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Optimisation of sugar concentration and fermentation temperature to produce a low sugar green tea-flavoured water kefir beverage

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DEDICATION

*This thesis is dedicated to my mother and father,
Ravinder Kour and Manjit Singh,
and my brother, Jaspreet Singh.*

Thank you all for giving wings to my dreams.

Abstract

Water kefir is a refreshing, self-carbonated, slightly sweet, low-alcoholic beverage characterised by a mildly acidic taste and yeasty aroma. Water kefir is prepared on a household scale or semi-industrial scale by inoculating sugar substrate with starter culture i.e., water kefir grains, consisting of a multispecies consortium of symbiotic lactic acid bacteria and yeast embedded in a polysaccharide matrix. Some of the microorganisms found in the kefir grains are recognized as probiotics.

The improved health awareness of consumers and popular trends such as healthy hydration, low added sugar, lactose intolerance and natural products are driving the research and development of non-dairy beverages which has created a niche market for fermented beverages such as water kefir. Previous research on water kefir was mainly focused on the identification and role of microorganisms present in the kefir grains. In recent years, growing interest in fermented lactose-free water kefir have led to the exploration of various sugar substrates (raw sugar, white sugar, brown sugar, molasses and honey), plant based substrates, dried fruits (apricot, dates, figs and raisin) and fermentation factors such as sugar concentration, temperature, kefir grain concentration and fermentation time. The aim of this study was to optimise the fermentation conditions (temperature and sugar concentration) to produce a novel, dairy-free low added sugar water kefir beverage using green tea.

The production of green tea flavoured water kefir with low added sugar was optimised by investigating the effect of sugar concentration (3%, 4% w/v) and fermentation temperature (22°C and 25°C) which were conducted in three phases. Phase I enumerated the microflora in the water kefir grains starter culture (lactic acid bacteria and yeast). During Phase II, physicochemical analysis (pH, titratable acidity, total soluble solids, and colour), microbiological analysis and consumer sensory analysis of four different formulations were evaluated to understand the effect of sugar concentration and fermentation temperature, with the aim of selecting an optimised green tea water kefir beverage. Phase III analysed the stability of the selected water kefir beverage during storage (4±1°C) for two weeks. HPLC analysis was used to determine concentrations of sugar, ethanol, organic acids, and antioxidants present in the selected beverage (WK2) from Phase II.

Lactic acid bacteria (7.80 ± 0.14 log cfu/g) and *S. cerevisiae* (7.25 ± 0.03 log cfu/g) were present in the water kefir grains starter culture. In phase II, during fermentation the pH and total soluble solids decreased ($p < 0.05$) with a concomitant increase ($p < 0.05$) in titratable acidity. The pH of the four formulations ranged between 3.30 ± 0.06 to 3.50 ± 0.00 , total soluble solids between 2.35 ± 0.07 - 3.82 ± 0.03 °Brix and the titratable acidity was 0.178 ± 0.01 - 0.228 ± 0.03 %. Results from Phase II showed that sugar concentration and fermentation temperature contributed to the physico-chemical and microbial properties of green tea water kefir. Increased metabolic activity of the microorganism was observed in samples prepared at high sugar concentrations and at the higher fermentation temperature. Water kefir sample (WK2) prepared at 22°C using the 4% (w/v) sugar concentration received the highest consumer sensory scores ($p < 0.05$), showed optimal physico-chemical results ($p < 0.05$) (pH (3.50 ± 0.00), T.A. (0.178 ± 0.01 %), TSS (3.82 ± 0.03 °B), colour L* (97.88 ± 0.03), colour a* (0.99 ± 0.09) and colour b* (3.15 ± 0.03), and microbiological results ($p < 0.05$) (viable cell count of LAB (8.50 ± 0.01 log cfu/mL) and *S. cerevisiae* (7.00 ± 0.04 log cfu/mL)). Therefore, water kefir beverage sample WK2 was selected for further studies in Phase III. In Phase III, the viable cell counts of lactic acid bacteria (8.42 ± 0.00 log cfu/mL) and yeast (6.85 ± 0.00 log cfu/mL) decreased ($p < 0.05$) during refrigerated storage (4±1°C) for two weeks.

The overall sensory acceptability of green tea water kefir remained high during the two week cold storage period. Results showed it is possible to prepare a low sugar, low alcohol, probiotic green tea water kefir beverage containing no sucrose (0%, w/v), glucose ($0.94\pm 0.04\%$, w/v), fructose ($1.45\pm 0.04\%$, w/v), ethanol ($0.67\pm 0.06\%$, v/v) and lactic acid ($0.626\pm 0.00\%$, v/v). Higher concentrations of antioxidants such as gallic acid (3.88 ± 0.13 $\mu\text{g/ml}$), epigallocatechin (89.03 ± 4.45 $\mu\text{g/ml}$), epigallocatechin-3-gallate (122.81 ± 2.79 $\mu\text{g/ml}$), epicatechin-3-gallate (25.67 ± 1.10 $\mu\text{g/ml}$), caffeine (74.44 ± 0.52 $\mu\text{g/ml}$), and theobromine (4.24 ± 0.03 $\mu\text{g/ml}$) were found in green tea water kefir beverage (WK2) at the end of two weeks storage compared to 28 d cold storage black tea water kefir (except gallic acid 10 $\mu\text{g/ml}$, epigallocatechin 4.1 $\mu\text{g/ml}$, epigallocatechin-3-gallate 3 $\mu\text{g/ml}$, caffeine 7 $\mu\text{g/ml}$, epicatechin-3-gallate 2 $\mu\text{g/ml}$, theobromine 6.90 $\mu\text{g/ml}$) (Subardjo, 2017). Presence of probiotic microorganisms and antioxidants in green tea water kefir may confer health benefits when consumed regularly.

To the author's knowledge, this is the first time that green tea (0.4%, w/v) has been used to prepare a novel, lactose-free, low added sugar (4%, w/v) green tea flavoured water kefir beverage. The fermentation process involved kefir grains (5%, w/v) at 22°C for 144 h yielding green tea flavoured water kefir beverage with optimal physico-chemical, microbial, sensory properties that meets the stipulated regulations of non-alcoholic beverages and brewed soft drinks. Further studies should be conducted to investigate the antioxidant activity of the beverage produced. Additionally, isolation and identification of the strains of water kefir grains should be conducted which could help in better understanding the microbial interactions of the kefir grains.

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List of Abbreviations

a*	redness-greenness
A.	<i>Acetobacter</i>
AAB	acetic acid bacteria
ABTS	2,2'-azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid
ADH	Alcohol dehydrogenase
ADP	Adenosine diphosphate
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemistry
ATP	Adenosine triphosphate
b*	Yellowness-blueness
B.	<i>Bifidobacterium</i>
CFU	Colony forming per Unit
d	Day
D.	<i>Dekkera</i>
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Epicatechin
ECG	Epicatechin gallate
EFSA	European Food Safety Authority
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
EMP	Emden-Meyerhof-Parnas
EPS	Exopolysaccharide
FAO	Food and Agriculture Organization
FRAP	Ferric-reducing antioxidant power analysis
FSANZ	Food Standards Australia New Zealand
g	gram
GA	Gallic acid
GC	Gallocatechin
GCG	Gallocatechin gallate
GRAS	Generally Regarded As Safe
GTF	Glucosyltransferase
h	hour
HPLC	High performance liquid chromatography
KHP	Potassium hydrogen phthalate solution
L	Litre
L.	<i>Lactobacillus</i>
LAB	Lactic acid bacteria
Lc.	<i>Leuconostoc</i>
LDH	Lactate dehydrogenase

M	Molarity
mg	milligram
mL	millilitre
mm	millimetre
MRS	de Man, Rogosa and Sharpe
NADP	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NZ	New Zealand
PDC	Pyruvate decarboxylase
PDH	Pyruvate dehydrogenase
PKP	Phosphoketolase pathway
<i>S.</i>	<i>Saccharomyces</i>
SD	Standard deviation
Spp.	Species (plural)
St. D	Storage day
T.A.	Titrateable acidity
TCA	Tricarboxylic acid
TSS	Total soluble solids
V	Volume
v/v	Volume per volume
VBNC	Viable state of not culturable cells
w/v	Weight per volume
WHO	World Health Organisation
YGC	Yeast Glucose Chloramphenicol
<i>Z.</i>	<i>Zygorulasporea</i>

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1. Introduction

1.1 Background

The global market for fermented foods and beverages is expected to increase to about USD \$1000 billion in the forecast period 2023-2030 (Meticulous Research, 2023). Among the fermented beverages, functional beverages, especially probiotic products, are of a major interest in this sector. The functional beverage market is expected to be worth nearly USD \$200 billion with a projected annual growth of about 7% during the 2022-2029 forecast period (Meticulous Research, 2022). Dairy-based beverages hold a major share of the segment, followed by non-dairy beverages dominated by fruit-based probiotic drinks (Ilango & Antony, 2021; Mordor Intelligence, 2023; Rasika et al., 2021). Fermented products such as kombucha, kimchi, switchel, kvas, and kefir are gaining popularity in the Asia-Pacific, Europe and North America regions (Coriolis, 2020; Mordor Intelligence, 2023; Meticulous Research, 2023; Nielson-Stowell, 2020).

Water kefir is an artisanal beverage produced by inoculating water kefir grains (6-20%, w/v) into a sugar solution (5-30%, w/v) that undergoes fermentation for 3-8 days at ambient temperature (21-30°C) under anaerobic conditions (Laureys & De Vuyst, 2014; Laureys et al., 2018; Magalhaes et al., 2010; Pendon et al., 2022; Verce et al., 2019). Fresh or dried fruits may be added as a source of micronutrients and flavouring (Cufaoglu & Erdinc, 2023; Laureys et al., 2018; Tireki, 2022). The fermenting grains are then removed from the fermented beverage by sieving and are reused (back-slopping) in the next fermentation process. The main metabolites produced during the fermentation of water kefir are lactic acid, acetic acid, ethanol, carbon dioxide and various aromatic compounds (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017). Production of organic acids helps in the acidification of the beverage, and improving its shelf-life, carbon dioxide helps in carbonation while aromatic compounds add to the sensory profile of the kefir beverages (Fiorda et al., 2017; Magalhaes et al., 2010; M'hir et al., 2021; Pendon et al., 2022).

Water kefir is a lightly carbonated, slightly sweet, acidic, and low-alcohol drink (0.06-3%, ABV) with a fruity taste and aroma (Corona et al., 2016; Laureys & De Vuyst, 2014; Magalhaes et al., 2010). The kefir grains (starter culture) used to ferment the beverage consist primarily of a symbiotic consortium of lactic acid bacteria (LAB) and yeast. However, small quantities of acetic acid bacteria (AAB) may be embedded in the kefir grain polysaccharide matrix (Gulitz et al., 2011; Marsh et al., 2013). The consortium of fermenting microorganisms present in the kefir grains

influences the physico-chemical and sensory characteristics of the fermentation process (Laureys & De Vuyst, 2017). The microbial composition of water kefir grains vary depending on geographical origin and diversity, substrate type, substrate concentration, nutrient concentration, fermentation time and temperature that influences the type and concentration of end-metabolites produced (Laureys & De Vuyst, 2014, Laureys et al., 2019; Laureys et al., 2021; Lynch et al., 2021; Magalhaes et al., 2010; Pendon et al., 2022; Randazzo et al., 2016; Spizzirri et al., 2023). The most common starter culture microorganisms used in water kefir fermentation are LAB species: *Lactobacillus (L.) paracasei*, *L. hilgardii*, *L. nagelii*, and *Bifidobacterium (B.) aquikefiri* and the yeast species *Saccharomyces (S.) cerevisiae*, and *Zygorulasporea (Z.) florentina* (Gulitz et al., 2011; Laureys & De Vuyst, 2017).

Previous research on water kefir has mainly focused on the identification and role of the microorganisms present in the kefir grains (da Miguel et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013; Martinez-Torres et al., 2017). Recent studies have explored various sugar substrates (Destro et al., 2019; Laureys & De Vuyst, 2014), plant-based substrates (Bueno et al., 2021; Corona et al., 2016; Darvishzadeh et al., 2021; Koh et al., 2018; Ozcelik et al., 2021; Randazzo et al., 2016), and tea-based substrates (Subardjo, 2017; Isrianto, 2020). Additionally, the role of factors such as sugar concentration, fermentation temperature and time, and concentration of kefir grains have also been studied to better understand the water kefir fermentation process (Laureys & De Vuyst, 2014; Laureys et al., 2019; Magalhaes et al., 2010; Martinez-Torres et al., 2017; Spizzirri et al., 2023; Stadie et al., 2013). The versatility of sugary kefir grains to ferment different substrates makes it feasible to explore new flavours, provide added value to the by-products and produce novel, functional and probiotic kefir beverages (Bueno et al., 2021; Fiorda et al., 2017; Spizzirri et al., 2023).

Compared to the popular and traditional dairy-based kefir, research and innovation in water kefir is still in an early stage. Presently, water kefir is produced on a household or semi-industrial scale whereas milk kefir has been successfully industrialised (Guzel-Seydim et al., 2021; Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Pendon et al., 2022; Spizzirri et al., 2023). From a commercial standpoint, it is important to understand the production process and role of fermentation factors such as fermentation temperature and sugar concentration to aid the

optimisation and upscaling of water kefir beverages as these factors have a direct impact on the overall quality of the end-products.

According to the Australia New Zealand Food Standard (FSANZ), water kefir is categorised under Standard 2.6.2 non-alcoholic beverages and brewed soft drinks. Water kefir is described as a fermented beverage prepared from water with sugar and one or more extracts or infusions of fruit or vegetables (FSANZ, 2022). It should not contain more than 1.15 % alcohol by volume (Cufaoglu & Erdinc, 2023; Moretti et al., 2022). However, water kefir beverages produced using fruits and vegetable substrates can sometimes have a higher than acceptable alcohol content (2-4%, v/v) due to the presence of fermentable sugars (Randazzo et al., 2016). In addition to this, fermentation factors such as the added sugar concentration (5-30%, w/v) have a direct impact on the ethanol concentration (0.02-2.00 %) produced in the kefir (Hsieh et al., 2012; Laureys and De Vuyst, 2014; Martinez-Torres et al., 2017; Pendon et al., 2022). Therefore, more research is required into substrate type and reducing added sugar concentrations, to ensure lower ethanol levels resulting in the production of water kefir beverages that meet the stipulated regulations for non-alcoholic beverages.

Tea based substrates such as green tea should be explored as a substitute in kefir fermentation for fruit and vegetables juices which are considered ideal products for delivering probiotic bacteria to lactose-intolerant consumers (Istrati et al., 2018; Mantzourani et al., 2019). Green tea is a non-fermented tea rich in polyphenolic compounds (catechins), amino acids, phenolic acids, micronutrients and other nutrients. Green tea is popularly consumed due to its various health benefits such as anti-diabetes, anti-obesity, anti-inflammatory, anti-photoaging, neuroprotection, stimulation of the cardiovascular system and central nervous system (Chacko et al., 2010; Musial et al., 2020; Pervin et al., 2018; Prasanth et al., 2019). Green tea has previously been used as a substrate for fermented beverages such as kombucha (Oliveira et al., 2023; Wang, 2018) and dairy based kefir (Karagozlu et al., 2017). However, unlike fruit juice substrates, green tea itself does not contain fermentable sugar enabling the concentration of added sugars to be controlled to produce a low sugar fermented beverage. Research and innovation in fermented beverages that cater to the needs of lactose intolerance, desire for clean label and healthy hydration are being explored in new product development. Therefore, making green tea of particular interest to explore its novel potential as a substrate in water kefir fermentation (Mordor Intelligence, 2023; Parida,

2022; Prasanth et al., 2019; Randazzo et al., 2016). To the author's knowledge, there is scanty if any information on the use of green tea to prepare non-alcoholic water kefir. Therefore, this study aimed to optimise the sugar concentration and fermentation temperature in the production of low-sugar green-tea non-alcoholic water kefir beverage.

1.2 Aim and Objectives

The overall aim of this study was to optimise the fermentation and development of a novel low sugar green tea water kefir beverage using water kefir grains as the starter culture.

Objectives

1. To enumerate the lactic acid bacteria and yeast (*Saccharomyces cerevisiae*) content of water kefir grains;
2. To determine the effect of temperature and sugar concentration on the physico-chemical, microbiological, and sensory properties of the green tea water kefir beverage by:
 - a) measuring pH, colour, total soluble solids, and analysing the titratable acidity;
 - b) enumerating the lactic acid bacteria and *Saccharomyces cerevisiae*;
 - c) evaluating the sensory characteristics of the fermented kefir beverages using focus group and consumer sensory evaluations;
3. To determine the ethanol, sugars (sucrose, fructose, glucose), organic acid (lactic acid and acetic acid), and antioxidant levels of the optimised formulation of green tea water kefir beverage during fermentation and storage (4°C) using high-performance liquid chromatography (HPLC).

2. Literature Review

2.1 Water kefir

2.1.1 Origin

Kefir is an ancient dairy-based fermented beverage (Moretti et al., 2022) thought to have originated in the Caucasus Mountains of Russia (Leite et al., 2013; Prado et al., 2015). Kefir milk is widely consumed for its various health benefits and is prepared using milk kefir grains, also known as kefiran (Lynch et al., 2021; Oboturova et al., 2022). Water kefir grains are reported to be available worldwide, but their real origins remain unknown (Gulitz et al., 2011; Laureys & De Vuyst, 2014). However, there are two popular theories on the origin of water kefir grains. The earliest report suggests the presence of water kefir grain in 1855 as a ginger beer plant, brought to Western Europe by a Crimean soldier (Ward, 1892). The second theory emerged in Mexico where the polysaccharide kefir grains, named "Tibi" or "Tibicos" are spontaneously formed on the pads of the *Opuntia* cactus (Lutz, 1899). It is highly possible that water kefir grains might have different origins (Waldherr et al., 2010). Vayssier. (1978) first reported the product as "sugary kefir grains", to differentiate them from the kefir granules that ferment milk (Bergmann et al., 2010; Pidoux, 1989).

2.1.2 Production process

Water kefir is a traditional, artisanal, self-carbonated, fermented beverage characterised by a slightly sweet, acidic, fruity flavour with low alcohol (Magalhaes et al., 2010; Stadie et al., 2013). Unlike milk kefir which is produced at the industrial scale (Lynch et al., 2021), defined cultures in water kefir fermentation have not been extensively studied and are not widely available (Lynch et al., 2021; Xu et al., 2018). Water kefir is usually produced at a household or semi-industrial scale by inoculating water kefir grains into a sugar solution that undergoes anaerobic fermentation for 3-8 days at ambient temperature (Laureys & De Vuyst, 2014; Laureys et al., 2018; Magalhaes et al., 2010; Pendon et al., 2022; Verce et al., 2019). After fermentation, water liquor is separated from the dried fruits and kefir grains through sieving and the recovered grains are re-used for the next fermentation using the back-slopping procedure. The ability of the grains to grow through the back-slopping process makes it feasible to produce the drinks on a household scale and allows the passing of the grain from one household to another (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017). The generalised production of water kefir beverage is shown in Figure 2.1.

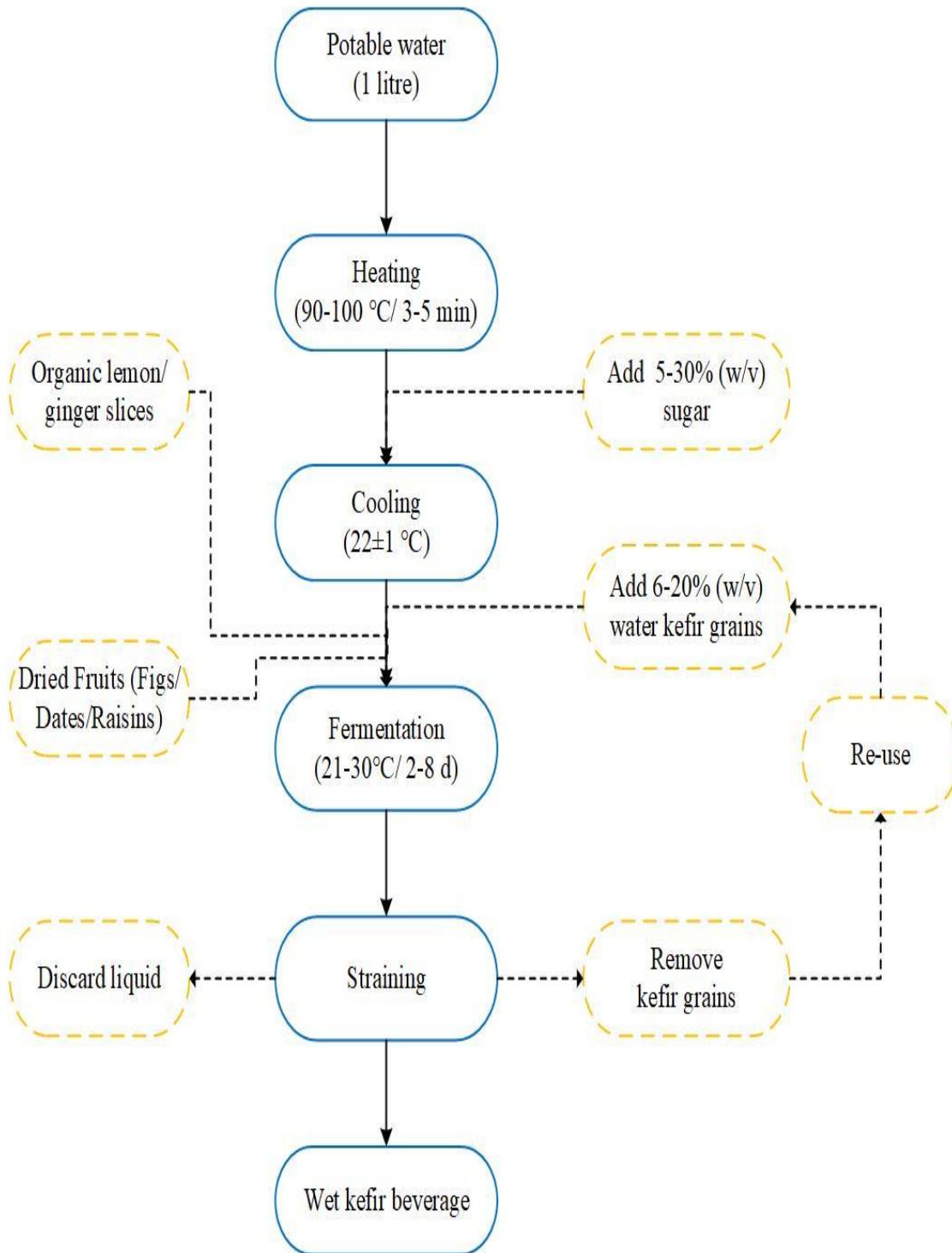


Figure 2.1: Overview of water kefir production process

Source: Laureys & De Vuyst. (2014); Laureys et al. (2018); Magalhaes et al. (2010); Stadie et al. (2013); Pendon et al. (2022)

Unfiltered raw sugar or sucrose is the main substrate in water kefir, which acts as the major carbon source (Amore & Faraco, 2013; Destro et al., 2019; Hamad, 2011; Tavares et al., 2023). The insoluble water kefir grains act as the inocula, which consists of a complex consortium of lactic acid bacteria, bifidobacteria, sometimes acetic acid bacteria in very small quantities and yeast embedded in the exopolysaccharide matrix (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Magalhaes et al., 2020; Marsh et al., 2013). During fermentation, some microorganisms permeate into the beverage where they grow by metabolising sucrose and produce a range of organic acids and other compounds. During fermentation, the pH decreases, thereby affecting physico-chemical and sensory profiles. (Fels et al., 2018; Laureys et al., 2018; Waldherr et al., 2010). Added dried fruits (figs, apricots, raisins, and dates) or organic lemon slices provide additional micronutrients and flavour (Cufaoglu & Erdinc, 2023; Gulitz et al., 2011; Laureys et al., 2018; Lynch et al., 2021; Pendon et al., 2022; Tireki, 2022).

2.1.3 Water kefir grains and the microbiota

Water kefir grains comprise a unique ecosystem of bacteria and yeast living in a symbiotic association anchored to the dextran exopolysaccharide matrix (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017). The grains are amorphous, compact, brittle, gelatinous, water-insoluble, granular, and vary in colour ranging from white to brownish depending on the colour of the substrate medium (Guzel-Seydim et al., 2021; Laureys et al., 2018; Moretti et al., 2022; Pendon et al., 2022; Waldherr et al., 2010). The water kefir grain is shown in Figure 2.2 and Figure 2.3 shows scanning electron microscopy images of the water kefir grain.



Figure 2.2: Water kefir grains

Notes: right image= water kefir grains after fermentation in white sugar solution; left image= water kefir grains after fermentation in brown sugar solution.

Source: Cufaoglu & Erdinc. (2023)

Figure 2.3: Scanning electron microscopy images of a water kefir grain

Notes: A= water kefir grain ($\times 50$); B= A cut water kefir grain showing exposed face of the internal surface (smooth area) and microorganisms on the external surface ($\times 250$); C= Rod-shaped and elongated yeast cells on the water kefir grain surface ($\times 1500$) and D= Bacilli bacteria on the water kefir grain surface ($\times 1500$).

Source: Lynch et al. (2021)

To date, research involving water kefir has chiefly focused on the isolation and identification of the microbial composition of the kefir grains. Various culture-dependent and culture-independent studies suggest the common presence of bacteria (lactic acid bacteria and sometimes acetic acid bacteria) and yeast in the kefir grains (Gulitz et al., 2011; Gulitz et al., 2013; Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Marsh et al., 2013; Martinez-Torres et al., 2017; Verce et al., 2019). It is estimated that the microorganisms in water kefir are present in a ratio of 10^8 cfu/g lactic acid bacteria, 10^6 - 10^8 cfu/g acetic acid bacteria, and 10^6 - 10^7 cfu/g yeasts (Gulitz et al., 2011). The consortium of microorganisms present in the kefir grains influences the characteristics of the fermentation process (Laureys & De Vuyst, 2017). Furthermore, the microbial composition of the water kefir grain varies depending on the geographical origin of kefir grains and fermentation conditions (substrate type, substrate concentration, nutrient composition, fermentation temperature and fermentation time) used in the production of water kefir beverages (Laureys & De Vuyst, 2017; Laureys et al., 2018; Luang-In et al., 2018; Martinez-Torres et al., 2017; Moretti et al., 2022; Pendon et al., 2022; Yerlikaya et al., 2022). Moreover, variations in microbial diversity from the same kefir grains have also been reported (Gamba et al., 2021).

The bacterial population of the grains usually contain gram-positive homo/heterofermentative lactic acid bacteria (LAB) from the genera *Zymomonas*, genus *Lactobacillus*, *Lactococcus*, *Leuconostoc* and sometimes gram-negative acetic acid bacteria (AAB) have also been found in small quantities (Marsh et al., 2013). The presence of *Bifidobacterium* in water kefir was first reported as the species *Bifidobacterium (B.) psychraerophilum* (Gulitz et al., 2013), and subsequently confirmed by Laureys & De Vuyst. (2014). The commonly found bacterial species in water kefir grains consist of *Lactobacillus (L.) casei/paracasei*, *L. nagelii*, *L. hilgardii*, *L. hordei*, *Leuconostoc (Lc.) mesenteroides*, *Acetobacter (A.) fabarum*, and *A. loveniensis* while the yeast population of water kefir grains are reported to consist of *Saccharomyces*, *Candida*, *Pichia*, *Hanseniaspora*, *Dekkera*, and *Zygosaccharomyces*. The dominant species of yeast in water kefir are *Saccharomyces (S.) cerevisiae*, *Zygorhizula (Z.) florentina*, and *Dekkera (D.) bruxellensis* (da Miguel et al., 2011; Gulitz et al., 2011; Gulitz et al., 2013; Laureys & De Vuyst, 2017; Magalhaes et al., 2010; Martinez-Torres et al., 2017; Verce et al., 2019). Some of the microorganisms commonly found in water kefir grains are shown in Table 2.1.

Table 2.1: Microbiota isolated from water kefir

Microorganisms	References
Bacteria	
<i>Acetobacter fabarum</i>	Gulitz et al., 2011; Gulitz et al., 2013
<i>Acetobacter loveniensis</i>	da Miguel et al., 2011; Magalhaes et al., 2010
<i>Acetobacter tropicalis</i>	Martinez-Torres et al., 2017
<i>Acetobacter okinawensis</i>	Martinez-Torres et al., 2017
<i>Acetobacter orientalis</i>	Gulitz et al., 2011; Martinez-Torres et al., 2017
<i>Bifidobacterium aquikefiri</i>	Laureys & De Vuyst, 2017; Verce et al., 2019
<i>Bifidobacterium crudilactis/psychraerophilum</i>	Gulitz et al., 2013; Laureys & De Vuyst, 2014
<i>Gluconobacter cerinus</i>	Laureys & De Vuyst, 2017
<i>Glucobacter liquefaciens</i>	da Miguel et al., 2011
<i>Glucobacter rosens/oxydans</i>	Laureys & De Vuyst, 2017
<i>Khuyvernomyces lactis</i>	Magalhaes et al., 2010
<i>Lactobacillus casei/ paracasei</i>	Gulitz et al., 2011; Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Martinez-Torres et al., 2017
<i>Lactobacillus buchneri/ parabuchneri</i>	da Miguel et al., 2011; Magalhaes et al., 2010
<i>Lactobacillus ghanensis</i>	Martinez-Torres et al., 2017
<i>Lactobacillus harbinensis</i>	Laureys & De Vuyst, 2014; Verce et al., 2019
<i>Lactobacillus helveticus</i>	da Miguel et al., 2011
<i>Lactobacillus hilgardii</i>	Gulitz et al., 2011; Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Martinez-Torres et al., 2017; Verce et al., 2019
<i>Lactobacillus hordei</i>	Gulitz et al., 2011; Verce et al., 2019; Xu et al., 2019a
<i>Lactobacillus kefiri</i>	da Miguel et al., 2011
<i>Lactobacillus mali</i>	Laureys & De Vuyst, 2014; Koh et al., 2018
<i>Lactobacillus nagelii</i>	Gulitz et al., 2011; Laureys & De Vuyst, 2017; Verce et al., 2019
<i>Lactobacillus satsumensis</i>	da Miguel et al., 2011
<i>Lactobacillus sunkii</i>	da Miguel et al., 2011
<i>Lactococcus lactis</i>	Magalhaes et al., 2010
<i>Leuconostoc mesenteroides</i>	Gulitz et al., 2011; Laureys & De Vuyst, 2017; Verce et al., 2019
<i>Leuconostoc citreum</i>	Gulitz et al., 2011
Yeast	
<i>Candida californica</i>	Martinez-Torres et al., 2017
<i>Dekkera bruxellensis</i>	Laureys & De Vuyst, 2014; Verce et al., 2019
<i>Hanseniaspora valbyensis</i>	Gulitz et al., 2011
<i>Kazachstania aerobia</i>	da Miguel et al., 2011
<i>Lachancea fermentati</i>	Gulitz et al., 2011
<i>Pichia caribbica</i>	da Miguel et al., 2011
<i>Pichia fermentati</i>	da Miguel et al., 2011
<i>Pichia membranifaciens</i>	da Miguel et al., 2011, Martinez-Torres et al., 2017
<i>Saccharomyces cerevisiae</i>	da Miguel et al., 2011; Gulitz et al., 2011; Laureys & De Vuyst, 2014, Laureys & De Vuyst, 2017; Verce et al., 2019
<i>Zygorulaspora florentina</i>	Gulitz et al., 2011; Laureys & De Vuyst., 2017

2.1.4 Exopolysaccharide production in water kefir

Bacteria can produce polysaccharides by the action of the enzyme glucosyltransferase (GTF), commonly called glucan sucrose (Waldherr et al., 2010). This enzyme is found in several genera of lactic acid bacteria namely *Lactobacillus* and *Leuconostoc* (Laureys & De Vuyst, 2014). The lactic acid bacteria species *L. hilgardii* is mainly associated with the production of exopolysaccharide and grain growth during water kefir fermentation (Laureys & De Vuyst, 2014; Martinez-Torres et al., 2017; Pidoux, 1989; Waldherr et al., 2010). The enzyme GTF secreted by *L. hilgardii* produces a high level of dextrans in the presence of sucrose (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Waldherr et al., 2010). GTF is activated due to bacterial acidification, therefore as the pH decreases, the exopolysaccharide (EPS) yield increases (Waldherr et al., 2010).

The water-insoluble sugary kefir grain exopolysaccharide is a homopolysaccharide composed of α 1,6-linked glucose polymer called dextran (α - glucans) (Coma et al., 2019; Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Waldherr et al., 2010). The linear dextran backbone may be branched to varying extends at the O2, O3 and O4 positions (α (1,2,6-), (1,3,6-), (1,4,6-) glucose units respectively), with additional α (1,3-) and α (1,4-) glucose units (Fels et al., 2018; Xu et al., 2018). The presence of α (1,3-) linkages in the glucan produced by *L. hilgardii* is responsible for the water-insoluble property of the kefir grains (Fels et al., 2018). However, not all kefir grain from strains of *L. hilgardii* can produce EPS (Gulitz et al., 2011; Laureys & De Vuyst, 2014)

Other EPS-producing lactic acid bacteria isolated from kefir grain include *L. nagelii*, *L. hordei*, *Lc. mesenteroides*, and *Lc. citreum* (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Stadie, 2013). The role of LAB (*L. hordei*, *L. nagelii*, and *L. mesenteroides*) in EPS production upon co-cultivation with *S. cerevisiae* was investigated where all three lactic acid bacteria produced glucan (dextran) while only *L. hordei* induced yeast aggregation on the hydrophilic slides (Xu et al., 2018). Structurally, glucans from *L. nagelii* and *L. hordei* strains showed (1,3,6-) linked glucose linkage in higher proportions than (1,4,6-) and (1,2,6-) linked glucose whereas *Lc. citreum* resulted in EPS with a higher portion of (1,4,6-) and (1,3,6-) linked glucose without the presence of any of (1,2,6-) linked glucose. Thus, *L. nagelii* and *L. hordei* show abundant branching at O3 while *Lc. citreum* shows major dextran branching at O4 along with notable branching at position O3. Therefore, this structural organisation of dextran produced by *L.*

hordei accounts for the induction of *S. cerevisiae* aggregation and network formation on the hydrophilic surface that helps in biofilm formation (Xu et al., 2018). Typical gel-producing lactic acid bacteria in water kefir are shown in Figure 2.4. The structural differences between the EPS of water kefir suggest that multiple microorganisms are involved in the production of soluble and insoluble exopolysaccharides (Fels et al., 2018; Xu et al., 2018).

The EPS concentration found in the kefir liquor for a strong EPS-producer ranges between 10-20 g/L and for a very strong EPS-producer could reach up to 32.5 g/L (Stadie, 2013). Moreover, the bacterial species *L. hilgardii* produces a very high exopolysaccharide concentration (30.8-32.5 g/L). The mass of water kefir in the grains increases during the first 24 hours of fermentation (16.4 g to 28.6 g) and the accumulation of fructose in the water kefir liquor indicates that the grains contain glucan (Laureys and De Vuyst, 2014). *L. hilgardii* showed a 2.7-fold increase in the wet weight of kefir grains during 96 h of fermentation (Martinez-Torres et al., 2017).

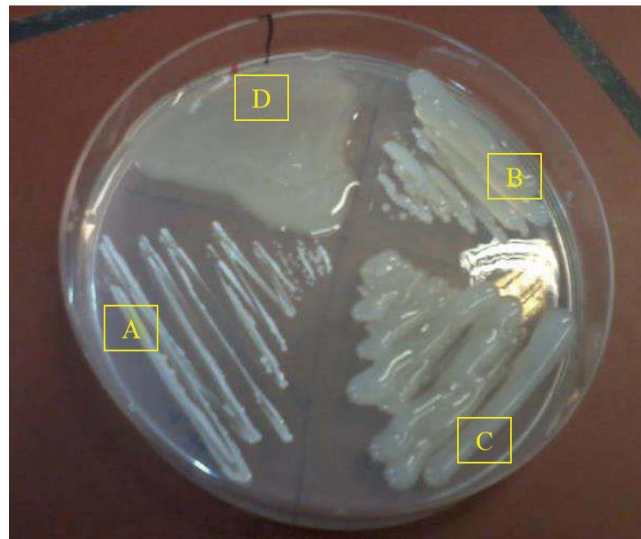


Figure 2.4: EPS production from lactic acid bacteria in water kefir grains

Notes: A = no EPS production (*L. nagelii*); B = slight EPS production (*L. nagelii*); C = strong EPS production (*L. hordei*); D = very strong EPS production (*Lc. citreum*) on sucrose containing agar plate.

Source: Stadie. (2013)

2.1.5 Interaction between the microbiotas of kefir grain

Lactic acid bacteria and yeast are the microbes most commonly found during water kefir fermentation (Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Stadie et al., 2013). The first studies investigating the interactions between lactic acid bacteria *L. hilgardii* and yeast *S. florentina* in water kefir suggested parasitism (Leroi & Pidoux, 1993a; Leroi and Pidoux, 1993b). The *S. florentina* promotes the growth of *L. hilgardii* by acidification and releasing metabolites such as carbon dioxide, pyruvate, propionate, acetate, and succinate while the growth and alcoholic fermentation of *S. florentina* reduced significantly after 72 h in the presence of *L. hilgardii*. The species *Saccharomyces florentina* had been re-classified as *Zygorulasporea florentina* (Kurtzman, 2003). In addition to this, negative interactions between the *L. hilgardii* and *Candida lambica* were observed (Leroi & Pidoux, 1993b). Contrary to this, recent studies indicate a mutual relationship between lactic acid bacteria and yeast upon co-cultivation (Stadie et al., 2013; Xu et al., 2019b)

The mutual relationship of dominant species of LAB (*L. hordei* and *L. naghelii*) and yeast (*Z. florentina* and *S. cerevisiae*) in water kefir are shown in Figure 2.5. In co-cultivation, the lactic acid bacteria induces yeast (*Z. florentina* and *S. cerevisiae*) to support LAB (*L. hordei* and *L. nagelii*) by the production of essential amino acids, vitamin B₆ and arginine. The lactic acid bacteria *L. hordei* and *L. nagelii* are auxotrophic for some amino acids and vitamin B₆, respectively in pure culture. In return, the LAB supports the growth of yeast by the acidification of the medium through the production of lactic acid and acetic acid. However, co-cultivation with *Z. florentina* had a higher impact on the growth of LAB than *S. cerevisiae* indicating *Z. florentina* as a dominant yeast in the water kefir grains (Stadie et al., 2013). Similarly, in another study co-cultivation of *L. hordei* and *S. cerevisiae* showed a mutualistic relationship (Xu et al., 2019b). Water kefir represents a complex and demanding habitat for the growth of lactic acid bacteria with high sugar concentration and a low concentration of essential nutrients. The microorganisms (LAB and yeast) in water kefir adapt to a symbiotic and environmental relationship for their survival (Stadie et al., 2013; Xu et al., 2019a; Xu et al., 2019b).

Figure 2.5: Microbial interactions of representative cultivable water kefir isolates

Source: Stadie et al. (2013).

2.1.6 Sugar metabolism in water kefir

i. Lactic acid bacteria

Lactic acid bacteria are a heterogeneous group of microorganisms characterised as gram-positive, non-sporing and catalase-negative that produce lactic acid as the main metabolic end-product during sugar fermentation (Imran et al., 2012; Reddy et al., 2008). The lactic acid produced helps to prevent the growth of pathogenic and spoilage microorganisms in fermented food products. Therefore, the ability to prolong the shelf life of fermented products makes lactic acid bacteria of particular interest in fermentation (Ravyts et al., 2012). LAB have been granted ‘Generally Regarded As Safe’ (GRAS) status and contains genera such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Streptococcus*, *Oenococcus*, *Pediococcus* and *Weissella* (Ravyts et al., 2012; Reddy et al., 2008). In water kefir, typical LAB species belonging to the genera *Lactobacillus*, *Leuconostoc*, and *Lactococcus* can be found. (Gulitz et al., 2013; Marsh et al., 2013; Waldherr et al., 2010).

Metabolism of sugars by lactic acid bacteria is divided into three groups i.e. homofermentative/homolactic fermentation, heterofermentative/heterolactic fermentation and facultative heterofermentative. Homofermentative LAB (*Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus* and some *Lactobacillus*) metabolise hexoses via glycolysis/Embden-Meyerhof-Parnas’s pathway (EMP) and lactate dehydrogenase (LDH) producing lactic acid as the

sole end-product (more than 85% lactic acid from glucose) (Carballo, 2012; Ganzle, 2015; Stadie, 2013; Reddy et al., 2008). On the other hand, heterofermentative LAB (*Leuconostoc*, *Oenococcus*, and some *Lactobacillus* strains) metabolise hexoses and pentoses via Pentose phosphate pathway (PKP) and reduction of acetyl-P producing lactate (50% from glucose), ethanol and CO₂ (Carballo, 2012; Ganzle, 2015; Reddy et al., 2008; Zaunmuller et al., 2006). Carbohydrate metabolisms by homofermentative and heterofermentative lactic acid bacteria are depicted in Figure 2.6.

Figure 2.6: Sugar metabolism by lactic acid bacteria

Source: Carballo. (2012)

Facultative heterofermentative metabolism means homo or heterolactic fermentation depending on the abundance of fermentable sugar in the solution where microorganisms (*L. hordei* and *L. plantarum*) exhibit homolactic fermentation when the culture medium has a high concentration of carbohydrates. However, in limited sugar concentration, the homolactic behaviour is shifted to heterolactic fermentation. This shift in fermentation from homofermentative to heterofermentative metabolism, also known as mixed-acid fermentation, depends on the fructose-1,6-bisphosphate concentration. Fructose-1,6-bisphosphate activates LDH in the lactic acid bacteria and inhibits nicotinamide adenine dinucleotide phosphate (NADP)-dependent glucose-6-phosphate dehydrogenase. At low concentration of fructose-1,6-bisphosphate, the heterofermentative pathway might take over in the homofermentative species (Carballo., 2012; Garrigues et al., 1997; Zannmuller et al., 2006). The lactic acid bacteria of water kefir belonging to the above-mentioned three groups of sugar metabolism are shown in Table 2.2.

Table 2.2: LAB species found in water kefir

Homofermentative LAB	Heterofermentative LAB	Facultative Heterofermentative LAB
<i>L. casei</i>	<i>L. hilgardii</i>	<i>L. hordei</i>
<i>L. nagelii</i>	<i>Lc. citreum</i>	<i>L. plantarum</i>
<i>L. hordei</i>	<i>Lc. Mesenteriodes</i>	<i>Lc. lactis</i>
<i>L. satsumensis</i>		
<i>Lc. Lactis</i>		

Source: Garrigues et al. (1997); Stadie. (2013); Xu et al. (2019 a); Zannmuller et al. (2006)

ii. Yeast

Typical species of yeast found in water kefir are *S. cerevisiae*, *Z. florentina*, and *D. bruxellensis* (Gulitz et al., 2013; Laureys & De Vuyst, 2017; Magalhaes et al., 2010). The *S. cerevisiae* commonly found in water kefir grains is a crab-tree positive, facultative anaerobic yeast which can metabolise glucose both aerobically and anaerobically (van Dijken et al., 1993; Walker & Stewart, 2016). In yeast, glucose is converted into pyruvate through glycolysis which can be degraded either through the respiration pathway or fermentation pathway. In the respiration pathway, pyruvate dehydrogenase (PDH) catalyses the conversion of pyruvate into Acetyl-coenzyme A, which is then oxidized to CO₂ via the tricarboxylic acid (TCA) cycle. While in the fermentation pathway, pyruvate is converted to acetaldehyde by the action of pyruvate decarboxylase (PDC) and further reduced to ethanol by alcohol dehydrogenase (ADH) (Pfeiffer & Morley, 2014; Van Dijken et al., 1993; Rodrigues et al., 2006). The sugar metabolism of yeast is shown in Figure 2.7.

Figure 2.7: Carbohydrate metabolism in yeast

Source: van Dijken et al. (1993)

2.1.7 Physicochemical and microbiological changes in water kefir beverage during fermentation

i. pH and organic acids

During the fermentation of water kefir, as a function of time, the correlation between the pH and the organic acids are inversely proportional to each other where a decrease in pH results in the increased production of organic acids (da Costa et al., 2018; Jayabalan et al., 2014). The pH of water kefir decreases from 5.31 to 3.47 after 48 h and then slowly decreases to 3.26 after 120 h of fermentation while the acetic acid increases from 1.21 g L⁻¹ to 2.66 g L⁻¹ after 48 h and the lactic acid increases from 0.03 g L⁻¹ to 10.96 g L⁻¹ after 120 h (Tu et al., 2019). Previous studies suggest that the rate of pH decrease is controlled by the fermentation temperature (Azi et al., 2021; Puerari et al., 2012), buffering capacity and calcium concentration of the fermentation medium (Laureys et al., 2018; Laureys et al., 2019). Whereas the substrate type affects the concentration of organic acids (Corona et al., 2016; Ozcelik et al., 2021; Randazzo et al., 2016).

ii. Sugar and total soluble solids (TSS, °Brix)

As the fermentation of water kefir proceeds, the concentration of sugar and total soluble solids (°Brix) decreases (Corona et al., 2016; Magalhaes et al., 2010; Randazzo et al., 2016). The main substrate, sucrose, is hydrolysed into glucose and fructose by the action of yeast invertase thus, increasing the concentration of the reducing sugar while decreasing the sucrose concentration (Magalhaes et al., 2010; Pfeiffer & Morley, 2014; Stadie, 2013; Tu et al., 2019). The concentration of sucrose in Brazilian water kefir decreased (40 mg/mL to 28 mg/mL) after 24 h of fermentation, while glucose (1mg/ml to 35 mg/ml) and fructose concentrations (1.5mg/ml-38mg/ml) increased (Magalhaes et al., 2010). Similar results have been previously reported for water kefir (Laureys & De Vuyst, 2014; Stadie, 2013; Tu et al., 2019). However, previous studies suggest that monosaccharide reduction and decrease in TSS of water kefir beverages depends on substrate type (Corona et al., 2016; Randazzo et al., 2016; Tu et al., 2019) and fermentation temperature as the higher the fermentation temperature, the greater the metabolism of sugars (higher reduction in TSS and sucrose) in the beverage samples (Loncar et al., 2006; Mu, 2019; Sabokbar & Khodaiyan, 2015; Subardjo, 2017).

iii. Colour

The colour parameters of water kefir beverages change during fermentation, with the lightness (L^*) increasing during fermentation (Corona et al., 2016; Randazzo et al., 2016; Subardjo, 2017). Variation in the increase and decrease of colour parameter a^* (redness-greenness) and b^* (yellowness-blueness) has been observed in previous studies of water kefir (Corona et al., 2016; Randazzo et al., 2016; Tireki, 2022). The change of colour may occur due to the biotransformation or degradation of polyphenols (thearubigin and theaflavins) into smaller compounds in the acidic environment by the action of enzymes excreted by the microorganisms (Chu & Chen, 2006; Jayabalan et al., 2007; Jayabalan et al., 2014).

iv. Ethanol and volatile compounds

Sugar metabolism during water kefir fermentation by the microbiota embedded in the kefir grains leads to the formation of ethanol, sugar alcohols and various volatile compounds (Koh et al., 2018; Laureys & De Vuyst, 2014; Loncar et al., 2013; Magalhaes et al., 2010; Ozcelik et al., 2021; Verce et al., 2019). Ethanol concentrations of between 1-4% (v/v) have been reported for kefir beverages (Dwiloka et al., 2020; Randazzo et al., 2016; Setyawardani et al., 2014). Water kefir beverages prepared using fruit juices have been reported to have ethanol concentrations of between 1.03-4.96 % (v/v) (Randazzo et al., 2016) and vegetable based-water kefir beverages between 0.09-3.0 % (v/v) after 48 h of fermentation (Corona et al., 2016). The concentration of ethanol produced during water kefir fermentation depends on factors such as substrate used, substrate concentration and fermentation temperature. For example, water kefir beverages prepared at 25°C contained 3.6 % ethanol (v/v) while that prepared at 10°C had lower ethanol of only 0.36 % (v/v) (Puerari et al., 2012). The Australia New Zealand Food Standard (FSANZ) stipulates that water kefir should not contain more than 1.15 % alcohol by volume (FSANZ, 2022; Cufaoglu & Erdinc, 2023; Moretti et al., 2022). Therefore, further research is required to produce products with consistently low alcohol that meet the stipulated regulations.

Apart from ethanol, sugar alcohols such as mannitol have also been found in water kefir ranging between 1.0-8.0 g L⁻¹ after 72 h of fermentation (Stadie et al., 2013). Several volatile organic compounds including acids, alcohols, aldehydes, esters, ketones, phenols, sulphur compounds, aromatic hydrocarbons and terpenes have been detected following the fermentation of water kefir (Corona et al., 2016; Laureys & De Vuyst, 2014; Randazzo et al., 2016). Of these, esters such as

isoamyl acetate, ethyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate produced by the yeast are thought to contribute the fruity/floral aroma of the water kefir (Corona et al., 2016; Laureys and De Vuyst, 2014; Laureys and De Vuyst, 2017; Laureys et al., 2021).

v. **Viable cells of lactic acid bacteria and yeast**

As previously mentioned in Section 2.1.3, the microbial composition of kefir grain is complex, unique, and diverse depending on the country of origin and fermentation conditions. However, lactic acid bacteria and *S. cerevisiae* are most frequently found in water kefir (da Miguel et al., 2011; Gulitz et al., 2011; Marsh et al., 2013). During fermentation, an increase in the viable cell count of LAB and yeast has been observed in Brazilian water kefir beverage after 24 h of fermentation where the initial count of LAB increases from 6.82 log CFU/ml to 8.32 log CFU/ml while that of yeasts increases from 5.63 log CFU/ml to 7.31 log CFU/ml (Magalhaes et al., 2010). An increase in viable cell counts of LAB and yeast have been reported in previous studies of water kefir (Corona et al., 2016; da Miguel et al., 2011; Gulitz et al., 2011; Laureys et al., 2018; Randazzo et al., 2016). However, variations in the microbial count of LAB and yeast from the eight regions of Brazilian kefir grains have been observed ranging between 6.04±0.03-9.18±0.01 log CFU/g and 5.92±0.08-8.30±0.01 log CFU/g respectively (da Miguel et al., 2011). In addition to the place of origin, fermentation temperature also influences the viable count of microorganisms in kefir beverage where higher temperature results in higher viable cell counts of kefir microorganisms (Darvishzadeh et al., 2021; Sabokbar & Khodaiyan, 2015)

2.1.8 Health Benefits of water kefir

A. Functional foods and Probiotics

Functional foods provide additional health benefits along with basic nutrition due to the presence of bioactive compounds and probiotics (Mantzourani et al., 2019). Probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit to the host” (FAO/WHO, 2002). Additionally, the live microorganism needs to be present in sufficient amounts in the food ($\approx 10^6$ – 10^7 cfu/g) to exhibit their beneficial properties (Terpou et al., 2019). Regular consumption of probiotics is associated with various health benefits such as good heart health by the reduction in cholesterol, improved immune system response, anti-carcinogenic and anti-hypersensitive system (Mantzourani et al., 2018; Rasika et al., 2021). Most probiotic

microorganisms are lactic acid bacteria belonging to *Lactobacillus* spp. and *Bifidobacteria* (Rasika et al., 2021), although other types of microorganisms are also identified namely *Enterococcus faecalis*, *Lactococcus lactis*, and *Saccharomyces boulardii* (Istrati et al., 2018). Commonly found probiotic species in water kefir are *Bifidobacterium psychraerophilum/crudilactis* (Gulitz et al., 2013; Laureys & De Vuyst, 2014; Laureys et al., 2016; Marsh et al., 2013), *S. cerevisiae* and lactic acid bacteria species (*L. harbinensis*, *L. paracasei*, *L. plantarum*, *L. mali*, and *Lactococcus lactis*) (da Miguel et al., 2011; Gulitz et al., 2011; Koh et al., 2018; Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Marsh et al., 2013; Talib et al., 2019). *In-vitro* studies of *Lactobacillus mali* K8 isolated from water kefir grains showed potential probiotic activity of high α -glucosidase inhibitory activity (antidiabetic), antibiotic resistance and resistance to gastrointestinal stress (Koh et al., 2018). Similarly, probiotic activity in lactic acid bacteria (*L. harbinensis*, *L. paracasei* and *L. plantarum*) isolated from Malaysian water kefir has also been reported (Talib et al., 2019).

B. Antioxidant Activity

Antioxidant activity of water kefir is attributed to the presence of probiotic, symbiotic, consortium of multispecies, organic acids and bioactive compounds (flavonoids, peptides, and phenolic acids) (Alsayadi et al 2013; Kumar et al., 2021; Mechmeche et al., 2019; Talib et al., 2019; Tu et al., 2019). Production of bioactive peptides (amides and aromatic compounds) in water kefir prepared from a substrate of tomato seed protein isolate has been previously reported and the medium also displayed high radical scavenging activity (Mechmeche et al., 2019). Similarly, high antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing power) is also observed in water kefir beverage and isolated ESP respectively from *Acetobacter pasteurianus* (Luang-In et al., 2018). In another study, a high antioxidant activity (DPPH radical scavenging activity 9.88-63.17%, ascorbate oxidation inhibition 6.08-25.57%, ferric reducing power 0.315-1.969) was observed in water kefir sample concentration of 0.125-5 mg/ml in which antioxidant activity is directly proportional to the concentration of kefir sample (Alsayadi et al 2013). Similarly, a high antioxidant capacity in water kefir beverages has been previously observed with different substrates such as soy-based (Azi et al., 2021; Tu et al., 2021), Russian olive water kefir (Darvishzadeh et al., 2021), fruit juice-substrate (Cornelian cherry, hawthorn, red plum, roseship and pomegranate) (Ozcelik et al., 2021) and milk based green or black tea kefir (Karagozlu et al., 2017).

C. Antimicrobial activity

The antimicrobial activity of water kefir is associated with the production of compounds such as bacteriocins, organic acids, ethanol, and bioactive compounds by the microbes during fermentation (Karagozlu et al., 2018; Rodrigues et al., 2005; Silva et al., 2009). Water kefir grains have been reported to exhibit antimicrobial activity and inhibit the growth of a wide range of pathogenic microorganisms such as *Bacillus cereus*, *Candida albicans*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella Typhi*, *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus salivarius* (Karagozlu et al., 2018; Rodrigues et al., 2005; Silva et al., 2009). The antimicrobial activity of water kefir beverage prepared using molasses, demerara sugar and brown sugar was studied where brown sugar showed the highest antimicrobial activity against the pathogenic microorganisms and the results also suggest that the size of inhibition halo of pathogenic microorganisms is influenced by the duration of the fermentation period, type of carbohydrate source and concentration of sugar (Silva et al., 2009). Similarly, antimicrobial activity in dairy-based kefir supplemented with 2% and 4% of tea infusion (black tea and green tea) were studied during 21 d of cold storage where green tea kefir (2%) showed higher antimicrobial activity (Karagozlu et al., 2018). The inhibition of the fungus *Aspergillus flavus* by water kefir in ensiled sorghum grains has also been demonstrated previously (Gonda et al., 2019).

2.2 Tea

Tea is the second most widely consumed non-alcoholic beverage worldwide after water (Bosch et al., 2015; Jiang et al., 2019; Prasanth et al., 2019). Tea is an infusion produced from different parts of the plant (leaves, buds, or delicate stems) *Camellia sinensis* (L) O. Kuntze. (Ji et al., 2017; Prasanth et al., 2019). Originating from China and India, tea is grown in over 30 countries with favourable tropical and subtropical climatic conditions (Juneja et al., 2013; Reygaert, 2018; Saeed et al., 2017; Wilson and Clifford, 2012; Xing et al., 2019). Approximately three billion kilograms of tea is produced and consumed worldwide per year (Hayat et al., 2015; Khan & Mukhtar, 2013), with China, India, Kenya and Sri Lanka being the major producers (Chang, 2015; Prasanth et al., 2019).

Tea was used in traditional Chinese medicine (2737 BC) before being actively adopted as a beverage (Saeed et al., 2017; Vuong, 2014; Yang & Liu, 2013). Additionally, tea is considered a functional beverage containing polyphenols, caffeine, minerals, and trace amounts of vitamins, amino acids, and carbohydrates. However, the amounts of these secondary metabolites in the tea vary depending on the type of tea (Hamilton-Miller, 1995; Wei et al., 2018; Weisburger, 1997; Wierzejska, 2014; Wilson and Clifford, 2012). Previous studies suggest that regular consumption of tea is associated with health benefits such as maintenance of the cardiovascular system, weight loss, and decreasing the risk of cancer and neurodegenerative diseases (Chacko et al., 2010; Lorenzo & Munekata, 2016; Wierzejska, 2014; Wilson and Clifford, 2012; Zhou et al., 2016). Tea is commonly used in the preparation of the fermented beverage kombucha as its nutrient content helps support the growth of the kombucha starter culture (Jayabalan et al., 2007; Jayabalan et al., 2014).

Apart from the growth environment, horticultural and harvesting practices, processing of tea leaves (drying and fermentation methods) and degree of oxidation play a crucial role in the quality and type of tea being produced (Cabrera et al., 2006; Chacko et al., 2010; Pervin et al., 2018). Based on the manufacturing process, teas are classified into five categories comprising six types, namely, non-fermented (green tea and yellow tea), slightly fermented (white tea), semi-fermented (oolong tea), fully fermented (black tea), and post-fermented (dark tea) (Jiang et al., 2019; Rao & Ramalakshmi, 2011; Reygaert, 2018; Tan et al., 2017). Among the six types of tea, black tea, green tea, and oolong tea are the most popular. About 78% of tea produced

worldwide is black tea which is widely consumed in Western countries followed by green tea (20%) which is popular in Asia, the United States and Europe. The remaining 2% of production belongs to oolong tea, particularly popular in China and Taiwan (Chacko et al., 2010; Juneja et al., 2013; Yang & Liu, 2013).

2.2.1 Green tea

Green tea has become increasingly popular among consumers and researchers due to its various health properties including anti-cancer, anti-obesity, anti-diabetes, anti-inflammatory, anti-microbial, anti-photoaging, neuroprotection, stimulation of the cardiovascular system and central nervous system (Abe & Inoue, 2021; Bosch et al., 2015; Chacko et al., 2010; Hamilton-Miller, 1995; Pervin et al., 2018; Prasanth et al., 2019; Rao & Ramalakshmi, 2011; Reygaert, 2018; Xing et al., 2019). The health benefits of green tea consumption are well-researched and are attributed to its chemical composition including polyphenolic compounds, especially flavanols, also known as catechins (Musial et al., 2020; Pervin et al., 2018; Prasanth et al., 2019). Green tea is a type of unfermented tea manufactured by heating or steaming the freshly harvested tea leaves at a high temperature to inactivate the heat-labile enzyme polyphenol oxidase thereby preventing oxidative fermentation and breakdown of colour pigments followed by pressing and drying. As a result, the natural structure of phenolic polyphenol compounds as well as the overall composition in green tea are maintained (Abe & Inoue, 2021; Chacko et al., 2010; Prasanth et al., 2019; Xing et al., 2019). The manufacturing process of green tea is shown in Figure 2.8. Green tea is considered the most predominant source of catechins among different types of teas, and other dietary foods such as chocolate, red grapes, wine, and apples (Bancirova, 2010; Cabrera et al., 2006; Zaveri, 2006).

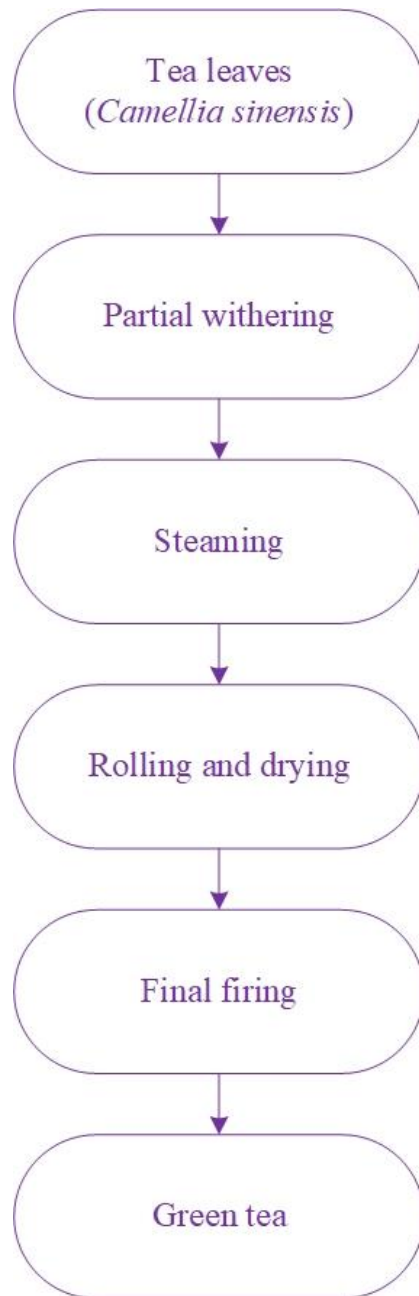


Figure 2.8: Manufacturing process of green tea

Source: Cabrera et al. (2006)

2.2.2 Chemical composition of green tea

The chemical composition of green tea varies depending on the climate, season, variety, age of leaves, horticultural practices, and position of the tea leaf on the harvested shoot (Cabrera et al, 2006; Musial et al., 2020; Prasanth et al., 2019; Siniya & Mishra, 2008). The chemical composition of green tea is complex comprising more than ten groups of compounds including polyphenols (30% dry weight), and protein (15-20% dry weight) mainly from enzymes and amino acids (4-7 % dry weight). In addition to the chemical compounds shown in Table 2.3, green tea contains trace amounts of sterols (stigmasterol), vitamins (A, B₂, B₃, C, E and K), phenolic acids (gallic acid, chlorogenic acid and caffeic acid) and volatile compounds such as aldehydes, alcohol, esters, and hydrocarbons (Cabrera et al., 2006; Chacko et al., 2010; Musial et al., 2020; Prasanth et al., 2019). Of these, catechins belonging to the polyphenol group play a crucial role in conferring the biological activity of tea (Bansal et al., 2013). The addition of green tea in water kefir fermentation can provide amino acids, micronutrients and catechins helping in the growth of kefir grain microbiota and improving the nutritional availability of kefir beverages.

Table 2.3: Chemical composition and compounds in green tea

Chemical composition	Chemical compound	% Dried weight of tea leaf
Polyphenols	Catechins Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (ECG), Epigallocatechin gallate (EGCG), Gallocatechin (GC), Galocatechin gallate (GCG)	30
	Flavonols Caempherol, Chlorogenic acid, Coumarylquinic acid, Quercetin, Myricetin, Theogallin	
Fibre		26
Proteins	Enzymes	15-20
Amino acids	Theanine or 5-N-ethylglutamine, Glutamic acid, Tryptophan, Glycine, Serine, Aspartic acid, Tyrosine, Valine, Leucine, Threonine, Arginine, Lysine	4
Carbohydrates	Glucose, Fructose, Sucrose, Cellulose, Pectin	7
Lipids	Linoleic and a-linolenic acids	7
Minerals	Calcium, magnesium, chromium, manganese, Iron, Copper, Zinc, Molybdenum, Selenium, sodium, phosphorous, cobalt, nickel, potassium, fluorine, and aluminium	5
Alkaloids	Caffeine, theobromine and theophylline	3-4
Pigments	Chlorophyll, carotenoids	2

Source: (Cabrera et al, 2006; Chacko et al., 2010; Musial et al., 2020; Prasanth et al., 2019).

i. 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 (Alcazar et al., 2007; Chacko et al., 2010). There are 26 kinds of free amino acids found in tea leaves (Li et al., 2017) among which theanine or 5-N-ethyl glutamine ($\geq 50\%$) (Alcazar et al., 2007; Juneja et al., 1999), arginine, glycine, leucine, lysine, serine, threonine, tryptophan, tyrosine, valine, aspartic acid and glutamic acid are commonly found in green tea (Cabrera et al., 2006; Chacko et al., 2010; Li et al., 2017). These amino acids provide an important nitrogen source for the growth of lactic acid bacteria and yeast during fermentation.

L-theanine (glutamic acid γ -ethyl amide; 5-N-ethyl glutamine) the predominant amino acid unique to green tea was discovered in 1949 and is responsible for the exotic and umami taste of tea (Jiang et al., 2019; Saeed et al., 2017). Theanine is available in the free form (non-protein) which is an important amino acid for the biosynthesis of polyphenols (Alcazar et al., 2007). Additionally, available scientific studies (*in vitro*, *in vivo* and *ex vivo*) speculate that consumption of L-theanine is presumably associated with several health benefits (Figure 2.9) of promoting physiological, neurological and biological functions such as anti-tumour properties (Liu et al., 2009; Zhang et al., 2013), stress management (Juneja et al., 1999; Kimura et al., 2007; Lu et al., 2004) and exhibiting neuroprotective functions (Cho et al., 2008; Kim et al., 2009; Sumathi et al., 2015; William et al., 2020). Therefore, improving cognitive functions, mood and concentration (Juneja et al., 1999; Saeed et al., 2017; William et al., 2020).

Figure 2.9: Beneficial effects associated to green tea's amino acid theanine
Source: Saeed et al. (2017)

ii. Phenolic polyphenols

Phenolic polyphenols, including flavanols and flavonoids, are the most abundant constituents in green tea, representing 30% dry weight of leaves (Chacko et al., 2010; Musial et al., 2020; Jakubczyk et al., 2020). Catechins (flavan-3-ols) belonging to the group of flavonoids are the major bioactive polyphenolic compound found in green tea which is presumably associated with various health benefits such as antioxidant activity, anti-tumour, anti-cancer, anti-diabetic, anti-obesity, antiphotaging, anti-hypertensive, anti-inflammatory, anti-proliferative, anti-thrombogenic, antimicrobial and confers protection against neurodegenerative and cardiovascular diseases. (Abe & Inoue, 2021; Chacko et al., 2010; Cabrera et al., 2006; Musial et al., 2020; Pervin et al., 2018; Prasanth et al., 2019; Reygaert, 2018; Xing et al., 2019).

There are four major catechins found in green tea namely (–)-epigallocatechin-3-gallate (EGCG), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epicatechin (EC) (Musial et al., 2020; Pervin et al., 2018). The structure of green tea catechins is shown in Figure 2.10. Among these, EGCG is the most abundant and well-researched bioactive catechin, making up 60% of the total catechins in green tea, followed by EGC (~20%), ECG (~14%), and EC (6%) (Cabrera et al., 2006; Reygaert, 2018). However, the brewing conditions such as infusion time and temperature of the water make a difference to the content of polyphenols, in particular, catechins present in tea. (Cabrera et al, 2006; Musial et al., 2020; Oh et al., 2021; Reygaert, 2018). According to the European Food Safety Authority (EFSA), 126 mg of catechins are present per 100 mL of green tea (Prasanth et al., 2019).

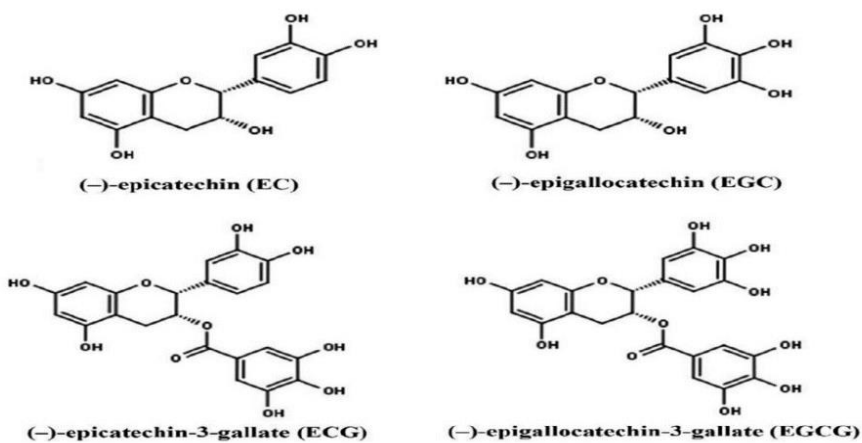


Figure 2.10: Structure of catechins found in green tea
Source: Li et al. (2020)

iii. Alkaloids

The predominant alkaloids in green tea leaves are methylxanthines including caffeine (1,3,5-trimethylxanthine), and two minors isomeric dimethylxanthines (theobromine and theophylline) together making up 3-4% (dry weight) of the total composition (Chacko et al., 2010) with approximately 1.5-4% caffeine, 0.2-0.4% theobromine and ~0.02% theophylline (Rao & Ramalakshmi, 2011; Schulz et al., 1999). The structure of alkaloids is shown in Figure 2.11.

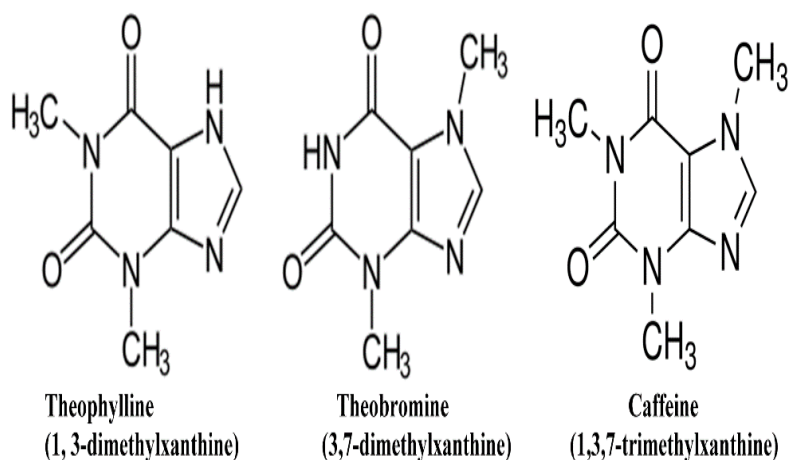


Figure 2.11: Chemical structure of methylxanthines

Source: Johnson et al. (2012)

Caffeine acts on the central nervous system, stimulates alertness, decreases fatigue and facilitates the association of ideas (Cabrera et al., 2006; Monteiro et al., 2016). Some of these activities caused by caffeine are influenced by theophylline which induces psychoactive activity, vasodilatory effects, and respiratory stimulant effects (Cabrera et al, 2006; Jankech et al., 2019). Additionally, caffeine in combination with L-theanine improves brain function, cognition, and mood (Kim et al., 2009; Saeed et al., 2017). Theobromine is a bitter alkaloid commonly found in the cacao plant (Xia et al., 2013). Although, theobromine is chemically similar to the other two methylxanthines its stimulating effect on the central nervous system is considerably less (Jankech et al., 2019).

iv. Phenolic acids

The phenolic acids present in green tea include gallic acid (GA; Figure 2.12), quinic, chlorogenic acid and caffeic acid, of which gallic acid (3,4,5-trihydroxybenzoic acid) has the most important role (Cabrera et al., 2006; Dufresne & Farnworth, 2001). Gallic acid has strong antioxidant properties and exhibits radical scavenging activity, metal ion chelation, inhibits lipid peroxidation, maintains endogenous defence systems, and shows anti-cancer properties (Badhani et al., 2015; Cabrera et al., 2006). The gallic acid content is higher in black tea as compared to green tea due to the fermentation process during which gallic acid is liberated from catechins (Cabrera et al., 2006; Jiang et al., 2019; Kongpichitchoke et al., 2016).

Figure 2.12: Chemical structure of gallic acid

Source: Fernandes & Salgado. (2016)

2.3 Summary

Water kefir is a probiotic, lactose-free, self-carbonated, artisanal beverage prepared by inoculating kefir grains into a sugary solution. The water kefir grains consist of a unique and complex consortium of symbiotic microorganisms that varies from country to country. The physico-chemical and organoleptic characteristics of water kefir are controlled by factors such as inoculum concentration, nutrient concentration, substrate type and fermentation conditions. The complexity of microbial interactions in kefir grains and their versatility to ferment various substrates make water kefir, of particular interest for research and innovation. Previous studies have shown that the utilisation of high concentrations of added sugar (5-30%, w/v) and fruit-based substrates (fermentable sugars) result in increased production of ethanol, above the stipulated regulations for non-alcoholic beverages. Therefore, the exploration of novel substrates and the reduction of added sugar concentration is an area of interest in reducing ethanol levels in water kefir. In this regard, tea-based substrates especially green tea should be explored in water kefir fermentation as a substitute for fruit and vegetable-based substrates. Green tea is rich in polyphenolic compounds (catechins), amino acids, phenolic acids, and micronutrients. The presence of these bioactive compounds in green tea is associated with health benefits such as anti-diabetes, anti-inflammatory, anti-photoaging and neuroprotection. Therefore, the incorporation of green tea in water kefir may help promote the growth of kefir grain microbiota and improve the nutritional composition of the kefir beverage. In comparison to fruit-based substrates, green tea does not naturally contain fermentable sugars. This allows the producer to control a fermentation factor that being the amount of sugar added, thus facilitating the production of a low-sugar, non-alcoholic water kefir beverage.

3. Materials and Methods

3.1 Experimental design

The development of a green tea water kefir beverage for optimising the sucrose concentration and fermentation temperature was conducted in three-integrated experimental phases.

Phase I: Propagation and microbial enumeration of water kefir grains

Phase I consisted with the enumeration of the kefir starter culture and propagation of the water kefir grains (Section 3.3.1). Microbial analysis of the kefir grains was conducted to determine the presence of lactic acid bacteria and yeast *S. cerevisiae* (Section 3.3.4-i). The LAB and the yeast *S. cerevisiae* were enumerated on de Man, Rogosa and Sharpe (MRS) agar and yeast extract glucose chloramphenicol (YGC) agar, respectively (Appendix B). All samples were analysed in duplicate, and the experiments were replicated twice.

Phase II: Effect of sugar concentration and temperature on the fermentation of green tea water kefir beverage

In phase two, four formulations were prepared using the multi-level general full factorial design of Minitab version 19 statistical software (Minitab Inc., Pennsylvania State University, USA). Phase two investigated the effect of sugar (sucrose) concentration and fermentation temperature to select the most promising formulation for producing a novel low sugar, shelf-stable green tea water kefir beverage with characteristic physico-chemical and sensory properties. Physico-chemical characteristics included pH, total soluble solids (TSS), titratable acidity (T.A.), and colour. LAB and *S. cerevisiae* of the four water kefir formulations were also determined. Aliquots (30 mL) of each of the four formulations of the water kefir beverage were withdrawn at an interval of 24 h during fermentation for physico-chemical and microbial analysis (Section 3.3.3, Section 3.3.4 respectively). A consumer sensory analysis of the fermented beverage was conducted (Section 3.3.5). The fermentation conditions consisted of two sugar concentrations (3% and 4%, w/v), two fermentation temperatures (22°C and 25°C) while the primary fermentation period (0-72 h) and bottling fermentation period (72-144 h) were kept constant. The General Linear Model analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) between the means of the data and the Tukey's test was used to separate significant results. All the samples were analysed

in duplicate, and the experiments were replicated twice. The experimental parameters of the four formulations of water kefir beverages are shown in Table 3.1.

Table 3.1 Experimental conditions for preparation of water kefir beverages.

Formulation #	Sample code	Fermentation temperature (°C)	% Sucrose (w/v)
1	WK1-3/22	22	3
2	WK2-4/22	22	4
3	WK3-3/25	25	3
4	WK4-4/25	25	4

Phase III: Analysis of final green tea water kefir beverage during fermentation and storage (4°C)

The most promising formulation from phase two was selected and prepared for use in phase three (final) to determine the stability of the product during storage at 4°C for two weeks. The samples were analysed for microbiological and physico-chemical characteristics as previously described in phase II. Additionally, the fermented green tea water kefir beverage was analysed for antioxidants, organic acids, sugar concentration, and ethanol content during fermentation for 144 h and storage for two weeks.

3.2 Raw materials

Activated starter culture (water kefir grains) was supplied by Forage and Ferment Limited (Auckland, NZ). Organic green tea was purchased from Naturally Organic, Albany, New Zealand; brown sugar (Chelsea Refinery, Auckland, New Zealand), organic medjool dates (Bard Valley Natural Delights, USA) (Appendix A) and ginger was purchased from Pak 'n' Save, Albany, New Zealand.

3.3 Methodology

3.3.1 Propagation of water kefir grains (Phase I)

In this study, water kefir grains were propagated according to Laureys & De Vuyst. (2014) with minor modifications and information given by Forage and Ferment Limited, Auckland, NZ. The culture was propagated by continuous back-slopping until the desired quantities of grains were obtained.

One litre of potable water was heated at 90°C using a kettle (Necessities, China). Unpurified sugar (50 g) was added into a litre of food grade plastic beaker (Thermo fisher, NZ). Then the hot water was transferred making the volume to 1 litre. The sugar was dissolved completely by stirring using a clean wooden or stainless spoon as recommended by the supplier. The mixture was cooled to room temperature (22°C) and the temperature of the sugared solution was measured using a temperature thermocouple (Fluke 51, Fisher Scientific, NZ). The cooled sugared solution (50 g/L) was transferred to one-litre clip-top glass jar (Living & Co, NZ) and water kefir grains (100 g/L) were added to the jar. Following the same procedure, another one-litre glass jar with sugared solution was prepared and the two formulations were fermented simultaneously at 22°C and 25°C for 24 h in the water bath (Grant, Global Science, NZ). Thereafter, the kefir grains were separated using a nylon kitchen strainer (200 mesh) (Muskaan, India) and the liquid was discarded. The propagation process was repeated twice to ensure strong adaptation and increased biomass of the kefir grains (Gulitz et al., 2011). Once the desired amounts of water kefir grains were obtained, the growth of the grains was maintained by fermentation for 48-72 h. In the case of low kefir grain biomass, medjool dates and refined cane sugar were added as sources of nutrients (amino acids, minerals, and vitamins) to boost the growth of water kefir grains (Laureys et al., 2018; Lynch et al., 2021; Tavares et al., 2023). The propagation of water kefir grains used is shown in Figure 3.1. All the chemicals and reagents used were of reagent grade or higher.

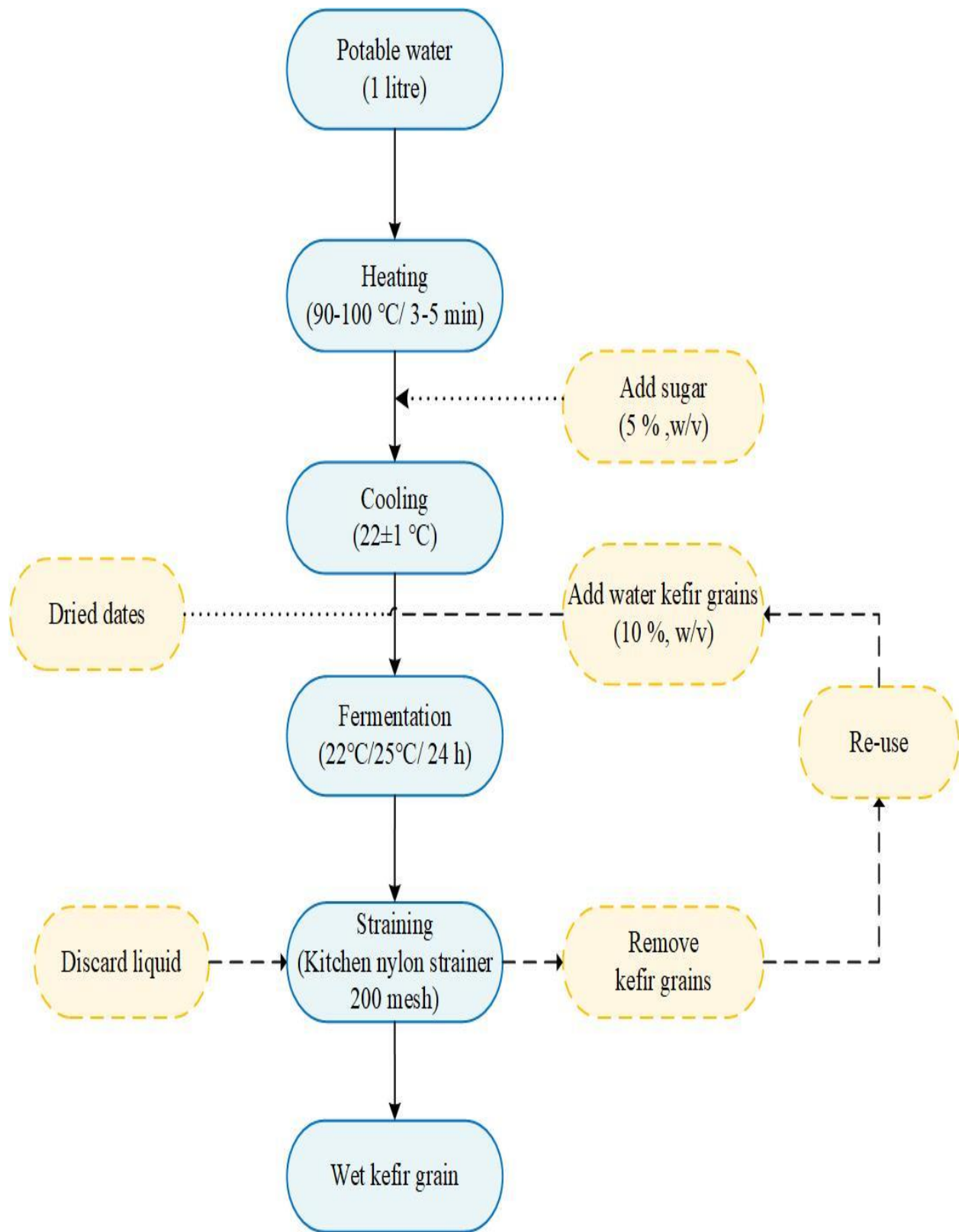


Figure 3.1 Propagation of water kefir grains by back-slopping process.

3.3.2 Preparation of green tea water kefir beverage (Phase II)

The water kefir beverage was prepared according to previous studies (Gulitz et al., 2011; Laureys & De Vuyst, 2014 & Waldherr et al., 2010) with minor modifications (Figure 3.2). Four formulations (Table 3.1, Section 3.1) of green tea water kefir beverage were prepared using organic green tea, organic dates, brown sugar, water kefir grains and organic peeled ginger. One litre of potable water was heated at 90°C for 2-3 minutes using a kettle (Necessities, China). Green tea (0.4%, w/v) was infused for 3-4 minutes in a beaker (Jayabalan et al., 2014; Wang, 2018). The tea leaves were removed by sieving, and the tea solution was transferred into beakers containing unrefined sugar (3% and 4%, w/v). Unrefined raw sugar was allowed to dissolve completely by stirring using a clean wooden or stainless spoon and the solutions were cooled to room temperature (22±1°C). The temperature of the sucrose-green tea solution was measured using a temperature thermocouple. Water kefir grains (5%, w/v) and 0.5% (w/v) of each medjool dates and fresh peeled ginger were added into four, one-litre clip-top glass jars (Laureys et al., 2018; Lynch et al., 2021). The brewed green tea-sugar solution was transferred into the glass jars and allowed to ferment in the water bath (Grant, Global Science, NZ) at 22°C and 25°C for 72 h, designated as the primary fermentation. The kefir grains, dates and ginger slices were removed at the end of 72 h using a 200-mesh (74 µm) kitchen nylon strainer (Muskaan, India). The recovered water kefir beverage was re-filtered through 400-mesh (38 µm) kitchen ultra-fine nylon mesh strainer (Grandado, NZ) and transferred into 500-mL amber flip-top bottles (Mangrove Jacks, NZ) and fermented at both 22°C and 25°C for an additional period of 72 h (secondary/bottled fermentation) for the development of flavour and carbonation (Pendon et al., 2022).

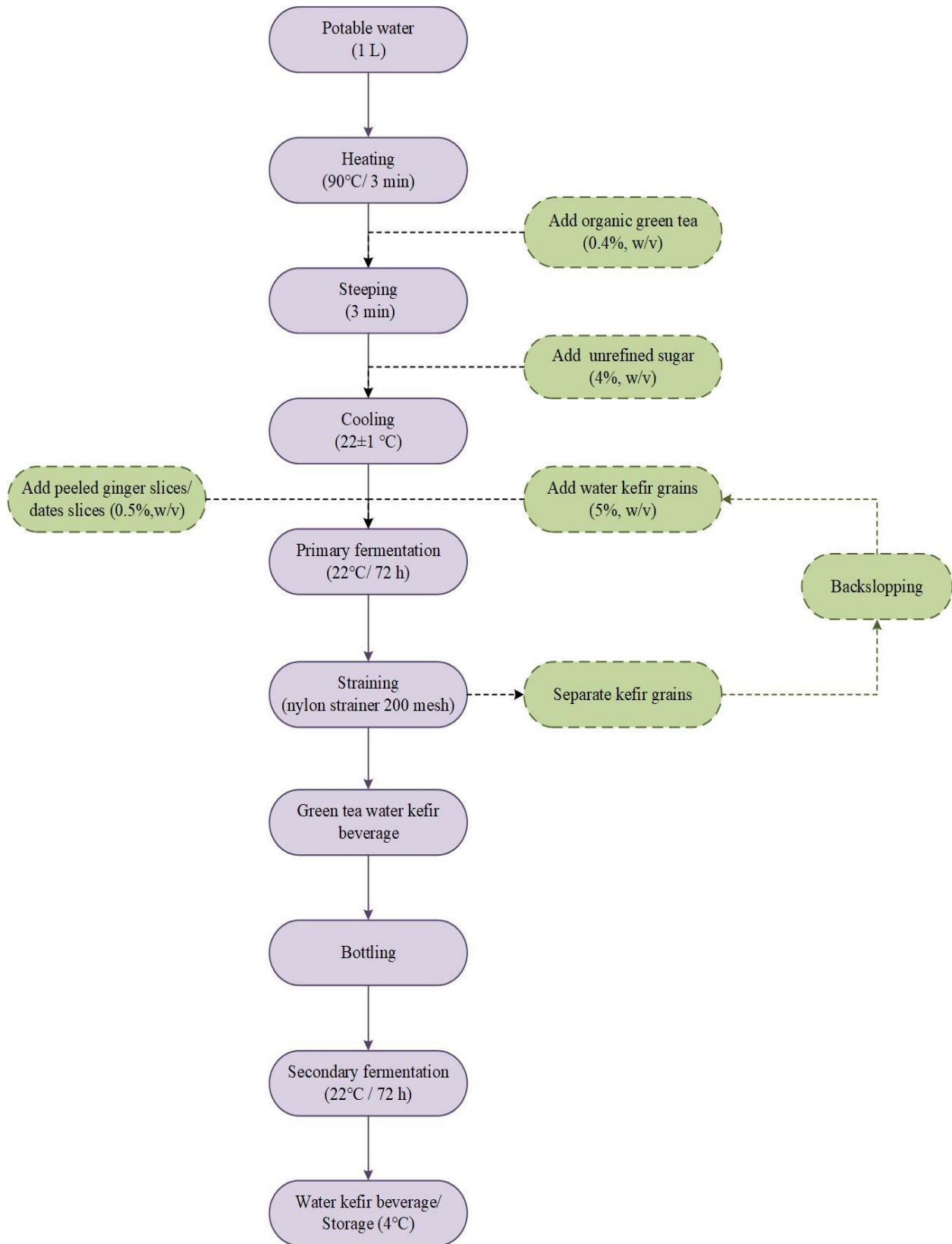


Figure 3.2 Preparation of green tea water kefir beverage.

3.3.3 Physico-chemical characteristics of water kefir

i. Measurement of pH

The pH of different water kefir formulations was directly measured based on the AOAC method 981.12 (AOAC, 2005 d) using a digital pH meter (Sartorius PB-20, USA) equipped with a glass electrode. The pH meter was calibrated using standard buffer solutions (pH 2.0, 4.0, 7.0) (LabServ, Thermofisher, NZ) before each measurement. The pH electrode was rinsed with distilled water between each measurement.

ii. Measurement of total soluble solids

Total soluble solids content was determined at ambient temperature ($22\pm 1^\circ\text{C}$) using a digital refractometer (Atago PR-101, Japan) previously calibrated with distilled water following the AOAC method 932.12 (AOAC, 2005 a). Two-three drops of the sample were transferred onto the glass prism of the digital refractometer using a dropper and the reading was recorded as °Brix (°B).

iii. Determination of titratable acidity (T.A.)

Standardisation of 0.1 M sodium hydroxide

Sodium hydroxide (0.1 M) was standardised following the AOAC standard method 936.16 (AOAC, 2005 b). The reagents used for the standardisation of sodium hydroxide were: 0.1 M sodium hydroxide (NaOH) (Univar, AjaxFinechem Pty Ltd, NZ), distilled water, potassium hydrogen phthalate solution (KHP) (Univar, AjaxFinechem Pty Ltd, NZ), and 1% phenolphthalein solution. One gram (1 g) potassium hydrogen phthalate (KHP) (Fisher Scientific, UK) was dried in an oven (Contherm, NZ) at 120°C for 2 h and then cooled in an air-tight desiccator (Bel-Art Product, US) with fresh desiccant for 60 min. Of the dried KHP, 0.20 g were weighed in a dry 250-mL Erlenmeyer flask on an analytical balance (A&D, 15905508, Korea), allowed to dissolve completely and then made up to 50 mL with distilled water. Three-four drops of 1% phenolphthalein solution were added and the mixture was swirled to mix thoroughly. The KHP solution was titrated against 0.1M NaOH solution until the first persistent light pink colour was observed and the volume (mL) of the NaOH titrated was recorded. The concentration of the NaOH solution was calculated using Equation 1.

$$M \text{ NaOH (mol/L)} = \frac{m \text{ KHP (g)} \times 1000}{V \text{ NaOH (mL)} \times \text{MW KHP (g/mol)}} \quad (1)$$

where M NaOH = molarity of NaOH (mol/L), m KHP = mass of KHP (g); V NaOH = volume used in the titration (mL), MW KHP = molecular weight of KHP = 204.23 (g/mol).

Determination of titratable acidity in water kefir

The acidity of the water kefir samples was determined following the AOAC standard method 947.05 (AOAC, 2005 c). Five grams of water kefir beverage sample were weighed in a 250-mL Erlenmeyer flask and mixed thoroughly with 10-mL distilled water. Three-four drops of 1% phenolphthalein solution were added to the solution and swirled to mix thoroughly. The acid-base titrations of the diluted samples were carried out against standardised 0.1 M NaOH until the first persistent light pink colour was observed, the volume (mL) of titre was recorded, and the concentration of lactic acid was calculated using Equation 2.

$$\% \text{ Lactic acid} = \frac{0.1 \text{ M NaOH (mol/L)} \times V \text{ NaOH (L)} \times \text{MW Lactic acid (g/mol)} \times 100}{m \text{ sample (g)}} \quad (2)$$

where M NaOH = molarity of NaOH (mol/L), V NaOH = volume of NaOH used in titration; MW Lactic acid (g/ mol) = molecular weight of lactic acid (90.08); 1 mL of sample \approx 1 g of sample

iv. Measurement of colour

The colour of the green tea water kefir samples was measured with the Minolta colourimeter (Chroma Meter CR-400, Minolta, Osaka, Japan) according to the CIElab chroma coordinates L*, a*, b* (Nielsen, 2017; Randazzo et al., 2016) where L* represents lightness (0-100) with 0 being black and 100 being white. The coordinate a* measures the red (+) and green (-); a decrease of a* indicates the colour changes from red to green while b* accounts for the yellow (+) and blue (-) spectrum of colour and a decrease of b* reflects a change in colour towards blue. The colourimeter was calibrated prior to use following the manufacturer's instructions. The water kefir sample (3 mL) was transferred into a 4-mL colourimeter plastic cuvette (Sigma Aldrich, NZ) and measured directly under the conditions recommended by the manufacturer and the corresponding L*, a*, b*

values were immediately recorded. For each sample, the measurements were performed in duplicate, and the experiment was replicated twice.

v. Analysis of sugars

The concentrations of sucrose, glucose, and fructose in green tea water kefir beverage were determined by the gold standard of high-performance liquid chromatography (HPLC) according to Stadie. (2013) with minor modifications. The HPLC system was equipped with a Rezex RCM-Monosaccharide RCM Ca²⁺ (8% cross-linked resin) column (300 × 7.8 mm) (Phenomenex, NZ) at 85°C using a Shimadzu HPLC system model LC-20AD XR (Shimadzu Corp, Japan), consisting of a degassing unit (DGU-20A5R), an autosampler (SIL-30AC), a column oven (CTO-20AC) and a refractive index detector (RID-20A). Distilled water (Milli-Q water) was used as the mobile phase at a flow rate of 0.6 mL/min which was previously filtered through a 0.22 µm nylon membrane filter (Merck Millipore Ltd, Ireland) and degassed to remove air bubbles using an ultrasonic bath (Bandelin, Germany). Prior to analysis, sugar standards were prepared using sucrose (84097) ≥ 99.5% (Sigma-Aldrich, NZ), fructose (F0127) ≥ 99% (Sigma-Aldrich, NZ), glucose (67528) ≥ 99.5% (Sigma-Aldrich, NZ), and distilled water. The concentrations of the standard solutions of sucrose, glucose and fructose were 0.05%, 0.1%, 0.5%, 1%, 2%, 4%, and 7%, (w/v). All the sugar standards and water kefir beverage samples were filtered through a 0.22-µm nylon membrane filter (33 mm) using a 5-mL syringe (Terumo, AU) into 2-mL vials (Shimadzu Corp, Japan). After generating the standard graph plots, the filtered beverage samples (10 µL) were injected into the HPLC column through an auto-sampler for chromatographic analysis. All analyses were performed in duplicate. The sugars were identified based on their respective retention times and then quantified by comparing the peak areas to the standard plots generated by the sugar standards. Peak areas were obtained using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan).

vi. Analysis of organic acids and ethanol

The concentration of lactic acid and ethanol in green tea water kefir beverage samples were determined by HPLC according to Stadie. (2013) with minor modifications. The HPLC system was described in Section 3.3.3 (v). The separation of organic acids (lactic acid and acetic acid) and ethanol was carried out on a Rezex ROA-Organic Acid H⁺ (8% cross-linked resin) column

(150×7.8 mm) (Phenomenex, NZ) at 60°C. Sulphuric acid (0.005 N) (Fisher Scientific, UK) was used as the mobile phase set at 0.6 mL/min flow rate and previously filtered through a 0.22- μ m membrane filter and degassed in an ultrasonic bath. The standard organic acids used were lactic acid (252476) \geq 95% (Sigma Aldrich, NZ), acetic acid (2789) \geq 99.5% (Fisher Scientific, UK) and absolute ethanol (UN1170) \geq 99.8% (Sigma Aldrich, NZ). The concentrations of the standard solutions of lactic acid and acetic acid were 0.01%, 0.05%, 0.1%, 1% and 2%, (w/v). The concentrations of the standard solutions of ethanol were 0.01%, 0.1%, 0.5%, 0.8%, 1%, 2% and 3% (w/v). Prior to analysis, standard solutions and test samples were filtered through 0.22 μ m syringe filters and kept in 2-mL vials for chromatographic analysis. Automatic injections (10 μ L) were performed in duplicate. Individual organic acids and ethanol were identified and quantified by comparison of their retention times and peak areas, to the respective standards. Peak areas were integrated using Shimadzu LC Solutions Software. The quantification of ethanol using Rezex ROA-Organic acid H⁺ column and refractive index detector through HPLC in place of Gas Chromatography-Flame ionization detection (GC-FID) (Kostic et al., 2021; Laureys & Vuyst, 2014) was employed due to the enhanced operational efficiency, productivity, and flexibility in analysing complex sample matrices. This analytical approach facilitates the simultaneous quantification of ethanol, acetic acid, and non-volatile organic acids such as lactic acid (Ahmed et al., 2023; Joseph et al., 2022; Lai et al., 2016; Lopez & Gomes, 1996; Nguyen et al., 2010; Xu et al., 2019a).

vii. Analysis of antioxidants

Analysis of the phenolic compounds (EGCG, ECG, EGC and gallic acid) and methylxanthine (caffeine and theobromine) in green tea water kefir beverages were analysed according to Cao et al. (2019) with minor modifications. The analysis was carried out using a reversed-phase HPLC system model LC-20AD (Shimadzu UFLC, Shimadzu Prominence, Japan) consisting of a degasser (DGU-20A5), an autosampler (SIL20AC HT), a column oven (CTO-20AC), a fluorescence detector (RF-20A XS) and a diode array detector (SPD-M20A). The acids were separated by a 5- μ m Grace Smart RP18 column (250 ×4.6 mm) (Grace, USA) at 18°C. A discontinuous gradient (Table 7) was run from 100% mobile phase A (0.1% trifluoroacetic acid in Milli-Q water) to 100% mobile phase B (0.1% trifluoroacetic acid in acetonitrile) at a flow rate of 0.75 mL/min. Gallic acid and (+)-catechin (1 mg/mL) standard stock solutions were prepared in Milli-Q water while

epicatechin standard solution (1 mg/mL) was prepared in 70% ethanol solution (v/v). Before analysis, standard solutions and test samples were filtered through 0.22 µm syringe filters and kept in 2 mL vials for chromatographic analysis. After the injection of the standards, the water kefir samples were auto-injected (20 µL) and the phenolic acids were detected at 270 nm. The samples were analysed in duplicate. The compounds were identified based on their retention times and the peaks were integrated using Shimadzu LC Solution Software (Shimadzu Prominence, Japan) followed by quantifying the compounds based on peak areas. The phenolic content of water kefir was quantified using HPLC due to its comparative advantage of accuracy, specificity, identification, reproducibility, quantification, and sensitivity over alternative methods such as Folin-Ciocalteu reagent based on colourimetric assay and various total antioxidant capacity (TAC) assay including ABTS, DPPH and FRAP. Moreover, TAC assay lacks to identify the specific phenolic compounds contributing to the overall antioxidant capacity in the beverage/food matrix (Bunzel & Schendel, 2017; Li et al., 2005; Tu et al., 2019; Zou et al., 2021).

Table 3.2 HPLC gradient program used to analyse catechin and gallic acid

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100.0	0.0
15	91.5	8.5
20	90.0	10.0
28	89.0	11.0
36	87.1	12.9
46	50.0	50.0
53	0.0	100.0
63	100.0	0.0
78	100.0	0.0

Note: Mobile phase A: 0.1% TFA in Milli-Q water; Mobile B: 0.1% TFA in acetonitrile.

3.3.4 Microbiological Analysis

i. Microbiota of water kefir grains starter culture (Phase 1)

The LAB and the yeast, *S. cerevisiae* were enumerated on de Man, Rogosa and Sharpe (MRS) agar and yeast extract glucose chloramphenicol (YGC) agar (Appendix B), respectively (Laureys & De Vuyst, 2014). The media were prepared according to the manufacturer's instructions and sterilised in an autoclave (Astell Scientific, UK) at 121°C for 15 min. The medium for enumeration of LAB

was supplemented with 0.1% cycloheximide to inhibit the growth of yeast and fungi (Hsieh et al., 2012). 10 g of water kefir grains were diluted with 90 g of 0.1% peptone water (Merck, Germany) and then mechanically homogenised in a stomacher laboratory blender (Stomacher, Global Science, NZ) for 5 min. Suitable serial dilutions were prepared by mixing 1 mL of the microbial suspension with 9 mL of peptone water. The serial dilutions were mixed using the vortex mixer (Labinco BV, Netherlands) for 15 s, followed by pour plating in duplicates (Gulitz et al., 2011, Koh et al., 2018). The MRS agar plates were incubated (Clayson, IM1000, AU) at 30°C for 3 days under anaerobic condition using the AnaeroGen™ pack (AN0035A) (Thermo Scientific, NZ) whereas the YGC agar plates were incubated at 25°C for 5 days (Laureys & De Vuyst, 2014). Isolates of the colonies were gram-stained and examined under oil immersion of the microscope (Carl Zeis, Germany) (Said et al., 2018).

ii. Enumeration of lactic acid bacteria in green tea water kefir beverages

Lactic acid bacteria in water kefir beverages were enumerated according to Laureys & De Vuyst. (2014), using MRS agar (Oxiod, UK). The media preparation and pour plating of serially diluted samples were described in Section 3.3.4 (i). Solidified agar plates were incubated (Clayson, IM1000, AU) anaerobically using AnaeroGen™ pack (AN0035A) (Thermo Scientific, NZ) at 30°C for 3 days. Grown colonies were enumerated using a colony counter (Bibbyscientific, UK) and results were reported as log colony-forming units per mL of sample.

iii. Enumeration of yeast in water kefir beverages

Viable cell counts of *Saccharomyces cerevisiae* in green tea water kefir beverages were enumerated using YGC agar (Merck, Germany) as described by Laureys & De Vuyst. (2014) and Atalar. (2019). The agar was prepared according to the manufacturer's instructions (Astell Scientific, UK). The samples were inoculated by pour-plating as described in Section 3.3.4 (i). The solidified agar plates were incubated aerobically at 25°C for 3 days in the incubator (Clayson IM1000, AU). Grown colonies were counted using a colony counter.

3.3.5 Sensory evaluation

Sensory evaluation of food and beverage is a scientific method used to measure, analyse, and interpret the characteristics of food and beverage as perceived through the sense of sight, smell, touch, taste, and hearing (Lyon, 1992; Lawless & Heymann, 2010). The sensory characteristics of

the water kefir beverage were determined according to Corona et al. (2016). Two types of sensory methods were used, focus group and consumer sensory evaluations. Before the focus group discussions and consumer sensory evaluation, the panellists were given an information sheet (Appendix D) describing the study and a copy of Massey University Human Ethics Application (4000022992).

Consumer sensory evaluation

Consumer sensory evaluation of four formulations of green tea water kefir beverage was conducted under white light in sensory booths of the Sensory Evaluation Laboratory at Massey University, Albany. The panellists were randomly recruited at the university by email and through posters. A 9-point hedonic scale (1= dislike extremely; 9 = like extremely) was used to evaluate the appearance, aroma, flavour, sourness, sweetness, and overall acceptance of the water kefir beverage samples. The results of sensory evaluation were used to aid the selection of the most promising formulation of green tea water kefir beverage in phase II. Thirty (30) consumer sensory panellists participated in each sensory evaluation session which was replicated twice.

Focus group sensory evaluation

Focus group sensory evaluation of the fermented water kefir beverages was conducted during cold storage of the beverages for two weeks (Phase III) (Dinella et al., 2022). Focus groups of 8-10 sensory panellists consisting of staff and university students who were familiar with fermented water kefir beverages evaluated the products. All the beverage samples were stored at $4\pm 1^{\circ}\text{C}$ for 24 hours prior to the evaluation. Each beverage sample (15 mL) was served in a 20-mL plastic cup coded with 3-digit random numbers. Each panellist was required to rinse their palate with water before evaluating each sample. The samples were evaluated in the Product Development Laboratory at Massey University, Albany Campus.

3.4 Analysis of Data

Water kefir beverage samples were analysed in duplicate, and the experiments were replicated twice. Minitab version 19 Statistical Software (Minitab Inc., State College, PA, USA) and Microsoft® Excel 2016 (Microsoft Inc., Santa, CA, USA) were used for data analysis. Data were expressed as mean \pm standard deviation (SD) and all the graphs were generated using OriginLab 2018 (OriginLab, Massachusetts, USA). One-Way Analysis of Variance (ANOVA) was used to determine the effect of fermentation temperature, sugar concentration on the physico-chemical properties (colour, pH, TSS, T.A, sugars, lactic acid, and ethanol) except antioxidants (descriptive analysis), microbiology (lactic acid bacteria and *S. cerevisiae*) and sensory characteristics of the fermented green tea water kefir beverages at $p < 0.05$. Tukey's multiple comparison test was used to separate significant differences (95% C.I.) between the means of the sample data. The statistical outputs are shown in Appendix G.

4. Results and Discussion

4.1 Phase 1: Water kefir grain starter culture

The activated starter culture i.e., water kefir grains (Figure 4.1) used in this study were provided by Forage and Ferment (Clevedon, New Zealand). The grains were a yellowish-white colour with an elastic, brittle, and irregular granular structure (Laureys et al., 2018; Waldherr et al., 2010). Water kefir grains are insoluble in water as their exopolysaccharide is composed of insoluble α -glucan dextrans (α -1-6 linked glucose polymer with 1-3 linked side chains) (Laureys & De Vuyst, 2014; Laureys & De Vuyst, 2017; Waldherr et al., 2010). Lactic acid bacteria species such as *L. hilgardii* (Fels et al., 2018; Pidoux, 1989; Waldherr et al., 2010), *L. nagelii*, *L. hordei*, *Lc. mesenteroides*, and *Lc. citreum* are commonly associated with the production of exopolysaccharide and kefir grain growth during fermentation (Gulitz et al., 2011).



Figure 4.1: Water kefir grain

(Image was captured by Redmi 9T, Xiaomi Inc., China)

Previous studies have shown water kefir grains to consist predominantly of the genus *Acetobacter*, *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, and *Zygorhizula* (Gulitz et al., 2011; Magalhaes et al., 2010; Marsh et al., 2013; Verce et al., 2019). However, the microbial composition of kefir grains is variable depending on the geographical region, climatic conditions, and fermentation conditions (e.g., temperature, substrate type, substrate concentration, micronutrient composition, nitrogen, and calcium concentration) used (Fiorda et al., 2017; Gamba et al., 2021; Laureys et al., 2018; Laureys et al., 2019; Laureys et al., 2021; Lynch et al., 2021; Martinez-Torres et al., 2017; Sarikkha et al., 2015; Zannini et al., 2022). According to the supplier (Forage and Ferment, New

Zealand) the water kefir grains used in this study consisted predominantly of lactic acid bacteria and *S. cerevisiae* (Laureys et al., 2021; Magalhaes et al., 2010). Colonies grown on MRS agar were gram-positive and catalase-negative (Appendix C), indicating the presence of *Lactobacillus* species. Similar results have been found in previous studies of water kefir grains (Leite et al., 2013; Said et al., 2018).

In this study, viable cell counts for *Lactobacillus* species and *S. cerevisiae* were 7.80 ± 0.14 log cfu/g and 7.25 ± 0.03 log cfu/g respectively, after 24 h fermentation at 22°C. According to the international food standards of the Codex Alimentarius, kefir should contain a minimum level of 10^7 cfu/mL bacteria and 10^4 cfu/mL yeast (FAO/WHO, 2011). Thus, the microbial population of the kefir starter culture obtained from the present study falls within the microbial criterion standard (da Miguel et al., 2011; Gulitz et al., 2011).

4.2 Phase II: Effect of fermentation temperature and concentration of sugar on green tea water kefir beverages

4.2.1 pH and titratable acidity

The mean pH and mean titratable acidity of green tea water kefir beverages containing 3% and 4% brown sugar were determined at 24 h intervals during fermentation for 144 h at 22°C and 25°C (Figure 4.2). During fermentation the pH value decreased due to the increase in organic acids (titratable acidity) produced as a result of substrate metabolism by the starter culture in the fermented beverages (da Costa et al., 2018; Jayabalan et al., 2014; Limbad et al., 2023; Tavares et al., 2023). The initial pH ranged between 4.96 ± 0.18 - 5.05 ± 0.04 and decreased ($p < 0.05$) after 24 h of fermentation to 3.89 ± 0.05 - 4.11 ± 0.01 (Appendix E). Magalhaes et al. (2010) have reported similar results (pH 4.1 ± 0.1) after 24 h of brown sugar water kefir fermentation. Thereafter, the pH decreased gradually until fermentation was completed (144 h). The final pH (144 h) of the green tea water kefir beverages lies between 3.30 ± 0.06 to 3.50 ± 0.00 which were similar to whey based-pomegranate juice kefir beverage fermented for 36 h (pH 3.40 ± 0.02 to 3.61 ± 0.02) (Sabokbar & Khodaiyan, 2015), black tea water kefir fermented for 72 h (pH 3.31 ± 0.03 to 3.50 ± 0.02) (Subardjo, 2017) and other studies of water kefir (Laureys & De Vuyst., 2014; Tavares et al., 2023; Tu et al., 2019). Results showed significant differences ($p < 0.05$) in pH between the samples (Appendix G.1). The lowest pH was observed in sample WK3 (3.30 ± 0.06) prepared at 25°C using 3% (w/v) sugar and highest for WK2 (3.50 ± 0.06) fermented at 22°C using 4% (w/v) brown sugar.

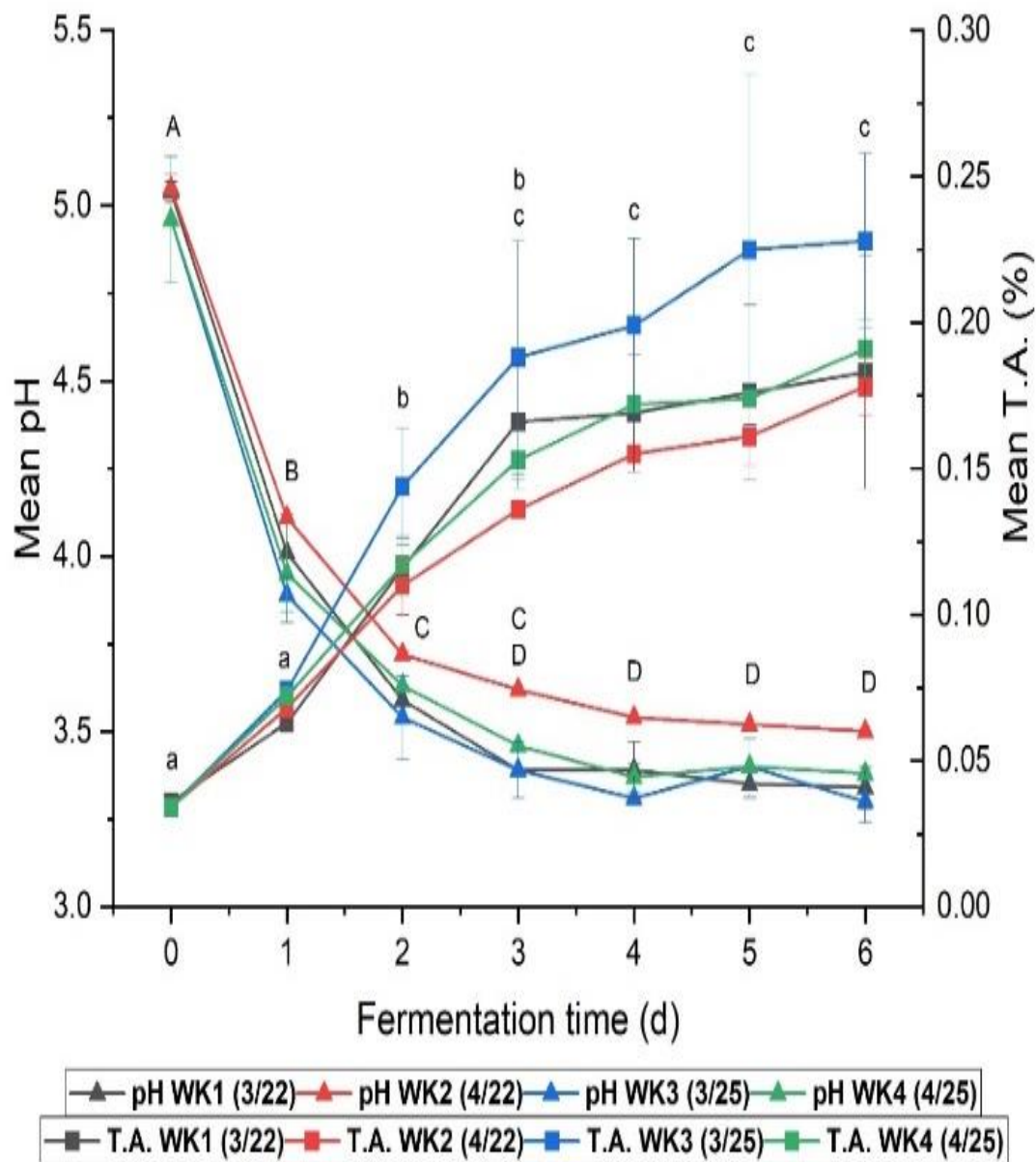


Figure 4.2: Mean pH and titratable acidity of green tea water kefir beverages during fermentation for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; T.A. = Titratable Acidity; ABCD = significant mean difference in the pH were observed in the four formulations throughout fermentation ($p < 0.05$); abc= significant mean difference in the T.A. were observed in the four formulations throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

The initial titratable acidity in fermented green tea beverages ranged between 0.034 ± 0.00 - 0.036 ± 0.00 % and gradually increased during fermentation to 0.178 ± 0.01 - 0.228 ± 0.03 %. Results showed significant differences ($p < 0.05$) in T.A. of the samples (Appendix G.2). The highest T.A. was observed for WK3 (0.228 ± 0.03 %) prepared at 25°C while the lowest was for WK2 (0.178 ± 0.01 %) fermented at 22°C . The T.A. of the beverages were low compared to black tea water kefir beverage which ranged between 0.225 ± 0.01 - 0.320 ± 0.01 % (Subardjo, 2017) and other published studies (Mu, 2019; Ozcelik et al., 2021; Pablo & Cimafranca, 2022; Randazzo et al., 2016; Sabokbar & Khodaiyan, 2015).

The pH and the concentration of organic acids produced depends on the geographical origin of the kefir grains, its microbial diversity, substrate type, substrate concentration, buffering capacity, calcium concentration, nutrient composition and fermentation conditions used (Laureys & De Vuyst, 2017; Laureys et al., 2018; Lynch et al., 2021; Puerari et al., 2012; Randazzo et al., 2016; Zannini et al., 2022). The present data indicated that fermentation temperature influenced the pH, and the titratable acidity of the fermented water kefir beverage as low fermentation temperature can decrease the metabolic activities of the microorganisms. Hence, low concentrations of T.A. and pH are produced as compared to beverages prepared at higher temperatures (Azi et al., 2021; Caro et al., 2015; Dimitreli & Antoniou, 2011; Loncar et al., 2006; Puerari et al., 2012; Sabokbar & Khodaiyan, 2015; Tzavaras et al., 2022).

4.2.2 Total soluble solids ($^{\circ}\text{Brix}$)

Sucrose is the main carbon source added during the fermentation of green tea water kefir. Additionally, fruits or dried fruits such as figs, raisins, apricots, or dates can be added as a source of micronutrients and have a favourable effect on the growth of the microorganisms (Cufaoglu & Erdinc, 2023; Laureys et al., 2018; Pendon et al., 2022; Tireki, 2022). In this study, medjool dates were used. Figure 4.3 shows that the TSS of all the green tea kefir beverage samples decreased ($p < 0.05$) during 144 h of fermentation (Appendix G.3). This decrease occurs as the water kefir microorganisms (starter culture) break down the sucrose into glucose and fructose due to the action of the enzyme invertase produced by the yeast during fermentation (Magalhaes et al., 2010; Pfeiffer & Morley, 2014; Stadie, 2013; Tu et al., 2019) and conversion of the monosaccharides to organic acids by LAB which contribute to the chemical and sensory characteristics of the product (Laureys & De Vuyst, 2014; Limbad et al., 2023; Stadie et al., 2013).

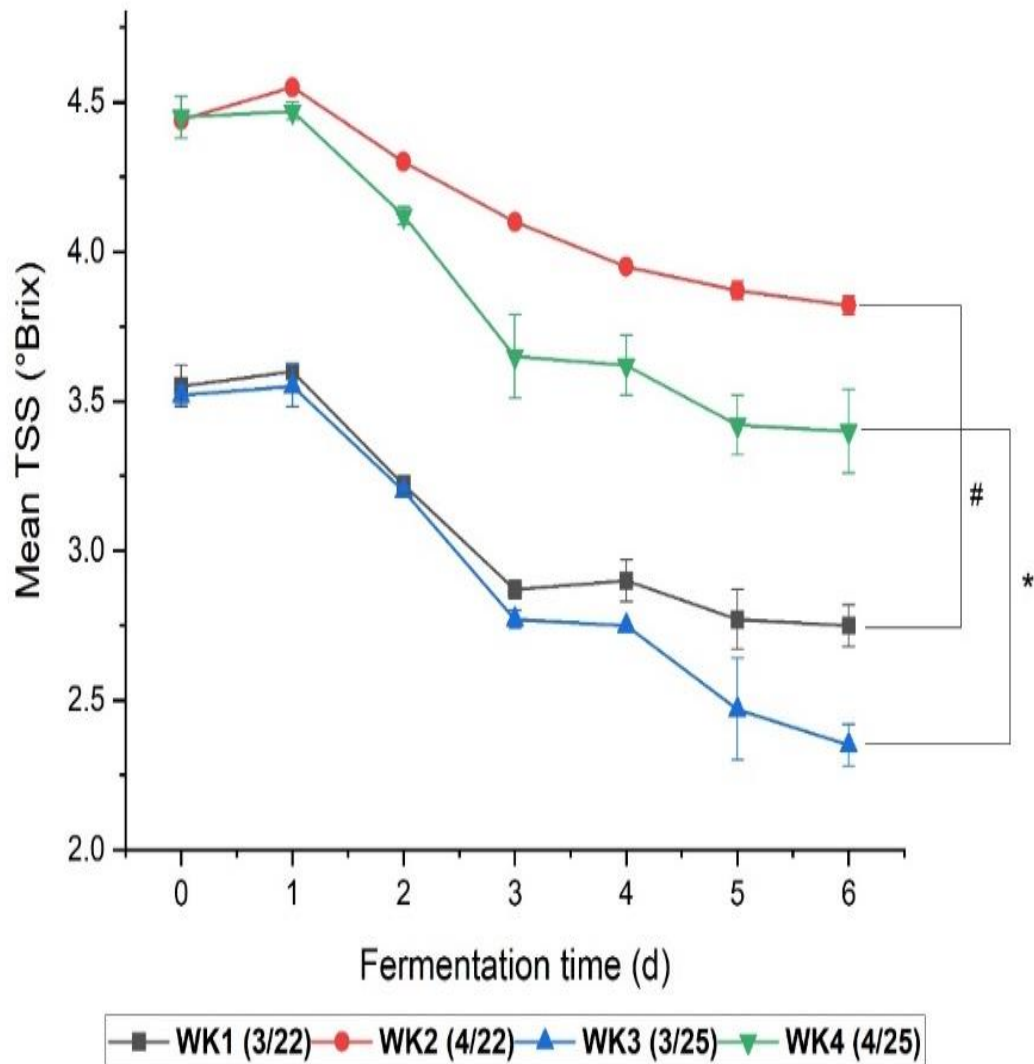


Figure 4.3: Mean total soluble solids (°Brix) of green tea water kefir beverages during fermentation for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; * = Higher reduction in TSS observed in formulations prepared at 25°C throughout fermentation ($p < 0.05$); # = significant mean difference observed in formulations prepared at 22°C throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

The total soluble solids increased slightly after 24 h of fermentation which may be due to the absorption of sugar released from the date slices (Tireki, 2022). The increase in Brix could be due to gel formation between free calcium present in the water and pectin of the microbially degraded medjool dates (Tireki, 2021). Thereafter, the TSS reduced ($p < 0.05$) throughout the 72 h fermentation period due to the activity of the starter culture. Afterwards, a gradual decrease ($p < 0.05$) in total soluble solids was observed during the maturation/bottled period (72 h-144 h). The reason for the continued decrease in TSS during the bottled phase after the removal of the water kefir grains may be due to the activity of residual kefir grain particles ($< 38 \mu\text{m}$) left in the liquor after re-filtration (Section 3.3.2). At the end of 144 h, the reduction in TSS ranged between 2.35 ± 0.07 - 3.82 ± 0.03 Brix. Similar results (3.97 Brix) were observed in soy whey water kefir fermented for 120 h (Tu et al., 2019) and vegetable juices-based water kefir (Carrot 3.38 ± 0.10 , melon 3.83 ± 0.06 and strawberry 2.47 ± 0.06 Brix) (Corona et al., 2016). In the present study, sample WK3 (2.35 ± 0.07) and WK4 (3.4 ± 0.14) both fermented at 25°C using 3% and 4% (w/v) sugar concentration respectively resulted in higher reductions in total soluble solids than sample WK1 (2.75 ± 0.07) and sample WK2 (3.82 ± 0.03) fermented at 22°C . Fermentation temperature had a direct effect on decreasing the total soluble solids. Therefore, higher the fermentation temperature, the greater the metabolism of sugars in beverage samples (Loncar et al., 2006; Mu, 2019; Sabokbar & Khodaiyan, 2015; Subardjo, 2017).

4.2.3 Colour

The colour and appearance of a product impacts consumer preference and product acceptability (Chaturvedula & Prakash, 2011; Pathare et al., 2013). Figure 4.4 shows the change in lightness (L^*) observed in green tea water kefir beverages during the fermentation period. The change in lightness ($p < 0.05$) were significant only for sample WK2 and sample WK4 prepared using 4% (w/v) sugar concentration fermented at 22°C and 25°C respectively. The lightness of samples prepared using 3% (w/v) sugar concentration (sample WK1 & sample WK3) did not change ($p > 0.05$) (Appendix G.4). The initial lightness of the four formulations ranged between 95.88 ± 0.03 - 96.89 ± 0.26 . The final L^* value were between 97.2 ± 0.07 - 98.49 ± 0.00 . The results obtained for beverages prepared using 4% (w/v) sugar concentration were similar to previously published studies where the lightness (L^*) of the beverage increased during water kefir fermentation (Corona et al., 2016; Randazzo et al., 2016). The change in lightness likely occurred

due to the degradation of the polyphenols (thearubigin and theaflavins) into smaller compounds by the activity of microorganisms present in the starter culture (Chu & Chen, 2006; Haslam, 2003; Jayabalan et al., 2014).

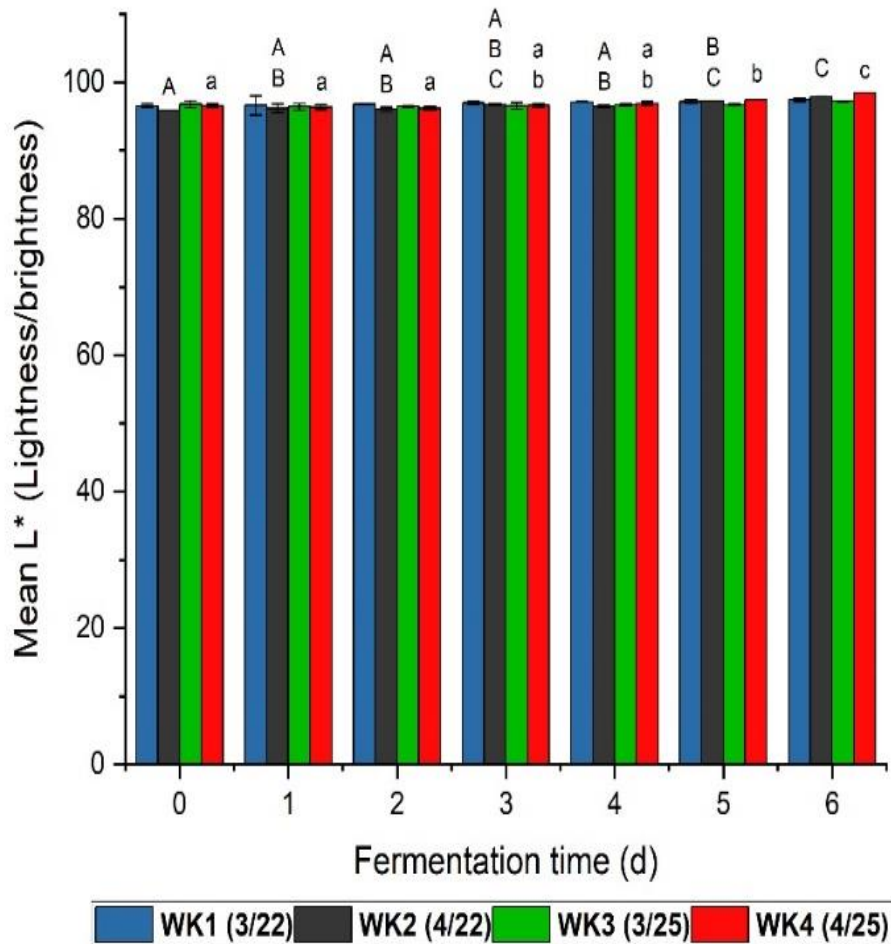


Figure 4.4: Mean L* of green tea water kefir beverages during fermentation for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; ABC = significant mean difference in the L* value of the formulations (WK2) throughout fermentation ($p < 0.05$); abc = significant mean difference in the L* value of the formulations (WK4) throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

The effects of fermentation temperature and sugar concentration on redness-greenness (a^*) and yellowness-blueness (b^*) are shown in Figures 4.5 and 4.6, respectively. Values of a^* and b^* for

all the samples increased ($p < 0.05$) during fermentation (Appendix G.5 and G.6 respectively). The initial a^* of all the water kefir sample ranged between 0.23 ± 0.03 - 0.39 ± 0.00 and increased to 0.99 ± 0.02 - 1.71 ± 0.16 . The initial b^* ranged between 1.01 ± 0.02 - 1.62 ± 0.03 and increased to 1.79 ± 0.00 - 3.15 ± 0.01 during fermentation. These results agree with Corona et al. (2016) where the value of a^* and b^* increased during fermentation for water kefir beverages prepared using vegetable juice. Moreover, the results suggest that during fermentation polyphenols may undergo biochemical transformation in an acidic environment which may affect the colour change of the fermented green tea beverage (Chu & Chen, 2006).

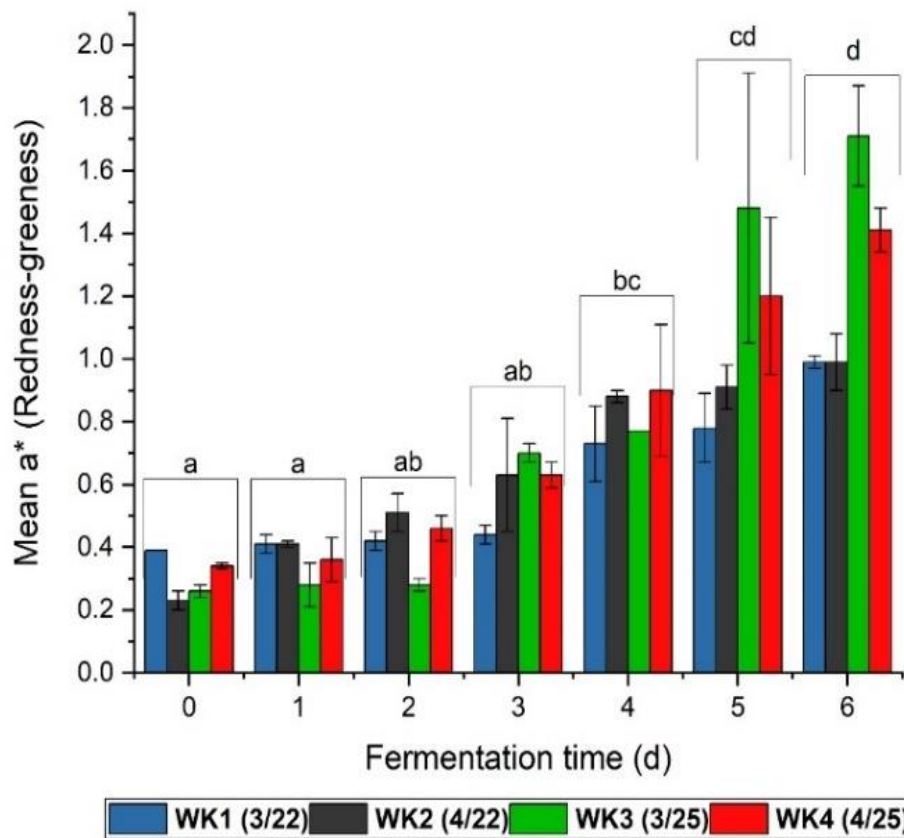


Figure 4.5: Mean a^* of green tea water kefir beverages during fermentation for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD ($n=4$); d = day; abcd = significant mean difference in the a^* value observed in all of the formulations throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

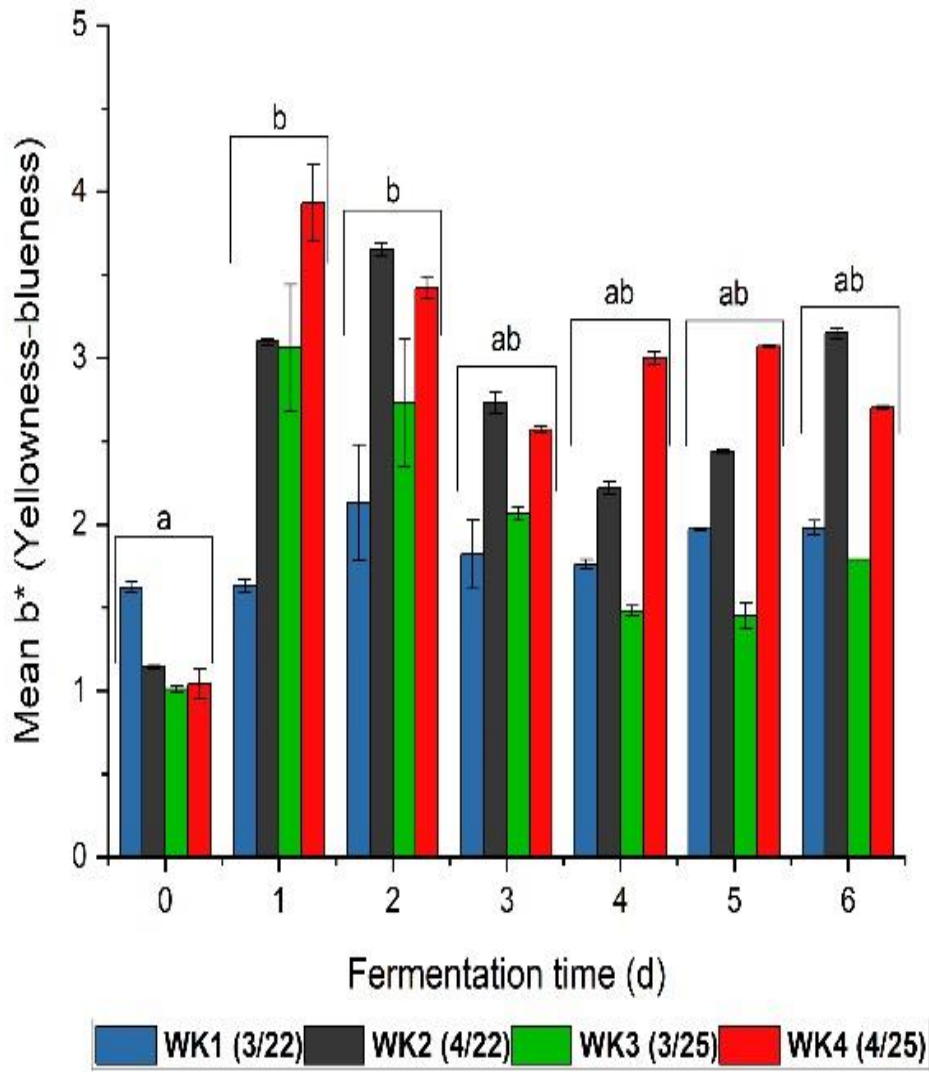


Figure 4.6: Mean b* of green tea water kefir beverages during fermentation for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; ab = significant mean difference in the b* value observed in all of the formulations throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

4.2.4 Microbiological analysis

The growth pattern of LAB and *S. cerevisiae* in green tea water kefir beverages during fermentation are shown in Figure 4.7 and 4.8, respectively. The LAB and yeast showed steady growth up to 72 h of fermentation. Viable cell counts of LAB increased ($p < 0.05$) throughout fermentation while the growth of yeast cells increased up to 72 h of fermentation and then decreased ($p < 0.05$). Increased growth of LAB and yeast during fermentation indicates a mutual existence, referred to as a symbiotic relationship which is attributed to the production of essential nutrients such as acetate, succinate, amino acid, and vitamin B₆ by yeast required for the growth of LAB. In return, the LAB support growth of yeast by the acidification of the medium through the production of organic acids (Leroi & Pidoux, 1993b; Magalhaes et al., 2010; Stadie et al., 2013).

The viable cell counts ($p < 0.05$) (Appendix G.7) of lactic acid bacteria increased steadily from (0 h) 6.88 ± 0.01 - 7.07 ± 0.07 log cfu/mL to 8.5 ± 0.01 - 8.73 ± 0.05 log cfu/mL at the end of the fermentation (144 h). These results agree with published studies that reported increased viable cell counts of lactic acid bacteria in kefir beverages during fermentation (Bau et al., 2015; Corona et al., 2016; Gulitz et al., 2011; Laureys et al., 2018; Magalhaes et al., 2010; da Miguel et al., 2011). Additionally, the fermentation temperature ($p < 0.05$) influenced the growth of lactobacilli such that water kefir beverages fermented at 25°C had higher viable counts (sample WK3: 8.66 ± 0.09 log cfu/mL and sample WK4: 8.73 ± 0.05 log cfu/mL) compared to kefir beverages prepared at 22°C (sample WK1: 8.56 ± 0.04 log cfu/mL and sample WK2: 8.5 ± 0.01 log cfu/mL). These results suggest that fermentation at 25°C was more favourable than 22°C for the growth of lactobacilli. Similar results were reported in previous studies where kefir beverages fermented at higher temperature had higher lactobacilli cell counts (Sabokbar & Khodaiyan, 2015; Darvishzadeh et al., 2021).

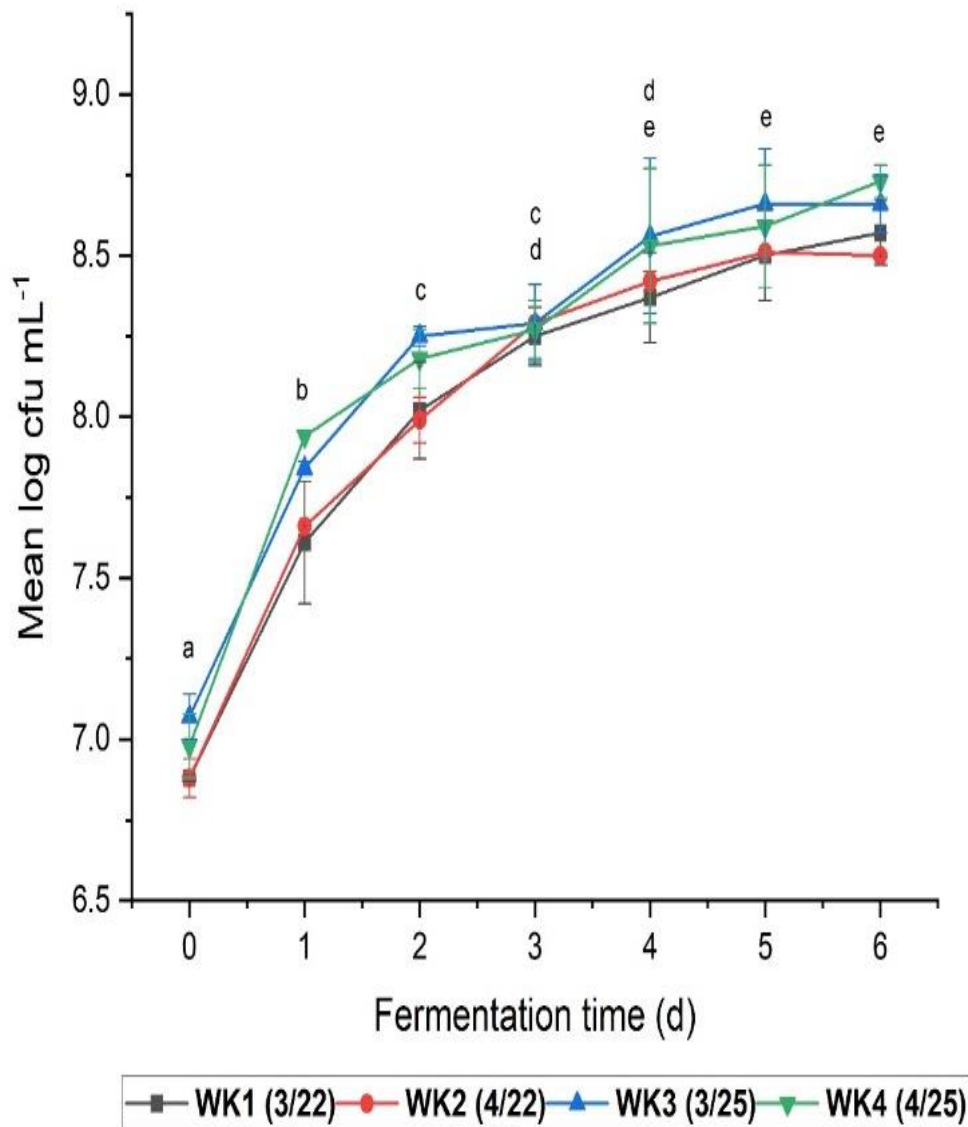


Figure 4.7: Mean log cfu/ml of Lactic acid bacteria during fermentation of green tea water kefir beverages for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; abcde = significant mean difference in the viable cell count of LAB were observed in all of the four formulations throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

As shown in Figure 4.8, the initial viable yeast cell counts in all beverage samples ranged from 6.02 ± 0.01 - 6.08 ± 0.03 log cfu/mL and increased ($p < 0.05$) (Appendix G.8) to 7.19 ± 0.07 - 7.27 ± 0.00 log cfu/mL after fermentation for 72 h and then decreased ($p < 0.05$) during bottled fermentation (72 h-144 h) to 6.97 ± 0.10 - 7.07 ± 0.14 log cfu/mL. The results obtained were in a similar range as previously published studies of water kefir (da Miguel et al., 2011; Gulitz et al., 2011; Laureys et al., 2018; Magalhaes et al., 2010). The increase in yeast cell numbers in the first 72 h may be due to acid tolerance of *Saccharomyces cerevisiae* (Kubo et al., 2014; Stadie et al., 2013). However, the decreased growth of yeast after 72 h may be attributed to the removal of water kefir grains (Section 3.3.2) and subsequent competition for nutrients in a demanding habitat of the low sugar filtered water kefir beverage which likely influenced the microbial growth (Leroi & Pidoux, 1993b; Magalhaes et al., 2010; Mantzourani et al., 2022; Laureys et al., 2018). Compared to the yeast cell counts, the predominance of LAB was observed throughout the period of fermentation in the water kefir beverages which is in line with previously published data (Bourrie et al., 2016; Laureys & De Vuyst, 2014; Lynch et al., 2021; Magalhaes et al., 2010)

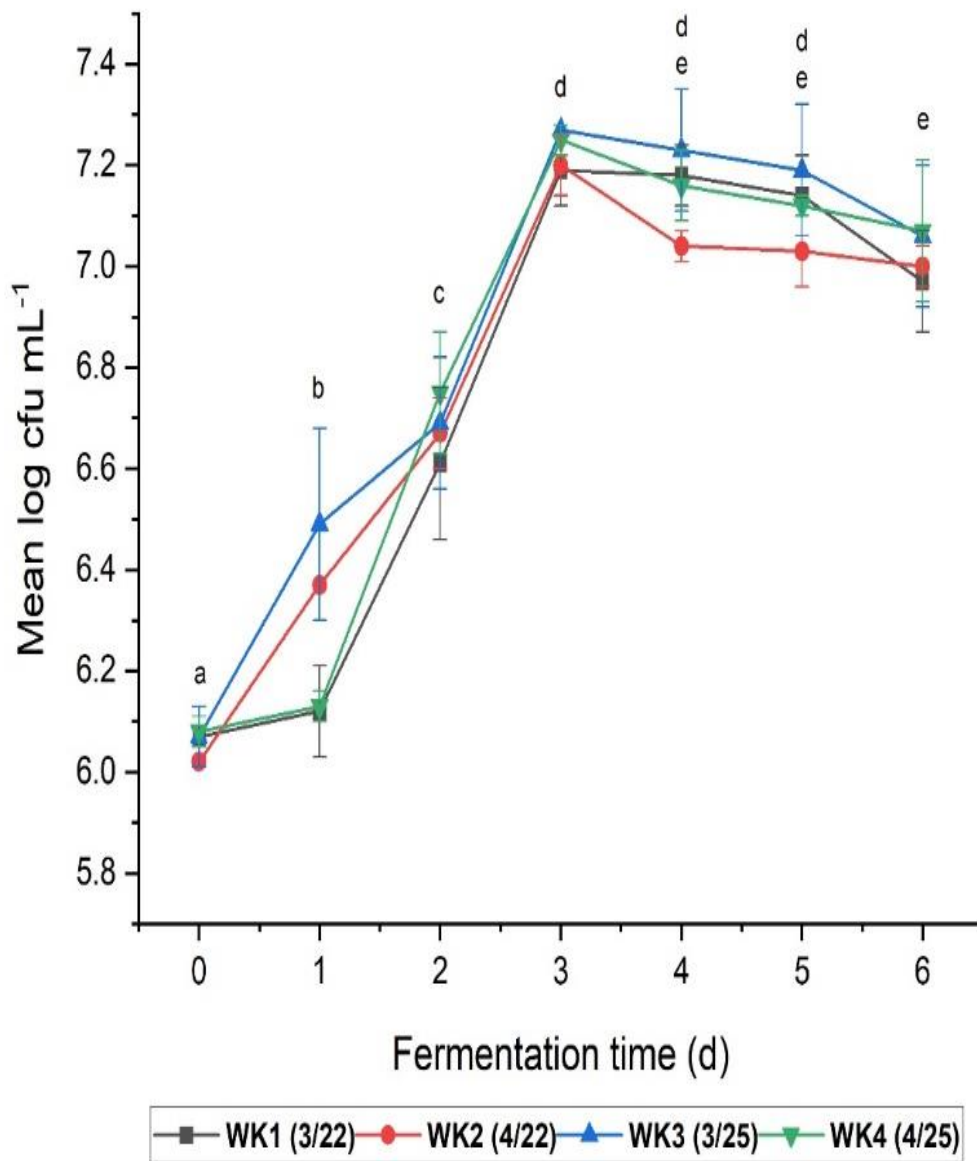


Figure 4.8: Mean log cfu/ml of *Saccharomyces cerevisiae* during fermentation of green tea water kefir beverages for 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; 0-3 fermentation time (days)= fermentation period; 4-6 bottled fermentation time (days)= maturation period after bottling without starter culture for 72 h; Error bars = \pm SD (n=4); d = day; abcde = significant mean difference in the viable cell count of yeast were observed in all of the four formulations throughout fermentation ($p < 0.05$); experiments were conducted in duplicate and replicated twice.

4.2.5 Consumer sensory evaluation

Four green tea water kefir formulations were evaluated for appearance, aroma, flavour, sweetness, sourness, and overall acceptability at the end of maturation (144 h). The samples were evaluated by consumer panellists (n=30) using a 9-point hedonic rating scale and the experiments were replicated twice (Appendix E). The fermented green tea beverages are shown in Figure 4.9 and the sensory scores of the four formulations are shown in Figure 4.10. Sugar concentration and fermentation temperature ($p < 0.05$) played a crucial role in the overall sensory attributes of the water kefir formulations (Appendix G.9).

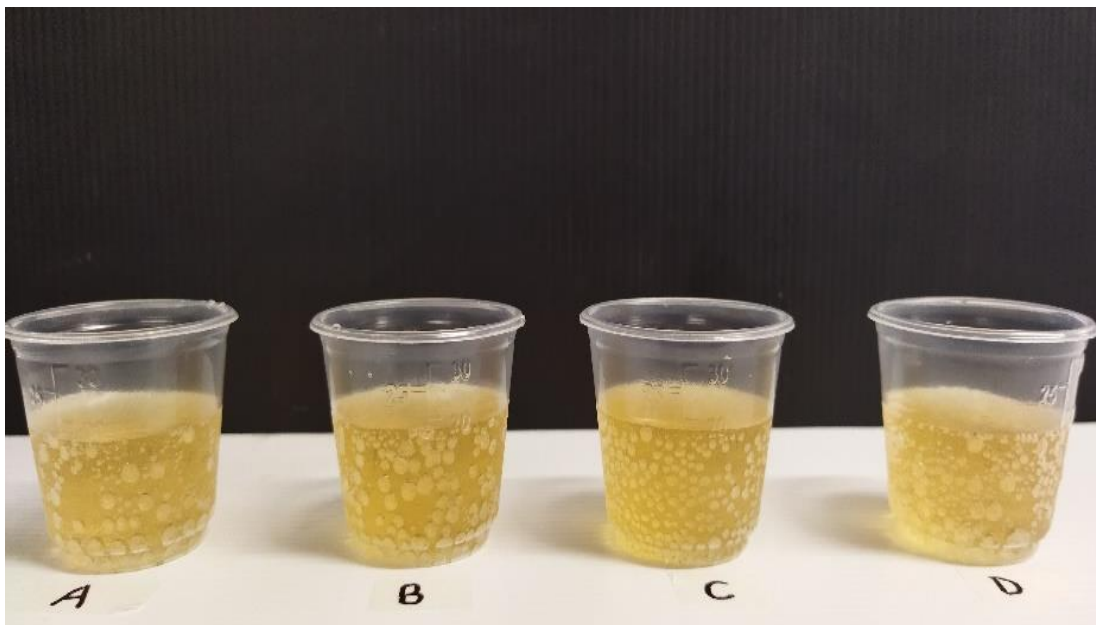


Figure 4.9 Green tea water kefir beverages

(Image was captured by Redmi 9T, Xiaomi Inc., China)

Notes: Sample A (WK1) = green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample B (WK2) = green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample C (WK3) = green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample D (WK4) = green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C

Of the four formulations, the green tea water kefir sample (WK2) fermented with 4% (w/v) brown sugar at 22°C received the highest consumer sensory scores for appearance (6.56 ± 0.04), sweetness (6.68 ± 0.11), sourness (6.51 ± 0.21), flavour (6.66 ± 0.32), aroma (6.7 ± 0.04) and overall acceptability (6.9 ± 0.14). Sample WK2 had a visible evolution of gas bubbles, which may have impacted the fizziness of the beverage. Sample WK2 was described by the panellists as, “a natural, refreshing, low-sugar drink, with a perfect balance of sweetness, acidity, and green tea-ginger flavour, and as

a well-fermented drink with yeasty aroma, rich flavour profile, sweeter drink compared to the other beverage samples with no bad aftertaste”. Water kefir sample (WK4) fermented with 4% (w/v) brown sugar at 25°C received the second highest scores for appearance (6.13 ± 0.04), sweetness (6.05 ± 0.16), sourness (6.20 ± 0.32), flavour (6.25 ± 0.35), aroma (6.16 ± 0.00) and overall acceptability (6.21 ± 0.35). This drink was described by the panellists as, “a refreshing, sparkly, low sugar, high acidity, and strongly fermented drink having a characteristic yeasty aroma but an overpowering ginger flavour and aftertaste”. The yeasty aroma of the green tea water kefir beverages may be due to the esters produced by the water kefir yeast (Corona et al., 2016; Nambou et al., 2014). Isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate are ester compounds which may contribute to the fruity and floral notes of fermented water kefir beverages (Corona et al., 2016; Fiorda et al., 2017; Laureys & De Vuyst, 2014; Randazzo et al., 2016).

The low scoring beverage samples (WK1 and WK3) were prepared using 3% (w/v) brown sugar at 22°C and 25°C respectively. Sample WK1 was described by the panellists as, “having a mild flavour, low sugar, low acidity, lacking good carbonation and fermentation. The drink did not have a well-rounded sensory profile”. Water kefir sample (WK3) received the lowest consumer sensory scores for appearance (5.56 ± 0.47), sweetness (4.48 ± 0.44), sourness (4.98 ± 0.58), flavour (4.88 ± 0.35), aroma (5.16 ± 0.28) and overall acceptability (4.9 ± 0.47). The drink was described by the panellists as, “a refreshing, fermented, carbonated and most acidic/sour of the four formulations. It had low sweetness compared to WK1, had a strong ginger flavour and an off-flavour/bitter aftertaste due to the high acidity/sourness”.

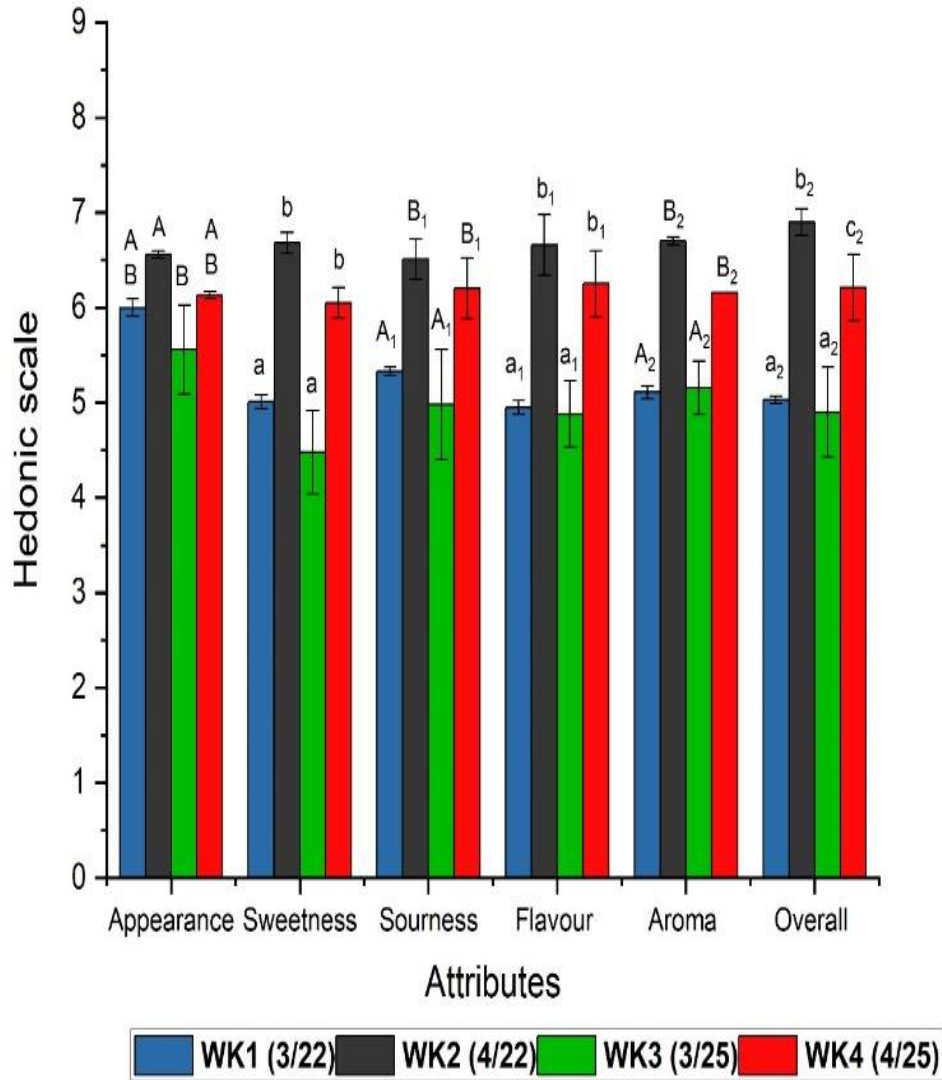


Figure 4.10: Mean consumer sensory evaluation scores of green tea water kefir beverages at the end of fermentation 144 h

Notes: Sample WK1= green tea water kefir containing 3% (w/v) brown sugar fermented at 22°C; Sample WK2= green tea water kefir containing 4% (w/v) brown sugar fermented at 22°C; Sample WK3= green tea water kefir containing 3% (w/v) brown sugar fermented at 25°C; Sample WK4= green tea water kefir containing 4% (w/v) brown sugar fermented at 25°C; Error bars = \pm SD (n=30); ab/a₁b₁/a₂b₂c₂ = significant mean difference in the sensory attributes (sweetness, flavour and overall acceptability respectively) were observed in all of the four formulations (p<0.05); AB/A₁B₁/A₂B₂ = significant mean difference in the sensory attributes (appearance, sourness and aroma respectively) were observed in all of the four formulations (p<0.05); experiments were conducted in duplicate and replicated twice.

4.2.6 Summary – Phase II

Results from phase II of this study showed that different sugar concentrations (3% and 4%, w/v) and fermentation temperatures (22°C and 25°C) contributed to the physicochemical, microbiological, and sensory profiles of the fermented ginger-green tea water kefir beverages. Increased metabolic activity of the microorganism was observed in samples prepared at high sugar concentrations and the higher fermentation temperature. However, kefir beverages prepared at the higher temperature, with the low sugar concentration were highly acidic and the least preferred by the sensory panellists. Water kefir sample (WK2) prepared at 22°C using the 4% (w/v) sugar concentration received the highest consumer sensory scores ($p < 0.05$), showed optimal physicochemical ($p < 0.05$) (pH (3.50 ± 0.00), T.A. (0.178 ± 0.01 %), TSS (3.82 ± 0.03 °B), colour L* (97.88 ± 0.03), colour a* (0.99 ± 0.09) and colour b* (3.15 ± 0.03) and microbiological results ($p < 0.05$) (viable cell count of LAB (8.50 ± 0.01 log cfu/mL) and *S. cerevisiae* (7.00 ± 0.04 log cfu/mL)). Therefore, water kefir beverage sample WK2 was selected for further studies.

4.3 Phase III: Analysis of the beverage with the most promising formulation during fermentation and storage (4±1°C)

4.3.1 Part I: Concentrations of ethanol, sugar, organic acids, and antioxidants in the most promising formulation (WK2) of green tea water kefir beverage during fermentation and storage

The purpose of part I of the study was to analyse the ethanol content, sugar, organic acids, and antioxidant composition of the most promising formulation (WK2) during 144 h of fermentation at 22°C and storage at 4°C for two weeks (Appendix F).

4.3.1.1 Ethanol content

The ethanol concentrations in the most promising green tea water kefir beverage selected from Phase II (WK2-4%/22°C) during fermentation and storage (4°C) for two weeks are shown in Figure 4.11 and Figure 4.12, respectively. From 0 to 24 h, the ethanol content increased ($p < 0.05$) from 0% to $0.14 \pm 0.02\%$ (v/v) (Appendix G.10). The ethanol content of the green tea water kefir beverage continued to increase until the removal of the kefir grains/bottling at 72 h ($0.36 \pm 0.02\%$, v/v). During bottled fermentation (72 h-144 h), the ethanol content initially decreased slightly but then continued to increase ($p < 0.05$) until the end of fermentation (144 h). These fluctuations in ethanol concentration are likely due to the decrease in viable cell counts of *S. cerevisiae* after 72 h, but the concomitant presence of a high viable cell count of LAB, as reported in Section 4.2.4. The ethanol concentration at the end of fermentation (144 h) was $0.53 \pm 0.12\%$ (v/v), which is similar to that observed in Brazilian milk kefir (0 to 0.32 ± 0.04 mg/mL) after 24 h of fermentation (Leite et al., 2013) and other studies of kefir beverages (Magalhaes et al., 2010; Zajsek & Gorsek, 2010). However, depending on the substrate type and substrate concentration during water kefir fermentation, higher concentrations of ethanol (1-4%) have been found (Dwiloka et al., 2020; Randazzo et al., 2016; Setyawardani et al., 2014).

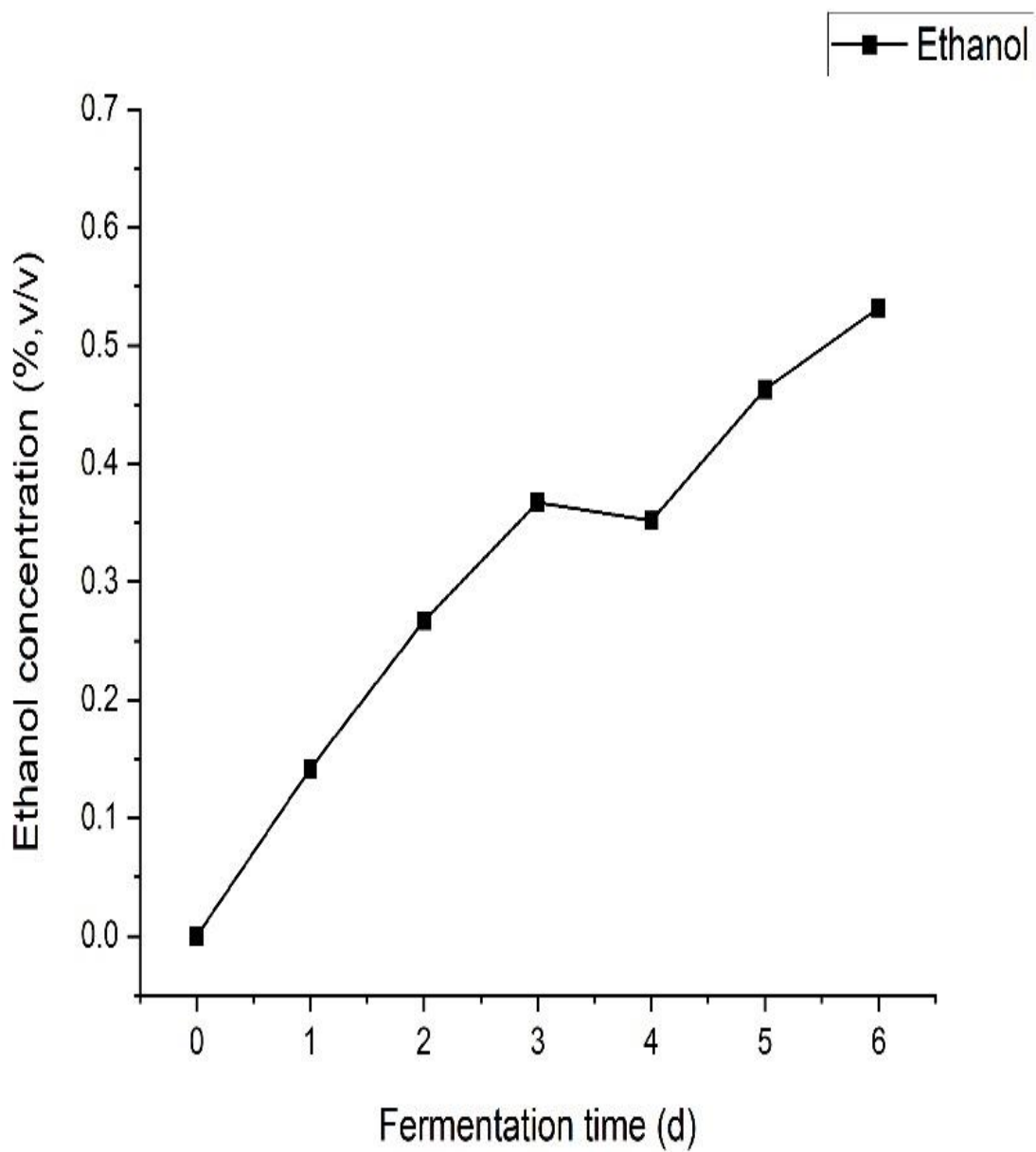


Figure 4.11: Mean ethanol concentration (% v/v) in green tea water kefir beverage (WK2) during fermentation for 144 h

Notes: d = day; experiments were conducted in duplicate and replicated twice.

During the two-week storage period, the concentration of ethanol continued to increase, although the rate of increase was lower than during fermentation. This suggested that the storage temperature influenced the metabolic activity of the yeast (Setyawardani & Sumarmono, 2015; Velicanski et al., 2014). The ethanol concentration increased ($p < 0.05$) from 0.62 ± 0.06 % at storage day (St. D-7) to 0.67 ± 0.06 % (v/v) at the end of two weeks (St. D-14) (Appendix G.11). Increasing ethanol concentrations in kefir beverages during cold storage (21d and 23 d) have been previously reported (Kok-Tas et al., 2013; Yildiz-Akgul et al., 2018 respectively). The results obtained are similar to published studies where low ethanol concentrations ($< 1\%$) were found during the storage period (Bueno et al., 2021; Kazakos et al., 2016; Koh et al., 2018; Leite et al., 2013; Mantzourani et al., 2022).

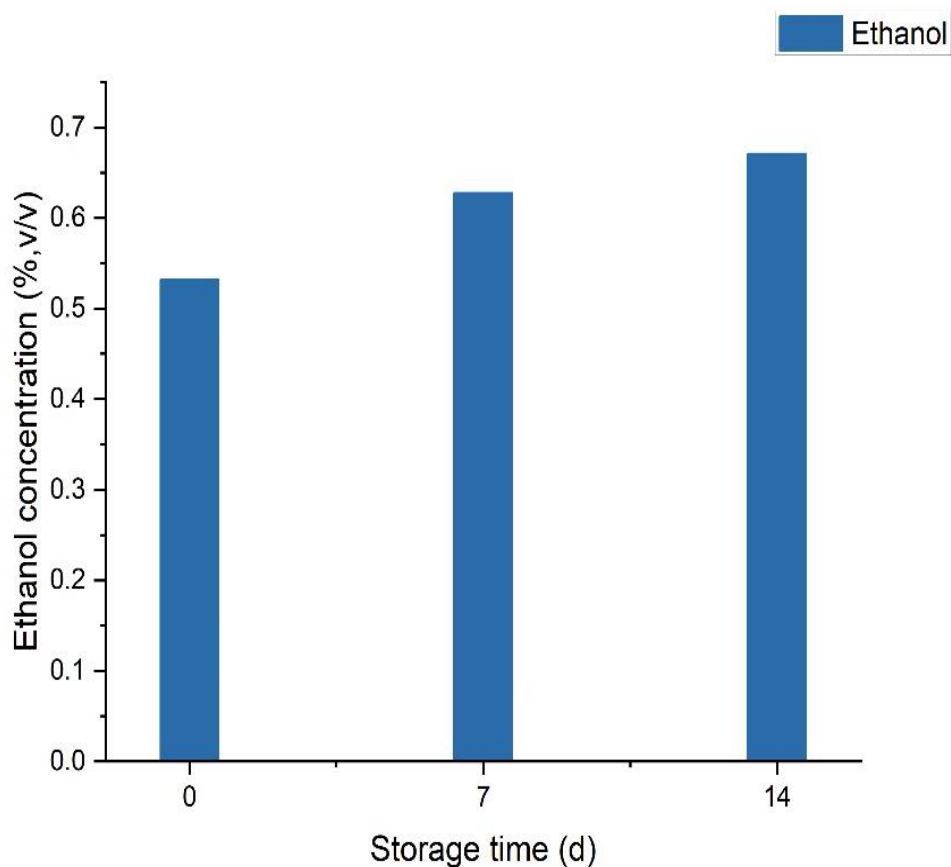


Figure 4.12: Mean ethanol concentration (% v/v) in green tea water kefir beverage (WK2) during two weeks of storage at $4 \pm 1^\circ\text{C}$

Notes: d = day; experiments were conducted in duplicate and replicated twice.

4.3.1.2 Sugars

The sugar composition of the most promising green tea water kefir beverage during fermentation, is shown in Figure 4.13. At the onset of fermentation, the concentrations of sucrose, glucose and fructose were $3.53 \pm 0.00\%$ (w/v), $1.27 \pm 0.30\%$ (w/v) and $0.58 \pm 0.03\%$ (w/v) respectively. Sucrose decreased ($p < 0.05$) during the first 24 h ($2.00 \pm 1.12\%$, w/v) of fermentation to 0% (w/v) at 48 h. The concentration of fructose increased ($p < 0.05$) up to $1.77 \pm 0.21\%$ (w/v) at 96 h. After this time, the concentration of fructose gradually decreased ($p < 0.05$) until the end of the fermentation period ($1.57 \pm 0.09\%$, w/v). The concentration of glucose did not differ during fermentation ($p > 0.05$), with the final concentration at 144 h being $1.19 \pm 0.21\%$ (w/v) (Appendix G.12). The presence of glucose and fructose in the green tea kefir beverage during the fermentation period indicated that the monosaccharides were being produced from the degradation of sucrose by the action of kefir yeast (Dwiloka et al., 2020; Laurey & De Vuyst, 2014; Stadie et al., 2013).

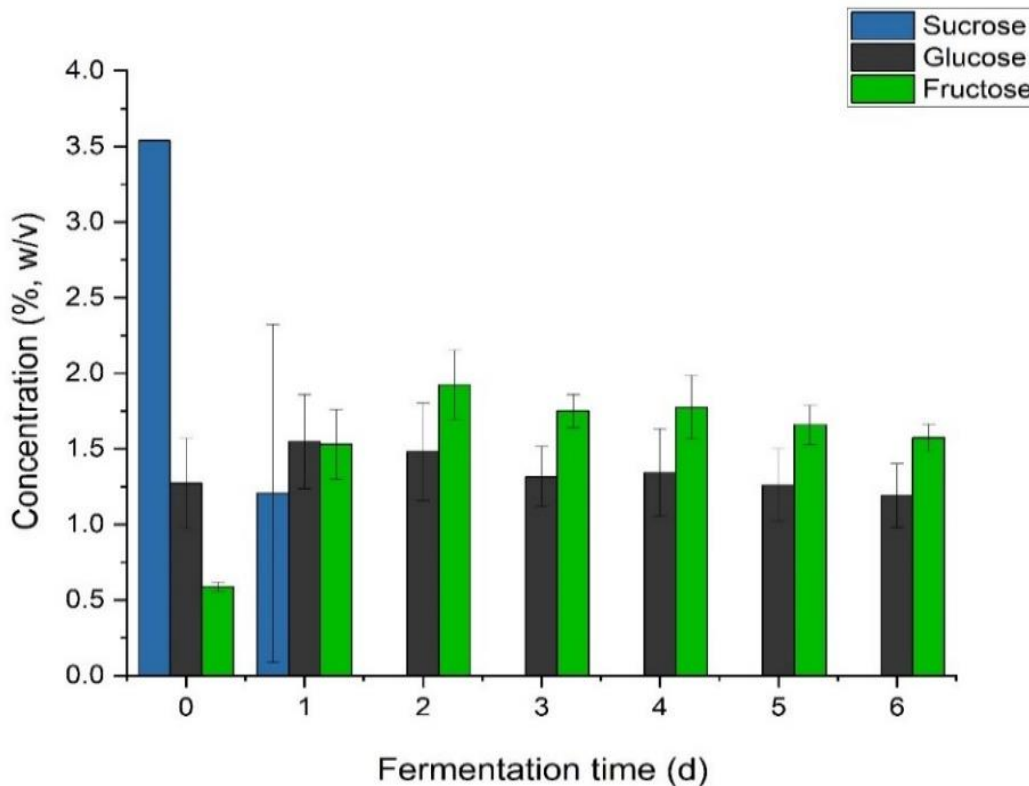


Figure 4.13: Mean concentration (% w/v) of sugars in green tea water kefir beverage (WK2) during the 144 h fermentation period

Notes: Error bars = \pm SD (n=4); d = day; experiments were conducted in duplicate and replicated twice.

The levels of the monosaccharides (glucose and fructose) did not change ($p>0.05$) during the storage period (Appendix G.13), as shown in Figure 4.14. The fact that no change in the monosaccharide concentrations were observed in green tea kefir beverage during storage suggested that the residual microbiota in the water kefir liquor were not metabolising the monosaccharides during cold storage. This could be due to a decrease in the viable LAB and yeast cells during cold storage as mentioned in Section 4.3.2.4. Additionally, in the present study, the concentration of glucose was lower than fructose throughout the storage period, which likely indicates the preference of yeast to ferment glucose than fructose (Berthels et al., 2004; Xu et al., 2019a).

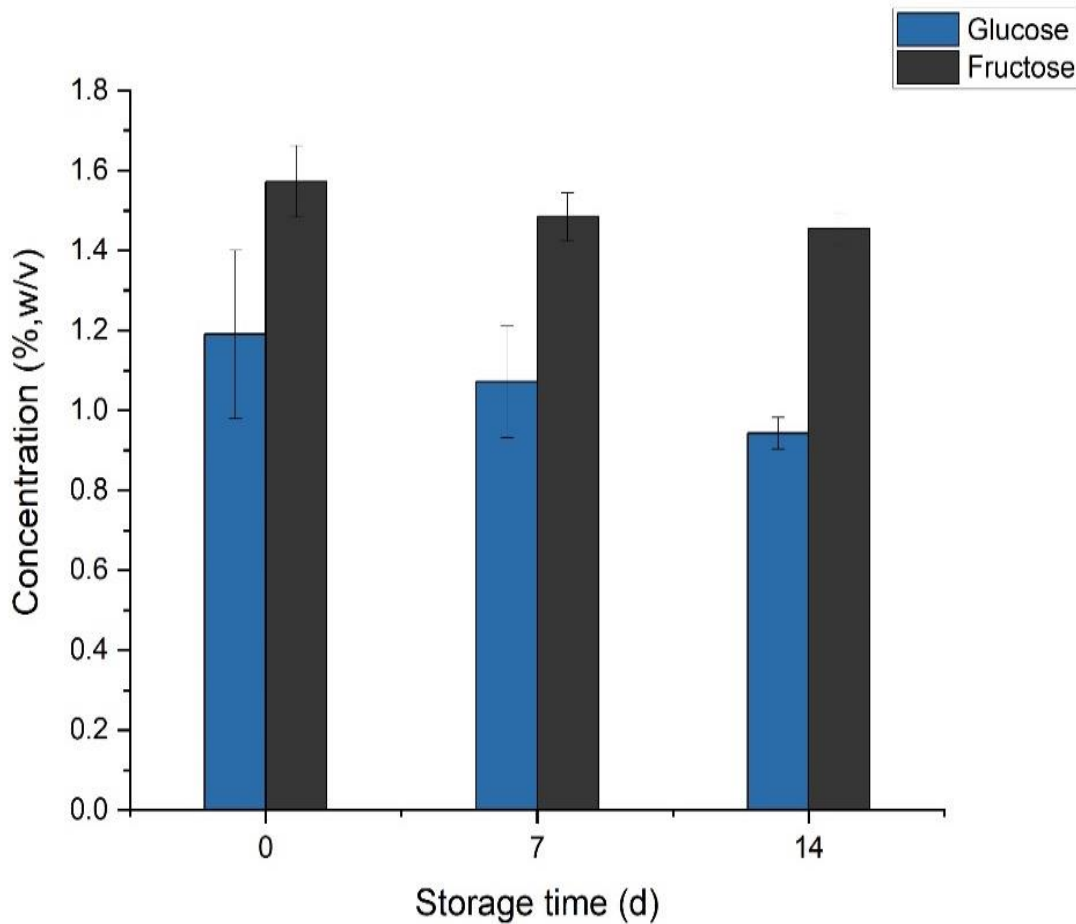


Figure 4.14: Mean concentration (% w/v) of sugars in green tea water kefir beverage (WK2) during the two week storage period at $4\pm 1^\circ\text{C}$

Notes: Error bars = \pm SD (n=4); d = day; experiments were conducted in duplicate and replicated twice.

4.3.1.3 Organic acids

Changes in the lactic acid concentration during the fermentation and storage periods are shown in Figure 4.15 and Figure 4.16, respectively. Lactic acid was detected in green tea water kefir after 48 h of fermentation ($0.181\pm 0.00\%$, v/v). Concentrations of lactic acid increased ($p < 0.05$) steadily until the completion of fermentation at 144 h ($0.625\pm 0.00\%$, v/v) (Limbad et al., 2023). Lactic acid concentrations of green tea water kefir were lower compared to milk-based kefir (0.44 g/100 mL) fermented for 18 h (Hikmetoglu et al., 2020) and whey-based-pomegranate kefir beverage fermented for 36 h (4.6 ± 0.1 - 5.9 ± 0.15 g/L) (Sabokbar & Khodaiyan, 2014). The presence of lactic acid is desirable as it aids in flavour development and prolongs the shelf life of fermented kefir beverages (Bengoa et al., 2019; Magalhaes et al., 2010; M'hir et al., 2021; Walsh et al., 2016).

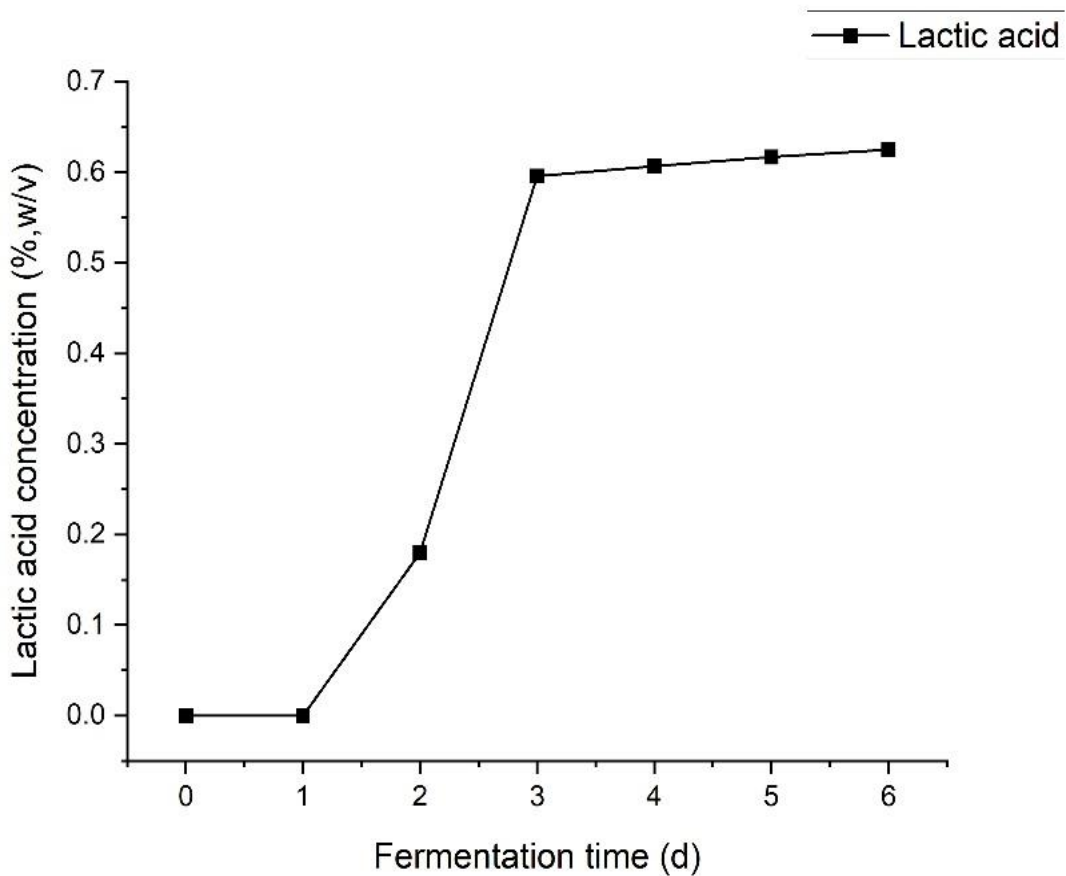


Figure 4.15: Mean concentration (% w/v) of lactic acid in green tea water kefir beverage (WK2) during fermentation for 144 h

Notes: d = day; experiments were conducted in duplicate and replicated twice.

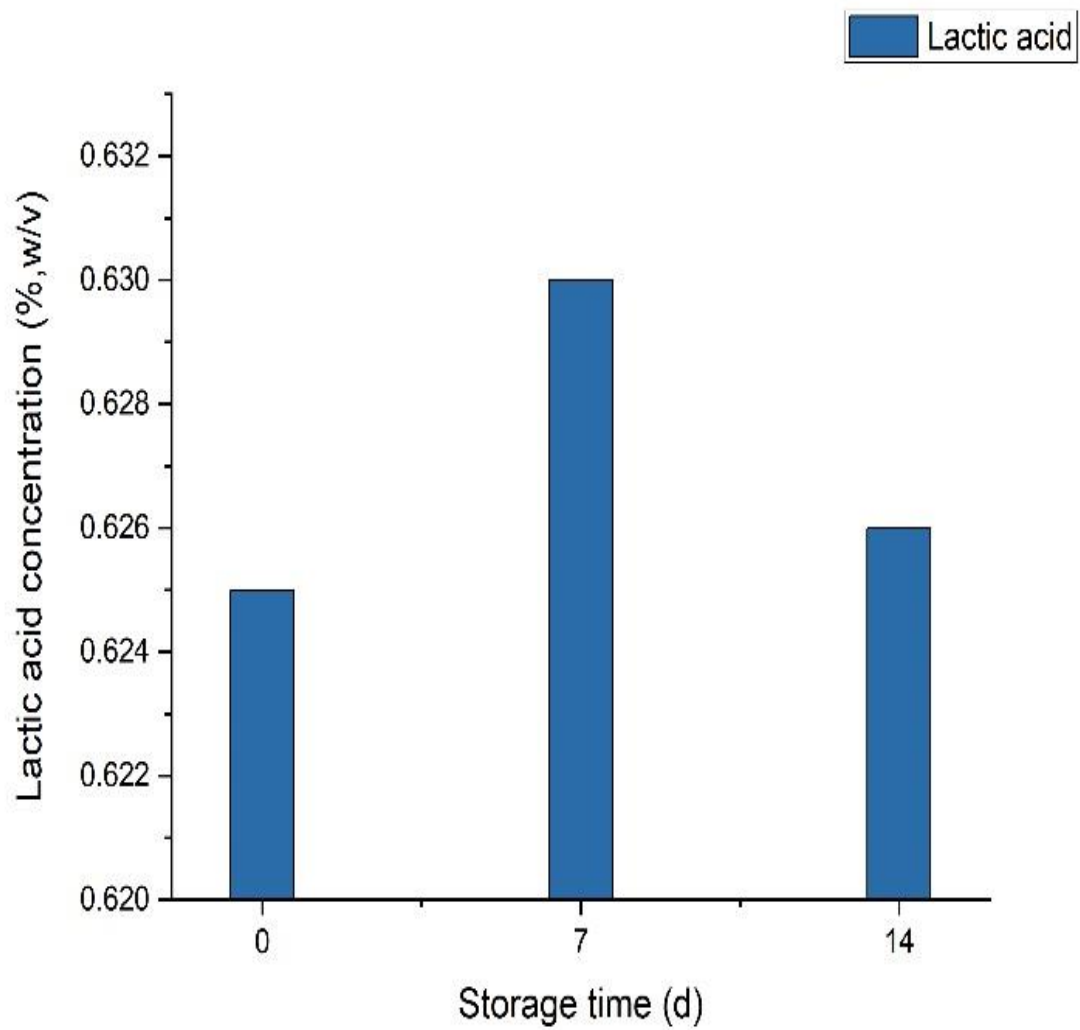


Figure 4.16: Mean concentration (% w/v) of lactic acid in green tea water kefir beverage (WK2) during storage period of two week at $4\pm 1^{\circ}\text{C}$

Notes: d = day; experiments were conducted in duplicate and replicated twice

No significant differences ($p>0.05$) in lactic acid concentration were found during the two week cold storage period, with the final concentration of lactic acid being $0.626\pm 0.00\%$ (v/v) (Appendix G.15). The lactic acid concentration of kefir during fermentation and storage depends on the substrate and type of starter culture used (Bueno et al., 2021; Saadi et al., 2017; Yildiz-Akgul et al., 2018). For example, the lactic acid concentration of milk-based kefir during 7 d storage was 0.51 g/100 ml (Hikmetoglu et al., 2020), while red pitaya/ apple pulp water kefir after 14 d storage was 0.48 ± 0.01 - 0.73 ± 0.01 g/L (Bueno et al., 2021), and after 16 d of refrigerated storage was 0.507 ± 0.0 - 0.658 ± 0.02 g/100 g (Yildiz-Akgul et al., 2018) and in pumpkin puree water kefir was 0.2-0.9 g/L during 28 d of cold storage (Koh et al., 2018).

The absence of acetic acid in green tea water kefir may be due to the microbial composition of the water kefir grains. The kefir grains vary depending on the geographical region and the fermentation process used (Martinez-Torres et al., 2017; Pendon et al., 2022; Zannini et al., 2022). Acetic acid bacteria may be present in low quantities, as found in various culture-dependent and culture-independent studies (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Laureys et al., 2017; Magalhaes et al., 2010; Marsh et al., 2013). The absence of acetic acid throughout fermentation and storage may also be due to the anaerobic fermentation conditions (Laureys et al., 2018; Laureys et al., 2021), as AAB remain in a dormant but viable state of not culturable cells (VBNC) in the absence of oxygen (Laureys et al., 2017). High concentrations of acetic acid are undesirable in water kefir due to the associated pH reduction, leading to sour sensorial profiles of beverages and the gradual decline of grain growth over the multiple back-slopping periods that take place as a result of excessive acidic stress (Laureys et al., 2017; Laureys et al., 2018; Laureys et al., 2021).

4.3.1.4 Antioxidant content

Figure 4.17 and Figure 4.18 show the concentration of phenolic compounds in green tea water kefir, which were analysed at the end of fermentation (144 h) and end of a two-week storage period (St. D-14). At the end of fermentation, the concentration of gallic acid, EGC, EGCG and ECG were 4.08 ± 0.24 $\mu\text{g/ml}$, 80.99 ± 1.35 $\mu\text{g/ml}$, 118.07 ± 4.68 $\mu\text{g/ml}$ and 23.33 ± 1.13 $\mu\text{g/ml}$ respectively. The concentration of caffeine and theobromine (methylxanthine) were 70.57 ± 1.53 $\mu\text{g/ml}$ and 4.06 ± 0.04 $\mu\text{g/ml}$ respectively. Following the two-week storage period, the concentration of gallic acid, EGC, EGCG, ECG, caffeine, and theobromine were 3.88 ± 0.13 $\mu\text{g/ml}$, 89.03 ± 4.45 $\mu\text{g/ml}$, 122.81 ± 2.79 $\mu\text{g/ml}$, 25.67 ± 1.10 $\mu\text{g/ml}$, 74.44 ± 0.52 $\mu\text{g/ml}$, 4.24 ± 0.03 $\mu\text{g/ml}$ respectively. The

antioxidant levels in green tea water kefir during cold storage were higher compared to black tea water kefir (Subardjo, 2017) and low compared to green tea kombucha (Wang, 2018), coffeeberry extract enriched kombucha (Essawet et al., 2015) and pulse supplemented dairy kefir (Saadi et al., 2017). This may be due to the low initial substrate concentrations (green tea 0.4%, w/v and brown sugar 4%, w/v) (Section 3.3.2) and differences in substrate, starter culture and fermentation conditions used compared to previous studies (Darvishzadeh et al., 2021; Karagozlu et al., 2017; Zannini et al., 2022). Results showed that the concentration of gallic acid decreased and catechin (EGCG and EGC) and caffeine increased while ECG and theobromine did not change. Increase in the concentration of EGC, EGCG and caffeine during storage may occur due to enzymatic degradation and biotransformation of other phenolic compounds by kefir microorganisms in an acidic environment (Amirdivani & Baba, 2015; Darvishzadeh et al., 2021; Jayabalan et al., 2007; Jayabalan et al., 2008; Jayabalan et al., 2014; Talib et al., 2019; Tu et al., 2019). These results suggested that green tea water kefir could be stored for two weeks under cold storage ($4\pm 1^{\circ}\text{C}$) without loss of phenolic compounds.

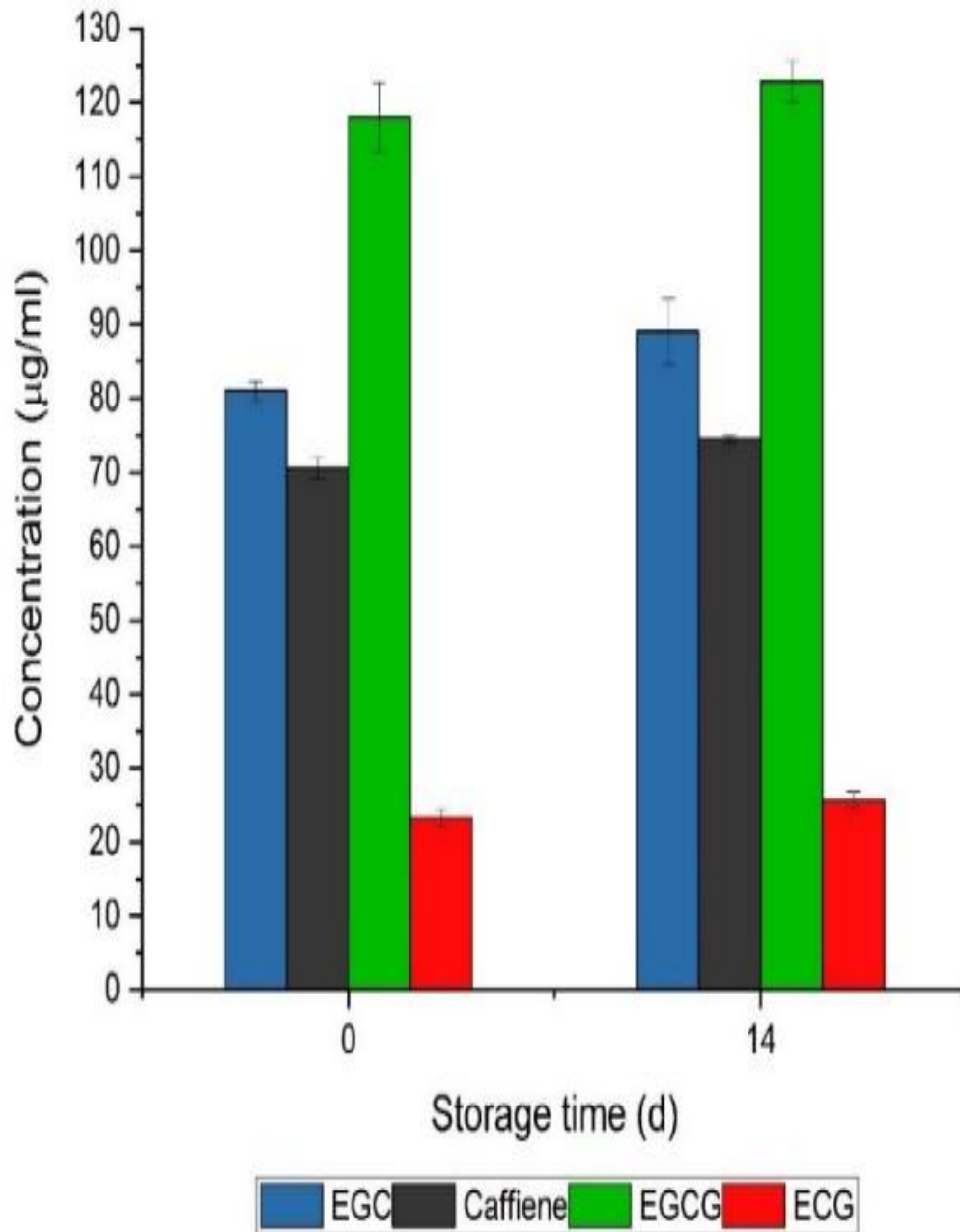


Figure 4.17: Mean concentration ($\mu\text{g/ml}$) of phenolic compounds in green tea water kefir beverage (WK2) at the end of fermentation (144 h) and end of storage period (St. D-14)

Notes: EGC= Epigallocatechin; EGCG= epigallocatechin gallate; ECG = epicatechin gallate; 0= end of fermentation period (144 h); 14= end of storage period (St. D-14); Error bars = \pm SD (n=4); d = day; experiments were conducted in duplicate and replicated twice.

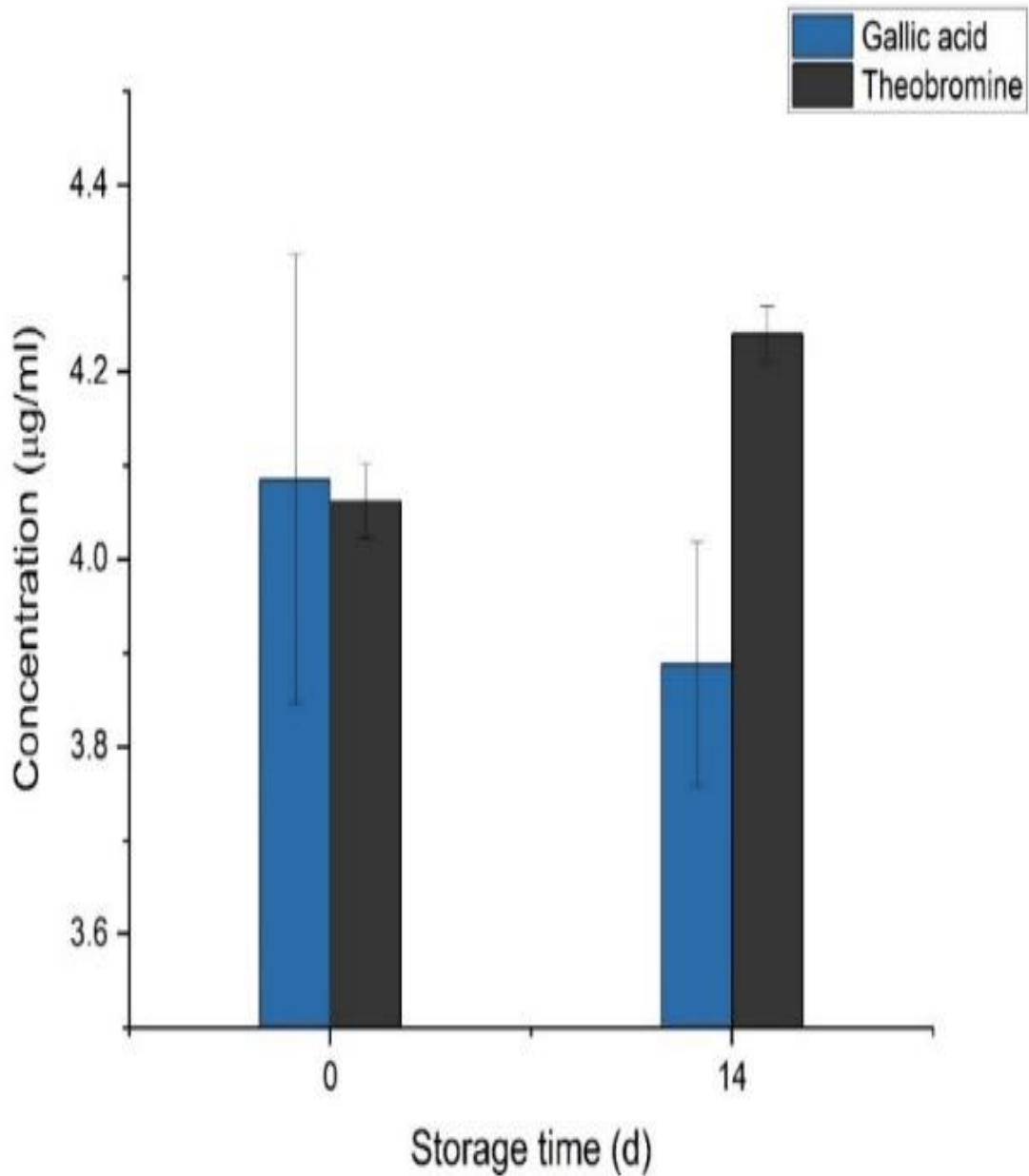


Figure 4.18: Mean concentration ($\mu\text{g/ml}$) of phenolic compounds in green tea water kefir beverage (WK2) during end of fermentation period (144 h) and end of storage period (St. D-14)

Notes: 0= end of fermentation period (144 h); 14= end of storage period (St. D-14); Error bars = \pm SD (n=4); d = day; experiments were conducted in duplicate and replicated twice.

4.3.1.5 Summary- Phase III part I

During fermentation, the concentration of sucrose decreased ($p < 0.05$) while that of fructose and lactic acid increased ($p < 0.05$). During the refrigerated storage period ($4 \pm 1^\circ\text{C}$), the concentration of glucose ($0.94 \pm 0.04\%$, w/v), fructose ($1.45 \pm 0.04\%$, w/v), lactic acid ($0.626 \pm 0.00\%$, v/v), and antioxidants (ECG 25.67 ± 1.10 $\mu\text{g/ml}$ and theobromine 4.24 ± 0.03 $\mu\text{g/ml}$) remained stable while that of caffeine (74.44 ± 0.52 $\mu\text{g/ml}$), EGCG (122.81 ± 2.79 $\mu\text{g/ml}$) and EGC (89.03 ± 4.45 $\mu\text{g/ml}$) increased. The ethanol content increased ($p < 0.05$) during fermentation and storage with a final concentration of $0.67 \pm 0.06\%$ (v/v).

4.3.2 Part II: Stability of final green tea water kefir formulation during refrigerated storage at 4±1°C

The purpose of part II was to investigate the stability of microbiological and physicochemical parameters of the beverage WK2 during storage at 4±1°C for 14 d.

4.3.2.1 Total soluble solids (°Brix)

The total soluble solids in green tea water kefir beverage during 14 days of storage (4±1°C) is shown in Figure 4.19. TSS gradually decreased ($p < 0.05$) from 3.82±0.10 (St. D-0) to 3.72±0.03°B, with a final TSS of 3.62±0.03°B after 2 weeks of storage (Appendix G.16). In previous studies, similar reductions in TSS were observed during cold storage (Abdolmaleki et al., 2015; Leite et al., 2013; Mu, 2019; Subardjo, 2017; Wang, 2018). Moreover, the reduction in brix indicated that the residual microflora (LAB and yeast) in the kefir beverages were still active and metabolising residual sugar in the beverage (Bueno et al., 2021), as mentioned in Section 4.3.2.4. The low brix concentration observed for the green tea water kefir produced meets one of the research objectives: to produce a low-sugar fermented beverage.

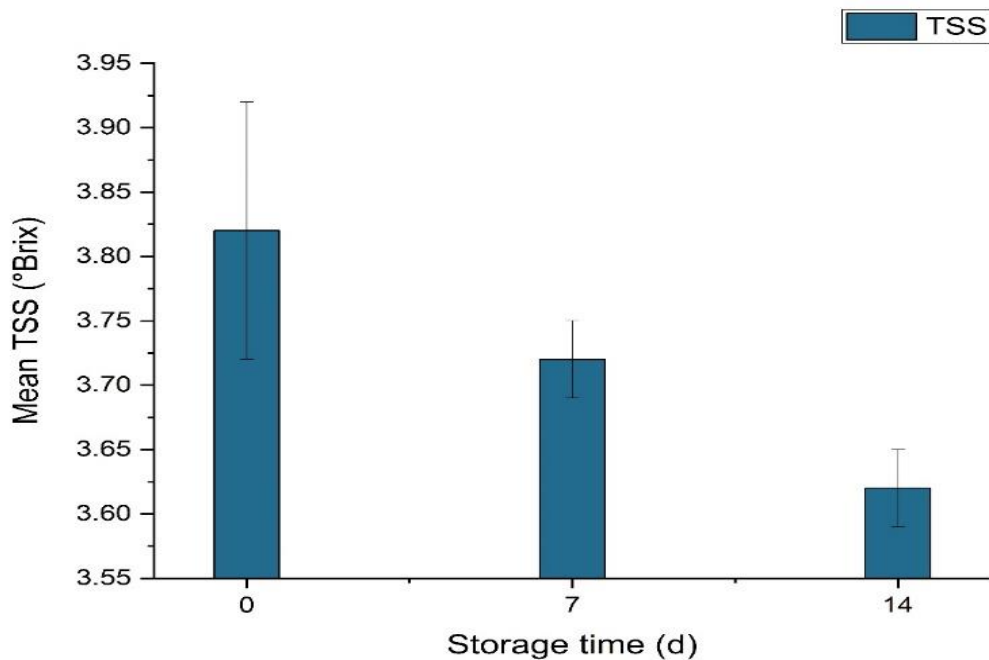


Figure 4.19: Mean TSS (°Brix) of green tea water kefir beverage (WK2) during storage period of two weeks at 4±1°C

Notes: TSS= Total soluble solids; Error bars = ± SD (n=4); d = day; experiments were conducted in duplicate and replicated twice.

4.3.2.2 pH and titratable acidity

Changes in the pH and T.A. of green tea water kefir beverage during cold storage for two weeks are shown in Figure 4.20. As expected, the pH of the water kefir beverage decreased ($p < 0.05$) with a simultaneous increase in acidity, as discussed previously (Section 4.2.1) (Saadi et al., 2017). Compared to storage day 0 (pH 3.51 ± 0.00), the pH decreased ($p < 0.05$) from 3.47 ± 0.00 (St. D-7) to 3.42 ± 0.00 after 2 weeks of storage. Similar results were reported following the refrigerated storage of Cornelian cherry-flavoured water kefir, with the pH decreasing from 3.53 ± 0.00 to 3.41 ± 0.02 after one week of storage (Ozcelik et al., 2021).

In the current study, T.A. increased ($p < 0.05$) from $0.179 \pm 0.00\%$ to $0.199 \pm 0.00\%$ by the end of storage (Appendix G.17). The T.A. in the current study was high compared to goat milk kefir ($0.154 \pm 0.00\%$) after 20 d of cold storage (Setyawardani & Sumarmono, 2015) but low compared to cow/oat milk-based kefir (0.48 ± 0.02 - $0.84 \pm 0.00\%$) during 21 d of refrigerated storage (Dinkci et al., 2015) and of red pitaya/apple pulp water kefir (0.45 - 0.53 g/100 ml) after 28 d of storage (Bueno et al., 2021). The differences in the results are likely due to differences in the substrate matrix, kefir grain concentration and fermentation conditions employed (Gronnevik et al., 2011; Saadi et al., 2017). Reduction in pH and the concomitant increase in T.A. during storage suggested post-metabolism of sugar into organic acids due to the activity of microorganisms (LAB and *S. cerevisiae*) (Corona et al., 2016; da Costa et al., 2019; dos Santos et al., 2019; Leite et al., 2013; Limbad et al., 2023; Tu et al., 2019).

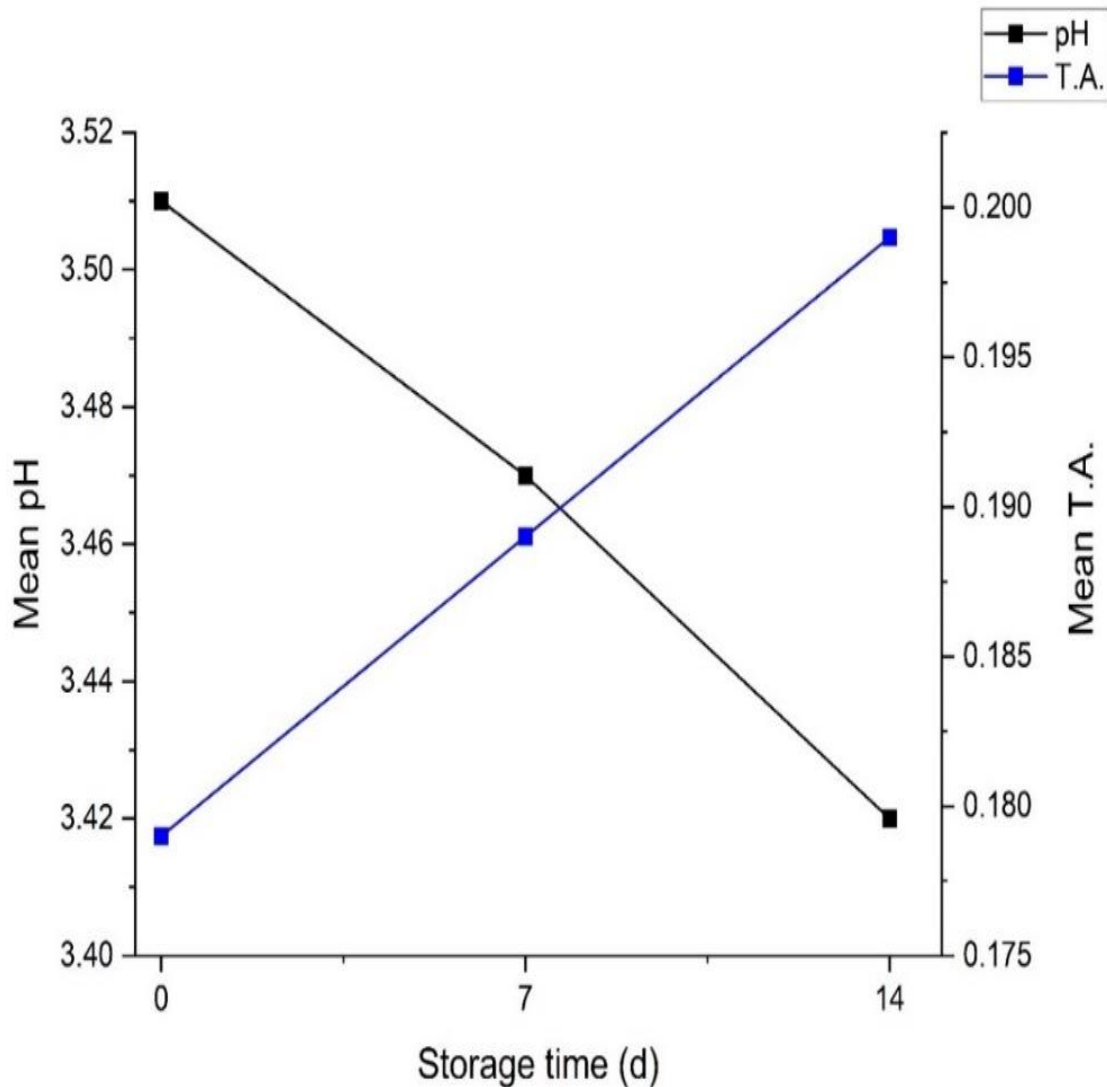


Figure 4.20: Mean pH and titratable acidity of green tea water kefir beverage (WK2) during storage period of two weeks at $4\pm 1^{\circ}\text{C}$

Notes: T.A.= Titratable acidity; d = day; experiments were conducted in duplicate and replicated twice.

4.3.2.3 Colour

Figure 4.21 shows the colour changes of the green tea water kefir beverage during storage. The lightness (L^*) value and redness-greenness (a^*) value increased ($p < 0.05$) during storage. The L^* value showed fluctuating changes, increasing ($p < 0.05$) from 98.10 ± 0.04 (St. D-0) to 99.17 ± 0.03 (St. D-7) and decreasing to 98.27 ± 0.18 after two weeks of storage (St. D-14). The a^* value gradually increased ($p < 0.05$) from 0.99 ± 0.03 (St. D-0) to 1.97 ± 0.06 at the end of storage (Appendix G.18). In contrast, the yellowness-blueness (b^*) value of the water kefir beverage

decreased ($p < 0.05$) from 3.17 ± 0.03 (St. D-0) to 2.32 ± 0.08 (St. D-14) during storage. Similar results have been previously observed in fermented kefir beverages (Hrnjez et al., 2014; Lopusiewicz et al., 2019; Lopusiewicz et al., 2020). The changes observed in the colour parameters during cold storage in the current study can be attributed to oxidation of colour pigments and the decrease in pH (Lopusiewicz et al., 2019).

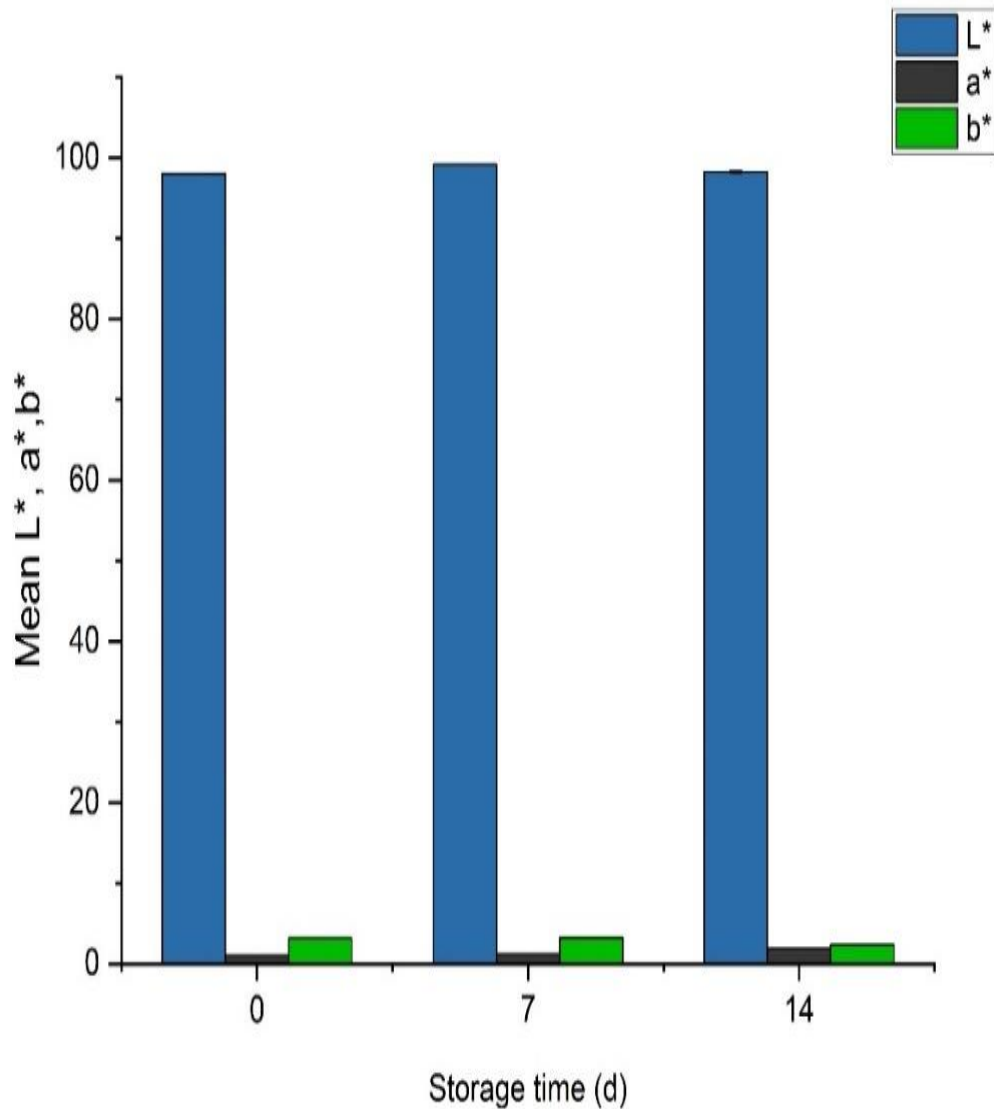


Figure 4.21: Mean colour (L^* , a^* , b^*) of green tea water kefir beverage (WK2) during storage period of two weeks at $4 \pm 1^\circ\text{C}$

Notes: L^* = Lightness, a^* = redness-greenness; b^* = yellowness-blueness; experiments were conducted in duplicate and replicated twice; d = day.

4.3.2.4 Microbiological analysis

Viable LAB and *S. cerevisiae* cell counts in the green tea water kefir beverage during 14 d storage (4°C), are shown in Figure 4.22. The LAB and yeast cell counts were 8.51±0.00 log cfu/mL and 7.02±0.01 log cfu/mL, respectively, at the beginning of storage and gradually decreased ($p < 0.05$) to 8.42±0.00 log cfu/mL and 6.85±0.00 log cfu/mL respectively, after 2-weeks refrigerated storage (4±1°C) (Appendix G.19). The decrease in viable LAB cell counts during storage was likely due to the depleted nutrient availability, bioconversion of sucrose to monosaccharides and acidification of the kefir beverages (da Costa et al., 2018; Dwiloka et al., 2020; Fiorda et al., 2017; Jeong et al., 2013; Laureys & De Vuyst, 2014; Lopusiewicz et al., 2020). Despite the observed decrease, the viable cell counts obtained in the present study do fall within the requirement of a probiotic beverage ($>10^6$ cfu/ml) (Terpou et al., 2019). Similar viable cell count results were reported for LAB-fermented pomegranate juice during one month of cold storage (7.2 log cfu/ml) (Mantzourani et al., 2022) and other kefir studies (Irigoyen et al., 2005; Kok-Tas et al., 2013; Lopusiewicz et al., 2020; Pinto et al., 2021; Saadi et al., 2017).

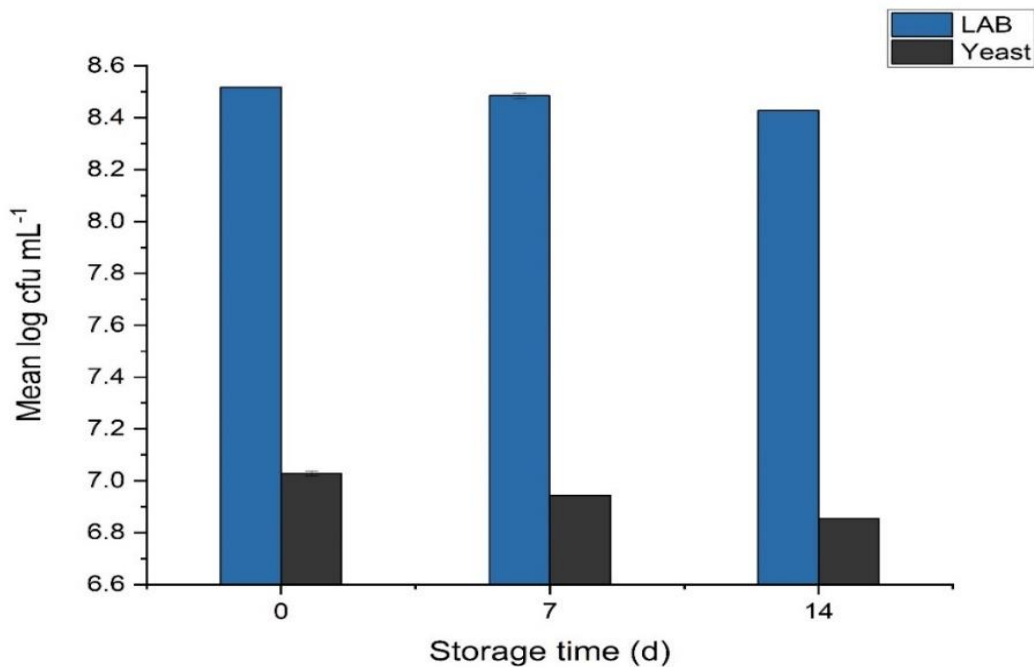


Figure 4.22: Mean viable cell count of lactic acid bacteria and yeast in green tea water kefir beverage (WK2) during storage period of two weeks at 4±1°C

Notes: d = day; experiments were conducted in duplicate and replicated twice.

4.3.2.5 Focus group sensory analysis

The green tea water kefir beverage was evaluated for sensory attributes (appearance, aroma, flavour, sourness, sweetness, and overall acceptance) during two weeks of refrigerated storage ($4\pm 1^{\circ}\text{C}$), using a 9-point hedonic scale (Figure 4.23). Sweetness was the only sensory attribute which changed significantly ($p < 0.05$) during the storage period (Appendix G.20). Previously published studies have also documented comparable decreases in sweetness during storage (Hrnjez et al., 2014; Subardjo, 2017; Wang, 2018). The observed decrease in sweetness aligns with the decrease in TSS during storage as mentioned in Section 4.3.2.1.

At the beginning of fermentation, mean sensory attribute scores for the green tea beverages were as follows, appearance (7.81 ± 0.08), sweetness (8.00 ± 0.17), sourness (7.75 ± 0.35), flavour (7.50 ± 0.00), aroma (7.50 ± 0.17) and overall acceptability (7.62 ± 0.35). The beverage was described by the panellists as, “a refreshing, fermented, fizzy drink with a balanced profile of flavour, sweetness, and acidity. A low sugar drink with no unexpected aftertaste”. Following the 2 week storage period, the beverage was described by the focus group panellists as, “a carbonated, fermented drink with lower sugar compared to storage day 0 but with an overall well-balanced flavour, sweetness, and acidity. No unexpected aftertaste or overpowering flavour of ginger or green tea were observed”. Moreover, an increase in the carbonation/fizziness was observed throughout storage period. Results from the current study suggested that green tea water kefir could be stored for two weeks under cold storage ($4\pm 1^{\circ}\text{C}$) without loss of sensory profile.

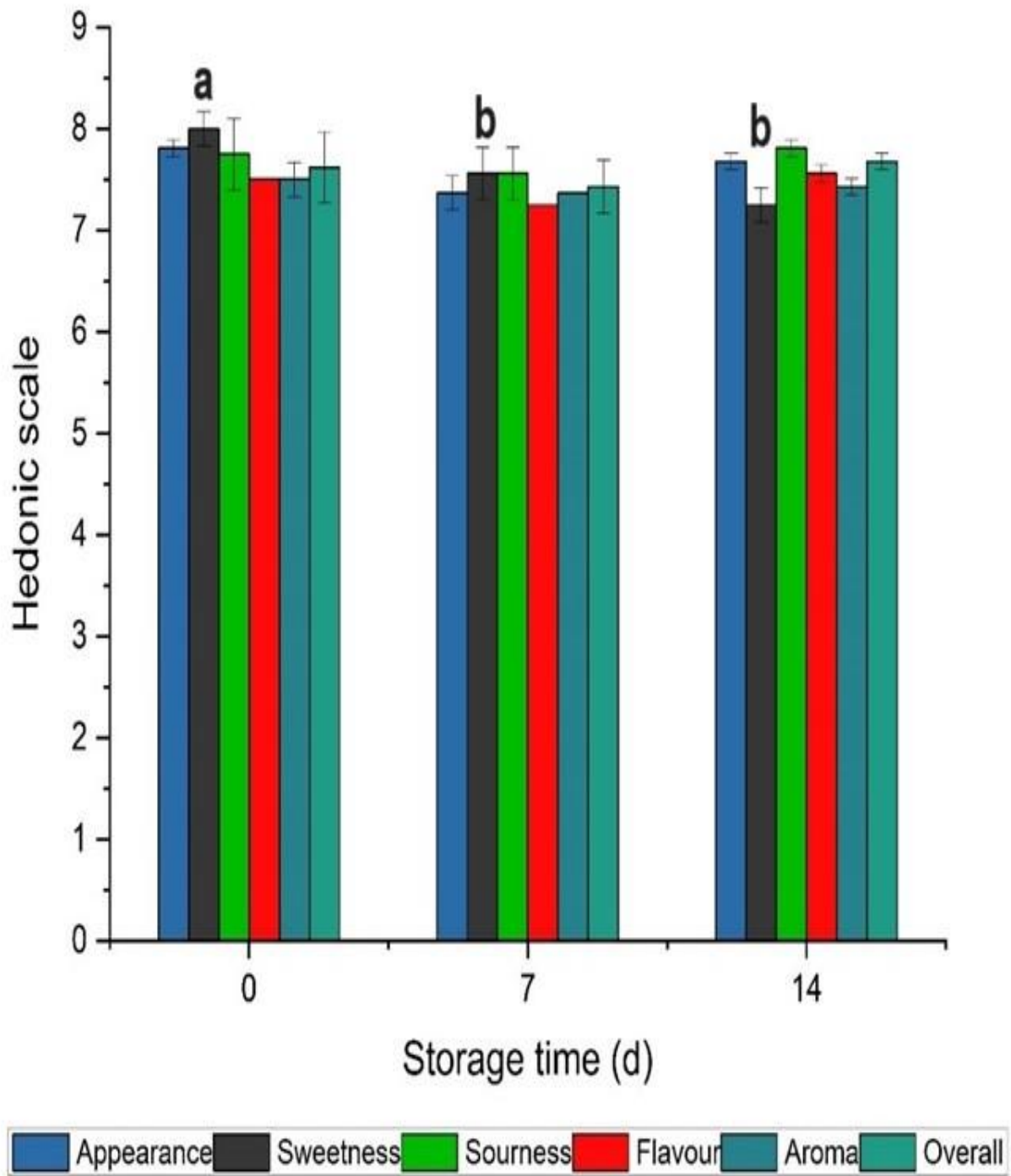


Figure 4.23: Mean sensory scores of green tea water kefir beverage (WK2) during storage period of two weeks at $4\pm 1^{\circ}\text{C}$

Notes: a,b= significant changes in sweetness throughout storage period ($p < 0.05$); Error bars = \pm SD ($n=4$); d = day; experiments were conducted in duplicate and replicated twice.

4.3.2.6 Summary- Phase III part II

At the end of the storage period, pH (3.42 ± 0.00), total soluble solids (3.62 ± 0.03 °B), colour b^* (2.32 ± 0.08), viable LAB (8.42 ± 0.00 log cfu/mL) and yeast cell counts (6.85 ± 0.00 log cfu/mL) decreased ($p < 0.05$) while colour (L^* 98.27 ± 0.18 and a^* 1.97 ± 0.06), titratable acidity and the ethanol concentration increased ($p < 0.05$). The results from the sensory evaluation suggested that the fermented green tea water kefir beverage was stable during storage for two weeks at 4°C.

5. Overall Conclusion

Green tea was used as a fermentation substrate to develop a novel, dairy-free, low sugar water kefir. Fermentation temperature and sugar concentration had a significant effect ($p < 0.05$) on the physico-chemical and organoleptic properties of the water kefir beverage. The most promising beverage based on the physico-chemical, microbiological, and sensory characteristics was green tea water kefir beverage (WK2) fermented with 4% (w/v) sugar at 22°C for 144 h. During refrigerated storage for two weeks, pH, total soluble solids, viable cell count of LAB and yeast decreased ($p < 0.05$), while colour (L^* and a^*), titratable acidity and the ethanol concentration increased ($p < 0.05$). The concentration of lactic acid, glucose and fructose remained stable during storage. The mean focus group sensory scores remained constant during storage but with a significant decrease in sweetness observed. Presence of the antioxidants at the end of storage period ($4 \pm 1^\circ\text{C}$) in the green tea water kefir suggested that the beverage may exert beneficial effects on human health when consumed regularly.

6. Recommendations

In the present study, the species or strains of the microorganism embedded in the water kefir grains were not identified. The microbial community of water kefir grains is complex depending on the substrate habitat and country of origin (Gamba et al., 2021; Laureys et al., 2018; Laureys et al., 2019; Laureys et al., 2021; Lynch et al., 2021; Martinez-Torres et al., 2017; da Miguel et al., 2011) and may contain strains with probiotic characteristics (Koh et al., 2018; Laureys & De Vuyst, 2014; Leite et al., 2015; Stadie et al., 2013). Therefore, isolation and identification of water kefir grains will help in the better understanding of microbial interactions and be useful for the control of the production process as well as the development of water kefir with other substrates.

Two levels of fermentation temperatures and sugar concentrations were investigated for the optimum fermentation condition of the green tea water kefir beverage. Additional fermentation temperatures, sugar concentrations and flavouring substrates may be considered in future studies for novel product development, by-product utilization, product differentiation and better understanding of the kefir fermentation process for upscaling of commercialization process.

The results from this study showed the presence of antioxidants in green tea water kefir. Additional studies should be carried out in order to confirm the antioxidant activity of water kefir, such as investigating the scavenging activities on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, ferric reducing ability of plasma (FRAP) and ABTS radical scavenging ability (Alsayadi et al., 2013; Talib et al., 2019; Tu et al., 2019). Further, *in vivo or in vitro* studies to investigate the beneficial health effects of these antioxidants could also be carried out (Prasanth et al., 2019; Rocha-Gomes et al., 2018).

7. References

- Abdolmaleki, F., Assadi, M. A., & Akbarirad, H. (2015). Assessment of beverages made from milk, soyamilk, and whey using Iranian kefir starter culture. *International Journal of Dairy Technology*, 68(3), 441-447.
- Abe, S. K., & Inoue, M. (2021). Green tea and cancer and cardiometabolic diseases: a review of the current epidemiological evidence. *European Journal of Clinical Nutrition*, 75(6), 865-876.
- Ahmed, M. E., Hammam, A. R., Ali, A. E. F., Alsaleem, K. A., Elfaruk, M. S., Kamel, D. G., & Moneeb, A. H. (2023). Measurement of carbohydrates and organic acids in varieties of cheese using high-performance liquid chromatography. *Food Science & Nutrition*, 11(5), 2081-2085.
- Alcazar, A., Ballesteros, O., Jurado, J., Pablos, F., Martin, M., Vilches, J., & Navalon, A. (2007). Differentiation of green, white, black, Oolong, and Pu-erh teas according to their free amino acids content. *Journal of Agricultural and Food Chemistry*, 55(15), 5960-5965.
- Alsayadi, M., Al Jawfi, Y., Belarbi, M., & Sabri, F. Z. (2013). Antioxidant potency of water kefir. *The Journal of Microbiology, Biotechnology and Food Sciences*, 2(6), 2444.
- Amirdivani, S., & Baba, A. S. H. (2015). Green tea yogurt: major phenolic compounds and microbial growth. *Journal of Food Science and Technology*, 52(7), 4652-4660.
- Amore, A., & Faraco, V. (2013). Physical and chemical factors affecting fermentation in food processing. In C. R. Soccol, A. Pandey, & C. Larroche (Eds.), *Fermentation Processes Engineering in the Food Industry* (pp. 47-73). Boca Raton, Florida: CRC Press.
- AOAC. (2005a). AOAC Official Method 932.14 Solids in Syrups. In: *American Organisation of Analytical Chemist International*, 18. Gaithersburg, Maryland, USA.
- AOAC. (2005b). AOAC Official Method 936.16 Standard Solution of Sodium Hydroxide Standard Potassium Hydrogen Phthalate Method. In: *American Organisation of Analytical Chemist International*, 18. Gaithersburg, Maryland, USA.
- AOAC. (2005c). AOAC Official Method 947.05 Acidity of Milk Titrimetric Method. In: *American Organisation of Analytical Chemist International*, 18. Gaithersburg, Maryland, USA.
- AOAC. (2005d). AOAC Official Method 981.12 pH of Acidified Foods. In: *American Organisation of Analytical Chemist International*, 18. Gaithersburg, Maryland, USA.

- Atalar, I. (2019). Functional kefir production from high pressure homogenised hazelnut milk. *LWT-Food Science and Technology*, 107, 256-263.
- Azi, F., Tu, C., Meng, L., Zhiyu, L., Cherinet, M. T., Ahmadullah, Z., & Dong, M. (2021). Metabolite dynamics and phytochemistry of a soy whey-based beverage bio-transformed by water kefir consortium. *Food Chemistry*, 342, 128225.
- Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. *The Royal Society Of Chemistry Advances*, 5(35), 27540-27557.
- Bancirova, M. (2010). Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. *Food Research International*, 43(5), 1379-1382.
- Bansal, S., Choudhary, S., Sharma, M., Kumar, S. S., Lohan, S., Bhardwaj, V. & Jyoti, S. (2013). Tea: a native source of antimicrobial agents. *Food Research International*, 53(2), 568-584.
- Bau, T. R., Garcia, S., Ida, E. I., & Bau, T. R. (2015). Changes in soymilk during fermentation with kefir culture: oligosaccharides hydrolysis and isoflavone aglycone production. *International Journal of Food Sciences & Nutrition*, 66(8), 845-850.
- Bengoa, A.A., Iraporda, C., Garrote, G.L., Abraham, A.G. (2019). Kefir micro-organisms: Their role in grain assembly and health properties of fermented milk. *Journal of Applied Microbiology*, 126, 686–700.
- Bergmann, R. S. D. O., Pereira, M. A., Veiga, S. M. O. M., Schneedorf, J. M., Oliveira, N. D. M. S., & Fiorini, J. E. (2010). Microbial profile of a kefir sample preparations: grains in natura and lyophilized and fermented suspension. *Food Science and Technology*, 30, 1022-1026.
- Berthels, N., Cordero Otero, R., Bauer, F., Thevelein, J., & Pretorius, I. (2004). Discrepancy in glucose and fructose utilisation during fermentation by *Saccharomyces cerevisiae* wine yeast strains. *FEMS Yeast Research*, 4(7), 683-689.
- Bosch, R., Philips, N., Suárez-Pérez, J. A., Juarranz, A., Devmurari, A., Chalensouk-Khaosaat, J., & González, S. (2015). Mechanisms of photoaging and cutaneous photocarcinogenesis, and photoprotective strategies with phytochemicals. *Antioxidants*, 4(2), 248-268.
- Bourrie, B.C.T.; Willing, B.P.; Cotter, P.D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontier in Microbiology*, 7, 647.
- Bueno, R. S., Ressutte, J. B., Hata, N. N., Henrique-Bana, F. C., Guergoletto, K. B., de Oliveira, A. G., & Spinosa, W. A. (2021). Quality and shelf-life assessment of a new beverage

- produced from water kefir grains and red pitaya. *LWT- Food Science and Technology*, 140, 110770.
- Bunzel, M., & Schendel, R. R. (2017). Determination of total phenolics and antioxidant capacity in food and ingredients. *Food Analysis*, 455-468.
- Cabrera, C., Artacho, R., Gimenez, R. (2006). Beneficial effects of green tea—A review. *Journal of the American College of Nutrition*, 25, 79–99.
- Cao, Q.Q., Zou, C., Zhang, Y.H., Du, Q.Z., Yin, J.F., Shi, J., Xu, Y.Q. (2019). Improving the taste of autumn green tea with tannase. *Food Chemistry*, 277, 432-437.
- Carballo, J. (2012). The role of fermentation reactions in the generation of flavor and aroma of foods. *Fermentation: Effects on food properties*, 51-83.
- Caro Velez, C. A., & Leon Pelaez, A. M. (2015). Antifungal capacity of cell-free supernatants obtained from fermentation of a substrate of brown sugar with water kefir grains. *Revista Colombiana de Biotecnología*, 17(2), 22-31.
- Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: a literature review. *Chinese Medicine*, 5(1), 1-9.
- Chang, K. (2015). World tea production and trade: Current and future development. *A publication by the Food and Agricultural Organization of the United Nations, Rome. Available online at www.fao.org (date accessed 29 May 2016).*
- Chaturvedula, V. S. P., & Prakash, I. (2011). The aroma, taste, color and bioactive constituents of tea. *Journal of Medicinal Plants Research*, 5(11), 2110-2124.
- Cho, H.S., Kim, S., Lee, S.Y., Park, J. A., Kim, S.J., & Chun, H. S. (2008). Protective effect of the green tea component, L-theanine on environmental toxins-induced neuronal cell death. *Neurotoxicology*, 29(4), 656-662.
- Chu, S., & Chen, C. (2006). Effects of origins and fermentation time on the antioxidant activities of kombucha. *Food Chemistry*, 98, 502-507.
- Coma, M. E., Peltzer, M. A., Delgado, J. F., & Salvay, A. G. (2019). Water kefir grains as an innovative source of materials: Study of plasticiser content on film properties. *European Polymer Journal*, 120, 109234.
- Coriolis. (2020). Opportunities In New Zealand non-alcoholic beverages: Part of emerging growth opportunities, *Food and Beverage Information Project*.

- Corona, O., Randazzo, W., Miceli, A., Guarcello, R., Francesca, N., Erten, H., Settanni, L. (2016). Characterization of kefir-like beverages produced from vegetable juices. *LWT-Food Science and Technology*, 66, 572-581.
- Cufaoglu, G., & Erdinc, A. N. (2023). An alternative source of probiotics: Water kefir. *Food Frontiers*, 4(1), 21-31.
- da Costa, M. R., de Alencar, E. R., dos Santos Leandro, E., Mendonça, M. A., & de Souza Ferreira, W. F. (2018). Characterization of the kefir beverage produced from yam (*Colocasia esculenta L.*), sesame seed (*Sesamum indicum L.*) and bean (*Phaseolus vulgaris L.*) extracts. *Journal of Food Science and Technology*, 55(12), 4851-4858.
- da Miguel M. G. C. P., Cardoso, P. G., Magalhaes, K. T., & Schwan, R. F. (2011). Profile of microbial communities present in tibico (sugary kefir) grains from the different Brazilian States. *World Journal of Microbiology and Biotechnology*, 27(8), 1875-1884.
- Darvishzadeh, P., Orsat, V., & Martinez, J. L. (2021). Process optimization for development of a novel water kefir drink with high antioxidant activity and potential probiotic properties from Russian olive fruit (*Elaeagnus angustifolia*). *Food and Bioprocess Technology*, 14, 248-260.
- Destro, T.M., Prates, D.D.F., Watanabe, L.S., Garcia, S., Biz, G. & Spinosa, W.A. (2019) Organic brown sugar and jaboticaba pulp influence on water kefir fermentation. *Ciência E Agrotecnologia*, 43.
- Dimitreli, G., & Antoniou, K. D. (2011). Effect of incubation temperature and caseinates on the rheological behaviour of Kefir. *Procedia Food Science*, 1, 583-588.
- Dinkci, N., Kesenkas, H., Korel, F., & Kinik, O. (2015). An innovative approach: cow/oat milk based kefir. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka*, 65(3), 177-186.
- Dinnella, C., Pierguidi, L., Spinelli, S., Borgogno, M., Toschi, T. G., Predieri, S., & Monteleone, E. (2022). Remote testing: Sensory test during Covid-19 pandemic and beyond. *Food Quality and Preference*, 96, 104437.
- dos Santos, D. C., de Oliveira Filho, J. G., Santana, A. C. A., de Freitas, B. S. M., Silva, F. G., Takeuchi, K. P., & Egea, M. B. (2019). Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, sensory, and technological characteristics. *LWT-Food Science and Technology*, 104, 30-37.

- Dufresne, C. J., & Farnworth, E. R. (2001). A review of latest research findings on the health promotion properties of tea. *The Journal of Nutritional Biochemistry*, 12(7), 404-421.
- Dwiloka, B., Rizqiatia, H., Setiana, B.E. (2020). Physicochemical and sensory characteristics of green coconut (*Cocos nucifera L.*) water kefir. *International Journal of Food Studies*, 9, 346–359.
- Essawet, N., Cvetkovic, D., Velicanski, A., Canadanovic-Brunet, J., Vulic, J., Maksimovic, V., & Markov, S. (2015). Polyphenols and antioxidant activities of kombucha beverage enriched with coffeeberry extract. *Chemical Industry and Chemical Engineering Quarterly*, 21(3), 399-409.
- FAO/WHO. (2002). Guidelines for the evaluation of probiotics in food. Report of a Joint Food and Agriculture Organization/World Health Organization Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.
- FAO/WHO. (2011). Milk and Milk Products. In CODEX Standard for Fermented Milks (CODEX STAN 243-2003) (pp. 6-16). Rome, Italy: *World Health Organization and Food Agriculture Organization of the United Nations*.
- Fels, L., Jakob, F., Vogel, R. F., & Wefers, D. (2018). Structural characterization of the exopolysaccharides from water kefir. *Carbohydrate Polymers*, 189, 296-303.
- Fernandes, F. H. A., & Salgado, H. R. N. (2016). Gallic acid: review of the methods of determination and quantification. *Critical reviews in analytical chemistry*, 46(3), 257-265.
- Fiorda, F. A., de Melo Pereira, G. V., Thomaz-Soccol, V., Rakshit, S. K., Pagnoncelli, M. G. B., de Souza Vandenberghe, L. P., & Soccol, C. R. (2017). Microbiological, biochemical, and functional aspects of sugary kefir fermentation-A review. *Food Microbiology*, 66, 86-95.
- FSANZ. (2022). *Food Standards Code Legislation. Standard 2.6.2 Non-alcoholic beverages and brewed soft drink. Australia New Zealand Food Standards.*
<https://www.legislation.gov.au/F2015L00465/latest/details>
- Gamba, R.R., Koyanagi, T., Peláez, A.L., De Antoni, G., Enomoto, T., 2021. Changes in microbiota during multiple fermentation of kefir in different sugar solutions revealed by high-throughput sequencing. *Current Microbiology*. 78(6), 2406–2413.
- Ganzle, M. G. (2015). Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in Food Science*, 2, 106-117.

- Garrigues, C., Loubiere, P., Lindley, N. D., & Cocaign-Bousquet, M. (1997). Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: predominant role of the NADH/NAD⁺ ratio. *Journal of Bacteriology*, *179* (17), 5282-5287.
- Gonda, M., Garmendia, G., Rufo, C., León Peláez, Á., Wisniewski, M., Droby, S., & Vero, S. (2019). Biocontrol of *Aspergillus flavus* in ensiled sorghum by water kefir microorganisms. *Microorganisms*, *7*(8), 253.
- Gronnevik, H., Marit, F., Naruhus, J.A. (2011). Microbiological and chemical properties of Norwegian kefir during storage, *International Dairy Journal*, *1*, 1-6.
- Gulitz, A., Stadie, J., Erhmann, M. A., Ludwig, W., & Vogel, R. F. (2013). Comparative phylobiomic analysis of the bacterial community of water kefir by 16S rRNA gene amplicon sequencing and ARDRA analysis. *Journal of Applied Microbiology*, *114*, 1082-1091.
- Gulitz, A., Stadie, J., Wenning, M., Ehrmann, M. A., & Vogel, R. F. (2011). The microbial diversity of water kefir. *International Journal of Food Microbiology*, *151*(3), 284-288.
- Guzel-Seydim, Z. B., Gokirmakli, c., & Greene, A. K. (2021). A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends in Food Science & Technology*, *113*, 42-53.
- Hamad, H. S. (2011). Factors affecting the growth of microorganism in food. In R. Bhat., A. K. Alias., & G. Palivath (Eds.). *Progress in Food Preservation* (pp 405- 427). Chicester: John Wilet & Sons.
- Hamilton-Miller, J. (1995). Antimicrobial properties of tea (*Camellia sinensis L.*). *Antimicrobial Agents and Chemotherapy*, *39*(11), 2375-2377.
- Haslam, E. (2003). Thoughts on thearubigins. *Phytochemistry*, *64*, 61-73.
- Hayat, K., Iqbal, H., Malik, U., Bilal, U., & Mushtaq, S. (2015). Tea and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition*, *55*(7), 939-954.
- Hikmetoglu, M., Sogut, E., Sogut, O., Gokirmakli, C., & Guzel-Seydim, Z. (2020). Changes in carbohydrate profile in kefir fermentation. *Bioactive Carbohydrates and Dietary Fibre*, *23*, 100220.
- Hrnjez, D., Vastag, Z., Milanovic, S., Vukic, V., Ilicic, M., Popovic, L., & Kanuric, K. (2014). The biological activity of fermented dairy products obtained by kombucha and conventional starter cultures during storage. *Journal of Functional Foods*, *10*, 336-345.

- Hsieh, H., Wang, S., Chen, T., Huang, Y., & Chen, M. (2012). Effect of cow's and goat's milk as fermentation media on the microbial ecology of sugary kefir grains. *International Journal of Food Microbiology*, *157*, 73-81.
- Ilango, S., & Antony, U. (2021). Probiotic microorganisms from non-dairy traditional fermented foods. *Trends in Food Science & Technology*, *118*, 617-638.
- Imran, M., Desmaures, N., & Vernoux, J.-P. (2012). Complex microbial communities as part of fermented food ecosystems and beneficial properties. *Fermentation*, 21-64: CRC Press.
- Irigoyen, A., Arana, I., Castiella, M., Torre, P., & Ibanez, F. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry*, *90*(4), 613-620.
- Isrianto, P. L. (2020). Kefir of Keji Beling Tea (*Strobilanthes crispus*) as Functional Beverage for Glucose intolerance. *International Journal of Applied Biology*, *4*(2), 31-36.
- Istrati, D. I., Pricop, E. M., Profir, A. G., & Vizireanu, C. (2018). Fermented functional beverages. *Functional Foods*, 403.
- Jakubczyk, K., Kochman, J., Kwiatkowska, A., Kałduńska, J., Dec, K., Kawczuga, D., & Janda, K. (2020). Antioxidant properties and nutritional composition of matcha green tea. *Foods*, *9*(4), 483.
- Jankech, T., Maliarová, M., & Martinka, N. (2019). Determination of methylxanthines in tea samples by HPLC method. *Nova Biotechnologica et Chimica*, *18*(2), 124-132.
- Jayabalan, R., Malbaa, A. R. V., Lonaar, E. S., Vitas, J. S., & Sathish kumar, M. (2014). A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 538-550.
- Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chemistry*, *102*, 392-398.
- Jayabalan, R., Subathradevi, P., Marimuthu, S., Sathishkumar, M., & Swaminathan, K. (2008). Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, *1*, 1-8.
- Jeong, S. H., Lee, S. H., Jung, J. Y., Choi, E. J. & Jeon, C. O. (2013). Microbial succession and metabolite changes during long-term storage of kimchi. *Journal of Food Science*, *78*(5), M763–M769.

- Ji, H.G., Lee, Y.R., Lee, M.S., Hwang, K. H., Kim, E.H., Park, J. S., & Hong, Y.S. (2017). Metabolic phenotyping of various tea (*Camellia sinensis L.*) cultivars and understanding of their intrinsic metabolism. *Food Chemistry*, 233, 321-330.
- Jiang, H., Yu, F., Qin, L., Zhang, N., Cao, Q., Schwab, W., Song, C. (2019). Dynamic change in amino acids, catechins, alkaloids, and gallic acid in six types of tea processed from the same batch of fresh tea (*Camellia sinensis L.*) leaves. *Journal of Food Composition and Analysis*, 77, 28-38.
- Johnson, I. M., Prakash, H., Prathiba, J., Raghunathan, R., & Malathi, R. (2012). Spectral analysis of naturally occurring methylxanthines (theophylline, theobromine and caffeine) binding with DNA. *Plos One*, 7(12), e50019.
- Joseph, J. A., Akkermans, S., & Van Impe, J. F. (2022). Processing method for the quantification of methanol and ethanol from bioreactor samples using gas chromatography–flame ionization detection. *Acs Omega*, 7(28), 24121-24133.
- Juneja, L. R., Chu, D.C., Okubo, T., Nagato, Y., & Yokogoshi, H. (1999). L-theanine a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science & Technology*, 10(6-7), 199-204.
- Juneja, L. R., Kapoor, M. P., Okubo, T., & Rao, T. (2013). *Green tea polyphenols: nutraceuticals of modern life*: CRC press.
- Karagozlu, C., Unal, G., Akalin, A. S., Akan, E., & Kinik, O. (2017). The effects of black and green tea on antioxidant activity and sensory characteristics of kefir. *Agro Food Industry Hi Tech*, 28, 77-80.
- Karagozlu, C., Unal, G., Akalin, A. S., Akan, E., & Kinik, O. (2018). The supplementary effect of black and green tea infusion on antimicrobial activities of kefir. *Food and Health*, 4(2), 124-131.
- Kazakos, S., Mantzourani, I., Nouska, C., Alexopoulos, A., Bezirtzoglou, E., Bekatorou, A., Varzakas, T. (2016). Production of low alcohol fruit beverages through fermentation of pomegranate and orange juices with kefir grains. *Current Research in Nutrition and Food Science*, 4(1), 19-26.
- Khan, N., & Mukhtar, H. (2013). Tea and Health: Studies in Humans. *Current Pharmaceutical Design*, 19(34), 6141–6147.

- Kim, T. I., Lee, Y. K., Park, S. G., Choi, I. S., Ban, J. O., Park, H. K., Oh, K. W. (2009). L-Theanine, an amino acid in green tea, attenuates β -amyloid-induced cognitive dysfunction and neurotoxicity: reduction in oxidative damage and inactivation of ERK/p38 kinase and NF- κ B pathways. *Free Radical Biology and Medicine*, 47(11), 1601-1610.
- Kimura, K., Ozeki, M., Juneja, L. R., & Ohira, H. (2007). L-Theanine reduces psychological and physiological stress responses. *Biological Psychology*, 74(1), 39-45.
- Koh, W. Y., Utra, U., Ahmad, R., Rather, I. A., & Park, Y.-H. (2018). Evaluation of probiotic potential and anti-hyperglycemic properties of a novel *Lactobacillus* strain isolated from water kefir grains. *Food Science and Biotechnology*, 27(5), 1369-1376.
- Kok-Tas, T., Seydim, A. C., Ozer, B., & Guzel-Seydim, Z. B. (2013). Effects of different fermentation parameters on quality characteristics of kefir. *Journal of Dairy Science*, 96(2), 780-789.
- Kongpichitchoke, T., Chiu, M.T., Huang, T.C., & Hsu, J.L. (2016). Gallic acid content in Taiwanese teas at different degrees of fermentation and its antioxidant activity by inhibiting PKC δ activation: *in vitro* and *in silico* studies. *Molecules*, 21(10), 1346.
- Kostić, E., Vujović, M., & Milosavljević, B. (2021). Validation of a method for ethanol analysis in biological and non-biological samples and its toxicological application. *HEMIJSKA INDUSTRIJA (Chemical Industry)*, 75(3), 175-183.
- Kubo, R., Ohta, K., Funakawa, S., Kitabatake, N., Araki, S., & Izawa, S. (2014). Isolation of lactic acid-tolerant *Saccharomyces cerevisiae* from Cameroonian alcoholic beverage. *Journal of Bioscience and Bioengineering*, 118(6), 657-660.
- Kumar, M. R., Yeap, S. K., Mohamad, N. E., Abdullah, J. O., Masarudin, M. J., Khalid, M., & Alitheen, N. B. (2021). Metagenomic and phytochemical analyses of kefir water and its subchronic toxicity study in BALB/c mice. *BMC Complementary Medicine and Therapies*, 21(1), 1-15.
- Kurtzman, C. P. (2003). Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the *Saccharomycetaceae*, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygorulasporea*. *FEMS Yeast Research*, 4(3), 233-245.

- Lai, B., Plan, M. R., Hodson, M. P., & Krömer, J. O. (2016). Simultaneous determination of sugars, carboxylates, alcohols and aldehydes from fermentations by high performance liquid chromatography. *Fermentation*, 2(1), 6.
- Laureys, D., & De Vuyst, L. (2014). Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fermentation. *Applied and Environmental Microbiology*, 80 (8), 2564-2572.
- Laureys, D., & De Vuyst, L. (2017). The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. *Journal of Applied Microbiology*, 122(3), 719-732.
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2018). Oxygen and diverse nutrients influence the water kefir fermentation process. *Food Microbiology*, 73, 351-361.
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2019). The buffer capacity and calcium concentration of water influence the microbial species diversity, grain growth, and metabolite production during water kefir fermentation. *Frontiers in Microbiology*, 10, 2876.
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2019). The buffer capacity and calcium concentration of water influence the microbial species diversity, grain growth, and metabolite production during water kefir fermentation. *Frontiers in Microbiology*, 10, 2876.
- Laureys, D., Cnockaert, M., De Vuyst, L., & Vandamme, P. (2016). *Bifidobacterium aquikefiri* sp. nov., isolated from water kefir. *International Journal of Systematic and Evolutionary Microbiology*, 66(3), 1281-1286.
- Laureys, D., Leroy, F., Hauffman, T., Raes, M., Aerts, M., Vandamme, P., & De Vuyst, L. (2021). The type and concentration of inoculum and substrate as well as the presence of oxygen impact the water kefir fermentation process. *Frontiers in Microbiology*, 12, 628599.
- Laureys, D., Leroy, F., Hauffman, T., Raes, M., Aerts, M., Vandamme, P., & De Vuyst, L. (2021). The type and concentration of inoculum and substrate as well as the presence of oxygen impact the water kefir fermentation process. *Frontiers in Microbiology*, 12, 628599.
- Laureys, D., Van Jean, A., Dumont, J., & De Vuyst, L. (2017). Investigation of the instability and low water kefir grain growth during an industrial water kefir fermentation process. *Applied Microbiology and Biotechnology*, 101(7), 2811-2819.
- Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices* (Vol. 2). New York: Springer.

- Leite, A. M. d. O., Miguel, M. A. L., Peixoto, R. S., Rosado, A. S., Silva, J. T., & Paschoalin, V. M. F. (2013). Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. *Brazilian Journal of Microbiology*, *44*(2), 341-349.
- Leroi, F., & Pidoux, M. (1993a). Characterization of interactions between *Lactobacillus hilgardii* and *Saccharomyces florentinus* isolated from sugary kefir grains. *Journal of Applied Bacteriology*, *74*(1), 54-60.
- Leroi, F., & Pidoux, M. (1993b). Detection of interactions between yeasts and lactic acid bacteria isolated from sugary kefir grains. *Journal of Applied Bacteriology*, *74*(1), 48-53.
- Li, J. W., Ding, S. D., & Ding, X. L. (2005). Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. *Process Biochemistry*, *40*(11), 3607-3613.
- Li, J., Fu, B., Huo, D., Hou, C., Yang, M., Shen, C., Yang, P. (2017). Discrimination of Chinese teas according to major amino acid composition by a colorimetric IDA sensor. *Sensors and Actuators B: Chemical*, *240*, 770-778.
- Limbad, M., Gutierrez-Maddox, N., Hamid, N., Kantono, K., Liu, T., & Young, T. (2023). Microbial and chemical changes during fermentation of coconut water kefir beverage. *Applied Sciences*, *13*(12), 7257.
- Liu, Q., Duan, H., Luan, J., Yagasaki, K., & Zhang, G. (2009). Effects of theanine on growth of human lung cancer and leukemia cells as well as migration and invasion of human lung cancer cells. *Cytotechnology*, *59*, 211-217.
- Loncar, E., Djuric, M., Malbasa, R., Kolarov, L., & Klasnja, M. (2006). Influence of working conditions upon kombucha conducted fermentation of black tea. *Food and Bioproducts Processing*, *84*(3), 186-192.
- López, E. F., & Gómez, E. F. (1996). Simultaneous determination of the major organic acids, sugars, glycerol, and ethanol by HPLC in grape musts and white wines. *Journal of Chromatographic Science*, *34*(5), 254-257.
- Lopusiewicz, L., Drozłowska, E., Siedlecka, P., Męzynska, M., Bartkowiak, A., Sienkiewicz, M., & Kwiatkowski, P. (2019). Development, characterization, and bioactivity of non-dairy kefir-like fermented beverage based on flaxseed oil cake. *Foods*, *8*(11), 544.
- Lopusiewicz, L., Drozłowska, E., Trocer, P., Kwiatkowski, P., Bartkowiak, A., Gefrom, A., Sienkiewicz, M., (2020). The effect of fermentation with kefir grains on the physicochemical

- and antioxidant properties of beverages from blue lupin (*Lupinus angustifolius L.*) seeds. *Molecules*, 25, 57-91.
- Lorenzo, J. M., & Munekata, P. E. S. (2016). Phenolic compounds of green tea: Health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine*, 6(8), 709-719.
- Lu, K., Gray, M. A., Oliver, C., Liley, D. T., Harrison, B. J., Bartholomeusz, C. F., & Nathan, P. J. (2004). The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans. *Human Psychopharmacology: Clinical and Experimental*, 19(7), 457-465.
- Luang-In, V., Saengha, W., Yotchaisarn, M., Halaslova, M., Udomwong, P., & Deeseenthum, S. (2018). Microbial strains and bioactive exopolysaccharide producers from Thai water kefir. *Microbiology and Biotechnology Letters*, 46(4), 403-415.
- Lutz, M. L. (1899). Recherches biologiques sur la constitution du Tibi. *Bulletin de la Societe Mycologique de France*, 15, 68-72
- Lynch, K. M., Wilkinson, S., Daenen, L., & Arendt, E. K. (2021). An update on water kefir: Microbiology, composition and production. *International Journal of Food Microbiology*, 345, 109128.
- Lyon, B. G. (1992). Laboratory Methods for Sensory Analysis of Food. | Poste, L.M., Mackie, D.A., Butler, G., and Larmond, E., *Laboratory Methods for Sensory Analysis of Food*. Research Branch, Agriculture Canada, Publication 1864/E. 1991. 91 pp.
- M'hir, S., Filannino, P., Mejri, A., Tlais, A. Z. A., Di Cagno, R., & Ayed, L. (2021). Functional exploitation of carob, oat flour, and whey permeate as substrates for a novel kefir-like fermented beverage: An optimized formulation. *Foods*, 10(2), 294.
- Magalhaes, K. T., Pereira, G. d. M., Dias, D. R., & Schwan, R. F. (2010). Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. *World Journal of Microbiology and Biotechnology*, 26(7), 1241-1250.
- Mantzourani, I., Kazakos, S., Terpou, A., Alexopoulos, A., Bezirtzoglou, E., Bekatorou, A., & Plessas, S. (2019). Potential of the probiotic *Lactobacillus plantarum ATCC 14917* strain to produce functional fermented pomegranate juice. *Foods*, 8(1), 4.
- Mantzourani, I., Kazakos, S., Terpou, A., Mallouchos, A., Kimbaris, A., Alexopoulos, A., Plessas, S. (2018). Assessment of volatile compounds evolution, antioxidant activity, and total

- phenolics content during cold storage of pomegranate beverage fermented by *Lactobacillus paracasei* K5. *Fermentation*, 4 (4), 95.
- Mantzourani, I., Terpou, A., Bekatorou, A., Plessas, S. (2022). Valorisation of lactic acid fermentation of pomegranate juice by an acid tolerant and potentially probiotic LAB isolated from kefir grains. *Fermentation*, 8, 142-154.
- Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2013). Sequence-based analysis of the microbial composition of water kefir from multiple sources. *FEMS Microbiology Letters*, 348(1), 79-85.
- Martinez-Torres, A., Gutierrez-Ambrocio, S., Heredia-del-Orbe, P., Villa-Tanaca, L., & Hernandez-Rodriguez, C. (2017). Inferring the role of microorganisms in water kefir fermentations. *International Journal of Food Science & Technology*, 52(2), 559-571.
- Mechmeche, M., Ksontini, H., Hamdi, M., & Kachouri, F. (2019). Production of bioactive peptides in tomato seed protein isolate fermented by water kefir culture: optimization of the fermentation conditions. *International Journal of Peptide Research and Therapeutics*, 25, 137-150.
- Meticulous Research. (2022). Functional beverages market by type (Energy drinks, sports drinks, dairy beverages), application (Health & wellness, weight management), distribution channel (Supermarkets, convenience stores, E-commerce), and geography - Global Forecast to 2029. <https://www.meticulousresearch.com/product/functional-beverages-market-5407>
- Meticulous Research. (2023). Fermented foods and beverages market by product (Fermented foods {Dairy products, vegetables}, fermented beverages {Alcoholic, non-alcoholic}), ingredient (Probiotics, amino acids), and distribution channel (Convenience stores, E-commerce) - Global Forecast to 2030. <https://www.meticulousresearch.com/product/fermented-foods-and-beverages-market-5469>
- Monteiro, J. P., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2016). Structure-bioactivity relationships of methylxanthines: Trying to make sense of all the promises and the drawbacks. *Molecules*, 21(8), 974.
- Mordor Intelligence. (2023). Fermented foods and beverages market- growth, trends, and forecast (2023-2028). <https://www.mordorintelligence.com/industry-reports/fermented-foods-beverages-market>

- Moretti, A. F., Moure, M. C., Quinoy, F., Esposito, F., Simonelli, N., Medrano, M., & León-Peláez, Á. (2022). Water kefir, a fermented beverage containing probiotic microorganisms: From ancient and artisanal manufacture to industrialized and regulated commercialization. *Future Foods*, 5, 100123.
- Mu, X. (2019). *Fermentation of water kefir beverage containing Ziziphus jujuba Mill. syrup: a thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology, Massey University, Albany, New Zealand* (Doctoral dissertation, Massey University).
- Musial, C., Kuban-Jankowska, A., & Gorska-Ponikowska, M. (2020). Beneficial properties of green tea catechins. *International Journal of Molecular Sciences*, 21(5), 1744.
- Nambou, K., Gao, C., Zhou, F., Guo, B., Ai, L., & Wu, Z. J. (2014). A novel approach of direct formulation of defined starter cultures for different kefir-like beverage production. *International Dairy Journal*, 34(2), 237-246.
- Nguyen, N., McGinley, M., & Mott, J. (2010). Faster real-time response to bacterial infection of bioethanol fermentation using a short Rezex™ ROA column. *Phenomenex*. <https://www.phenomenex.com/documents/2020/11/04/23/59/faster-realtime-response-to-bacterial-infection-of-bioethanol-fermentation-using-a-short-rezex-roa-c>
- Nielsen, S. S. (2017). Correction to: Food Analysis Fifth Edition. *Food Analysis*, C1-C3.
- Nielson-Stowell, A. (2020) Retail sales trends for fermented food and beverage. *The Fermentation Association*. <https://fermentationassociation.org/retail-sales-trends-for-fermented-food-and-beverage>
- Oboturova, N., Evdokimov, I., Kulikova, I., Bratsikhin, A., & Bogueva, D. (2022). Traditional foods of the North Caucasus region. In *Nutritional and Health Aspects of Traditional and Ethnic Foods of Eastern Europe* (pp. 69-91). Academic Press.
- Oh, J.H., Lee, C.Y., Lee, Y.E., Yoo, S.H., Chung, J.O., Rha, C.S., Shim, S.M. (2021). Profiling of *in vitro* bioaccessibility and intestinal uptake of flavonoids after consumption of commonly available green tea types. *Molecules*, 26(6), 1518.
- Oliveira, J. T., da Costa, F. M., da Silva, T. G., Simões, G. D., dos Santos Pereira, E., da Costa, P. Q., Pieniz, S. (2023). Green tea and kombucha characterization: Phenolic composition, antioxidant capacity and enzymatic inhibition potential. *Food Chemistry*, 408, 135206.

- Ozcelik, F., Akan, E. & Kinik, O. (2021) Use of Cornelian cherry, hawthorn, red plum, roship and pomegranate juices in the production of water kefir beverages. *Food Bioscience*, 42, 101219.
- Pablo, A. G. P., & Cimafranca, L. C. (2022). Development of a water kefir beverage made from jackfruit processing by-product and its potential probiotic property. *Philippine Journal of Science*, 151(5), 1589-1598.
- Parida, M. (2022). Functional beverages: A modern gateway to healthy lifestyle. *The Pharma Innovation Journal*, 11(7), 2227-2235
- Pathare, P. B., Opara, U. L., & Al-Said, F. A.J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, 6(1), 36-60.
- Pendon, M. D., Bengoa, A. A., Iraporda, C., Medrano, M., Garrote, G. L., & Abraham, A. G. (2022). Water kefir: Factors affecting grain growth and health-promoting properties of the fermented beverage. *Journal of Applied Microbiology*, 133(1), 162-180.
- Pervin, M., Unno, K., Ohishi, T., Tanabe, H., Miyoshi, N., & Nakamura, Y. (2018). Beneficial effects of green tea catechins on neurodegenerative diseases. *Molecules*, 23(6), 1297.
- Pfeiffer, T., & Morley, A. (2014). An evolutionary perspective on the Crabtree effect. *Frontiers in Molecular Biosciences*, 1(17), 1-6.
- Pidoux, M. (1989). The microbial flora of sugary kefir grain (the gingerbeer plant): biosynthesis of the grain from *Lactobacillus hilgardii* producing a polysaccharide gel. *MIRCEN Journal of Applied Microbiology and Biotechnology*, 5(2), 223-238.
- Pinto, L. C., de Oliveira, T. P., de Souza, R., Santos, N. B. F., Santos, L. F. P., de Assis Santos, A., & Lopes, M. V. (2021). Probiotic kefir-fermented beverage-based *Colocasia esculenta* L.: Development, characterization, and microbiological stability during chilled storage. *Journal of Food Processing and Preservation*, 45(2), e15113.
- Prado, M. R., & Blandón, L. M., Vandenberghe L.P., Rodrigues, C., Castro, G.R., & Thomaz-Soccol, V. (2015). *Milk kefir: composition, microbial cultures, biological activities, and related products*. *Frontier Microbiology*, 6(1177), 10-3389.
- Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (*Camellia sinensis*) in antiphotaging, stress resistance, neuroprotection, and autophagy. *Nutrients*, 11(2), 474.

- Puerari, C., Magalhaes, K. T., & Schwan, R. F. (2012). New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. *Food Research International*, 48, 634-640.
- Randazzo, W., Corona, O., Guarcello, R., Francesca, N., Germana, M. A., Erten, H., Settanni, L. (2016). Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiology*, 54, 40-51.
- Rao, L. J. M., & Ramalakshmi, K. (Eds.). (2011). Recent trends in soft beverages. CRC Press.
- Rasika, D., Vidanarachchi, J. K., Luiz, S. F., Azeredo, D. R. P., Cruz, A. G., & Ranadheera, C. S. (2021). Probiotic Delivery through Non-Dairy Plant-Based Food Matrices. *Agriculture*, 11(7), 599.
- Ravyts, F., Vuyst, L. D., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences*, 12 (4), 356-367.
- Reddy, G., Altaf, M., Naveena, B., Venkateshwar, M., & Kumar, E. V. (2008). Amylolytic bacterial lactic acid fermentation—a review. *Biotechnology advances*, 26 (1), 22-34.
- Reygaert, W. C. (2018). Green tea catechins: Their use in treating and preventing infectious diseases. *BioMed Research International*, 2018.
- Rocha-Gomes, A., Escobar, A., Soares, J. S., Silva, A. A. d., Dessimoni-Pinto, N. A. V., & Riul, T. R. (2018). Chemical composition and hypocholesterolemic effect of milk kefir and water kefir in Wistar rats. *Revista de Nutricao*, 31(2), 137-145.
- Rodrigues, F. S., Ludovico, P., & Leao, C. (2006). Sugar metabolism in yeasts: an overview of aerobic and anaerobic glucose catabolism. *Springer*.
- Rodrigues, K. L., Caputo, L. R. G., Carvalho, J. C. T., Evangelista, J., & Schneedorf, J. M. (2005). Antimicrobial and healing activity of kefir and kefir extract. *International Journal of Antimicrobial Agents*, 25 (5), 404-408.
- Saadi, L. O., Zaidi, F., Oomah, B. D., Haros, M., Yebra, M. J., & Hosseinian, F. (2017). Pulse ingredients supplementation affects kefir quality and antioxidant capacity during storage. *LWT- Food Science and Technology*, 86, 619-626.
- Sabokbar, N., & Khodaiyan, F. (2015). Characterization of pomegranate juice and whey based novel beverage fermented by kefir grains. *Journal of Food Science & Technology*, 52 (6), 2711-3718.

- Saeed, M., Naveed, M., Arif, M., Kakar, M. U., Manzoor, R., Abd El-Hack, M. E., Munjal, A. (2017). Green tea (*Camellia sinensis*) and L-theanine: Medicinal values and beneficial applications in humans-A comprehensive review. *Biomedicine & Pharmacotherapy*, *95*, 1260-1275.
- Said, N., Fahrodi, D., Malaka, R., & Maruddin, F. (2018). The characteristics of lactic acid bacteria isolated from Indonesian commercial kefir grain. *Malaysian Journal of Microbiology*, *14*, 632-639.
- Sarikkha, P., Nitorisavut, R., Poljungreed I., & Boonyarattanakalin, S. (2015). Identification of bacteria and yeast communities in a Thai sugary kefir by Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analyses. *The Journal of Industrial Technology*, *11*(2), 25-39.
- Schulz, H., Engelhardt, U. H., Wegent, A., Drews, H. H., & Lapczynski, S. (1999). Application of near-infrared reflectance spectroscopy to the simultaneous prediction of alkaloids and phenolic substances in green tea leaves. *Journal of Agricultural and Food Chemistry*, *47*(12), 5064-5067.
- Setyawardani, T., & Sumarmono, J. (2015). Chemical and microbiological characteristics of goat milk kefir during storage under different temperatures. *Journal of the Indonesian Tropical Animal Agriculture*, *40*(3), 183-188.
- Setyawardani, T., Rahardjo, A. H., Sulistyowati, M., & Wasito, S. (2014). Physiochemical and organoleptic features of goat milk kefir made of different kefir grain concentration on controlled fermentation. *Animal Production*, *16*(1).
- Silva, K. R., Rodrigues, S. A., Filho, L. X., & Lima, A. S. (2009). Antimicrobial activity of broth fermented with kefir grains. *Applied Biochemistry and Biotechnology*, *152*, 316-325.
- Sinija, V., & Mishra, H. N. (2008). Green tea: Health benefits. *Journal of Nutritional & Environmental Medicine*, *17*(4), 232-242.
- Spizzirri, U. G., Loizzo, M. R., Aiello, F., Prencipe, S. A., & Restuccia, D. (2023). Non-dairy kefir beverages: formulation, composition, and main features. *Journal of Food Composition and Analysis*, 105130.
- Stadie, J. (2013). *Metabolic activity and symbiotic interaction of bacteria and yeasts in water kefir* (Doctoral dissertation, Technische Universitat Munchen) Germany.

- Stadie, J., Gulitz, A., Ehrmann, M. A., & Vogel, R. F. (2013). Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiology*, 35(2), 92-98.
- Subardjo, M. V. K. (2017). *Black tea water kefir beverage: a thesis submitted in partial fulfilment of the requirement for the degree of Master of Food Technology, Massey University, Albany, New Zealand* (Doctoral dissertation, Massey University).
- Sumathi, T., Shobana, C., Thangarajeswari, M., & Usha, R. (2015). Protective effect of L-theanine against aluminium induced neurotoxicity in cerebral cortex, hippocampus, and cerebellum of rat brain—histopathological, and biochemical approach. *Drug and Chemical Toxicology*, 38(1), 22-31.
- Talib, N., Mohamad, N. E., Yeap, S. K., Hussin, Y., Aziz, M. N. M., Masarudin, M. J., Alitheen, N. B. (2019). Isolation and characterization of *Lactobacillus spp.* from kefir samples in Malaysia. *Molecules*, 24 (14), 2606.
- Tan, J., Engelhardt, U. H., Lin, Z., Kaiser, N., & Maiwald, B. (2017). Flavonoids, phenolic acids, alkaloids and theanine in different types of authentic Chinese white tea samples. *Journal of Food Composition and Analysis*, 57, 8-15.
- Tavares, P. P. L. G., Mamona, C. T. P., Nascimento, R. Q., dos Anjos, E. A., de Souza, C. O., Almeida, R. C. d. C., Magalhaes-Guedes, K. T. (2023). Non-Conventional Sucrose-Based Substrates: Development of Non-Dairy Kefir Beverages with Probiotic Potential. *Fermentation*, 9(4), 384.
- Terpou, A., Papadaki, A., Lappa, I.K., Kachrimanidou, V., Bosnea, L.A., Kopsahelis, N., 2019. Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients* 11, 15-91.
- Tireki, S. (2021). A review on packed non-alcoholic beverages: Ingredients, production, trends and future opportunities for functional product development. *Trends in Food Science & Technology*, 112, 442-454.
- Tireki, S. (2022). Physicochemical and Sensory Parameters of Vegan Water Kefir Beverages Fermented with Different Fruits. *The European Journal of Research and Development*, 2(4), 1-12.

- Tu, C., Azi, F., Huang, J., Xu, X., Xing, G., & Dong, M. (2019). Quality and metagenomic evaluation of a novel functional beverage produced from soy whey using water kefir grains. *LWT- Food Science and Technology*, *113*, 108258.
- Tzavaras, D., Papadelli, M., & Ntaikou, I. (2022). From milk kefir to water kefir: Assessment of fermentation processes, microbial changes and evaluation of the produced beverages. *Fermentation*, *8*(3), 135.
- van Dijken, J. P., Weusthuis, R. A., & Pronk, J. T. (1993). Kinetics of growth and sugar consumption in yeasts. *Antonie van leeuwenhoek*, *63* (3-4), 343-352.
- Vayssier, Y. (1978). Le kefir: analyse qualitative et quantitative. *Revue Laitiere Francaise*, *361*, 73-75.
- Velicanski, A. S., Cvetkovic, D. D., Markov, S. L., Saponjac, T., Vesna, T., & Vulic, J. J. (2014). Antioxidant and antibacterial activity of the beverage obtained by fermentation of sweetened lemon balm (*Melissa officinalis L.*) tea with symbiotic consortium of bacteria and yeasts. *Food Technology and Biotechnology*, *52*(4), 420-429.
- Verce, M., De Vuyst, L., & Weckx, S. (2019). Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel *Oenococcus* species. *Frontiers in Microbiology*, *10*, 479.
- Vuong, Q. V. (2014). Epidemiological evidence linking tea consumption to human health: a review. *Critical Reviews in Food Science and Nutrition*, *54*(4), 523-536.
- Waldherr, F. W., Doll, V. M., Meibner, D., & Vogel, R. F. (2010). Identification and characterization of a glucan-producing enzyme from *Lactobacillus hilgardii* TMW 1.828 involved in granule formation of water kefir. *Food Microbiology*, *27*(5), 672-678.
- Walker, G. M., & Stewart, G. G. (2016). *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages*, *2*(4), 30.
- Walsh, A. M., Crispie, F., Kilcawley, K., O'Sullivan, O., O'Sullivan, M. G., Claesson, M. J., & Cotter, P. D. (2016). Microbial succession and flavour production in the fermented dairy beverage kefir. *MSystems*, *1*(5), 101-128.
- Wang, X. (2018). *Development and characteristics of green tea kombucha: A thesis presented in partial fulfilment of the requirements for The Degree of Master of Food Technology at Massey University, Albany, New Zealand* (Doctoral dissertation, Massey University).

- Ward, H. M. (1892). The ginger-beer plant, and the organisms composing it: a contribution to the study of fermentation-yeasts and bacteria. *Philosophical Transactions of the Royal Society of London*, 183, 125-197.
- Wei, C., Yang, H., Wang, S., Zhao, J., Liu, C., Gao, L., She, G. (2018). Draft genome sequence of *Camellia sinensis* var. *sinensis* provides insights into the evolution of the tea genome and tea quality. *Proceedings of the National Academy of Sciences*, 115(18), E4151-E4158.
- Weisburger, J. H. (1997). Tea and health: a historical perspective. *Cancer letters*, 114(1-2), 315-317.
- Wierzejska, R. (2014). Tea and health—A review of the current state of knowledge. *Przegląd Epidemiological*, 68, 595–599.
- Williams, J. L., Everett, J. M., D’Cunha, N. M., Sergi, D., Georgousopoulou, E. N., Keegan, R. J., Naumovski, N. (2020). The effects of green tea amino acid L-theanine consumption on the ability to manage stress and anxiety levels: a systematic review. *Plant Foods for Human Nutrition*, 75(1), 12-23.
- Willson, K. C., & Clifford, M. N. (2012). *Tea: cultivation to consumption*: Springer Science & Business Media.
- Xia, Z., Ni, Y., & Kokot, S. (2013). Simultaneous determination of caffeine, theophylline and theobromine in food samples by a kinetic spectrophotometric method. *Food Chemistry*, 141(4), 4087-4093.
- Xing, L., Zhang, H., Qi, R., Tsao, R., & Mine, Y. (2019). Recent advances in the understanding of the health benefits and molecular mechanisms associated with green tea polyphenols. *Journal of Agricultural and Food Chemistry*, 67(4), 1029-1043.
- Xu, D., Bechtner, J., Behr, J., Eisenbach, L., Geibler, A. J., & Vogel, R. F. (2019a). Lifestyle of *Lactobacillus hordei* isolated from water kefir based on genomic, proteomic, and physiological characterization. *International Journal of Food Microbiology*, 290, 141-149.
- Xu, D., Behr, J., Geißler, A. J., Bechtner, J., Ludwig, C., & Vogel, R. F. (2019b). Label-free quantitative proteomic analysis reveals the lifestyle of *Lactobacillus hordei* in the presence of *Saccharomyces cerevisiae*. *International Journal of Food Microbiology*, 294, 18-26.
- Xu, D., Fels, L., Wefers, D., Behr, J., Jakob, F., & Vogel, R. F. (2018). *Lactobacillus hordei* dextrans induce *Saccharomyces cerevisiae* aggregation and network formation on hydrophilic surfaces. *International Journal of Biological Macromolecules*, 115, 236-242.

- Yang, J., & Liu, R. H. (2013). The phenolic profiles and antioxidant activity in different types of tea. *International Journal of Food Science & Technology*, *48*(1), 163-171.
- Yerlikaya, O., Akan, E., & Kinik, O. (2022). The metagenomic composition of water kefir microbiota. *International Journal of Gastronomy and Food Science*, *30*, 100621.
- Yildiz-Akgul, F., Yetisemiyen, A., Senel, E., & Yildirim, Z. (2018). Microbiological, physicochemical, and sensory characteristics of kefir produced by secondary fermentation. *Mljekarstvo/Dairy*, *68*(3), 201-213.
- Zajsek, K. & Gorsek, A. (2010). Mathematical modelling of ethanol production by mixed kefir grains yeast population as a function of temperature variations. *Biochemical Engineering Journal*, *49*(1), 7-12.
- Zannini, E., Lynch, K. M., Nyhan, L., Sahin, A. W., O'Riordan, P., Luk, D., Arendt, E. K. (2022). Influence of substrate on the fermentation characteristics and culture-dependent microbial composition of water kefir. *Fermentation*, *9*(1), 28.
- Zaunmuller, T., Eichert, M., Richter, H., & Uden, G. (2006). Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. *Applied Microbiology and Biotechnology*, *72* (3), 421-429.
- Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sciences*, *78*(18), 2073-2080.
- Zhang, L., Zhang, Z. Z., Lu, Y. N., Zhang, J. S., & Preedy, V. R. (2013). L-Theanine from green tea: Transport and effects on health. *Tea in Health and Disease Prevention*, 425-435.
- Zhou, Q., Li, H., Zhou, J., Ma, Y., Wu, T., & Ma, H. (2016). Green tea, black tea consumption and risk of the endometrial cancer: a systematic review and metaanalysis. *Archives of Gynaecology and Obstetrics*, *293*, 143-155.
- Zou, C., Li, R. Y., Chen, J. X., Wang, F., Gao, Y., Fu, Y. Q., & Yin, J. F. (2021). Zijuan tea-based kombucha: Physicochemical, sensorial, and antioxidant profile. *Food Chemistry*, *363*, 130322.

Appendix

A. Nutritional Composition of Medjool dates

Table A.1 Nutritional composition of 100 % organic medjool dates (Bard Valley Natural Delights, USA) (Net weight = 227 g) as per nutritional panel of the package.

Composition	Average quantity per serving	Average quantity per 100g
Energy	570 kJ	1240 kJ
Protein	1.1 g	2.4 g
Fat, total	0.1 g	0.2 g
-saturated	<0.1 g	<0.1 g
Carbohydrate	30.4 g	66.0 g
-sugar	25.8 g	56 g
Dietary fiber, total	4.2 g	9.2 g
Sodium	0.7 mg	1.5 mg
Potassium	281 mg	611 mg

Serving per package: 5

Serving size: 46 g

B. Composition of media for microbiological analysis

Table B.1 List of ingredients of the media

Medium	Ingredients	Composition (g/L)
MRS agar (CM0361) Oxoid	Peptone	10.0
	‘Lab-Lemco’ Powder	8.0
	Yeast extract	4.0
	Glucose	20.0
	Di-potassium hydrogen phosphate	2.0
	Sodium acetate 3H ₂ O	5.0
	Tri-ammonium citrate	2.0
	Magnesium sulphate 7H ₂ O	0.2
	Magnesium sulphate 4H ₂ O	0.05
	Agar	10.0
	Sorbitan mono-oleate	1.0 (mL)
YGC agar (1.16000.0500) Merck KGaA	Yeast extract	5.0
	D (+) glucose	20.0
	Chloramphenicol	0.1
	Agar	14.9

C. Gram staining of water kefir grains starter culture

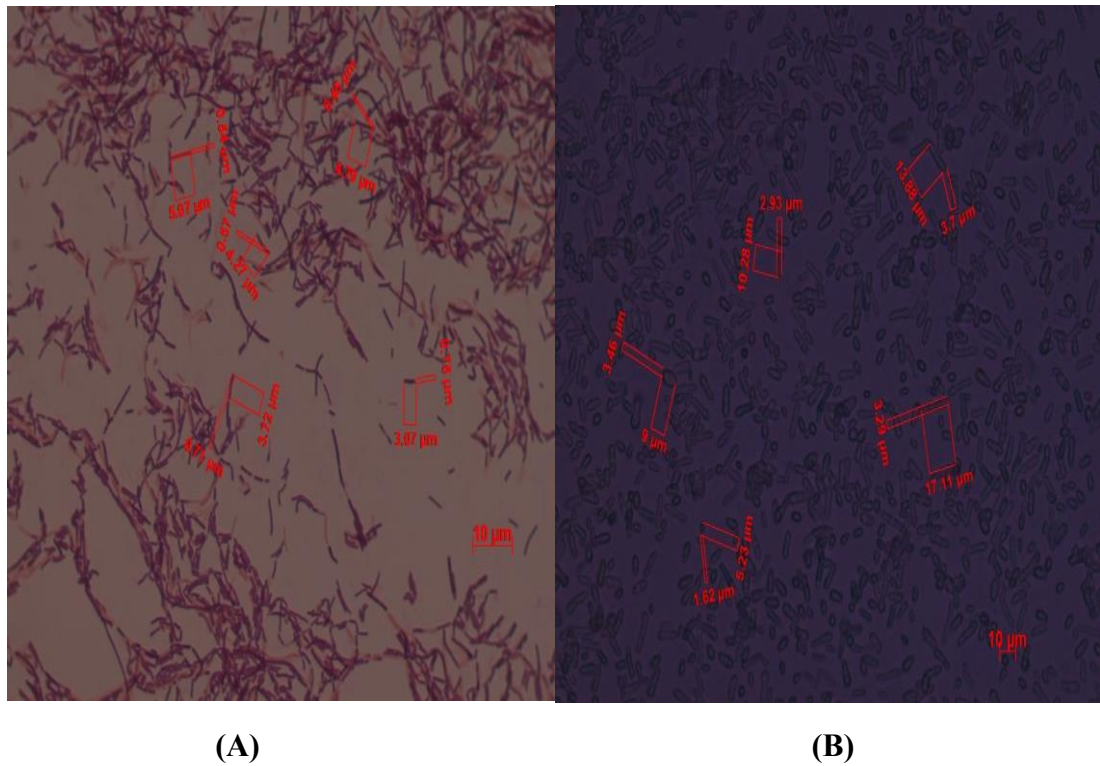


Figure C.1 Observation of cell morphology using the gram staining method, (A)= Bacterial cell morphology under Carl Zeis (model HBO 50/AC, Germany) transmission light microscope magnification (x100); (B)= Yeast cell morphology under Carl Zeis (model HBO 50/AC, Germany) transmission light microscope magnification (x100).

Table C.1 Microbiological analysis of water kefir grains (starter culture)

Parameters	Trial 1		Trial 2		Mean±SD
	Log cfu/g	Log cfu/g	Log cfu/g	Log cfu/g	
<i>Lactobacillus</i> sp.	7.707	7.991	7.778	7.763	7.809±0.14
<i>Saccharomyces cerevisiae</i>	7.217	7.255	7.283	7.264	7.254±0.03

D. Sensory evaluation questionnaire

INFORMATION SHEET

Introduction

I am Vishaldeep Kour, a Master of Food Technology student at the School of Food and Advanced Technology (SFAT), Albany, Massey University. My research investigates the optimisation of low sugar water kefir fermentation to produce consistent high-quality products which meet the stipulated regulations.

You are being invited to evaluate the sensory characteristics of freshly fermented water kefir. The objective of this part of the study is to evaluate the level of acceptance of the prepared fermented water kefir by consumers.

Participant involvement

The trial involves tasting and evaluating four types of organic fermented green tea water kefir. Your participation will take 5-7 minutes. The fermented water kefir contains the following ingredients: organic green tea, organic dates, ginger, brown sugar, natural water kefir grains (starter culture), boiled and cooled water.


You should not participate if you are allergic or may be 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 benefits 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 the postgraduate degree research project. In case you wish to receive a summary of the findings once the data analysis has been completed, please provide your email address.

You have no obligation to accept this invitation. If you decide 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 your participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researchers.

Project Contacts

- Vishaldeep Kour (Master of Food Technology student): 
- Dr. Tony Mutukumira (supervisor): a.n.mutukumira@massey.ac.nz
- Dr. Kay Rutherford (Co-supervisor): K.J.Rutherford@massey.ac.nz

This project has been evaluated by peer review and judged to be low risk (Massey University Ethics 4000022992). Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named above is 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 researcher(s), please contact Professor Craig Johnson, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz

Ethics Approved Number: 4000022992

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 in the Information Sheet.

Signature: _____

Date: _____

Full Name (Printed): _____

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 water before and between samples.

Note: Each sample must be evaluated on a separate form

PRODUCT: Low sugar fermented green-tea flavoured water kefir

Sample code:

Attribute	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
Appearance/ Colour									
Sweetness									
Acidity/ Sourness									
Flavour									
Aroma									
Overall Acceptance									

- **Would you buy this product?**
Yes/No
- **Comments:**

E. Data analysis of Phase II

Table E.1 Mean of four formulations of green tea water kefir for physiochemical and microbiological parameters.

Sample code	Fermentation Period (d)	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	S. cerevisiae log cfu/mL
WK1	0	5.04	3.55	0.036	96.50	0.39	1.62	6.88	6.07
	1	4.01	3.60	0.063	96.60	0.41	1.63	7.61	6.12
	2	3.59	3.22	0.116	96.78	0.42	2.13	8.02	6.61
	3	3.39	2.87	0.166	96.96	0.44	1.81	8.25	7.19
	4	3.39	2.90	0.169	97.10	0.73	1.76	8.37	7.18
	5	3.35	2.77	0.176	97.19	0.78	1.97	8.50	7.14
	6	3.34	2.75	0.183	97.42	0.99	1.98	8.56	6.97
WK2	0	5.05	4.44	0.034	95.88	0.23	1.14	6.88	6.02
	1	4.11	4.55	0.068	96.20	0.41	3.10	7.66	6.37
	2	3.72	4.30	0.110	96.02	0.51	2.65	7.99	6.67
	3	3.62	4.10	0.136	96.72	0.63	2.73	8.29	7.20
	4	3.54	3.95	0.155	96.44	0.88	2.22	8.42	7.04
	5	3.52	3.87	0.161	97.25	0.91	2.44	8.51	7.03
	6	3.50	3.82	0.178	97.88	0.99	3.15	8.50	7.00
WK3	0	4.96	3.52	0.034	96.77	0.26	1.01	7.07	6.07
	1	3.89	3.55	0.074	96.41	0.28	3.06	7.84	6.49
	2	3.54	3.20	0.144	96.47	0.28	2.73	8.25	6.69
	3	3.39	2.77	0.188	96.55	0.70	2.06	8.29	7.27
	4	3.31	2.75	0.199	96.65	0.77	1.48	8.56	7.23
	5	3.40	2.47	0.225	96.70	1.48	1.45	8.66	7.19
	6	3.30	2.35	0.228	97.20	1.71	1.79	8.66	7.06
WK4	0	4.96	4.45	0.034	96.53	0.34	1.04	6.98	6.08
	1	3.95	4.47	0.072	96.36	0.36	3.93	7.94	6.13
	2	3.63	4.12	0.117	96.17	0.46	3.42	8.18	6.75
	3	3.46	3.65	0.153	96.62	0.63	2.57	8.27	7.25
	4	3.37	3.62	0.172	96.89	0.90	3.00	8.53	7.16
	5	3.40	3.42	0.174	97.44	1.20	3.07	8.59	7.12
	6	3.38	3.40	0.191	98.49	1.41	2.70	8.73	7.07

Notes: Sample code WK1 = 3%/22°C, WK2 = 4%/22°C, WK3 = 3%/25°C, WK4 = 4%/25°C; Fermentation time: 0 = 0 h, 1 = 24 h, 2 = 48 h, 3 = 72 h, 4 = 96 h, 5 = 120 h, 6 = 144 h; 0-3 d = Fermentation period with starter culture; 3-6 d = bottled fermentation without starter culture.

Table E.2 Raw data of phase II

Sample code	Fermentation Period (d)	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	<i>S. cerevisiae</i> log cfu/mL
WK1	0	1	5.06	3.5	0.042	96.47	0.30	1.81	6.87	6.04
WK1	0	1	5.09	3.5	0.034	96.96	0.48	1.38	6.86	6.14
WK1	0	2	5.00	3.6	0.034	94.71	0.37	1.76	6.89	6.10
WK1	0	2	5.04	3.6	0.034	97.88	0.42	1.54	6.89	6.11
WK1	1	1	4.02	3.6	0.059	96.21	0.41	1.74	7.80	6.09
WK1	1	1	3.98	3.6	0.059	95.01	0.46	1.46	7.71	6.03
WK1	1	2	4.03	3.6	0.068	97.05	0.40	1.88	7.45	6.21
WK1	1	2	4.02	3.6	0.068	98.13	0.37	1.44	7.49	6.17
WK1	2	1	3.59	3.2	0.111	96.75	0.38	2.08	7.96	6.73
WK1	2	1	3.58	3.2	0.102	96.69	0.42	1.68	7.87	6.71
WK1	2	2	3.66	3.3	0.127	96.86	0.50	2.06	8.16	6.55
WK1	2	2	3.53	3.2	0.127	96.82	0.40	2.71	8.11	6.46
WK1	3	1	3.45	3.0	0.154	97.19	0.48	1.91	8.18	7.23
WK1	3	1	3.46	2.8	0.136	96.45	0.46	2.04	8.20	7.28
WK1	3	2	3.46	2.8	0.170	97.13	0.41	1.39	8.31	7.12
WK1	3	2	3.22	2.9	0.205	97.09	0.44	1.94	8.33	7.17
WK1	4	1	3.45	3.0	0.148	97.06	0.54	1.81	8.19	7.22
WK1	4	1	3.46	2.9	0.153	97.05	0.74	1.77	8.35	7.23
WK1	4	2	3.44	2.8	0.153	96.97	0.60	1.80	8.69	7.17
WK1	4	2	3.23	2.9	0.221	97.35	1.05	1.67	8.28	7.12
WK1	5	1	3.36	2.7	0.161	97.47	0.64	1.98	8.45	7.06
WK1	5	1	3.41	2.7	0.145	96.51	0.76	1.95	8.63	7.09
WK1	5	2	3.43	2.8	0.162	96.54	0.65	2.00	8.72	7.24
WK1	5	2	3.21	2.9	0.238	98.24	1.07	1.97	8.50	7.16
WK1	6	1	3.36	2.7	0.153	96.87	0.86	2.04	8.53	6.75
WK1	6	1	3.41	2.7	0.153	97.63	1.16	1.98	8.53	7.05
WK1	6	2	3.38	2.8	0.187	97.83	0.90	1.94	8.62	6.92
WK1	6	2	3.21	2.8	0.239	97.37	1.04	1.96	8.56	7.10

Sample code	Fermentation Time (d)	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	<i>S. cerevisiae</i> log cfu/mL
WK2	0	1	5.07	4.5	0.034	95.19	0.23	0.77	6.85	5.99
WK2	0	1	5.10	4.4	0.034	96.53	0.29	1.51	6.83	6.03
WK2	0	2	5.01	4.5	0.034	95.30	0.20	0.84	6.96	6.05
WK2	0	2	5.02	4.5	0.034	96.51	0.22	1.47	6.89	6.02
WK2	1	1	4.10	4.5	0.068	95.28	0.37	3.10	7.63	6.44
WK2	1	1	4.10	4.5	0.068	96.24	0.47	3.15	7.68	6.31
WK2	1	2	4.11	4.5	0.068	96.21	0.37	3.09	7.65	6.36
WK2	1	2	4.12	4.5	0.068	97.08	0.43	3.09	7.71	6.39
WK2	2	1	3.72	4.3	0.094	96.15	0.53	3.59	7.96	6.63
WK2	2	1	3.70	4.3	0.102	95.41	0.41	3.64	7.92	6.60
WK2	2	2	3.73	4.3	0.127	96.78	0.53	3.65	8.02	6.75
WK2	2	2	3.73	4.3	0.119	95.76	0.59	3.72	8.07	6.70
WK2	3	1	3.62	4.1	0.136	96.68	0.53	2.80	8.27	7.12
WK2	3	1	3.62	4.1	0.128	96.57	0.47	2.77	8.32	7.20
WK2	3	2	3.63	4.1	0.145	97.00	0.80	2.65	8.29	7.21
WK2	3	2	3.61	4.1	0.136	96.66	0.72	2.73	8.30	7.28
WK2	4	1	3.54	4.0	0.154	96.76	0.84	2.35	8.41	6.90
WK2	4	1	3.54	3.9	0.145	96.46	0.96	2.17	8.39	7.13
WK2	4	2	3.54	4.0	0.170	97.05	0.90	2.24	8.42	7.05
WK2	4	2	3.54	3.9	0.153	95.50	0.82	2.14	8.49	7.08
WK2	5	1	3.52	3.8	0.153	96.73	0.95	2.51	8.53	7.10
WK2	5	1	3.52	3.9	0.145	97.83	0.77	2.39	8.52	7.06
WK2	5	2	3.52	3.9	0.178	96.83	1.19	2.52	8.49	7.08
WK2	5	2	3.52	3.9	0.170	97.64	0.73	2.34	8.51	6.88
WK2	6	1	3.49	3.8	0.168	97.82	0.93	3.12	8.48	6.96
WK2	6	1	3.51	3.8	0.170	98.00	1.20	3.12	8.51	6.99
WK2	6	2	3.50	3.8	0.187	97.85	0.89	3.16	8.52	7.02
WK2	6	2	3.51	3.9	0.188	97.88	0.96	3.19	8.51	7.05

Sample code	Fermentation Time (d)	Replication	pH	TSS (°Brix)	T.A. (%)	Color L*	Color a*	Color b*	LAB log cfu/mL	<i>S. cerevisiae</i> log cfu/mL
WK3	0	1	5.10	3.5	0.034	96.42	0.23	0.86	7.11	6.13
WK3	0	1	5.08	3.5	0.034	96.34	0.26	1.13	7.14	6.11
WK3	0	2	4.82	3.5	0.034	96.66	0.25	0.90	7.01	6.06
WK3	0	2	4.85	3.6	0.034	97.68	0.30	1.17	7.02	5.99
WK3	1	1	3.85	3.6	0.076	95.88	0.21	3.18	7.76	6.78
WK3	1	1	3.86	3.6	0.076	96.27	0.25	3.50	7.89	6.48
WK3	1	2	3.93	3.5	0.068	96.87	0.30	2.89	7.83	6.38
WK3	1	2	3.94	3.5	0.076	96.63	0.38	2.69	7.89	6.32
WK3	2	1	3.43	3.2	0.153	96.62	0.21	2.40	8.22	6.81
WK3	2	1	3.48	3.2	0.170	96.19	0.40	2.52	8.25	6.78
WK3	2	2	3.63	3.2	0.128	96.63	0.21	2.95	8.29	6.62
WK3	2	2	3.63	3.2	0.127	96.44	0.32	3.07	8.27	6.59
WK3	3	1	3.31	2.7	0.239	96.69	0.77	1.98	8.18	7.43
WK3	3	1	3.35	2.8	0.196	97.01	0.58	2.20	8.23	7.12
WK3	3	2	3.45	2.8	0.153	96.33	0.86	1.93	8.37	7.29
WK3	3	2	3.46	2.8	0.162	96.19	0.60	2.13	8.39	7.26
WK3	4	1	3.27	2.7	0.239	96.61	0.82	1.56	8.42	7.35
WK3	4	1	3.33	2.8	0.212	96.90	0.71	1.35	8.36	7.30
WK3	4	2	3.41	2.7	0.179	96.38	0.79	1.58	8.77	7.12
WK3	4	2	3.42	2.8	0.170	96.74	0.76	1.44	8.72	7.16
WK3	5	1	3.25	2.3	0.297	96.61	1.83	1.46	8.49	7.32
WK3	5	1	3.31	2.4	0.238	96.67	1.75	1.33	8.59	7.27
WK3	5	2	3.40	2.6	0.179	96.71	1.18	1.77	8.76	7.12
WK3	5	2	3.40	2.6	0.188	96.82	1.16	1.26	8.81	7.09
WK3	6	1	3.23	2.2	0.282	97.00	1.44	1.84	8.57	6.98
WK3	6	1	3.29	2.4	0.230	97.50	1.75	1.76	8.63	6.94
WK3	6	2	3.35	2.4	0.196	97.36	1.78	1.79	8.83	7.07
WK3	6	2	3.34	2.4	0.205	96.94	1.89	1.80	8.64	7.28

Sample code	Fermentation Time (d)	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	<i>S. cerevisiae</i> log cfu/mL
WK4	0	1	5.08	4.4	0.034	96.95	0.37	0.99	6.97	6.11
WK4	0	1	5.10	4.4	0.034	96.50	0.30	1.24	6.84	6.12
WK4	0	2	4.82	4.5	0.034	96.31	0.37	0.89	7.07	6.07
WK4	0	2	4.84	4.5	0.034	96.37	0.33	1.07	7.05	6.06
WK4	1	1	3.95	4.4	0.068	96.00	0.20	4.28	7.99	6.06
WK4	1	1	4.00	4.5	0.068	96.23	0.41	3.92	7.88	6.26
WK4	1	2	3.96	4.5	0.077	96.93	0.39	3.70	8.00	6.16
WK4	1	2	3.95	4.5	0.077	96.31	0.44	3.84	7.90	6.06
WK4	2	1	3.62	4.1	0.111	96.49	0.52	3.49	8.20	7.12
WK4	2	1	3.64	4.2	0.102	96.23	0.46	3.44	8.03	7.1
WK4	2	2	3.63	4.1	0.128	95.99	0.42	3.32	8.26	7.21
WK4	2	2	3.64	4.1	0.137	95.97	0.44	3.43	8.24	7.24
WK4	3	1	3.46	3.7	0.136	96.86	0.51	2.57	8.18	7.28
WK4	3	1	3.46	3.8	0.144	96.05	0.70	2.61	8.22	7.27
WK4	3	2	3.47	3.5	0.170	96.77	0.64	2.51	8.33	7.29
WK4	3	2	3.47	3.6	0.161	96.83	0.70	2.59	8.36	7.17
WK4	4	1	3.41	3.7	0.162	96.77	0.76	3.03	8.32	6.89
WK4	4	1	3.40	3.7	0.170	96.64	1.35	3.05	8.40	6.82
WK4	4	2	3.43	3.5	0.187	96.23	0.78	2.99	8.73	6.63
WK4	4	2	3.44	3.6	0.170	97.94	0.73	2.95	8.78	6.67
WK4	5	1	3.40	3.5	0.170	97.45	1.50	3.13	8.44	7.10
WK4	5	1	3.42	3.5	0.170	97.38	1.26	3.00	8.46	7.17
WK4	5	2	3.40	3.3	0.178	97.50	1.00	3.11	8.70	7.12
WK4	5	2	3.40	3.4	0.178	97.43	1.04	3.06	8.77	7.08
WK4	6	1	3.39	3.5	0.179	98.42	1.31	2.72	8.71	6.95
WK4	6	1	3.39	3.5	0.179	98.57	1.42	2.70	8.66	6.99
WK4	6	2	3.38	3.3	0.204	98.48	1.74	2.71	8.85	7.188
WK4	6	2	3.36	3.3	0.204	98.52	1.20	2.67	8.70	7.162

Notes: Sample code WK1 = 3%/22°C, WK2 = 4%/22°C, WK3 = 3%/25°C, WK4 = 4%/25°C; Fermentation time: 0 = 0 h, 1 = 24 h, 2 = 48 h, 3 = 72 h, 4 = 96 h, 5 = 120 h, 6 = 144 h; 0-3 d = Fermentation period with starter culture; 3-6 d = bottled fermentation without starter culture.

Table E.3 Consumer sensory evaluation scores of green tea beverages at the end of fermentation (144 h) (phase II)

Panelist	Code	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall	Panelist	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall
1	WK1	6	6	5	5	4	5	1	7	5	5	5	4	5
2	WK1	8	7	7	7	7	7	2	6	5	4	4	6	6
3	WK1	7	8	3	6	7	5	3	6	5	4	7	2	5
4	WK1	5	6	6	6	6	6	4	8	3	5	1	1	1
5	WK1	5	6	5	5	6	5	5	5	4	5	4	3	4
6	WK1	6	7	7	7	5	7	6	6	5	6	6	5	6
7	WK1	5	4	3	2	2	2	7	5	6	1	2	2	2
8	WK1	6	6	5	5	5	6	8	5	3	3	3	3	3
9	WK1	5	6	6	6	6	5	9	5	7	6	7	4	7
10	WK1	5	6	7	7	6	7	10	5	7	7	7	5	7
11	WK1	6	6	4	3	5	5	11	8	6	7	2	4	4
12	WK1	6	5	6	5	4	6	12	4	6	7	6	8	6
13	WK1	4	5	7	6	6	6	13	5	5	5	6	6	6
14	WK1	3	3	2	2	2	2	14	5	3	6	2	7	4
15	WK1	7	6	7	6	6	6	15	7	5	7	5	6	6
16	WK1	6	6	6	6	6	6	16	8	6	7	8	7	7
17	WK1	8	4	4	4	4	2	17	7	7	6	7	7	7
18	WK1	5	3	3	3	3	3	18	7	6	8	8	6	7
19	WK1	8	7	7	6	7	7	19	7	5	7	6	8	6
20	WK1	8	5	4	6	6	5	20	7	3	4	4	7	3
21	WK1	7	3	5	6	5	6	21	4	2	5	2	5	3
22	WK1	7	2	4	2	2	2	22	4	5	5	6	5	5
23	WK1	8	7	8	8	8	8	23	5	4	4	4	3	4
24	WK1	7	4	5	4	7	6	24	6	7	6	7	7	6
25	WK1	7	3	6	5	4	6	25	8	2	2	2	5	4
26	WK1	5	2	6	2	4	3	26	4	7	7	4	4	5
27	WK1	7	7	6	6	6	6	27	6	8	5	6	6	6
28	WK1	6	3	4	4	5	3	28	6	5	7	6	6	6
29	WK1	5	3	6	5	4	4	29	7	3	4	3	6	3
30	WK1	4	6	5	5	4	5	30	5	4	6	7	7	6

Notes: Sample code WK1 = 3%/22°C

Panellist	Code	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall	Panelist	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall
1	WK2	8	8	8	7	8	8	1	6	8	7	8	8	8
2	WK2	8	6	5	6	5	7	2	5	6	6	7	6	6
3	WK2	7	7	7	8	8	7	3	7	8	7	7	8	8
4	WK2	5	6	4	5	8	6	4	7	7	7	7	6	8
5	WK2	6	7	6	7	7	8	5	7	6	7	7	5	8
6	WK2	6	6	6	6	6	6	6	5	7	7	7	7	7
7	WK2	6	6	7	5	8	7	7	5	6	5	7	6	8
8	WK2	6	7	7	7	7	7	8	7	7	8	8	8	8
9	WK2	6	6	6	6	6	6	9	8	7	7	7	6	7
10	WK2	6	6	6	6	7	6	10	7	8	6	7	7	8
11	WK2	6	7	6	7	6	7	11	8	7	9	8	9	8
12	WK2	6	7	7	7	7	7	12	7	7	7	7	7	7
13	WK2	5	7	7	7	6	7	13	6	5	6	7	6	6
14	WK2	8	8	8	8	8	8	14	6	6	5	6	6	6
15	WK2	7	7	7	6	6	7	15	7	6	6	6	6	6
16	WK2	6	6	6	7	7	7	16	7	6	6	7	6	6
17	WK2	6	6	6	6	6	6	17	6	7	7	6	7	7
18	WK2	8	7	7	7	8	7	18	8	7	7	8	7	7
19	WK2	6	6	5	7	6	6	19	5	6	5	5	6	6
20	WK2	6	6	5	5	6	6	20	5	7	7	6	5	6
21	WK2	6	6	5	5	6	6	21	5	7	7	7	6	7
22	WK2	6	5	5	5	5	5	22	8	7	6	6	6	6
23	WK2	5	6	6	5	5	6	23	7	6	7	6	6	6
24	WK2	7	7	7	7	7	7	24	5	6	5	6	6	6
25	WK2	9	9	9	9	9	9	25	7	7	7	7	7	7
26	WK2	8	7	6	5	6	6	26	7	7	7	7	7	7
27	WK2	7	6	6	6	6	7	27	7	7	7	7	7	7
28	WK2	7	6	6	6	7	7	28	7	7	7	7	7	7
29	WK2	6	7	8	8	8	8	29	8	8	7	8	8	8
30	WK2	7	7	7	7	7	7	30	8	7	8	8	8	8

Notes: WK2 = 4%/22°C,

Panellist	Code	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall	Panelist	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall
1	WK3	4	5	6	6	6	6	1	7	4	5	5	5	5
2	WK3	6	8	7	7	5	6	2	7	4	5	5	6	5
3	WK3	3	2	2	2	2	2	3	3	4	5	4	6	5
4	WK3	7	8	6	5	8	7	4	5	4	4	5	5	6
5	WK3	7	5	8	4	4	3	5	6	3	3	3	6	3
6	WK3	4	3	3	3	3	3	6	7	2	2	2	2	2
7	WK3	8	6	4	6	5	6	7	6	4	5	4	4	3
8	WK3	7	3	6	7	5	7	8	5	5	4	4	4	4
9	WK3	7	2	4	3	4	3	9	5	3	3	3	4	4
10	WK3	7	4	5	6	7	5	10	5	6	7	6	3	6
11	WK3	6	4	4	6	8	6	11	5	6	6	6	5	6
12	WK3	3	6	4	5	5	4	12	8	2	6	2	6	3
13	WK3	8	3	2	3	6	3	13	6	4	6	4	5	4
14	WK3	4	6	6	6	6	6	14	5	6	6	6	6	7
15	WK3	4	3	4	4	4	3	15	6	6	8	6	7	6
16	WK3	4	4	4	4	4	4	16	7	6	7	5	6	6
17	WK3	6	2	5	4	6	5	17	8	7	7	8	7	7
18	WK3	5	3	4	5	6	5	18	6	6	5	6	6	6
19	WK3	4	5	3	2	3	3	19	7	6	7	7	7	6
20	WK3	5	3	3	3	4	3	20	7	6	4	6	6	5
21	WK3	3	4	4	6	6	6	21	4	3	2	4	2	3
22	WK3	3	3	3	3	5	3	22	8	4	8	8	7	8
23	WK3	5	3	4	5	6	5	23	4	4	4	5	5	4
24	WK3	7	5	3	4	4	4	24	5	6	7	5	4	6
25	WK3	5	2	2	2	2	2	25	6	7	7	7	6	6
26	WK3	5	5	6	6	5	5	26	8	3	3	6	6	7
27	WK3	6	1	8	5	5	3	27	4	8	8	6	7	6
28	WK3	4	5	6	6	5	6	28	7	6	7	6	7	6
29	WK3	5	6	5	5	4	6	29	5	4	5	3	5	5
30	WK3	5	6	6	6	6	7	30	5	5	6	7	6	7

Notes: WK3 = 3%/25°C

Panelist	Code	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall	Panelist	Appearance	Sweetness	Acidity	Flavor	Aroma	Overall
1	WK4	7	6	6	6	6	7	1	7	7	7	8	7	7
2	WK4	6	6	7	7	7	6	2	9	9	9	9	9	9
3	WK4	9	9	9	9	9	9	3	5	6	7	7	6	7
4	WK4	6	6	5	6	5	6	4	4	6	5	6	7	6
5	WK4	7	7	7	6	6	6	5	5	6	7	7	5	6
6	WK4	6	7	7	6	5	6	6	5	5	6	6	5	5
7	WK4	8	7	7	7	8	7	7	8	5	4	6	7	5
8	WK4	4	6	6	6	6	6	8	4	5	4	5	5	5
9	WK4	6	5	4	4	6	3	9	8	4	8	7	5	7
10	WK4	8	8	8	8	8	7	10	7	5	6	5	5	6
11	WK4	8	8	8	8	8	8	11	7	5	6	6	6	6
12	WK4	6	5	6	6	6	6	12	7	7	8	8	8	8
13	WK4	5	5	5	6	5	5	13	6	5	5	5	5	5
14	WK4	6	2	7	4	6	4	14	4	4	6	5	5	5
15	WK4	6	7	5	6	8	7	15	8	8	9	8	9	8
16	WK4	4	4	5	5	5	5	16	5	6	6	6	5	6
17	WK4	3	3	2	3	6	3	17	5	5	4	5	4	5
18	WK4	6	6	6	6	6	6	18	7	6	7	7	7	7
19	WK4	5	5	4	5	6	5	19	4	4	6	7	5	7
20	WK4	6	7	5	6	6	6	20	8	6	6	6	7	6
21	WK4	7	7	7	7	7	7	21	5	6	6	7	6	6
22	WK4	8	7	7	7	6	7	22	5	6	4	6	6	7
23	WK4	3	4	3	4	4	4	23	4	7	7	6	4	5
24	WK4	6	8	8	7	8	7	24	7	7	7	4	4	6
25	WK4	7	5	6	6	6	7	25	5	6	6	6	6	7
26	WK4	6	6	7	5	5	5	26	6	9	7	7	7	7
27	WK4	5	5	5	6	5	5	27	8	9	9	9	9	9
28	WK4	7	6	6	6	4	6	28	7	8	8	8	8	8
29	WK4	7	5	6	6	5	7	29	8	8	8	8	8	8
30	WK4	7	6	5	6	7	6	30	5	5	5	5	5	5

Notes: WK4 = 4%/25°C

Table E.4 Physicochemical and microbiological characteristics of green tea water kefir beverage (WK2) during storage period (4°C) (raw data of phase III part II)

Sample code	Storage time (d)	Replication	pH	TSS (°Brix)	T.A. (%)	Color L*	Color a*	Color b*	LAB log cfu/ml	S. cerevisiae log cfu/ml
WK2	1	1	3.52	3.9	0.172	98.41	0.94	3.19	8.519	7.040
WK2	1	1	3.51	3.9	0.187	97.73	1.1	3.27	8.520	7.035
WK2	1	2	3.52	3.8	0.171	97.88	0.95	3.09	8.518	7.010
WK2	1	2	3.51	3.7	0.187	98.39	0.99	3.13	8.519	7.030
WK2	2	1	3.48	3.8	0.197	99.16	1.21	3.27	8.484	6.942
WK2	2	1	3.47	3.7	0.188	99.24	1.11	3.19	8.502	6.958
WK2	2	2	3.48	3.8	0.187	99.39	1.38	3.23	8.474	6.956
WK2	2	2	3.48	3.7	0.188	98.9	1.06	3.12	8.481	6.933
WK2	3	1	3.43	3.7	0.195	98.42	1.89	2.22	8.440	6.857
WK2	3	1	3.42	3.6	0.195	98.39	1.96	2.31	8.421	6.846
WK2	3	2	3.42	3.6	0.204	98.09	2.04	2.56	8.438	6.865
WK2	3	2	3.42	3.6	0.204	98.2	2.01	2.22	8.416	6.849

Notes: Sample code WK2 = 4%/22°C; Storage time: 1 = St. D-0, 2 = St. D-7, 3 = St. D-14; St. D= storage day; d= day

Table E.5 Focus group sensory evaluation scores for green tea water kefir beverage (WK2) during storage (4°C) (raw data of phase III, part II)

Time (d)	Panellists	Replication	Appearance	Sweetness	Sourness	Flavour	Aroma	Overall acceptability
St. D-0	1	1	7	8	8	7	7	7
	2	1	8	8	9	8	8	8
	3	1	8	8	8	7	7	8
	4	1	8	8	8	8	8	8
	5	1	8	8	7	7	7	8
	6	1	8	8	9	8	9	8
	7	1	8	8	7	8	8	8
	8	1	8	9	8	7	7	8
	1	2	7	8	8	7	8	7
	2	2	8	7	8	8	8	8
	3	2	7	8	7	7	7	7
	4	2	8	8	8	8	7	7
	5	2	9	9	8	8	8	8
	6	2	8	7	7	7	7	7
	7	2	7	8	7	7	7	7
	8	2	8	8	7	8	7	8
St. D-7	1	1	7	7	7	7	7	7
	2	1	8	8	8	7	8	8
	3	1	7	7	7	7	8	8
	4	1	7	8	7	7	6	8
	5	1	9	9	8	8	8	8
	6	1	8	8	8	8	8	8
	7	1	7	8	7	7	7	7
	8	1	7	7	7	7	7	7
	1	2	7	7	7	7	7	7
	2	2	8	8	9	8	8	8
	3	2	7	7	7	7	7	7
	4	2	7	7	8	7	8	7
	5	2	9	8	8	7	7	8
	6	2	6	7	7	7	7	7
	7	2	7	7	8	7	8	7
	8	2	7	8	8	8	7	7
St. D-14	1	1	8	8	9	8	8	8
	2	1	8	7	8	8	8	8
	3	1	7	6	7	7	7	7
	4	1	8	7	7	7	7	7
	5	1	9	8	9	8	8	9
	6	1	8	7	8	8	7	8
	7	1	7	7	7	7	7	7
	8	1	7	7	7	7	7	7
	1	2	8	7	9	8	8	8
	2	2	7	9	9	9	9	9
	3	2	8	7	8	8	7	8
	4	2	7	7	7	7	7	7
	5	2	8	8	8	7	8	8
	6	2	8	7	7	7	7	7
	7	2	8	7	8	8	7	8
	8	2	7	7	7	7	7	7

Notes: Sample code WK2 = 4%/22°C; St. D= storage day; d= day

F. HPLC Data

F.1 HPLC Standard curve

F.1.1 Ethanol

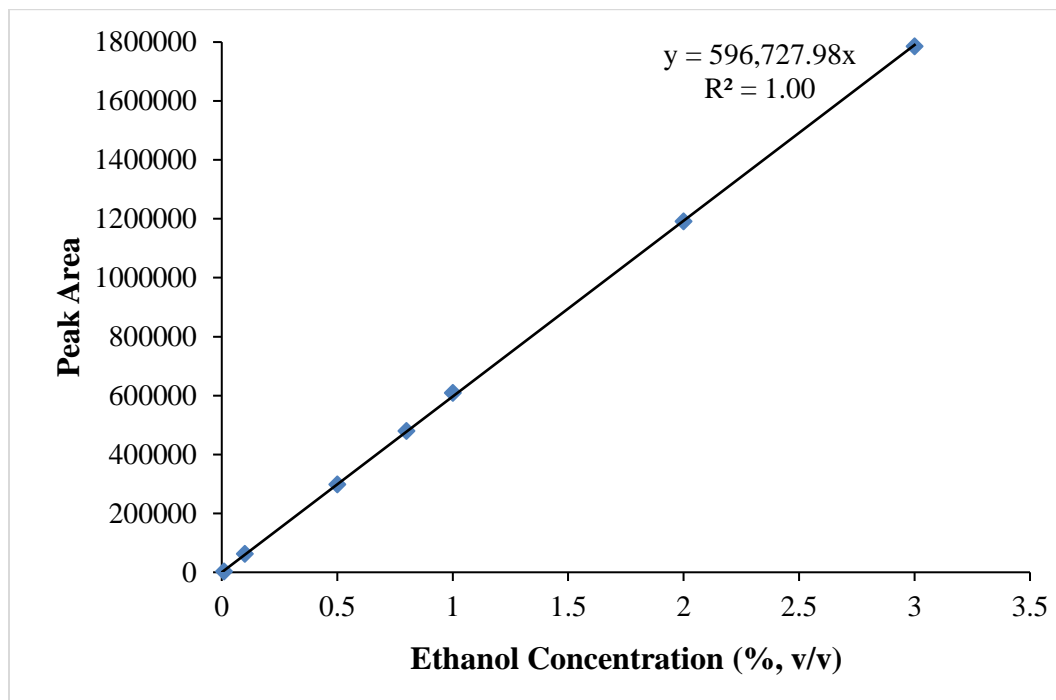


Table F.1 HPLC data for ethanol standards peak area and retention time

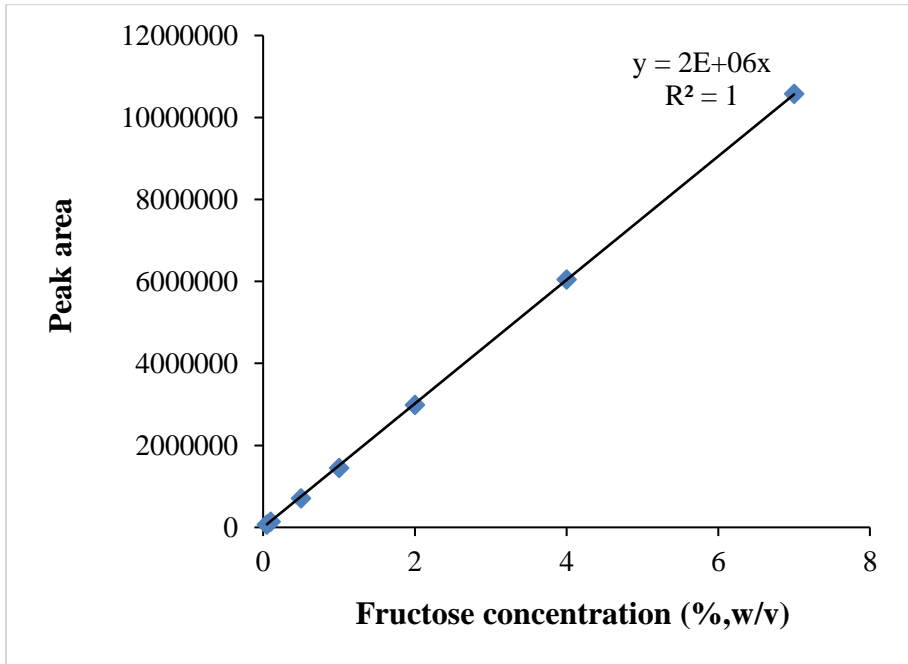
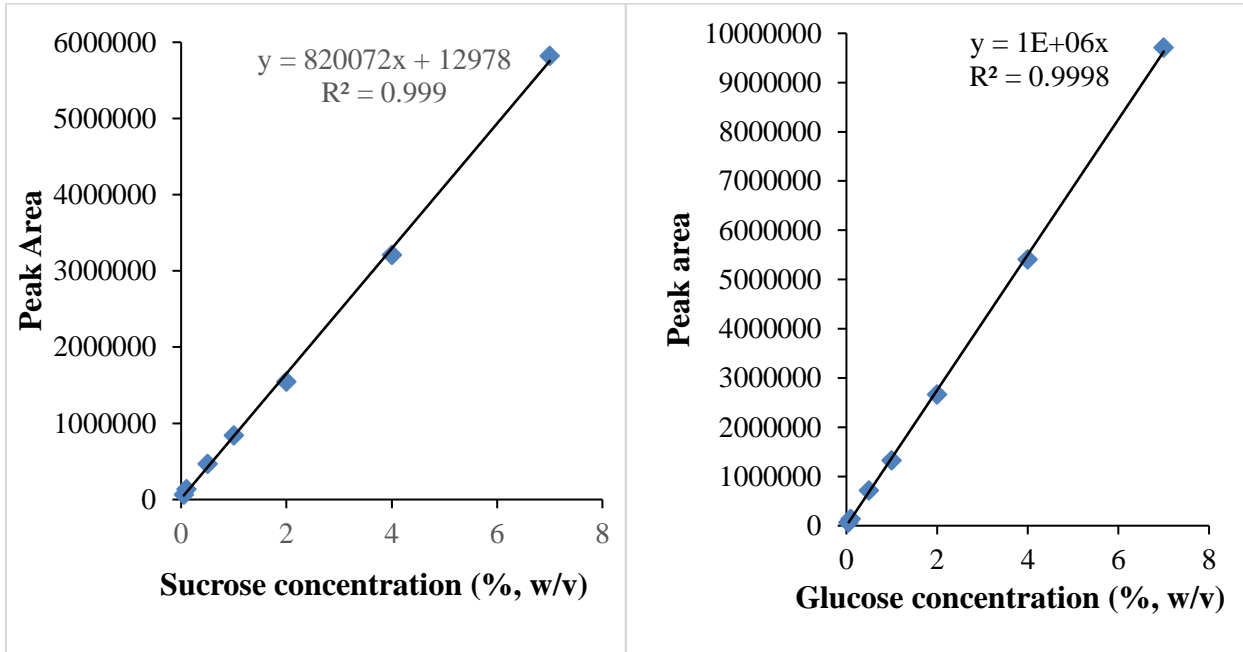
Standard	Concentration (% v/v)	Mean peak area	Mean retention time (min)
Ethanol	0.01	3362	21.789
	0.1	63448	
	0.5	299080	
	0.8	479959	
	1	608985	
	2	1192032	
	3	1786124	

Table F.2 HPLC data of ethanol concentration in green tea water kefir beverage (WK2) during fermentation (144 h) and storage period of two weeks (4±1°C) (raw data of phase III part I)

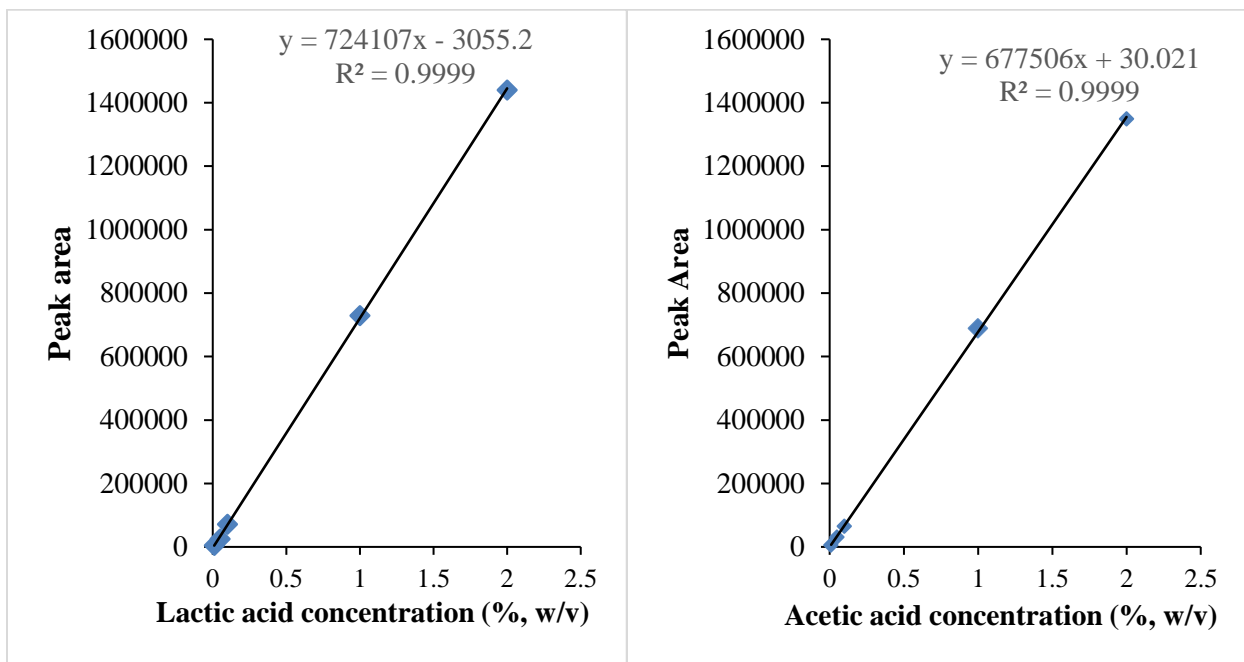
Sample code	Fermentation time	Replication	Peak area	Ethanol concentration (%, v/v)
WK2	1	1	nd	nd
WK2	1	1	nd	nd
WK2	1	2	nd	nd
WK2	1	2	nd	nd
WK2	2	1	75762	0.122
WK2	2	1	75547	0.121
WK2	2	2	97453	0.158
WK2	2	2	103293	0.168
WK2	3	1	139925	0.229
WK2	3	1	140974	0.231
WK2	3	2	181528	0.299
WK2	3	2	188572	0.311
WK2	4	1	176542	0.291
WK2	4	1	232633	0.385
WK2	4	2	242789	0.402
WK2	4	2	236975	0.393
WK2	5	1	175826	0.29
WK2	5	1	214296	0.354
WK2	5	2	233200	0.386
WK2	5	2	228896	0.379
WK2	6	1	168802	0.278
WK2	6	1	305138	0.507
WK2	6	2	320705	0.533
WK2	6	2	321451	0.535
WK2	7	1	252566	0.419
WK2	7	1	328247	0.546
WK2	7	2	354644	0.59
WK2	7	2	344657	0.574
WK2	8	1	369101	0.615
WK2	8	1	329914	0.549
WK2	8	2	393730	0.656
WK2	8	2	414991	0.692
WK2	9	1	399322	0.665
WK2	9	1	353149	0.588
WK2	9	2	427953	0.714
WK2	9	2	429989	0.717

Notes: Sample code WK2 = 4%/22°C; Fermentation time: 1=0 h, 2=24 h, 3=48 h, 4=72 h, 5=96 h, 6=120 h, 7=144 h; Storage time: 7= St. D-0, 8 = St. D-7, 9 = St. D-14; St. D= storage day; d= day; nd= not detected

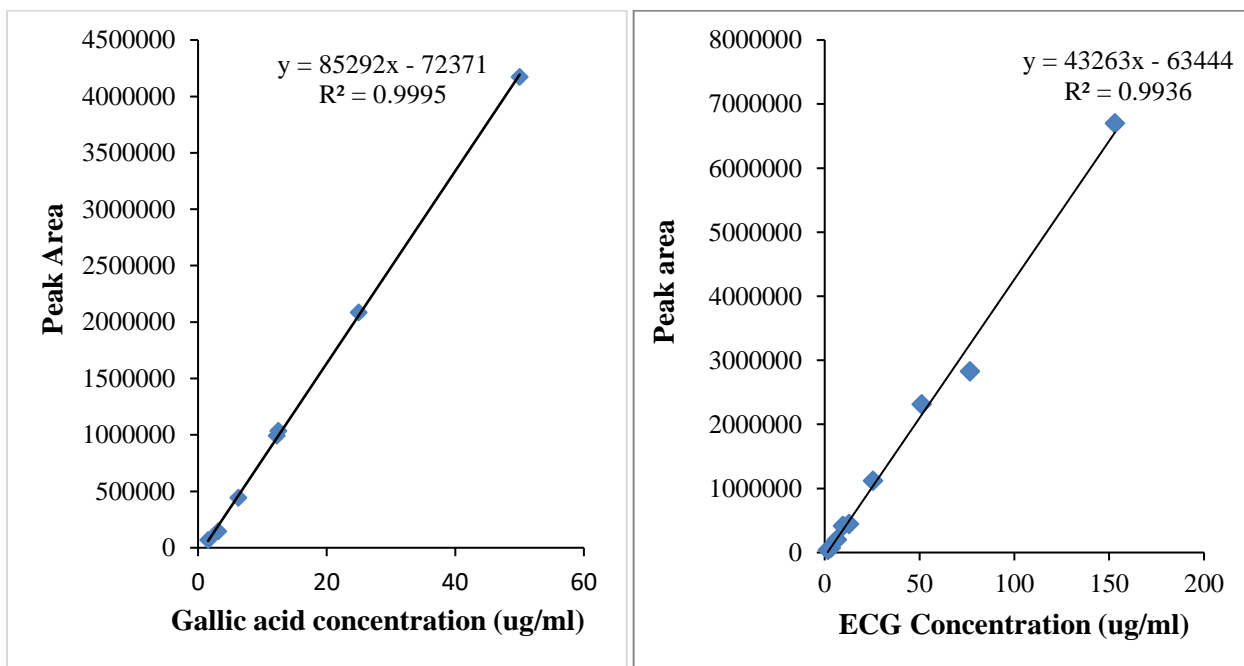
F.1.2 Sugars

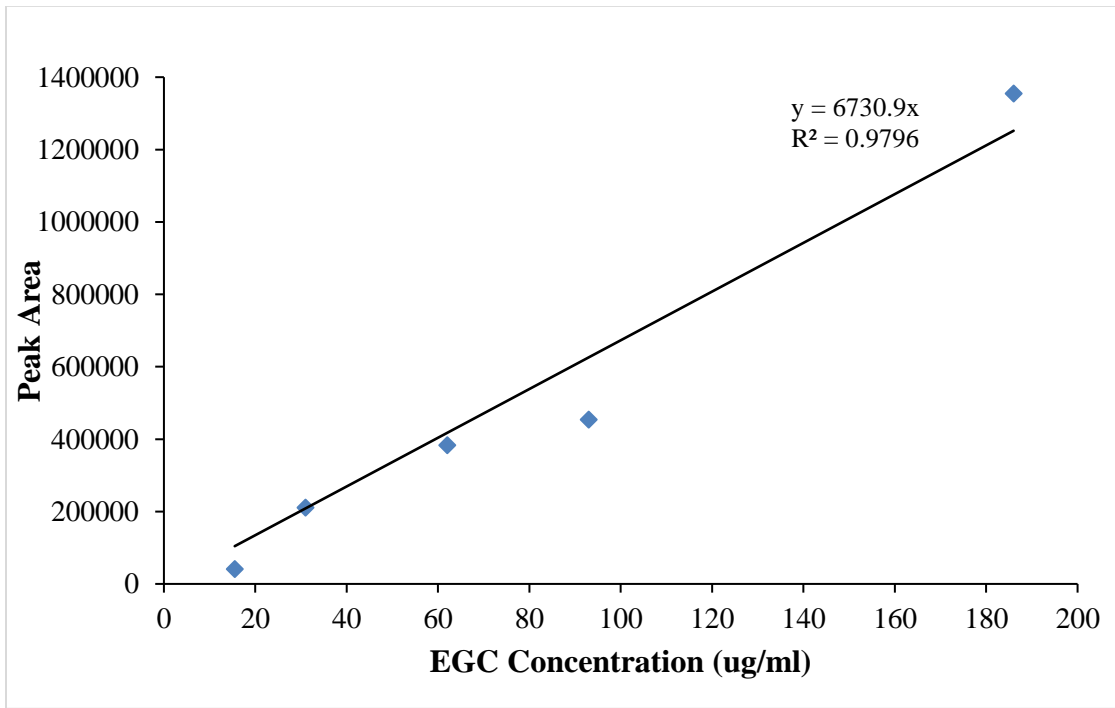
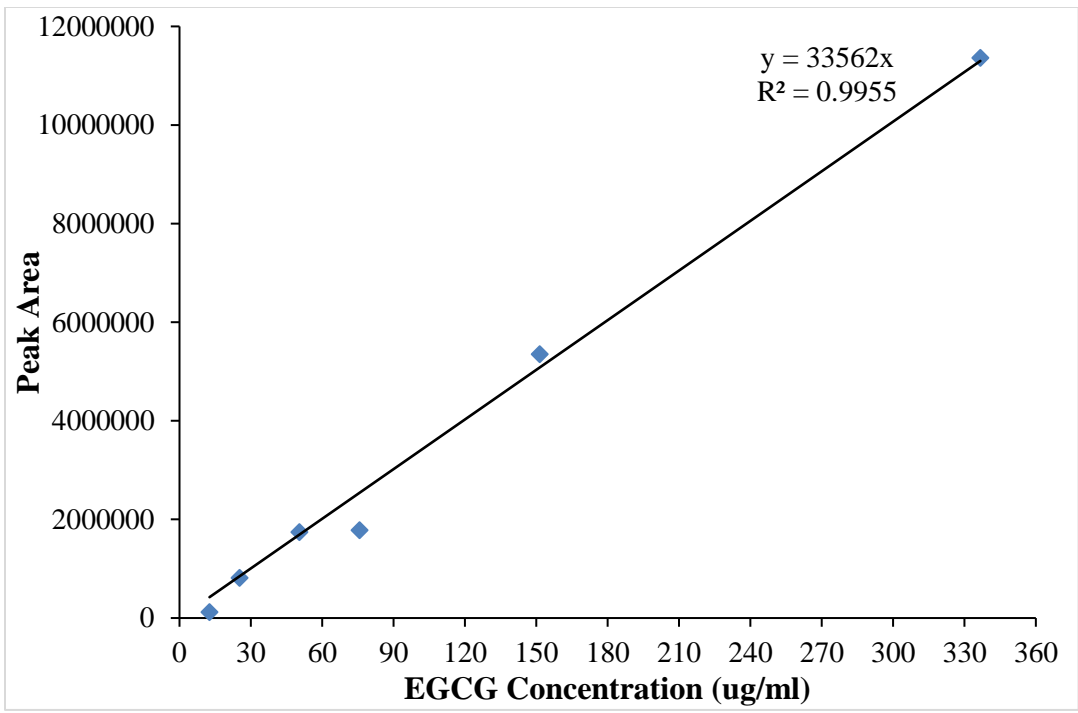


F.1.3 Organic acids



F.1.4 Antioxidants





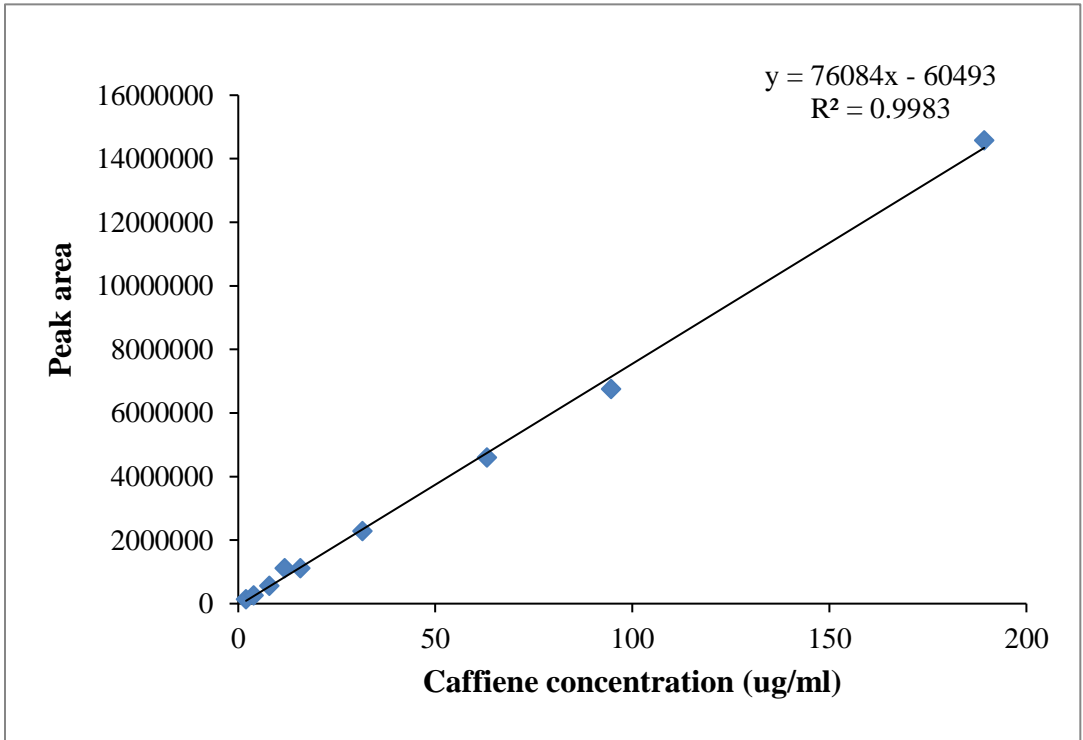
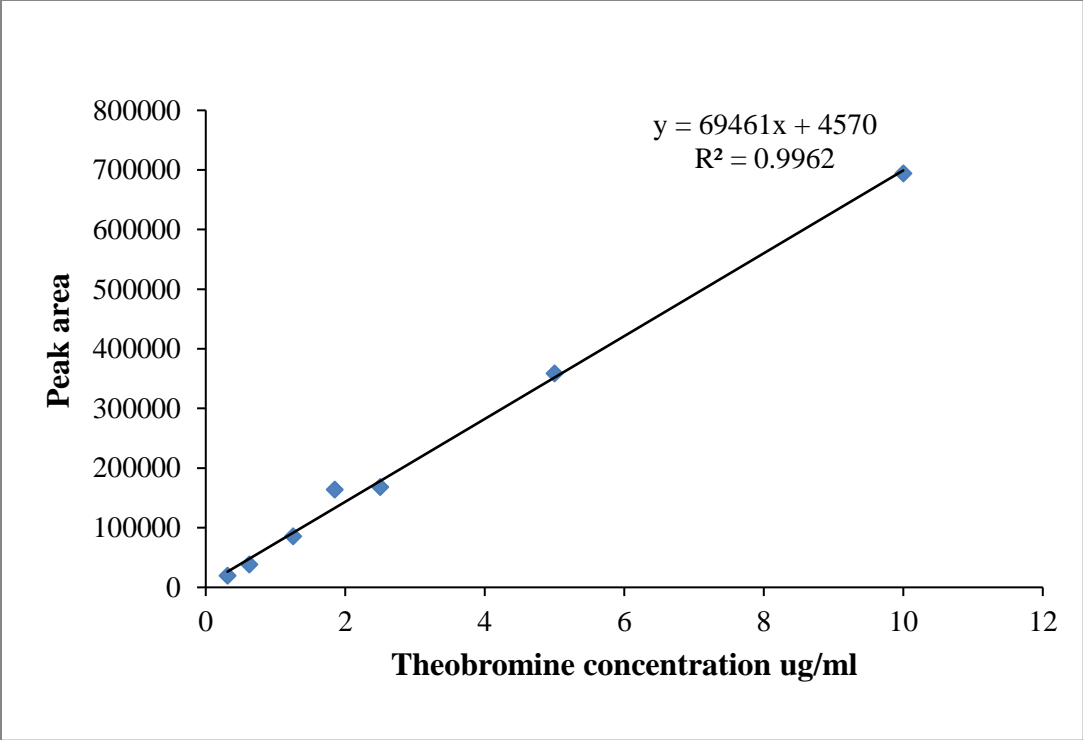


Table F.3 HPLC data for sugar and organic acids standard peak area and retention time

Standard	Concentration (%, w/v)	Mean peak area	Mean retention time (min)
Sucrose	0.05	66875	8.84
	0.1	137315	
	0.5	471580	
	1	844737	
	2	1548141	
	4	3212413	
	7	5823847	
Fructose	0.05	66610	13.03
	0.1	136001	
	0.5	705212	
	1	1449259	
	2	2986513	
	4	6047938	
	7	10577500	
Glucose	0.05	66484	10.68
	0.1	141101	
	0.5	718263	
	1	1329825	
	2	2666982	
	4	5411135	
	7	9709857	
Lactic acid	0.01	4998	13.75
	0.05	25198	
	0.1	72074	
	1	729777	
	2	1440855	
Acetic acid	0.01	5899	15.69
	0.05	31215	
	0.1	65946	
	1	688071	
	2	1349938	

Table F.4 HPLC data for antioxidants standard peak area and retention time

Standard	Concentration (ug/ml)	Mean peak area	Mean retention time (min)
Gallic acid	1.5625	69391	10.37
	3.125	143363	
	6.25	441072	
	12.25	991285	
	12.50	1033820	
	25.00	2084619	
	50.00	4170648	
Theobromine	0.3125	19336	19.06
	0.625	38113	
	1.25	85486	
	1.85	163914	
	2.50	168253	
	5.00	358854	
	10.00	694049	
EGC	15.5	40606	23.56
	31.0	210808	
	62.0	382630	
	93.0	453695	
	186	1354598	
Caffeine	1.9718	143613	26.85
	3.9437	263180	
	7.8875	562941	
	11.830	1114170	
	15.775	1117822	
	31.55	2282045	
	63.10	4601252	
	94.65	6750158	
	189.30	14576170	
EGCG	12.625	120222	31.56
	25.25	816730	
	50.50	1740096	
	75.75	1782312	
	151.50	5348665	
	336.67	11358432	
ECG	1.593	39201	45.43
	3.187	67721	
	6.375	198359	
	9.56	416260	
	12.75	442760	
	25.50	1117332	
	51	2311741	
	76.5	2824388	
	153	6697546	

Table F.5 HPLC data of sugar concentrations in green tea water kefir beverage (WK2) during fermentation (144 h) and storage period of two weeks (4±1°C) (raw data of phase III part I)

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	2957270	3.774	1565258	1.228	768688	0.551
1	1	1	2598114	3.304	2149700	1.716	797557	0.573
1	1	2	2899685	3.526	1494771	1.102	873515	0.599
1	1	2	2917402	3.548	1413352	1.043	918632	0.629
1	2	1	1964567	2.474	1884332	1.495	1607489	1.179
1	2	1	2076160	1.53	2506360	2.014	2148053	1.584
1	2	2	368671	0.449	1836390	1.349	2496730	1.67
1	2	2	304911	0.372	1824834	1.341	2522363	1.687
1	3	1	nd	0	2205149	1.763	2844667	2.105
1	3	1	nd	0	2200343	1.759	2903014	2.149
1	3	2	nd	0	1644623	1.211	2582550	1.726
1	3	2	nd	0	1620268	1.193	2563720	1.714
1	4	1	nd	0	1851213	1.467	2389374	1.764
1	4	1	nd	0	1910621	1.517	2586496	1.912
1	4	2	nd	0	1533420	1.13	2489846	1.665
1	4	2	nd	0	1565597	1.153	2481655	1.66
1	5	1	nd	0	2147044	1.714	2786254	2.061
1	5	1	nd	0	1822413	1.443	2479120	1.831
1	5	2	nd	0	1481286	1.092	2380448	1.593
1	5	2	nd	0	1526449	1.125	2410017	1.612
1	6	1	nd	0	1995534	1.588	2496073	1.844
1	6	1	nd	0	1640920	1.292	2250983	1.661
1	6	2	nd	0	1438758	1.062	2322692	1.555
1	6	2	nd	0	1482946	1.094	2359100	1.579
1	7	1	nd	0	1882634	1.493	2318409	1.711
1	7	1	nd	0	1533961	1.202	2076160	1.53
1	7	2	nd	0	1394664	1.03	2266807	1.518
1	7	2	nd	0	1404600	1.037	2290183	1.533
1	8	1	nd	0	1404089	1.094	1950073	1.436
1	8	1	nd	0	1618536	1.273	2133135	1.573
1	8	2	nd	0	1319092	0.975	2219846	1.487
1	8	2	nd	0	1280299	0.947	2157730	1.446
1	9	1	nd	0	1250704	0.925	2128856	1.427
1	9	1	nd	0	1375175	1.016	2282137	1.528
1	9	2	nd	0	1238927	0.917	2144303	1.437
1	9	2	nd	0	1239412	0.917	2133814	1.43

Notes: Sample code WK2 = 4%/22°C; Fermentation time: 1=0 h, 2=24 h, 3=48 h,4=72 h, 5=96 h, 6=120 h, 7=144 h; Storage time: 7 = St. D-0, 8 = St. D-7, 9 = St. D-14; St. D= storage day; d= day; nd= not detected

Table F.6 HPLC data of organic acids concentrations in green tea water kefir beverage (WK2) during fermentation (144 h) and storage period of two weeks (4±1°C) (raw data of phase III part I)

Sample code	Fermentation time	Replication	Lactic acid		Acetic acid	
			Peak area	Concentration (% w/v)	Peak area	Concentration (% w/v)
WK2	1	1	nd	0	nd	nd
WK2	1	1	nd	0	nd	nd
WK2	1	2	nd	0	nd	nd
WK2	1	2	nd	0	nd	nd
WK2	2	1	nd	0	nd	nd
WK2	2	1	nd	0	nd	nd
WK2	2	2	nd	0	nd	nd
WK2	2	2	nd	0	nd	nd
WK2	3	1	nd	0	nd	nd
WK2	3	1	128044	0.181	nd	nd
WK2	3	2	128044	0.181	nd	nd
WK2	3	2	128040	0.179	nd	nd
WK2	4	1	128044	0.181	nd	nd
WK2	4	1	427549	0.595	nd	nd
WK2	4	2	427549	0.595	nd	nd
WK2	4	2	432451	0.601	nd	nd
WK2	5	1	427549	0.595	nd	nd
WK2	5	1	435411	0.606	nd	nd
WK2	5	2	434094	0.604	nd	nd
WK2	5	2	441108	0.613	nd	nd
WK2	6	1	435411	0.606	nd	nd
WK2	6	1	441108	0.613	nd	nd
WK2	6	2	440234	0.612	nd	nd
WK2	6	2	450246	0.626	nd	nd
WK2	7	1	444901	0.619	nd	nd
WK2	7	1	450035	0.626	nd	nd
WK2	7	2	450035	0.626	nd	nd
WK2	7	2	452466	0.629	nd	nd
WK2	8	1	446090	0.62	nd	nd
WK2	8	1	452033	0.628	nd	nd
WK2	8	2	459494	0.639	nd	nd
WK2	8	2	453697	0.631	nd	nd
WK2	9	1	448494	0.624	nd	nd
WK2	9	1	443203	0.616	nd	nd
WK2	9	2	462191	0.643	nd	nd
WK2	9	2	448025	0.623	nd	nd

Notes: Sample code WK2 = 4%/22°C; Fermentation time: 1=0 h, 2=24 h, 3=48 h, 4=72 h, 5=96 h, 6=120 h, 7=144 h; Storage time: 7 = St. D-0, 8 = St. D-7, 9 = St. D-14; St. D= storage day; d= day; nd= not detected

Table F.7 HPLC data of organic acids concentrations in green tea water kefir beverage (WK2) during end of fermentation (144 h) and end of storage period (St. D-14) (raw data of phase III part I)

Sample code	Fermentation time (d)	Replication	Gallic acid		Theobromine		EGC	
			Peak area	Concentration (ug/ml)	Peak area	Concentration (ug/ml)	Peak area	Concentration (ug/ml)
WK2	1	1	256525	3.855	281717	4.056	489758	77.677
WK2	1	1	265844	3.965	287297	4.136	552292	86.226
WK2	1	2	293800	4.293	278712	4.013	504598	79.706
WK2	1	2	288253	4.227	280773	4.042	509374	80.358
WK2	2	1	255079	3.838	291527	4.196	600726	92.848
WK2	2	1	246805	3.741	295504	4.253	591092	91.531
WK2	2	2	272998	4.049	298471	4.295	527374	82.819
WK2	2	2	262448	3.925	295166	4.248	572063	88.929
Sample code	Fermentation time (d)	Replication	Caffeine		EGCG		ECG	
			Peak area	Concentration (ug/ml)	Peak area	Concentration (ug/ml)	Peak area	Concentration (ug/ml)
WK2	1	1	5409758	72.247	4886553	144.35	1348886	32.857
WK2	1	1	5320325	71.077	3232740	98.425	592289	15.421
WK2	1	2	5183227	69.283	3468493	104.97	819374	20.648
WK2	1	2	5215518	69.706	4173389	124.55	982702	24.414
WK2	2	1	5597933	74.708	4423627	131.5	1224557	29.990
WK2	2	1	5500095	73.428	3656024	110.18	918289	22.929
WK2	2	2	5626494	75.081	3728807	112.2	877430	21.987
WK2	2	2	5586101	74.553	4635721	137.39	1129568	27.800

Notes: Sample code WK2 = 4%/22°C; 1= end of fermentation (144 h/St. D-0), 2= end of storage (St. D-14); St. D= storage day; d= day

G. Statistic output

G.1 Statistical analysis of pH of green tea water kefir beverage during fermentation period.

One-way ANOVA: pH versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time (d)	6	9.4100	1.56834	224.97	0.000
Error	21	0.1464	0.00697		
Total	27	9.5564			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0834951	98.47%	98.03%	97.28%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	5.0475	0.0377	(4.9607, 5.1343)
1	4	4.0125	0.0222	(3.9257, 4.0993)
2	4	3.5900	0.0535	(3.5032, 3.6768)
3	4	3.3975	0.1184	(3.3107, 3.4843)
4	4	3.3950	0.1103	(3.3082, 3.4818)
5	4	3.3525	0.0995	(3.2657, 3.4393)
6	4	3.3400	0.0891	(3.2532, 3.4268)

Pooled StDev = 0.0834951

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
0	4	5.0475	A
1	4	4.0125	B
2	4	3.5900	C
3	4	3.3975	D
4	4	3.3950	D
5	4	3.3525	D
6	4	3.3400	D

Means that do not share a letter are significantly different.

One-way ANOVA: pH versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time (d)	6	7.60054	1.26676	3941.02	0.000
Error	21	0.00675	0.00032		
Total	27	7.60729			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0179284	99.91%	99.89%	99.84%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	5.0500	0.0424	(5.0314, 5.0686)
1	4	4.10750	0.00957	(4.08886, 4.12614)
2	4	3.72000	0.01414	(3.70136, 3.73864)
3	4	3.62000	0.00816	(3.60136, 3.63864)
4	4	3.540	0.000	(3.521, 3.559)
5	4	3.520	0.000	(3.501, 3.539)
6	4	3.50250	0.00957	(3.48386, 3.52114)

Pooled StDev = 0.0179284

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
0	4	5.0500	A
1	4	4.10750	B
2	4	3.72000	C
3	4	3.62000	D
4	4	3.540	E
5	4	3.520	E
6	4	3.50250	E

Means that do not share a letter are significantly different.

One-way ANOVA: pH versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time (d)	6	8.6182	1.43637	187.50	0.000
Error	21	0.1609	0.00766		
Total	27	8.7791			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0875255	98.17%	97.64%	96.74%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	4.9625	0.1480	(4.8715, 5.0535)
1	4	3.8950	0.0465	(3.8040, 3.9860)
2	4	3.5425	0.1031	(3.4515, 3.6335)
3	4	3.3925	0.0741	(3.3015, 3.4835)
4	4	3.3575	0.0709	(3.2665, 3.4485)
5	4	3.3400	0.0735	(3.2490, 3.4310)
6	4	3.3025	0.0550	(3.2115, 3.3935)

Pooled StDev = 0.0875255

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
0	4	4.9625	A
1	4	3.8950	B
2	4	3.5425	C
3	4	3.3925	C D
4	4	3.3575	C D
5	4	3.3400	D
6	4	3.3025	D

Means that do not share a letter are significantly different.

One-way ANOVA: pH versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time (d)	6	7.88044	1.31341	383.21	0.000
Error	21	0.07197	0.00343		
Total	27	7.95241			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0585438	99.09%	98.84%	98.39%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	4.9600	0.1506	(4.8991, 5.0209)
1	4	3.9650	0.0238	(3.9041, 4.0259)
2	4	3.63250	0.00957	(3.57163, 3.69337)
3	4	3.46500	0.00577	(3.40413, 3.52587)
4	4	3.42000	0.01826	(3.35913, 3.48087)
5	4	3.40500	0.01000	(3.34413, 3.46587)
6	4	3.38000	0.01414	(3.31913, 3.44087)

Pooled StDev = 0.0585438

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
0	4	4.9600	A
1	4	3.9650	B
2	4	3.63250	C
3	4	3.46500	D
4	4	3.42000	D
5	4	3.40500	D
6	4	3.38000	D

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (pH) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	8.3703	1.39506	191.32	0.000
Error	21	0.1531	0.00729		
Total	27	8.5235			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0853913	98.20%	97.69%	96.81%

Means

Factor	N	Mean	StDev	95% CI
0	4	5.0025	0.0492	(4.9137, 5.0913)
1	4	3.9900	0.0938	(3.9012, 4.0788)
2	4	3.6200	0.0762	(3.5312, 3.7088)
3	4	3.4650	0.1085	(3.3762, 3.5538)
4	4	3.4025	0.0978	(3.3137, 3.4913)
5	4	3.4175	0.0723	(3.3287, 3.5063)
6	4	3.3800	0.0864	(3.2912, 3.4688)

Pooled StDev = 0.0853913

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
0	4	5.0025	A
1	4	3.9900	B
2	4	3.6200	C
3	4	3.4650	C D
5	4	3.4175	D
4	4	3.4025	D
6	4	3.3800	D

Means that do not share a letter are significantly different.

G.2 Statistical analysis of T.A. of green tea water kefir beverage during fermentation period.

One-way ANOVA: T.A. versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.08488	0.014147	17.47	0.000
Error	21	0.01700	0.000810		
Total	27	0.10189			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0284557	83.31%	78.54%	70.33%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.03600	0.00400	(0.00641, 0.06559)
1	4	0.06350	0.00520	(0.03391, 0.09309)
2	4	0.11675	0.01239	(0.08716, 0.14634)
3	4	0.1663	0.0293	(0.1367, 0.1958)
4	4	0.1687	0.0349	(0.1392, 0.1983)
5	4	0.1765	0.0417	(0.1469, 0.2061)
6	4	0.1830	0.0406	(0.1534, 0.2126)

Pooled StDev = 0.0284557

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.1830	A
5	4	0.1765	A B
4	4	0.1687	A B
3	4	0.1663	A B
2	4	0.11675	B C
1	4	0.06350	C D
0	4	0.03600	D

Means that do not share a letter are significantly different.

One-way ANOVA: T.A. versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.067310	0.011218	107.26	0.000
Error	21	0.002197	0.000105		
Total	27	0.069507			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0102272	96.84%	95.94%	94.38%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.03400	0.00000	(0.02337, 0.04463)
1	4	0.06800	0.00000	(0.05737, 0.07863)
2	4	0.11050	0.01515	(0.09987, 0.12113)
3	4	0.13625	0.00695	(0.12562, 0.14688)
4	4	0.15550	0.01047	(0.14487, 0.16613)
5	4	0.16150	0.01515	(0.15087, 0.17213)
6	4	0.17825	0.01072	(0.16762, 0.18888)

Pooled StDev = 0.0102272

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.17825	A
5	4	0.16150	A
4	4	0.15550	A B
3	4	0.13625	B
2	4	0.11050	C
1	4	0.06800	D
0	4	0.03400	E

Means that do not share a letter are significantly different.

One-way ANOVA: T.A. versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.13887	0.023146	21.87	0.000
Error	21	0.02223	0.001059		
Total	27	0.16110			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0325355	86.20%	82.26%	75.47%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.03400	0.00000	(0.00017, 0.06783)
1	4	0.07400	0.00400	(0.04017, 0.10783)
2	4	0.1445	0.0208	(0.1107, 0.1783)
3	4	0.1875	0.0390	(0.1537, 0.2213)
4	4	0.2000	0.0317	(0.1662, 0.2338)
5	4	0.2255	0.0543	(0.1917, 0.2593)
6	4	0.2282	0.0386	(0.1944, 0.2621)

Pooled StDev = 0.0325355

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.2282	A
5	4	0.2255	A
4	4	0.2000	A B
3	4	0.1875	A B
2	4	0.1445	B C
1	4	0.07400	C D
0	4	0.03400	D

Means that do not share a letter are significantly different.

One-way ANOVA: T.A. versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.082589	0.013765	111.93	0.000
Error	21	0.002582	0.000123		
Total	27	0.085172			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0110895	96.97%	96.10%	94.61%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.03400	0.00000	(0.02247, 0.04553)
1	4	0.07250	0.00520	(0.06097, 0.08403)
2	4	0.11950	0.01589	(0.10797, 0.13103)
3	4	0.15275	0.01552	(0.14122, 0.16428)
4	4	0.17225	0.01053	(0.16072, 0.18378)
5	4	0.17400	0.00462	(0.16247, 0.18553)
6	4	0.19150	0.01443	(0.17997, 0.20303)

Pooled StDev = 0.0110895

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.19150	A
5	4	0.17400	A B
4	4	0.17225	A B
3	4	0.15275	B
2	4	0.11950	C
1	4	0.07250	D
0	4	0.03400	E

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (T.A.) versus Fermentation time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	0.091037	0.015173	44.68	0.000
Error	21	0.007132	0.000340		
Total	27	0.098169			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0184288	92.74%	90.66%	87.08%

Means

Factor	N	Mean	StDev	95% CI
0	4	0.034500	0.001000	(0.015338, 0.053662)
1	4	0.06925	0.00486	(0.05009, 0.08841)
2	4	0.12175	0.01515	(0.10259, 0.14091)
3	4	0.1608	0.0219	(0.1416, 0.1799)
4	4	0.17375	0.01839	(0.15459, 0.19291)
5	4	0.1840	0.0281	(0.1648, 0.2032)
6	4	0.1950	0.0226	(0.1758, 0.2142)

Pooled StDev = 0.0184288

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
6	4	0.1950	A
5	4	0.1840	A
4	4	0.17375	A
3	4	0.1608	A B
2	4	0.12175	B
1	4	0.06925	C
0	4	0.034500	C

Means that do not share a letter are significantly different.

G.3 Statistical analysis of total soluble solids (°Brix) of green tea water kefir beverage during fermentation period.

One-way ANOVA: TSS versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	3.1471	0.524524	107.46	0.000
Error	21	0.1025	0.004881		
Total	27	3.2496			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0698638	96.85%	95.94%	94.39%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	3.5500	0.0577	(3.4774, 3.6226)
1	4	3.600	0.000	(3.527, 3.673)
2	4	3.2250	0.0500	(3.1524, 3.2976)
3	4	2.8750	0.0957	(2.8024, 2.9476)
4	4	2.9000	0.0816	(2.8274, 2.9726)
5	4	2.7750	0.0957	(2.7024, 2.8476)
6	4	2.7500	0.0577	(2.6774, 2.8226)

Pooled StDev = 0.0698638

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	3.600	A
0	4	3.5500	A
2	4	3.2250	B
4	4	2.9000	C
3	4	2.8750	C
5	4	2.7750	C
6	4	2.7500	C

Means that do not share a letter are significantly different.

One-way ANOVA: TSS versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	1.89714	0.316190	204.31	0.000
Error	21	0.03250	0.001548		
Total	27	1.92964			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0393398	98.32%	97.83%	97.01%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	4.4750	0.0500	(4.4341, 4.5159)
1	4	4.500	0.000	(4.459, 4.541)
2	4	4.300	0.000	(4.259, 4.341)
3	4	4.100	0.000	(4.059, 4.141)
4	4	3.9500	0.0577	(3.9091, 3.9909)
5	4	3.8750	0.0500	(3.8341, 3.9159)
6	4	3.8250	0.0500	(3.7841, 3.8659)

Pooled StDev = 0.0393398

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	4.500	A
0	4	4.4750	A
2	4	4.300	B
3	4	4.100	C
4	4	3.9500	D
5	4	3.8750	D E
6	4	3.8250	E

Means that do not share a letter are significantly different.

One-way ANOVA: TSS versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	5.6371	0.939524	148.91	0.000
Error	21	0.1325	0.006310		
Total	27	5.7696			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0794325	97.70%	97.05%	95.92%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	3.5250	0.0500	(3.4424, 3.6076)
1	4	3.5500	0.0577	(3.4674, 3.6326)
2	4	3.200	0.000	(3.117, 3.283)
3	4	2.7750	0.0500	(2.6924, 2.8576)
4	4	2.7500	0.0577	(2.6674, 2.8326)
5	4	2.4750	0.1500	(2.3924, 2.5576)
6	4	2.3500	0.1000	(2.2674, 2.4326)

Pooled StDev = 0.0794325

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	3.5500	A
0	4	3.5250	A
2	4	3.200	B
3	4	2.7750	C
4	4	2.7500	C
5	4	2.4750	D
6	4	2.3500	D

Means that do not share a letter are significantly different.

One-way ANOVA: TSS versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	5.1771	0.862857	106.59	0.000
Error	21	0.1700	0.008095		
Total	27	5.3471			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0899735	96.82%	95.91%	94.35%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	4.4500	0.0577	(4.3564, 4.5436)
1	4	4.4750	0.0500	(4.3814, 4.5686)
2	4	4.1250	0.0500	(4.0314, 4.2186)
3	4	3.6500	0.1291	(3.5564, 3.7436)
4	4	3.6250	0.0957	(3.5314, 3.7186)
5	4	3.4250	0.0957	(3.3314, 3.5186)
6	4	3.4000	0.1155	(3.3064, 3.4936)

Pooled StDev = 0.0899735

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	4.4750	A
0	4	4.4500	A
2	4	4.1250	B
3	4	3.6500	C
4	4	3.6250	C D
5	4	3.4250	D E
6	4	3.4000	E

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (TSS) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1, WK2, WK3, WK4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	7.227	2.4090	14.49	0.000
Error	24	3.990	0.1663		
Total	27	11.217			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.407751	64.43%	59.98%	51.58%

Means

Sample code	N	Mean	StDev	95% CI
WK1	7	3.094	0.363	(2.776, 3.412)
WK2	7	4.147	0.288	(3.829, 4.465)
WK3	7	2.944	0.485	(2.626, 3.262)
WK4	7	3.876	0.464	(3.558, 4.194)

Pooled StDev = 0.407751

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2	7	4.147	A
WK4	7	3.876	A
WK1	7	3.094	B
WK3	7	2.944	B

Means that do not share a letter are significantly different.

Notes: WK1 = 3%/22°C, WK2 = 4%/22°C, WK3 = 3%/25°C, WK4 = 4%/25°C

G.4 Statistical analysis of colour (L*) of green tea water kefir beverage during fermentation period.

One-way ANOVA: Colour (L*) versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	2.627	0.4379	0.68	0.670
Error	21	13.586	0.6470		
Total	27	16.214			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.804345	16.20%	0.00%	0.00%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	96.505	1.332	(95.669, 97.341)
1	4	96.600	1.320	(95.764, 97.436)
2	4	96.7800	0.0753	(95.9436, 97.6164)
3	4	96.965	0.346	(96.129, 97.801)
4	4	97.1075	0.1666	(96.2711, 97.9439)
5	4	97.190	0.830	(96.354, 98.026)
6	4	97.425	0.415	(96.589, 98.261)

Pooled StDev = 0.804345

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	97.425	A
5	4	97.190	A
4	4	97.1075	A
3	4	96.965	A
2	4	96.7800	A
1	4	96.600	A
0	4	96.505	A

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (L*) versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	12.509	2.0848	6.53	0.001
Error	21	6.707	0.3194		
Total	27	19.215			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.565119	65.10%	55.13%	37.95%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	95.882	0.738	(95.295, 96.470)
1	4	96.202	0.735	(95.615, 96.790)
2	4	96.025	0.587	(95.437, 96.613)
3	4	96.7275	0.1879	(96.1399, 97.3151)
4	4	96.442	0.673	(95.855, 97.030)
5	4	97.257	0.558	(96.670, 97.845)
6	4	97.8875	0.0789	(97.2999, 98.4751)

Pooled StDev = 0.565119

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	97.8875	A
5	4	97.257	A B
3	4	96.7275	A B C
4	4	96.442	B C
1	4	96.202	B C
2	4	96.025	B C
0	4	95.882	C

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (L*) versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	1.647	0.2745	2.19	0.085
Error	21	2.636	0.1255		
Total	27	4.283			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.354295	38.45%	20.87%	0.00%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	96.775	0.618	(96.407, 97.143)
1	4	96.412	0.432	(96.044, 96.781)
2	4	96.470	0.206	(96.102, 96.838)
3	4	96.555	0.369	(96.187, 96.923)
4	4	96.657	0.220	(96.289, 97.026)
5	4	96.7025	0.0885	(96.3341, 97.0709)
6	4	97.200	0.273	(96.832, 97.568)

Pooled StDev = 0.354295

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	97.200	A
0	4	96.775	A
5	4	96.7025	A
4	4	96.657	A
3	4	96.555	A
2	4	96.470	A
1	4	96.412	A

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (L*) versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	15.447	2.5746	18.10	0.000
Error	21	2.987	0.1422		
Total	27	18.434			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.377119	83.80%	79.17%	71.20%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	96.532	0.289	(96.140, 96.925)
1	4	96.368	0.397	(95.975, 96.760)
2	4	96.170	0.244	(95.778, 96.562)
3	4	96.627	0.387	(96.235, 97.020)
4	4	96.895	0.734	(96.503, 97.287)
5	4	97.4400	0.0497	(97.0479, 97.8321)
6	4	98.4975	0.0634	(98.1054, 98.8896)

Pooled StDev = 0.377119

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	98.4975	A
5	4	97.4400	B
4	4	96.895	B C
3	4	96.627	B C
0	4	96.532	C
1	4	96.368	C
2	4	96.170	C

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (Colour L*) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	6.116	1.0193	8.66	0.000
Error	21	2.471	0.1177		
Total	27	8.587			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.343034	71.22%	63.00%	48.84%

Means

Factor	N	Mean	StDev	95% CI
0	4	96.420	0.380	(96.063, 96.777)
1	4	96.3925	0.1648	(96.0358, 96.7492)
2	4	96.360	0.337	(96.003, 96.717)
3	4	96.7125	0.1791	(96.3558, 97.0692)
4	4	96.770	0.287	(96.413, 97.127)
5	4	97.145	0.315	(96.788, 97.502)
6	4	97.748	0.570	(97.391, 98.104)

Pooled StDev = 0.343034

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
6	4	97.748	A
5	4	97.145	A B
4	4	96.770	B
3	4	96.7125	B
0	4	96.420	B
1	4	96.3925	B
2	4	96.360	B

Means that do not share a letter are significantly different.

G.5 Statistical analysis of colour (a*) of green tea water kefir beverage during fermentation period.

One-way ANOVA: Colour (a*) versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	1.3401	0.22335	12.83	0.000
Error	21	0.3655	0.01741		
Total	27	1.7056			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.131932	78.57%	72.45%	61.90%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.3925	0.0763	(0.2553, 0.5297)
1	4	0.4100	0.0374	(0.2728, 0.5472)
2	4	0.4250	0.0526	(0.2878, 0.5622)
3	4	0.4475	0.0299	(0.3103, 0.5847)
4	4	0.732	0.228	(0.595, 0.870)
5	4	0.780	0.201	(0.643, 0.917)
6	4	0.9900	0.1371	(0.8528, 1.1272)

Pooled StDev = 0.131932

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.9900	A
5	4	0.780	A
4	4	0.732	A B
3	4	0.4475	B C
2	4	0.4250	C
1	4	0.4100	C
0	4	0.3925	C

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (a*) versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	1.9515	0.32526	22.48	0.000
Error	21	0.3039	0.01447		
Total	27	2.2554			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.120297	86.53%	82.68%	76.05%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.2350	0.0387	(0.1099, 0.3601)
1	4	0.4100	0.0490	(0.2849, 0.5351)
2	4	0.5150	0.0755	(0.3899, 0.6401)
3	4	0.6300	0.1556	(0.5049, 0.7551)
4	4	0.8800	0.0632	(0.7549, 1.0051)
5	4	0.910	0.210	(0.785, 1.035)
6	4	0.9950	0.1396	(0.8699, 1.1201)

Pooled StDev = 0.120297

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.9950	A
5	4	0.910	A
4	4	0.8800	A B
3	4	0.6300	B C
2	4	0.5150	C
1	4	0.4100	C D
0	4	0.2350	D

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (a*) versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	8.5223	1.42039	49.27	0.000
Error	21	0.6054	0.02883		
Total	27	9.1277			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.169786	93.37%	91.47%	88.21%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.2600	0.0294	(0.0835, 0.4365)
1	4	0.2850	0.0733	(0.1085, 0.4615)
2	4	0.2850	0.0926	(0.1085, 0.4615)
3	4	0.7025	0.1352	(0.5260, 0.8790)
4	4	0.7700	0.0469	(0.5935, 0.9465)
5	4	1.480	0.360	(1.303, 1.657)
6	4	1.7150	0.1930	(1.5385, 1.8915)

Pooled StDev = 0.169786

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	1.7150	A
5	4	1.480	A
4	4	0.7700	B
3	4	0.7025	B
1	4	0.2850	C
2	4	0.2850	C
0	4	0.2600	C

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (a*) versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	4.3450	0.72416	23.18	0.000
Error	21	0.6559	0.03123		
Total	27	5.0009			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.176733	86.88%	83.14%	76.68%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.3425	0.0340	(0.1587, 0.5263)
1	4	0.3600	0.1086	(0.1762, 0.5438)
2	4	0.4600	0.0432	(0.2762, 0.6438)
3	4	0.6375	0.0896	(0.4537, 0.8213)
4	4	0.905	0.297	(0.721, 1.089)
5	4	1.200	0.230	(1.016, 1.384)
6	4	1.417	0.233	(1.234, 1.601)

Pooled StDev = 0.176733

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	1.417	A
5	4	1.200	A B
4	4	0.905	B C
3	4	0.6375	C D
2	4	0.4600	D
1	4	0.3600	D
0	4	0.3425	D

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (Colour a*) versus Fermentation time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	3.4282	0.57137	15.44	0.000
Error	21	0.7772	0.03701		
Total	27	4.2054			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.192385	81.52%	76.24%	67.14%

Means

Factor	N	Mean	StDev	95% CI
0	4	0.3050	0.0733	(0.1050, 0.5050)
1	4	0.3650	0.0614	(0.1650, 0.5650)
2	4	0.4175	0.0988	(0.2175, 0.6175)
3	4	0.6000	0.1117	(0.4000, 0.8000)
4	4	0.8200	0.0829	(0.6200, 1.0200)
5	4	1.093	0.312	(0.892, 1.293)
6	4	1.275	0.351	(1.075, 1.475)

Pooled StDev = 0.192385

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
6	4	1.275	A
5	4	1.093	A B
4	4	0.8200	B C
3	4	0.6000	C D
2	4	0.4175	C D
1	4	0.3650	D
0	4	0.3050	D

Means that do not share a letter are significantly different.

G.6 Statistical analysis of colour (b*) of green tea water kefir beverage during fermentation period.

One-way ANOVA: Colour (b*) versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.8837	0.14729	2.86	0.034
Error	21	1.0805	0.05145		
Total	27	1.9643			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.226834	44.99%	29.27%	2.21%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	1.6225	0.1997	(1.3866, 1.8584)
1	4	1.630	0.216	(1.394, 1.866)
2	4	2.133	0.427	(1.897, 2.368)
3	4	1.820	0.292	(1.584, 2.056)
4	4	1.7625	0.0640	(1.5266, 1.9984)
5	4	1.9750	0.0208	(1.7391, 2.2109)
6	4	1.9800	0.0432	(1.7441, 2.2159)

Pooled StDev = 0.226834

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
2	4	2.133	A
6	4	1.9800	A
5	4	1.9750	A
3	4	1.820	A
4	4	1.7625	A
1	4	1.630	A
0	4	1.6225	A

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (b*) versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	15.7816	2.63027	100.50	0.000
Error	21	0.5496	0.02617		
Total	27	16.3312			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.161776	96.63%	95.67%	94.02%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	1.147	0.397	(0.979, 1.316)
1	4	3.1075	0.0287	(2.9393, 3.2757)
2	4	3.6500	0.0535	(3.4818, 3.8182)
3	4	2.7375	0.0650	(2.5693, 2.9057)
4	4	2.2250	0.0933	(2.0568, 2.3932)
5	4	2.4400	0.0891	(2.2718, 2.6082)
6	4	3.1475	0.0340	(2.9793, 3.3157)

Pooled StDev = 0.161776

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
2	4	3.6500	A
6	4	3.1475	B
1	4	3.1075	B C
3	4	2.7375	C D
5	4	2.4400	D E
4	4	2.2250	E
0	4	1.147	F

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (b*) versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	12.930	2.15492	45.08	0.000
Error	21	1.004	0.04781		
Total	27	13.933			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.218648	92.79%	90.74%	87.19%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	1.0150	0.1576	(0.7876, 1.2424)
1	4	3.065	0.353	(2.838, 3.292)
2	4	2.735	0.325	(2.508, 2.962)
3	4	2.0600	0.1262	(1.8326, 2.2874)
4	4	1.4825	0.1078	(1.2551, 1.7099)
5	4	1.455	0.226	(1.228, 1.682)
6	4	1.7975	0.0330	(1.5701, 2.0249)

Pooled StDev = 0.218648

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	3.065	A
2	4	2.735	A
3	4	2.0600	B
6	4	1.7975	B C
4	4	1.4825	C D
5	4	1.455	C D
0	4	1.0150	D

Means that do not share a letter are significantly different.

One-way ANOVA: Colour (b*) versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	19.6844	3.28074	239.57	0.000
Error	21	0.2876	0.01369		
Total	27	19.9720			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.117022	98.56%	98.15%	97.44%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	1.0475	0.1480	(0.9258, 1.1692)
1	4	3.935	0.247	(3.813, 4.057)
2	4	3.4200	0.0716	(3.2983, 3.5417)
3	4	2.5700	0.0432	(2.4483, 2.6917)
4	4	3.0050	0.0443	(2.8833, 3.1267)
5	4	3.0750	0.0580	(2.9533, 3.1967)
6	4	2.7000	0.0216	(2.5783, 2.8217)

Pooled StDev = 0.117022

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	3.935	A
2	4	3.4200	B
5	4	3.0750	C
4	4	3.0050	C
6	4	2.7000	D
3	4	2.5700	D
0	4	1.0475	E

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (Colour b*) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	8.466	1.4110	3.33	0.018
Error	21	8.901	0.4238		
Total	27	17.367			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.651033	48.75%	34.10%	8.89%

Means

Factor	N	Mean	StDev	95% CI
0	4	1.202	0.284	(0.526, 1.879)
1	4	2.930	0.955	(2.253, 3.607)
2	4	2.982	0.690	(2.306, 3.659)
3	4	2.295	0.426	(1.618, 2.972)
4	4	2.115	0.664	(1.438, 2.792)
5	4	2.232	0.689	(1.556, 2.909)
6	4	2.405	0.633	(1.728, 3.082)

Pooled StDev = 0.651033

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
2	4	2.982	A
1	4	2.930	A
6	4	2.405	A B
3	4	2.295	A B
5	4	2.232	A B
4	4	2.115	A B
0	4	1.202	B

Means that do not share a letter are significantly different.

G.7 Statistical analysis of viable cell counts of LAB in green tea water kefir beverage during fermentation period.

One-way ANOVA: LAB (log cfu/ml) versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	9.0046	1.50076	89.66	0.000
Error	21	0.3515	0.01674		
Total	27	9.3561			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.129380	96.24%	95.17%	93.32%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.87750	0.01500	(6.74297, 7.01203)
1	4	7.6125	0.1694	(7.4780, 7.7470)
2	4	8.0250	0.1338	(7.8905, 8.1595)
3	4	8.2550	0.0759	(8.1205, 8.3895)
4	4	8.377	0.218	(8.243, 8.512)
5	4	8.5750	0.1229	(8.4405, 8.7095)
6	4	8.5600	0.0424	(8.4255, 8.6945)

Pooled StDev = 0.129380

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
5	4	8.5750	A
6	4	8.5600	A
4	4	8.377	A B
3	4	8.2550	B C
2	4	8.0250	C
1	4	7.6125	D
0	4	6.87750	E

Means that do not share a letter are significantly different.

One-way ANOVA: LAB (log cfu/ml) versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	8.54580	1.42430	854.12	0.000
Error	21	0.03502	0.00167		
Total	27	8.58082			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0408358	99.59%	99.48%	99.27%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.8825	0.0574	(6.8400, 6.9250)
1	4	7.6675	0.0350	(7.6250, 7.7100)
2	4	7.9925	0.0660	(7.9500, 8.0350)
3	4	8.2950	0.0208	(8.2525, 8.3375)
4	4	8.4288	0.0421	(8.3863, 8.4712)
5	4	8.51250	0.01708	(8.47004, 8.55496)
6	4	8.50500	0.01732	(8.46254, 8.54746)

Pooled StDev = 0.0408358

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
5	4	8.51250	A
6	4	8.50500	A
4	4	8.4288	A
3	4	8.2950	B
2	4	7.9925	C
1	4	7.6675	D
0	4	6.8825	E

Means that do not share a letter are significantly different.

One-way ANOVA: LAB (log cfu/ml) versus Fermentation time (d) of sample WK3 (3/25°C)

One-way ANOVA: LAB (log cfu/ml) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	7.9354	1.32257	95.04	0.000
Error	21	0.2923	0.01392		
Total	27	8.2277			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.117969	96.45%	95.43%	93.69%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	7.0700	0.0648	(6.9473, 7.1927)
1	4	7.8425	0.0618	(7.7198, 7.9652)
2	4	8.2575	0.0299	(8.1348, 8.3802)
3	4	8.2925	0.1034	(8.1698, 8.4152)
4	4	8.568	0.207	(8.445, 8.690)
5	4	8.6625	0.1486	(8.5398, 8.7852)
6	4	8.6675	0.1127	(8.5448, 8.7902)

Pooled StDev = 0.117969

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	8.6675	A
5	4	8.6625	A
4	4	8.568	A
3	4	8.2925	B
2	4	8.2575	B
1	4	7.8425	C
0	4	7.0700	D

Means that do not share a letter are significantly different.

One-way ANOVA: LAB (log cfu/ml) versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	8.4565	1.40942	81.30	0.000
Error	21	0.3640	0.01734		
Total	27	8.8206			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.131665	95.87%	94.69%	92.66%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.9825	0.1044	(6.8456, 7.1194)
1	4	7.9425	0.0613	(7.8056, 8.0794)
2	4	8.1825	0.1047	(8.0456, 8.3194)
3	4	8.2725	0.0862	(8.1356, 8.4094)
4	4	8.557	0.231	(8.421, 8.694)
5	4	8.5925	0.1672	(8.4556, 8.7294)
6	4	8.7300	0.0829	(8.5931, 8.8669)

Pooled StDev = 0.131665

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	8.7300	A
5	4	8.5925	A
4	4	8.557	A B
3	4	8.2725	B C
2	4	8.1825	C D
1	4	7.9425	D
0	4	6.9825	E

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (LAB) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	8.3074	1.38456	133.97	0.000
Error	21	0.2170	0.01033		
Total	27	8.5244			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.101659	97.45%	96.73%	95.47%

Means

Factor	N	Mean	StDev	95% CI
0	4	6.9525	0.0914	(6.8468, 7.0582)
1	4	7.7625	0.1541	(7.6568, 7.8682)
2	4	8.1100	0.1252	(8.0043, 8.2157)
3	4	8.27500	0.01915	(8.16929, 8.38071)
4	4	8.4700	0.0898	(8.3643, 8.5757)
5	4	8.5650	0.0751	(8.4593, 8.6707)
6	4	8.6125	0.1024	(8.5068, 8.7182)

Pooled StDev = 0.101659

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
6	4	8.6125	A
5	4	8.5650	A
4	4	8.4700	A B
3	4	8.27500	B C
2	4	8.1100	C
1	4	7.7625	D
0	4	6.9525	E

Means that do not share a letter are significantly different.

G.8 Statistical analysis of viable cell counts of yeast in green tea water kefir beverage during fermentation period.

One-way ANOVA: Yeast (log cfu/ml) versus Fermentation time (d) of sample WK1 (3/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	5.6745	0.945757	104.46	0.000
Error	21	0.1901	0.009054		
Total	27	5.8647			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0951503	96.76%	95.83%	94.24%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.0975	0.0419	(5.9986, 6.1964)
1	4	6.1250	0.0806	(6.0261, 6.2239)
2	4	6.6125	0.1297	(6.5136, 6.7114)
3	4	7.2000	0.0698	(7.1011, 7.2989)
4	4	7.1850	0.0507	(7.0861, 7.2839)
5	4	7.1375	0.0802	(7.0386, 7.2364)
6	4	6.9550	0.1563	(6.8561, 7.0539)

Pooled StDev = 0.0951503

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
3	4	7.2000	A
4	4	7.1850	A
5	4	7.1375	A B
6	4	6.9550	B
2	4	6.6125	C
1	4	6.1250	D
0	4	6.0975	D

Means that do not share a letter are significantly different.

One-way ANOVA: Yeast (log cfu/ml) versus Fermentation time (d) of sample WK2 (4/22°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	4.4291	0.738182	151.76	0.000
Error	21	0.1021	0.004864		
Total	27	4.5312			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0697444	97.75%	97.10%	95.99%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.0225	0.0250	(5.9500, 6.0950)
1	4	6.3750	0.0545	(6.3025, 6.4475)
2	4	6.6700	0.0678	(6.5975, 6.7425)
3	4	7.2025	0.0655	(7.1300, 7.2750)
4	4	7.0400	0.0990	(6.9675, 7.1125)
5	4	7.0300	0.1013	(6.9575, 7.1025)
6	4	7.0050	0.0387	(6.9325, 7.0775)

Pooled StDev = 0.0697444

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
3	4	7.2025	A
4	4	7.0400	B
5	4	7.0300	B
6	4	7.0050	B
2	4	6.6700	C
1	4	6.3750	D
0	4	6.0225	E

Means that do not share a letter are significantly different.

One-way ANOVA: Yeast (log cfu/ml) versus Fermentation time (d) of sample WK3 (3/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	5.0123	0.83538	47.93	0.000
Error	21	0.3660	0.01743		
Total	27	5.3783			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.132021	93.19%	91.25%	87.90%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.0725	0.0624	(5.9352, 6.2098)
1	4	6.490	0.204	(6.353, 6.627)
2	4	6.7000	0.1111	(6.5627, 6.8373)
3	4	7.2750	0.1271	(7.1377, 7.4123)
4	4	7.2325	0.1100	(7.0952, 7.3698)
5	4	7.2000	0.1122	(7.0627, 7.3373)
6	4	7.0695	0.1523	(6.9322, 7.2068)

Pooled StDev = 0.132021

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
3	4	7.2750	A
4	4	7.2325	A
5	4	7.2000	A
6	4	7.0695	A
2	4	6.7000	B
1	4	6.490	B
0	4	6.0725	C

Means that do not share a letter are significantly different.

One-way ANOVA: Yeast (log cfu/ml) versus Fermentation time (d) of sample WK4 (4/25°C)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	5.8483	0.974712	142.15	0.000
Error	21	0.1440	0.006857		
Total	27	5.9923			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0828079	97.60%	96.91%	95.73%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	6.0900	0.0294	(6.0039, 6.1761)
1	4	6.1350	0.0957	(6.0489, 6.2211)
2	4	7.1675	0.0680	(7.0814, 7.2536)
3	4	7.2525	0.0556	(7.1664, 7.3386)
4	4	6.7525	0.1228	(6.6664, 6.8386)
5	4	7.1175	0.0386	(7.0314, 7.2036)
6	4	7.0700	0.1169	(6.9839, 7.1561)

Pooled StDev = 0.0828079

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
3	4	7.2525	A
2	4	7.1675	A
5	4	7.1175	A
6	4	7.0700	A
4	4	6.7525	B
1	4	6.1350	C
0	4	6.0900	C

Means that do not share a letter are significantly different.

One-way ANOVA: Sample code (Yeast) versus Fermentation time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	6	5.1783	0.863048	115.64	0.000
Error	21	0.1567	0.007463		
Total	27	5.3350			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0863892	97.06%	96.22%	94.78%

Means

Factor	N	Mean	StDev	95% CI
0	4	6.0600	0.0271	(5.9702, 6.1498)
1	4	6.2775	0.1828	(6.1877, 6.3673)
2	4	6.6800	0.0577	(6.5902, 6.7698)
3	4	7.2275	0.0386	(7.1377, 7.3173)
4	4	7.1525	0.0806	(7.0627, 7.2423)
5	4	7.1200	0.0668	(7.0302, 7.2098)
6	4	7.0250	0.0480	(6.9352, 7.1148)

Pooled StDev = 0.0863892

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
3	4	7.2275	A
4	4	7.1525	A B
5	4	7.1200	A B
6	4	7.0250	B
2	4	6.6800	C
1	4	6.2775	D
0	4	6.0600	E

Means that do not share a letter are significantly different.

G.9 Statistical analysis of sensory evaluation of green tea water kefir beverage during fermentation period.

One-way ANOVA: Appearance versus Sample code

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	30.53	10.178	5.74	0.001
Error	236	418.40	1.773		
Total	239	448.93			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.33150	6.80%	5.62%	3.62%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	6.000	1.302	(5.661, 6.339)
WK2 (4/22)	60	6.567	1.031	(6.228, 6.905)
WK3 (3/25)	60	5.567	1.466	(5.228, 5.905)
WK4 (4/25)	60	6.133	1.478	(5.795, 6.472)

Pooled StDev = 1.33150

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.567	A
WK4 (4/25)	60	6.133	A B
WK1 (3/22)	60	6.000	A B
WK3 (3/25)	60	5.567	B

Means that do not share a letter are significantly different.

One-way ANOVA: Sweetness versus Sample code

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	177.4	59.128	28.49	0.000
Error	236	489.8	2.075		
Total	239	667.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.44063	26.59%	25.65%	24.08%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	5.017	1.642	(4.650, 5.383)
WK2 (4/22)	60	6.6833	0.7700	(6.3169, 7.0497)
WK3 (3/25)	60	4.483	1.672	(4.117, 4.850)
WK4 (4/25)	60	6.050	1.489	(5.684, 6.416)

Pooled StDev = 1.44063

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.6833	A
WK4 (4/25)	60	6.050	A
WK1 (3/22)	60	5.017	B
WK3 (3/25)	60	4.483	B

Means that do not share a letter are significantly different.

One-way ANOVA: Acidity/ Sourness versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	93.08	31.028	14.17	0.000
Error	236	516.90	2.190		
Total	239	609.98			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47995	15.26%	14.18%	12.36%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	5.333	1.548	(4.957, 5.710)
WK2 (4/22)	60	6.517	1.017	(6.140, 6.893)
WK3 (3/25)	60	4.983	1.742	(4.607, 5.360)
WK4 (4/25)	60	6.200	1.516	(5.824, 6.576)

Pooled StDev = 1.47995

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.517	A
WK4 (4/25)	60	6.200	A
WK1 (3/22)	60	5.333	B
WK3 (3/25)	60	4.983	B

Means that do not share a letter are significantly different.

One-way ANOVA: Flavour versus Sample code

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	147.9	49.315	23.58	0.000
Error	236	493.6	2.092		
Total	239	641.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.44624	23.06%	22.08%	20.43%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	4.950	1.845	(4.582, 5.318)
WK2 (4/22)	60	6.667	0.951	(6.299, 7.034)
WK3 (3/25)	60	4.883	1.552	(4.516, 5.251)
WK4 (4/25)	60	6.250	1.284	(5.882, 6.618)

Pooled StDev = 1.44624

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.667	A
WK4 (4/25)	60	6.250	A
WK1 (3/22)	60	4.950	B
WK3 (3/25)	60	4.883	B

Means that do not share a letter are significantly different.

One-way ANOVA: Aroma versus Sample code

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	108.7	36.237	18.45	0.000
Error	236	463.4	1.964		
Total	239	572.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.40135	19.00%	17.97%	16.23%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	5.117	1.698	(4.760, 5.473)
WK2 (4/22)	60	6.700	0.997	(6.344, 7.056)
WK3 (3/25)	60	5.167	1.440	(4.810, 5.523)
WK4 (4/25)	60	6.167	1.380	(5.810, 6.523)

Pooled StDev = 1.40135

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.700	A
WK4 (4/25)	60	6.167	A
WK3 (3/25)	60	5.167	B
WK1 (3/22)	60	5.117	B

Means that do not share a letter are significantly different.

One-way ANOVA: Overall acceptability versus Sample code

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	WK1 (3/22), WK2 (4/22), WK3 (3/25), WK4 (4/25)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	166.5	55.515	29.58	0.000
Error	236	442.9	1.877		
Total	239	609.5			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.36995	27.33%	26.40%	24.84%

Means

Sample code	N	Mean	StDev	95% CI
WK1 (3/22)	60	5.033	1.646	(4.685, 5.382)
WK2 (4/22)	60	6.900	0.838	(6.552, 7.248)
WK3 (3/25)	60	4.900	1.548	(4.552, 5.248)
WK4 (4/25)	60	6.217	1.303	(5.868, 6.565)

Pooled StDev = 1.36995

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
WK2 (4/22)	60	6.900	A
WK4 (4/25)	60	6.217	B
WK1 (3/22)	60	5.033	C
WK3 (3/25)	60	4.900	C

Means that do not share a letter are significantly different.

G.10 Statistical analysis of ethanol content of green tea water kefir beverage WK2 (4%/22°C) during 144 h of fermentation.

One-way ANOVA: Ethanol versus Fermentation time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.7266	0.121106	13.10	0.000
Error	21	0.1941	0.009242		
Total	27	0.9207			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0961350	78.92%	72.90%	62.53%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.000000	0.000000	(-0.099962, 0.099962)
1	4	0.2323	0.1918	(0.1323, 0.3322)
2	4	0.2675	0.0436	(0.1675, 0.3675)
3	4	0.3677	0.0516	(0.2678, 0.4677)
4	4	0.3522	0.0437	(0.2523, 0.4522)
5	4	0.4633	0.1242	(0.3633, 0.5632)
6	4	0.5323	0.0777	(0.4323, 0.6322)

Pooled StDev = 0.0961350

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping		
6	4	0.5323	A		
5	4	0.4633	A	B	
3	4	0.3677	A	B	C
4	4	0.3522	A	B	C
2	4	0.2675		B	C
1	4	0.2323			C
0	4	0.000000			D

Means that do not share a letter are significantly different.

G.11 Statistical analysis of ethanol content of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: Ethanol versus Storage time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.04036	0.020179	4.51	0.044
Error	9	0.04027	0.004475		
Total	11	0.08063			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0668936	50.05%	38.95%	11.21%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	0.5323	0.0777	(0.4566, 0.6079)
7	4	0.6280	0.0613	(0.5523, 0.7037)
14	4	0.6710	0.0602	(0.5953, 0.7467)

Pooled StDev = 0.0668936

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	4	0.6710	A
7	4	0.6280	A B
0	4	0.5323	B

Means that do not share a letter are significantly different.

G.12 Statistical analysis of sugar concentration of green tea water kefir beverage WK2 (4%/22°C) during 144 h of fermentation.

One-way ANOVA: Sucrose versus Fermentation time (Days)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	43.028	7.1714	48.70	0.000
Error	21	3.092	0.1472		
Total	27	46.120			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.383722	93.30%	91.38%	88.08%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	3.5380	0.1921	(3.1390, 3.9370)
1	4	1.206	0.997	(0.807, 1.605)
2	4	0.000000	0.000000	(-0.398997, 0.398997)
3	4	0.000000	0.000000	(-0.398997, 0.398997)
4	4	0.000000	0.000000	(-0.398997, 0.398997)
5	4	0.000000	0.000000	(-0.398997, 0.398997)
6	4	0.000000	0.000000	(-0.398997, 0.398997)

Pooled StDev = 0.383722

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
0	4	3.5380	A
1	4	1.206	B
6	4	0.000000	C
5	4	0.000000	C
4	4	0.000000	C
3	4	0.000000	C
2	4	0.000000	C

Means that do not share a letter are significantly different.

One-way ANOVA: Glucose versus Fermentation time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	0.3917	0.06528	0.86	0.540
Error	21	1.5943	0.07592		
Total	27	1.9860			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.275536	19.72%	0.00%	0.00%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	1.272	0.306	(0.986, 1.559)
1	4	1.550	0.317	(1.263, 1.836)
2	4	1.482	0.323	(1.195, 1.768)
3	4	1.317	0.204	(1.030, 1.603)
4	4	1.344	0.293	(1.057, 1.630)
5	4	1.259	0.242	(0.972, 1.546)
6	4	1.191	0.217	(0.904, 1.477)

Pooled StDev = 0.275536

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
1	4	1.550	A
2	4	1.482	A
4	4	1.344	A
3	4	1.317	A
0	4	1.272	A
5	4	1.259	A
6	4	1.191	A

Means that do not share a letter are significantly different.

One-way ANOVA: Fructose versus Fermentation time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
 Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	4.6717	0.77862	27.08	0.000
Error	21	0.6038	0.02875		
Total	27	5.2755			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.169565	88.55%	85.28%	79.65%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.5880	0.0336	(0.4117, 0.7643)
1	4	1.530	0.238	(1.354, 1.706)
2	4	1.923	0.236	(1.747, 2.100)
3	4	1.7503	0.1180	(1.5739, 1.9266)
4	4	1.774	0.220	(1.598, 1.951)
5	4	1.6597	0.1309	(1.4834, 1.8361)
6	4	1.5730	0.0922	(1.3967, 1.7493)

Pooled StDev = 0.169565

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
2	4	1.923	A
4	4	1.774	A B
3	4	1.7503	A B
5	4	1.6597	A B
6	4	1.5730	A B
1	4	1.530	B
0	4	0.5880	C

Means that do not share a letter are significantly different.

G.13 Statistical analysis of sugar of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: Glucose versus Storage time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.1218	0.06092	2.56	0.131
Error	9	0.2139	0.02376		
Total	11	0.3357			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.154158	36.29%	22.13%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	1.191	0.217	(1.016, 1.365)
7	4	1.0722	0.1482	(0.8979, 1.2466)
14	4	0.9438	0.0483	(0.7694, 1.1181)

Pooled StDev = 0.154158

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	4	1.191	A
7	4	1.0722	A
14	4	0.9438	A

Means that do not share a letter are significantly different.

One-way ANOVA: Fructose versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.02982	0.014908	3.03	0.098
Error	9	0.04425	0.004916		
Total	11	0.07406			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0701174	40.26%	26.98%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	1.5730	0.0922	(1.4937, 1.6523)
7	4	1.4855	0.0624	(1.4062, 1.5648)
14	4	1.4555	0.0485	(1.3762, 1.5348)

Pooled StDev = 0.0701174

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	4	1.5730	A
7	4	1.4855	A
14	4	1.4555	A

Means that do not share a letter are significantly different.

G.14 Statistical analysis of lactic acid concentration of green tea water kefir beverage WK2 (4%/22°C) during 144 h of fermentation.

One-way ANOVA: Lactic acid versus Fermentation time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Fermentation time (d)	7	0, 1, 2, 3, 4, 5, 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Fermentation time (d)	6	2.17405	0.362342	31121.42	0.000
Error	21	0.00024	0.000012		
Total	27	2.17430			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0034122	99.99%	99.99%	99.98%

Means

Fermentation time (d)	N	Mean	StDev	95% CI
0	4	0.000000	0.000000	(-0.003548, 0.003548)
1	4	0.000000	0.000000	(-0.003548, 0.003548)
2	4	0.180500	0.001000	(0.176952, 0.184048)
3	4	0.59650	0.00300	(0.59295, 0.60005)
4	4	0.60725	0.00395	(0.60370, 0.61080)
5	4	0.61750	0.00645	(0.61395, 0.62105)
6	4	0.62525	0.00377	(0.62170, 0.62880)

Pooled StDev = 0.00341216

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Fermentation time (d)	N	Mean	Grouping
6	4	0.62525	A
5	4	0.61750	A
4	4	0.60725	B
3	4	0.59650	C
2	4	0.180500	D
1	4	0.000000	E
0	4	0.000000	E

Means that do not share a letter are significantly different.

G.15 Statistical analysis of lactic acid of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: Lactic acid versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.03358	0.01679	1.01	0.403
Error	9	0.14997	0.01666		
Total	11	0.18355			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.129085	18.30%	0.14%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	0.62525	0.00377	(0.47924, 0.77126)
7	4	0.63050	0.00635	(0.48449, 0.77651)
14	4	0.516	0.223	(0.370, 0.662)

Pooled StDev = 0.129085

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
7	4	0.63050	A
0	4	0.62525	A
14	4	0.516	A

Means that do not share a letter are significantly different.

G.16 Statistical analysis of TSS of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: TSS (°Brix) versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.08167	0.040833	8.17	0.009
Error	9	0.04500	0.005000		
Total	11	0.12667			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0707107	64.47%	56.58%	36.84%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	3.8250	0.0957	(3.7450, 3.9050)
7	4	3.7500	0.0577	(3.6700, 3.8300)
14	4	3.6250	0.0500	(3.5450, 3.7050)

Pooled StDev = 0.0707107

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	4	3.8250	A
7	4	3.7500	A B
14	4	3.6250	B

Means that do not share a letter are significantly different.

G.17 Statistical analysis of pH and T.A. of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: pH versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.017317	0.008658	311.70	0.000
Error	9	0.000250	0.000028		
Total	11	0.017567			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0052705	98.58%	98.26%	97.47%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	3.51500	0.00577	(3.50904, 3.52096)
7	4	3.47750	0.00500	(3.47154, 3.48346)
14	4	3.42250	0.00500	(3.41654, 3.42846)

Pooled StDev = 0.00527046

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	4	3.51500	A
7	4	3.47750	B
14	4	3.42250	C

Means that do not share a letter are significantly different.

One-way ANOVA: T.A. (%) versus Storage time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.000821	0.000411	9.53	0.006
Error	9	0.000388	0.000043		
Total	11	0.001209			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0065638	67.93%	60.80%	42.98%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	0.17925	0.00896	(0.17183, 0.18667)
7	4	0.19000	0.00469	(0.18258, 0.19742)
14	4	0.19950	0.00520	(0.19208, 0.20692)

Pooled StDev = 0.00656379

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	4	0.19950	A
7	4	0.19000	A B
0	4	0.17925	B

Means that do not share a letter are significantly different.

G.18 Statistical analysis of colour (L*, a*, b*) of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: Colour L* versus Storage time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	2.6402	1.32011	21.00	0.000
Error	9	0.5658	0.06287		
Total	11	3.2061			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.250743	82.35%	78.43%	68.62%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	98.102	0.349	(97.819, 98.386)
7	4	99.172	0.205	(98.889, 99.456)
14	4	98.2750	0.1572	(97.9914, 98.5586)

Pooled StDev = 0.250743

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
7	4	99.172	A
14	4	98.2750	B
0	4	98.102	B

Means that do not share a letter are significantly different.

One-way ANOVA: Colour a* versus Storage time (d)

Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	2.15287	1.07643	109.10	0.000
Error	9	0.08880	0.00987		
Total	11	2.24167			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0993311	96.04%	95.16%	92.96%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	0.9950	0.0733	(0.8826, 1.1074)
7	4	1.1900	0.1412	(1.0776, 1.3024)
14	4	1.9750	0.0656	(1.8626, 2.0874)

Pooled StDev = 0.0993311

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	4	1.9750	A
7	4	1.1900	B
0	4	0.9950	B

Means that do not share a letter are significantly different.

One-way ANOVA: Colour b* versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	1.9686	0.98432	81.91	0.000
Error	9	0.1081	0.01202		
Total	11	2.0768			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.109621	94.79%	93.64%	90.74%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	3.1700	0.0783	(3.0460, 3.2940)
7	4	3.2025	0.0640	(3.0785, 3.3265)
14	4	2.3275	0.1607	(2.2035, 2.4515)

Pooled StDev = 0.109621

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
7	4	3.2025	A
0	4	3.1700	A
14	4	2.3275	B

Means that do not share a letter are significantly different.

G.19 Statistical analysis of microbiological growth of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: LAB (log cfu/ml) versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.016635	0.008318	86.69	0.000
Error	9	0.000864	0.000096		
Total	11	0.017499			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0097951	95.07%	93.97%	91.23%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	8.51900	0.00082	(8.50792, 8.53008)
7	4	8.48525	0.01193	(8.47417, 8.49633)
14	4	8.42875	0.01204	(8.41767, 8.43983)

Pooled StDev = 0.00979512

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	4	8.51900	A
7	4	8.48525	B
14	4	8.42875	C

Means that do not share a letter are significantly different.

One-way ANOVA: S. cerevisiae (log cfu/ml) versus Storage time (d)**Method**

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.060989	0.030494	236.54	0.000
Error	9	0.001160	0.000129		
Total	11	0.062149			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0113541	98.13%	97.72%	96.68%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	4	7.02875	0.01315	(7.01591, 7.04159)
7	4	6.94725	0.01187	(6.93441, 6.96009)
14	4	6.85425	0.00854	(6.84141, 6.86709)

Pooled StDev = 0.0113541

Tukey Pairwise Comparisons**Grouping Information Using the Tukey Method and 95% Confidence**

Storage time (d)	N	Mean	Grouping
0	4	7.02875	A
7	4	6.94725	B
14	4	6.85425	C

Means that do not share a letter are significantly different.

G.20 Statistical analysis of focus group sensory attributes of green tea water kefir beverage WK2 (4%/22°C) during storage (4±1°C) for two weeks

One-way ANOVA: Appearance versus Storage time (d)**Method**

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	1.625	0.8125	1.86	0.167
Error	45	19.625	0.4361		
Total	47	21.250			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.660387	7.65%	3.54%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	7.813	0.544	(7.480, 8.145)
7	16	7.375	0.806	(7.042, 7.708)
14	16	7.688	0.602	(7.355, 8.020)

Pooled StDev = 0.660387

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	16	7.813	A
14	16	7.688	A
7	16	7.375	A

Means that do not share a letter are significantly different.

One-way ANOVA: Sweetness versus Storage time (d)

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	4.542	2.2708	6.03	0.005
Error	45	16.938	0.3764		
Total	47	21.479			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.613505	21.14%	17.64%	10.28%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	8.000	0.516	(7.691, 8.309)
7	16	7.563	0.629	(7.254, 7.871)
14	16	7.250	0.683	(6.941, 7.559)

Pooled StDev = 0.613505

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	16	8.000	A
7	16	7.563	A B
14	16	7.250	B

Means that do not share a letter are significantly different.

One-way ANOVA: Sourness/Acidity versus Storage time (d)
Method

Null hypothesis All means are equal
 Alternative hypothesis Not all means are equal
 Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.5417	0.2708	0.52	0.597
Error	45	23.3750	0.5194		
Total	47	23.9167			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.720725	2.26%	0.00%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	7.750	0.683	(7.387, 8.113)
7	16	7.563	0.629	(7.200, 7.925)
14	16	7.813	0.834	(7.450, 8.175)

Pooled StDev = 0.720725

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	16	7.813	A
0	16	7.750	A
7	16	7.563	A

Means that do not share a letter are significantly different.

One-way ANOVA: Flavour versus Storage time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.8750	0.4375	1.52	0.229
Error	45	12.9375	0.2875		
Total	47	13.8125			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.536190	6.33%	2.17%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	7.500	0.516	(7.230, 7.770)
7	16	7.250	0.447	(6.980, 7.520)
14	16	7.563	0.629	(7.293, 7.832)

Pooled StDev = 0.536190

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	16	7.563	A
0	16	7.500	A
7	16	7.250	A

Means that do not share a letter are significantly different.

One-way ANOVA: Aroma versus Storage time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.1250	0.06250	0.16	0.853
Error	45	17.6875	0.39306		
Total	47	17.8125			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.626941	0.70%	0.00%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	7.500	0.632	(7.184, 7.816)
7	16	7.375	0.619	(7.059, 7.691)
14	16	7.438	0.629	(7.122, 7.753)

Pooled StDev = 0.626941

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
0	16	7.500	A
14	16	7.438	A
7	16	7.375	A

Means that do not share a letter are significantly different.

One-way ANOVA: Overall acceptability versus Storage time (d)

Method

Null hypothesis All means are equal
Alternative hypothesis Not all means are equal
Significance level $\alpha = 0.05$
Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Storage time (d)	3	0, 7, 14

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time (d)	2	0.5417	0.2708	0.81	0.453
Error	45	15.1250	0.3361		
Total	47	15.6667			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.579751	3.46%	0.00%	0.00%

Means

Storage time (d)	N	Mean	StDev	95% CI
0	16	7.625	0.500	(7.333, 7.917)
7	16	7.438	0.512	(7.146, 7.729)
14	16	7.688	0.704	(7.396, 7.979)

Pooled StDev = 0.579751

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Storage time (d)	N	Mean	Grouping
14	16	7.688	A
0	16	7.625	A
7	16	7.438	A

Means that do not share a letter are significantly different.