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BLACK TEA WATER KEFIR BEVERAGE

A Thesis submitted in partial fulfilment of the requirement for the degree of
Master of Food Technology

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ABSTRACT

Fermented foods and beverages play an important role in the human diet as they provide essential nutrients as well as contribute towards prevention of diseases. Lactic acid bacteria and yeasts are a major group of microorganisms associated with fermented products. Some of the microorganisms, known as probiotics, confer health properties to human health. Thus, many different types of fermented foods and beverages containing probiotics are produced around the world to support wellness and health. In recent years, there has been increased interest in the development of fermented functional plant-based foods and beverages due to a surge in scientific research of the products. Further, there is evidence that probiotic microorganisms can grow well in plant-based substrates.

Water kefir is a sparkling fermented beverage with an acidic, sweet, slightly alcoholic taste, and a yeasty flavour. Water kefir fermentation can be achieved by the inoculation of water kefir grains as a starter culture into a solution containing sugar. Kefir grains consist of a symbiotic starter culture of lactic acid bacteria (LAB) and yeasts contained in a polysaccharide matrix. Microorganisms present in kefir grains are recognized as probiotics. The majority of previous studies have focused on the isolation and identification of water kefir cultures responsible for fermentation. There is, therefore, scanty information on the fermentation of plant-based water kefir beverages. The main objective of this study was to develop fermented black tea beverage using water kefir grains as a starter culture.

Fermentation of black tea infusions as single and mixed substrate with carrot juice using water kefir grains were investigated. Microflora of water kefir grains used consisted of symbiotic starter culture of lactic acid bacteria (*Lactococcus* spp. and *Lactobacillus* spp.) and a yeast (*Saccharomyces cerevisiae*). The study was conducted in three main phases. The first phase investigated the effect of sucrose concentration (5% and 10%) and fermentation temperature (25°C and 30°C) in black tea water kefir fermentation for 72 h. Meanwhile, the effect of added carrot juice (5%, 10%, and 15%) on kefir beverage during secondary fermentation (24 h) at 25°C was investigated in the second phase. The stability of the final black tea water kefir beverage formulation during storage (4°C) for four weeks was investigated in phase three. Samples of black tea water kefir beverages were subjected to various analyses during fermentation and storage (4°C) for 4 weeks: titratable acidity, total soluble solids (°Brix), colour, viable cell counts of constituent starter culture, sensory evaluation, sugars, organic acids, antioxidants, and pH was also measured.

Results showed that fermentation temperature, sugar concentration, and carrot juice concentration contributed to the physico-chemical and microbiological characteristic as well as sensory properties of the product. In phases one and two, pH and total soluble solids (°Brix) decreased, while titratable acidity and cell counts of LAB and yeasts increased during fermentation of the products. LAB and yeasts were able to grow in black tea and addition of carrot juice into the beverages slightly increased their growth. The best fermentation conditions based on physico-chemical and sensory properties were kefir beverage containing sugar (10%) and carrot juice (10%) fermented at 25°C for 96 h. In phase three, the growth and survival of *Lactococcus* spp. and *Lactobacillus* spp. were low during storage of the product (4°C) while *Saccharomyces cerevisiae* maintained high cell numbers (7.03 ± 0.07 log cfu/ml) at the end of storage (28 days). Results showed the possibility to produce low sugar water kefir beverage containing $0.08 \pm 0.01\%$ (w/v) sucrose, $1.55 \pm 0.04\%$ (w/v) glucose, and $2.93 \pm 0.20\%$ (w/v) fructose. The fermented kefir beverage also contained $0.202 \pm 0.02\%$ (w/v) lactic acid, $0.114 \pm 0.03\%$ (w/v) acetic acid and some antioxidants (gallic acid, ECG, EGC, EGCG, theobromine and caffeine) which may be beneficial to human health. There was significant difference ($p < 0.05$) in the colour (L^* , a^* , b^*) of the fermented beverages during storage (4°C).

Black tea water kefir beverage containing 10% sugar and 10% carrot juice fermented at 25°C for 96 h was well-liked by consumer sensory panellists.

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LIST OF ABBREVIATIONS

a*	=	redness-greenness
AAB	=	Acetic acid bacteria
ACK	=	Acetate kinase
Acs	=	Acetyl-coenzyme A synthetase
Adh	=	Alcohol dehydrogenase
ADP	=	Adenosine diphosphate
Ald	=	Acetaldehyde dehydrogenase
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemist
ATP	=	Adenosine triphosphate
B	=	Bifidobacterium
b*	=	yellowness-blueness
BOP	=	Broken orange pekoe
BT	=	Black tea
CFU	=	Colony forming per Unit
CTC	=	Crush-Tear-Curl
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
EC	=	Epicatechin
ECG	=	Epicatechin gallate
ECP	=	Endless chain pressure
EGC	=	Epigallocatechin
EGCG	=	Epigallocatechin gallate
EMP	=	Emden-Meyerhoff-Parnas
EPS	=	Exopolysaccharides
F	=	Fanning
FADH	=	Flavin adenine dinucleotide
FAO	=	Food and Agriculture Organization
FBD	=	Fluid bed dried
FSANZ	=	Food Standards Australia New Zealand
g	=	Gram
GABA	=	Gamma-Amino Butyric Acid

GAP	=	Glyceraldehyde-3P
GRAS	=	Generally Recognized As Safe
GC	=	Gas chromatography
GI	=	Gastrointestinal
H	=	Hour
HIV	=	Human Immunodeficiency Virus
HPLC	=	High performance liquid chromatography
L	=	Litre
L*	=	Lightness
LAB	=	Lactic acid bacteria
Lb.	=	Lactobacillus
Lc.	=	Leuconostoc
LDH	=	Lactate dehydrogenase
Min	=	Minute
ml	=	milliliter
mg	=	milligram
mm	=	millimeter
NaCl	=	Sodium chloride
NAD	=	Nicotinamide adenine dinucleotide
NADH	=	Nicotinamide adenine dinucleotide hydride
OP	=	Orange pekoe
ORS	=	Oral rehydration solution
OXPHOS	=	Oxidative phosphorylation
PCR	=	Polymerase chain reaction
Pdc	=	Pyruvate decarboxylase
Pdh	=	Pyruvate-dehydrogenase complex
PFL	=	Pyruvate formate-lyase
PKP	=	Phospho-ketolase pathway
PTA	=	Phospho-trans-acetylase
SD	=	Standard deviation
spp	=	species (plural)
TSS	=	Total soluble solids
T.A.	=	Titrateable Acidity
TCA	=	Tricarboxylic acid

TF	=	theaflavins
TFA	=	trifluoroacetic acid
TR	=	thearubigins
MRS	=	de Man, Rogosa and Sharpe
VOC	=	Volatile Organic Compounds
v/v	=	volume per volume
WHO	=	World Health Organization
w/v	=	weight per volume
YGC	=	Yeast Glucose Chloramphenicol

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1. INTRODUCTION

1.1 Background

Food fermentation is one of the most ancient and economical ways of food processing and food preservation which has been used by humans for centuries (Adams & Nout, 2001; Campbell-Platt, 1994; Kabak & Dobson, 2011). Fermentation of foods and beverages involve bacteria, yeasts, or moulds which working singularly or together convert a variety of raw foods to fermented products (Blandino et al., 2003; Nout, 2003; Nout, 2005). Microbial and enzymatic transformations which occur during fermentation change the sensory profile (appearance, aroma, flavour, and texture) and nutritional value of the raw material to produce desirable food product properties and nutrients (Campbell-Platt, 1994; Steinkraus, 1996). Fermentation also enhances the digestibility of foods and improves food safety by destruction of undesirable substances present in raw foods such as phytates and tannins (Adams & Mitchell, 2002; Sharma & Kapoor, 1996; Simango, 1997).

In recent years, there has been an increased demand for the production and consumption of fermented foods and beverages due to their health beneficial effects (enhance immune system, prevent chronic diseases), nutritional properties (improve the availability of essential amino acids and vitamins), and sensory profile (enhance organoleptic quality of the food) (Campbell-Platt, 1994; Leroy & De Vuyst, 2004; Sabokbar & Khodaiyan, 2015). The fermented products provide 20-40% of food supply globally (Campbell-Platt, 1994). A wide variety of raw ingredients such as milk, meat, fish, vegetables, fruits, tea, and cereals are used as substrates in the fermentation and transform into fermented foods and beverages including sauerkraut, kombucha, tempeh, beers, wine, sausages, yogurt, cheese, and kefir (Ahmad & Amer, 2013; Blandino et al., 2003; Corona et al., 2016; Dai et al., 2013; Dimitrovski et al., 2015; Jayabalan et al., 2014; Kabak & Dobson, 2011; Leite et al., 2013). However, there is a growing consumer interest in plant-based and non-dairy based fermented products due to health challenges such as milk protein allergies, lactose intolerance, high fat, and cholesterol content which pose major hurdles to dairy-based fermented products (Kumar,

Vijayendra & Reddy, 2015; Prado et al., 2008; Rivera-Espinoza & Gallardo-Navarro, 2010; Vasudha & Mishra, 2013). Therefore, there is need to search for novel non-dairy foods and beverages to meet the needs of consumers affected by components in milk (Bernat et al., 2015; Gawkowski & Chikindas, 2013; Mridula & Sharma, 2015). In this regards, water kefir has emerged as a strong candidate to existing non-dairy fermented beverages (Corona et al., 2016; Laureys & De Vuyst, 2014; Randazzo et al., 2016).

Kefir is derived from Turkish word *keyif* which means pleasant taste or good feeling (Kurmann, Rasic, & Kroger, 1992; Tamime, 2006). Typically, kefir is an acidic fermented beverages with slight alcoholic taste and yeasty flavor which originates from the Caucasian and Eastern Europe (Farnworth, 2005; Garcia et al., 2006; Gronnevik, Falstad, & Narvhus, 2011; Tratnik et al., 2006). Historically, there are two types of kefir beverages; dairy kefir and sugary kefir which are fermented using kefir grains as a starter culture (Pidoux, 1989). The characteristic of dairy kefir grains are yellowish-white, cauliflower-shaped, and gelatinous (Pidoux 1989; Waldherr et al., 2010). Milk kefir is usually made from animal-based milks (cow, sheep, goats, and buffalo) (Cais-Sokolinska, et al., 2015; Gul et al., 2015; Tratnik et al., 2006). However, it can be also produced from non-animal milk sources such as rice milk (Sirirat, & Jelena, 2010), walnut milk (Cui et al., 2013), and soymilk (Abdolmaleki, Assadi & Akbarirad, 2015; Ba, Garcia, & Ida, 2013). However, kefir beverages have been also made from solution containing sugars and the fermentation is induced by grains which are transparent, mucilaginous, and elastic (Pidoux 1989; Waldherr et al., 2010). Therefore, it may be referred to as sugary kefir, tibico, or water kefir in order to differentiate it from dairy kefir (Bergmann et al., 2010; Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Pidoux, 1989).

Water kefir is sparkling fermented beverage with an acidic, sweet, slightly alcoholic taste, and a yeasty flavour. The beverage is fermented by kefir grains, a symbiotic starter culture of lactic acid bacteria (LAB) and yeasts contained in a polysaccharide matrix (Laureys & De Vuyst, 2014; Puerari, Magalhaes, & Schwan, 2012). Fermented water kefir has a distinct aroma profile derived from a mixture of organic acids, ethanol, carbon dioxide and other flavour compounds such as ethyl decanoate and ethyl hexanoate (Corona et al., 2016; Laureys & De Vuyst, 2014; Randazzo et al., 2016). The unique flavour is a consequence of the symbiotic metabolic activity of several lactic

acid bacteria and yeasts (Laureys & De Vuyst, 2014; Otles & Cagindi, 2003). Several bacteria and yeasts which are present in kefir grains are recognised as probiotics confer beneficial effects on health, thereby promoting wellness and reducing the risk of diseases such as diarrhoea and colon cancer (Otles & Cagindi, 2003; Parvez, et al., 2006). Typically, water kefir can be produced from any solution containing fermentable sugar such as sucrose (Laureys & De Vuyst, 2014), molasses (Bernat et al., 2015), vegetable juice (Corona et al., 2016) and fruit juice (Randazzo et al., 2016). At present, consumer demand for non-dairy fermented products has increased (Prado et al., 2008; Rivera-Espinoza & Gallardo-Navarro, 2010; Vasudha & Mishra, 2013) and many studies have been conducted to develop fermented foods and beverages from various food matrices including fruits and vegetables (Bernat et al., 2014; Mridula & Sharma, 2015; Randazzo et al., 2016; Salmeron et al., 2015). Further, there is growing interest in developing plant-based fermented beverages utilizing tea (black tea, green tea) and carrot as fermentation medium (Corona et al., 2016; Essawet et al., 2015; Fu et al., 2014; Kiros et al., 2016). This new product development may give an alternative to the consumers and might widen the choice for the consumption of fermented products (Prado et al., 2008; Vasuda & Mishra, 2013).

Tea (*Camellia sinensis*) is one of the most consumed beverages in the world (Da Silva Pinto, 2013; Hayat et al., 2015). It is produced from the leaves of *Camellia sinensis* which originated in South East Asia and is now being widely cultivated in more than 30 countries (Higdon & Frei, 2003; Li et al., 2013). There are four major types of tea based on the fermentation process of the tea leaves: white tea, oolong tea, green tea, and black tea (Horzic et al., 2009; McKay & Blumberg, 2002). Among the four types, black tea is the most popular form around the world and accounts for 78% of the total tea consumed (Kraujalytė, Pelvan, & Alasalvar, 2016; Li et al., 2013). Furthermore, black tea has shown diverse health benefits such as reduced risk of chronic diseases particularly cardiovascular diseases and cancers (Bancirova, 2010; Gardner et al., 2007; Higdon & Frei, 2003; Ruxton, 2008). The health beneficial effects of black tea have been attributed to the antioxidant properties of polyphenolic compounds such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), theaflavins (TF), and thearubigins (TR). In addition, black tea has important source of other nutrients including amino acids (2-4% dry weight of leaves) and minerals (2-5% dry weight of leaves) (Balentine et al., 1997; Fernandez et al., 2002;

Higdon & Frei, 2003; Li et al, 2013; Rao & Ramalakshmi, 2011). Furthermore, black tea is reported to have no adverse effects on lactic acid bacteria during fermentation (Jaziri et al., 2009; Najgebauer-Lejko et al, 2011; Zhao & Shah, 2014). Thus, black tea is a suitable ingredient to be used as a fermentation medium and transform into fermented products with enhanced nutritional and functional quality.

Carrot (*Daucus carota*) is a root vegetable with high nutritional value as it contains carbohydrates, proteins, vitamins (A, B, C, E, and K), and minerals (calcium, potassium, phosphorus, sodium, and iron) (Kun et al., 2008; Sharma et al., 2012). Further, the root contains valuable carotenoids, especially β -carotene (Demir, Acar & Bahceci, 2004; Leja et al., 2013) which gives natural pigment of bright orange colour (Quitão-Teixeira et al., 2008) and provides health properties including free radical scavengers, anti-mutagenic, immune-enhancers, and protective effects against cancers (Briviba et al., 2004; Sharma et al., 2012). Additionally, carrot is recognized as healthy food products and does not have allergenic effect. Thus, carrot frequently consumed by consumers suffering from milk protein allergy or lactose intolerance (Kun et al., 2008). With consumer's interest in functional foods, the use of carrot in fermented products has created new opportunities for product development (Alwis et al., 2016; Amany et al., 2012; Cakmakci et al., 2014; Kiros et al., 2016; Madora, Takalani, & Mashau, 2016; Tamminen et al., 2013). Therefore, the use of carrot juice as a natural flavouring and colouring agent as well as a source of fermentable sugars in black tea water kefir beverages may be beneficial due to its good sensory properties and high nutritional value (Kiros et al., 2016; Madora et al., 2016; Sharma et al., 2012).

Previous studies have focused on the isolation and identification of water kefir grains starter cultures responsible for fermentation (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013; Miguel et al., 2011). There is however scanty information on fermentation of tea-based water kefir beverages. Thus, fermentation of mixed black tea and carrot juice with water kefir grains is novel research which will create new consumer products.

1.2 Aim and Objectives

Aim:

The overall aim of the project was to develop fermented black tea beverage using water kefir grains as a starter culture.

Objectives:

1. To investigate the effect of fermentation temperature, concentrations of sucrose, and carrot juice on the physico-chemical, microbiological, and sensory properties of black tea water kefir beverage.
2. To characterise physico-chemical properties of fermented black tea water kefir beverage by determining colour, total soluble solids (TSS), pH, and titratable acidity during fermentation and storage at 4°C.
3. To characterise microbial growth profiles of water kefir grains by enumerating lactic acid bacteria (*Lactobacillus* spp., *Lactococcus* spp.) and yeast (*Saccharomyces cerevisiae*) in black tea water kefir beverage during fermentation and storage at 4°C.
4. To determine sugars (sucrose, fructose, and glucose), organic acids (lactic acid and acetic acid), and antioxidants (gallic acid, EGC, ECG, EGCG, theobromine, and caffeine) content of black tea water kefir beverage during fermentation and storage at 4°C using high performance liquid chromatography (HPLC).
5. To conduct consumer sensory evaluation of prepared black tea water kefir beverage.

2. LITERATURE REVIEW

2.1 Food fermentation

Fermentation is one of the oldest food processing technologies which has been used by human for thousands of years for the production of foods and beverages with desirable properties (Prajapati & Nair, 2003; Smid & Hugenholtz, 2010). The term ‘fermentation’ is derived from the Latin word *fervere* which means “to boil”. It describes the action of yeast on fruit extracts or malted grains producing carbon dioxide bubbles which may be viewed as boiling during the production of alcoholic beverages (Stanbury, Whitaker, & Hall, 1995). In food processing, fermentation is defined as a transformation process of organic substances involving selected microorganisms (bacteria, yeasts, or moulds) under specified conditions for the production of a range of products containing metabolites such as organic acids, ethanol, or carbon dioxide which can suppress the growth of undesirable microorganisms (Adams & Nout, 2001; Bamforth, 2005). There are several roles of fermentation in food processing. According to Steinkraus (1996), there are five roles of food fermentation which include (1) improving food safety through formation of inhibitory metabolites such as organic acids (lactic acid, acetic acid, and propionic acid), ethanol, carbon dioxide, reutrin, and bacteriocins to suppress growth of pathogenic bacteria (Caplice & Fitzgerald, 1999); (2) enhancing the sensory properties of the food through development of a diverse of flavour, aroma, and texture (Lacroix et al., 2010); (3) enriching nutritional composition of the food through the synthesis of essential amino acids, fatty acids, and vitamins (van Boekel et al., 2010); (4) decreasing cooking times and fuel requirements (Simango, 1997), as well as (5) providing natural ways of improving the digestibility of food. Fermentation also has detoxification function and destruct undesirable substances present in raw foods such as phytates and tannins (Sharma & Kapoor, 1996).

Nowadays, fermented foods and beverages are regarded as part of our daily diets. The main substrates used in commercial production of fermented products are milk, meat, fish, cereals, fruits, and vegetables, yielding over 500 varieties of fermented foods and beverages worldwide (Kabak & Dobson, 2011). Among the fermented products, foods

and beverages containing probiotics have gained high interest to consumers (Kandylis et al., 2015; Stanton et al., 2005).

2.2 Probiotics

Probiotics has been a subject of interest for the last few decades in the food research and development field (Prado et al., 2008). The word probiotic derived from the Greek language, *pro* and *bios*, means “for life” (Gismondo, Drago, & Lombardi, 1999; Guarner et al., 2005). According to FAO and WHO (2001) probiotics are defined as “live microorganisms which when administered in adequate amounts confer a beneficial effect on the host health”. Thus, to exert beneficial effect, the probiotics must be alive and available in high numbers, at least reach a minimum level of 10^6 colony forming per unit (CFU) of products at the time of consumption (Bansal et al., 2016; FAO/WHO, 2002; Madureira et al., 2011).

There are several criteria that are required for a microorganism to be qualified and considered as probiotics; (1) it must be non-pathogenic, innocuous to the host, and generally regarded as safe (GRAS); (2) present in high amounts in the product and retain viability during storage; (3) resistance to gastric acid and bile toxicity (survive in the gastrointestinal (GI) tract), as well as (4) adherence to human intestinal cells, able to multiply in and colonise the gut (Collins, Thornton, & O’Sullivan, 1998; Havenaar & Huis, 1992; Gismondo et al., 1999; Parvez et al., 2006). Common probiotic microorganisms involved in food fermentations are lactic acid bacteria (LAB) from genera *Lactobacillus* and *Bifidobacterium* (Stiles & Holzapfel, 1997; Tamang et al., 2016; Zhang & Cai, 2014). However, some species belonging to the genera of *Bacillus*, *Escherichia*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Propionibacterium*, *Saccharomyces*, and *Streptococcus* are also recognised as probiotics due to their health-promoting effects (Czerucka, & Rampal, 2002; Gismondo et al., 1999; Hatoum, Labrie, & Fliss, 2012; Parvez et al., 2006; Prado et al., 2008). The list of probiotic microorganisms can be seen in Table 2.1.

Table 2.1 Probiotic microorganisms

<i>Bacillus</i>	<i>Bacillus cereus</i> , <i>Bacillus clausii</i> , <i>Bacillus pumilus</i>
<i>Bifidobacterium</i>	<i>Bf. adolescentis</i> , <i>Bf. animalis</i> , <i>Bf. breve</i> , <i>Bf. bifidum</i> , <i>Bf. infantis</i> , <i>Bf. lactis</i> , <i>Bf. longum</i> .
<i>Escherichia</i>	<i>Escherichia coli</i> nissle
<i>Enterococcus</i>	<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>
<i>Lactococcus</i>	<i>Lactococcus lactis</i> sp. <i>cremoris</i> , <i>Lactococcus lactis</i> sp. <i>lactis</i>
<i>Lactobacillus</i>	<i>Lb. acidophilus</i> , <i>Lb. amylovorus</i> , <i>Lb. brevis</i> , <i>Lb. casei</i> , <i>Lb. casei</i> <i>sp. rhamnosus</i> , <i>Lb. delbrueckii</i> sp. <i>bulgaricus</i> , <i>Lb. fermentum</i> , <i>Lb. gasseri</i> , <i>Lb. helveticus</i> , <i>Lb. johnsonii</i> , <i>Lb. lactis</i> , <i>Lb. paracasei</i> , <i>Lb. plantarum</i> , <i>Lb. reuteri</i> .
<i>Leuconostoc</i>	<i>Leuconostoc mesenteroides</i>
<i>Propionibacterium</i>	<i>Propionibacterium freudenreichii</i>
<i>Saccharomyces</i>	<i>Saccharomyces boulardii</i>
<i>Streptococcus</i>	<i>Streptococcus salivarius</i> sp. <i>thermophilus</i> , <i>Streptococcus</i> <i>cremoris</i> , <i>Streptococcus diacetylactis</i> .

Source: Czerucka, & Rampal, (2002); Gismondo et al., (1999); Hatoum, Labrie, & Fliss, (2012); Parvez et al., (2006); Prado et al., (2008)

2.2.1 Health benefits of probiotics

Several studies have been carried out to provide evidence related to the health benefits of probiotics which include relief to diarrhoea, immune enhancement, and alleviation of lactose intolerance (Chapman, Gibson, & Rowland, 2011; Gismondo et al., 1999; Parvez et al., 2006; Vandenplas, Huys, & Daube, 2015).

2.2.1.1 Diarrhoea

Diarrhoea is a major manifestation of gastrointestinal illness which occurs when there is an imbalance between water and electrolyte absorption and secretion thus decreasing stool consistency (Schiller, 2012). Rehydration by replacing the fluids and electrolyte loss with oral rehydration solution (ORS) is a common therapy for treating diarrhoea (Ozuah, Avner, & Stein, 2002). However, it does not reduce the stool frequency or decrease the duration of diarrhoea. Therefore, use of probiotics is suitable alternative to alleviate diarrhoea symptoms (Cucchiara et al., 2002). Probiotic microorganisms which have shown some promising results in treating diarrhoea for children are *Lactobacillus* GG, *Lactobacillus casei*, *Lactobacillus reuteri*, *Saccharomyces boulardii*, and *Bifidobacterium bifidum* (Gismondo et al., 1999; Guandalini, 2011; Weichselbaum, 2010). Probiotics are able to adhere to human intestinal cell, multiply, colonise the gastrointestinal tract and compete with pathogenic bacteria. Probiotics might also inhibit harmful bacteria growth by producing bacteriocins, binding toxins and secreting enzymes to neutralize the bacterial toxins. Further, they will enhance the intestinal barrier function, competitive mechanism for adhesion, creating unfavourable environment for the pathogenic bacteria, and modulating the local immune system indirectly (Nagpal et al., 2012; Parvez et al., 2006; Vandenplas et al., 2015). Sharif et al., (2016) reported the alleviation of diarrhoea symptoms by using probiotic yeast, *Saccharomyces boulardii* in combination with oral rehydration solution (ORS). The probiotic was administered once daily to the children with acute diarrhoea (Sharif et al., 2016). Another study reported that *Lactobacillus* GG administered at a dosage of 10^{10} cfu per day for five days resulted in shorter duration and improvement in stool consistency in children with diarrhoea (Aggarwal et al., 2014).

2.2.1.2 Immunity system enhancement

Probiotics can enhance the immune responses of the host by stimulating the production of cytokines in lymphocytes, increasing natural killer cell activity as well as phagocytosis, and increasing levels of immunoglobulins in blood (Nagpal et al., 2012). Falasca et al., (2015) reported that patients infected with HIV has shown an enhanced immune response through reduction of inflammatory status by consuming one bottle of fermented milk containing *Lactobacillus casei* Shirota twice a day for four weeks.

However, probiotics need to be consumed daily as the beneficial effect can disappear once the intake of probiotics has stopped (Moro-Garcia et al., 2013).

2.2.1.3 Lactose intolerance

Lactose intolerance is defined as a symptom of gastrointestinal distress characterized by bloating, abdominal pain, and nausea caused by lactase deficiency or lactose malabsorption after ingestion of dairy based products (Brown-Esters, Namara, & Savaiano, 2012; Misselwitz et al., 2013). The undigested lactose transit to the small and large intestine, subsequently fermented by colonic microflora and produce hydrogen, methane, and carbon dioxide, thus creating the potential for the symptoms (He et al., 2006). Use of probiotics specifically *Lactobacillus acidophilus*, has been shown to improve lactose digestion and alleviates the symptoms of lactose intolerance when orally administered for four weeks (Kim & Gilliland, 1983; Pakdaman et al., 2016). There are two possible roles of probiotics in managing lactose intolerance. Firstly, the release of microbial enzyme, β -galactosidase, into the small intestine to support lactose digestion and secondly, the probiotics themselves may alter the intestinal pH, modify the colonic bacteria, or affect the sensitivity of the subject and thus alleviate the symptoms of lactose intolerance (de Vrese et al., 2001; Lomer, Parkes, & Sanderson, 2008).

2.2.2 Non-dairy probiotic products

Probiotic products are usually distributed and commercialized in the form of dairy-based fermented products (Kandylis et al., 2016). However, there are some health challenges associated to dairy-based probiotics products including lactose intolerance, milk protein allergy, and the cholesterol content (Kumar et al., 2015). Furthermore, there is also ongoing trend of vegetarianism throughout the world which demands for plant-based probiotic products (Bansal et al., 2016; Granato et al., 2010). Among the non-dairy based fermented products, there are a wide variety of non-dairy fermented beverages produced around the world such as bushera, boza, hardaliye, kombucha, mahewu, pozol, and togwa which play important roles in the human diet (Prado et al., 2008). These fermented products are made from botanical sources including fruits, vegetables, cereals, legumes, and tea (Bansal et al., 2016; Prado et al., 2008; Vasudha &

Mishra, 2013). In addition, there is another vegetarian probiotic beverage which has been assayed for probiotic properties called water kefir which seems to be a good candidate (Corona et al., 2016; Randazzo et al., 2016).

2.3 Water kefir

Water kefir grains are reported to be available worldwide. Water kefir grains and their origins still remains unknown (Gulitz et al., 2011; Laureys & De Vuyst, 2014). There are various synonyms for water kefir grains such as “ginger beer plants” that were brought back from the Crimean war in 1955 by English soldiers (Hesseltine, 1965; Ward, 1892), “Tibi grains” or “Tibico” found in Mexico as granules on leaves of a Mexican plant (*Opuntia*) (Lutz, 1899), “California bees”, African bees”, Ale nuts”, “Balm of Gilead”, “Japanese Beer Seed” (Kebler, 1921) and “Sugary kefir grains” (Pidoux, 1989; Pidoux et al., 1990). Water kefir grains are the main ingredient in the fermentation of water kefir beverage (Pidoux, 1989).

Water kefir is a refreshing, self-carbonated fermented beverage which is made from solution containing sucrose. It has acidic, slightly sweet with an alcoholic taste (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013; Pidoux, 1989). Traditionally, water kefir is cultured by adding 5-10% (w/v) of wet water kefir grains in 5-10% (w/v) sucrose solution (Gulitz et al., 2013; Gulitz et al., 2011; Magalhaes et al., 2010; Waldherr, Doll, Meißner, & Vogel, 2010b) and fermented at room temperature (20-25°C) for 24-72 hours (Laureys & De Vuyst, 2014; Pidoux, 1989; Reiß, 1990). Lengkey and Balia (2014) reported that kefir beverage which was produced with 10% kefir grains gave the best results in terms of pH and lactic acid production. Dried or fresh fruit and some slices of lemon are usually supplemented to this sugar solution to improve its flavour (Pidoux et al., 1990; Waldherr et al., 2010b). The general processing of water kefir beverage is outlined in Figure 2.1

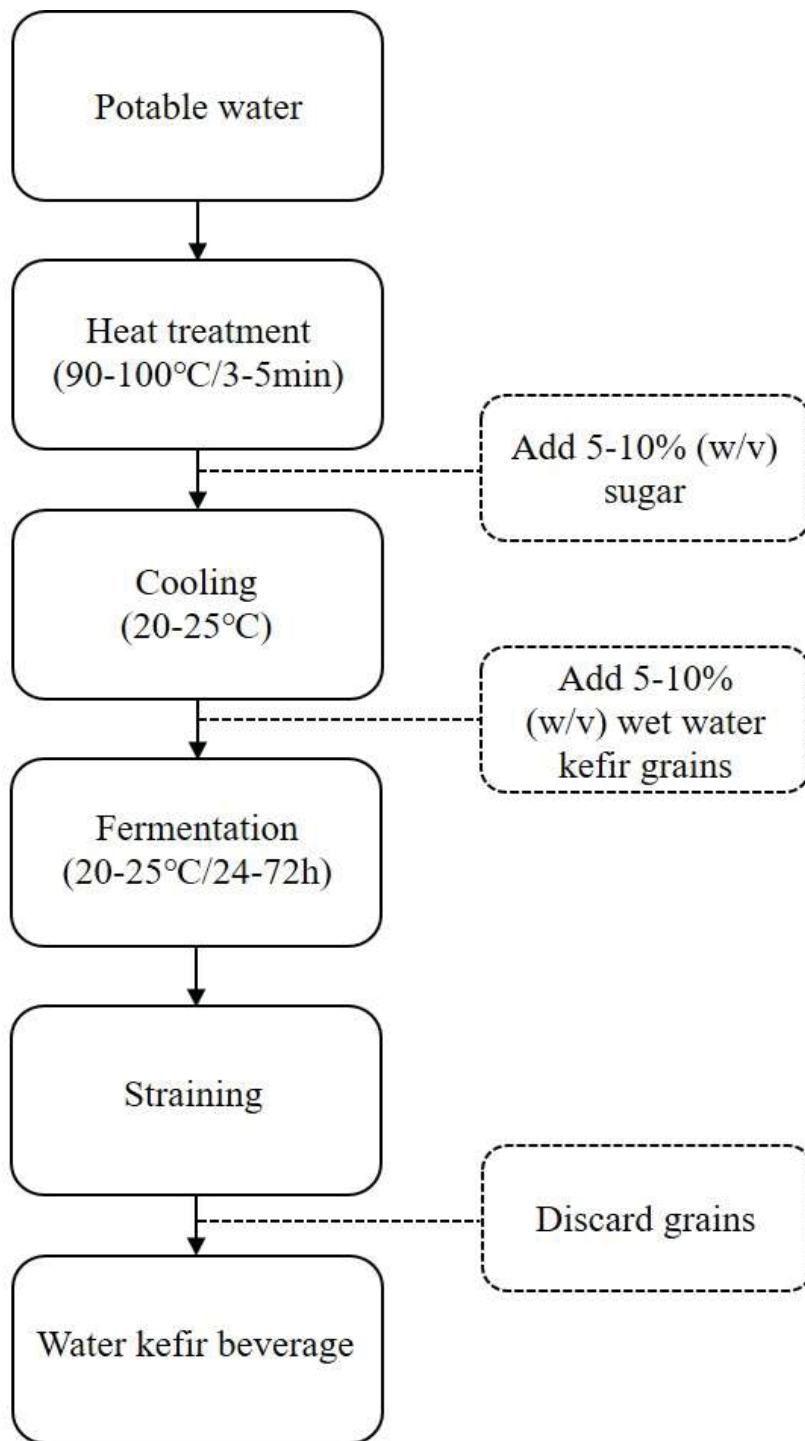


Figure 2.1 Overview of manufacturing of water kefir beverage
Source: Gulitz et al., (2013); Gulitz et al., (2011); Magalhaes et al., (2010)

2.4 Water kefir grains microflora

The microbiota of water kefir grains is a combination of bacteria and yeast that live in symbiosis in a polysaccharide matrix (Franzetti et al., 1998; Galli et al., 1995; Gulitz et al., 2011; Gulitz et al., 2013; Horisberger, 1969; Laureys & De Vuyst, 2014; Lutz, 1899; Marsh et al., 2013; Moinas et al., 1980; Neve & Heller 2002; Pidoux, 1989; Ward, 1892). The microorganisms are embedded in transparent, elastic, and crystal-like shape of 5-20 mm in diameter consisting of insoluble dextran with α -1,6-linked glucose and α -1,3-branching (Galli et al., 1995; Horisberger, 1969; Pidoux, 1989). The water kefir grain is shown in Figure 2.2.



Figure 2.2 Water kefir grains

Source: Stadie, (2013)

Several investigations have been published on microbial diversity of water kefir grains where various types of bacteria and yeasts have been isolated and identified from water kefir consortia (Gulitz et al., 2013; Gulitz et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013). Some of the microorganisms commonly present in water kefir grains are shown in Table 2.2. Microbial composition of water kefir grains is variable (Gulitz et al., 2013; Gulitz et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013). The microflora of water kefir is estimated to contain 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 bacterial content of the grains usually contains various Gram positive homo- and hetero- fermentative lactic acid bacteria (LAB) from genus *Lactobacillus*, *Lactococcus*, *Leuconostoc* and Gram negative acetic acid bacteria (AAB) from genus *Acetobacter*. Recently, the genus *Bifidobacterium* in water kefir grains has been reported with part of the sequences being assigned to *Bifidobacterium psychraerophilum* (Gulitz et al., 2013; Laureys & De Vuyst, 2014). The yeast content of the grains usually comprises *Saccharomyces*, *Candida*, *Hanseniaspora*, *Dekkera*, and *Zygosaccharomyces* (Galli et al., 1995; Gulitz et al., 2011; Gulitz et al., 2013; Laureys & De Vuyst, 2014; Marsh et al., 2013; Pidoux, 1989). The dominant species of bacteria in water kefir grains are *Lactobacillus hordei*, *Lactobacillus nagelii*, *Leuconostoc mesenteroides*, *Leuconostoc citreum*, *Acetobacter fabarum* (Gulitz et al., 2013; Gulitz et al., 2011), *Lactobacillus casei* (Franzetti et al., 1998; Galli et al., 1995; Laureys & De Vuyst, 2014; Pidoux, 1989), *Lactobacillus hilgardii* (Laureys & De Vuyst, 2014; Pidoux, 1989; Waldherr et al., 2010a), *Lactobacillus brevis* (Horiberger, 1969; Moinas et al., 1980) while the predominant yeast are *Zygorulaspora florentina* (Gulitz et al., 2011), *Saccharomyces cerevisiae* (Gulitz et al., 2011; Laureys & De Vuyst, 2014), *Dekkera bruxellensis* (Laureys & De Vuyst, 2014), and *Zymomonas* (Marsh et al., 2013).

Table 2.2 Isolated bacteria and yeasts in water kefir grains

Microorganisms	References
Bacteria	
<i>Acetobacter fabarum</i>	Gulitz et al., 2011
<i>Acetobacter orientalis</i>	Gulitz et al., 2011
<i>Bifidobacterium psychraerophilum</i>	Gulitz et al., 2013; Laureys & De Vuyst, 2014
<i>Enterobacter hormachei</i>	Waldherr, 2010a
<i>Gluconobacter frateurii</i>	Waldherr, 2010a
<i>Lactobacillus brevis</i>	Horisberger, 1969; Moinas et al., 1980
<i>Lactobacillus buchneri</i>	Galli et al., 1995
<i>Lactobacillus casei</i> subsp. <i>casei</i>	Franzetti et al., 1998; Galli et al., 1995; Laureys & De Vuyst, 2014; Pidoux, 1989
<i>Lactobacillus casei</i> subsp. <i>pseudopantarum</i>	Galli et al., 1995
<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>	Pidoux, 1989
<i>Lactobacillus collinoides</i>	Galli et al., 1995
<i>Lactobacillus fructiovorans</i>	Galli et al., 1995
<i>Lactobacillus hilgardii</i>	Laureys & De Vuyst, 2014; Pidoux, 1989; Waldherr, 2010a
<i>Lactobacillus harbinensis</i>	Laureys & De Vuyst, 2014
<i>Lactobacillus hordei</i>	Gulitz et al., 2011; Gulitz et al., 2013
<i>Lactobacillus nagelii</i>	Gulitz et al., 2011; Gulitz et al., 2013
<i>Lactobacillus plantarum</i>	Pidoux, 1989
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	Pidoux, 1989
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Moinas et al., 1980; Pidoux, 1989; Waldherr, 2010a
<i>Leuconostoc citreum</i>	Gulitz et al., 2011
<i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>	Pidoux, 1989
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	Galli et al., 1995; Waldherr, 2010a
Yeasts	
<i>Candida lambica</i>	Pidoux, 1989
<i>Candida valida</i>	Pidoux, 1989
<i>Dekkera bruxellensis</i>	Laureys & De Vuyst, 2014
<i>Hanseniaspora valbynesis</i>	Galli et al., 1995; Neve & Heller, 2002; Pidoux, 1989;
<i>Hanseniaspora vinalis</i>	Galli et al., 1995; Pidoux, 1989;
<i>Hanseniaspora yalbensis</i>	Franzetti et al., 1998
<i>Kloeckera apiculata</i>	Franzetti et al., 1998; Pidoux, 1989;
<i>Saccharomyces bayanus</i>	Waldherr, 2010a
<i>Saccharomyces cerevisiae</i>	Franzetti et al., 1998; Galli et al., 1995; Laureys & De Vuyst, 2014; Moinas et al., 1980;
<i>Saccharomyces florentinus</i>	Galli et al., 1995
<i>Saccharomyces pretoriensis</i>	Galli et al., 1995
<i>Zygosaccharomyces florentinus</i>	Pidoux, 1989; Neve & Heller, 2002
<i>Zygorhizula florentina</i>	Gulitz et al., 2011

2.5 Symbiotic interactions of bacteria and yeasts in water kefir fermentation

The association between lactic acid bacteria and yeasts has been described as symbiosis by many authors (Franzetti et al., 1998; Pidoux & Leroi, 1993a; Pidoux & Leroi, 1993b; Stadie, Gulitz, Ehrmann, & Vogel, 2013; Ward, 1892). Yeast and bacteria are thought to be in a symbiotic relationship which shares their by-products as energy source or growth stimulant to survive or multiply in the same environment (Lopitz-Otsoa, Rementería, Elguezabal, & Garaziar, 2006). Yeasts not only can provide nutrients such as vitamins and amino acids for bacteria growth but also can utilize the metabolites produced by bacteria as an energy source (Feuillat et al., 1977; Wood & Hodge, 1985). The first synergism of lactic acid bacteria and yeasts isolated from water kefir has been investigated by Pidoux & Leroi (1993b) involving *Lactobacillus hilgardii* and *Saccharomyces florentinus*. *Saccharomyces florentinus* has been reclassified as *Zygorulaspora florentina* (Kurtzman, 2003). In mixed culture, *Lactobacillus hilgardii* grew better in the presence of *Zygorulaspora florentina* (Pidoux & Leroi, 1993b). *Zygorulaspora florentina* produces CO₂, pyruvate, propionate, acetate, and succinate which favourable to *Lactobacillus hilgardii*. Furthermore, the ability of *Zygorulaspora florentina* to hydrolyse sucrose into glucose and fructose induces the growth of *Lactobacillus hilgardii* as the simple sugars were metabolized by the bacteria rapidly (Pidoux & Leroi, 1993a). However, the growth of *Zygorulaspora florentina* as well as ethanol production was significantly declined after 72 hours due to *Lactobacillus hilgardii* fermentation (Pidoux & Leroi, 1993b) but the growth of *Zygorulaspora florentina* was not inhibited by *Lactobacillus hilgardii* when the initial yeast: bacteria ratio exceeded 1% (Essia Ngang, Letourneau, & Villa; Pidoux & Leroi, 1993a).

Stadie et al., (2013) investigated the interactions between *Zygorulaspora florentina*, *Saccharomyces cerevisiae*, *Lactobacillus hordei*, and *Lactobacillus nagelii* in water kefir by comparing the growth of main representative water kefir isolates in a model system in co-cultivation and pure culture. In this study, Stadie et al., (2013) delineates the interactions between lactobacilli and yeasts as symbiotic mutualism. *Zygorulaspora florentina* and *Saccharomyces cerevisiae* can provide essential nutrients which are needed by lactobacilli to grow. However, *Zygorulaspora florentina* has higher influence than *Saccharomyces cerevisiae*. Growth of *Lactobacillus*

hordei is supported by the release of vitamin B6 from yeasts while *Lactobacillus nagelii* is improved in growth by the disposal of essential amino acids produced by yeasts. The release of yeasts metabolites for the lactobacilli is due to acidification stimulation of the medium by lactobacilli which produce lactic acid and acetic acid during fermentation (Stadie et al., 2013). Further, the growth of yeast is improved with decreasing pH (Stadie et al., 2013; Vosti & Joslyn, 1954). An overview of the metabolic interaction of lactobacilli and yeasts from water kefir isolates is shown in Figure 2.3.

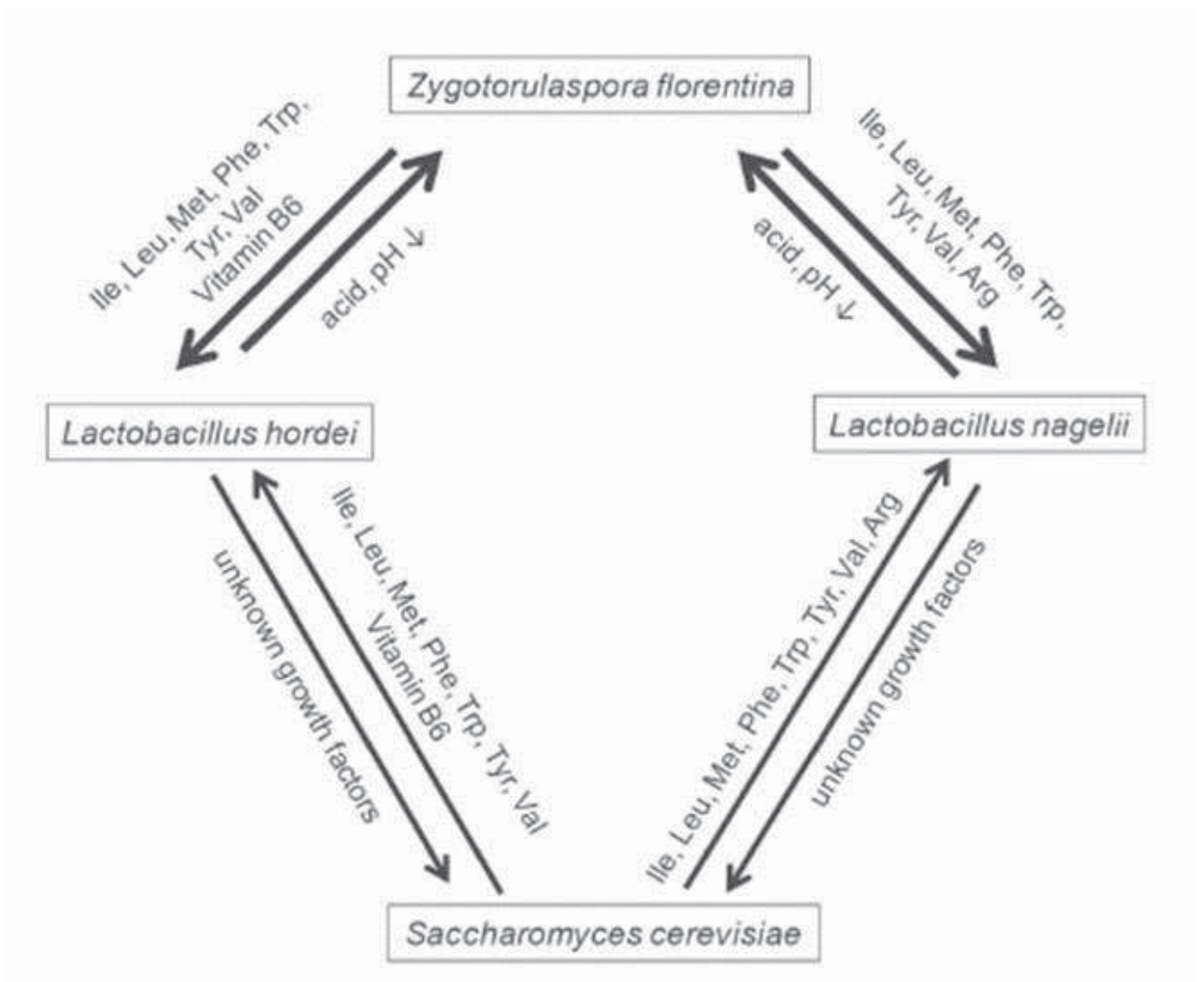


Figure 2.3 Interactions of main representative cultivable water kefir isolates
Source: Stadie et al., (2013).

2.6 Sugar metabolism by water kefir microorganisms

2.6.1 Water kefir lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are a heterogeneous group of microorganisms which use carbohydrates as primary carbon and energy source to produce lactic acid as the major end-product (Jay, 1992). LAB are Gram-positive (+) bacteria, acid tolerant, and facultative anaerobic microorganism. Typical LAB species belonging to the genera *Lactobacillus*, *Leuconostoc*, and *Lactococcus* can be found in water kefir (Gulitz et al., 2013; Moinas et al., 1980; Pidoux, 1989; Waldherr, 2010a). Metabolism of sugars by lactic acid bacteria is divided into two types, homofermentative and heterofermentative LAB (Figure 2.4 and Figure 2.5). Homo-fermentative LAB (*Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus* and some *Lactobacillus*) produce mainly lactic acid and hetero-fermentative LAB (*Leuconostoc*, *Oenococcus*, and certain *Lactobacillus*) yield a large variety of fermentation products such as acetic acid, ethanol, carbon dioxide, and formic acid (Kleerebezem & Hugenholtz, 2003). Homofermentative LAB ferment sugars via the Emden Meyerhof Parnas (EMP) pathway to pyruvate, which converts 1 mole of glucose into 2 moles of lactic acid as the final product by lactate dehydrogenase (LDH) (Figure 2.4). Under certain conditions such carbon limitation, homolactic metabolism can be shifted to a mixed-acid metabolism which is characterized by the production of formate, acetate, ethanol, and CO₂ in addition to lactate. There are several possible pathways for acetate production. Pyruvate can be metabolized anaerobically into acetate by pyruvate-formate-lyase (PFL), phosphor-trans-acetylase (PTA), and acetate kinase (ACK) or aerobically by the pyruvate dehydrogenase complex, PTA, and ACK (Kleerebezem & Hugenholtz, 2003).

In contrast, heterofermentative bacteria produce large amounts of lactate, CO₂ and ethanol from glucose using the phosphoketolase pathway (PKP). Fermentation of pentoses (xylose, ribose) leads to the formation of pyruvate and acetyl-P and their subsequent conversion to lactate and acetate, respectively. Hexoses (glucose, fructose, and mannose) in these bacteria can be converted to lactate, CO₂, and ethanol. CO₂ is a product of 6-P-gluconate degradation, which occurs during conversion of hexoses to pentoses. The specific enzyme of the heterofermentative pathway, D-xylulose-5P phosphoketolase, catalyzes the conversion of xylulose-5P to glyceraldehyde-3P (GAP) and acetyl-P. The GAP enters the EMP pathway leading to the production of lactate,

whereas acetyl-P is converted into ethanol (Figure 2.5) (Kleerebezem & Hugenholtz, 2003).

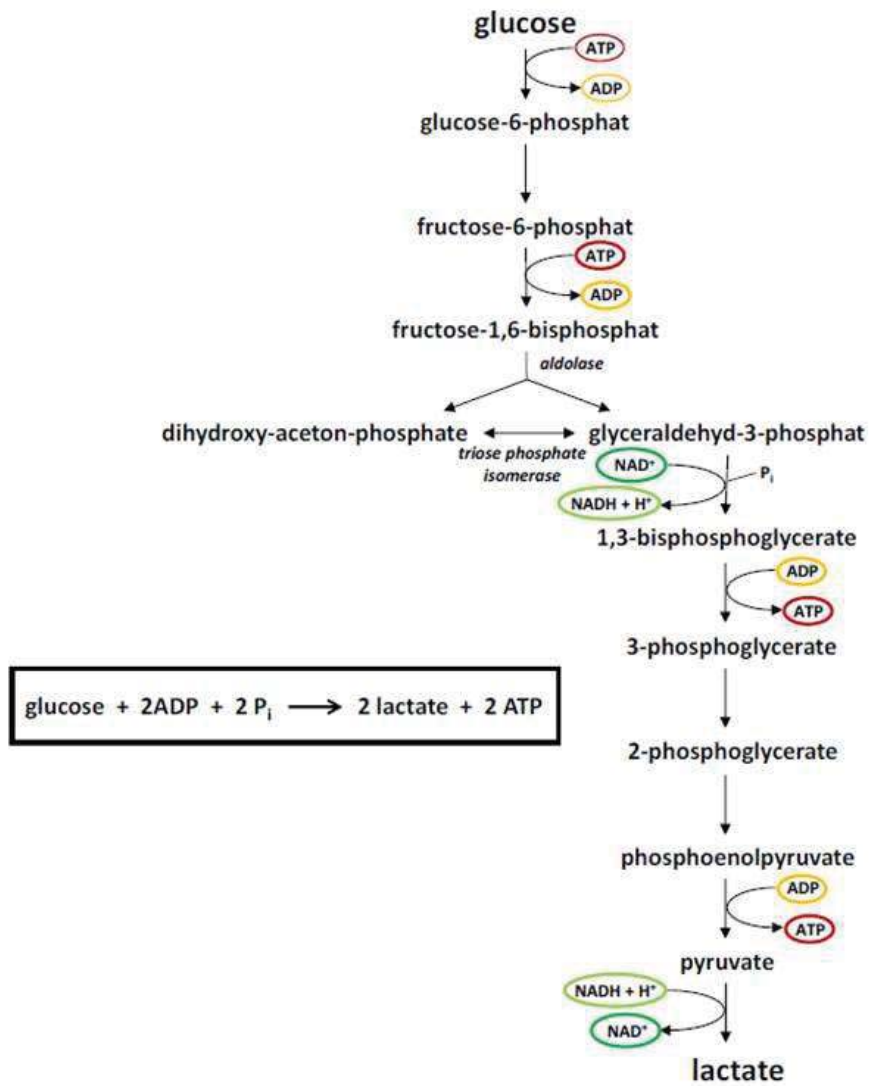


Figure 2.4 Sugar metabolisms by homofermentative lactic acid bacteria
 Source: Stadie, (2013)

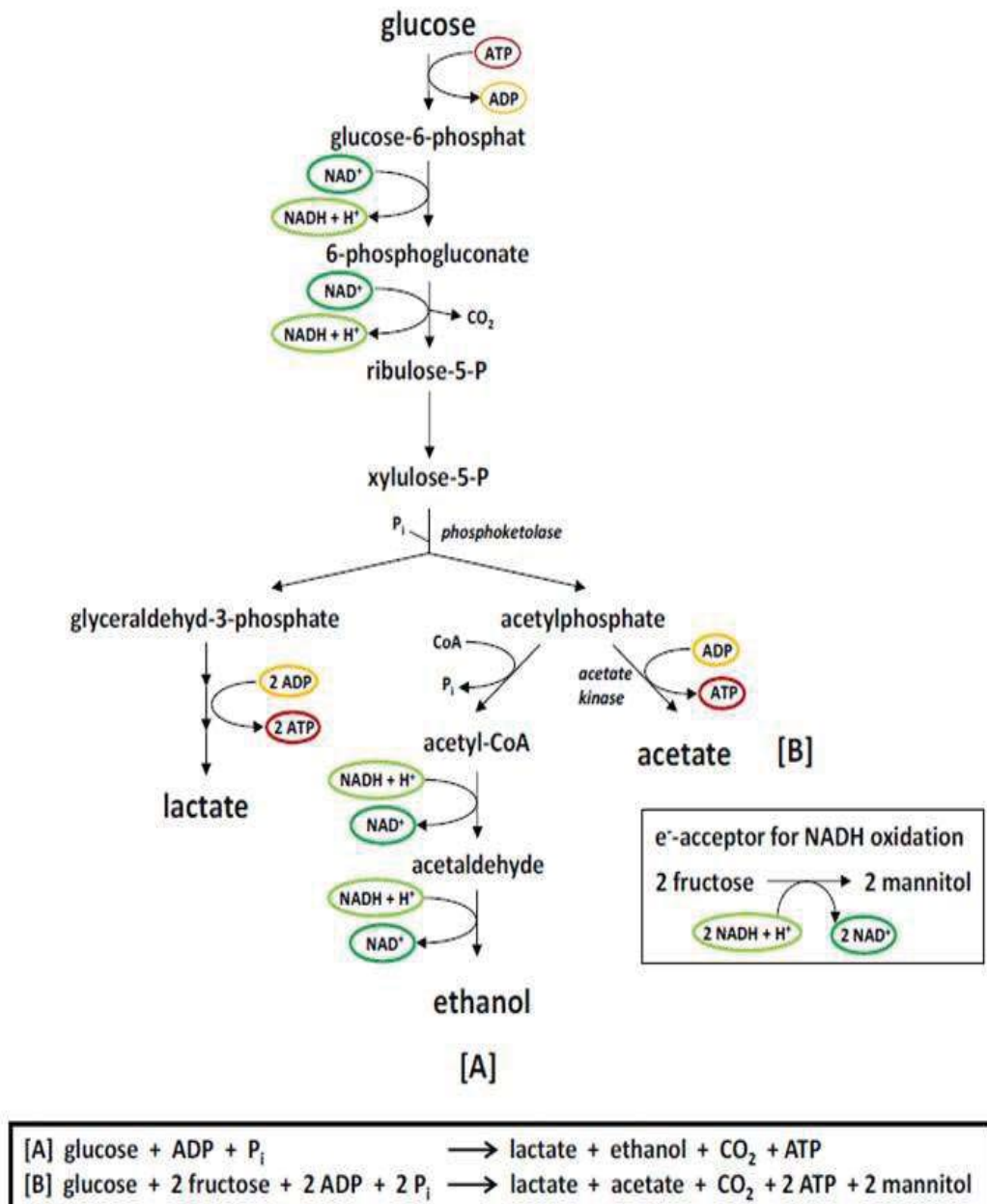


Figure 2.5 Sugar metabolism by heterofermentative lactic acid bacteria

Source: Stadie, (2013)

Table 2.3 Homo- and hetero-fermentative species of LAB in water kefir

Homofermentative LAB	Heterofermentative LAB
<i>Lactobacillus hordei</i>	<i>Lactobacillus hilgardii</i>
<i>Lactobacillus nagelii</i>	<i>Leuconostoc mesenteroides</i>
<i>Lactobacillus casei</i>	<i>Leuconostoc citreum</i>

Source: Gulitz et al., (2013).

2.6.2 Water kefir yeasts

Typical yeasts in water kefir grains belong to the genera *Saccharomyces*, *Zygorulasporea* (Gulitz et al., 2013), *Dekkera* (Laureys & De Vuyst, 2014), *Candida*, and *Hanseniaspora* (Pidoux, 1989). *Saccharomyces cerevisiae* commonly found in water kefir grains, is a facultative anaerobic yeast which able to metabolise glucose aerobically and anaerobically (Pronk et al., 1996). Further, *Saccharomyces cerevisiae* is known as Crabtree positive yeast (De Deken, 1966; Rodrigues et al., 2006). The phenomenon, in which suppression of respiration by high glucose concentration, is called Crabtree effect (De Deken, 1966). As glucose levels rise, pyruvate is diverted away from citric acid cycle into ethanol synthesis by conversion to acetaldehyde and CO₂ by pyruvate decarboxylase. To overcome this phenomenon (Crabtree effect) sugar may be added in stages to the fermentation vessel (Pfeiffer & Morley, 2014). Anaerobically, yeasts ferment glucose to ethanol which is commonly called alcoholic fermentation (Rodrigues et al., 2006).

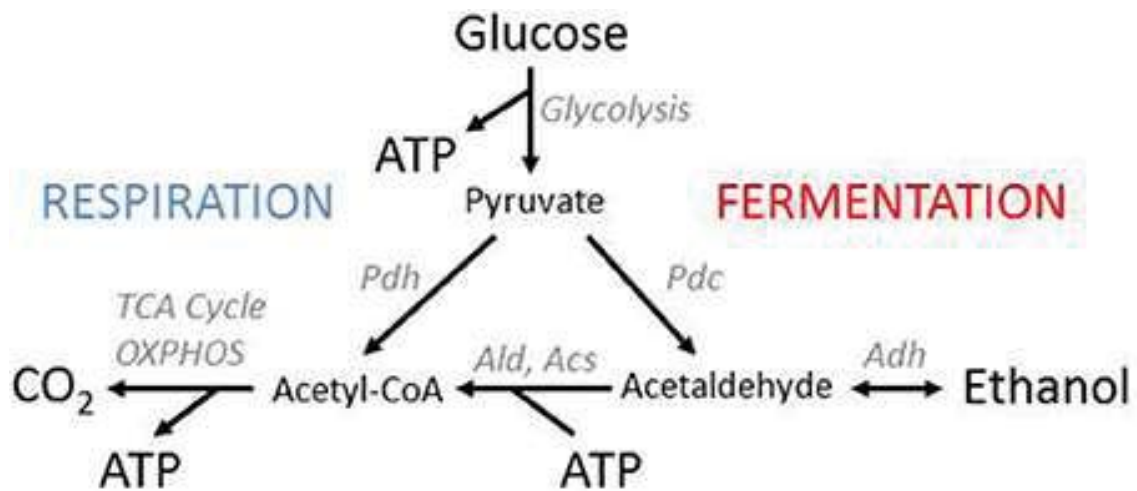


Figure 2.6 Sugar metabolism by yeasts

Source: Pfeiffer & Morley, (2014)

Water kefir medium commonly contains sucrose. The disaccharide sucrose is hydrolysed by yeast invertase to fructose and glucose (Pronk et al., 1996; Rodrigues et al., 2006). Glucose are converted into pyruvate through glycolysis. Further, pyruvate can be degraded either through the respiration pathway or fermentation pathway (Berg

et al., 2002). In the respiration pathway, pyruvate is transformed by pyruvate dehydrogenase (Pdh) into acetyl-Coenzyme A, which is then oxidized in the tricarboxylic acid (TCA) cycle to CO₂. While in fermentation, pyruvate is converted to acetaldehyde by pyruvate decarboxylase (Pdc) and acetaldehyde is then reduced to ethanol catalysed by alcohol dehydrogenase (Adh). Further, acetaldehyde is subsequently converted into acetyl-Coenzyme A, the substrate for the citric acid cycle, by acetaldehyde dehydrogenase (Ald) and acetyl-CoA synthetase (Acs) (Pfeiffer & Morley, 2014; Pronk et al., 1996;). In the presence of oxygen and absence of repression pyruvate can be metabolised to CO₂ and energy in form of ATP (Pfeiffer & Morley, 2014). The sugar metabolism of yeast can be seen in Figure 2.6.

2.7 Exopolysaccharides (EPS) production by water kefir microorganisms

Bacteria can produce polysaccharides by the action of enzyme glucosyl transferase and excreted extracellularly. The microbial polysaccharides are called exopolysaccharides (EPS). The enzyme glucosyl transferase are found in several genera of lactic acid bacteria such as *Lactobacillus* and *Leuconostoc* and high levels of dextran can be produced by this enzyme (Waldherr et al., 2010). Water kefir grains are described to consist of dextran and some LAB presence in water kefir grains are capable of producing EPS or gel (Stadie, 2013). *Lactobacillus hilgardii* is considered important for the grain formation in water kefir grains (Pidoux, 1989). The EPS derived from *Lactobacillus hilgardii* is a single polysaccharide which has characteristics of a dextran structure (α -(1,6)-glucose, α -(1,3,6)-glucose, terminal- α -glucose, and α -(1,4,6)-glucose with additional α -(1,3)-glucose (Pidoux et al., 1990). The presence of α -(1,3)-glucose in the dextran structure make the polysaccharide insoluble in water. In addition, dextran with a high percentage of α -(1,3)-branching are described to be insoluble (Cote & Skory, 2012; Shukla et al., 2011).

Gulitz et al., (2011) and Stadie (2013) reported that there are other lactic acid bacteria in water kefir grains, which have the ability to produce EPS including *Lactobacillus nagelii*, *Lactobacillus hordei*, *Leuconostoc mesenteroides*, and *Leuconostoc citreum*. The bacteria produced a glucan as indicated by glucose monomer content of the EPS. Stadie (2013) reported that EPS concentration detected in liquid medium could range between 9.2-32.5 g/l. In addition, a strong EPS-producer can produce 10-20 g/L EPS,

whereas about 30g/L EPS can be produced by a very strong EPS-producer. Further, *Lactobacillus hilgardii* produced very high EPS with 30.8-32.5 g/L EPS (Stadie, 2013). Typical gel-producing lactic acid bacteria in water kefir is shown in Figure 2.7. According to Laureys and De Vuyst, (2104), water kefir grains mass increased during 24 hours fermentation from 16.4 g to 28.6 g and the accumulation of fructose in the water kefir liquor indicated that the grains contained glucan.

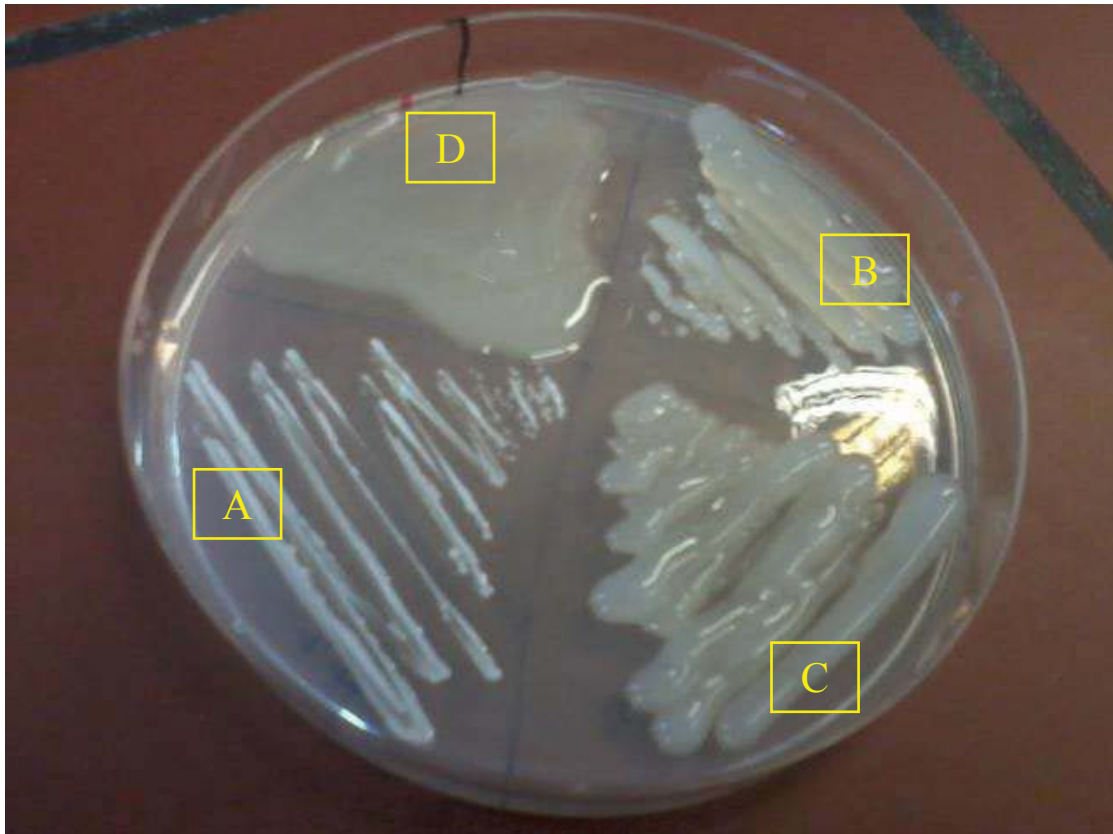


Figure 2.7 EPS production from lactic acid bacteria in water kefir grains

Source: Stadie, (2013)

A = no EPS productionn (*Lb. nagelii*, TMW 1.1825); B = slight EPS production (*Lb. nagelii*, TMW 1.1826); C = strong EPS production (*Lb. hordei*, TMW 1.1907); D = very strong EPS production (*Lc. citreum*, TMW 2.1194) on sucrose containing agar plates.

2.8 Chemical composition of water kefir beverages

The chemical composition of water kefir beverages is variable and not well-described. The type of substrates and the microbial composition of the grains will affect its sensory

and chemical properties of the final product (Ottles & Cagindi, 2003). The main metabolites of water kefir formed during fermentation are lactic acid and ethanol while acetic acid, glycerol, and mannitol are produced in low concentrations (Laureys & De Vuyst, 2014). In water kefir beverage, the total content of sucrose was 1.2 g/l after 24 hours of fermentation (Laureys & De Vuyst, 2014). The amount of ethanol and lactic acid content after 72 hours of fermentation was 20.3 g/l and 4.9 g/l, respectively (Laureys & De Vuyst, 2014). In addition to organic acids and ethanol which are generated during fermentation, there are volatile compounds that present and impart aroma to water kefir. Laureys and De Vuyst, (2014) reported that the most prevalent volatile aroma compounds are ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate with levels of 13.40 mg/l, 0.11 mg/l, 0.37 mg/l, 3.44 mg/l, and 1.40 mg/l, respectively. However, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate give the highest impact on the aroma of the final product, contributing to its fruity and floral notes.

2.9 Physico-chemical and microbiological changes during water kefir fermentation

2.9.1 Colour

The colour of water kefir beverages significantly changed during the fermentation (Corona et al., 2016; Randazzo et al., 2016). The lightness of the product which is indicated with L* value generally increased during water kefir fermentation (Corona et al., 2016; Randazzo et al., 2016). Jayabalan et al., (2007) also reported the similar colour changes pattern in the fermentation of tea beverage (kombucha) which involving bacteria and yeast during fermentation. Changes of colour in kombucha may occur due to the biotransformation or degradation of polyphenols, such as thearubigin and theaflavins into smaller chemical structure in the acidic environment by enzymes excreted by microorganism (Chu & Chen, 2006; Jayabalan et al., 2014).

2.9.2 Sugar and total soluble solids (TSS, °Brix)

Concentration of sugars may change during water kefir fermentation. The major substrate, sucrose, is hydrolysed into monosaccharides (glucose and fructose) by the enzyme, invertase, which is produced by yeasts during fermentation (Magalhaes et al.,

2010; Stadie et al., 2013). The decrease in sucrose concentration correlates with the decrease of total soluble solids (°Brix) (Corona et al., 2016; Magalhaes et al., 2010; Randazzo et al., 2016). Laureys and De Vuyst (2014) reported that the level of sucrose gradually decreased from 47.5 g/l to 1.2 g/l during 24 hours of fermentation. This result agrees with another study which reported that concentration of sucrose at 40 mg/ml decreased to 28 mg/ml after 24 hours of fermentation (Magalhaes et al., 2010). Stadie et al., (2013) reported that the sucrose was fermented and concentrations of fructose and glucose increased. However, the concentration of glucose decreased after 36 hours and the content of fructose reduced after 60 hours fermentation (Stadie et al., 2013).

2.9.3 pH and organic acids

The production of organic acids during water kefir fermentation rapidly decreased pH of the product (Laureys & De Vuyst, 2014; Magalhaes et al., 2010). According to Laureys & De Vuyst, (2014), the pH of water kefir beverage decreased from 4.26 to 3.45 after 72 hours fermentation and then the pH decreased slowly to 3.35 after 192 hours of fermentation. Stadie et al., (2013) has also reported that the pH decreased rapidly from 6.5 to 3.5 within 48 hours of fermentation. While the pH decreased gradually, the concentration of organic acids including lactic acid and acetic acid increased from 0.7 g/l to 4.9 g/l and 0.1 g/l to 1.0 g/l, respectively after 72 hours fermentation (Laureys & De Vuyst, 2014). Other studies have shown that during water kefir fermentation, concentration of lactic acid increased up to 3.7 g/l within 96 hours of fermentation (Stadie et al., 2013) and acetic acid increased and reached 1.4 mg/ml after 24 hours fermentation (Magalhaes et al., 2010). The amount of lactic acid and acetic acid in the final product can be varied depending on the type of substrates used. A water kefir beverage made from fruit juices including apple, grape, kiwi, pomegranate, pear, and quince had lactic acid and acetic acid concentration between 0.02-1.00 g/l and 0.06-0.16 g/l, respectively after 48 hours fermentation (Randazzo et al., 2016). Corona et al. (2016) also reported that levels of lactic acid and acetic acid of water kefir beverages made from vegetable juices were diverse at the end of 48 hours fermentation, ranging between 0.58-4.81 g/l and 0.03-1.90 g/, respectively. The concentration of lactic acid and acetic acid in cocoa kefir beverages reached 1.0-5.5 g/l and 0.5-1.0 g/l, respectively, after 72 hours fermentation (Puerari et al., 2012). These observations indicated that the simultaneous process of sugar metabolism by homofermentative and heterofermentative

bacteria occurred and were followed by the production of lactic acid and acetic acid (Magalhaes et al., 2010; Puerari et al., 2012).

2.9.4 Ethanol, sugar alcohol and volatile compounds

In water kefir fermentation, ethanol, sugar alcohol such as mannitol, and volatile compounds are also produced. During fermentation, concentration of ethanol increased linearly and reached 20.3 g/l after 72 hours (Laureys & De Vuyst, 2014). Corona et al., (2016) reported that the amount of ethanol increased and ranged between 0.06-3% (v/v) in the final product. Similar results have been reported with the concentration of ethanol increasing up to 4.96% (v/v) after 48 hours fermentation depending on the source of the fermentation medium (Randazzo et al., 2016). Regarding sugar alcohol such mannitol, the concentration increased ranging between 1.0-8.0 g/l after 72 hours of fermentation (Stadie et al., 2013). Different compositions of volatile compounds were detected after water kefir fermentation. This therefore showed that volatile compounds increased during fermentation (Corona et al., 2016; Laureys & De Vuyst, 2014; Randazzo et al., 2016). Corona et al., (2016) and Randazzo et al., (2016) indicated that esters were responsible for the unique aroma of water kefir beverages. Laureys and De Vuyst (2014) reported that isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were the major esters that dominated the aroma of water kefir which contributed to its fruity and floral notes.

2.9.5 Viable cells of lactic acid bacteria and yeasts

There are different types of lactic acid bacteria and yeast present in water kefir grains. However, lactic acid bacteria from the genus *Lactobacillus* and yeasts from genus *Saccharomyces* were the most frequently found in water kefir grains and beverages (Gulitz et al., 2011; Marsh et al., 2013; Miguel et al., 2011). Viable cells count of lactic acid bacteria and yeasts generally increase during fermentation (Corona et al., 2016; Randazzo et al., 2016). The initial count of lactic acid bacteria in water kefir beverage was 6.82 log CFU/ml and reached 8.32 log CFU/ml by the end 24 hours of fermentation. Meanwhile, the number of yeasts was 5.63 log CFU/ml which increased to 7.31 log CFU/ml after 24 hours fermentation (Magalhaes et al., 2010). Laureys and De Vuyst (2014) reported that viable counts of lactic acid bacteria and yeasts in water

kefir grains were higher in water kefir liquor. Viable cells counts of lactic acid bacteria and yeasts in water kefir grains were 8.2 log CFU/g and 7.4 CFU/g, respectively and 6.9 log CFU/ml and 6.3 log/ml in water kefir liquor, respectively. Further, the composition and abundance of microflora in water kefir grains also may vary depending on its origin. Gulitz et al., (2011) recorded that viable cell counts of lactic acid bacteria and yeast from three water kefir grains were 8.11-8.2 log CFU/g and 6.76-7.43 log CFU/g, respectively.

2.10 Beneficial effect of water kefir

2.10.1 Antimicrobial activity

Water kefir grains have been reported to have antimicrobial activity against pathogenic microorganisms and effectively inhibit a wide variety of microorganisms as such *Candida albicans*, *Salmonella Typhi*, *Shigella sonnei*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Streptococcus pyogenes*, *Streptococcus salivarius*, and *Pseudomonas aeruginosa* (Rodrigues et al., 2005; Silva et al., 2009). The inhibition halo zone of water kefir against several pathogenic microorganisms is shown in Table 2.4. The antimicrobial activity of water kefir is associated with the production of organic acids, peptides (bacteriocins), carbondioxide, and ethanol. These compounds may have beneficial effects in the reduction of food borne pathogens.

Table 2.4 Antimicrobial activity of fermented broth by water kefir grains

Target microorganism	Ø of inhibition zone (mm)
<i>Streptococcus pyogenes</i>	27.2
<i>Streptococcus salivarius</i>	24.9
<i>Staphylococcus aureus</i>	30.0
<i>Pseudomonas aeruginosa</i>	30.2
<i>Salmonella typhi</i>	25.6
<i>Escherichia coli</i>	28.4
<i>Listeria monocytogenes</i>	29.3
<i>Candida albicans</i>	28.0
<i>Shigella sonnei</i>	12.0

Source: Rodrigues et al., (2005) & Silvia et al., (2009)

2.10.2 Antioxidant potency

Water kefir beverage is the results of fermentation of sugar solution by water kefir grains which consist of lactic acid bacteria and yeasts. During fermentation, there are many important substances have been produced. Muneer Alsayadi et al., (2012) investigated the antioxidant activity of water kefir by determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, inhibition of ascorbate autoxidation and reducing power activity. Water kefir showed great ability to DPPH-scavenging, ranging from 9.88-63.17% and inhibiting ascorbate oxidation at 6.08-25.57% (Muneer Alsayadi et al., 2012). The high ability of water kefir for inhibition ascorbate autoxidation is associated with lactic acid bacteria and yeasts which also can refer to their simultaneously existing and their intracellular and extracellular metabolites. Furthermore, water kefir showed high ability to reduce Fe^{3+} /ferricyanide complex to the ferrous form. The reducing power increased as the concentration of water kefir increased (Muneer Alsayadi et al., 2012). Muneer Alsayadi et al., (2012) concluded that water kefir can be a source of natural antioxidants with good potential to health.

2.11 Tea (*Camellia sinensis*)

Tea is produced from the dried young tender leaves, shoots, or leaf stalks of the plant *Camellia sinensis* (Anggraini et al., 2016; Balentine, Wiseman, & Bouwens, 1997; Hayat et al., 2015) which originated from China (Carr, 1972; Sharangi, 2009). Tea is one of the most popular and widely consumed beverages in the world after water (Lin et al., 2014; Najgebauer-Lejko, 2014; Owuor, 2014). About three billion kilograms of tea is produced yearly and consumed by two thirds of the world's population (Gupta, Saha, & Giri, 2002; Hayat et al., 2015; Khan & Mukhtar, 2013). Over the years, tea has been regarded as a healthy beverage due to its wide variety of antioxidants compound (Hayat et al., 2015; Higdon & Frei, 2003; Lambert, 2013) which could bring benefits for human health and prevent chronic diseases such as cancer and cardiovascular diseases (Bushman, 1998; Higdon & Frei, 2003; Lambert, 2013; Zhou et al., 2016). Recently, tea consumption has continuously increased not only because of its health benefits but also its pleasant flavour (Lin et al., 2014; Owuor, 2014).

There are many different types of tea derived from the leaves of *Camellia sinensis* which can be classified based on the processing methods into four main groups. These are white tea, green tea, oolong tea, and black tea (Chang et al., 2004; Lin et al., 2010; Owuor, 2014). Various tea processing methods are outlined in Figure 2.6. Of the total amount of tea produced and consumed worldwide, black tea is the major tea accounting for over 78% which is usually consumed in Western countries (Cabrera, Gimenez, & Lopez, 2003; Seeram et al., 2006) whereas 20% is green tea which is popular in Asian countries and oolong tea 2% which is produced and preferred mainly in China and Taiwan (Chang et al., 2004; Lin et al., 2010).

2.11.1 Black tea

Black tea is the most popular and widely consumed tea around the world (Chang et al., 2004) therefore there are multifarious black tea products in the market such as tea bag, flavoured tea, and iced tea (Rao & Ramalakshmi, 2011). Black tea also can be used as raw ingredient in the making of fermented tea as a variety of value-added products and these include kombucha tea and cider tea (Dufresne & Farnworth, 2000; Jayabalan et al., 2014; Rao & Ramalakshmi, 2011). Black tea is the result of biochemical oxidation of polyphenols in crushed young tender leaves involving multistep enzymatic oxidation process in the presence of oxygen that is terminated by drying (Hara et al., 1995; Owuor, 2014; Rao & Ramalakshmi, 2011). The basic principle of manufacturing black tea is shown in Figure 2.8.

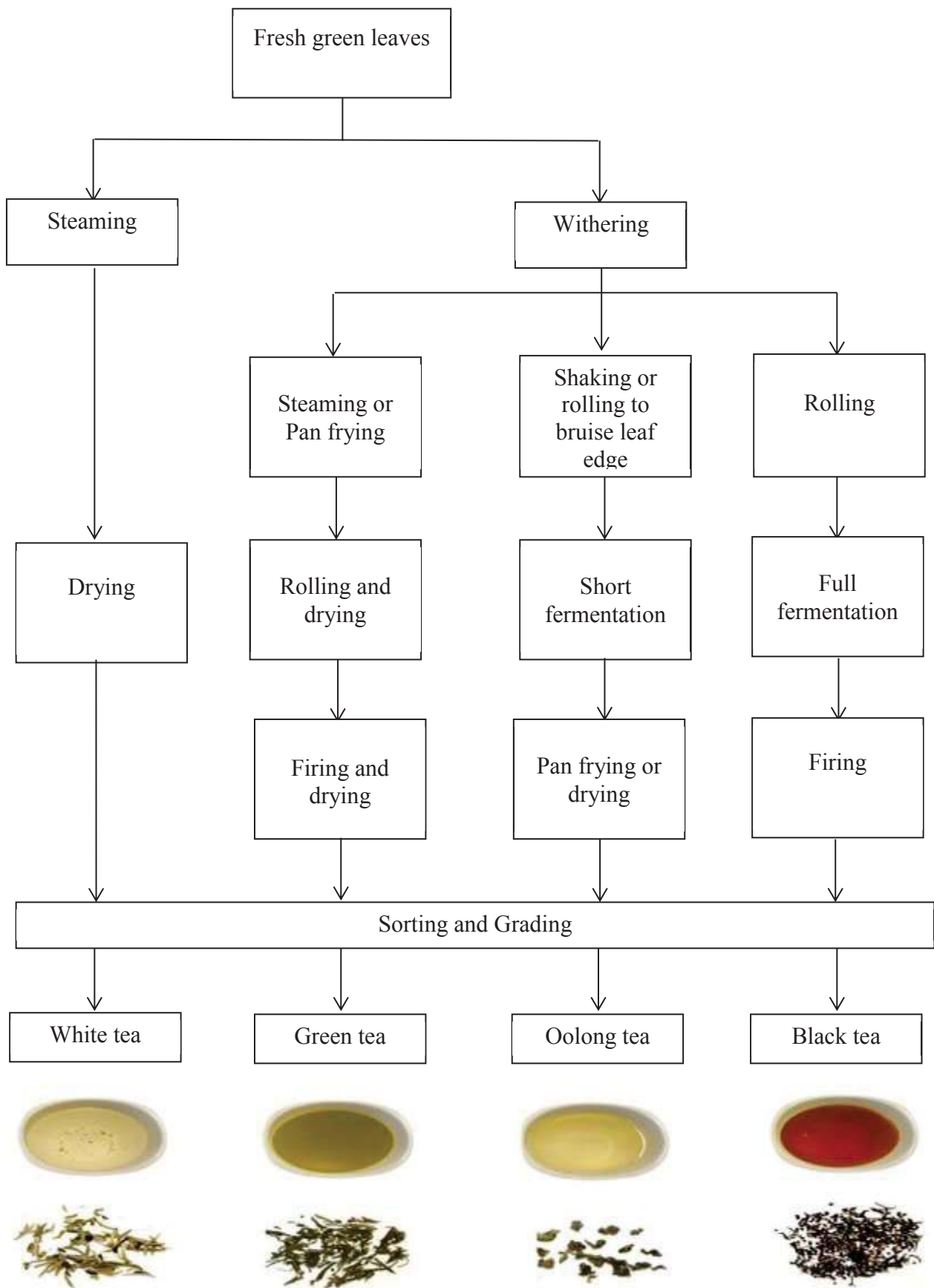


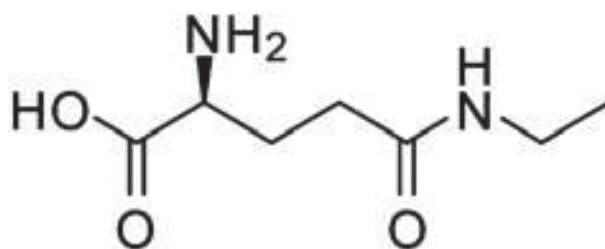
Figure 2.8 Processing methods of varieties of tea

Source: Owuor (2014)

2.11.2 Major chemical composition of black tea leaves

2.11.2.1 Amino acids

Black tea contains about 2-3% amino acids (Rao & Ramalakshmi, 2011; Sharma & Jagan, 2009) including alanine, arginine, asparagine, aspartic acids, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, theanine, threonine, and tyrosine (Table 2.6). Theanine is the most abundant amino acids present in black tea which constitutes about 50-60% of total free amino acids in black tea (de Mejia, Ramirez-Mares, & Puangpraphant, 2009; Rao & Ramalakshmi, 2011) and is known as the only free amino acids found in tea. It presents a unique form, L-theanine (γ -N-ethylglutamine) (Figure 2.9) which is responsible for the sweet and savoury taste (Sari & Velioglu, 2013) and is also a constituent of thearubigin which is responsible for the color of the tea brews (Rao & Ramalakshmi, 2011). Theanine is reported to improve the brain's ability to concentrate, learn, and memorize. Further, it has been demonstrated to increase serotonin, gamma-amino butyric acid (GABA), and dopamine levels in the brain which impart neuroprotective effects (Syu et al., 2008).



L-Theanine

Figure 2.9 Chemical structure of L-theanine

Source: Yang et al., (2013)

Table 2.5 Amino acids levels in black tea

Amino acids	mg/g (dry mass)	Amino acids	mg/g (dry mass)
Alanine	0.14-0.81	Leucine	0.11-0.52
Arginine	0.07-0.62	Phenylalanine	0.16-1.34
Asparagine	0.25-1.64	Serine	0.22-1.12
Aspartic acid	0.30-1.79	Theanine	0.49-4.12
Glutamine	0.47-2.28	Threonine	0.07-0.41
Histidine	0.07-1.41	Tyrosine	0.10-1.45
Isoleucine	0.16-0.65		

Source: Alcazar et al., (2007)

2.11.2.2 Alkaloids

Caffeine, theobromine and theophylline are methylxanthine compounds in black tea which are classified as alkaloids (Figure 2.10) (Li et al., 2013). Caffeine is the richest alkaloid among the three xanthine molecules in black tea. Lin et al., (2014) reported that the concentration of caffeine in black tea infusion was 43.07 mg/100 ml (Lin et al., 2014). Caffeine constitutes 3-4% of the dry weight of black tea leaves which provides a significant contribution to the briskness and creamy properties of tea brew (Horzic et al., 2009; Sharma & Jagan, 2009) while theobromine and theophylline are present in low quantities (Sharma & Jagan, 2009). Horzic et al., (2009) reported the concentrations of caffeine, theobromine and theophylline content in the black tea infusion at 293.97 mg/l, 45.93 mg/l, and 9.27 mg/l, respectively. The amount of caffeine, theobromine, and theophylline in black tea infusion were 4.002-8.657 mg/100mg, 0.190-2.607 mg/100mg, and 0.088-1.079 mg/100mg, respectively and this result was obtained from different brands of black tea (Tokusoglu, Unal, & Balaban, 2008). Further, the brewing conditions such as time, temperature, and the amount of tea leaves or powder used also affect the alkaloid content of black tea beverage (Astill et al., 2001). Horzic et al., (2009) studied that higher temperature used for black tea brewing resulted in higher amounts of caffeine, theobromine, and theophylline in the black tea infusions.

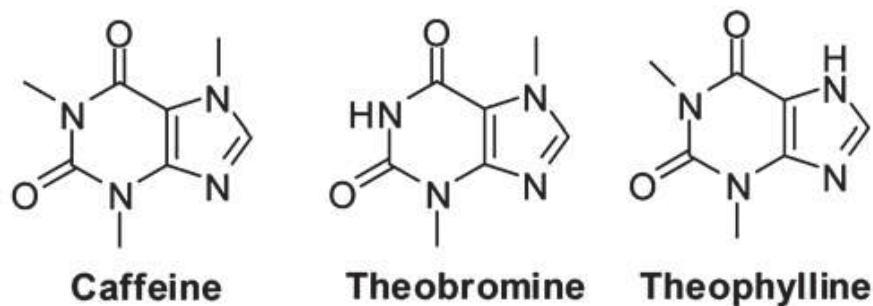


Figure 2.10 Chemical structure of caffeine, theobromine, and theophylline

Source: Li et al., (2013)

2.11.2.3 Phenolic polyphenols

Phenolic polyphenols is the most abundant compound found in tea leaves and it constitutes about 30% on dry weight basis. Among the polyphenols, flavanols which are known as catechins represent major portion in tea polyphenols (Łuczaj & Skrzydlewska, 2005). There are four major derivatives of catechins, epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (Figure 2.11) (Rao & Ramalakshmi, 2011). Tokusoglu et al., (2008) reported the range value of catechin, EGCG, EGC, ECG, and EC in black tea infusions were 0.107-0.286 mg/100mg, 0.356-5.972 mg/100mg, 0.260-4.298 mg/100mg, 0.208-1.338 mg/100mg, and 0.114-5.023 mg/100mg, respectively. The amount of phenolic concentration in black tea infusion was influenced by the infusion time, temperature, and also the origin of the black tea leaves (Kelebek, 2016; Ramalho et al., 2013).

Due to the oxidation process of catechins by enzymes polyphenol oxidase in black tea manufacturing during fermentation, about 75% of the catechins compounds are oxidised and polymerized into quinones generating theaflavins (TF) and thearubigins (TR) (Figure 2.12) (Łuczaj & Skrzydlewska, 2005; Sharma & Jagan, 2009). TF and TR are responsible for the colour of black tea in which TF gives red-orange or yellowish-brown colour and TR provides a reddish-brown colour (Leung et al., 2001; Li et al., 2013; Sharma & Jagan, 2009). During fermentation, there are four major TFs formed shown in Figure 2.13 while TR formed is shown in Figure 2.10. Kelebek (2016) reported the concentration of TF in black tea infusion at between 23.71-48.25 mg/l. This result was

obtained from different infusion time and brewing temperature used. The higher brewing temperature and the longer infusion time resulted in higher TF levels (Kelebek, 2016). The concentration of TR in black tea infusion was in a range of 101.61-127.01 mg/g (Alasalvar et al., 2013). This result may vary due to the variety of tea and brewing techniques used (Peterson et al., 2004). The TR formation mechanism is illustrated in Figure 2.14.

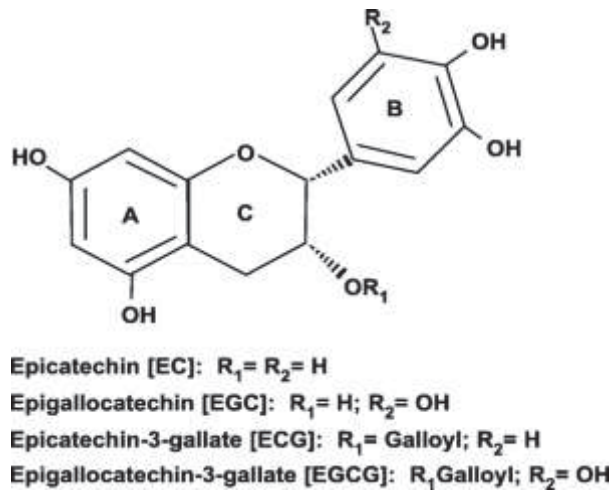


Figure 2.11 Structure of catechins and its derivatives in tea leaves

Source: Luczaj & Skrzydlweska, (2005)

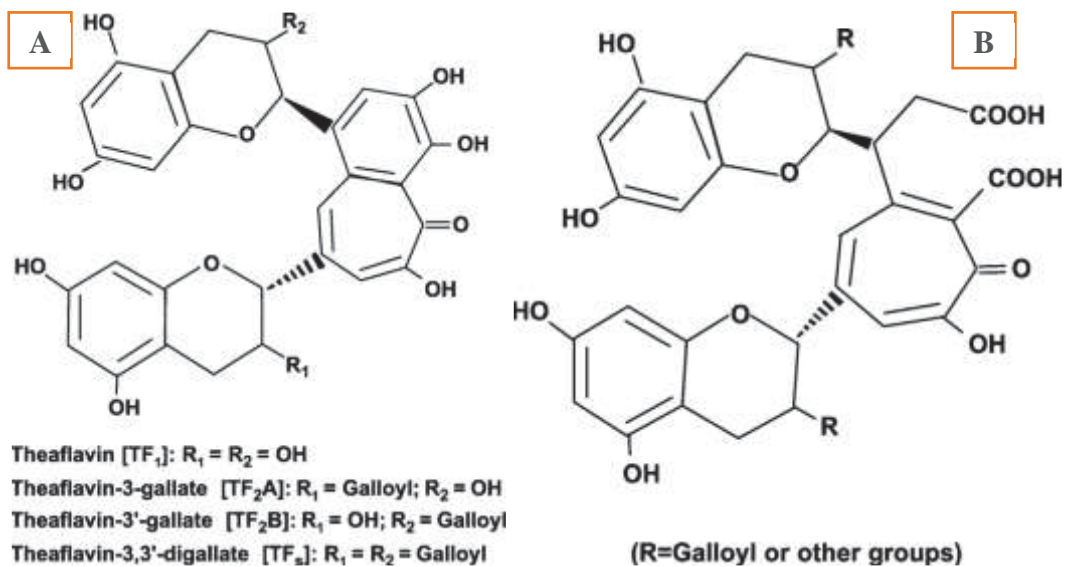


Figure 2.12 Structure of (A) theaflavins and (B) thearubigins

Source: Luczaj & Skrzydlweska, (2005)

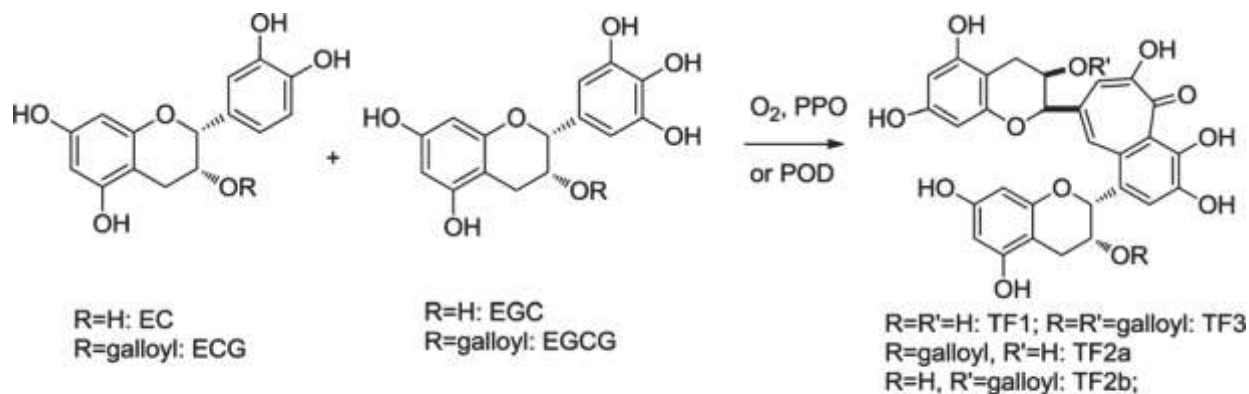


Figure 2.13 The flavins formed from catechins during fermentation of tea

Source: Li et al., (2005)

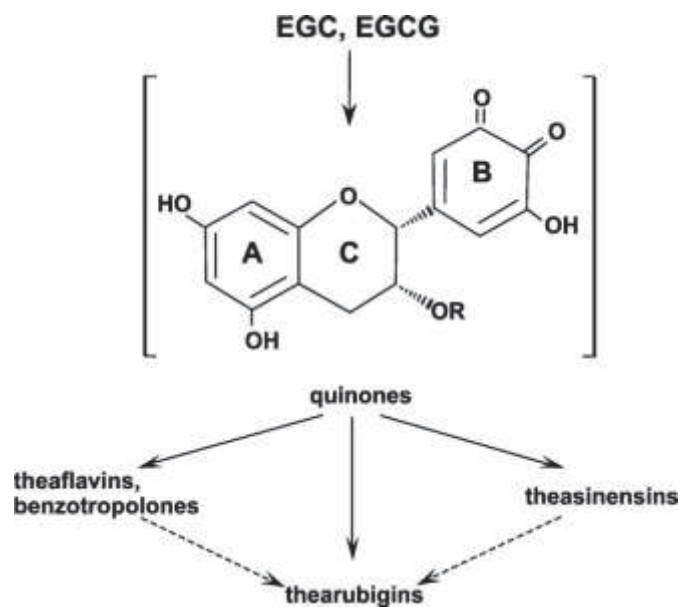


Figure 2.14 Mechanism for the formation of thearubigins

Source: Luczaj & Skrzydlweska, (2005)

2.11.2.4 Phenolic acids

Gallic acid and theogallin (Figure 2.15) are the most abundant simple polyphenols present in tea and its amount increases during oxidation in black tea manufacturing due to its liberation from catechins gallates (Harbowy & Balentine, 1997). Hilal and Engelhardt (2007) reported that gallic acid and theogallin concentration in black tea was about 0.16-0.60 mg/g and 0.1-1.0 mg/g, respectively.

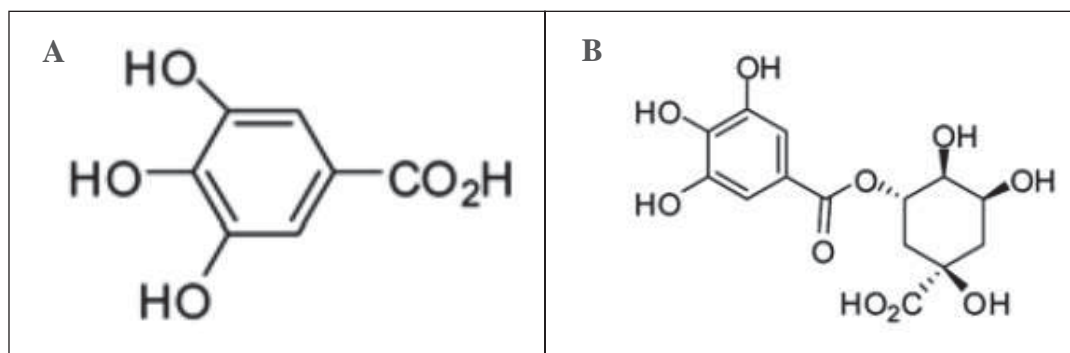


Figure 2.15 Structure of (A) gallic acid and (B) theogallin.

Source: Li et al., (2005)

2.11.3 Manufacturing process of black tea

There are five stages involved in the processing of black tea from fresh tea leaves which include withering, rolling, fermentation, firing, and sieving. Each step in the manufacturing process play important role to produce high quality of black tea product (Rao & Ramalakshmi, 2011).

2.11.3.1 Withering

Freshly picked tea leaves normally contains 75-85% moisture content which needs to be reduced to 50-65% (Bokuchava & Skobeleva, 1980). The partial reduction of water content in tea leaves is called withering. The purpose of withering is not only to remove a third of the moisture in fresh tea leaves but it also renders the tea leaves soft and pliable as a physical conditioning for subsequent processing (Hayat et al., 2015). There are two types of withering methods, traditional and modern withering (Bokuchava &

Skobeleva, 1980). The common method for withering is based on the traditional process in which tea leaves are spread on large troughs from steel, wood, or brick which fitted with a mesh that is designed to allow airflow through the leaf. Natural or traditional withering process takes 18-20 hours depending on environmental conditions (Rao & Ramalakshmi, 2011). Therefore, many tea producing factories use modern withering with large troughs which are ventilated by large ventilators and requires about 6-8 hours. The shortened withering time is adequate for the tea leaf under undergo sufficient physical, chemical, and biochemical changes to produce high quality tea withering (Bokuchava & Skobeleva, 1980). Temperature, relative humidity, and duration of withering process have significant effects on the chemical reactions (Table 2.6) that take place in the tea leaves to produce good quality of final products (Bokuchava & Skobeleva, 1980; Obanda et al., 2004).

Table 2.6 Biochemical changes during withering of black tea

Chemical changes in tea leaf	Effect of changes
Increase in amino acids	Precursors of flavour compounds and contribution to colour and aroma of the tea
Increase in activity of tea leaf enzymes (β -glucosidase and invertase)	Indication of withering time
Increase in essential oils	Contribution to aroma of tea
Decrease in catechin content	Indication of oxidation and contribution to colour of the tea

Source: Bokuchava & Skobeleva, (1980); Obanda et al., (2004)

2.11.3.2 Rolling

Rolling is the second stage in black tea manufacturing which is usually called maceration or leaf distortion process (Rao & Ramalakshmi, 2011). In the rolling step, tea leaves are twisted and broken to allow the leaves to macerate and juices to express. During this stage, juice which contains tannin and catechin come into contact with enzymes, polyphenol oxidase, in the tea leaves and then triggers the fermentation process (Bokuchava & Skobeleva, 1980). There are two methods of rolling in black tea processing, orthodox and Crush-Tear-Curl (CTC) method. Among these two methods,

CTC style is commonly used in black tea factories as it gives high yield and is cost-effective (Rao & Ramalakshmi, 2011). In the CTC style, the tea leaves undergo crushing, tearing, and curling in between two metal cylinders with serrated surfaces thus facilitating direct contact between the juice and the air. Further, the tea produced by CTC method produces more colour and increases theaflavin content (Cloughley et al., 1981; Owuor et al., 1989).

2.11.3.3 Fermentation

The basic principle of manufacturing black tea is “fermentation” which refers to the enzymatic browning reaction induced by oxidative enzymes primarily, o-diphenol oxidase (Bokuchava & Skobeleva, 1980; Rao & Ramalakshmi, 2011). This step is very important and it is influenced by temperature, humidity, and presence of oxygen during fermentation. In the fermentation process, rolled tea leaves are spread in layers of 4-5 cm and remain there for 2-4 hours at 20-30°C with relative humidity 96-98% and under constant oxygen supply. The fermentation process used depends on the variety of tea and rolling technique used (Bokuchava & Skobeleva, 1980). Physicochemical changes occur during black tea fermentation, including aroma, colour, and taste of the tea. The colour of the tea leaves change from green to a coppery-red shade and improves the taste and aroma of the tea. Oxidation process leads to the decreasing of tannin which is responsible for the bitter taste and develop an astringent taste of the tea. Further, grassy odour gradually changes to the typical pleasant aroma of black tea (Bokuchava & Skobeleva, 1980; Owuor, 2014; Rao & Ramalakshmi, 2011).

2.11.3.4 Firing or Drying

The objective of firing or drying is to inactivate enzymatic activity, thereby terminating fermentation and to reduce moisture level of the fermented tea leaves. This process is accomplished by exposing the fermented tea leaves to hot air (88-93°C) in two stages. The first firing is performed to reduce moisture content to 18-20% while the second firing reduces to 3-4% (Bokuchava & Skobeleva, 1980; Rao & Ramalakshmi, 2011). There are two methods of drying, endless chain pressure (ECP) and fluid bed drier (FBD). In the ECP dryer, the inlet air temperature is 88-91°C and exhaust hot air temperature ranges between 49-54°C. Whereas, in the FBD inlet temperature is about

127°C and outlet temperature ranges between 88-93°C. ECP has been used in black tea manufacturing for decades and is more efficient in terms of energy consumption (Bokuchava & Skobeleva, 1980; Temple & van Boxtel, 2000). Apart from removal of moisture content, several changes occur at this stage including increase of amino acids, volatile compounds and browning reactions and loss of soluble carbohydrates (Owuor, 2014).

2.11.3.5 Sorting and grading

Sorting and grading are important stage for the marketing of tea. Sorting is used to remove tea fibers and stalks which can be done manually by hand-picking or mechanically by using sorting machine. The grading stage is a classification of tea leaf according to size by passing tea through mechanical oscillating sieves. Stalks and fibre particles are removed electro-statically. Major grades of black tea are orange pekoe (OP), broken orange pekoe (BOP), fanning (F), and dust (lowest grade of tea) (Bokuchava & Skobeleva, 1980; Owuor, 2014; Rao & Ramalakshmi, 2011).

2.11.4 Preparation of black tea solution as a fermentation medium

Several studies have reported the use of black tea infusions in fermented foods and beverages either as a supplement or as the main media including kombucha (Jayabalan, Marimuthu, & Swaminathan, 2007), yogurt (Jaziri et al., 2009), and Chinese steamed bread (Zhu, Sakulnak, & Wang, 2016). There are several methods for making tea brew. Hajiaghaalipour et al., (2016) reported that black tea infusions can be made by steeping for about 5 minutes with hot water (100°C) resulting in the retention of high levels of antioxidants. Further, the amount of black tea leaves used in making tea brews varies from 0.4-10% (Chen & Liu, 2000; Jayabalan et al., 2007; Jaziri et al., 2009; Sreeramulu, Zhu, & Knol, 2000; Zhu et al., 2016). Watawana et al., (2015) reviewed the general amount of tea leaves used in tea-based fermented beverages at about 5 grams per litre. Colour characteristic of black tea infusion has L^* , a^* , and b^* values of 71.24 ± 0.09 , 12.62 ± 0.21 , and 60.51 ± 0.16 , respectively, (Lin et al., 2014). However, infusion time and temperature influence the colour properties of black tea infusion with L^* , a^* , and b^* values ranging between 60.59 ± 0.05 - 71.30 ± 0.01 , 5.41 ± 0.01 - 15.30 ± 0.02 , and $32.55 \pm$

0.01-34.32± 0.03. Higher infusion temperature and longer infusion time resulted in higher a* and b* but low in L* values (Kelebek, 2016).

2.11.5 Changes in content of antioxidant compounds during fermentation of tea-based fermented beverages

Jayabalan et al., (2007) reported that catechin compounds especially EC, ECG, EGC, and EGCG decreased during 9 days of fermentation of tea-based fermented beverage. Further, Sun, Li, & Chen (2015) indicated that gallic acid concentration declined during tea-based fermentation. Changes in the stability of phenolic compounds can be caused by degradation of phenolic compounds in acidic environments (Jayabalan et al., 2008) also the unstable of phenolic content during fermentation may occur due to the biotransformation of the phenolic compounds by enzymes excreted by microorganisms (bacteria and yeasts) (Zhu et al., 2007). Phenolic compounds are combined or bound with sugar in natural forms, thus reducing their availability to microorganism. However, during fermentation, enzymes are released which hydrolyse complexes of phenolics into soluble-free phenols that biologically more active and readily absorbed. Thus, the concentration of phenolic compounds decreases as fermentation time increases (Adetuyi & Ibrahim, 2014).

2.12 Carrot (*Daucus carota*)

Carrot (*Daucus carota*) belongs to the family *Apiaceae* and is one of the most known root vegetables cultivated throughout the world. Carrot is a highly valued vegetable due to its nutritive value and bioactive compounds (Hart & Scott, 1995). The consumption of carrot and its products have increased recently due to their recognition as an important source of natural antioxidants carotenoids which can provide significant health-promoting benefits such as anticancer activity, prevent against cardiovascular diseases, and promote growth and development of human organ, especially of children and young people (Briviba et al., 2004; da Silva Dias, 2014; Sharma et al., 2012). Carrots can be processed into several products such as cake, candy, dehydrated, pickle, and juices (Sharma et al., 2012). Carrot juice is the most popular non-alcoholic beverages. The steady increase in carrot juice production and consumption has also been reported (Schieber, Stintzing, & Carle, 2001).

2.12.1 Chemical composition of carrot

Carrot contains water, carbohydrates, protein, fat, dietary fibers, vitamins, minerals, and phytonutrients (Table 2.7). However, the value of chemical composition in carrot may vary depend on the variety of the root vegetable (Raees-ul & Prasad, 2015). Sugars identified in carrot comprise sucrose (4.5-6.1 g/100g), glucose (1.3-1.7 g/100g), and fructose (1.4-1.7 g/100g) which gives a slightly sweet taste (Bach et al., 2015).

Table 2.7 Chemical composition of carrot

Composition	(%)
Water	86-89
Carbohydrates	9.58-10.6
Sugars	5.4-7.5
Fiber	0.6-2.9
Protein	0.6-2.0
Fat	0.2-0.7
Vitamin	(mg/100g)
B1 (Thiamine)	0.04
B2 (Riboflavin)	0.02
B3 (Niacin)	0.2
C (Ascorbic acid)	4
Minerals	(mg/100g)
Potassium (K)	240
Calcium (Ca)	34-80
Phosphorus (P)	25-53
Natrium (Na)	40
Magnesium (Mg)	9
Iron (Fe)	0.4-2.2
Zinc (Zn)	0.2
Copper (Cu)	0.02
Phytonutrients	(mg/100g)
Carotenes	5.33-54.8

Source: Sharma et al., (2012)

2.12.2 Carrot juice processing

There are several stages in the production of carrot juice including washing, peeling and cutting, blanching, extraction, filtration or centrifugation, heating, and cooling (Figure 2. 16) (Sharma et al., 2009; Sherafati, Kalbasi-Ashtari, & Mousavi, 2013). Washing, peeling, and cutting are aimed to remove dust and reduce soil contamination (Sherafati et al., 2013). In carrot juice processing, blanching is necessary in order to inactivate the enzymes and soften the tissues of carrots for maximum juice extraction while heat treatment is aimed to reduce microbial population (Sharma et al., 2012). In processing of carrot juice, a coagulum may be formed when the carrot is heated to about 82°C (180°F). Therefore, acidification of carrot juice with food grade acid such as acetic, citric, malic, or lactic to the pH of 5.3-5.5 is important to reduce the formation of coagulum (Paul, 1975).

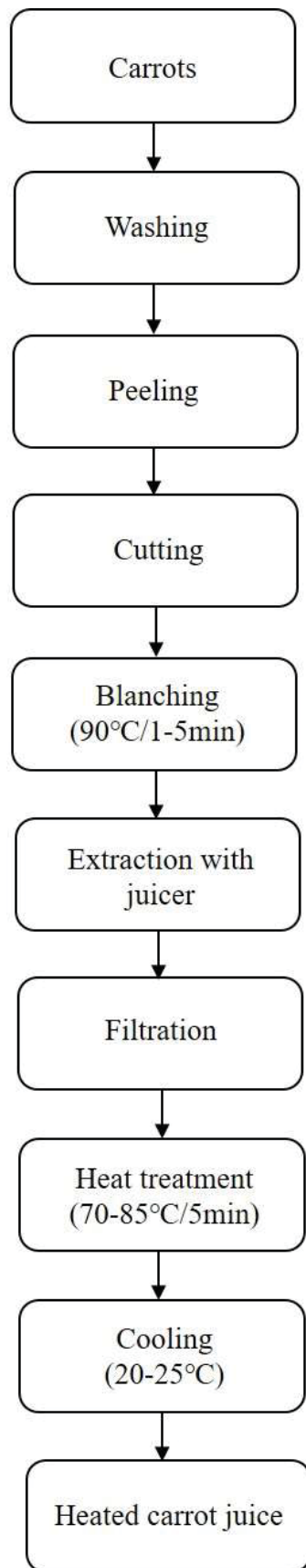


Figure 2.16 Carrot juice processing

Source: Sharma et al., (2009)

2.12.3 Changes on components in carrot juice during fermentation

There are microbial, chemical, and physical changes occur during carrot juice fermentation. Kun et al., (2008) reported that *Bifidobacterium* strains such as *B. lactis* Bb-12, *B. bifidum* B7.1 and *B. bifidum* B3.2 were capable to grow in carrot juice medium and their viable cell number increased from 10^8 cfu/ml to 10^{10} cfu/ml after 24 h fermentation at 37°C. This result was in agreement to Tamminen et al., (2013) who reported that viable cell population of *Lactobacillus* and *Bifidobacterium* increased during 18 h fermentation of carrot juice. In addition, Corona et al., (2016) and Sakamoto et al., (1996) also reported that lactic acid bacteria grew well in carrot juice medium and viable cell counts increased during fermentation. Further, Rafiq et al., (2016) suggested that carrot juice can serve as a suitable media for probiotic bacteria growth.

Degradation of some components in raw material such decrease in pH and sugars are characteristic features of lactic acid fermentation (Bergqvist et al., 2005). During fermentation of carrot juice with lactic acid bacteria, the amount of sugars including sucrose, glucose and fructose decreased (Rafiq et al., 2016; Sakamoto et al., 1996). However, Kun et al., (2008) investigated that sucrose and glucose content decreased as fermentation time increased while fructose concentration did not change in carrot juice fermentation with *Bifidobacterium*. This may due to different capability of *Bifidobacterium* with other lactic acid bacteria in carbohydrate utilization (Wang et al., 2003). Hydrolysis of sugars by lactic acid bacteria induces an appreciable decrease in pH of carrot juice due to organic acids formation during fermentation (Alwis et al., 2016; Bergqvist et al., 2005; Kun et al., 2008; Rafiq et al., 2016; Sakamoto et al., 1996; Tamminen et al., 2013). Bergqvist et al., (2005) reported that there were five organic acids detected in fermented carrot juice including lactic acid, acetic acid, tartaric acid, propionic acid, and citric acid. Among five organic acids previously described, lactic acid was the dominant acid produced in carrot juice fermentation. Similar result was reported by Corona et al., (2016). During carrot juice fermentation, the amount of carotenoids both α -carotene and β -carotene decreased (Kun et al., 2008; Sakamoto et al., 1996). This degradation could be due to bacteria metabolism and also may be affected by fermentation conditions such as temperature and pH (Kun et al., 2008; Panda & Ray, 2007). In addition, lactic acid fermentation can degrade metal chelates

such phytate during carrot juice fermentation as a result of microbial or plant enzymatic activities (Bergvist et al., 2005; Reddy & Pierson, 1994). Therefore, mineral availability may be improved by lactic acid fermentation (Svanberg et al., 1990). Bergvist et al., (2005) reported that concentration of phytate in carrot juice diminished completely after fermentation and iron solubility improved up to 30 fold. This results were in agreement with Bergvist et al., (2006) and Rakin et al., (2007).

Fermentation may also cause colour change of carrot juice (Sakamoto et al., 1996). Corona et al., (2016) reported that the lightness or L* value of carrot juice increased in water kefir fermentation. In addition, a* (redness-greenness) and b* (yellowness-blueness) value of carrot juice also increased after fermentation. This results was similar to Sakamoto et al., (1996). The colour change in fermented carrot juice may be attributed to the change in carotenoids due to acidification during fermentation by lactic acid bacteria (Minguez-Mosquera & Gandul-Rojas, 1994; Sakamoto et al., 1996).

3. MATERIALS AND METHODS

3.1 Experimental design

In this study, the development of black tea water kefir beverage were conducted in four integrated experimental phases comprising of a preliminary, first, secondary, and third phase. The preliminary phase involved the confirmation of the microflora (lactic acid bacteria and yeasts) of water kefir grains starter culture used in the study. de Man, Rogosa and Sharpe (MRS) agar and M17 agar were used to enumerate *Lactobacillus* spp. and *Lactococcus* spp., respectively, while yeast extract glucose chloramphenicol agar (YGC) was used to enumerate *Saccharomyces cerevisiae*. The first phase aimed to investigate the effect concentration of sucrose and fermentation temperature, as well as selecting the most promising formulation for black tea water kefir beverage. The effect of added carrot juice into the most promising kefir formulation was investigated at second phase and the third phase determined the stability of the final kefir beverage formulation during storage at 4°C for four weeks.

A 2² factorial design was used to investigate the effect concentration of sucrose and fermentation temperature in the preparation of black tea water kefir beverage. The design involved two variables with two levels for each variable: concentration of sucrose (5% and 10%) and fermentation temperature (25°C and 30°C). The four coded treatments are shown in Table 3.1. The most promising formulation of black tea water kefir beverages from the first phase was selected and used in the second phase. In the second phase, each sample was subjected to 2 steps of fermentation, comprising of primary fermentation for 72 hours and secondary fermentation with added carrot juice for 24 hours. Three levels concentration of carrot juice (5%, 10%, and 15%) were added to the fermenting base to initiate secondary fermentation, which produced gas (Phung, 2015; Martinez-Rodriguez & Pueyo, 2008). In the first and second phases, each sample was analysed for mesophilic viable cell counts, acidity (pH and titratable acidity), total soluble solids (TSS) analysis, and colour was measured during fermentation. Consumer sensory evaluation was conducted for the final product after 72 and 96 hours fermentation in the first and second phases, respectively. The most promising formulation from second phase was selected and used in the third (final) phase to

determine the stability of the product during storage 4°C for four weeks. The samples were analysed for microbiological and physico-chemical characteristic previously described. All the samples were analysed in duplicate and the experiments were replicated three times.

Table 3.1 Experimental design of treatments for screening potential formulations for fermentation of black tea water kefir beverage

Experiment number	Experimental code	Concentration of sucrose (% w/v)	Fermentation temperature (°C)
1	BT/5/25 (A)	5	25
2	BT/5/30 (B)	5	30
3	BT/10/25 (C)	10	25
4	BT/10/30 (D)	10	30

Notes: BT = black tea

3.2 Description of fermentation variables

3.2.1 Concentration of sucrose

The rate of microbial growth is important in food fermentations (Amore & Faraco, 2013). It is therefore essential to create favourable growth conditions to achieve desired fermentation results (Pederson, 1971). One of the intrinsic factors that affect microbial growth is nutrient content. The major nutrient used by microorganism as source of energy is carbohydrates such as sugar (Amore & Faraco, 2013; Hamad, 2011). In this experiment, sucrose was added to black tea water kefir beverage at two levels, 5% and 10% (Hsieh et al., 2012; Magalhaes et al., 2010; Marsh et al., 2013).

3.2.2 Fermentation temperature

Temperature plays an important role in fermentations as it creates a favourable environment for growth of fermenting microorganisms (Amore & Faraco, 2013). Fermentation temperature of a food product influences the rate of fermentation and the species of organisms involved (Pederson, 1971). In this study, black tea water kefir beverage was fermented at 25°C and 30°C (Cui et al., 2013; Magalhaes et al., 2010; Marsh et al., 2013).

3.3 Raw materials

Ingredients used for preparing tea water kefir beverage were sourced from local suppliers. Certified organic black tea 443 purchased from Naturally Organic, Albany, New Zealand and was used as the medium for fermentation; certified organic raw sugar 4522 (Chelsea Refinery, Auckland, New Zealand) was purchased from New World, Albany, New Zealand was used as source of carbon, and water kefir grains (starter culture) were obtained from a commercial traditional household production (Healthy Brews, New Zealand). Certified organic raw carrot 571 (Willowmere Organic Farms Ltd., Canterbury, New Zealand) was purchased from Naturally Organic, Albany, New Zealand and was used as source of flavour for the final product.

3.4 Methods

3.4.1 Preparation of water kefir grains starter culture

In this study, the water kefir grains (starter culture) were prepared according to Magalhaes et al., (2010) with minor modification. 1 L of potable water was heated at 90°C on a hot plate (13, Roband, Australia) and then 50 g of sucrose were added and dissolved completely. The mixture was cooled to room temperature (25-30°C) and water kefir grains (100 g/L) were added to the cooled sucrose solution (50 g/L) then the mixture was fermented at 25°C for 24 h in a water bath (Grant, Global Science, NZ) (Magalhaes et al., 2010; Marsh et al., 2013). After 24 h fermentation, the supernatant was discarded and the water kefir grains were washed with potable water (20°C) and strained using a kitchen nylon strainer (200 mesh) (Muskaan, India). Propagation of water kefir grains in sucrose solution was repeated twice under the same conditions to allow adaptation (Gulitz et al., 2011). The procedure for the preparation of water kefir grains is shown in Figure 3.1.

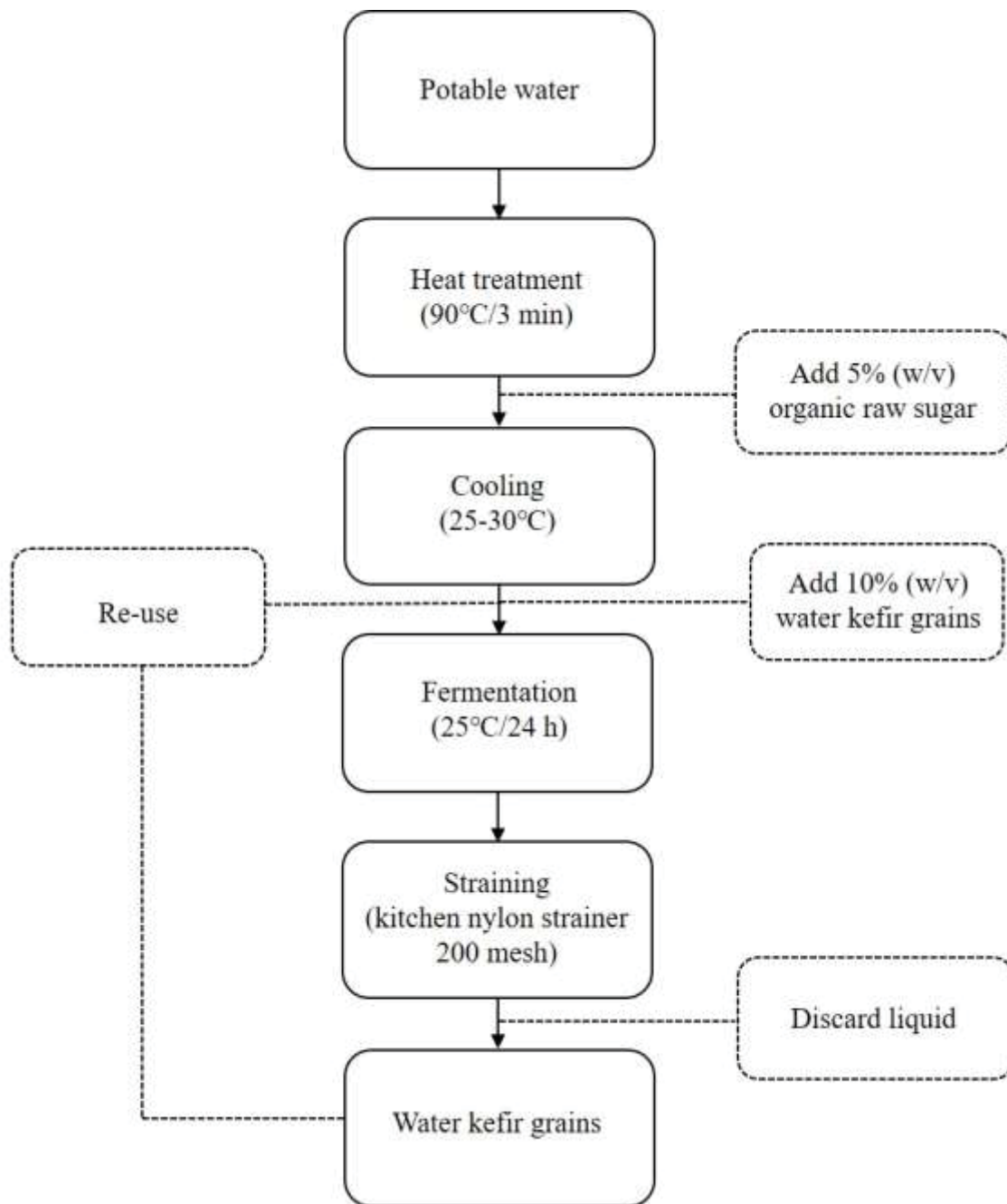


Figure 3.1 Laboratory preparation of water kefir grains

3.4.2 Preparation of black tea water kefir beverage

Black tea water kefir beverage was prepared using organic black tea, organic raw sugar, and water kefir grains. One L of potable water was heated at 90°C for 3 min, and then 0.5% of organic black tea (5 g) (w/v) was added (Jayabalan et al., 2014) and allowed to infuse (steep) for 3 min (Hajiaghaalipour, Sanusi, & Kanthimathi, 2016). Organic black

tea leaves were removed from the infusion, and then sucrose (5% or 10%) was added to the black tea solution. The solution was stirred until completely dissolved. Two hundreds (200 ml) sucrose-black tea solution were dispensed into sterile labelled glass jars (230 ml) and allowed to cool to room temperature (25-30°C). The temperature of sucrose-black tea solution was measured by a temperature thermocouple (Fluke 51, Fisher Scientific, NZ). After cooling, 10% (w/v) of water kefir grains were added to each sample. The samples were then fermented in a water bath (Grant, Global Science, NZ) at selected fermentation temperatures, 25°C or 30°C for 72 h. The preparation of black tea water kefir beverage is shown in Figure 3.2.

3.4.3 Preparation of organic carrot juice

Carrot juice was prepared according to the method of Kiros et al., (2016) with minor modifications. Raw organic carrots (*Daucus carota*) were thoroughly washed with potable water, peeled with hand-fruit peeler (Dalson, Dalsonware Pty. Ltd., Australia) to remove dirt or reduce soil contamination (Sherafati et al., 2013) and cut longitudinally into halves with kitchen knife (Staysharp® Wiltshire, New Zealand). The carrots were blanched at 90°C for 1 min to tenderize carrot tissues and inactivate pectinase and peroxidase enzymes (Salwa et al., 2004; Sherafati et al., 2013). Carrot juice was extracted using a juice extractor (N/1000-juicer, F.E.D Australia & New Zealand). Fresh carrot juice was filtered through a kitchen nylon strainer (200 mesh) (Muskaan, India) to separate coarse materials. The extracted carrot juice (200 ml) was filled into a sterile glass jar (230 ml) and pasteurised at 75°C for 5 min in a water bath (Grant, Global Science, NZ), followed by cooling to room temperature (20°C). The preparation of carrot juice is shown in Figure 3.2.

3.4.4 Preparation for secondary fermentation

After three days of fermentation of black tea water kefir beverage, pasteurised carrot juice (5%, 10%, or 15% (w/v)) was added into the promising formulation of black tea water kefir beverage and the samples were fermented for another 24 hours at 25°C.

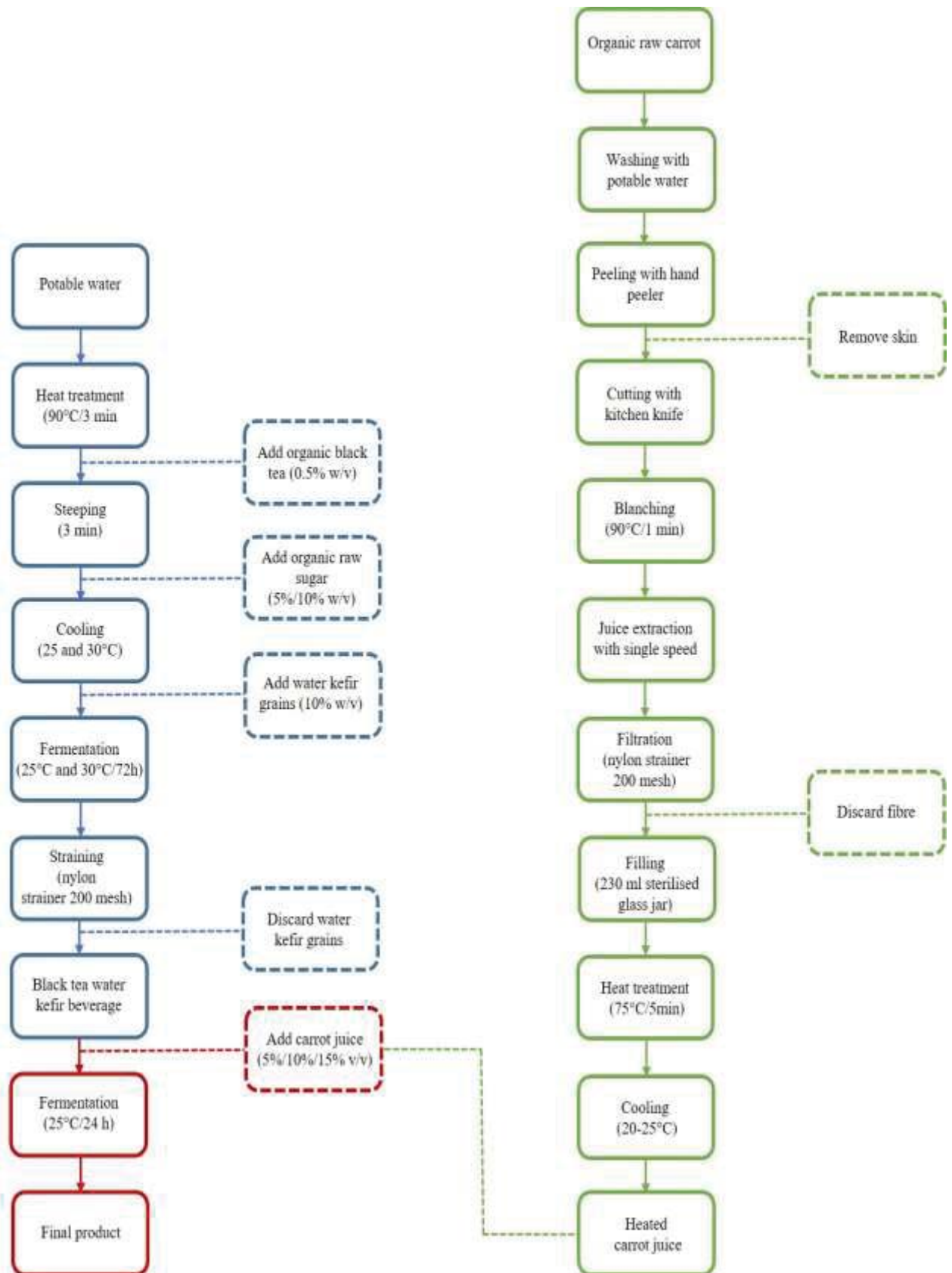


Figure 3.2 Preparation of black tea water kefir beverage and pasteurised carrot juice

3.5 Characterisation of black tea water kefir beverages

3.5.1 Acidity of black tea water kefir beverage

The acidity level of black tea water kefir beverage contributes to the sensory profile of the product (Laurey & De Vuyst, 2014; Magalhaes et al., 2010) and the keeping quality of the beverage (Puerari et al., 2012; Stadie et al., 2013). Titratable acidity and pH of black tea water kefir beverages were determined.

3.5.1.1 Titratable acidity

Standardisation of 0.1 M sodium hydroxide

Sodium hydroxide (0.1 M) was standardised following the AOAC standard method (AOAC, 936.16, 1990). The reagents used for 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. 0.15-0.20 g of dried KHP on Erlenmeyer flask were measured with an analytical balance (Sartorius CP 225 D, USA) and 50 ml distilled water were added to dissolve KHP completely. 4-6 drops of 1% phenolphthalein solution were added to the KHP solution and the mixture was swirled thoroughly. The 0.1 M NaOH solution was standardised against KHP solution until the first persistent pink colour and volume of titre NaOH (ml) was recorded. The test titrations were repeated until results were concordant, and the concentration of prepared NaOH solution was calculated using equation 1.

$$M \text{ NaOH} = \frac{m \text{ KHP}}{V \text{ NaOH} \times 0.204229} \dots\dots\dots(1)$$

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

Determination of acidity in black tea water kefir beverage

Determination of the acidity level of black tea water kefir beverage was achieved following the AOAC standard method (AOAC 947.05, 2005). Ten grams (10 g) of black tea water kefir beverage samples were measured on an analytical balance (Sartorius CP 225 D, USA) and mixed thoroughly with 20 ml of distilled water. About 2 ml (2-3 drops) of 1% phenolphthalein solution were added to the mixed solution and then thoroughly mixed by swirling. Titrations of the diluted samples were carried out against standardised 0.1 M NaOH until the test samples had the first persistent light pink colour or reach pH 8.3 was observed. The volume of titre (ml of NaOH) used in the titration was recorded and the concentration of lactic acid was calculated using equation (2). The titration was done in duplicate and the experiment was repeated three times.

$$\% \text{ Lactic acid} = \frac{\text{volume of NaOH required (ml)} \times 0.0090}{\text{sample weight (g)}} \times 100 \dots\dots\dots(2)$$

1 mL 0.1M NaOH = 0.009 g lactic acid; 1 mL of sample \approx 1 g of sample.

3.5.1.2 Measurement of pH

pH was obtained by direct measurement of black tea water kefir samples using a digital pH meter (Sartorius PB-20, USA) equipped with a glass electrode following the AOAC method 981.12 (AOAC, 2005). Prior to each measurement, the pH was calibrated using standard buffer solutions at pH 4.0, 7.0, and 10.0 (LabServ, Thermofisher, NZ). The pH electrode was rinsed with distilled water after each measurement. pH measurement was done in duplicate and the experiment was conducted in triplicate.

3.5.2 Determination of total soluble solids (TSS)

Determination of total soluble solids content (TSS) was performed according to the AOAC method 932.12 (AOAC, 1990). The soluble solids content was measured using a digital refractometer (Atago PR-101, Japan) and the results were recorded as °Brix.

3.5.3 Determination of sugars

Concentrations of sucrose, glucose, and fructose in black tea water kefir beverage was analysed by high performance liquid chromatography (HPLC) according to Stadie (2013) with minor modifications. Analyses of the sugars were carried out using a HPLC model LC-10AT (Shimadzu Corp, Japan) equipped with an auto injector (SIL-10A, Shimadzu Corp, Japan), column oven (CTO-10AS, Shimadzu Corp, Japan), system controller (SCL-10A, Shimadzu Corp, Japan) and a dual detection system consisting of a Refractive Index (RI) detector (RID-10A, Shimadzu Corp, Japan) and an Ultra Violet (UV) detector (SPD-10A, Shimadzu Corp, Japan). A Rezex RCM-Monosaccharide, RCM Ca²⁺ (8% cross-linked resin) column (300 x 7.8 mm) was used for the analysis at 80°C. Distilled water (Milli-Q water) was used as the mobile phase at a flow rate of 0.6ml/min. The distilled water was filtered through nylon membrane filter 0.22 µm with diameter of 47 mm (Membrane-solutions, USA) and degassed to remove air bubbles with ultrasonic bath (Bandelin Sonorex Super RK510, Germany). The samples were withdrawn by 3 ml syringe (Terumo, Australia), filtered through 0.20 µm syringe filters (Terumo, Australia) and then kept in 2 ml vials (Shimadzu Corp, Japan). One µl (1 µl) of filtered sample was injected into an auto-sampler for chromatographic analysis and performed in duplicate. Individual sugars were identified and quantified by comparison of their retention times and peak areas, respectively, to the standard sugars. Peak areas were integrated using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). The quantification of sugars was performed using calibration curves obtained from the standard. Sugar standards comprising glucose (67528) ≥ 99.5% (Sigma Aldrich, NZ), fructose (F0127) ≥99% (Sigma Aldrich, NZ), and sucrose (84097) ≥ 99.5% (Sigma Aldrich, NZ) were prepared in distilled water and filtered through a 0.2 µm filter using a syringe. Standard concentrations of sucrose were 0.01%, 0.1%, 1.25%, 5%, and 10% (w/v), whereas for glucose and fructose, the concentrations were 0.1%, 1.25%, 3%, 5%, and 10% (w/v).

3.5.4 Determination of organic acids

Lactic acid and acetic acid in black tea water kefir beverage were analysed using HPLC equipped with a UV detector according to Stadie (2013) with minor modifications. The HPLC model LC-10AT (Shimadzu Corp, Japan) equipped with an auto injector (SIL-10A, Shimadzu Corp, Japan), column oven (CTO-10AS, Shimadzu Corp, Japan), system controller (SCL-10A, Shimadzu Corp, Japan) and a dual detection system consisting of a Refractive Index (RI) detector (RID-10A, Shimadzu Corp, Japan) and an Ultra Violet (UV) detector (SPD-10A, Shimadzu Corp, Japan) was used for the analyses. A separation Rezex ROA-Organic Acid (8% cross-linked resin) column (300 x 7.8 mm) was used for the analyses at 85°C. Sulphuric acid (Fisher Scientific, UK) (0.005 N) was used as the mobile phase at flow rate of 0.6ml/min. Prior to analysis, standard solutions and test samples were filtered through 0.20 µm syringe filters (Terumo, Australia) and kept in 2 ml vials (Shimadzu Corp, Japan) for chromatographic analysis. Automatic injections (10 µl) were performed in duplicate. Standards organic acids used comprised lactic acid (252476) ≥ 95% (Sigma Aldrich, NZ) and acetic acid (2789) ≥ 99.5% (Fisher scientific, UK). Individual organic acids were identified and quantified by comparison of their retention times and peak areas, respectively, to the standard. Peak areas were integrated using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). The quantification of organic acids was performed using calibration curves obtained from the standard compounds. The concentrations of lactic acid used were 0.01%, 0.05%, 0.07%, 0.1%, 0.3%, 0.5% (w/v) and concentrations of acetic acid standard used were 0.01%, 0.03%, 0.05%, 0.07%, 0.1%, and 0.3% (w/v).

3.5.5 Determination of antioxidants

Determination of phenolic (gallic acid, ECG, EGC, and EGCG) and methylxanthine (caffeine and theobromine) compounds in black tea water kefir beverage was analysed by HPLC using the method described by Yao et al. (2004) with minor modifications. The phenolic and methylxanthine compounds were separated by reversed phase HPLC (Shimadzu UFLC, Shimadzu Prominence, Japan) equipped with two pumps (LC-20AD), an auto sampler (SIL-20ACHT), and an SPD-M20A photodiode array detector. A 5 µm Grace Smart RP18 column (250 x 4.6 mm) (Grace Davison Discovery Sciences, Deerfield, IL, USA) was used at 18°C. Two mobile phases were used, mobile

phase A (0.1% Trifluoroacetic acid (TFA) in Milli-Q water) and mobile phase B (0.1% TFA in acetonitrile) was set at a flow rate of 0.75ml/min. The phenolic and methylxanthine compounds were detected at 270 nm and then identified and quantified based on their respective retention times and peak areas. Peak areas were integrated using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). Prior to analysis, standard solutions and samples were filtered through 0.20 µm filter (Terumo, Australia) and kept in 2 ml vials (Shimadzu Corp, Japan). Automatic injections of samples and standards (20µl) were performed in triplicate.

3.5.6 Determination of colour

The colour of black tea water kefir was measured using a Konica Minolta spectrophotometer (CM-5, Japan) according to the L*, a*, b* colour system (Kurtmann et al., 2009). The spectrophotometer was calibrated prior to use following manufacturer's instruction. Black tea water kefir beverage (3ml) was transferred into 4 ml spectrophotometer plastic cuvettes (Sigma Aldrich, NZ) and the sample was illuminated with D65 artificial daylight (10° standard angle) according to conditions provided by the manufacturer. The colour of the test sample was measured directly and the results were recorded immediately after measurement. The colour of black tea water kefir beverage was measured in duplicate and the experiment was repeated three times. In this study, the colour measurement resulted in the generation of L*, a*, b* values. According to MacDougall, (2002), the colour space has three values; a* value, which measures redness (+) or greenness (-); an increase in a* value represents a change in colour towards redness, b* value measures yellowness (+) or blueness (-); an increase in the b* value reflects a change in colour towards yellow, and L* value measures brightness or lightness (+) or darkness (-); a decrease in L* value represents a change in colour towards darkness.

3.5.7 Microbiological analysis

3.5.7.1 Microflora of water kefir grains starter culture

Enumeration of LAB and yeasts in water kefir grains starter culture was carried out by plating serial dilutions on specific culture media according to Laureys & De Vuyst (2014) and Magalhaes et al., (2010). According to the supplier (David Jones, Healthy

Brews, New Zealand) water kefir grains used in this study predominantly contained LAB (*Lactobacillus* spp. and *Lactococcus* spp.) and yeast (*Saccharomyces cerevisiae*). LAB were enumerated on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid, UK) and on M17 agar (Oxoid, UK) to separate *Lactobacillus* spp. and *Lactococcus* spp., respectively. All media for enumeration of LAB were supplemented with 0.1% of cycloheximide (Oxoid, UK) to inhibit growth of yeast and fungi (Hsieh et al., 2012). Yeasts were enumerated on yeast extract-glucose-chloramphenicol agar (YGC) (Merck, Germany). M17 agar plates were incubated at 37°C for 48 h under aerobic conditions and, 72 h for bacterial growth on MRS agar under anaerobic conditions using an Anaerogen pack (AN0035A) (Mitsubishi Gas Chemical Company Inc., Japan). Yeasts were grown under aerobic conditions at 25°C for 5 days. Isolates of the grown colonies were further characterized by Gram staining and catalase testing. Gram-positive and catalase-negative isolates were considered as presumptive LAB (Leite et al., 2015).

3.5.7.2 Enumeration of *Lactococcus* spp. in fermented black tea water kefir beverage

Enumeration of *Lactococcus* spp. bacteria was conducted according to Magalhaes et al., (2010). M17 agar (CM0785, Oxoid, UK) plates were prepared according to the manufacturer's instructions. Ten millilitre (10 ml) of test sample were sub-sampled and 90 ml of sterilised 0.1% peptone water (Merck, Germany) were added to the test portion in a stomacher bag (Global Science, NZ) to obtain a 10⁻¹ dilution. The mixture was blended for 2 min using a stomacher laboratory blender (Stomacher, Global Science, NZ). Suitable dilutions of the test samples were pour-plated in duplicate. After solidification of agar, the prepared Petri dishes were inverted and incubated (Clayson IM1000R, Australia) at 37°C for 48 h. The grown colonies were counted using a colony counter (Bibbyscientific, UK). Gram staining and catalase test were conducted on the grown colonies (*Lactococcus* spp. is a Gram positive coccus and catalase negative; Figure B.1 in Appendix B).

3.5.7.3 Enumeration of *Lactobacillus* spp. in fermented black tea water kefir beverage

Viable cell counts of *Lactobacillus* spp. were enumerated using de Man, Rogosa and Sharpe (MRS) agar (CM0361, Oxoid, UK) following the method of Laureys & De Vuyst (2014). The medium was prepared according to manufacturer's instructions. Of the dry medium, 24.80 g of dehydrated media were suspended in 400 ml distilled water and sterilised in an autoclave (Astell Scientific, UK) at 121°C for 15 min. The samples were inoculated and pour plating previously described in section 3.5.7.2. The agar plates were inverted and incubated (Clayson IM1000R, Australia) at anaerobic conditions using Anaerogen pack (AN0035A) (Mitsubishi Gas Chemical Company Inc., Japan) at 37°C for 72 h. Grown colonies were counted using a colony counter (Bibbyscientific, UK). Gram staining and catalase test were conducted to on the grown colonies (*Lactobacillus* spp. is a Gram positive rods and catalase negative; Figure B.2 in Appendix B).

3.5.7.4 Enumeration of *Saccharomyces cerevisiae* in fermented black tea water kefir beverage

Viable cell counts of yeast were enumerated using Yeast extract Glucose Chloramphenicol (YGC) agar (1.16000.0500, Merck KGaA, Germany) following the method of Laureys & De Vuyst (2014). The medium was prepared according to manufacturer's instructions. Of the dry medium, 16 g of dehydrated media were suspended in 400 ml distilled water and sterilised in an autoclave (Astell Scientific, UK) at 121°C for 15 min. The samples were inoculated and pour plating previously described in section 3.5.7.2. The agar plates were inverted and incubated (Clayson IM1000R, Australia) at aerobic conditions at 25°C for 5 days. Grown colonies were counted using a colony counter (Bibbyscientific, UK).

3.5.8 Sensory evaluation

Sensory evaluation of food and beverage is a scientific method used to analyze and interpret characteristics of food and beverage as perceived through the sense of sight, smell, touch, taste, and hearing (Kemp, Hollowood, & Hort, 2009; Lawless &

Heymann, 2010). The role of sensory evaluation is not only to give opportunity for consumers to evaluate sensory characteristics of food and beverage, but it also provides an insight into the development and commercial strategy of products to producers (Kemp et al., 2009). The method and materials used in the sensory evaluation were based on Corona et al., (2010) with minor modifications.

Consumer panelists at Massey University, Albany were randomly recruited by email communications to evaluate the sensory attributes of black tea water kefir beverages. The results were used to aid the selection of the most promising formulation. Prior to the sensory evaluation, participants were requested to complete an Ethic Form approved by Massey University Human Ethics Committees (4000016138), regarding the content of the study and privacy of the participants.

A 9-point hedonic scale (1-9) was used to evaluate each attribute (appearance, odour, flavour, sourness, sweetness) and overall acceptance of black tea water kefir beverage samples (Meilgaard, Civille, & Carr, 2006) (Appendix C). The evaluation was conducted in Sensory Evaluation and Product Development Laboratory under white light. Fifteen millilitres (15 ml) of refrigerated (4°C) black tea water kefir beverage samples were served in 25 ml transparent plastic cups coded with 3-digit random codes. The random numbers were obtained from Snedecor and Cochran (1980). Each panellist was required to rinse their palate with potable water before tasting each sample.

3.6 Analysis of data

Experiments were carried out in three replications and each sample was analysed in duplicate. Data were analysed by statistical test using procedures of the SPSS Version 22 (IBMTM Company, USA). Data were expressed as mean \pm standard deviation (SD) and were tested for normality and homogeneity using the Kolmogorov-Smirnov and Levene Test at 95% confidence levels. Non-normal distributed data (non-parametric) were Log transformed to obtain normality. Normal distributed data were further analysed by One-way Analysis of Variance (ANOVA). One way ANOVA was used to investigate the effect concentration of sucrose, fermentation temperature, and concentration of carrot juice to the physico-chemical, microbiological, and sensory properties of the product at $\alpha=0.05$. Tukey's multi-comparison test was applied to

separate significant different between groups mean values at 95% confidence interval (Field, 2013; O'Mahony, 1986). Kruskal-Wallis test was used to analyse non-parametric data. The statistical outputs are shown in Appendix F.

4. RESULTS AND DISCUSSION

4.1 Water kefir grains starter culture

In this study, water kefir grains were purchased from Healthy Brews (New Zealand), a local commercial household producer. The kefir grains (Figure 4.1) had elastic, brittle, and irregular structures, with diameters ranging from 3 to 20 mm. Other researchers (Pidoux, 1989; Miguel et al., 2011; Waldherr et al., 2010) have reported similar results. The grains were insoluble in water because they mainly consisted of insoluble dextran (α -1-6 linked glucose polymer) (Horisberger, 1969). The EPS-producing LAB such as *Lactobacillus hordei*, *Lactobacillus nagelii*, and *Leuconostoc mesenteroides* was responsible for producing the dextran (30.8-32.5 g/L EPS) (Pidoux, 1989; Stadie, 2013; Waldherr et al., 2010).



Figure 4.1 Water kefir grains

(Image was captured by Iphone 4S, Apple Inc., USA)

According to the supplier (Healthy Brews, NZ), the microflora of water kefir grains used in present study were mainly composed of *Lactococcus* spp., *Lactobacillus* spp. and *Saccharomyces cerevisiae*. Gram-staining and catalase test showed that colonies grown on MRS agar and M17 agar were Gram positive and catalase negative (Appendix

B). The predominant microorganisms described here were in agreement to Magalhaes, et al., 2010. According to Gulitz et al., (2011), water kefir grains might consist predominantly of the genus *Lactobacillus*, *Leuconostoc*, *Acetobacter*, *Saccharomyces*, and *Zygorhizula*. The diversity of LAB and yeasts in water kefir grains may be influenced by their geographical origin and fermentation media (Fiorda et al., 2016; Gulitz et al., 2011; Hsieh et al., 2012; Marsh et al., 2013; Miguel et al., 2011; Plessas et al., 2017; Sarikkha et al., 2015). Viable cell counts of *Lactococcus* spp., *Lactobacillus* spp., and *Sacharomyces cerevisiae* were 6.62 ± 0.18 log cfu/g, 7.34 ± 0.14 log cfu/g, and 6.38 ± 0.34 log cfu/g, respectively after 24 h fermentation at 25°C. Results of the present study (Appendix D) were lower than previously reported where viable cell counts were 8.32 log cfu/g, 8.41 log cfu/g and 7.31 log cfu/g for *Lactococcus*, *Lactobacillus*, and yeasts, respectively (Magalhaes et al., 2010). According to Miguel et al., (2011) the microbial population of water kefir grains varied between 6.04-9.18 log cfu/g for LAB and 5.92-8.30 log cfu/g for yeasts. Thus, the population of microorganisms of water kefir grains in this study were within the range to those previously reported.

4.2 Effect of fermentation temperature and concentration of sugar on black tea water kefir beverages

4.2.1 Acidity

Acidity (pH and titratable acidity (T.A.)) of black tea water kefir beverages containing 5% and 10% sugar was determined during fermentation for 72 h at 25°C and 30°C (Figures 4.2 and 4.3). In general, the pH of black tea water kefir beverage inoculated with water kefir grains decreased ($p < 0.05$) while T.A. increased steadily as the fermentation time increased. The initial pH of the samples ranged between 5.54 ± 0.02 - 5.59 ± 0.07 decreasing rapidly during the first 24 h to 4.02 ± 0.10 - 4.28 ± 0.14 (Appendix D) depending on type of fermentation conditions and then gradually decreased during fermentation for 72 h (Figures 4.2 and 4.3). Garcia et al., (2006) and Sabokbar & Khodaiyan, (2015) reported similar results. The range of final pH of the fermented beverages obtained in this study (3.31 ± 0.03 to 3.50 ± 0.02) were similar to Laureys & de Vuyst (2014) who reported final pH of water kefir beverage of 3.45 ± 0.01 after fermentation for 72 h.

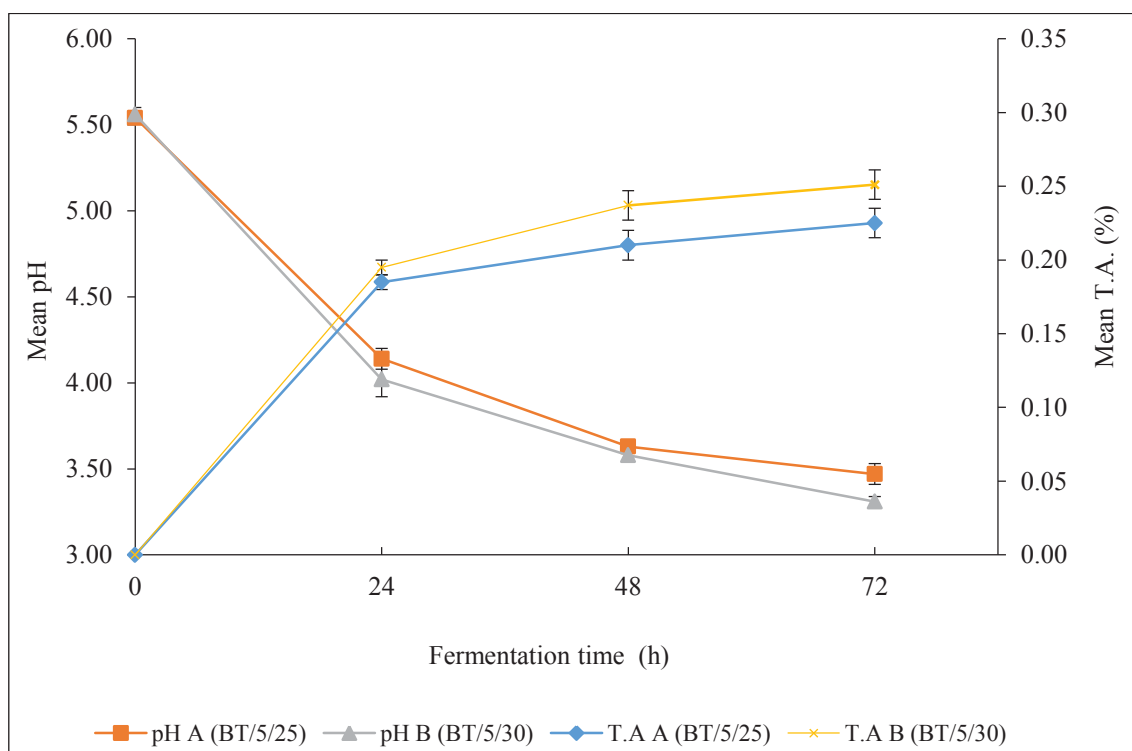


Figure 4.2 Mean pH and titratable acidity of black tea water kefir beverages during fermentation for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; Error bars = \pm SD (n=6); T.A. = Titratable Acidity.

Figure 4.2 and Figure 4.3 show the fermented beverage containing 5% (w/v) sugar (30°C) had the lowest pH (3.31 ± 0.03) while the sample containing 10% (w/v) sugar (25°C) (3.50 ± 0.02) had the highest pH. Samples B and D fermented at 30°C resulted in lower pH than samples A and C which fermented at 25°C. These results indicated that fermentation temperature affect microbial activities during their metabolism. Loncar et al., (2006) and Puerari et al., (2012) reported similar results. According to Dimitreli & Antoniou (2011), low fermentation temperature can reduce activities of the microorganisms. In addition, Leroi & Courcoux, (1996) indicated that fermentation temperature had impact on lactic acid production in mixed cultures with yeast to produce high lactic acid production.

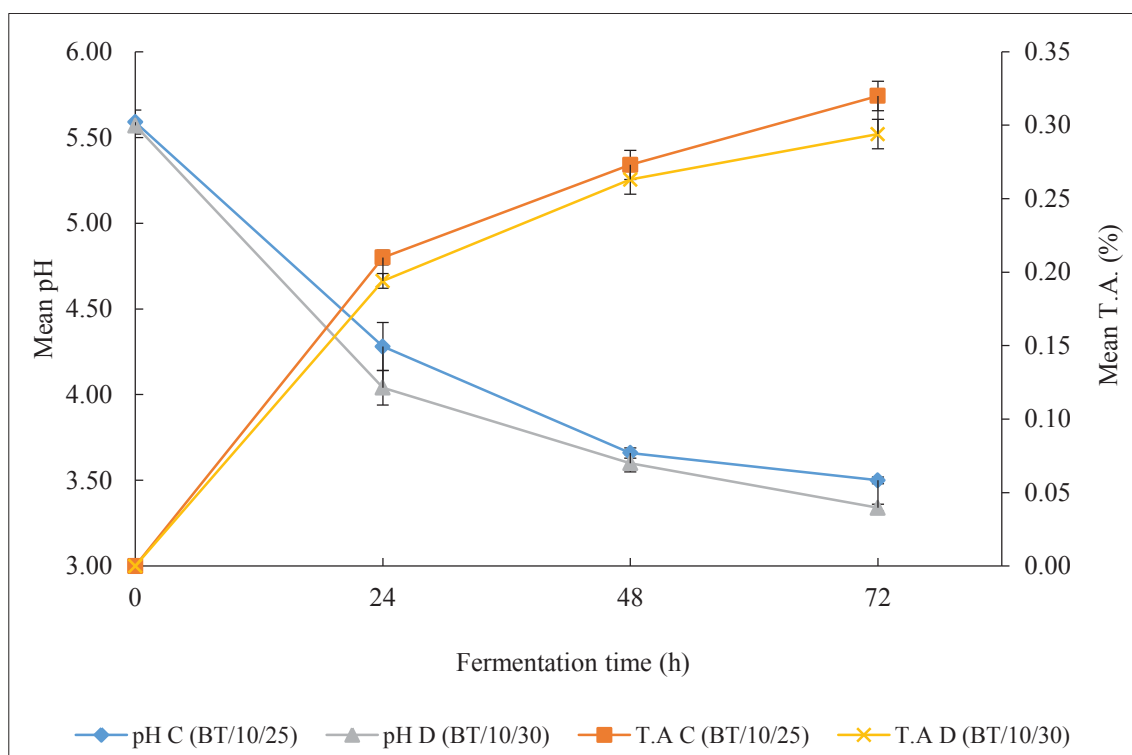


Figure 4.3 Mean pH and titratable acidity of black tea water kefir during fermentation for 72 h

Notes: Samples C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=6); T.A. = Titratable Acidity.

In the first phase, T.A. concentration in all samples increased from 0 to 0.225-0.320% during fermentation. The increase in T.A. was concomitant with decrease in pH (Figure 4.2 and Figure 4.3). Fermentation of water kefir resulted in acid production such as lactic acid and acetic acid (Laureys & De Vuyst, 2014; Stadie et al., 2013). Therefore, increase in T.A. may be attributed to the conversion of sugars in black tea water kefir beverages to organic acids by LAB via either the Embden Meyerhof Parnas (EMP) (homofermentative) or phosphoketolose pathway (heterofermentative) pathways (Hamad, 2011; Kleerebezem & Hugenholtz, 2003). The amount of acid produced in water kefir depends on the growth of lactic acid bacteria or type of starter culture used (Kazakos et al., 2016; Puerari et al., 2012). In the present study, starter culture used was mainly composed of genera *Lactobacillus*, *Lactococcus*, and *Saccharomyces*.

Results showed significant difference ($p < 0.05$) of T.A. concentration between samples at 72 h fermentation (Appendix F). Sample A had the lowest T.A. ($0.225 \pm 0.01\%$) while sample C had the highest T.A. ($0.320 \pm 0.01\%$) (Figure 4.2 and Figure 4.3). Results of present study suggested that the concentration of sugar may have influenced the T.A. concentration of the final products. Samples C and D containing 10% (w/v) sugar had higher concentration of T.A. than samples A and B containing 5% (w/v) sugar. These results are in agreement with Malbasa et al., (2008) who reported that higher concentration of sugar (molasses) resulted in higher lactic acid production in fermented black tea beverage (kombucha).

4.2.2 Total soluble solids (°Brix)

Sucrose is the main substrate added in the black tea water kefir fermentation (section 3.2.1). The concentration of sucrose was measured by a digital refractometer and the results were recorded as total soluble solids (°Brix). Figure 4.4 shows that total soluble solids of all samples decreased ($p < 0.05$) during fermentation for 72 h. Microorganisms (starter culture) metabolised added sugar in black tea water kefir beverages during fermentation which may influenced the total soluble solids content of all samples (Stadie et al., 2013).

Total soluble solids reduction ranged from 1.65 (sample A) to 2.65 (sample D) during fermentation (Figure 4.4). Decrease in total soluble solids during the fermentation may due to metabolism of sucrose into glucose and fructose by enzyme invertase of the yeast (Dickinson & Kruckeberg, 2006; Feldmann, 2005; Pidoux & Leroi, 1993a) and conversion of the monosaccharides to organic acids by LAB (Laureys & De Vuyst, 2014; Stadie et al., 2013). In the present study, sample B (2.25) and sample D (2.65) fermented at 30°C resulted in higher reductions in total soluble solids than sample A (1.65) and sample C (2.05) fermented at 25°C. Fermentation temperature of 30°C could affect more on total soluble solids changes as compared to 25°C. According to Loncar et al., (2006), metabolism of sugars was greater in fermented black tea beverage samples obtained at higher temperature.

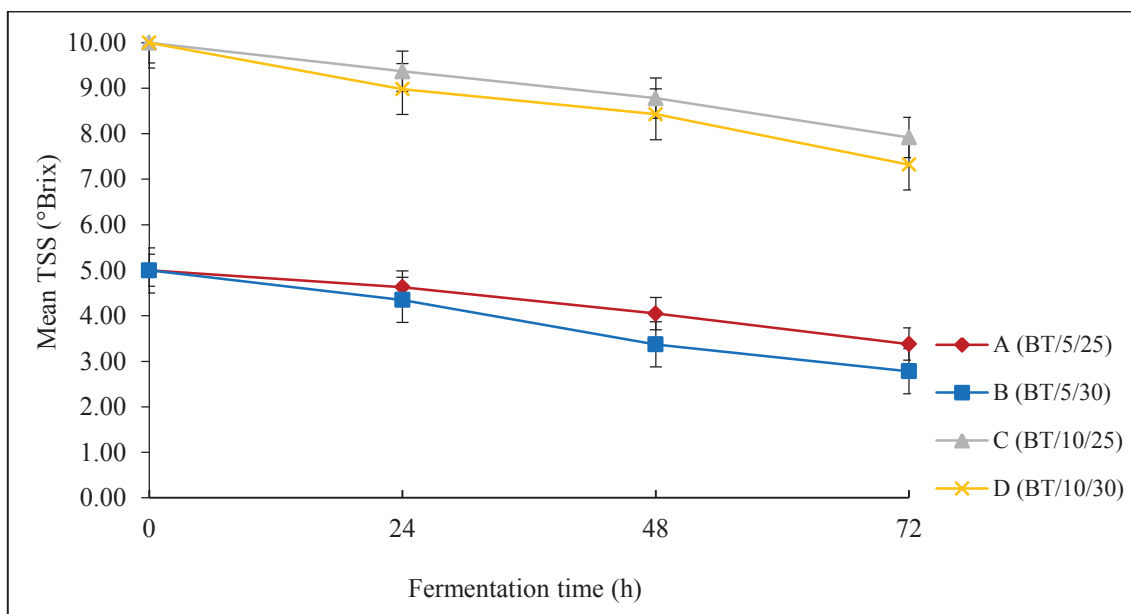


Figure 4.4 Mean total soluble solids (°Brix) of black tea water kefir beverages during fermentation for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=6); TSS = Total Soluble Solids.

4.2.3 Colour

Colour is one of the key attributes of tea (Chaturvedula & Prakash, 2011). Colour of black tea infusion is influenced by theaflavins and thearubigins that are responsible for the red-orange and red-brown colour, respectively (Leung et al., 2001; Li et al., 2013; Sharma & Jagan, 2009). Therefore, it is important to analyse the colour of black tea water kefir beverage. In this study, L^* , a^* , and b^* values of black tea solution were 76.54 ± 0.49 , 7.57 ± 0.10 , and 54.02 ± 0.20 , respectively (Appendix D). The results were contrary to the study by Li et al., (2014) who reported L^* , a^* , and b^* values of 71.24 ± 0.09 , 12.62 ± 0.21 , and 60.51 ± 0.16 , respectively. These differences may be attributed to the effects of infusion time and temperature used. Kelebek, (2016) and Peterson et al., (2004) reported that higher brewing temperature and longer infusion time resulted in higher theaflavins and thearubigins concentration in black tea infusion which may influence the colour of black tea.

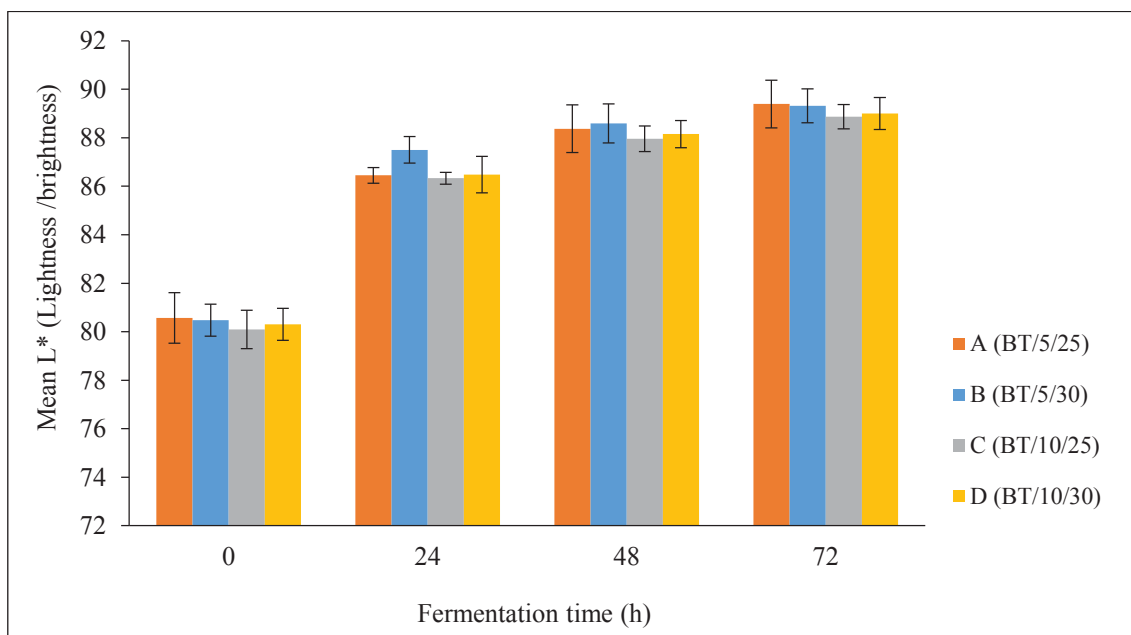


Figure 4.5 Mean L* of black tea water kefir beverages during fermentation for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=6).

Changes in the colour of black tea water kefir beverages were observed during fermentation (Figures 4.5, 4.6, and 4.7). The L* of all samples increased during fermentation for 72 h ($p < 0.05$). There was rapid increased of L* during the first 24 h fermentation and then the lightness/brightness steadily increased as the pH decreased (Figure 4.5). Increase of L* indicated that the colour of the product became lighter probably due to the suppression of ionization or destruction of the structure of thearubigins (Haslam, 2003). However, the lightness of all the samples at 72 h was not different ($p > 0.05$). Figure 4.5 showed that all the fermented samples had similar L* values, ranging between 88.87 ± 0.50 to 89.39 ± 0.98 .

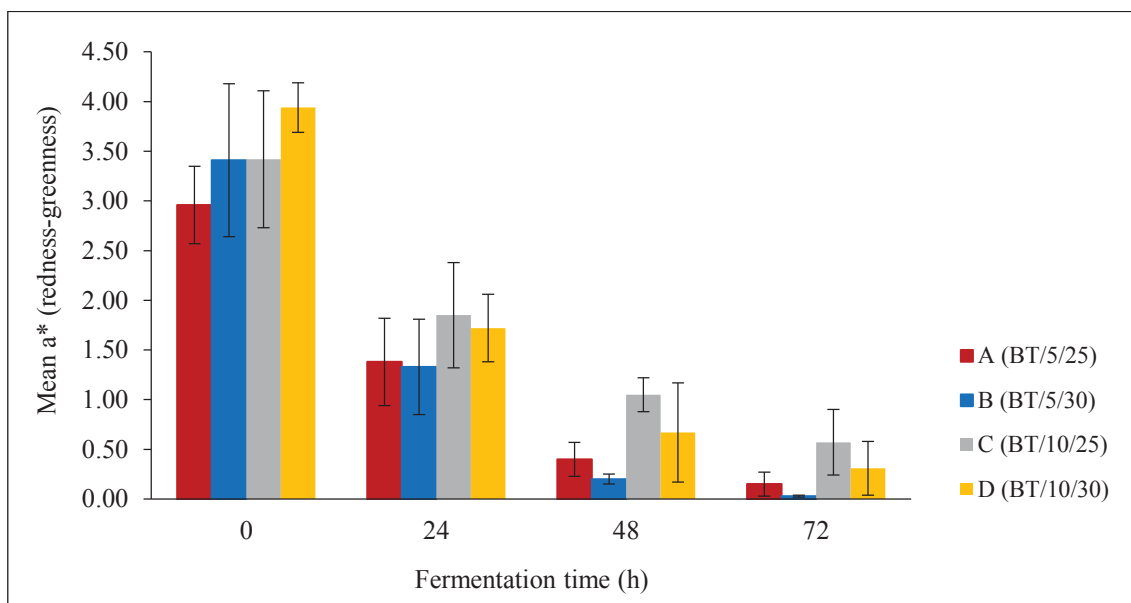


Figure 4.6 Mean a^* of black tea water kefir beverages during fermentation for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=6).

Effect of fermentation temperature and sugar concentration on redness-greenness (a^*) and yellowness-blueness (b^*) are shown in Figure 4.6 and Figure 4.7. Values of a^* and b^* for all the samples decreased ($p < 0.05$) during fermentation for 72 h. This result was in agreement with Corona et al., (2016) who reported decreased of a^* and b^* in kefir beverage made from strawberry juice during fermentation. Redness-greenness of all samples were different ($p < 0.05$) at the end of fermentation (Appendix F) while yellowness-blueness which ranged from 28.63 ± 1.66 to 29.85 ± 1.66 were not different ($p > 0.05$) (Appendix F). Sample C had the highest a^* value (0.57 ± 0.33) and sample B had the lowest a^* value (0.03 ± 0.01) (Appendix D). Based on results shown in Figure 4.6, it is assumed that fermentation temperature and sugar concentration influenced the redness-greenness of the final product. In addition, polyphenols may undergo transformation due to biochemical reaction in acidic environment during fermentation, which may affect the colour change in fermented black tea beverage (Cu & Chen, 2006).

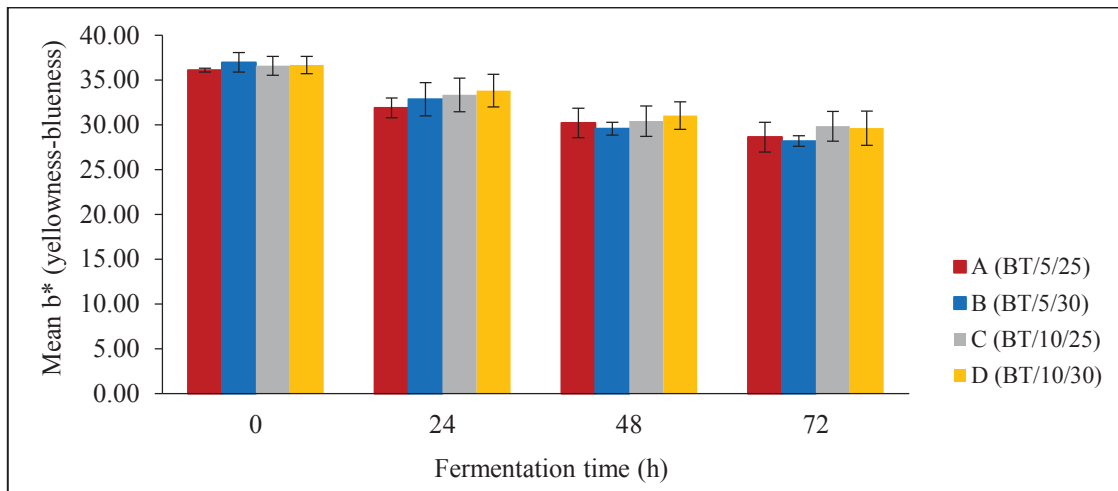


Figure 4.7 Mean b^* of black tea water kefir beverages during fermentation for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=6).

4.2.4 Microbiological analysis

The comparative growth pattern of *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* during fermentation for 72 h are shown in Figures 4.8, 4.9 and 4.10. In this phase, fermentation temperature and sugar concentration had significant effects on microbial growth during fermentation for 72 h ($p < 0.05$). All the starter bacteria showed steady growth up to 48 h fermentation (Figures 4.8, 4.9 and 4.10). Viable cell counts of *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* increased ($p < 0.05$) during fermentation up to 48 h and then decreased. Increased population of LAB and yeasts during fermentation may indicate that symbiotic mutualism metabolism (Stadie et al., 2013). According to Stadie et al., (2013), cell yield of water kefir lactobacilli and yeast increased significantly in mixed-culture compared to pure single cultures. Leroi & Pidoux, (1993b) reported that growth of *Lactobacillus hilgardii* was stimulated in the presence of *Saccharomyces florentinus*. This may be attributed by the excretion of essential nutrients such as propionate, succinate, amino acids and vitamin B6 by yeasts favourable to lactobacilli (Abbas, 2006; Leroi & Pidoux, 1993b; Stadie et al., 2013). The stimulation occurred in the presence of lactobacilli, which acidified the medium and support the growth of yeast

(Stadie et al., 2013). A study by Stadie et al., (2013) indicated that growth of yeasts improved at a starting pH of 4.00

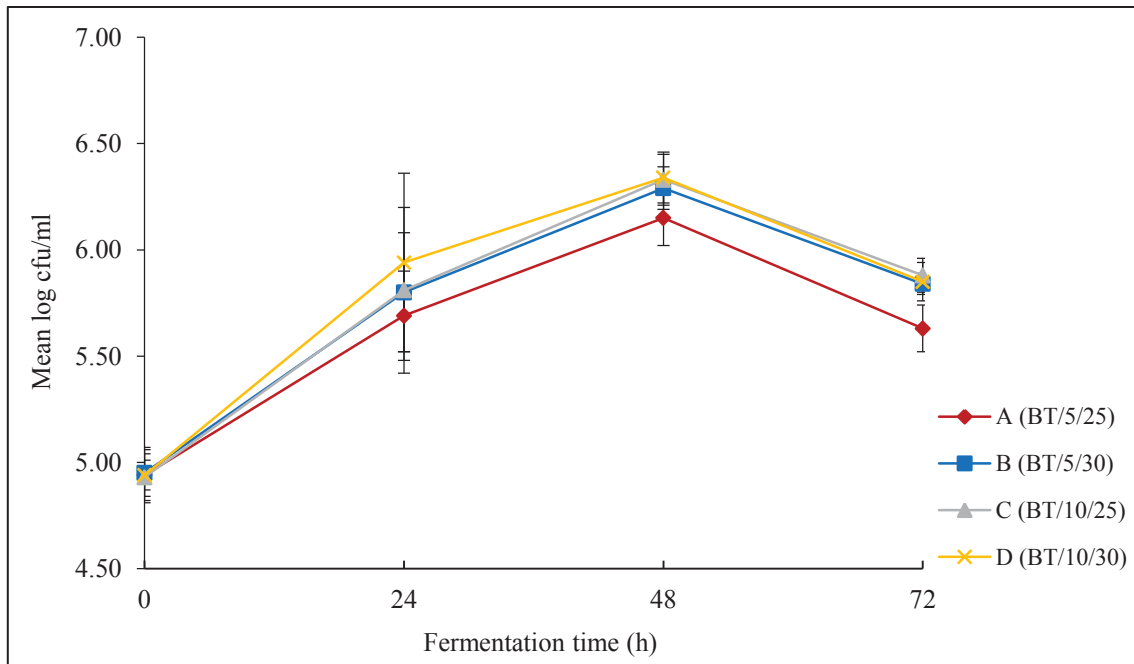


Figure 4.8 Mean log cfu/ml of *Lactococcus* spp. during fermentation of black tea water kefir beverages for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=3).

In this phase, viable cell counts of *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* increased only by 1.21-1.40 log cfu/ml, 0.65-0.76 log cfu/ml, and 0.81-1.07 log cfu/ml, respectively, during fermentation for 48 h. There was a slight decrease of microorganisms after 48 h fermentation in all samples. Viable cell counts for *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* decreased in the range between 0.45-0.52 log cfu/ml, 0.55-0.83 log cfu/ml, and 0.03-0.11 log cfu/ml, respectively. These results may be attributed to low pH and competition for nutrients that can influence the microbial growth (Leroi & Pidoux, 1993b). Similar results were reported by Garcia et al., (2006) in which *Lactococcus* spp. and *Lactobacillus* spp. decreased after 8 h fermentation. However, Bau et al., (2015) reported increased viable cell counts of lactic acid bacteria in kefir beverage during fermentation. The final pH of

all the fermented black tea water kefir samples were between 3.31 ± 0.03 - 3.50 ± 0.02 and Garcia et al., (2006) reported the final pH of milk kefir beverage at 3.88 ± 0.08 . Results in the studies previously reported were lower than the study by Bau et al., (2015) who reported a final pH of 4.97 at the end of fermentation.

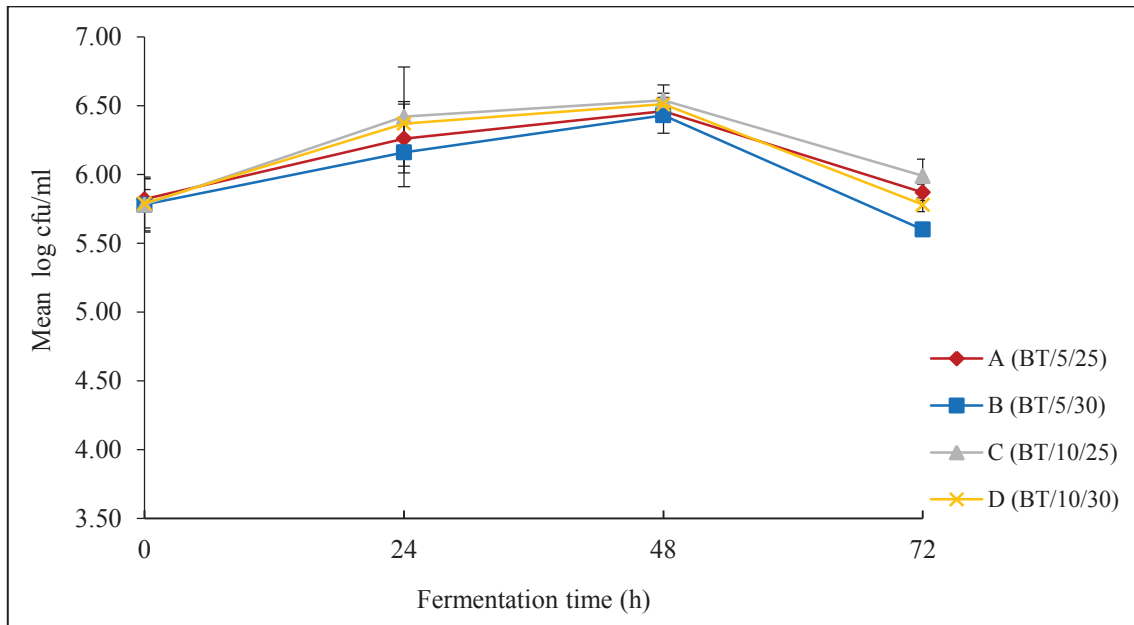


Figure 4.9 Mean log cfu/ml of *Lactobacillus* spp. during fermentation of black tea water kefir beverages for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=3).

Of the fermented black tea water kefir beverages, sample C had the highest viable cell counts of *Lactococcus* spp. and *Lactobacillus* spp., while sample D had the highest counts of *Saccharomyces cerevisiae* at the end of fermentation (Figures 4.8, 4.9, and 4.10). Sample A (5.87 ± 0.06 log cfu/ml) and C (5.99 ± 0.12 log cfu/ml) fermented at 25°C had higher viable cell counts of *Lactobacillus* spp. at the end of fermentation than sample B (5.60 ± 0.02 log cfu/ml) and D (5.78 ± 0.05 log cfu/ml) fermented at 30°C. The results suggest that fermentation at 25°C was more favourable for the growth of lactobacilli. Similar results were reported by Sabokbar & Khodaiyan (2015) in which kefir beverage fermented at 25°C had higher lactobacilli cell counts than kefir beverage fermented at 19°C.

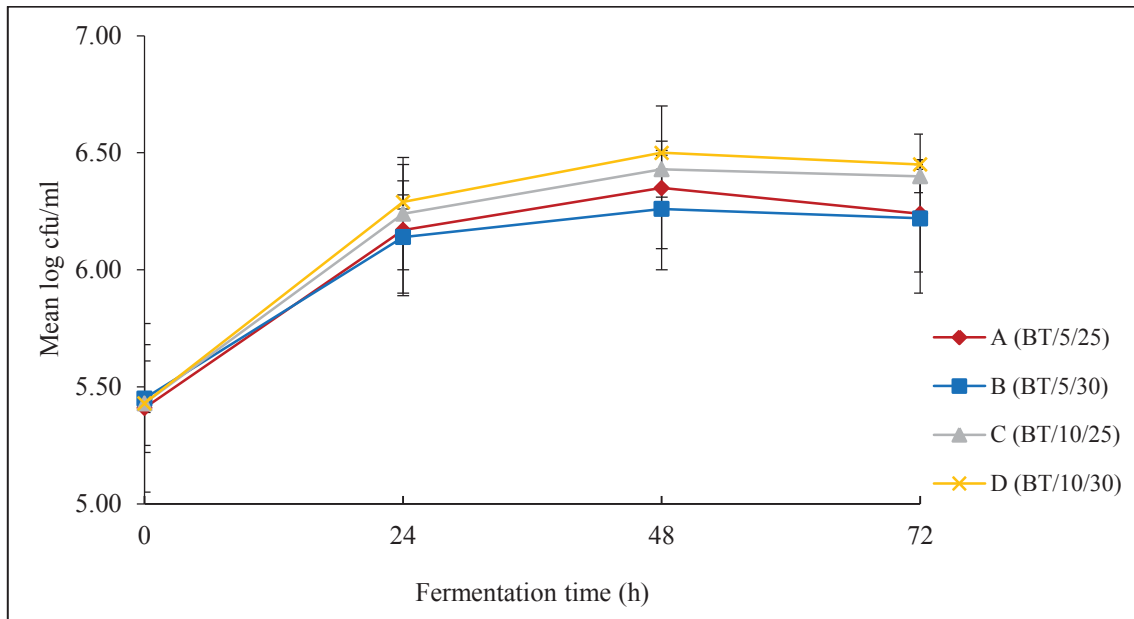


Figure 4.10 Mean log cfu/ml of *Saccharomyces cerevisiae* during fermentation of black tea water kefir beverages for 72 h

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Error bars = \pm SD (n=3).

The initial viable cell counts of yeast counts in all samples ranged from 5.41 ± 0.36 - 5.45 ± 0.23 log cfu/ml increased ($p < 0.05$) to 6.26 ± 0.17 - 6.50 ± 0.01 log cfu/ml after fermentation for 48 h at 25°C and 30°C and then decreased slightly. Population of *Saccharomyces cerevisiae* in all samples were not different ($p > 0.05$) at the end of fermentation (Appendix F). Walsh & Martin, (1977) reported that the optimum temperature for *Saccharomyces cerevisiae* was in the range of 30-35°C. However, *Saccharomyces cerevisiae* can grow at 15-35°C depend on the strains, whereas some strains performed better at high temperature while other strains at low temperature (Torija et al., 2003).

Among the starter cultures, *Saccharomyces cerevisiae* had the highest viable cell counts at 72 h (Figure 4.8, 4.9, and 4.10). High cell counts of yeasts may due to acid tolerance of *Saccharomyces cerevisiae* (Kubo et al., 2014). According to Stadie et al., (2013), the growth of *Saccharomyces cerevisiae* was not influenced by the acidification of the

medium. However, the growth rate of *Saccharomyces cerevisiae* decrease as the pH decreased starting from pH of 3.50 to 3.00 (Fleet & Heard, 1993).

4.2.5 Sensory evaluation

Sensory evaluation was conducted to examine the sensory attributes of black tea water kefir beverage at the end of fermentation. Figure 4.11 shows the results of sensory evaluation of black tea water kefir beverages. Fermentation temperature and sugar concentration did not affect the appearance and odour properties of black tea water kefir beverage samples. The scores of appearance and odour for all the samples ranged from 6.63 ± 0.86 - 6.52 ± 0.91 and 6.13 ± 0.95 - 6.50 ± 1.00 , respectively (Appendix D). However, there were differences ($p < 0.05$) of flavour, sourness, sweetness, and overall acceptability among the samples.

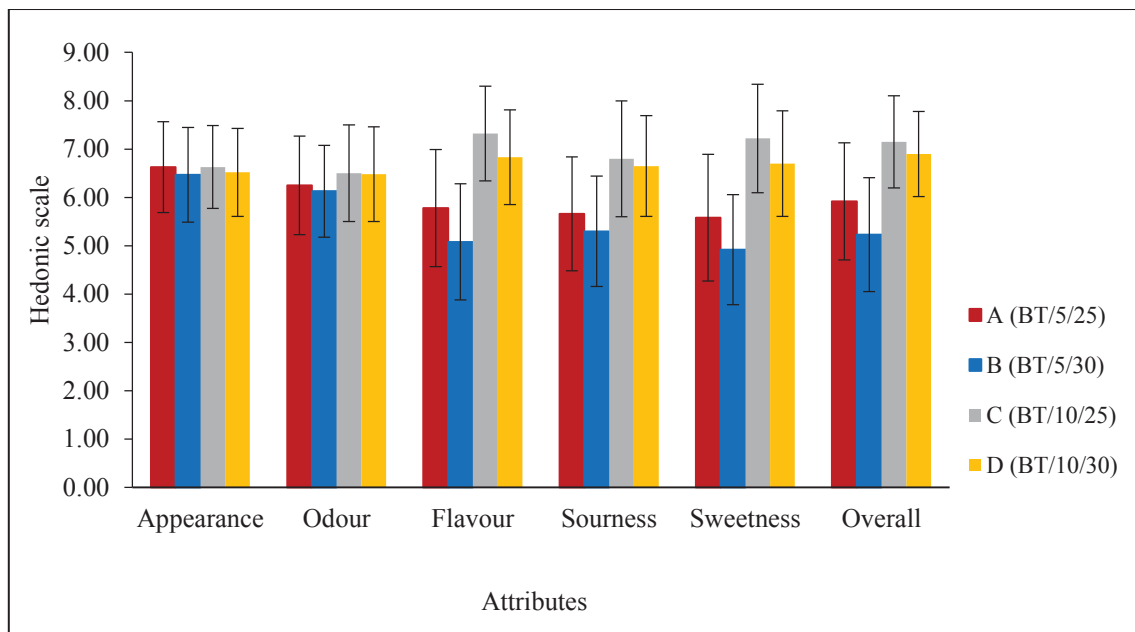


Figure 4.11 Mean sensory evaluation scores of black tea water kefir beverages at the end of fermentation (72 h).

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose; Hedonic scaling: 1-9 with 1 as lowest and 9 the highest; Error bars = \pm SD (n=60).

The odour of the black tea water kefir beverages may be influenced by the esters production of kefir yeasts (Nambou et al., 2014). According to Magalhaes et al., (2011), increase in esters during kefir fermentation is a common phenomenon. Isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate are esters compounds which contribute to the fruity and floral notes of the fermented water kefir beverages (Corona et al., 2016; Laurey & de Vuyst, 2014; Randazzo et al., 2016).



Figure 4.12 Black tea water kefir beverages

(Image was captured by iPhone 4S, Apple Inc., USA).

Notes: Samples A (BT/5/25) = fermented (25°C) black tea water kefir beverage containing 5% sucrose; B (BT/5/30) = fermented (30°C) black tea water kefir beverage containing 5% sucrose; C (BT/10/25) = fermented (25°C) black tea water kefir beverage containing 10% sucrose; D (BT/10/30) = fermented (30°C) black tea water kefir beverage containing 10% sucrose.

4.2.6 Conclusions

Results in this phase showed that fermentation temperature and sugar concentration influenced the physio-chemical, microbiological as well as sensory properties of the product. Sample C (containing 10% sugar) fermented at 25°C produced more gas bubbles, had the highest lactic acid concentration ($0.320\pm 0.01\%$) and the highest cell counts of *Lactococcus* spp. ($5.88 \log \text{ cfu/ml}$) and *Lactobacillus* spp. ($5.99 \log \text{ cfu/ml}$) at the end of fermentation. Further, sample C received the highest consumer sensory scores compared to the other samples. The most promising formulation in phase 1 based on physico-chemical, microbiological, and sensory characteristic was sample C, which will be further investigated in subsequent phases.

4.3 Effect of carrot juice concentrations on black tea water kefir beverages

4.3.1 Acidity

In phase 2, carrot juice was added at 72 h into black tea water kefir beverage (formulation C10) selected in the phase 1. Fermentation after addition of carrot juice was described as secondary fermentation. Three levels of carrot juice concentrations (5%, 10%, 15%) were added to three samples C5, C10, and C15, respectively. All the samples had no significant difference in pH ($p>0.05$), which ranged from 3.45 ± 0.11 to 3.47 ± 0.11 after fermentation for 72 h (Figure 4.13). pH of samples C5, C10, and C15 increased to 3.91 ± 0.29 , 4.14 ± 0.31 , and 4.26 ± 0.37 , respectively, after addition of carrot juice at 72 h (Appendix D) and then decreased ($p<0.05$) to 3.72 ± 0.33 , 3.91 ± 0.44 , and 3.94 ± 0.45 , respectively during secondary fermentation (Figure 4.13). Production of organic acids such as lactic acid and acetic acid by LAB during kefir fermentation decreased pH (Corona et al., 2016; Garcia et al., 2006; Stadie et al., 2013). However, pH of the samples were not different ($p>0.05$) at 96 h fermentation (Appendix F).

pH of all samples decreased ($p<0.05$) while T.A. increased during fermentation for 96 h (Figure 4.13). The sample containing higher carrot juice concentration produced higher amount of T.A. (Figure 4.13). The results were in agreement with Madora et al., (2016) who reported increased acidity in low fat yogurt when the concentration of carrot juice was increased. However, the results are contrary to Kiros et al., (2016), who reported decreased titratable acidity in yogurt after addition of carrot juice. Discrepancies between results of this study and previous reports may attributed to different types of fermented products, fermentation temperature, and microorganisms (starter culture) used which may influence the acidity of the fermented product. Yogurt fermentation consist of only lactic acid bacteria while kefir fermentation has a symbiotic culture of lactic acid bacteria and yeast (Tamime & Robinson, 1999; Stadie et al., 2013). Further, Sakamoto et al., (1996) reported that different types of lactic acid bacteria produced lactic acid at different amount from 0.23% to 0.48% after fermentation of carrot juice for 20 h. In this study, sample C15 had the highest T.A. ($0.417\pm 0.01\%$) (Appendix D). However, there was no difference ($p>0.05$) in T.A. between samples C10 and C15 (Appendix F).

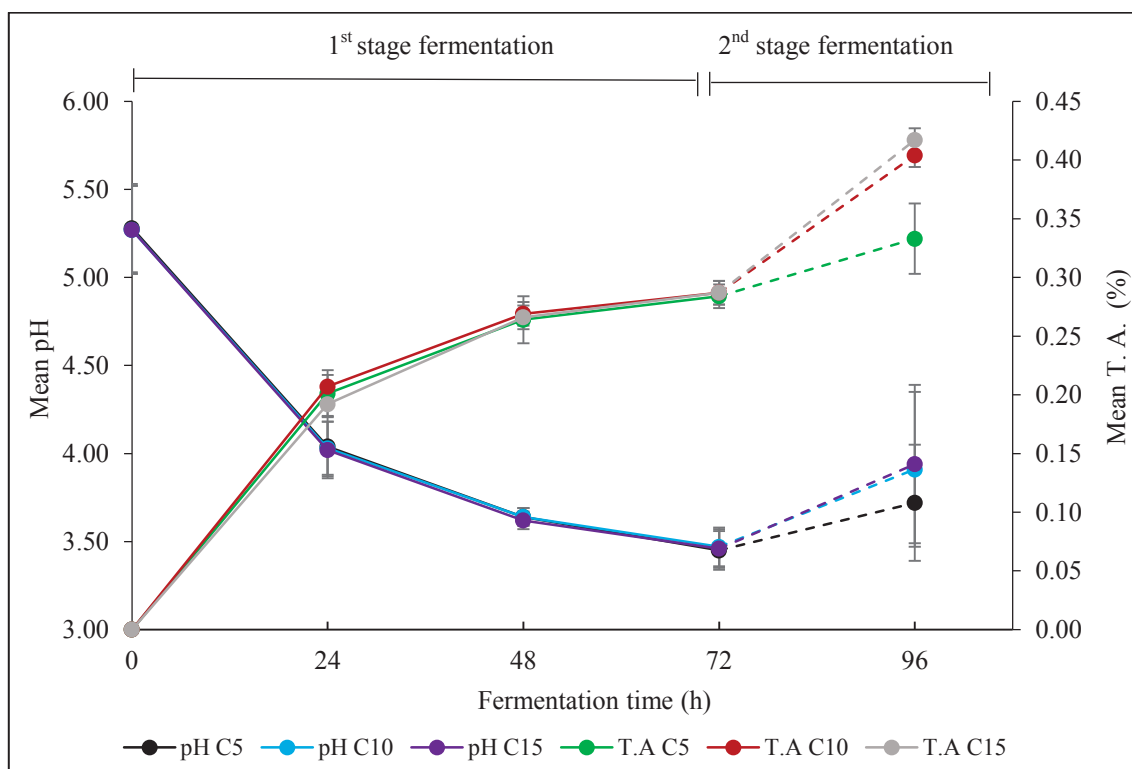


Figure 4.13 Mean pH and titratable acidity of black tea water kefir beverages with and without added carrot juice during fermentation

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = ± SD (n=6); T.A. = Titratable Acidity.

4.3.2 Total soluble solids (°Brix)

Total soluble solids of samples C5, C10, and C15 increased by 0.23, 0.25, and 0.25°Brix, respectively, after addition of carrot juice at 72 h (Appendix D). Carrot juice contains carbohydrates such as sucrose, glucose, and fructose (Bach et al., 2015), thus addition of carrot juice contributed to the increase of total soluble solids in the samples (Madora et al., 2016). Total soluble solids for all samples decreased ($p < 0.05$) during fermentation for 96 h (Figure 4.14). Decreased in total soluble solids for all the samples suggested that lactic acid bacteria and yeasts in water kefir grains metabolised the sugars present in black tea water kefir beverages into organic compounds (Laureys & de Vuyst, 2014; Stadie et al., 2013). According to Li (2006), fermentation of carrot using a

mixed culture of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* resulted in faster sugar metabolism than a single culture of *Lactobacillus plantarum*.

Figure 4.14 shows that presence of higher carrot juice concentration in samples resulted in lower TSS ($^{\circ}$ Brix) in black tea water kefir beverages at the end of fermentation. This result was similar to Kiros et al., (2016) who reported that total solids of yogurt decreased with increased concentration of carrot juice after fermentation. Sample C15 had the lowest total soluble solids at $6.75 \pm 0.21^{\circ}$ Brix while sample C5 had the highest TSS at $7.33 \pm 0.09^{\circ}$ Brix (Appendix D). However, there was not difference ($p > 0.05$) of total soluble solids between samples C10 and C15 (Appendix F).

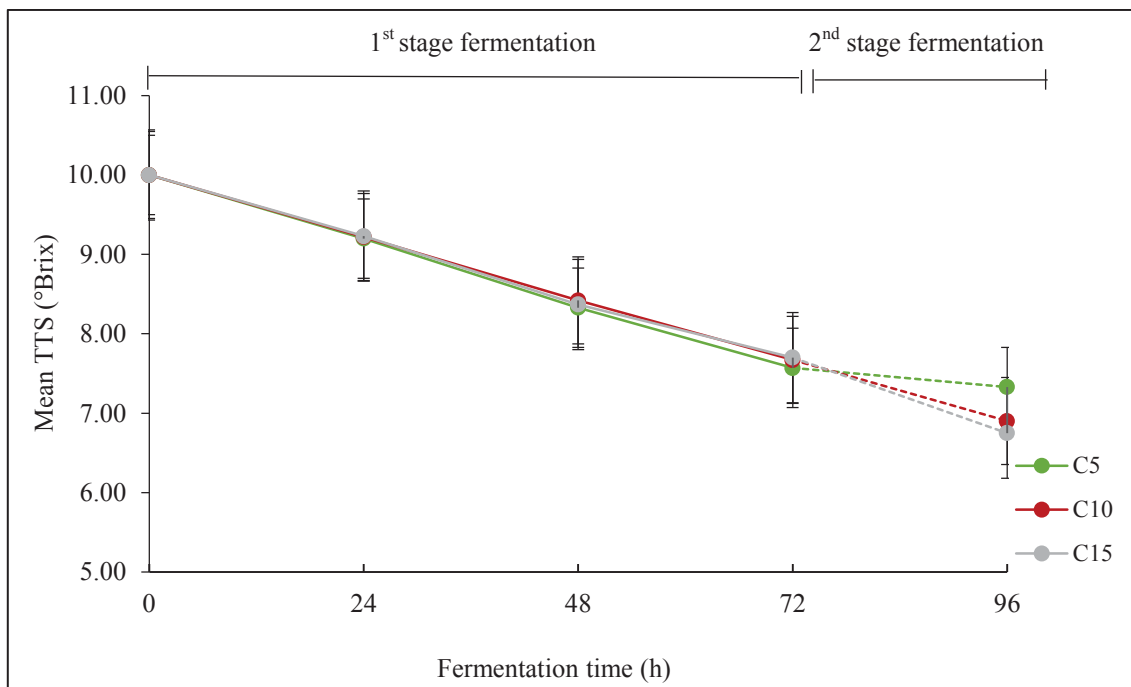


Figure 4.14 Mean total soluble solids ($^{\circ}$ Brix) of black tea water kefir beverages with and without added carrot juice during fermentation

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25 $^{\circ}$ C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25 $^{\circ}$ C for 24 h with added carrot juice (---); Error bars = \pm SD (n=6); TSS = Total Soluble Solids.

4.3.3 Colour

Colour of pasteurised carrot juice used in this study had L^* , a^* , and b^* values of 0.56 ± 0.15 , 2.19 ± 0.73 , and 0.87 ± 0.26 , respectively (Appendix D). This result was different to Sakamoto et al., (1996) which reported the colour of carrot juice with L^* , a^* , and b^* values of 27.81, 10.71, and 15.57, respectively. According to Bao & Chang (1994), Chen et al., (1995), and Khalil et al., (2015), different processing conditions in carrot juice production can influence the colour of carrot juice. Goncalves et al., (2010) reported that the colour of carrot (L^* , a^* , and b^*) decreased with increased processing time and temperature. In addition, heat treatment induces modifications in carotenoids resulting in darker carrot juice which is reflected by low L^* , a^* , and b^* values (Ahmed et al., 2002; Chen et al., 1995). Madora et al., (2016) reported that fresh carrot had higher L^* , a^* , and b^* values compared to heated carrot powder.

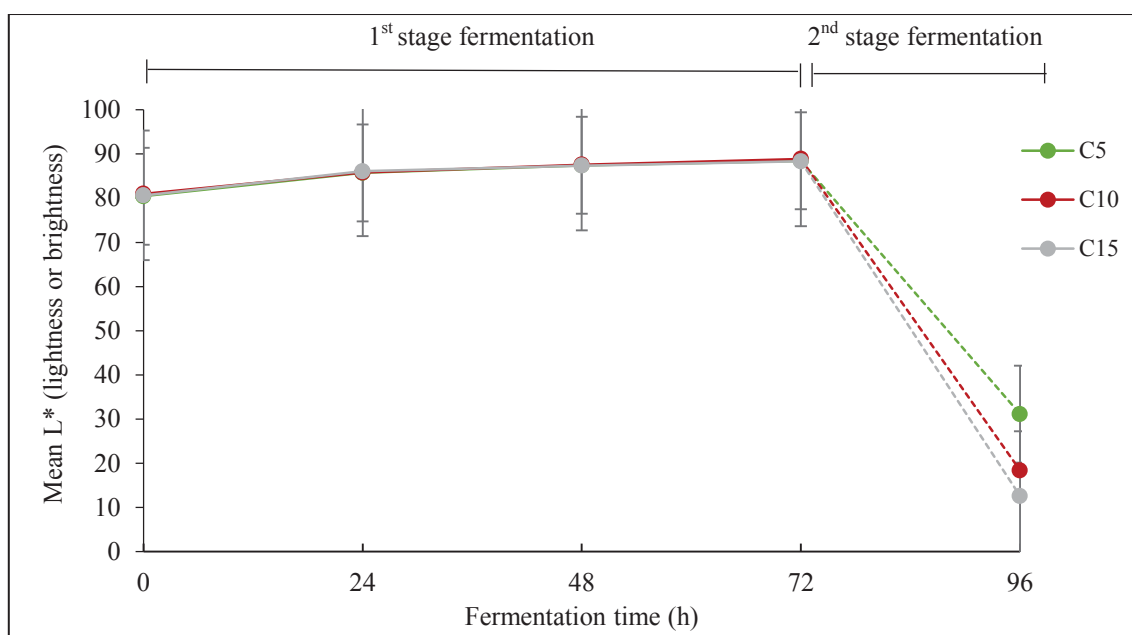


Figure 4.15 Mean L^* of black tea water kefir beverages with and without added carrot juice during fermentation

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=6).

L* values of all samples increased during 72 h fermentation and then decreased ($p < 0.05$) after secondary fermentation for 24 h (Figure 4.15). The lightness among the samples were difference ($p < 0.05$) at 96 h fermentation. Results suggest that concentration of carrot juice may have affected the lightness of the products during secondary fermentation. After addition of carrot juice at 72 h, the lightness of samples C5, C10, and C15 were 43.61 ± 2.87 , 25.77 ± 3.59 , and 16.78 ± 3.72 , respectively, which decreased to 31.12 ± 0.72 , 18.39 ± 0.86 , 12.59 ± 0.80 , respectively, after secondary fermentation for 24 h fermentation (Appendix D). Sample C15 with added 15% carrot juice concentration had the lowest L* value while C5 containing 5% carrot juice had the highest lightness after secondary fermentation (Figure 4.15). This result was similar to Madora et al., (2016) who reported that the lightness of yogurt decreased as the concentration of carrot powder was increased.

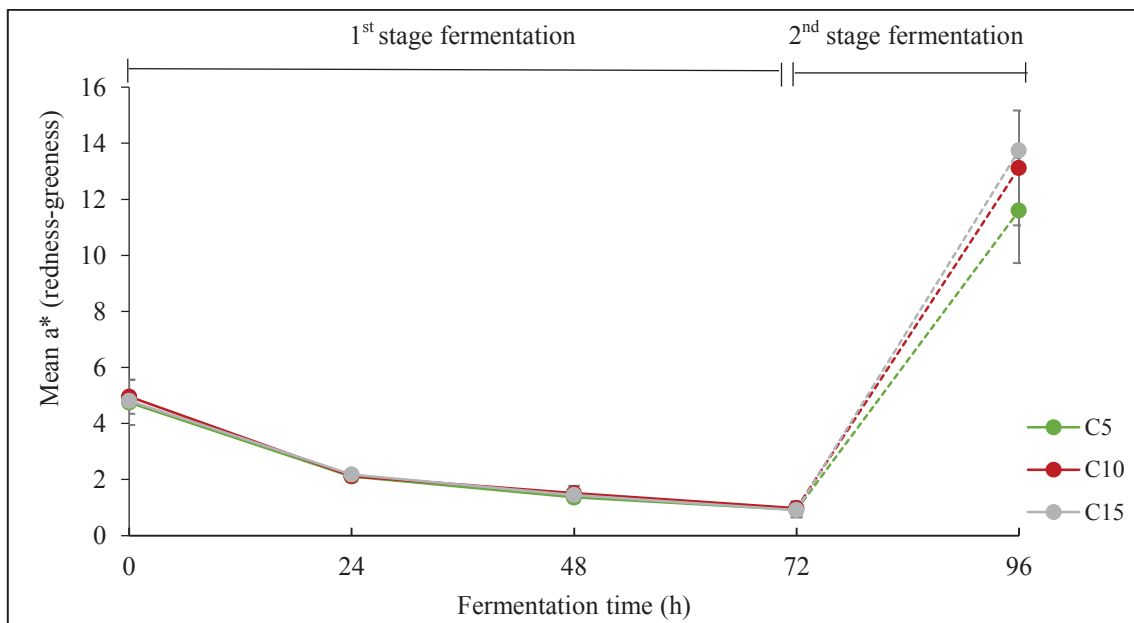


Figure 4.16 Mean a* of black tea water kefir beverages with and without added carrot juice during fermentation

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=6).

Figure 4.16 shows that a* values decreased during 72 h fermentation and then increased after secondary fermentation. Increase of a* values in all the samples after addition of

carrot juice may due to the presence of carotenoid compounds such as β -carotene which is responsible for red-orange colour in carrot (Quitao-Teixeira et al., 2008). Redness-greenness (a^*) of samples C5, C10, and C15 were 16.02 ± 2.56 , 19.12 ± 2.14 , and 19.44 ± 1.71 , respectively, after addition of carrot juice which decreased to 11.60 ± 1.88 , 13.12 ± 2.05 , and 13.75 ± 2.06 , respectively after secondary fermentation for 24 h (Appendix D). Decreased a^* values during secondary fermentation has the same trend with the decreased a^* values in the initial fermentation for 72 h (Figure 4.16). Although, a^* values decreased during fermentation, there was no difference ($p > 0.05$) in a^* values among the samples at 96 h fermentation. The results indicated that the concentration of carrot juice might not have affected the redness-greenness of fermented black tea water kefir beverage samples.

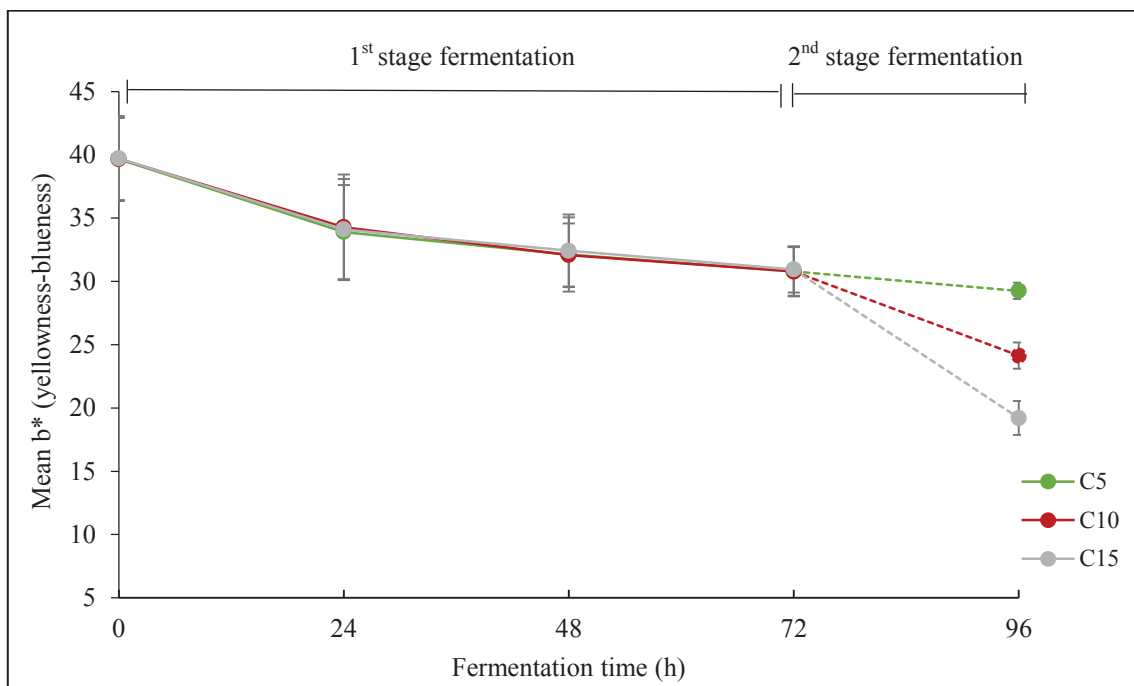


Figure 4.17 Mean b^* of black tea water kefir beverages with and without added carrot juice during fermentation

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=6).

In phase 2 , b* values for samples C5, C10, and C15 were 30.78±1.97, 30.79±1.93, and 30.95±1.82, respectively, after fermentation for 72 h and then decreased to 30.10±0.08, 29.33±0.52, and 28.64±0.92, respectively, after addition of carrot juice (Appendix D). The results showed that the sample containing higher concentration of carrot juice resulted in lower b* value. During secondary fermentation, yellowness-blueness (b*) of all samples continued to decrease (Figure 4.17) and there were differences (p<0.05) of b* values among the samples at 96 h fermentation (Appendix F). This result indicates that concentration of carrot juice may influence the yellowness-blueness of the beverages. According to Minguez-Mosquera & Gandul-Rojas, (1994) and Sakamoto et al., (1996), colour change of fermented carrot juice may be attributed to structural changes of carotenoids due to acidification during fermentation.

4.3.4 Microbiological analysis

Effects of different concentration of carrot juice on viable cell counts of *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* in black tea water kefir beverage during fermentation are presented in Figures 4.18, 4.19, and 4.20. There were slight increases in cell numbers of *Lactococcus* spp. and *Lactobacillus* spp. after secondary fermentation for 24 h (Figures 4.18 and 4.19). The outcome is in agreement with El-Abasy et al., (2012) who reported increased cell counts of LAB after addition of carrot juice to yogurt.

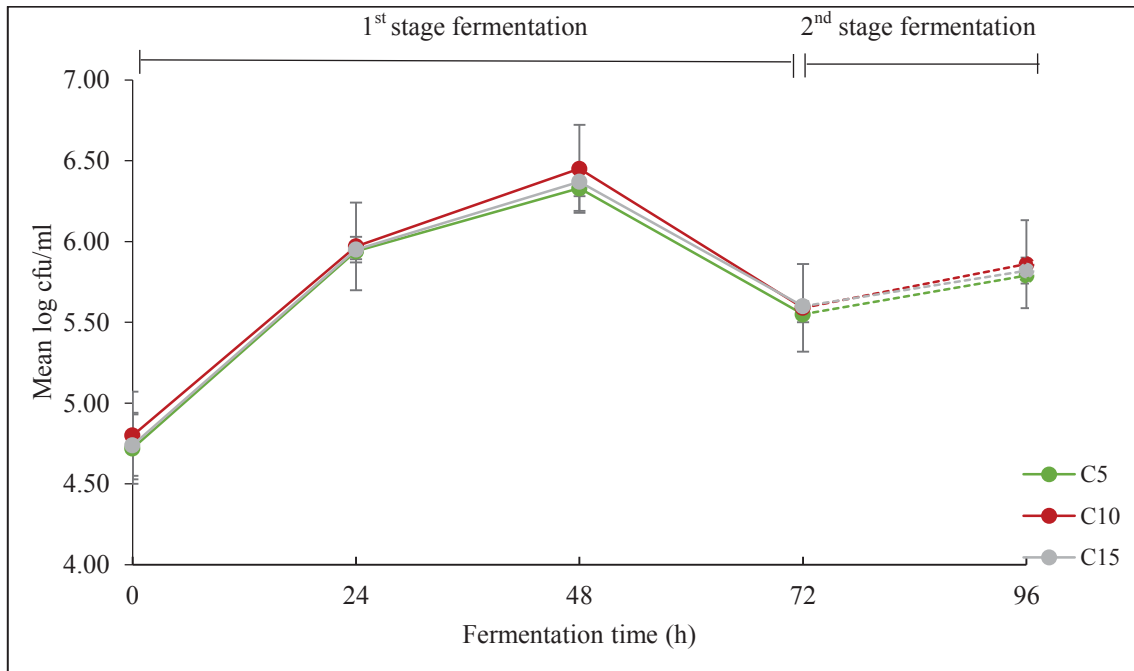


Figure 4.18 Mean log cfu/ml of *Lactococcus* spp. during fermentation of black tea water kefir beverages with and without added carrot juice

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=3).

Carrot juice used in this study had pH of 6.55 ± 0.08 and addition of the carrot juice increased pH of the samples (Appendix D). The fermentation medium became less acidic and favourable for the growth of LAB. Figures 4.18 and 4.19 showed that all the samples containing carrot juice had higher viable cell counts at 96 h compared to 72 h. The addition of carrot juice to black tea water kefir beverage improved the growth of *Lactococcus* spp. and *Lactobacillus* spp. which may be due to increased pH of the samples after addition of carrot juice that created a favourable environment for growth of *Lactococcus* spp. and *Lactobacillus* spp. In addition, sugar and nutrient content in carrot juice could provide additional carbon and energy source to lactic acid bacteria during fermentation (Kun et al., 2008).

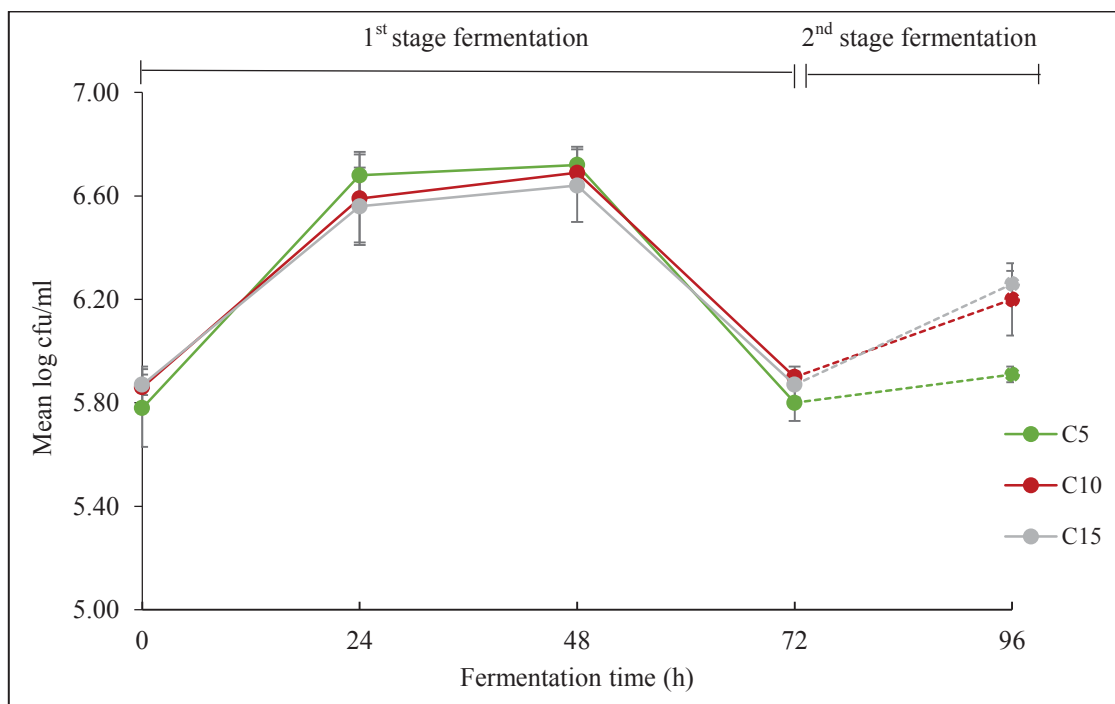


Figure 4.19 Mean log cfu/ml of *Lactobacillus* spp. during fermentation of black tea water kefir beverages with and without added carrot juice

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=3).

In general, *Lactococcus* spp and *Lactobacillus* spp. growth was slow which was shown by the increase of cell numbers ranging from 0.22-0.27 log cfu/ml and 0.11-0.39 log cfu/ml, respectively, after secondary fermentation for 24 h. There was no difference ($p>0.05$) in the population of *Lactococcus* spp. while there were differences ($p<0.05$) of *Lactobacillus* spp. cell counts among the samples at 96 h (Appendix F). Samples C10 and C15 had the highest cell counts of *Lactococcus* spp., and *Lactobacillus* spp., respectively, with 5.86 ± 0.01 log cfu/ml and 6.26 ± 0.05 log cfu/ml, respectively, after secondary fermentation. However, viable cell counts of *Lactobacillus* spp. in sample C10 (6.20 ± 0.14 log cfu/ml) was not different ($p>0.05$) to sample C15

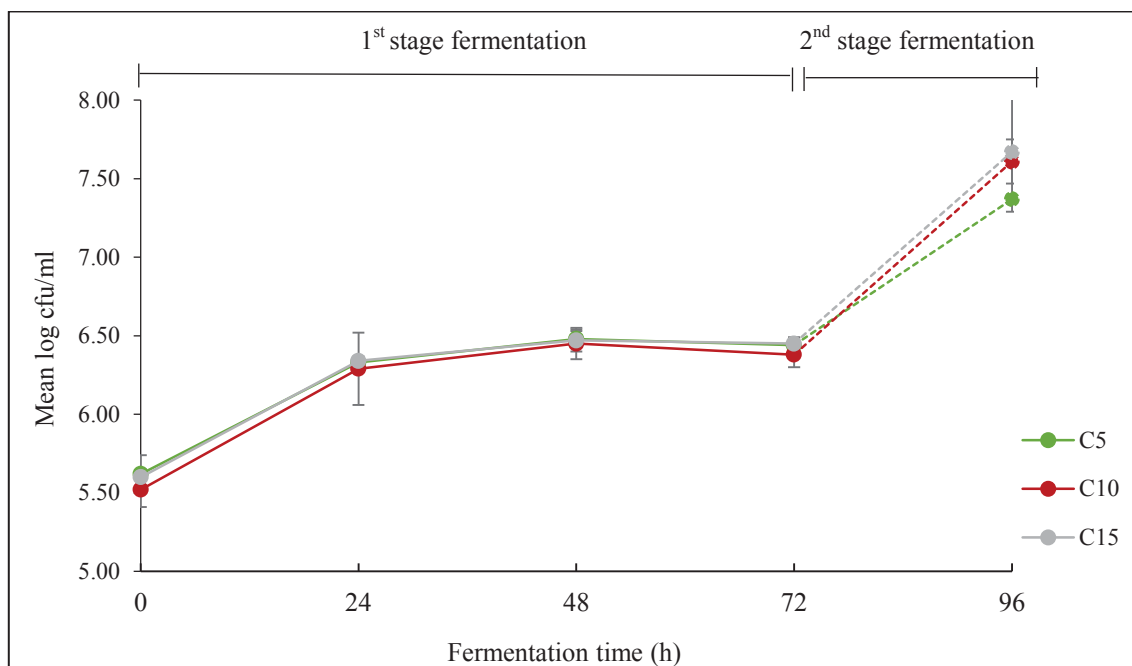


Figure 4.20 Mean log cfu/ml of *Saccharomyces cerevisiae* during fermentation of black tea water kefir beverages with and without added carrot juice

Notes: Samples C5 = fermented black tea water kefir beverage containing 5% carrot juice; C10 = fermented black tea water kefir beverage containing 10% carrot juice; C15 = fermented black tea water kefir beverage containing 15% carrot juice; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=3).

Cell numbers of *Saccharomyces cerevisiae* increased ($p < 0.05$) during secondary fermentation for all samples (Figure 4.20). At 72 h, viable cell counts of yeast in samples C5, C10, and C15 were 6.44 ± 0.05 , 6.38 ± 0.08 , 6.45 ± 0.04 , respectively, which then increased ($p < 0.05$) to 7.37 ± 0.03 , 7.61 ± 0.14 , and 7.67 ± 0.38 , respectively. Higher concentration of carrot juice may have contributed to increase growth of *Saccharomyces cerevisiae* (Figure 4.20).

4.3.5 Sensory evaluation

Results of sensory evaluation of black tea water kefir beverages containing carrot juice are shown in Figure 4.21. Results showed that different levels of carrot juice concentration did not affect ($p > 0.05$) the sourness and sweetness of the samples. However, there were significant differences ($p < 0.05$) among the samples in appearance,

odour, flavour, and overall acceptability parameter. In phase 2, fermented black tea water kefir containing carrot juice samples had pH below of 4.00 and TSS of 8.0°Brix. Thus, scores for sourness and sweetness ranged from 5.85±1.35-6.17±1.36 and 6.07±1.62-6.58±1.61, respectively, which indicate that consumer panellists liked the sourness and sweetness of the products (Appendix D).

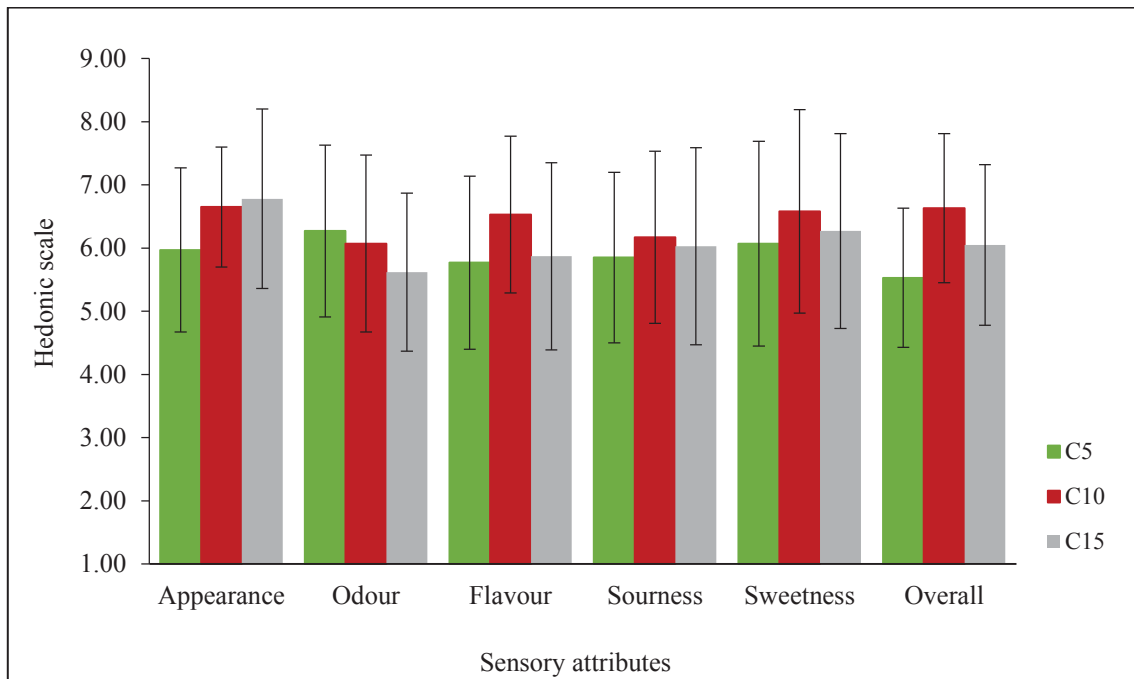


Figure 4.21 Mean sensory scores of black tea water kefir beverages with added carrot juice

Notes: Samples C5 = fermented (25°C) black tea water kefir beverage containing 5% carrot juice; C10 = fermented (25°C) black tea water kefir beverage containing 10% carrot juice; C15= fermented (25°C) black tea water kefir beverage containing 15% carrot juice; Error bars = ± SD (n=60); Hedonic scale = 1-9 with 1 as lowest and 9 the highest.

Black tea water kefir beverage with 10% carrot juice (sample C10) had the highest score for all the sensory attributes, except for the appearance, as compared to other samples (Figure 4.21). However, there was no difference ($p>0.05$) in appearance, odour, sourness, and sweetness between samples C10 and C15. Sensory score for appearance of samples C5, C10, and C15 were 5.97±1.30, 6.65±0.95, and 6.78±1.42, respectively. Fermented black tea water kefir containing higher concentration of carrot juice had higher scores for appearance due to darker and deeper orange colour of the sample (Figure 4.22) which may be more appealing to the consumer sensory panellist (Figure

4.21). Sample C10 containing 10% carrot juice had more gas bubbles, which probably contributes to the overall sensory profile of the product (Figure 4.21).



Figure 4.22 Physical appearance of black tea water kefir beverages with added carrot juice at different levels of concentration.

Notes: Samples C5 = fermented (25°C) black tea water kefir beverage containing 5% carrot juice; C10 = fermented (25°C) black tea water kefir beverage containing 10% carrot juice; C15 = fermented (25°C) black tea water kefir beverage containing 15% carrot juice.

4.3.6 Conclusions

Results of phase 2 indicated that different levels concentration of carrot juice contributed to the physio-chemical, microbiological and sensory properties of the products. Sample C15 with 15% carrot juice had the highest lactic acid concentration and the lowest total soluble solids. Sample C15 also had the highest counts for *Lactobacillus* spp. and *Saccharomyces cerevisiae* at the end of fermentation (96 h). However, samples C10 and C15 were not significantly different ($p < 0.05$). The former formulation (C10) received the highest sensory scores by consumer sensory panellists. Based on results of phase 2, the most promising formulation was sample C10 which will further be investigated for product stability.

4.4 Concentrations of sugars, organic acids, and antioxidants in final formulation (C10) of black tea water kefir beverage during fermentation

4.4.1 Sugars and organic acids content

Sugars and organic acids compounds in black tea water kefir beverage during fermentation are shown in Figure 4.23. Sucrose decreased ($p < 0.05$) during the first 24 h of fermentation and gradually decreased up to 72 h. Overall, sucrose concentration of the black tea water kefir beverage decreased during 96 h of fermentation. Concentration of sucrose of the fermented black tea water kefir was $1.39 \pm 0.22\%$ (w/v) at 72 h and increased to $3.11 \pm 1.09\%$ (w/v) after addition of 10% carrot juice (Appendix D). Concentration of sucrose continued to decrease during secondary fermentation to $1.62 \pm 0.08\%$ (w/v).

Glucose and fructose were present in fermented black tea water kefir during fermentation, suggesting that, the monosaccharides were produced from the degradation of sucrose by kefir yeast (Laurey & de Vuyst, 2014; Leroi & Pidoux, 1993b; Stadie et al., 2013). The two monosaccharides (glucose and fructose) increased from $0.46 \pm 0.13\%$ (w/v) and $0.34 \pm 0.15\%$ (w/v), respectively, to $0.97 \pm 0.03\%$ (w/v) and $1.58 \pm 0.17\%$ (w/v), respectively, within 72 h fermentation. Stadie et al., (2013) reported similar results in which the concentrations of glucose and fructose increased within 48 h of fermentation. The addition of 10% carrot juice at 72 h increased glucose and fructose to $1.08 \pm 0.14\%$ (w/v) and $1.72 \pm 0.06\%$ (w/v), respectively, and the two sugars slightly decreased after second stage of fermentation (Figure 4.23).

Lactic and acetic acid concentrations increased significantly ($p < 0.05$) during fermentation for 96 h (Figure 4.23). The two organic acids peaked at $0.255 \pm 0.04\%$ (w/v) and $0.148 \pm 0.03\%$ (w/v), respectively, by end of secondary fermentation at 96 h. Concentration of lactic acid in the black tea water kefir beverage was lower than 4.90 g/L which was reported by Laurey & de Vuyst (2014). The difference in concentration of lactic acid between this study and previous work may be related to the variations of microflora composition in kefir grains and substrate (Franzetti et al., 1998; Hsieh et al., 2012). Production of lactic acid and acetic acid in water kefir fermentation indicated the

existence of homo-fermentative and hetero-fermentative pathways by LAB during fermentation (Puerari et al., 2012; Stadie et al., 2013).

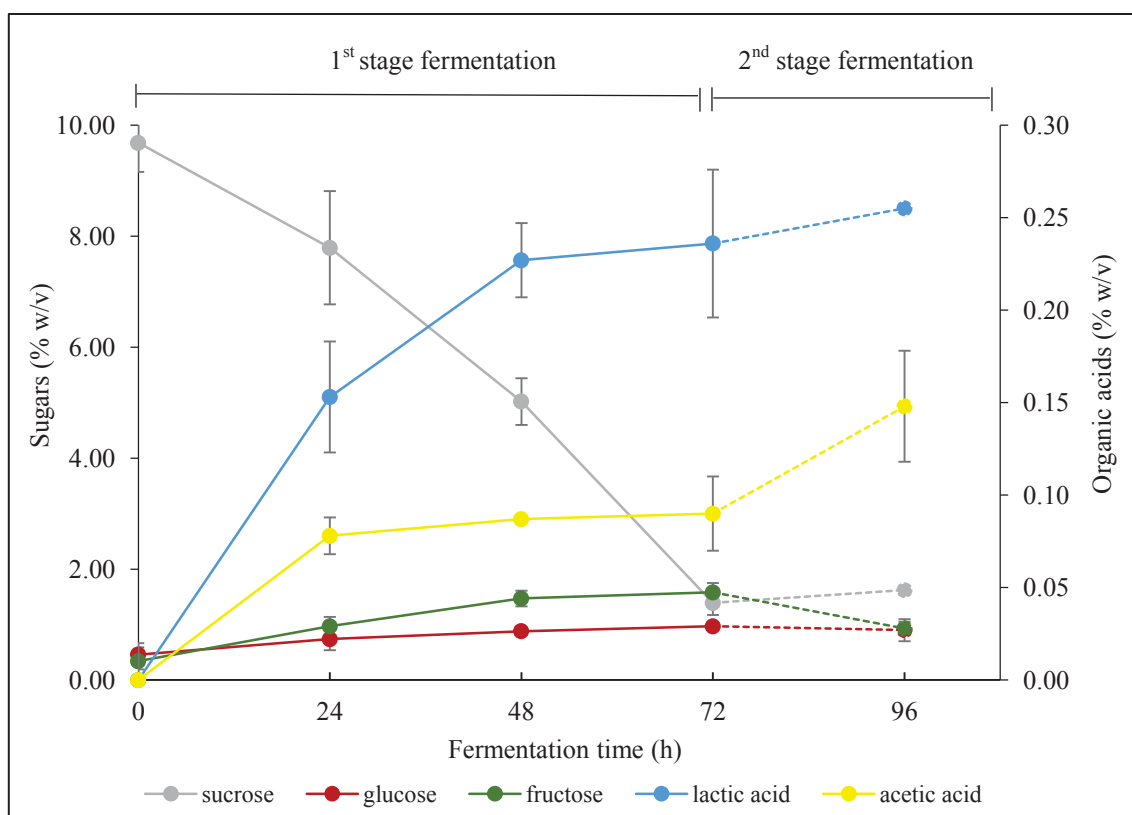


Figure 4.23 Concentration of sugars and organic acids during fermentation of black tea water kefir beverage

Notes: 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—). 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = ± SD (n=6).

4.4.2 Antioxidants

Changes in phenolic compounds concentration of black tea water kefir beverage during fermentation are shown in Figure 4.24. Gallic acid and EGC decreased significantly ($p < 0.05$) while EGCG and ECG progressively increased from the first 24 h to 72 h fermentation, then decreased at 96 h. Further, theobromine and caffeine also decreased significantly ($p < 0.05$) during fermentation (Figure 4.25). At the end of fermentation, concentration of gallic acid, EGC, EGCG, ECG, theobromine, and caffeine were 9.76 ± 0.42 $\mu\text{g/ml}$, 5.09 ± 0.50 $\mu\text{g/ml}$, 3.15 ± 1.69 $\mu\text{g/ml}$, 3.92 ± 2.64 $\mu\text{g/ml}$, 6.57 ± 0.30 $\mu\text{g/ml}$, and 76.80 ± 2.68 $\mu\text{g/ml}$, respectively.

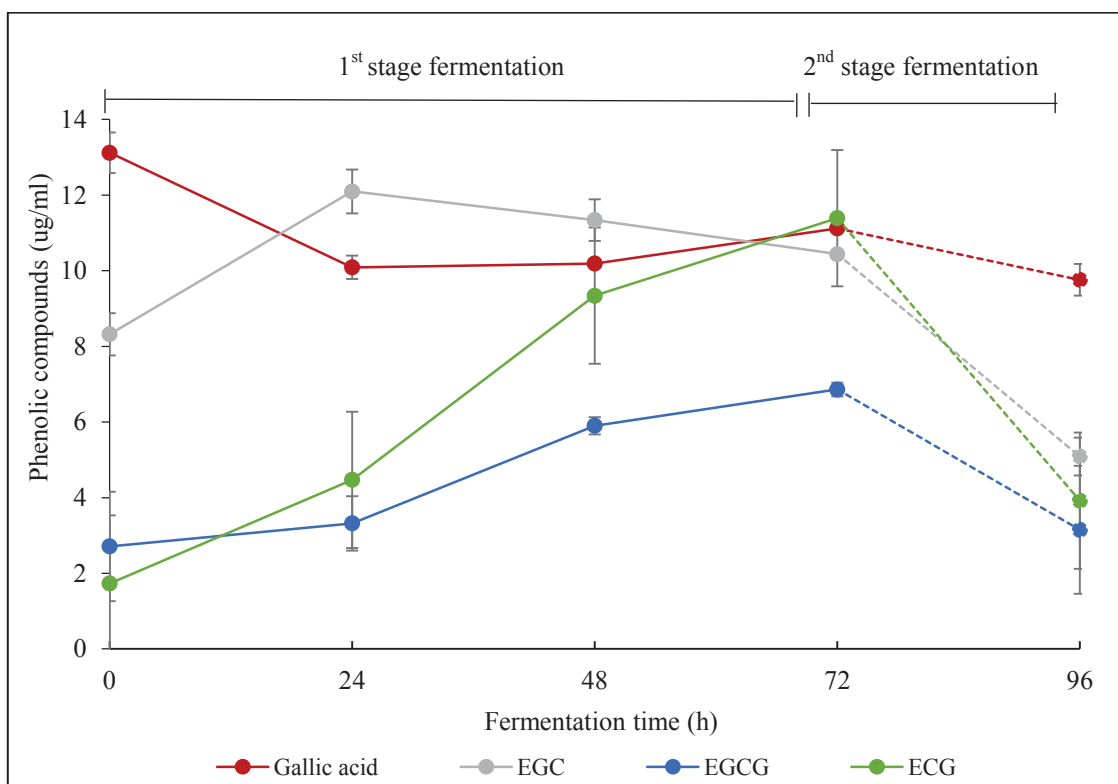


Figure 4.24 Concentration of phenolic compounds in black tea water kefir beverage during fermentation

Notes: EGC = epigallocatechin; EGCG = epigallocatechin gallate; ECG = epicatechin gallate; 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—). 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = ± SD (n=6).

Gallic acid and caffeine concentrations were lower than those reported by Essawet et al., (2015) who indicated concentration of gallic acid and caffeine in tea beverage fermented (kombucha) were $19.46 \pm 0.58 \mu\text{g/ml}$ and $82.33 \pm 3.05 \mu\text{g/ml}$, respectively, at the end of fermentation. However, concentrations of EGC, EGCG, and ECG in black tea water kefir beverage were higher than Essawet et al., (2015). Changes in gallic acid, EGC, EGCG, ECG, theobromine, and caffeine concentrations during fermentation may occur due to the biotransformation of the phenolic compounds by enzymes of LAB and yeasts during fermentation (Zhu et al., 2007). Phenolic compounds have been reported to degrade into smaller compounds during fermentation (Amirdivani & Baba, 2015). An acidic environment may be catalytic to degrade of complex phenolic compounds (Jayabalan et al., 2007 and 2008).

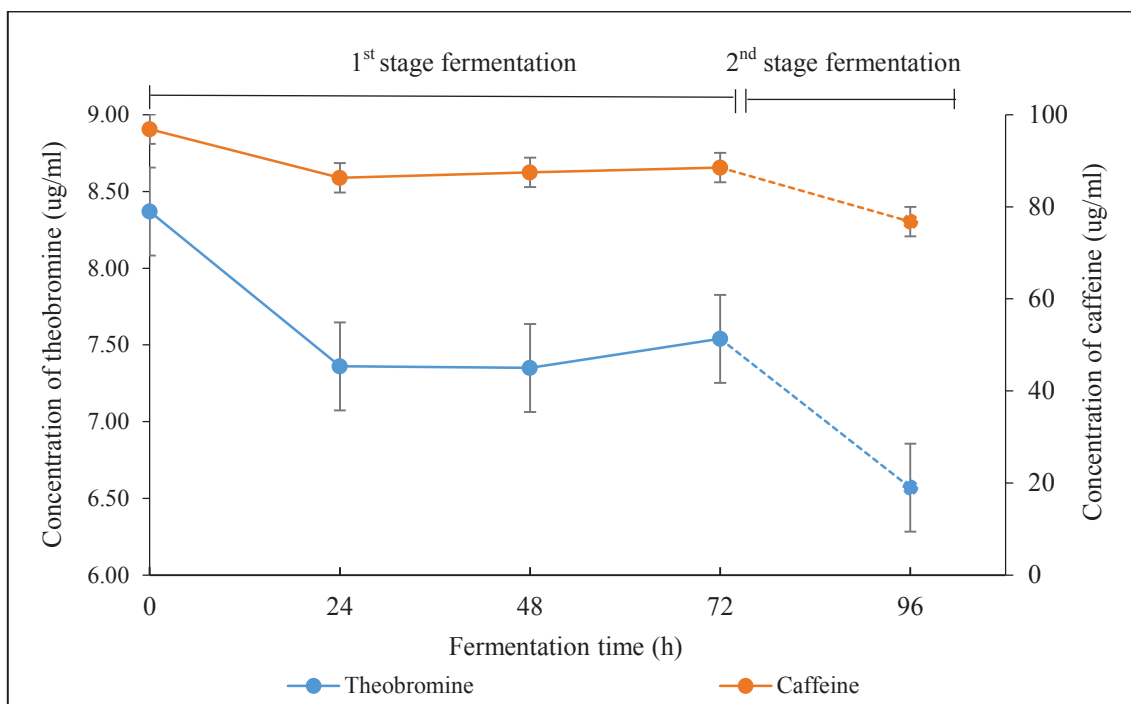


Figure 4.25 Concentration of methylxanthine (theobromine and caffeine) compounds in black tea water kefir beverage during fermentation

Notes: 1st stage fermentation conditions = 25°C for 72 h without added carrot juice (—); 2nd stage fermentation conditions = 25°C for 24 h with added carrot juice (---); Error bars = \pm SD (n=6).

4.5 Stability of black tea water kefir beverage with added 10% carrot juice during storage (4°C)

4.5.1 Acidity

pH and titratable acidity (% T.A.) of black tea-carrot juice water kefir beverage during storage at 4°C are presented in Figure 4.26. As expected, pH of kefir beverage decreased with concomitant increased of titratable acidity. pH decreased ($p < 0.05$) from 3.98 ± 0.10 (day 0) to 3.68 ± 0.10 (day 28) as a consequence of organic acid production (Yilmaz et al., 2006). The reduction of pH indicated the metabolism of sugars to organic acids by kefir starter culture during storage period.

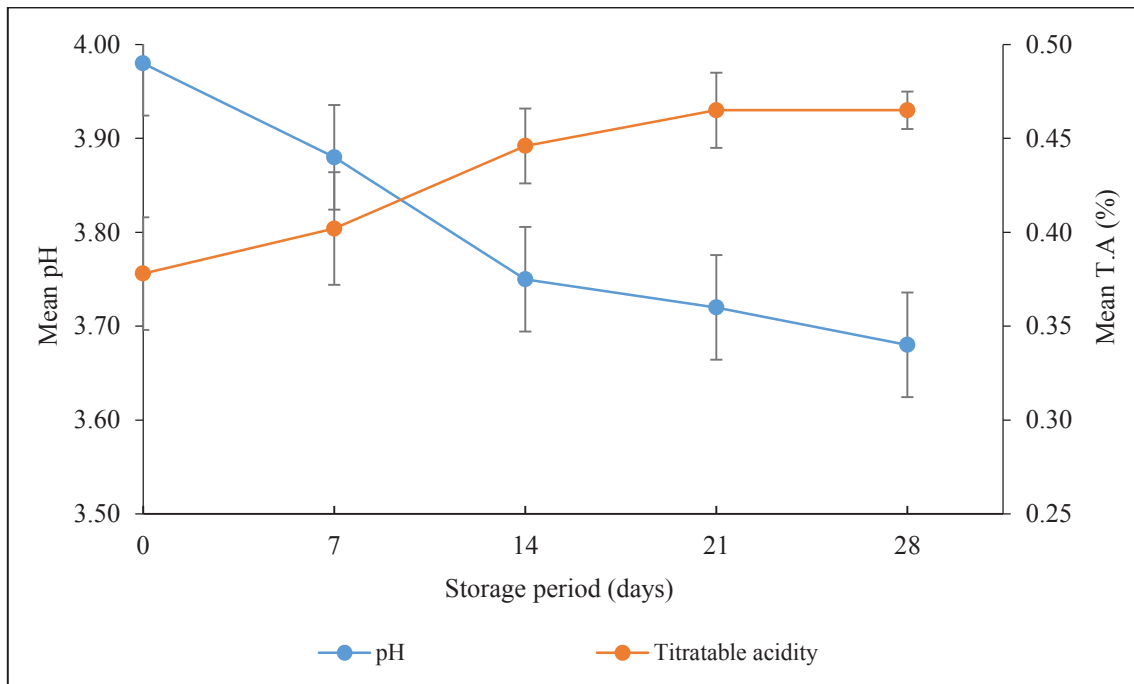


Figure 4.26 Mean pH and titratable acidity of black tea-carrot juice water kefir beverage during storage (4°C)

Notes: T.A. = Titratable Acidity; Error bars = \pm SD (n=6)

Chemical changes such as pH and T.A. in kefir beverage occur during cold storage (Leite et al., 2013). The pH of black tea-carrot juice water kefir beverage at the end of storage was higher than that reported by Kazakos et al., (2016) in pomegranate (3.11 ± 0.05) and orange kefir beverage (3.19 ± 0.05) at day 28. The discrepancy in pH obtained in this study and previous report may due to the use of different fermentation substrate, as well as the initial lower pH (3.8) of pomegranate and orange juice than tea (5.86 ± 0.03) or carrot juice (6.55 ± 0.08) (Appendix D). Concentration of kefir grains used as starter culture may impact on the growth rate of microorganisms (Cui et al., 2013). A study by Irigoyen et al., (2003), pH of kefir beverage was affected by the initial concentration of kefir grains inoculated into kefir beverage.

4.5.2 Total soluble solids (°Brix)

Figure 4.27 shows reduction of total soluble solids in black tea-carrot juice water kefir beverage during storage for 28 days at 4°C. Total soluble solids decreased ($p < 0.05$)

from $7.10 \pm 0.10^\circ\text{Brix}$ to $5.7 \pm 0.18^\circ\text{Brix}$. The results indicate that lactic acid bacteria and yeast were still active and fermented the residual sucrose in black tea water kefir beverage during cold storage. The fermentation process was characterized by continued production and accumulation of organic acids from 0.378 ± 0.03 to $0.465 \pm 0.01\%$ (w/v) as well as decreased pH (Figure 4.26).

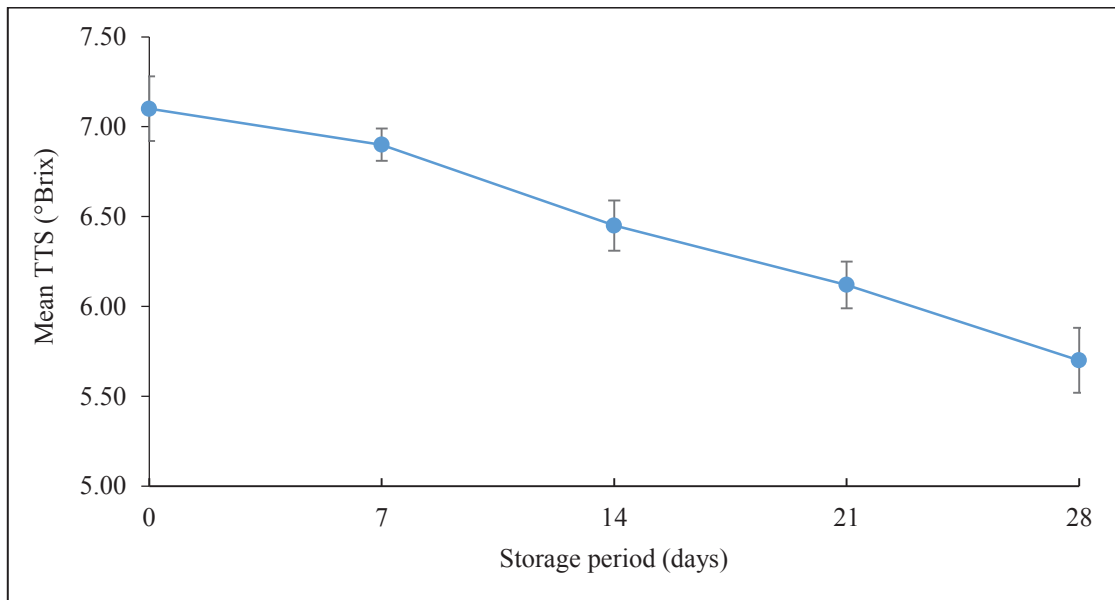


Figure 4.27 Mean total soluble solids (TSS) of black tea-carrot juice water kefir beverage during storage (4°C)

Notes: Error bars = \pm SD (n=6).

Similar results were reported by Abdolmaleki et al., (2015) who indicated significant reductions of carbohydrates content in milk and soya kefir beverage during storage. However, results of this study were not in agreement with Irigoyen et al., (2005) who reported that lactose concentration remained constant during storage for 14 days. Type of substrate and its compound may be responsible for the growth of microorganisms. According to Hsieh et al., (2012), kefir grains contain a large variety of different microorganisms that adapt to different environments. In addition, fermentation substrate significantly affects the growth and changes of microbial ecology in kefir grains which can influence the chemical composition of kefir beverage (Hsieh et al., 2012).

4.5.3 Colour

Colour of products may impact the perception of consumers (Chung et al., 2016). Therefore, measurement of colour during storage is important. Colour parameters of black tea-carrot juice water kefir beverage during storage are shown in Figure 4.28. L^* , a^* , and b^* decreased from 20.16 ± 2.06 - 9.9 ± 1.49 , 14.70 ± 0.88 - 11.21 ± 1.19 , and 23.97 ± 0.23 - 18.89 ± 0.48 , ($p < 0.05$) during refrigerated storage (Appendix D). However, all colour parameters were stable after three weeks of storage. Decreased of b^* values were similar to Hrnjez et al., (2014) who reported yellowness-blueness changes in fermented milk inoculated with mixed starter culture (LAB-yeast) during cold storage. Colour changes of kefir beverages during storage was similar to Cakmakci et al., (2014) who reported that addition of carrot juice to yogurt significantly affected the colour of yogurt during storage

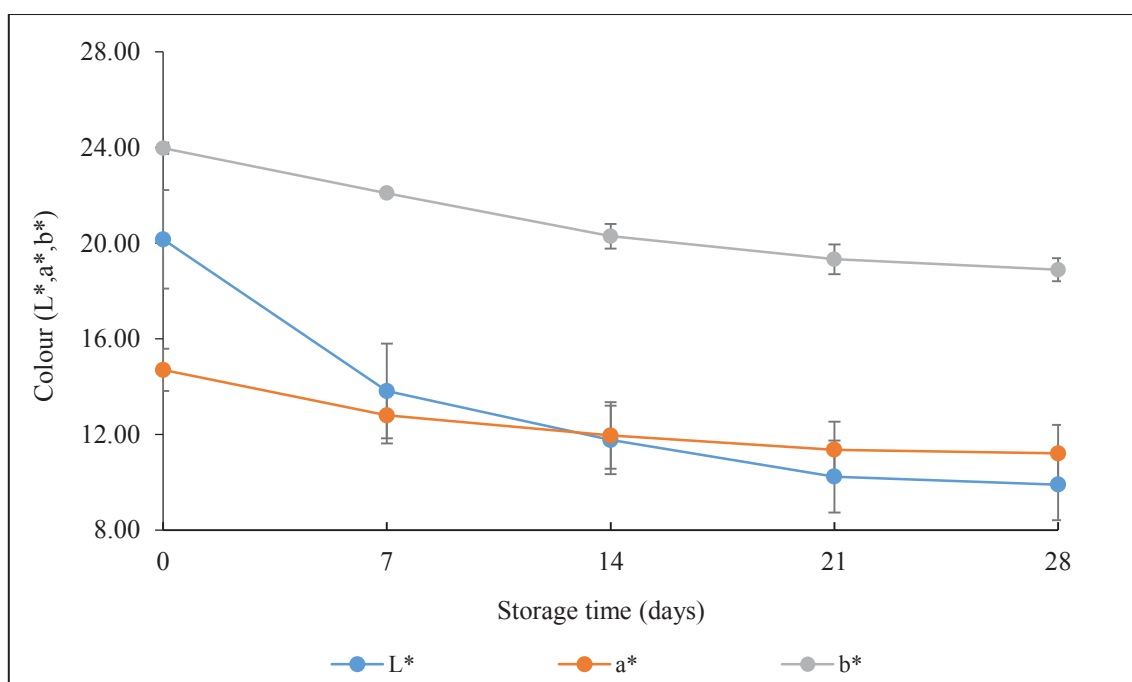


Figure 4.28 Colour of black tea-carrot juice water kefir beverage during storage (4°C)

Notes: L^* =lightness; a^* = redness-greenness; b^* = yellowness-blueness; Error bars = \pm SD (n=6).

4.5.4 Microbiological analysis

Viable cell counts of lactic acid bacteria and yeast of fermented black tea-carrot juice water kefir during storage are presented in Figure 4.29. The microbiological analysis was conducted during storage to determine the survival of LAB during storage. Lactic acid bacteria contained in the water kefir grains used in this study may be categorized as probiotics bacteria. According to FAO/WHO, (2002), viable cell counts of probiotic should meet minimum standard of 10^6 cfu per gram or millilitre at the time of consumption. Probiotics are live microorganisms that confer beneficial effects on health when administered in appropriate amounts (Donkor et al., 2007).

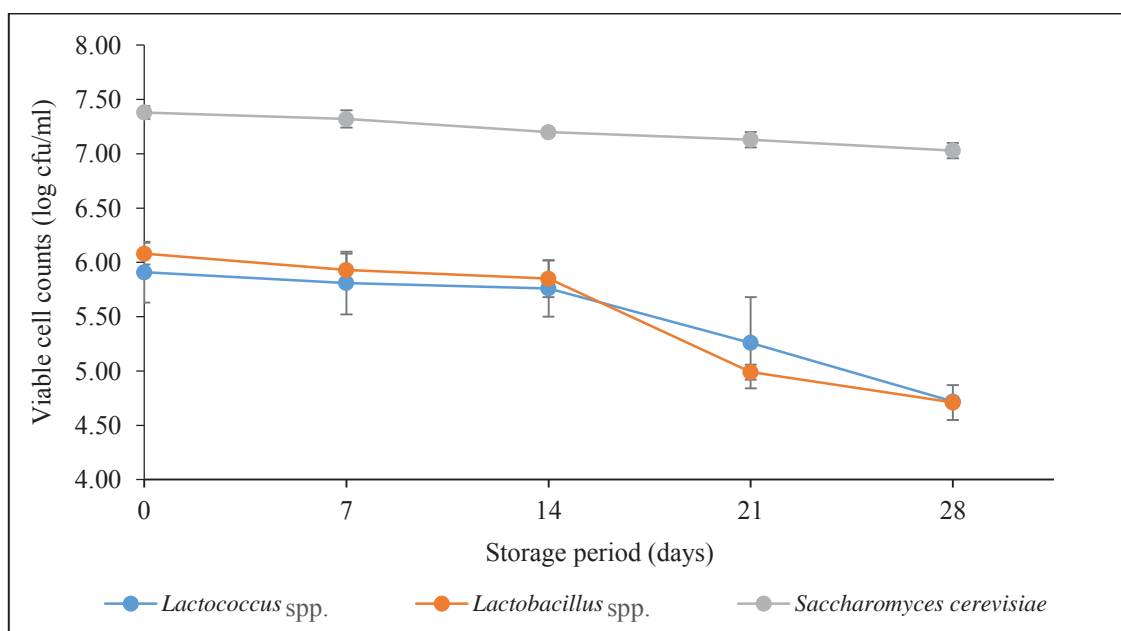


Figure 4.29 Viable cells counts of kefir microorganisms in black tea-carrot juice water kefir beverage during storage (4°C)

Notes: Error bars = \pm SD (n=6).

Viable cells of *Lactococcus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae* were 5.91 ± 0.28 log cfu/ml, 6.08 ± 0.10 log cfu/ml, and 7.38 ± 0.06 log cfu/ml, respectively, at day 0 (Appendix D). The population of *Lactococcus* spp. and *Lactobacillus* spp. decreased slightly within the first two weeks and then decreased ($p < 0.05$) to 4.72 ± 0.16 log cfu/ml and 4.71 ± 0.04 log cfu/ml, respectively, at day 28 of

storage. This result was similar to Abdolmaleki et al., (2015), Gronnevik et al., (2011), and Kazakos et al., (2016) who reported significant decreases of lactococci and lactobacilli during cold storage. The reason could be attributed to the poor survival of some lactic acid bacteria in the stressful condition such very low pH (3.5) (Mousavi et al., 2011; Sheehan et al., 2007). According to Costa et al., (2017), lack of nutrients during storage may also affect the growth of microorganisms. The accumulation of metabolites in the product can inhibit the growth or survival of microorganisms. To support the survival of microorganisms in the final products, it is common to add prebiotics such as inulin and fructooligosaccharides (FOS) (Waldemar et al., 2011). A study by Oliveira and Jurkiewicz (2009) reported that fermented drink with added inulin and acacia gum contained about 10^7 cfu/g of probiotic microorganisms for 22 days of storage.

Viable cells counts of *Saccharomyces cerevisiae* in black tea-carrot juice water kefir beverage decreased during storage (Figure 4.29), contrary to Abdolmaleki et al., (2015) and Gronnevik et al., (2011) who recorded increases during storage. Cell population of kefir yeast in the fermented black tea water kefir beverage was 7.03 ± 0.07 log cfu/ml at day 28 of storage which meet the minimum standard of yeast content (10^4 cfu/g) for kefir (Codex, 2003). Thus, the survival of yeast was better than LAB, which probably due to LAB are more sensitive to low pH than yeast (Irigoyen et al., 2005).

4.5.5 Sugars and organic acids content

Sucrose concentration in this kefir beverage continued to decrease ($p < 0.05$) while glucose and fructose increased during storage (Figure 4.30). The decrease of sucrose, was expected as the disaccharide is hydrolysed to glucose and fructose moieties by the enzyme invertase which is excreted by yeasts (Stadie et al., 2013). Acetic acid concentrations were stable for the first two weeks and then gradually decreased while lactic acid concentration decreased significantly ($p < 0.05$) during refrigerated storage (Figure 4.30), probably due to reduction of LAB during storage (Figure 4.29). The amount of lactic acid decreased during storage which may be caused by the metabolism of the pyruvate by yeast. Yeast metabolise pyruvate to other organic compounds such as ethanol and CO_2 (Paucean et al., 2012).

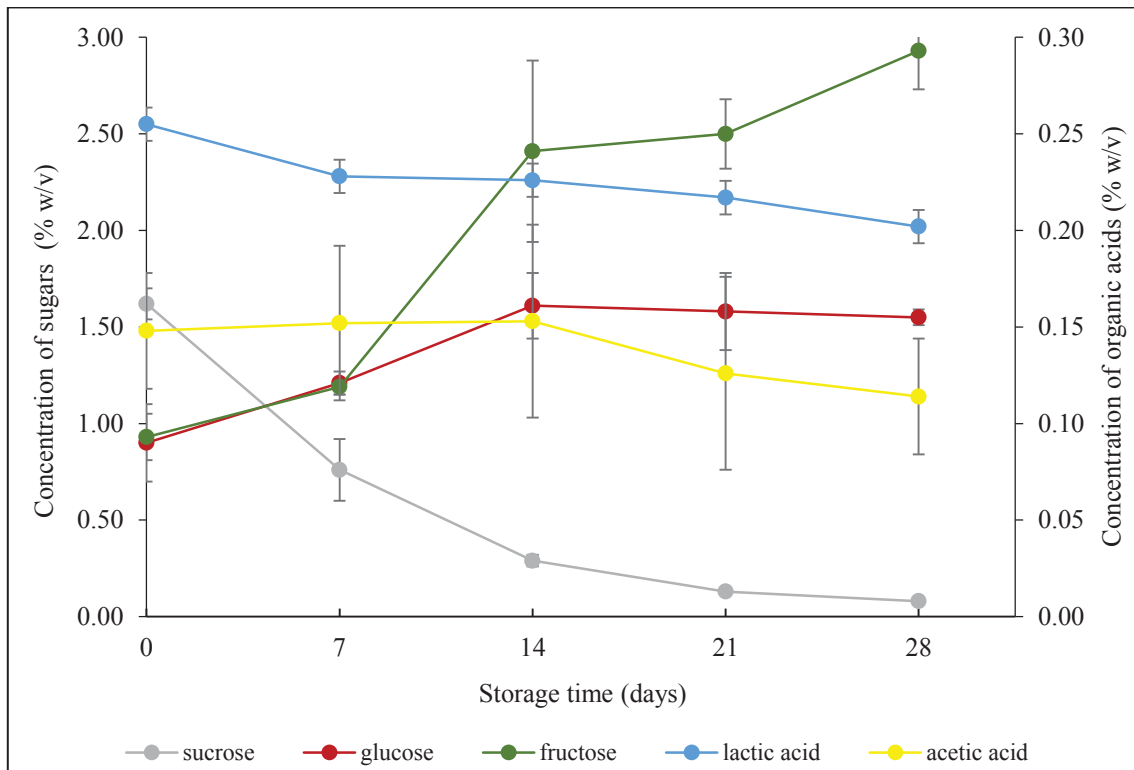


Figure 4.30 Concentration of sugars and organic acids in black tea-carrot juice water kefir beverage during storage (4°C)

Notes: Error bars = \pm SD (n=6).

4.5.6 Concentration of antioxidants

Concentration of phenolic compounds in black tea-carrot juice water kefir beverage during refrigerated storage are presented in Figure 4.31. The amount of gallic acid increased while EGC, EGCGC, and ECG had decreased at the end of storage. Gallic acid is released from catechin by LAB (*Lactobacillus hilgardii* 5w) (Alberto et al., 2004). Concentration of theobromine and caffeine were variable during storage (Figure 4.32). Theobromine and caffeine decreased during the first two weeks of storage and then increased in the last two weeks. Amirdivani & Baba, (2014) reported fluctuation of phenolic compounds in green tea yogurt during 28 days of storage.

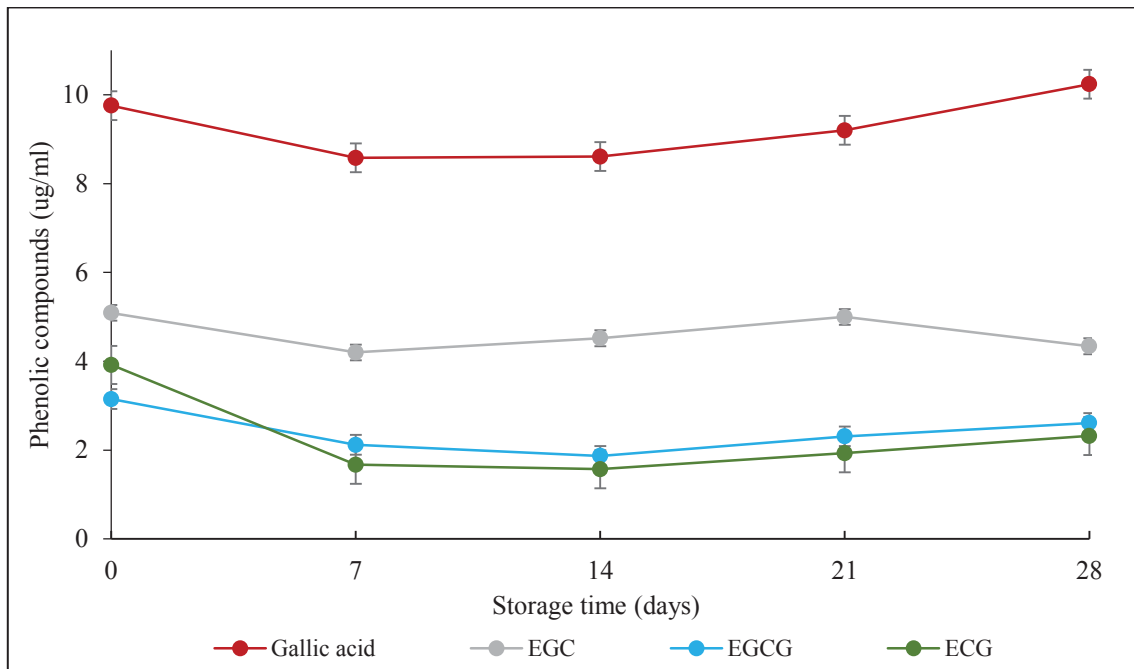


Figure 4.31 Concentration of phenolic compounds in black tea-carrot juice water kefir beverage during storage (4°C)

Notes: EGC = epigallocatechin; EGCG = epigallocatechin gallate; ECG = epicatechin gallate; Error bars = \pm SD (n=6).

Phenolic compounds decreased during storage which may be attributed to the degradation of complex polyphenols to small molecules by enzymes liberated by bacteria and yeast in acidic environment or complexion with other molecules (Amirdivani & Baba, 2015; Jayabalan et al., 2008; Kalt et al., 1999; Rawel et al., 2003). Lactic acid bacteria are presumable capable of metabolising phenolic compounds to other metabolites (Alberto et al., 2004; Tabasco et al., 2011). *Lactobacillus* spp. has been reported to metabolise phenolic compounds in food (Rodriguez et al., 2009).

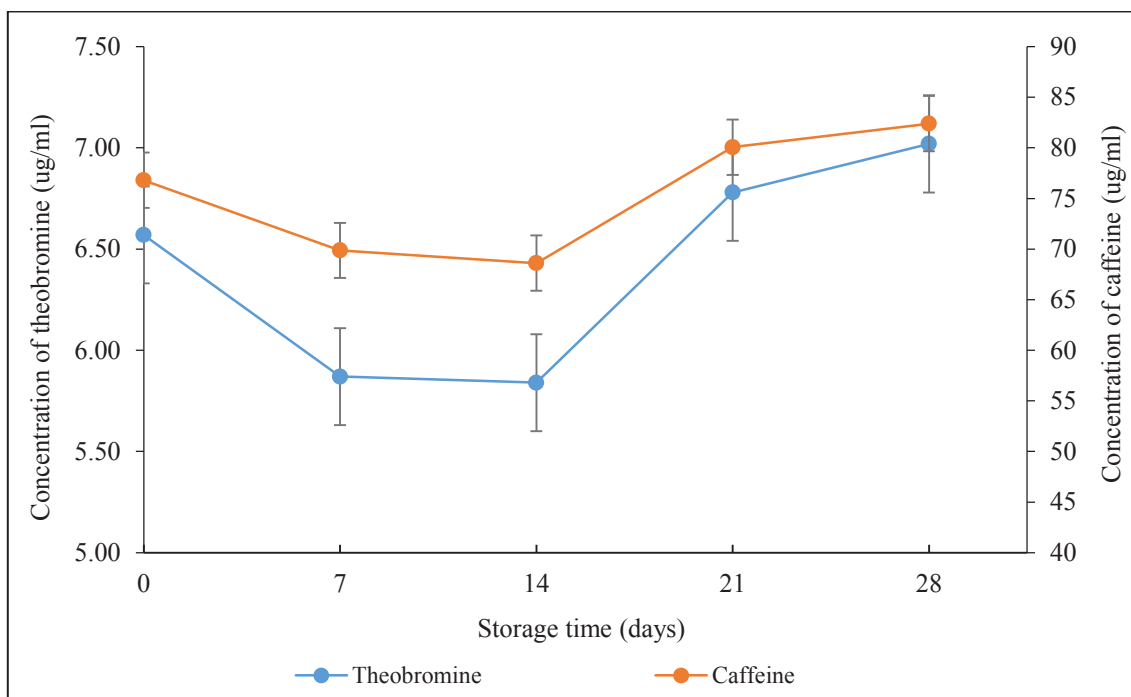


Figure 4.32 Concentration of methylxanthine (theobromine and caffeine) compounds in black tea water kefir beverage during storage (4°C)

Notes: Error bars = \pm SD (n=6).

4.5.7 Sensory evaluation

Sensory evaluation of black tea-carrot juice water kefir beverage during storage is shown in Figure 4.33. Sensory scores for odour, flavour, sourness, sweetness, and overall acceptability parameter were stable for the first two weeks of storage and then steadily decreased. However, sensory scores for appearance were stable up to 21 days of storage and then decreased (Figure 4.33). Results of sensory evaluation were similar to Kilic et al., (1999) who reported decrease of sensory scores during storage. However, Katsiari et al., (2002) reported no significant effect on sensory attributes of yogurt. Discrepancies of results between the present study and previous studies may be related to differences in microflora in kefir and yogurt as well as base formulations. Results of sensory evaluation suggested that black tea-carrot juice water kefir beverage could be stored for two weeks under cold storage (4°C) without loss of sensory properties.

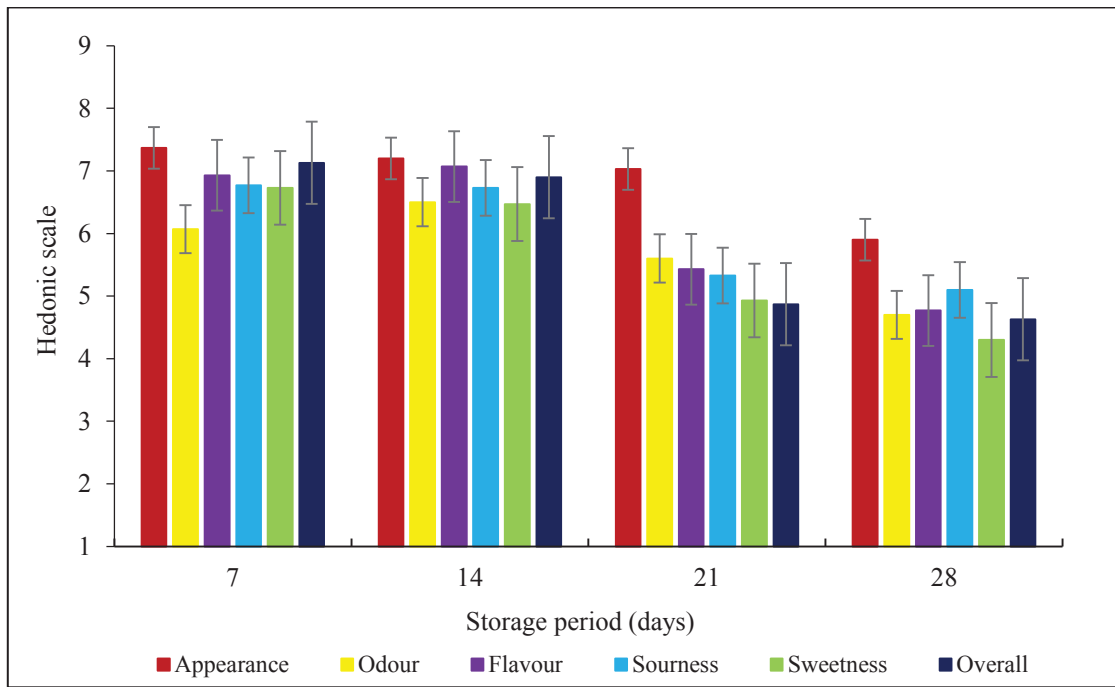


Figure 4.33 Sensory scores of black tea water kefir beverage during storage

Notes: Error bars = \pm SD (n=30).

4.5.8 Conclusions

Viable cell counts in black tea water kefir beverage containing 10% carrot juice were stable during cold storage (4°C) for 14 days and the product was well-liked by a sensory consumer panel using hedonic rating scale. TSS, pH, colour, and concentration of organic acids in the product decreased during storage while concentration of sugars and antioxidants were variable.

5. OVERALL CONCLUSIONS

Black tea infusion and carrot juice can be used as fermentation medium to produce water kefir beverage. The most promising fermentation based on physico-chemical and sensory characteristics was black tea water kefir beverage fermented with 10% sugar and 10% carrot juice at 25°C for 96 h. The addition of carrot juice contributed to a slight increase in the growth of microorganisms during secondary fermentation. During refrigerated storage, colour, pH, total soluble solids, sucrose, organic acids, viable cell counts of lactic acid bacteria and yeasts decreased while titratable acidity, glucose and fructose increased. Black tea water kefir beverage containing carrot juice was well-liked by consumer sensory panellists and the fermented black tea-carrot juice water kefir beverage also contained some antioxidants beneficial to human health.

6. RECOMMENDATIONS

The following recommendations are suggested for future research:

1. The lactic acid bacteria cell counts in the black tea water kefir beverage containing carrot juice were low during storage at 4°C. To support the survival of microorganisms during storage, prebiotics may be added (Oliveira & Jurkiewicz, 2009; Waldemar et al., 2011).
2. In this study, water kefir grains were used as a starter culture. Maintain a stable and constant kefir grains starter culture for kefir beverage production is high challenge and risk of contamination, due to complex microbiological composition in water kefir grains (Guzel-Seydim et al., 2005; N'Guessan et al., 2016; Papavasiliou et al., 2008). Therefore, development of freeze dried starter culture based on the predominant lactic acid bacteria and yeasts from kefir grains may be an alternative method to preserve the inoculum. Use of freeze dried starter culture is convenient and will enable to produce consistent and safe products.

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APPENDIX

A. Composition of agar media for microbiological analyses

Table A.1 List of agar media and its ingredients

Product name and Brand	Ingredients	Composition (g)
MRS agar (CM0361), Oxoid	Peptone	10.0
	Lab-Lemco powder	8.0
	Yeast extract	4.0
	Hydrogen phosphate	2.0
	Sodium acetate 3H ₂ O	5.0
	Tri-ammonium citrate	2.0
	Magnesium sulphate 7H ₂ O	0.2
	Manganese sulphate 4H ₂ O	0.05
	Agar	10.0
M17 agar (CM0785), Oxoid	Tryptone	5.0
	Soya peptone	5.0
	Meat digest	5.0
	Yeast extract	2.5
	Ascorbic acid	0.5
	Magnesium sulphate	0.25
	di-sodium-β-glycerophosphate	19.0
	Agar	11.0
YGC agar (1.16000.0500), Merck KGaA	Yeast extract	5.0
	D(+) glucose	20
	Chloramphenicol	0.1
	Agar	14.9

B. Gram-staining of water kefir grains starter culture

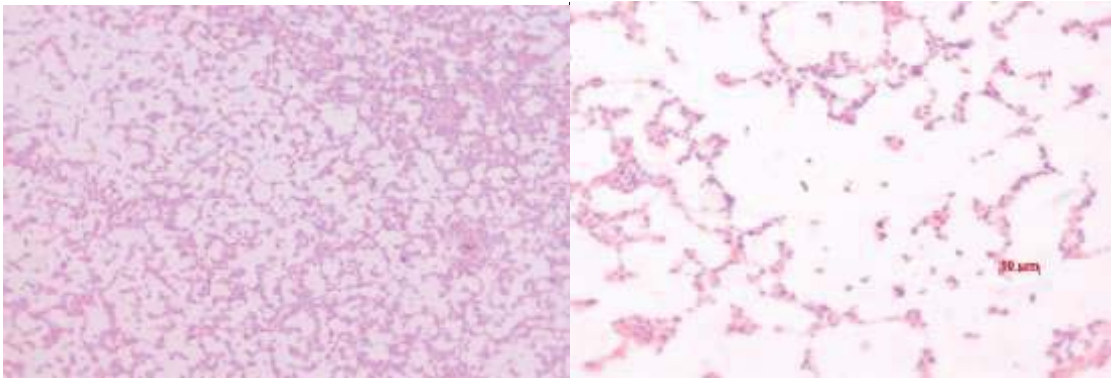


Figure B.1 Gram staining of *Lactococcus* spp. grown on M17 agar under oil immersion (100 x magnifications) using Carl Zeiss (model HBO 50/AC, Germany) transmission light microscope.



Figure B.2 Gram staining of *Lactobacillus* spp. grown on MRS agar under oil immersion (100 x magnifications) using Carl Zeiss (model HBO 50/AC, Germany) transmission light microscope.

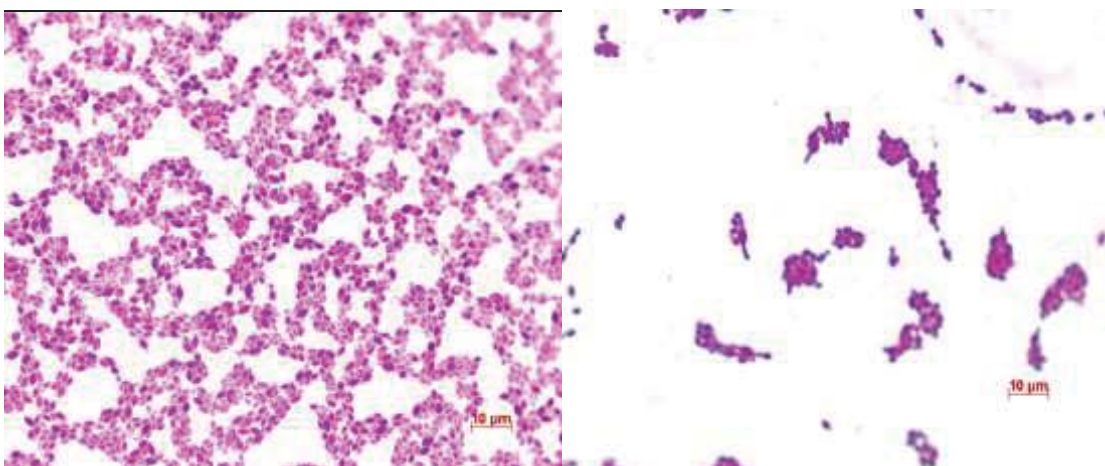


Figure B.3 Gram staining of yeast grown on YGC agar under oil immersion (100 x magnifications) using Carl Zeiss (model HBO 50/AC, Germany) transmission light microscope.

C. Sensory evaluation questioner

ETHICS notification: 4000016138

INFORMATION SHEET

Introduction

I am Maria Subardjo, a Master Food Technology candidate at School of Food and Nutrition (SFN), Albany, Massey University. This study is part of my research project and may contribute to the development of organic fermented black tea water kefir. You are therefore invited to take part in a study that evaluates the sensory characteristics of organic fermented black tea water kefir. The aim of this sensory evaluation is to evaluate the level of acceptance of organic fermented tea water kefir by potential consumers.

Participant involvement

The trial involves tasting and evaluating four types of organic fermented black tea water kefir beverage. Your participation will take 5 to 10 minutes. The organic fermented black tea water kefir you will taste may contain all or some of the following ingredients: organic black tea, commercial starter cultures (kefir grains), and traces of organic sugar.

You should not take part 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 beliefs which may prevent you from consuming the foods under consideration.

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

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

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

Project Contacts

- Maria Subardjo (Master student)-vina.kania@gmail.com
- Dr Tony Muraokunura (supervisor) - a.n.muraokunura@massey.ac.nz

ETHICS notification: 4000016138

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

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

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 conditions set out in the Information Sheet

Signature: Date:

Full Names (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: Organic Fermented Black Tea 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									
Odour									
Flavour									
Sourness									
Sweetness									
Overall Acceptability									

D. Data Analysis

Table D.1 Microbiology analysis of water kefir grains starter culture

Parameter	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Mean \pm SD
	cfu/g	cfu/g	cfu/g	log cfu/g	log cfu/g	log cfu/g	
<i>Lactococcus</i> sp. on M17 agar	3.85x10 ⁶	2.91x10 ⁶	6.50x10 ⁶	6.59	6.46	6.81	6.62 \pm 0.18
<i>Lactobacillus</i> sp. on MRS agar	2.48x10 ⁷	2.80x10 ⁷	1.51x10 ⁷	7.39	7.45	7.18	7.34 \pm 0.14
<i>Saccharomyces cerevisiae</i> on YGS agar	1.10x10 ⁶	2.42x10 ⁶	5.25x10 ⁶	6.04	6.38	6.72	6.38 \pm 0.34

Table D.2 Characteristic of black tea infusions

Parameter	Trial 1	Trial 2	Trial 3	Mean \pm SD
Black tea without water kefir grains				
Colour				
L*	77.12	76.03	76.51	76.49
a*	7.45	7.61	7.65	7.64
b*	54.04	53.79	54.27	54.22
pH				
	5.85	5.85	5.90	5.88
				5.86 \pm 0.03

Table D.3 Characteristic of pasteurised carrot juice

Parameter	Trial 1	Trial 2	Trial 3	Mean \pm SD
Colour				
L*	0.39	0.55	0.72	0.76
a*	1.29	2.36	2.79	2.78
b*	0.50	0.95	1.07	1.18
pH				
	6.46	6.58	6.60	6.63
				6.55 \pm 0.08
TSS(°Brix)				
	9.4	9.3	9.6	9.6
				9.45 \pm 0.12

Table D.4 Raw data phase 1

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S.cerevisiae</i> log CFU/ml
1	1	1	5.55	nd	5.0	81.64	3.45	36.12	4.98	5.74	5.07
1	1	1	5.56	nd	5.0	81.62	3.44	36.11			
1	1	2	5.51	nd	5.1	79.65	2.84	36.26	4.98	5.86	5.38
1	1	2	5.54	nd	5.1	79.11	2.54	35.79			
1	1	3	5.53	nd	5.0	80.45	2.66	36.00	4.87	5.87	5.79
1	1	3	5.55	nd	5.0	80.93	2.80	36.36			
1	2	1	4.09	0.180	4.6	86.52	1.88	30.78	5.90	5.98	6.18
1	2	1	4.11	0.180	4.7	86.43	1.94	30.99			
1	2	2	4.11	0.189	4.7	86.50	1.00	33.08	5.49	6.48	6.39
1	2	2	4.10	0.189	4.6	86.99	0.90	33.47			
1	2	3	4.20	0.180	4.6	86.05	1.26	31.51	5.66	6.30	5.94
1	2	3	4.22	0.189	4.6	86.21	1.30	31.55			
1	3	1	3.61	0.207	4.0	89.60	0.21	28.13	6.05	6.50	6.35
1	3	1	3.67	0.198	4.0	89.51	0.23	28.23			
1	3	2	3.64	0.207	4.1	87.34	0.47	30.57	6.11	6.39	6.16
1	3	2	3.60	0.216	4.1	88.34	0.34	30.88			
1	3	3	3.63	0.216	4.1	87.86	0.54	31.72	6.29	6.48	6.53
1	3	3	3.65	0.216	4.0	87.54	0.62	31.75			
1	4	1	3.43	0.216	3.5	90.15	0.03	26.46	5.75	5.81	6.24
1	4	1	3.41	0.225	3.5	90.47	0.00	26.70			
1	4	2	3.44	0.225	3.3	89.62	0.14	28.89	5.62	5.93	6.28
1	4	2	3.44	0.234	3.3	89.73	0.15	29.39			
1	4	3	3.53	0.225	3.4	88.18	0.30	30.13	5.52	5.88	6.20
1	4	3	3.55	0.225	3.3	88.20	0.27	30.21			

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
2	1	1	5.56	nd	5.0	81.28	3.90	37.11	4.98	5.59	5.17
2	1	1	5.61	nd	5.0	81.11	3.93	37.13			
2	1	2	5.55	nd	5.1	79.91	3.87	38.13	5.03	5.78	5.47
2	1	2	5.50	nd	5.1	79.73	3.93	38.08			
2	1	3	5.57	nd	5.0	80.03	2.44	35.70	4.83	5.98	5.72
2	1	3	5.54	nd	5.0	80.74	2.39	35.68			
2	2	1	4.00	0.198	4.2	86.98	1.88	30.80	5.47	5.88	6.14
2	2	1	4.03	0.198	4.2	86.67	1.65	30.85			
2	2	2	3.91	0.189	4.4	88.03	1.50	32.57	5.98	6.35	6.38
2	2	2	3.91	0.189	4.3	87.60	1.42	33.17			
2	2	3	3.91	0.198	4.4	87.98	0.88	35.40	5.94	6.27	5.91
2	2	3	3.91	0.198	4.4	87.71	0.63	34.34			
2	3	1	3.62	0.234	3.2	89.64	0.13	28.72	6.19	6.57	6.26
2	3	1	3.60	0.225	3.2	89.59	0.14	28.71			
2	3	2	3.58	0.243	3.4	87.91	0.20	29.70	6.29	6.38	6.01
2	3	2	3.60	0.243	3.4	88.24	0.24	29.84			
2	3	3	3.56	0.234	3.4	88.15	0.26	30.19	6.38	6.32	6.50
2	3	3	3.57	0.243	3.5	88.03	0.20	30.27			
2	4	1	3.33	0.252	2.7	90.10	0.04	27.49	5.87	5.58	6.20
2	4	1	3.35	0.252	2.6	89.97	0.02	27.47			
2	4	2	3.28	0.243	2.8	88.53	0.01	28.55	5.87	5.60	6.19
2	4	2	3.30	0.243	2.8	88.46	0.02	28.20			
2	4	3	3.31	0.252	2.9	89.30	0.03	28.69	5.77	5.63	6.25
2	4	3	3.29	0.261	2.9	89.55	0.04	28.79			

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
3	1	1	5.66	nd	10.0	81.10	4.00	35.73	5.04	5.57	5.12
3	1	1	5.70	nd	10.0	80.99	3.99	35.69			
3	1	2	5.54	nd	9.9	79.41	2.52	36.31	4.93	5.78	5.35
3	1	2	5.58	nd	9.9	79.38	2.55	36.02			
3	1	3	5.54	nd	10.0	79.55	3.73	37.96	4.81	5.98	5.82
3	1	3	5.53	nd	10.0	80.13	3.70	37.88			
3	2	1	4.28	0.207	9.4	86.00	2.44	31.20	5.36	6.07	6.32
3	2	1	4.26	0.207	9.4	86.44	2.48	31.11			
3	2	2	4.15	0.216	9.3	86.60	1.89	33.65	6.03	6.79	6.36
3	2	2	4.10	0.216	9.3	86.41	1.72	33.40			
3	2	3	4.43	0.207	9.4	86.05	1.33	35.51	6.04	6.41	6.04
3	2	3	4.45	0.207	9.4	86.47	1.26	35.15			
3	3	1	3.65	0.270	8.8	87.78	0.84	29.19	6.20	6.67	5.41
3	3	1	3.63	0.261	8.8	87.68	0.86	29.23			
3	3	2	3.63	0.270	8.7	87.31	1.07	30.31	6.37	6.47	6.33
3	3	2	3.65	0.279	8.7	87.95	1.06	33.42			
3	3	3	3.67	0.279	8.8	88.15	1.26	29.11	5.43	6.47	6.56
3	3	3	3.70	0.279	8.9	88.87	1.20	31.27			
3	4	1	3.52	0.333	8.0	89.18	0.36	28.43	5.90	5.92	6.40
3	4	1	3.51	0.333	8.0	89.09	0.37	27.39			
3	4	2	3.52	0.315	7.9	88.02	0.94	30.01	5.80	6.12	6.42
3	4	2	3.50	0.324	7.9	88.50	1.05	31.65			
3	4	3	3.48	0.306	7.9	89.30	0.39	31.33	5.95	5.92	6.39
3	4	3	3.49	0.306	7.8	89.12	0.30	30.27			

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
4	1	1	5.57	nd	10.0	80.22	4.13	35.87	5.09	5.61	5.09
4	1	1	5.60	nd	10.0	80.75	4.14	35.83			
4	1	2	5.57	nd	9.9	80.34	3.83	38.14	4.90	5.79	5.42
4	1	2	5.54	nd	9.9	79.05	3.63	37.11			
4	1	3	5.55	nd	10.0	80.91	3.72	37.20	4.83	5.98	5.77
4	1	3	5.57	nd	10.0	80.55	4.20	35.90			
4	2	1	3.98	0.198	9.0	86.30	2.10	31.46	5.47	6.20	6.38
4	2	1	4.00	0.189	9.1	86.09	2.13	31.54			
4	2	2	3.97	0.189	9.0	86.84	1.35	34.76	6.07	6.51	6.47
4	2	2	3.96	0.189	9.0	85.48	1.37	34.65			
4	2	3	4.14	0.198	8.9	87.71	1.66	35.37	6.28	6.39	6.03
4	2	3	4.19	0.198	8.9	86.46	1.68	35.15			
4	3	1	3.64	0.261	8.4	88.48	0.10	29.78	6.21	6.61	6.46
4	3	1	3.63	0.261	8.4	88.20	0.12	28.71			
4	3	2	3.52	0.252	8.5	87.60	0.74	32.61	6.40	6.47	6.46
4	3	2	3.57	0.261	8.5	87.37	0.59	32.54			
4	3	3	3.64	0.270	8.4	88.81	1.26	31.37	6.43	6.47	6.58
4	3	3	3.60	0.270	8.4	88.46	1.18	31.15			
4	4	1	3.31	0.288	7.5	89.20	0.10	27.16	5.94	5.83	6.43
4	4	1	3.33	0.288	7.5	89.25	0.11	27.20			
4	4	2	3.34	0.297	7.3	88.15	0.64	31.11	5.76	5.74	6.45
4	4	2	3.36	0.306	7.2	88.20	0.66	31.13			
4	4	3	3.33	0.297	7.2	89.59	0.15	30.62	5.85	5.77	6.46
4	4	3	3.35	0.288	7.2	89.62	0.17	30.58			

Note:

Product treatment (1:BT5%/25C, 2:BT5%/30C, 3:BT10%/25C, 4:BT10%/30C); Fermentation time (1:0hour, 2:24hours, 3:48hours, 4:72hours)
nd = not detected

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

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

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

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

Table D.5 Raw data phase 2

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S.cerevisiae</i> log CFU/ml
C5	1	1	5.05	nd	9.8	80.54	4.60	36.94	4.48	5.75	5.48
C5	1	1	5.05	nd	9.9	80.49	4.61	36.89			
C5	1	2	5.58	nd	10.0	80.03	3.99	43.79	4.93	5.65	5.66
C5	1	2	5.59	nd	10.0	80.03	3.87	43.81			
C5	1	3	5.18	nd	9.9	80.77	5.74	38.26	4.74	5.94	5.70
C5	1	3	5.20	nd	10.0	80.77	5.70	38.26			
C5	2	1	4.07	0.180	9.2	85.76	2.19	31.32	5.95	6.64	6.31
C5	2	1	4.07	0.180	9.2	85.68	2.21	31.27			
C5	2	2	4.22	0.207	9.2	86.09	1.72	38.70	5.88	6.62	6.32
C5	2	2	4.19	0.207	9.1	86.09	2.13	38.70			
C5	2	3	3.84	0.216	9.3	85.34	2.19	31.75	5.98	6.79	6.36
C5	2	3	3.84	0.216	9.2	85.34	2.19	31.66			
C5	3	1	3.69	0.243	8.3	87.51	1.55	30.27	6.41	6.72	6.53
C5	3	1	3.70	0.243	8.3	86.46	1.52	30.19			
C5	3	2	3.63	0.288	8.2	88.05	1.15	35.93	6.16	6.65	6.50
C5	3	2	3.63	0.270	8.2	88.04	1.15	35.90			
C5	3	3	3.59	0.270	8.5	87.31	1.38	30.22	6.41	6.79	6.40
C5	3	3	3.59	0.270	8.5	87.29	1.42	30.24			
C5	4	1	3.56	0.261	7.7	87.88	1.27	29.14	5.49	5.74	6.45
C5	4	1	3.56	0.270	7.6	88.90	1.25	29.10			
C5	4	2	3.48	0.297	7.5	88.75	0.82	33.28	5.59	5.80	6.38
C5	4	2	3.48	0.297	7.5	88.70	0.83	33.27			
C5	4	3	3.31	0.288	7.6	88.22	0.66	29.95	5.57	5.88	6.45
C5	4	3	3.31	0.288	7.5	88.25	0.67	29.94			
C5	5	1	3.96	0.360	7.5	30.68	13.21	29.95	5.77	5.91	7.41
C5	5	1	3.96	0.360	7.4	30.70	13.14	29.82			
C5	5	2	3.28	0.342	7.3	30.61	9.25	28.57	5.78	5.94	7.36
C5	5	2	3.30	0.333	7.3	30.64	9.18	28.41			
C5	5	3	3.90	0.297	7.2	32.07	12.39	29.37	5.82	5.88	7.35
C5	5	3	3.90	0.306	7.3	32.04	12.42	29.43			

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
C10	1	1	5.03	nd	9.9	81.63	4.59	5.6.45	4.59	5.86	5.48
C10	1	1	5.05	nd	10.0	80.61	4.58	36.83			
C10	1	2	5.58	nd	9.9	80.01	4.62	43.79	4.81	5.78	5.44
C10	1	2	5.60	nd	9.9	80.12	4.45	44.04			
C10	1	3	5.19	nd	10.0	81.82	5.75	38.25	4.99	5.94	5.65
C10	1	3	5.19	nd	10.0	81.80	5.74	38.26			
C10	2	1	4.05	0.198	9.2	85.39	2.14	31.45	5.93	6.73	6.32
C10	2	1	4.05	0.189	9.2	86.41	2.14	31.46			
C10	2	2	4.18	0.216	9.2	85.19	2.08	39.65	6.03	6.41	6.04
C10	2	2	4.20	0.216	9.1	85.16	2.12	39.64			
C10	2	3	3.85	0.216	9.3	86.31	2.09	31.72	5.96	6.64	6.51
C10	2	3	3.85	0.207	9.3	86.31	2.17	31.75			
C10	3	1	3.70	0.252	8.4	88.01	1.26	30.77	6.48	6.67	6.38
C10	3	1	3.70	0.261	8.3	88.96	1.26	30.75			
C10	3	2	3.60	0.279	8.3	86.61	1.82	35.32	6.45	6.71	6.41
C10	3	2	3.60	0.279	8.3	87.57	1.83	35.27			
C10	3	3	3.63	0.270	8.6	87.22	1.45	30.15	6.42	6.69	6.56
C10	3	3	3.63	0.270	8.6	87.24	1.43	30.25			
C10	4	1	3.57	0.279	7.6	89.32	1.25	29.34	5.62	5.95	6.45
C10	4	1	3.56	0.270	7.6	89.35	1.19	29.23			
C10	4	2	3.50	0.297	7.9	88.98	0.99	33.27	5.61	5.89	6.40
C10	4	2	3.50	0.297	7.9	87.95	1.02	33.27			
C10	4	3	3.33	0.288	7.5	88.86	0.73	29.89	5.54	5.87	6.40
C10	4	3	3.33	0.288	7.5	88.80	0.67	29.76			
C10	5	1	4.19	0.405	6.8	17.35	13.97	23.44	5.85	6.26	7.53
C10	5	1	4.19	0.405	6.9	17.38	13.94	23.58			
C10	5	2	3.34	0.423	6.9	18.52	10.52	23.44	5.86	6.04	7.77
C10	5	2	3.34	0.405	6.7	18.55	10.55	23.42			
C10	5	3	4.18	0.396	7.0	19.25	14.93	25.46	5.88	6.31	7.53
C10	5	3	4.19	0.387	7.1	19.30	14.82	25.47			

Product Treatment	Fermentation Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
C15	1	1	5.03	nd	9.9	80.62	4.59	36.92	4.52	5.90	5.60
C15	1	1	5.03	nd	9.9	80.47	4.60	36.88			
C15	1	2	5.58	nd	10.0	80.09	4.44	44.04	4.83	5.82	5.55
C15	1	2	5.58	nd	10.0	81.01	3.81	43.93			
C15	1	3	5.20	nd	9.8	80.79	5.72	38.26	4.88	5.87	5.66
C15	1	3	5.20	nd	9.9	80.80	5.70	38.31			
C15	2	1	4.02	0.180	9.2	86.68	2.17	31.29	5.94	6.72	6.32
C15	2	1	4.02	0.180	9.2	86.43	2.22	31.47			
C15	2	2	4.19	0.189	9.3	85.44	2.15	39.29	5.88	6.53	6.34
C15	2	2	4.19	0.189	9.2	85.42	2.17	39.22			
C15	2	3	3.84	0.207	9.2	86.33	2.18	31.65	6.04	6.44	6.37
C15	2	3	3.84	0.207	9.3	86.34	2.20	31.71			
C15	3	1	3.69	0.252	8.3	87.43	1.53	30.25	6.27	6.60	6.40
C15	3	1	3.69	0.252	8.4	86.98	1.56	31.76			
C15	3	2	3.57	0.279	8.3	87.68	1.42	36.08	6.44	6.80	6.53
C15	3	2	3.57	0.270	8.3	87.54	1.43	36.03			
C15	3	3	3.61	0.270	8.4	87.29	1.38	30.21	6.41	6.52	6.49
C15	3	3	3.61	0.270	8.5	87.32	1.39	30.25			
C15	4	1	3.56	0.279	7.5	88.72	1.29	29.92	5.62	5.92	6.45
C15	4	1	3.56	0.279	7.5	88.69	1.26	29.30			
C15	4	2	3.49	0.297	8.0	88.45	0.76	33.28	5.57	5.83	6.42
C15	4	2	3.49	0.288	8.0	88.52	0.85	33.28			
C15	4	3	3.32	0.288	7.6	87.36	0.66	30.01	5.61	5.85	6.49
C15	4	3	3.32	0.288	7.6	88.37	0.66	29.91			
C15	5	1	4.01	0.423	6.6	12.05	13.88	19.82	5.82	6.22	7.37
C15	5	1	4.01	0.423	6.6	12.08	13.95	19.80			
C15	5	2	3.41	0.432	7.0	12.06	11.34	17.45	5.73	6.24	7.61
C15	5	2	3.41	0.423	7.1	12.10	11.39	17.55			
C15	5	3	4.40	0.396	6.6	13.62	15.97	20.32	5.90	6.32	7.67
C15	5	3	4.40	0.405	6.6	13.61	15.96	20.27			

Note:

Product treatment (C5: black tea water kefir with 5% carrot juice; C10: black tea water kefir with 10% carrot juice; C15: black tea water kefir with 15% carrot juice

Fermentation time (1:0hour, 2:24hours, 3:48hours, 4:72hours, 5:96hours) nd = not detected

Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability	Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	C5	4	5	7	6	7	7	31	C5	7	9	8	8	8	8
2	C5	7	7	7	7	7	7	32	C5	3	5	6	6	7	6
3	C5	6	6	5	5	4	5	33	C5	6	5	6	5	6	6
4	C5	7	5	8	4	7	7	34	C5	7	7	7	8	6	6
5	C5	8	8	6	6	6	7	35	C5	6	5	4	5	3	4
6	C5	7	3	4	5	3	3	36	C5	5	5	7	6	6	7
7	C5	4	5	4	4	4	4	37	C5	6	7	7	6	6	6
8	C5	5	6	7	7	7	8	38	C5	6	6	8	6	7	8
9	C5	7	6	6	7	7	7	39	C5	6	8	8	8	8	7
10	C5	6	7	6	6	4	6	40	C5	8	7	6	6	7	7
11	C5	4	6	6	5	4	6	41	C5	5	6	8	8	8	7
12	C5	4	3	5	6	5	4	42	C5	8	6	5	7	6	6
13	C5	6	7	7	6	7	6	43	C5	6	8	7	5	7	7
14	C5	6	4	2	7	2	3	44	C5	8	7	8	7	8	8
15	C5	8	5	4	5	5	4	45	C5	5	5	6	6	6	6
16	C5	8	9	9	5	9	9	46	C5	5	5	7	6	7	7
17	C5	5	6	7	7	7	7	47	C5	5	7	6	4	4	5
18	C5	6	6	6	4	6	6	48	C5	5	5	5	4	4	5
19	C5	6	6	6	5	7	7	49	C5	4	6	4	4	5	4
20	C5	7	6	5	6	6	6	50	C5	5	6	4	6	6	6
21	C5	7	6	9	8	9	8	51	C5	6	6	8	8	8	8
22	C5	7	9	7	4	4	6	52	C5	7	9	4	5	8	5
23	C5	6	7	8	6	7	7	53	C5	5	7	7	8	8	7
24	C5	7	6	9	8	9	8	54	C5	5	7	6	4	4	5
25	C5	8	5	4	5	5	4	55	C5	5	5	5	4	4	5
26	C5	8	9	9	5	9	9	56	C5	4	6	4	4	5	4
27	C5	6	6	6	4	6	6	57	C5	5	6	4	6	6	6
28	C5	5	6	6	7	6	6	58	C5	6	6	8	8	5	6
29	C5	4	7	7	6	6	9	59	C5	7	9	4	5	5	5
30	C5	8	6	4	4	6	6	60	C5	5	7	7	8	5	7

Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability	Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	C10	5	6	6	7	7	7	31	C10	7	7	7	7	6	7
2	C10	6	6	6	5	5	6	32	C10	7	7	8	8	8	8
3	C10	7	7	7	5	6	6	33	C10	7	2	5	5	3	5
4	C10	7	4	8	5	6	7	34	C10	7	8	7	7	4	5
5	C10	8	8	8	7	8	7	35	C10	5	5	4	5	3	4
6	C10	5	3	4	4	5	5	36	C10	6	4	3	3	4	3
7	C10	7	7	7	7	8	7	37	C10	6	5	7	6	6	7
8	C10	6	4	6	6	6	7	38	C10	7	6	7	7	6	7
9	C10	6	7	8	9	9	8	39	C10	8	5	8	8	8	7
10	C10	6	6	4	6	4	5	40	C10	7	6	6	6	6	6
11	C10	5	7	7	6	6	7	41	C10	6	6	7	8	6	7
12	C10	7	3	6	6	6	6	42	C10	8	6	7	7	8	8
13	C10	6	7	7	6	6	6	43	C10	6	7	9	8	9	9
14	C10	7	6	7	3	7	7	44	C10	8	7	7	8	7	7
15	C10	8	4	6	5	7	6	45	C10	5	4	4	4	4	4
16	C10	7	7	8	5	8	8	46	C10	7	5	4	4	5	4
17	C10	6	6	5	6	5	5	47	C10	6	7	7	7	9	7
18	C10	6	6	6	7	8	7	48	C10	6	8	7	7	9	7
19	C10	5	5	4	5	5	5	49	C10	6	6	7	7	8	8
20	C10	5	5	4	6	6	5	50	C10	8	5	6	6	7	7
21	C10	8	7	8	7	8	8	51	C10	7	5	6	7	8	7
22	C10	7	8	4	3	2	4	52	C10	8	8	7	6	8	8
23	C10	6	6	7	7	6	7	53	C10	7	8	8	6	8	8
24	C10	8	7	8	7	8	8	54	C10	6	7	7	7	7	7
25	C10	8	6	6	5	7	6	55	C10	6	8	7	7	7	7
26	C10	7	7	8	5	8	8	56	C10	6	6	7	7	6	8
27	C10	6	6	6	7	8	7	57	C10	8	5	6	5	7	7
28	C10	6	6	8	9	8	7	58	C10	7	5	6	7	8	7
29	C10	7	6	7	7	6	8	59	C10	8	8	7	5	7	6
30	C10	8	7	6	6	6	7	60	C10	7	8	8	6	8	6

Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability	Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	C15	7	5	6	5	6	6	31	C15	7	8	7	8	7	7
2	C15	8	6	6	7	6	7	32	C15	6	6	7	7	6	6
3	C15	5	3	3	4	3	3	33	C15	6	4	4	4	6	6
4	C15	6	5	8	5	6	7	34	C15	7	7	8	9	8	8
5	C15	8	8	8	5	7	7	35	C15	6	5	6	6	4	6
6	C15	3	2	2	5	5	3	36	C15	7	6	6	5	7	6
7	C15	6	4	4	3	5	5	37	C15	8	4	4	4	7	5
8	C15	8	5	4	4	5	4	38	C15	7	4	4	6	4	4
9	C15	5	6	7	9	9	7	39	C15	8	5	8	8	8	7
10	C15	6	6	7	6	6	6	40	C15	8	6	6	7	6	6
11	C15	6	6	6	7	6	7	41	C15	4	6	7	5	7	5
12	C15	8	4	4	6	6	5	42	C15	7	5	6	5	6	6
13	C15	7	6	6	4	4	5	43	C15	8	5	5	7	7	7
14	C15	7	6	4	5	5	4	44	C15	8	8	7	8	8	8
15	C15	9	5	6	8	8	7	45	C15	5	4	4	4	6	4
16	C15	7	7	9	5	9	9	46	C15	6	5	7	5	7	6
17	C15	8	5	6	7	6	6	47	C15	9	5	5	6	7	7
18	C15	5	6	6	7	7	7	48	C15	8	4	5	8	7	7
19	C15	8	6	5	5	5	6	49	C15	6	6	7	6	6	7
20	C15	3	5	3	5	5	4	50	C15	4	5	4	5	3	4
21	C15	7	7	7	7	7	7	51	C15	7	6	7	8	8	7
22	C15	7	9	8	8	7	8	52	C15	8	6	4	7	8	7
23	C15	7	6	6	4	6	6	53	C15	7	7	4	2	3	5
24	C15	7	7	7	7	7	7	54	C15	9	5	5	6	7	7
25	C15	9	5	6	5	8	7	55	C15	8	4	5	8	7	8
26	C15	7	7	9	5	9	9	56	C15	6	6	7	6	6	7
27	C15	5	6	6	7	7	7	57	C15	4	5	4	6	3	6
28	C15	8	6	7	9	7	7	58	C15	7	6	7	8	8	7
29	C15	7	6	6	5	5	8	59	C15	8	6	4	7	8	7
30	C15	7	6	6	6	6	8	60	C15	7	7	4	6	3	6

Table D.6 Raw data phase 3

Product Treatment	Storage Time	Replication	pH	Lactic acid (%)	TTS (°Brix)	Colour (L*)	Colour (a*)	Colour (b*)	<i>Lactococcus</i> spp. log CFU/ml	<i>Lactobacillus</i> spp. log CFU/ml	<i>S. cerevisiae</i> log CFU/ml
C10	1	1	3.88	0.342	6.9	19.43	14.72	24.29	5.89	5.99	7.40
C10	1	1	3.88	0.342	6.9	19.55	14.74	24.13	5.84	5.97	7.35
C10	1	2	4.10	0.387	7.1	22.78	13.67	24.07	6.26	6.04	7.42
C10	1	2	4.09	0.387	7.1	22.66	13.73	23.88	6.21	6.10	7.46
C10	1	3	3.96	0.405	7.3	18.24	15.66	23.73	5.58	6.20	7.32
C10	1	3	3.95	0.405	7.3	18.27	15.68	23.72	5.65	6.18	7.31
C10	2	1	3.72	0.360	6.8	13.02	12.10	22.29	5.74	5.73	7.34
C10	2	1	3.74	0.360	6.8	13.00	12.14	22.10	5.63	5.77	7.31
C10	2	2	4.03	0.414	6.9	16.32	11.92	22.13	6.19	5.93	7.39
C10	2	2	4.05	0.414	6.9	16.33	12.01	21.93	6.16	6.00	7.42
C10	2	3	3.85	0.432	7.0	12.10	14.33	22.04	5.54	6.06	7.20
C10	2	3	3.86	0.432	7.0	12.14	14.29	22.05	5.59	6.10	7.24
C10	3	1	3.69	0.414	6.3	11.25	11.36	20.67	5.66	5.67	7.16
C10	3	1	3.67	0.423	6.3	11.19	11.54	20.49	5.60	5.61	7.21
C10	3	2	3.88	0.450	6.6	13.52	10.76	20.69	6.13	5.88	7.18
C10	3	2	3.88	0.450	6.6	13.45	10.68	20.58	6.06	5.99	7.20
C10	3	3	3.69	0.468	6.5	10.33	13.68	19.61	5.58	5.99	7.21
C10	3	3	3.70	0.468	6.4	10.46	13.72	19.67	5.53	5.97	7.19
C10	4	1	3.65	0.450	5.9	9.44	11.22	19.85	5.13	5.00	7.19
C10	4	1	3.65	0.450	6.0	9.46	11.38	19.79	4.99	4.90	7.07
C10	4	2	3.83	0.459	6.2	12.11	10.12	19.66	5.83	5.07	7.19
C10	4	2	3.85	0.459	6.2	12.23	10.05	19.57	5.76	4.93	7.20
C10	4	3	3.65	0.486	6.2	9.12	12.70	18.55	4.97	4.99	7.04
C10	4	3	3.68	0.486	6.2	9.09	12.69	18.53	4.89	5.07	7.09
C10	5	1	3.63	0.459	5.7	9.11	11.15	18.91	4.68	4.59	7.12
C10	5	1	3.62	0.459	5.7	9.07	11.27	18.75	4.74	4.49	7.02
C10	5	2	3.79	0.459	5.9	11.88	9.87	18.67	4.77	4.73	7.05
C10	5	2	3.82	0.459	5.9	11.76	9.88	19.62	4.72	4.66	7.05
C10	5	3	3.59	0.477	5.5	8.79	12.53	18.22	4.76	4.87	6.92
C10	5	3	3.61	0.477	5.5	8.80	12.56	19.17	4.66	4.92	6.98

Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability	Panelist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	C10	6	6	6	6	6	8	1	C10	8	6	8	8	8	6
2	C10	8	5	6	7	6	8	2	C10	5	5	7	6	6	6
3	C10	7	4	6	5	5	8	3	C10	8	9	9	7	8	6
4	C10	6	5	7	7	6	8	4	C10	6	7	8	8	8	6
5	C10	8	8	8	8	7	8	5	C10	7	8	8	7	8	7
6	C10	8	5	8	7	8	7	6	C10	8	5	4	7	6	8
7	C10	7	5	7	6	7	8	7	C10	7	7	7	8	8	7
8	C10	6	4	5	6	6	8	8	C10	8	6	9	8	8	6
9	C10	3	4	2	4	3	7	9	C10	7	7	6	5	5	3
10	C10	8	6	9	8	8	7	10	C10	7	6	7	7	7	8
11	C10	8	8	9	9	9	6	11	C10	6	7	7	7	6	9
12	C10	8	8	7	7	7	5	12	C10	6	5	5	5	5	7
13	C10	8	7	9	7	8	7	13	C10	7	6	6	7	5	8
14	C10	7	6	7	7	7	6	14	C10	8	7	7	6	5	8
15	C10	8	8	7	6	4	7	15	C10	8	7	8	6	6	6
16	C10	7	7	7	5	8	7	16	C10	9	6	8	7	6	7
17	C10	8	4	3	7	8	5	17	C10	6	5	5	5	5	5
18	C10	8	4	7	6	6	7	18	C10	7	6	6	7	5	7
19	C10	7	7	8	8	8	6	19	C10	8	7	7	6	5	8
20	C10	8	6	7	7	2	7	20	C10	8	7	8	6	6	6
21	C10	8	6	9	8	8	7	21	C10	9	6	8	7	6	8
22	C10	8	8	9	9	9	8	22	C10	8	9	9	7	8	9
23	C10	8	8	7	7	7	8	23	C10	6	7	8	8	8	7
24	C10	8	7	9	7	8	8	24	C10	7	8	8	7	8	8
25	C10	7	6	7	7	7	7	25	C10	8	5	4	7	6	8
26	C10	8	8	7	6	4	5	26	C10	6	5	5	5	5	6
27	C10	7	7	7	5	8	7	27	C10	7	6	6	7	5	7
28	C10	8	4	3	7	8	8	28	C10	8	6	8	8	8	5
29	C10	8	4	7	6	6	8	29	C10	5	5	7	6	6	7
30	C10	7	7	8	8	8	8	30	C10	8	9	9	7	8	8

Panellist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability	Panellist	Product treatment	Appearance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	C10	7	6	4	6	3	3	1	C10	7	4	4	6	3	4
2	C10	5	5	4	6	3	5	2	C10	5	5	4	5	3	5
3	C10	7	5	5	7	4	3	3	C10	7	4	5	7	4	4
4	C10	6	4	7	6	4	4	4	C10	6	4	4	6	4	4
5	C10	7	5	5	2	5	5	5	C10	7	4	5	2	5	5
6	C10	7	5	5	7	6	5	6	C10	7	3	5	7	6	3
7	C10	7	7	7	6	5	3	7	C10	5	7	4	5	5	3
8	C10	8	6	5	6	5	4	8	C10	5	4	5	6	5	4
9	C10	7	7	2	5	5	3	9	C10	7	7	2	5	5	4
10	C10	7	6	3	7	5	3	10	C10	7	6	3	7	5	3
11	C10	6	4	3	7	6	3	11	C10	6	4	3	5	4	4
12	C10	6	5	7	5	5	5	12	C10	6	5	4	5	5	5
13	C10	7	6	5	7	5	3	13	C10	7	5	5	7	4	3
14	C10	8	7	7	5	5	5	14	C10	5	7	4	5	5	5
15	C10	8	7	7	5	6	6	15	C10	5	7	5	5	4	6
16	C10	9	6	7	3	6	7	16	C10	5	6	5	3	4	7
17	C10	6	5	3	5	5	5	17	C10	6	5	3	5	5	5
18	C10	7	6	7	7	5	7	18	C10	7	4	7	7	5	5
19	C10	8	7	5	4	5	5	19	C10	5	4	5	4	4	5
20	C10	8	7	7	6	6	6	20	C10	5	2	7	4	5	6
21	C10	7	6	5	3	4	4	21	C10	7	4	5	3	4	4
22	C10	8	5	5	7	5	5	22	C10	4	5	5	7	5	5
23	C10	6	4	7	4	5	7	23	C10	6	4	7	4	5	5
24	C10	7	5	5	3	5	5	24	C10	7	5	5	3	3	5
25	C10	8	5	7	5	6	5	25	C10	5	3	5	5	5	5
26	C10	6	5	7	4	5	6	26	C10	5	5	7	4	3	6
27	C10	7	6	7	7	5	7	27	C10	7	4	6	7	5	5
28	C10	8	6	3	4	5	5	28	C10	8	6	3	4	3	5
29	C10	5	5	7	6	4	7	29	C10	5	3	7	6	4	4
30	C10	8	5	5	5	5	5	30	C10	3	5	4	4	2	5

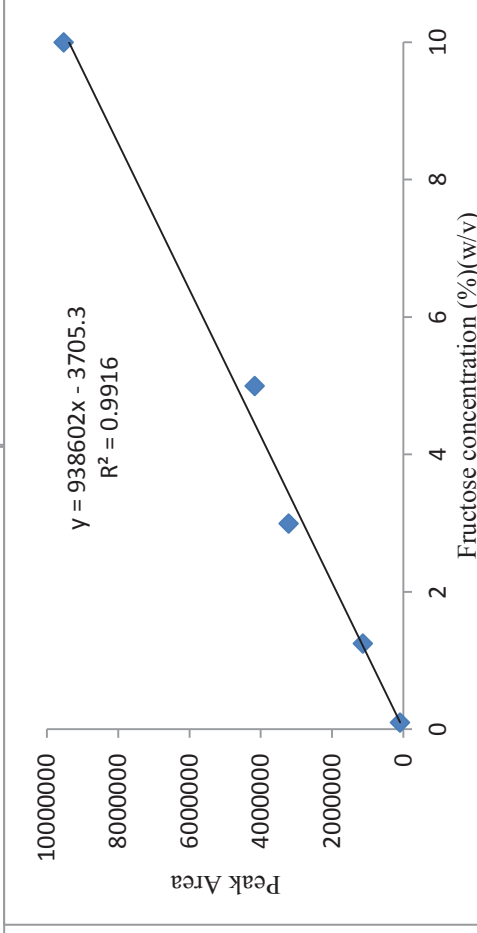
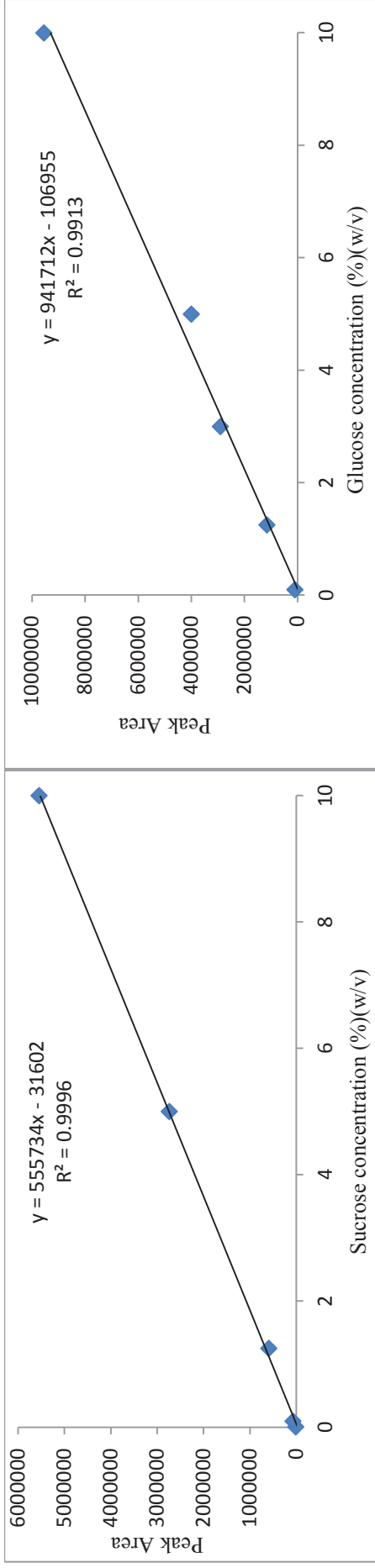
Table D.7 Physico-chemical characteristic of black tea water kefir beverage after carrot juice addition

Product code	pH	Total soluble solids	Colour		
			L*	a*	b*
C5	3.91±0.29	7.80±0.09	43.61±2.87	16.02±2.56	30.10±0.08
C10	4.14±0.31	7.92±0.12	25.77±3.59	19.12±2.14	29.33±0.52
C15	4.36±0.37	7.95±0.16	16.78±3.72	19.44±1.71	28.64±0.92

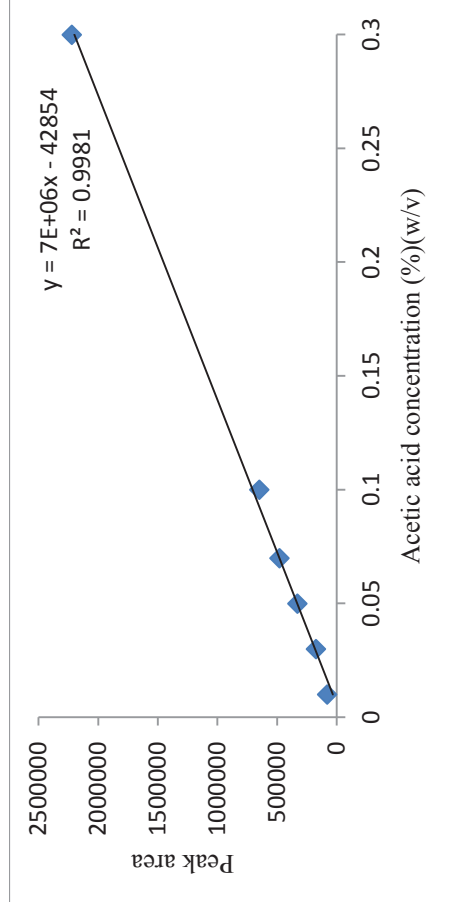
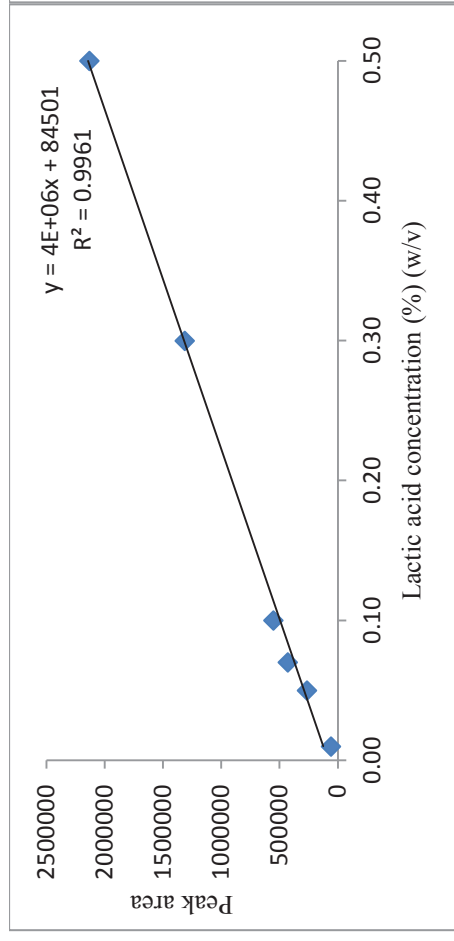
Table D.8 Sugars and organic acids content of black tea water kefir beverage after 10% carrot juice addition

Product code	Sugars (% w/v)			Organic acids (% w/v)	
	Sucrose	Glucose	Fructose	Lactic acid	Acetic acid
C10	3.11±1.09	1.08±0.14	1.72±0.06	0.169±0.03	0.094±0.00

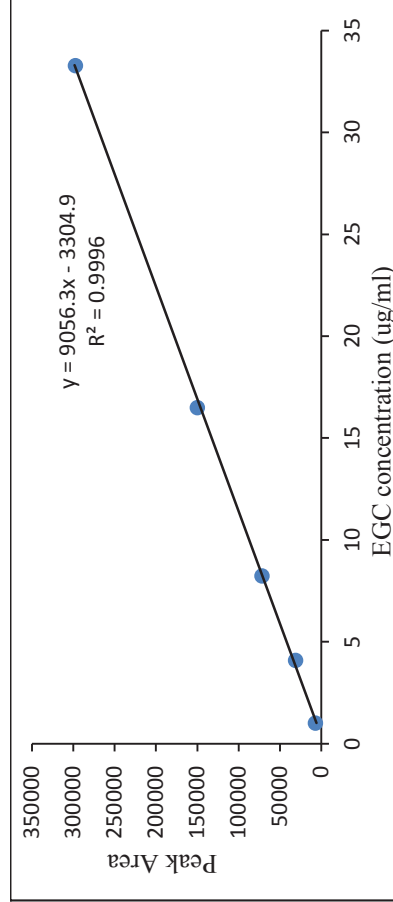
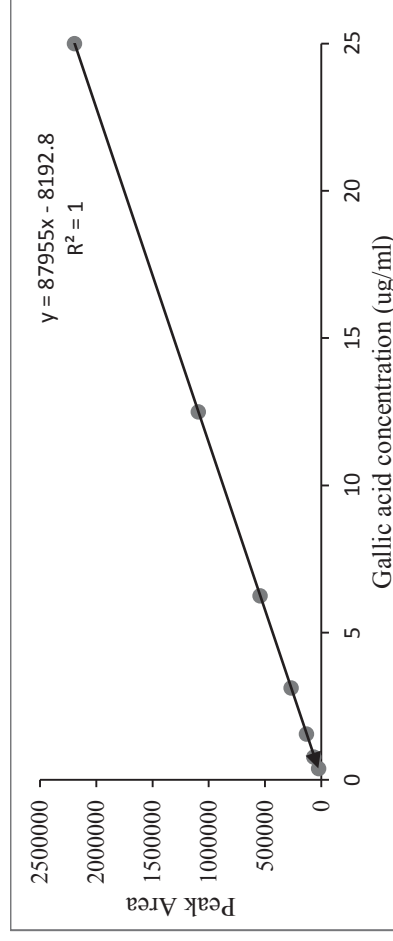
E. HPLC data
E.1 HPLC standard curve
E.1.1 Sugars



E.1.2 Organic acids



E.1.3 Antioxidants



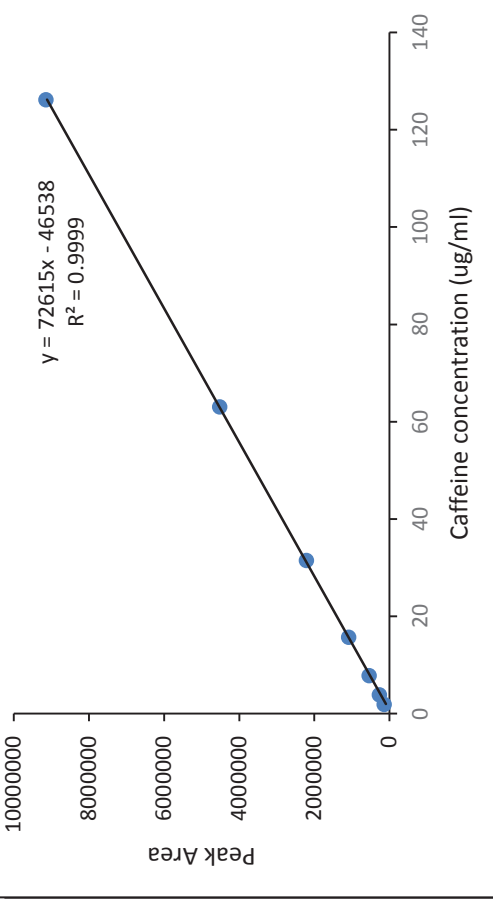
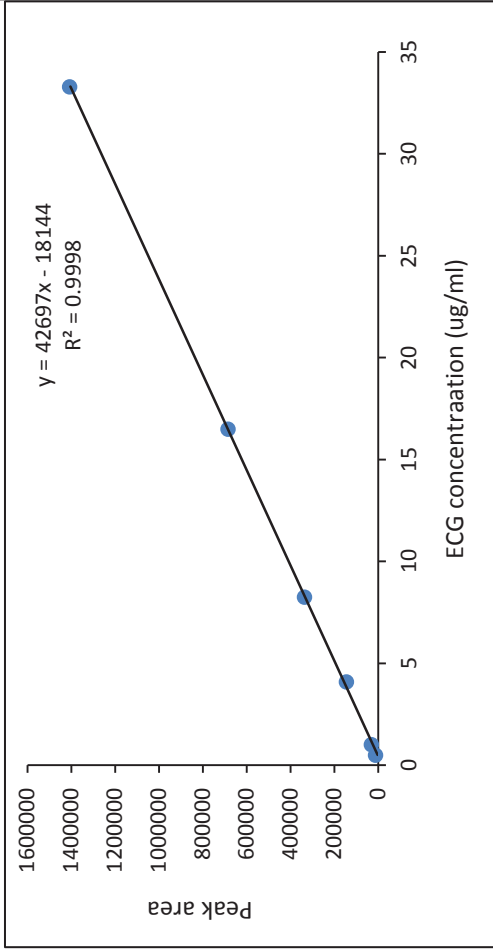
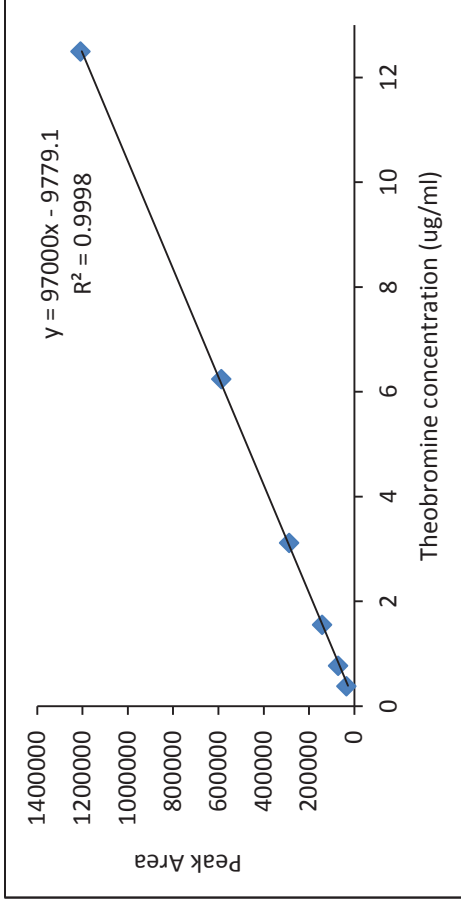
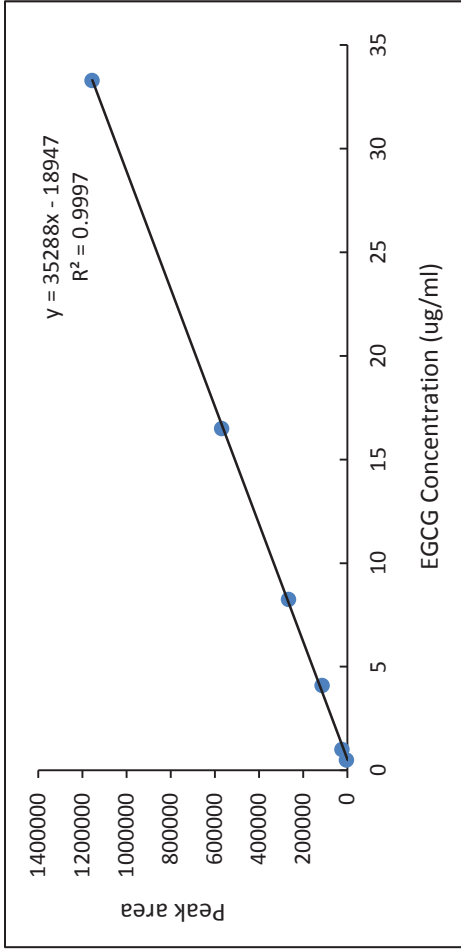


Table E. 1 HPLC raw data results for sugars

Product code	Fermentation Time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)
C10	1	1	5835600	10.56	279583	0.41	578035	0.62
C10	1	1	5566276	10.07	132664	0.25	319449	0.34
C10	1	2	5124430	9.28	322354	0.46	172949	0.19
C10	1	2	5126336	9.28	307073	0.44	331117	0.36
C10	1	3	5254861	9.51	408241	0.55	247212	0.27
C10	1	3	5191264	9.40	492217	0.64	240931	0.26
C10	2	1	4692012	8.50	839885	1.01	1083182	1.16
C10	2	1	4683608	8.48	734443	0.89	1076643	1.15
C10	2	2	4471386	8.10	636637	0.79	954921	1.02
C10	2	2	3476559	6.31	554055	0.70	683089	0.73
C10	2	3	4471386	8.10	418353	0.56	791031	0.85
C10	2	3	4768087	8.64	349252	0.48	862131	0.92
C10	3	1	2949548	5.36	722623	0.88	1485200	1.59
C10	3	1	2955209	5.37	661403	0.82	1490431	1.59
C10	3	2	2737558	4.98	671098	0.83	1235371	1.32
C10	3	2	2323163	4.24	678233	0.83	1256525	1.34
C10	3	3	2805247	5.10	765380	0.93	1510865	1.61
C10	3	3	2780765	5.06	804395	0.97	1265009	1.35
C10	4	1	590458	1.12	804783	0.97	1391277	1.49
C10	4	1	585568	1.11	831952	1.00	1451281	1.55
C10	4	2	850942	1.59	825339	0.99	1770643	1.89
C10	4	2	830642	1.55	824266	0.99	1546102	1.65
C10	4	3	761559	1.43	780288	0.94	1365083	1.46
C10	4	3	810290	1.51	778109	0.94	1369816	1.46
C10	5	1	885078	1.65	958552	1.13	682865	0.73
C10	5	1	887899	1.65	914433	1.08	830159	0.89
C10	5	2	939960	1.75	806638	0.97	983484	1.05
C10	5	2	850282	1.59	672229	0.83	990049	1.06
C10	5	3	820190	1.53	595424	0.75	866936	0.93
C10	5	3	834855	1.56	493310	0.64	855224	0.92

Product code	Storage Time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)
C10	1	1	885078	1.65	958552	1.13	682865	0.73
C10	1	1	887899	1.65	914433	1.08	830159	0.89
C10	1	2	939960	1.75	806638	0.97	983484	1.05
C10	1	2	850282	1.59	672229	0.83	990049	1.06
C10	1	3	820190	1.53	595424	0.75	866936	0.93
C10	1	3	834855	1.56	493310	0.64	855224	0.92
C10	2	1	493703	0.95	1042830	1.22	1078221	1.15
C10	2	1	475280	0.91	1061360	1.24	1079028	1.15
C10	2	2	424510	0.82	989772	1.16	1118587	1.20
C10	2	2	319178	0.63	985365	1.16	1094149	1.17
C10	2	3	308873	0.61	978286	1.15	1169812	1.25
C10	2	3	308886	0.61	1131044	1.31	1148270	1.23
C10	3	1	112251	0.26	1541558	1.75	2671291	2.85
C10	3	1	111571	0.26	1608411	1.82	2680630	2.86
C10	3	2	108973	0.25	1271354	1.46	1808515	1.93
C10	3	2	143307	0.31	1293987	1.49	2627823	2.80
C10	3	3	145555	0.32	1238611	1.43	1900770	2.03
C10	3	3	148659	0.32	1496639	1.70	1874131	2.00
C10	4	1	40366	0.13	1486376	1.69	2221318	2.37
C10	4	1	40967	0.13	1468666	1.67	2165054	2.31
C10	4	2	41466	0.13	1143746	1.33	2252419	2.40
C10	4	2	41067	0.13	1147551	1.33	2344235	2.50
C10	4	3	44798	0.14	1528927	1.74	2633323	2.81
C10	4	3	39790	0.13	1523888	1.73	2441218	2.60
C10	5	1	9495	0.07	1336200	1.53	2699471	2.88
C10	5	1	9839	0.07	1368574	1.57	2703961	2.88
C10	5	2	10549	0.08	1309747	1.50	2631683	2.81
C10	5	2	18895	0.09	1318923	1.51	2646039	2.82
C10	5	3	20117	0.09	1393849	1.59	2690667	2.87
C10	5	3	21217	0.10	1400978	1.60	3127330	3.34

Table E.2 HPLC raw data results for organic acids

Product code	Fermentation Time	Replication	Lactic acid		Acetic acid	
			Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)
C10	1	1	87083	0.00	nd	0.00
C10	1	1	83259	0.00	nd	0.00
C10	1	2	84236	0.00	nd	0.00
C10	1	2	84188	0.00	nd	0.00
C10	1	3	85818	0.00	nd	0.00
C10	1	3	85632	0.00	nd	0.00
C10	2	1	574214	0.122	423579	0.067
C10	2	1	770826	0.172	447045	0.070
C10	2	2	751827	0.167	547733	0.084
C10	2	2	613917	0.132	448595	0.070
C10	2	3	719374	0.159	580142	0.089
C10	2	3	758721	0.169	581796	0.089
C10	3	1	798227	0.178	582751	0.089
C10	3	1	953794	0.217	549570	0.085
C10	3	2	1059382	0.244	553517	0.085
C10	3	2	1191916	0.277	563718	0.087
C10	3	3	953928	0.217	580715	0.089
C10	3	3	999116	0.229	572863	0.088
C10	4	1	1172278	0.272	571057	0.088
C10	4	1	1101177	0.254	599417	0.092
C10	4	2	1020497	0.234	419444	0.066
C10	4	2	974588	0.223	499417	0.077
C10	4	3	954555	0.218	700822	0.106
C10	4	3	947332	0.216	724933	0.110
C10	5	1	1376175	0.323	1271396	0.188
C10	5	1	880273	0.199	1166152	0.173
C10	5	2	1048898	0.241	759014	0.115
C10	5	2	1094880	0.253	943790	0.141
C10	5	3	1127452	0.261	925587	0.138
C10	5	3	1092946	0.252	903211	0.135

Product code	Storage Time	Replication	Lactic acid		Acetic acid	
			Peak area	Concentration (%) (w/v)	Peak area	Concentration (%) (w/v)
C10	1	1	1376175	0.323	1271396	0.188
C10	1	1	880273	0.199	1166152	0.173
C10	1	2	1048898	0.241	759014	0.115
C10	1	2	1094880	0.253	943790	0.141
C10	1	3	1127452	0.261	925587	0.138
C10	1	3	1092946	0.252	903211	0.135
C10	2	1	962375	0.219	859737	0.129
C10	2	1	828661	0.186	731243	0.111
C10	2	2	989151	0.226	1017208	0.151
C10	2	2	1243437	0.290	1426302	0.210
C10	2	3	978457	0.223	1192311	0.176
C10	2	3	980391	0.224	900213	0.135
C10	3	1	1145938	0.265	1293219	0.191
C10	3	1	1068243	0.246	1356285	0.200
C10	3	2	864854	0.195	629345	0.096
C10	3	2	1074881	0.248	602729	0.092
C10	3	3	888403	0.201	1102141	0.164
C10	3	3	886213	0.200	1198810	0.177
C10	4	1	1281997	0.299	1020199	0.152
C10	4	1	1209975	0.281	1127656	0.167
C10	4	2	911002	0.207	1009759	0.150
C10	4	2	914633	0.208	1035937	0.154
C10	4	3	700283	0.154	346967	0.056
C10	4	3	702133	0.154	499039	0.077
C10	5	1	973301	0.222	837326	0.126
C10	5	1	960805	0.219	950308	0.142
C10	5	2	899112	0.204	985404	0.147
C10	5	2	900419	0.204	733187	0.111
C10	5	3	812332	0.182	502344	0.078
C10	5	3	807120	0.181	533290	0.082

Table E.3 HPLC raw data results for antioxidants

Product code	Fermentation Time	Replication	Gallic acid		Theobromine		Caffeine	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
C10	1	1	1191359	13.64	821969	8.57	7242037	100.37
C10	1	1	1191811	13.64	828928	8.65	7248069	100.46
C10	1	1	1183333	13.55	822668	8.58	7224993	100.14
C10	1	2	1106879	12.68	781424	8.16	6728656	93.30
C10	1	2	1098097	12.58	776259	8.10	6767102	93.83
C10	1	2	1104118	12.65	781773	8.16	6714001	93.10
C10	2	1	903842	10.37	689553	7.21	6107539	84.75
C10	2	1	904402	10.38	686354	7.18	6117948	84.89
C10	2	1	904338	10.37	691972	7.23	6125516	85.00
C10	2	2	856853	9.84	718913	7.51	6323338	87.72
C10	2	2	850432	9.76	718800	7.51	6311017	87.55
C10	2	2	857190	9.84	721761	7.54	6326351	87.76
C10	3	1	885168	10.16	698360	7.30	6322595	87.71
C10	3	1	885470	10.16	698521	7.30	6319862	87.67
C10	3	1	887445	10.18	700005	7.32	6313045	87.58
C10	3	2	886343	10.17	706836	7.39	6289948	87.26
C10	3	2	892854	10.24	708242	7.40	6290403	87.27
C10	3	2	889658	10.21	707237	7.39	6292194	87.29
C10	4	1	974308	11.17	687138	7.18	6282911	87.16
C10	4	1	974803	11.18	688944	7.20	6281229	87.14
C10	4	1	972426	11.15	690206	7.22	6278356	87.10
C10	4	2	966729	11.08	722809	7.55	6482177	89.91
C10	4	2	966729	11.08	722664	7.55	6481498	89.90
C10	4	2	964924	11.06	723671	7.56	6486673	89.97
C10	5	1	883618	10.14	653138	6.83	5709857	79.27
C10	5	1	883524	10.14	654131	6.84	5710817	79.29
C10	5	1	882541	10.13	653266	6.84	5702114	79.17
C10	5	2	817655	9.39	601400	6.30	5354343	74.38
C10	5	2	812607	9.33	599345	6.28	5352499	74.35
C10	5	2	818998	9.40	602690	6.31	5351086	74.33

Product code	Fermentation Time	Replication	EGC		EGCG		ECG	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
C10	1	1	68269	7.90	127367	4.15	70753	2.08
C10	1	1	76697	8.83	121278	3.97	73042	2.14
C10	1	1	75251	8.67	120964	3.96	72213	2.12
C10	1	2	77244	8.89	29929	1.39	39691	1.35
C10	1	2	72086	8.32	31096	1.42	36624	1.28
C10	1	2	69602	8.05	26657	1.29	41645	1.40
C10	2	1	102770	11.71	77378	2.73	39848	1.36
C10	2	1	100412	11.45	71351	2.56	39166	1.34
C10	2	1	101888	11.62	78806	2.77	42318	1.42
C10	2	2	112346	12.77	129837	4.22	188347	4.84
C10	2	2	111493	12.68	120872	3.96	187721	4.82
C10	2	2	108912	12.39	112032	3.71	177676	4.59
C10	3	1	95276	10.89	180342	5.65	433364	10.57
C10	3	1	93944	10.74	185805	5.80	433102	10.57
C10	3	1	95389	10.90	184225	5.76	427018	10.43
C10	3	2	103536	11.80	200347	6.21	329225	8.14
C10	3	2	103712	11.82	198712	6.17	331646	8.19
C10	3	2	104626	11.92	186095	5.81	329114	8.13
C10	4	1	90728	10.38	231938	7.11	553338	13.38
C10	4	1	90808	10.39	226562	6.96	548883	13.28
C10	4	1	90792	10.39	225588	6.93	548334	13.27
C10	4	2	92209	10.55	218938	6.74	385872	9.46
C10	4	2	91228	10.44	214428	6.61	387026	9.49
C10	4	2	91927	10.52	220843	6.80	384411	9.43
C10	5	1	39938	4.77	38931	1.64	46010	1.50
C10	5	1	38289	4.59	36626	1.57	46768	1.52
C10	5	1	37905	4.55	37915	1.61	46335	1.51
C10	5	2	47108	5.57	149380	4.77	251218	6.31
C10	5	2	46493	5.50	147074	4.70	248004	6.23
C10	5	2	47227	5.58	144371	4.63	256038	6.42

Product code	Storage Time	Replication	Gallic acid		Theobromine		Caffeine	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
C10	1	1	883618	10.14	653138	6.83	5709857	79.27
C10	1	1	883524	10.14	654131	6.84	5710817	79.29
C10	1	1	882541	10.13	653266	6.84	5702114	79.17
C10	1	2	817655	9.39	601400	6.30	5354343	74.38
C10	1	2	812607	9.33	599345	6.28	5352499	74.35
C10	1	2	818998	9.40	602690	6.31	5351086	74.33
C10	2	1	695983	8.01	529517	5.56	4835768	67.24
C10	2	1	698445	8.03	523327	5.50	4835675	67.23
C10	2	1	687926	7.91	530551	5.57	4844971	67.36
C10	2	2	801737	9.21	592588	6.21	5214020	72.44
C10	2	2	795183	9.13	591522	6.20	5217132	72.49
C10	2	2	797096	9.16	592975	6.21	5214994	72.46
C10	3	1	908859	10.43	650204	6.80	5824716	80.85
C10	3	1	847789	9.73	652557	6.83	5826487	80.88
C10	3	1	906477	10.40	650623	6.81	5821969	80.82
C10	3	2	612684	7.06	463377	4.88	4046356	56.36
C10	3	2	611310	7.04	462496	4.87	4047120	56.37
C10	3	2	609870	7.03	463237	4.88	4045572	56.35
C10	4	1	687403	7.91	521182	5.47	4749581	66.05
C10	4	1	684516	7.88	534426	5.61	4906070	68.20
C10	4	1	683913	7.87	506138	5.32	4738360	65.89
C10	4	2	916892	10.52	748294	7.82	6735954	93.40
C10	4	2	916586	10.51	749080	7.82	6740946	93.47
C10	4	2	915982	10.51	750753	7.84	6734969	93.39
C10	5	1	706889	8.13	534180	5.61	5078292	70.58
C10	5	1	748105	8.60	557917	5.85	4894332	68.04
C10	5	1	743578	8.55	554859	5.82	4892539	68.02
C10	5	2	1052781	12.06	793351	8.28	6925401	96.01
C10	5	2	1048858	12.02	794204	8.29	6912349	95.83
C10	5	2	1056032	12.10	793679	8.28	6914329	95.86

Product code	Storage Time	Replication	EGC		EGCG		ECG	
			Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)
C10	1	1	39938	4.77	38931	1.64	46010	1.50
C10	1	1	38289	4.59	36626	1.57	46768	1.52
C10	1	1	37905	4.55	37915	1.61	46335	1.51
C10	1	2	47108	5.57	149380	4.77	251218	6.31
C10	1	2	46493	5.50	147074	4.70	248004	6.23
C10	1	2	47227	5.58	144371	4.63	256038	6.42
C10	2	1	44173	5.24	63351	2.33	40965	1.38
C10	2	1	42964	5.11	62448	2.31	40003	1.36
C10	2	1	43177	5.13	63723	2.34	43410	1.44
C10	2	2	25591	3.19	42597	1.74	64882	1.94
C10	2	2	25812	3.22	40227	1.68	64092	1.93
C10	2	2	26416	3.28	39487	1.66	64395	1.93
C10	3	1	55889	6.54	80822	2.83	43957	1.45
C10	3	1	54410	6.37	81727	2.85	45936	1.50
C10	3	1	52315	6.14	80175	2.81	45685	1.49
C10	3	2	21337	2.72	49866	1.95	51900	1.64
C10	3	2	21467	2.74	46284	1.85	53109	1.67
C10	3	2	20512	2.63	44556	1.80	53549	1.68
C10	4	1	42058	5.01	61351	2.28	42547	1.42
C10	4	1	41240	4.92	62609	2.31	42876	1.43
C10	4	1	41416	4.94	64600	2.37	43611	1.45
C10	4	2	43228	5.14	60488	2.25	84900	2.41
C10	4	2	43183	5.13	61563	2.28	87033	2.46
C10	4	2	40805	4.87	65475	2.39	85050	2.42
C10	5	1	48957	5.77	24376	1.23	79795	2.29
C10	5	1	52278	6.14	20638	1.12	72877	2.13
C10	5	1	54018	6.33	28534	1.35	80450	2.31
C10	5	2	20353	2.61	75361	2.67	93767	2.62
C10	5	2	19983	2.57	64182	2.36	92849	2.60
C10	5	2	20148	2.59	79801	2.80	97086	2.70

Table E.4 HPLC data results for sugar, organic acids, and antioxidants standard peak area and retention time

Standards	Concentration (%) (w/v)	Mean peak area	Mean retention time (minutes)
Sucrose	0.01	6654.50	9.588
	0.10	65186.33	
	1.25	586004.67	
	5.00	2734985.67	
	10.00	5540965.33	
Glucose	0.10	94697.33	11.348
	1.25	1152097.17	
	3.00	2896778.67	
	5.00	3995407.00	
	10.00	9548369.5	
Fructose	0.10	93730.17	14.564
	1.25	1139844.00	
	3.00	3214290.17	
	5.00	4171139.17	
	10.00	9524415.67	
Lactic acid	0.01	57937.25	16.826
	0.05	262783.25	
	0.07	428923.25	
	0.10	552412.50	
	0.30	1312902.25	
	0.50	2130791.50	
Acetic acid	0.01	79631.00	19.241
	0.03	175027.50	
	0.05	330634.00	
	0.07	478516.25	
	0.10	647461.50	
	0.30	2219378.30	

Standards	Concentration (ug/ml)	Mean Peak Area	Mean Retention time (minutes)
Gallic acid	0.39	21975.00	12.754
	0.78	64148.00	
	1.56	131419.00	
	3.125	265495.00	
	6.25	541251.00	
	12.50	1090112.00	
	25.00	2191269.00	
Theobromine	0.39	33890.00	20.173
	0.78	70543.00	
	1.56	140769.00	
	3.125	287104.00	
	6.25	587135.00	
	12.5	1208579.00	
EGC	1.02	6861.00	26.383
	4.10	30895.00	
	8.25	71316.00	
	16.50	149572.00	
	33.30	296915.00	
Caffeine	1.97	130186.00	28.798
	3.94	261639.00	
	7.88	532569.00	
	15.78	1075974.00	
	31.55	2203223.00	
	63.1	4516188.00	
	126.2	9138697.00	
EGCG	0.5	3522.00	36.964
	1.02	24221.00	
	4.1	114108.00	
	8.25	266069.00	
	16.5	569404.00	
	33.3	1155760.00	
ECG	0.5	10688	53.567
	1.02	29925	
	4.1	143656	
	8.25	335397	
	16.5	684194	
	33.3	1405811	

F. Statistic Output

F.1.1 Statistical analysis of pH of black tea water kefir beverage samples during 72 hours of fermentation

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
pH_BT5_25C	0 Hour	6	5.5400	.01789	.00730	5.5212	5.5588	5.51	5.56
	24 Hour	6	4.1383	.05636	.02301	4.0792	4.1975	4.09	4.22
	48 Hour	6	3.6333	.02582	.01054	3.6062	3.6604	3.60	3.67
	72 Hour	6	3.4667	.05820	.02376	3.4056	3.5277	3.41	3.55
	Total	24	4.1946	.83371	.17018	3.8425	4.5466	3.41	5.56
pH_BT5_30C	0 Hour	6	5.5550	.03619	.01478	5.5170	5.5930	5.50	5.61
	24 Hour	6	4.0217	.10362	.04230	3.9129	4.1304	3.91	4.15
	48 Hour	6	3.5817	.02401	.00980	3.5565	3.6069	3.56	3.62
	72 Hour	6	3.3100	.02608	.01065	3.2826	3.3374	3.28	3.35
	Total	24	4.1171	.88845	.18136	3.7419	4.4922	3.28	5.61
pH_BT10_25C	0 Hour	6	5.5917	.07167	.02926	5.5165	5.6669	5.53	5.70
	24 Hour	6	4.2783	.14219	.05805	4.1291	4.4275	4.10	4.45
	48 Hour	6	3.6550	.02665	.01088	3.6270	3.6830	3.63	3.70
	72 Hour	6	3.5033	.01633	.00667	3.4862	3.5205	3.48	3.52
	Total	24	4.2571	.84455	.17239	3.9005	4.6137	3.48	5.70
pH_BT10_30C	0 Hour	6	5.5667	.02066	.00843	5.5450	5.5883	5.54	5.60
	24 Hour	6	4.0400	.08899	.04041	3.9361	4.1439	3.96	4.19
	48 Hour	6	3.6000	.04775	.01949	3.5499	3.6501	3.52	3.64
	72 Hour	6	3.3367	.01751	.00715	3.3183	3.3550	3.31	3.36
	Total	24	4.1358	.88361	.18037	3.7627	4.5089	3.31	5.60

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
pH_BT5_25C	Between Groups	15.949	3	5.316	2816.609	.000
	Within Groups	.038	20	.002		
	Total	15.987	23			
pH_BT5_30C	Between Groups	18.089	3	6.030	1812.937	.000
	Within Groups	.067	20	.003		
	Total	18.155	23			
pH_BT10_25C	Between Groups	16.273	3	5.424	824.066	.000
	Within Groups	.132	20	.007		
	Total	16.405	23			
pH_BT10_30C	Between Groups	17.894	3	5.965	1861.968	.000
	Within Groups	.064	20	.003		
	Total	17.958	23			

pH_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72 Hour	6	3.4667			
48 Hour	6		3.6333		
24 Hour	6			4.1383	
0 Hour	6				5.5400
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72 Hour	6	3.3100			
48 Hour	6		3.5817		
24 Hour	6			4.0217	
0 Hour	6				5.5550
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72 Hour	6	3.5033			
48 Hour	6		3.6550		
24 Hour	6			4.2783	
0 Hour	6				5.5917
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72 Hour	6	3.3367			
48 Hour	6		3.6000		
24 Hour	6			4.0400	
0 Hour	6				5.5667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
pH_BT5_25C	0 Hour	24 Hour	1.40167 [*]	.02508	.000	1.3315	1.4719
		48 Hour	1.90667 [*]	.02508	.000	1.8365	1.9769
		72 Hour	2.07333 [*]	.02508	.000	2.0031	2.1435
	24 Hour	0 Hour	-1.40167 [*]	.02508	.000	-1.4719	-1.3315
		48 Hour	.50500 [*]	.02508	.000	.4348	.5752
		72 Hour	.67167 [*]	.02508	.000	.6015	.7419
	48 Hour	0 Hour	-1.90667 [*]	.02508	.000	-1.9769	-1.8365
		24 Hour	-.50500 [*]	.02508	.000	-.5752	-.4348
		72 Hour	.16667 [*]	.02508	.000	.0965	.2369
	72 Hour	0 Hour	-2.07333 [*]	.02508	.000	-2.1435	-2.0031
		24 Hour	-.67167 [*]	.02508	.000	-.7419	-.6015
		48 Hour	-.16667 [*]	.02508	.000	-.2369	-.0965
pH_BT5_30C	0 Hour	24 Hour	1.53333 [*]	.03330	.000	1.4401	1.6265
		48 Hour	1.97333 [*]	.03330	.000	1.8801	2.0665
		72 Hour	2.24500 [*]	.03330	.000	2.1518	2.3382
	24 Hour	0 Hour	-1.53333 [*]	.03330	.000	-1.6265	-1.4401
		48 Hour	.44000 [*]	.03330	.000	.3468	.5332
		72 Hour	.71167 [*]	.03330	.000	.6185	.8049
	48 Hour	0 Hour	-1.97333 [*]	.03330	.000	-2.0665	-1.8801
		24 Hour	-.44000 [*]	.03330	.000	-.5332	-.3468
		72 Hour	.27167 [*]	.03330	.000	.1785	.3649
	72 Hour	0 Hour	-2.24500 [*]	.03330	.000	-2.3382	-2.1518
		24 Hour	-.71167 [*]	.03330	.000	-.8049	-.6185
		48 Hour	-.27167 [*]	.03330	.000	-.3649	-.1785
pH_BT10_25C	0 Hour	24 Hour	1.31333 [*]	.04684	.000	1.1822	1.4444
		48 Hour	1.93667 [*]	.04684	.000	1.8056	2.0678
		72 Hour	2.08833 [*]	.04684	.000	1.9572	2.2194
	24 Hour	0 Hour	-1.31333 [*]	.04684	.000	-1.4444	-1.1822
		48 Hour	.62333 [*]	.04684	.000	.4922	.7544
		72 Hour	.77500 [*]	.04684	.000	.6439	.9061
	48 Hour	0 Hour	-1.93667 [*]	.04684	.000	-2.0678	-1.8056
		24 Hour	-.62333 [*]	.04684	.000	-.7544	-.4922
		72 Hour	.15167 [*]	.04684	.020	.0206	.2828
	72 Hour	0 Hour	-2.08833 [*]	.04684	.000	-2.2194	-1.9572
		24 Hour	-.77500 [*]	.04684	.000	-.9061	-.6439
		48 Hour	-.15167 [*]	.04684	.020	-.2828	-.0206
pH_BT10_30C	0 Hour	24 Hour	1.52667 [*]	.03268	.000	1.4352	1.6181
		48 Hour	1.96667 [*]	.03268	.000	1.8752	2.0581
		72 Hour	2.23000 [*]	.03268	.000	2.1385	2.3215
	24 Hour	0 Hour	-1.52667 [*]	.03268	.000	-1.6181	-1.4352
		48 Hour	.44000 [*]	.03268	.000	.3485	.5315
		72 Hour	.70333 [*]	.03268	.000	.6119	.7948
	48 Hour	0 Hour	-1.96667 [*]	.03268	.000	-2.0581	-1.8752
		24 Hour	-.44000 [*]	.03268	.000	-.5315	-.3485
		72 Hour	.26333 [*]	.03268	.000	.1719	.3548
	72 Hour	0 Hour	-2.23000 [*]	.03268	.000	-2.3215	-2.1385
		24 Hour	-.70333 [*]	.03268	.000	-.7948	-.6119
		48 Hour	-.26333 [*]	.03268	.000	-.3548	-.1719

*. The mean difference is significant at the 0.05 level.

F.1.2 Statistical analysis of pH of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

pH72Hour

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5%25C	6	3.4667	.05820	.02376	3.4056	3.5277	3.41	3.55
BT5%30C	6	3.3100	.02608	.01065	3.2826	3.3374	3.28	3.35
BT10%25C	6	3.5033	.01633	.00667	3.4862	3.5205	3.48	3.52
BT10%30C	6	3.3367	.01751	.00715	3.3183	3.3550	3.31	3.36
Total	24	3.4042	.08997	.01837	3.3662	3.4422	3.28	3.55

ANOVA

pH72Hour

Test of Homogeneity of Variances

pH72Hour				
Levene Statistic	df1	df2	Sig.	
7.979	3	20	.001	

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.163	3	.054	46.834	.000
Within Groups	.023	20	.001		
Total	.186	23			

pH72Hour

Tukey HSD^a

Multiple Comparisons

Dependent Variable: pH72Hour

Tukey HSD

i) Treatments	j) Treatments	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5%25C	BT10%30C	-.15667	.01966	.000	.1016	-.2117
	BT10%25C	-.03667	.01966	.274	-.0917	.0184
	BT10%30C	.13000	.01966	.000	.0750	.1850
BT5%30C	BT10%25C	-.15667	.01966	.000	-.2117	-.1016
	BT10%30C	-.18333	.01966	.000	-.2484	-.1333
	BT10%25C	-.02667	.01966	.540	-.0817	.0284
BT10%25C	BT5%25C	.03667	.01966	.274	-.0184	.0817
	BT5%30C	.13333	.01966	.000	.1383	.2484
	BT10%30C	.19667	.01966	.000	.1116	.2217
BT10%30C	BT5%25C	-.13000	.01966	.000	-.1850	-.0750
	BT5%30C	.02667	.01966	.540	-.0284	.0817
	BT5%25C	-.16667	.01966	.000	-.2217	-.1116

*. The mean difference is significant at the 0.05 level.

Subset for alpha = 0.05

Treatments	N	1	2
BT5%30C	6	3.3100	
BT10%30C	6	3.3367	
BT5%25C	6		3.4667
BT10%25C	6		3.5033
Sig.		.540	.274

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Test Statistics^{a,b}

pH72Hour

Chi-Square	18.391
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable: Treatments

Ranks

	Treatments	N	Mean Rank
pH72Hour	BT5%25C	6	17.50
	BT5%30C	6	4.67
	BT10%25C	6	19.50
	BT10%30C	6	8.33
	Total	24	

F.2.1 Statistical analysis of lactic acid of black tea water kefir beverage samples during 72 hours of fermentation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Lacticacid_BT5_25C	0Hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24Hours	6	.1845	.00493	.00201	.1793	.1897	.18	.19
	48Hours	6	.2100	.00735	.00300	.2023	.2177	.20	.22
	72Hours	6	.2250	.00569	.00232	.2190	.2310	.22	.23
	Total	24	.1549	.09266	.01891	1.157	.1940	.00	.23
Lacticacid_BT5_30C	0Hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24Hours	6	.1950	.00465	.00190	.1901	.1999	.19	.20
	48Hours	6	.2370	.00735	.00300	.2293	.2447	.23	.24
	72Hours	6	.2505	.00677	.00277	.2434	.2576	.24	.26
	Total	24	.1706	.10291	.02101	1.272	.2141	.00	.26
Lacticacid_BT10_25C	0Hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24Hours	6	.2100	.00465	.00190	.2051	.2149	.21	.22
	48Hours	6	.2730	.00735	.00300	.2653	.2807	.26	.28
	72Hours	6	.3195	.01241	.00506	.3065	.3325	.31	.33
	Total	24	.2006	.12500	.02552	1.478	.2534	.00	.33
Lacticacid_BT10_30C	0Hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24Hours	6	.1935	.00493	.00201	.1883	.1987	.19	.20
	48Hours	6	.2625	.00677	.00277	.2554	.2696	.25	.27
	72Hours	6	.2940	.00735	.00300	.2863	.3017	.29	.31
	Total	24	.1875	.11676	.02383	1.382	.2368	.00	.31

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lacticacid_BT5_25C	Between Groups	.197	3	.066	2371.829	.000
	Within Groups	.001	20	.000		
	Total	.197	23			
Lacticacid_BT5_30C	Between Groups	.243	3	.081	2666.185	.000
	Within Groups	.001	20	.000		
	Total	.244	23			
Lacticacid_BT10_25C	Between Groups	.358	3	.119	2081.314	.000
	Within Groups	.001	20	.000		
	Total	.359	23			
Lacticacid_BT10_30C	Between Groups	.313	3	.104	3359.710	.000
	Within Groups	.001	20	.000		
	Total	.314	23			

Lacticacid_BT10_25C

		Subset for alpha = 0.05			
HOURL	N	1	2	3	4
Tukey HSD ^a	0Hour	6	.0000		
	24Hours	6		.2100	
	48Hours	6			.2730
	72Hours	6			.3195
	Sig.	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Lacticacid_BT5_25C

		Subset for alpha = 0.05			
HOURL	N	1	2	3	4
Tukey HSD ^a	0Hour	6	.0000		
	24Hours	6		.1845	
	48Hours	6			.2100
	72Hours	6			.2250
	Sig.	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Lacticacid_BT5_30C

		Subset for alpha = 0.05			
HOURL	N	1	2	3	4
Tukey HSD ^a	0Hour	6	.0000		
	24Hours	6		.1950	
	48Hours	6			.2370
	72Hours	6			.2505
	Sig.	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Lacticacid_BT10_30C

		Subset for alpha = 0.05			
HOURL	N	1	2	3	4
Tukey HSD ^a	0Hour	6	.0000		
	24Hours	6		.1935	
	48Hours	6			.2625
	72Hours	6			.2940
	Sig.	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) HOUR	(J) HOUR	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lacticacid_BT5_25C	0Hour	24Hours	-.184500*	.003037	.000	-.193000	-.176000
		48Hours	-.210000*	.003037	.000	-.218500	-.201500
		72Hours	-.225000*	.003037	.000	-.233500	-.216500
	24Hours	0Hour	.184500*	.003037	.000	.176000	.193000
		48Hours	-.025500*	.003037	.000	-.034000	-.017000
		72Hours	-.040500*	.003037	.000	-.049000	-.032000
	48Hours	0Hour	.210000*	.003037	.000	.201500	.218500
		24Hours	.025500*	.003037	.000	.017000	.034000
		72Hours	-.015000*	.003037	.000	-.023500	-.006500
	72Hours	0Hour	.225000*	.003037	.000	.216500	.233500
		24Hours	.040500*	.003037	.000	.032000	.049000
		48Hours	.015000*	.003037	.000	.006500	.023500
Lacticacid_BT5_30C	0Hour	24Hours	-.195000*	.003182	.000	-.20391	-.18609
		48Hours	-.237000*	.003182	.000	-.24591	-.22809
		72Hours	-.250500*	.003182	.000	-.25941	-.24159
	24Hours	0Hour	.195000*	.003182	.000	.18609	.20391
		48Hours	-.042000*	.003182	.000	-.05091	-.03309
		72Hours	-.055500*	.003182	.000	-.06441	-.04659
	48Hours	0Hour	.237000*	.003182	.000	.22809	.24591
		24Hours	.042000*	.003182	.000	.03309	.05091
		72Hours	-.013500*	.003182	.002	-.02241	-.00459
	72Hours	0Hour	.250500*	.003182	.000	.24159	.25941
		24Hours	.055500*	.003182	.000	.04659	.06441
		48Hours	.013500*	.003182	.002	.00459	.02241
Lacticacid_BT10_25C	0Hour	24Hours	-.210000*	.004373	.000	-.22224	-.19776
		48Hours	-.273000*	.004373	.000	-.28524	-.26076
		72Hours	-.319500*	.004373	.000	-.33174	-.30726
	24Hours	0Hour	.210000*	.004373	.000	.19776	.22224
		48Hours	-.063000*	.004373	.000	-.07524	-.05076
		72Hours	-.109500*	.004373	.000	-.12174	-.09726
	48Hours	0Hour	.273000*	.004373	.000	.26076	.28524
		24Hours	.063000*	.004373	.000	.05076	.07524
		72Hours	-.046500*	.004373	.000	-.05874	-.03426
	72Hours	0Hour	.319500*	.004373	.000	.30726	.33174
		24Hours	.109500*	.004373	.000	.09726	.12174
		48Hours	.046500*	.004373	.000	.03426	.05874
Lacticacid_BT10_30C	0Hour	24Hours	-.193500*	.003217	.000	-.20250	-.18450
		48Hours	-.262500*	.003217	.000	-.27150	-.25350
		72Hours	-.294000*	.003217	.000	-.30300	-.28500
	24Hours	0Hour	.193500*	.003217	.000	.18450	.20250
		48Hours	-.069000*	.003217	.000	-.07800	-.06000
		72Hours	-.100500*	.003217	.000	-.10950	-.09150
	48Hours	0Hour	.262500*	.003217	.000	.25350	.27150
		24Hours	.069000*	.003217	.000	.06000	.07800
		72Hours	-.031500*	.003217	.000	-.04050	-.02250
	72Hours	0Hour	.294000*	.003217	.000	.28500	.30300
		24Hours	.100500*	.003217	.000	.09150	.10950
		48Hours	.031500*	.003217	.000	.02250	.04050

*. The mean difference is significant at the 0.05 level.

F.2.2 Statistical analysis of lactic acid of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

lacticacid_log72h								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5%25C	6	-.6479	.01099	.00449	-.6595	-.6364	-.67	-.63
BT5%30C	6	-.6013	.01172	.00478	-.6136	-.5890	-.61	-.58
BT10%25C	6	-.4958	.01687	.00689	-.5135	-.4781	-.51	-.48
BT10%30C	6	-.5318	.01078	.00440	-.5431	-.5204	-.54	-.51
Total	24	-.5692	.06165	.01258	-.5952	-.5432	-.67	-.48

Test of Homogeneity of Variances

lacticacid_log72h			
Levene Statistic	df1	df2	Sig.
1.575	3	20	.227

ANOVA

lacticacid_log72h					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.084	3	.029	170.151	.000
Within Groups	.003	20	.000		
Total	.087	23			

Multiple Comparisons

Dependent Variable: lacticacid_log72h

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5%25C	BT5%30C	-.04661*	.00741	.000	-.0674	-.0259
	BT10%25C	-.15213*	.00741	.000	-.1729	-.1314
	BT10%30C	-.11617*	.00741	.000	-.1369	-.0954
BT5%30C	BT5%25C	.04661*	.00741	.000	.0259	.0674
	BT10%25C	-.10552*	.00741	.000	-.1263	-.0848
	BT10%30C	-.06956*	.00741	.000	-.0903	-.0488
BT10%25C	BT5%25C	.15213*	.00741	.000	.1314	.1729
	BT5%30C	.10552*	.00741	.000	.0848	.1263
	BT10%30C	.03596*	.00741	.001	.0152	.0567
BT10%30C	BT5%25C	.11617*	.00741	.000	.0954	.1369
	BT5%30C	.06956*	.00741	.000	.0488	.0903
	BT10%25C	-.03596*	.00741	.001	-.0567	-.0152

*. The mean difference is significant at the 0.05 level.

lacticacid_log72h

Tukey HSD^a

Subset for alpha = 0.05					
Treatments	N	1	2	3	4
BT5%25C	6	-.6479			
BT5%30C	6		-.6013		
BT10%30C	6			-.5318	
BT10%25C	6				-.4958
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

F.3.1 Statistical analysis of total soluble solids (°Brix) of black tea water kefir beverage samples during 72 hours of fermentation

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TTS_Brix_BT5_25C	0hour	6	5.0333	.05164	.02108	4.9791	5.0875	5.00	5.10
	24hours	6	4.6333	.05164	.02108	4.5791	4.6875	4.60	4.70
	48hours	6	4.0500	.05477	.02236	3.9925	4.1075	4.00	4.10
	72hours	6	3.3833	.09832	.04014	3.2802	3.4865	3.30	3.50
	Total	24	4.2750	.63878	.13039	4.0053	4.5447	3.30	5.10
TTS_Brix_BT5_30C	0hour	6	5.0333	.05164	.02108	4.9791	5.0875	5.00	5.10
	24hours	6	4.3167	.09832	.04014	4.2135	4.4198	4.20	4.40
	48hours	6	3.3500	.12247	.05000	3.2215	3.4785	3.20	3.50
	72hours	6	2.7833	.11690	.04773	2.6606	2.9060	2.60	2.90
	Total	24	3.8708	.89029	.18173	3.4949	4.2468	2.60	5.10
TTS_Brix_BT10_25C	0hour	6	9.9667	.05164	.02108	9.9125	10.0209	9.90	10.00
	24hours	6	9.3667	.05164	.02108	9.3125	9.4209	9.30	9.40
	48hours	6	8.7833	.07528	.03073	8.7043	8.8623	8.70	8.90
	72hours	6	7.9167	.07528	.03073	7.8377	7.9957	7.80	8.00
	Total	24	9.0083	.77511	.15922	8.6810	9.3356	7.80	10.00
TTS_Brix_BT10_30C	0hour	6	9.9667	.05164	.02108	9.9125	10.0209	9.90	10.00
	24hours	6	8.9833	.07528	.03073	8.9043	9.0623	8.90	9.10
	48hours	6	8.4333	.05164	.02108	8.3791	8.4875	8.40	8.50
	72hours	6	7.3167	.14720	.06909	7.1622	7.4711	7.20	7.50
	Total	24	8.6750	.98168	.20038	8.2805	9.0895	7.20	10.00

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
TTS_Brix_BT5_25C	Between Groups	9.295	3	3.098	688.519	.000
	Within Groups	.090	20	.005		
	Total	9.385	23			
TTS_Brix_BT5_30C	Between Groups	18.025	3	6.008	586.165	.000
	Within Groups	.205	20	.010		
	Total	18.230	23			
TTS_Brix_BT10_25C	Between Groups	13.735	3	4.578	1098.800	.000
	Within Groups	.083	20	.004		
	Total	13.818	23			
TTS_Brix_BT10_30C	Between Groups	22.002	3	7.334	898.027	.000
	Within Groups	.163	20	.008		
	Total	22.165	23			

TTS_Brix_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72hours	6	3.3833			
48hours	6		4.0500		
24hours	6			4.6333	
0hour	6				5.0333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

TTS_Brix_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72hours	6	2.7833			
48hours	6		3.3500		
24hours	6			4.3167	
0hour	6				5.0333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

TTS_Brix_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72hours	6	7.9167			
48hours	6		8.7833		
24hours	6			9.3667	
0hour	6				9.9667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

TTS_Brix_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
72hours	6	7.3167			
48hours	6		8.4333		
24hours	6			8.9833	
0hour	6				9.9667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TTS_Brix_BT5_25C	0hour	24hours	.40000*	.03873	.000	.2916	.5084
		48hours	.98333*	.03873	.000	.8749	1.0917
		72hours	1.65000*	.03873	.000	1.5416	1.7584
	24hours	0hour	-.40000*	.03873	.000	-.5084	-.2916
		48hours	.58333*	.03873	.000	.4749	.6917
		72hours	1.25000*	.03873	.000	1.1416	1.3584
	48hours	0hour	-.98333*	.03873	.000	-1.0917	-.8749
		24hours	-.58333*	.03873	.000	-.6917	-.4749
		72hours	.66667*	.03873	.000	.5583	.7751
	72hours	0hour	-1.65000*	.03873	.000	-1.7584	-1.5416
		24hours	-1.25000*	.03873	.000	-1.3584	-1.1416
		48hours	-.66667*	.03873	.000	-.7751	-.5583
TTS_Brix_BT5_30C	0hour	24hours	.71667*	.05845	.000	.5531	.8803
		48hours	1.68333*	.05845	.000	1.5197	1.8469
		72hours	2.25000*	.05845	.000	2.0864	2.4136
	24hours	0hour	-.71667*	.05845	.000	-.8803	-.5531
		48hours	.96667*	.05845	.000	.8031	1.1303
		72hours	1.53333*	.05845	.000	1.3697	1.6969
	48hours	0hour	-1.68333*	.05845	.000	-1.8469	-1.5197
		24hours	-.96667*	.05845	.000	-1.1303	-.8031
		72hours	.56667*	.05845	.000	.4031	.7303
	72hours	0hour	-2.25000*	.05845	.000	-2.4136	-2.0864
		24hours	-1.53333*	.05845	.000	-1.6969	-1.3697
		48hours	-.56667*	.05845	.000	-.7303	-.4031
TTS_Brix_BT10_25C	0hour	24hours	.60000*	.03727	.000	.4957	.7043
		48hours	1.18333*	.03727	.000	1.0790	1.2876
		72hours	2.05000*	.03727	.000	1.9457	2.1543
	24hours	0hour	-.60000*	.03727	.000	-.7043	-.4957
		48hours	.58333*	.03727	.000	.4790	.6876
		72hours	1.45000*	.03727	.000	1.3457	1.5543
	48hours	0hour	-1.18333*	.03727	.000	-1.2876	-1.0790
		24hours	-.58333*	.03727	.000	-.6876	-.4790
		72hours	.86667*	.03727	.000	.7624	.9710
	72hours	0hour	-2.05000*	.03727	.000	-2.1543	-1.9457
		24hours	-1.45000*	.03727	.000	-1.5543	-1.3457
		48hours	-.86667*	.03727	.000	-.9710	-.7624
TTS_Brix_BT10_30C	0hour	24hours	.98333*	.05217	.000	.8373	1.1294
		48hours	1.53333*	.05217	.000	1.3873	1.6794
		72hours	2.65000*	.05217	.000	2.5040	2.7960
	24hours	0hour	-.98333*	.05217	.000	-1.1294	-.8373
		48hours	.55000*	.05217	.000	.4040	.6960
		72hours	1.66667*	.05217	.000	1.5206	1.8127
	48hours	0hour	-1.53333*	.05217	.000	-1.6794	-1.3873
		24hours	-.55000*	.05217	.000	-.6960	-.4040
		72hours	1.11667*	.05217	.000	.9706	1.2627
	72hours	0hour	-2.65000*	.05217	.000	-2.7960	-2.5040
		24hours	-1.66667*	.05217	.000	-1.8127	-1.5206
		48hours	-1.11667*	.05217	.000	-1.2627	-.9706

*. The mean difference is significant at the 0.05 level.

F.3.2 Statistical analysis of total soluble solids (°Brix) of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

TTS_Brix_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5_25C	6	1.6500	.13784	.05627	1.5053	1.7947	1.50	1.80
BT5_30C	6	2.2500	.12247	.05000	2.1215	2.3785	2.10	2.40
BT10_25C	6	2.0500	.08367	.03416	1.9622	2.1378	2.00	2.20
BT10_30C	6	2.6500	.13784	.05627	2.5053	2.7947	2.50	2.80
Total	24	2.1500	.38561	.07871	1.9872	2.3128	1.50	2.80

ANOVA

TTS_Brix_72H

Test of Homogeneity of Variances				Sum of Squares	df	Mean Square	F	Sig.
Levene Statistic	df1	df2	Sig.	Between Groups	3	1.040	69.333	.000
1.333	3	20	.292	Within Groups	20	.015		
				Total	23			

Multiple Comparisons

Dependent Variable: TTS_Brix_72H

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5_25C	BT5_30C	-.60000*	.07071	.000	-.7979	-.4021
	BT10_25C	-.40000*	.07071	.000	-.5979	-.2021
	BT10_30C	-1.00000*	.07071	.000	-1.1979	-.8021
BT5_30C	BT5_25C	.60000*	.07071	.000	.4021	.7979
	BT10_25C	.20000*	.07071	.047	.0021	.3979
	BT10_30C	-.40000*	.07071	.000	-.5979	-.2021
BT10_25C	BT5_25C	.40000*	.07071	.000	.2021	.5979
	BT5_30C	-.20000*	.07071	.047	-.3979	-.0021
	BT10_30C	-.60000*	.07071	.000	-.7979	-.4021
BT10_30C	BT5_25C	1.00000*	.07071	.000	.8021	1.1979
	BT5_30C	.40000*	.07071	.000	.2021	.5979
	BT10_25C	.60000*	.07071	.000	.4021	.7979

*. The mean difference is significant at the 0.05 level.

TTS_Brix_72H

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
BT5_25C	6	1.6500			
BT10_25C	6		2.0500		
BT5_30C	6			2.2500	
BT10_30C	6				2.6500
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

F.4.1 Statistical analysis of colour (L*, a*, b*) of black tea water kefir beverage samples during 72 hours of fermentation

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Colour_L_BT5_25C	0Hour	6	80.5667	1.03638	.42305	79.4792	81.6542	79.11	81.64
	24hours	6	86.4500	.32158	.13128	86.1125	86.7875	86.05	86.99
	48hours	6	88.3650	.99216	.40096	87.3343	89.3957	87.34	89.60
	72hours	6	89.3917	.97905	.39970	88.3642	90.4191	88.18	90.47
	Total	24	86.1933	3.58455	.73169	84.6797	87.7070	79.11	90.47
Colour_L_BT5_30C	0Hour	6	80.4667	.66232	.27039	79.7716	81.1617	79.73	81.28
	24hours	6	87.4950	.55219	.22543	86.9155	88.0745	86.67	88.03
	48hours	6	88.5933	.79932	.32632	87.7545	89.4322	87.91	89.64
	72hours	6	89.3183	.89970	.36565	88.5840	90.0526	88.46	90.10
	Total	24	86.4663	3.65723	.74653	84.9240	88.0126	79.73	90.10
Colour_L_BT10_25C	0Hour	6	80.0933	.78610	.32092	79.2684	80.9183	79.38	81.10
	24hours	6	86.3283	.34425	.09971	86.0720	86.5847	86.00	86.60
	48hours	6	87.9567	.52868	.21583	87.4018	88.5115	87.31	88.87
	72hours	6	88.8683	.50026	.20423	88.3433	89.3933	88.02	89.30
	Total	24	85.8117	3.53553	.72169	84.3187	87.3046	79.38	89.30
Colour_L_BT10_30C	0Hour	6	80.3033	.66448	.27126	79.6060	81.0006	79.05	80.91
	24hours	6	86.4800	.75198	.30700	85.6908	87.2692	85.46	87.71
	48hours	6	88.1533	.55748	.22759	87.5693	88.7384	87.37	88.81
	72hours	6	89.0017	.66295	.27065	88.3059	89.6974	88.15	89.62
	Total	24	85.9646	3.53096	.72075	84.4936	87.4756	79.05	89.62

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Colour_L_BT5_25C	3.015	3	20	.054
Colour_L_BT5_30C	.722	3	20	.591
Colour_L_BT10_25C	2.727	3	20	.071
Colour_L_BT10_30C	.128	3	20	.943

ANOVA						
		Type of Sum of Squares	df	Mean Square	F	Sig.
Colour_L_BT5_25C	Between Groups	388.024	3	93.241	328.424	.000
	Within Groups	15.502	20	.775		
	Total	398.527	23			
Colour_L_BT5_30C	Between Groups	298.273	3	99.424	212.436	.000
	Within Groups	8.360	20	.418		
	Total	307.633	23			
Colour_L_BT10_25C	Between Groups	331.463	3	110.821	316.629	.000
	Within Groups	4.037	20	.202		
	Total	335.500	23			
Colour_L_BT10_30C	Between Groups	277.970	3	92.657	210.910	.000
	Within Groups	8.786	20	.439		
	Total	286.756	23			

Colour_L_BT5_25C

Tukey HSD^a

Subset for alpha = 0.05				
Hour	N	1	2	3
0Hour	6	80.5667		
24hours	6		86.4500	
48hours	6			88.3650
72hours	6			89.3917
Sig.		1.000	1.000	.214

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_L_BT5_30C

Tukey HSD^a

Subset for alpha = 0.05				
Hour	N	1	2	3
0Hour	6	80.4667		
24hours	6		87.4950	
48hours	6		88.5933	88.5933
72hours	6			89.3183
Sig.		1.000	.052	.287

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_L_BT10_25C

Tukey HSD^a

Subset for alpha = 0.05					
Hour	N	1	2	3	4
0Hour	6	80.0933			
24hours	6		86.3283		
48hours	6			87.9567	
72hours	6				88.8683
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_L_BT10_30C

Tukey HSD^a

Subset for alpha = 0.05				
Hour	N	1	2	3
0Hour	6	80.3033		
24hours	6		86.4800	
48hours	6			88.1533
72hours	6			89.0017
Sig.		1.000	1.000	.153

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Colour_L_BT5_25C	0Hour	24hours	-5.88333*	.50830	.000	-7.3060	-4.4606
		48hours	-7.79833*	.50830	.000	-9.2210	-6.3756
		72hours	-8.82500*	.50830	.000	-10.2477	-7.4023
	24hours	0Hour	5.88333*	.50830	.000	4.4606	7.3060
		48hours	-1.91500*	.50830	.006	-3.3377	-.4923
		72hours	-2.94167*	.50830	.000	-4.3644	-1.5190
	48hours	0Hour	7.79833*	.50830	.000	6.3756	9.2210
		24hours	1.91500*	.50830	.006	.4923	3.3377
		72hours	-1.02667	.50830	.214	-2.4494	.3960
	72hours	0Hour	8.82500*	.50830	.000	7.4023	10.2477
		24hours	2.94167*	.50830	.000	1.5190	4.3644
		48hours	1.02667	.50830	.214	-.3960	2.4494
Colour_L_BT5_30C	0Hour	24hours	-7.02833*	.39497	.000	-8.1338	-5.9228
		48hours	-8.12667*	.39497	.000	-9.2322	-7.0212
		72hours	-8.85167*	.39497	.000	-9.9572	-7.7462
	24hours	0Hour	7.02833*	.39497	.000	5.9228	8.1338
		48hours	-1.09833	.39497	.052	-2.2038	.0072
		72hours	-1.82333*	.39497	.001	-2.9288	-.7178
	48hours	0Hour	8.12667*	.39497	.000	7.0212	9.2322
		24hours	1.09833	.39497	.052	-.0072	2.2038
		72hours	-.72500	.39497	.287	-1.8305	.3805
	72hours	0Hour	8.85167*	.39497	.000	7.7462	9.9572
		24hours	1.82333*	.39497	.001	.7178	2.9288
		48hours	.72500	.39497	.287	-.3805	1.8305
Colour_L_BT10_25C	0Hour	24hours	-6.23500*	.31720	.000	-7.1228	-5.3472
		48hours	-7.86333*	.31720	.000	-8.7511	-6.9755
		72hours	-8.77500*	.31720	.000	-9.6628	-7.8872
	24hours	0Hour	6.23500*	.31720	.000	5.3472	7.1228
		48hours	-1.62833*	.31720	.000	-2.5161	-.7405
		72hours	-2.54000*	.31720	.000	-3.4278	-1.6522
	48hours	0Hour	7.86333*	.31720	.000	6.9755	8.7511
		24hours	1.62833*	.31720	.000	.7405	2.5161
		72hours	-.91167*	.31720	.043	-1.7995	-.0239
	72hours	0Hour	8.77500*	.31720	.000	7.8872	9.6628
		24hours	2.54000*	.31720	.000	1.6522	3.4278
		48hours	.91167*	.31720	.043	.0239	1.7995
Colour_L_BT10_30C	0Hour	24hours	-6.17667*	.38267	.000	-7.2477	-5.1056
		48hours	-7.85000*	.38267	.000	-8.9211	-6.7789
		72hours	-8.69833*	.38267	.000	-9.7694	-7.6273
	24hours	0Hour	6.17667*	.38267	.000	5.1056	7.2477
		48hours	-1.67333*	.38267	.002	-2.7444	-.6023
		72hours	-2.52167*	.38267	.000	-3.5927	-1.4506
	48hours	0Hour	7.85000*	.38267	.000	6.7789	8.9211
		24hours	1.67333*	.38267	.002	.6023	2.7444
		72hours	-.84833	.38267	.153	-1.9194	.2227
	72hours	0Hour	8.69833*	.38267	.000	7.6273	9.7694
		24hours	2.52167*	.38267	.000	1.4506	3.5927
		48hours	.84833	.38267	.153	-.2227	1.9194

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Colour_a_BT5_25C	0Hour	6	2.9550	.9644	1.6091	2.5414	3.3886	2.54	3.45
	24hours	6	1.3800	.43800	.17981	.8204	1.8396	.90	1.94
	48hours	6	.4017	.18822	.08867	.2261	.5782	.21	.62
	72hours	6	1.483	.12156	.04963	.0208	.2759	.08	.30
	Total	24	1.2213	1.16238	.23727	.7304	1.7121	.08	3.45
Colour_a_BT5_30C	0Hour	6	3.4100	.77121	.31484	2.6007	4.2193	2.39	3.93
	24hours	6	1.3297	.47631	.19445	.8268	1.8265	.63	1.88
	48hours	6	.1950	.05206	.02125	.1404	.2498	.13	.26
	72hours	6	.0267	.01211	.00464	.0140	.0304	.01	.04
	Total	24	1.2396	1.44171	.29429	.6388	1.8484	.01	3.93
Colour_a_BT10_25C	0Hour	6	3.4150	.69319	.28299	2.6975	4.1425	2.52	4.00
	24hours	6	1.8533	.52972	.21463	1.3016	2.4050	1.28	2.48
	48hours	6	1.0483	.17183	.07007	.8862	1.2284	.84	1.26
	72hours	6	.5683	.33367	.13822	.2182	.9185	.38	1.05
	Total	24	1.7213	1.18888	.24264	1.2193	2.2332	.38	4.00
Colour_a_BT10_30C	0Hour	6	3.9417	.34506	.10025	3.6945	4.1988	3.63	4.20
	24hours	6	1.7150	.33969	.13868	1.3585	2.0715	1.35	2.13
	48hours	6	.6650	.49935	.20386	.1410	1.1890	.18	1.26
	72hours	6	.3050	.26853	.10963	.0232	.5888	.18	.66
	Total	24	1.6587	1.48483	.30905	1.0298	2.2838	.18	4.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour_a_BT5_25C	Between Groups	29.124	3	9.708	99.501	.000
	Within Groups	1.951	20	.098		
	Total	31.075	23			
Colour_a_BT5_30C	Between Groups	43.684	3	14.561	70.644	.000
	Within Groups	4.122	20	.206		
	Total	47.806	23			
Colour_a_BT10_25C	Between Groups	28.010	3	9.337	41.603	.000
	Within Groups	4.488	20	.224		
	Total	32.498	23			
Colour_a_BT10_30C	Between Groups	48.210	3	16.070	129.361	.000
	Within Groups	2.485	20	.124		
	Total	50.695	23			

Colour_a_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.1483		
48hours	6	.4017		
24hours	6		1.3800	
0Hour	6			2.9550
Sig.		.511	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.0267		
48hours	6	.1950		
24hours	6		1.3267	
0Hour	6			3.4100
Sig.		.917	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.5683		
48hours	6	1.0483		
24hours	6		1.8533	
0Hour	6			3.4150
Sig.		.323	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.3050		
48hours	6	.6650		
24hours	6		1.7150	
0Hour	6			3.9417
Sig.		.317	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Colour_a_BT5_25C	0Hour	24hours	1.57500*	.18034	.000	1.0702	2.0798
		48hours	2.55333*	.18034	.000	2.0486	3.0581
		72hours	2.80667*	.18034	.000	2.3019	3.3114
	24hours	0Hour	-1.57500*	.18034	.000	-2.0798	-1.0702
		48hours	.97833*	.18034	.000	.4736	1.4831
		72hours	1.23167*	.18034	.000	.7269	1.7364
	48hours	0Hour	-2.55333*	.18034	.000	-3.0581	-2.0486
		24hours	-.97833*	.18034	.000	-1.4831	-.4736
		72hours	.25333	.18034	.511	-.2514	.7581
	72hours	0Hour	-2.80667*	.18034	.000	-3.3114	-2.3019
		24hours	-1.23167*	.18034	.000	-1.7364	-.7269
		48hours	-.25333	.18034	.511	-.7581	.2514
Colour_a_BT5_30C	0Hour	24hours	2.08333*	.26212	.000	1.3497	2.8170
		48hours	3.21500*	.26212	.000	2.4813	3.9487
		72hours	3.38333*	.26212	.000	2.6497	4.1170
	24hours	0Hour	-2.08333*	.26212	.000	-2.8170	-1.3497
		48hours	1.13167*	.26212	.002	.3980	1.8653
		72hours	1.30000*	.26212	.000	.5663	2.0337
	48hours	0Hour	-3.21500*	.26212	.000	-3.9487	-2.4813
		24hours	-1.13167*	.26212	.002	-1.8653	-.3980
		72hours	.16833	.26212	.917	-.5653	.9020
	72hours	0Hour	-3.38333*	.26212	.000	-4.1170	-2.6497
		24hours	-1.30000*	.26212	.000	-2.0337	-.5663
		48hours	-.16833	.26212	.917	-.9020	.5653
Colour_a_BT10_25C	0Hour	24hours	1.56167*	.27351	.000	.7961	2.3272
		48hours	2.36667*	.27351	.000	1.6011	3.1322
		72hours	2.84667*	.27351	.000	2.0811	3.6122
	24hours	0Hour	-1.56167*	.27351	.000	-2.3272	-.7961
		48hours	.80500*	.27351	.037	.0395	1.5705
		72hours	1.28500*	.27351	.001	.5195	2.0505
	48hours	0Hour	-2.36667*	.27351	.000	-3.1322	-1.6011
		24hours	-.80500*	.27351	.037	-1.5705	-.0395
		72hours	.48000	.27351	.323	-.2855	1.2455
	72hours	0Hour	-2.84667*	.27351	.000	-3.6122	-2.0811
		24hours	-1.28500*	.27351	.001	-2.0505	-.5195
		48hours	-.48000	.27351	.323	-1.2455	.2855
Colour_a_BT10_30C	0Hour	24hours	2.22667*	.20349	.000	1.6571	2.7962
		48hours	3.27667*	.20349	.000	2.7071	3.8462
		72hours	3.63667*	.20349	.000	3.0671	4.2062
	24hours	0Hour	-2.22667*	.20349	.000	-2.7962	-1.6571
		48hours	1.05000*	.20349	.000	.4804	1.6196
		72hours	1.41000*	.20349	.000	.8404	1.9796
	48hours	0Hour	-3.27667*	.20349	.000	-3.8462	-2.7071
		24hours	-1.05000*	.20349	.000	-1.6196	-.4804
		72hours	.36000	.20349	.317	-.2096	.9296
	72hours	0Hour	-3.63667*	.20349	.000	-4.2062	-3.0671
		24hours	-1.41000*	.20349	.000	-1.9796	-.8404
		48hours	-.36000	.20349	.317	-.9296	.2096

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Colour_b_BT5_25C	0Hour	8	36.1067	.19977	80155	35.8970	36.3163	35.79	36.36
	24hours	6	31.8967	1.11484	45513	30.7267	33.0666	30.76	33.47
	48hours	6	30.2133	1.64176	67026	28.4904	31.9363	28.13	35.75
	72hours	6	28.6300	1.66265	67666	26.8649	30.3751	26.46	30.21
	Total	24	31.7117	3.09465	63169	30.4049	33.0184	26.46	36.36
Colour_b_BT5_30C	0Hour	6	36.9717	1.08627	44347	35.8917	38.1116	35.68	38.13
	24hours	6	32.8550	1.84964	75511	30.9139	34.7961	30.80	35.40
	48hours	6	29.5717	69660	28439	28.8406	30.3027	28.71	30.27
	72hours	6	28.1983	59122	24136	27.5779	28.8188	27.47	28.79
	Total	24	31.8992	3.62193	73932	30.3698	33.4286	27.47	38.13
Colour_b_BT10_25C	0Hour	6	36.5983	1.04819	42792	35.4983	37.6983	35.69	37.96
	24hours	6	33.3367	1.87814	76675	31.3657	35.3077	31.11	35.51
	48hours	6	30.4217	1.66586	69233	28.8420	32.2014	29.11	33.42
	72hours	6	29.8467	1.65531	67578	28.1095	31.5838	27.39	31.65
	Total	24	32.5508	3.12274	63743	31.2322	33.8695	27.39	37.96
Colour_b_BT10_30C	0Hour	6	36.6750	.95643	39046	35.6713	37.6787	35.83	38.14
	24hours	6	33.8217	1.81724	74189	31.8146	35.7267	31.46	35.37
	48hours	6	31.0267	1.53957	62853	29.4110	32.6423	28.71	32.61
	72hours	6	29.8333	1.91461	70164	27.8241	31.8426	27.16	31.13
	Total	24	32.7882	3.13919	64079	31.4636	34.1147	27.16	38.14

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour_b_BT5_25C	Between Groups	186.552	3	62.184	36.887	.000
	Within Groups	33.716	20	1.686		
	Total	220.268	23			
Colour_b_BT5_30C	Between Groups	274.544	3	91.515	67.341	.000
	Within Groups	27.180	20	1.359		
	Total	301.723	23			
Colour_b_BT10_25C	Between Groups	173.074	3	57.691	22.531	.000
	Within Groups	51.211	20	2.561		
	Total	224.285	23			
Colour_b_BT10_30C	Between Groups	175.389	3	58.463	22.808	.000
	Within Groups	51.266	20	2.563		
	Total	226.654	23			

Colour_b_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	28.6300		
48hours	6	30.2133	30.2133	
24hours	6		31.8967	
0Hour	6			36.1067
Sig.		.183	.145	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	28.1983		
48hours	6	29.5717		
24hours	6		32.8550	
0Hour	6			36.9717
Sig.		.207	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	29.8467		
48hours	6	30.4217		
24hours	6		33.3367	
0Hour	6			36.5983
Sig.		.924	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	29.6333		
48hours	6	31.0267		
24hours	6		33.8217	
0Hour	6			36.6750
Sig.		.452	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Colour_b_BT5_25C	0Hour	24hours	4.21000*	.74962	.000	2.1118	6.3082
		48hours	5.89333*	.74962	.000	3.7952	7.9915
		72hours	7.47667*	.74962	.000	5.3785	9.5748
	24hours	0Hour	-4.21000*	.74962	.000	-6.3082	-2.1118
		48hours	1.68333	.74962	.145	-.4148	3.7815
		72hours	3.26667*	.74962	.002	1.1685	5.3648
	48hours	0Hour	-5.89333*	.74962	.000	-7.9915	-3.7952
		24hours	-1.68333	.74962	.145	-3.7815	.4148
		72hours	1.58333	.74962	.183	-.5148	3.6815
	72hours	0Hour	-7.47667*	.74962	.000	-9.5748	-5.3785
		24hours	-3.26667*	.74962	.002	-5.3648	-1.1685
		48hours	-1.58333	.74962	.183	-3.6815	.5148
Colour_b_BT5_30C	0Hour	24hours	4.11667*	.67305	.000	2.2328	6.0005
		48hours	7.40000*	.67305	.000	5.5162	9.2838
		72hours	8.77333*	.67305	.000	6.8895	10.6572
	24hours	0Hour	-4.11667*	.67305	.000	-6.0005	-2.2328
		48hours	3.28333*	.67305	.000	1.3995	5.1672
		72hours	4.65667*	.67305	.000	2.7728	6.5405
	48hours	0Hour	-7.40000*	.67305	.000	-9.2838	-5.5162
		24hours	-3.28333*	.67305	.000	-5.1672	-1.3995
		72hours	1.37333	.67305	.207	-.5105	3.2572
	72hours	0Hour	-8.77333*	.67305	.000	-10.6572	-6.8895
		24hours	-4.65667*	.67305	.000	-6.5405	-2.7728
		48hours	-1.37333	.67305	.207	-3.2572	.5105
Colour_b_BT10_25C	0Hour	24hours	3.26167*	.92386	.010	.6759	5.8475
		48hours	6.17667*	.92386	.000	3.5909	8.7625
		72hours	6.75167*	.92386	.000	4.1659	9.3375
	24hours	0Hour	-3.26167*	.92386	.010	-5.8475	-.6759
		48hours	2.91500*	.92386	.024	.3292	5.5008
		72hours	3.49000*	.92386	.006	.9042	6.0758
	48hours	0Hour	-6.17667*	.92386	.000	-8.7625	-3.5909
		24hours	-2.91500*	.92386	.024	-5.5008	-.3292
		72hours	.57500	.92386	.924	-2.0108	3.1608
	72hours	0Hour	-6.75167*	.92386	.000	-9.3375	-4.1659
		24hours	-3.49000*	.92386	.006	-6.0758	-.9042
		48hours	-.57500	.92386	.924	-3.1608	2.0108
Colour_b_BT10_30C	0Hour	24hours	2.85333*	.92435	.027	.2661	5.4405
		48hours	5.64833*	.92435	.000	3.0611	8.2355
		72hours	7.04167*	.92435	.000	4.4545	9.6289
	24hours	0Hour	-2.85333*	.92435	.027	-5.4405	-.2661
		48hours	2.79500*	.92435	.031	.2078	5.3822
		72hours	4.18833*	.92435	.001	1.6011	6.7755
	48hours	0Hour	-5.64833*	.92435	.000	-8.2355	-3.0611
		24hours	-2.79500*	.92435	.031	-5.3822	-.2078
		72hours	1.39333	.92435	.452	-1.1939	3.9805
	72hours	0Hour	-7.04167*	.92435	.000	-9.6289	-4.4545
		24hours	-4.18833*	.92435	.001	-6.7755	-1.6011
		48hours	-1.39333	.92435	.452	-3.9805	1.1939

*. The mean difference is significant at the 0.05 level.

F.4.2 Statistical analysis of colour (L*, a*, b*) of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

Colour_L_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5%25C	6	89.3917	.97905	.39970	88.3642	90.4191	88.18	90.47
BT5%30C	6	89.3183	.69970	.28565	88.5840	90.0526	88.46	90.10
BT10%25C	6	88.8683	.50026	.20423	88.3433	89.3933	88.02	89.30
BT10%30C	6	89.0017	.66295	.27065	88.3059	89.6974	88.15	89.62
Total	24	89.1450	.71679	.14631	88.8423	89.4477	88.02	90.47

Test of Homogeneity of Variances

Colour_L_72H

Levene Statistic	df1	df2	Sig.
1.481	3	20	.250

ANOVA

Colour_L_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.128	3	.376	.703	.581
Within Groups	10.689	20	.534		
Total	11.817	23			

Multiple Comparisons

Dependent Variable: Colour_L_72H

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5%25C	BT5%30C	.07333	.42208	.998	-1.1081	1.2547
	BT10%25C	.52333	.42208	.610	-.6581	1.7047
	BT10%30C	.39000	.42208	.792	-.7914	1.5714
BT5%30C	BT5%25C	-.07333	.42208	.998	-1.2547	1.1081
	BT10%25C	.45000	.42208	.713	-.7314	1.6314
	BT10%30C	.31667	.42208	.875	-.8647	1.4981
BT10%25C	BT5%25C	-.52333	.42208	.610	-1.7047	.6581
	BT5%30C	-.45000	.42208	.713	-1.6314	.7314
	BT10%30C	-.13333	.42208	.989	-1.3147	1.0481
BT10%30C	BT5%25C	-.39000	.42208	.792	-1.5714	.7914
	BT5%30C	-.31667	.42208	.875	-1.4981	.8647
	BT10%25C	.13333	.42208	.989	-1.0481	1.3147

Colour_L_72H

Tukey HSD^a

Treatments	N	Subset for alpha = 0.05
		1
BT10%25C	6	88.8683
BT10%30C	6	89.0017
BT5%30C	6	89.3183
BT5%25C	6	89.3917
Sig.		.610

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Descriptives

Log_colour_a_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5%25C	5	-.8584	.39977	.17878	-1.3548	-.3620	-1.52	-.52
BT5%30C	6	-1.6194	.23018	.09397	-1.8610	-1.3779	-2.00	-1.40
BT10%25C	6	-.3022	.23550	.09614	-.5493	-.0550	-.52	.02
BT10%30C	6	-.6544	.37168	.15174	-1.0444	-.2643	-1.00	-.18
Total	23	-.8586	.58206	.12137	-1.1103	-.6069	-2.00	.02

ANOVA

Test of Homogeneity of Variances

Log_colour_a_72H

Levene Statistic	df1	df2	Sig.
.822	3	19	.498

Log_colour_a_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.581	3	1.860	18.880	.000
Within Groups	1.872	19	.099		
Total	7.454	22			

Multiple Comparisons

Dependent Variable: Log_colour_a_72H

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5%25C	BT5%30C	.76101*	.19008	.004	.2265	1.2955
	BT10%25C	-.55627*	.19008	.040	-1.0907	-.0218
	BT10%30C	-.20404	.19008	.709	-.7385	.3304
BT5%30C	BT5%25C	-.76101*	.19008	.004	-1.2955	-.2265
	BT10%25C	-1.31728*	.18124	.000	-1.8269	-.8077
	BT10%30C	-.96506*	.18124	.000	-1.4747	-.4555
BT10%25C	BT5%25C	.55627*	.19008	.040	.0218	1.0907
	BT5%30C	1.31728*	.18124	.000	.8077	1.8269
	BT10%30C	.35223	.18124	.244	-.1574	.8618
BT10%30C	BT5%25C	.20404	.19008	.709	-.3304	.7385
	BT5%30C	.96506*	.18124	.000	.4555	1.4747
	BT10%25C	-.35223	.18124	.244	-.8618	.1574

*. The mean difference is significant at the 0.05 level.

Log_colour_a_72H

Tukey HSD^{a,b}

Treatments	N	Subset for alpha = 0.05		
		1	2	3
BT5%30C	6	-1.6194		
BT5%25C	5		-.8584	
BT10%30C	6		-.6544	-.6544
BT10%25C	6			-.3022
Sig.		1.000	.695	.262

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.714.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Descriptives

Colour_b_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5%25C	6	28.6300	1.66285	.67886	26.8849	30.3751	26.46	30.21
BT5%30C	6	28.1983	.59122	.24136	27.5779	28.8188	27.47	28.79
BT10%25C	6	29.8467	1.65531	.67578	28.1095	31.5838	27.39	31.65
BT10%30C	6	29.6333	1.91461	.78164	27.6241	31.6426	27.16	31.13
Total	24	29.0771	1.59952	.32650	28.4017	29.7525	26.46	31.65

ANOVA

Colour_b_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.243	3	3.748	1.575	.227
Within Groups	47.602	20	2.380		
Total	58.845	23			

Multiple Comparisons

Dependent Variable: Colour_b_72H

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5%25C	BT5%30C	.43167	.89071	.962	-2.0614	2.9247
	BT10%25C	-1.21667	.89071	.534	-3.7097	1.2764
	BT10%30C	-1.00333	.89071	.678	-3.4964	1.4897
BT5%30C	BT5%25C	-.43167	.89071	.962	-2.9247	2.0614
	BT10%25C	-1.64833	.89071	.280	-4.1414	.8447
	BT10%30C	-1.43500	.89071	.395	-3.9280	1.0580
BT10%25C	BT5%25C	1.21667	.89071	.534	-1.2764	3.7097
	BT5%30C	1.64833	.89071	.280	-.8447	4.1414
	BT10%30C	.21333	.89071	.995	-2.2797	2.7064
BT10%30C	BT5%25C	1.00333	.89071	.678	-1.4897	3.4964
	BT5%30C	1.43500	.89071	.395	-1.0580	3.9280
	BT10%25C	-.21333	.89071	.995	-2.7064	2.2797

Colour_b_72H

Tukey HSD^a

Treatments	N	Subset for alpha = 0.05
		1
BT5%30C	6	28.1983
BT5%25C	6	28.6300
BT10%30C	6	29.6333
BT10%25C	6	29.8467
Sig.		.280

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Ranks

	Treatments	N	Mean Rank
Colour_b_72H	BT5%25C	6	10.50
	BT5%30C	6	9.00
	BT10%25C	6	15.67
	BT10%30C	6	14.83
	Total	24	

Test Statistics^{a,b}

Colour_b_72H	
Chi-Square	3.807
df	3
Asymp. Sig.	.283

a. Kruskal Wallis Test

b. Grouping Variable: Treatments

F.5.1 Statistical analysis of microbiological growth of black tea water kefir beverage samples during 72 hours of fermentation

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Viable_cell_count_Lactococcus_BT5_25C	0hour	3	4.9433	.00351	.03667	4.7856	5.1011	4.87	4.98
	24hours	3	5.6833	.30508	.11893	5.1716	6.1950	5.49	5.90
	48hours	3	6.1500	.12490	.07211	5.8397	6.4603	6.05	6.29
	72hours	3	5.6300	.11533	.06688	5.3435	5.9165	5.52	5.75
	Total	12	5.6017	.46470	.13415	5.3064	5.8969	4.87	6.29
Viable_cell_count_Lactococcus_BT5_30C	0hour	3	4.9467	.10408	.06009	4.6881	5.2052	4.83	5.03
	24hours	3	5.7967	.28361	.16374	5.0921	6.5013	5.47	5.98
	48hours	3	6.2867	.39504	.05487	6.0506	6.5228	6.19	6.36
	72hours	3	5.8367	.05774	.03333	5.6932	5.9801	5.77	5.87
	Total	12	5.7167	.24234	.15133	5.3836	6.0498	4.83	6.36
Viable_cell_count_Lactococcus_BT10_25C	0hour	3	4.9267	.11504	.06642	4.6409	5.2124	4.81	5.04
	24hours	3	5.8100	.38974	.22502	4.8418	6.7782	5.36	6.04
	48hours	3	6.3333	.11930	.06988	6.0370	6.6297	6.20	6.43
	72hours	3	5.8833	.07638	.04410	5.6636	6.0731	5.60	5.95
	Total	12	5.7383	.28306	.16254	5.3806	6.0981	4.81	6.43
Viable_cell_count_Lactococcus_BT10_30C	0hour	3	4.9400	.13454	.07767	4.6058	5.2742	4.83	5.09
	24hours	3	5.9400	.42036	.24269	4.8958	6.9842	5.47	6.28
	48hours	3	6.3467	.11930	.06988	6.0503	6.6430	6.21	6.43
	72hours	3	5.8500	.09000	.05196	5.6264	6.0736	5.78	5.94
	Total	12	5.7692	.27243	.16525	5.4055	6.1328	4.83	6.43

ANOVA							
				Sum of Squares	df	Mean Square	Sig.
Viable_cell_count_Lactococcus_BT5_25C	Between Groups	(Combined)		2.225	3	.742	.39357
		Linear Term	Contrast	.958	1	.958	.50824
			Deviation	1.267	2	.634	.33623
	Within Groups			.151	8	.019	
	Total			2.375	11		
Viable_cell_count_Lactococcus_BT5_30C	Between Groups	(Combined)		2.816	3	.939	.36238
		Linear Term	Contrast	1.498	1	1.498	.57813
			Deviation	1.318	2	.659	.25435
	Within Groups			.207	8	.026	
	Total			3.023	11		
Viable_cell_count_Lactococcus_BT10_25C	Between Groups	(Combined)		3.117	3	1.039	.22440
		Linear Term	Contrast	1.727	1	1.727	.37305
			Deviation	1.390	2	.695	.15009
	Within Groups			.370	8	.046	
	Total			3.487	11		
Viable_cell_count_Lactococcus_BT10_30C	Between Groups	(Combined)		3.170	3	1.057	.19467
		Linear Term	Contrast	1.476	1	1.476	.27187
			Deviation	1.694	2	.847	.15607
	Within Groups			.434	8	.054	
	Total			3.604	11		

Viable_cell_count_Lactococcus_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	4.9433		
72hours	3		5.6300	
24hours	3		5.6833	
48hours	3			6.1500
Sig.		1.000	.962	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactococcus_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	4.9467		
24hours	3		5.7967	
72hours	3		5.8367	
48hours	3			6.2867
Sig.		1.000	.989	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactococcus_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	4.9267	
24hours	3		5.8100
72hours	3		5.8833
48hours	3		6.3333
Sig.		1.000	.069

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactococcus_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	4.9400	
72hours	3		5.8500
24hours	3		5.9400
48hours	3		6.3467
Sig.		1.000	.115

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Viable_cell_count_Lactococcus_BT5_25C	0hour	24hours	-.74000*	.11208	.001	-1.0989	-.3811
		48hours	-1.20667*	.11208	.000	-1.5656	-.8478
		72hours	-.68667*	.11208	.001	-1.0456	-.3278
	24hours	0hour	.74000*	.11208	.001	.3811	1.0989
		48hours	-.46667*	.11208	.013	-.8256	-.1078
		72hours	.05333	.11208	.962	-.3056	.4122
	48hours	0hour	1.20667*	.11208	.000	.8478	1.5656
		24hours	.46667*	.11208	.013	.1078	.8256
		72hours	.52000*	.11208	.007	.1611	.8789
	72hours	0hour	.68667*	.11208	.001	.3278	1.0456
		24hours	-.05333	.11208	.962	-.4122	.3056
		48hours	-.52000*	.11208	.007	-.8789	-.1611
Viable_cell_count_Lactococcus_BT5_30C	0hour	24hours	-.85000*	.13142	.001	-1.2709	-.4291
		48hours	-1.34000*	.13142	.000	-1.7609	-.9191
		72hours	-.89000*	.13142	.001	-1.3109	-.4691
	24hours	0hour	.85000*	.13142	.001	.4291	1.2709
		48hours	-.49000*	.13142	.024	-.9109	-.0691
		72hours	-.04000	.13142	.989	-.4609	.3809
	48hours	0hour	1.34000*	.13142	.000	.9191	1.7609
		24hours	.49000*	.13142	.024	.0691	.9109
		72hours	.45000*	.13142	.037	.0291	.8709
	72hours	0hour	.89000*	.13142	.001	.4691	1.3109
		24hours	.04000	.13142	.989	-.3809	.4609
		48hours	-.45000*	.13142	.037	-.8709	-.0291
Viable_cell_count_Lactococcus_BT10_25C	0hour	24hours	-.88333*	.17569	.004	-1.4460	-.3207
		48hours	-1.40667*	.17569	.000	-1.9693	-.8440
		72hours	-.95667*	.17569	.003	-1.5193	-.3940
	24hours	0hour	.88333*	.17569	.004	.3207	1.4460
		48hours	-.52333	.17569	.069	-1.0860	.0393
		72hours	-.07333	.17569	.974	-.6360	.4893
	48hours	0hour	1.40667*	.17569	.000	.8440	1.9693
		24hours	.52333	.17569	.069	-.0393	1.0860
		72hours	.45000	.17569	.123	-.1126	1.0126
	72hours	0hour	.95667*	.17569	.003	.3940	1.5193
		24hours	.07333	.17569	.974	-.4893	.6360
		48hours	-.45000	.17569	.123	-1.0126	.1126
Viable_cell_count_Lactococcus_BT10_30C	0hour	24hours	-1.00000*	.19023	.003	-1.6092	-.3908
		48hours	-1.40667*	.19023	.000	-2.0159	-.7975
		72hours	-.91000*	.19023	.006	-1.5192	-.3008
	24hours	0hour	1.00000*	.19023	.003	.3908	1.6092
		48hours	-.40667	.19023	.220	-1.0159	.2025
		72hours	.09000	.19023	.963	-.5192	.6992
	48hours	0hour	1.40667*	.19023	.000	.7975	2.0159
		24hours	.40667	.19023	.220	-.2025	1.0159
		72hours	.49667	.19023	.115	-.1125	1.1059
	72hours	0hour	.91000*	.19023	.006	.3008	1.5192
		24hours	-.09000	.19023	.963	-.6992	.5192
		48hours	-.49667	.19023	.115	-1.1059	.1125

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Viable_cell_count_Lactobacillus_BT5_25C	0hour	3	5.8233	.07234	.04177	5.6436	6.0030	5.74	5.87
	24hours	3	6.2533	.25325	1.4621	5.6242	6.8824	5.98	6.48
	48hours	3	6.4567	.05859	.03383	6.3111	6.6022	6.39	6.50
	72hours	3	5.8300	.21633	1.2490	5.2926	6.3674	5.65	6.07
	Total	12	6.0908	.32174	.09288	5.8864	6.2953	5.65	6.50
Viable_cell_count_Lactobacillus_BT5_30C	0hour	3	5.7833	.18502	1.1280	5.2989	6.2678	5.59	5.98
	24hours	3	6.1667	.25146	1.4518	5.5420	6.7913	5.88	6.35
	48hours	3	6.4233	.13051	.07535	6.0991	6.7475	6.32	6.57
	72hours	3	5.7933	.14468	.08353	5.4339	6.1527	5.70	5.96
	Total	12	6.0417	.32298	.09324	5.8364	6.2469	5.59	6.57
Viable_cell_count_Lactobacillus_BT10_25C	0hour	3	5.7767	.20502	1.1837	5.2674	6.2800	5.57	5.98
	24hours	3	6.4233	.26019	2.0795	5.5286	7.3181	6.07	6.79
	48hours	3	6.5367	.11547	.06667	6.2498	6.8235	6.47	6.67
	72hours	3	5.9067	.18475	1.0667	5.4477	6.3656	5.80	6.12
	Total	12	6.1688	.39376	1.1367	5.9107	6.4110	5.57	6.79
Viable_cell_count_Lactobacillus_BT10_30C	0hour	3	5.4267	.34005	1.9633	4.5819	6.2714	5.09	5.77
	24hours	3	6.2933	.23245	1.3421	5.7159	6.8708	6.03	6.47
	48hours	3	6.5000	.06928	.04000	6.3279	6.6721	6.46	6.58
	72hours	3	5.8067	.16862	.09735	5.3978	6.2255	5.69	6.00
	Total	12	6.0067	.47784	1.3794	5.7031	6.3103	5.09	6.59

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Viable_cell_count_Lactobacillus_BT5_25C	Between Groups	.899	3	.300	10.028	.004
	Within Groups	.239	8	.030		
	Total	1.139	11			
Viable_cell_count_Lactobacillus_BT5_30C	Between Groups	.889	3	.290	8.323	.008
	Within Groups	.278	8	.035		
	Total	1.148	11			
Viable_cell_count_Lactobacillus_BT10_25C	Between Groups	1.267	3	.422	7.706	.010
	Within Groups	.438	8	.055		
	Total	1.705	11			
Viable_cell_count_Lactobacillus_BT10_30C	Between Groups	2.106	3	.702	13.838	.002
	Within Groups	.406	8	.051		
	Total	2.512	11			

Viable_cell_count_Lactobacillus_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.8233	
72hours	3	5.8300	
24hours	3	6.2533	6.2533
48hours	3		6.4567
Sig.		.062	.511

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactobacillus_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.7833	
72hours	3	5.7933	
24hours	3	6.1667	6.1667
48hours	3		6.4233
Sig.		.131	.390

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactobacillus_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.7767		
72hours	3	5.9067	5.9067	
24hours	3		6.4233	6.4233
48hours	3			6.5367
Sig.		.902	.101	.931

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Lactobacillus_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.4267		
72hours	3	5.8067	5.8067	
24hours	3		6.2933	6.2933
48hours	3			6.5000
Sig.		.242	.110	.686

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Viable_cell_count_Lactobacillus_BT5_25C	0hour	24hours	-.43000	.14119	.062	-.8821	.0221
		48hours	-.63333*	.14119	.009	-1.0855	-.1812
		72hours	-.00667	.14119	1.000	-.4588	.4455
	24hours	0hour	.43000	.14119	.062	-.0221	.8821
		48hours	-.20333	.14119	.511	-.6555	.2488
		72hours	.42333	.14119	.067	-.0288	.8755
	48hours	0hour	.63333*	.14119	.009	.1812	1.0855
		24hours	.20333	.14119	.511	-.2488	.6555
		72hours	.62667*	.14119	.009	.1745	1.0788
	72hours	0hour	.00667	.14119	1.000	-.4455	.4588
		24hours	-.42333	.14119	.067	-.8755	.0288
		48hours	-.62667*	.14119	.009	-1.0788	-.1745
Viable_cell_count_Lactobacillus_BT5_30C	0hour	24hours	-.38333	.15233	.131	-.8712	.1045
		48hours	-.64000*	.15233	.013	-1.1278	-.1522
		72hours	-.01000	.15233	1.000	-.4978	.4778
	24hours	0hour	.38333	.15233	.131	-.1045	.8712
		48hours	-.25667	.15233	.390	-.7445	.2312
		72hours	.37333	.15233	.144	-.1145	.8612
	48hours	0hour	.64000*	.15233	.013	.1522	1.1278
		24hours	.25667	.15233	.390	-.2312	.7445
		72hours	.63000*	.15233	.014	.1422	1.1178
	72hours	0hour	.01000	.15233	1.000	-.4778	.4978
		24hours	-.37333	.15233	.144	-.8612	.1145
		48hours	-.63000*	.15233	.014	-1.1178	-.1422
Viable_cell_count_Lactobacillus_BT10_25C	0hour	24hours	-.64667*	.19115	.039	-1.2588	-.0345
		48hours	-.76000*	.19115	.017	-1.3721	-.1479
		72hours	-.13000	.19115	.902	-.7421	.4821
	24hours	0hour	.64667*	.19115	.039	.0345	1.2588
		48hours	-.11333	.19115	.931	-.7255	.4988
		72hours	.51667	.19115	.101	-.0955	1.1288
	48hours	0hour	.76000*	.19115	.017	.1479	1.3721
		24hours	.11333	.19115	.931	-.4988	.7255
		72hours	.63000*	.19115	.044	.0179	1.2421
	72hours	0hour	.13000	.19115	.902	-.4821	.7421
		24hours	-.51667	.19115	.101	-1.1288	.0955
		48hours	-.63000*	.19115	.044	-1.2421	-.0179
Viable_cell_count_Lactobacillus_BT10_30C	0hour	24hours	-.86667*	.18389	.007	-1.4556	-.2778
		48hours	-1.07333*	.18389	.002	-1.6622	-.4844
		72hours	-.38000	.18389	.242	-.9689	.2089
	24hours	0hour	.86667*	.18389	.007	.2778	1.4556
		48hours	-.20667	.18389	.686	-.7956	.3822
		72hours	.48667	.18389	.110	-.1022	1.0756
	48hours	0hour	1.07333*	.18389	.002	.4844	1.6622
		24hours	.20667	.18389	.686	-.3822	.7956
		72hours	.69333*	.18389	.023	.1044	1.2822
	72hours	0hour	.38000	.18389	.242	-.2089	.9689
		24hours	-.48667	.18389	.110	-1.0756	.1022
		48hours	-.69333*	.18389	.023	-1.2822	-.1044

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Viable_cell_count_Scorevisae_BT5_25C	0hour	5.4133	.36116	.20951	4.5182	6.3105	5.07	6.79
	24hours	6.1700	.22517	.13000	5.6107	6.7293	5.94	6.39
	48hours	6.3467	.18592	.10682	5.9970	6.8093	6.16	6.53
	72hours	6.2400	.04000	.02309	6.1400	6.3394	6.20	6.28
	Total	12	6.0425	.43326	.12507	5.7672	6.3178	5.07
Viable_cell_count_Scorevisae_BT5_30C	0hour	5.4533	.27538	.15899	4.7693	6.1374	5.17	5.72
	24hours	6.1433	.22592	.13589	5.5595	6.7271	5.91	6.38
	48hours	6.2567	.24992	.14146	5.6480	6.8653	6.01	6.50
	72hours	6.2133	.03215	.01858	6.1335	6.2932	6.19	6.25
	Total	12	6.0167	.39092	.11259	5.7689	6.2645	5.17
Viable_cell_count_Scorevisae_BT10_25C	0hour	5.4300	.35679	.20599	4.5437	6.3103	5.12	5.92
	24hours	6.2400	.17436	.10068	5.8059	6.6731	6.04	6.36
	48hours	6.1000	.60652	.35133	4.5883	7.6117	5.41	6.58
	72hours	6.4033	.07528	.00982	6.3054	6.4413	6.39	6.42
	Total	12	6.0433	.49538	.14301	5.7386	6.3591	5.12
Viable_cell_count_Scorevisae_BT10_30C	0hour	5.4267	.34025	.19633	4.5816	6.2714	5.09	5.77
	24hours	6.2933	.29245	.13421	5.7150	6.8706	6.03	6.47
	48hours	6.5000	.06928	.04000	6.3278	6.6721	6.46	6.58
	72hours	6.4467	.07528	.00982	6.4087	6.4846	6.43	6.46
	Total	12	6.1667	.46720	.14058	5.8972	6.4701	5.09

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Viable_cell_count_Scorevisae_BT5_25C	Between Groups	1.631	3	.544	10.022	.004
	Within Groups	.434	8	.054		
	Total	2.065	11			
Viable_cell_count_Scorevisae_BT5_30C	Between Groups	1.289	3	.430	8.945	.006
	Within Groups	.384	8	.048		
	Total	1.673	11			
Viable_cell_count_Scorevisae_BT10_25C	Between Groups	1.643	3	.548	4.147	.048
	Within Groups	1.056	8	.132		
	Total	2.699	11			
Viable_cell_count_Scorevisae_BT10_30C	Between Groups	2.259	3	.753	17.245	.001
	Within Groups	.349	8	.044		
	Total	2.609	11			

Viable_cell_count_Scorevisae_BT5_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.4133	
24hours	3		6.1700
72hours	3		6.2400
48hours	3		6.3467
Sig.		1.000	.791

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Scorevisae_BT5_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.4533	
24hours	3		6.1433
72hours	3		6.2133
48hours	3		6.2567
Sig.		1.000	.918

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Scorevisae_BT10_25C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.4300	
48hours	3	6.1000	6.1000
24hours	3	6.2400	6.2400
72hours	3		6.4033
Sig.		.097	.742

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Viable_cell_count_Scorevisae_BT10_30C

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.4267	
24hours	3		6.2933
72hours	3		6.4467
48hours	3		6.5000
Sig.		1.000	.637

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Viable_cell_count_Screev isae_BT5_25C	0hour	24hours	-.75667*	.19016	.017	-1.3656	-.1477
		48hours	-.93333*	.19016	.005	-1.5423	-.3244
		72hours	-.82667*	.19016	.011	-1.4356	-.2177
	24hours	0hour	.75667*	.19016	.017	.1477	1.3656
		48hours	-.17667	.19016	.791	-.7856	.4323
		72hours	-.07000	.19016	.982	-.6790	.5390
	48hours	0hour	.93333*	.19016	.005	.3244	1.5423
		24hours	.17667	.19016	.791	-.4323	.7856
		72hours	.10667	.19016	.941	-.5023	.7156
	72hours	0hour	.82667*	.19016	.011	.2177	1.4356
		24hours	.07000	.19016	.982	-.5390	.6790
		48hours	-.10667	.19016	.941	-.7156	.5023
Viable_cell_count_Screev isae_BT5_30C	0hour	24hours	-.69000*	.17895	.020	-1.2631	-.1169
		48hours	-.80333*	.17895	.009	-1.3764	-.2303
		72hours	-.76000*	.17895	.012	-1.3331	-.1869
	24hours	0hour	.69000*	.17895	.020	.1169	1.2631
		48hours	-.11333	.17895	.918	-.6864	.4597
		72hours	-.07000	.17895	.978	-.6431	.5031
	48hours	0hour	.80333*	.17895	.009	.2303	1.3764
		24hours	.11333	.17895	.918	-.4597	.6864
		72hours	.04333	.17895	.995	-.5297	.6164
	72hours	0hour	.76000*	.17895	.012	.1869	1.3331
		24hours	.07000	.17895	.978	-.5031	.6431
		48hours	-.04333	.17895	.995	-.6164	.5297
Viable_cell_count_Screev isae_BT10_25C	0hour	24hours	-.81000	.29671	.097	-1.7602	.1402
		48hours	-.67000	.29671	.187	-1.6202	.2802
		72hours	-.97333*	.29671	.045	-1.9235	-.0232
	24hours	0hour	.81000	.29671	.097	-.1402	1.7602
		48hours	.14000	.29671	.963	-.8102	1.0902
		72hours	-.16333	.29671	.944	-1.1135	.7868
	48hours	0hour	.67000	.29671	.187	-.2802	1.6202
		24hours	-.14000	.29671	.963	-1.0902	.8102
		72hours	-.30333	.29671	.742	-1.2535	.6468
	72hours	0hour	.97333*	.29671	.045	.0232	1.9235
		24hours	.16333	.29671	.944	-.7868	1.1135
		48hours	.30333	.29671	.742	-.6468	1.2535
Viable_cell_count_Screev isae_BT10_30C	0hour	24hours	-.86667*	.17064	.004	-1.4131	-.3202
		48hours	-1.07333*	.17064	.001	-1.6198	-.5269
		72hours	-1.02000*	.17064	.001	-1.5664	-.4736
	24hours	0hour	.86667*	.17064	.004	.3202	1.4131
		48hours	-.20667	.17064	.637	-.7531	.3398
		72hours	-.15333	.17064	.806	-.6998	.3931
	48hours	0hour	1.07333*	.17064	.001	.5269	1.6198
		24hours	.20667	.17064	.637	-.3398	.7531
		72hours	.05333	.17064	.989	-.4931	.5998
	72hours	0hour	1.02000*	.17064	.001	.4736	1.5664
		24hours	.15333	.17064	.806	-.3931	.6998
		48hours	-.05333	.17064	.989	-.5998	.4931

*. The mean difference is significant at the 0.05 level.

F.5.2 Statistical analysis of microbiological growth of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

Viable_cell_count_Lactococcus_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5_25C	3	5.6300	.11533	.06658	5.3435	5.9165	5.52	5.75
BT5_30C	3	5.8367	.05774	.03333	5.6932	5.9801	5.77	5.87
BT10_25C	3	5.8833	.07638	.04410	5.6936	6.0731	5.80	5.95
BT10_30C	3	5.8500	.09000	.05196	5.6264	6.0736	5.76	5.94
Total	12	5.8000	.12799	.03695	5.7187	5.8813	5.52	5.95

Test of Homogeneity of Variances

Viable_cell_count_Lactococcus_72H

Levene Statistic	df1	df2	Sig.
.332	3	8	.803

ANOVA

Viable_cell_count_Lactococcus_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.119	3	.040	5.194	.028
Within Groups	.061	8	.008		
Total	.180	11			

Viable_cell_count_Lactococcus_72H

Tukey HSD^a

Treatments	N	Subset for alpha = 0.05	
		1	2
BT5_25C	3	5.6300	
BT5_30C	3	5.8367	5.8367
BT10_30C	3	5.8500	5.8500
BT10_25C	3		5.8833
Sig.		.059	.911

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Descriptives

Viable_cell_count_Lactobacillus_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5_25C	3	5.8733	.06028	.03480	5.7236	6.0231	5.81	5.93
BT5_30C	3	5.6033	.02517	.01453	5.5408	5.6658	5.58	5.63
BT10_25C	3	5.9867	.11547	.06667	5.6998	6.2735	5.92	6.12
BT10_30C	3	5.7800	.04583	.02646	5.6662	5.8938	5.74	5.83
Total	12	5.8108	.15837	.04572	5.7102	5.9115	5.58	6.12

Test of Homogeneity of Variances

Viable_cell_count_Lactobacillus_72H

Levene Statistic	df1	df2	Sig.
3.705	3	8	.061

ANOVA

Viable_cell_count_Lactobacillus_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.236	3	.079	16.006	.001
Within Groups	.039	8	.005		
Total	.276	11			

Multiple Comparisons

Dependent Variable: Viable_cell_count_Lactobacillus_72H

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5_25C	BT5_30C	.27000*	.05730	.007	.0865	.4535
	BT10_25C	-.11333	.05730	.272	-.2968	.0702
	BT10_30C	.09333	.05730	.416	-.0902	.2768
BT5_30C	BT5_25C	-.27000*	.05730	.007	-.4535	-.0865
	BT10_25C	-.38333*	.05730	.001	-.5668	-.1998
	BT10_30C	-.17667	.05730	.059	-.3602	.0068
BT10_25C	BT5_25C	.11333	.05730	.272	-.0702	.2968
	BT5_30C	.38333*	.05730	.001	.1998	.5668
	BT10_30C	.20667*	.05730	.028	.0232	.3902
BT10_30C	BT5_25C	-.09333	.05730	.416	-.2768	.0902
	BT5_30C	.17667	.05730	.059	-.0068	.3602
	BT10_25C	-.20667*	.05730	.028	-.3902	-.0232

*. The mean difference is significant at the 0.05 level.

Viable_cell_count_Lactobacillus_72H

Tukey HSD^a

Treatments	N	Subset for alpha = 0.05		
		1	2	3
BT5_30C	3	5.6033		
BT10_30C	3	5.7800	5.7800	
BT5_25C	3		5.8733	5.8733
BT10_25C	3			5.9867
Sig.		.059	.416	.272

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Descriptives

Viable_cell_count_Scerevisae_72H

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
BT5_25C	3	6.2400	.04000	.02309	6.1406	6.3394	6.20	6.28
BT5_30C	3	6.2133	.03215	.01856	6.1335	6.2932	6.19	6.25
BT10_25C	3	6.4033	.01528	.00882	6.3654	6.4413	6.39	6.42
BT10_30C	3	6.4467	.01528	.00882	6.4087	6.4846	6.43	6.46
Total	12	6.3258	.10791	.03115	6.2573	6.3944	6.19	6.46

ANOVA

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
1.101	3	8	.403

Viable_cell_count_Scerevisae_72H

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.122	3	.041	52.427	.000
Within Groups	.006	8	.001		
Total	.128	11			

Multiple Comparisons

Dependent Variable: Viable_cell_count_Scerevisae_72H

Tukey HSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BT5_25C	BT5_30C	.02667	.02273	.659	-.0461	.0995
	BT10_25C	-.16333*	.02273	.000	-.2361	-.0905
	BT10_30C	-.20667*	.02273	.000	-.2795	-.1339
BT5_30C	BT5_25C	-.02667	.02273	.659	-.0995	.0461
	BT10_25C	-.19000*	.02273	.000	-.2628	-.1172
	BT10_30C	-.23333*	.02273	.000	-.3061	-.1605
BT10_25C	BT5_25C	.16333*	.02273	.000	.0905	.2361
	BT5_30C	.19000*	.02273	.000	.1172	.2628
	BT10_30C	-.04333	.02273	.298	-.1161	.0295
BT10_30C	BT5_25C	.20667*	.02273	.000	.1339	.2795
	BT5_30C	.23333*	.02273	.000	.1605	.3061
	BT10_25C	.04333	.02273	.298	-.0295	.1161

*. The mean difference is significant at the 0.05 level.

Viable_cell_count_Scerevisae_72H

Tukey HSD^a

Treatments	N	Subset for alpha = 0.05	
		1	2
BT5_30C	3	6.2133	
BT5_25C	3	6.2400	
BT10_25C	3		6.4033
BT10_30C	3		6.4467
Sig.		.659	.298

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

F.6.1 Statistical analysis of sensory evaluation of black tea water kefir beverage samples at 72 hours of fermentation

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Appearance	BT5_25C	60	6.6333	.93820	.12112	6.3910	6.8757	5.00	9.00
	BT5_30C	60	6.4667	.98233	.12682	6.2129	6.7204	5.00	9.00
	BT10_25C	60	6.6333	.86292	.11140	6.4104	6.8562	5.00	9.00
	BT10_30C	60	6.5167	.91117	.11763	6.2813	6.7520	5.00	9.00
	Total	240	6.5625	.92176	.05950	6.4453	6.6797	5.00	9.00
Odour	BT5_25C	60	6.2500	1.01889	.13154	5.9868	6.5132	5.00	8.00
	BT5_30C	60	6.1333	.94719	.12228	5.8886	6.3780	5.00	8.00
	BT10_25C	60	6.5000	1.00000	.12910	6.2417	6.7583	5.00	8.00
	BT10_30C	60	6.4833	.96536	.12463	6.2340	6.7327	5.00	8.00
	Total	240	6.3417	.98945	.06387	6.2158	6.4675	5.00	8.00
Flavour	BT5_25C	60	5.7833	1.20861	.15603	5.4711	6.0956	4.00	9.00
	BT5_30C	60	5.0833	1.19734	.15458	4.7740	5.3926	3.00	8.00
	BT10_25C	60	7.3167	.98276	.12687	7.0628	7.5705	6.00	9.00
	BT10_30C	60	6.7000	1.07829	.13921	6.4214	6.9786	5.00	9.00
	Total	240	6.2208	1.40426	.09064	6.0423	6.3994	3.00	9.00
Sourness	BT5_25C	60	5.6667	1.15958	.14970	5.3671	5.9662	4.00	9.00
	BT5_30C	60	5.3000	1.13943	.14710	5.0057	5.5943	4.00	9.00
	BT10_25C	60	6.8000	1.20451	.15550	6.4888	7.1112	5.00	9.00
	BT10_30C	60	6.6500	1.03866	.13409	6.3817	6.9183	5.00	9.00
	Total	240	6.1042	1.29756	.08376	5.9392	6.2692	4.00	9.00
Sweetness	BT5_25C	60	5.5833	1.30568	.16856	5.2460	5.9206	4.00	9.00
	BT5_30C	60	4.9167	1.13931	.14708	4.6224	5.2110	3.00	8.00
	BT10_25C	60	7.2167	1.12131	.14476	6.9270	7.5063	5.00	9.00
	BT10_30C	60	6.5667	1.16977	.15102	6.2645	6.8689	5.00	9.00
	Total	240	6.0708	1.47475	.09519	5.8833	6.2584	3.00	9.00
Overall	BT5_25C	60	5.9167	1.21141	.15839	5.6037	6.2296	4.00	9.00
	BT5_30C	60	5.2333	1.18417	.15288	4.9274	5.5392	3.00	8.00
	BT10_25C	60	7.1500	.95358	.12311	6.9037	7.3963	6.00	9.00
	BT10_30C	60	6.9000	.87721	.11325	6.6734	7.1266	5.00	9.00
	Total	240	6.3000	1.31040	.08459	6.1334	6.4666	3.00	9.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	1.279	3	.426	499	.684
	Within Groups	201.783	236	.855		
	Total	203.063	239			
Odour	Between Groups	5.817	3	1.939	2.005	.114
	Within Groups	226.167	236	.967		
	Total	233.983	239			
Flavour	Between Groups	174.946	3	58.315	46.448	.000
	Within Groups	296.350	236	1.256		
	Total	471.296	239			
Sourness	Between Groups	97.213	3	32.404	25.058	.000
	Within Groups	305.183	236	1.293		
	Total	402.396	239			
Sweetness	Between Groups	187.713	3	62.571	44.467	.000
	Within Groups	332.083	236	1.407		
	Total	519.796	239			
Overall	Between Groups	142.033	3	47.344	41.634	.000
	Within Groups	288.367	236	1.137		
	Total	430.400	239			

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Appearance	.356	3	236	.785
Odour	.832	3	236	.477
Flavour	.333	3	236	.802
Sourness	.736	3	236	.531
Sweetness	.613	3	236	.666
Overall	2.499	3	236	.060

Appearance

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	
BT5_30C	60	6.4667	
BT10_30C	60	6.5167	
BT5_25C	60	6.6333	
BT10_25C	60	6.6333	
Sig.		.757	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Odour

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	
BT5_30C	60	6.1333	
BT5_25C	60	6.2500	
BT10_30C	60	6.4833	
BT10_25C	60	6.5000	
Sig.		.175	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Flavour

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
BT5_30C	60	5.0833			
BT5_25C	60		5.7833		
BT10_30C	60			6.7000	
BT10_25C	60				7.3167
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Sweetness

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
BT5_30C	60	4.9167			
BT5_25C	60		5.5833		
BT10_30C	60			6.5667	
BT10_25C	60				7.2167
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Sourness

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
BT5_30C	60	5.3000	
BT5_25C	60	5.6667	
BT10_30C	60		6.6500
BT10_25C	60		6.8000
Sig.		.292	.888

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Overall

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05		
		1	2	3
BT5_30C	60	5.2333		
BT5_25C	60		5.9167	
BT10_30C	60			6.9000
BT10_25C	60			7.1500
Sig.		1.000	1.000	.574

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Appearance	BTS_25C	BTS_30C	16667	16882	.757	-2701	6035
		BT10_25C	00000	16882	1.000	-4368	4368
		BT10_20C	11667	16882	.900	-3201	5535
	BTS_30C	BTS_25C	-16667	16882	.757	-6035	2701
		BT10_25C	-16667	16882	.757	-6035	2701
		BT10_30C	-05000	16882	.991	-4668	3668
	BT10_25C	BTS_25C	00000	16882	1.000	-4368	4368
		BTS_30C	16667	16882	.757	-2701	8035
		BT10_30C	11667	16882	.900	-3201	5535
	BT10_30C	BTS_25C	-11667	16882	.900	-5535	3201
		BTS_30C	05000	16882	.991	-3668	4668
		BT10_25C	-11667	16882	.900	-5535	3201
Odor	BTS_25C	BTS_30C	11667	17952	.916	-3478	5812
		BT10_25C	-25000	17952	.595	-7145	2145
		BT10_20C	-23333	17952	.564	-6978	2312
	BTS_30C	BTS_25C	-11667	17952	.916	-5812	3478
		BT10_25C	-36667	17952	.175	-8712	8978
		BT10_30C	-35000	17952	.210	-8145	1145
	BT10_25C	BTS_25C	25000	17952	.595	-2145	7145
		BTS_30C	36667	17952	.175	-8978	8712
		BT10_30C	81667	17952	1.000	-4478	4812
	BT10_30C	BTS_25C	23333	17952	.564	-2312	6978
		BTS_30C	35000	17952	.210	-1145	8145
		BT10_25C	-01667	17952	1.000	-4812	4478
Favour	BTS_25C	BTS_30C	70000*	20459	.004	1708	12294
		BT10_25C	-1.53333*	20459	.000	-2.0627	-1.0040
		BT10_30C	-0.91667*	20459	.000	-1.4460	-.3873
	BTS_30C	BTS_25C	-70000*	20459	.004	-1.2294	-1.1708
		BT10_25C	-2.23333*	20459	.000	-2.7627	-1.7040
		BT10_30C	-1.61667*	20459	.000	-2.1460	-1.0873
	BT10_25C	BTS_25C	1.53333*	20459	.000	1.0040	2.0627
		BTS_30C	2.23333*	20459	.000	1.7040	2.7627
		BT10_30C	81667*	20459	.015	.0873	1.1460
	BT10_30C	BTS_25C	91667*	20459	.000	.3873	1.4460
		BTS_30C	1.61667*	20459	.000	1.0873	2.1460
		BT10_25C	-61667*	20459	.015	-1.1460	-.0873
Sourness	BTS_25C	BTS_30C	36667	20762	.292	-1705	9039
		BT10_25C	-1.13333*	20762	.000	-1.6705	-.5961
		BT10_30C	-.98333*	20762	.000	-1.5205	-.4461
	BTS_30C	BTS_25C	-36667	20762	.292	-.9039	1705
		BT10_25C	-1.50000*	20762	.000	-2.0372	-.9628
		BT10_30C	-1.35000*	20762	.000	-1.8872	-.8128
	BT10_25C	BTS_25C	1.13333*	20762	.000	.5961	1.6705
		BTS_30C	1.50000*	20762	.000	.8628	2.0372
		BT10_30C	15000	20762	.888	-.3872	.6872
	BT10_30C	BTS_25C	98333*	20762	.000	4461	1.5205
		BTS_30C	1.35000*	20762	.000	.8128	1.8872
		BT10_25C	-15000	20762	.888	-.6872	.3872
Sweetness	BTS_25C	BTS_30C	66667*	21657	.012	1663	12270
		BT10_25C	-1.63333*	21657	.000	-2.1937	-1.0730
		BT10_30C	-.98333*	21657	.000	-1.5437	-.4230
	BTS_30C	BTS_25C	-66667*	21657	.012	-1.2270	-.1663
		BT10_25C	-2.30000*	21657	.000	-2.8604	-1.7396
		BT10_30C	-1.85000*	21657	.000	-2.2104	-1.0896
	BT10_25C	BTS_25C	1.63333*	21657	.000	1.0730	2.1937
		BTS_30C	2.30000*	21657	.000	1.7396	2.8604
		BT10_30C	65000*	21657	.016	.0896	1.2104
	BT10_30C	BTS_25C	98333*	21657	.000	4230	1.5437
		BTS_30C	1.65000*	21657	.000	1.0896	2.2104
		BT10_25C	-65000*	21657	.016	-1.2104	-.0896
Overall	BTS_25C	BTS_30C	68333*	18469	.003	1796	1.1871
		BT10_25C	-1.23333*	18469	.000	-1.7371	-.7296
		BT10_30C	-.88333*	18469	.000	-1.4871	-.4796
	BTS_30C	BTS_25C	-.68333*	18469	.003	-1.1871	-.1796
		BT10_25C	-1.81667*	18469	.000	-2.4204	-1.4129
		BT10_30C	-1.66667*	18469	.000	-2.1704	-1.1529
	BT10_25C	BTS_25C	1.23333*	18469	.000	.7296	1.7371
		BTS_30C	1.81667*	18469	.000	1.4129	2.4204
		BT10_30C	25000	18469	.574	-.2538	.7538
	BT10_30C	BTS_25C	98333*	18469	.000	4796	1.4871
		BTS_30C	1.66667*	18469	.000	1.1529	2.1704
		BT10_25C	-.25000	18469	.574	-.7538	.2538

*. The mean difference is significant at the 0.05 level.

F.7.1 Statistical analysis of pH of black tea water kefir beverage with added carrot juice samples during 96 hours of fermentation

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
pH_C5	0hour	6	5.2750	.24825	.10135	5.0145	5.5355	5.05	5.59
	24hours	6	4.0383	.16534	.06750	3.8648	4.2118	3.84	4.22
	48hours	6	3.6383	.04750	.01939	3.5885	3.6882	3.59	3.70
	72hours	6	3.4500	.11419	.04662	3.3302	3.5698	3.31	3.56
	96hours	6	3.7167	.33164	.13539	3.3686	4.0647	3.28	3.96
	Total	30	4.0237	.69228	.12639	3.7652	4.2822	3.28	5.59
pH_C10	0hour	6	5.2733	.25445	.10388	5.0063	5.5404	5.03	5.60
	24hours	6	4.0300	.15297	.06245	3.8695	4.1905	3.85	4.20
	48hours	6	3.6433	.04590	.01874	3.5952	3.6915	3.60	3.70
	72hours	6	3.4650	.10858	.04433	3.3511	3.5789	3.33	3.57
	96hours	6	3.9050	.43766	.17868	3.4457	4.3643	3.34	4.19
	Total	30	4.0633	.68521	.12510	3.8075	4.3192	3.33	5.60
pH_C15	0hour	6	5.2700	.25187	.10283	5.0057	5.5343	5.03	5.58
	24hours	6	4.0167	.15655	.06391	3.8524	4.1810	3.84	4.19
	48hours	6	3.6233	.05465	.02231	3.5660	3.6807	3.57	3.69
	72hours	6	3.4567	.11039	.04507	3.3408	3.5725	3.32	3.56
	96hours	6	3.9400	.44605	.18210	3.4719	4.4081	3.41	4.40
	Total	30	4.0613	.68784	.12558	3.8045	4.3182	3.32	5.58

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH_C5	Between Groups	12.827	4	3.207	74.838	.000
	Within Groups	1.071	25	.043		
	Total	13.898	29			
pH_C10	Between Groups	12.148	4	3.037	51.722	.000
	Within Groups	1.468	25	.059		
	Total	13.616	29			
pH_C15	Between Groups	12.210	4	3.053	50.526	.000
	Within Groups	1.510	25	.060		
	Total	13.721	29			

pH_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	3.4500		
48hours	6	3.6383		
96hours	6	3.7167	3.7167	
24hours	6		4.0383	
0hour	6			5.2750
Sig.		.261	.094	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	3.4650		
48hours	6	3.6433	3.6433	
96hours	6		3.9050	
24hours	6		4.0300	
0hour	6			5.2733
Sig.		.709	.072	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

pH_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	3.4567		
48hours	6	3.6233	3.6233	
96hours	6		3.9400	
24hours	6		4.0167	
0hour	6			5.2700
Sig.		.765	.071	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I Hour)	(J Hour)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
pH_C5	0hour	24hours	1.23667 [*]	.11951	.000	.8857	1.5877	
		48hours	1.83667 [*]	.11951	.000	1.2857	1.9877	
		72hours	1.82500 [*]	.11951	.000	1.4740	2.1760	
		96hours	1.55833 [*]	.11951	.000	1.2073	1.9093	
	24hours	0hour	-1.23667 [*]	.11951	.000	-1.5877	-.8857	
		48hours	.40000 [†]	.11951	.020	.0490	.7510	
		72hours	.58833 [†]	.11951	.000	.2373	.9393	
		96hours	.32167	.11951	.084	-.0293	.6727	
	48hours	0hour	-1.83667 [*]	.11951	.000	-1.9877	-1.2857	
		24hours	-.40000 [†]	.11951	.020	-.7510	-.0490	
		72hours	-.18833	.11951	.526	-.1627	.5393	
		96hours	-.07833	.11951	.964	-.4293	.2727	
	72hours	0hour	-1.82500 [*]	.11951	.000	-2.1760	-1.4740	
		24hours	-.58833 [†]	.11951	.000	-.9393	-.2373	
		48hours	-.18833	.11951	.526	-.5393	.1627	
		96hours	-.26667	.11951	.201	-.6177	.0843	
	96hours	0hour	-1.55833 [*]	.11951	.000	-1.9093	-1.2073	
		24hours	-.32167	.11951	.084	-.6727	.0293	
		48hours	.07833	.11951	.964	-.2727	.4293	
		72hours	.26667	.11951	.201	-.0843	.6177	
	pH_C10	0hour	24hours	1.24333 [*]	.13990	.000	.8325	1.6542
			48hours	1.63000 [†]	.13990	.000	1.2191	2.0409
			72hours	1.80833 [†]	.13990	.000	1.3975	2.2192
			96hours	1.36833 [†]	.13990	.000	.9575	1.7792
24hours		0hour	-1.24333 [*]	.13990	.000	-1.6542	-.8325	
		48hours	-.38667	.13990	.072	-.0242	.7975	
		72hours	.56500 [†]	.13990	.004	.1541	.9759	
		96hours	1.2500	.13990	.897	-.2859	.5359	
48hours		0hour	-1.63000 [†]	.13990	.000	-2.0409	-1.2191	
		24hours	-.38667	.13990	.072	-.7975	.0242	
		72hours	.17833	.13990	.709	-.2325	.5892	
		96hours	-.26167	.13990	.358	-.6725	.1492	
72hours		0hour	-1.80833 [†]	.13990	.000	-2.2192	-1.3975	
		24hours	-.56500 [†]	.13990	.004	-.9759	-.1541	
		48hours	-.17833	.13990	.709	-.5892	.2325	
		96hours	-.44000 [†]	.13990	.032	-.8509	-.0291	
96hours		0hour	-1.36833 [†]	.13990	.000	-1.7792	-.9575	
		24hours	-.12500	.13990	.897	-.5359	.2859	
		48hours	.26167	.13990	.358	-.1492	.6725	
		72hours	.44000 [†]	.13990	.032	.0291	.8509	
pH_C15		0hour	24hours	1.25333 [*]	.14191	.000	.8366	1.6701
			48hours	1.84667 [*]	.14191	.000	1.2299	2.0634
			72hours	1.81333 [*]	.14191	.000	1.3966	2.2301
			96hours	1.33000 [†]	.14191	.000	.9132	1.7468
	24hours	0hour	-1.25333 [*]	.14191	.000	-1.6701	-.8366	
		48hours	-.39333	.14191	.071	-.0234	.6101	
		72hours	-.56000 [†]	.14191	.005	-.1432	.9768	
		96hours	.07667	.14191	.982	-.3401	.4934	
	48hours	0hour	-1.84667 [*]	.14191	.000	-2.0634	-1.2299	
		24hours	-.39333	.14191	.071	-.8101	.0234	
		72hours	-.16667	.14191	.765	-.2501	.5834	
		96hours	-.31667	.14191	.201	-.7334	1.001	
	72hours	0hour	-1.81333 [*]	.14191	.000	-2.2301	-1.3966	
		24hours	-.56000 [†]	.14191	.005	-.9768	-.1432	
		48hours	-.16667	.14191	.765	-.5834	.2501	
		96hours	-.48333 [†]	.14191	.017	-.9001	-.0666	
	96hours	0hour	-1.33000 [†]	.14191	.000	-1.7468	-.9132	
		24hours	-.07667	.14191	.982	-.4934	.3401	
		48hours	-.31667	.14191	.201	-.1001	.7334	
		72hours	.48333 [†]	.14191	.017	.0666	.9001	

* The mean difference is significant at the 0.05 level.

F.7.2 Statistical analysis of pH of black tea water kefir beverage with added carrot juice samples at 96 hours of fermentation

Descriptives

pH_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Carrot5%	6	3.7167	.33164	.13539	3.3686	4.0647	3.28	3.96
Carrot10%	6	3.9050	.43766	.17868	3.4457	4.3643	3.34	4.19
Carrot15%	6	3.9400	.44605	.18210	3.4719	4.4081	3.41	4.40
Total	18	3.8539	.39672	.09351	3.6566	4.0512	3.28	4.40

ANOVA

Test of Homogeneity of Variances				pH_96hours				
pH_96hours				Sum of Squares	df	Mean Square	F	Sig.
Levene Statistic	df1	df2	Sig.	Between Groups	.173	2	.087	.519
.497	2	15	.618	Within Groups	2.502	15	.167	
				Total	2.676	17		

Multiple Comparisons

Dependent Variable: pH_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Carrot5%	Carrot10%	-.18833	.23582	.710	-.8009	.4242
	Carrot15%	-.22333	.23582	.620	-.8359	.3892
Carrot10%	Carrot5%	.18833	.23582	.710	-.4242	.8009
	Carrot15%	-.03500	.23582	.988	-.6475	.5775
Carrot15%	Carrot5%	.22333	.23582	.620	-.3892	.8359
	Carrot10%	.03500	.23582	.988	-.5775	.6475

pH_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
Carrot5%	6	3.7167
Carrot10%	6	3.9050
Carrot15%	6	3.9400
Sig.		.620

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

F.8.1 Statistical analysis of lactic acid concentration of black tea water kefir beverage with added carrot juice samples during 96 hours of fermentation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
La_C5	0hour	6	.0000	.00000	.00000	.0000	.0000	.00	
	24hours	6	.2010	.01676	.00684	.1834	.2186	.18	.22
	48hours	6	.2640	.01770	.00722	.2454	.2826	.24	.29
	72hours	6	.2835	.01479	.00604	.2680	.2990	.26	.30
	96hours	6	.3330	.02670	.01090	.3050	.3610	.30	.36
	Total	30	.2163	.11922	.02177	.1718	.2608	.00	.36
La_C10	0hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24hours	6	.2070	.01136	.00465	.1951	.2189	.19	.22
	48hours	6	.2685	.01052	.00430	.2575	.2795	.25	.28
	72hours	6	.2865	.01052	.00430	.2755	.2975	.27	.30
	96hours	6	.4035	.01196	.00488	.3909	.4161	.39	.42
	Total	30	.2331	.13537	.02471	.1826	.2836	.00	.42
La_C15	0hour	6	.0000	.00000	.00000	.0000	.0000	.00	.00
	24hours	6	.1920	.01230	.00502	.1791	.2049	.18	.21
	48hours	6	.2655	.01102	.00450	.2539	.2771	.25	.28
	72hours	6	.2865	.00677	.00277	.2794	.2936	.28	.30
	96hours	6	.4170	.01355	.00553	.4028	.4312	.40	.43
	Total	30	.2322	.13958	.02548	.1801	.2843	.00	.43

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
La_C5	Between Groups	.405	4	.101	331.513	.000
	Within Groups	.008	25	.000		
	Total	.412	29			
La_C10	Between Groups	.529	4	.132	1338.156	.000
	Within Groups	.002	25	.000		
	Total	.531	29			
La_C15	Between Groups	.562	4	.141	1399.960	.000
	Within Groups	.003	25	.000		
	Total	.565	29			

La_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05			
		1	2	3	4
0hour	6	.0000			
24hours	6		.2010		
48hours	6			.2640	
72hours	6			.2835	
96hours	6				.3330
Sig.		1.000	1.000	.327	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000

La_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05				
		1	2	3	4	5
0hour	6	.0000				
24hours	6		.2070			
48hours	6			.2685		
72hours	6				.2865	
96hours	6					.4035
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000

La_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05				
		1	2	3	4	5
0hour	6	.0000				
24hours	6		.1920			
48hours	6			.2655		
72hours	6				.2865	
96hours	6					.4170
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
La_C5	0hour	24hours	-.20100*	.01008	.000	-.2306	-.1714	
		48hours	-.26400*	.01008	.000	-.2936	-.2344	
		72hours	-.28350*	.01008	.000	-.3131	-.2539	
		96hours	-.33300*	.01008	.000	-.3626	-.3034	
	24hours	0hour	.20100*	.01008	.000	.1714	.2306	
		48hours	-.06300*	.01008	.000	-.0926	-.0334	
		72hours	-.08250*	.01008	.000	-.1121	-.0529	
		96hours	-.13200*	.01008	.000	-.1616	-.1024	
	48hours	0hour	.26400*	.01008	.000	.2344	.2936	
		24hours	.06300*	.01008	.000	.0334	.0926	
		72hours	-.01950	.01008	.327	-.0491	.0101	
		96hours	-.06900*	.01008	.000	-.0986	-.0394	
	72hours	0hour	.28350*	.01008	.000	.2539	.3131	
		24hours	.08250*	.01008	.000	.0529	.1121	
		48hours	.01950	.01008	.327	-.0101	.0491	
		96hours	-.04950*	.01008	.000	-.0791	-.0199	
	96hours	0hour	.33300*	.01008	.000	.3034	.3626	
		24hours	.13200*	.01008	.000	.1024	.1616	
		48hours	.06900*	.01008	.000	.0394	.0986	
		72hours	.04950*	.01008	.000	.0199	.0791	
	La_C10	0hour	24hours	-.20700*	.00574	.000	-.2239	-.1901
			48hours	-.26850*	.00574	.000	-.2854	-.2516
			72hours	-.28650*	.00574	.000	-.3034	-.2696
			96hours	-.40350*	.00574	.000	-.4204	-.3866
		24hours	0hour	.20700*	.00574	.000	.1901	.2239
			48hours	-.06150*	.00574	.000	-.0784	-.0446
			72hours	-.07950*	.00574	.000	-.0964	-.0626
			96hours	-.19650*	.00574	.000	-.2134	-.1796
		48hours	0hour	.26850*	.00574	.000	.2516	.2854
			24hours	.06150*	.00574	.000	.0446	.0784
			72hours	-.01800*	.00574	.032	-.0349	-.0011
			96hours	-.13500*	.00574	.000	-.1519	-.1181
		72hours	0hour	.28650*	.00574	.000	.2696	.3034
			24hours	.07950*	.00574	.000	.0626	.0964
			48hours	.01800*	.00574	.032	.0011	.0349
			96hours	-.11700*	.00574	.000	-.1339	-.1001
96hours		0hour	.40350*	.00574	.000	.3866	.4204	
		24hours	.19650*	.00574	.000	.1796	.2134	
		48hours	.13500*	.00574	.000	.1181	.1519	
		72hours	.11700*	.00574	.000	.1001	.1339	
La_C15		0hour	24hours	-.19200*	.00579	.000	-.2090	-.1750
			48hours	-.26550*	.00579	.000	-.2825	-.2485
			72hours	-.28650*	.00579	.000	-.3035	-.2695
			96hours	-.41700*	.00579	.000	-.4340	-.4000
		24hours	0hour	.19200*	.00579	.000	.1750	.2090
			48hours	-.07350*	.00579	.000	-.0905	-.0565
			72hours	-.09450*	.00579	.000	-.1115	-.0775
			96hours	-.22500*	.00579	.000	-.2420	-.2080
		48hours	0hour	.26550*	.00579	.000	.2485	.2825
			24hours	.07350*	.00579	.000	.0565	.0905
			72hours	-.02100*	.00579	.010	-.0380	-.0040
			96hours	-.15150*	.00579	.000	-.1685	-.1345
		72hours	0hour	.28650*	.00579	.000	.2695	.3035
			24hours	.09450*	.00579	.000	.0775	.1115
			48hours	.02100*	.00579	.010	.0040	.0380
			96hours	-.13050*	.00579	.000	-.1475	-.1135
	96hours	0hour	.41700*	.00579	.000	.4000	.4340	
		24hours	.22500*	.00579	.000	.2080	.2420	
		48hours	.15150*	.00579	.000	.1345	.1685	
		72hours	.13050*	.00579	.000	.1135	.1475	

*. The mean difference is significant at the 0.05 level.

F.8.2 Statistical analysis of lactic acid concentration of black tea water kefir beverage with added carrot juice samples at 96 hours of fermentation

Descriptives

La_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Carrot5%	6	.3330	.02670	.01090	.3050	.3610	.30	.36
Carrot10%	6	.4035	.01196	.00488	.3909	.4161	.39	.42
Carrot15%	6	.4170	.01355	.00553	.4028	.4312	.40	.43
Total	18	.3845	.04174	.00984	.3637	.4053	.30	.43

ANOVA

Test of Homogeneity of Variances

La_96hours				La_96hours					
Levene Statistic	df1	df2	Sig.	Sum of Squares	df	Mean Square	F	Sig.	
2.893	2	15	.087	Between Groups	.024	2	.012	35.234	.000
				Within Groups	.005	15	.000		
				Total	.030	17			

Multiple Comparisons

Dependent Variable: La_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Carrot5%	Carrot10%	-.07050*	.01075	.000	-.0984	-.0426
	Carrot15%	-.08400*	.01075	.000	-.1119	-.0561
Carrot10%	Carrot5%	.07050*	.01075	.000	.0426	.0984
	Carrot15%	-.01350	.01075	.440	-.0414	.0144
Carrot15%	Carrot5%	.08400*	.01075	.000	.0561	.1119
	Carrot10%	.01350	.01075	.440	-.0144	.0414

*. The mean difference is significant at the 0.05 level.

La_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
Carrot5%	6	.3330	
Carrot10%	6		.4035
Carrot15%	6		.4170
Sig.		1.000	.440

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

F.9.1 Statistical analysis of total soluble solids of black tea water kefir beverage with added carrot juice samples during 96 hours of fermentation

		Descriptives							
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Brix_C5	0hour	6	9.0333	.08165	.03333	8.8476	10.0190	9.80	10.00
	24hours	6	9.2000	.06325	.02582	8.1336	9.2664	9.10	9.30
	48hours	6	8.3333	1.3661	.55570	6.1900	8.4767	8.20	8.50
	72hours	6	7.5667	.08165	.03333	7.4810	7.6534	7.50	7.70
	96hours	6	7.3333	.10328	.04216	7.2249	7.4417	7.20	7.50
	Total	30	8.4733	1.00067	.18270	8.0987	8.8470	7.20	10.00
Brix_C10	0hour	6	9.9500	.05477	.02236	9.8825	10.0075	9.90	10.00
	24hours	6	9.2167	.07528	.03073	9.1377	9.2957	9.10	9.30
	48hours	6	8.4167	1.4720	.56009	6.3622	8.5711	8.30	8.60
	72hours	6	7.6667	1.8619	.77601	7.4753	7.8621	7.50	7.90
	96hours	6	6.9000	1.4142	.55274	6.7510	7.0484	6.70	7.10
	Total	30	8.4300	1.10708	.20212	8.0160	8.8434	6.70	10.00
Brix_C15	0hour	6	9.9167	.07528	.03073	9.8377	9.9957	9.80	10.00
	24hours	6	9.2333	.05164	.02108	9.1791	9.2875	9.20	9.30
	48hours	6	8.3667	.08165	.03333	8.2810	8.4534	8.30	8.50
	72hours	6	7.7000	2.3664	.96681	7.4517	7.9483	7.50	8.00
	96hours	6	6.7500	2.3452	.95574	6.5039	6.9961	6.00	7.10
	Total	30	8.3933	1.14287	.20866	7.9666	8.8205	6.00	10.00

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Brix_C5	Between Groups	28.805	4	7.201	771.571	.000
	Within Groups	.233	25	.009		
	Total	29.039	29			
Brix_C10	Between Groups	35.118	4	8.780	516.441	.000
	Within Groups	.425	25	.017		
	Total	35.543	29			
Brix_C15	Between Groups	37.249	4	9.312	369.530	.000
	Within Groups	.630	25	.025		
	Total	37.879	29			

Brix_C5						
Tukey HSD ^a						
Subset for alpha = 0.05						
Hour	N	1	2	3	4	5
96hours	6	7.3333				
72hours	6		7.5667			
48hours	6			8.3333		
24hours	6				9.2000	
0hour	6					9.0333
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Brix_C10						
Tukey HSD ^a						
Subset for alpha = 0.05						
Hour	N	1	2	3	4	5
96hours	6	6.9000				
72hours	6		7.6667			
48hours	6			8.4167		
24hours	6				9.2167	
0hour	6					9.9500
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Brix_C15						
Tukey HSD ^a						
Subset for alpha = 0.05						
Hour	N	1	2	3	4	5
96hours	6	6.7500				
72hours	6		7.7000			
48hours	6			8.3667		
24hours	6				9.2333	
0hour	6					9.9167
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Brix_C5	0hour	24hours	.73333*	.05578	.000	.5895	.8971	
		48hours	1.60000*	.05578	.000	1.4362	1.7638	
		72hours	2.36667*	.05578	.000	2.2029	2.5305	
		96hours	2.60000*	.05578	.000	2.4362	2.7638	
	24hours	0hour	-.73333*	.05578	.000	-.8971	-.5695	
		48hours	.86667*	.05578	.000	.7029	1.0305	
		72hours	1.63333*	.05578	.000	1.4695	1.7971	
		96hours	1.86667*	.05578	.000	1.7029	2.0305	
	48hours	0hour	-1.60000*	.05578	.000	-1.7638	-1.4362	
		24hours	-.86667*	.05578	.000	-1.0305	-.7029	
		72hours	.76667*	.05578	.000	.6029	.9305	
		96hours	1.00000*	.05578	.000	.8362	1.1638	
	72hours	0hour	-2.36667*	.05578	.000	-2.5305	-2.2029	
		24hours	-1.63333*	.05578	.000	-1.7971	-1.4695	
		48hours	-.76667*	.05578	.000	-.9305	-.6029	
		96hours	.23333*	.05578	.003	.0695	.3971	
	96hours	0hour	-2.60000*	.05578	.000	-2.7638	-2.4362	
		24hours	-1.86667*	.05578	.000	-2.0305	-1.7029	
		48hours	-1.00000*	.05578	.000	-1.1638	-.8362	
		72hours	-.23333*	.05578	.003	-.3971	-.0695	
	Brix_C10	0hour	24hours	.73333*	.07528	.000	.5123	.9544
			48hours	1.53333*	.07528	.000	1.3123	1.7544
			72hours	2.28333*	.07528	.000	2.0623	2.5044
			96hours	3.05000*	.07528	.000	2.8289	3.2711
24hours		0hour	-.73333*	.07528	.000	-.9544	-.5123	
		48hours	.80000*	.07528	.000	.5789	1.0211	
		72hours	1.55000*	.07528	.000	1.3289	1.7711	
		96hours	2.31667*	.07528	.000	2.0956	2.5377	
48hours		0hour	-1.53333*	.07528	.000	-1.7544	-1.3123	
		24hours	-.80000*	.07528	.000	-1.0211	-.5789	
		72hours	.75000*	.07528	.000	.5289	.9711	
		96hours	1.51667*	.07528	.000	1.2956	1.7377	
72hours		0hour	-2.28333*	.07528	.000	-2.5044	-2.0623	
		24hours	-1.55000*	.07528	.000	-1.7711	-1.3289	
		48hours	-.75000*	.07528	.000	-.9711	-.5289	
		96hours	.76667*	.07528	.000	.5456	.9877	
96hours		0hour	-3.05000*	.07528	.000	-3.2711	-2.8289	
		24hours	-2.31667*	.07528	.000	-2.5377	-2.0956	
		48hours	-1.51667*	.07528	.000	-1.7377	-1.2956	
		72hours	-.76667*	.07528	.000	-.9877	-.5456	
Brix_C15		0hour	24hours	.68333*	.09165	.000	.4142	.9525
			48hours	1.55000*	.09165	.000	1.2808	1.8192
			72hours	2.21667*	.09165	.000	1.9475	2.4858
			96hours	3.16667*	.09165	.000	2.8975	3.4358
	24hours	0hour	-.68333*	.09165	.000	-.9525	-.4142	
		48hours	.86667*	.09165	.000	.5975	1.1358	
		72hours	1.53333*	.09165	.000	1.2642	1.8025	
		96hours	2.48333*	.09165	.000	2.2142	2.7525	
	48hours	0hour	-1.55000*	.09165	.000	-1.8192	-1.2808	
		24hours	-.86667*	.09165	.000	-1.1358	-.5975	
		72hours	.66667*	.09165	.000	.3975	.9358	
		96hours	1.61667*	.09165	.000	1.3475	1.8858	
	72hours	0hour	-2.21667*	.09165	.000	-2.4858	-1.9475	
		24hours	-1.53333*	.09165	.000	-1.8025	-1.2642	
		48hours	-.66667*	.09165	.000	-.9358	-.3975	
		96hours	.95000*	.09165	.000	.6808	1.2192	
	96hours	0hour	-3.16667*	.09165	.000	-3.4358	-2.8975	
		24hours	-2.48333*	.09165	.000	-2.7525	-2.2142	
		48hours	-1.61667*	.09165	.000	-1.8858	-1.3475	
		72hours	-.95000*	.09165	.000	-1.2192	-.6808	

*. The mean difference is significant at the 0.05 level.

F.9.2 Statistical analysis of total soluble solids of black tea water kefir beverage with added carrot juice samples at 96 hours of fermentation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Carrot5%	6	7.3333	.10328	.04216	7.2249	7.4417	7.20	7.50
Carrot10%	6	6.9000	.14142	.05774	6.7516	7.0484	6.70	7.10
Carrot15%	6	6.7500	.23452	.09574	6.5039	6.9961	6.60	7.10
Total	18	6.9944	.29995	.07070	6.8453	7.1436	6.60	7.50

ANOVA

Test of Homogeneity of Variances				Brix_96hours					
Levene Statistic	df1	df2	Sig.	Sum of Squares	df	Mean Square	F	Sig.	
4.145	2	15	.037	Between Groups	1.101	2	.551	19.280	.000
				Within Groups	.428	15	.029		
				Total	1.529	17			

Multiple Comparisons

Dependent Variable: Brix_96hours
Tukey HSD

(i) Treatment	(j) Treatment	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Carrot5%	Carrot0%	.43333*	.09756	.001	.1799	.6868
	Carrot5%	-.58333*	.09756	.000	-.3299	-.8368
Carrot10%	Carrot5%	-.43333*	.09756	.001	-.6868	-.1799
	Carrot5%	.15000	.09756	.302	-.1034	.4034
Carrot15%	Carrot5%	-.58333*	.09756	.000	-.8368	-.3299
	Carrot10%	-.15000	.09756	.302	-.4034	.1034

*. The mean difference is significant at the 0.05 level.

Brix_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
Carrot15%	6	6.7500	
Carrot10%	6	6.9000	
Carrot5%	6		7.3333
Sig.		.302	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Kruskal-Wallis Test

	Ranks		
	Treatment	N	Mean Rank
Brix_96hours	Carrot5%	6	15.50
	Carrot10%	6	7.83
	Carrot15%	6	5.17
	Total	18	

Test Statistics^{a,b}

Brix_96hours	
Chi-Square	12.333
df	2
Asymp. Sig.	.002

a. Kruskal Wallis Test

b. Grouping Variable:
Treatment

F.10.1 Statistical analysis of colour (L*, a*, and b* value) of black tea water kefir beverage with added carrot juice samples during 96 hours of fermentation

Descriptives									
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Colour_L_C5	0hour	6	80.4383	33660	13741	80.0951	80.7916	80.03	80.77
	24hours	6	85.7167	33637	13732	85.3637	86.0697	85.34	86.09
	48hours	6	87.4433	58936	24061	86.8248	88.0619	86.46	88.05
	72hours	6	88.4500	39314	16050	88.0374	88.8626	87.88	88.90
	96hours	6	31.1233	72240	29492	30.3652	31.8814	30.61	32.07
	Total	30	74.8343	2330987	407321	88.3037	82.9650	30.61	88.90
Colour_L_C10	0hour	6	80.9983	85040	34717	80.1059	81.8908	80.01	81.82
	24hours	6	85.7950	60695	24779	85.1580	86.4320	85.16	86.41
	48hours	6	87.6017	80963	33029	86.7526	88.4507	86.61	88.96
	72hours	6	88.8767	50914	20786	88.3424	89.4110	87.95	89.35
	96hours	6	18.3917	86161	35175	17.4875	19.2959	17.35	19.30
	Total	30	72.3327	2757471	503443	82.0361	82.6292	17.35	89.35
Colour_L_C15	0hour	6	80.6300	32131	13117	80.2928	80.9672	80.09	81.01
	24hours	6	86.1067	53917	22012	85.5408	86.6725	85.42	86.68
	48hours	6	87.3733	24064	98824	87.1208	87.6259	86.98	87.68
	72hours	6	88.3517	50436	20590	87.8224	88.8810	87.36	88.72
	96hours	6	12.5867	79674	32527	11.7505	13.4228	12.05	13.62
	Total	30	71.0097	2983870	544778	59.8617	82.1516	12.05	88.72

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Colour_L_C5	Between Groups	14427.929	4	3606.982	14425.428	.000
	Within Groups	6.251	25	.250		
	Total	14434.180	29			
Colour_L_C10	Between Groups	22036.836	4	5509.209	10025.104	.000
	Within Groups	13.739	25	.550		
	Total	22050.574	29			
Colour_L_C15	Between Groups	25813.390	4	6453.348	24061.400	.000
	Within Groups	6.705	25	.268		
	Total	25820.095	29			

Colour_L_C5

Tukey HSD^a

Subset for alpha = 0.05

Hour	N	1	2	3	4	5
96hours	6	31.1233				
0hour	6		80.4383			
24hours	6			85.7167		
48hours	6				87.4433	
72hours	6					88.4500
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Colour_L_C10

Tukey HSD^a

Subset for alpha = 0.05

Hour	N	1	2	3	4	5
96hours	6	18.3917				
0hour	6		80.9983			
24hours	6			85.7950		
48hours	6				87.6017	
72hours	6					88.8767
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Colour_L_C15

Tukey HSD^a

Subset for alpha = 0.05

Hour	N	1	2	3	4	5
96hours	6	12.5867				
0hour	6		80.6300			
24hours	6			86.1067		
48hours	6				87.3733	
72hours	6					88.3517
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Colour_L_C5	0hour	24hours	-5.27833*	.28870	.000	-6.1262	-4.4305	
		48hours	-7.00500*	.28870	.000	-7.8529	-6.1571	
		72hours	-8.01167*	.28870	.000	-8.8595	-7.1638	
		96hours	49.31500*	.28870	.000	48.4671	50.1629	
		96hours	5.27833*	.28870	.000	4.4305	6.1262	
	24hours	0hour	5.27833*	.28870	.000	4.4305	6.1262	
		48hours	-1.72667*	.28870	.000	-2.5745	-.8788	
		72hours	-2.73333*	.28870	.000	-3.5812	-1.8855	
		96hours	54.59333*	.28870	.000	53.7455	55.4412	
	48hours	0hour	7.00500*	.28870	.000	6.1571	7.8529	
		24hours	1.72667*	.28870	.000	.8788	2.5745	
		72hours	-1.00667*	.28870	.014	-1.8545	-.1588	
		96hours	56.32000*	.28870	.000	55.4721	57.1679	
	72hours	0hour	8.01167*	.28870	.000	7.1638	8.8595	
		24hours	2.73333*	.28870	.000	1.8855	3.5812	
		48hours	1.00667*	.28870	.014	.1588	1.8545	
		96hours	57.32667*	.28870	.000	56.4788	58.1745	
	96hours	0hour	-49.31500*	.28870	.000	-50.1629	-48.4671	
		24hours	-54.59333*	.28870	.000	-55.4412	-53.7455	
		48hours	-56.32000*	.28870	.000	-57.1679	-55.4721	
		72hours	-57.32667*	.28870	.000	-58.1745	-56.4788	
	Colour_L_C10	0hour	24hours	-4.79667*	.42800	.000	-6.0536	-3.5397
			48hours	-6.60333*	.42800	.000	-7.8603	-5.3464
			72hours	-7.87833*	.42800	.000	-9.1353	-6.6214
96hours			62.60667*	.42800	.000	61.3497	63.8636	
96hours			4.79667*	.42800	.000	3.5397	6.0536	
24hours		0hour	4.79667*	.42800	.000	3.5397	6.0536	
		48hours	-1.80667*	.42800	.002	-3.0636	-.5497	
		72hours	-3.08167*	.42800	.000	-4.3386	-1.8247	
		96hours	67.40333*	.42800	.000	66.1464	68.6603	
48hours		0hour	6.60333*	.42800	.000	5.3464	7.8603	
		24hours	1.80667*	.42800	.002	.5497	3.0636	
		72hours	-1.27500*	.42800	.046	-2.5320	-.0180	
		96hours	69.21000*	.42800	.000	67.9530	70.4670	
72hours		0hour	7.87833*	.42800	.000	6.6214	9.1353	
		24hours	3.08167*	.42800	.000	1.8247	4.3386	
		48hours	1.27500*	.42800	.046	.0180	2.5320	
		96hours	70.48500*	.42800	.000	69.2280	71.7420	
96hours		0hour	-62.60667*	.42800	.000	-63.8636	-61.3497	
		24hours	-67.40333*	.42800	.000	-68.6603	-66.1464	
		48hours	-69.21000*	.42800	.000	-70.4670	-67.9530	
		72hours	-70.48500*	.42800	.000	-71.7420	-69.2280	
Colour_L_C15		0hour	24hours	-5.47667*	.29900	.000	-6.3548	-4.5985
			48hours	-6.74333*	.29900	.000	-7.6215	-5.8652
			72hours	-7.72167*	.29900	.000	-8.5998	-6.8435
	96hours		68.04333*	.29900	.000	67.1652	68.9215	
	96hours		5.47667*	.29900	.000	4.5985	6.3548	
	24hours	0hour	5.47667*	.29900	.000	4.5985	6.3548	
		48hours	-1.26667*	.29900	.002	-2.1448	-.3885	
		72hours	-2.24500*	.29900	.000	-3.1231	-1.3669	
		96hours	73.52000*	.29900	.000	72.6419	74.3981	
	48hours	0hour	6.74333*	.29900	.000	5.8652	7.6215	
		24hours	1.26667*	.29900	.002	.3885	2.1448	
		72hours	-.97833*	.29900	.024	-1.8565	-.1002	
		96hours	74.78667*	.29900	.000	73.9085	75.6648	
	72hours	0hour	7.72167*	.29900	.000	6.8435	8.5998	
		24hours	2.24500*	.29900	.000	1.3669	3.1231	
		48hours	.97833*	.29900	.024	.1002	1.8565	
		96hours	75.76500*	.29900	.000	74.8869	76.6431	
	96hours	0hour	-68.04333*	.29900	.000	-68.9215	-67.1652	
		24hours	-73.52000*	.29900	.000	-74.3981	-72.6419	
		48hours	-74.78667*	.29900	.000	-75.6648	-73.9085	
		72hours	-75.76500*	.29900	.000	-76.6431	-74.8869	

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Colour_a_C5	0hour	8	4.7517	86953	35045	3.9021	5.6912	3.87	5.74
	24hours	8	2.1050	16055	67779	1.5050	2.3050	1.72	2.21
	48hours	8	1.3617	17543	87162	1.1776	1.5458	1.15	1.55
	72hours	8	.9167	27551	11248	.6275	1.2058	.86	1.27
	96hours	8	11.5983	187925	76678	8.6272	12.5694	9.18	12.21
	Total	38	4.1467	4.11038	75135	2.6100	5.6833	.69	13.21
Colour_a_C10	0hour	8	4.9550	81471	25095	4.3039	5.6001	4.43	5.75
	24hours	8	2.1233	83396	81382	2.0879	2.1589	2.09	2.17
	48hours	8	1.5083	25826	10543	1.2373	1.7794	1.29	1.83
	72hours	8	.9750	23544	99812	.7279	1.2221	.67	1.25
	96hours	8	13.1217	234577	83019	10.9748	15.2686	10.52	14.93
	Total	38	4.5567	4.67118	85284	2.7924	6.2809	.67	14.93
Colour_a_C15	0hour	8	4.8100	75492	30828	4.0177	5.6023	3.81	5.72
	24hours	8	2.1817	82483	81014	2.1356	2.2677	2.15	2.23
	48hours	8	1.4517	87521	83070	1.3727	1.5308	1.38	1.56
	72hours	8	.9133	26911	11893	.6099	1.2167	.69	1.29
	96hours	8	13.7483	206141	84157	11.5950	15.9117	11.34	15.97
	Total	38	4.6210	4.82377	88895	2.7824	6.4598	.69	15.97

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour_a_C5	Between Groups	469.505	4	117.376	135.659	.000
	Within Groups	21.631	25	.865		
	Total	491.136	29			
Colour_a_C10	Between Groups	609.346	4	152.337	182.533	.000
	Within Groups	23.432	25	.937		
	Total	632.778	29			
Colour_a_C15	Between Groups	678.514	4	169.629	172.766	.000
	Within Groups	24.546	25	.982		
	Total	703.060	29			

Colour_a_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.9167		
48hours	6	1.3617		
24hours	6	2.1050		
0hour	6		4.7517	
96hours	6			11.5983
Sig.		.208	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.9750		
48hours	6	1.5083		
24hours	6	2.1233		
0hour	6		4.9550	
96hours	6			13.1217
Sig.		.271	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	6	.9133		
48hours	6	1.4517		
24hours	6	2.1817		
0hour	6		4.8100	
96hours	6			13.7483
Sig.		.206	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Colour_a_C5	0hour	24hours	2.64667 [*]	.53704	.000	1.0695	4.2239
		48hours	3.39000 [*]	.53704	.000	1.8128	4.9672
		72hours	3.83500 [*]	.53704	.000	2.2578	5.4122
		96hours	-6.84667 [*]	.53704	.000	-8.4239	-5.2695
	24hours	0hour	-2.64667 [*]	.53704	.000	-4.2239	-1.0695
		48hours	.74333	.53704	.643	-.8339	2.3205
		72hours	1.18833	.53704	.208	-.3889	2.7655
		96hours	-9.49333 [*]	.53704	.000	-11.0705	-7.9161
	48hours	0hour	-3.39000 [*]	.53704	.000	-4.9672	-1.8128
		24hours	-.74333	.53704	.643	-2.3205	.8339
		72hours	.44500	.53704	.919	-1.1322	2.0222
		96hours	-10.23667 [*]	.53704	.000	-11.8139	-8.6595
	72hours	0hour	-3.83500 [*]	.53704	.000	-5.4122	-2.2578
		24hours	-1.18833	.53704	.208	-2.7655	.3889
		48hours	-.44500	.53704	.919	-2.0222	1.1322
		96hours	-10.68167 [*]	.53704	.000	-12.2589	-9.1045
	96hours	0hour	6.84667 [*]	.53704	.000	5.2695	8.4239
		24hours	9.49333 [*]	.53704	.000	7.9161	11.0705
		48hours	10.23667 [*]	.53704	.000	8.6595	11.8139
		72hours	10.68167 [*]	.53704	.000	9.1045	12.2589
Colour_a_C10	0hour	24hours	2.83167 [*]	.55895	.000	1.1901	4.4732
		48hours	3.44667 [*]	.55895	.000	1.8051	5.0882
		72hours	3.98000 [*]	.55895	.000	2.3384	5.6216
		96hours	-8.16667 [*]	.55895	.000	-9.8082	-6.5251
	24hours	0hour	-2.83167 [*]	.55895	.000	-4.4732	-1.1901
		48hours	.81500	.55895	.805	-1.0266	2.2566
		72hours	1.14833	.55895	.271	-.4932	2.7899
		96hours	-10.99833 [*]	.55895	.000	-12.6399	-9.3568
	48hours	0hour	-3.44667 [*]	.55895	.000	-5.0882	-1.8051
		24hours	-.81500	.55895	.805	-2.2566	1.0266
		72hours	-.53333	.55895	.873	-1.1082	2.1749
		96hours	-11.61333 [*]	.55895	.000	-13.2549	-9.9718
	72hours	0hour	-3.98000 [*]	.55895	.000	-5.6216	-2.3384
		24hours	-1.14833	.55895	.271	-2.7899	.4932
		48hours	-.53333	.55895	.873	-2.1749	1.1082
		96hours	-12.14667 [*]	.55895	.000	-13.7882	-10.5051
	96hours	0hour	8.16667 [*]	.55895	.000	6.5251	9.8082
		24hours	10.99833 [*]	.55895	.000	9.3568	12.6399
		48hours	11.61333 [*]	.55895	.000	9.9718	13.2549
		72hours	12.14667 [*]	.55895	.000	10.5051	13.7882
Colour_a_C15	0hour	24hours	2.62833 [*]	.57208	.001	.9482	4.3085
		48hours	3.35833 [*]	.57208	.000	1.6782	5.0385
		72hours	3.89667 [*]	.57208	.000	2.2165	5.5768
		96hours	-8.93833 [*]	.57208	.000	-10.6185	-7.2582
	24hours	0hour	-2.62833 [*]	.57208	.001	-4.3085	-.9482
		48hours	.73000	.57208	.708	-.9501	2.4101
		72hours	1.26833	.57208	.206	-.4118	2.9485
		96hours	-11.56667 [*]	.57208	.000	-13.2468	-9.8865
	48hours	0hour	-3.35833 [*]	.57208	.000	-5.0385	-1.6782
		24hours	-.73000	.57208	.708	-2.4101	.9501
		72hours	.53833	.57208	.878	-1.1418	2.2185
		96hours	-12.29667 [*]	.57208	.000	-13.9768	-10.6165
	72hours	0hour	-3.89667 [*]	.57208	.000	-5.5768	-2.2165
		24hours	-1.26833	.57208	.206	-2.9485	.4118
		48hours	-.53833	.57208	.878	-2.2185	1.1418
		96hours	-12.83500 [*]	.57208	.000	-14.5151	-11.1549
	96hours	0hour	8.93833 [*]	.57208	.000	7.2582	10.6185
		24hours	11.56667 [*]	.57208	.000	9.8865	13.2468
		48hours	12.29667 [*]	.57208	.000	10.6165	13.9768
		72hours	12.83500 [*]	.57208	.000	11.1549	14.5151

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Colour_b_C5	0hour	6	39.6583	3.26407	1.33255	36.2329	43.0838	36.89	43.81
	24hours	6	33.9000	3.72272	1.51980	29.9932	37.8068	31.27	38.70
	48hours	6	32.1250	2.83585	1.19856	29.0440	35.2060	30.19	35.93
	72hours	6	30.7800	1.96757	.80326	28.7152	32.8448	29.10	33.28
	96hours	6	29.2583	1.63707	.68008	28.5698	29.9269	28.41	29.95
	Total	30	33.1443	4.45454	.81328	31.4810	34.8077	28.41	43.81
Colour_b_C10	0hour	6	39.6750	3.34441	1.36535	36.1653	43.1847	36.83	44.04
	24hours	6	34.2783	4.15890	1.69786	29.9138	38.6428	31.45	39.65
	48hours	6	32.0850	2.49929	1.02033	29.4622	34.7078	30.15	35.32
	72hours	6	30.7933	1.93431	.78968	28.7634	32.8233	29.23	33.27
	96hours	6	24.1350	1.03181	.42123	23.0522	25.2178	23.42	25.47
	Total	30	32.1933	5.75673	1.05103	30.0437	34.3429	23.42	44.04
Colour_b_C15	0hour	6	39.7233	3.35892	1.37127	36.1984	43.2483	36.88	44.04
	24hours	6	34.1050	3.99194	1.62970	29.9157	38.2943	31.29	39.29
	48hours	6	32.4300	2.86931	1.17139	29.4188	35.4412	30.21	36.08
	72hours	6	30.9500	1.82244	.74401	29.0375	32.8625	29.30	33.28
	96hours	6	16.4200	4.03672	1.64798	12.1837	20.6563	11.44	20.32
	Total	30	30.7257	8.45933	1.54445	27.5669	33.8844	11.44	44.04

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour_b_C5	Between Groups	388.400	4	97.100	12.978	.000
	Within Groups	187.046	25	7.482		
	Total	575.446	29			
Colour_b_C10	Between Groups	763.386	4	190.847	24.137	.000
	Within Groups	197.671	25	7.907		
	Total	961.057	29			
Colour_b_C15	Between Groups	1799.910	4	449.978	40.857	.000
	Within Groups	275.336	25	11.013		
	Total	2075.247	29			

Colour_b_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
96hours	6	29.2583		
72hours	6	30.7800	30.7800	
48hours	6	32.1250	32.1250	
24hours	6		33.9000	
0hour	6			39.6583
Sig.		.387	.306	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
96hours	6	24.1350		
72hours	6		30.7933	
48hours	6		32.0850	
24hours	6		34.2783	
0hour	6			39.6750
Sig.		1.000	.233	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
96hours	6	16.4200		
72hours	6		30.9500	
48hours	6		32.4300	
24hours	6		34.1050	34.1050
0hour	6			39.7233
Sig.		1.000	.483	.050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Colour_h_C5	0hour	24hours	5.75833 ^a	1.57922	.010	1.1204	10.3963	
		48hours	7.53333 ^a	1.57922	.001	2.8954	12.1713	
		72hours	8.87833 ^a	1.57922	.000	4.2404	13.5163	
		96hours	10.40000 ^a	1.57922	.000	5.7620	15.0380	
	24hours	0hour	-5.75833 ^a	1.57922	.010	-10.3963	-1.1204	
		48hours	1.77500	1.57922	.782	-2.8630	6.4130	
		72hours	3.12000	1.57922	.306	-1.5180	7.7580	
		96hours	4.64167 ^a	1.57922	.050	.0037	9.2796	
	48hours	0hour	-7.53333 ^a	1.57922	.001	-12.1713	-2.8954	
		24hours	-1.77500	1.57922	.782	-6.4130	2.8630	
		72hours	1.34500	1.57922	.912	-3.2930	5.8830	
		96hours	2.86667	1.57922	.387	-1.7713	7.5046	
	72hours	0hour	-8.87833 ^a	1.57922	.000	-13.5163	-4.2404	
		24hours	-3.12000	1.57922	.306	-7.7580	1.5180	
		48hours	-1.34500	1.57922	.912	-5.9830	3.2930	
		96hours	1.52167	1.57922	.869	-3.1163	6.1596	
	96hours	0hour	-10.40000 ^a	1.57922	.000	-15.0380	-5.7620	
		24hours	-4.64167 ^a	1.57922	.050	-9.2796	-.0037	
		48hours	-2.86667	1.57922	.387	-7.5046	1.7713	
		72hours	-1.52167	1.57922	.869	-6.1596	3.1163	
	Colour_b_C10	0hour	24hours	5.39667 ^a	1.62346	.021	6288	10.1646
			48hours	7.59000 ^a	1.62346	.001	2.8221	12.3579
			72hours	8.88167 ^a	1.62346	.000	4.1138	13.6496
			96hours	15.54000 ^a	1.62346	.000	10.7721	20.3079
24hours		0hour	-5.39667 ^a	1.62346	.021	-10.1646	-.6288	
		48hours	2.19333	1.62346	.663	-2.5746	6.9612	
		72hours	3.48500	1.62346	.233	-1.2829	8.2529	
		96hours	10.14333 ^a	1.62346	.000	5.3754	14.9112	
48hours		0hour	-7.59000 ^a	1.62346	.001	-12.3579	-2.8221	
		24hours	-2.19333	1.62346	.663	-6.9612	2.5746	
		72hours	1.28167	1.62346	.929	-3.4762	8.0596	
		96hours	7.95000 ^a	1.62346	.000	3.1821	12.7179	
72hours		0hour	-8.88167 ^a	1.62346	.000	-13.6496	-4.1138	
		24hours	-3.48500	1.62346	.233	-8.2529	1.2829	
		48hours	-1.28167	1.62346	.929	-6.0596	3.4762	
		96hours	6.65833 ^a	1.62346	.003	1.8904	11.4262	
96hours		0hour	-15.54000 ^a	1.62346	.000	-20.3079	-10.7721	
		24hours	-10.14333 ^a	1.62346	.000	-14.9112	-5.3754	
		48hours	-7.95000 ^a	1.62346	.000	-12.7179	-3.1821	
		72hours	-6.65833 ^a	1.62346	.003	-11.4262	-1.8904	
Colour_b_C15		0hour	24hours	5.61833	1.91602	.050	-.0088	11.2455
			48hours	7.29333 ^a	1.91602	.007	1.6662	12.9205
			72hours	8.77333 ^a	1.91602	.001	3.1462	14.4005
			96hours	23.30333 ^a	1.91602	.000	17.6762	28.9305
	24hours	0hour	-5.61833	1.91602	.050	-11.2455	.0088	
		48hours	1.67500	1.91602	.904	-3.9521	7.3021	
		72hours	3.15500	1.91602	.483	-2.4721	8.7821	
		96hours	17.68500 ^a	1.91602	.000	12.0579	23.3121	
	48hours	0hour	-7.29333 ^a	1.91602	.007	-12.9205	-1.6662	
		24hours	-1.67500	1.91602	.904	-7.3021	3.9521	
		72hours	1.48000	1.91602	.936	-4.1471	7.1071	
		96hours	16.01000 ^a	1.91602	.000	10.3829	21.6371	
	72hours	0hour	-8.77333 ^a	1.91602	.001	-14.4005	-3.1462	
		24hours	-3.15500	1.91602	.483	-8.7821	2.4721	
		48hours	-1.48000	1.91602	.936	-7.1071	4.1471	
		96hours	14.53000 ^a	1.91602	.000	8.9029	20.1571	
	96hours	0hour	-23.30333 ^a	1.91602	.000	-28.9305	-17.6762	
		24hours	-17.68500 ^a	1.91602	.000	-23.3121	-12.0579	
		48hours	-16.01000 ^a	1.91602	.000	-21.6371	-10.3829	
		72hours	-14.53000 ^a	1.91602	.000	-20.1571	-8.9029	

*. The mean difference is significant at the 0.05 level.

F.10.2 Statistical analysis of colour (L*, a*, and b* value) of black tea water kefir beverage with added carrot juice samples at 96 hours of fermentation

Descriptives

Colour_L_96hours								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	6	31.1233	.72240	.29492	30.3652	31.8814	30.61	32.07
carrot10%	6	18.3917	.86161	.35175	17.4875	19.2959	17.35	19.30
carrot15%	6	12.5867	.79674	.32527	11.7505	13.4228	12.05	13.62
Total	18	20.7006	8.00110	1.88588	16.7217	24.6794	12.05	32.07

Test of Homogeneity of Variances

Colour_L_96hours				
Levene Statistic	df1	df2	Sig.	
.079	2	15	.924	

ANOVA

Colour_L_96hours					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1076.603	2	538.401	852.121	.000
Within Groups	9.495	15	.633		
Total	1086.098	17			

Multiple Comparisons

Dependent Variable: Colour_L_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carrot10%	12.73167*	.45935	.000	11.5385	13.9248
	carrot15%	18.53667*	.45935	.000	17.3435	19.7298
carrot10%	carrot5%	-12.73167*	.45935	.000	-13.9248	-11.5385
	carrot15%	5.80500*	.45935	.000	4.6118	6.9982
carrot15%	carrot5%	-18.53667*	.45935	.000	-19.7298	-17.3435
	carrot10%	-5.80500*	.45935	.000	-6.9982	-4.6118

*. The mean difference is significant at the 0.05 level.

Colour_L_96hours

Tukey HSD^a

Subset for alpha = 0.05				
Treatment	N	1	2	3
carrot15%	6	12.5867		
carrot10%	6		18.3917	
carrot5%	6			31.1233
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Descriptives

Colour_a_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	6	11.5983	1.87825	.76679	9.6272	13.5694	9.18	13.21
carrot10%	6	13.1217	2.04577	.83518	10.9748	15.2686	10.52	14.93
carrot15%	6	13.7483	2.06141	.84157	11.5850	15.9117	11.34	15.97
Total	18	12.8228	2.09318	.49337	11.7819	13.8637	9.18	15.97

ANOVA

Test of Homogeneity of Variances				Colour_a_96hours					
Colour_a_96hours				Sum of Squares	df	Mean Square	F	Sig.	
Levene Statistic	df1	df2	Sig.	Between Groups	14.672	2	7.336	1.840	.193
.047	2	15	.954	Within Groups	59.812	15	3.987		
				Total	74.484	17			

Multiple Comparisons

Dependent Variable: Colour_a_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carrot10%	-1.52333	1.15289	.406	-4.5179	1.4713
	carrot15%	-2.15000	1.15289	.183	-5.1446	.8446
carrot10%	carrot5%	1.52333	1.15289	.406	-1.4713	4.5179
	carrot15%	-.62667	1.15289	.851	-3.6213	2.3679
carrot15%	carrot5%	2.15000	1.15289	.183	-.8446	5.1446
	carrot10%	.62667	1.15289	.851	-2.3679	3.6213

Colour_a_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
carrot5%	6	11.5983
carrot10%	6	13.1217
carrot15%	6	13.7483
Sig.		.183

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Descriptives

Colour_b_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	6	29.2583	.63707	.26008	28.5898	29.9269	28.41	29.95
carrot10%	6	24.1350	1.03181	.42123	23.0522	25.2178	23.42	25.47
carrot15%	6	16.4200	4.03672	1.64798	12.1837	20.6563	11.44	20.32
Total	18	23.2711	5.89124	1.38858	20.3415	26.2008	11.44	29.95

ANOVA

Test of Homogeneity of Variances

Colour_b_96hours

Levene Statistic	df1	df2	Sig.
12.105	2	15	.001

Colour_b_96hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	501.185	2	250.593	42.316	.000
Within Groups	88.828	15	5.922		
Total	590.013	17			

Multiple Comparisons

Dependent Variable: Colour_b_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carrot10%	5.12333*	1.40498	.006	1.4740	8.7727
	carrot15%	12.83833*	1.40498	.000	9.1890	16.4877
carrot10%	carrot5%	-5.12333*	1.40498	.006	-8.7727	-1.4740
	carrot15%	7.71500*	1.40498	.000	4.0656	11.3644
carrot15%	carrot5%	-12.83833*	1.40498	.000	-16.4877	-9.1890
	carrot10%	-7.71500*	1.40498	.000	-11.3644	-4.0656

*. The mean difference is significant at the 0.05 level.

Colour_b_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05		
		1	2	3
carrot15%	6	16.4200		
carrot10%	6		24.1350	
carrot5%	6			29.2583
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Ranks

	Treatment	N	Mean Rank
Colour_b_96hours	carrot5%	6	15.50
	carrot10%	6	9.50
	carrot15%	6	3.50
	Total	18	

Test Statistics^{a,b}

	Colour_b_96 hours
Chi-Square	15.174
df	2
Asymp. Sig.	.001

a. Kruskal Wallis Test

b. Grouping Variable: Treatment

F.11.1 Statistical analysis of microbiological analysis of black tea water kefir beverage with added carrot juice samples during 96 hours of fermentation

Descriptives									
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Lactococcus_C5	0hour	3	4.7167	.22591	.13043	4.1555	5.2778	4.48	4.93
	24hours	3	5.9367	.05132	.02963	5.8092	6.0641	5.88	5.98
	48hours	3	6.3267	.14434	.08333	5.9681	6.6852	6.16	6.41
	72hours	3	5.5500	.05292	.03055	5.4186	5.6814	5.49	5.59
	96hours	3	5.7900	.02046	.01528	5.7243	5.8557	5.77	5.82
	Total	15	5.6640	.56539	.14598	5.3509	5.9771	4.48	6.41
Lactococcus_C10	0hour	3	4.7967	.20033	.11566	4.2990	5.2943	4.59	4.99
	24hours	3	5.9733	.05132	.02963	5.8459	6.1008	5.93	6.03
	48hours	3	6.4533	.02517	.01453	6.3908	6.5158	6.43	6.48
	72hours	3	5.5900	.04359	.02517	5.4817	5.6983	5.54	5.62
	96hours	3	5.8633	.01528	.00882	5.8254	5.9013	5.85	5.88
	Total	15	5.7353	.57092	.14741	5.4192	6.0515	4.59	6.48
Lactococcus_C15	0hour	3	4.7433	.19502	.11260	4.2689	5.2278	4.52	4.88
	24hours	3	5.9533	.08083	.04667	5.7525	6.1541	5.88	6.04
	48hours	3	6.3733	.09074	.05239	6.1479	6.5987	6.27	6.44
	72hours	3	5.6000	.02646	.01528	5.5343	5.6657	5.57	5.62
	96hours	3	5.8167	.08505	.04910	5.6054	6.0279	5.73	5.90
	Total	15	5.6973	.56635	.14623	5.3837	6.0110	4.52	6.44

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Lactococcus_C5	Between Groups	4.319	4	1.080	69.221	.000
	Within Groups	.156	10	.016		
	Total	4.475	14			
Lactococcus_C10	Between Groups	4.472	4	1.118	122.776	.000
	Within Groups	.091	10	.009		
	Total	4.563	14			
Lactococcus_C15	Between Groups	4.369	4	1.092	89.922	.000
	Within Groups	.121	10	.012		
	Total	4.490	14			

Lactococcus_C5						
Tukey HSD ^a						
Hour	N	Subset for alpha = 0.05				
		1	2	3	4	
0hour	3	4.7167				
72hours	3		5.5500			
96hours	3			5.7900		
24hours	3				5.9367	
48hours	3					6.3267
Sig.		1.000	.206	.820	1.000	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Lactococcus_C10						
Tukey HSD ^a						
Hour	N	Subset for alpha = 0.05				
		1	2	3	4	
0hour	3	4.7967				
72hours	3		5.5900			
96hours	3			5.8633		
24hours	3				5.9733	
48hours	3					6.4533
Sig.		1.000	1.000	.634	1.000	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Lactococcus_C15						
Tukey HSD ^a						
Hour	N	Subset for alpha = 0.05				
		1	2	3	4	
0hour	3	4.7433				
72hours	3		5.6000			
96hours	3			5.8167		
24hours	3				5.9533	
48hours	3					6.3733
Sig.		1.000	.190	.574	1.000	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	() Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Lactococcus_C5	0hour	24hours	-1.22000*	10198	.000	-1.5556	-.8844	
		48hours	-1.61000*	10198	.000	-1.9456	-1.2744	
		72hours	-.83333*	10198	.000	-1.1690	-.4977	
		96hours	-1.07333*	10198	.000	-1.4090	-.7377	
	24hours	0hour	1.22000*	10198	.000	.8844	1.5556	
		48hours	-.39000*	10198	.022	-.7256	-.0544	
		72hours	.38667*	10198	.023	.0510	.7223	
		96hours	.14667	10198	.620	-.1890	.4823	
	48hours	0hour	1.61000*	10198	.000	1.2744	1.9456	
		24hours	.39000*	10198	.022	.0544	.7256	
		72hours	.77667*	10198	.000	.4410	1.1123	
		96hours	.53667*	10198	.003	.2010	.8723	
	72hours	0hour	.83333*	10198	.000	.4977	1.1690	
		24hours	-.38667*	10198	.023	-.7223	-.0510	
		48hours	-.77667*	10198	.000	-1.1123	-.4410	
		96hours	-.24000	10198	.206	-.5756	.0956	
	96hours	0hour	1.07333*	10198	.000	.7377	1.4090	
		24hours	-.14667	10198	.620	-.4823	.1890	
		48hours	-.53667*	10198	.003	-.8723	-.2010	
		72hours	.24000	10198	.206	-.0956	.5756	
	Lactococcus_C10	0hour	24hours	-1.17667*	07792	.000	-1.4331	-.9202
			48hours	-1.65667*	07792	.000	-1.9131	-1.4002
			72hours	-.79333*	07792	.000	-1.0498	-.5369
			96hours	-1.06667*	07792	.000	-1.3231	-.8102
24hours		0hour	1.17667*	07792	.000	.9202	1.4331	
		48hours	-.48000*	07792	.001	-.7364	-.2236	
		72hours	.38333*	07792	.004	.1269	.6398	
		96hours	.11000	07792	.634	-.1464	.3664	
48hours		0hour	1.65667*	07792	.000	1.4002	1.9131	
		24hours	.48000*	07792	.001	.2236	.7364	
		72hours	.86333*	07792	.000	.6069	1.1198	
		96hours	.59000*	07792	.000	.3336	.8464	
72hours		0hour	.79333*	07792	.000	.5369	1.0498	
		24hours	-.38333*	07792	.004	-.6398	-.1269	
		48hours	-.86333*	07792	.000	-1.1198	-.6069	
		96hours	-.27333*	07792	.036	-.5298	-.0169	
96hours		0hour	1.06667*	07792	.000	.8102	1.3231	
		24hours	-.11000	07792	.634	-.3664	.1464	
		48hours	-.59000*	07792	.000	-.8464	-.3336	
		72hours	.27333*	07792	.036	.0169	.5298	
Lactococcus_C15		0hour	24hours	-1.21000*	08999	.000	-1.5062	-.9138
			48hours	-1.63000*	08999	.000	-1.9262	-1.3338
			72hours	-.85667*	08999	.000	-1.1528	-.5605
			96hours	-1.07333*	08999	.000	-1.3695	-.7772
	24hours	0hour	1.21000*	08999	.000	.9138	1.5062	
		48hours	-.42000*	08999	.006	-.7162	-.1238	
		72hours	.35333*	08999	.019	.0572	.6495	
		96hours	.13667	08999	.574	-.1595	.4328	
	48hours	0hour	1.63000*	08999	.000	1.3338	1.9262	
		24hours	.42000*	08999	.006	.1238	.7162	
		72hours	.77333*	08999	.000	.4772	1.0695	
		96hours	.55667*	08999	.001	.2605	.8528	
	72hours	0hour	.85667*	08999	.000	.5605	1.1528	
		24hours	-.35333*	08999	.019	-.6495	-.0572	
		48hours	-.77333*	08999	.000	-1.0695	-.4772	
		96hours	-.21667	08999	.190	-.5128	.0795	
	96hours	0hour	1.07333*	08999	.000	.7772	1.3695	
		24hours	-.13667	08999	.574	-.4328	.1595	
		48hours	-.55667*	08999	.001	-.8528	-.2605	
		72hours	.21667	08999	.190	-.0795	.5128	

* The mean difference is significant at the 0.05 level

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Lactobacillus_C5	0hour	3	5.7800	.14731	.08505	5.4141	6.1459	5.65	5.94
	24hours	3	6.6833	.09292	.05384	6.4525	6.9141	6.62	6.79
	48hours	3	6.7200	.07000	.04041	6.5461	6.8939	6.65	6.79
	72hours	3	5.8067	.07024	.04055	5.6322	5.9811	5.74	5.88
	96hours	3	5.9100	.03000	.01732	5.8355	5.9845	5.88	5.94
	Total	15	6.1800	.44990	.11616	5.9309	6.4291	5.65	6.79
Lactobacillus_C10	0hour	3	5.8600	.08000	.04619	5.6613	6.0587	5.78	5.94
	24hours	3	6.5933	.16503	.09528	6.1834	7.0033	6.41	6.73
	48hours	3	6.6900	.02000	.01155	6.6403	6.7397	6.67	6.71
	72hours	3	5.9033	.04163	.02404	5.7999	6.0068	5.87	5.95
	96hours	3	6.2033	.14364	.08293	5.8465	6.5602	6.04	6.31
	Total	15	6.2500	.36553	.09438	6.0476	6.4524	5.78	6.73
Lactobacillus_C15	0hour	3	5.8633	.04041	.02333	5.7629	5.9637	5.82	5.90
	24hours	3	6.5633	.14295	.08253	6.2082	6.9184	6.44	6.72
	48hours	3	6.6400	.14422	.08327	6.2817	6.9983	6.52	6.80
	72hours	3	5.6267	.36964	.21341	4.7084	6.5449	5.20	5.85
	96hours	3	6.2600	.05292	.03055	6.1286	6.3914	6.22	6.32
	Total	15	6.1907	.43729	.11291	5.9485	6.4328	5.20	6.80

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactobacillus_C5	Between Groups	2.752	4	.688	83.756	.000
	Within Groups	.082	10	.008		
	Total	2.834	14			
Lactobacillus_C10	Between Groups	1.758	4	.439	38.958	.000
	Within Groups	.113	10	.011		
	Total	1.871	14			
Lactobacillus_C15	Between Groups	2.312	4	.578	15.856	.000
	Within Groups	.365	10	.036		
	Total	2.677	14			

Lactobacillus_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05	
		1	2
0hour	3	5.7800	
72hours	3	5.8067	
96hours	3	5.9100	
24hours	3		6.6833
48hours	3		6.7200
Sig.		.446	.986

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Lactobacillus_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.8600		
72hours	3	5.9033		
96hours	3		6.2033	
24hours	3			6.5933
48hours	3			6.6900
Sig.		.986	1.000	.796

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Lactobacillus_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
72hours	3	5.6267		
0hour	3	5.8633	5.8633	
96hours	3		6.2600	6.2600
24hours	3			6.5633
48hours	3			6.6400
Sig.		.574	.156	.182

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Hour	(J) Hour	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Lactobacillus_C5	0hour	24hours	-.90333 [*]	.07400	.000	-1.1469	-.6598	
		48hours	-.94000 [*]	.07400	.000	-1.1835	-.6965	
		72hours	-.02667	.07400	.996	-.2702	.2169	
		96hours	-.13000	.07400	.446	-.3735	.1135	
		24hours	.90333 [*]	.07400	.000	.6598	1.1469	
	24hours	0hour	-.02667	.07400	.986	-.2802	.2069	
		72hours	.87667 [*]	.07400	.000	.6331	1.1202	
		96hours	.77333 [*]	.07400	.000	.5298	1.0169	
		48hours	.94000 [*]	.07400	.000	.6965	1.1835	
		24hours	.02667	.07400	.986	-.2069	.2802	
	48hours	0hour	.91333 [*]	.07400	.000	.6698	1.1569	
		72hours	.81000 [*]	.07400	.000	.5665	1.0535	
		96hours	.02667	.07400	.996	-.2169	.2702	
		24hours	-.87667 [*]	.07400	.000	-1.1202	-.6331	
		48hours	-.91333 [*]	.07400	.000	-1.1569	-.6698	
	72hours	0hour	-.10333	.07400	.643	-.3469	.1402	
		96hours	-.13000	.07400	.446	-.3735	.1135	
		24hours	-.77333 [*]	.07400	.000	-1.0169	-.5298	
		48hours	-.81000 [*]	.07400	.000	-1.0535	-.5665	
		72hours	.10333	.07400	.643	-.1402	.3469	
	Lactobacillus_C10	0hour	24hours	-.73333 [*]	.08672	.000	-1.0187	-.4479
			48hours	-.83000 [*]	.08672	.000	-1.1154	-.5446
			72hours	-.04333	.08672	.986	-.3287	.2421
			96hours	-.34333 [*]	.08672	.018	-.6287	-.0579
24hours			.73333 [*]	.08672	.000	.4479	1.0187	
24hours		0hour	-.09667	.08672	.796	-.3821	.1887	
		72hours	.69000 [*]	.08672	.000	.4046	.9754	
		96hours	.39000 [*]	.08672	.008	.1046	.6754	
		48hours	.83000 [*]	.08672	.000	.5446	1.1154	
		24hours	.09667	.08672	.796	-.1887	.3821	
48hours		0hour	.78667 [*]	.08672	.000	.5013	1.0721	
		72hours	.48667 [*]	.08672	.002	.2013	.7721	
		96hours	.04333	.08672	.986	-.2421	.3287	
		24hours	-.69000 [*]	.08672	.000	-.9754	-.4046	
		48hours	-.78667 [*]	.08672	.000	-1.0721	-.5013	
72hours		0hour	-.30000 [*]	.08672	.038	-.5854	-.0146	
		96hours	.34333 [*]	.08672	.018	.0579	.6287	
		24hours	-.39000 [*]	.08672	.008	-.6754	-.1046	
		48hours	-.48667 [*]	.08672	.002	-.7721	-.2013	
		72hours	.30000 [*]	.08672	.038	.0146	.5854	
Lactobacillus_C15		0hour	24hours	-.70000 [*]	.15591	.008	-1.2131	-.1869
			48hours	-.77667 [*]	.15591	.004	-1.2898	-.2636
			72hours	.23667	.15591	.574	-.2764	.7498
			96hours	-.39667	.15591	.156	-.9098	.1164
	24hours		.70000 [*]	.15591	.008	.1869	1.2131	
	24hours	0hour	-.07667	.15591	.986	-.5898	.4364	
		72hours	.93667 [*]	.15591	.001	.4236	1.4498	
		96hours	.30333	.15591	.355	-.2098	.8164	
		48hours	.77667 [*]	.15591	.004	.2636	1.2898	
		24hours	.07667	.15591	.986	-.4364	.5898	
	48hours	0hour	1.01333 [*]	.15591	.001	.5002	1.5264	
		72hours	.38000	.15591	.182	-.1331	.8931	
		96hours	-.39667	.15591	.156	-.9098	.1164	
		24hours	-.30333	.15591	.355	-.8164	.2098	
		48hours	-.38000	.15591	.182	-.8931	.1331	
	72hours	0hour	-.63333 [*]	.15591	.015	-1.1464	-.1202	
		96hours	-.39667	.15591	.156	-.9098	.1164	
		24hours	-.30333	.15591	.355	-.8164	.2098	
		48hours	-.38000	.15591	.182	-.8931	.1331	
		72hours	.63333 [*]	.15591	.015	.1202	1.1464	

*. The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Scerevisiae_C5	0hour	3	5.6133	.11719	.06766	5.3222	5.9044	5.48	5.70
	24hours	3	6.3300	.02648	.01528	6.2643	6.3957	6.31	6.36
	48hours	3	6.4767	.06807	.03930	6.3076	6.6458	6.40	6.53
	72hours	3	6.4267	.04041	.02333	6.3263	6.5271	6.38	6.45
	96hours	3	7.3733	.03215	.01856	7.2935	7.4532	7.35	7.41
	Total	15	6.4440	.58233	.15036	6.1215	6.7665	5.48	7.41
Scerevisiae_C10	0hour	3	5.5233	.11150	.06438	5.2463	5.8003	5.44	5.65
	24hours	3	6.2900	.23643	.13650	5.7027	6.8773	6.04	6.51
	48hours	3	6.4500	.09644	.05568	6.2104	6.6896	6.38	6.56
	72hours	3	6.4167	.02887	.01667	6.3450	6.4884	6.40	6.45
	96hours	3	7.6100	.13856	.08000	7.2658	7.9542	7.53	7.77
	Total	15	6.4580	.70140	.18110	6.0696	6.8464	5.44	7.77
Scerevisiae_C15	0hour	3	5.6033	.05508	.03180	5.4665	5.7401	5.55	5.66
	24hours	3	6.3433	.02917	.01453	6.2808	6.4058	6.32	6.37
	48hours	3	6.4733	.06658	.03844	6.3079	6.6387	6.40	6.53
	72hours	3	6.4533	.03512	.02028	6.3661	6.5406	6.42	6.49
	96hours	3	7.5500	.15875	.09165	7.1557	7.9443	7.37	7.67
	Total	15	6.4847	.64738	.16715	6.1262	6.8432	5.55	7.67

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Scerevisiae_C5	Between Groups	4.704	4	1.176	270.558	.000
	Within Groups	.043	10	.004		
	Total	4.748	14			
Scerevisiae_C10	Between Groups	6.692	4	1.673	85.650	.000
	Within Groups	.195	10	.020		
	Total	6.887	14			
Scerevisiae_C15	Between Groups	5.798	4	1.450	209.881	.000
	Within Groups	.069	10	.007		
	Total	5.867	14			

Scerevisiae_C5

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.6133		
24hours	3		6.3300	
72hours	3		6.4267	
48hours	3		6.4767	
96hours	3			7.3733
Sig.		1.000	.119	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Scerevisiae_C10

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.5233		
24hours	3		6.2900	
72hours	3		6.4167	
48hours	3		6.4500	
96hours	3			7.6100
Sig.		1.000	.640	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Scerevisiae_C15

Tukey HSD^a

Hour	N	Subset for alpha = 0.05		
		1	2	3
0hour	3	5.6033		
24hours	3		6.3433	
72hours	3		6.4533	
48hours	3		6.4733	
96hours	3			7.5500
Sig.		1.000	.369	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Hour	(j) Hour	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Scerevisiae_C5	0hour	24hours	-.71667*	.05383	.000	-.8938	-.5395	
		48hours	-.86333*	.05383	.000	-1.0405	-.6862	
		72hours	-.81333*	.05383	.000	-.9905	-.6362	
		96hours	-1.76000*	.05383	.000	-1.9372	-1.5828	
	24hours	0hour	.71667*	.05383	.000	.5395	.8938	
		48hours	-.14667	.05383	.119	-.3238	.0305	
		72hours	-.09667	.05383	.426	-.2738	.0805	
		96hours	-1.04333*	.05383	.000	-1.2205	-.8662	
	48hours	0hour	.86333*	.05383	.000	.6862	1.0405	
		24hours	.14667	.05383	.119	-.0305	.3238	
		72hours	.05000	.05383	.879	-.1272	.2272	
		96hours	-.89667*	.05383	.000	-1.0738	-.7195	
	72hours	0hour	.81333*	.05383	.000	.6362	.9905	
		24hours	.09667	.05383	.426	-.0805	.2738	
		48hours	-.05000	.05383	.879	-.2272	.1272	
		96hours	-.94667*	.05383	.000	-1.1238	-.7695	
	96hours	0hour	1.76000*	.05383	.000	1.5828	1.9372	
		24hours	1.04333*	.05383	.000	.8662	1.2205	
		48hours	.89667*	.05383	.000	.7195	1.0738	
		72hours	.94667*	.05383	.000	.7695	1.1238	
	Scerevisiae_C10	0hour	24hours	-.76667*	.11411	.000	-1.1422	-.3911
			48hours	-.92667*	.11411	.000	-1.3022	-.5511
			72hours	-.89333*	.11411	.000	-1.2689	-.5178
			96hours	-2.08667*	.11411	.000	-2.4622	-1.7111
		24hours	0hour	.76667*	.11411	.000	.3911	1.1422
			48hours	-.16000	.11411	.640	-.5356	.2156
			72hours	-.12667	.11411	.798	-.5022	.2489
			96hours	-1.32000*	.11411	.000	-1.6956	-.9444
48hours		0hour	.92667*	.11411	.000	.5511	1.3022	
		24hours	.16000	.11411	.640	-.2156	.5356	
		72hours	.03333	.11411	.998	-.3422	.4089	
		96hours	-1.16000*	.11411	.000	-1.5356	-.7844	
72hours		0hour	.89333*	.11411	.000	.5178	1.2689	
		24hours	.12667	.11411	.798	-.2489	.5022	
		48hours	-.03333	.11411	.998	-.4089	.3422	
		96hours	-1.19333*	.11411	.000	-1.5689	-.8178	
96hours		0hour	2.08667*	.11411	.000	1.7111	2.4622	
		24hours	1.32000*	.11411	.000	.9444	1.6956	
		48hours	1.16000*	.11411	.000	.7844	1.5356	
		72hours	1.19333*	.11411	.000	.8178	1.5689	
Scerevisiae_C15		0hour	24hours	-.74000*	.06786	.000	-.9633	-.5167
			48hours	-.87000*	.06786	.000	-1.0933	-.6467
			72hours	-.85000*	.06786	.000	-1.0733	-.6267
			96hours	-1.94667*	.06786	.000	-2.1700	-1.7233
		24hours	0hour	.74000*	.06786	.000	.5167	.9633
			48hours	-.13000	.06786	.369	-.3533	.0933
			72hours	-.11000	.06786	.517	-.3333	.1133
			96hours	-1.20667*	.06786	.000	-1.4300	-.9833
	48hours	0hour	.87000*	.06786	.000	.6467	1.0933	
		24hours	.13000	.06786	.369	-.0933	.3533	
		72hours	.02000	.06786	.998	-.2033	.2433	
		96hours	-1.07667*	.06786	.000	-1.3000	-.8533	
	72hours	0hour	.85000*	.06786	.000	.6267	1.0733	
		24hours	.11000	.06786	.517	-.1133	.3333	
		48hours	-.02000	.06786	.998	-.2433	.2033	
		96hours	-1.09667*	.06786	.000	-1.3200	-.8733	
	96hours	0hour	1.94667*	.06786	.000	1.7233	2.1700	
		24hours	1.20667*	.06786	.000	.9833	1.4300	
		48hours	1.07667*	.06786	.000	.8533	1.3000	
		72hours	1.09667*	.06786	.000	.8733	1.3200	

*. The mean difference is significant at the 0.05 level.

F.11.2 Statistical analysis of microbiological analysis of black tea water kefir beverage with added carrot juice samples at 96 hours of fermentation

Descriptives

Lactococcus_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	3	5.7900	.02646	.01528	5.7243	5.8557	5.77	5.82
carro10%	3	5.8633	.01528	.00882	5.8254	5.9013	5.85	5.88
carrot15%	3	5.8167	.08505	.04910	5.6054	6.0279	5.73	5.90
Total	9	5.8233	.05545	.01848	5.7807	5.8660	5.73	5.90

Test of Homogeneity of Variances

Lactococcus_96hours

Levene Statistic	df1	df2	Sig.
2.328	2	6	.179

ANOVA

Lactococcus_96hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.008	2	.004	1.518	.293
Within Groups	.016	6	.003		
Total	.025	8			

Multiple Comparisons

Dependent Variable: Lactococcus_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carro10%	-.07333	.04260	.273	-.2040	.0574
	carrot15%	-.02667	.04260	.812	-.1574	.1040
carro10%	carrot5%	.07333	.04260	.273	-.0574	.2040
	carrot15%	.04667	.04260	.551	-.0840	.1774
carrot15%	carrot5%	.02667	.04260	.812	-.1040	.1574
	carro10%	-.04667	.04260	.551	-.1774	.0840

Lactococcus_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
carrot5%	3	5.7900
carrot15%	3	5.8167
carro10%	3	5.8633
Sig.		.273

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Descriptives

Lactobacillus_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	3	5.9100	.03000	.01732	5.8355	5.9845	5.88	5.94
carro10%	3	6.2033	.14364	.08293	5.8465	6.5602	6.04	6.31
carrot15%	3	6.2600	.05292	.03055	6.1286	6.3914	6.22	6.32
Total	9	6.1244	.18042	.06014	5.9858	6.2631	5.88	6.32

ANOVA

Test of Homogeneity of Variances

Lactobacillus_96hours

Levene Statistic	df1	df2	Sig.
5.516	2	6	.044

Lactobacillus_96hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.212	2	.106	13.053	.007
Within Groups	.049	6	.008		
Total	.260	8			

Multiple Comparisons

Dependent Variable: Lactobacillus_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carro10%	-.29333*	.07354	.017	-.5190	-.0677
	carrot15%	-.35000*	.07354	.007	-.5756	-.1244
carro10%	carrot5%	.29333*	.07354	.017	.0677	.5190
	carrot15%	-.05667	.07354	.733	-.2823	.1690
carrot15%	carrot5%	.35000*	.07354	.007	.1244	.5756
	carro10%	.05667	.07354	.733	-.1690	.2823

*. The mean difference is significant at the 0.05 level.

Lactobacillus_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
carrot5%	3	5.9100	
carro10%	3		6.2033
carrot15%	3		6.2600
Sig.		1.000	.733

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Ranks

Lactobacillus_96hours	Treatment	N	Mean Rank
	carrot5%		3
carro10%		3	6.33
carrot15%		3	6.67
Total		9	

Test Statistics^{a,b}

Lactobacillus_96hours	
Chi-Square	5.422
df	2
Asymp. Sig.	.066

a. Kruskal-Wallis Test

b. Grouping Variable: Treatment

Descriptives

Scerevisiae_96hours

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
carrot5%	3	7.3733	.03215	.01856	7.2935	7.4532	7.35	7.41
carro10%	3	7.6100	.13856	.08000	7.2658	7.9542	7.53	7.77
carrot15%	3	7.5500	.15875	.09165	7.1557	7.9443	7.37	7.67
Total	9	7.5111	.15070	.05023	7.3953	7.6270	7.35	7.77

ANOVA

Scerevisiae_96hours

Test of Homogeneity of Variances				Sum of Squares	df	Mean Square	F	Sig.
Scerevisiae_96hours	Levene Statistic	df1	df2					
	4.106	2	6	.091	2	.045	2.999	.125
				.091	6	.015		
				.182	8			

Multiple Comparisons

Dependent Variable: Scerevisiae_96hours

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
carrot5%	carro10%	-.23667	.10048	.123	-.5450	.0716
	carrot15%	-.17667	.10048	.261	-.4850	.1316
carro10%	carrot5%	.23667	.10048	.123	-.0716	.5450
	carrot15%	.06000	.10048	.827	-.2483	.3683
carrot15%	carrot5%	.17667	.10048	.261	-.1316	.4850
	carro10%	-.06000	.10048	.827	-.3683	.2483

Scerevisiae_96hours

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
carrot5%	3	7.3733
carrot15%	3	7.5500
carro10%	3	7.6100
Sig.		.123

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

F.12.1 Statistical analysis of sensory evaluation of black tea water kefir beverage with added carrot juice samples

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Appearance	carrot5%	60	5.9667	1.30146	.16802	5.6305	6.3029	3.00	8.00
	carrot10%	60	6.6500	.95358	.12311	6.4037	6.8963	5.00	8.00
	carrot15%	60	6.7833	1.41531	.18272	6.4177	7.1489	3.00	9.00
	Total	180	6.4667	1.28333	.09565	6.2779	6.6554	3.00	9.00
Odour	carrot5%	60	6.2667	1.36378	.17806	5.9144	6.6190	3.00	9.00
	carrot10%	60	6.0667	1.40056	.18081	5.7049	6.4285	2.00	8.00
	carrot15%	60	5.6167	1.24997	.16137	5.2938	5.9396	2.00	9.00
	Total	180	5.9833	1.35974	.10135	5.7833	6.1833	2.00	9.00
Flavour	carrot5%	60	5.7667	1.36998	.17686	5.4128	6.1206	2.00	9.00
	carrot10%	60	6.5333	1.24147	.16027	6.2126	6.8540	4.00	9.00
	carrot15%	60	5.8667	1.47828	.19085	5.4848	6.2485	2.00	9.00
	Total	180	6.0556	1.40121	.10444	5.8495	6.2616	2.00	9.00
Sourness	carrot5%	60	5.8500	1.35077	.17438	5.5011	6.1989	4.00	8.00
	carrot10%	60	6.1667	1.35505	.17494	5.8166	6.5167	3.00	9.00
	carrot15%	60	6.0333	1.56191	.20164	5.6299	6.4368	2.00	9.00
	Total	180	6.0167	1.42396	.10614	5.8072	6.2261	2.00	9.00
Sweetness	carrot5%	60	6.0867	1.62467	.20974	5.6470	6.4864	2.00	9.00
	carrot10%	60	6.5833	1.60815	.20761	6.1679	6.9988	2.00	9.00
	carrot15%	60	6.2667	1.53895	.19868	5.8691	6.6642	3.00	9.00
	Total	180	6.3056	1.59642	.11899	6.0708	6.5404	2.00	9.00
Overall_acceptability	carrot5%	60	5.5333	1.09648	.14155	5.2501	5.8166	3.00	7.00
	carrot10%	60	6.6333	1.17843	.15213	6.3289	6.9378	3.00	9.00
	carrot15%	60	6.0500	1.26792	.16369	5.7225	6.3775	3.00	8.00
	Total	180	6.0722	1.25974	.09390	5.8869	6.2575	3.00	9.00

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Appearance	2.321	2	177	.101
Odour	.259	2	177	.772
Flavour	.596	2	177	.552
Sourness	.986	2	177	.375
Sweetness	.181	2	177	.834
Overall_acceptability	.515	2	177	.598

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	23.033	2	11.517	7.501	.001
	Within Groups	271.767	177	1.535		
	Total	294.800	179			
Odour	Between Groups	13.300	2	6.650	3.705	.027
	Within Groups	317.650	177	1.795		
	Total	330.950	179			
Flavour	Between Groups	20.844	2	10.422	5.580	.004
	Within Groups	330.600	177	1.868		
	Total	351.444	179			
Sourness	Between Groups	3.033	2	1.517	.746	.476
	Within Groups	359.917	177	2.033		
	Total	362.950	179			
Sweetness	Between Groups	8.144	2	4.072	1.609	.203
	Within Groups	448.050	177	2.531		
	Total	456.194	179			
Overall_acceptability	Between Groups	36.344	2	18.172	12.985	.000
	Within Groups	247.717	177	1.400		
	Total	284.061	179			

Appearance

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
carrot5%	60	5.9667	
carrot10%	60		6.6500
carrot15%	60		6.7833
Sig.		1.000	.826

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Odour

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
carrot15%	60	5.6167	
carrot10%	60	6.0667	6.0667
carrot5%	60		6.2667
Sig.		.160	.693

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Flavour

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05	
		1	2
carrot5%	60	5.7667	
carrot15%	60	5.8667	
carrot10%	60		6.5333
Sig.		.915	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Sourness

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
carrot5%	60	5.8500
carrot15%	60	6.0333
carrot10%	60	6.1667
Sig.		.445

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Sweetness

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05
		1
carrot5%	60	6.0667
carrot15%	60	6.2667
carrot10%	60	6.5833
Sig.		.180

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Overall_acceptability

Tukey HSD^a

Treatment	N	Subset for alpha = 0.05		
		1	2	3
carrot5%	60	5.5333		
carrot15%	60		6.0500	
carrot10%	60			6.6333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 60.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Appearance	carrot5%	carrot10%	-.68333*	.22623	.008	-1.2181	-.1486
		carrot15%	-.81667*	.22623	.001	-1.3514	-.2819
	carrot10%	carrot5%	.68333*	.22623	.008	.1486	1.2181
		carrot15%	-.13333	.22623	.826	-.6681	.4014
	carrot15%	carrot5%	.81667*	.22623	.001	.2819	1.3514
		carrot10%	.13333	.22623	.826	-.4014	.6681
Odour	carrot5%	carrot10%	.20000	.24458	.693	-.3781	.7781
		carrot15%	.65000*	.24458	.023	.0719	1.2281
	carrot10%	carrot5%	-.20000	.24458	.693	-.7781	.3781
		carrot15%	.45000	.24458	.160	-.1281	1.0281
	carrot15%	carrot5%	-.65000*	.24458	.023	-1.2281	-.0719
		carrot10%	-.45000	.24458	.160	-1.0281	.1281
Flavour	carrot5%	carrot10%	-.76667*	.24952	.007	-1.3564	-.1769
		carrot15%	-.10000	.24952	.915	-.6898	.4898
	carrot10%	carrot5%	.76667*	.24952	.007	.1769	1.3564
		carrot15%	.66667*	.24952	.022	.0769	1.2564
	carrot15%	carrot5%	.10000	.24952	.915	-.4898	.6898
		carrot10%	-.66667*	.24952	.022	-1.2564	-.0769
Sourness	carrot5%	carrot10%	-.31667	.26035	.445	-.9320	.2987
		carrot15%	-.18333	.26035	.761	-.7987	.4320
	carrot10%	carrot5%	.31667	.26035	.445	-.2987	.9320
		carrot15%	.13333	.26035	.866	-.4820	.7487
	carrot15%	carrot5%	.18333	.26035	.761	-.4320	.7987
		carrot10%	-.13333	.26035	.866	-.7487	.4820
Sweetness	carrot5%	carrot10%	-.51667	.29048	.180	-1.2032	.1699
		carrot15%	-.20000	.29048	.771	-.8866	.4866
	carrot10%	carrot5%	.51667	.29048	.180	-.1699	1.2032
		carrot15%	.31667	.29048	.521	-.3699	1.0032
	carrot15%	carrot5%	.20000	.29048	.771	-.4866	.8866
		carrot10%	-.31667	.29048	.521	-1.0032	.3699
Overall_acceptability	carrot5%	carrot10%	-1.10000*	.21599	.000	-1.6105	-.5895
		carrot15%	-.51667*	.21599	.047	-1.0272	-.0062
	carrot10%	carrot5%	1.10000*	.21599	.000	.5895	1.6105
		carrot15%	.58333*	.21599	.021	.0728	1.0938
	carrot15%	carrot5%	.51667*	.21599	.047	.0062	1.0272
		carrot10%	-.58333*	.21599	.021	-1.0938	-.0728

*. The mean difference is significant at the 0.05 level.

F.13. Statistical analysis of sugars, organic acids, and antioxidants content of black tea water kefir beverage with added 10% carrot juice samples during 96 hours fermentation

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Sucrose	0hour	6	9.6833	.52033	.21243	9.1373	10.2294	9.28	10.56
	24hours	6	8.0217	.86756	.35418	7.1112	8.9321	6.31	8.64
	48hours	6	5.0183	.41378	.16893	4.5841	5.4526	4.24	5.37
	72hours	6	1.3850	.21575	.08808	1.1586	1.6114	1.11	1.59
	96hours	6	1.6217	.07910	.03229	1.5387	1.7047	1.53	1.75
	Total	30	5.1460	3.41837	.62411	3.8696	6.4224	1.11	10.56
Glucose	0hour	6	.4583	.13227	.05400	.3195	.5971	.25	.64
	24hours	6	.7383	.19974	.08154	.5287	.9479	.48	1.01
	48hours	6	.8767	.06186	.02525	.8117	.9416	.82	.97
	72hours	6	.9717	.02639	.01078	.9440	.9994	.94	1.00
	96hours	6	.9000	.19246	.07857	.6980	1.1020	.64	1.13
	Total	30	.7890	.22640	.04134	.7045	.8735	.25	1.13
Fructose	0hour	6	.3400	.15007	.06126	.1825	.4975	.19	.62
	24hours	6	.9717	.17058	.06964	.7927	1.1507	.73	1.16
	48hours	6	1.4667	.14292	.05835	1.3167	1.6167	1.32	1.61
	72hours	6	1.5833	.16657	.06800	1.4085	1.7981	1.46	1.89
	96hours	6	.9300	.12063	.04933	.8032	1.0568	.73	1.06
	Total	30	1.0583	.47219	.08621	.8920	1.2347	.19	1.89

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Sucrose	Between Groups	.332.635	4	83.159	333.321	.000
	Within Groups	6.237	25	.249		
	Total	338.872	29			
Glucose	Between Groups	.992	4	.248	12.527	.000
	Within Groups	.495	25	.020		
	Total	1.486	29			
Fructose	Between Groups	5.894	4	1.474	64.408	.000
	Within Groups	.572	25	.023		
	Total	6.466	29			

Sucrose

Tukey HSD^a

Subset for alpha = 0.05					
Fermentation_time	N	1	2	3	4
72hours	6	1.3850			
96hours	6	1.6217			
48hours	6		5.0183		
24hours	6			8.0217	
0hour	6				9.6833
Sig.		.922	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Glucose

Tukey HSD^a

Subset for alpha = 0.05			
Fermentation_time	N	1	2
0hour	6	.4583	
24hours	6		.7383
48hours	6		.8767
96hours	6		.9000
72hours	6		.9717
Sig.		1.000	.057

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Fructose

Tukey HSD^a

Subset for alpha = 0.05				
Fermentation_time	N	1	2	3
0hour	6	.3400		
96hours	6		.9300	
24hours	6		.9717	
48hours	6			1.4667
72hours	6			1.5833
Sig.		1.000	.969	.672

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Fermentation_time	(J) Fermentation_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Sucrose	0hour	24hours	1.66167 [*]	.28838	.000	.8147	2.5086	
		48hours	4.66500 [*]	.28838	.000	3.8181	5.5119	
		72hours	8.29833 [*]	.28838	.000	7.4514	9.1453	
		96hours	8.06167 [*]	.28838	.000	7.2147	8.9086	
	24hours	0hour	-1.66167 [*]	.28838	.000	-2.5086	-.8147	
		48hours	3.00333 [*]	.28838	.000	2.1564	3.8503	
		72hours	6.63667 [*]	.28838	.000	5.7897	7.4836	
		96hours	6.40000 [*]	.28838	.000	5.5531	7.2469	
	48hours	0hour	-4.66500 [*]	.28838	.000	-5.5119	-3.8181	
		24hours	-3.00333 [*]	.28838	.000	-3.8503	-2.1564	
		72hours	3.63333 [*]	.28838	.000	2.7864	4.4803	
		96hours	3.39667 [*]	.28838	.000	2.5497	4.2436	
	72hours	0hour	-8.29833 [*]	.28838	.000	-9.1453	-7.4514	
		24hours	-6.63667 [*]	.28838	.000	-7.4836	-5.7897	
		48hours	-3.63333 [*]	.28838	.000	-4.4803	-2.7864	
		96hours	-.23667	.28838	.922	-1.0836	.6103	
	96hours	0hour	-8.06167 [*]	.28838	.000	-8.9086	-7.2147	
		24hours	-6.40000 [*]	.28838	.000	-7.2469	-5.5531	
		48hours	-3.39667 [*]	.28838	.000	-4.2436	-2.5497	
		72hours	.23667	.28838	.922	-.6103	1.0836	
	Glucose	0hour	24hours	-.28000 [*]	.08122	.016	-.5185	-.0415
			48hours	-.41833 [*]	.08122	.000	-.8569	-.1798
			72hours	-.51333 [*]	.08122	.000	-.7519	-.2748
			96hours	-.44167 [*]	.08122	.000	-.6802	-.2031
24hours		0hour	.28000 [*]	.08122	.016	.0415	.5185	
		48hours	-.13833	.08122	.450	-.3769	1.002	
		72hours	-.23333	.08122	.057	-.4719	.0052	
		96hours	-.16167	.08122	.299	-.4002	.0769	
48hours		0hour	.41833 [*]	.08122	.000	.1798	.6569	
		24hours	.13833	.08122	.450	-.1002	.3769	
		72hours	-.09500	.08122	.768	-.3335	.1435	
		96hours	-.02333	.08122	.998	-.2619	.2152	
72hours		0hour	.51333 [*]	.08122	.000	.2748	.7519	
		24hours	.23333	.08122	.057	-.0052	.4719	
		48hours	.09500	.08122	.768	-.1435	.3335	
		96hours	.07167	.08122	.901	-.1669	.3102	
96hours		0hour	.44167 [*]	.08122	.000	.2031	.6802	
		24hours	.16167	.08122	.299	-.0769	.4002	
		48hours	.02333	.08122	.998	-.2152	.2619	
		72hours	-.07167	.08122	.901	-.3102	.1669	
Fructose		0hour	24hours	-.63167 [*]	.08733	.000	-.8881	-.3752
			48hours	-1.12667 [*]	.08733	.000	-1.3831	-.8702
			72hours	-1.24333 [*]	.08733	.000	-1.4998	-.9869
			96hours	-.59000 [*]	.08733	.000	-.8465	-.3335
	24hours	0hour	.63167 [*]	.08733	.000	.3752	.8881	
		48hours	-.49500 [*]	.08733	.000	-.7515	-.2385	
		72hours	-.61167 [*]	.08733	.000	-.8681	-.3552	
		96hours	.04167	.08733	.989	-.2148	.2981	
	48hours	0hour	1.12667 [*]	.08733	.000	.8702	1.3831	
		24hours	.49500 [*]	.08733	.000	.2385	.7515	
		72hours	-.11667	.08733	.672	-.3731	.1398	
		96hours	.53667 [*]	.08733	.000	.2802	.7931	
	72hours	0hour	1.24333 [*]	.08733	.000	.9869	1.4998	
		24hours	.61167 [*]	.08733	.000	.3552	.8681	
		48hours	.11667	.08733	.672	-.1398	.3731	
		96hours	.65333 [*]	.08733	.000	.3869	.9098	
	96hours	0hour	.59000 [*]	.08733	.000	.3335	.8465	
		24hours	-.04167	.08733	.989	-.2981	.2148	
		48hours	-.53667 [*]	.08733	.000	-.7931	-.2802	
		72hours	-.65333 [*]	.08733	.000	-.9098	-.3869	

* The mean difference is significant at the 0.05 level.

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean				
					Lower Bound	Upper Bound	Minimum	Maximum	
Lactic_acid									
0hour	6	.0000	.00000	.00000	.0000	.0000	.000	.001	
24hours	6	.15350	.021211	.028659	.10124	.15776	.122	.172	
48hours	6	.22700	.032845	.013400	.19253	.26147	.178	.277	
72hours	6	.23617	.022436	.038159	.21282	.29971	.216	.272	
96hours	6	.25483	.040012	.016335	.21284	.29682	.198	.323	
Total	30	.17430	.038539	.017591	.13751	.21109	.000	.323	
Acetic_acid									
0hour	6	.00000	.00000	.00000	.0000	.0000	.000	.001	
24hours	6	.07817	.010285	.034101	.06739	.08894	.067	.099	
48hours	6	.08717	.001835	.005749	.08524	.08909	.085	.089	
72hours	6	.08993	.015788	.038653	.07222	.10748	.060	.119	
96hours	6	.14933	.026949	.015102	.12005	.17861	.115	.199	
Total	30	.08070	.020136	.029153	.06199	.09942	.000	.188	

Lactic_acid

TukeyHSD^a

Fermentation_time	N	Subset for alpha = 0.05		
		1	2	3
0hour	6	.00000		
24hours	6		.15350	
48hours	6			.22700
72hours	6			.23617
96hours	6			.25483
Sig.		1.000	1.000	.422

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactic_acid	Between Groups	.263	4	.066	90.634	.000
	Within Groups	.018	25	.001		
	Total	.282	29			
Acetic_acid	Between Groups	.067	4	.017	75.341	.000
	Within Groups	.006	25	.000		
	Total	.073	29			

Acetic_acid

TukeyHSD^a

Fermentation_time	N	Subset for alpha = 0.05		
		1	2	3
0hour	6	.00000		
24hours	6		.07817	
48hours	6			.08717
72hours	6			.08993
96hours	6			.14933
Sig.		1.000	.002	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000

Multiple Comparisons

Tukey HSD

Dependent Variable	(i) Fermentation_time	(j) Fermentation_time	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Lactic_acid	0hour	24hours	-153500*	015563	.000	-19921	-10779	
		48hours	-227000*	015563	.000	-27271	-18129	
		72hours	-236167*	015563	.000	-28187	-19046	
		96hours	-254833*	015563	.000	-30054	-20913	
	24hours	0hour	153500*	015563	.000	10779	19921	
		48hours	-073500*	015563	.001	-11921	-02779	
		72hours	-082667*	015563	.000	-12837	-03696	
		96hours	-101333*	015563	.000	-14704	-05563	
	48hours	0hour	227000*	015563	.000	18129	27271	
		24hours	073500*	015563	.001	02779	11921	
		72hours	-009167	015563	.975	-05487	03854	
		96hours	-027833	015563	.402	-07354	01787	
	72hours	0hour	236167*	015563	.000	19046	28187	
		24hours	082667*	015563	.000	03696	12837	
		48hours	009167	015563	.975	-03854	05487	
		96hours	-018667	015563	.752	-06437	02704	
	96hours	0hour	254833*	015563	.000	20913	30054	
		24hours	101333*	015563	.000	05563	14704	
		48hours	027833	015563	.402	-01787	07354	
		72hours	018667	015563	.752	-02704	06437	
	Acetic_acid	0hour	24hours	-078167*	008629	.000	-10351	-05283
			48hours	-087167*	008629	.000	-11251	-06183
			72hours	-089833*	008629	.000	-11517	-06449
			96hours	-148333*	008629	.000	-17367	-12299
24hours		0hour	078167*	008629	.000	05283	10351	
		48hours	-009000	008629	.833	-03434	01634	
		72hours	-011667	008629	.662	-03701	01367	
		96hours	-070167*	008629	.000	-09551	-04483	
48hours		0hour	087167*	008629	.000	06183	11251	
		24hours	009000	008629	.833	-01634	03434	
		72hours	-002667	008629	.998	-02801	02267	
		96hours	-061167*	008629	.000	-08851	-03583	
72hours		0hour	089833*	008629	.000	06449	11517	
		24hours	011667	008629	.662	-01367	03701	
		48hours	002667	008629	.998	-02267	02801	
		96hours	-058500*	008629	.000	-08384	-03316	
96hours		0hour	148333*	008629	.000	12299	17367	
		24hours	070167*	008629	.000	04483	09551	
		48hours	061167*	008629	.000	03583	08851	
		72hours	058500*	008629	.000	03316	08384	

* The mean difference is significant at the 0.05 level

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Gallic_acid	30	10.8557	1.28004	9.33	13.64
EGC	30	9.4860	2.59333	4.55	12.77
EGCG	30	4.3867	1.96047	1.29	7.11
ECG	30	5.8857	4.19886	1.28	13.38
Theobromine	30	7.4053	.61442	6.28	8.65
Caffeine	30	87.1873	6.84203	74.33	100.46
Fermentation_time	30	3.0000	1.43839	1.00	5.00

Test Statistics^{a,b}

	Gallic_acid	EGC	EGCG	ECG	Theobromine	Caffeine
Chi-Square	24.769	26.832	22.838	22.457	22.384	22.800
df	4	4	4	4	4	4
Asymp. Sig.	.000	.000	.000	.000	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: Fermentation_time

Ranks

	Fermentation_time	N	Mean Rank
Gallic_acid	ohour	6	27.50
	24hours	6	11.00
	48hours	6	12.50
	72hours	6	21.50
	96hours	6	5.00
	Total	30	
	EGC	ohour	6
24hours		6	26.00
48hours		6	23.00
72hours		6	15.50
96hours		6	3.50
Total		30	
EGCG		ohour	6
	24hours	6	10.08
	48hours	6	21.50
	72hours	6	27.50
	96hours	6	11.00
	Total	30	
	ECG	ohour	6
24hours		6	9.00
48hours		6	23.00
72hours		6	26.00
96hours		6	12.50
Total		30	
Theobromine		ohour	6
	24hours	6	14.92
	48hours	6	15.50
	72hours	6	16.08
	96hours	6	3.50
	Total	30	
	Caffeine	ohour	6
24hours		6	13.50
48hours		6	16.00
72hours		6	17.00
96hours		6	3.50
Total		30	

F.14.1 Statistical analysis of pH, titratable acidity, and totals soluble solids of black tea water kefir beverage with added 10% carrot juice samples during refrigerated storage

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
day0	6	3.9767	.09771	.03989	3.8741	4.0792	3.88	4.10
day7	6	3.8750	.13982	.05708	3.7283	4.0217	3.72	4.05
day14	6	3.7517	.09988	.04078	3.6468	3.8565	3.67	3.88
day21	6	3.7183	.09517	.03885	3.6185	3.8182	3.65	3.85
day28	6	3.6767	.10073	.04112	3.5710	3.7824	3.59	3.82
Total	30	3.7997	.15060	.02749	3.7434	3.8559	3.59	4.10

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
.434	4	25	.783

Multiple Comparisons

Dependent Variable: pH_duringstorage
Tukey HSD

(I) Storage_time	(J) Storage_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
day0	day7	-.10167	.06233	.492	-.0814	.2847
	day14	-.22500*	.06233	.011	-.0419	.4081
	day21	-.25833*	.06233	.003	-.0753	.4414
	day28	-.30000*	.06233	.001	-.1169	.4831
day7	day0	.10167	.06233	.492	-.2847	.0814
	day14	.12333	.06233	.305	-.0597	.3064
	day21	.15667	.06233	.120	-.0264	.3397
	day28	.19833*	.06233	.029	.0153	.3814
day14	day0	.22500*	.06233	.011	-.4081	-.0419
	day7	.12333	.06233	.305	-.3064	.0597
	day21	.03333	.06233	.983	-.1497	.1164
	day28	-.07500	.06233	.750	-.1081	.2581
day21	day0	-.25833*	.06233	.003	-.4414	-.0753
	day7	-.15667	.06233	.120	-.3397	.0264
	day14	-.03333	.06233	.983	-.2164	.1497
	day28	.04167	.06233	.961	-.1414	.2247
day28	day0	-.30000*	.06233	.001	-.4831	-.1169
	day7	-.19833*	.06233	.029	-.3814	-.0153
	day14	-.07500	.06233	.750	-.2581	.1081
	day21	-.04167	.06233	.961	-.2247	.1414

*. The mean difference is significant at the 0.05 level.

pH_duringstorage

Tukey HSD^a

Storage_time	N	Subset for alpha = 0.05		
		1	2	3
day28	6	3.6767		
day21	6	3.7183	3.7183	
day14	6	3.7517	3.7517	
day7	6		3.8750	3.8750
day0	6			3.9767
Sig.		.750	.120	.492

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Kruskal-Wallis Test

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
TA_duringstorage	30	.43110	.042038	.342	.486
Storage_time	30	3.0000	1.43839	1.00	5.00

Ranks

	Storage_time	N	Mean Rank
TA_duringstorage	day0	6	4.83
	day7	6	9.00
	day14	6	17.67
	day21	6	22.50
	day28	6	23.50
Total		30	

Test Statistics^{a,b}

	TA_duringstor age
Chi-Square	21.464
df	4
Asymp. Sig.	.000

a. Kruskal Wallis Test

b. Grouping Variable:
Storage_time

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
day0	6	7.1000	.17889	.07303	6.9123	7.2877	6.90	7.30
day7	6	6.9000	.08944	.03651	6.8061	6.9939	6.80	7.00
day14	6	6.4500	.13784	.05627	6.3053	6.5947	6.30	6.60
day21	6	6.1167	.13292	.05426	5.9772	6.2562	5.90	6.20
day28	6	5.7000	.17889	.07303	5.5123	5.8877	5.50	5.90
Total	30	6.4533	.53610	.09788	6.2532	6.6535	5.50	7.30

ANOVA

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
.759	4	25	.562

TTS_duringstorage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.791	4	1.948	89.624	.000
Within Groups	.543	25	.022		
Total	8.335	29			

Multiple Comparisons

Dependent Variable: TTS_duringstorage
Tukey HSD

(I) Storage_time	(J) Storage_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
day0	day7	.2000	.09511	.163	-.0500	.4500
	day14	.6500	.08511	.000	.4000	.9000
	day21	.98333	.08511	.000	.7334	1.2333
	day28	1.4000	.08511	.000	1.1500	1.6500
day7	day0	-.2000	.09511	.163	-.4500	.0500
	day14	-.4500	.08511	.000	-.7000	-.2000
	day21	-.78333	.08511	.000	-.8334	-.7333
	day28	-1.2000	.08511	.000	-.9500	-1.4500
day14	day0	-.6500	.08511	.000	-.9000	-.4000
	day7	-.4500	.08511	.000	-.7000	-.2000
	day21	-.33333	.08511	.005	-.6834	-.5833
	day28	.7500	.08511	.000	.5000	1.0000
day21	day0	-.98333	.08511	.000	-1.2333	-.7334
	day7	-.78333	.08511	.000	-1.0333	-.5334
	day14	-.33333	.08511	.005	-.5833	-.0834
	day28	.41667	.08511	.000	.1667	.6666
day28	day0	-1.4000	.08511	.000	-1.6500	-.1500
	day7	-1.2000	.08511	.000	-1.4500	-.9500
	day14	-.7500	.08511	.000	-1.0000	-.5000
	day21	-.41667	.08511	.000	-.6666	-.1667

*. The mean difference is significant at the 0.05 level.

TTS_duringstorage

Tukey HSD^a

Storage_time	N	Subset for alpha = 0.05			
		1	2	3	4
day28	6	5.7000			
day21	6		6.1167		
day14	6			6.4500	
day7	6				6.9000
day0	6				7.1000
Sig.		1.000	1.000	1.000	.163

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000

F.14.2 Statistical analysis of colour (L*, a*, b*) of black tea water kefir beverage with added 10% carrot juice samples during refrigerated storage

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
Colour_L_duringstorage	day0	6	20.1550	2.66290	.84217	17.9901	22.3199	16.24	22.76
	day7	6	13.8183	1.98208	.60918	11.7383	15.8984	12.10	16.33
	day14	6	11.7000	1.43192	.58458	10.1973	13.2027	10.33	13.52
	day21	6	10.2417	1.50215	.61325	8.6651	11.8181	9.05	12.23
	day28	6	9.9017	1.49232	.60924	8.3356	11.4678	8.79	11.88
	Total	30	13.1633	4.14130	.75609	11.6169	14.7097	8.79	22.76
Colour_a_duringstorage	day0	6	14.7000	.80187	.32990	13.7749	15.6251	13.67	15.88
	day7	6	12.7983	1.17346	.47906	11.5689	14.0298	11.02	14.33
	day14	6	11.9567	1.29074	.52777	10.4972	13.4162	10.68	13.72
	day21	6	11.3600	1.16946	.47743	10.1327	12.5873	10.05	13.70
	day28	6	11.2100	1.19470	.48774	9.9962	12.4638	9.67	12.56
	Total	30	12.4050	1.69455	.30938	11.7722	13.0378	9.87	15.88
Colour_b_duringstorage	day0	6	23.9700	.23074	.09420	23.7279	24.2121	23.72	24.29
	day7	6	22.0900	.11950	.04879	21.9646	22.2154	21.93	22.29
	day14	6	20.2850	.50500	.20617	19.7550	20.8150	19.61	20.69
	day21	6	19.3250	.81581	.25145	18.6786	19.9714	18.53	19.85
	day28	6	18.8900	.47510	.19295	18.3914	19.3886	18.22	19.62
	Total	30	20.9120	1.95707	.35791	20.1812	21.6428	18.22	24.29

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Colour_L_duringstorage	.805	4	25	.533
Colour_a_duringstorage	.632	4	25	.644
Colour_b_duringstorage	5.176	4	25	.004

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour_L_duringstorage	Between Groups	423.778	4	105.944	35.881	.000
	Within Groups	73.598	25	2.944		
	Total	497.381	29			
Colour_a_duringstorage	Between Groups	46.957	4	11.739	8.873	.000
	Within Groups	34.416	25	1.377		
	Total	81.373	29			
Colour_b_duringstorage	Between Groups	106.435	4	26.609	143.425	.000
	Within Groups	4.638	25	.185		
	Total	111.073	29			

Colour_L_duringstorage

Tukey HSD^a

Subset for alpha = 0.05				
Storage_time	N	1	2	3
day28	6	9.9017		
day21	6	10.2417		
day14	6	11.7000	11.7000	
day7	6		13.8183	
day0	6			20.1550
Sig.		.387	.236	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_a_duringstorage

Tukey HSD^a

Subset for alpha = 0.05			
Storage_time	N	1	2
day28	6	11.2100	
day21	6	11.3600	
day14	6	11.9567	
day7	6	12.7983	12.7983
day0	6		14.7000
Sig.		.164	.066

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Colour_b_duringstorage

Tukey HSD^a

Subset for alpha = 0.05					
Storage_time	N	1	2	3	4
day28	6	18.8900			
day21	6	19.3250			
day14	6		20.2850		
day7	6			22.0900	
day0	6				23.9700
Sig.		.424	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Test Statistics^{a,b}

Colour_b_duringstorage	
Chi-Square	25.548
df	4
Asymp. Sig.	.000

a. Kruskal-Wallis Test

b. Grouping Variable:
Storage_time

Ranks

	Storage_time	N	Mean Rank
Colour_b_duringstorage	day0	6	27.50
	day7	6	21.50
	day14	6	14.50
	day21	6	8.50
	day28	6	5.50
Total		30	

Multiple Comparisons

Tukey HSD

Dependant Variable	(I) Storage_time	(J) Storage_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Colour_l_duringstorage	day0	day7	6.33667*	.99056	.000	3.4275	9.2458	
		day14	8.45500*	.99056	.000	5.5459	11.3641	
		day21	9.91333*	.99056	.000	7.0042	12.8225	
		day28	10.25333*	.99056	.000	7.3442	13.1625	
	day7	day0	-6.33667*	.99056	.000	-9.2458	-3.4275	
		day14	-2.11833	.99056	.236	-.7908	5.0275	
		day21	-3.57667*	.99056	.011	-.6675	6.4658	
		day28	-3.91667*	.99056	.005	1.0075	6.8258	
	day14	day0	-8.45500*	.99056	.000	-11.3641	-5.5459	
		day7	-2.11833	.99056	.236	-5.0275	.7908	
		day21	-1.45833	.99056	.589	-1.4508	4.3675	
		day28	-1.79833	.99056	.387	-1.1108	4.7075	
	day21	day0	-9.91333*	.99056	.000	-12.8225	-7.0042	
		day7	-3.57667*	.99056	.011	-6.4658	-.6675	
		day14	-1.45833	.99056	.589	-4.3675	1.4508	
		day28	-.34000	.99056	.997	-2.5691	3.2491	
	day28	day0	-10.25333*	.99056	.000	-13.1625	-7.3442	
		day7	-3.91667*	.99056	.005	-6.8258	-1.0075	
		day14	-1.79833	.99056	.387	-4.7075	1.1108	
		day21	-.34000	.99056	.997	-3.2491	2.5691	
	Colour_a_duringstorage	day0	day7	1.90167	.67741	.066	-.0878	3.8911
			day14	2.74333*	.67741	.004	.7539	4.7328
			day21	3.34000*	.67741	.000	1.3505	5.3295
			day28	3.49000*	.67741	.000	1.5005	5.4795
day7		day0	-1.90167	.67741	.066	-3.8911	.0878	
		day14	.84167	.67741	.727	-1.1478	2.8311	
		day21	1.43833	.67741	.242	-.5511	3.4278	
		day28	1.58833	.67741	.164	-.4011	3.5778	
day14		day0	-2.74333*	.67741	.004	-4.7328	-.7539	
		day7	-.84167	.67741	.727	-2.8311	1.1478	
		day21	-.59667	.67741	.901	-1.3928	2.5861	
		day28	-.74667	.67741	.804	-1.2428	2.7361	
day21		day0	-3.34000*	.67741	.000	-5.3295	-1.3505	
		day7	-1.43833	.67741	.242	-3.4278	.5511	
		day14	-.59667	.67741	.901	-2.5861	1.3928	
		day28	-.15000	.67741	.999	-1.8395	2.1395	
day28		day0	-3.49000*	.67741	.000	-5.4795	-1.5005	
		day7	-1.58833	.67741	.164	-3.5778	.4011	
		day14	-.74667	.67741	.804	-2.7361	1.2428	
		day21	-1.50000	.67741	.999	-2.1395	1.8395	
Colour_b_duringstorage		day0	day7	1.88000*	.24868	.000	1.1497	2.6103
			day14	3.68500*	.24868	.000	2.9547	4.4153
			day21	4.64500*	.24868	.000	3.9147	5.3753
			day28	5.08000*	.24868	.000	4.3497	5.8103
	day7	day0	-1.88000*	.24868	.000	-2.6103	-1.1497	
		day14	1.80500*	.24868	.000	1.0747	2.5353	
		day21	2.76500*	.24868	.000	2.0347	3.4953	
		day28	3.20000*	.24868	.000	2.4697	3.9303	
	day14	day0	-3.68500*	.24868	.000	-4.4153	-2.9547	
		day7	-1.80500*	.24868	.000	-2.5353	-1.0747	
		day21	.96000*	.24868	.006	.2297	1.6903	
		day28	1.39500*	.24868	.000	.6647	2.1253	
	day21	day0	-4.64500*	.24868	.000	-5.3753	-3.9147	
		day7	-2.76500*	.24868	.000	-3.4953	-2.0347	
		day14	-.96000*	.24868	.006	-1.6903	-.2297	
		day28	-.43500	.24868	.424	-.2953	1.1653	
	day28	day0	-5.08000*	.24868	.000	-5.8103	-4.3497	
		day7	-3.20000*	.24868	.000	-3.9303	-2.4697	
		day14	-1.39500*	.24868	.000	-2.1253	-.6647	
		day21	-.43500	.24868	.424	-1.1653	.2953	

* The mean difference is significant at the 0.05 level.

F.14.3 Statistical analysis of microbiological analysis of black tea water kefir beverage with added 10% carrot juice samples during refrigerated storage

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lactococcus_duringstorage	day0	6	5.9050	.28077	.11462	5.6104	6.1996	5.58	6.26
	day7	6	5.8083	.26173	.11808	5.5022	6.1145	5.54	6.18
	day14	6	5.7600	.26374	.10767	5.4832	6.0368	5.53	6.13
	day21	6	5.2617	.42088	.17182	4.8200	5.7033	4.89	5.93
	day28	6	4.7217	.04401	.01797	4.6755	4.7679	4.66	4.77
	Total	30	5.4913	.52511	.09567	5.2953	5.6874	4.66	6.26
Lactobacillus_duringstorage	day0	6	6.0800	.08654	.03841	5.9787	6.1813	5.87	6.20
	day7	6	5.9317	.15258	.06228	5.7716	6.0917	5.73	6.15
	day14	6	5.8517	.18888	.06845	5.6733	6.0301	5.61	5.98
	day21	6	4.9933	.07005	.02860	4.9108	5.0868	4.80	5.07
	day28	6	4.7100	.16458	.06718	4.5373	4.8927	4.48	4.92
	Total	30	5.8133	.57611	.10518	5.5982	5.7285	4.48	6.20
S_cerevisiae_duringstorage	day0	6	7.3767	.05855	.02421	7.3142	7.4392	7.31	7.46
	day7	6	7.3167	.08501	.03471	7.2275	7.4059	7.20	7.42
	day14	6	7.1917	.01941	.00792	7.1713	7.2120	7.16	7.21
	day21	6	7.1300	.07127	.02910	7.0552	7.2048	7.04	7.28
	day28	6	7.0233	.08831	.02789	6.9516	7.0950	6.92	7.12
	Total	30	7.2077	.14248	.02601	7.1545	7.2609	6.92	7.46

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Lactococcus_duringstorage	5.711	4	25	.002
Lactobacillus_duringstorage	2.020	4	25	.122
S_cerevisiae_duringstorage	2.805	4	25	.047

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactococcus_duringstorage	Between Groups	.594	4	.1468	17.878	.000
	Within Groups	2.003	20	.080		
	Total	2.597	24			
Lactobacillus_duringstorage	Between Groups	8.158	4	2.0395	122.485	.000
	Within Groups	.467	20	.023		
	Total	8.625	24			
S_cerevisiae_duringstorage	Between Groups	.484	4	.121	28.967	.000
	Within Groups	.104	20	.004		
	Total	.588	24			

Lactococcus_duringstorage

Tukey HSD^a

Subset for alpha = 0.05

Storage_time	N	1	2	3		
day28	6	4.7217				
day21	6		5.2617			
day14	6			5.7600		
day7	6				5.8083	
day0	6					5.9050
Sig.		1.000	1.000	.904		

Laetobacillus_duringstorage

Tukey HSD^a

Subset for alpha = 0.05

Storage_time	N	1	2	3		
day28	6	4.7100				
day21	6		4.9933			
day14	6			5.0517		
day7	6				5.9317	
day0	6					6.0800
Sig.		1.000	1.000	.055		

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 8.000.

S_cerevisiae_duringstorage

Tukey HSD^a

Subset for alpha = 0.05

Storage_time	N	1	2	3		
day28	6	7.0233				
day21	6		7.1300			
day14	6			7.1917		
day7	6				7.3167	
day0	6					7.3767
Sig.		.659	.486	.507		

Ranks

	Storage_time	N	Mean Rank
Lactococcus_duringstorage	day0	6	22.92
	day7	6	19.83
	day14	6	18.42
	day21	6	12.83
	day28	6	3.58
	Total	30	
S_cerevisiae_duringstorage	day0	6	26.83
	day7	6	22.67
	day14	6	14.50
	day21	6	10.17
	day28	6	4.33
	Total	30	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

Test Statistics^{a,b}

	Lactococcus_duringstorage	S_cerevisiae_duringstorage
Chi-Square	18.074	24.241
df	4	4
Asymp. Sig.	.001	.000

a. Kruskal Wallis Test
b. Grouping Variable: Storage_time

Multiple Comparisons

Tukey HSD

Dependent Variable	I) Storage_time	J) Storage_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Lactococcus_duringstorage	day0	day7	.09667	.16584	.976	-.3904	.5837	
		day14	1.4500	.16584	.904	-.3421	.6321	
		day21	.64333*	.16584	.006	.1563	1.1304	
		day28	1.18333*	.16584	.000	.6963	1.6704	
	day7	day0	-.09667	.16584	.976	-.5837	.3904	
		day14	.04833	.16584	.998	-.4387	.5354	
		day21	.54667*	.16584	.022	.0596	1.0337	
		day28	1.08667*	.16584	.000	.5996	1.5737	
	day14	day0	-.14500	.16584	.904	-.6321	.3421	
		day7	-.04833	.16584	.998	-.5354	.4387	
		day21	.49833*	.16584	.043	.0113	.9854	
		day28	1.03833*	.16584	.000	.5513	1.5254	
	day21	day0	-.64333*	.16584	.006	-1.1304	-.1563	
		day7	-.54667*	.16584	.022	-1.0337	-.0596	
		day14	-.49833*	.16584	.043	-.9854	-.0113	
		day28	.54000*	.16584	.025	.0529	1.0271	
	day28	day0	-1.18333*	.16584	.000	-1.6704	-.6963	
		day7	-1.08667*	.16584	.000	-1.5737	-.5996	
		day14	-1.03833*	.16584	.000	-1.5254	-.5513	
		day21	-.54000*	.16584	.025	-1.0271	-.0529	
	Lactobacillus_duringstorage	day0	day7	1.4833	.07893	.354	-.0835	.3802
			day14	2.2833	.07893	.055	-.0035	.4602
			day21	1.08667*	.07893	.000	.8548	1.3185
			day28	1.37000*	.07893	.000	1.1382	1.6018
day7		day0	-.14833	.07893	.354	-.3802	.0835	
		day14	.08000	.07893	.847	-.1518	.3118	
		day21	.93833*	.07893	.000	.7065	1.1702	
		day28	1.22167*	.07893	.000	.9898	1.4535	
day14		day0	-.22833	.07893	.055	-.4602	.0035	
		day7	-.08000	.07893	.847	-.3118	.1518	
		day21	.85833*	.07893	.000	.6265	1.0902	
		day28	1.14167*	.07893	.000	.9098	1.3735	
day21		day0	-1.08667*	.07893	.000	-1.3185	-.8548	
		day7	-.93833*	.07893	.000	-1.1702	-.7065	
		day14	-.85833*	.07893	.000	-1.0902	-.6265	
		day28	.28333	.07893	.011	.0515	.5152	
day28		day0	-1.37000*	.07893	.000	-1.6018	-1.1382	
		day7	-1.22167*	.07893	.000	-1.4535	-.9898	
		day14	-1.14167*	.07893	.000	-1.3735	-.9098	
		day21	-.28333*	.07893	.011	-.5152	-.0515	
S_cerevisiae_duringstorage		day0	day7	.06000	.03732	.507	-.0496	.1696
			day14	1.6500*	.03732	.000	.0754	.2946
			day21	2.4667*	.03732	.000	.1370	.3563
			day28	.35333	.03732	.000	.2437	.4630
	day7	day0	-.06000	.03732	.507	-.1696	.0496	
		day14	1.2500*	.03732	.020	.0154	.2346	
		day21	1.8667*	.03732	.000	.0770	.2963	
		day28	.29333	.03732	.000	.1837	.4030	
	day14	day0	-.18500*	.03732	.000	-.2946	-.0754	
		day7	-.12500*	.03732	.020	-.2346	-.0154	
		day21	.06187	.03732	.480	-.0480	.1713	
		day28	1.6833*	.03732	.001	.0587	.2780	
	day21	day0	-.24667*	.03732	.000	-.3563	-.1370	
		day7	-.18667*	.03732	.000	-.2963	-.0770	
		day14	-.06187	.03732	.480	-.1713	.0480	
		day28	1.0667	.03732	.059	-.0030	.2163	
	day28	day0	-.35333*	.03732	.000	-.4630	-.2437	
		day7	-.29333*	.03732	.000	-.4030	-.1837	
		day14	-.16833*	.03732	.001	-.2780	-.0587	
		day21	-1.0667	.03732	.059	-.2163	.0030	

* The mean difference is significant at the 0.05 level.

F.14.4 Statistical analysis of sugars, organic acids, and antioxidants of black tea water kefir beverage with added 10% carrot juice samples during refrigerated storage

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Sucrose_duringstorage	30	.5757	.58891	.07	1.75
Glucose_duringstorage	30	1.3693	.31281	.64	1.82
Fructose_duringstorage	30	1.9930	.83185	.73	3.34
Storage_time	30	3.0000	1.43839	1.00	5.00

Ranks

	Storage_time	N	Mean Rank
Sucrose_duringstorage	day0	6	27.50
	day7	6	21.50
	day14	6	15.50
	day21	6	9.50
	day28	6	3.50
	Total	30	
Glucose_duringstorage	day0	6	3.50
	day7	6	9.50
	day14	6	22.17
	day21	6	21.83
	day28	6	20.50
	Total	30	
Fructose_duringstorage	day0	6	3.50
	day7	6	9.50
	day14	6	19.00
	day21	6	18.75
	day28	6	26.75
	Total	30	

Test Statistics^{a,b}

	Sucrose_duringstorage	Glucose_duringstorage	Fructose_duringstorage
Chi-Square	28.033	22.427	25.517
df	4	4	4
Asymp. Sig.	.000	.000	.000

a. Kruskal Wallis Test

b. Grouping Variable: Storage_time

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lactic_duringstorage	day0	6	.25483	.040012	.016335	.21284	.29682	.199	.323
	day7	6	.22800	.033864	.013825	.19246	.26354	.186	.290
	day14	6	.22583	.030551	.012472	.19377	.25789	.195	.265
	day21	6	.21717	.061545	.025126	.15258	.28175	.154	.299
	day28	6	.20200	.017539	.007160	.18359	.22041	.181	.222
	Total	30	.22557	.040605	.007413	.21040	.24073	.154	.323
Acetic_duringstorage	day0	6	.14833	.026949	.011002	.12005	.17661	.115	.188
	day7	6	.15200	.035889	.014652	.11434	.18966	.111	.210
	day14	6	.15333	.047580	.019424	.10340	.20327	.092	.200
	day21	6	.12600	.046943	.019164	.07674	.17526	.056	.167
	day28	6	.11433	.029480	.012035	.08340	.14527	.078	.147
	Total	30	.13880	.039034	.007127	.12422	.15338	.056	.210

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Lactic_duringstorage	1.992	4	25	.127
Acetic_duringstorage	1.558	4	25	.216

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactic_duringstorage	Between Groups	.009	4	.002	1.436	.252
	Within Groups	.039	25	.002		
	Total	.048	29			
Acetic_duringstorage	Between Groups	.007	4	.002	1.264	.310
	Within Groups	.037	25	.001		
	Total	.044	29			

Lactic_duringstorage

Tukey HSD^a

Storage_time	N	Subset for alpha = 0.05
day28	6	.20200
day21	6	.21717
day14	6	.22583
day7	6	.22800
day0	6	.25483
Sig.		.172

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Acetic_duringstorage

Tukey HSD^a

Storage_time	N	Subset for alpha = 0.05
day28	6	.11433
day21	6	.12600
day0	6	.14833
day7	6	.15200
day14	6	.15333
Sig.		.417

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Storage_time	(J) Storage_time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
Lactic_duringstorage	day0	day7	.026833	.022769	.763	-.04004	.09370	
		day14	.029000	.022769	.709	-.03787	.09587	
		day21	.037667	.022769	.479	-.02920	.10454	
		day28	.052833	.022769	.172	-.01404	.11970	
	day7	day0	-.026833	.022769	.763	-.09370	.04004	
		day14	.002167	.022769	1.000	-.06470	.06904	
		day21	.010833	.022769	.989	-.05604	.07770	
		day28	.026000	.022769	.783	-.04087	.09287	
	day14	day0	-.029000	.022769	.709	-.09587	.03787	
		day7	-.002167	.022769	1.000	-.06904	.06470	
		day21	.008667	.022769	.995	-.05820	.07554	
		day28	.023833	.022769	.831	-.04304	.09070	
	day21	day0	-.037667	.022769	.479	-.10454	.02920	
		day7	-.010833	.022769	.989	-.07770	.05604	
		day14	-.008667	.022769	.995	-.07554	.05820	
		day28	.015167	.022769	.962	-.05170	.08204	
	day28	day0	-.052833	.022769	.172	-.11970	.01404	
		day7	-.026000	.022769	.783	-.09287	.04087	
		day14	-.023833	.022769	.831	-.09070	.04304	
		day21	-.015167	.022769	.962	-.08204	.05170	
	Acetic_duringstorage	day0	day7	-.003667	.022137	1.000	-.06868	.06135
			day14	-.005000	.022137	.999	-.07001	.06001
			day21	.022333	.022137	.849	-.04268	.08735
			day28	.034000	.022137	.550	-.03101	.09901
		day7	day0	.003667	.022137	1.000	-.06135	.06868
			day14	-.001333	.022137	1.000	-.06635	.06368
			day21	.026000	.022137	.765	-.03901	.09101
			day28	.037667	.022137	.451	-.02735	.10268
day14		day0	.005000	.022137	.999	-.06001	.07001	
		day7	.001333	.022137	1.000	-.06368	.06635	
		day21	.027333	.022137	.732	-.03768	.09235	
		day28	.039000	.022137	.417	-.02601	.10401	
day21		day0	-.022333	.022137	.849	-.08735	.04268	
		day7	-.026000	.022137	.765	-.09101	.03901	
		day14	-.027333	.022137	.732	-.09235	.03768	
		day28	.011667	.022137	.984	-.05335	.07668	
day28		day0	-.034000	.022137	.550	-.09901	.03101	
		day7	-.037667	.022137	.451	-.10268	.02735	
		day14	-.039000	.022137	.417	-.10401	.02601	
		day21	-.011667	.022137	.984	-.07668	.05335	

Kruskal-Wallis Test

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Gallic_duringstorage	30	9.2777	1.45041	7.03	12.10
EGC_duringstorage	30	4.6297	1.30425	2.57	6.54
EGCG_duringstorage	30	2.3493	.92916	1.12	4.77
ECG_duringstorage	30	2.3047	1.42837	1.36	6.42
Theobromine_duringstorage	30	6.3910	1.03490	4.87	8.29
Caffeine_duringstorage	30	75.5460	11.80882	56.35	96.01
Storage_time	30	3.8000	1.43839	1.00	5.00

Test Statistics^{a,b}

	Gallic_duringstorage	EGC_duringstorage	EGCG_duringstorage	ECG_duringstorage	Theobromine_duringstorage	Caffeine_duringstorage
Chi-Square	5.095	779	2.404	10.163	8.503	4.727
df	4	4	4	4	4	4
Asymp. Sig.	.278	.941	.662	.038	.165	.316

a. Kruskal-Wallis Test

b. Grouping Variable: Storage_time

Ranks

	Storage_time	N	Mean Rank
Gallic_duringstorage	day0	6	19.00
	day7	6	10.92
	day14	6	12.00
	day21	6	15.58
	day28	6	20.00
	Total	30	
EGC_duringstorage	day0	6	17.00
	day7	6	13.42
	day14	6	16.75
	day21	6	16.08
	day28	6	14.25
	Total	30	
EGCG_duringstorage	day0	6	17.00
	day7	6	12.92
	day14	6	18.50
	day21	6	16.92
	day28	6	12.17
	Total	30	
ECG_duringstorage	day0	6	19.92
	day7	6	9.83
	day14	6	11.00
	day21	6	13.75
	day28	6	23.00
	Total	30	
Theobromine_duringstorage	day0	6	19.92
	day7	6	10.50
	day14	6	11.08
	day21	6	16.08
	day28	6	19.92
	Total	30	
Caffeine_duringstorage	day0	6	18.50
	day7	6	10.50
	day14	6	12.50
	day21	6	16.33
	day28	6	19.67
	Total	30	

F.14.5 Statistical analysis of sensory evaluation of black tea water kefir beverage with added 10% carrot juice during refrigerated storage

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Appearance	day7	30	7.3667	1.06620	19468	6.9685	7.7548	3.00	8.00
	day14	30	7.2000	1.06350	19417	6.9029	7.5971	5.00	9.00
	day21	30	7.0333	.96431	17606	6.6733	7.3934	5.00	9.00
	day28	30	5.9000	1.15520	21091	5.4686	6.3314	3.00	8.00
	Total	120	6.8750	1.19918	10947	6.6592	7.0918	3.00	9.00
Odour	day7	30	6.6667	1.50707	27515	5.5038	6.6294	4.00	8.00
	day14	30	6.5000	1.22474	22391	6.0427	6.9573	5.00	9.00
	day21	30	5.6000	.93218	17019	5.2519	5.9481	4.00	7.00
	day28	30	4.7000	1.29055	23562	4.2181	5.1819	2.00	7.00
	Total	120	5.7167	1.40535	12866	5.4819	5.9714	2.00	9.00
Flavour	day7	30	6.9333	1.77984	32495	6.2687	7.5979	2.00	9.00
	day14	30	7.0667	1.43679	26232	6.5302	7.6032	4.00	9.00
	day21	30	5.4333	1.59056	29040	4.9394	6.0273	2.00	7.00
	day28	30	4.7667	1.33089	24299	4.2697	5.2636	2.00	7.00
	Total	120	6.0500	1.81428	16562	5.7221	6.3779	2.00	9.00
Sourness	day7	30	6.7667	1.16511	21272	6.3316	7.2017	4.00	9.00
	day14	30	6.7333	.94443	17243	6.3807	7.0860	5.00	8.00
	day21	30	5.3333	1.44636	26407	4.7933	5.8734	2.00	7.00
	day28	30	5.1000	1.42272	25975	4.5687	5.6313	2.00	7.00
	Total	120	5.9833	1.46662	13388	5.7182	6.2484	2.00	9.00
Sweetness	day7	30	6.7333	1.72073	31416	6.0908	7.3759	2.00	9.00
	day14	30	6.4667	1.27937	23358	5.9889	6.9444	5.00	8.00
	day21	30	4.9033	.78462	14331	4.6402	5.2264	3.00	6.00
	day28	30	4.3000	.91539	16713	3.9582	4.6418	2.00	6.00
	Total	120	5.6083	1.58933	14509	5.3210	5.8956	2.00	9.00
Overall acceptability	day7	30	7.1333	.97320	17768	6.7699	7.4967	5.00	8.00
	day14	30	6.9000	1.29588	23659	6.4161	7.3839	3.00	9.00
	day21	30	4.8667	1.35762	24790	4.3596	5.3737	3.00	7.00
	day28	30	4.6333	.96431	17606	4.2733	4.9934	3.00	7.00
	Total	120	5.8933	1.62016	14790	5.5905	6.1762	3.00	9.00

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	39.692	3	13.231	11.677	.000
	Within Groups	121.433	116	1.033		
	Total	171.125	119			
Odour	Between Groups	63.550	3	17.833	11.312	.000
	Within Groups	182.967	116	1.578		
	Total	238.367	119			
Flavour	Between Groups	115.233	3	38.411	16.117	.000
	Within Groups	278.467	116	2.383		
	Total	391.700	119			
Sourness	Between Groups	71.367	3	23.789	14.849	.000
	Within Groups	184.600	116	1.591		
	Total	255.967	119			
Sweetness	Between Groups	125.092	3	41.697	27.561	.000
	Within Groups	175.560	116	1.513		
	Total	300.592	119			
Overall acceptability	Between Groups	155.767	3	51.922	38.461	.000
	Within Groups	156.600	116	1.350		
	Total	312.367	119			

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Appearance	1.089	3	116	.357
Odour	2.505	3	116	.063
Flavour	.639	3	116	.592
Sourness	2.363	3	116	.075
Sweetness	8.775	3	116	.000
Overall acceptability	1.317	3	116	.272

Appearance

Tukey HSD ^a			
Storage period	N	Subset for alpha = 0.05	
		1	2
day28	30	5.9000	
day21	30		7.0333
day14	30		7.2000
day7	30		7.3667
Sig.		1.000	.620

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 30.000.

Odour

Tukey HSD ^a				
Storage period	N	Subset for alpha = 0.05		
		1	2	3
day28	30	4.7000		
day21	30		5.6000	
day7	30		6.0667	6.0667
day14	30		6.5000	
Sig.		1.000	.477	.542

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 30.000.

Flavour

Tukey HSD ^a			
Storage period	N	Subset for alpha = 0.05	
		1	2
day28	30	4.7000	
day21	30	5.7333	
day7	30		6.9333
day14	30		7.0667
Sig.		.343	.987

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 30.000.

Sourness				Sweetness			
Tukey HSD ^a				Tukey HSD ^a			
Storage_period	N	Subset for alpha = 0.05		Storage_period	N	Subset for alpha = 0.05	
		1	2			1	2
day28	30	5.1000		day28	30	4.3000	
day7	30	6.3333		day7	30	4.8333	
day14	30		8.7333	day14	30		6.4000
day7	30		6.7667	day7	30		6.7333
Sig.		.890	1.000	Sig.		.196	.035

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000

Overall_acceptability			
Tukey HSD ^a			
Storage_period	N	Subset for alpha = 0.05	
		1	2
day28	30	4.6333	
day7	30	4.0889	
day14	30		6.9000
day7	30		7.1333
Sig.		.064	.064

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000

Multiple Comparisons							
Tukey HSD							
Dependent Variable	(I) Storage_period	(J) Storage_period	Mean Difference (I - J)			95% Confidence Interval	
			Mean Diff.	Sig.	Lower Bound	Upper Bound	
Appearance	day7	day4	.8887	.2749	.038	-.5497	.9681
		day21	.3333	.2749	.028	-.8891	1.0487
		day28	1.46667	.2749	.008	.7903	2.1831
	day14	day7	-.8887	.2749	.038	-.8891	.5497
		day21	.8887	.2749	.038	-.5497	.9681
		day28	1.30000	.2749	.008	.5838	2.0164
	day21	day7	-.3333	.2749	.038	-.9497	.3031
		day14	-.8887	.2749	.038	-.8891	.5497
		day28	1.13333	.2749	.008	.4108	1.8497
	day28	day7	-1.46667	.2749	.008	-2.1831	-.7503
		day14	-1.30000	.2749	.008	-2.0164	-.5838
		day21	-1.13333	.2749	.008	-1.8497	-.4108
Bitter	day7	day4	-.4333	.3249	.542	-1.2784	.4117
		day21	.46667	.3249	.477	-.3764	1.3117
		day28	1.36667	.3249	.006	.5216	2.2117
	day14	day7	-.4333	.3249	.542	-.4117	1.2784
		day21	.90000	.3249	.032	.0650	1.7450
		day28	1.80000	.3249	.008	.9058	2.6450
	day21	day7	-.46667	.3249	.477	-.9117	.3784
		day14	-.90000	.3249	.032	-1.7450	-.0950
		day28	.90000	.3249	.032	.0650	1.7450
	day28	day7	-1.36667	.3249	.006	-2.2117	-.5216
		day14	-1.80000	.3249	.008	-2.6450	-.9550
		day21	-1.90000	.3249	.032	-1.7450	-.0950
Fruity	day7	day4	-.3333	.3889	.887	-1.1724	.5057
		day21	1.00000	.3889	.001	.4610	2.5390
		day28	2.16667	.3889	.008	1.1276	3.2057
	day14	day7	-.3333	.3889	.887	-.9057	1.1724
		day21	1.03333	.3889	.008	.5943	2.6724
		day28	2.30000	.3889	.008	1.2610	3.3390
	day21	day7	-.50000	.3889	.001	-2.5080	-.4610
		day14	-1.03333	.3889	.008	-2.6724	-.5943
		day28	.88887	.3889	.343	-.3724	1.7057
	day28	day7	-2.16667	.3889	.008	-3.2057	-1.1276
		day14	-2.30000	.3889	.008	-3.3380	-1.2610
		day21	-.88887	.3889	.243	-1.7057	.3724
Sourness	day7	day4	.8333	.3252	1.000	.6157	.8824
		day21	1.43333	.3252	.008	.5848	2.2824
		day28	1.66667	.3252	.008	.6178	2.6157
	day14	day7	-.8333	.3252	1.000	-.8824	.6157
		day21	1.40000	.3252	.008	.5510	2.2480
		day28	1.63333	.3252	.008	.7848	2.4824
	day21	day7	-1.43333	.3252	.008	-2.2804	-.5843
		day14	-1.40000	.3252	.008	-2.2480	-.5510
		day28	-.2333	.3252	.888	-.6157	1.0624
	day28	day7	-1.66667	.3252	.008	-2.6157	-.8178
		day14	-1.63333	.3252	.008	-2.4824	-.7843
		day21	-.23333	.3252	.888	-.6157	1.0624
Sweetness	day7	day14	-.2889	.3179	.839	-.5612	1.0945
		day21	1.80000	.3179	.008	.8722	2.6278
		day28	2.43333	.3179	.008	1.6885	3.2612
	day14	day7	-.2889	.3179	.839	-.5612	1.0945
		day21	1.53333	.3179	.008	.7055	2.3612
		day28	2.16667	.3179	.008	1.3288	2.9945
	day21	day7	-1.80000	.3179	.008	-2.6278	-.9722
		day14	-1.53333	.3179	.008	-2.3612	-.7055
		day28	-.8333	.3179	.188	-.5612	1.4612
	day28	day7	-2.43333	.3179	.008	-3.2612	-1.6055
		day14	-2.16667	.3179	.008	-2.9945	-1.3288
		day21	-.8333	.3179	.188	-.5612	1.4612
Overall_acceptability	day7	day21	.2333	.3000	.864	-.5487	1.0153
		day28	2.26667	.3000	.008	1.4947	3.0487
		day28	2.50000	.3000	.008	1.1180	3.2820
	day14	day7	-.2333	.3000	.864	-1.0153	.5487
		day21	2.03333	.3000	.008	1.2019	2.8153
		day28	2.26667	.3000	.008	1.4947	3.0487
	day21	day7	-2.26667	.3000	.008	-3.0487	-1.4847
		day14	-2.0333	.3000	.008	-2.8153	-1.2019
		day28	.2333	.3000	.864	-.5487	1.0153
	day28	day7	-2.50000	.3000	.008	-3.2820	-1.7100
		day14	-2.26667	.3000	.008	-3.0487	-1.4847
		day21	-.2333	.3000	.864	-1.0153	.5487

* The mean difference is significant at the 0.05 level.