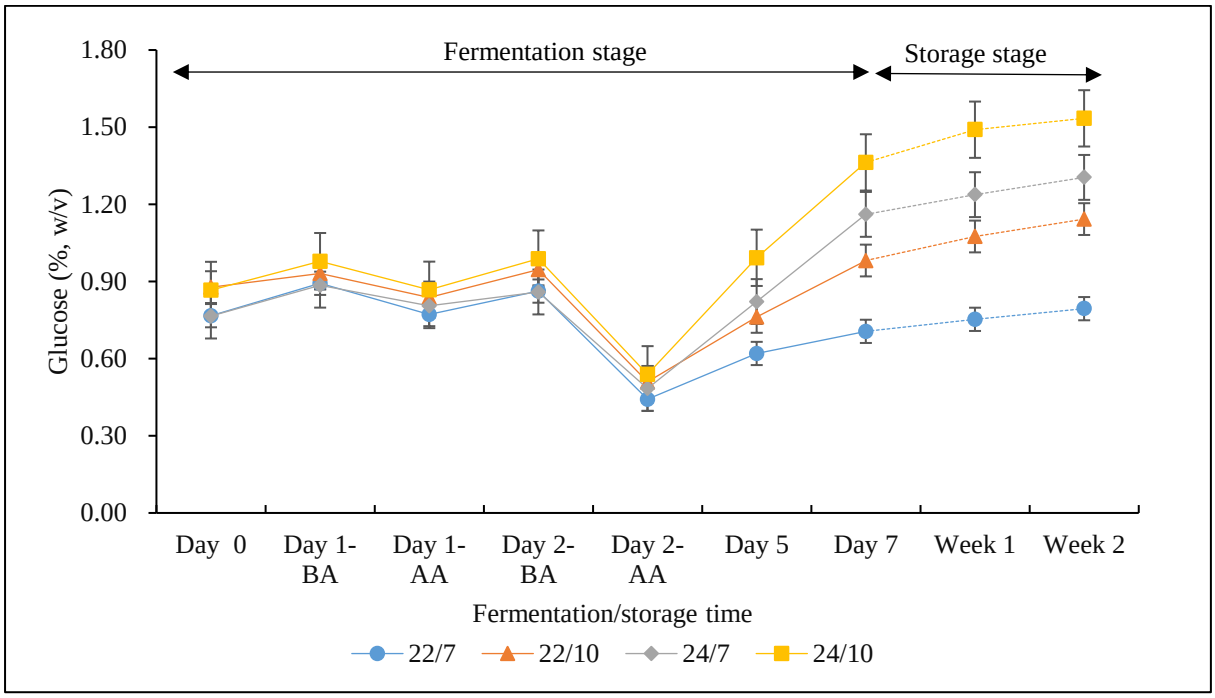

Figure 4.19 Concentration (%) of glucose in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

At the end of fermentation, sucrose, glucose and fructose concentrations (%) in the four kombucha samples were significantly different ($p<0.05$). Samples fermented at 24°C had lower sucrose levels (4.04±0.36 for 24/7; 6.48±0.25 for 24/10) than samples fermented at 22°C (5.00±0.53 for 22/7; 7.50±0.32 for 22/10). Sample 24/10 had the highest glucose (1.36±0.04) and fructose (1.46±0.09) levels whereas sample 22/7 had the lowest (0.71±0.01 of glucose; 0.44±0.04 of fructose). These results indicate that both the fermentation temperature and sugar concentration had significant effects ($p<0.05$) on the sucrose, glucose and fructose levels in the

final kombucha product and therefore on the microbial activities of the kombucha culture. Lončar et al. (2014) studied the kinetics of saccharide fermentation by kombucha, and reported lower levels of sucrose and higher levels of glucose and fructose in kombucha fermented at 30°C compared to kombucha fermented at 22°C. These results suggest that the higher fermentation temperature and higher sugar concentrations increased the metabolism of the carbohydrate, and hence increased the hydrolysis of sucrose into glucose and fructose, resulting in the lower sucrose and higher glucose and fructose levels observed (Figure 4.19 and 4.20).

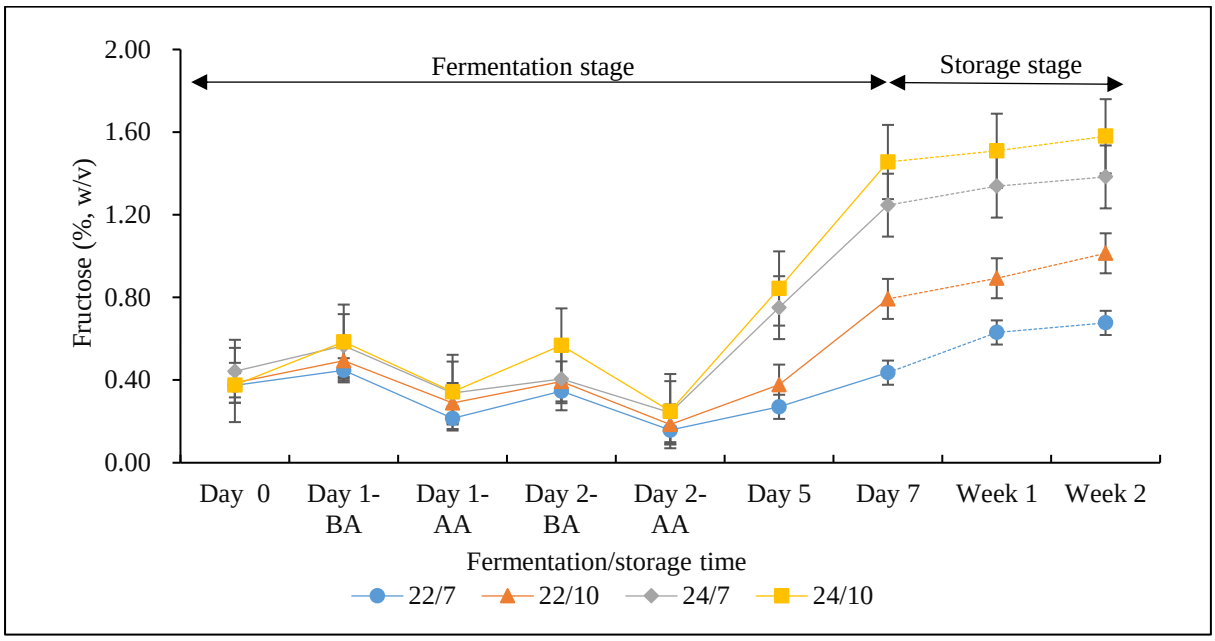

Figure 4.20 Concentration (%) of fructose in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

As can be seen in Figure 4.18, 4.19 and 4.20, sucrose decreased steadily, and glucose and fructose increased during cold-storage (4 °C). At the end of storage (Week 2), sucrose levels in the four samples ranged from 3.16±0.40 to 5.87±0.21% (w/v), while glucose and fructose levels ranged from 0.79±0.05 to 1.53±0.03% (w/v) and 0.68±0.09 to 1.58±0.08% (w/v), respectively. Changes in the sugar concentrations suggest that the metabolism of sugars by the kombucha symbiotic consortium were still continuing, albeit at slower rates under the storage conditions used.

4.3.6 Ethanol

Yeast in the kombucha culture has been shown to be responsible for the enzymatic hydrolysis of sucrose into fructose and glucose, and then these two sugars (monosaccharides) are metabolised into ethanol and carbon dioxide (Lončar et al., 2013). The effects of sugar concentration and fermentation temperature on the ethanol content of green tea kombucha are shown in Figure 4.21.

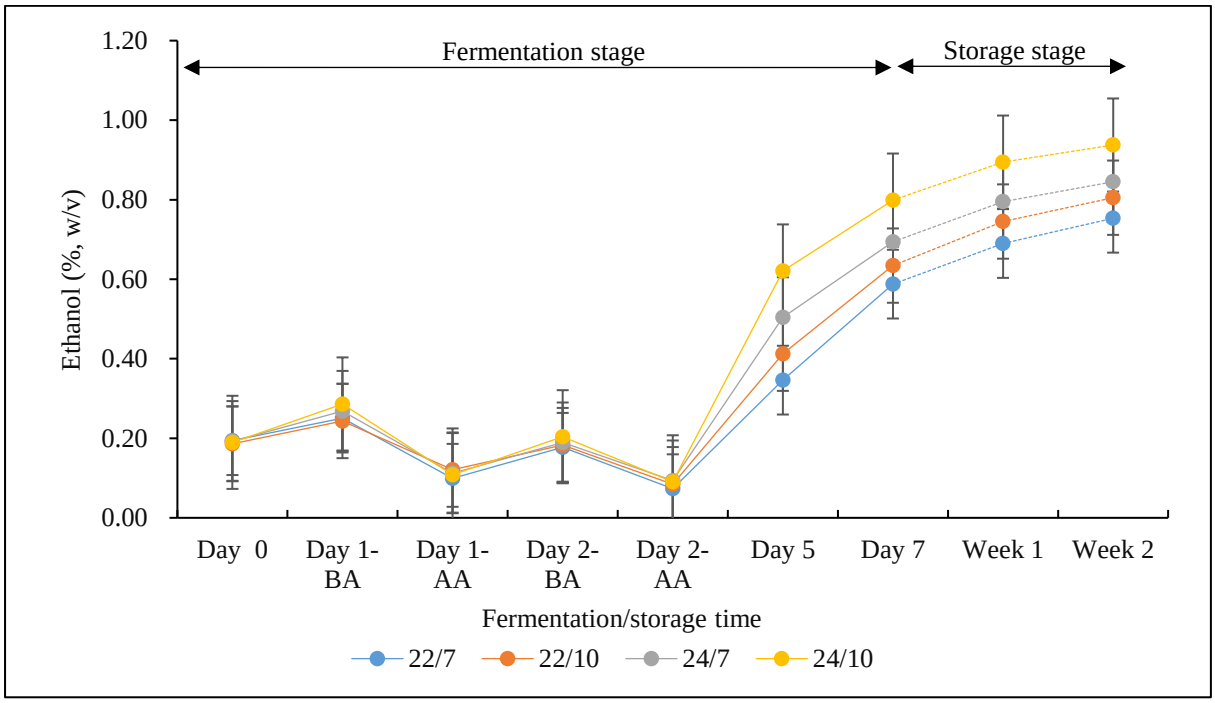

Figure 4.21 Concentration (%) of ethanol in green tea kombucha during fermentation at 22°C and 24°C for 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD. ; experiments were replicated twice.

The concentrations of ethanol in the four green tea kombucha treatments increased after one day of fermentation and decreased after addition of sweetened green tea. The lowest concentration of ethanol in all treatments was 0.07±0.01-0.09±0.01% (w/v), observed on Day 2-AA, after which the rate of ethanol generation increased to achieve a level of 0.59±0.02-0.81±0.03% (w/v) at the end of fermentation. The concentration of the ethanol in these kombucha samples were much lower than that reported by Chakravorty (2016), where ethanol increased from an initial level of 0.46% to 2.8% after 7 days of fermentation. However, the

ethanol concentration in the final product in the current study was similar to that of Chen and Liu (2000), who reported an ethanol content of around 0.5% after 10 days of incubation at 24±3℃. The diversity in the ethanol content in these reports may be due to differences in the initial sugar concentration and also the different yeast cultures used in the kombucha production (Dijken et al., 1993).

Samples fermented at 24℃ generated more ethanol than samples incubated at 22℃, with concentrations of ethanol in sample 24/7 and 24/10 being 0.69±0.05% and 0.81±0.03% respectively at the completion of fermentation, which is significantly higher ($p<0.05$) than that detected in samples 22/7 (0.59±0.02) and 22/10 (0.63±0.01). Similar results were shown by Lončar et al. (2006), who reported the ethanol content in kombucha fermented at 30℃ (4 g/L) was significantly higher than in the sample (2.8 g/L) incubated at 22℃. These phenomena suggest that incubation temperature has a significant effect on the rate of metabolism of the symbiotic association in kombucha which influences the rate of production of ethanol.

During refrigerated storage, the ethanol content continued to increase, although the generation rate was lower than that during fermentation. This suggests that yeast metabolism was being influenced by the temperature used and slowed down during storage. In addition, it has been reported that high acidity and lower sugar content can inhibit the metabolic activity of yeasts and ethanol production. Thus, the low pH in the kombucha samples during storage was probably another reason for the low generation of ethanol (Velićanski et al., 2013) during this period.

4.3.7 Microbiology

The growth of yeast and acetic acid bacteria in green tea kombucha beverages during fermentation and storage are shown in Figures 4.22 and 4.23 respectively. Between Day 0 and Day 2-AA, there was no obvious difference in the microbiological growth of the four samples, with all cell counts increasing after incubation and decreasing after the addition of sweetened green tea. From Day 2-AA to Day 7, in samples fermented at 22°C, cell counts of both yeasts and acetic acid bacteria generally increased until fermentation was completed, while the cell counts of yeasts and acetic acid bacteria in samples 24/7 and 24/10 increased until Day 5, thereafter decreasing to the end of fermentation.

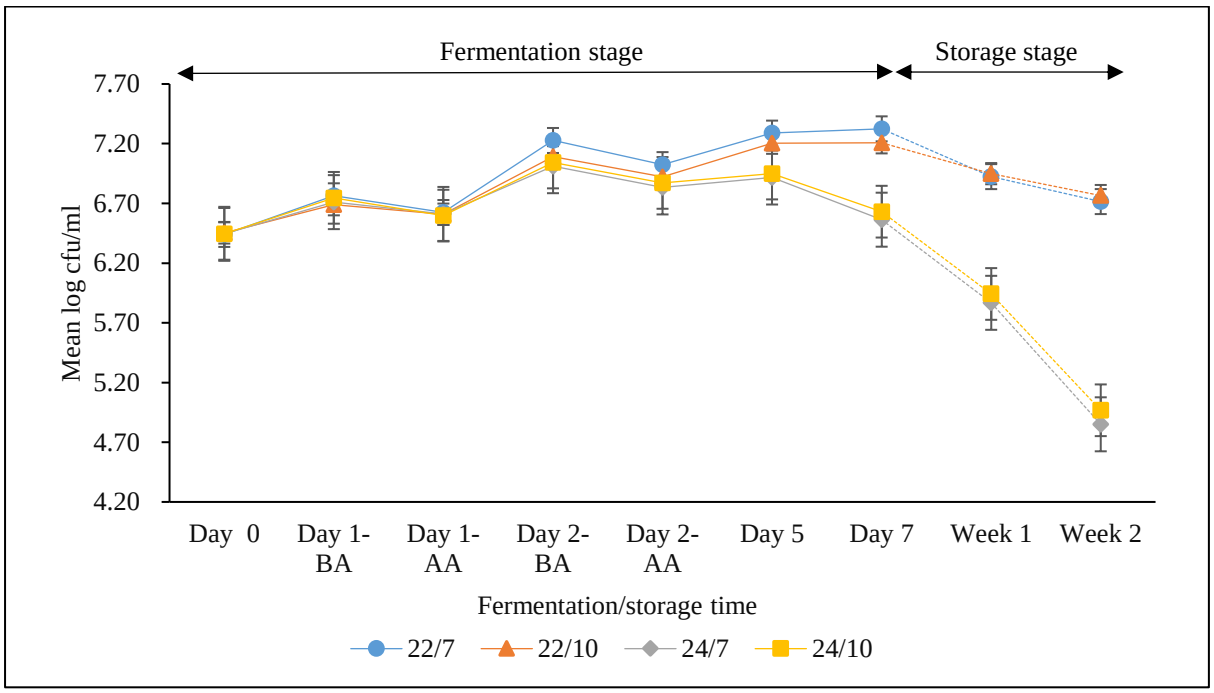

Figure 4.22 Mean log cfu/ml of yeast in green tea kombucha during fermentation at 22°C and 24°C 7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

During Day 2-AA to Day 7, both yeasts and acetic acid bacteria cell numbers (log cfu/ml) in the green tea kombucha beverages fermented at 22℃ (22/7, 22/10) were higher than those in samples (24/7 and 24/10) incubated at 24℃. At Day 7, samples 22/7 (7.27±0.19 log cfu/ml) and 22/10 (7.21±0.20 log cfu/ml) had significantly ($p<0.05$) higher yeast cell counts than samples fermented at 24℃ (6.46±0.21 for 24/7,6.53±0.15 for 24/10). Similar results were also obtained for the acetic acid bacteria cell counts, in which samples fermented at 22℃ contained 6.32±0.24 log cfu/ml and 6.24±0.19 log cfu/ml, compared with samples 24/7 and 24/10 which only contained 5.36±0.34 log cfu/ml and 5.52±0.19 log cfu/ml at the end of fermentation. These results demonstrate that the fermentation temperature had a significant ($p<0.05$) effect on the growth of the microorganisms, with 22℃ providing more favourable growth of AAB and yeasts compared to 24℃. However, no significant difference ($p > 0.05$) in cell counts of yeast

and acetic acid bacteria at the end of fermentation was found between samples containing 7 % or 10% sugar.

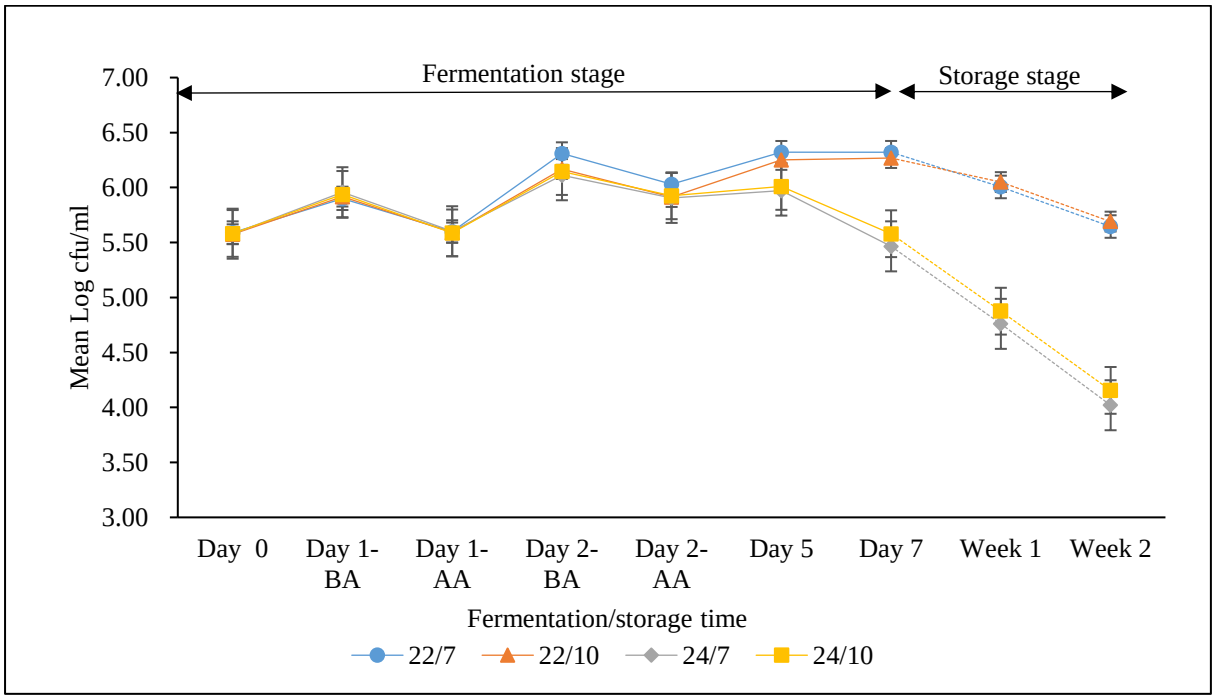

Figure 4.23 Mean log cfu/ml of acetic acid bacteria in green tea kombucha during fermentation at 22°C and 24°C for7 days and storage at 4°C for 2 weeks

Notes: BA = before addition of green tea; AA = after addition of green tea; Samples 22/7 = 7% sugared green tea kombucha fermented at 22°C; 22/10 = 10% sugared green tea kombucha fermented at 22°C; 24/7 = 7% sugared green tea kombucha fermented at 24°C; 24/10 = 10% sugared green tea kombucha fermented at 24°C; Week 1 = storage at 4°C for 1 week; Week 2 = storage at 4°C for 2 weeks; n=4; Error bars = ±SD; experiments were replicated twice.

During storage at 4°C, the quantities of yeasts and acetic acid bacteria cells in all green tea kombucha samples steadily decreased, with yeast numbers decreasing to 4.60±0.90-6.77±0.16 log cfu/ml, and acetic acid bacteria numbers reducing to 3.53±0.97-5.69±0.18 log cfu/ml. These findings are in accordance with the observations of Fu (2013), who reported that the survival rate of acetic acid bacteria and yeast was 54.09% and 73.97% respectively, after 10 days storage at 4°C, with both yeast and AAB cell numbers steadily decreasing during a 14 days storage period at 4°C.

4.3.8 Sensory evaluation

Sensory evaluation of the beverages produced in this phase was conducted at the end of the fermentation (Day 7), and after storage for 1 and 2 weeks, with the most promising formulation

being selected by an informal focus group comprised of 5 panellists. During the sensory evaluation, the appearance, flavour, sourness, sweetness and overall acceptability of green tea kombucha beverages were compared and discussed.

For the fresh kombucha products (four sample treatments, fermented for 7 days), the appearance of the four different kombucha samples were all described as a yellow clear liquid with visible gas bubbles, and no obvious visual difference in colour was observed between samples. The gas bubbles in the beverages were generated by the yeast in the kombucha cultures, with samples fermented at 22℃ having more bubbles than those fermented at 24℃: This result may be attributed to the kombucha products fermented at 22℃ having more viable yeast cells (Section 4.3.7). A vinegary and fruity smell was detected from all samples, however the samples fermented at 24℃ (24/7 and 24/10) had a stronger vinegary and sour smell than samples fermented at 22℃ (22/7 and 22/10). The level of sourness detected was greater in the samples fermented at 24℃ (24/10 and 24/7) than those fermented at 22℃ (22/7 and 22/10), while more sweetness was detected in samples containing 10% sugar (22/10, 24/10) than samples containing 7% sugar (22/7, 24/7). This result is in agreement with the titratable acidity (TA) results (Section 4.3.1) and TSS (Section 4.3.2), where the TA of samples 24/10 and 24/7 were greater than samples 22/7 and 22/10; and the samples containing 10% sugar had higher TSS than samples containing 7% sugar. Sample 24/7 had the lowest level of sweetness and sample 22/10 had the highest. Based on the overall acceptability, samples fermented at 24℃ were found to be over-fermented and were deemed too acidic; sample 22/7 was well-balanced in terms of sweetness and sourness and was favoured by the panellists; while 22/10 was described as having more sweetness than sourness, but was described as being too sweet for the panellists. Hence the preferred sample at the end of fermentation was the kombucha sample containing 7% sugar and fermented at 22℃ for 7 days.

During the storage period, there was no obvious visual difference in the colour distinguished by the panel in the samples stored for 1 or 2 weeks. Compared to the fresh green tea kombucha produced after 7 days of fermentation, the sourness detected by both smell and taste increased with storage. As the samples were stored in sealed containers, more fizziness was detected in the stored samples, particularly in the samples fermented at 22℃. This increase in fizziness was appealing to the panellists.

4.3.9 Conclusion

Results from this phase of experiments indicated that the fermentation temperature and sugar concentration both influenced the physico-chemical, microbiological and sensory attributes of the green tea kombucha products. Samples fermented at higher temperatures had higher acid and ethanol levels, and lower TSS and cell counts. The sample containing 7% sugar and fermented at 22℃ was well-balanced in terms of sweetness and sourness, had more gas bubbles, the highest yeast and acetic acid bacteria cell counts at the end of fermentation, as well as being stable during storage. Therefore, sample 22/7 was deemed to be the most promising and stable formulation from Phase 3 experiments, based on the physico-chemical, microbiological and sensory properties, and was utilised for further stability studies in Phase 4.

4.4 Antibacterial capability of fermented (22°C) green tea kombucha containing 7% sugar

Green tea kombucha containing 7% sugar and fermented at 22°C for 7 days was tested for antibacterial capability using the disc diffusion method. *Escherichia.coli* 111, *Listeria monocytogenes* 15E03-1, *Salmonella typhimurium* ESR3479, *Staphylococcus aureus* MU-A57 and *Pseudomonas aeruginosa* MU-A26 were used to test for the antibacterial capacity of the green tea kombucha. The mean inhibition diameter for each pathogen is shown in Table 4.1.

Table 4.1 Antibacterial activities of the most promising green tea kombucha

Target microorganism	**Inhibition zone Ø (mm)**
Escherichia coli 111	11.5 ± 0.5
Listeria monocytogenes 15E03-1	11.8 ± 0.4
Pseudomonas aeruginosa MU-A26	11.5 ± 0.4
Salmonella typhimurium ESR3479	11.0 ± 0.7
Staphylococcus aureus MU-A57	11.3 ± 0.4

Notes: n = 4; experiments were replicated twice.

The mean inhibition zones of the five pathogens tested against green tea kombucha were similar, with an average diameter of 11.4±0.6 mm. The inhibition zones were smaller than

those described by Battikh (2013), who reported inhibition zone diameters for *E. coli*, *L. monocytogenes*, *S. typhimurium*, *S. aureus* and *Ps. aeruginosa* were 14.5 ± 0.7, 21.5 ± 2.1, 18.0 ± 0.4, 14.0 ± 1.4 and 12.0 ± 0.0 mm respectively, when tested against green tea kombucha fermented at room temperature for 21 days. However, the results of this study were similar to the study of Aleksandra (2014), who fermented kombucha with lemon balm at 28 ± 1°C for 7 days. In that study, the inhibition diameter for *E. coli*, *L. monocytogenes*, *S. typhimurium* and *P. aeruginosa* were 11.3, 11.0, 11.7 and 11.7 mm, respectively.

The antibacterial activities of kombucha are mainly due to the low pH and the accumulation of organic acids during fermentation, which consist primarily of acetic acid, gluconic acid and lactic acid (Ayed & Hamdi, 2015). Aleksandra (2014) studied the antibacterial activities of kombucha samples with different titratable acidity, and found that the beverages with higher acidity resulted in larger inhibition zones (especially against Gram-positive bacteria strains). Two main mechanisms for the inhibition of the bacterial growth by acids were reported by Velicanski et al. (2014). First, the inhibition may be caused by the accumulation of the dissociated anion acid to toxic levels, and also by cytoplasmic acidification. These observations suggest that the antibacterial potential of kombucha beverages is directly associated with the concentration of organic acids (Velicanski et al., 2014).

Some studies (Greenwalt et al., 1998; Battikh et al., 2013; Velicanski et al., 2014) have reported that neutralized kombucha also had antibacterial capacity against several Gram-negative bacteria, such as *E. coli* and *Salmonella* sp. The findings indicated that the antibacterial activities of kombucha was not only due to the presence of organic acids, but was also attributable to biologically active components present in the product, such as proteins, enzymes, phenolic compounds and bacteriocins, which are either substrates or the metabolic products from fermentation (Sreeramulu, 2000; Sreeramulu, 2001; Battikh et al., 2013).

The differences in the inhibition zones between this study and other findings may also be due to the differences in the fermentation conditions and compositional differences in the media for production of kombucha, which may influence the final composition of the kombucha, particularly acetic acid, the main antibacterial compound (Battikh et al., 2013).

4.5 Phase 4: Characteristics of fermented green tea kombucha during storage for 4 weeks at 4°C

From the results of Phases 2 and 3, the most promising fermentation conditions based on physico-chemical, microbial and sensory characteristics were green tea kombucha containing 7% sugar fermented at 22℃ for 7 days. Thus, the characteristics of this beverage during 4 weeks of cold-storage (4℃) were investigated in this phase.

4.5.1 pH and titratable acidity

The changes in pH and titratable acidity (TA) of green tea kombucha beverage during storage (4°C) for 4 weeks are shown in Figure 4.24. The pH decreased from 3.12±0.01 to 3.03±0.04 in Week 1 and remained stable for the rest of the storage period, while the TA increased. The stability of the pH during Weeks 2 to 4 may be due to the buffering effect of carbon dioxide, which is produced by the yeast remaining in kombucha beverage (Malbaša et al., 2011; Kallel et al., 2012; Chakravorty et al., 2016).

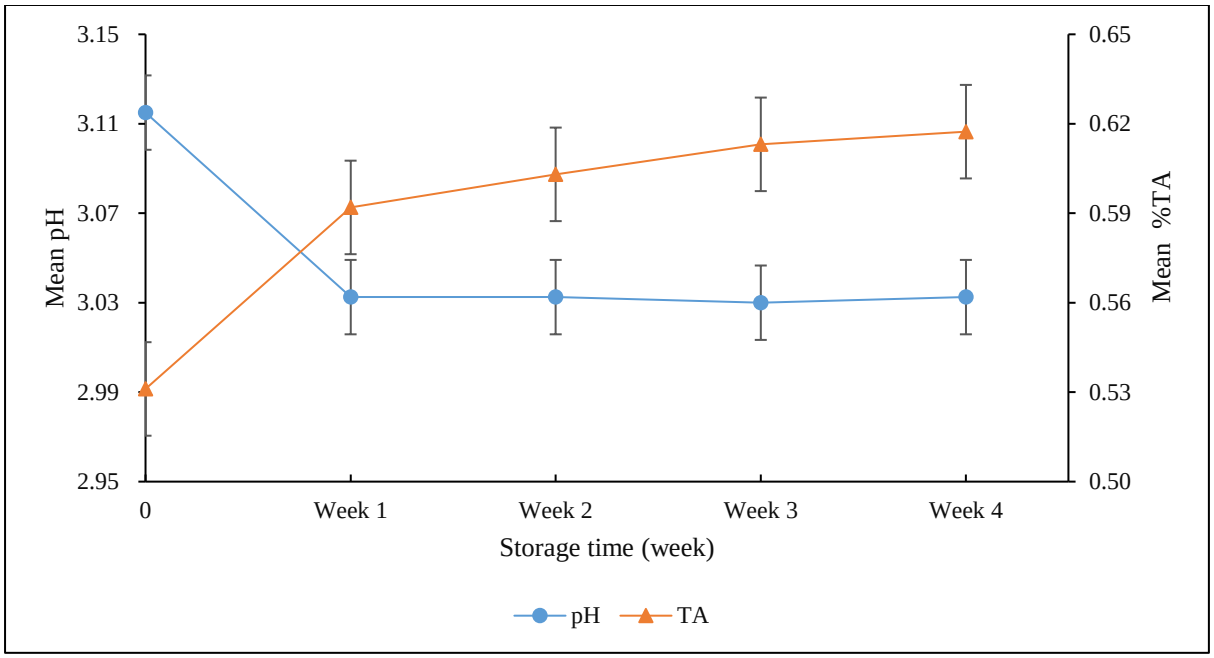

Figure 4.24 Mean pH and titratable acidity (%) of green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: TA = Titratable acidity; n=4; Error bars = ±SD; experiments were replicated twice.

Titratable acidity in green tea kombucha increased from 0.53±0.01 to 0.59±0.03 during the first week of storage, and thereafter increased to 0.62±0.02 by the end of the storage. The increase of titratable acidity was concomitant with the increase of organic acids in Section 4.5.4. The increased acidity observed during storage was caused by the fermentation of sugars to organic acids by the kombucha microorganisms in the beverage (Chen & Liu, 2000). Similar results were reported in a study of fermented kombucha yoghurt, where the pH of the product decreased from 4.54 to 4.10 during cold-storage (Hrnjez at al., 2014). The differences in pH between the products were likely attributable to the different fermentation substrates and conditions as well as the initial pH (Lončar et al., 2006). Chemical changes such as the increase in TA and decrease in pH have also been observed in cold-stored kefir beverage, which is a similar fermented drink to kombucha (Leite et al., 2013). The decrease in pH and increase in TA during storage suggest that the kombucha culture was still active during this period.

4.5.2 Total soluble solids (TSS)

Figure 4.25 shows the reduction of total soluble solids (TSS) in green tea kombucha beverage during storage at 4℃ for 4 weeks. TSS decreased significantly ($p<0.05$) from 7.40±0.10 to 6.80±0.00 ºBrix during storage, which indicated the metabolism of residual sugar by yeasts and acetic acid bacteria in green tea kombucha beverage during cold-storage. The reduction of TSS was concomitant with the increase of TA (0.53±0.01 to 0.62±0.02) and decrease in pH (3.12±0.01 to 3.03±0.04) (Figure 4.24).

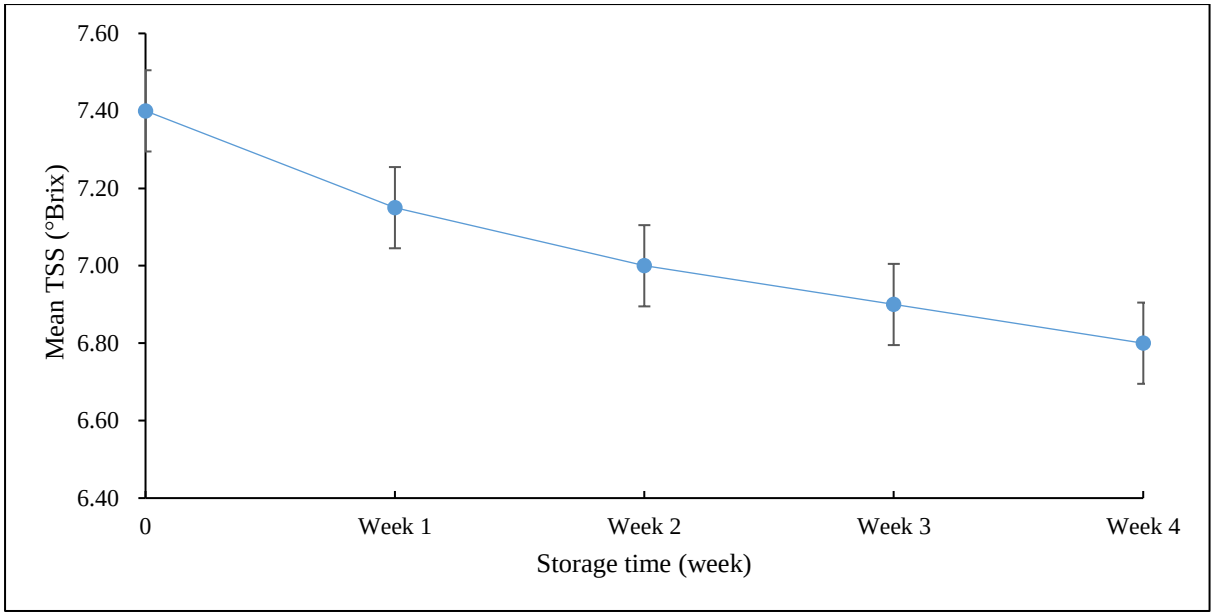

Figure 4.25 Mean total soluble solids (TSS) of green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: Error bars = ±SD; TSS = Total Soluble Solids; n=4; experiments were replicated twice.

Available information suggests that there is a gap in the research on sugar metabolism in kombucha during storage. However, changes in residual sugars during storage have been studied in mixed-culture fermented kefir beverages. From the report of Gronnevik et al. (2011), the carbohydrate content in fermented kefir was significantly reduced after 3 weeks of refrigerated storage, which is in agreement with our study. However, Irigoyen et al. (2005) obtained different results, with the lactose concentration remaining constant during storage. The differences in these results may be attributed to the differences in substrates, which affect the metabolism of sugars and growth of microorganisms (Hsieh et al., 2012).

4.5.3 Colour

Colour is one of the key attributes of green tea kombucha, which may impact on the perception of consumers (Chung et al., 2016). Changes of colour in green tea kombucha beverage during cold-storage for 4 weeks are shown in Figure 4.26. The brightness/lightness (L*) of the beverage increased from 85.82±2.22 to 89.17±2.04 during storage, but the changes were not significant ($p>0.05$). A similar result was reported by Hrnjez (2015) following the fermentation of dairy product with kombucha starter culture, with no change in lightness/brightness (L*) of the kombucha yoghurt during 14 days of storage at 4±1°C (Hrnjez at al., 2014).

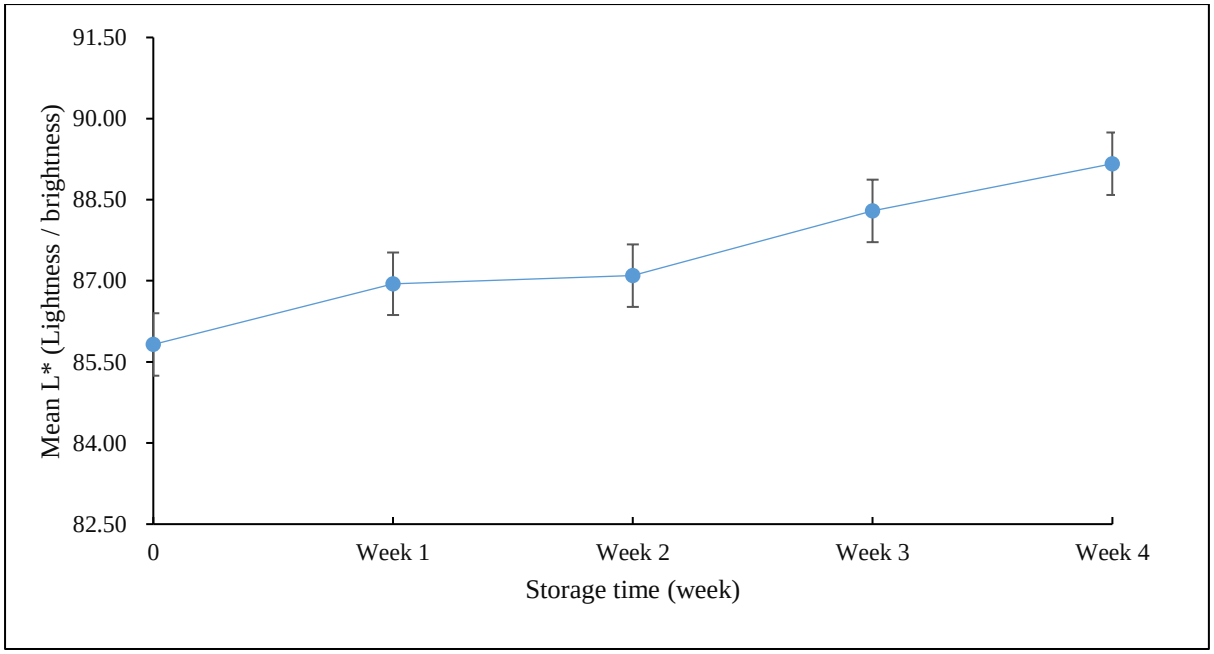

Figure 4.26 Mean brightness/Lightness (L*) of green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

During 4 weeks of cold-storage, a* (redness-greenness) and b* (yellowness-blueness) decreased significantly ($p<0.05$) from 0.49±0.06 to 0.27±0.08 and 15.79±0.69 to 13.13±0.40, respectively (Figure 4.27). These results were similar to that of Hrnjez (2014), who reported a significant reduction in a* and b* during storage of fermented kombucha dairy product for 14 days.

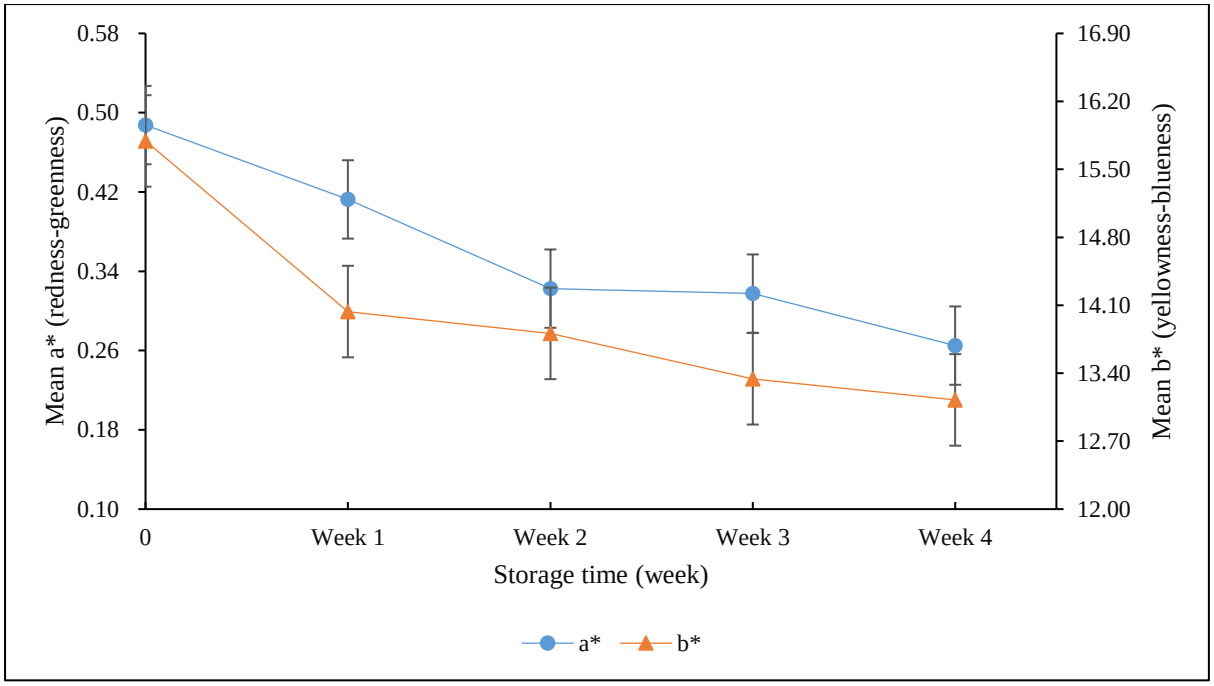

Figure 4.27 Mean redness-greenness (a*) and yellowness-blueness (b*) of green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD.

4.5.4 Organic acids

The comparative increases in gluconic acid and acetic acid content in green tea kombucha during cold-storage for 4 weeks are presented in Figure 4.28. Both gluconic acid and acetic acid increased steadily ($p<0.05$) during storage. Gluconic acid increased from 0.35±0.03 to 0.41±0.01 % (w/v) over the 4 week storage period, while acetic acid increased from 0.31±0.00 to 0.37±0.01 % (w/v). The generation of organic acids was attributed to the metabolism of residual sugars to acids by yeasts and acetic acid bacteria in the kombucha beverage (Hrnjez et al., 2014). As can be seen from Figure 4.28, the rate of increase of the acids decreased from Week 1, suggesting that the metabolic activities of the kombucha microorganisms slowed with concomitant decrease in cell numbers of yeasts and acetic acid bacteria (Section 4.5.8).

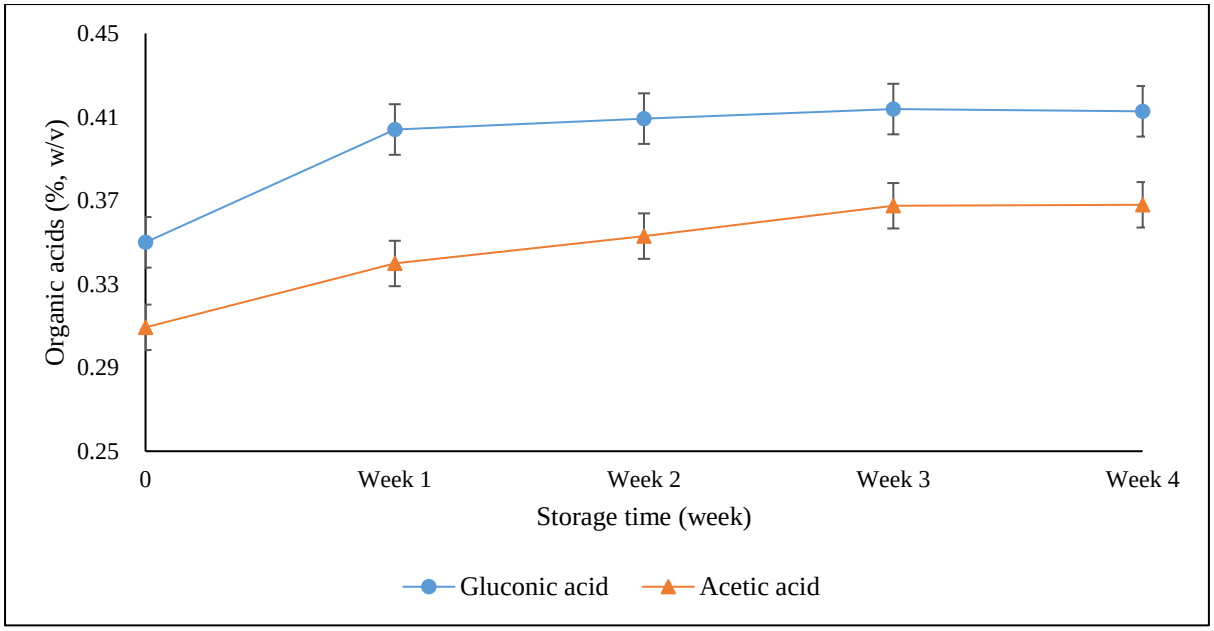

Figure 4.28 Concentration (%) of organic acids in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

4.5.5 Sugars

The changes in the concentrations of sucrose, glucose and fructose in green tea kombucha during refrigerated storage are shown in Figure 4.29. Sucrose decreased steadily from 4.52±0.12 to 2.75±0.03 % (w/v), while glucose and fructose continued to increase from 0.67±0.06 % (w/v) and 0.44±0.05 % (w/v) to 1.51±0.22 % (w/v) and 1.42±0.22 % (w/v), respectively. This result was expected as sucrose is hydrolysed into glucose and fructose by the enzyme invertase, which is secreted by yeast (Chen & Liu, 2000).

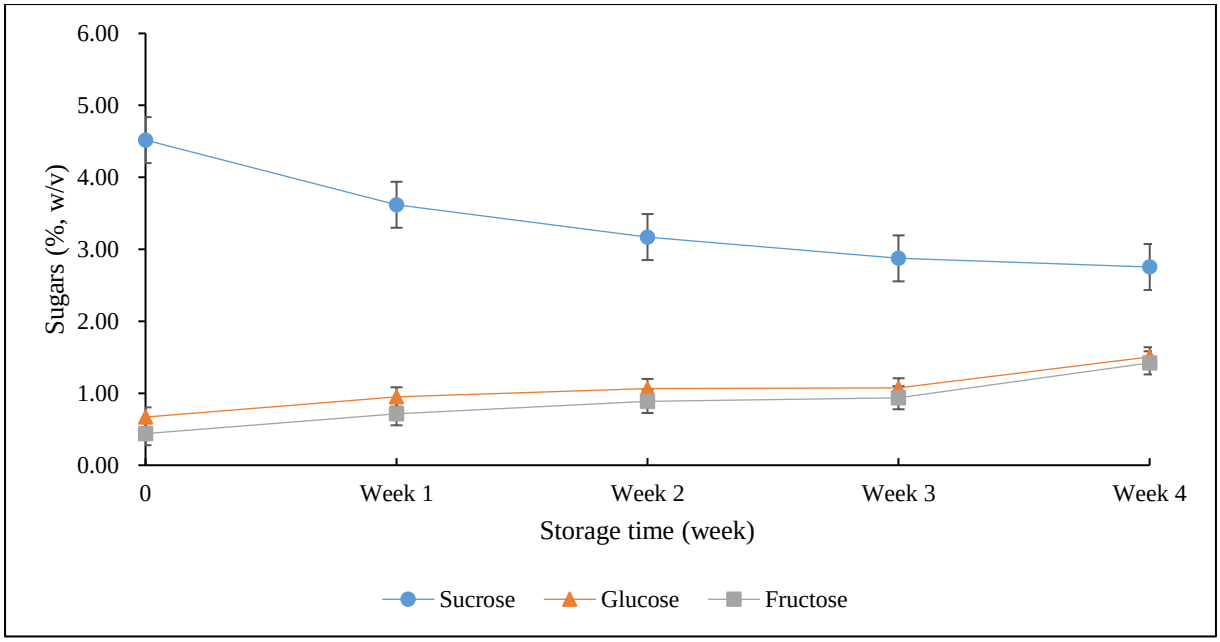

Figure 4.29 Concentration (%) of sugars in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.\

4.5.6 Ethanol

The ethanol content in fermented green tea kombucha beverage during 4 weeks of storage at 4°C is shown in Figure 4.30. Ethanol increased significantly ($p<0.05$) from 0.70±0.02 to 0.99±0.02 % (w/v) after 4 weeks of cold-storage. A similar pattern of increased ethanol content during cold-storage of a mixed-culture fermented beverage was reported by Guzel-Seydim et al. (2000), with the content increasing from 0.4 µg/g to 0.8 µg/g after 21 days of storage. The increase in ethanol content during storage was due to sugar metabolism by yeasts. During cold-storage, glucose is hydrolysed to pyruvate, which is then converted to acetaldehyde. The acetaldehyde is then hydrolysed to ethanol through the yeast fermentation pathway (Bai et al., 2008; Pfeiffer & Morley, 2014).

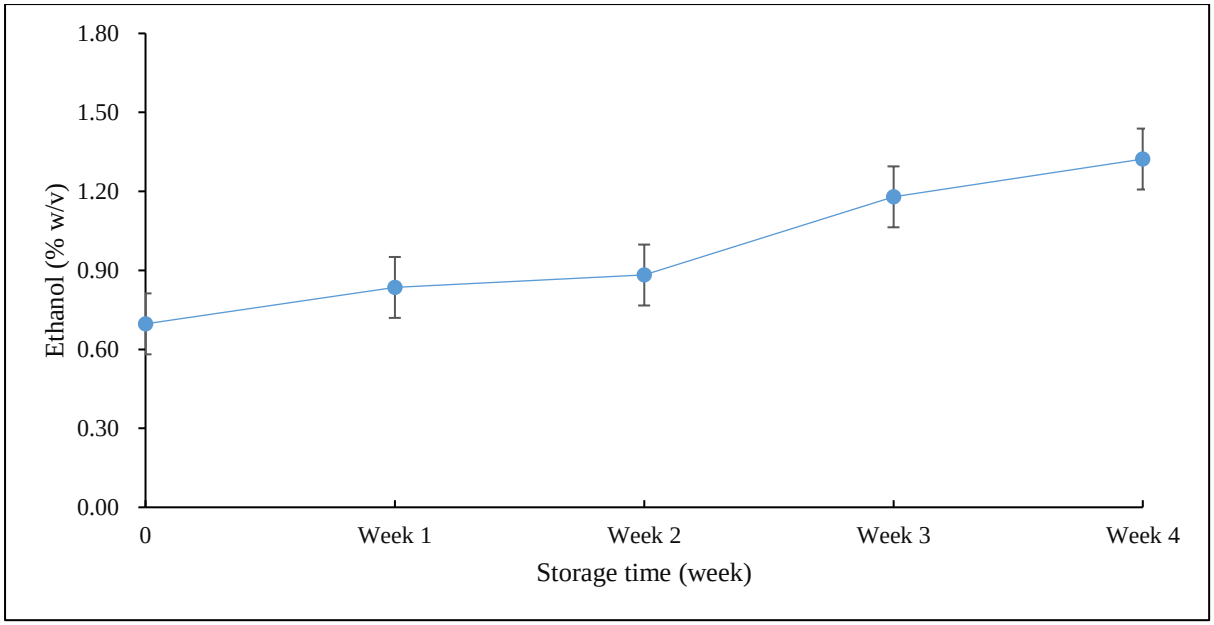

Figure 4.30 Concentration (%) of ethanol in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

4.5.7 Antioxidants

The content of phenolic compounds in green tea kombucha during cold-storage is shown in Figure 4.31. Phenolic compounds are reported to have positive effects on the prevention of various type of cancers and to have healing properties (Stoner & Mukhtar, 1995; Prabhu & Landau, 2001; Yang, Maliakal & Meng, 2002; Jayabalan, Marimuthu & Swaminathan, 2007). The phenolic compounds analysed in the kombucha beverage were gallic acid, epigallocatechin (EGC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), which were likely derived from the green tea. The concentrations of gallic acid, EGC, EGCG and ECG were 5.7±0.04 µg/ml, 130.89±6.86 µg/ml, 152.26±39.70 µg/ml and 41.11±16.23 µg/ml respectively, at the beginning of the storage period. The concentrations of gallic acid, EGC and ECG increased while EGCG decreased during 4 weeks of storage, however the changes in EGCG and ECG were not significant ($p>0.05$). Tu et al. (2005) have reported that catechins (EGC, ECG and EGCG) are stable at pH 3.6-5.6, however the pH in the present green tea kombucha was lower, at pH 3.03-3.13. The low pH may explain the variable concentration of phenolic compounds during storage. Under acidic conditions, acid-sensitive microbial cells might release catechins (ECG), which may have contributed to the observed increase in ECG levels during storage (Jayabalan et al., 2007).

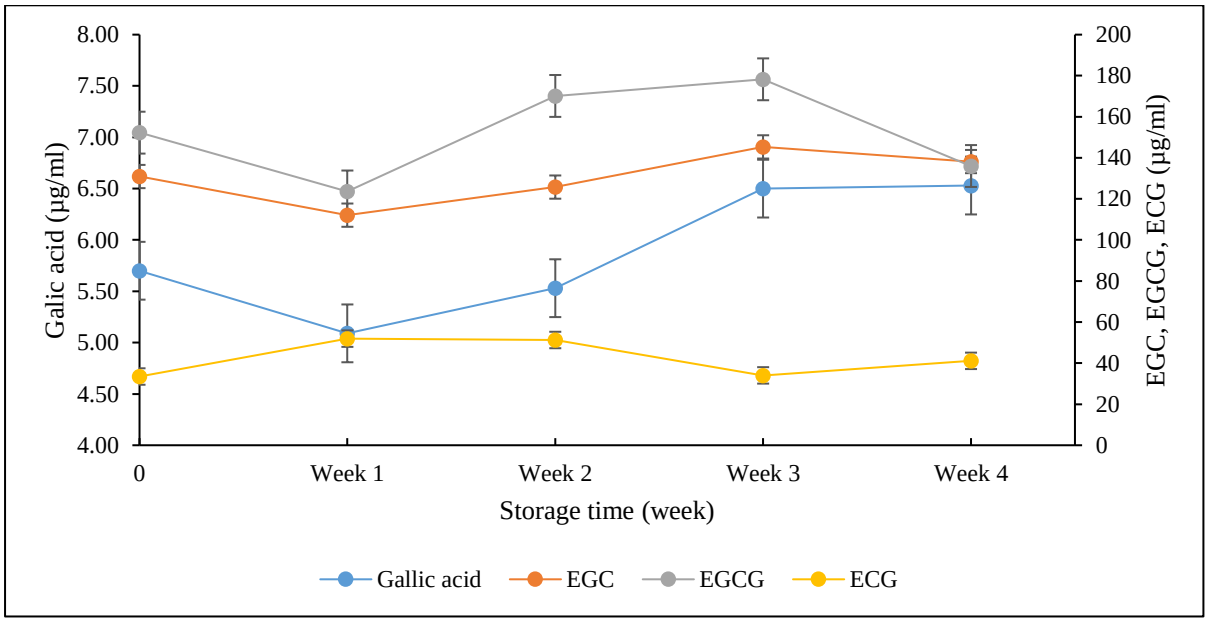

Figure 4.31 Concentration of phenolic compounds in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: EGC=epigallocatechin; EGCG=epigallocatechin gallate; ECG=epicatechin gallate; n=4; Error bars = ±SD; experiments were replicated twice.

Variable changes ($p<0.05$) in methylxanthine levels (caffeine and theobromine) in green tea kombucha were observed during storage (Figure 4.32). Caffeine and theobromine decreased during the first week, thereafter increased to 116.12±0.45 µg/ml and 7.35±0.17 µg/ml at Week 3. At the end of storage, the concentrations of caffeine and theobromine were 110.40±2.37 µg/ml and 7.07±0.04 µg/ml respectively. Fluctuations in levels of phenolic compounds in green tea yogurt were also reported by Amirdivani & Baba (2014) during 28 days of storage. The variable changes of antioxidants in green tea kombucha may be due to biotransformations of the phenolic compounds by enzymes from the acetic acid bacteria and yeast during storage (Jayabalan et al, 2014).

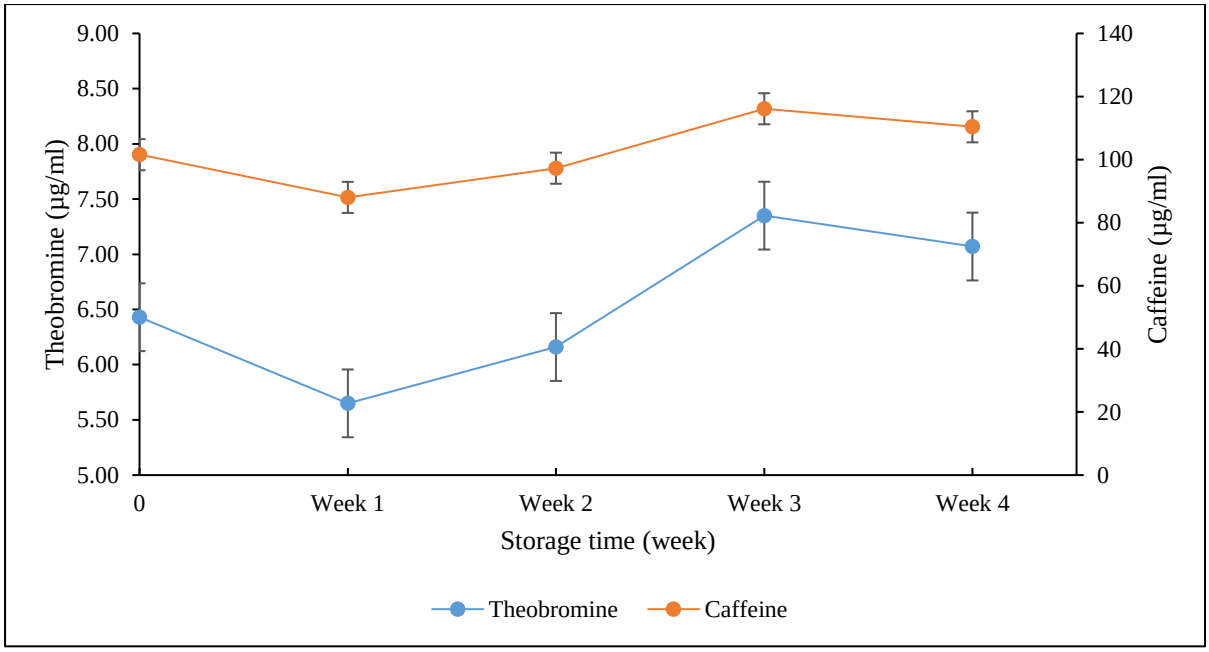

Figure 4.32 Concentration of phenolic compounds in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

4.5.8 Microbiology

Cell counts of yeast and acetic acid bacteria (AAB) in green tea kombucha decreased during storage at 4℃ (Figure 4.33). Viable yeasts cells and AAB in green tea kombucha were 7.04±0.14 log cfu/ml and 6.08±0.13 log cfu/ml respectively at the beginning of storage, and decreased ($p<0.05$) to 5.45±0.24 and 5.03±0.07 respectively, at the end of 4 weeks of refrigerated storage. Fu et al. (2014) have reported similar results in green tea kombucha, where the viable yeast cells decreased from around 1.4×10^7 cfu/ml at day 0 to 5×10^6 cfu/ml at day 14, while AAB decreased from 9.3×10^6 cfu/ml to 3.4×10^6 cfu/ml during the same period (Fu et al., 2014).

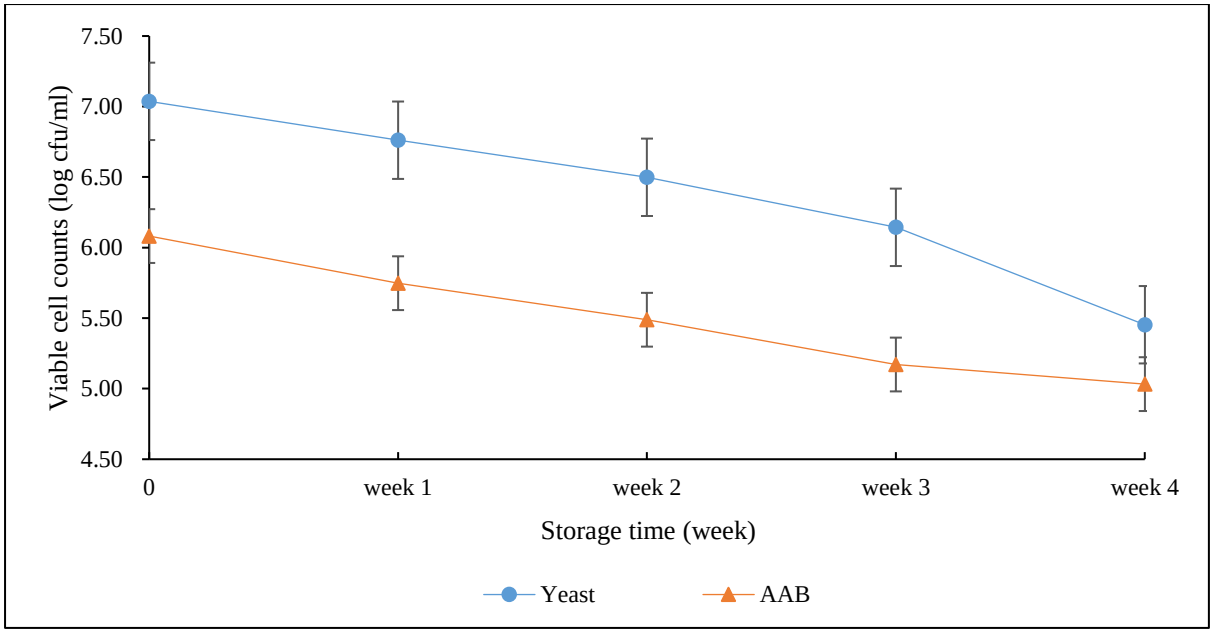

Figure 4.33 Mean log cfu/ml of yeast and acetic acid bacteria in green tea kombucha beverages during storage at 4°C for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

The reduction of yeast and AAB in green tea kombucha was probably due to the stressful acidic environment (Chen & Liu, 2000). The optimum pH for the growth of yeasts is between 5.4 to 6.3, while the pH in the beverage during storage was only 3.03-3.12 (Section 4.5.1). Low pH and depleted nutrients during storage may inhibit the growth and survival of yeasts and AAB (Mousavi et al., 2011; Sheehan et al., 2007).

4.5.9 Sensory evaluation

A 9-point hedonic scale was used for sensory evaluation of the fermented kombucha beverage during 4 weeks of storage (4°C). A panel of 60 consumer sensory panellists evaluated the green tea kombucha samples for appearance, aroma, flavour, sweetness, sourness and overall product acceptability at the end of the fermentation and during storage period. The results of consumer sensory evaluation of Phase 4 are shown in Figure 4.34. The evaluated sensory attributes of green tea kombucha were stable during the first 2 weeks of storage. This result was in agreement with Hrnjez (2015) who reported no difference in the appearance, aroma, flavour, taste and overall preference scores of fermented kombucha dairy product during 14 days of storage. However, the sensory attributes decreased in the last 2 weeks of storage, with aroma

and sourness receiving the lowest sensory scores at Week 4. The aroma and sourness probably contributed to the lower overall acceptability of green tea kombucha at the end of storage.

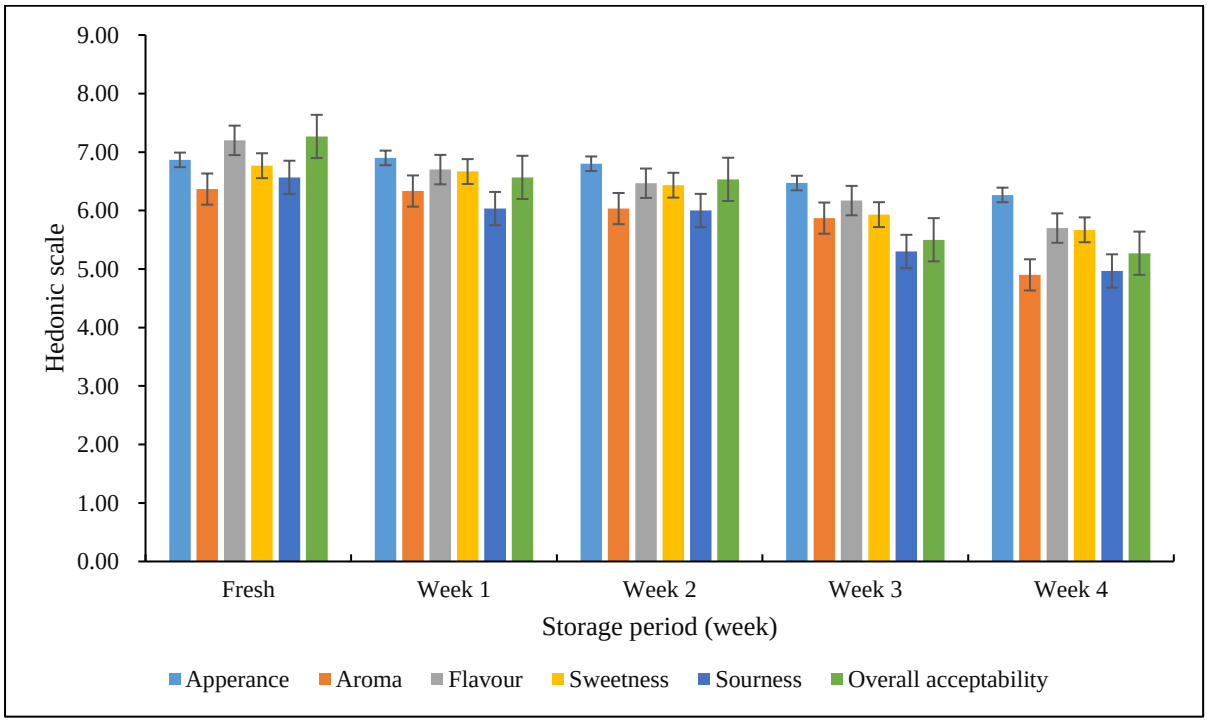

Figure 4.34 Sensory evaluation scores of green tea kombucha beverages during storage at 4℃ for 4 weeks

Notes: n=4; Error bars = ±SD; experiments were replicated twice.

4.5.10 Conclusion

Green tea kombucha containing 7% sugar and fermented at 22℃ was stable during refrigerated storage for 4 weeks and the product received high sensory scores for overall product acceptability. Viable cell counts, TSS, pH, colour, sucrose, and in the product decreased, while TA, concentration of organic acids, ethanol, glucose and fructose in green tea kombucha increased during storage. Results of the sensory evaluation suggest that the green tea kombucha beverage could be stored for 14 days at 4℃ without significant loss of sensory attributes.

5. Overall conclusions

Fermentation time, temperature and concentration of sugar had significant effects ($P<0.05$) on the production of a high quality green tea kombucha. Fermented kombucha containing 7% sugar and fermented at 22℃ for 7 days received the highest overall acceptability sensory scores from consumer panellists. The fermented green tea kombucha exhibited antibacterial activities against *Escherichia coli* 111, *Listeria monocytogenes* 15E03-1, *Salmonella typhimurium* ESR3479, *Staphylococcus aureus* MU-A57 and *Pseudomonas aeruginosa* MU-A26. Appreciable amounts of antioxidants were detected in the fermented green tea kombucha, indicating that the beverage may exert beneficial effects on human health after consumption. During storage at 4℃ for 4 weeks, the colour of the kombucha was stable and the overall acceptability of this beverage remained high.

6. Recommendations

In the current study, the strains of microorganisms in fermented kombucha was not analysed. Information on the species or strains of microorganisms responsible for the fermentation would be useful for the control of the production process as well as the development of kombucha with other substrates (Teoh, Heard & Cox, 2004).

In order to improve the sensory properties and health benefits of kombucha, medicinal herbs, such as lemon balm, peppermint, thyme and sage could be added to kombucha. The herbs contain essential oils which may have additional antimicrobial and anti-cancerogenic activities (Velićanski, Cvetković & Markov, 2013; Velićanski et al., 2014). In addition, fruit juices such as grape juice or apple juice could also be added to kombucha, which may extend the range of the flavour (Liamkaew, Chattrawanit & Danvirutai, 2016; Ayed, Abid & Hamdi, 2017).

The use of phytochemicals naturally present in food or food extracts as antioxidants and functional foods has become a global trend (Jayabalan et al., 2014). The results from this study showed that green tea kombucha contained high levels of antioxidants. In order to confirm the antioxidant activity of kombucha, additional studies should be carried out such as investigating the scavenging activities on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, superoxide radical and inhibitory activity against hydroxyl radical-mediated linoleic acid (Jayabalan et al., 2008; Hrnjez et al., 2014; Fu et al., 2014). Further, human or animals studies to investigate the beneficial health effects of these antioxidants could also be carried (Rietveld & Wiseman, 2003).

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Appendix

A. Composition of agar media for microbiological analysis

Table A.1 List of ingredients in agar media

Product name and brand	Ingredients	Content (g/L)
YGC agar (1.16000.0500), Merck KGaA	Yeast extract	5.0
	D(+) glucose	20
	Chloramphenicol	0.1
	Agar	14.9
YPM agar	Yeast extract	5.0
	Peptone	3.0
	Mannitol	25.0
	Agar	12.0
MRS agar (CM0361), Oxoid	Peptone	10.0
	Lab-Lemco powder	8.0
	Yeast extract	4.0
	Hydrogen phosphate	2.0
	Sodium acetate $3H_20$	5.0
	Tri-ammonium citrate	2.0
	Magnesium sulphate 7 H_20	0.2
	Manganese sulphate $4H_20$	0.05
	Agar	10.0

B. Sensory evaluation questioneer

INFORMATION SHEET

Introduction

I am Xiaolei Wang, a Master of Food Technology student at School of Food and Nutrition (SFN), Albany, Massey University. My research project is optimization and characteristics of green tea kombucha, so this study is part of my project and will contribute to the development of green tea kombucha. You are therefore invited to take part in a study that evaluates the sensory characteristics of organic fermented green tea kombucha. The objective of this sensory evaluation is to evaluate the level of acceptance of organic fermented green tea kombucha by potential consumers.

Participant involvement

This study involves tasting and evaluating an organic fermented green tea kombucha, it may take you 5-10 minutes. The green tea kombucha that you will taste may contain all or some of following ingredients: green tea, cane sugar, starter cultures (kombucha strains) and kombucha vinegar.

You should not participate if you are allergic or may affected by the consumption of any of the listed ingredients. In the unlikely event of any adverse reaction, medical assistance will be provided. You may advise one of the researchers of any potentially relevant cultural, religious or ethical beliefs which may prevent you from consuming the food under consideration.

The information collected in this study will not be linked to any individual's identity and will be used to complete my postgraduate degree research project. In case you wish to receive a summary of the findings once data analysis has been completed, please provide your email address.

You may under no obligation to accept this invitation. If you decided to participate, you have the right to:

- Decline to answer any particular questions;
- Withdraw from the study (at any time);
- Ask any questions about the study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researchers.

Ethics Approval Number 4000017046

Project Contacts

- Xiaolei Wang (Master of Food Technology student) - daisy152517@gmail.com
- Dr Tony Mutukumira (supervisor) – a.n.mutukumira@massey.ac.nz

This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named above are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you wish to raise with someone other than the researcher(s), please contact Professor John O'Neill, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz.

Ethics Approval Number 4000017046

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction.

I understand that I have the right to withdraw from the study at any time and decline my answers.

I agree to voluntarily participate in this study under the condition set out the Information Sheet.

Signature: _____ **Date:** _____

Full Name (Printed): _____

Ethics Approval Number 4000017046

SENSORY ACCEPTANCE TEST

You will be given four coded samples. For each of the following characteristics, please taste the sample and indicate how much you like/dislike it by ticking [✓] in the appropriate box.

You may taste the sample more than once. Please rinse your mouth with green tea kombucha and between samples.

Note: Each samples must be evaluated on a separate form.

PROJECT: Organic Green Tea Kombucha **SAMPLE CODE:**

Attribute	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Nether like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance/ Color									
Odour									
Flavour									
Sweetness									
Sourness									
Overall acceptability									

Ethics Approval Number 4000017046

C. Data Analysis

Table C.1 Microbial profile analysis of kombucha starter culture

Parameter	Trial 1		Trial 2		Trial 1		Trial 2		Mean ± SD
	Replication 1	Replication 2	Replication 1	Replication 2	Replication 1	Replication 2	Replication 1	Replication 2	
	cfu/ml	cfu/ml	cfu/ml	cfu/ml	log cfu/ml	log cfu/ml	log cfu/ml	log cfu/ml	
Acetic acid bacteria	1.58E+07	1.62E+07	1.12E+07	1.18E+07	7.20	7.21	7.05	7.07	7.13±0.07
Yeast	1.32E+06	1.41E+06	1.02E+06	1.10E+06	6.12	6.15	6.08	6.04	6.08±0.06
Lactic acid bacteria	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

Note: Nd = Not detected

Table C.2 Raw data in Phase 2

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
1	1	1	3.40	0.29	7.30	84.70	0.25	17.57	6.34	5.95
1	1	1	3.40	0.29	7.30	84.88	0.27	17.56	6.36	5.97
1	1	2	3.31	0.35	8.20	85.41	0.11	15.16	5.97	5.32
1	1	2	3.28	0.35	8.20	85.27	0.08	15.17	5.98	5.26
1	2	1	3.33	0.33	7.10	88.45	0.13	16.02	6.65	5.73
1	2	1	3.32	0.33	7.10	88.30	0.12	16.02	6.61	5.74
1	2	2	3.27	0.39	8.00	88.65	0.11	13.82	6.12	5.83
1	2	2	3.25	0.39	8.00	88.54	0.11	13.83	6.14	5.80
1	3	1	3.40	0.22	8.00	86.99	0.19	17.32	6.40	5.53
1	3	1	3.41	0.22	8.00	86.88	0.20	17.31	6.41	5.52
1	3	2	3.35	0.30	8.50	86.77	0.30	17.13	5.77	5.26
1	3	2	3.35	0.30	8.50	86.86	0.31	17.10	5.76	5.28
1	4	1	3.35	0.28	7.70	88.01	0.14	16.61	7.05	6.09
1	4	1	3.36	0.28	7.60	88.41	0.14	16.63	7.06	6.10
1	4	2	3.27	0.37	8.50	90.86	0.25	16.11	6.49	5.66
1	4	2	3.25	0.36	8.50	87.41	0.27	16.08	6.57	5.72
1	5	1	3.57	0.15	8.50	85.41	0.32	19.73	6.88	5.93
1	5	1	3.57	0.16	8.50	85.33	0.34	19.83	6.86	5.94
1	5	2	3.52	0.22	8.80	86.25	0.31	19.73	6.42	5.46
1	5	2	3.50	0.22	8.80	86.72	0.28	19.79	6.43	5.47
1	6	1	3.27	0.37	8.10	87.90	0.30	16.91	7.21	6.20
1	6	1	3.29	0.38	8.10	87.89	0.30	16.94	7.18	6.22
1	6	2	3.21	0.41	8.00	88.44	0.26	16.91	7.00	6.05
1	6	2	3.20	0.40	8.00	87.79	0.25	16.94	7.10	6.05
1	7	1	3.16	0.48	7.70	88.22	0.28	15.99	6.57	5.64
1	7	1	3.17	0.49	7.70	88.41	0.28	16.02	6.56	5.63
1	7	2	2.97	0.68	7.60	88.32	0.18	14.72	6.08	5.25
1	7	2	3.00	0.68	7.60	88.77	0.14	14.78	6.06	5.26
1	8	1	2.95	0.68	7.50	89.52	0.21	14.66	5.59	4.62
1	8	1	2.97	0.68	7.50	88.39	0.20	14.64	5.58	4.64
1	8	2	2.80	0.88	7.50	89.99	0.17	14.33	5.21	4.06
1	8	2	2.83	0.88	7.50	89.74	0.19	14.32	5.25	4.05

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
2	1	1	3.41	0.29	8.40	83.67	0.25	17.55	6.38	5.93
2	1	1	3.41	0.29	8.40	83.08	0.27	17.54	6.39	5.97
2	1	2	3.31	0.35	9.20	86.61	0.10	15.13	5.94	5.26
2	1	2	3.30	0.35	9.20	86.56	0.11	15.10	5.98	5.30
2	2	1	3.31	0.33	8.20	88.08	0.10	15.95	6.54	5.70
2	2	1	3.30	0.33	8.20	87.98	0.10	15.94	6.58	5.83
2	2	2	3.25	0.39	9.00	89.20	0.10	13.72	6.04	5.81
2	2	2	3.26	0.39	9.00	89.14	0.10	13.72	6.03	5.88
2	3	1	3.40	0.21	9.90	87.06	0.18	17.45	6.51	5.67
2	3	1	3.39	0.21	9.90	87.03	0.15	17.48	6.53	5.71
2	3	2	3.36	0.30	10.60	87.23	0.31	16.29	5.78	5.27
2	3	2	3.36	0.29	10.60	87.13	0.28	16.32	5.76	5.24
2	4	1	3.35	0.28	9.60	88.72	0.12	16.55	6.93	6.06
2	4	1	3.36	0.28	9.60	88.83	0.11	16.56	6.90	6.04
2	4	2	3.27	0.37	10.50	88.91	0.25	15.32	6.20	5.55
2	4	2	3.27	0.37	10.50	88.81	0.24	15.30	6.28	5.63
2	5	1	3.55	0.15	11.00	86.68	0.29	19.72	6.76	5.84
2	5	1	3.56	0.15	11.00	86.65	0.31	19.78	6.82	5.86
2	5	2	3.49	0.22	11.50	85.90	0.30	18.08	6.15	5.31
2	5	2	3.51	0.22	11.50	85.61	0.28	18.09	6.17	5.34
2	6	1	3.23	0.41	10.30	87.88	0.25	15.87	7.25	6.25
2	6	1	3.22	0.42	10.30	87.95	0.24	15.87	7.26	6.21
2	6	2	3.20	0.42	11.10	90.06	0.28	15.06	6.62	5.78
2	6	2	3.23	0.42	11.10	90.07	0.27	15.66	6.60	5.76
2	7	1	3.15	0.50	10.00	88.39	0.23	15.21	6.69	5.71
2	7	1	3.14	0.51	10.00	88.36	0.22	15.24	6.67	5.69
2	7	2	3.00	0.67	10.80	90.98	0.19	14.53	6.09	5.31
2	7	2	2.99	0.67	10.80	90.99	0.18	14.51	6.10	5.29
2	8	1	2.90	0.74	9.80	89.69	0.19	14.06	5.69	4.78
2	8	1	2.92	0.74	9.80	89.67	0.20	14.08	5.63	4.79
2	8	2	2.85	0.88	10.70	90.25	0.16	14.03	5.35	4.17
2	8	2	2.82	0.88	10.70	90.21	0.15	14.05	5.36	4.16

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
3	1	1	3.42	0.28	7.30	84.24	0.25	17.59	6.39	5.98
3	1	1	3.42	0.29	7.30	84.14	0.23	17.55	6.34	5.92
3	1	2	3.30	0.35	8.20	86.51	0.11	15.03	5.91	5.32
3	1	2	3.29	0.36	8.20	86.35	0.09	15.04	5.99	5.28
3	2	1	3.31	0.36	7.20	89.29	0.09	16.16	6.62	5.75
3	2	1	3.32	0.36	7.20	89.23	0.08	16.14	6.65	5.84
3	2	2	3.23	0.41	8.00	88.50	0.10	13.24	6.00	5.79
3	2	2	3.24	0.42	8.00	88.62	0.09	13.28	5.99	5.77
3	3	1	3.40	0.21	8.00	87.37	0.18	17.20	6.49	5.61
3	3	1	3.41	0.22	8.00	87.36	0.16	17.21	6.51	5.72
3	3	2	3.36	0.30	8.50	87.76	0.26	16.89	5.84	5.24
3	3	2	3.35	0.31	8.50	87.80	0.27	16.89	5.85	5.23
3	4	1	3.30	0.32	7.70	89.07	0.15	16.35	6.83	6.12
3	4	1	3.29	0.31	7.70	89.04	0.12	16.45	6.82	6.15
3	4	2	3.23	0.41	8.50	89.93	0.16	14.94	6.23	5.50
3	4	2	3.21	0.41	8.50	90.00	0.18	14.94	6.25	5.51
3	5	1	3.53	0.18	8.50	86.84	0.28	19.13	6.69	5.82
3	5	1	3.54	0.18	8.50	86.40	0.29	19.13	6.67	5.85
3	5	2	3.48	0.22	8.80	87.77	0.25	17.45	5.99	5.23
3	5	2	3.48	0.21	8.80	87.72	0.27	17.45	5.97	5.21
3	6	1	3.26	0.38	7.60	89.51	0.25	15.54	6.60	5.45
3	6	1	3.24	0.39	7.60	89.56	0.24	15.59	6.61	5.40
3	6	2	3.19	0.48	7.60	90.43	0.26	14.32	5.92	4.85
3	6	2	3.16	0.48	7.60	90.33	0.26	14.36	5.94	4.87
3	7	1	3.14	0.50	7.20	91.52	0.19	14.08	6.06	4.93
3	7	1	3.15	0.50	7.20	91.56	0.20	14.11	6.05	4.97
3	7	2	2.97	0.67	7.20	91.56	0.18	13.74	5.32	4.33
3	7	2	2.98	0.67	7.20	91.47	0.18	13.77	5.37	4.32
3	8	1	3.01	0.67	7.00	91.91	0.18	13.76	5.07	3.41
3	8	1	2.98	0.67	7.00	91.87	0.16	13.76	5.05	3.38
3	8	2	2.90	0.74	7.00	92.02	0.15	12.83	4.31	3.71
3	8	2	2.87	0.74	7.00	91.78	0.14	12.83	4.25	3.73

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
4	1	1	3.41	0.20	8.40	83.86	0.25	17.65	6.38	6.02
4	1	1	3.41	0.20	8.40	83.72	0.27	17.76	6.34	5.99
4	1	2	3.28	0.36	9.20	86.17	0.09	15.13	5.98	5.30
4	1	2	3.30	0.35	9.20	86.30	0.1	15.11	5.92	5.32
4	2	1	3.31	0.36	8.10	88.13	0.09	15.97	6.74	5.85
4	2	1	3.30	0.36	8.10	88.84	0.11	16.02	6.75	5.88
4	2	2	3.22	0.43	9.00	87.47	0.11	13.71	6.03	5.88
4	2	2	3.23	0.44	9.00	87.86	0.09	13.71	6.01	5.85
4	3	1	3.42	0.22	9.90	86.10	0.17	17.71	6.59	5.81
4	3	1	3.38	0.21	9.90	86.18	0.21	17.74	6.53	5.82
4	3	2	3.34	0.31	10.60	87.68	0.29	16.23	5.88	5.25
4	3	2	3.34	0.31	10.60	87.60	0.24	16.24	5.94	5.22
4	4	1	3.32	0.30	9.50	88.06	0.15	16.39	6.90	6.13
4	4	1	3.34	0.31	9.50	88.20	0.16	16.35	6.88	6.09
4	4	2	3.21	0.41	10.50	90.14	0.17	14.85	6.39	5.55
4	4	2	3.21	0.41	10.50	89.80	0.18	14.77	6.38	5.56
4	5	1	3.53	0.17	11.00	86.45	0.28	19.12	6.71	5.88
4	5	1	3.54	0.17	11.00	86.37	0.28	19.17	6.72	5.91
4	5	2	3.47	0.22	11.50	87.55	0.3	16.87	6.18	5.23
4	5	2	3.48	0.23	11.50	87.67	0.29	16.88	6.16	5.20
4	6	1	3.21	0.42	10.00	89.01	0.25	14.93	6.66	5.63
4	6	1	3.23	0.42	10.00	89.04	0.23	14.96	6.65	5.65
4	6	2	3.14	0.50	11.00	89.69	0.26	13.79	6.07	4.91
4	6	2	3.14	0.49	11.00	89.76	0.28	13.79	6.08	4.89
4	7	1	3.11	0.53	9.50	90.84	0.2	13.61	5.92	4.76
4	7	1	3.13	0.53	9.50	90.56	0.2	13.68	5.88	4.75
4	7	2	2.95	0.69	10.50	89.79	0.21	13.50	5.27	4.30
4	7	2	2.94	0.69	10.50	89.72	0.2	13.56	5.25	4.30
4	8	1	2.87	0.74	9.40	91.83	0.19	12.97	4.74	3.23
4	8	1	2.84	0.74	9.40	91.37	0.17	12.85	4.76	3.27
4	8	2	2.86	0.85	10.20	89.88	0.15	12.33	4.23	3.69
4	8	2	2.85	0.85	10.20	89.90	0.17	12.32	4.19	3.66

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Fermentation time: 1 = Day 0, 2 = Day 1-BA, 3 = Day 1-AA, 4 = Day 2-BA, 5 = Day 2-AA; 6 = Day 7, 7 = Day 10, 8 = Day 14.

Table C.3 Raw data in Phase 3 during fermentation for 7 days

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (°Brix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
1	1	1	3.39	0.29	8.60	86.68	0.25	16.91	6.31	5.36
1	1	1	3.40	0.29	8.60	86.55	0.26	16.93	6.33	5.39
1	1	2	3.27	0.31	8.40	86.07	0.19	17.60	6.59	5.80
1	1	2	3.26	0.31	8.40	85.64	0.22	17.77	6.53	5.81
1	2	1	3.25	0.36	8.50	87.67	0.20	16.60	6.51	5.75
1	2	1	3.25	0.36	8.50	87.66	0.21	16.63	6.61	5.73
1	2	2	3.21	0.42	8.10	87.25	0.17	16.41	6.98	6.09
1	2	2	3.24	0.43	8.10	87.12	0.17	16.45	6.95	6.02
1	3	1	3.35	0.18	8.70	85.49	0.31	18.86	6.33	5.42
1	3	1	3.38	0.18	8.70	85.57	0.29	18.88	6.36	5.43
1	3	2	3.30	0.21	8.50	83.09	0.37	19.21	6.88	5.79
1	3	2	3.30	0.21	8.50	83.11	0.36	19.21	6.92	5.76
1	4	1	3.31	0.20	8.50	89.79	0.2	18.06	7.23	6.42
1	4	1	3.31	0.20	8.50	89.72	0.17	18.11	7.25	6.39
1	4	2	3.28	0.25	8.40	87.11	0.33	18.47	7.20	6.21
1	4	2	3.28	0.25	8.40	87.06	0.28	18.50	7.23	6.20
1	5	1	3.55	0.10	8.80	84.23	0.48	20.41	7.05	6.11
1	5	1	3.55	0.10	8.80	84.02	0.47	20.43	7.00	6.14
1	5	2	3.48	0.13	8.80	82.89	0.43	20.57	7.05	5.98
1	5	2	3.47	0.13	8.80	82.67	0.45	20.56	7.00	5.89
1	6	1	3.26	0.31	8.10	85.54	0.39	18.54	7.45	6.49
1	6	1	3.26	0.30	8.10	85.50	0.35	18.57	7.47	6.53
1	6	2	3.25	0.30	8.00	83.78	0.32	19.17	7.15	6.11
1	6	2	3.24	0.30	8.00	83.64	0.34	19.20	7.09	6.15
1	7	1	3.20	0.43	7.60	86.35	0.26	18.09	7.48	6.61
1	7	1	3.21	0.43	7.60	86.39	0.31	18.07	7.47	6.49
1	7	2	3.16	0.46	7.40	86.22	0.29	17.91	7.16	6.08
1	7	2	3.15	0.46	7.40	86.12	0.25	17.91	7.19	6.10

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
2	1	1	3.39	0.29	9.80	86.08	0.23	16.51	6.33	5.31
2	1	1	3.38	0.29	9.80	86.08	0.26	16.57	6.35	5.32
2	1	2	3.28	0.31	9.70	86.28	0.19	17.07	6.56	5.82
2	1	2	3.26	0.32	9.70	86.00	0.22	17.76	6.57	5.85
2	2	1	3.24	0.36	9.60	87.60	0.2	16.14	6.45	5.73
2	2	1	3.24	0.36	9.60	87.45	0.21	16.17	6.44	5.78
2	2	2	3.22	0.43	9.10	88.08	0.15	16.14	6.94	6.07
2	2	2	3.23	0.43	9.10	88.04	0.12	16.24	6.93	6.09
2	3	1	3.38	0.18	10.80	85.66	0.31	18.54	6.34	5.37
2	3	1	3.36	0.18	10.80	85.59	0.34	18.56	6.32	5.38
2	3	2	3.31	0.21	10.60	84.59	0.32	18.95	6.89	5.79
2	3	2	3.31	0.21	10.60	84.02	0.3	18.88	6.89	5.83
2	4	1	3.30	0.22	10.60	90.01	0.23	17.46	7.16	6.34
2	4	1	3.30	0.21	10.60	90.22	0.21	17.53	7.14	6.35
2	4	2	3.30	0.29	10.50	88.06	0.24	17.79	7.03	5.97
2	4	2	3.30	0.29	10.50	87.83	0.23	17.76	7.04	6.02
2	5	1	3.53	0.11	11.40	84.08	0.44	19.93	6.95	5.99
2	5	1	3.53	0.11	11.40	84.00	0.41	19.96	6.97	5.88
2	5	2	3.44	0.16	11.30	83.31	0.43	19.55	6.91	5.91
2	5	2	3.43	0.16	11.30	83.01	0.46	19.64	6.86	5.88
2	6	1	3.26	0.32	10.70	85.29	0.33	18.22	7.42	6.42
2	6	1	3.28	0.32	10.70	85.19	0.35	18.23	7.40	6.40
2	6	2	3.27	0.34	10.70	85.29	0.33	17.88	7.00	6.09
2	6	2	3.28	0.34	10.70	85.55	0.34	17.90	6.99	6.09
2	7	1	3.16	0.46	10.50	86.75	0.26	17.65	7.43	6.43
2	7	1	3.14	0.47	10.50	86.85	0.26	17.67	7.39	6.43
2	7	2	3.10	0.48	10.30	86.75	0.23	16.58	7.00	6.11
2	7	2	3.12	0.48	10.30	86.95	0.29	16.53	7.01	6.10

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
3	1	1	3.41	0.29	8.60	86.85	0.21	17.00	6.36	5.34
3	1	1	3.38	0.29	8.60	86.69	0.21	17.03	6.32	5.31
3	1	2	3.28	0.30	8.40	86.42	0.23	17.64	6.53	5.82
3	1	2	3.28	0.30	8.40	86.18	0.24	17.70	6.57	5.85
3	2	1	3.21	0.39	8.50	88.32	0.14	16.33	6.46	5.77
3	2	1	3.21	0.39	8.50	88.32	0.15	16.38	6.46	5.77
3	2	2	3.19	0.43	7.80	87.94	0.15	16.23	6.94	6.13
3	2	2	3.20	0.43	7.80	87.81	0.18	16.28	6.98	6.16
3	3	1	3.35	0.20	8.80	85.29	0.32	18.09	6.38	5.51
3	3	1	3.35	0.20	8.80	85.26	0.34	18.13	6.38	5.49
3	3	2	3.28	0.23	8.30	86.45	0.27	18.33	6.85	5.69
3	3	2	3.28	0.23	8.30	85.78	0.25	18.40	6.84	5.72
3	4	1	3.27	0.31	8.50	88.16	0.2	17.51	7.10	6.29
3	4	1	3.28	0.31	8.50	88.10	0.22	17.46	7.08	6.34
3	4	2	3.26	0.36	8.20	87.68	0.2	17.24	6.95	5.92
3	4	2	3.25	0.36	8.20	87.52	0.19	17.46	6.92	5.89
3	5	1	3.45	0.15	8.80	85.61	0.4	19.57	6.87	6.04
3	5	1	3.45	0.15	8.80	85.46	0.39	19.54	6.92	6.05
3	5	2	3.40	0.19	8.70	84.39	0.42	19.31	6.79	5.78
3	5	2	3.38	0.19	8.70	84.48	0.39	19.37	6.76	5.76
3	6	1	3.24	0.34	7.80	86.85	0.4	16.91	6.98	6.15
3	6	1	3.24	0.34	7.80	86.92	0.37	16.92	7.02	6.13
3	6	2	3.19	0.39	7.50	86.83	0.27	17.22	6.83	5.80
3	6	2	3.20	0.39	7.50	86.22	0.26	17.38	6.84	5.81
3	7	1	3.08	0.56	7.30	86.08	0.28	15.86	6.67	5.62
3	7	1	3.07	0.55	7.30	86.35	0.27	15.86	6.67	5.59
3	7	2	3.11	0.53	7.00	89.74	0.21	16.41	6.44	5.31
3	7	2	3.11	0.53	7.00	89.79	0.19	16.44	6.47	5.34

Product treatment	Fermentation time	Replication	pH	Acetic acid (%)	TSS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
4	1	1	3.38	0.29	9.80	86.11	0.22	16.58	6.32	5.33
4	1	1	3.40	0.29	9.80	86.14	0.22	16.57	6.31	5.30
4	1	2	3.27	0.31	9.70	86.24	0.18	17.65	6.57	5.86
4	1	2	3.29	0.31	9.70	86.04	0.21	17.75	6.58	5.84
4	2	1	3.21	0.38	9.50	87.96	0.18	15.99	6.49	5.72
4	2	1	3.22	0.38	9.50	87.60	0.17	15.99	6.52	5.74
4	2	2	3.14	0.47	9.10	87.75	0.18	16.35	7.02	6.14
4	2	2	3.14	0.47	9.10	87.76	0.13	16.28	6.96	6.15
4	3	1	3.35	0.19	10.70	85.03	0.32	17.74	6.41	5.53
4	3	1	3.36	0.19	10.70	85.01	0.32	17.73	6.39	5.50
4	3	2	3.27	0.25	10.60	85.69	0.31	17.29	6.74	5.63
4	3	2	3.27	0.25	10.60	85.31	0.28	17.35	6.85	5.69
4	4	1	3.27	0.31	10.50	88.71	0.24	17.09	7.10	6.21
4	4	1	3.29	0.31	10.50	88.80	0.21	17.26	7.09	6.20
4	4	2	3.25	0.36	10.50	88.04	0.23	16.49	6.97	6.07
4	4	2	3.25	0.36	10.50	87.84	0.21	16.08	7.00	6.10
4	5	1	3.44	0.15	11.30	84.85	0.42	19.16	6.91	6.04
4	5	1	3.45	0.15	11.30	85.21	0.44	19.16	6.92	6.02
4	5	2	3.41	0.18	11.30	83.99	0.39	19.09	6.83	5.83
4	5	2	3.40	0.18	11.30	83.68	0.43	19.16	6.83	5.81
4	6	1	3.23	0.35	10.40	86.24	0.37	16.57	7.03	6.18
4	6	1	3.22	0.35	10.40	86.24	0.36	16.53	7.03	6.17
4	6	2	3.21	0.44	10.40	86.34	0.25	16.47	6.89	5.84
4	6	2	3.22	0.44	10.40	86.37	0.35	16.47	6.85	5.85
4	7	1	3.02	0.59	10.10	87.24	0.27	16.14	6.68	5.81
4	7	1	3.03	0.59	10.10	87.12	0.22	15.81	6.68	5.83
4	7	2	3.06	0.57	10.00	88.01	0.21	15.19	6.57	5.36
4	7	2	3.05	0.57	10.00	88.11	0.29	15.23	6.59	5.32

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Fermentation time: 1 = Day 0, 2 = Day 1-BA, 3 = Day 1-AA, 4 = Day 2-BA, 5 = Day 2-AA; 6 = Day 5, 7 = Day 7.

Table C.4 Raw data in Phase 3 during storage for 2 weeks

Product treatment	Storage time	Replication	pH	Acetic acid (%)	TTS (°Brix)	Colour (L)	Colour (a)	Colour (b)	Yeast log CFU/ml	Acetic acid bacteria log CFU/ml
1	1	1	3.16	0.45	7.20	86.54	0.23	16.15	7.13	6.25
1	1	1	3.17	0.45	7.20	86.51	0.25	16.17	7.16	6.23
1	1	2	3.13	0.52	7.20	87.74	0.15	16.58	6.71	5.78
1	1	2	3.13	0.52	7.20	87.84	0.19	16.58	6.70	5.76
1	2	1	3.15	0.46	6.90	87.21	0.18	16.11	6.85	5.83
1	2	1	3.17	0.46	6.90	87.18	0.15	16.12	6.78	5.85
1	2	2	3.11	0.54	7.10	88.84	0.11	15.50	6.65	5.44
1	2	2	3.09	0.54	7.10	88.68	0.04	15.52	6.58	5.46
2	1	1	3.12	0.50	10.20	87.50	0.17	16.07	7.04	6.28
2	1	1	3.14	0.50	10.20	87.47	0.14	16.08	7.14	6.26
2	1	2	3.09	0.58	10.00	88.45	0.19	15.15	6.85	5.84
2	1	2	3.11	0.58	10.00	88.51	0.23	15.15	6.77	5.82
2	2	1	3.10	0.50	9.90	87.39	0.10	15.59	6.90	5.87
2	2	1	3.12	0.50	9.90	87.26	0.10	15.51	6.95	5.89
2	2	2	3.06	0.59	9.90	90.78	0.09	14.27	6.60	5.49
2	2	2	3.09	0.59	9.90	90.64	0.13	14.28	6.61	5.51
3	1	1	3.06	0.59	7.20	88.92	0.18	14.05	5.97	5.00
3	1	1	3.05	0.59	7.20	88.78	0.16	14.37	5.99	5.00
3	1	2	2.97	0.69	6.90	90.06	0.14	14.97	5.76	4.52
3	1	2	2.99	0.69	6.90	90.09	0.14	14.90	5.75	4.53
3	2	1	3.07	0.59	7.00	90.39	0.09	13.58	5.09	4.31
3	2	1	3.04	0.59	7.00	90.35	0.08	13.59	5.01	4.33
3	2	2	2.98	0.70	6.80	91.06	0.10	13.91	4.68	3.73
3	2	2	2.96	0.70	6.80	91.09	0.08	13.80	4.62	3.71
4	1	1	3.02	0.64	10.00	88.30	0.18	14.73	6.10	5.15
4	1	1	3.03	0.64	10.00	88.20	0.19	14.74	6.03	5.16
4	1	2	2.95	0.74	9.80	89.74	0.15	13.00	5.79	4.59
4	1	2	2.94	0.74	9.80	89.69	0.17	13.01	5.85	4.60
4	2	1	3.01	0.65	9.80	90.09	0.11	13.85	5.14	4.48
4	2	1	3.03	0.64	9.80	90.13	0.11	13.83	5.11	4.49
4	2	2	2.93	0.75	9.70	90.12	0.06	12.46	4.83	3.82
4	2	2	2.91	0.75	9.70	90.17	0.09	12.46	4.79	3.83

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Storage time: 1 = Week 1, 2 = Week 2.

Table C.5 Raw data in Phase 4

Product treatment	Storage time	Replication	pH	Acetic acid (%)	TTS (ºBrix)	Colour (L)	Colour (a)	Colour (b)	Yeast (log CFU/ml)	Acetic acid bacteria (log CFU/ml)
22/7	0	1	3.13	0.52	7.30	83.68	0.55	16.46	7.19	6.22
22/7	0	1	3.12	0.52	7.30	83.52	0.54	16.51	7.20	6.24
22/7	0	2	3.10	0.54	7.50	88.06	0.45	15.09	6.89	5.96
22/7	0	2	3.11	0.54	7.50	88.03	0.41	15.11	6.87	5.91
22/7	1	1	3.05	0.56	7.10	85.69	0.48	14.72	6.72	5.83
22/7	1	1	3.06	0.56	7.10	85.08	0.48	14.76	6.93	5.81
22/7	1	2	3.01	0.62	7.20	88.44	0.33	13.62	6.76	5.64
22/7	1	2	3.01	0.62	7.20	88.57	0.36	13.04	6.62	5.72
22/7	2	1	3.07	0.58	7.00	85.54	0.44	14.52	6.36	5.37
22/7	2	1	3.04	0.58	7.00	85.48	0.41	14.50	6.39	5.38
22/7	2	2	3.00	0.63	7.00	88.67	0.22	13.11	6.54	5.60
22/7	2	2	3.02	0.63	7.00	88.69	0.22	13.11	6.70	5.60
22/7	3	1	3.05	0.59	6.90	86.10	0.42	13.77	6.22	5.18
22/7	3	1	3.07	0.59	6.90	86.13	0.42	13.79	6.09	5.16
22/7	3	2	3.01	0.64	6.90	90.47	0.21	12.91	6.16	5.18
22/7	3	2	2.99	0.64	6.90	90.47	0.22	12.90	6.11	5.16
22/7	4	1	3.07	0.60	6.80	87.09	0.35	13.52	5.34	5.05
22/7	4	1	3.08	0.60	6.80	87.16	0.34	13.52	5.06	4.90
22/7	4	2	3.00	0.63	6.80	91.21	0.19	12.73	5.67	5.11
22/7	4	2	2.98	0.64	6.80	91.20	0.18	12.73	5.74	5.08

Note: Storage time: 1 = Week 1, 2 Week 2, 3 = Week 3, 4 = Week 4.

Panellist	Product code	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Panellist	Product code	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	1	7	7	7	7	8	8	31	1	7	7	7	7	7	7
2	1	7	6	7	7	4	7	32	1	8	6	6	5	6	7
3	1	9	6	8	8	8	8	33	1	6	6	7	8	6	6
4	1	6	6	7	8	6	7	34	1	4	7	7	6	4	6
5	1	7	6	7	6	4	7	35	1	5	4	8	8	8	8
6	1	6	8	8	8	7	7	36	1	7	7	6	6	4	7
7	1	6	5	5	5	5	6	37	1	9	7	7	5	7	8
8	1	6	6	6	6	6	6	38	1	6	8	7	7	7	7
9	1	5	8	4	5	4	5	39	1	7	6	7	7	4	7
10	1	6	8	8	7	7	7	40	1	7	5	4	6	6	5
11	1	8	5	8	8	8	8	41	1	6	8	8	8	7	8
12	1	6	9	8	6	6	7	42	1	9	8	8	9	8	8
13	1	7	7	4	6	6	4	43	1	6	6	6	6	6	6
14	1	5	7	7	6	4	6	44	1	5	3	8	8	7	7
15	1	7	6	7	7	7	6	45	1	7	7	7	7	8	8
16	1	4	3	8	8	7	7	46	1	6	9	8	6	6	6
17	1	5	4	8	8	8	8	47	1	8	6	8	5	7	9
18	1	9	8	8	9	8	9	48	1	7	4	7	7	7	7
19	1	6	8	8	8	8	8	49	1	7	4	8	7	7	8
20	1	7	4	8	7	7	7	50	1	8	7	8	7	8	7
21	1	8	6	8	5	7	8	51	1	6	8	8	8	8	8
22	1	9	7	7	5	7	8	52	1	6	5	5	5	5	6
23	1	8	6	4	5	6	7	53	1	5	8	5	5	4	5
24	1	7	7	8	8	7	8	54	1	8	4	8	6	7	8
25	1	8	7	9	9	9	9	55	1	7	8	8	5	5	7
26	1	8	4	8	6	7	8	56	1	8	6	8	8	8	8
27	1	8	7	8	6	6	7	57	1	8	5	8	8	8	7
28	1	8	7	8	7	8	6	58	1	7	7	8	8	7	8
29	1	6	8	8	5	5	9	59	1	8	7	7	6	6	7
30	1	7	4	7	7	7	6	60	1	8	7	9	9	9	8

Panellist	Product code	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Panellist	Product code	Appearance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	2	8	4	6	6	6	4	31	2	6	6	7	7	6	6
2	2	8	6	6	5	7	7	32	2	6	8	9	8	8	9
3	2	5	6	6	7	6	6	33	2	6	7	8	4	8	6
4	2	7	7	8	6	4	8	34	2	8	9	7	7	8	8
5	2	8	7	8	4	6	8	35	2	8	4	8	8	7	8
6	2	8	7	9	8	9	9	36	2	6	2	6	7	6	6
7	2	8	6	4	6	5	3	37	2	5	7	7	7	6	7
8	2	6	5	6	7	5	5	38	2	4	8	8	9	6	7
9	2	9	6	5	5	4	4	39	2	6	6	6	6	6	6
10	2	6	7	6	7	4	5	40	2	5	9	8	8	8	8
11	2	9	6	7	5	5	6	41	2	6	6	5	6	4	7
12	2	8	7	7	7	6	7	42	2	8	6	5	5	4	4
13	2	6	7	4	6	6	6	43	2	7	7	7	5	6	7
14	2	7	5	5	9	4	4	44	2	7	5	5	7	5	5
15	2	8	5	5	7	8	7	45	2	6	6	6	8	4	7
16	2	6	6	8	7	6	7	46	2	8	8	7	6	8	8
17	2	8	7	7	6	8	8	47	2	9	4	6	6	5	5
18	2	5	6	7	5	6	7	48	2	8	7	9	8	9	9
19	2	7	5	6	7	6	6	49	2	8	7	7	6	7	7
20	2	6	6	7	7	6	7	50	2	9	6	7	7	5	6
21	2	6	6	7	6	4	7	51	2	6	7	6	6	6	6
22	2	9	3	8	8	8	8	52	2	5	6	7	5	6	6
23	2	3	8	8	9	6	7	53	2	7	6	6	5	7	6
24	2	5	9	8	8	8	8	54	2	8	6	4	7	7	7
25	2	7	7	8	5	6	7	55	2	7	6	8	6	6	7
26	2	7	7	6	8	3	6	56	2	8	7	6	7	4	5
27	2	8	8	9	8	8	9	57	2	8	6	6	6	4	5
28	2	5	6	6	8	7	6	58	2	8	7	8	6	5	6
29	2	8	7	7	6	6	7	59	2	8	5	5	9	4	6
30	2	6	8	7	7	8	8	60	2	6	6	7	7	6	7

Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	3	6	8	6	7	4	6	1	3	8	4	4	4	3	4
2	3	6	6	7	8	7	8	2	3	4	8	7	7	8	7
3	3	8	4	6	6	2	4	3	3	5	6	5	5	7	7
4	3	6	7	8	7	7	7	4	3	9	8	5	5	4	6
5	3	7	8	7	5	6	6	5	3	7	6	8	7	8	8
6	3	8	8	9	8	8	9	6	3	6	6	7	4	7	6
7	3	8	7	7	6	7	7	7	3	4	8	8	8	6	7
8	3	5	6	7	5	6	6	8	3	8	6	7	7	6	7
9	3	7	8	7	7	7	8	9	3	7	5	5	5	4	6
10	3	8	8	7	8	8	7	10	3	9	6	7	7	7	7
11	3	7	6	7	8	6	8	11	3	5	7	6	6	5	6
12	3	8	8	7	6	7	7	12	3	6	3	7	7	7	7
13	3	4	4	6	6	3	6	13	3	8	5	6	8	5	6
14	3	7	5	7	7	5	7	14	3	9	7	7	7	6	8
15	3	5	8	8	7	8	8	15	3	8	6	7	5	8	7
16	3	4	5	6	6	5	6	16	3	7	7	7	8	5	7
17	3	8	5	7	6	6	6	17	3	5	7	6	8	3	6
18	3	8	5	6	6	6	6	18	3	9	8	7	6	7	7
19	3	8	7	8	8	8	9	19	3	5	3	5	6	3	4
20	3	6	6	8	8	7	8	20	3	7	6	7	8	8	7
21	3	8	6	5	6	5	6	21	3	6	7	3	2	4	4
22	3	8	6	5	3	6	6	22	3	8	8	7	8	8	8
23	3	5	4	5	6	5	5	23	3	9	8	8	8	6	9
24	3	7	5	3	5	5	4	24	3	7	6	8	7	8	8
25	3	8	1	3	5	4	3	25	3	7	7	8	7	7	8
26	3	5	7	7	7	8	7	26	3	6	3	5	4	5	5
27	3	8	3	6	6	6	6	27	3	7	2	5	6	2	2
28	3	6	7	6	7	6	7	28	3	3	5	7	7	8	7
29	3	8	6	7	7	6	7	29	3	7	7	7	8	7	7
30	3	7	7	6	6	6	6	30	3	8	6	8	8	8	8

Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	4	6	5	6	5	4	5	31	4	5	4	5	6	4	4
2	4	5	5	4	5	5	5	32	4	7	6	6	7	5	6
3	4	6	6	7	7	2	2	33	4	6	7	7	7	6	6
4	4	7	7	5	6	5	5	34	4	8	4	6	6	6	6
5	4	6	5	4	5	6	4	35	4	7	6	6	6	5	6
6	4	7	4	5	4	2	3	36	4	5	5	5	6	5	5
7	4	9	7	7	2	7	6	37	4	9	6	7	7	6	7
8	4	7	6	7	6	6	6	38	4	4	4	5	5	4	4
9	4	8	5	7	3	5	5	39	4	6	7	8	8	7	7
10	4	7	6	7	7	8	7	40	4	7	6	6	5	4	5
11	4	2	5	5	5	5	5	41	4	8	8	8	6	7	7
12	4	5	6	6	8	5	6	42	4	3	3	5	4	2	3
13	4	5	5	7	5	3	4	43	4	6	5	5	5	5	5
14	4	7	6	9	4	5	5	44	4	6	8	7	6	4	6
15	4	7	5	6	6	5	6	45	4	7	6	6	7	6	6
16	4	7	4	7	8	5	7	46	4	8	7	7	8	6	7
17	4	5	4	6	8	6	6	47	4	5	7	5	4	4	4
18	4	6	5	6	6	6	6	48	4	8	5	6	5	5	5
19	4	7	6	6	5	8	7	49	4	7	4	5	5	5	5
20	4	4	5	6	6	7	6	50	4	9	7	7	7	4	6
21	4	5	5	6	7	5	4	51	4	8	3	4	4	3	3
22	4	7	8	7	8	5	6	52	4	6	5	6	6	5	5
23	4	8	6	6	7	5	6	53	4	7	7	7	6	7	7
24	4	9	5	7	7	5	7	54	4	4	6	6	6	7	6
25	4	8	6	5	6	4	6	55	4	3	5	6	6	6	6
26	4	8	8	8	6	8	8	56	4	7	6	6	7	5	5
27	4	7	7	5	6	5	5	57	4	6	6	7	6	6	6
28	4	6	7	6	7	5	5	58	4	8	7	8	6	7	6
29	4	6	6	6	5	6	6	59	4	7	6	7	6	7	6
30	4	7	6	6	8	6	6	60	4	7	5	6	5	6	5

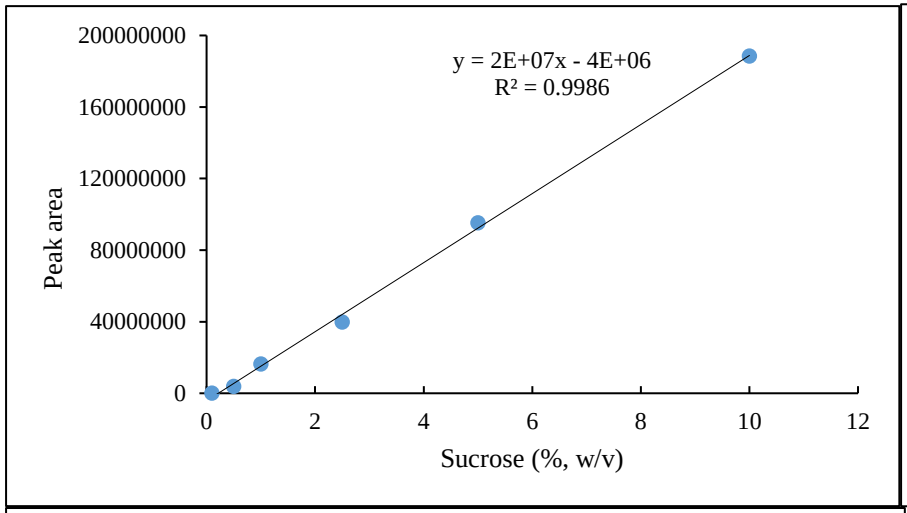
Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability	Panellist	Product code	Apperance	Aroma	Flavour	Sweetness	Sourness	Overall acceptability
1	5	6	6	6	5	4	5	31	5	3	2	4	3	4	3
2	5	8	7	6	6	4	6	32	5	6	4	5	7	4	5
3	5	6	6	6	7	2	3	33	5	9	6	6	6	5	6
4	5	4	8	4	5	4	4	34	5	4	5	6	5	7	6
5	5	2	4	3	3	3	3	35	5	2	3	6	7	6	5
6	5	5	4	8	7	7	7	36	5	7	5	6	6	5	5
7	5	8	9	6	6	4	6	37	5	8	6	7	7	4	6
8	5	7	2	6	4	3	4	38	5	6	1	3	3	2	2
9	5	5	6	6	7	4	6	39	5	5	5	7	6	7	6
10	5	8	6	7	7	8	7	40	5	8	7	8	8	7	8
11	5	6	5	5	5	4	5	41	5	5	6	5	6	6	6
12	5	7	3	8	4	4	4	42	5	7	5	5	5	4	5
13	5	6	4	6	7	7	7	43	5	7	3	4	3	5	3
14	5	7	4	6	6	7	6	44	5	8	7	6	4	7	5
15	5	8	6	4	5	5	6	45	5	6	6	7	6	6	6
16	5	8	9	9	7	7	7	46	5	5	6	5	7	4	5
17	5	6	2	6	7	6	5	47	5	6	7	7	7	6	6
18	5	2	1	5	5	4	3	48	5	6	5	6	6	6	6
19	5	5	4	4	6	5	4	49	5	5	5	6	5	5	5
20	5	8	7	4	5	4	4	50	5	7	5	5	3	5	4
21	5	9	1	6	7	6	5	51	5	7	6	5	7	4	7
22	5	6	4	4	5	6	6	52	5	8	3	4	3	4	4
23	5	6	4	7	8	8	8	53	5	6	5	6	7	5	6
24	5	7	8	8	6	7	6	54	5	7	3	7	7	6	7
25	5	4	6	6	6	6	6	55	5	4	2	5	6	5	5
26	5	7	5	5	5	5	6	56	5	9	8	8	8	6	7
27	5	7	5	5	5	4	5	57	5	6	5	6	7	4	6
28	5	7	3	3	4	2	4	58	5	8	5	5	4	6	5
29	5	7	3	4	3	2	3	59	5	7	6	6	6	3	5
30	5	6	5	8	7	7	7	60	5	6	5	5	5	1	3

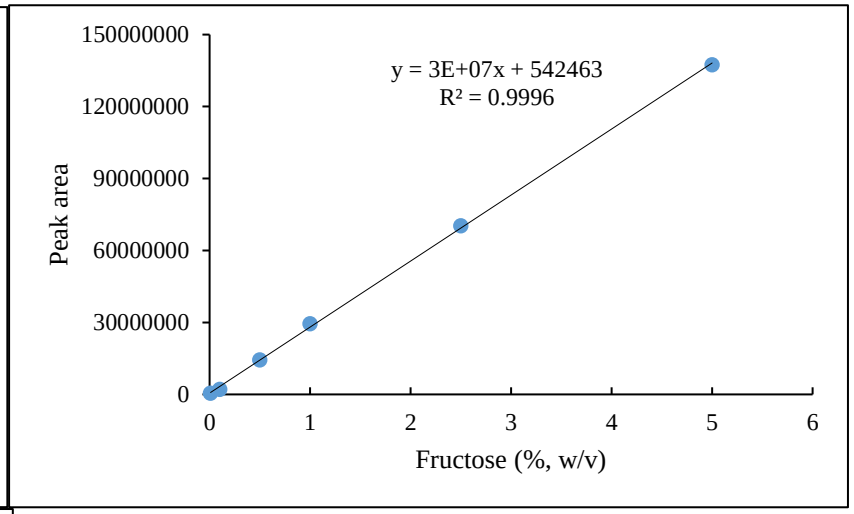
Note: Product code: 1 = fresh green tea kombucha; 2 = green tea kombucha stored at 4℃ for 1 week; 3 = green tea kombucha stored at 4℃ for 3 weeks; 4 = green tea kombucha stored at 4℃ for 3 weeks; 5 = green tea kombucha stored at 4℃ for 4 week.

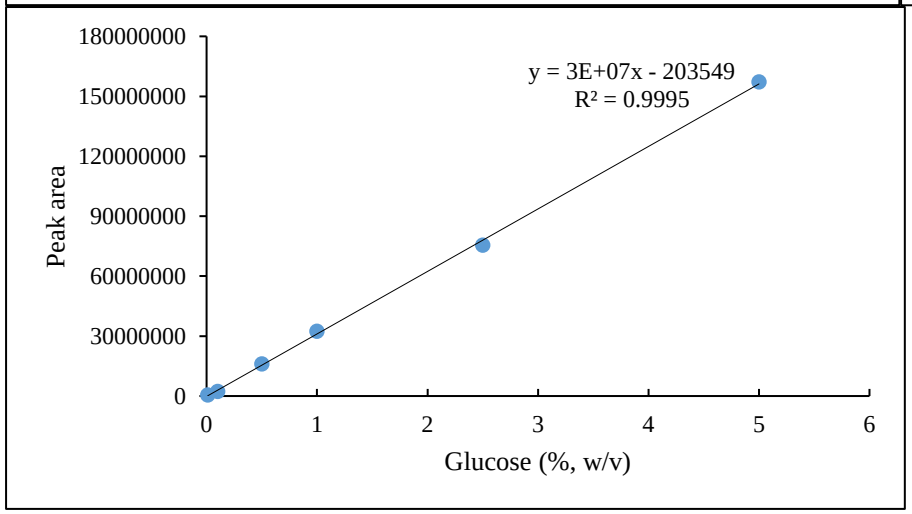
D. HPLC data

D.1 HPLC standard curve

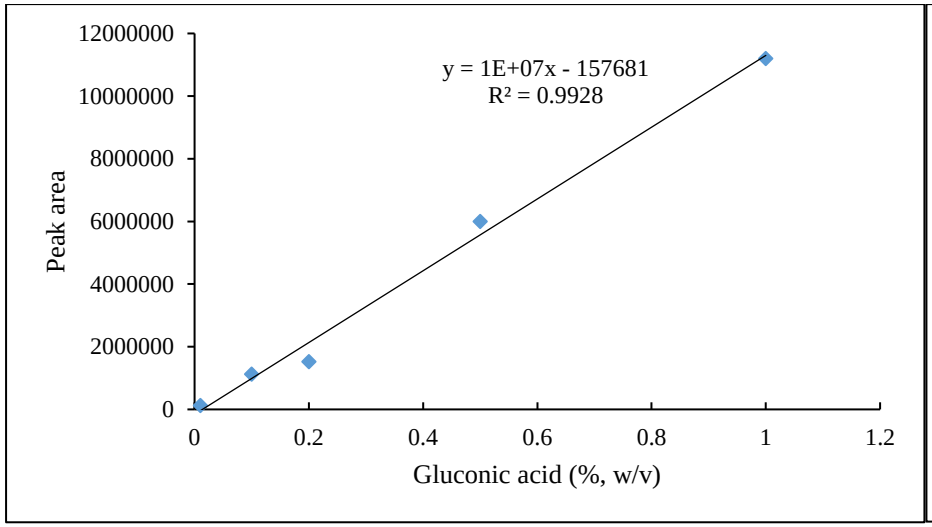
D1.1 Sugars

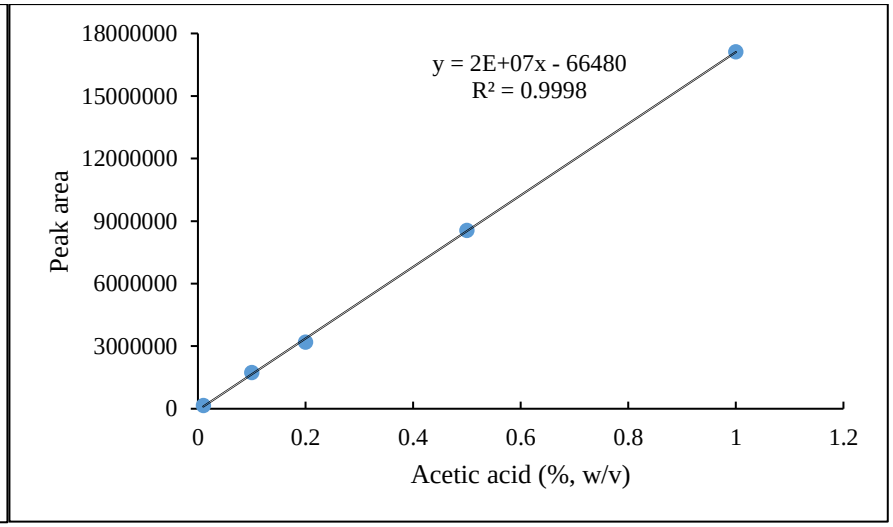




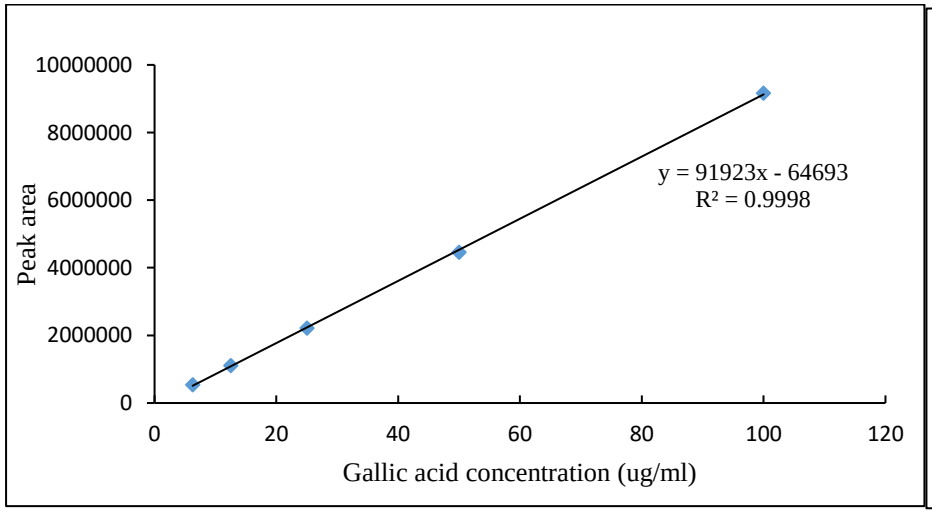


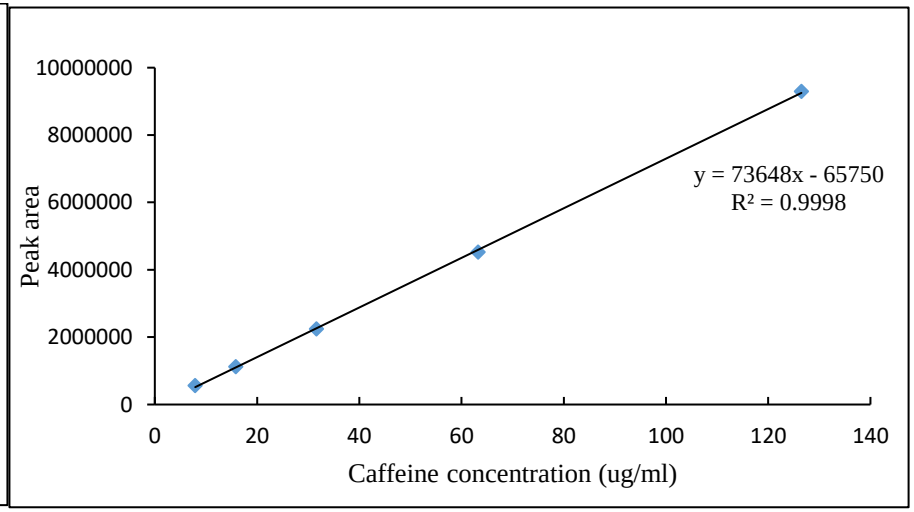
D1.2 Organic acids

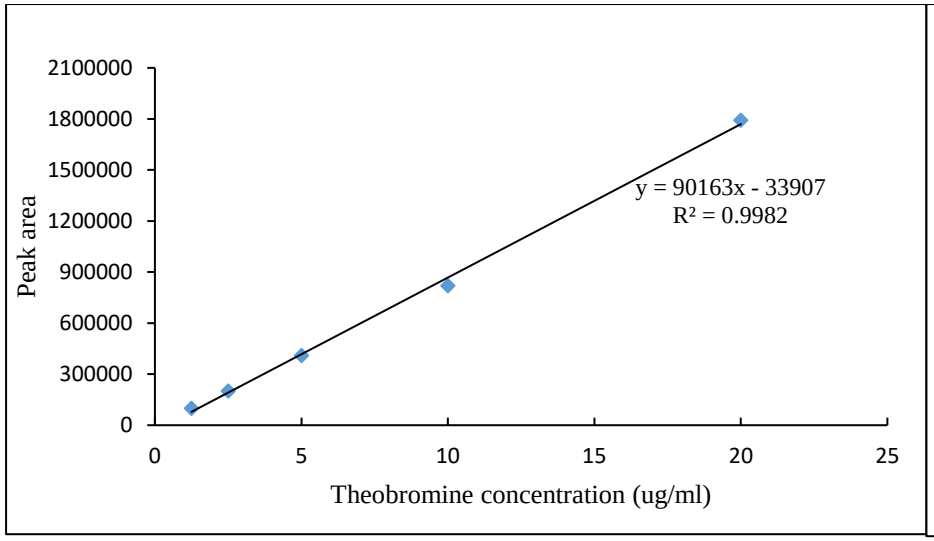


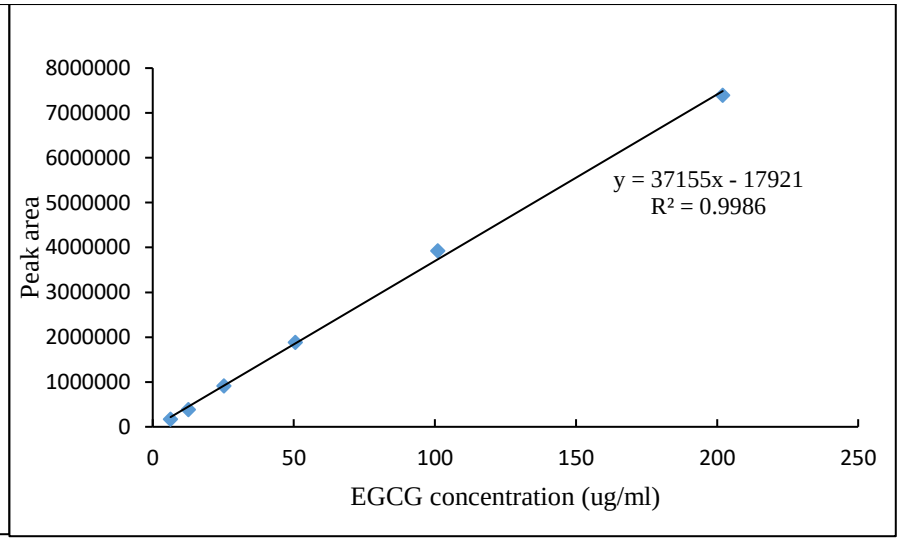


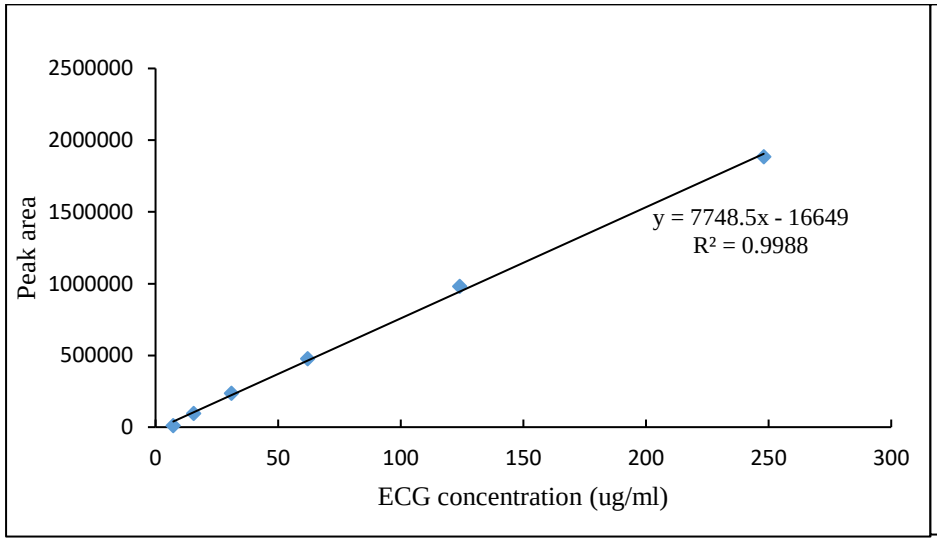
D1.3 Antioxidants






Peak area
2100000
1800000
1500000
1200000
900000
600000
300000
0
0
5
10
15
20
25
Theobromine concentration (ug/ml)
y = 90163x - 33907
R² = 0.9982


Peak area
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
50
100
150
200
250
EGCG concentration (ug/ml)
y = 37155x - 17921
R² = 0.9986


Peak area
2500000
2000000
1500000
1000000
500000
0
0
50
100
150
200
250
300
ECG concentration (ug/ml)
y = 7748.5x - 16649
R² = 0.9988

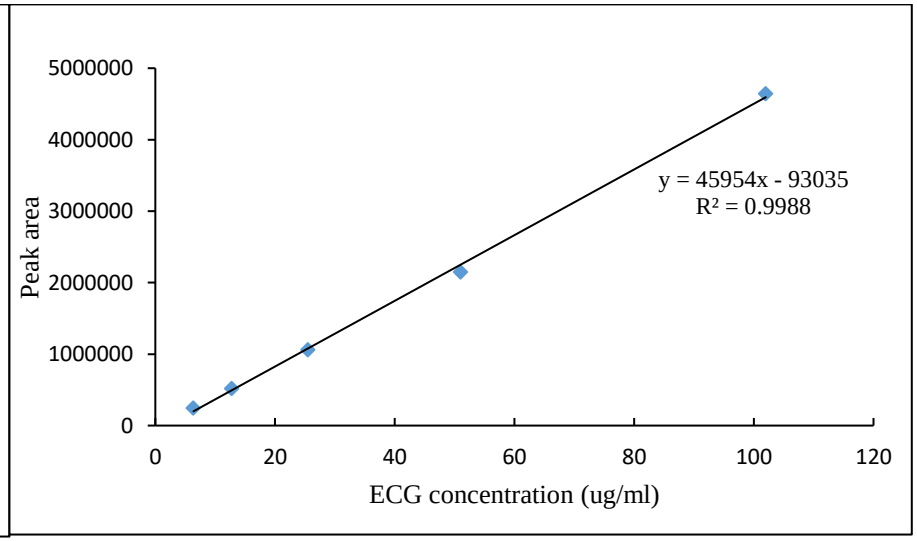

Peak area
5000000
4000000
3000000
2000000
1000000
0
0
20
40
60
80
100
120
ECG concentration (ug/ml)
y = 45954x - 93035
R² = 0.9988

Table D.1 HPLC raw data of sugars in Phase 3 during fermentation for 7 days

Product treatment	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
1	1	1	159871648	8.19	27667468	0.93	15233576	0.49
1	1	1	160011819	8.20	26976969	0.91	15155498	0.49
1	1	2	144873057	7.44	18384308	0.62	8276445	0.26
1	1	2	145509062	7.48	18210335	0.61	8471406	0.26
1	2	1	150362795	7.72	34569056	1.16	17977776	0.58
1	2	1	147796347	7.59	34016344	1.14	17712544	0.57
1	2	2	133572557	6.88	18881882	0.64	10103253	0.32
1	2	2	133743436	6.89	18881124	0.64	10064480	0.32
1	3	1	167605356	8.58	28276567	0.95	9858858	0.31
1	3	1	167762374	8.59	28131378	0.94	9854198	0.31
1	3	2	149995479	7.70	17692398	0.60	4100538	0.12
1	3	2	149422565	7.67	17668608	0.60	4068411	0.12
1	4	1	161233069	8.26	29840344	1.00	13184145	0.42
1	4	1	160952005	8.25	29902250	1.00	13173449	0.42
1	4	2	136614121	7.03	21190626	0.71	8969680	0.28
1	4	2	136497382	7.02	21776405	0.73	8425020	0.26
1	5	1	176405326	9.02	14223636	0.48	6339584	0.19
1	5	1	176273032	9.01	14213515	0.48	6340549	0.19
1	5	2	169226598	8.66	11910137	0.40	4238665	0.12
1	5	2	165148702	8.46	11918422	0.40	4235220	0.12
1	6	1	128552709	6.63	19021363	0.64	7675661	0.24
1	6	1	128555446	6.63	18928754	0.64	7672895	0.24
1	6	2	100675661	5.23	17915118	0.60	9638226	0.30
1	6	2	100716478	5.24	17741710	0.60	9650232	0.30
1	7	1	107273443	5.56	20705332	0.70	12621320	0.40
1	7	1	106150923	5.51	20551544	0.69	12504870	0.40
1	7	2	85446040	4.47	21427482	0.72	14667138	0.47
1	7	2	85507158	4.48	21240493	0.71	14675373	0.47

Product treatment	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
2	1	1	178098679	9.10	32272950	1.08	15081986	0.48
2	1	1	178184285	9.11	32296612	1.08	15260887	0.49
2	1	2	164472207	8.42	19992736	0.67	9013378	0.28
2	1	2	164391709	8.42	20000327	0.67	9195338	0.29
2	2	1	162414391	8.32	33638024	1.13	17707397	0.57
2	2	1	162755389	8.34	33771078	1.13	17786162	0.57
2	2	2	165299255	8.46	21795023	0.73	12885180	0.41
2	2	2	165814412	8.49	21745988	0.73	13003971	0.42
2	3	1	201002672	10.25	29066023	0.98	10554900	0.33
2	3	1	200520688	10.23	29174354	0.98	10572161	0.33
2	3	2	202983395	10.35	20826219	0.70	7932946	0.25
2	3	2	202592506	10.33	20661158	0.70	7806010	0.24
2	4	1	184314713	9.42	33396237	1.12	14242296	0.46
2	4	1	185794135	9.49	33668565	1.13	13310898	0.43
2	4	2	182451520	9.32	22839667	0.77	10913753	0.35
2	4	2	182343824	9.32	22883699	0.77	10934321	0.35
2	5	1	215346804	10.97	17010213	0.57	7199317	0.22
2	5	1	212002357	10.80	17188429	0.58	7102407	0.22
2	5	2	205782566	10.49	13121272	0.44	5028067	0.15
2	5	2	204819413	10.44	13043720	0.44	5046379	0.15
2	6	1	164631021	8.43	20713540	0.70	10739774	0.34
2	6	1	164925320	8.45	20708693	0.70	10788198	0.34
2	6	2	173964551	8.90	24434469	0.82	12989584	0.41
2	6	2	173909219	8.90	24797530	0.83	13019690	0.42
2	7	1	152456970	7.82	27929343	0.94	23052049	0.75
2	7	1	152495067	7.82	27929983	0.94	23181715	0.75
2	7	2	139702368	7.19	30263642	1.02	25537297	0.83
2	7	2	139435064	7.17	30854925	1.04	25541326	0.83

Product treatment	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
3	1	1	157960945	8.10	28248744	0.95	17860896	0.58
3	1	1	158636027	8.13	28443537	0.95	17993971	0.58
3	1	2	148416973	7.62	17216883	0.58	9724437	0.31
3	1	2	148183340	7.61	17154258	0.58	9718256	0.31
3	2	1	142882773	7.34	34381828	1.15	22605950	0.74
3	2	1	141609675	7.28	34137416	1.14	22242590	0.72
3	2	2	132917039	6.85	18333326	0.62	12578621	0.40
3	2	2	133433030	6.87	18612460	0.63	12781007	0.41
3	3	1	166814325	8.54	29834993	1.04	14506765	0.47
3	3	1	166491179	8.52	29759633	1.03	14406704	0.46
3	3	2	154942280	7.95	17256712	0.58	6874192	0.21
3	3	2	153668588	7.88	17107272	0.58	6870596	0.21
3	4	1	159075058	8.15	30893327	1.00	16595374	0.54
3	4	1	158553264	8.13	30640357	1.00	16569210	0.53
3	4	2	139989931	7.20	21307722	0.72	8873833	0.28
3	4	2	139913626	7.20	21386128	0.72	8853234	0.28
3	5	1	173864518	8.89	16741145	0.56	7663346	0.24
3	5	1	173682838	8.88	16651492	0.56	7668704	0.24
3	5	2	158269948	8.11	11929030	0.40	7963897	0.25
3	5	2	158444157	8.12	11932504	0.40	7981818	0.25
3	6	1	99311147	5.17	24123664	0.81	19945970	0.65
3	6	1	99375452	5.17	24119459	0.81	19935737	0.65
3	6	2	88144473	4.61	24846627	0.84	26548317	0.87
3	6	2	86309075	4.52	24733662	0.83	25797096	0.84
3	7	1	83863041	4.39	34616645	1.16	35424140	1.16
3	7	1	84044497	4.40	34847477	1.17	35541071	1.17
3	7	2	69614250	3.68	34497849	1.16	40416412	1.33
3	7	2	69552107	3.68	34535715	1.16	40369259	1.33

Product treatment	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
4	1	1	177536635	9.08	32405305	1.09	17363732	0.56
4	1	1	177652098	9.08	32406908	1.09	17421179	0.56
4	1	2	162075216	8.30	19313898	0.65	6287268	0.19
4	1	2	161443983	8.27	19105765	0.64	6245670	0.19
4	2	1	162847650	8.34	36430048	1.22	24113177	0.79
4	2	1	162780290	8.34	36499302	1.22	24104171	0.79
4	2	2	155583551	7.98	21831989	0.73	12150591	0.39
4	2	2	155900662	8.00	21913483	0.74	12101547	0.39
4	3	1	194836319	9.94	31670956	1.06	14891185	0.48
4	3	1	195720757	9.99	31486296	1.06	15246904	0.49
4	3	2	194260205	9.91	19590538	0.66	6605933	0.20
4	3	2	194022690	9.90	20570332	0.69	6554945	0.20
4	4	1	188280998	9.61	33791931	1.13	19858683	0.64
4	4	1	185257808	9.46	33035909	1.11	19535021	0.63
4	4	2	177429703	9.07	25460319	0.86	15448605	0.50
4	4	2	177193998	9.06	25579792	0.86	15414345	0.50
4	5	1	214482107	10.92	17982525	0.61	9381716	0.29
4	5	1	213652625	10.88	17930249	0.60	9069389	0.28
4	5	2	205390407	10.47	14032270	0.47	6830132	0.21
4	5	2	205898096	10.49	13937129	0.47	6852889	0.21
4	6	1	142992721	7.35	30735469	1.03	23582421	0.77
4	6	1	143186282	7.36	30820740	1.03	23653480	0.77
4	6	2	136760225	7.04	28350439	0.95	28032688	0.92
4	6	2	136735336	7.04	28338705	0.95	28091105	0.92
4	7	1	130654973	6.73	39533753	1.32	41830622	1.38
4	7	1	130751584	6.74	39153950	1.31	41017335	1.35
4	7	2	120602878	6.23	41996907	1.41	46892797	1.55
4	7	2	120751941	6.24	42061300	1.41	47069581	1.55

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Fermentation time: 1 = Day 0, 2 = Day 1-BA, 3 = Day 1-AA, 4 = Day 2-BA, 5 = Day 2-AA; 6 = Day 5, 7 = Day 7.

Table D.2 HPLC raw data of sugars in Phase 3 during storage for 2 weeks

Product treatment	Storage time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
1	1	1	89621351	4.68	21300986	0.72	16977746	0.55
1	1	1	89757372	4.69	21677215	0.73	16948743	0.55
1	1	2	70661258	3.73	23276462	0.78	21940572	0.71
1	1	2	70589433	3.73	23279637	0.78	21958869	0.71
1	2	1	74032923	3.90	22109343	0.74	18180074	0.59
1	2	1	74048085	3.90	22119650	0.74	18195464	0.59
1	2	2	58316092	3.12	25194208	0.85	23273088	0.76
1	2	2	59524535	3.18	25080868	0.84	23715771	0.77
2	1	1	131283506	6.76	30844527	1.03	26437864	0.86
2	1	1	134652966	6.93	30901572	1.04	27135380	0.89
2	1	2	122155809	6.31	33246128	1.11	27884353	0.91
2	1	2	122227321	6.31	33197387	1.11	27838814	0.91
2	2	1	117714292	6.09	33619680	1.13	30734163	1.01
2	2	1	117480546	6.07	33595122	1.13	30726493	1.01
2	2	2	109269673	5.66	34538635	1.16	31128909	1.02
2	2	2	109293763	5.66	34546439	1.16	31203220	1.02
3	1	1	74665586	3.93	36510729	1.22	38775776	1.27
3	1	1	75517414	3.98	36530079	1.22	38807070	1.28
3	1	2	59555206	3.18	37317875	1.25	42580306	1.40
3	1	2	59571073	3.18	37337398	1.25	42626361	1.40
3	2	1	67270120	3.56	38672122	1.30	39584136	1.30
3	2	1	67213321	3.56	38668007	1.30	39611274	1.30
3	2	2	51316655	2.77	39221653	1.31	44418397	1.46
3	2	2	51282047	2.76	39194657	1.31	44543537	1.47
4	1	1	115337031	5.97	43679256	1.46	43862566	1.44
4	1	1	115373522	5.97	43736813	1.46	43974735	1.45
4	1	2	109153895	5.66	45480241	1.52	47708168	1.57
4	1	2	109252697	5.66	45111252	1.51	47776561	1.57
4	2	1	106025421	5.50	45121961	1.51	45804281	1.51
4	2	1	106223305	5.51	45189617	1.51	45431767	1.50
4	2	2	100968214	5.25	46018868	1.54	50320955	1.66
4	2	2	100817301	5.24	46974073	1.57	50180257	1.65

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Storage time: 1 = Week 1, 2 = Week 2.

Table D.3 HPLC raw data of sugars in Phase 4

Product treatment	Storage time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
22/7	1	1	83741129	4.39	17852989	0.60	12121709	0.39
22/7	1	1	83713791	4.39	17848211	0.60	12120498	0.39
22/7	1	2	89010883	4.65	21919893	0.74	15338755	0.49
22/7	1	2	88978734	4.65	21927339	0.74	15334053	0.49
22/7	2	1	66181930	3.51	26799024	0.90	21586840	0.70
22/7	2	1	66176646	3.51	26792598	0.90	21549142	0.70
22/7	2	2	70584924	3.73	29631316	0.99	22436580	0.73
22/7	2	2	70591632	3.73	29761790	1.00	22539842	0.73
22/7	3	1	58358921	3.12	30947143	1.04	24835262	0.81
22/7	3	1	58317684	3.12	30990903	1.04	24823951	0.81
22/7	3	2	60022111	3.20	32963321	1.11	29469376	0.96
22/7	3	2	60951331	3.25	32022916	1.07	29481030	0.96
22/7	4	1	52194639	2.81	31934248	1.07	27444133	0.90
22/7	4	1	52792922	2.84	31211332	1.05	25557400	0.83
22/7	4	2	54503034	2.93	32030960	1.07	30961900	1.01
22/7	4	2	54477007	2.92	32907327	1.10	30875739	1.01
22/7	5	1	50480617	2.72	37546044	1.26	35958246	1.18
22/7	5	1	50455186	2.72	37455689	1.26	35935954	1.18
22/7	5	2	51729532	2.79	52439056	1.75	50036207	1.65
22/7	5	2	51681228	2.78	52373284	1.75	50937649	1.68

Note: Storage time: 1 = Week 1, 2 Week 2, 3 = Week 3, 4 = Week 4.

Table D.4 HPLC raw data of organic acids in Phase 3 during fermentation for 7 days

Product treatment	Fermentation time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
1	1	1	1677008	0.18	3295696	0.17
1	1	1	1660571	0.18	3215240	0.16
1	1	2	931511	0.11	3211961	0.16
1	1	2	816392	0.10	3270933	0.17
1	2	1	2029353	0.22	4170091	0.21
1	2	1	2068782	0.22	4109090	0.21
1	2	2	1298050	0.15	2246704	0.12
1	2	2	1205186	0.14	2281752	0.12
1	3	1	909625	0.11	2044681	0.11
1	3	1	890378	0.10	1955600	0.10
1	3	2	654055	0.08	2322683	0.12
1	3	2	490199	0.06	1915792	0.10
1	4	1	1561695	0.17	2578656	0.13
1	4	1	1315174	0.15	2009442	0.10
1	4	2	866771	0.10	2780651	0.14
1	4	2	807688	0.10	2742774	0.14
1	5	1	399641	0.06	1020134	0.05
1	5	1	509185	0.07	980927	0.05
1	5	2	286405	0.04	1269617	0.07
1	5	2	271330	0.04	1285186	0.07
1	6	1	1144399	0.13	3253748	0.17
1	6	1	1129628	0.13	3291245	0.17
1	6	2	865170	0.10	2865955	0.15
1	6	2	886774	0.10	2856689	0.15
1	7	1	2571872	0.27	5749162	0.29
1	7	1	2648541	0.28	5587454	0.28
1	7	2	2245399	0.24	5565926	0.28
1	7	2	2165815	0.23	5556654	0.28

Product treatment	Fermentation time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
2	1	1	1706616	0.19	3330417	0.17
2	1	1	1630291	0.18	3203514	0.16
2	1	2	914802	0.11	3491369	0.18
2	1	2	860456	0.10	3131403	0.16
2	2	1	2077858	0.22	4074294	0.21
2	2	1	2037335	0.22	3448626	0.18
2	2	2	1665353	0.18	3963292	0.20
2	2	2	1562944	0.17	3721849	0.19
2	3	1	930097	0.11	2068445	0.11
2	3	1	946644	0.11	2041266	0.11
2	3	2	528093	0.07	1769530	0.09
2	3	2	516278	0.07	1827823	0.09
2	4	1	1542567	0.17	2594205	0.13
2	4	1	1420061	0.16	2339954	0.12
2	4	2	879476	0.10	2939703	0.15
2	4	2	779796	0.09	2640267	0.14
2	5	1	387975	0.05	1044223	0.06
2	5	1	443630	0.06	1003023	0.05
2	5	2	304321	0.05	1803506	0.09
2	5	2	458303	0.06	1820416	0.09
2	6	1	1111540	0.13	2644313	0.14
2	6	1	1084826	0.12	2581573	0.13
2	6	2	1682985	0.18	3691892	0.19
2	6	2	1686148	0.18	3549836	0.18
2	7	1	3110351	0.33	5626505	0.28
2	7	1	2815790	0.30	5610781	0.28
2	7	2	2513522	0.27	5739585	0.29
2	7	2	2491253	0.26	5832521	0.29

Product treatment	Fermentation time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
3	1	1	1673391	0.18	3119898	0.16
3	1	1	1728701	0.19	2947468	0.15
3	1	2	920722	0.11	3549037	0.18
3	1	2	873860	0.10	3444677	0.18
3	2	1	2734662	0.29	4112058	0.21
3	2	1	2687712	0.28	4068589	0.21
3	2	2	1461380	0.16	4959622	0.25
3	2	2	1458287	0.16	4835085	0.25
3	3	1	1298051	0.15	2065463	0.11
3	3	1	1245767	0.14	2037653	0.11
3	3	2	657631	0.08	2538207	0.13
3	3	2	593235	0.08	2414412	0.12
3	4	1	2258120	0.24	3424280	0.17
3	4	1	2260903	0.24	3413988	0.17
3	4	2	1197743	0.14	4476427	0.23
3	4	2	1034824	0.12	4114513	0.21
3	5	1	911143	0.11	1518825	0.08
3	5	1	833388	0.10	1478197	0.08
3	5	2	375785	0.05	2193040	0.11
3	5	2	435025	0.06	2212107	0.11
3	6	1	1701164	0.19	3208047	0.16
3	6	1	1609343	0.18	3020190	0.15
3	6	2	2324832	0.25	4413498	0.22
3	6	2	2337248	0.25	4267474	0.22
3	7	1	3404485	0.36	6822090	0.34
3	7	1	3485311	0.36	6397053	0.32
3	7	2	4706362	0.49	8030877	0.40
3	7	2	4714803	0.49	8419332	0.42

Product treatment	Fermentation time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
4	1	1	1722088	0.19	3212341	0.16
4	1	1	1673223	0.18	3142416	0.16
4	1	2	978450	0.11	3549037	0.18
4	1	2	857152	0.10	3444677	0.18
4	2	1	2876628	0.30	4183533	0.21
4	2	1	2837767	0.30	4089204	0.21
4	2	2	1335448	0.15	5310458	0.27
4	2	2	1326350	0.15	5127019	0.26
4	3	1	1186764	0.13	1881609	0.10
4	3	1	1165785	0.13	2095937	0.11
4	3	2	620799	0.08	2875304	0.15
4	3	2	551423	0.07	2792924	0.14
4	4	1	2219386	0.24	3767732	0.19
4	4	1	2096587	0.23	3579876	0.18
4	4	2	1230868	0.14	4629400	0.23
4	4	2	1164350	0.13	4510667	0.23
4	5	1	1088904	0.12	1539958	0.08
4	5	1	1066310	0.12	1372283	0.07
4	5	2	239013	0.04	2006893	0.10
4	5	2	350201	0.05	2083246	0.11
4	6	1	1988524	0.21	3769609	0.19
4	6	1	2036171	0.22	3696824	0.19
4	6	2	2679244	0.28	3714874	0.19
4	6	2	2672446	0.28	3538979	0.18
4	7	1	3784836	0.39	7253264	0.37
4	7	1	3826789	0.40	7021369	0.35
4	7	2	5870807	0.60	8625113	0.43
4	7	2	5924555	0.61	8468704	0.43

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Fermentation time: 1 = Day 0, 2 = Day 1-BA, 3 = Day 1-AA, 4 = Day 2-BA, 5 = Day 2-AA; 6 = Day 5, 7 = Day 7.

Table D.5 HPLC raw data of organic acids in Phase 3 during storage for 2 weeks

Product treatment	Storage time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
1	1	1	3367734	0.35	5937491	0.30
1	1	1	3273201	0.34	5966145	0.30
1	1	2	3015283	0.32	7028689	0.35
1	1	2	2973113	0.31	7082275	0.36
1	2	1	3437843	0.36	6017803	0.30
1	2	1	3448581	0.36	6042449	0.31
1	2	2	3085378	0.32	7271415	0.37
1	2	2	3077200	0.32	7244877	0.37
2	1	1	3553125	0.37	5945017	0.30
2	1	1	3505632	0.37	5967106	0.30
2	1	2	3086882	0.32	7400012	0.37
2	1	2	3050891	0.32	7439026	0.38
2	2	1	3682178	0.38	5974398	0.30
2	2	1	3563499	0.37	5872713	0.30
2	2	2	3162266	0.33	7479833	0.38
2	2	2	3172253	0.33	7486392	0.38
3	1	1	3803405	0.40	7954478	0.40
3	1	1	3801093	0.40	7960413	0.40
3	1	2	5920092	0.61	9822862	0.49
3	1	2	5945820	0.61	9857466	0.50
3	2	1	3981239	0.41	7948267	0.40
3	2	1	3974102	0.41	7978151	0.40
3	2	2	6098713	0.63	9884692	0.50
3	2	2	6099266	0.63	9930014	0.50
4	1	1	3954754	0.41	8569317	0.43
4	1	1	3906763	0.41	8524072	0.43
4	1	2	7092382	0.73	9970741	0.50
4	1	2	7020596	0.72	9912168	0.50
4	2	1	4071782	0.42	8564127	0.43
4	2	1	3986240	0.41	8505158	0.43
4	2	2	7059777	0.72	9966118	0.50
4	2	2	7127577	0.73	10154862	0.51

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Storage time: 1 = Week 1, 2 = Week 2.

Table D.6 HPLC raw data of organic acids in Phase 4

Product treatment	Storage time	Replication	Gluconic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
22/7	1	1	3076168	0.32	6146568	0.31
22/7	1	1	3077449	0.32	6140347	0.31
22/7	1	2	3608968	0.38	6202210	0.31
22/7	1	2	3645451	0.38	5996126	0.30
22/7	2	1	3677903	0.38	6390550	0.32
22/7	2	1	3621739	0.38	6305405	0.32
22/7	2	2	4124069	0.41	7121095	0.36
22/7	2	2	4102570	0.43	7135606	0.36
22/7	3	1	3737946	0.39	6553756	0.33
22/7	3	1	3718664	0.39	6551499	0.33
22/7	3	2	4149015	0.43	7444458	0.38
22/7	3	2	4132251	0.43	7462897	0.38
22/7	4	1	3879163	0.40	7097798	0.36
22/7	4	1	3840159	0.40	7111934	0.36
22/7	4	2	4155371	0.43	7417082	0.37
22/7	4	2	4140954	0.43	7479588	0.38
22/7	5	1	3881971	0.40	7125812	0.36
22/7	5	1	3885878	0.40	7188923	0.36
22/7	5	2	4085125	0.42	7425214	0.37
22/7	5	2	4157995	0.43	7491119	0.38

Note: Storage time: 1 = Week 1, 2 Week 2, 3 = Week 3, 4 = Week 4.

Table D.7 HPLC raw data of antioxidants in Phase 4

Product treatment	Storage time	Replication	Gallic acid		Theobromine		EGC	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
22/7	1	1	459201	5.70	575805	6.76	1050589	137.73
22/7	1	1	464775	5.76	576880	6.77	1050828	137.77
22/7	1	2	454334	5.65	515704	6.10	943695	123.94
22/7	1	2	457005	5.68	515138	6.09	945189	124.13
22/7	2	1	372380	4.75	464296	5.53	834688	109.87
22/7	2	1	373365	4.77	465231	5.54	836014	110.04
22/7	2	2	433337	5.42	486082	5.77	867864	114.15
22/7	2	2	434940	5.44	485040	5.76	868279	114.21
22/7	3	1	402671	5.08	491908	5.83	884776	116.34
22/7	3	1	403482	5.09	492253	5.84	885439	116.42
22/7	3	2	485037	5.98	550620	6.48	1030889	135.19
22/7	3	2	484346	5.97	551766	6.50	1029772	135.05
22/7	4	1	521520	6.38	643940	7.52	1089744	142.79
22/7	4	1	521891	6.38	644243	7.52	1087622	142.51
22/7	4	2	545580	6.64	614535	7.19	1131737	148.21
22/7	4	2	542681	6.61	613629	7.18	1128413	147.78
22/7	5	1	525680	6.42	599988	7.03	1019440	133.71
22/7	5	1	526265	6.43	599482	7.02	1020875	133.90
22/7	5	2	541146	6.59	607071	7.11	1086154	142.32
22/7	5	2	549309	6.68	606935	7.11	1089539	142.76

Product treatment	Storage time	Replication	Caffeine		EGCG		ECG	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
22/7	1	1	7782161	106.56	7120253	192.12	2545698	57.42
22/7	1	1	7729645	105.85	7108610	191.81	2538120	57.26
22/7	1	2	7067763	96.86	4160807	112.47	1049642	24.87
22/7	1	2	7078130	97.00	4167557	112.65	1050889	24.89
22/7	2	1	6207340	85.18	5640233	152.29	2089684	47.50
22/7	2	1	6214362	85.27	5654931	152.68	2080726	47.30
22/7	2	2	6629300	90.91	3507503	94.88	856279	20.66
22/7	2	2	6618416	90.76	3496795	94.60	856808	20.67
22/7	3	1	6543793	89.75	5933074	160.17	2114189	48.03
22/7	3	1	6549465	89.82	5932783	160.16	2108494	47.91
22/7	3	2	7651953	104.79	6676967	180.19	2418762	54.66
22/7	3	2	7651025	104.78	6671362	180.04	2410486	54.48
22/7	4	1	8511700	116.47	5929141	160.06	1911990	43.63
22/7	4	1	8525720	116.66	5920223	159.82	1885986	43.07
22/7	4	2	8453529	115.68	7282882	196.50	2689998	60.56
22/7	4	2	8453114	115.67	7286571	196.60	2684921	60.45
22/7	5	1	7914691	108.36	4982141	134.57	1449517	33.57
22/7	5	1	7871138	107.77	4998878	135.02	1442728	33.42
22/7	5	2	8199211	112.22	5085322	137.35	1443568	33.44
22/7	5	2	8274335	113.24	5076160	137.10	1447125	33.52

Note: Storage time: 1 = Week 1, 2 Week 2, 3 = Week 3, 4 = Week 4.

Table D.6 HPLC data of sugars, organic acids and antioxidants standards peak area and retention time

Standards	Concentration (%,w/v)	Mean peak area	Mean retention time (min)
Sucrose	0.1	81320.5	9.172
	0.5	3877005	
	1	16357247.5	
	2.5	39702132	
	5	95154400.5	
	10	188435119	
Glucose	0.01	466903	10.912
	0.1	2240686	
	0.5	15982034.5	
	1	32419380.5	
	2.5	75478902.5	
	5	157128690	
Fructose	0.01	485159.5	14.429
	0.1	2017389	
	0.5	14306559.5	
	1	29425197.5	
	2.5	70335507	
	5	137416709	
Acetic acid	0.01	154725	16.262
	0.1	1732070	
	0.2	3193531.5	
	0.5	8554366	
	1	17114644.5	
Gluconic acid	0.01	118877	25.776
	0.1	1119876.5	
	0.2	1520355	
	0.5	5994581	
	1	11195890	

Standards	Concentration (ug/ml)	Mean peak area	Mean retention time (min)
Gallic acid	6.25	539533.5	12.0144
	12.5	1104691	
	25	2219533	
	50	4460960.5	
	100	9161958.5	
Theobromine	1.25	99893.5	19.8658
	2.5	201660.5	
	5	410374	
	10	820194.5	
	20	1792171	
Caffine	7.91	556625.5	28.168
	15.81	1115833	
	31.625	2243614	
	63.25	4517072	
	126.5	9288655	
EGC	7.25	9679	25.3485
	15.5	94420.5	
	31	234935.5	
	62	476157.5	
	124	980555	
	248	1883715	
ECG	6.375	244436	50.8454
	12.75	518172	
	25.5	1061777	
	51	2148423.5	
	102	4643693	
EGCG	6.3125	168306.5	35.003
	12.625	383962	
	25.25	915165	
	50.5	1884134.5	
	101	3924681	
	202	7392375.5	

E. GC data

E.1 GC standard curve of ethanol

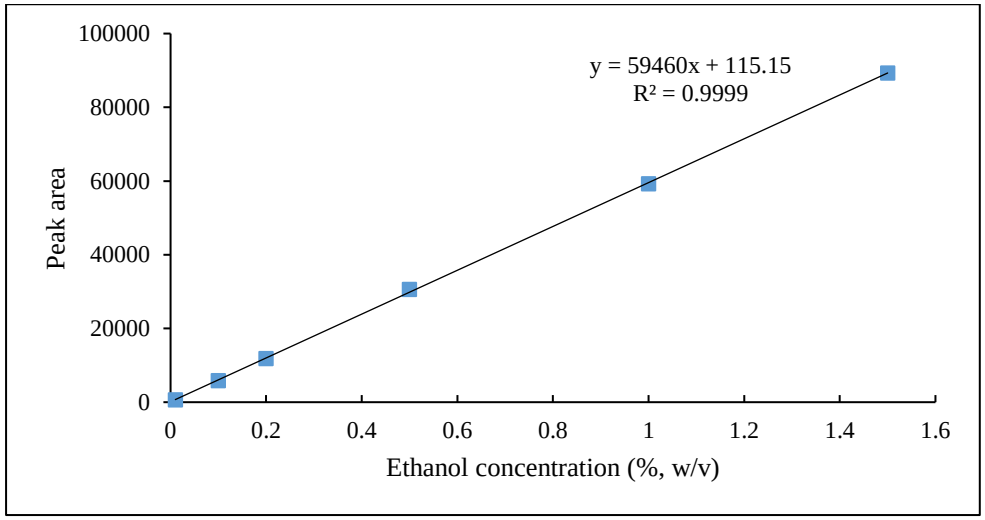


Table E.1 GC raw data of ethanol in Phase 3 during fermentation for 7 days

Product treatment	Fermentation time	Replication	Peak area	Ethanol concentration (%, w/v)	Product treatment	Fermentation time	Replication	Peak area	Ethanol concentration (%, w/v)
1	1	1	9252.1	0.15	2	1	1	9257.0	0.15
1	1	1	10572.8	0.18	2	1	1	9154.7	0.15
1	1	2	12323.2	0.21	2	1	2	12853.8	0.21
1	1	2	14456.9	0.24	2	1	2	13574.0	0.23
1	2	1	13869.8	0.23	2	2	1	13299.6	0.22
1	2	1	12862.6	0.21	2	2	1	11507.6	0.19
1	2	2	17134.8	0.29	2	2	2	17305.5	0.29
1	2	2	16247.8	0.27	2	2	2	16271.0	0.27
1	3	1	6130.0	0.10	2	3	1	5756.1	0.09
1	3	1	5923.8	0.10	2	3	1	5278.3	0.09
1	3	2	6067.8	0.10	2	3	2	10742.2	0.18
1	3	2	6012.9	0.10	2	3	2	7508.3	0.12
1	4	1	11463.0	0.19	2	4	1	10691.5	0.18
1	4	1	11608.0	0.19	2	4	1	10973.9	0.18
1	4	2	9589.7	0.16	2	4	2	11681.2	0.19
1	4	2	9990.4	0.17	2	4	2	10629.4	0.18
1	5	1	5069.1	0.08	2	5	1	4555.4	0.07
1	5	1	5136.6	0.08	2	5	1	4503.1	0.07
1	5	2	3982.1	0.07	2	5	2	6010.3	0.10
1	5	2	3708.0	0.06	2	5	2	5488.6	0.09
1	6	1	23292.1	0.39	2	6	1	28563.7	0.48
1	6	1	21787.6	0.36	2	6	1	26624.0	0.45
1	6	2	18558.2	0.31	2	6	2	21741.5	0.36
1	6	2	19167.8	0.32	2	6	2	21696.2	0.36
1	7	1	36165.7	0.61	2	7	1	39013.4	0.65
1	7	1	33727.4	0.57	2	7	1	36682.2	0.61
1	7	2	34875.6	0.58	2	7	2	38194.4	0.64
1	7	2	35469.2	0.59	2	7	2	37429.1	0.63

Product treatment	Fermentation time	Replication	Peak area	Ethanol concentration (%, w/v)	Product treatment	Fermentation time	Replication	Peak area	Ethanol concentration (%, w/v)
3	1	1	9414.0	0.16	4	1	1	9982.6	0.17
3	1	1	9323.0	0.15	4	1	1	8921.3	0.15
3	1	2	13684.1	0.23	4	1	2	13545.6	0.23
3	1	2	13888.9	0.23	4	1	2	13163.8	0.22
3	2	1	13123.5	0.22	4	2	1	13512.9	0.23
3	2	1	15552.3	0.26	4	2	1	12423.1	0.21
3	2	2	17540.3	0.29	4	2	2	20346.0	0.34
3	2	2	18144.9	0.30	4	2	2	22008.0	0.37
3	3	1	6586.4	0.11	4	3	1	5677.1	0.09
3	3	1	6168.2	0.10	4	3	1	6001.1	0.10
3	3	2	8543.8	0.14	4	3	2	8543.8	0.14
3	3	2	5880.9	0.10	4	3	2	5880.9	0.10
3	4	1	12815.4	0.21	4	4	1	12515.6	0.21
3	4	1	13108.0	0.22	4	4	1	12546.9	0.21
3	4	2	9971.6	0.17	4	4	2	12140.5	0.20
3	4	2	9572.9	0.16	4	4	2	11759.9	0.20
3	5	1	5499.2	0.09	4	5	1	5104.2	0.08
3	5	1	5533.7	0.09	4	5	1	5134.0	0.08
3	5	2	6156.7	0.10	4	5	2	5647.8	0.09
3	5	2	5558.5	0.09	4	5	2	6111.9	0.10
3	6	1	36654.6	0.61	4	6	1	38613.8	0.65
3	6	1	36397.4	0.61	4	6	1	39943.9	0.67
3	6	2	22780.3	0.38	4	6	2	34125.2	0.57
3	6	2	24519.3	0.41	4	6	2	35425.6	0.59
3	7	1	39013.4	0.75	4	7	1	48824.6	0.82
3	7	1	36682.2	0.73	4	7	1	49088.2	0.82
3	7	2	38194.4	0.67	4	7	2	46795.9	0.79
3	7	2	37429.1	0.63	4	7	2	45958.5	0.77

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Fermentation time: 1 = Day 0, 2 = Day 1-BA, 3 = Day 1-AA, 4 = Day 2-BA, 5 = Day 2-AA; 6 = Day 5, 7 = Day 7.

Table E.2 GC raw data of ethanol in Phase 3 during storage for 2 weeks

Product treatment	Storage time	Replication	Peak area	Ethanol concentration (%, w/v)
1	1	1	41519.1	0.70
1	1	1	41554.2	0.70
1	1	2	41341.2	0.69
1	1	2	40125.0	0.67
1	2	1	46689.1	0.78
1	2	1	45107.2	0.76
1	2	2	43763.5	0.73
1	2	2	44052.4	0.74
2	1	1	45508.9	0.76
2	1	1	44337.6	0.74
2	1	2	43581.8	0.73
2	1	2	44250.7	0.74
2	2	1	48328.6	0.81
2	2	1	48807.6	0.82
2	2	2	47137.4	0.79
2	2	2	47434.3	0.80
3	1	1	49868.0	0.84
3	1	1	49590.9	0.83
3	1	2	44928.2	0.75
3	1	2	45560.0	0.76
3	2	1	53052.3	0.89
3	2	1	53728.4	0.90
3	2	2	47958.0	0.80
3	2	2	47353.3	0.79
4	1	1	55565.5	0.93
4	1	1	55613.4	0.93
4	1	2	51746.9	0.87
4	1	2	50493.7	0.85
4	2	1	57268.4	0.96
4	2	1	57895.4	0.97
4	2	2	59652.5	0.92
4	2	2	59326.8	0.90

Note: Product treatment: 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 22/10; Storage time: 1 = Week 1, 2 = Week 2.

Table E.3 GC raw data of ethanol in Phase 4

Product treatment	Storage time	Replication	Peak area	Ethanol concentration (%, w/v)
22/7	0	1	43814.4	0.73
22/7	0	1	41751.8	0.70
22/7	0	2	40025.0	0.67
22/7	0	2	40612.1	0.68
22/7	1	1	49457.4	0.83
22/7	1	1	49292.1	0.83
22/7	1	2	47326.0	0.79
22/7	1	2	48518.4	0.82
22/7	2	1	51539.7	0.86
22/7	2	1	52480.5	0.88
22/7	2	2	52654.3	0.88
22/7	2	2	53941.8	0.91
22/7	3	1	55130.4	0.93
22/7	3	1	54518.1	0.91
22/7	3	2	56511.9	0.95
22/7	3	2	55710.0	0.93
22/7	4	1	57809.4	0.97
22/7	4	1	57232.8	0.96
22/7	4	2	59965.4	1.01
22/7	4	2	59073.4	1.00

Note: Storage time: 1 = Week 1, 2 = Week 2, 3 = Week 3, 4 = Week 4.

Table E.4 GC data of ethanol standards peak area and retention time

Standards	Concentration (%, w/v)	Mean peak area	Mean retention time (min)
Ethanol	0.01	632.5	0.754
	0.1	5843.25	
	0.2	11864.35	
	0.5	30618.8	
	1	59239.35	
	1.5	89305.16	

F. Antibacterial activities analysis

Table F.1 Antibacterial inhibition zone diameters (mm)

Parameter	Trail 1	Trail 2	Trail 1	Trail 2	Mean ± SD
	mm	mm	mm	mm	
Escherichia coli 111	12	11	11	12	11.5±0.5
Listeria monocytogenes 15E03-1	11.5	11.5	12.5	11.5	11.8±0.4
Pseudomonas aeruginosa MU-A26	11.5	12	11.5	11	11.5±0.4
Salmonella typhimurium ESR3479	11	10	11	12	11.0±0.7
Staphylococcus aureus MU-A57	12	11	11	11	11.3±0.4

G. Statistic output

G.1 Statistical analysis of pH of green tea kombucha samples during 14 days of fermentation

One-way ANOVA: pH versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.91973	0.306575	57.26	0.000
Error	12	0.06425	0.005354		
Total	15	0.98398			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0731722	93.47%	91.84%	88.39%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	3.5400	0.0356	(3.4603, 3.6197)
6	4	3.2425	0.0443	(3.1628, 3.3222)
7	4	3.0750	0.1047	(2.9953, 3.1547)
8	4	2.8875	0.0850	(2.8078, 2.9672)

Pooled StDev = 0.0731722

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	3.5400	A
6	4	3.2425	B
7	4	3.0750	C
8	4	2.8875	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: pH versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.91515	0.305050	111.77	0.000
Error	12	0.03275	0.002729		
Total	15	0.94790			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0522414	96.54%	95.68%	93.86%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	3.5275	0.0330	(3.4706, 3.5844)
6	4	3.22000	0.01414	(3.16309, 3.27691)
7	4	3.0700	0.0868	(3.0131, 3.1269)
8	4	2.8725	0.0457	(2.8156, 2.9294)

Pooled StDev = 0.0522414

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	3.5275	A
6	4	3.22000	B
7	4	3.0700	C
8	4	2.8725	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: pH versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.72125	0.240417	56.18	0.000
Error	12	0.05135	0.004279		
Total	15	0.77260			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0654153	93.35%	91.69%	88.18%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	3.5075	0.0320	(3.4362, 3.5788)
6	4	3.2125	0.0457	(3.1412, 3.2838)
7	4	3.0600	0.0983	(2.9887, 3.1313)
8	4	2.9400	0.0658	(2.8687, 3.0113)

Pooled StDev = 0.0654153

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	3.5075	A
6	4	3.2125	B
7	4	3.0600	C
8	4	2.9400	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: pH versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.91027	0.303423	87.37	0.000
Error	12	0.04167	0.003473		
Total	15	0.95194			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0589315	95.62%	94.53%	92.22%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	3.5050	0.0351	(3.4408, 3.5692)
6	4	3.1800	0.0469	(3.1158, 3.2442)
7	4	3.0325	0.1014	(2.9683, 3.0967)
8	4	2.85500	0.01291	(2.79080, 2.91920)

Pooled StDev = 0.0589315

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	3.5050	A
6	4	3.1800	B
7	4	3.0325	C
8	4	2.85500	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

G.2 Statistical analysis of T.A of green tea kombucha samples during 14 days of fermentation

One-way ANOVA: T.A versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.77788	0.259294	36.61	0.000
Error	12	0.08499	0.007082		
Total	15	0.86287			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0841558	90.15%	87.69%	82.49%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.1875	0.0377	(0.0958, 0.2792)
6	4	0.39000	0.01826	(0.29832, 0.48168)
7	4	0.5846	0.1151	(0.4929, 0.6763)
8	4	0.7800	0.1155	(0.6883, 0.8717)

Pooled StDev = 0.0841558

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	0.7800	A
7	4	0.5846	B
6	4	0.39000	C
5	4	0.1875	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: T.A versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.83955	0.279851	68.39	0.000
Error	12	0.04910	0.004092		
Total	15	0.88865			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0639666	94.47%	93.09%	90.18%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.1850	0.0404	(0.1153, 0.2547)
6	4	0.41750	0.00500	(0.34781, 0.48719)
7	4	0.5881	0.0904	(0.5184, 0.6578)
8	4	0.8100	0.0808	(0.7403, 0.8797)

Pooled StDev = 0.0639666

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	0.8100	A
7	4	0.5881	B
6	4	0.41750	C
5	4	0.1850	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: T.A versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.57485	0.191617	52.08	0.000
Error	12	0.04415	0.003679		
Total	15	0.61900			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0606561	92.87%	91.08%	87.32%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.1975	0.0206	(0.1314, 0.2636)
6	4	0.4325	0.0550	(0.3664, 0.4986)
7	4	0.5850	0.0981	(0.5189, 0.6511)
8	4	0.7050	0.0404	(0.6389, 0.7711)

Pooled StDev = 0.0606561

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	0.7050	A
7	4	0.5850	A
6	4	0.4325	B
5	4	0.1975	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: T.A versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.76728	0.255759	67.42	0.000
Error	12	0.04552	0.003794		
Total	15	0.81280			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0615916	94.40%	93.00%	90.04%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.1975	0.0320	(0.1304, 0.2646)
6	4	0.4575	0.0435	(0.3904, 0.5246)
7	4	0.6115	0.0907	(0.5444, 0.6786)
8	4	0.7950	0.0635	(0.7279, 0.8621)

Pooled StDev = 0.0615916

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	0.7950	A
7	4	0.6115	B
6	4	0.4575	C
5	4	0.1975	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

G.3 Statistical analysis of total soluble solids (°Brix) of green tea kombucha samples during 14 days of fermentation

One-way ANOVA: TSS (ºBrix) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	3.1675	1.05583	115.18	0.000
Error	12	0.1100	0.00917		
Total	15	3.2775			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0957427	96.64%	95.80%	94.03%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	8.6500	0.1732	(8.5457, 8.7543)
6	4	8.0500	0.0577	(7.9457, 8.1543)
7	4	7.6500	0.0577	(7.5457, 7.7543)
8	4	7.500	0.000	(7.396, 7.604)

Pooled StDev = 0.0957427

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
5	4	8.6500	A		
6	4	8.0500		B	
7	4	7.6500			C
8	4	7.500			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14, TSS = total soluble solids.

One-way ANOVA: TSS (ºBrix) versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	2.340	0.7800	4.00	0.035
Error	12	2.340	0.1950		
Total	15	4.680			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.441588	50.00%	37.50%	11.11%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	11.250	0.289	(10.769, 11.731)
6	4	10.700	0.462	(10.219, 11.181)
7	4	10.400	0.462	(9.919, 10.881)
8	4	10.250	0.520	(9.769, 10.731)

Pooled StDev = 0.441588

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping	
5	4	11.250	A	
6	4	10.700	A	B
7	4	10.400	A	B
8	4	10.250		B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14, TSS = total soluble solids.

One-way ANOVA: TSS (ºBrix) versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	6.48750	2.16250	288.33	0.000
Error	12	0.09000	0.00750		
Total	15	6.57750			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0866025	98.63%	98.29%	97.57%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	8.6500	0.1732	(8.5557, 8.7443)
6	4	7.600	0.000	(7.506, 7.694)
7	4	7.200	0.000	(7.106, 7.294)
8	4	7.000	0.000	(6.906, 7.094)

Pooled StDev = 0.0866025

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	8.6500	A
6	4	7.600	B
7	4	7.200	C
8	4	7.000	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14, TSS = total soluble solids.

One-way ANOVA: TSS (ºBrix) versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	5.008	1.6692	6.93	0.006
Error	12	2.890	0.2408		
Total	15	7.898			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.490748	63.41%	54.26%	34.94%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	11.250	0.289	(10.715, 11.785)
6	4	10.500	0.577	(9.965, 11.035)
7	4	10.000	0.577	(9.465, 10.535)
8	4	9.800	0.462	(9.265, 10.335)

Pooled StDev = 0.490748

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping	
5	4	11.250	A	
6	4	10.500	A	B
7	4	10.000		B
8	4	9.800		B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14, TSS = total soluble solids.

G.4 Statistical analysis of colour (L, a*, b*) of green tea kombucha samples during 14 days of fermentation

One-way ANOVA: Colour (L) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	25.821	8.6071	31.43	0.000
Error	12	3.287	0.2739		
Total	15	29.108			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.523337	88.71%	85.89%	79.93%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	85.928	0.673	(85.357, 86.498)
6	4	88.005	0.294	(87.435, 88.575)
7	4	88.430	0.240	(87.860, 89.000)
8	4	89.410	0.707	(88.840, 89.980)

Pooled StDev = 0.523337

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
8	4	89.410	A		
7	4	88.430	A	B	
6	4	88.005		B	
5	4	85.928			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (L) versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	35.28	11.759	11.19	0.001
Error	12	12.61	1.051		
Total	15	47.89			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.02517	73.66%	67.08%	53.18%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	86.210	0.539	(85.093, 87.327)
6	4	88.990	1.242	(87.873, 90.107)
7	4	89.680	1.507	(88.563, 90.797)
8	4	89.955	0.318	(88.838, 91.072)

Pooled StDev = 1.02517

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	89.955	A
7	4	89.680	A
6	4	88.990	A
5	4	86.210	B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (L) versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	55.141	18.3804	104.08	0.000
Error	12	2.119	0.1766		
Total	15	57.260			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.420231	96.30%	95.37%	93.42%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	87.183	0.674	(86.725, 87.640)
6	4	89.957	0.490	(89.500, 90.415)
7	4	91.5275	0.0427	(91.0697, 91.9853)
8	4	91.8950	0.0995	(91.4372, 92.3528)

Pooled StDev = 0.420231

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	91.8950	A
7	4	91.5275	A
6	4	89.957	B
5	4	87.183	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (L) versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	32.767	10.9224	22.18	0.000
Error	12	5.908	0.4923		
Total	15	38.675			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.701669	84.72%	80.90%	72.84%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	87.010	0.695	(86.246, 87.774)
6	4	89.375	0.405	(88.611, 90.139)
7	4	90.227	0.558	(89.463, 90.992)
8	4	90.745	1.005	(89.981, 91.509)

Pooled StDev = 0.701669

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
8	4	90.745	A
7	4	90.227	A
6	4	89.375	A
5	4	87.010	B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (a*) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.03547	0.011823	7.08	0.005
Error	12	0.02003	0.001669		
Total	15	0.05549			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0408503	63.91%	54.89%	35.85%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.3125	0.0250	(0.2680, 0.3570)
6	4	0.2775	0.0263	(0.2330, 0.3220)
7	4	0.2200	0.0712	(0.1755, 0.2645)
8	4	0.19250	0.01708	(0.14800, 0.23700)

Pooled StDev = 0.0408503

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping	
5	4	0.3125	A	
6	4	0.2775	A	B
7	4	0.2200		B
8	4	0.19250		B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (a*) versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.034875	0.011625	28.47	0.000
Error	12	0.004900	0.000408		
Total	15	0.039775			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0202073	87.68%	84.60%	78.10%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.29500	0.01291	(0.27299, 0.31701)
6	4	0.26000	0.01826	(0.23799, 0.28201)
7	4	0.2050	0.0238	(0.1830, 0.2270)
8	4	0.1750	0.0238	(0.1530, 0.1970)

Pooled StDev = 0.0202073

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping	
5	4	0.29500	A	
6	4	0.26000	A	
7	4	0.2050		B
8	4	0.1750		B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (a*) versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.035000	0.011667	60.87	0.000
Error	12	0.002300	0.000192		
Total	15	0.037300			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0138444	93.83%	92.29%	89.04%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.27250	0.01708	(0.25742, 0.28758)
6	4	0.25250	0.00957	(0.23742, 0.26758)
7	4	0.18750	0.00957	(0.17242, 0.20258)
8	4	0.15750	0.01708	(0.14242, 0.17258)

Pooled StDev = 0.0138444

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	0.27250	A
6	4	0.25250	A
7	4	0.18750	B
8	4	0.15750	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (a*) versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	0.033125	0.011042	54.08	0.000
Error	12	0.002450	0.000204		
Total	15	0.035575			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0142887	93.11%	91.39%	87.76%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	0.28750	0.00957	(0.27193, 0.30307)
6	4	0.2550	0.0208	(0.2394, 0.2706)
7	4	0.20250	0.00500	(0.18693, 0.21807)
8	4	0.17000	0.01633	(0.15443, 0.18557)

Pooled StDev = 0.0142887

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	0.28750	A
6	4	0.2550	B
7	4	0.20250	C
8	4	0.17000	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (b*) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	64.421	21.4737	152.36	0.000
Error	12	1.691	0.1409		
Total	15	66.112			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.375416	97.44%	96.80%	95.45%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	19.7700	0.0490	(19.3610, 20.1790)
6	4	16.9250	0.0173	(16.5160, 17.3340)
7	4	15.378	0.725	(14.969, 15.786)
8	4	14.4875	0.1879	(14.0785, 14.8965)

Pooled StDev = 0.375416

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	19.7700	A
6	4	16.9250	B
7	4	15.378	C
8	4	14.4875	D

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (b*) versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	54.566	18.1885	58.78	0.000
Error	12	3.713	0.3094		
Total	15	58.279			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.556264	93.63%	92.04%	88.67%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	18.918	0.962	(18.312, 19.523)
6	4	15.615	0.383	(15.009, 16.221)
7	4	14.873	0.407	(14.267, 15.478)
8	4	14.0550	0.0208	(13.4490, 14.6610)

Pooled StDev = 0.556264

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
5	4	18.918	A		
6	4	15.615		B	
7	4	14.873		B	C
8	4	14.0550			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (b*) versus Fermentation time (sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	59.342	19.7807	44.73	0.000
Error	12	5.306	0.4422		
Total	15	64.649			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.664986	91.79%	89.74%	85.41%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	18.290	0.970	(17.566, 19.014)
6	4	14.953	0.708	(14.228, 15.677)
7	4	13.9250	0.1971	(13.2006, 14.6494)
8	4	13.295	0.537	(12.571, 14.019)

Pooled StDev = 0.664986

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
5	4	18.290	A		
6	4	14.953		B	
7	4	13.9250		B	C
8	4	13.295			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Colour (b*) versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	66.517	22.1724	38.81	0.000
Error	12	6.856	0.5713		
Total	15	73.373			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.755845	90.66%	88.32%	83.39%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	18.010	1.311	(17.187, 18.833)
6	4	14.368	0.667	(13.544, 15.191)
7	4	13.5875	0.0763	(12.7641, 14.4109)
8	4	12.618	0.341	(11.794, 13.441)

Pooled StDev = 0.755845

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
5	4	18.010	A		
6	4	14.368		B	
7	4	13.5875		B	C
8	4	12.618			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

G.5 Statistical analysis of microbiological growth of green tea kombucha samples during 14 days of fermentation

One-way ANOVA: Yeast (log cfu/ml) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	6.2953	2.09842	42.44	0.000
Error	12	0.5933	0.04944		
Total	15	6.8886			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.222361	91.39%	89.23%	84.69%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	6.645	0.256	(6.402, 6.887)
6	4	7.1245	0.0943	(6.8822, 7.3667)
7	4	6.318	0.285	(6.076, 6.561)
8	4	5.407	0.206	(5.165, 5.650)

Pooled StDev = 0.222361

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
6	4	7.1245	A
5	4	6.645	B
7	4	6.318	B
8	4	5.407	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Yeast log cfu/ml versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	4.269	1.4231	13.68	0.000
Error	12	1.248	0.1040		
Total	15	5.518			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.322521	77.38%	71.72%	59.78%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	6.476	0.364	(6.124, 6.827)
6	4	6.933	0.370	(6.582, 7.285)
7	4	6.388	0.338	(6.036, 6.739)
8	4	5.5061	0.1795	(5.1547, 5.8574)

Pooled StDev = 0.322521

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
6	4	6.933	A
5	4	6.476	A
7	4	6.388	A
8	4	5.5061	B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Yeast log cfu/ml versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	7.101	2.3670	13.80	0.000
Error	12	2.059	0.1716		
Total	15	9.160			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.414193	77.52%	71.91%	60.04%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	6.331	0.405	(5.879, 6.782)
6	4	6.270	0.387	(5.818, 6.721)
7	4	5.700	0.410	(5.249, 6.151)
8	4	4.670	0.451	(4.219, 5.121)

Pooled StDev = 0.414193

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	6.331	A
6	4	6.270	A
7	4	5.700	A
8	4	4.670	B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: Yeast log cfu/ml versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	9.989	3.3296	29.84	0.000
Error	12	1.339	0.1116		
Total	15	11.328			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.334057	88.18%	85.22%	78.98%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	6.443	0.315	(6.079, 6.807)
6	4	6.365	0.335	(6.001, 6.729)
7	4	5.580	0.370	(5.216, 5.944)
8	4	4.479	0.313	(4.116, 4.843)

Pooled StDev = 0.334057

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	6.443	A
6	4	6.365	A
7	4	5.580	B
8	4	4.479	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14.

One-way ANOVA: AAB (log cfu/ml) versus Fermentation time (Sample 22/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	6.9745	2.32483	38.59	0.000
Error	12	0.7229	0.06024		
Total	15	7.6974			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.245435	90.61%	88.26%	83.30%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	5.700	0.271	(5.433, 5.967)
6	4	6.1304	0.0930	(5.8630, 6.3978)
7	4	5.445	0.220	(5.177, 5.712)
8	4	4.343	0.332	(4.075, 4.610)

Pooled StDev = 0.245435

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
6	4	6.1304	A		
5	4	5.700	A	B	
7	4	5.445		B	
8	4	4.343			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14; AAB = acetic acid bacteria.

One-way ANOVA: AAB log cfu/ml versus Fermentation time (Sample 22/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	5.038	1.67922	19.55	0.000
Error	12	1.031	0.08591		
Total	15	6.069			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.293110	83.01%	78.76%	69.80%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	5.587	0.303	(5.268, 5.907)
6	4	5.999	0.265	(5.680, 6.319)
7	4	5.499	0.230	(5.180, 5.819)
8	4	4.475	0.358	(4.156, 4.794)

Pooled StDev = 0.293110

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
6	4	5.999	A
5	4	5.587	A
7	4	5.499	A
8	4	4.475	B

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14; AAB = acetic acid bacteria.

One-way ANOVA: AAB log cfu/ml versus Fermentation time (Sample 24/7)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	8.758	2.9193	29.15	0.000
Error	12	1.202	0.1001		
Total	15	9.960			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.316462	87.93%	84.92%	78.55%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	5.528	0.356	(5.183, 5.873)
6	4	5.143	0.327	(4.798, 5.487)
7	4	4.636	0.363	(4.291, 4.981)
8	4	3.5575	0.1883	(3.2127, 3.9022)

Pooled StDev = 0.316462

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping		
5	4	5.528	A		
6	4	5.143	A	B	
7	4	4.636		B	
8	4	3.5575			C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14; AAB = acetic acid bacteria.

One-way ANOVA: AAB log cfu/ml versus Fermentation time (Sample 24/10)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time	4	5, 6, 7, 8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time	3	10.462	3.4873	29.73	0.000
Error	12	1.408	0.1173		
Total	15	11.870			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.342514	88.14%	85.17%	78.91%

Means

Fermentation time	N	Mean	StDev	95% CI
5	4	5.554	0.394	(5.181, 5.927)
6	4	5.272	0.429	(4.899, 5.645)
7	4	4.528	0.262	(4.155, 4.901)
8	4	3.463	0.247	(3.090, 3.836)

Pooled StDev = 0.342514

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time	N	Mean	Grouping
5	4	5.554	A
6	4	5.272	A
7	4	4.528	B
8	4	3.463	C

Means that do not share a letter are significantly different.

Note: Fermentation time 5 = Day 2 AA; 6 = Day 7; 7 = Day 10; 8 = Day 14; AAB = acetic acid bacteria.

G.6 Statistical analysis of pH of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: pH versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.042019	0.014006	24.45	0.000
Error	12	0.006875	0.000573		
Total	15	0.048894			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0239357	85.94%	82.42%	75.00%

Means

Sample code	N	Mean	StDev	95% CI
1	4	3.1800	0.0294	(3.1539, 3.2061)
2	4	3.1300	0.0258	(3.1039, 3.1561)
3	4	3.0925	0.0206	(3.0664, 3.1186)
4	4	3.04000	0.01826	(3.01392, 3.06608)

Pooled StDev = 0.0239357

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping		
1	4	3.1800	A		
2	4	3.1300	A	B	
3	4	3.0925		B	
4	4	3.04000			C

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

G.7 Statistical analysis of T.A. of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: T.A. versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.046519	0.015506	95.35	0.000
Error	12	0.001952	0.000163		
Total	15	0.048470			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0127527	95.97%	94.97%	92.84%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.44363	0.01574	(0.42974, 0.45752)
2	4	0.47301	0.00812	(0.45912, 0.48690)
3	4	0.54281	0.01180	(0.52892, 0.55670)
4	4	0.57914	0.01406	(0.56525, 0.59303)

Pooled StDev = 0.0127527

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
4	4	0.57914	A
3	4	0.54281	B
2	4	0.47301	C
1	4	0.44363	D

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10; T.A. = Titratable acidity.

G.8 Statistical analysis of total soluble solids (°Brix) of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: TSS (ºBrix) versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	34.1300	11.3767	758.44	0.000
Error	12	0.1800	0.0150		
Total	15	34.3100			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.122474	99.48%	99.34%	99.07%

Means

Sample code	N	Mean	StDev	95% CI
1	4	7.5000	0.1155	(7.3666, 7.6334)
2	4	10.4000	0.1155	(10.2666, 10.5334)
3	4	7.1500	0.1732	(7.0166, 7.2834)
4	4	10.0500	0.0577	(9.9166, 10.1834)

Pooled StDev = 0.122474

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
2	4	10.4000	A
4	4	10.0500	B
1	4	7.5000	C
3	4	7.1500	D

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10; TSS = Total soluble solids.

G.9 Statistical analysis of colour (L, a*, b*) of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: Colour L versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	7.215	2.405	2.14	0.149
Error	12	13.500	1.125		
Total	15	20.715			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.06066	34.83%	18.54%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
1	4	86.2700	0.1236	(85.1145, 87.4255)
2	4	86.8250	0.0957	(85.6695, 87.9805)
3	4	87.99	2.05	(86.83, 89.15)
4	4	87.620	0.512	(86.465, 88.775)

Pooled StDev = 1.06066

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
3	4	87.99	A
4	4	87.620	A
2	4	86.8250	A
1	4	86.2700	A

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: Colour a* versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.003569	0.001190	0.99	0.431
Error	12	0.014425	0.001202		
Total	15	0.017994			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0346711	19.83%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.2775	0.0275	(0.2397, 0.3153)
2	4	0.2600	0.0245	(0.2222, 0.2978)
3	4	0.2375	0.0443	(0.1997, 0.2753)
4	4	0.2475	0.0386	(0.2097, 0.2853)

Pooled StDev = 0.0346711

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
1	4	0.2775	A
2	4	0.2600	A
4	4	0.2475	A
3	4	0.2375	A

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: Colour b* versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	13.520	4.5068	24.45	0.000
Error	12	2.212	0.1843		
Total	15	15.732			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.429314	85.94%	82.43%	75.01%

Means

Sample code	N	Mean	StDev	95% CI
1	4	17.9950	0.0985	(17.5273, 18.4627)
2	4	17.108	0.638	(16.640, 17.575)
3	4	16.142	0.326	(15.675, 16.610)
4	4	15.593	0.462	(15.125, 16.060)

Pooled StDev = 0.429314

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
1	4	17.9950	A	
2	4	17.108	A	
3	4	16.142		B
4	4	15.593		B

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

G.10 Statistical analysis of microbiological growth of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: Yeast (log cfu/ml) versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	1.8255	0.60849	23.36	0.000
Error	12	0.3125	0.02604		
Total	15	2.1380			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.161379	85.38%	81.73%	74.01%

Means

Sample code	N	Mean	StDev	95% CI
1	4	7.3243	0.1728	(7.1485, 7.5001)
2	4	7.207	0.234	(7.032, 7.383)
3	4	6.5635	0.1259	(6.3877, 6.7394)
4	4	6.6306	0.0590	(6.4548, 6.8064)

Pooled StDev = 0.161379

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
1	4	7.3243	A	
2	4	7.207	A	
4	4	6.6306		B
3	4	6.5635		B

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: AAB (log cfu/ml) versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	2.4171	0.80568	15.15	0.000
Error	12	0.6382	0.05318		
Total	15	3.0552			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.230610	79.11%	73.89%	62.87%

Means

Sample code	N	Mean	StDev	95% CI
1	4	6.321	0.271	(6.070, 6.572)
2	4	6.2683	0.1883	(6.0171, 6.5196)
3	4	5.4650	0.1626	(5.2138, 5.7162)
4	4	5.580	0.278	(5.329, 5.831)

Pooled StDev = 0.230610

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
1	4	6.321	A	
2	4	6.2683	A	
4	4	5.580		B
3	4	5.4650		B

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10; AAB = Acetic acid bacteria.

G.11 Statistical analysis of organic acids level of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: Gluconic acid versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.15765	0.052549	9.84	0.001
Error	12	0.06409	0.005341		
Total	15	0.22174			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0730819	71.10%	63.87%	48.61%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.2566	0.0238	(0.1769, 0.3362)
2	4	0.2890	0.0292	(0.2094, 0.3687)
3	4	0.4235	0.0731	(0.3439, 0.5032)
4	4	0.5009	0.1208	(0.4213, 0.5806)

Pooled StDev = 0.0730819

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping		
4	4	0.5009	A		
3	4	0.4235	A	B	
2	4	0.2890		B	C
1	4	0.2566			C

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: Acetic acid versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.03979	0.013265	13.10	0.000
Error	12	0.01215	0.001012		
Total	15	0.05194			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0318160	76.61%	70.77%	58.43%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.28406	0.00452	(0.24940, 0.31872)
2	4	0.28844	0.00520	(0.25378, 0.32310)
3	4	0.3742	0.0481	(0.3395, 0.4089)
4	4	0.3954	0.0411	(0.3608, 0.4301)

Pooled StDev = 0.0318160

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
4	4	0.3954	A	
3	4	0.3742	A	
2	4	0.28844		B
1	4	0.28406		B

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

G.12 Statistical analysis of sugars level of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: Sucrose versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	28.359	9.4531	49.04	0.000
Error	12	2.313	0.1928		
Total	15	30.672			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.439045	92.46%	90.57%	86.59%

Means

Sample code	N	Mean	StDev	95% CI
1	4	5.005	0.613	(4.526, 5.483)
2	4	7.501	0.373	(7.022, 7.979)
3	4	4.038	0.415	(3.560, 4.517)
4	4	6.485	0.289	(6.007, 6.963)

Pooled StDev = 0.439045

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
2	4	7.501	A
4	4	6.485	B
1	4	5.005	C
3	4	4.038	D

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: Glucose versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.93259	0.310862	224.19	0.000
Error	12	0.01664	0.001387		
Total	15	0.94923			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0372371	98.25%	97.81%	96.88%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.70616	0.01397	(0.66559, 0.74673)
2	4	0.9816	0.0512	(0.9410, 1.0222)
3	4	1.16093	0.00522	(1.12037, 1.20150)
4	4	1.3630	0.0519	(1.3224, 1.4036)

Pooled StDev = 0.0372371

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
4	4	1.3630	A
3	4	1.16093	B
2	4	0.9816	C
1	4	0.70616	D

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

One-way ANOVA: Fructose versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	2.51238	0.837461	137.72	0.000
Error	12	0.07297	0.006081		
Total	15	2.58536			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0779809	97.18%	96.47%	94.98%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.4358	0.0406	(0.3509, 0.5208)
2	4	0.7929	0.0467	(0.7079, 0.8778)
3	4	1.2465	0.0945	(1.1616, 1.3315)
4	4	1.4553	0.1075	(1.3704, 1.5403)

Pooled StDev = 0.0779809

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
4	4	1.4553	A
3	4	1.2465	B
2	4	0.7929	C
1	4	0.4358	D

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

G.13 Statistical analysis of ethanol level of green tea kombucha samples at 7 days of fermentation

One-way ANOVA: ethanol versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	1, 2, 3, 4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.10890	0.036299	29.90	0.000
Error	12	0.01457	0.001214		
Total	15	0.12347			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0348449	88.20%	85.25%	79.02%

Means

Sample code	N	Mean	StDev	95% CI
1	4	0.58769	0.01737	(0.54973, 0.62565)
2	4	0.63429	0.01685	(0.59633, 0.67225)
3	4	0.6941	0.0550	(0.6562, 0.7321)
4	4	0.8081	0.0353	(0.7702, 0.8461)

Pooled StDev = 0.0348449

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping		
4	4	0.8081	A		
3	4	0.6941		B	
2	4	0.63429		B	C
1	4	0.58769			C

Means that do not share a letter are significantly different.

Note: Sample code 1 = 22/7, 2 = 22/10, 3 = 24/7, 4 = 24/10.

G.14 Statistical analysis of pH of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: pH versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.02213	0.005533	4.96	0.009
Error	15	0.01672	0.001115		
Total	19	0.03886			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0333916	56.96%	45.48%	23.48%

Means

Storage time	N	Mean	StDev	95% CI
1	4	3.11500	0.01291	(3.07941, 3.15059)
2	4	3.0325	0.0263	(2.9969, 3.0681)
3	4	3.0325	0.0299	(2.9969, 3.0681)
4	4	3.0300	0.0365	(2.9944, 3.0656)
5	4	3.0325	0.0499	(2.9969, 3.0681)

Pooled StDev = 0.0333916

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
1	4	3.11500	A
5	4	3.0325	B
3	4	3.0325	B
2	4	3.0325	B
4	4	3.0300	B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.15 Statistical analysis of T.A. of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: T.A. versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.01968	0.004919	7.26	0.002
Error	15	0.01017	0.000678		
Total	19	0.02984			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0260332	65.93%	56.85%	39.44%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.53108	0.01376	(0.50333, 0.55882)
2	4	0.5920	0.0369	(0.5642, 0.6197)
3	4	0.6030	0.0266	(0.5753, 0.6308)
4	4	0.6131	0.0270	(0.5854, 0.6409)
5	4	0.6174	0.0201	(0.5896, 0.6451)

Pooled StDev = 0.0260332

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
5	4	0.6174	A
4	4	0.6131	A
3	4	0.6030	A
2	4	0.5920	A
1	4	0.53108	B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.16 Statistical analysis of total soluble solids (°Brix) of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: TSS (ºBrix) versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.88000	0.220000	66.00	0.000
Error	15	0.05000	0.003333		
Total	19	0.93000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0577350	94.62%	93.19%	90.44%

Means

Storage time	N	Mean	StDev	95% CI
1	4	7.4000	0.1155	(7.3385, 7.4615)
2	4	7.1500	0.0577	(7.0885, 7.2115)
3	4	7.000	0.000	(6.938, 7.062)
4	4	6.900	0.000	(6.838, 6.962)
5	4	6.800	0.000	(6.738, 6.862)

Pooled StDev = 0.0577350

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	4	7.4000	A		
2	4	7.1500		B	
3	4	7.000			C
4	4	6.900			C D
5	4	6.800			D

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4; TSS = total soluble solids

G.17 Statistical analysis of colour (L*, a*, b*) of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Colour L* versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	26.72	6.680	1.33	0.304
Error	15	75.37	5.024		
Total	19	102.09			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.24152	26.17%	6.49%	0.00%

Means

Storage time	N	Mean	StDev	95% CI
1	4	85.82	2.57	(83.43, 88.21)
2	4	86.945	1.819	(84.556, 89.334)
3	4	87.095	1.830	(84.706, 89.484)
4	4	88.29	2.51	(85.90, 90.68)
5	4	89.16	2.36	(86.78, 91.55)

Pooled StDev = 2.24152

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
5	4	89.16	A
4	4	88.29	A
3	4	87.095	A
2	4	86.945	A
1	4	85.82	A

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Colour a* versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.1250	0.031245	3.28	0.040
Error	15	0.1430	0.009533		
Total	19	0.2680			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0976388	46.64%	32.41%	5.13%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.4875	0.0685	(0.3834, 0.5916)
2	4	0.4125	0.0789	(0.3084, 0.5166)
3	4	0.3225	0.1190	(0.2184, 0.4266)
4	4	0.3175	0.1184	(0.2134, 0.4216)
5	4	0.2650	0.0926	(0.1609, 0.3691)

Pooled StDev = 0.0976388

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping	
1	4	0.4875	A	
2	4	0.4125	A	B
3	4	0.3225	A	B
4	4	0.3175	A	B
5	4	0.2650		B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Colour b* versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	17.784	4.4461	8.98	0.001
Error	15	7.427	0.4951		
Total	19	25.211			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.703655	70.54%	62.69%	47.63%

Means

Storage time	N	Mean	StDev	95% CI
1	4	15.793	0.800	(15.043, 16.542)
2	4	14.035	0.848	(13.285, 14.785)
3	4	13.810	0.808	(13.060, 14.560)
4	4	13.342	0.505	(12.593, 14.092)
5	4	13.125	0.456	(12.375, 13.875)

Pooled StDev = 0.703655

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
1	4	15.793	A
2	4	14.035	B
3	4	13.810	B
4	4	13.342	B
5	4	13.125	B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.18 Statistical analysis of microbiological growth of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Yeast (log cfu/ml) versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	6.0217	1.50543	42.87	0.000
Error	15	0.5267	0.03511		
Total	19	6.5484			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.187386	91.96%	89.81%	85.70%

Means

Storage time	N	Mean	StDev	95% CI
1	4	7.0369	0.1807	(6.8372, 7.2366)
2	4	6.7614	0.1297	(6.5617, 6.9611)
3	4	6.4985	0.1558	(6.2988, 6.6982)
4	4	6.1439	0.0590	(5.9442, 6.3436)
5	4	5.453	0.314	(5.254, 5.653)

Pooled StDev = 0.187386

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	4	7.0369	A		
2	4	6.7614	A	B	
3	4	6.4985		B	C
4	4	6.1439			C
5	4	5.453			D

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: AAB (log cfu/ml) versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	2.9068	0.72671	58.60	0.000
Error	15	0.1860	0.01240		
Total	19	3.0929			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.111361	93.99%	92.38%	89.31%

Means

Storage time	N	Mean	StDev	95% CI
1	4	6.0820	0.1700	(5.9633, 6.2007)
2	4	5.7479	0.0845	(5.6292, 5.8666)
3	4	5.4888	0.1308	(5.3702, 5.6075)
4	4	5.17156	0.01406	(5.05288, 5.29024)
5	4	5.0324	0.0929	(4.9137, 5.1511)

Pooled StDev = 0.111361

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
1	4	6.0820	A
2	4	5.7479	B
3	4	5.4888	C
4	4	5.17156	D
5	4	5.0324	D

Means that do not share a letter are significantly different.

Notess: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4; AAB = acetic acid bacteria.

G.19 Statistical analysis of organic acids level of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Gluconic acid versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.010930	0.002733	5.18	0.008
Error	15	0.007917	0.000528		
Total	19	0.018848			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0229746	57.99%	46.79%	25.32%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.3517	0.0326	(0.3272, 0.3762)
2	4	0.3993	0.0231	(0.3748, 0.4238)
3	4	0.4092	0.0238	(0.3847, 0.4337)
4	4	0.41379	0.01599	(0.38931, 0.43828)
5	4	0.41271	0.01471	(0.38823, 0.43720)

Pooled StDev = 0.0229746

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping	
4	4	0.41379	A	
5	4	0.41271	A	
3	4	0.4092	A	
2	4	0.3993	A	B
1	4	0.3517		B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Acetic acid versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.009464	0.002366	6.85	0.002
Error	15	0.005183	0.000346		
Total	19	0.014646			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0185879	64.61%	55.18%	37.09%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.30930	0.00438	(0.28949, 0.32911)
2	4	0.3399	0.0229	(0.3201, 0.3597)
3	4	0.3530	0.0312	(0.3332, 0.3728)
4	4	0.36750	0.00957	(0.34769, 0.38731)
5	4	0.36796	0.01080	(0.34815, 0.38777)

Pooled StDev = 0.0185879

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping	
5	4	0.36796	A	
4	4	0.36750	A	
3	4	0.3530	A	
2	4	0.3399	A	B
1	4	0.30930		B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.20 Statistical analysis of sugars level of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Sucrose versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	8.1714	2.04285	211.58	0.000
Error	15	0.1448	0.00966		
Total	19	8.3162			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0982603	98.26%	97.79%	96.90%

Means

Storage time	N	Mean	StDev	95% CI
1	4	4.5181	0.1521	(4.4133, 4.6228)
2	4	3.6192	0.1273	(3.5145, 3.7239)
3	4	3.1706	0.0649	(3.0659, 3.2753)
4	4	2.8746	0.0589	(2.7699, 2.9793)
5	4	2.7543	0.0357	(2.6496, 2.8591)

Pooled StDev = 0.0982603

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	4	4.5181	A		
2	4	3.6192		B	
3	4	3.1706			C
4	4	2.8746			D
5	4	2.7543			D

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Glucose versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	1.4519	0.36298	19.49	0.000
Error	15	0.2793	0.01862		
Total	19	1.7313			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.136464	83.87%	79.56%	71.32%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.6697	0.0784	(0.5243, 0.8151)
2	4	0.9483	0.0559	(0.8029, 1.0938)
3	4	1.0645	0.0320	(0.9191, 1.2099)
4	4	1.0742	0.0232	(0.9287, 1.2196)
5	4	1.505	0.287	(1.360, 1.651)

Pooled StDev = 0.136464

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
5	4	1.505	A		
4	4	1.0742		B	
3	4	1.0645		B	
2	4	0.9483		B	C
1	4	0.6697			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Fructose versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	2.0746	0.51864	26.33	0.000
Error	15	0.2954	0.01970		
Total	19	2.3700			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.140340	87.53%	84.21%	77.84%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.4395	0.0619	(0.2900, 0.5891)
2	4	0.71619	0.01777	(0.56662, 0.86575)
3	4	0.8870	0.0894	(0.7374, 1.0366)
4	4	0.9389	0.0888	(0.7893, 1.0885)
5	4	1.422	0.280	(1.273, 1.572)

Pooled StDev = 0.140340

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
5	4	1.422	A		
4	4	0.9389		B	
3	4	0.8870		B	
2	4	0.71619		B	C
1	4	0.4395			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.21 Statistical analysis of ethanol level of green tea kombucha samples during 4 weeks of storage

One-way ANOVA: ethanol versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.198358	0.049590	103.18	0.000
Error	15	0.007209	0.000481		
Total	19	0.205568			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0219231	96.49%	95.56%	93.77%

Means

Storage time	N	Mean	StDev	95% CI
1	4	0.6969	0.0281	(0.6735, 0.7202)
2	4	0.81639	0.01882	(0.79303, 0.83976)
3	4	0.8825	0.0206	(0.8591, 0.9059)
4	4	0.93049	0.01636	(0.90713, 0.95386)
5	4	0.9850	0.0238	(0.9616, 1.0084)

Pooled StDev = 0.0219231

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
5	4	0.9850	A
4	4	0.93049	B
3	4	0.8825	C
2	4	0.81639	D
1	4	0.6969	E

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.22 Statistical analysis of antioxidants content in green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Gallic acid versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	6.328	1.58204	17.61	0.000
Error	15	1.348	0.08984		
Total	19	7.676			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.299737	82.44%	77.76%	68.79%

Means

Storage time	N	Mean	StDev	95% CI
1	4	5.6952	0.0483	(5.3758, 6.0147)
2	4	5.093	0.385	(4.774, 5.413)
3	4	5.533	0.513	(5.213, 5.852)
4	4	6.5012	0.1414	(6.1818, 6.8207)
5	4	6.5304	0.1263	(6.2110, 6.8498)

Pooled StDev = 0.299737

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping	
5	4	6.5304	A	
4	4	6.5012	A	
1	4	5.6952		B
3	4	5.533		B
2	4	5.093		B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: EGC versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	2564.2	641.04	14.52	0.000
Error	15	662.3	44.16		
Total	19	3226.5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6.64492	79.47%	74.00%	63.51%

Means

Storage time	N	Mean	StDev	95% CI
1	4	130.89	7.92	(123.81, 137.97)
2	4	112.07	2.44	(104.99, 119.15)
3	4	125.75	10.82	(118.67, 132.83)
4	4	145.32	3.09	(138.24, 152.40)
5	4	138.18	5.05	(131.09, 145.26)

Pooled StDev = 6.64492

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
4	4	145.32	A		
5	4	138.18	A	B	
1	4	130.89	A	B	
3	4	125.75		B	C
2	4	112.07			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: EGCG versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	8317	2079.2	2.74	0.068
Error	15	11383	758.9		
Total	19	19700			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
27.5480	42.22%	26.81%	0.00%

Means

Storage time	N	Mean	StDev	95% CI
1	4	152.3	45.8	(122.9, 181.6)
2	4	123.6	33.3	(94.3, 153.0)
3	4	170.14	11.52	(140.78, 199.50)
4	4	178.2	21.1	(148.9, 207.6)
5	4	136.013	1.418	(106.654, 165.371)

Pooled StDev = 27.5480

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
4	4	178.2	A
3	4	170.14	A
1	4	152.3	A
5	4	136.013	A
2	4	123.6	A

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: ECG versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	1282	320.6	2.28	0.109
Error	15	2107	140.4		
Total	19	3389			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
11.8509	37.84%	21.26%	0.00%

Means

Storage time	N	Mean	StDev	95% CI
1	4	41.11	18.74	(28.48, 53.74)
2	4	34.03	15.44	(21.40, 46.66)
3	4	51.27	3.81	(38.64, 63.90)
4	4	51.93	9.91	(39.30, 64.56)
5	4	33.4850	0.0688	(20.8552, 46.1148)

Pooled StDev = 11.8509

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
4	4	51.93	A
3	4	51.27	A
1	4	41.11	A
2	4	34.03	A
5	4	33.4850	A

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Theobromine versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	7.577	1.89421	26.87	0.000
Error	15	1.057	0.07048		
Total	19	8.634			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.265485	87.76%	84.49%	78.23%

Means

Storage time	N	Mean	StDev	95% CI
1	4	6.430	0.390	(6.148, 6.713)
2	4	5.6461	0.1333	(5.3632, 5.9290)
3	4	6.162	0.379	(5.879, 6.444)
4	4	7.3533	0.1922	(7.0703, 7.6362)
5	4	7.0680	0.0466	(6.7851, 7.3510)

Pooled StDev = 0.265485

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
4	4	7.3533	A		
5	4	7.0680	A		
1	4	6.430		B	
3	4	6.162		B	C
2	4	5.6461			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Caffeine versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	1940.6	485.14	19.88	0.000
Error	15	366.1	24.41		
Total	19	2306.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.94019	84.13%	79.90%	71.79%

Means

Storage time	N	Mean	StDev	95% CI
1	4	101.57	5.36	(96.30, 106.83)
2	4	88.03	3.24	(82.76, 93.29)
3	4	97.28	8.66	(92.02, 102.55)
4	4	116.117	0.518	(110.852, 121.382)
5	4	110.40	2.74	(105.13, 115.66)

Pooled StDev = 4.94019

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping			
4	4	116.117	A			
5	4	110.40	A	B		
1	4	101.57		B	C	
3	4	97.28			C	D
2	4	88.03				D

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

G.23 Statistical analysis of sensory evaluation in green tea kombucha samples during 4 weeks of storage

One-way ANOVA: Appearance versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	0.62400	0.156000	23.40	0.002
Error	5	0.03333	0.006667		
Total	9	0.65733			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0816497	94.93%	90.87%	79.72%

Means

Storage time	N	Mean	StDev	95% CI
1	2	6.8667	0.0943	(6.7183, 7.0151)
2	2	6.9000	0.0471	(6.7516, 7.0484)
3	2	6.800	0.141	(6.652, 6.948)
4	2	6.467	0.000	(6.318, 6.615)
5	2	6.2667	0.0471	(6.1183, 6.4151)

Pooled StDev = 0.0816497

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping	
2	2	6.9000	A	
1	2	6.8667	A	
3	2	6.800	A	
4	2	6.467		B
5	2	6.2667		B

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Aroma versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	2.8507	0.71267	32.07	0.001
Error	5	0.1111	0.02222		
Total	9	2.9618			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.149071	96.25%	93.25%	84.99%

Means

Storage time	N	Mean	StDev	95% CI
1	2	6.333	0.283	(6.062, 6.604)
2	2	6.333	0.000	(6.062, 6.604)
3	2	6.0333	0.0943	(5.7624, 6.3043)
4	2	5.7000	0.0471	(5.4290, 5.9710)
5	2	4.900	0.141	(4.629, 5.171)

Pooled StDev = 0.149071

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	2	6.333	A		
2	2	6.333	A		
3	2	6.0333	A	B	
4	2	5.7000		B	
5	2	4.900			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Flavour versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	2.53600	0.634000	1426.50	0.000
Error	5	0.00222	0.000444		
Total	9	2.53822			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0210819	99.91%	99.84%	99.65%

Means

Storage time	N	Mean	StDev	95% CI
1	2	7.200	0.000	(7.162, 7.238)
2	2	6.700	0.000	(6.662, 6.738)
3	2	6.4667	0.0471	(6.4283, 6.5050)
4	2	6.167	0.000	(6.128, 6.205)
5	2	5.700	0.000	(5.662, 5.738)

Pooled StDev = 0.0210819

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
1	2	7.200	A
2	2	6.700	B
3	2	6.4667	C
4	2	6.167	D
5	2	5.700	E

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Sourness versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	1.78622	0.446556	75.83	0.000
Error	5	0.02944	0.005889		
Total	9	1.81567			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0767391	98.38%	97.08%	93.51%

Means

Storage time	N	Mean	StDev	95% CI
1	2	6.7667	0.0943	(6.6272, 6.9062)
2	2	6.6500	0.0236	(6.5105, 6.7895)
3	2	6.4333	0.0943	(6.2938, 6.5728)
4	2	5.9333	0.0471	(5.7938, 6.0728)
5	2	5.6667	0.0943	(5.5272, 5.8062)

Pooled StDev = 0.0767391

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	2	6.7667	A		
2	2	6.6500	A	B	
3	2	6.4333		B	
4	2	5.9333			C
5	2	5.6667			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Sweetness versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	3.24622	0.81156	67.63	0.000
Error	5	0.06000	0.01200		
Total	9	3.30622			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.109545	98.19%	96.73%	92.74%

Means

Storage time	N	Mean	StDev	95% CI
1	2	6.5667	0.0471	(6.3676, 6.7658)
2	2	6.033	0.000	(5.834, 6.232)
3	2	6.000	0.236	(5.801, 6.199)
4	2	5.3000	0.0471	(5.1009, 5.4991)
5	2	4.967	0.000	(4.768, 5.166)

Pooled StDev = 0.109545

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	2	6.5667	A		
2	2	6.033		B	
3	2	6.000		B	
4	2	5.3000			C
5	2	4.967			C

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.

One-way ANOVA: Overall versus Storage time

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	4	4.95556	1.23889	929.17	0.000
Error	5	0.00667	0.00133		
Total	9	4.96222			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0365148	99.87%	99.76%	99.46%

Means

Storage time	N	Mean	StDev	95% CI
1	2	7.1333	0.0471	(7.0670, 7.1997)
2	2	6.567	0.000	(6.500, 6.633)
3	2	6.5333	0.0471	(6.4670, 6.5997)
4	2	5.500	0.000	(5.434, 5.566)
5	2	5.2667	0.0471	(5.2003, 5.3330)

Pooled StDev = 0.0365148

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping
1	2	7.1333	A
2	2	6.567	B
3	2	6.5333	B
4	2	5.500	C
5	2	5.2667	D

Means that do not share a letter are significantly different.

Notes: storage time 1 = 0, 2 = Week 1, 3 = Week 2, 4 = Week 3, 5 = Week 4.