Fermentation of Water Kefir Beverage Containing *Ziziphus Jujuba* Mill. Syrup

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DEDICATION

This thesis is dedicated to my mother and father,
Zhen Chen and Chunjing Mu,
and my husband, Yangyi Liu.

Thank you all for helping to give me the life I love today.

ABSTRACT

Water kefir is a self-carbonated, low sugar beverage with a mildly sour taste fermented by a microbial multispecies of kefir dominated by lactic acid bacteria (LAB) and yeasts. The fermented beverage is popular due to its pleasant sensory characteristics and perceived health benefits. The presence of probiotics and antioxidants in the water kefir confer health benefits to consumers when consumed in sufficient amounts. The major antioxidant in jujube fruit is rutin which is related to the reducing of high-density lipoprotein cholesterol and fasting blood sugar in patients with diabetes mellitus.

The fermented beverage (water kefir) is produced mainly at household and by small-scale artisans whereby sucrose is normally added as a source of carbon for the fermentation. Due to consumer demand for foods containing low calories and carbohydrates, there is an incentive to produce products with reduced amount of added sugar including fermented water kefir. Jujube contains a high amount of sugars. This study investigated the potential of using syrup extracted from *Ziziphus jujuba* Mill. (jujube) to partially replace added sucrose used for water kefir fermentation.

Several water-bath methods were investigated for the extraction of the jujube syrup. The most efficient method consisted of 650 mL extraction water and the mixture (jujube and water) was extracted at 70°C. The syrup obtained by this method was subjected to further studies. Two concentrations of the jujube syrup (10%, 20%, v/v) and two fermentation temperatures (25°C, 27°C) were used for the jujube water kefir fermentation. Various analyses and measurements were conducted on the beverage during fermentation and storage (4°C). The beverages were analysed for sugar, acidity, antioxidants, titratable acid, while pH, colour, and total soluble solids were measured. Microbiological analyses of the beverages were also conducted. The beverages were subjected to sensory evaluation by an informal focus group and by consumer sensory panellists using a 9-point hedonic rating scale.

The beverage with higher syrup concentration (%, v/v) contained higher total soluble solids and was darker than the sample containing a lower concentration of syrup ($p<0.05$). By the end of the fermentation period (72 h), the beverage with higher syrup concentration had higher cell counts of LAB and yeast. No differences ($p>0.05$) were observed between the total soluble solids of the beverages fermented at 25°C and 27°C. The fermented (27°C) jujube water kefir beverage (2.5% organic raw sugar, w/v; 20% jujube syrup, v/v for stage 1 fermentation) with added jujube syrup (20% v/v) in stage 2 was selected as the most promising formulation by consumer sensory panellists. At the end of the fermentation (72 h), the selected beverage contained ethanol (3.37±0.13% v/v), sucrose (0.17±0.03% w/v), glucose (0.92±0.14% w/v), fructose (1.44±0.08% w/v), lactic acid (0.14±0.00% w/v), acetic acid (0.37±0.02% w/v), and rutin (6.26±0.16% w/v). The high concentration of ethanol may be attributed to the conversion of lactic acid into ethanol by the LAB. After storage for 21 days (4°C), yeast counts had decreased ($p<0.05$) while LAB counts had decreased by about one log. The concentrations of the sugars and acetic acid had decreased whereas the concentrations of ethanol, rutin and lactic acid increased. Meanwhile, the overall acceptability sensory scores of the beverage had decreased after storage for 21 days (4°C). The results of this study indicated the potential of producing a low sugar jujube water kefir using reduced added sugar and jujube syrup. However, more research is required to reduce the ethanol content of the beverage to meet the requirement for low alcohol product in New Zealand. Also, further research is required to improve the stability of the beverage during refrigerated storage.

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LIST OF ABBREVIATIONS

a*	Redness-greeness
AAB	Acetic acid bacteria
ABTS	2,2’-azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid
Acs	Acetyl-CoA synthetase
Adh	Alcohol dehydrogenase
Ald	Acetaldehyde dehydrogenase
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
ATP	Adenosine triphosphate
b*	Yellowness-blueness
CFU	Colony forming per Unit
d	Day
DJ	Dried jujube
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Epicatechin
EMP	Emden-Meyerhoff-Parnas
EPS	Exopolysaccharides
FAO	Food and Agriculture Organization
FBS	Fasting blood sugar
FRAP	Ferric-reducing antioxidant power analysis
FSANZ	Food Standards Australia New Zealand
g	Gram
GC	Gas chromatography
h	Hour
HDL	High-density lipoprotein cholesterol
HepG	Human hepatocellular carcinoma cells

HePS	Heteropolysaccharides
HoPS	Homopolysaccharides
HPLC	High performance liquid chromatography
IBD	Inflammentory bowel disease
L	Litre
L*	Lightness
LAB	Lactic acid bacteria
LDH	Lactate dehydrogenase
M	Molarity
min	Minute
mL	Millilitre
mm	Millimetre
MRS	de Man, Rogosa and Sharpe
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydride
PCR	Polymerase chain reaction
Pdc	Pyruvate decarboxylase
Pdh	Pyruvate-dehydrogenase complex
PKP	Phosphoketolase pathway
S.	*Saccharomyces*
SD	Standard deviation
spp.	species (plural)
T.A.	Titratable acidity
TCA	Tricarboxylic acid
TSS	Total soluble solid
V	Volume
WHO	World Health Organization
YGC	Yeast Glucose Chloramphenicol

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Chapter 1. INTRODUCTION

1.1 Background

Fermentation, the oldest food preservation method after drying, has been used as a biotechnology to improve the shelf-life of the food products and sensory characteristics for more than 6000 years (Bhalla, 2016). The increasing popularity of fermented food is also attributed to their improved nutritional value (Charalampopoulos & Webb, 2013; Nair & Prajapati, 2008). The term "fermentation" is derived from the Latin word, *fevere*, which means "to ferment" (Tekluu, Gebremariam, Aregai, & Saripalli, 2015). From a biochemical perspective, fermentation is defined as a metabolic process that derives energy from organic compounds such as carbohydrates without the engagement of the exogenous oxidising agent (Arasaradnam et al., 2009; Ray & Joshi, 2014).

Fermented foods originated several thousand years ago when microorganisms were incidentally introduced into foods, and the Indian subcontinent appears to be the origin of the art of the technology (Bhalla, 2016; Nair & Prajapati, 2008). The roles of fermentation include: (1) preserving food by reducing water activity and producing inhibitory metabolites (organic acid, carbon dioxide, ethanol) (Gaggia, Di Gioia, Baffoni, & Biavati, 2011); (2) improving food safety by detoxifying and inhibiting pathogens (Ray & Joshi, 2014); (3) biologically enriching the nutritional value with vitamins, proteins, essential amino acids and fatty acids (Steinkraus, 1995); (4) improving the sensory quality of the food by developing diverse aromas, flavours, and textures (Sicard & Legras, 2011; Steinkraus, 1995); (5) naturally improving the digestibility of food due to the presence of probiotics (Hasan, Sultan, & Mar-E-Um, 2014); and (6) decreasing fuel requirement and cooking times (Steinkraus, 1995). Therefore, fermentation makes the foods diverse and palatable by changing sensory properties as well as enhancing the aroma and flavour (Campbell-Platt, 1994; Hasan et al., 2014). Moreover, fermented foods improve the availability of vitamins and essential amino acids, which can be more nutritious than the unfermented counterparts (Hasan et al., 2014).

Currently, there are different categories of fermented products across the world including dairy, meat, seafood, cereal, legume, and fruit and vegetable (Abu-Ghannam & Rajauria, 2015; Bhalla, 2016; Josephsen & Jespersen, 2004). Due to lactose-intolerance as well as the unfavourable cholesterol in fermented dairy products, there is an increasing demand for non-dairy fermented products (Ranadheera, Baines, & Adams, 2010). Apart from lactose-intolerance, the ongoing trend of vegetarianism worldwide and the requirement for the cold-storage environment also contributed to the development of vegetarian fermented products (Abu-Ghannam & Rajauria, 2015; Ranadheera et al., 2010; Soccol et al., 2010). Vegetarian fermented products can be divided into several groups based on the raw material. Bread, *dosa*, soy sauce, *sufu* are cereal- and legume-based fermented foods which are important sources of proteins and carbohydrates to the diet. *Gari* and *fufu* are fermented plant root products traditionally made in the West African countries. Sauerkraut, pickled vegetables, olives and *kimchi* come under the fruit- and vegetable-based fermentation category (Abu-Ghannam & Rajauria, 2015; Hansen, 2002; Nair & Prajapati, 2008).

Among the non-dairy fermented products, there are different varieties of non-dairy fermented beverages made from botanical sources such as cereals, legumes, fruits and vegetables (Vasudha & Mishra, 2013). Non-dairy beverages are not new, as there are several traditional products available around the world including *bushera*, *haria*, *mahewu*, *hardaliye*, and kombucha (Kandylis, Pissaridi, Bekatorou, Kanellaki, & Koutinas, 2016). Recently, a non-dairy fermented beverage, water kefir, has generated interest in countries such as USA, France, Brazil, and Japan due to its health benefits and sensory profile (Farnworth, 2006; Sarkar, 2007; Zhou, Liu, Jiang, & Dong, 2009).

Water kefir, also known as sugary kefir, is a self-carbonated beverage produced by fermenting sugar solution with kefir grains which consist of microorganisms and polysaccharides (Davidović, Miljković, Antonović, Rajilić-Stojanović, & Dimitrijević-Branković, 2015; Randazzo et al., 2016). The microbial population of water kefir include mainly lactic acid bacteria (LAB) (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*), acetic acid bacteria (AAB) (*Acetobacter*) and yeasts (*Saccharomyces*, *Candida*, *Zygosaccharomyces*) (Gulitz, Stadie, Wenning, Ehrmann, & Vogel, 2011; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013; Miguel, Cardoso, Magalhães, & Schwan, 2011). Some microbial strains of the starter cultures of water

kefir beverages have been reported to have probiotic functions (Gulitz et al., 2011; Magalhaes, Pereira, Dias, & Schwan, 2010). Moreover, organic acids, exopolysaccharides, polypeptides and other compounds produced by the microorganism in water kefir also help to improve health (Koh, Utra, Ahmad, Rather, & Park, 2018). Health benefits such as anti-inflammatory, antioxidant, antimicrobial, and healing activities have been attributed to the consumption of water kefir beverages (Alsayadi, Al Jawfi, Belarbi, & Sabri, 2013; Moreira et al., 2008; Rodrigues et al., 2016; Rodrigues, Caputo, Carvalho, Evangelista, & Schneedorf, 2005). Therefore, consumers can benefit from consuming water kefir beverage (Fiorda et al., 2017).

Traditionally, water kefir is fermented with sugar solution at the first stage of fermentation, and dried fruit or fruit juice is added mainly for flavour at the second stage (Koh et al., 2017; Randazzo et al., 2016). Recently, alternative water kefir beverages have been developed such as "*Tapache*" which is fermented with brown sugar, pineapple and cinnamon, and "*Kefir d'uva*", a grape-based kefir beverage (Fiorda et al., 2017). Water kefir beverages can also be prepared with vegetables such as ginger, carrots, onions and fennels (Fiorda et al., 2017; Koh et al., 2017; Miguel et al., 2011). However, there are limited studies on fermentation of water kefir beverage using natural fruit syrup. Due to its high sugar content, the natural fruit syrup can not only be added to supplement the carbon source for fermentation but also added for the flavour of the water kefir beverage (Chniti et al., 2017; Tang, Shi, & Aleid, 2013). Chinese jujube (*Ziziphus jujuba* Mill.), abundant in the tropical and subtropical regions of Asia, is not only a profitable fruit but also a favourable material for extraction of polysaccharides due to the high sugar content (Li, Ding, & Ding, 2005; Li, Fan, Ding, & Ding, 2007). Further, its nutritional content such as vitamin C, phenolic compounds and abundant minerals makes it a potential resource for water kefir fermentation (Li et al., 2005). Therefore, this study investigated the fermentation of water kefir beverage containing *Ziziphus jujuba* Mill. (jujube) syrup.

1.2 Aim and objectives

Aim

The overall aim of this study was to investigate the potential of producing fermented water kefir beverage with added *Ziziphus jujuba* Mill. (jujube) syrup as a supplement of carbon source.

Objectives

1. To determine the optimum extraction conditions of jujube syrup using the water-bath method;
2. To determine the optimum fermentation temperature and jujube syrup concentration for the preparation of jujube water kefir beverage by:
    a. Measuring pH, total soluble solids (TSS), colour;
    b. Analysing titratable acidity (T.A.) and microbial content (lactic acid bacteria and *Saccharomyces cerevisiae*);
    c. Conducting consumer sensory evaluation;
3. To analyse ethanol, sugars (sucrose, fructose, glucose), organic acids (lactic acid, acetic acid), antioxidants (gallic acid, catechin, epicatechin, rutin) of the final formulation of jujube water kefir beverage during fermentation and three-week storage (4°C) using gas chromatography (GC) and high performance liquid chromatography (HPLC), respectively;
4. To characterise the physicochemical properties (pH, TSS, T.A., colour) and microbiological content (lactic acid bacteria and *Saccharomyces cerevisiae*) of jujube water kefir during storage (4°C) for three weeks, and,
5. To conduct consumer sensory evaluation of prepared jujube water kefir beverage during storage (4°C) for three weeks.

Chapter 2. LITERATURE REVIEW

2.1 Food fermentation

Fermentation occurs when microorganisms consume susceptible organic substances as part of their metabolic processes (Caplice & Fitzgerald, 1999). In food processing, fermentation is defined as a transformation process of organic substrates involving selected microorganisms (bacteria, yeasts, or moulds) under aerobic or anaerobic conditions to produce a range of products containing metabolites such as alcohol, carbon dioxide or organic acids, which can suppress the growth of undesirable microorganisms (Bamforth, 2008; Fellows, 2009). The term "fermented foods" describes a particular class of food products produced from raw or cooked material of plant or animal origin by microorganism(s). The substrates (food products) can be produced by natural (spontaneous) fermentation or by adding pure or mixed culture with known characteristics (Chakrabarty, Sharma, & Tamang, 2010; Hasan et al., 2014; Tekluu et al., 2015). The fungal fruit bodies or mushrooms that can be directly consumed are also included (Campbell-Platt, 1994).

The production and preservation of foods by fermentation is one of the oldest food processing technologies (Caplice & Fitzgerald, 1999). *Dahi*, a coagulated sour milk eaten as a food item in the Indian subcontinent was developed around 6000 BC (Ray & Joshi, 2014). Cheese, another type of fermented dairy product, was discovered around 8000 years ago in the valleys of the rivers Tigris and Euphrates (Nair & Prajapati, 2008; Ray & Joshi, 2014). Later, due to the lack of safe drinking water, alcoholic fermentation which included conversion of grapes into wine, and barley to beer were developed by the Sumerians and Egyptians during the period 4000-2000 BC (Campbell-Platt, 1994; Ray & Joshi, 2014). During the same period, the Egyptians utilised the residue left from barley fermentation to produce raised bread which later became to dominate the European diet (Ray & Joshi, 2014). In the east and south-east Asia, the chief cereal, rice, was fermented to *lao-chao*, and based on the concept of having a balanced meal, fermented vegetables were developed about thousands years ago in this region (Campbell-Platt, 1994). *Kimchi*, a typical traditional fermented vegetable, is eaten nearly every day in Korea (Campbell-Platt, 1994; Ray &

Joshi, 2014). Soy sauce and fermented fish pastes are important sources of amino acids and proteins. These ancient practices can be traced back to 3000 years ago (Campbell-Platt, 1994).

Different fermentation technologies were transferred from one region to another as people migrated (Campbell-Platt, 1994). By studying the chemical and biological basis of fermentation as well as the developments in food processing technology, there is a wide range of fermented products across the world, such as bread, cheese, wine, pickles, sausages, and yoghurt. Therefore, food fermentation offers considerable potential for new product development (Charalampopoulos & Webb, 2013).

2.2 Non-dairy fermented beverages

Due to the lactose intolerance and the ongoing trend of vegetarianism worldwide, there is an increasing demand for non-dairy fermented products (Abu-Ghannam & Rajauria, 2015). Among the non-dairy fermented products, the (non-dairy) fermented beverages made from botanical sources such as cereals, legumes, fruits and vegetables provide an alternative source of probiotics for vegetarians and/or vegans and lactose intolerant consumers (Fiorda et al., 2017; Vasudha & Mishra, 2013).

2.2.1 Cereal-based fermented beverages

Cereal grains are an important source of protein, carbohydrates, vitamins, minerals, and also contain water-soluble fibre, oligosaccharides and resistant starch (Vasudha & Mishra, 2013). A number of non-dairy fermented cereal beverages have been developed throughout history. Bushera, a traditional beverage produced in Uganda, is fermented with sorghum and millet grains for 1-6 days at ambient temperature (Vasudha & Mishra, 2013). *Haria* is a rice-based fermented beverage with 2-3% alcohol content consumed in East-Central India, while another rice-based fermented beverage, *chicha*, is characterised as an acidic non-alcoholic beverage (Kandylis et al., 2016). *Mahewu* (*amahewu*), an African sour beverage made from maize porridge was reported to

be dominated by predominant *Lactococcus lactis* subsp. *lactis* (Kandylis et al., 2016; Vasudha & Mishra, 2013).

2.2.2 Fruit and vegetable-based fermented beverages

Fruit juice contains beneficial nutrients and has a pleasant taste profile for all the age groups. *Hardaliye* is a well-known lactic acid fermented beverage in Turkey produced since ancient times, and it is made from red grape or grape juice, crushed mustard seeds, and benzoic acid (Vasudha & Mishra, 2013). Kombucha, a traditional beverage, is a plant-based beverage produced by fermenting sugared tea using a symbiotic culture of bacteria and yeast (Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014). It is reported that kombucha may provide numerous health benefits including preventing cardiovascular diseases, promoting digestive functions and improving resistance against cancer (Dufresne & Farnworth, 2000; Jayabalan et al., 2014). Recently, a non-dairy fermented beverage similar to kombucha but requires a shorter fermentation time, named water kefir, is gaining popularity worldwide among consumers and researchers due to its sensory profile and nutritional properties (Fiorda et al., 2017).

2.3 Water kefir

Water kefir, also known as sugary kefir, is a naturally carbonated drink with a mildly sour taste that is fermented by a microbial multispecies community (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Magalhaes et al., 2010). Traditionally, the 5-10% (w/v) kefir grains are added into a solution containing 3-10% (w/v) sucrose, dried fruits and slices of lemon may be added to provide flavour. The fermentation takes place at room temperature (20°-27°C) for 24-72 hours (Gulitz, Stadie, Ehrmann, Ludwig, & Vogel, 2013; Marsh et al., 2013; Reiß, 1990; Waldherr, Doll, Meißner, & Vogel, 2010). Optimal pH for kefir (3.95) can be achieved by using 10% kefir grains as the starter culture (Lengkey & Balia, 2014). An overview of a generalised traditional production process of water kefir beverage is shown in Figure 2.1.

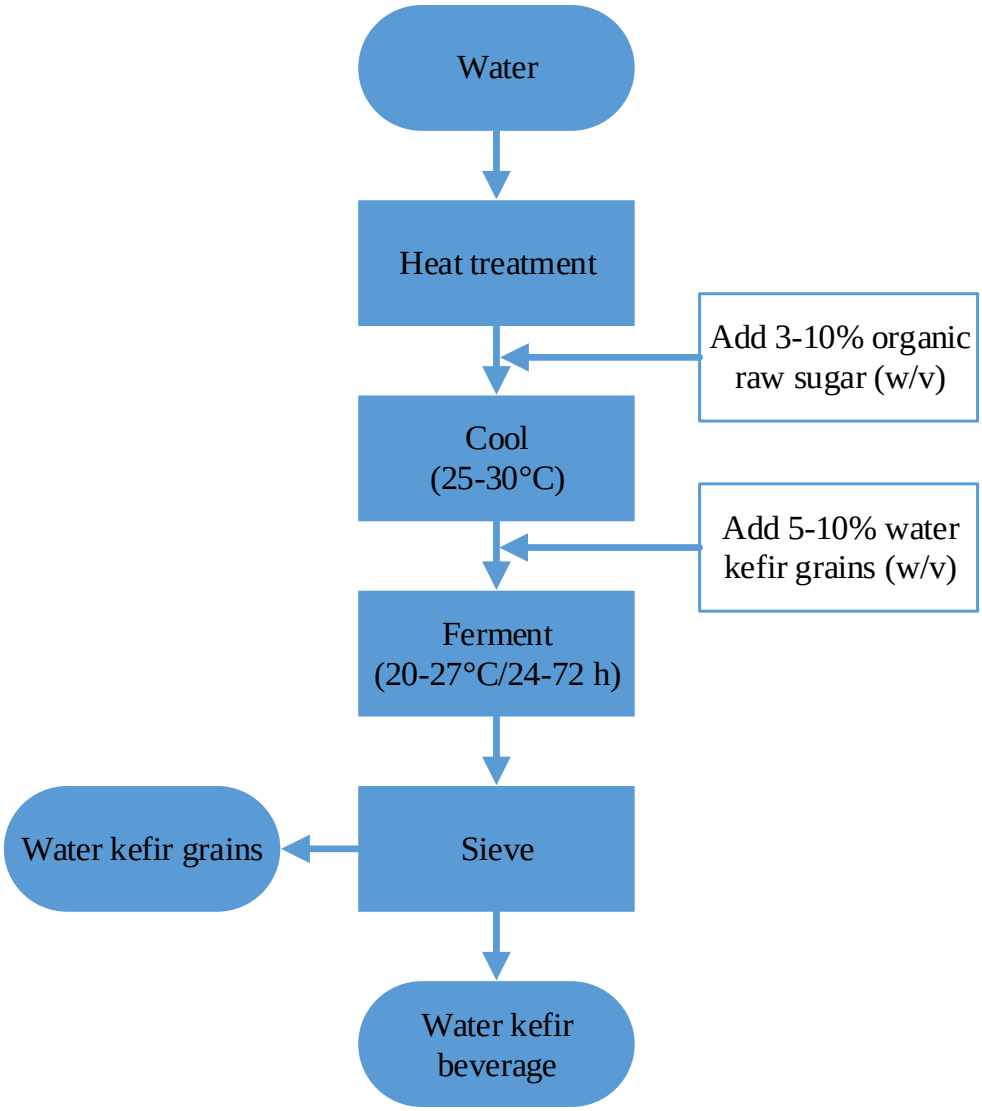

Figure 2.1 Generalised traditional production process of water kefir beverage

Source: Gulitz et al. (2013); Marsh et al. (2013); Reiß (1990); Waldherr et al. (2010)

2.3.1 Origin and distribution of water kefir

The origin of water kefir is not unknown. However, kefir grains were first linked to "ginger beer plants", which were brought by English soldiers from the Crimen War in 1855 (Fiorda et al., 2017; Ward, 1892). Lutz (1899) described a similar system, "Tibi", which originated from Mexican prickly pear cactus (*Optunia*) (Fiorda et al., 2017; Gulitz et al., 2011). At the end of 19th century, it was called "grains vivantes" in Paris, and around 1930, water kefir was well-known in

Switzerland under the name “ferment de raisins” (Reiß, 1990). Vayssier (1978) first reported the product as “sugary kefir grains”, and Pidoux (1989) used this term to discriminate them from the grains fermenting milk. Besides the terms mentioned here, water kefir grains have also been called as “African bees”, “Ale nuts”, “Balm of Gilead”, “Bèbées”, “California bees”, “Japanese Beer Seeds”, and “Tibico” (Gulitz et al., 2011; Neve & Heller, 2002; Waldherr et al., 2010).

Water kefir is mainly consumed in Brazil and Mexico. In Brazil, the beverage is mainly produced at homes (Magalhaes et al., 2010). However, with the expanding studies and more comprehensive understanding of water kefir, the popularity of the beverage has increased around the world. Previous studies showed the countries with high consumption of water kefir beverage include Canada, Mexico, and USA (North America); Malaysia, Thailand, and Japan (Asia); Argentina, Brazil, Chile and Peru (Latin America); and France, Greece, Netherlands, Norway, Portugal, Romaine, Russia, Spain, Turkey, and UK (Europe) (Farnworth, 2006; Sarkar, 2007; Zhou et al., 2009).

2.3.2 Water kefir grains and microbiota

The water kefir grains are whitish-to-translucent irregular particles with 8-10 mm diameter (Horisberger, 1969; Reiß, 1990). The grains consist of dextran, an α 1-6 linked glucose polymer with 1-3 linked side chains, produced by certain *Lactobacillus*, and/or *Leuconostoc* species (Gulitz et al., 2011; Horisberger, 1969; Laureys & De Vuyst, 2014). Waldherr et al. (2010) identified a strain of *Lactobacillus hilgardii* which is responsible for producing large amounts of the granule-forming dextran and characterised the glycosyltransferase for dextran production. The microbiota of grains is a combination of bacteria and yeasts which live in symbiosis in this polysaccharide matrix (Gulitz et al., 2011; Horisberger, 1969; Laureys & De Vuyst, 2014; Magalhaes et al., 2010). By scanning the grains and observing under transmission electron microscopy, Pidoux (1989) reported a low content of microorganisms in the inner part of the grain while the outer was covered by a network of pseudomycelia containing blastospores and stuck together or onto the bacteria colonies. The surface pseudomycelia might be ascribed to the *Candida* and other yeasts only retained in the spaces inside the matrix or in the cracks formed by the increased gas pressure during

fermentation. Reiß (1990) also reported that the grains are hollow due to the accumulated CO_2 during fermentation. Typical appearance of water kefir grains is shown in Figure 2.2.

Figure 2.2 Appearance of water kefir grains

Source: Stadie (2013)

The microbial species' diversity of water kefir consists of a stable microbiota of mainly AAB, LAB, and yeasts (Gulitz et al., 2013; Marsh et al., 2013; Miguel et al., 2011; Waldherr et al., 2010). By conducting culture-independent analysis (PCR-DGGE) on the Brazilian water kefir beverage, Magalhaes et al. (2010) reported the dominance of *Lactobacillus paracasei*, *Lactobacillus kefiri*, *Lactobacillus parabuchneri* and *Acetobacter lovaniensis* in the bacteria group and *Saccharomyces cerevisiae* and *Kluyveromyces lactis* in the yeast group. Moreover, the PCR-DGGE technique used in this study enabled the detection of *Acetobacter lovaniensis* and *Kazachstania aerobia* which were not described in the previous studies. Miguel et al. (2011) also investigated the profile of microbial communities in Brazilian water kefir samples and this study was the first to detect bacteria species of *Gluconobacter liquefaciens* and *Bacillus cereus* and yeast species *Pichia cecembensis*, *Pichia caribbica* and *Zygosaccharomyces fermentati* by PCR-DGGE analysis. Meanwhile, Gulitz et al. (2011) investigated the microbial consortia residing in three German water kefir grains and reported the predominant LAB bacteria comprising *Lactobacillus hordei*, *Lactobacillus nagelii*, *Leuconostoc mesenteroides*, *Hanseniaspora valbyensis*, *Lachancea*

fermentati, and *S. cerevisiae*, *Zygotorulaspora florentina* were the dominant yeasts. Another study on Thailand water kefir grains reported common (kefir) species such as *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Bacillus cereus*, and *S. cerevisiae* which were similar to the microorganisms reported in different countries (Sarikkha, Nitisoravut, Poljungreed, & Boonyarattanakalin, 2015).

The first comprehensive description of water kefir bacterial community by 16S rDNA amplicon sequencing was presented by Gulitz et al. (2013). In this study, a new milestone was set with the first detection of bifidobacteria in water kefir microbiota, and a large number of corresponding sequences found in all investigated water kefir samples showed that the bifidobacteria are part of the core species of kefir matrices. Later, by culture-independent analysis, Laureys, Cnockaert, De Vuyst, and Vandamme (2016) also detected *Bifidobacterium crudilactis* and *Bifidobacterium psychraerophilum* in water kefir grains from Belgium. Due to the unexpected presence of bifidobacteria in the tested water kefir samples and the difficulty in cultivating these species, there may be some underestimation of the role of bifidobacteria in other water kefir (Gulitz et al., 2013).

According to Fiorda et al. (2017), a high diversity was found in yeast species with the predominance of *Saccharomyces* species, while a more stable diversity of bacteria composition was observed. This may be attributed to the fact that the metabolism of yeasts is depended on the availability of carbon and energy (Sikander, 2007). Therefore, the high sucrose content in water kefir may promote the growth of *Saccharomyces* species which can convert sucrose to glucose and fructose by intervase so that the glucose act as a free metabolite for yeast cells (Sikander, 2007). However, there is high variability in the microbiota of different water kefir as it depends on the climatic and cultural conditions (Gulitz et al., 2013; Gulitz et al., 2011; Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Miguel et al., 2011). The isolated microorganisms from water kefir grains are shown in Table 2.1.

Table 2.1 Isolated microorganisms from water kefir grains

Bacteria	Reference	Yeast	Reference
Acetobacter fabarum	Gulitz et al. (2013); Gulitz et al. (2011); Laureys and De Vuyst (2014); Magalhaes et al. (2010); Miguel et al. (2011)	*Candida lambica*	Pidoux (1989)
Acetobacter lovaniensis	Laureys and De Vuyst (2014); Magalhaes et al. (2010); Miguel et al. (2011)	*Candida valida*	Pidoux (1989)
Acetobacter orientalis	Gulitz et al. (2013); Gulitz et al. (2011)	*Dekkera bruxellensis*	Laureys and De Vuyst (2014)
Bacillus cereus	Miguel et al. (2011)	*Hanseniaspora uvarum*	Fiorda et al. (2016)
Bifidobacterium aquikefiri sp. nov.	Laureys et al. (2016)	*Hanseniaspora valbynesis*	Gulitz et al. (2011)
Bifidobacterium crudilactis	Gulitz et al. (2013); Laureys and De Vuyst (2014)	*Issatchenkia orientalis*	Fiorda et al. (2016)
Bifidobacterium psychraerophilum	Gulitz et al. (2013); Laureys and De Vuyst (2014)	*Kazachstania aerobia*	Magalhaes et al. (2010)
Enterobacter ludwigii	Zanirati et al. (2015)	*Kazachstania unispora*	Puerari et al. (2012)
Gluconobacter frateuri	Gulitz et al. (2013)	*Kloeckera apiculate*	Pidoux (1989)
Gluconobacter liquefaciens	Miguel et al. (2011)	*Kluyveromyces lactis*	Magalhaes et al. (2010)
Klebsiella pneumoniae	Zanirati et al. (2015)	*Kluyveromyces marxianus*	Puerari et al. (2012)
Lactobacillus brevis	Laureys and De Vuyst (2014)	*Lanchancea fermentati*	Fiorda et al. (2016); Gulitz et al. (2011)
Lactobacillus buchneri	Magalhaes et al. (2010); Miguel et al. (2011)	*Lanchancea meyercii*	Magalhaes et al. (2010)
Lactobacillus casei subsp. *casei*	Pidoux (1989)	*Pichia caribbica*	Miguel et al. (2011)
Lactobacillus casei subsp. *rhamnosus*	Pidoux (1989)	*Pichia cecembensis*	Miguel et al. (2011)
Lactobacillus diolivorans	Laureys and De Vuyst (2014)	*Pichia kudriavzevii*	Fiorda et al. (2016)
Lactobacillus fermentum	Puerari, Magalhães, and Schwan (2012)	*Pichia membranifaciens*	Fiorda et al. (2016); Miguel et al. (2011)
Lactobacillus ghanensis	Gulitz et al. (2013); Laureys and De Vuyst (2014)	*Saccharomyces cerevisae*	Gulitz et al. (2011); Magalhaes et al. (2010); Miguel et al. (2011); Puerari et al. (2012)
Lactobacillus harbinensis	Laureys and De Vuyst (2014)	*Yarrowia lipolytica*	Miguel et al. (2011)
Lactobacillus helveticus	Miguel et al. (2011)	*Zygosaccharomyces fermentati*	Fiorda et al. (2016); Miguel et al. (2011)
Lactobacillus hilgardii	Gulitz et al. (2013); Gulitz et al. (2011); Laureys and De Vuyst (2014); Pidoux (1989); Waldherr et al. (2010)	*Zygosaccharomyces florentinus*	Pidoux (1989)
Lactobacillus hordei	Gulitz et al. (2013); Gulitz et al. (2011); Laureys and De Vuyst (2014)	*Zygotorulaspora florentina*	Gulitz et al. (2011)
Lactobacillus kefiranofaciens	Zanirati et al. (2015)		
Lactobacillus kefiri	Magalhaes et al. (2010); Miguel et al. (2011)		
Lactobacillus mali	Gulitz et al. (2013); Laureys and De Vuyst (2014)		
Lactobacillus nagelii	Gulitz et al. (2013); Gulitz et al. (2011); Laureys and De Vuyst (2014)		
Lactobacillus parabuchneri	Magalhaes et al. (2010)		
Lactobacillus paracasei subsp. *paracasei*	Magalhaes et al. (2010)		
Lactobacillus paracasei subsp. *tolerans*	Magalhaes et al. (2010)		
Lactobacillus parafarraginis	Zanirati et al. (2015)		
Lactobacillus perolens	Zanirati et al. (2015)		
Lactobacillus plantarum	Pidoux (1989); Puerari et al. (2012)		
Lactobacillus satsumensis	A Gulitz et al. (2013); Miguel et al. (2011)		
Lactobacillus sunkii	Miguel et al. (2011)		
Lactococcus lactis subsp. *cremoris*	Pidoux (1989)		
Lactococcus lactis subsp. *lactis*	Pidoux (1989)		
Leuconostoc citreum	Magalhaes et al. (2010)		
Leuconostoc mesenteroides subsp. *dextranicum*	Pidoux (1989)		
Lysinibacillus sphaericus	Fiorda et al. (2016)		
Oenococcus kitaharae	Zanirati et al. (2015)		
Oenococcus oeni	Zanirati et al. (2015)		

2.3.3 Microbial interactions during fermentation of water kefir

The symbiotic interactions in water kefir fermentation among yeast, LAB, and AAB have been described in many studies (Leroi & Pidoux, 1993a, 1993b; Stadie, 2013; Stadie, Gulitz, Ehrmann, & Vogel, 2013). Bacteria and yeasts are in a symbiotic relationship since they can survive or multiply by sharing their bioproducts which act as an energy source or growth stimulant, the symbiotic interactions in water kefir isolates can be considered as mutualism (Lopitz-Otsoa, Rementeria, Elguezabal, & Garaizar, 2006; Stadie et al., 2013). Leroi and Pidoux (1993b) were the first to report the synergism between *Lactobacillus hilgardii* and *Saccharomyces florentinus*, which has been reclassified as *Zygotorulaspora florentina* in water kefir (Stadie et al., 2013). It was reported that the acetate, succinate, pyruvate, propionate and CO_2 produced by *Zygotorulaspora florentina* supported better survival of *Lactobacillus hilgardii* which resulted in a significant increase in lactic acid production (Stadie et al., 2013). The overview of the interactions among representative water kefir microorganisms is shown in Figure 2.3.

Figure 2.3 Overview of the interactions among representative cultivable water kefir isolates
Source: Stadie et al. (2013)

Stadie et al. (2013) reported that the presence of both the lactobacilli (*Lactobacillus hordei* and *Lactobacillus nagelii*) showed positive effects in the growth of the two yeasts, *S. cerevisiae* and *Zygotorulaspora florentina*. This suggested that the co-cultivation of yeasts and lactobacilli can result in better growth of both lactobacilli and yeasts than the single cultivation of the individual organisms.

The acidification of the medium by lactobacilli supported the growth of the yeast (*Zygotorulaspora florentina*) while the essential nutrients produced by the yeasts improved the growth of lactobacilli (Stadie et al., 2013). The amino acids and vitamin B_6 released by yeasts promoted the growth of *Lactobacillus nagelii* and *Lactobacillus hordei*, respectively (Stadie et al., 2013). The release of arginine by yeast could be induced by co-cultivation, and the vitamin is essential for the growth of *Lactobacillus hordei*. According to Stadie et al. (2013), the improvement in the growth of microorganisms may vary based on the co-cultivated species. In their study, better growth of *Lactobacillus hordei* was observed when co-cultivated with *Zygotorulaspora florentina* than with *S. cerevisiae*. Later, Fiorda et al. (2017) reported that the separation of microbial kefir grains cultures can lead to non-active biochemical activity or non-growth of microorganisms in sugar solution.

Figure 2.4 Microbial metabolic activities during water kefir fermentation

Notes: LAB (Lactic Acid Bacteria); AAB (Acetic Acid Bacteria).

Source: Fiorda et al. (2017)

Besides the interactions between the lactobacilli and yeast, yeast metabolism can promote the growth of AAB and production of uric acid (Fiorda et al., 2017). Sucrose can be hydrolysed by yeast invertase into glucose and fructose, which can be utilised by the LAB as a carbon source, and the ethanol produced by yeast is converted to acetic acid by some heterofermentative AAB based on their alcohol dehydrogenase activity (Magalhaes et al., 2010; Rodrigues, Ludovico, & Leão, 2006). A simplified overview of the interactions is shown in the figure above (Figure 2.4).

2.3.4 Carbohydrate metabolism of water kefir microorganisms

2.3.4.1 Water kefir lactic acid bacteria

The breakdown of carbohydrates by different catabolic pathways provides ATP and other reducing equivalents as sources of energy for bacterial cells (Khandelwal, Gaspar, Crespo, & Upendra, 2016). Lactic acid bacteria (LAB) are gram-positive, non-sporulating, facultative anaerobic, rod-shape or cocci microorganisms which can produce lactic acid by using carbohydrates as an energy source (Khandelwal et al., 2016; Montet, Ray, & Zakhia-Rozis, 2014; Stadie, 2013). The resultant low pH restricts the growth of spoilage pathogenic flora such as *Escherichia coli*, *Salmonella* and *Staphylococcus* (Montet et al., 2014; Ross, Morgan, & Hill, 2002). Water kefir contains several species belonging to the genera *Lactobacillus, Leuconostoc* and *Lactococcus* (Gulitz et al., 2011; Magalhaes et al., 2010; Marsh et al., 2013; Sarikkha et al., 2015). Based on the fermentation patterns, the genus can be divided into two sub-groups: homo-fermentative and hetero-fermentative LAB, which correspond to homo-lactic metabolism and hetero-lactic metabolism (Figure 2.5) (Khandelwal et al., 2016; Montet et al., 2014; Reddy, Altaf, Naveena, Venkateshwar, & Kumar, 2008; Stadie, 2013).

Homo-fermentative LAB belonging to *Pediococcus*, *Streptococcus*, *Lactococcus* and certain *Lactobacillus* produce more than 85% lactic acid from glucose with the acid being the major product for glycolysis, or Embden-Meyerhof-Parnas (EMP) pathway in carbohydrate metabolism (Khandelwal et al., 2016; Montet et al., 2014; Reddy et al., 2008). Homo-fermentative LAB can ferment 1 mole of glucose into 2 moles of lactic acid by lactate dehydrogenase (LDH), producing

a net yield of ATP two moles per molecule (Reddy et al., 2008). Homo-fermentative LAB uses fructose 6-phosphate for the biosynthesis of mannitol (Khandelwal et al., 2016). Under nutrient-limited conditions, the homo-lactic glycolysis may be shifted to mixed acid fermentations, producing acetate, 2,3-butanediol, ethanol, diacetyl, and in some cases, mannitol (Ramsey, Hartke, & Huycke, 2014).

Figure 2.5 Carbohydrate metabolism of lactic acid bacteria

Source: Reddy et al. (2008)

Due to the lack of aldolase and triose phosphate isomerase, hetero-fermentative LAB such as *Leuconostoc*, *Oenococcus* and some *Lactobacillus* produce only 50% lactic acid with appreciable amounts of acetate, CO_2 and ethanol from glucose by 6-phosphogluconate/phosphoketolase pathway (Khandelwal et al., 2016; Montet et al., 2014; Reddy et al., 2008; Stadie, 2013). The hetero-fermentative LAB ferment one mole of glucose to produce one mole of lactic acid, ethanol and CO_2 respectively (Montet et al., 2014; Reddy et al., 2008). During this process, only one mole of ATP is produced per molecule, leading to lower growth of the fermenting microorganisms per mole of glucose metabolised (Reddy et al., 2008). Acetyl phosphate can be converted to acetate instead of ethanol by acetate kinase in the presence of electron-accepting sugar (fructose, citrate, malate, etc.), resulting in two moles of ATP (Montet et al., 2014; Stadie, 2013). Hetero-fermentative LAB uses fructose for mannitol biosynthesis instead of fructose-6-phosphate (Montet et al., 2014). Typical homo- and hetero-fermentative LAB in water kefir are shown in Table 2.2.

Table 2.2 Homo- and hetero-fermentative LAB in water kefir.

Homo-fermentative LAB	**Hetero-fermentative LAB**
Lactobacillus casei	*Lactobacillus hilgardii*
Lactobacillus hordei	*Leuconostoc citreum*
Lactobacillus nagelii	*Leuconostoc mesenteroides*
Lactobacillus satsumensis	

Source: Stadie (2013)

2.3.4.2 Water kefir yeast

Yeasts belonging to the genera such as *Saccharomyces*, *Candida*, *Lanchancea*, *Pichia* and *Hanseniaspora* have been reported in water kefir grains (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Marsh et al., 2013; Pidoux, 1989). *S. cerevisiae* is a facultative anaerobic yeast commonly found in the water kefir grains. The sugar composition of the media and oxygen availability are two important environmental conditions that strongly affect the yeast metabolism (Rodrigues et al., 2006). Depending on the conditions, the yeast may display either a fermentative or respiratory metabolism or even both (mixed respiratory-fermentative metabolism) (Rodrigues et al., 2006).

Pasteur effect and Crabtree effect are two frequently observed effects related to the sugar metabolism in *S. cerevisiae* (Pronk, Yde Steensma, & van Dijken, 1996; Rodrigues et al., 2006). The Pasteur effect refers to the inhibition of fermentation in the presence of oxygen due to the lower efficiency of ATP production compared with respiration (De Deken, 1966; Pronk et al., 1996; Rodrigues et al., 2006). However, the Pasteur effect can be only observed at resting-cell conditions or at low growth rates in sugar-limiting continuous culturing (Fiechter & Seghezzi, 1992; Rodrigues et al., 2006).

The Crabtree effect refers to the occurrence of fermentation in the presence of oxygen, and *S. cerevisiae* is regarded as Crabtree positive yeast which catabolise sugar mainly by fermentation (Bhalla, 2016; De Deken, 1966; Fiechter & Seghezzi, 1992; Hagman & Piškur, 2015). Water kefir is commonly prepared with sugar solutions which contain sucrose (Gulitz et al., 2011; Marsh et al., 2013; Waldherr et al., 2010). Sucrose is hydrolysed by yeast invertase to glucose and fructose, and the glucose is converted to pyruvate through glycolysis (Fiorda et al., 2017; Pfeiffer & Morley, 2014; Pronk et al., 1996; Rodrigues et al., 2006). At high sugar concentrations, the cell is switched into the fermentation process, in which the pyruvate is converted to acetaldehyde catalysed by pyruvate decarboxylase (Pdc) and finally transformed to ethanol by alcohol dehydrogenase (Adh), resulting in two moles of ATP per molecule. When the sugar concentration is low, the pyruvate is converted to acetyl-CoA through respiration pathway, which then enters the tricarboxylic acid (TCA) cycle and yielding 32 moles of ATP per molecule in mitochondrion (Bhalla, 2016; Pfeiffer & Morley, 2014; Pronk et al., 1996; van Dijken, Weusthuis, & Pronk, 1993). In addition, the acetyl-CoA can be converted from the acetaldehyde formed in the fermentative process with the aid of acetaldehyde dehydrogenase (Ald) and acetyl-CoA synthetase (Acs) (Pfeiffer & Morley, 2014; van Dijken et al., 1993). The metabolism of carbohydrates in yeast is shown in Figure 2.6. With the Crabtree effect, the sugar metabolism in yeast can be effectively regulated by inhibiting energy production with high sugar concentration. When the sugar supply is limited, pyruvate is shunted into the respiratory chain, resulting in the synthesis of 32 molecules of ATP per molecule of sugar (pyruvate), thereby overcoming the inhibition of energy production (Bhalla, 2016).

In water kefir, besides *S. cerevisiae*, other yeast species such as *Lanchancea*, *Pichia*, and *Hanseniaspora* also display a high fermentative capacity (Fiorda et al., 2017). In general, these are

predominant yeasts species at first while *S. cerevisiae* takes over at a later stage of fermentation (Morrissey, Davenport, Querol, & Dobson, 2004). Moreover, the enhanced sensory quality such as typical yeasty aroma and refreshing taste can also be attributed to different yeast species present in the water kefir (Fiorda et al., 2017; Magalhaes et al., 2010).

Figure 2.6 Modified metabolism of carbohydrates in yeasts
Source: van Dijken et al. (1993)

2.3.5 Exopolysaccharides (EPS) production by water kefir microorganisms

The microbial exopolysaccharides (EPS) are secreted polymers that can be released as extracellular slime from the cell to the surroundings or tightly attached to the cell surface (Zajšek, Kolar, & Goršek, 2011). Based on the biosynthesis mechanism and the chemical composition, the EPS can be classified as homopolysaccharides (HoPS) and heteropolysaccharides (HePS) (Ruas-Madiedo, Salazar, & Clara, 2009; Stadie, 2013). The HoPS consist of only one type of sugar monomer whereas the HePS is composed of different monomers with repeating precursor units, and the latter play important roles in the rheology and mouthfeel of fermented milk products

(Stadie, 2013). As discussed previously, water kefir yeast and bacteria are embedded in the exopolysaccharides matrix called water kefir grains, and the exopolysaccharides play major roles in water kefir grain formation (Fels, Jakob, Vogel, & Wefers, 2018; Koh et al., 2018). LAB are able to produce α-glucans and fructans from sucrose. (Fels et al., 2018; Ruas-Madiedo et al., 2009; Waldherr & Vogel, 2009). The enzymes dextransucrase and fructansucrases can cleave the glucose-fructose linkage of sucrose and transfer one of the monosaccharides to the growing polysaccharide chain (Ruas-Madiedo et al., 2009). The α-glucans produced by lactic acid bacteria can be classified based on the carbon involved in the linkage, and dextran, an α-1-6 linked glucose polymer, is the most frequently described α-glucans (Fels et al., 2018).

Presently, there are several reports on the formation of EPS in water kefir. Pidoux (1989) reported that the *Lactobacillus hilgardii* was the primary EPS producer in water kefir. Waldherr et al. (2010) also stated the role of *Lactobacillus hilgardii* as main the EPS-producer by comparing the EPS from water kefir grains and the EPS produced from *Lactobacillus hilgardii* culture. In addition, Waldherr et al. (2010) showed that with the supplement of sucrose, large slimy colonies could be produced by *Lactobacillus hilgardii* (Figure 2.7), and the slimy appearance of colonies can be regarded as the EPS. By cultivating the EPS-producing water kefir isolates in liquid medium with 8% sucrose, Stadie (2013) reported that the EPS concentration could range from 9.2-32.5 g/L. Among the tested strains, *Lactobacillus hilgardii* was demonstrated to be a strong EPS-producer which produced 32.5 g/L EPS after 48 h fermentation.

Figure 2.7 *Lactobacillus hilgardii* on mMRS agar without (left) and with a supplement of sucrose (right)

Source: Waldherr et al. (2010)

Gulitz et al. (2011) reported that 57 LAB strains belonging to *Lactobacillus casei, Lactobacillus hordei, Lactobacillus hilgardii, Lactobacillus nagelii* and *Leuconostoc mesenteroides* have potential to produce EPS. In contrast to Pidoux (1989) and Waldherr et al. (2010), none of the *Lactobacillus hilgardii* isolated in this research was able to produce EPS while some strains of *Lactobacillus nagelii, Lactobacillus hilgardii*, and *Leuconostoc Mesenteroides* were the major EPS producers. Laureys and De Vuyst (2014) reported that the *Lactobacillus hilgardii* could be the primary EPS producer, but not all the strains from water kefir can produce EPS. Moreover, the EPS-producing LAB in water kefir also includes *Lactobacillus brevis, Lactobacillus casei, Lactobacillus hordei, Lactobacillus nagelii* and *Leuconostoc Mesenteroides*. These different findings might be attributed to different fermentation conditions and the diverse microbial consortia present in variable sources of water kefir grains (Gulitz et al., 2013).

2.3.6 Chemical compositions of water kefir beverages

Since most of the available reports on water kefir focus on the microbial composition, limited sources are available for the chemical composition. The chemical properties of kefir may be affected by the microbial composition of the grains and the type of substrates (Fiorda et al., 2017; Otles & Cagindi, 2003). In water kefir fermentation, lactic acid and ethanol are the major end-products while some organic acids such as acetic acid and the useful metabolites including esters, mannitol, and glycerol are produced at a lower concentration (Fiorda et al., 2017; Laureys & De Vuyst, 2014). Laureys and De Vuyst (2014) reported that sucrose concentration dropped to 1.2 g/L after 24 h fermentation while ethanol and lactic acid increased to 20.3 g/L and 4.9 g/L respectively, after 72 h fermentation. Moreover, the volatile compounds such as ethyl acetate, ethyl decanoate, ethyl hexanoate, ethyl octanoate, and isoamyl acetate were also detected in the water kefir beverage. Laureys and De Vuyst (2014) showed that the fruity and floral aroma of the final product was strongly affected by ethyl decanoate, ethyl hexanoate, and ethyl octanoate.

2.3.7 Characteristic of water kefir beverage during fermentation

2.3.7.1 Concentration of sugars and total soluble solids in water kefir beverage

The changes in sugar and total soluble solids (TSS) have been reported in previous reports (Corona et al., 2016; Magalhaes et al., 2010; Randazzo et al., 2016; Stadie et al., 2013). During fermentation, the major carbon source, sucrose, is hydrolysed into glucose and fructose by the invertase present in the yeast (Stadie, 2013). The concentration of the soluble solids can be measured by a refractometer and recorded as °Brix (Corona et al., 2016; Magalhaes et al., 2010). By analysing the water kefir beverage fermented with added unrefined sugar, Magalhaes et al. (2010) reported reduced sucrose content from 40 mg/mL to 28 mg/mL after 24 h fermentation. Laureys and De Vuyst (2014) also reported decreased sucrose concentration during kefir fermentation. In their study, the sucrose content in the kefir fermented with unrefined cane sugar decreased from 47.5 g/L to 1.2 g/L after 24 h fermentation. The sucrose concentration in black tea water kefir developed by Subardjo (2017) also agreed with the previous reports, and the results showed that the TSS of the beverages prepared with different sugar concentrations decreased during fermentation for 72 h (Subardjo, 2017). As expected, the concentrations of the glucose and fructose increased as the sucrose was fermented, however, decreased release of glucose and fructose were reported with prolonged (60 h) fermentation (Stadie et al., 2013).

2.3.7.2 pH and organic acids

Due to the formation of the organic acids, the pH and the composition of organic acids of water kefir beverage may change during fermentation (Lengkey & Balia, 2014; Magalhaes et al., 2010; Stadie et al., 2013). Stadie et al. (2013) reported that the pH of water kefir decreased from 6.5 to 3.5 during 48 h of fermentation, and Laureys and De Vuyst (2014) reported a decrease in pH from 4.26 to 3.45 within 72 h fermentation. By monitoring the pH of cocoa kefir beverage fermented at 25°C, Puerari et al. (2012) found that the pH decreased markedly during the first 48 h and reached 3.8 after 72 h. In the study done by Subardjo (2017), the pH of all the four black tea water kefir beverages rapidly decreased within the first 24 h of fermentation, and then gradually decreased

until the end of fermentation at 96 h. With respect to the organic acids, Lengkey and Balia (2014) noted increased concentrations of lactic acid (from 0.7 g/L to 4.9 g/L) and acetic acid (from 0.1 g/L to 1.0 g/L). Puerari et al. (2012) obtained maximum concentration of lactic acid (1.0 g/L) and acetic acid (0.5 g/L) in cocoa kefir beverage fermented at 10°C after 72 h whereas cocoa kefir beverage fermented at 25°C contained higher lactic acid (5.55 g/L) and acetic acid (1.0 g/L) after 72 h fermentation. These differences in the organic acids may be attributed to different fermentation temperatures. In studies of water kefir beverages containing juices from carrot, fennel, melon, onion, strawberry and tomato, lactic acid ranged between 0.58-4.81 g/L, and 0.03-1.9 g/L for acetic acid (Corona et al., 2016). The juiced beverages were fermented at 25°C for 48 h. In addition, Randazzo et al. (2016) reported that lactic acid in kefir prepared with fruit juices ranged from 0.02-1.00 g/L and acetic acid ranged from 0.06-0.16 g/L. These results indicated that with the same fermentation temperature and time, the concentration of organic acids can be affected by different fermentation substrates.

2.3.7.3 Colour

During fermentation, the colour of water kefir beverage may change significantly (Subardjo, 2017). Randazzo et al. (2016) reported an increase in lightness (L^*) for all samples of fruit-based kefir beverages after fermentation. Subardjo (2017) also reported an increase in the lightness of kefir made from black tea during 72 h fermentation, whereas the redness-greenness (a^*) and the yellowness-blueness (b^*) decreased. Changes in the colour of black tea kefir beverage may be attributed to the degradation or biotransformation of polyphenols, which result in smaller chemical structures of the thearubigin and theaflavins in tea (Jayabalan et al., 2014).

2.3.7.4 Viable cells of lactic acid bacteria and yeasts

As mentioned previously, LAB belonging to the genus *Lactobacillus* and yeasts belonging to the genus *Saccharomyces* were the most frequently reported microorganisms in both water kefir grains and the fermented beverages (Gulitz et al., 2011; Laureys & De Vuyst, 2014; Miguel et al., 2011).

For the LAB and yeasts, the viable cells generally increased during 24 h fermentation, and the counts of LAB are commonly higher than those for the yeasts (Randazzo et al., 2016; Stadie et al., 2013). Magalhaes et al. (2010) reported increased LAB from 6.82 log CFU/mL to 8.32 log CFU/mL and the yeasts from 5.63 log CFU/mL to 7.31 log CFU/mL at the end of 24 h fermentation. Koh et al. (2017) enumerated 10^{12} and 10^{9} CFU/mL *Lactobacillus* and yeasts respectively in water kefir beverage prepared with pumpkin puree and brown sugar. Whereas, Subardjo (2017) reported 6.20±0.14 and 6.38±0.08 log CFU/mL *Lactobacillus* spp. and *S. cerevisiae* respectively, in black tea water kefir beverage containing sugar and carrot juice. A study by Laureys and De Vuyst (2014) showed that for both LAB and yeast, the counts in water kefir beverage were lower than those in water kefir grains. The results showed the LAB present in water kefir grains and water kefir beverage were 8.2 log CFU/mL and 6.9 log CFU/mL respectively whereas the viable cell counts for yeasts were 7.4 log CFU/mL 6.3 log CFU/mL respectively. However, the microbial composition and the corresponding viable cell counts may be different based on different origins of the water kefir grains and fermentation conditions (Gulitz et al., 2013).

2.3.7.5 Sugar alcohol, ethanol and volatile compounds

In addition to the organic acids, sugar alcohol, ethanol and volatile compounds are also produced in water kefir. Stadie (2013) reported that mannitol, a sugar alcohol produced during water kefir fermentation peaked 8.0 g/L within 72 h fermentation. Meanwhile, ethanol levels ranging from 0.06-3% (v/v) have been reported in water kefir fermentation (Corona et al., 2016), and can reach up to 4.96% (v/v) after 48 h fermentation (Randazzo et al., 2016). Puerari et al. (2012) reported 4.5 g/L ethanol in cocoa kefir beverage fermented at 10°C while a higher ethanol content (45 g/L) was obtained in the beverage fermented at 25°C. The difference in the concentration of organic acids may be attributed to different fermentation temperatures. Previous studies have reported the presence of several volatile compounds (ethyl decanoate, ethyl hexanoate, ethyl octanoate) in water kefir (Corona et al., 2016; Laureys & De Vuyst, 2014; Randazzo et al., 2016). The volatile compounds have been attributed to the unique aroma of the final fermented beverage (Laureys and De Vuyst (2014).

2.3.8 Safety of water kefir fermentation

Comparing to the cells in the solution, water kefir microorganisms which embedded in the polysaccharide matrix are more resistant to the physical and chemical stresses such as antibiotics, ozone treatment and ultraviolet radiation (UV) exposure (Magalhaes et al., 2010; Schneedorf, 2012). The kefir growth can be decreased with these stresses, however, none of them are able to completely stop the biomass production or break the grain structure after disturbances. Hence, kefir grains can retain their activity for a considerable time with proper storage because the strong resistances enable the microorganisms to recover to normal growth after exposures (Schneedorf, 2012).

Although the LAB and yeasts and their respective metabolites generally prevent the presence of pathogenic microorganisms in the fermentation process. However, improper handling and excessive washing of grains which may contaminate the products with pathogenic microorganisms or change the microbial community, and affect the quality of the beverage (Schneedorf, 2012). High sugar concentration or low inoculation rate may encourage the growth of mould or pathogenic bacteria (Fiorda et al., 2017). Despite the reliability of subculturing water kefir grains, however, the grains are more vulnerable to microbial contamination such as contamination with *Bacillus* spp., *Micrococcus* spp. and coliforms which may lead to spoilage of the beverage (Cetinkaya & Elal Mus, 2012; Mistry, 2004; Schneedorf, 2012). Grains preserved at the frozen temperatures (-20°C and -80°C) can produce good products without subculturing (Garrote, Abraham, & De Antoni, 1997).

2.3.9 Health benefits of water kefir beverage

2.3.9.1 Probiotics

Probiotics are live microorganisms that confer health benefits on the host when administered in certain amounts (FAO/WHO, 2002). The probiotic microorganisms used in commercial products are mainly the lactic acid bacteria (LAB) belonging to the genera *Lactobacillus* and

Bifidobacterium (Fleet & Balia, 2006; Heller, 2001; Parvez, Malik, Ah Kang, & Kim, 2006; Soccol et al., 2010). Based on different strains and the related mechanisms, the beneficial effects attributed to probiotics include improving intestinal health, enhancing immune response, enhancing the bioavailability of nutrients, reducing serum cholesterol, and increasing resistance to malignancy, certain cancers and infectious illness. The beneficial microorganisms can also reduce the symptoms and prevalence of lactose intolerance as well as increasing resistance to malignancy infectious illness (Kechagia et al., 2013; Parvez et al., 2006; Ranadheera et al., 2010; Soccol et al., 2010).

Several studies have reported potential probiotic microorganisms isolated from water kefir grains (Diosma, Romanin, Rey-Burusco, Londero, & Garrote, 2014; Gulitz et al., 2011; Zanirati et al., 2015). In water kefir, some microorganisms belonging to the genera *Lactobacillus*, such as *Lactobacillus casei*, *Lactobacillus diolivorans*, *Lactobacillus mali* and *Lactobacillus statsumensis* showed good tolerance of gastric acids and bile salts as well as strong antagonistic effects toward pathogens. Moreover, lactobacilli species demonstrated strong adherence of epithelial and mucosal cells (Fiorda, Pereira, Thomaz-Soccol, Medeiros, et al., 2016; Koh et al., 2018; Zanirati et al., 2015). Zanirati et al. (2015) suggested the use of probiotic *Lactobacillus* strains with other LAB and yeasts as starter cultures to produce water kefir with enhanced health benefits. Moreover, some yeast strains of *S. cerevisiae* and *Kluyveromyces marxianus* in water kefir grains have been reported to have probiotic characteristics (Diosma et al., 2014). Some probiotics in kefir have been reported to have anti-hyperglycaemic and anti-hyperlipidaemia activities (Alsayadi et al., 2014).

2.3.9.2 Antimicrobial activity

The ethanol, organic acids, bacteriocins (peptides) and other bioactive components produced by the microbes in water kefir grains have inhibitory properties against the growth of several pathogenic microbes which include *Candida albicans*, *Escherichia coli*, *Helicobacter pylori*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus salivarius* (Kandylis et al., 2016; Rodrigues et al., 2005; Silva, Rodrigues, Xavier Filho, & Lima, 2009). By measuring the size of the inhibition halo zone against pathogenic microorganisms, Silva et al. (2009) showed that

the antimicrobial activities of water kefir grains can be affected by several factors which include fermentation time, the concentration of carbon source and the water kefir grains prepared with unrefined sugar having the highest antimicrobial activities compared to use of demerara and molasses. In addition to the water kefir grains, the liquid part of water kefir also showed antibiotic activities (Rodrigues et al., 2005). The size of the inhibition halos against several pathogenic microorganisms by water kefir grains and the beverage are listed in Table 2.3.

Table 2.3 Mean diameters (mm) of inhibition zones of pathogens by water kefir grains and the beverage

Bacteria	**Water kefir grains**	**Water kefir**
Streptococcus pyogenes	29.0	27.2
Streptococcus salivarius	27.1	24.9
Staphylococcus aureus	28.3	30.0
*Pseudomonas **aeruginosa***	26.2	30.2
Salmonella typhimurium	26.8	25.6
Escherichia coli	26.0	28.4
Listeria monocytogenes	23.4	29.3
Candida albicans	23.2	28.0

Source: Rodrigues et al. (2005)

2.3.9.3 Antioxidant activity

One of the important health benefits of consuming water kefir beverages is the antioxidant activity of the products (Alsayadi et al., 2013; Fiorda, Pereira, Thomaz-Soccol, Medeiros, et al., 2016; Fiorda et al., 2017; Lengkey & Balia, 2014; Stadie, 2013). Previous studies have shown that water kefir exhibited high DPPH scavenging ability (9.88-63.17%), and the inhibition of ascorbate autoxidation (6.08-25.57%) was enhanced with increasing water kefir concentration (Alsayadi et al., 2013). The antioxidant activity of honey-based water kefir by determining the DPPH and ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical-scavenging activity has also been reported (Fiorda, Pereira, Thomaz-Soccol, Medeiros, et al. (2016) The antioxidant activity of kefir inoculated with different substrates all increased during fermentation, with honey-based kefir beverage demonstrating higher antioxidant capacity compared to the dairy-based kefir beverage. In addition to consuming the beverage, direct intake of the EPS produced by water kefir microorganisms has the potential to reduce the risk of cardiovascular diseases (Abu-Ghannam & Rajauria, 2015).

2.3.10 Manufacturing of water kefir beverage

Based on available reports, the majority of water kefir beverages are traditionally made at homes. Therefore, there is no published industrial process for the manufacturing of water kefir (Fiorda et al., 2017; Magalhaes et al., 2010). In the past, the major challenges related to the industrialised production were the transportation which may introduce foreign microorganisms, and contamination by the natural starter microbiota to start the fermentation (Fiorda et al., 2017; Schwan, Pereira, & Fleet, 2014). Therefore, there is a need to stabilise the microbial growth and control the microbiota present in different batches.

The evolution of yoghurt fermentation provides some clues for developing an industrial process for water kefir fermentation (Fiorda et al., 2017). In early times, yoghurt was only produced at household level due to the difficulties in transportation and relied on contamination by natural starter culture. The development of defined starter cultures with predictable characteristics, improvements in equipment design and better control of fermentation processes lead to the growth of large scale production of fermented milk beverages (Fiorda et al., 2017; Özer, 2010).

Fiorda et al. (2017) stated that the industrial process of water kefir production has never been established. However, the information about the production process (Figure 2.8) of water kefir beverage reported in the same paper contradicts this statement. In this process, kefir grains are directly added to the pasteurised and cooled sugar substrate and ferment in the vessel for 20-24 h at 25-30°C (Fiorda et al., 2017). Then, grains and the medium are separated by filtration using a sterile sieve. The grains are washed and stored in a cooling tank for reuse while the kefir beverages are stored at 4°C and distributed. According to Güzel-Seydim, Seydim, and Greene (2000), kefir beverages can be stored at refrigerated temperatures up to twenty days after fermenting at 25-30°C for 20-24 h. Furthermore, lyophilising the starter cultures containing yeast and LAB isolated from kefir fermentation is an alternative process for producing kefir beverage.

Figure 2.8 Production process of water kefir beverage

Source: Fiorda et al. (2017)

2.3.11 Alternative sources of substrates for water kefir fermentation

Unrefined sugar, such as brown sugar and raw sugar, is the most common substrate for water kefir fermentation due to the abundant minerals which assist the growth of microorganisms (Magalhaes et al., 2010; Marsh et al., 2013; Miguel et al., 2011). With the development of consumer knowledge on health and wellbeing, the use of alternative non-dairy substrates (Figure 2.9) in water kefir fermentation have been reported (Fiorda et al., 2017). Kefir beverages fermented with non-dairy substrates do not only have similar characteristics to the traditional water kefir beverages but also provide the possibility of producing new non-dairy probiotic beverages with natural ingredients and different flavours (Grønnevik, Falstad, & Narvhus, 2011; Miguel et al., 2011).

Figure 2.9 Sources of substrates for non-dairy kefir beverages

Source: Fiorda et al. (2017)

Several vegetable extracts which include ginger, onion, pumpkin, and carrot have been studied for the growth of water kefir grains (Fiorda et al., 2017). Ginger beer, which has been named from the ginger beer plant, can be produced by kefir fermentation of ginger extract (Pidoux, 1989). Compared to the fermentation in unrefined sugar, a slower fermentation was observed in ginger crystal, which resulted in higher ethanol content (Fiorda et al., 2017). Koh et al. (2017) reported that water kefir beverage fermented with 22.28% (w/v) pumpkin puree and 9.07% (w/v) brown sugar contained 10^{12} and 10^{9} CFU/mL *Lactobacillus* and yeasts, respectively. Subardjo (2017)

found that after 96 h fermentation at 25°C, black tea water kefir beverage prepared with 10% (w/v) sugar and 10% (w/v) carrot contained 6.20±0.14 and 6.38±0.08 log CFU/mL *Lactobacillus* and *S. cerevisiae* respectively. The black tea-carrot kefir formulation received the highest mean consumer sensory scores for product acceptance on the 9-point hedonic rating scale.

In the molasses category, syrup extracted from Piloncillo can be used in traditional kefir fermentation (Rubio, Lappe, Wacher, & Ulloa, 1993). Recently, water honey kefir fermentation produced a beverage with higher antioxidant and probiotic activities compared with the traditional kefir beverage (Fiorda, Pereira, Thomaz-Soccol, Medeiros, et al., 2016; Fiorda, Pereira, Thomaz-Soccol, Rakshit, & Soccol, 2016). In addition, Rodrigues et al. (2016) reported decreased ulcerogenic responses and inflammatory in animal studies.

Comparing to vegetables and molasses, fruits are the most diverse products among kefir fermentation substrates shown in Figure 2.9. Fruits are rich sources of many nutrients (proteins, amino acids, minerals, vitamins) which make them ideal substrates for the growth of kefir microorganisms (Randazzo et al., 2016). *Tapache*, produced with kefir grains, pineapples, unrefined sugars and cinnamon, is a popular fermented beverage in Latin America (Fiorda et al., 2017). In southern Italy, a grape-based kefir beverage named "*Kefir d'uva*" is a refreshing, acidic and slightly-carbonated product with low alcohol content (Moreno-Terrazas, Reyes-Morales, Huerta-Ochoa, Guerrero-Legarreta, & Vernon-Carter, 2001). In addition, typical fruits such as apples, prickly pears, kiwifruits and several region-specific fruits such as pomegranates and quinces have also been used for the preparation of kefir beverage (Kazakos et al., 2016; Randazzo et al., 2016; Sabokbar & Khodaiyan, 2015). In a study on kefir fermentation in Mediterranean fruit juices, Randazzo et al. (2016) reported the highest levels of rod-shaped LAB (8.0±0.2 log cfu/mL) and cocci LAB (8.3±0.7 log cfu/mL) in the kefir fermented with prickly pear fruit juice while the highest level of yeast (8.0±0.9 log cfu/mL) was obtained in the kefir prepared with pomegranate juice. The fermented beverage containing kiwifruit (89.51±0.15%) and pomegranate (88.04±0.43%) had the highest DPPH. Among the beverages analysed in their study, kefir fermented with apple juice obtained the highest overall sensory scores.

2.4 *Ziziphus jujuba* Mill.

2.4.1 Origin and distribution

Jujube (*Ziziphus jujuba* Mill.) belongs to the genus *Ziziphus* in the buckthorn family Rhamnaceae (Gao, Wu, & Wang, 2013). Jujube has been an indigenous fruit cultivated in China for more than 4000 years, and mainly distributed in the northwest region, the Yellow River Valley areas, and the eastern region (Ji et al., 2017). In the ancient Chinese medicine books, jujube was considered as an excellent herbal medicine and it was recognised as one of the five most valuable fruits (Chen et al., 2015; Chen et al., 2014). It has been used as the traditional Chinese medicine to treat fatigue, anorexia, and loose stools caused by deficiency syndromes of the spleen in women (Guo et al., 2010). Nowadays, jujube is widely cultivated not only in China but also in tropical and subtropical regions such as Australia, Europe, and southern and eastern Asia (Ji et al., 2017). However, China is the only country known to export jujube, and its cultivation area reached around 2.8 million hectares which produced over 7 million tonnes of jujube in 2014 (Ji et al., 2017). There are more than 700 jujube cultivars available in China (Guo et al., 2010). The picture of fresh jujube fruit was shown in Figure 2.10.

Figure 2.10 Photo of fully ripened fresh jujube (*Ziziphus jujuba* Mill.) fruit
Source: Ji et al. (2017)

2.4.2 Nutritional composition

Dried jujube has been commonly consumed as food for thousands of years. Due to its high nutraceutical and nutritional values, it has also been utilised as a food additive and flavouring (Li, Fan, et al., 2007). The nutritional composition of fresh jujube fruit is shown in Table 2.4.

Table 2.4 Nutritional composition of dried *Ziziphus jujuba cv. jinsixiaozao*

Type	Nutrients (units)	Content
Proximate composition (%)	Moisture	18.99
	Protein	5.01
	Soluble fibre	2.79
	Insoluble fibre	6.11
	Lipid	0.37
	Carbohydrate	81.62
Mineral (mg/100 g)	Calcium, Ca	65.20
	Iron, Fe	4.68
	Magnesium, Mg	39.70
	Phosphorus, P	110.00
	Potassium, K	79.20
	Sodium, Na	6.34
	Zinc, Zn	0.55
Vitamin (mg/100 g)	Vitamin C	359.00
	Thiamin	0.05
	Riboflavin	0.07

Source: Li, Fan, et al. (2007)

Jujube fruit can be utilised as a carbon supplement for fermentation as well as a good source for sugar extraction due to its high carbohydrate content. Li, Fan, et al. (2007) reported that the major sugars present in the tested jujube were fructose and glucose, with the concentration ranged from 18.6-42.9% and 19.2-27.2% of dry weight basis respectively. The fructose and glucose contents were found to be higher than the sucrose content in the jujube, and this may be attributed to that during the fruits ripening, the sucrose is hydrolysed by invertase when translocating from the leaves to the flesh (Li, Fan, et al., 2007; Wang & Camp, 2000).

The dietary fibre in jujube fruit may slow the digestion which contributes to regulating blood sugar level and controlling calorie intake due to the satiating effect (Gao et al., 2013). Gusakova et al.

(1999) identified 33 fatty acids with chain length range from 7 to 28 carbons in the dried pulp of jujube and reported that 16 of the identified fatty acids were related to the fragrance of the jujube. Different fatty acid compositions were detected at different developmental stages (Guil-Guerrero, Delgado, González, & Isasa, 2004). San and Yildirim (2010) found that the jujube fruits were rich in linoleic acid (omega-6), a lipid which is not able to be synthesised by the human body. In addition to linoleic, this study also reported the predominance of oleic, palmitic, and palmitoleic acids in the tested jujube fruit.

Jujube fruit is considered as good source of minerals (Gao et al., 2013). By investigating the mineral composition of five jujube cultivars, Li, Fan, et al. (2007) demonstrated the predominance of potassium (79.2-458 mg/100 g dry weight), phosphorus (59.3-110 mg/100 g dry weight), calcium (45.6-118 mg/100 g dry weight) and manganese (24.6-42.1 mg/100 g dry weight) in all tested jujube, whereas sodium, zinc and copper were present in lower concentrations. Selenium, a nutritionally significant element, was not found in the tested jujube. In regards to vitamin C, Li, Fan, et al. (2007) reported that high vitamin C content was detected in the tested jujube, with the concentration range from 192 to 359 mg/100 g dry weight. Besides the vitamins shown in Table 2.3, vitamin B-6, niacin, and vitamin A were also found in fresh jujube (Gao et al., 2013). Moreover, organic acids such as citric, succinic, malic acids and amino acids such as L-Ala, L-Asp, L-Glu were also detected in the jujube fruits (Choi, Ahn, Kozukue, Levin, & Friedman, 2011). Hence, the jujube fruit can be considered as a rich medium for the growth of water kefir microorganisms due to its sugar, minerals, vitamin C and amino acids content (Randazzo et al., 2016; Schwan, 1998).

2.4.3 Bioactive compounds

Recent pharmacological researche have shown that *Ziziphus jujuba* has various biological activities such as antioxidant activities, anti-inflammatory effects, immunological activities (Chi et al., 2015; Gao et al., 2011; Li et al., 2017). These pharmacological effects can be attributed to the bioactive compounds present in the jujube (Ji et al., 2017). The related bioactive compounds including phenolic compounds, polysaccharides, vitamin C, triterpenic acids, nucleosides,

nucleobases, α-tocopherol, carotene, and flavonoids have been identified in jujube fruit (Choi et al., 2011; Gao et al., 2013; Gao, Wu, Wang, Xu, & Du, 2012; Guo et al., 2015; Pawlowska, Camangi, Bader, & Braca, 2009; San & Yildirim, 2010; Shi, Zhang, Su, Zhou, & Li, 2018).

2.4.3.1 Phenolic compounds

Numerous studies have shown that the phenolic compounds present in foods are important to human health due to their antioxidant properties (Gao, Wu, Wang, et al., 2012; San & Yildirim, 2010; Liu et al., 2017). The phenolic compounds detected in jujube fruit were shown in Table 2.4. Hudina, Liu, Veberic, Stampar, and Colaric (2008) investigated the phenolic profile of eight dried jujube cultivars cultivated in China. Their study reported the considerable differences in the contents of phenolic compounds (in mg/100 g dried fruit) among different cultivars: cafferic acid (0.09-0.37), catechin (0.65-2.12), chlorogenic acid (0.22-0.95), epicatechin (0.48-5.13), and rutin (0.60-5.77). Choi et al. (2012) and Shi et al. (2018) also demonstrated the significant differences in the phenolic profiles among different jujube varieties with different maturity levels. By investigating the distribution of phenolic acid in different tissues of dried jujube fruit, Zhang, Jiang, Ye, Ye, and Ren (2010) detected 200.64 mg/kg dry weight gallic acid and 239.79 mg/kg dry weight protocatechuic acid in peel, whereas the concentrations were 70.12 mg/kg dry weight and 85.59 mg/kg dry weight in pulp, respectively.

Moreover, the fresh jujube was found to display the different phenolic profiles compare to the dried jujube fruit. Gao, Wu, Wang, et al. (2012) investigated the effect of drying of jujubes on the phenolic compounds using high-performance liquid chromatography (HPLC) analysis. In fresh jujube, rutin (27.0 mg/kg dry weight) and catechin (20.8 mg/kg dry weight) were found to present at higher concentrations compared to that of protocatechuic acid, vanillic acid, and ferulic acid. Results showed that microwave-dried jujube contained more than double of the concentration in fresh jujube (45.1 mg/kg of dry weight) while catechin was not detected in the sun-dried jujube. The concentration of rutin also decreased to 12.6 mg/kg dry weight after sun-drying. Furthermore, the gallic acid, which was not detected in the fresh jujube, increased to 12.7 mg/kg dry weight after freeze-drying (Gao, Wu, Wang, et al., 2012). By evaluating the antioxidant activities and jujube

composition using HPLC analysis, Liu et al. (2017) reported the presence of protocatechuic acid, catechol, *p*-hydroxybenzoic acid and vanilla acid in the water extract of *Ziziphus jujuba* cv. *jinsixiaozao*. However, catechin or rutin was not detected. This indicates that the thermal treatment may influence on the phenolic composition of jujube fruit. The phenolic compounds identified in jujube fruit are shown in Table 2.5.

Table 2.5 Phenolic compounds identified in jujube fruit

Phenolic compound	Reference
Apigenin-7-glucoside	(San & Yildirim, 2010)
Caffeic acid	(Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Catechin	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Catechol	(Liu et al., 2017)
Chlorogenic acid	(Shi et al., 2018; Zhang et al., 2010)
Cinnamic acid	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Ellagic acid	(Gao, Wu, Yu, et al., 2012)
Epicatechin	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Eriodictyol	(San & Yildirim, 2010)
Ferulic acid	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Gallic acid	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Kaempferol-glucosyl-rhamnoside	(Choi et al., 2011)
p-coumaric acid	(Gao, Wu, Wang, et al., 2012; Liu et al., 2017)
p-hydroxybenzoic acid	(Gao, Wu, Wang, et al., 2012; Liu et al., 2017)
Procyanidin B1	(Shi et al., 2018)
Procyanidin B2	(Choi et al., 2011; Shi et al., 2018)
Procyanidin B3	(Shi et al., 2018)
Protocatechuic acid	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012; Liu et al., 2017)
Quercetin	(Gao, Wu, Yu, et al., 2012; Shi et al., 2018)
Quercetin-3-galactoside	(Choi et al., 2011; Shi et al., 2018)
Quercetin-3-glucoside	(Shi et al., 2018)
Quercetin-3-robinobioside	(Pawlowska et al., 2009)
Quercetin-3-rutinoside	(Choi et al., 2011)
Rutin	(Gao, Wu, Wang, et al., 2012; Gao, Wu, Yu, et al., 2012)
Syringic acid	(San & Yildirim, 2010)
Vanillic acid	(Gao, Wu, Wang, et al., 2012; Liu et al., 2017)

2.4.3.2 Polysaccharides

One of the most abundant components in the *Ziziphus jujuba* are polysaccharides, which can be recognised as a major group of bioactive constituents (Ji et al., 2017). The structural characteristics of polysaccharide mainly include the monosaccharide composition, type of glycosyl linkage, molecular weight, etc. (Gao et al., 2013). Polysaccharides with different monosaccharide constituents have been identified in jujube fruit. The polysaccharides fractions in jujube fruit

isolated by Wang et al. (2015) were found to be comprised of arabinose, glucose, galactose, galacturonic acid, mannose and rhamnose. The result of *in vitro* antitumor test showed that both fractions inhibited the growth of human HepG2 cells, indicating the antitumor property of the isolated polysaccharides. Chang, Hsu, and Chen (2010) isolated three acid polysaccharide fractions and one neutral polysaccharide fraction from the jujube polysaccharides. Arabinose, galactose, glucose, mannose, rhamnose, and xylose were detected in the polysaccharide fraction based on the gas chromatography (GC) result. The isolated polysaccharides fractions present a more significant effect in scavenging superoxide anions than hydroxyls radicals, and the acidic polysaccharides were found to be more effective in chelating ferrous (Chang et al., 2010).

2.4.4 Ziziphus jujuba polysaccharide and syrup extraction

The polysaccharides are the structural components of the cell wall in *Ziziphus jujuba*, and the basic extraction methods are based on the mechanism of breaking the outer layer of cell wall without changing the structures of the jujube polysaccharides (Jin, Zhao, Huang, Xu, & Shang, 2012; Zhang, Cui, Cheung, & Wang, 2007). Hot or boiling water extraction is the most convenient method which is widely used in the industry (El-Nagga & El–Tawab, 2012). Li, Liu, Fan, Ai, and Shan (2011) extracted the polysaccharides by refluxing the jujube fruit with 95% ethanol in a water bath at 70°C followed by extraction with distilled water at 80°C for 3 h. Rotary evaporation was then used for concentrating the aqueous extract. As a result, the water extraction method requires long extraction time and high temperature, which result in low efficiency and may cause the degradation of nutritive components.

Different technologies such as microwave-assisted treatment and high-powered ultrasonic processing have been developed to improve the efficiency of extraction (El-Nagga & El–Tawab, 2012; Li, Ding, & Ding, 2007). The schematic overview of the jujube polysaccharide extraction is shown in Figure 2.11. Study showed that an ultrasonic-assisted extraction had a positive effect on the yield and purity of the extracted polysaccharides (Li, Ding, et al., 2007). The optimum extraction could be achieved with 45-53° extraction temperature, 31.7 W actual sonic power, 20:1 water/solid ratio and 20 min extraction time. The yield was found to increase by 20.2% of the hot-water extraction method, and the purity could also be improved with the short ultrasonic

application (Li, Ding, et al., 2007). Qu, Yu, Luo, Zhao, and Huang (2013) reported that the extraction with ultrasonic power of 120 W at 55°C for 15 min resulted in the best yield while the polysaccharides extracted with ultrasonic power of 80 W at 40°C for 15 min exhibited the best hydroxyl radical scavenging activity. The ultrasound can act on the cell wall and increase the accessibility and extractability of polysaccharides, which resulted in higher efficiency (Li, Ding, et al., 2007). In addition to the ultrasound-assisted method, microwave-assisted extraction is also employed to improve the extraction yield. El-Nagga and El–Tawab (2012) reported that the syrup extracted with the microwave method contained significantly high total sugars and total phenolics than the water extracted syrup. Rostami and Gharibzahedi (2016) found that with the 30:1 water/material ratio, the yield of polysaccharides extracted at 70°C for 60 min with 400 W microwave power could be maximized to 9.02%. Hence, with the assistance of microwave and ultrasound, the yield can be increased with a shorter processing time (Ji et al., 2017).

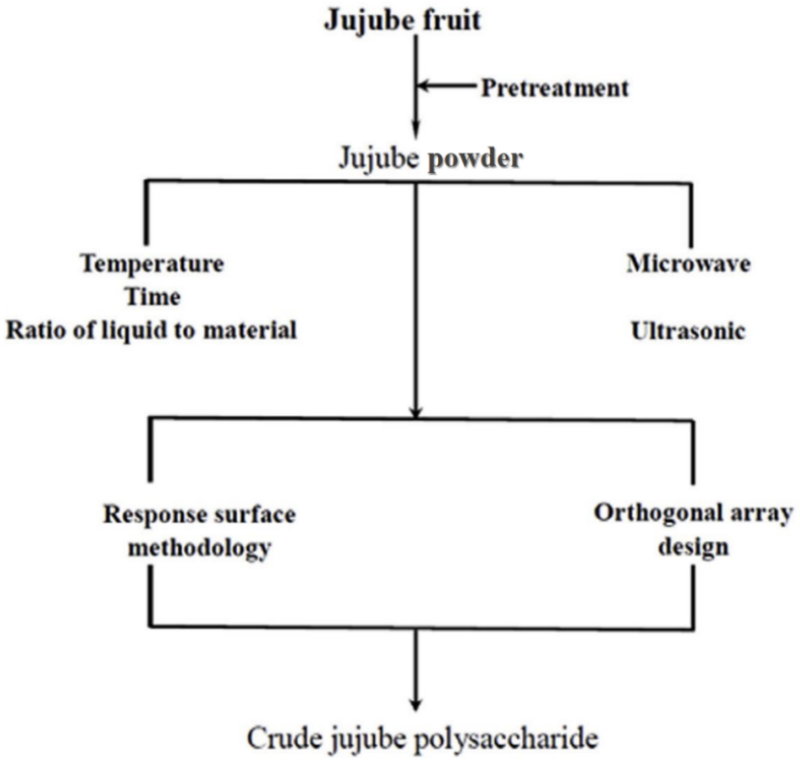

Figure 2.11 Modified schematic overview of *Ziziphus jujuba* Mill. polysaccharide extraction
Source: Ji et al. (2017)

2.4.5 Health benefits of jujube fruit

There are numerous studies investigated the biological activity of jujube fruit, however, most of these studies were done *in vitro* or based on the animal model, and human clinical evidence are still needed (Gao et al., 2013). The potential health benefits related to jujube such as antiobesity, antidiabetic activity, anti-inflammatory, antioxidant, gastrointestinal protective activity are shown in Figure 2.12.

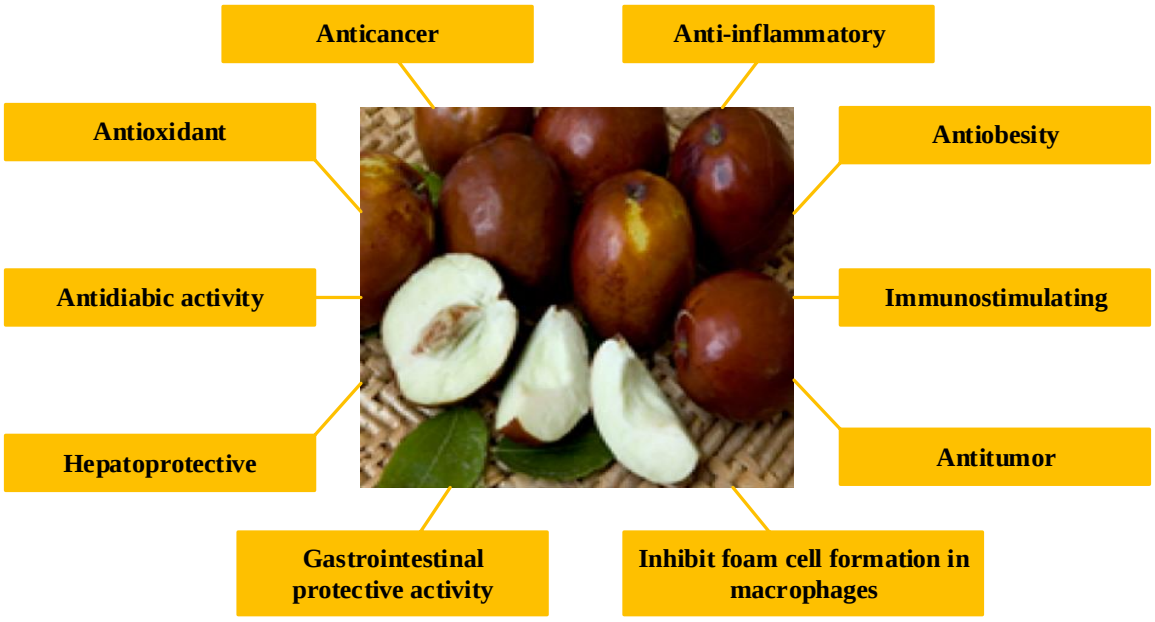

Figure 2.12 Pharmacological properties of jujube fruit

Source: Gao et al. (2013); Ji et al. (2017)

2.4.5.1 Antioxidant activity

The antioxidant compounds present in the fruit can help the human body to reduce the oxidative damage (Choi et al., 2011; Li et al., 2005). Many researchers have investigated the antioxidant capacity of jujube fruit, and the antioxidant capacity can be related to the compounds such as phenolics (as discussed in section 2.4.3.1) and vitamins (Li et al., 2005). Li, Fan, et al. (2007) evaluated the total antioxidant capacity using Ferric-reducing antioxidant power analysis (FRAP) and reported that among the tested five cultivars, the total antioxidant capacity ranged from 342-

1173 μmol/g FRAP. Gao, Wu, Yu, et al. (2012) measured the antioxidant activity by 2,2'-azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid (ABTS) assay and the 2,2-diphenyl-1-picylhydrazyl (DPPH) radical scavenging method. The results showed that higher antioxidative activities were obtained in fruits with higher antioxidant content. Recently, Liu et al. (2017) investigated the antioxidant activities of jujube water extract. The result from DPPH, ABTS and FRAP assay were 202.18, 60.13 and 4670 μmol Trolox per mL, respectively, indicating a significant antioxidant effect of jujube water extract.

2.4.5.2 Gastrointestinal protective property

Studies have reported that jujube fruits may have positive effects on maintaining gastrointestinal health. By using a hamster model, Huang, Yen, Sheu, and Chau (2008) found that consumption of jujube water-soluble carbohydrate concentrate (WSCC) (at least 40 mg/day) could effectively reduce the exposure of intestinal mucosa to the harmful substances such as toxic ammonia. Yue et al. (2015) also investigated the effect of jujube polysaccharides on the inflammatory bowel disease (IBD) using a rat model and reported that the jujube polysaccharides could significantly reduce the severity of colitis and the mucosal damage in colitis rats. Yue et al. (2015) stated that the protection from jujube polysaccharides may be attributed to the increased barrier function through the activation of AMP-activated protein kinase (AMPK). Hence, the jujube polysaccharides can produce anti-inflammatory effects by inhibiting the cytokine cascade as well as promote tissue repair through various growth factors (Huang et al., 2008; Ji et al., 2017).

Chapter 3. MATERIALS AND METHODS

3.1 Experimental design

This section describes the organisation of the design of the experiments. The development of fermented jujube water kefir was conducted in three integrated phases comprising (1) the optimisation of jujube fruit syrup extraction, (2) investigation of the effect of jujube syrup concentration and fermentation temperature on fermentation of jujube water kefir beverage; selection of the most promising formulation of jujube water kefir beverage, and (3) analysis of the final jujube water kefir beverage formulation during the fermentation and three-week storage at 4°C. All the experiments were replicated two times with duplicate analyses/measurements for all parameters expect in phase I, the mass and the volume of syrup were measured directly. All of the chemicals used in this experiment were of reagent grade or higher.

Phase I: Optimisation of jujube syrup extraction

The most efficient water-bath extraction procedure for jujube syrup using 2^2 factorial design was investigated in Phase I (El-Nagga & El–Tawab, 2012). In this phase, the experiments were organised in two stages. The first stage aimed to select the most efficient method for the extraction of jujube syrup. The factorial (2^2) included two variables (Table 3.1) with two categories in stage one, which were the type of rehydration of dried jujube fruit (rehydrated and non-rehydrated) and type of concentration of syrup by evaporation of water (evaporated and non-evaporated). In phase one, the mass and volume of the extracted jujube syrup were measured while total soluble solids (TSS) were analysed. The density of the syrup was calculated from the results of the mass and volume.

Table 3.1 Phase I stage I: Experimental design for the optimisation of jujube syrup extraction

Experiment	Experimental code	Type of rehydration	Type of concentration
1	S1 (NR/E)	Non-rehydrated	Evaporated
2	S2 (NR/NE)	Non-rehydrated	Non-evaporated
3	S3 (R/E)	Rehydrated	Evaporated
4	S4 (R/NE)	Rehydrated	Non-evaporated

Notes: NR/E – non-rehydrated, evaporated; NR/NE – non-rehydrated, non-evaporated; R/E – rehydrated, evaporated; R/NE – rehydrated, non-evaporated.

In stage I, under the non-evaporated category (Table 3.1), the volume of extracted syrup using the rehydration method (S4) (19.50±0.71 mL/100 g dried jujube) was lower than the quantity of the extract obtained using the non-rehydrated method (S2) (72.00±0.47 mL/100 g dried jujube). The TSS measured in °Brix of the extracted syrup were similar (Appendix D). Due to the low efficiency of the rehydration method for the extraction of jujube syrup, no further investigation was conducted using this technique. Comparing the syrup extracted by the non-rehydrated method, the total soluble solids of the syrup obtained using rehydration method increased after evaporation (S1) (39.75 ± 0.06 °Brix) (Appendix D), whereas the yield decreased slightly while the production time increased.

In stage II, the optimum extraction condition for jujube syrup was determined based on the most efficient (combined) method (S2) obtained in stage one. Thus, from stage one, the quanity of water for extraction (600 mL; 650 mL) and extraction temperature (70°C; 75°C) were the two variables selected, respectively (Table 3.2).

Table 3.2 Phase I stage II: Experimental design for the optimisation of jujube syrup extraction

Experiment	Experimental code	Volume of water (mL)	Extraction temperature (°C)
A	SA (600/70)	600	70
B	SB (600/75)	600	75
C	SC (650/70)	650	70
D	SD (650/75)	650	75

Notes: 600/70 – 600 mL, 70°C; 600/75 – 600 mL, 75°C; 650/70 – 650 mL, 70°C; 650/75 – 650 mL, 75°C.

Phase II: Effect of jujube syrup concentration and temperature on fermentation of jujube water kefir beverage

Phase II investigated the effect of jujube syrup concentration and fermentation temperature on the beverage to select the most promising fermented formulation. Two levels of syrup concentrations (10% and 20%) and two fermentation temperatures (25°C and 27°C) were the two variables used in the second phase (Table 3.3). The acidity (pH and titratable acidity), TSS, colour and viable cell counts of the beverage were determined during the first and second stages of fermentation. The most promising fermented formulation of the beverage was selected by conducting informal focus groups and consumer sensory evaluation after 72 h fermentation.

Table 3.3 Phase II: Screening of potential formulations for jujube water kefir beverage fermentation

Formulation	Experimental code	Fermentation temperature (°C)	Volume/concentration (mL, % v/v) of jujube syrup in 1st & 2nd stage fermentation medium
1	K1 (10/25)	25	20 mL, 10%
2	K2 (10/27)	27	20 mL, 10%
3	K3 (20/25)	25	40 mL, 20%
4	K4 (20/27)	27	40 mL, 20%

Note: 10/25 – 10%, 25°C; 10/27 – 10%, 27°C; 20/25 – 20%, 25°C; 20/27 – 20%, 27°C

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

In phase III, the experiment was separated into two parts. In part one, the selected fermented formulation of jujube water kefir was analysed for antioxidant, organic acids, sugar compositions, and ethanol content during fermentation. In part two, the stability (physicochemical and microbiological characteristics) of the final jujube water kefir beverage formulation was determined during storage (4°C) for three weeks.

3.2 Description of fermentation variables

3.2.1 Concentration of jujube syrup

In kefir fermentation, the fermenting culture interacts with the substrate in the presence of oxygen from the air, nutrients, water and acids (Hansen, 2002; Reddy et al., 2008). The primary activity of the culture is to convert the carbohydrates to desired metabolites such as alcohol, lactic acid, acetic acid or CO_2 (Hansen, 2002; Heller, 2001; Reddy et al., 2008). In this experiment, two levels (10%, 20%, v/v) of jujube syrup concentration were used as supplementary sources of energy (carbon) during fermentation.

3.2.2 Fermentation temperature

Temperature is one of the essential factors in food fermentation that affects the microbial ecology and the effectiveness of the fermentation (Torija, Rozes, Poblet, Guillamón, & Mas, 2003). According to the supplier (Cultures for Health, Raleigh, North Carolina, USA) of the culture used in this experiment, the optimum fermentation temperature was 27°C. However, water kefir has been successfully fermented at other temperatures such as 25°C (Alsayadi et al., 2013; Fiorda et al., 2017; Koh et al., 2017; Magalhaes et al., 2010; Marsh et al., 2013; Randazzo et al., 2016; Waldherr et al., 2010). Therefore, 25°C and 27°C were the selected fermentation temperatures for jujube water kefir beverage in the present study.

3.3 Raw materials

The starter culture used in the experiments was supplied as dried water kefir grains (Cultures for Health, Raleigh, North Carolina, USA) purchased online (www.simpleliving.co.nz) from Simple Living (Otahuhu, Auckland, New Zealand). Dried jujube fruit supplied by Laoling People's Government, Shandong, China served as the major source of carbon and flavour for jujube water kefir. The dried jujube was vacuum packed and delivered in the cardboard by airfreight. Certified organic raw sugar No. 4522 (Chelsea Refinery, Birkenhead, Auckland, New Zealand) was purchased from Countdown® supermarket, Birkenhead, New Zealand.

3.4 Methods

3.4.1 Extraction of jujube syrup

The syrup was extracted according to the method of El-Nagga and El–Tawab (2012) with minor modifications. For both the rehydration and non-rehydrated methods, the surface of the dried jujube fruit was cleaned with a dry towel to remove adhering dirt and then cut into pieces (~1 cm^3 cube) with a standard kitchen knife (Harrison & Lane, NZ).

3.4.1.1 Extraction of syrup with rehydration

Dried jujube (19% moisture content) fruit seeds were removed, and the pulp was chopped into pieces (~1 cm^3 cube) (Figure 3.1). The chopped dried jujube fruit pieces (300 g) were weighed (Sartorius, Germany) in a 2-L metal jar. One litre (1 L) of potable water was boiled using an electric kettle (Sunbean, NZ) and cooled to 80°C. The temperature of the boiled water was measured by a thermometer (RS Pro, RS-41, NZ). The dried jujube fruit pieces were rehydrated in 600 mL boiled water (80°C) for 1 h in a 70°C-water bath (Kim et al., 2012). The jujube solution was then blended using a ProfiCook blender (PC-UM 1006, Germany) set at mode 3 for 3 min and then transferred into the metal jar. The slurry was heated in a water bath (Julabo, PURA 14, Germany) and held at 70°C for 30 min followed by centrifugation (Sigma, 6-16KS, Germany) at 2549 g for 15 min (El-Nagga & El–Tawab, 2012). The supernatant was filtered using a plastic sieve (300 mesh) and collected in the tared cylinder (100 mL) for measuring the mass, volume and total soluble solids (section 3.5.1-3.5.2). The supernatant was then stored in sealed glass jars in a refrigerator at 4°C (Skope, A050110822, Germany) until required for further treatment and analysis.

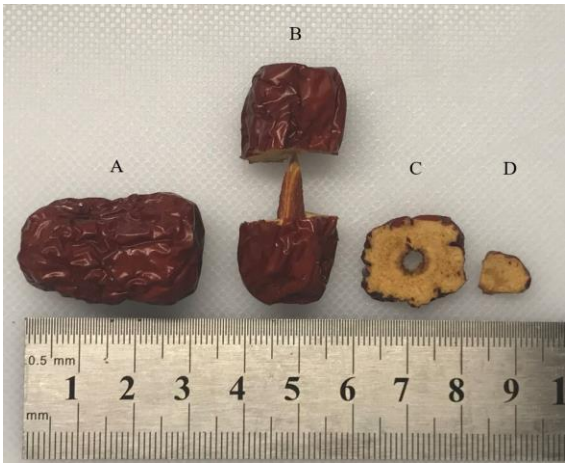

Figure 3.1 Dried jujube fruit (*Ziziphus jujuba* Mill.)

Notes: A) Dried jujube fruit; B) cut jujube with seed; C) cross section of cut jujube without seed; D) jujube piece.

3.4.1.2 Extraction of syrup without rehydration

Dried jujube was prepared as previously described. Chopped jujube pieces (300 g) were weighed (Sartorius, Germany), and 60 g (×5 portions) were blended in a coffee blender (Breville, BCG200, AU) for 15 s. The blended jujube was collected in a 2-L metal jar and 600 mL or 650 mL of boiled water (80°C) were added. The jujube solution was then heated in a water bath and held at 70°C or 75°C for 30 min. The heated jujube slurry was blended (ProfiCook, PC-UM 1006, Germany) on mode 3 for 3 min followed by centrifugation at 2549 × *g* for 15 min (El-Nagga & El–Tawab, 2012). The supernatant was filtered and stored as described in section 3.4.1.1.

3.4.1.3 Concentrating jujube syrup using a rotatory evaporator

The filtered jujube syrup (100 mL) was transferred into a 500-mL round bottom glass flask (Buchi, Switzerland) and concentrated in a rotatory evaporator (Buchi, R-3 HB, Switzerland) at 70°C under vacuum for 1 h at rotation setting of 5 (El-Nagga & El–Tawab, 2012). The concentrated jujube syrup was transferred into 100 mL tared cylinder to measure volume and mass (section 3.5.1-3.5.2). The concentrated jujube syrup was stored in sealed glass jars in the refrigerator (4°C) until required for analysis. An overview of the jujube syrup extraction methods is shown in Figure 3.2.

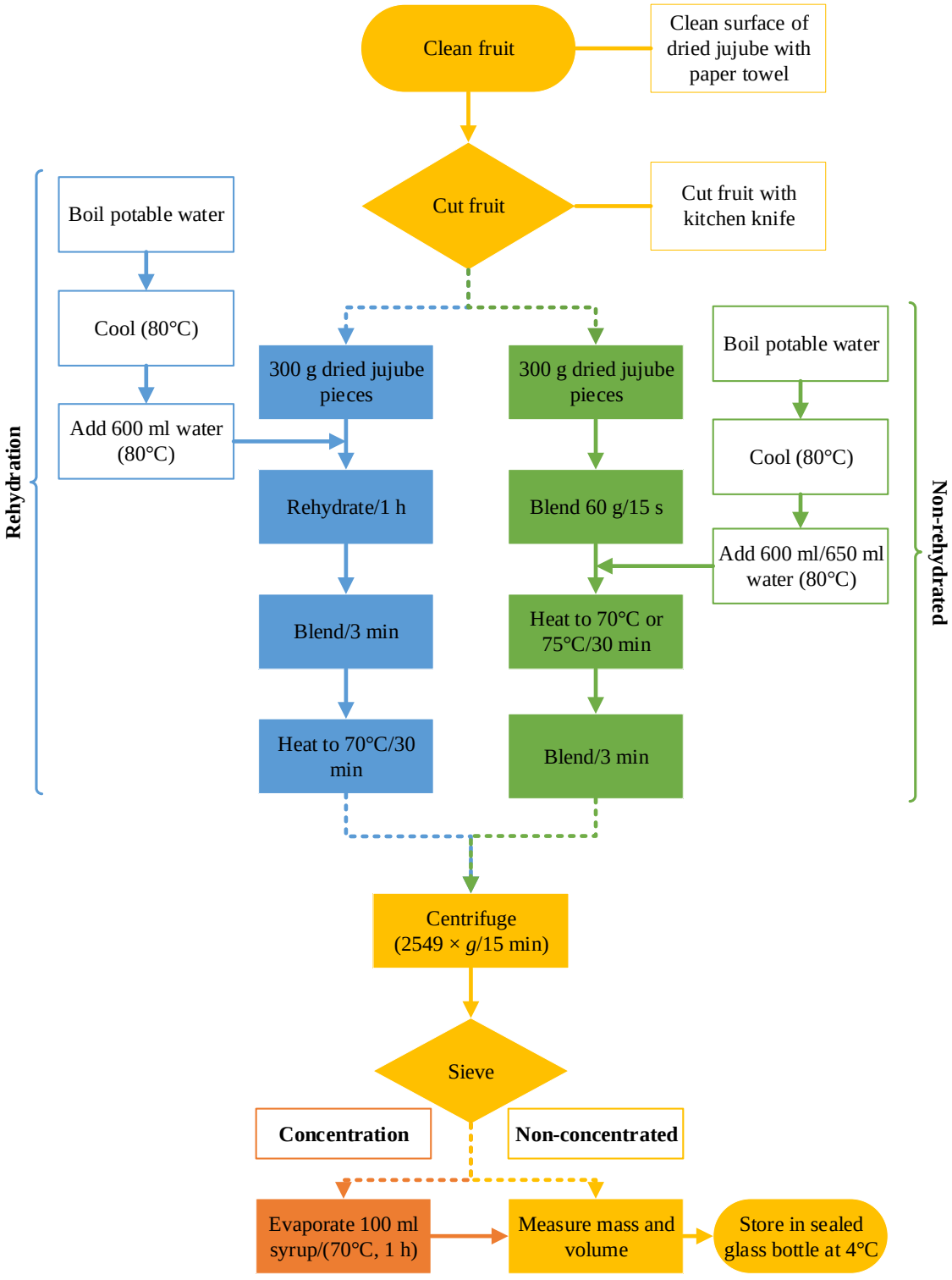

Figure 3.2 Overview of jujube syrup extraction used in this study

3.4.2 Activation of water kefir grains starter culture

Water kefir grains (kefir starter culture) were rehydrated according to the instructions of the supplier (Cultures for Health, Raleigh, North Carolina, USA). One litre (1 L) of potable water was boiled in a kettle (Sunbeam, NZ), and 5% (w/v) of organic raw sugar was added and dissolved completely by stirring using a clean wooden or stainless spoon as recommended by the supplier. The solution was cooled to 25-30°C, and the temperature was measured using a thermocouple (RS Pro, RS-41, NZ). Dried water kefir grains (5 g) were added and allowed to rehydrate in an incubator (Contherm, 06186, NZ) at 27°C for 96 h. Following activation, the water kefir grains were recovered by sieving using a plastic strainer (300 mesh) (ACQ Development Ltd, NZ) and then washed gently with potable water in the jar until the wash water was visibly clean.

3.4.3 Propagation of water kefir grains starter culture

The activated water kefir grains were propagated according to the method of Magalhaes et al. (2010) with minor modifications (Figure 3.3). Organic raw sugar (50 g) was added into 1 L of boiled potable water and stirred to dissolve completely. After the solution (50 g/L) had cooled to between 25-30°C, 100 g rehydrated water kefir grains were added. The mixture was allowed to ferment in an incubator at 27°C for 24 h. After 24 h, the water kefir grains were recovered by sieving (ACQ Development Ltd, NZ) and the supernatant was discarded. The recovered kefir grains were re-suspended in the sugar solution (50 g/L). This process (propagation) was repeated two times to facilitate strong adaptation and an increase in the number of kefir grains. The propagation of water kefir grains is summarised in Figure 3.3.

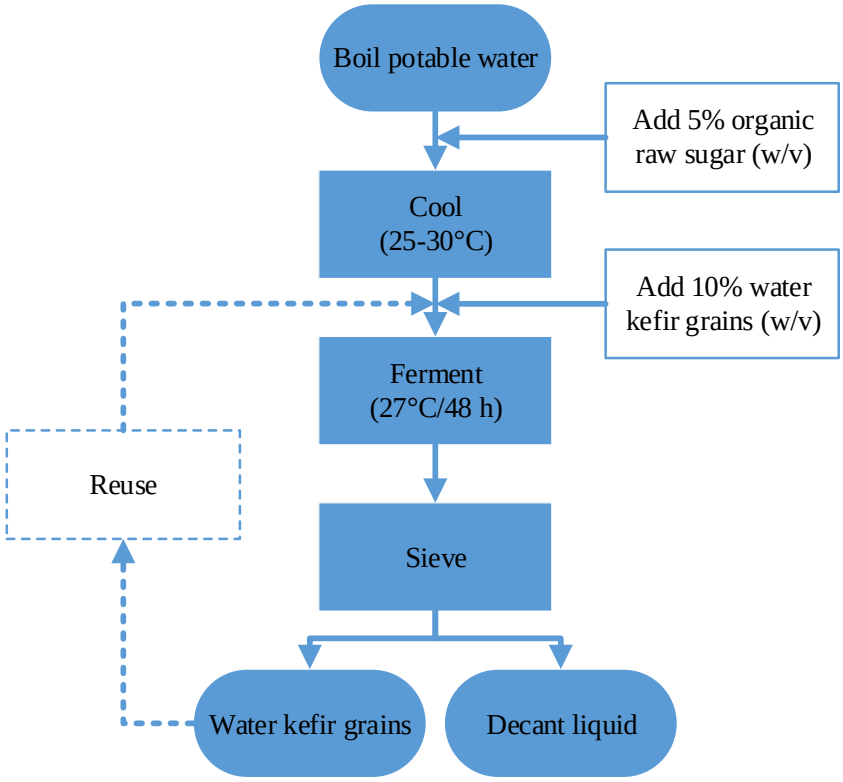

Figure 3.3 Laboratory propagation of water kefir grains

3.4.4 Preparation of jujube water kefir beverage (fermentation stages 1 and 2)

The jujube water kefir beverage was prepared using water kefir grains, organic raw sugar, and extracted jujube syrup. Twenty-five grammes (25 g) (2.5% w/v) organic raw sugar was transferred into 1 L boiled potable water and allowed to dissolve by stirring using a clean wooden spoon. The solution was cooled to 25-30°C, and then 200 mL sugar solution were transferred into 300-mL sterile glass jars. Ten percent (w/v) of water kefir grains (20 g) were added into the sugared solution, followed by adding the jujube syrup (10% v/v, 20 mL or 20% v/v, 40 mL). The mixtures were stirred. In the first stage of fermentation, the mixtures (samples) were fermented in the incubator for 48 h at 25°C or 27°C. After 48 h, the supernatants were recovered by sieving water kefir grains, and the grains were re-suspended in the sugar solution (50 g/L) for storage (4°C).

In the second stage of fermentation, the fermented jujube beverage from stage one was treated separately as follows (Figure 3.4): portions of 200 mL beverage fermented for 48 h in stage one

were transferred into 300-mL sterile glass jars. Jujube syrup (10% v/v, 20 ml or 20% v/v, 40 mL) was added to the 48-h fermented beverage recovered from stage one. The mixtures (samples) were sealed in the glass jar and fermented in the incubator for another 24 h at 25°C or 27°C. Samples (20 mL) were withdrawn every 24 h and stored at 4°C until required for microbial analysis (section 3.6.1), physicochemical analysis (section 3.6.2) and or sensory evaluation (3.6.3). The preparation and fermentation of jujube water kefir beverage are shown in Figure 3.4.

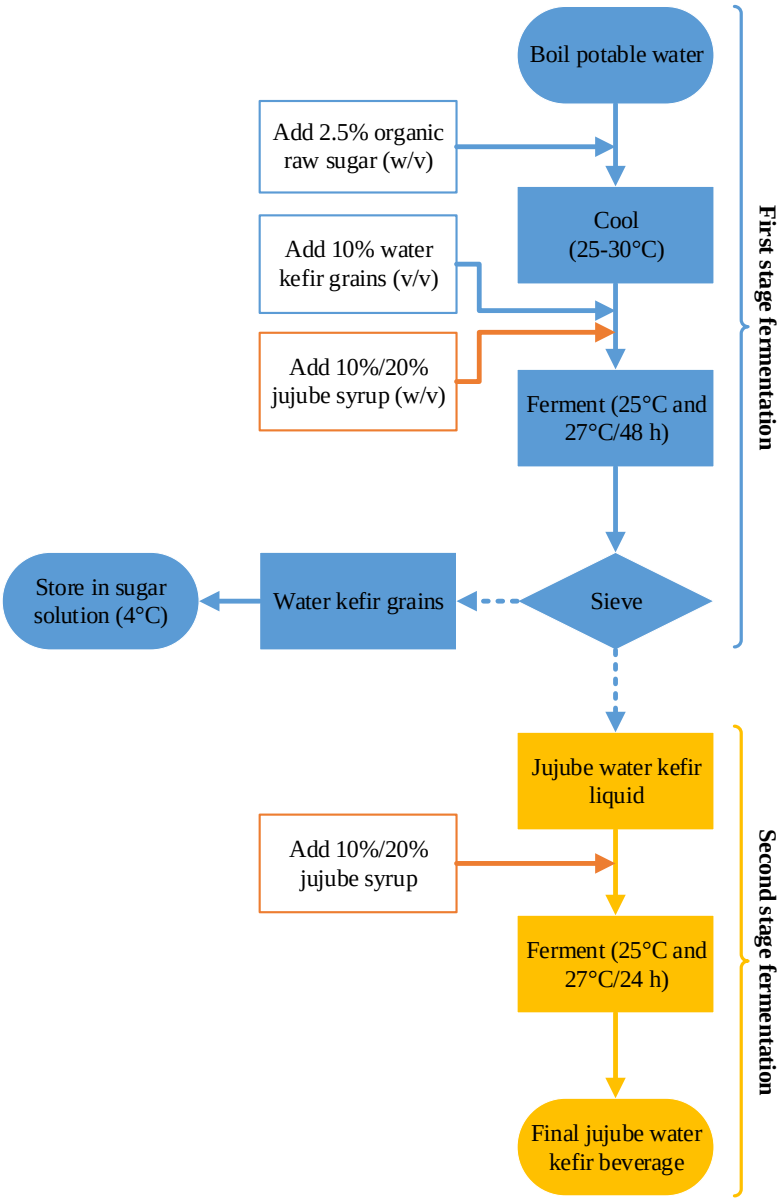

Figure 3.4 Preparation and fermentation of jujube water kefir beverage

Notes: dark blue - first stage fermentation; deep yellow - second stage fermentation.

3.5 Characterisation of extracted jujube syrup

3.5.1 Measurement of mass, volume and calculation of the density of jujube syrup

The mass and volume of the extracted jujube syrup were measured directly after collecting the filtered syrup in the tared cylinder as described in section 3.4.1. The mass of the extract was weighed on an analytical balance (Sartorius, 33207952, Germany), and the volume was measured in a measuring cylinder. The density of the jujube syrup was calculated using the data on the mass and volume.

3.5.2 Determination of total soluble solids (TSS)

Total soluble solids (TSS) were measured using a hand digital refractometer (Reichert, 13940000, USA) according to the AOAC Official Method 932.14 (AOAC, 2005a). To measure TSS, 0.5 mL of the jujube syrup were transferred onto the glass prism of the refractometer and the reading was recorded as °Brix.

3.5.3 Measurement of colour

A CM-5 Konica Minolta (Japan) spectrophotometer was used to measure the colour of the extracted jujube syrup based on the L*, a*, b* colour system (Pathare, Opara, & Al-Said, 2013). The spectrophotometer was calibrated using distilled water based on the manufacturer's instructions. The jujube syrup (3 mL) was slowly pipetted into 4-mL spectrophotometer plastic cuvettes to avoid generating air bubbles which interfere with the measurement. The colour of the sample was directly measured by illuminating D65 artificial daylight at a 10° standard angle, and the corresponding L*, a*, b* values were immediately recorded after the measurement. For each sample, a duplicate measurement was recorded. According to Pathare et al. (2013), the parameter a* represents the greenness (-) and redness (+) while b* represents the blueness (-) and yellowness (+). L* is a psychometric index of lightness which measures the black (-) or white (+).

3.6 Characterisation of jujube water kefir beverage

3.6.1 Physicochemical analysis

3.6.1.1 Measurement of total soluble solids (TSS) and colour

The total soluble solids were determined as previously described in section 3.5.2. The colour of the jujube water kefir beverage was measured using a Konica Minolta spectrophotometer (CM-5, Japan) as described in section 3.5.3.

3.6.1.2 Measurement of pH

The pH of jujube water kefir beverage was directly measured based on the AOAC method 981.12 (AOAC, 2005d) using a digital pH meter (Sartorius PB-20, USA) equipped with a glass electrode (Mettler Toledo, InLab®Expert Pro-ISM, NZ). The pH meter was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0) (LabServ, Thermofisher, NZ) before each measurement, and the electrode was rinsed with distilled water after each measurement.

3.6.1.3 Determination of titratable acidity

Standardisation of 0.1 M sodium hydroxide

The standardisation of sodium hydroxide (0.1 M) was done according to the AOAC Official Method 936.16 (AOAC, 2005b). One gram (1 g) potassium hydrogen phthalate (KHP) (Fisher Scientific, UK) was dried for 2 h at 120°C in the oven (Contherm, NZ) and then cooled in an air-tight desiccator (Bel-Art Product, US) with fresh desiccant for 60 min. Of the dried KHP, 0.2000 g were weighed in a dry 250-mL Erlenmeyer flask on an analytical balance (A&D, 15905508, Korea), then 50 mL distilled water was added to dissolve the powder (KHP) completely. A few drops (3-4) of phenolphthalein solution (1%) were added and the mixture was swirled to mix

thoroughly. The KHP solution was titrated against about 0.1 M NaOH (Fisher Scientific, UK) solution until the first tinge of a persistent faint pink colour and the volume (mL) of the titre (NaOH) was recorded. The titration was repeated until concordant results were obtained. The concentration of the NaOH solution was calculated using equation (1).

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

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

Determination of titratable acidity in jujube water kefir beverage

The acidity of the jujube water kefir beverage was determined following the AOAC standard method 947.05 (AOAC, 2005c). The beverage samples (5 g) were weighed in a 250-mL Erlenmeyer flask using an analytical balance (A&D, 15905508, Korea) and mixed completely with 10 mL distilled water. Three to four drops of phenolphthalein solution (1%) were added and the mixture was swirled to mix thoroughly. The diluted samples were titrated against the standardised NaOH solution until the first persistent faint pink colour was observed, and the volume of NaOH (mL) used in the titration was recorded. The lactic acid concentration was calculated using equation (2).

$$\%\ Lactic\ acid = \frac{0.1\ M\ NaOH\ (mol/L) \times V\ NaOH\ (L) \times 90.08(g/mol)}{m\ sample\ (g)} \times 100 \quad (2)$$

where V NaOH = volume of NaOH used in the titration; the molecular weight of lactic acid = 90.08 g/mol; 1 mL of sample ≈ 1 g sample.

3.6.1.4 Analysis of ethanol content

The ethanol concentration in the kefir beverage was analysed according to the method of Mapitse, Okatch, and Moshoeshoe (2014) using Shimadzu GC system model GC-17A (Shimadzu, Japan) with minor modifications. The temperature programme of the GC column during analysis of ethanol is shown in Figure 3.5.

The analysis was carried out on a DBWAX capillary column (30 m × 0.32 mm, 0.25 μm) (J&W Scientific, 2296882, US) at 40°C equipped with a CBM-102 communications bus module, an AOC power supply module, an AOC-20i auto-injector, and flame ionisation detector. The injection and detection temperatures were set at 150°C and 280°C respectively, and a 10:1 split ratio was used. The initial column temperature was set at 40°C and held for 4 min, the temperature was then increased to 250°C at 33.33°C/min and held for 5 min (Figure 3.5). The carrier gas used in GC analysis was nitrogen with a flow rate of 76 mL/min.

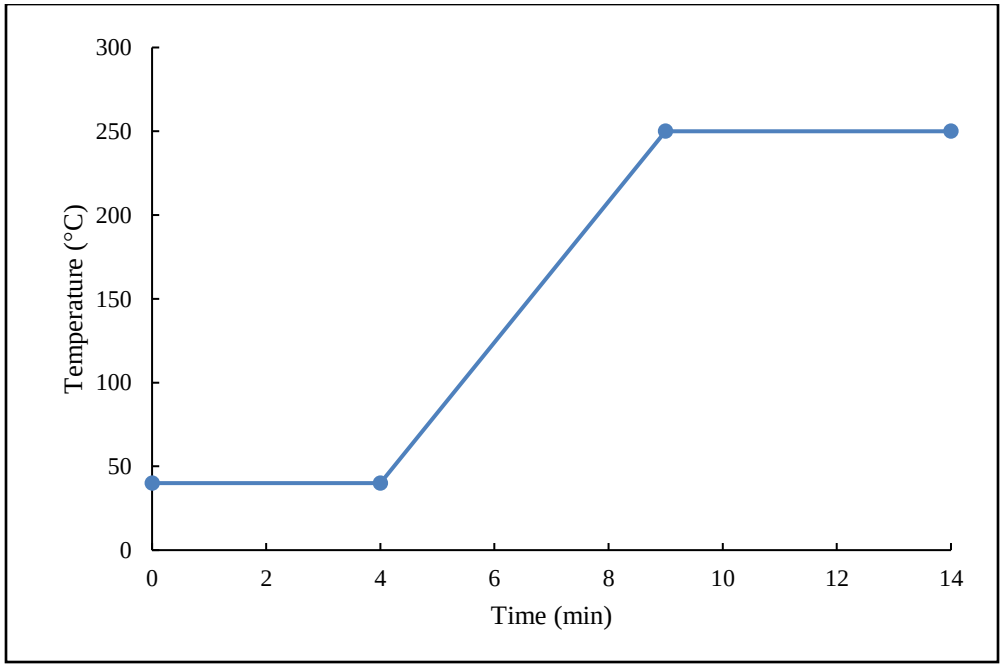

Figure 3.5 Temperature programme of the GC column during analysis of ethanol

The ethanol standards (0.01%, 0.1%, 0.4%, 0.5%, 0.8%, 1%, 2%) were prepared using ethanol (≥ 99.5%, BSPEL975.20) (Thermo Fisher Scientific, NZ) and distilled water (v/v). The jujube water kefir beverage was withdrawn using a 5-mL syringe (Terumo, AU) and filtered through a 0.22-µm filter unit (33 mm) (Merck Millipore Ltd, Ireland). For the standards and the filtered samples, 0.2 µl were injected into the GC injection port using a 10-µl syringe (Shimadzu, 221-34618, Japan). The standards were analysed first to generate the standard curve followed by the injection of samples. The ethanol was identified according to the retention time and the ethanol content was calculated based on peak area. Chromatographic data were recorded and integrated using Shimadzu GCsolution Software (Shimadzu, Japan). The standard curve was generated using Microsoft® Excel 2016 (Santa Rosa, CA, USA).

3.6.1.5 Analysis of sugars

The method described by Ann-Charlotte (2006) was used to identify and quantify sucrose, glucose and fructose in jujube water kefir beverage. Sugars were separated by HPLC performed on a Rezex RCM-Monosaccharide RCM Ca^{2+} (8% cross-linked resin) column (300 × 7.8 mm) (Phenomenex, NZ) at 85°C using a Shimadzu HPLC system model LC-20AD XR (Shimadzu Corp, Japan), consisting of a degassing unit (DGU-20A5R), an autosampler (SIL-30AC), a column oven (CTO-20AC), a diode array detector (SPD-M20A), a fluorescence detector (RF-20A XS), and a refractive index detector (RID-20A). The mobile phase used for sugar analysis was distilled water at 0.4 mL/min flow rate. Before use, the distilled water was filtered through a 0.22-µm nylon membrane (Merck Millipore Ltd, Ireland) and degassed in an ultrasonic bath (Bandelin, Germany). The sugar standards were prepared using sucrose (84097) ≥ 99.5% (Sigma-Aldrich, NZ), fructose (F0127) ≥ 99% (Sigma-Aldrich, NZ), glucose (67528) ≥ 99.5% (Sigma-Aldrich, NZ), and distilled water. The concentrations of the standard solutions of sucrose, glucose and fructose were 0.01%, 0.05%, 0.1%, 1.25%, 5%, 7%, and 10%. All the standards and samples were filtered through a 0.22-µm filter unit (33 mm) (Merck Millipore Ltd, Ireland) using a 5-mL syringe (Terumo, AU) into 2-mL vials (Shimadzu Corp, Japan). After generating the standard curves, the experimental samples were injected into the HPLC column. The sugars were identified based on their respective retention times and then quantified by comparing the peak areas to the standard curves generated by the sugar standards.

3.6.1.6 Analysis of organic acids

Lactic acid and acetic acid concentrations in jujube water kefir beverage samples were analysed by HPLC as described by Jekle, Houben, Mitzscherling, and Becker (2010) with minor modifications. The analyses were carried out using a Shimadzu HPLC system model LC-10AT VP equipped with an auto-injector (SIL-10A), a column oven (CTO-10AS VP), a system controller (SCL-10A VP), a dual detection system including an UV detector (SPD-10A VP) with 210 nm wavelength and a RI detector (RID-10A) (Shimadzu Corp, Japan). The separation of lactic acid and acetic acid was carried out on a Rezex ROA-Organic Acid H^+ (8% cross-linked resin) column (300 × 7.8 mm) (Phenomenex, NZ) at 60°C. The mobile phase used for organic acid analysis was the sulphuric acid solution (0.005 N) (AJA534) (Ajax Finechem, NZ) set at 0.6 mL/min flow rate. Prior to analysis, the mobile phase was filtered through a 0.22-µm membrane filter (Merck Millipore Ltd, Ireland) and degassed in an ultrasonic bath (Bandelin, Germany) to remove air bubbles. The standards comprising lactic acid (85%) (1012211) (Ajax Finechem, NZ) and acetic acid (≥ 99.7%) (1743468) (Fisher Scientific, UK) were prepared in distilled water. Standard concentrations of lactic acid used were 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, and 2% (w/v), whereas for acetic acid, the concentrations were 0.01%, 0.05%, 0.1%, 0.3%, 0.5%, 0.8%, and 1% (w/v). The experimental samples and acid standards were filtered through a 0.22-µm filter unit (33 mm) (Merk Millipore Ltd, Ireland) using a 5-mL syringe (Terumo, AU) and kept in 2-mL vials (Shimadzu Corp, Japan). The standards for the acids were injected first to generate a standard curve. The sugars were identified based on their respective retention times and the peaks were integrated using Shimadzu LC solution Software (Shimadzu Corp, Japan). The concentrations of the acids were determined based on the peak area of the standard curves generated by the standard compounds.

3.6.1.7 Analysis of antioxidants

The phenolic acids (catechins, epicatechin, gallic acid, and rutin) were analysed according to the method of Gao et al. (2011) and Zhang, Jiang, Ye, Ye, and Ren (2010) with minor modifications. The analyses were carried out using a reversed phase HPLC system model LC-20AD (Shimadzu UFLC, Shimadzu Prominence, Japan) consisting of a degasser (DGU-20A5), an autosampler (SIL-20AC HT), a column oven (CTO-20AC), a fluorescence detector (RF-20A XS) and a diode array detector (SPD-M20A). The acids were separated by a 5-µm Grace Smart RP18 column (250 ×4.6 mm) (Grace, USA) at 18°C. A discontinuous gradient (Table 3.4) was run from 100% mobile phase A (0.1% trifluoroacetic acid in Milli-Q water) to 100% mobile phase B (0.1% trifluoroacetic acid in acetonitrile) at a flow rate of 0.75 mL/min. Before analysis, samples were filtered through a 0.22-µm filter unit (Merck Millipore Ltd, Ireland) using a 5-mL syringe (Terumo, AU) into 2-mL vials (Shimadzu Corp, Japan). Gallic acid, (+)-catechin and rutin (1 mg/mL) standard stock solutions were prepared in Milli-Q water while epicatechin standard solution (1 mg/mL) was prepared in 70% ethanol solution (v/v). The samples were analysed following the injection of standards. Automatic injections (20 µl) were performed in duplicate, and the phenolic acids were detected at 270 nm. The compounds were identified based on their retention times and the peaks were integrated using Shimadzu LC Solution Software (Shimadzu Prominence, Japan) followed by quantifying the compounds based on peak areas.

Table 3.4 HPLC gradient programme used to analyse phenolic acids

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

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

3.6.2 Microbiological analysis

3.6.2.1 Microbiota of water kefir grains (starter culture)

The yeast, *S. cerevisiae* and total LAB in water kefir grains starter culture were enumerated according to Miguel et al. (2011) and Laureys and De Vuyst (2014). According to the supplier of the culture, the water kefir grains used in this research consisted mostly of LAB and the yeast, *S. cerevisiae* (Cultures for Health, Raleigh, North Carolina, USA), which agrees with the report of Gulitz et al. (2011). In this study, total LAB and *S. cerevisiae* were enumerated by plating suitable serial dilutions on de Man, Rogosa, and Sharp (MRS) agar (Oxoid, UK) and yeast extract-glucose-chloramphenicol (YGC) agar (Merck, Germany), respectively. The medium was prepared according to the manufacturer's instructions. Ten grams (10 g) of water kefir grains were weighed into a stomacher bag and 90 g of sterilised 0.1% peptone water (Merck, Germany) added to obtain a 10^{-1} dilution. The mixture was blended for 1 min using a stomacher blender (Stomacher, Global Science, NZ). Suitably diluted samples were mixed for 15 s using a vortex mixer (Labinco BV, Netherlands) and pour-plated (1 mL) in duplicate. After the agar plates had solidified, the plates were inverted and incubated. The MRS agar plates were incubated (Clayson, IM1000, AU) at 30°C for 3 days under anaerobic condition using AnaeroGen™ pack (AN0035A) (Thermo Scientific, NZ) while YGC agar plates were aerobically incubated at 25°C for 5 days. Isolates of the grown colonies were Gram stained and examined under oil-immersion of the microscope. The Gram stained cells were examined for morphology and Gram reactions (Leite et al., 2015).

3.6.2.2 Enumeration of Lactic Acid Bacteria in jujube water kefir beverage

Enumeration of the total LAB was conducted according to the method of Miguel et al. (2011) using the de Man, Rogosa, and Sharp (MRS) agar (Oxoid, UK). The medium was prepared based on the manufacturer's instructions. For plating, suitably diluted samples were mixed for 15 s using a vortex mixer (Labinco BV, Netherlands) followed by pour-plating 1 mL in duplicate. After the agar had solidified, the plates were inverted and incubated (Clayson, IM1000, AU) anaerobically using AnaeroGen™ pack (AN0035A) (Thermo Scientific, NZ) at 30°C for 3 days. A colony counter (Stuart®, R570001635, UK) was used to count the grown colonies.

3.6.2.3 Enumeration of Saccharomyces cerevisiae in jujube water kefir beverage

Enumeration of *S. cerevisiae* was conducted according to Laureys and De Vuyst (2014) using yeast extract glucose chloramphenicol (YGC) agar (Oxoid, UK). The medium was prepared according to the instructions of the manufacturer. The samples were inoculated by pour-plating as described in the preceding section. The solidified agar plates were inverted and then incubated aerobically at 25°C for 5 days in the incubator (Clayson IM1000, AU). Grown colonies were counted using a colony counter (Stuart®, R570001635, UK).

3.6.3 Sensory analysis

Sensory evaluation is a scientific method used to measure and analyse the characteristics of food and beverage by different sensory perceptions including sight, smell, touch, taste and hearing (Lawless & Heymann, 2010; Stone, 2012). The method described by Corona et al. (2016) was modified to evaluate the appearance, odour, flavour, sweetness, sourness and overall acceptability of jujube water kefir beverage. Two types of sensory methods were used, focus groups and consumer sensory evaluation. Prior to the focus group discussions and consumer sensory evaluation, the panellists were given an information sheet (Appendix C) describing the study and a copy of Massey University Human Ethics Application (4000018836) (Appendix C).

In this section, focus group discussions evaluated four fermented jujube water kefir samples. An informal focus group consists of a small number of people whose purpose is to discuss a particular topic or set of issues led by the researcher who acts as the moderator (Dawson, Manderson, & Tallo, 1992; Wilkinson & Silverman, 2004). The aim of conducting the informal focus group in this study was to discuss the preliminary attributes (appearance, odour, flavour, sweetness, sourness) of the jujube water kefir beverage (Uysal-Pala, Karagul-Yuceer, Pala, & Savas, 2006).

The kefir beverage was firstly evaluated by informal focus group discussions and then by consumer sensory evaluations at Massey University, Albany campus. Six experienced sensory panellists consisting of staff and university students were recruited to participate in the informal

focus group. The panellists were familiar with fermented water kefir beverages. All four beverages had been stored at 4 ± 1 °C for 24 h prior to the evaluation by a focus group. Each beverage sample (15 mL) was served in 20-mL clear plastic cups (Huhtamaki, NZ) coded with three-digit random numbers. The samples were evaluated by the focus group in the Product Development Laboratory at Massey University, Albany Campus.

In the second part, consumer sensory panellists evaluated the samples. The consumer sensory evaluation of the jujube water kefir beverage was conducted under white light in a sensory booth in the Sensory Evaluation Laboratory at Massey University, Albany, and the panellists were randomly recruited at the University by email correspondence and posters. Each beverage was prepared as previously described. A 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) was used to evaluate the attributes (appearance, odour, flavour, sweetness, sourness) and overall acceptance (Lim, 2011) (Appendix C) of each sample. The sensory panellists were required to clean the palate with water at ambient temperature (20 ± 1 °C) between evaluating each sample. The results of sensory evaluation were used to aid the selection of the most promising formulation of jujube water kefir beverage. Thirty (30) consumer sensory panellists participated in each sensory evaluation session.

3.7 Statistical data analysis

Minitab version 18 Statistical Software (Minitab Inc., State College, PA, USA) and Microsoft® Excel 2016 (Santa Rosa, CA, USA) were used for data analysis. Data were expressed as mean ± standard deviation (SD) and all the graphs were generated by Microsoft® Excel. One-way Analysis of Variance (ANOVA) was used to determine any significant ($p<0.05$) differences between the various parameters of syrup extraction (TSS, mass, volume, density) using different extraction methods, extraction temperatures and quantities of water for extraction. Data on the effects of jujube syrup concentration, fermentation temperature, storage time on the microbiological content (LAB and *S. cerevisiae*) and physicochemical (colour, pH, TSS, T.A., organic acids, sugars, ethanol, antioxidant) properties, and sensory profiles of jujube water beverage were also determined. Tukey's multiple comparison test was used to separate significant differences ($p<0.05$) between the means of sample data. The statistical outputs are shown in Appendix G.

Chapter 4. RESULTS AND DISCUSSION

4.1 Phase I: Optimisation of jujube syrup extraction

4.1.1 Stage I: Efficiency of jujube syrup extraction methods

The purpose of the stage I was to determine the optimum combination of methods for the extraction of jujube syrup.

4.1.1.1 Total soluble solids (°Brix) and volume of syrup

The efficiency of the extraction method was determined by measuring the quantity (volume) and the amount total soluble solids (TSS) of the syrup. The TSS and the volume of extracted jujube syrup from DJ fruit (Figure 4.1) using different combinations of extraction methods are shown in Figure 4.2. TSS is an index of the soluble solids concentration in a sample, and it is usually expressed in °Brix (percent sucrose by weight) (IFU, 2005). Syrup with the highest TSS (39.75±0.06 °Brix) was obtained using the non-rehydrated extraction method followed by evaporation (S1) of the extract. The TSS of S2 (22.65±0.06 °Brix) and S4 (22.63±0.05 °Brix) were lower ($p<0.05$) than S1 and no differences ($p>0.05$) were observed between samples S2 and S4.

Figure 4.1 Appearance of dried *Ziziphus jujuba* Mill. cv *jinsixiaozao* fruit used in this experiment

(Captured by iPhone 7 Plus, 12 megapixels Apple Inc., USA)

According to previous studies (Al-Farsi et al., 2007; El-Nagga & El–Tawab, 2012), the TSS of dates syrup increased up to 70 °Brix using a rotary evaporator. In the current study, rotary evaporation reduced the volume by evaporating the moisture content in the syrup thereby increasing the TSS. Figure 4.2 shows that the volume of the syrup extracted without rehydration and concentration (S2) was 71.95±0.47 mL/100 g DJ, which was the highest among the three samples, and the lowest volume (19.50±0.71 mL/100 g DJ) was the syrup extracted using rehydration method (S4) (Appendix D). During rehydration, water is first imbibed into the material, followed by swelling of the materials, resulting in leaching of solubles (Krokida & Marinos-Kouris, 2003; Lewicki, 1998). Therefore, the volume of water used for extraction was reduced to reduce the imbibition of water into the DJ, which may result in a lower syrup volume for S4. However, due to the lower yield of the rehydration method, no further investigation was conducted using this method.

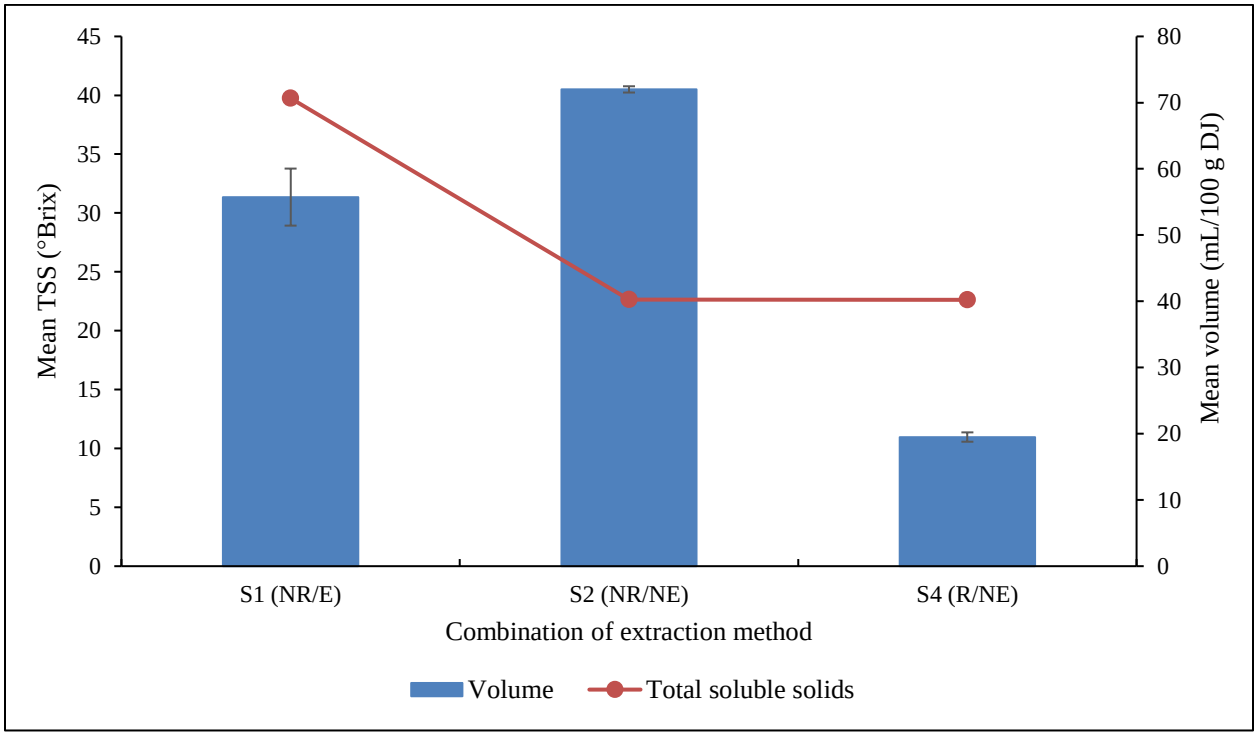

Figure 4.2 Mean total soluble solids (°Brix) and volume (mL/100 g DJ) of syrup extracted using different combinations of extraction methods

Notes: Samples S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated; DJ = dried jujube; Error bars for TSS = ± SD (n=4); Error bars for volume = ± SD (n=2); experiments were replicated twice.

4.1.1.2 Mass and density

The mass and density of extracted jujube syrup using different extraction methods are shown in Figure 4.3. The syrup extracted using non-rehydrated, non-evaporated method (S2) had higher ($p<0.05$) mass (77.64±0.49 g/100 g DJ) than S1 (64.42±5.00 g/100 g DJ) and S4 (21.02±0.76 g/100 g DJ) (Appendix D). The mass for S4 was the lowest among the three samples, which may be attributed to the imbibition of water into the dried fruit (Krokida & Marinos-Kouris, 2003).

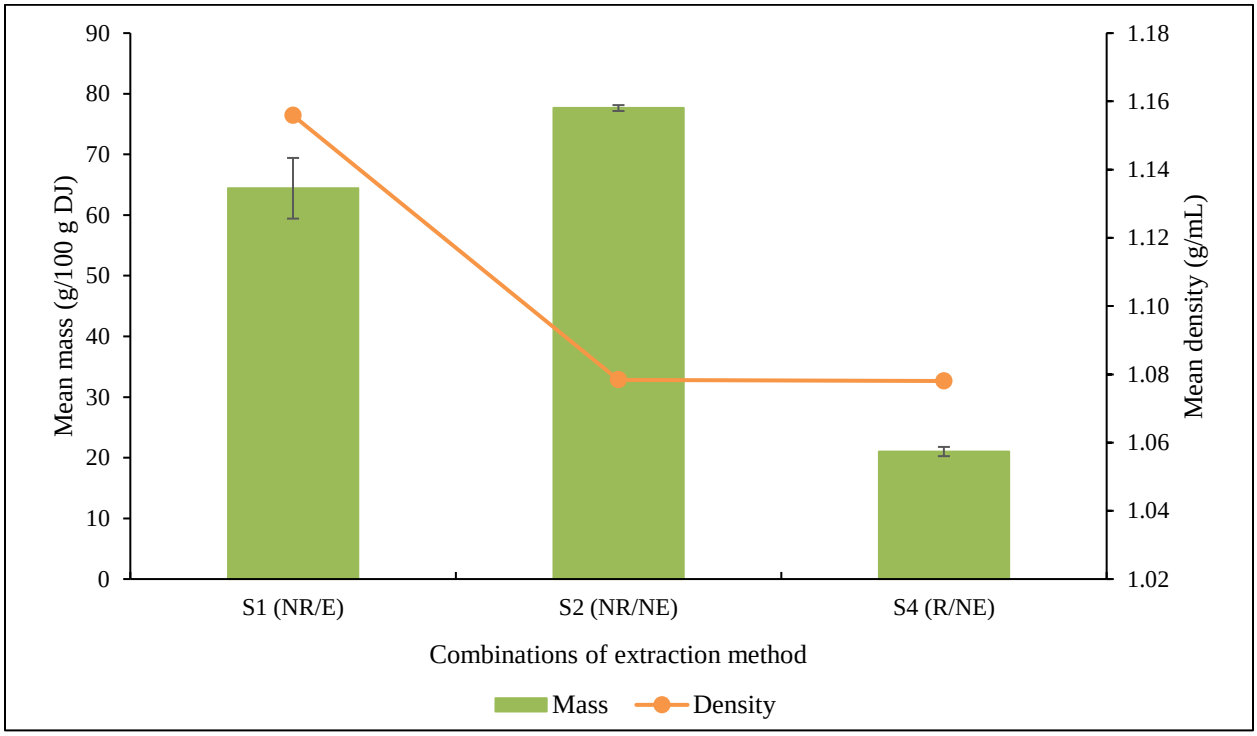

Figure 4.3 Mean mass (g/100 g DJ) and density (g/mL) of syrup extracted using different combinations of extraction methods

Notes: Samples S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated; DJ = dried jujube; Error bars = ± SD (n=2); experiments were replicated twice.

The results showed that the higher the TSS, the higher the density of the syrup, and this trend was consistent with the study by Zuritz et al. (2005). However, the calculated density of the syrup was lower than the data reported by IFU (2005). The TSS of S1 were 39.75±0.06 °Brix with a density of 1.16±0.00 g/mL whereas the TSS of S2 were 22.65±0.06 °Brix with a density of 1.08±0.00

g/mL (Appendix D). According to IFU (2005), the relative density of fruit juice with 39.7 °Brix is 1.17721, and 1.09465 for the fruit juice with 22.6 °Brix. Zuritz et al. (2005) reported that although the density was markedly impacted by the level of TSS, the density of the grape juices with the same TSS was different at different measuring temperatures. As the temperature of the grape juice with the same TSS increased, the density slightly decreased (Zuritz et al., 2005). Hence, the inconsistency between the TSS obtained from the present study and other studies may be attributed to the temperature fluctuations during measurement.

4.1.1.3 Summary – phase I stage I

This section summarises the most efficient combination of the methods used for the extraction of jujube syrup.

Results in phase I stage I showed that the rehydration of the DJ fruit significantly ($p<0.05$) decreased the yield and TSS of the syrup while evaporation significantly ($p<0.05$) increased the TSS. Although the evaporation resulted in a syrup with higher TSS, the volume and the mass of the syrup slightly decreased with increased production time. By comparing the production time, volume and TSS, S2 (non-rehydrated, non-evaporated) was further investigated. This sample had the highest yield (71.95±0.47 mL/100 g DJ) with 22.65±0.06 °Brix. The mass and density of S2 were 77.64±0.49 g/100 g DJ and 1.08±0.00 g/mL, respectively. The inconsistencies between the density of the extracted syrup and the density of the fruit juice with specific TSS reported in previous studies may be attributed to the temperature fluctuations during measurement.

4.1.2 Stage II: Selection of the optimum conditions (temperature and quantity of water) for jujube syrup extraction

The purpose of stage II was to determine the optimum extraction condition for jujube syrup based on the most efficient (combined) method (S2) obtained in stage I.

4.1.2.1 Total soluble solids (°Brix) and volume of syrup

The volume of extracted jujube syrup using different extraction conditions and the total soluble solids (TSS) of the extracts are shown in Figure 4.4. The lowest TSS (21.95±0.06 °Brix) and the highest yield (104.83±0.71 mL/100 g DJ) were obtained in the sample SC while the highest TSS (22.75±0.05 °Brix) and the lowest yield (73.00±0.47 mL/100 g DJ) were obtained in SB (Appendix D).

By comparing the syrup extracted at the same temperature, the TSS of the syrup extracted with 650 mL water were lower ($p<0.05$) than the syrup extracted with 600 mL water. TSS, expressed as °Brix, is an index of soluble solids concentration in the syrup (Javanmardi & Kubota, 2006). The high quantity of water used for extraction decreased the concentration of soluble solids, producing a syrup with low TSS and high yield. Moreover, by comparing the syrup extracted with the same amount of water, although the syrup extracted at 75°C showed slightly higher TSS than the one extracted at 70°C, the difference was not significant ($p>0.05$). Lee, Yusof, Hamid, and Baharin (2006) investigated the optimum conditions for extracting banana juice using the hot water extraction method. It was shown that the TSS of the banana juice increased as the extraction temperature increased. The result is in agreement with Lee et al. (2006). However, Lee et al. (2006) reported an increased yield of banana juice as the extraction temperature increased. The differences in the extracted yields may be attributed to the evaporation of water content during extraction. Although high extraction temperatures can increase the yield of extraction, high temperature may decrease the yield by increasing the hydrolysis of polysaccharides (Cai, Gu, & Tang, 2008).

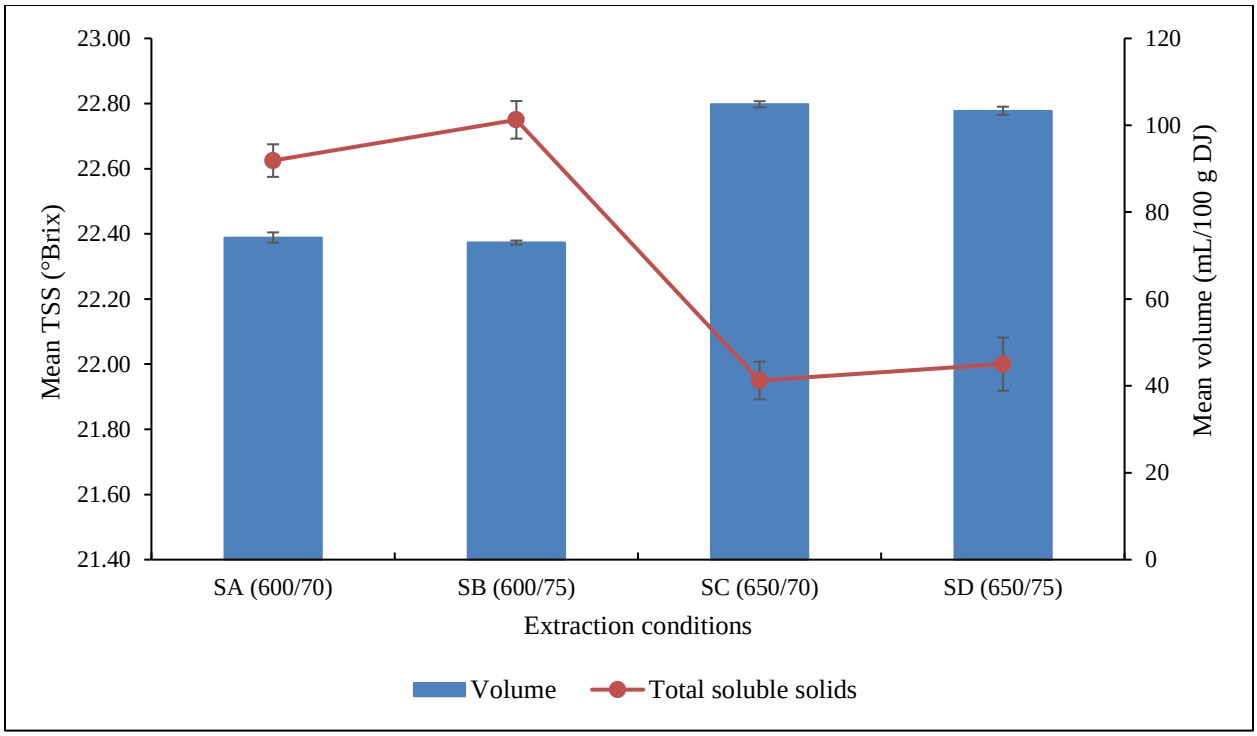

Figure 4.4 Mean total soluble solids (°Brix) and volume (mL) of syrup extracted using different extraction conditions

Notes: Sample SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C; DJ = dried jujube; Error bars for TSS = ± SD (n=4); Error bars for volume = ± SD (n=2); experiments were replicated twice.

4.1.2.2 Mass and density

Mass and density of extracted jujube syrup using different extraction conditions are shown in Figure 4.5. Results showed that the syrup extracted with a higher quantity of water had a higher mass. Syrup SC had the highest mass (112.35±0.75 g/100 g DJ), which was higher ($p<0.05$) than the mass of syrup SA (79.97±1.30 g/100 g DJ) and SB (78.81±0.54 g/100 g DJ). The increased syrup mass was caused by the increased quantity of water used for extraction. By comparing the syrup extracted with the same quantity of water, no differences ($p>0.05$) were observed between the samples of syrup extracted at 70°C and 75°C.

As mentioned previously, TSS is an index of the concentration of soluble solids in the syrup. Hence, the syrup with high TSS is expected to have a high density. Results from the present study

were in agreement with this statement. The highest density (1.0796±0.0004 g/mL) was obtained in syrup SB, which had the highest TSS (22.75±0.06 °Brix) among the four samples while syrup SC had the lowest density (1.07±0.00 g/mL) and the lowest TSS (21.95±0.06 °Brix) (Appendix D). According to IFU (2005), the relative density values of the fruit juice with 22.7 °Brix and 21.9 °Brix are 1.08610 and 1.09149, respectively. Comparing the data obtained from the present study and previous reports, the lower density may be attributed to the temperature fluctuations during measurement (Zuritz et al., 2005). Furthermore, the effect of the increased extraction temperature (70°C to 75°C) on the density of syrup was not significant ($p>0.05$). Nevertheless, extracting juice at high temperature may lead to detrimental changes on the sensory properties and degradation of nutrients such as rutin, and some organic acids (Buchner, Krumbein, Rohn, & Kroh, 2006; Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010; Lee & Coates, 2003).

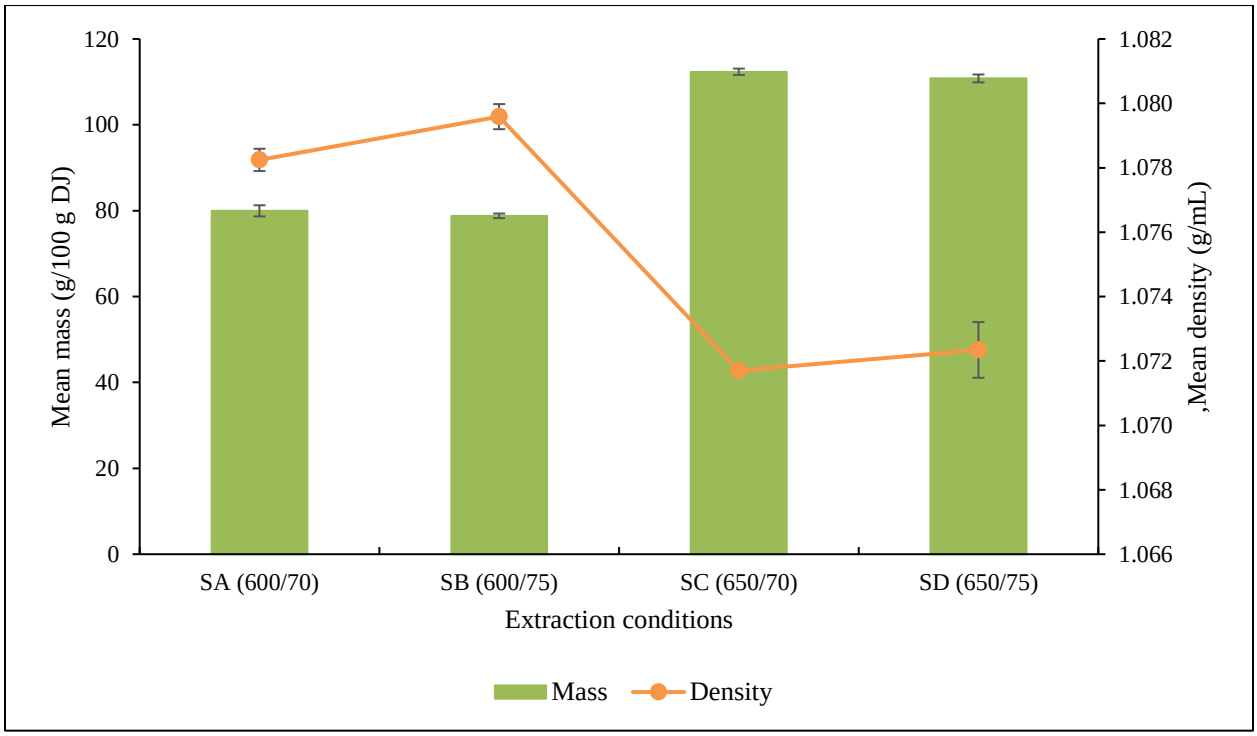

Figure 4.5 Mean mass (g/100g DJ) and density (g/mL) of syrup extracted using different extraction conditions

Notes: Sample SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C; DJ = dried jujube; Error bars = ± SD (n=2); experiments were replicated twice.

4.1.2.3 Summary – phase I stage II

This section summarises the optimum extraction conditions for jujube syrup developed based on the most efficient (combined) method (S2) obtained in stage I.

Results in phase I stage II showed that the mass and density of the extracted jujube syrup were influenced ($p<0.05$) by the quantity (volume) of water used for extraction, while the effect of the extraction temperature was negligible. The highest volume (104.83±0.71 mL/100 g DJ) was obtained from the syrup extracted using 650 mL water with 70°C extraction temperature (SC). No significant difference ($p>0.05$) was observed in the measured parameters between the syrup extracted at 70°C and 75°C. In order to avoid excessive heat and minimise the degradation of the nutrients, 70°C was selected as the syrup extraction temperature. Overall, the optimum quantity of water for extracting jujube syrup was 650 mL at 70°C using the non-rehydrated method.

4.2 Phase II: Effect of temperature (fermentation) and jujube syrup concentration on fermentation of jujube water kefir beverage

The purpose of phase II was to select the most promising formulation by investigating the effects of jujube syrup concentration and fermentation temperature on the jujube water kefir beverage.

4.2.1 Description of kefir grains (starter culture)

In the present study, the starter culture supplied as air-dried water kefir grains (Cultures for Health, Raleigh, North Carolina, USA) was purchased online from Simple Living (Otahuhu, Auckland, New Zealand, www.simpleliving.co.nz). The rehydrated kefir grains (Figure 4.6) had a yellowish-white colour with a brittle, elastic structure and irregular shapes. The diameter of the kefir grains ranged from 1 mm to 15 mm, which were similar to previous studies (Horisberger, 1969; Reiß, 1990; Waldherr et al., 2010). The grains are insoluble in water due to the presence of dextran (α 1-6 linked glucose polymer with 1-3 linked side chains), which is produced by some species of *Lactobacillus*, and/or *Leuconostoc* (Horisberger, 1969; Laureys & De Vuyst, 2014).

Figure 4.6 Water kefir grains used in this experiment

(Captured by iPhone 7 Plus (Apple Inc., USA), 12 megapixels)

According to the supplier (Culture for Health, Raleigh, North Carolina, USA), the microbiota of the water kefir grains mainly consisted of LAB and *S. cerevisiae*. Images of Gram stains showed that the colonies grown on both MRS and YGC agar were Gram-positive (Appendix B). The results obtained from the present study were similar to previous studies (Gulitz et al., 2011; Laureys & De Vuyst, 2017; Magalhaes et al., 2010). The genera *Lactobacillus*, *Acetobacter*, *Saccharomyces*, and *Kluyveromyces* were reported in Brazilian sugary kefir (Magalhaes et al. (2010). However, due to several factors such as cultural properties, climatic conditions and fermentation substrates, there are high variabilities in the microflora of water kefir grains (Gulitz et al., 2013; Hsieh, Wang, Chen, Huang, & Chen, 2012; Öner, Karahan, & Çakmakçı, 2010).

In the present study, viable cell counts of LAB and *S. cerevisiae* in water kefir grains were 7.17±0.06 cfu/g and 6.26±0.08 cfu/g respectively, after 24 h fermentation at 27°C (Appendix D). A study by Gulitz et al. (2011) reported LAB cell counts ranging from 1.3×10^8 – 1.6×10^8 cells/g, whereas the yeasts counts ranged from 5.8×10^6 – 2.7×10^7 cells/g. Miguel et al. (2011) studied the microbial population of water kefir grains from different origins. Their study reported counts of LAB ranging from 6.04±0.03-9.18±0.01 cfu/g while the yeasts ranged from 5.92±0.08-8.30±0.01 cfu/g. The viable cell counts of LAB and the yeast obtained in the present study are within the range of the microbial population reported by previous studies (Miguel et al., 2011; Subardjo, 2017). According to the international food standards of the Codex Alimentarius, kefir should contain a minimum level of 10^7 cfu/mL bacteria and 10^4 cfu/mL yeast (FAO/WHO, 2011). Thus,

the microbial population of the kefir starter culture obtained from the present study met the microbial criterion of the standard.

4.2.2 Total soluble solids (°Brix)

The concentration of total soluble solids was measured by a digital refractometer and the results were recorded as °Brix. The TSS of jujube water kefir beverage fermented with the kefir grains for 72 h is shown in Figure 4.7.

1st Stage Fermentation

Results showed that for all the samples (Figure 4.7), the TSS decreased ($p<0.05$) during the first stage of fermentation (0-48 h). The reduction in TSS may be attributed to the metabolism of monosaccharides into organic compounds during fermentation by the LAB. During fermentation, sucrose is hydrolysed to glucose and fructose by invertase present in the yeast (Khandelwal et al., 2016; Montet et al., 2014; Pfeiffer & Morley, 2014; Pronk et al., 1996). The monosaccharides are then metabolised into organic compounds, which contribute to the chemical and sensory characteristics of the product (Stadie, 2013). Glucose is readily metabolised by LAB during fermentation (Reddy et al., 2008). The slope (Figure 4.7) of the TSS indicates that TSS decreased rapidly within the first 24 h of the fermentation period and steadily decreased from 24 h to 48 h. A study on black tea water kefir beverage showed that the TSS of the beverage fermented with 5% (w/v) sugared tea at 27°C decreased steadily during the first stage fermentation, with the reduction of 0.8 °Brix in the first 24 h (Subardjo, 2017). In the present study, the reduction of the TSS of the beverage fermented with 2.5% (w/v) sucrose and 10% (v/v) jujube syrup at 27°C was 3.35 °Brix (Appendix D). Jujube fruit is rich in phosphorus, and it also contains vitamin C and other minerals such as calcium, potassium, and iron (Li, Fan, et al., 2007). The minerals are important for the growth of microorganisms, especially phosphorus which is essential for the synthesis of phospholipids and nucleic acid (Marsh et al., 2013; Randazzo et al., 2016). As a result, the steep decrease in the TSS of the jujube water kefir beverage may be attributed to the improved growth of kefir microorganisms due to the presence of minerals and vitamin C in the jujube syrup.

Furthermore, the steady decrease of TSS from 24 h to 48 h may be a reflection of the lower sugar concentration in the jujube syrup.

By comparing the beverage fermented (0-48 h) with the same amount of jujube syrup, sample K2 (2.65) and K4 (3.78) fermented at 27°C showed higher reductions in the TSS than sample K1 (2.60) and K3 (3.68) fermented at 25°C (Appendix D). This result is in agreement with Subardjo (2017) who reported that black tea kefir beverage fermented at 30°C had a higher reduction in TSS than the one fermented at 25°C.

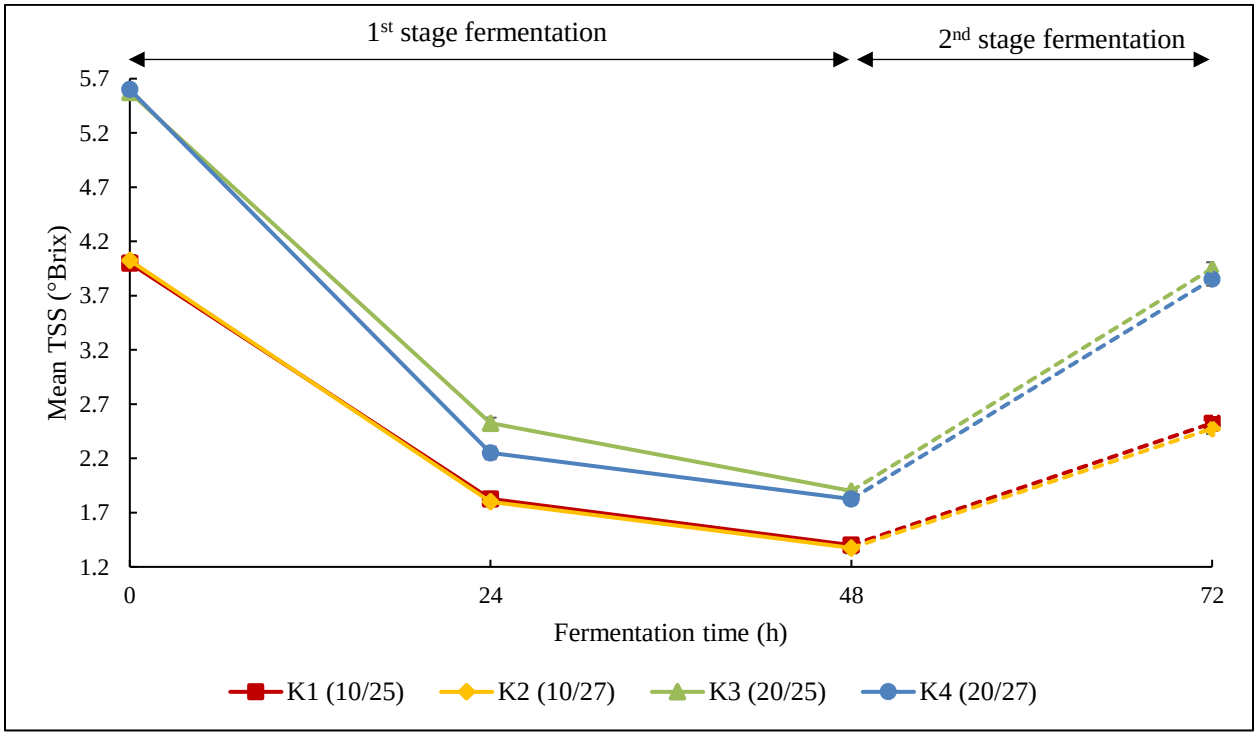

Figure 4.7 Mean total soluble solids (°Brix) of jujube water kefir beverages during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (—); 2nd stage fermentation (---); Error bars = ± SD (n=4); experiments were replicated twice.

2nd Stage Fermentation

In the second stage fermentation, TSS increased ($p<0.05$) in all samples. After the addition of jujube syrup at 48 h, the TSS of samples K1, K2, K3 and K4 increased by 1.13, 1.00, 2.05, and 2.03, respectively (Appendix D). It is normal practice to add a fresh source of carbohydrates after initial fermentation of kefir water beverage to initiate carbonation (Kwak, Park, & Kim, 1996; Subardjo, 2017). The carbohydrates in jujube may be responsible for the increase in the TSS of the beverage (Chen et al., 2015; El-Nagga & El–Tawab, 2012). However, the TSS of the beverages after the additional jujube syrup at stage 2 were lower than the TSS at 0 h, which suggested the metabolism of the kefir microorganisms after addition of the fresh source of carbohydrates. The highest TSS was obtained in sample K3 (3.95±0.06 °Brix) with the lowest TSS recorded in sample K1 (2.48±0.05 °Brix) (Appendix D). No differences ($p>0.05$) were observed between the TSS of the samples fermented at 25°C and 27°C. Moreover, the differences ($p<0.05$) in the TSS between the samples fermented with different concentrations of jujube syrup indicated that the TSS may be affected by the concentrations of the jujube syrup.

4.2.3 pH and titratable acidity

1st Stage Fermentation

First stage fermentation included the period 0-48 h. The pH and the T.A. of jujube water kefir beverages are shown in Figure 4.8 and Figure 4.9. During the first stage of fermentation (0-48 h), the pH of all the samples decreased ($p<0.05$). Meanwhile, in the first 24 h of fermentation, the initial pH of the beverages ranged from 4.85±0.01 to 4.96±0.01 followed by a marked decrease to 3.60±0.01-3.71±0.01 (Appendix D). This result was consistent with the rapid decrease in the TSS during the first stage of fermentation (0-24 h), (Figure 4.7). Magalhaes et al. (2010) also reported a marked decrease in the pH of sugary kefir from 5.6±0.1 to 4.1±0.1 during the first 24 h of fermentation. The rapid decrease in the pH reflects the metabolic activity of the microorganisms which produce organic acids including lactic acid, a major metabolite (Koh et al., 2017; Pidoux, 1989). In addition, the minerals and vitamin C present in the jujube fruit provide a rich environment

for the growth of kefir microorganisms, which may contribute to the rapid decrease of pH (Li, Fan, et al., 2007). The pH of the jujube water kefir beverage at the end of the first stage fermentation (48 h) ranged from 3.39±0.01 to 3.54±0.00 (Appendix D). The range of pH levels reported here is similar to the results reported by Randazzo et al. (2016) who developed a kiwifruit kefir beverage with pH of 3.48±0.03 after 48 h fermentation.

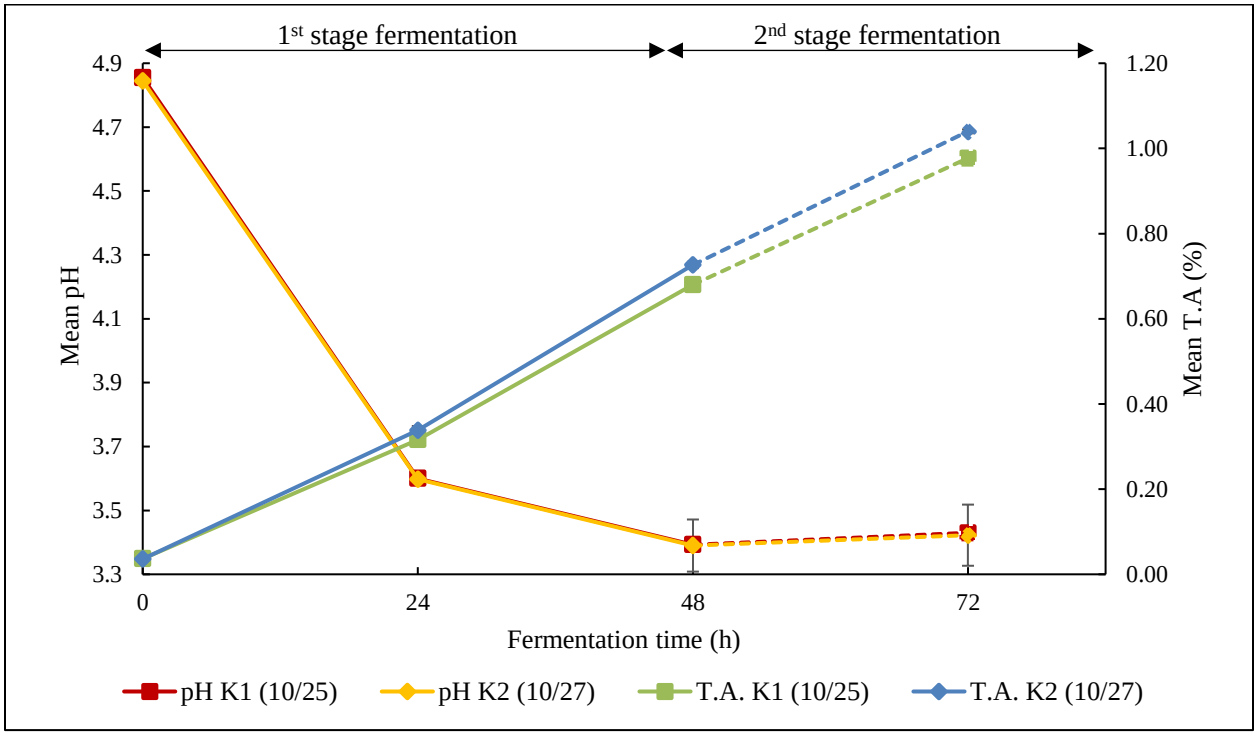

Figure 4.8 Mean pH and titratable acidity (T.A.) (%) of jujube water kefir beverages (K1 & K2) during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; 1st stage fermentation (—); 2nd stage fermentation (---); DJ = dried jujube; Error bars = ± SD (n=4); experiments were replicated twice.

The initial T.A. of all samples ranged from 0.04±0.00% to 0.06±0.00% (Figure 4.8 and 4.9). According to Gao, Wu, Yu, et al. (2012), the common organic acids found in jujube fruit are malic acid and citric acid, with succinic acid only being detected in some of the cultivars. Hence, the low concentration of T.A. may be due to the presence of organic acids. During fermentation for 48 h,

T.A. increased ($p<0.05$) as the pH decreased. During water kefir fermentation, the sugar is converted to organic acids by the microorganisms such as LAB and AAB present in the water kefir culture (Laureys & De Vuyst, 2014; Reddy et al., 2008; Stadie et al., 2013). Thus, the significant increase in T.A. may be explained by the conversion of sugars into organic acids such as acetic acid and lactic acid. However, the type and the amount of organic acids produced depends on the type of starter culture, fermentation conditions and the substrate used in the fermentation (Puerari et al., 2012; Randazzo et al., 2016). According to unpublished information provided by the supplier (Cultures for Health, Raleigh, North Carolina, USA), the starter culture used in this study contained *S. cerevisiae* and undefined LAB.

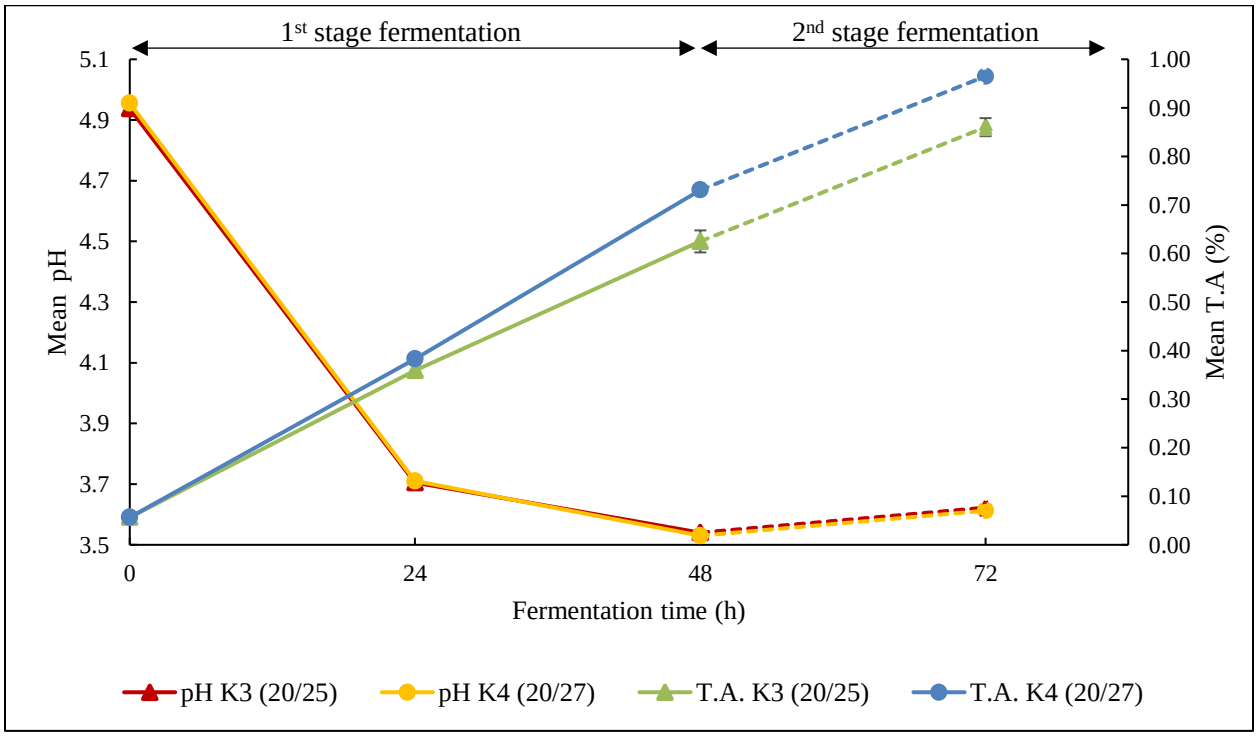

Figure 4.9 Mean pH and titratable acidity (T.A.) (%) of jujube water kefir beverages (K3 & K4) during fermentation for 72 h

Notes: Sample K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (—); 2nd stage fermentation (---); Error bars = ± SD (n=4); experiments were replicated twice.

2nd Stage Fermentation

Fermentation after addition (20%, v/v) of jujube syrup was described as the second stage fermentation. In stage 2 fermentation, the pH of all samples had increased ($p<0.05$) by end of fermentation at 48 h. The final pH of samples K1, K2, K3, and K4 were 3.43±0.01, 3.42±0.01, 3.62±0.01, and 3.61±0.01, respectively (Appendix D). Fermentation of these samples at a higher temperature (27°C) resulted in slightly lower pH levels of the beverages compared to samples fermented at 25°C. The results are in agreement with Subardjo (2017) who reported a lower pH of the black tea kefir beverage fermented at 30°C than the sample fermented at 25°C. The metabolism of the water kefir microorganisms may be affected by the fermentation temperature. According to Soupioni, Golfinopoulos, Kanellaki, and Koutinas (2013), higher fermentation temperature may increase the activities of the microorganisms thereby increasing the fermentation rate. Bensmira and Jiang (2012) also reported that the kefir grains fermented at a lower temperature contained low-density gel, which is more susceptible to deformation. However, in the present study, no differences ($p>0.05$) were observed in pH between samples fermented at 25°C and 27°C. Further, at the end of the fermentation, the pH of the beverage fermented with higher jujube syrup concentration was higher ($p<0.05$) than the one fermented with lower syrup concentration. The outcome is contrary to Bensmira and Jiang (2012) who reported higher pH in peanut-milk kefir beverage fermented with higher sugar concentration. The differences in the results obtained from this study (pH) and other studies may be attributed to the action of different types of starter culture, fermentation conditions, and fermentation substrates.

After the second stage fermentation (i.e. after 48 h), T.A. of all samples increased ($p<0.05$) compared to the T.A. obtained at 48 h. The highest T.A. was obtained in sample K1 (0.98±0.01%) with the lowest in sample K3 (0.86±0.00%). The samples fermented with higher syrup concentration resulted in a higher amount of T.A. (Figure 4.8 and Figure 4.9). This outcome is in disagreement with the study by Cui, Chen, Wang, and Han (2013) who reported lower T.A. in walnut milk kefir beverage with an increase in sucrose concentration. Differences in T.A. levels may be associated with using different fermentation media and starter cultures. Further, by comparing the beverage fermented with the same amount of jujube syrup, the sample fermented at 27°C had higher ($p<0.05$) amount of T.A. than the sample fermented at 25°C. Cui et al. (2013)

observed an increase in T.A. in the walnut milk kefir beverage when the fermentation temperature was increased from 25°C to 40°C. During milk kefir fermentation, Soupioni et al. (2013) also reported increased metabolic activities as temperature increased up to 30°C, which resulted in an increased lactose uptake. Therefore, the higher acidity may be associated with increased fermentation rate as the fermentation temperature is elevated (Soupioni et al., 2013).

4.2.4 Colour

In food and beverages, colour is an important attribute which impacts on consumer preferences (Pathare et al., 2013). Therefore, it is necessary to analyse the colour of jujube water kefir beverage. The colour of the food and beverages can be related to the microbial, physical, chemical, and biochemical changes during food processing (Pathare et al., 2013). In the present study, the L*, a* and b* values of the extracted jujube syrup were 41.50±0.37, 4.50±0.40, and 6.92±0.65, respectively (Appendix D). Elleuch et al. (2008) reported L*, a* and b* values of 31.71±0.57, 14.68±0.23, and 22.34±0.12, respectively for the extracted date pastes whereas the data reported by Sánchez-Zapata et al. (2011) were 32.44±0.74, 5.78±0.54, and 7.33±1.19, respectively. Different varieties of date fruit and extraction conditions were used, and therefore the differences were not unexpected (Sánchez-Zapata et al., 2011). Changes in the colour of jujube water kefir beverages are shown in Figure 4.10, Figure 4.11 and Figure 4.12.

1st Stage Fermentation

The L* values of all samples increased significantly ($p<0.05$) in the first stage of fermentation (0-48 h) (Figure 4.10). The steady increase in the L* value was observed as the total soluble solids decreased (section 4.2.2). For all samples, the highest L* value was obtained at 48 h fermentation which ranged from 61.59±0.09 to 74.19±0.34 (Appendix D). The increased L* value indicated that the colour of the beverage became brighter during fermentation, which may be the result of the microbial transformation of the polyphenols present in the jujube syrup during metabolic activities (Chu & Chen, 2006). Samples with a higher concentration of jujube syrup had a lower L* value,

which reflected the dark colour of the syrup (41.50±0.37). This, therefore, suggests that a higher concentration of jujube syrup may be responsible for the darker colour of the beverage. Elleuch et al. (2008) reported that the colour of the extracted jujube paste may be affected by the solubility of the pigments which could be responsible for the dark colour of the jujube fruit.

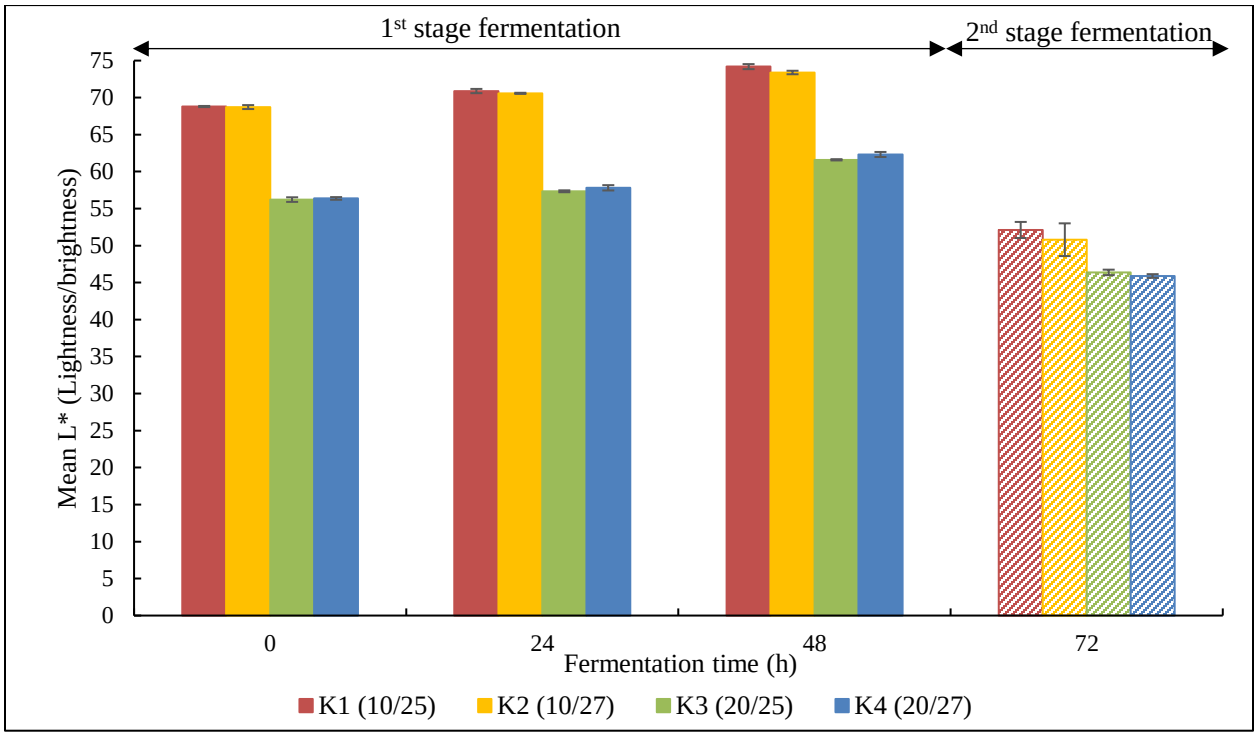

Figure 4.10 Mean L* of jujube water kefir beverages during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (solid fill); 2nd stage fermentation (pattern fill); Error bars = ± SD (n=4); experiments were replicated twice.

2nd Stage Fermentation

In the second stage fermentation, the L* value of all samples decreased significantly ($p<0.05$) after addition (that is, second addition) of jujube syrup at 48 h (Figure 4.10). As discussed previously, a decrease in the lightness of the beverage was probably caused by the addition of the jujube syrup at 48 h of fermentation. At the end of stage 2 fermentation (72 h), the lightness of the beverages K1, K2, K3, and K4 had decreased to 52.12±1.81, 50.80±2.21, 46.37±0.37, and 45.89±0.25, respectively (Appendix D). Samples (K1 and K2) with higher jujube syrup concentration had higher ($p<0.05$) L* value than the samples (K3 and K4) with lower jujube syrup concentration. However, there was no effect ($p>0.05$) of fermentation temperatures (25°C or 27°C) in the lightness (L*) of the beverages. Therefore, the results of this study suggest that the concentration of the jujube syrup had an effect on the lightness of the beverages at 72 h.

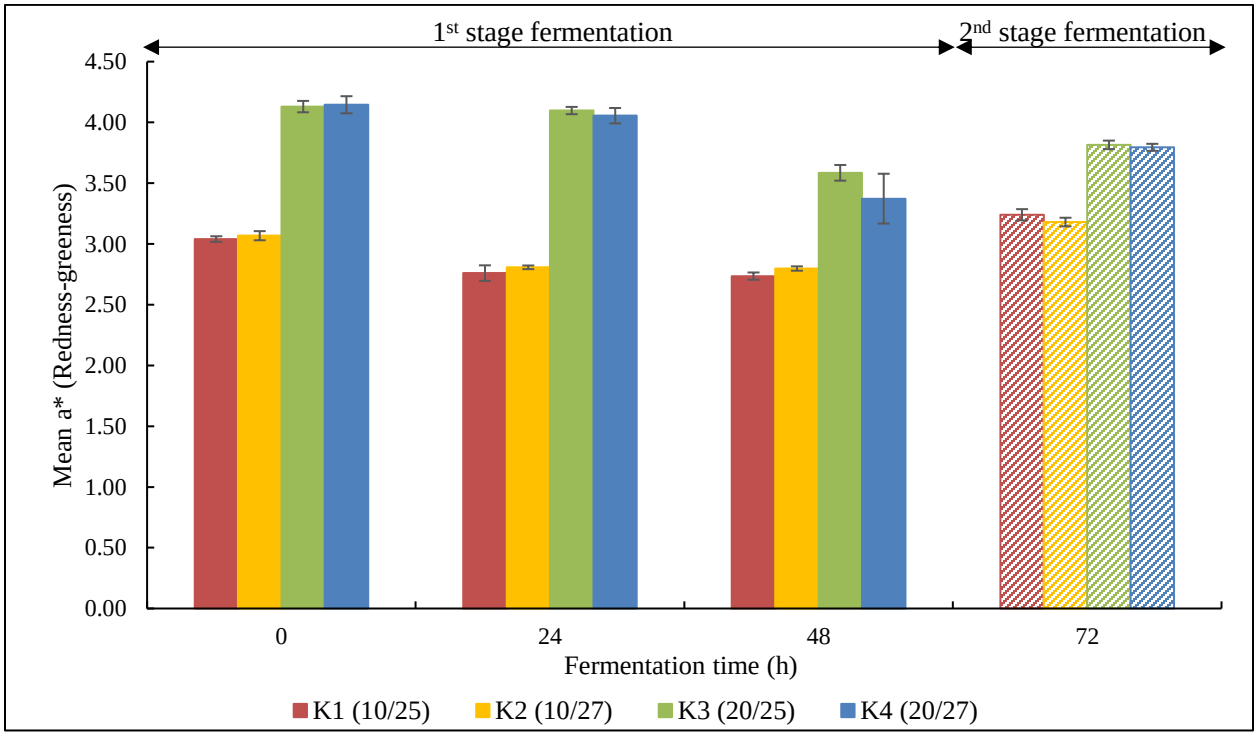

Figure 4.11 Mean a* of jujube water kefir beverages during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (solid fill); 2nd stage fermentation (pattern fill); Error bars = ± SD (n=4); experiments were replicated twice.

1st Stage Fermentation

During the first stage of fermentation, there was a decrease in both a* and b* values for all samples (Figure 4.11 and Figure 4.12). The positive a* and b* values represented the redness and yellowness of the samples, respectively (Pathare et al., 2013). The results were consistent with Corona et al. (2016) who reported that the redness and yellowness of the beverages fermented with strawberry juices decreased during fermentation. A rapid decrease in the redness was obtained in samples K3 and K4 from 24 h to 48 h, while a decrease in the a* value was more steady in samples K1 and K2 from 0 h to 48 h. At 48 h fermentation, the a* value of the samples ranged from 2.73±0.03 to 3.59±0.06 (Appendix D). In regards to the yellowness, the decrease in b* value was significant ($p<0.05$) for sample K1 and K2 during the 48 h fermentation period, whereas for K3 and K4, the decreases ($p<0.05$) were only observed from 24 h to 48 h during fermentation. At 48 h, the b* values of the samples ranged from 13.87±0.79 to 15.40±0.09 (Appendix D). The results on colour suggested that the structural changes of the carotenoids due to the acidification may be responsible for the colour change of the fruit juice (Sakamoto, Koguchi, Ishiguro, & Miyakawa, 1996). It is, therefore, reasonable to assume that the changes in the a* and b* values of the beverage may be attributed to the structural changes of the polyphenols and carotenoids during lactic acid fermentation (Chu & Chen, 2006; Sakamoto et al., 1996; Shi et al., 2018).

2nd Stage Fermentation

After the second stage of fermentation, the redness (a*) of all samples increased ($p<0.05$) whereas the yellowness (b*) decreased ($p<0.05$) compared to the samples fermented for 48 h. The highest a* value was obtained in sample K3 (3.81±0.04), while sample K2 (3.18±0.04) had the lowest redness. Although there were no differences ($p>0.05$) in a* values between the samples fermented at 25°C and 27°C, the beverage fermented with higher jujube syrup concentration had higher ($p<0.05$) a* value compared to the one fermented with lower jujube syrup. The redness of the jujube fruit is related to the β-carotene, lutein, and anthocyanin in the skin while the chlorophylls can affect the greenness of the fruit (Shi et al., 2018). Thus, the beverages containing higher amounts of jujube syrup may contain higher amounts of β-carotene, lutein, and anthocyanin, which

could lead to higher a* values of the beverages. For the yellowness of the beverages, K1 had the highest b* value (11.38±0.12) while the lowest was obtained in sample K4 (10.02±0.02). Similarly, the differences ($p<0.05$) in b* values were only obtained among the beverages fermented with different syrup concentrations. The sample containing higher syrup concentration had a higher b* value than the one fermented with lower syrup concentration. According to Tang et al. (2013), the yellowness of the date fruit may change to brown during the metabolism of tannins by polyphenol oxidase.

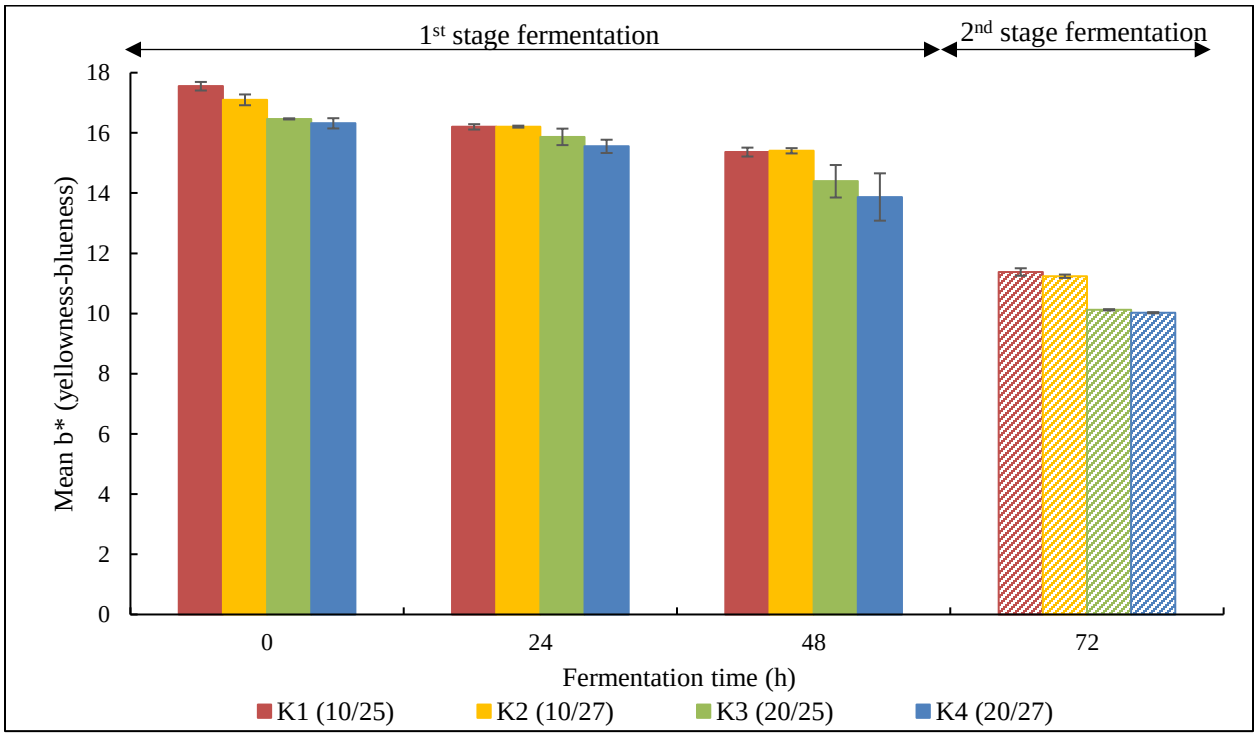

Figure 4.12 Mean b* of jujube water kefir beverages during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (solid fill); 2nd stage fermentation (pattern fill); Error bars = ± SD (n=4); experiments were replicated twice.

4.2.5 Microbiological analysis of the beverage

The growth pattern of LAB and *S. cerevisiae* during fermentation for 72 h are shown in Figure 4.13 and Figure 4.14, respectively. For all samples, the viable cell counts of LAB and *S. cerevisiae* increased ($p<0.05$) in the first 24 h of fermentation and then decreased from 24 h to 48 h. Co-cultivation of yeasts and lactobacilli is mutually beneficial as it improves the growth of both microorganisms than single cultivation, which is described as symbiotic (Stadie et al., 2013). In a study by Leroi and Pidoux (1993b), the growth of *Lactobacillus hilgardii* was supported by *Saccharomyces florentinus* while Stadie (2013) observed improved growth of *Zygotorulaspora florentina* in the presence of lactobacilli. According to Lengkey and Balia (2014), the LAB and yeasts are responsible for the lactic-alcoholic fermentation. Hence, the improved growth of both microorganisms is consistent with the rapid increase of the T.A. during the first 24 h of fermentation which was observed in this study (section 4.2.3).

1st Stage Fermentation

For all samples, the initial viable cell counts for LAB ranged from 6.64±0.05 to 6.66±0.03 log cfu/mL (Appendix D). At 24 h fermentation, the highest LAB cell counts were obtained in sample K4 (7.27±0.04 log cfu/mL) whereas the lowest were obtained in sample K1 (7.12±0.10 log cfu/mL). However, viable LAB cells for all samples slightly decreased by 0.09-0.14 log cfu/mL after 48 h fermentation. According to Leroi and Pidoux (1993a), the low pH and competition for nutrients can affect microbial growth. Laureys, Aerts, Vandamme, and De Vuyst (2018) reported that during fermentation, the nutrient source can also influence the pH through the release of buffering compounds. Their study showed that in water kefir fermentation, the low nutrient concentration may lead to slow fermentation, resulting in low metabolite concentration, high total residual carbohydrate concentration and pH (Laureys et al., 2018). Moreover, the growth of water kefir grains could be also supressed by excessive acidity regardless of the presence of EPS-producing *Lactobacillus hilgardii* (Laureys et al., 2018).

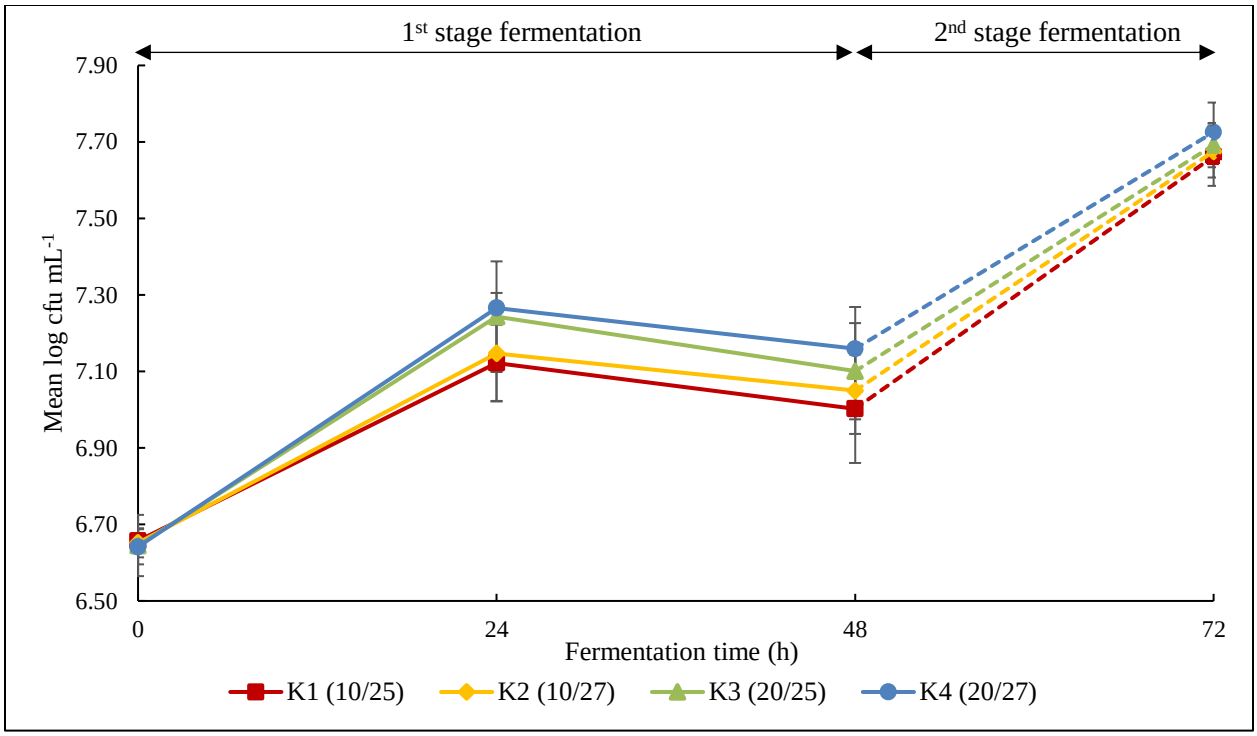

Figure 4.13 Mean log cfu/mL of LAB of jujube water kefir beverages during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (—); 2nd stage fermentation (---); Error bars = ± SD (n=4); experiments were replicated twice.

2nd Stage Fermentation

With the addition of fresh jujube syrup in the second stage of fermentation, the cell counts of the LAB reached the highest level in all samples at 72 h (Figure 4.13). This result is in agreement with Corona et al. (2016) who also reported a significant increase in the cell counts of LAB in the water kefir fermented with fruit juice after 72 h fermentation at 25°C. According to a study on the microbiota of a Zimbabwean fruit, *masau* (*Ziziphus mauritiana*), large amounts of LAB (9.20±0.58 log cfu/g) were found in the fermented *masau* whereas the cell counts were much lower in the ripe *masau* fruit (3.00±0.07 log cfu/g) (Nyanga et al., 2007). This indicated that *masau* could be considered as a favourable fermentation medium for LAB. With respect to this study, the

increase ($p<0.05$) in the LAB cell counts in all samples after the second stage fermentation may be attributed to the addition of jujube syrup, which provided a rich resource environment for the growth of LAB (Randazzo et al., 2016; Tang et al., 2013). At the end of the fermentation, the viable cell counts of LAB in samples K1, K2, K3, and K4 were 7.66±0.08, 7.68±0.07, 7.69±0.06, and 7.73±0.08 log cfu/mL, respectively (Appendix D). Comparing LAB counts in this study to the fermented *masau* fruit, the cell counts were lower in the jujube water kefir beverage. The discrepancy between this study and *masau* fermentation may be attributed to the loss of nutrients in the jujube syrup during high-temperature extraction. In addition, differences in the microbiota of the fermented *masau* fruit and fermented water kefir is another potential source of differences in the amount of LAB. The LAB found in the fermented *masau* fruit were mostly *Lactobacillus agilis* and *Lactobacillus plantarum*, with low amounts of *Lactobacillus bifermentus* and *Lactobacillus hilgardii*. Meanwhile *Lactobacillus casei*, *Lactobacillus hordei*, *Leuconostoc mesenteroides* have frequently been reported in water kefir (Nyanga et al., 2007; Stadie, 2013). Further, the traditional selective growth medium, MRS, was used in the present study. Zanirati et al. (2015) stated that some specific strains in water kefir may not grow on the MRS medium due to insufficient nutritional requirements, leading to the under-estimation of the actual amount of LAB in the samples.

At the end of the fermentation (72 h), beverages (containing the same amount of jujube syrup) fermented at 27°C (K2 and K4) had higher amounts of LAB compared to the beverages fermented at 25°C (K1 and K3). Puerari et al. (2012) reported that the proliferation of *Lactobacillus* sp. in water kefir grains can be improved at temperatures above 25°C. However, results from this study showed that syrup concentration and fermentation temperature had no effects ($p>0.05$) on the viable cell counts of LAB in the jujube water kefir beverage for 72 h fermentation.

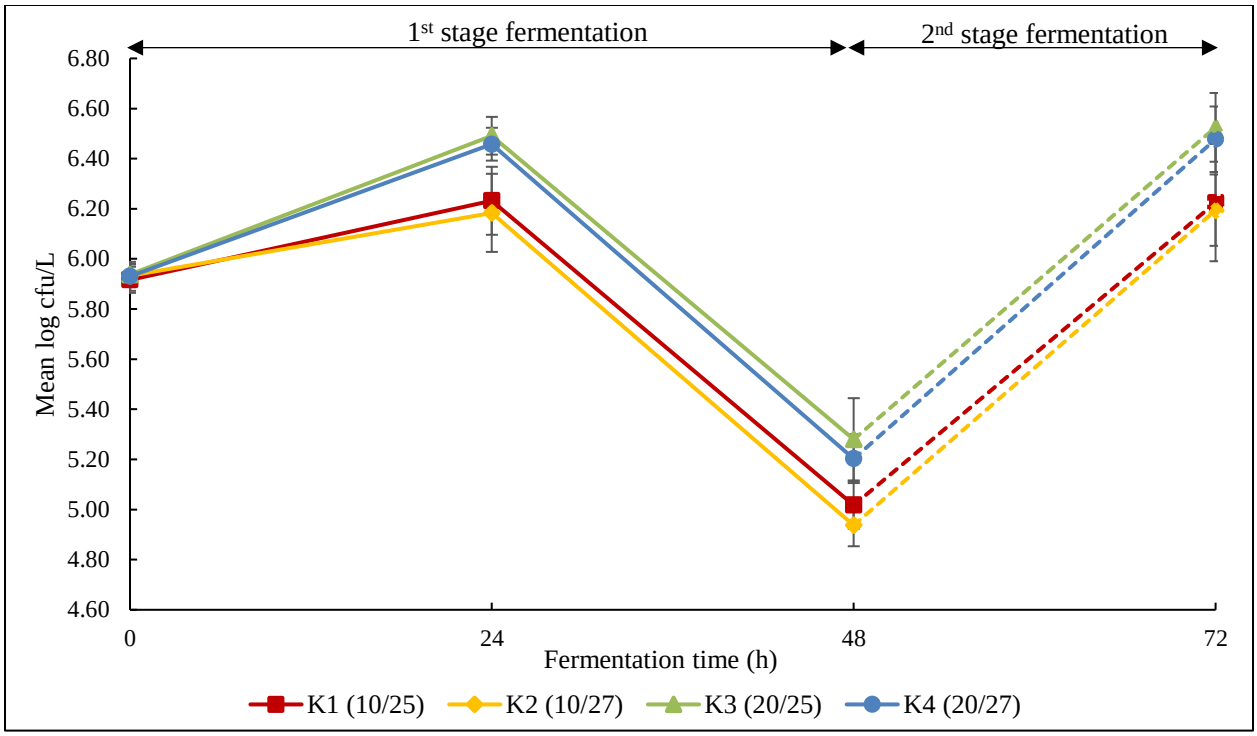

Figure 4.14 Mean log cfu/mL *Saccharomyces cerevisiae* of jujube water kefir beverage during fermentation for 72 h

Notes: Samples K1 (10/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K2 (10/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 10% jujube syrup for both fermentation stages; K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; 1st stage fermentation (—); 2nd stage fermentation (---); Error bars = ± SD (n=4); experiments were replicated twice.

1st Stage Fermentation

For all the samples, the initial viable cell counts for *S. cerevisiae* ranged from 5.92±0.05-5.94±0.04 log cfu/mL (Appendix D). The yeast cell counts of all the samples increased to 6.18±0.16-6.49±0.16 log cfu/mL at the end of 24 h fermentation and then decreased to the lowest levels (4.94±0.09-5.28±0.16 log cfu/mL) at 48 h fermentation. The increased counts of *S. cerevisiae* at 24 h (Figure 4.14) was consistent with the marked decrease in TSS and increased T.A. from 0 - 24 h (section 4.2.2 and 4.2.3). The rapid decrease in TSS may be attributed to the accelerated hydrolysis of sucrose into glucose and fructose by the increased growth of *S. cerevisiae* and the conversion of sugar into organic acids by LAB (Montet et al., 2014; Pfeiffer & Morley, 2014;

Pronk et al., 1996). The marked decrease in *S. cerevisiae* counts may be attributed to the low pH at 48 h (3.39±0.01 to 3.54±0.00). Previous studies reported that the reproduction of *Zygotorulaspora florentina* yeast was optimised with a starting level of pH 4, while the growth of *Zygotorulaspora florentina* decreased when the starting level was pH 3 (Stadie et al., 2013). Özer (2010) also reported that low pH (<4.0) may have a negative effect on the kefir due to the high acidity. Inhibition of *S. cerevisiae* during fermentation has also been attributed to osmotic pressure in solutions containing high TSS. Chniti et al. (2017) reported the inhibition of *S. cerevisiae* during the fermentation of yeasts in dates syrup containing high TSS due to the osmotic stress.

2nd Stage Fermentation

With the addition of jujube syrup at the second stage of fermentation, the cell counts of *S. cerevisiae* increased ($p<0.05$) in all the samples at 72 h relative to the cell counts obtained at 48 h. Comparing to the *S. cerevisiae* counts of samples obtained at 0 h, the yeast cells increased to 6.19±0.14-6.52±0.14 log cfu/mL after 72 h fermentation, with no significant ($p>0.05$) differences. Corona et al. (2016) also reported an increase in the microbial load of yeast in the water kefir beverage fermented with fruit juice after fermentation for 72 h at 25°C. A study on the microbiota of the *masau* (*Ziziphus mauritiana*) reported higher yeast counts in the fermented *masau* (9.30±0.40 log cfu/g) than in unripened *masau* fruit (3.90±0.04 log cfu/g) (Nyanga et al., 2007). This suggested that the *masau* fruit could be a suitable fermentation medium for *S. cerevisiae*. As a result, it can be assumed that the jujube syrup, which provided a nutrient-rich fermentation environment, led to the increase ($p<0.05$) in cell counts of *S. cerevisiae* at the end of second stage fermentation. At the end of fermentation (72 h), the viable counts of *S. cerevisiae* in K1, K2, K3 and K4 were 6.22±0.23, 6.19±0.14, 6.52±0.14, and 6.47±0.13 log cfu/mL, respectively (Appendix D). These results are consistent with Corona et al. (2016) who reported 6.7±0.4 log cfu/mL yeasts in the water kefir beverage containing carrot juice fermented for 72 h at 25°C.

At the end of the fermentation (72 h), with the same amount of jujube, samples (K1 and K3) fermented at 25°C had a slightly higher amount of *S. cerevisiae* than the sample fermented at 27°C. Hence, fermentation at 25°C may be more favourable for the growth of *S. cerevisiae*, which is

consistent with Puerari et al. (2012) who reported rapid growth of yeasts when the temperature was increased from 19°C to 25°. However, the results showed that the fermentation temperature and syrup concentration had no effects ($p>0.05$) on viable counts of *S. cerevisiae* in the jujube water kefir beverage at 72 h fermentation.

4.2.6 Sensory evaluation

The sensory evaluation for selecting the most promising jujube water kefir beverage consisted of two stages. In the first stage, informal focus groups comprising of six experienced sensory panellists who were familiar with water kefir beverages participated in the first stage of the sensory evaluation. In this stage, jujube water kefir beverages with four formulations were evaluated for appearance, aroma, flavour, sweetness, sourness and overall acceptability at the end of the fermentation (72 h).

4.2.6.1 Informal focus group evaluation

Among the four beverages, there were apparent sensory differences between samples containing different syrup concentrations while the sensory differences between the samples fermented at 25°C and 27°C were not obvious. The appearance of jujube water kefir beverage containing lower amounts of jujube syrup was described as light-yellow, and the sample containing a high amount of jujube was yellow-orange. Visible small gas bubbles were only detected in the beverage fermented with a higher concentration of syrup. Gas bubbles are desirable in fermented water kefir, which is attributed to the generation of carbon dioxide by yeast during fermentation (Reiß, 1990; Sicard & Legras, 2011). The produced carbon dioxide is related to the typical characteristic fizziness of the kefir beverage (Otles & Cagindi, 2003). The evolution of gas bubbles was consistent with the higher viable cell counts of *S. cerevisiae* obtained in the beverage with higher syrup concentration (section 4.2.5). A stronger vinegary aroma was noted in the samples fermented with less syrup, which was most likely due to the presence of higher acidity (section 4.2.3). All the samples were described as ‘refreshing beverage’ characterised by dates (jujube) flavour, with a more intense dates flavour in the beverage with higher syrup concentration. However, the

sourness in samples fermented with less syrup was described as overpowering, with a throat burn, astringency and pungency during the tasting. The sweetness of the beverage can be due to the residual sugar, and samples with higher TSS (3.85±0.06-3.95±0.06 °Brix) were perceived as sweeter than the samples with lower TSS (2.48±0.05-2.53±0.05 °Brix). The sensory changes are induced when LAB and yeasts metabolise sugars producing organic acids, which mask the sweetness, thereby increasing the sourness (Bamforth, 2008; Gaspar & Crespo, 2016). Due to the strong vinegary aroma, the absence of fizziness, and the overpowering acidity, samples containing lower amounts of jujube syrup received lower overall acceptability. No sensory differences were obtained between the beverages fermented at 25°C and 27°C. Therefore, the beverages fermented with 2.5% (w/v) organic raw sugar and 20% (v/v) jujube syrup in stage 1 fermentation with added jujube syrup (20%, v/v) in stage 2 fermentation (K3 and K4) had balanced flavour profiles and pleasant fizziness. Therefore, the two formulations were further investigated.

4.2.6.2 Consumer sensory evaluation

Following the informal focus group, the samples were evaluated by consumer panellists (n=30) using a 9-point hedonic rating scale. In the second stage of sensory evaluation, the beverages (2.5% organic raw sugar, w/v; 20%, jujube syrup, v/v) were fermented at 25°C and 27 °C in the stage 1 fermentation, and then jujube syrup (20%, v/v) was added in stage 2 fermentation.

Jujube water kefir beverage fermented at 27°C (K4) received higher consumer sensory scores for appearance (6.37±1.27), odour (6.33±1.21), flavour (6.37±1.30), sweetness (6.07±1.39), and overall acceptability (6.53±1.57) (Appendix D). In addition, sample K4 was characterised by visible evolution of gas bubbles, which may impact on the fizziness of the beverage (Figure 4.15).

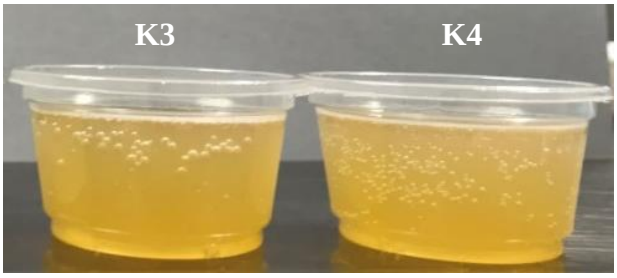

Figure 4.15 Jujube water kefir beverages (K3 & K4)

(Captured by iPhone 7 Plus (Apple Inc., USA), 12 megapixels)

Notes: Sample K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages.

Figure 4.16 shows the results of the consumer sensory evaluation of jujube water kefir beverages. The main difference between the two beverages was the sensory scores for odour. Odour can be simply regarded as a smell which may be pleasant or unpleasant (Mills, 1995). The beverage (K4) fermented at 27°C received higher scores for odour than sample K3 fermented at 25°C, but with no significant differences ($p>0.05$). The yeasty odour of kefir beverage is related to the esters (ethyl octanoate, ethyl decanoate, ethyl hexanoate, and isoamyl acetate) produced by the *S. cerevisiae* (Laureys & De Vuyst, 2014; Randazzo et al., 2016). Laureys et al. (2018) also reported that the high abundance of *S. cerevisiae* could lead to high ester concentrations. As a result, the insignificant difference in the odour of two beverages (K3 and K4) may be attributed to the non-significant difference in the viable cells of *S. cerevisiae* at the end of the fermentation (section 4.2.5).

According to van Wyk, Britz, and Myburgh (2002), the sourness and creaminess of kefir are increased due to increased metabolic activities of culture microorganisms at high temperature. These characteristics were reported by van Wyk et al. (2002) when the temperature was increased from 25°C to 35°C during the fermentation of milk kefir. However, in the present study, fermentation temperature had no apparent effect ($p>0.05$) on the overall sensory attributes of jujube water kefir beverage. The discrepancy may be attributed to the composition of different starter cultures and fermentation medium (van Wyk et al., 2002). The kefir grains used in kefir water fermentation contain a complex mixture of starter cultures with different growth

requirements (Gulitz et al., 2011; Miguel et al., 2011; Stadie, 2013; Waldherr et al., 2010). In a Brazilian water kefir beverage studied by Magalhaes et al. (2010), the dominant microorganisms were *Lactobacillus paracas*ei, *Lactobacillus kefiri, Acetobacter lovaniensis, S. cerevisiae* and *Kluyveromyces lactis*. In a separate study on Brazilian water kefir, Miguel et al. (2011), reported that the *Gluconobacter liquefaciens*, *Lactobacillus casei*, *Lactobacillus kefiri*, *S. cerevisiae* and *Pichia cecembensis* were the dominant microorganisms. In addition, nutrient concentration and nutrient source can affect the microbial species diversity and production of metabolites (Laureys et al., 2018). However, the low nutrient concentration favoured the growth of *Lactobacillus hilgardii* whereas the growth of *S. cerevisiae* and *Lactobacillus nagelii* were promoted with high nutrition concentration (Laureys et al., 2018).

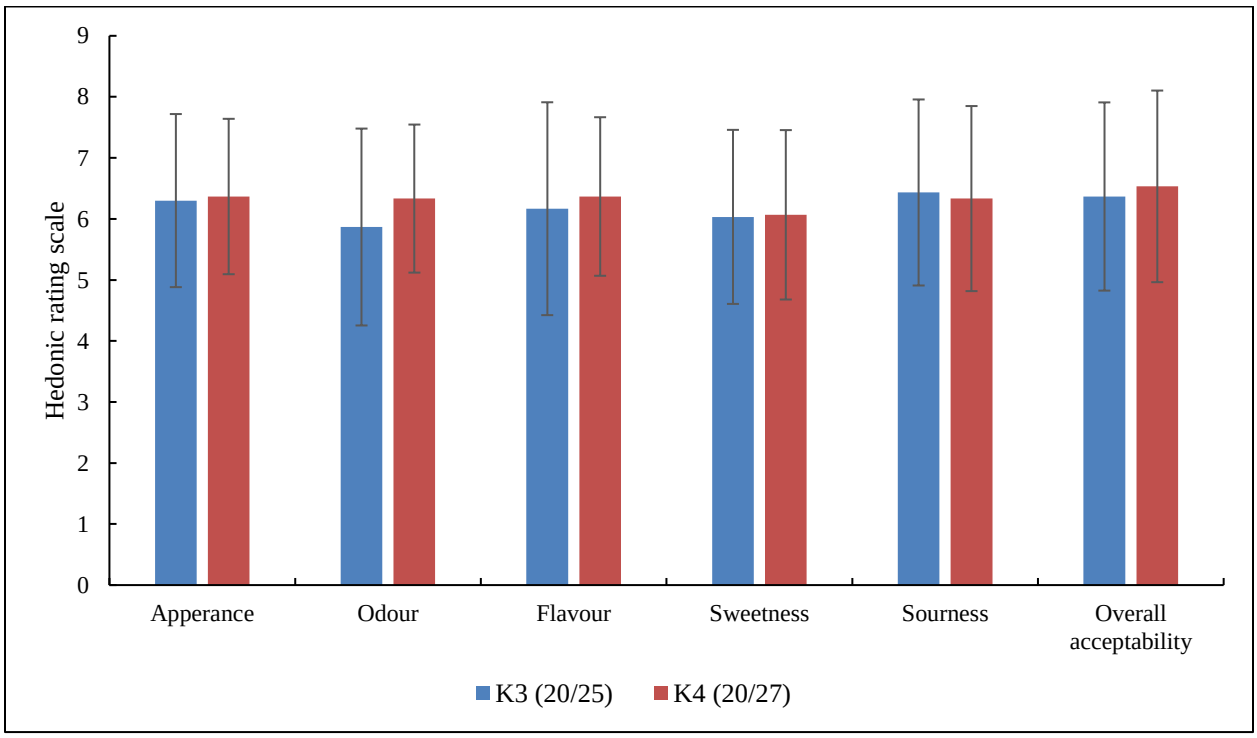

Figure 4.16 Mean consumer sensory evaluation scores of jujube water kefir beverages (K3 & K4) at the end of fermentation (72 h)

Notes: Sample K3 (20/25) = jujube water kefir beverage fermented at 25°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; K4 (20/27) = jujube water kefir beverage fermented at 27°C in 2.5% sugared water with addition of 20% jujube syrup for both fermentation stages; Hedonic scaling: 1-9 with 1 as lowest score and 9 as the highest; Error bars = ± SD (n=30).

4.2.7 Summary – phase II

This section summarises the effect of concentration of jujube syrup and fermentation temperature on the jujube water kefir beverage and the description of the most promising formulation.

Results in phase II of this study showed that different jujube concentrations and fermentation temperatures contributed to the physicochemical, microbiological and sensory profile of the fermented jujube water kefir beverages. The beverage (2.5% organic raw sugar, w/v; 10% jujube syrup, v/v) fermented at 27°C in stage 1 fermentation with added jujube syrup (10% v/v) in stage 2 fermentation (K2) contained the highest titratable acidity and the lowest total soluble solids. The highest counts of LAB at the end of fermentation (72 h) was obtained in the beverage (2.5% organic raw sugar, w/v; 20 % jujube syrup, v/v) fermented at 27°C stage 1 fermentation with added jujube syrup (20% v/v) in the stage 2 fermentation (K4) while the beverage fermentation at 25°C with the same formulation (K3) had the highest counts of *S. cerevisiae*. However, no apparent differences in the sensory characteristics of the two samples (K3 and K4) were found by the informal focus group. During consumer sensory evaluation, sample K4 received the highest mean sensory scores for overall acceptability and other attributes. Sample K4 was described as a refreshing carbonated beverage with acidic taste and a yeasty odour. Based on the results obtained in phase II, the most promising formulation was sample K4, which was subjected to further investigation.

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

Based on the results discussed in sections 4.2.2-4.2.6, sample K4, which was selected as the most promising formulation, was further investigated in phase III. The beverage (K4) was produced by 2-stage fermentation at 27 °C. The beverage was fermented in 2.5% (w/v) organic raw sugar and 20% (v/v) jujube syrup in stage 1 fermentation with the addition of 20% (v/v) jujube syrup in the stage 2 fermentation.

4.3.1 Part I: Concentrations of ethanol, sugar, organic acids and antioxidants in the final formulation of jujube water kefir beverage during fermentation

The purpose of part I of the study was to analyse the ethanol content, sugar, organic acids, and antioxidant compositions of the most promising formulation (K4) during 72 h fermentation at 27°C.

4.3.1.1 Ethanol content

S. cerevisiae, which exhibits strong fermentative metabolism, has the ability to hydrolyse sucrose into glucose and fructose, then metabolise the two sugars into carbon dioxide and ethanol (Hagman & Piškur, 2015; Pronk et al., 1996; Rodrigues et al., 2006). In water kefir, *S. cerevisiae* is primarily responsible for ethanol production (Corona et al., 2016; Laureys & De Vuyst, 2014; Magalhaes et al., 2010; Puerari et al., 2012). The concentration of ethanol in the most promising jujube water kefir beverage selected from Phase II (K4) during fermentation is shown in Figure 4.17.

From 0 h to 24 h, the ethanol content increased ($p<0.05$) from 0% to 2.47±0.02% (v/v) (Appendix E). The result is in agreement with Corona et al. (2016) who reported 2.56±0.62% (v/v) ethanol in fermented water kefir beverage containing melon juice after 24 h fermentation. In this study, the increase in ethanol content ($p<0.05$) might be caused by increased metabolic activities of *S. cerevisiae* as discussed in section 4.2.5. After 48 h, the concentration of ethanol decreased ($p<0.05$)

by 0.21% (v/v). In the present study, 1.25 log cfu/mL reduction in the viable cells counts of *S. cerevisiae* was observed from 24 h to 48 h in sample K4. Hence, the decrease in the ethanol content may be attributed to the reduction of viable *S. cerevisiae* counts. Magalhaes et al. (2010) also reported a gradual decrease in the ethanol content after 12 h fermentation. Beshkova, Simova, Frengova, Simov, and Dimitrov (2003) and Magalhaes et al. (2010) reported that in the presence of heterofermentative bacteria from genus *Acetobacter*, part of the ethanol may be first converted to acetaldehyde by the alcohol dehydrogenase and then to acetic acid by aldehyde dehydrogenase. This is attributed to the unique characteristic of AAB which can oxidise the alcohol into acetic acid (Guillamón & Mas, 2017). Hence, the presence of oxygen could increase the activity of AAB (Laureys et al., 2018).

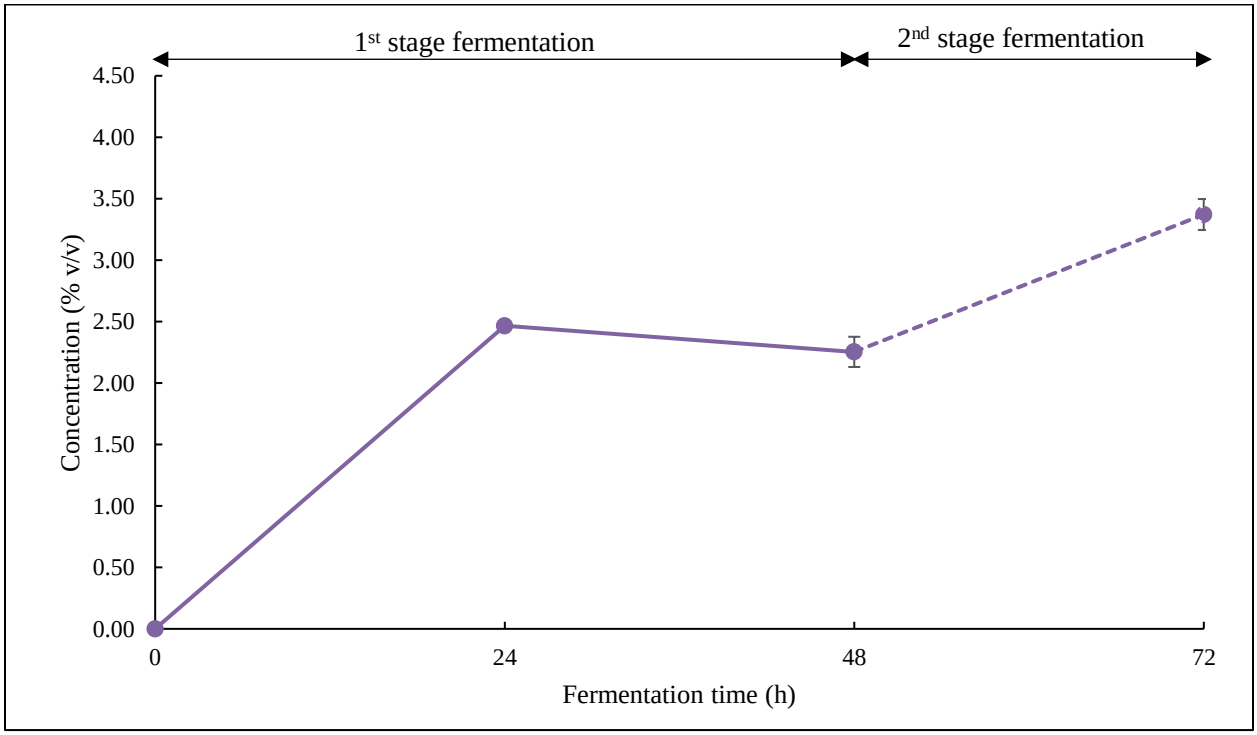

Figure 4.17 Mean concentration (%, v/v) of ethanol in jujube water kefir beverage (K4) during fermentation for 72 h

Notes: 1st stage fermentation conditions = 27°C for 48 h (—); 2nd stage fermentation conditions = 27°C for 24 h (---); Error bars = ± SD (n=4); experiments were replicated twice.

After the addition of jujube syrup at 48 h, the ethanol content reached the highest level, (3.37±0.13%, v/v) with further fermentation for 24 h (Appendix E). The increase in ethanol may be explained by the high cell counts of *S. cerevisiae* at 72 h (Figure 4.14). The high concentration of ethanol obtained in the present study was similar to Corona et al. (2016) who reported 3.00±0.14% (v/v) of ethanol in fermented carrot juice water kefir beverage. The ethanol concentration of the jujube water kefir beverage was however higher than the data reported by Laureys and De Vuyst (2014) (20.3±1.3 g/L), while lower than the reports by Puerari et al. (2012) (3.6% v/v) and Randazzo et al. (2016) (4.96% v/v). According to work on date syrup by Chniti et al. (2017), the ethanol production of some yeast strains was reported to be affected by the initial sugar concentration of the syrup. Previous studies showed that high ethanol concentration may be obtained by using osmotolerant yeasts (*Zygosaccharomyces rouxii*) in the fermentation of concentrated syrup. Whereas the efficiency of ethanol production may be reduced when using *S. cerevisiae* for concentrated syrup fermentation due to the high osmotic stress (Chniti et al., 2017).

Besides the yeasts, the LAB may also affect the ethanol concentration during fermentation. Some species of *Lactobacillus* and *Lactococcus* can convert acetaldehyde to ethanol through metabolism of the aldehyde by alcohol dehydrogenase, which may explain our high ethanol concentration (Beshkova et al., 2003; Puerari et al., 2012). At low concentration of dissolved oxygen, lactic acid may be converted to acetaldehyde (Drysdale & Fleet, 1988). Elferink et al. (2001) also proposed that under anaerobic conditions, the *Lactobacillus buchneri* and *Lactobacillus parabuchneri* may degrade the lactic acid to acetic acid and a small amount of ethanol without the external electron receptor. It is possible that the significant increase in the total LAB during the second stage fermentation (section 4.2.5) contributed to the increase in the ethanol content. As a result, the contradiction in the ethanol concentration between the present study and the previous studies may be attributed to different fermentation substrates and composition of starter culture (Yaman et al., 2010; Zhou et al., 2009).

Ethanol produced during fermentation is important for the sensory profile of water kefir. According to previous studies, the presence of ethanol and carbon dioxide provide a desirable yeasty aroma with exotic notes of the kefir beverage (Güzel-Seydim, Seydim, Greene, & Bodine, 2000).

4.3.1.2 Sugars

The composition of sugar in the most promising jujube water kefir beverage during fermentation is shown in Figure 4.18. Sucrose concentration was 2.93±0.02% (w/v) after the additions of 2.5% (w/v) organic raw sugar and 20% (v/v) jujube syrup at the beginning of fermentation (0 h) (Appendix F). The concentration of glucose (1.09±0.00% w/v) and fructose (0.98±0.01% w/v) at 0 h suggested that apart from sucrose, the extracted jujube syrup also contained these two monosaccharides. However, sucrose was not analysed before additions. Previous studies reported smaller amounts of sucrose in jujube fruit compared to fructose and glucose (Gao, Wu, Wang, et al., 2012; Li, Fan, et al., 2007). The relative sweetness of fructose is higher than sucrose and glucose due to the high strength of the hydrogen bond (Lee, 1987). Hence, the higher content of fructose in jujube fruit may contribute to the intense sweetness of the fruit.

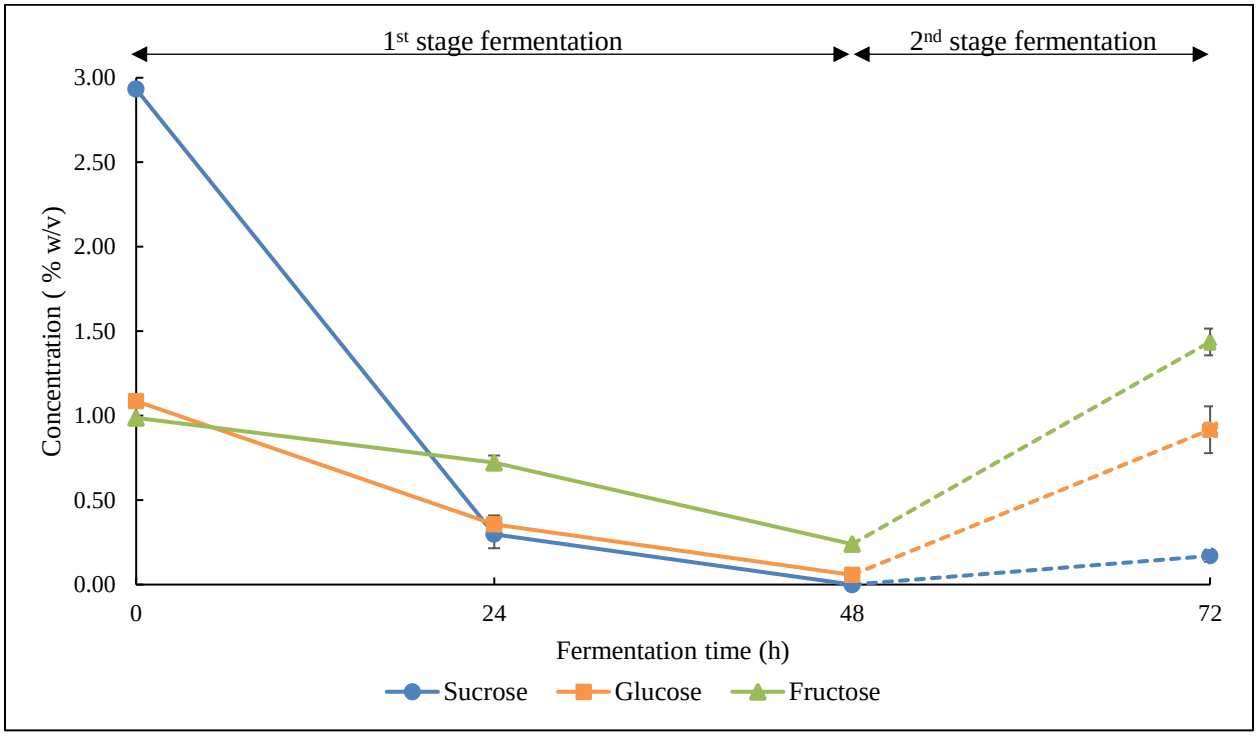

Figure 4.18 Mean concentration (%, w/v) of sugars in jujube water kefir beverage (K4) during fermentation for 72 h

Notes: 1st stage fermentation conditions = 27°C for 48 h (—); 2nd stage fermentation conditions = 27°C for 24 h (---); Error bars = ± SD (n=4); experiments were replicated twice.

During the first stage of fermentation (0 h to 48 h), sucrose decreased ($p<0.05$) to 0.30±0.08% (w/v) in the first 24 h and then gradually decreased to 0% (w/v) at 48 h. With the addition of jujube syrup in stage 2, the sucrose concentration increased to 0.17±0.03% (w/v) at 72 h fermentation. Overall, the sucrose content in jujube water kefir beverage decreased ($p<0.05$) during 72 h fermentation. The trends of sucrose concentration during fermentation are consistent with the pattern of TSS (section 4.2.2).

At the onset of the fermentation, the concentrations of glucose and fructose were similar. However, the differences in the concentrations of the sugars became apparent after 24 h fermentation (Figure 4.18). At 48 h, the concentration of glucose (0.06±0.00% w/v) and fructose (0.24±0.00% w/v) had decreased to the lowest level. During fermentation, sucrose is hydrolysed into glucose and fructose by the invertase present in the yeast, and the glucose and fructose are then utilised by LAB and the yeast (Montet et al., 2014; Pronk et al., 1996). According to Berthels, Cordero Otero, Bauer, Thevelein, and Pretorius (2004), *S. cerevisiae* displays a preference for glucose than fructose during fermentation. This is attributed to the two glucose transporters (Rgt2p and Snf3p) which act as glucose receptors to generate intracellular glucose signal. Hence, glucose signalling is a receptor-mediated process in yeast (Ozcan, Dover, Rosenwald, Wölfl, & Johnston, 1996). The fast depletion of glucose can lead to the differences between the concentrations of fructose and glucose. Therefore, the residual sugar in yeast-fermented products usually contains higher amounts of fructose than glucose if the initial fermentation media contain the same amounts of the two sugars (Berthels et al., 2004). The results from the present study are consistent with the findings by Berthels et al. (2004).

By the end of the second stage fermentation (72 h), the concentrations of sucrose (0.17±0.03%, w/v), glucose (0.92±0.14%, w/v), and fructose (1.44±0.09%, w/v) all increased ($p<0.05$) compared with the sugar concentrations at 48 h. These results are inconsistent with Stadie (2013) who reported decreased concentrations of glucose and sucrose after 48 h fermentation. The dissimilar results may be attributed to different microbial ecology and fermentation substrates (Hsieh et al., 2012). The increases ($p<0.05$) in all sugars can be attributed to the addition of jujube syrup, which may contain small amounts of sucrose, glucose and fructose (Guo et al., 2015).

4.3.1.3 Organic acids

The concentrations of organic acids in jujube water kefir beverage are shown in Figure 4.19. At 0 h, a small amount of lactic acid (<0.10% w/v) was detected. This result cannot be explained easily as there are no published data. During the first stage fermentation (0 h to 48 h), the lactic acid concentration had increased to 0.11±0.02% (w/v) at 48 h, while the acetic acid had increased ($p<0.05$) to 0.25±0.00% (w/v) (Appendix F).

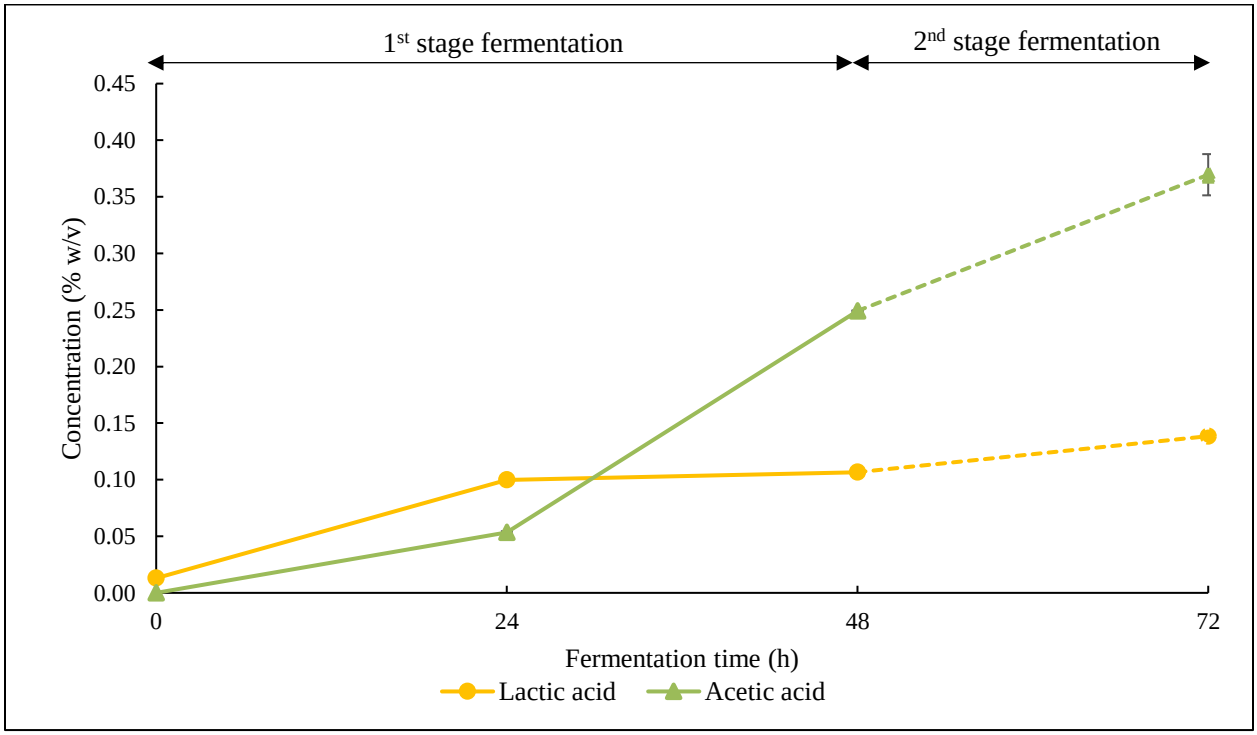

Figure 4.19 Mean concentration (%, w/v) of organic acids in jujube water kefir beverage (K4) during fermentation for 72 h

Notes: 1st stage fermentation conditions = 27°C for 48 h (—); 2nd stage fermentation conditions = 27°C for 24 h (---); Error bars = ± SD (n=4); experiments were replicated twice.

According to Pidoux (1989) and Koh et al. (2017), the increase in organic acids is the result of the metabolism of sugar by the LAB and yeast. The increase ($p<0.05$) in the organic acids from 0 to 24 h may be related to the increase ($p<0.05$) in the metabolic activities of viable cell counts of LAB and yeast (section 4.2.5). Corona et al. (2016) reported kefir beverage fermented with carrot juice which contained 4.81±0.65 g/L lactic acid and 1.90±0.71 g/L acetic acid after 48 h

fermentation. The results obtained from the present study were lower than that reported by Corona et al. (2016). Subardjo (2017) reported 0.23±0.03% (w/v) lactic acid and 0.09±0.00% (w/v) acetic acid in black tea-carrot water kefir beverage after 48 h fermentation. The variation in the concentrations of lactic acid and acetic acid may be attributed to differences in fermentation substrate, fermentation conditions and microbial composition of the kefir grains (Hsieh et al., 2012; Kök-Taş, Seydim, Özer, & Guzel-Seydim, 2013).

With the addition of jujube syrup at 48 h, the concentration of lactic acid (0.14±0.00% w/v) and acetic acid (0.37±0.02% w/v) reached peak levels at the end of second stage fermentation (72 h). Results showed that the final jujube water kefir beverage contained higher acetic acid than lactic acid (Figure 4.19). The acetic acid can be produced by AAB during kefir fermentation through oxidation of the alcohol or sugars (Guillamón & Mas, 2017). Hence, the presence of oxygen could lead to the proliferation of acetic acid bacteria (AAB) (Laureys et al., 2018). Moreover, ethanol is an important energy source for AAB (Laureys et al., 2018). As a result, the high ethanol concentration (section 4.3.1.1) may contribute to the growth of AAB and resulting in high acetic acid content.

4.3.1.4 Antioxidants

Previous studies have shown that jujube fruit contains variable amounts of phenolic compounds depending on the cultivar and growth conditions (Gao et al., 2013; Liu et al., 2017; San & Yildirim, 2010). In this study, only rutin was present at high concentration (2.39±0.01 μg/mL to 6.26±0.16 μg/mL) while the concentrations of gallic acid, catechin and epicatechin (EC) were all lower than 0.390625 μg/mL throughout the fermentation (data not shown). According to Li, Taylor, Ferruzzi, and Mauer (2012), the presence of lower amounts of catechins may be caused by accelerated degradation of catechins at 25-120°C. In this study, low concentrations of gallic acid, catechin and epicatechin may be attributed to the use of high temperature (70°C) during jujube syrup extraction. Rutin, a polyphenolic flavonoid, can protect the functional β-cells and prompt it to produce insulin (Kamalakkannan & Prince, 2006). Sattanathan, Dhanapal, Umarani, and Manavalan (2011) reported that supplementation of rutin could significantly lower the level of fasting blood sugar (FBS) and high-density lipoprotein cholesterol (HDL) in patients with diabetes mellitus.

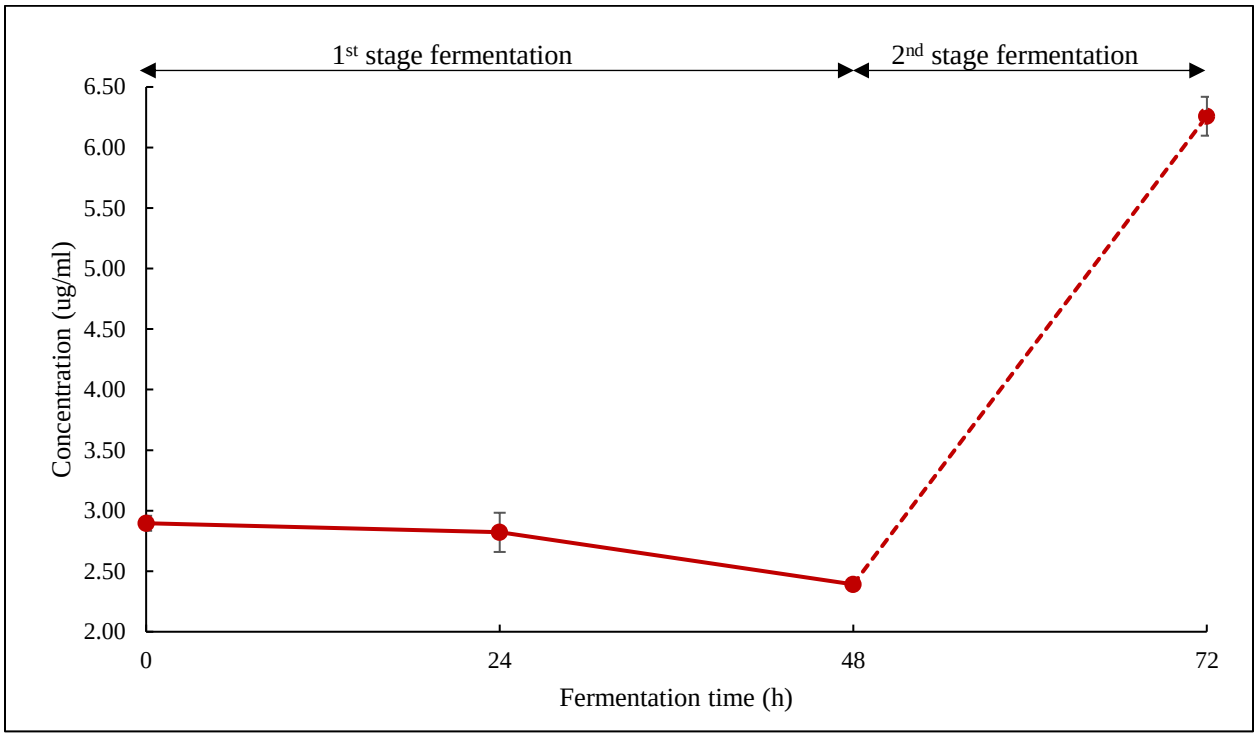

Figure 4.20 Mean concentration (µg/mL) of rutin in jujube water kefir beverage (K4) during fermentation for 72 h

Notes: 1st stage fermentation conditions = 27°C for 48 h (—); 2nd stage fermentation conditions = 27°C for 24 h (---); Error bars = ± SD (n=4); experiments were replicated twice.

The concentration of rutin in jujube water kefir beverage during fermentation is shown in Figure 4.20. The presence of high concentration of rutin in jujube fruit compared to other phenolic compounds has been previously reported (Hudina et al., 2008; San & Yildirim, 2010). At 0 h, the concentration of rutin was 2.90±0.06 µg/mL and then gradually decreased to 2.39±0.01 µg/mL by the end of the first stage fermentation (Appendix E). Amirdivani and Baba (2015) reported the degradation of phenolic compounds into smaller compounds during yoghurt fermentation. The degradation of the phenolic compounds can be related to the biotransformation of the phenolic compounds catalysed by the enzymes in LAB and yeast. Li et al. (2012) also reported that the low pH, which may be catalytic during the degradation of phenolic compounds can contribute to the loss of phenolic compounds.

After the addition of jujube syrup, the rutin content had increased ($p<0.05$) to 6.26±0.19 μg/mL at the end of fermentation (72 h). The significant increase in the rutin may be attributed to the high rutin content in the extracted jujube syrup, which confirms its high concentration in the jujube fruit (Choi et al., 2011; San & Yildirim, 2010).

4.3.1.5 Summary – phase III part I

This section summarises the ethanol content, sugar, organic acid, and antioxidant compositions of the beverage K4 during 72 h fermentation at 27°C.

Results in phase III part I showed that during the first stage fermentation (0-48 h), concentrations of the lactic acid and acetic acid increased while levels of sucrose, glucose, fructose, and rutin decreased. The ethanol content increased in the first 24 h and then decreased between 24-48 h. In the second stage fermentation (48-72 h), the concentrations of ethanol, sucrose, glucose and fructose increased ($p<0.05$). By the end of 72 h, the concentrations were 3.37±0.13% (v/v), 0.17±0.03% (w/v), 0.92±0.14% (w/v), and 1.43±0.08% (w/v), respectively. The beverage contained 6.26±0.16 μg/mL rutin and the concentrations of lactic acid and acetic acid were less than 0.5% (w/v).

4.3.2 Part II: Stability of final jujube water kefir beverage formulation during storage at 4°C

The purpose of part II was to investigate the stability of microbiological and physicochemical properties of the beverage K4 during storage at 4°C for 21 d.

4.3.2.1 Total soluble solids (°Brix)

The total soluble solids in jujube water kefir beverage during storage (4°C) for 21 days is shown in Figure 4.21. TSS decreased significantly ($p<0.05$) from 3.78±0.10 °Brix to 3.13±0.01 °Brix, suggesting that the LAB and yeast were still active to metabolise the residual sugar in the beverage during 4°C storage. The decrease in TSS is supported by the presence of LAB and yeast (section 4.3.2.4). However, the rate of reduction of the TSS was lower compared to the concentration of the TSS obtained during fermentation at 27°C (section 4.2.2). The metabolic rates of LAB and yeast are reduced at low temperature (Puerari et al., 2012).

A marked reduction in lactose levels in milk kefir after 28-d refrigerated storage was reported by Leite et al. (2013). A decrease in pyruvate was also reported by Güzel-Seydim, Seydim, and Greene et al. (2000) which indicated the metabolism of carbohydrate. In contrast, Irigoyen, Arana, Castiella, Torre, and Ibanez (2005) reported a stable lactose level in milk kefir during storage of the beverage for 14 days. The fermentation media is important for the microbial ecological profiles of water kefir and any changes in the microbial ecology may influence the growth of the grains which impact on the composition of the beverage (Beshkova et al., 2003; Hsieh et al., 2012; Irigoyen, Ortigosa, Torre, & Ibanez, 2003; Öner et al., 2010). Thus, the differences between the results from the present study and the previous studies in terms of the profile of TSS during storage may be attributed to variable fermentation substrates (Hsieh et al., 2012).

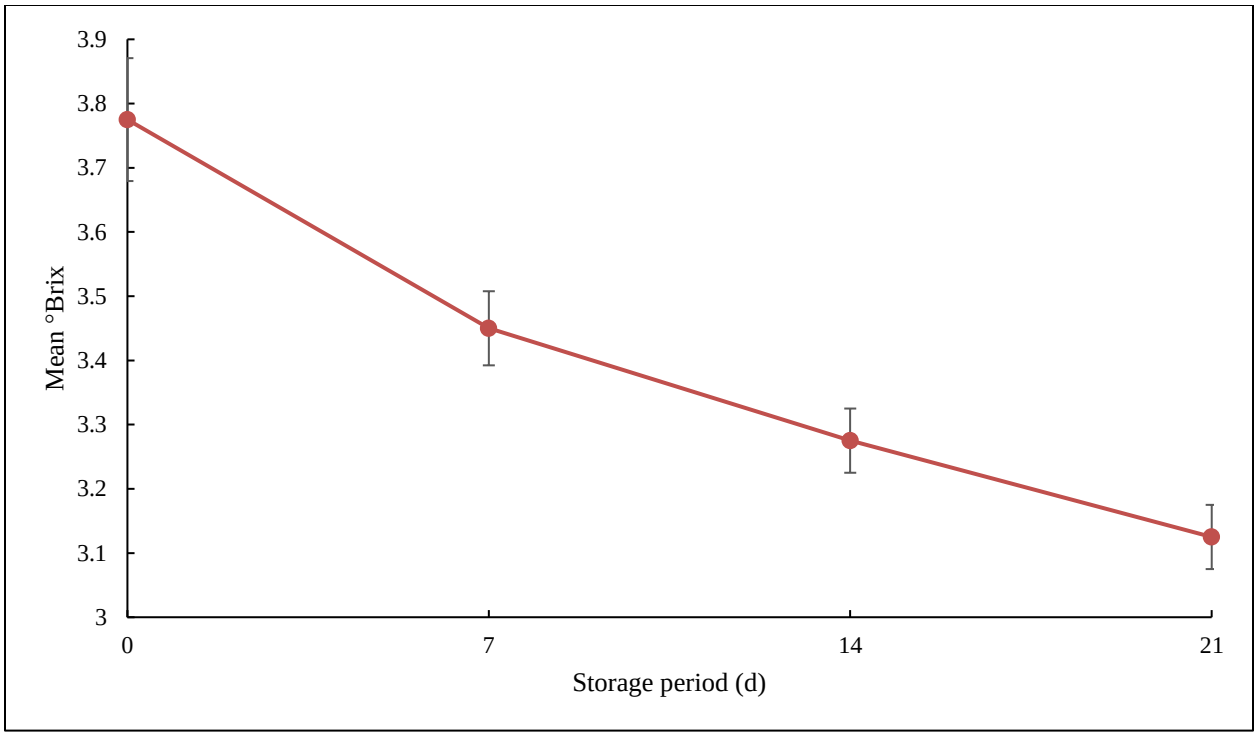

Figure 4.21 Mean total soluble solids (°Brix) of jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.2 pH and acidity

Figure 4.22 shows the pH and T.A. of jujube water kefir beverage during storage for 21 d (4°C). Compared to 0 d (pH 3.69±0.01), the pH decreased ($p<0.05$) to 3.65±0.01 at 21 d while T.A. increased from 0.97±0.01% to 0.99±0.01% by the end of storage (Appendix D).

The reduction in pH and increase in T.A. suggested post-metabolism of sugar to organic acids by the viable LAB and yeast during refrigerated storage. The increased acidity is consistent with the decrease in TSS as discussed previously (section 4.3.2.1). Similarly to TSS, the rate of acid production during storage was lower than the acidification during fermentation at 27°C (4.2.3). According to Irigoyen et al. (2003), the rate of acid production and the total capacity of acidification are clearly affected by the fermentation temperature.

A study by Cais-Sokolińska, Danków, and Pikul (2008) reported a progressive decrease of pH of beverage from 4.59 to 4.37 during storage for 21 d (6±1°C). Leite et al. (2013) reported similar pH results of a Brazilian kefir beverage, of which the pH progressively decreased ($p<0.05$) from 4.75±0.01 to 4.31±0.00 during storage at 4°C. The results of pH obtained in the present study are much lower than the levels reported by Leite et al. (2013). This may be attributed to the lower initial pH (0 h) of the jujube water kefir beverage K4 (4.96±0.01) compared to the Brazilian kefir beverage (6.55±0.01) reported by Leite et al. (2013). However, the results on pH are in agreement with Yaman, Elmalı, and Kamber (2010) who reported variable reductions in the pH of kefir beverages made with bovine milk, ewe milk and goat milk during cold storage for 7 d. Contrary to our findings and similar previous studies, Güzel-Seydim, Seydim, and Greene (2000) reported a non-significant ($p>0.05$) changes in the pH of kefir during 21-d cold storage. Again, the discrepancy may be ascribed to different fermentation conditions and fermentation substrates which can affect the physicochemical properties of the beverage (Irigoyen et al., 2003; Yaman et al., 2010).

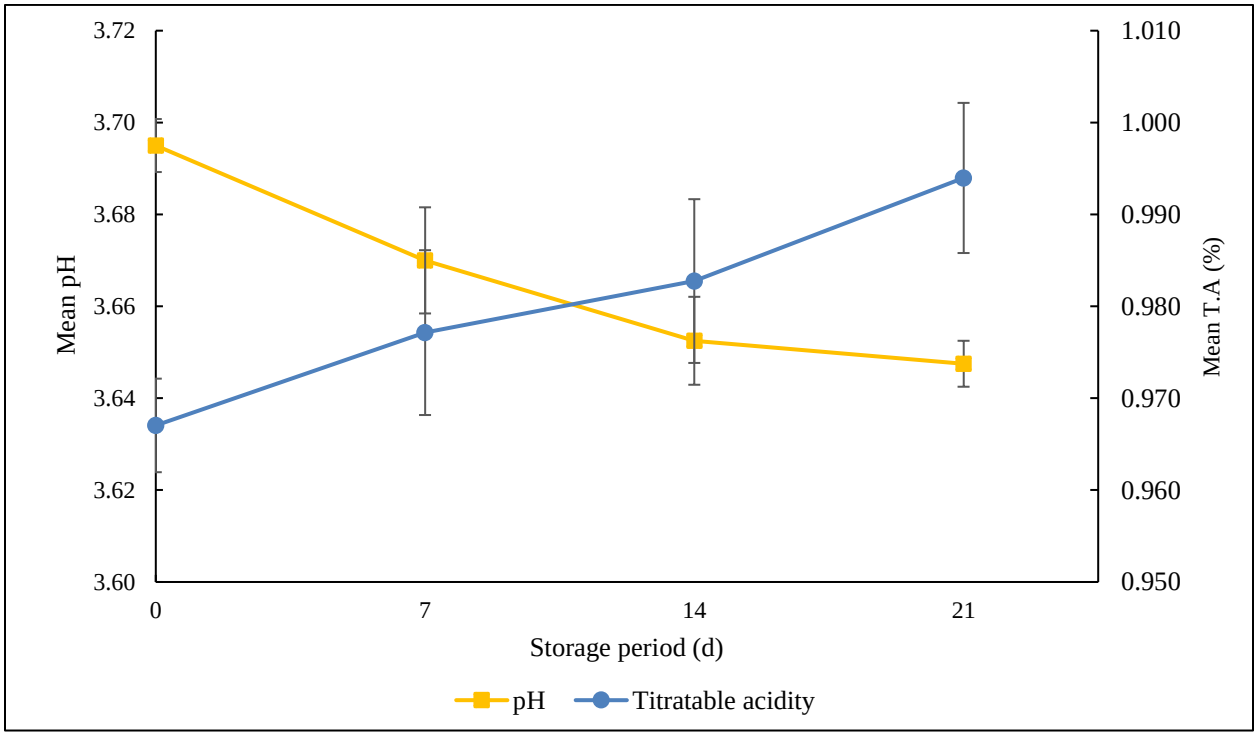

Figure 4.22 Mean pH and titratable acidity (%) of jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.3 Colour

The measurement of the colour of jujube water kefir beverage during storage (4°C) is important since colour impacts on the acceptance and likeness of the products by consumers (Pathare et al., 2013). Figure 4.23 shows colour changes of the jujube water kefir beverage during storage. The L* value (lightness) rapidly decreased ($p<0.05$) from 45.94±0.07 to 43.24±0.39 in the first 7 d and then gradually decreased to 41.13±0.18 by the end of storage (4°C) (Appendix D). The a* value (redness-greenness) also decreased from 3.61±0.02 to 2.61±0.10 during 21 d. A significant increase ($p<0.05$) in the b* value (yellowness-blueness) was observed from 0 d to 7 d, reaching a maximum of 14.12±0.09 at the end of the storage period (21 d). Fiorda, Pereira, Thomaz-Soccol, Rakshit, et al. (2016) reported a decrease in the a* value of the water kefir beverage after 21-d cold storage. Subardjo (2017) also reported a decrease in L* and a* values of the black tea-carrot juice water kefir beverage during cold-storage.

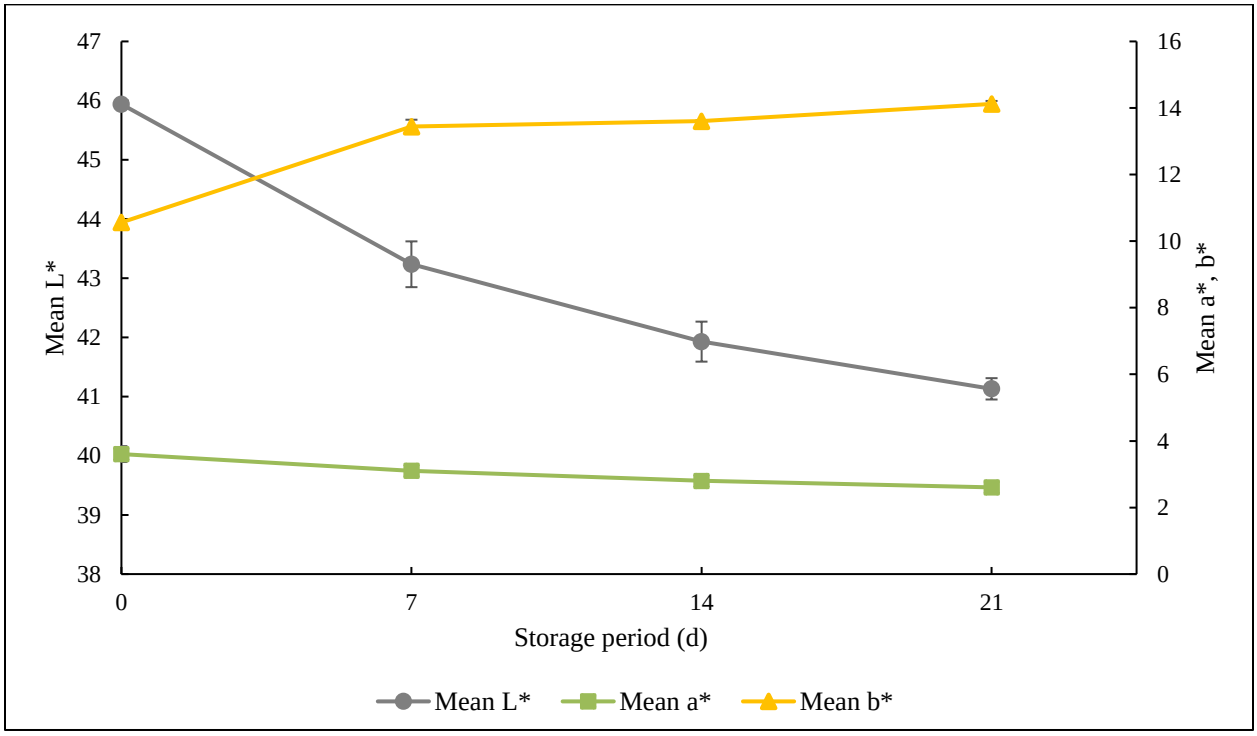

Figure 4.23 Colour of jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.4 Microbiological analysis of beverage

Figure 4.24 shows the viable cell counts of the LAB and *S. cerevisiae* in jujube water kefir beverage during 21-d storage (4°C). At 0 d, the viable cells of LAB and *S. cerevisiae* were 7.57±0.03 log cfu/mL and 6.57±0.08 log cfu/mL, respectively. The viable cells of LAB gradually decreased ($p<0.05$) to 6.56±0.03 log cfu/mL after 21-d storage while for *S. cerevisiae*, the cells markedly decreased by about 4.15 log cfu/mL and reached 2.42±0.44 log cfu/mL at the end of storage (Appendix D).

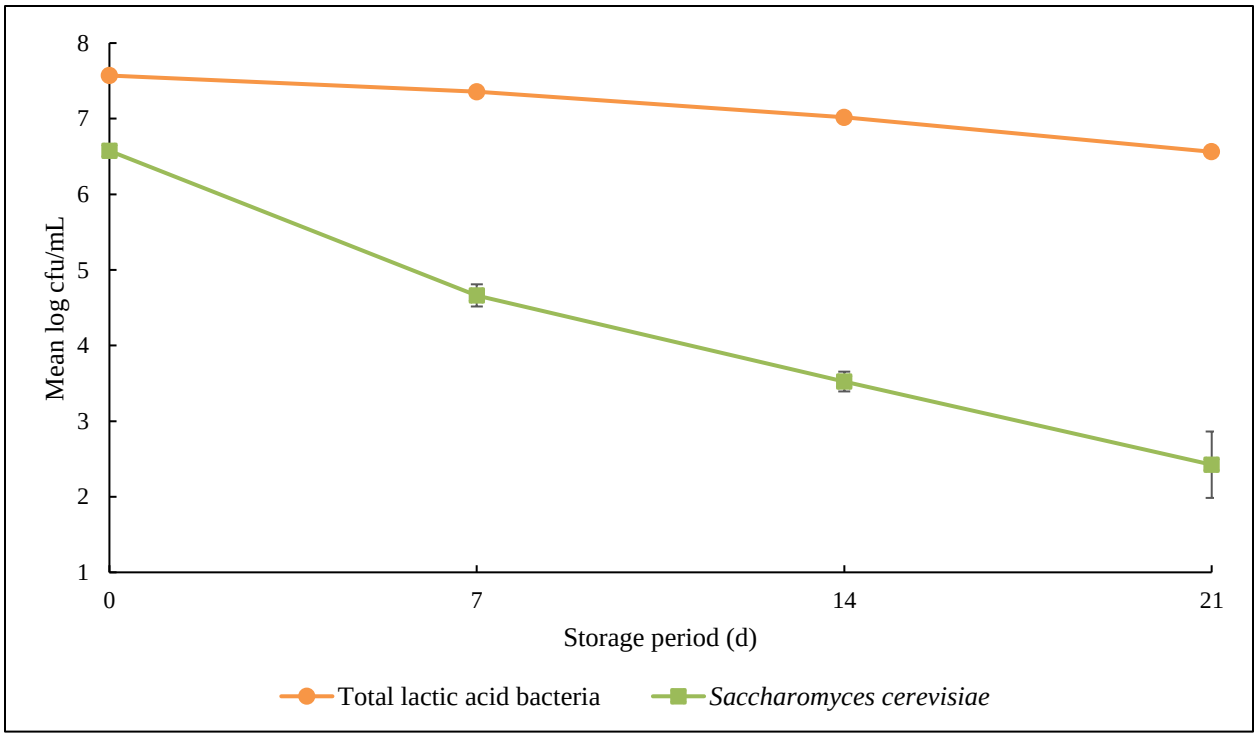

Figure 4.24 Mean viable cell counts (log cfu/mL) of microorganisms in jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

According to Koh et al. (2017), the accumulation of metabolites such as lactic acid may inhibit the growth and survival of some microorganisms during storage. Further, the decrease in the viable cell counts may be also due to the depleted nutrients and the low temperature of storage (Leroi &

Pidoux, 1993a; Puerari et al., 2012). Storage conditions were presumed to affect the microflora and the overall quality of the beverage (Sarkar, 2008). The results obtained from the present study are in agreement with the study by Grønnevik et al. (2011) and Irigoyen et al. (2005) who reported high decreases of the LAB during cold-storage of milk kefir beverages for four weeks. In contrast, Irigoyen et al. (2005) reported stable counts of yeasts and AAB in milk kefir while Grønnevik et al. (2011) observed increased yeast counts in Norwegian kefir after three weeks of cold storage. Meanwhile, Öner et al. (2010) and Leite et al. (2013) reported stable counts of *Lactobacillus* spp. and yeast in kefir. As previously mentioned, the discrepancies in the viable cells counts obtained from the present research and other studies may be attributed to the differences in the storage and fermentation conditions including the type of substrate and the microbial ecology (Hsieh et al., 2012; Öner et al., 2010).

In this study, the viable cell counts of LAB and *S. cerevisiae* were much lower (6.56±0.03 log cfu/mL LAB and 2.42±0.44 log cfu/mL *S. cerevisiae*) than the study by Leite et al. (2013) who reported around 6 log units of yeast and 10 log units of LAB in milk kefir during cold-storage. The presence of a high microbial population in milk kefir may be attributed to nutrient-rich of milk compared to the sugared jujube solution (Zanirati et al., 2015).

The high concentration of LAB is desirable for the water kefir beverage as some strains of LAB isolated from water kefir grains have exhibited probiotic activity (Gulitz et al., 2011; Zanirati et al., 2015). Also, kefir yeasts have been reported to possess potential probiotic features (Diosma et al., 2014). According to FAO/WHO (2002) and Kechagia et al. (2013), a probiotic product should contain at least 10^6 colony forming per unit (CFU) at the time of consumption. The jujube water kefir beverage produced in this study may be a potential probiotic beverage due to the presence of high and stable viable cells of LAB (10^6/mL) during the storage.

4.3.2.5 Ethanol content

The concentration of ethanol in jujube water kefir beverage during storage is shown in Figure 4.25. At the beginning of storage (0 d), the concentration of ethanol in jujube water kefir beverage was 3.52±0.33% (v/v) (Appendix E). From 0 d to 14 d, the ethanol content increased ($p<0.05$) to 4.41±0.21% (v/v), and stabilised from 14 d to 21 d ($p>0.05$). A similar result was reported by Koh et al. (2018) in which ethanol increased (0.2% to 0.8% v/v) in the pumpkin fruit puree fermented with kefir *Lactobacillus*. Kök-Taş et al. (2013) also reported a large increase in ethanol concentration of kefir beverage during 21-d cold storage. The increases in ethanol content and acidity (section 4.3.2.2) are consistent with the decrease in TSS (section 4.3.2.1). According to Koh et al. (2018), increased ethanol content may be attributed to the metabolism of the yeast and the heterofermentative LAB which fermented the residual sugar in the jujube water kefir beverage into ethanol and organic acids during storage. This result is in agreement with the high viable cell counts of the kefir microorganisms of this study reported in section 4.3.2.4.

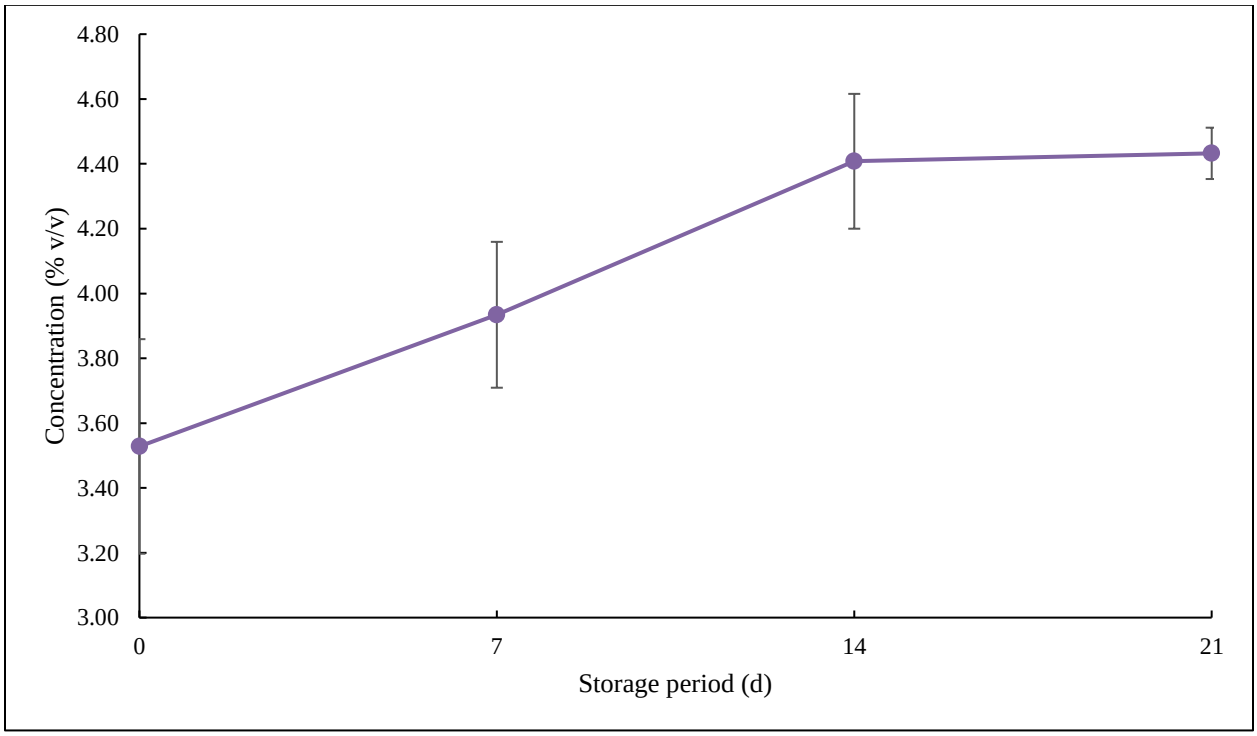

Figure 4.25 Mean concentration (%, v/v) of ethanol in jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.6 Sugars

During the storage period (4°C) of fermented jujube kefir beverage, the concentration of the three sugars decreased with different magnitudes (Figure 4.26). At 0 d, the concentration of sucrose, glucose, and fructose were 0.09±0.01% (w/v), 0.42±0.03% (w/v), and 1.44±0.08% (w/v), respectively (Appendix F). At 21 d, the concentrations of the sugars decreased to 0.00±0.00% (w/v), 0.03±0.00% (w/v), and 0.63±0.02% (w/v), respectively (Appendix F). The decrease in the sugar content suggested metabolic activities of viable cells in the jujube water kefir during storage, which was consistent with increased acidity and ethanol and the decreased TSS. Similar results were reported by Grønnevik et al. (2011) with decreases in glucose concentration in milk kefir beverage during cold storage. In the present study, glucose concentration was lower than fructose concentration throughout the storage period, which confirmed the preference of active yeast to ferment glucose than fructose (Berthels et al., 2004).

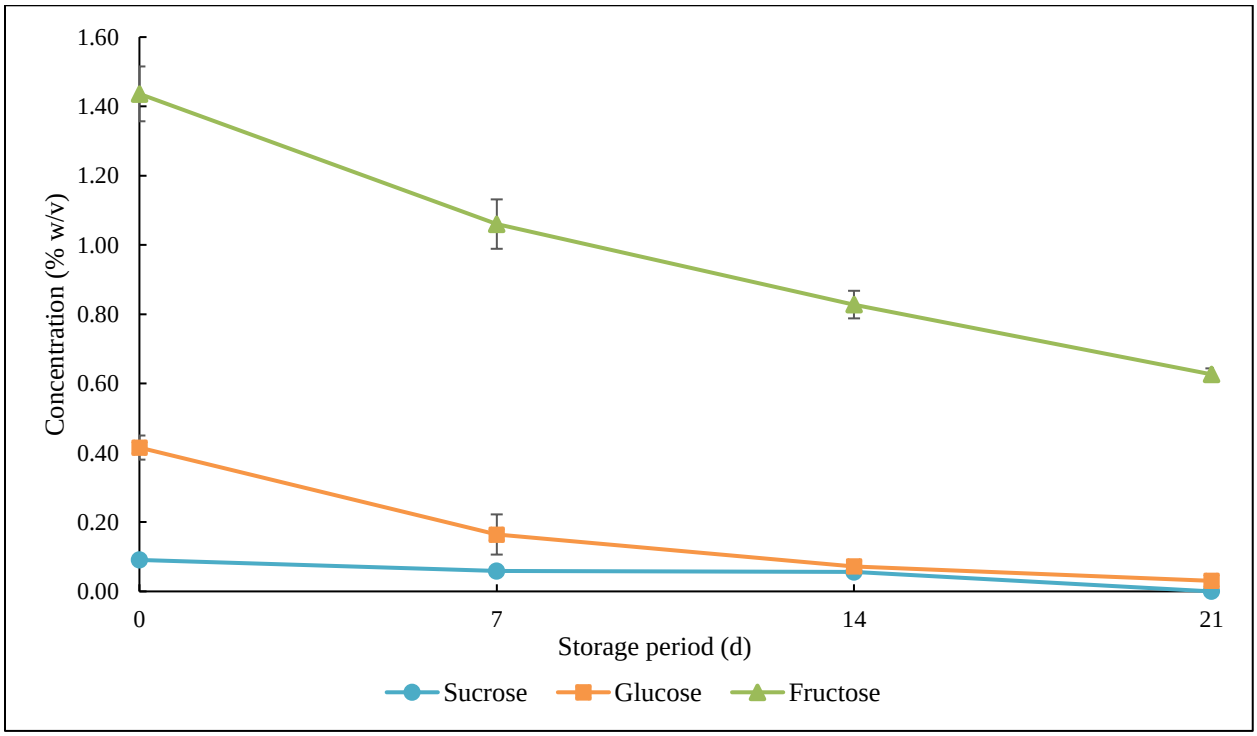

Figure 4.26 Mean concentration (%, w/v) of sugars in jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.7 Organic acids

Figure 4.27 shows the concentration of organic acids in jujube water kefir beverage during cold-storage at 4°C. The acetic acid content remained stable ($p>0.05$) while the concentration of lactic acid increased ($p<0.05$) from 0 d to 14 d and then stabilised after 14 d. At the end of the storage period (21 d), the concentrations of lactic acid and acetic acid were 0.22±0.01% (w/v) and 0.24±0.00% (w/v), respectively (Appendix F). The increase in the lactic acid may be attributed to the sugar metabolism by LAB (Khandelwal et al., 2016; Montet et al., 2014). This is in agreement with Leite et al. (2013) who reported increased lactic acid in Brazilian kefir beverage during 28-d cold storage. However, results are not agreement with Subardjo (2017) who reported decreased lactic acid and acetic acid concentrations in black tea-carrot juice kefir beverage during 28-d cold storage. The discrepancy in the organic acid concentration obtained in this study and previous work may be attributed to the different composition of microorganisms in kefir grains and fermentation medium (Hsieh et al., 2012).

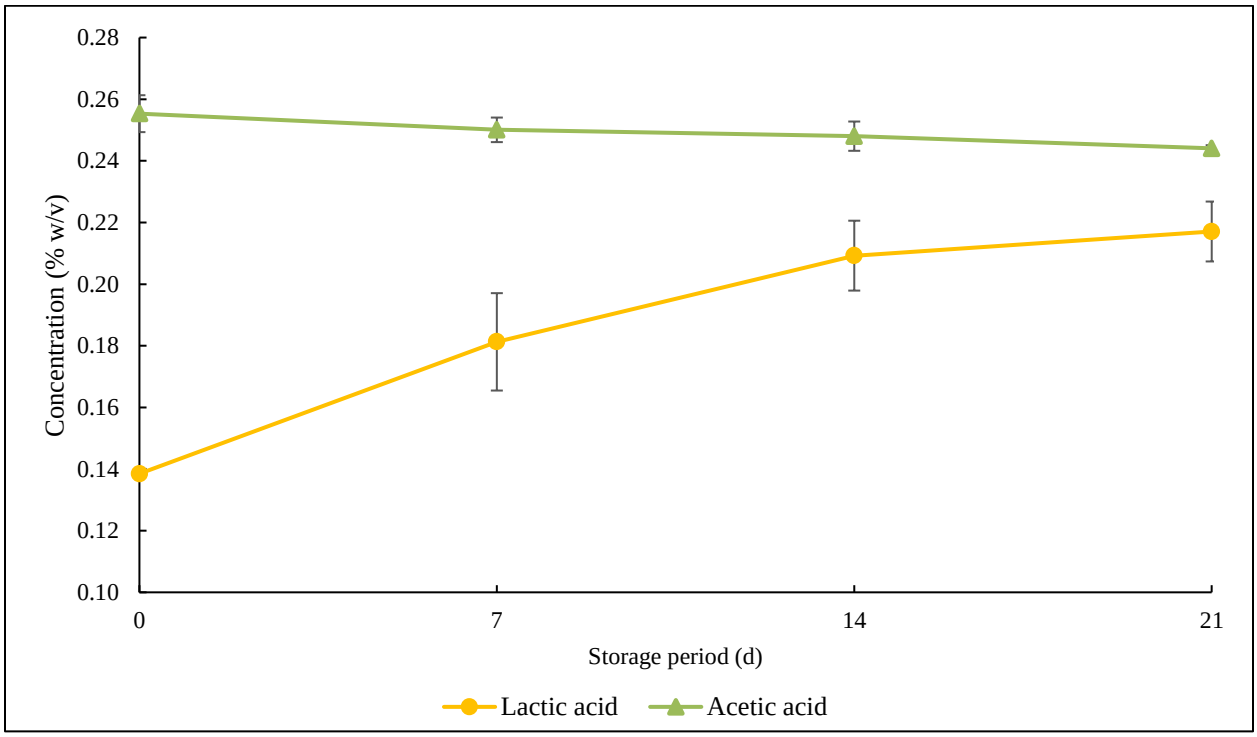

Figure 4.27 Mean concentration (%, w/v) of organic acids in jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.8 Antioxidants

Of the analysed antioxidants, the concentrations of gallic acid, catechin, and epicatechin were lower than 0.390625 µg/mL (data not shown) in the jujube water kefir beverage during storage, while a relatively large amount of rutin (Figure 4.28) was observed.

From 0 d to 21 d, the concentration of rutin increased ($p<0.05$) from 7.15±0.28 µg/mL to 11.06±0.14 µg/mL (Appendix F). The result is contrary to the study by Recamales, Sayago, González-Miret, and Hernanz (2006) who reported a decrease of the antioxidant in white wine during storage. As previously discussed, the general factors affecting fermentation may be attributed to the different fermentation substrates (Hsieh et al., 2012; Öner et al., 2010). Also, phenolic compounds can be hydrolysed and oxidised by light and high temperature thereby leading to their degradation (Cheynier, Rigaud, Souquet, Duprat, & Moutounet, 1990; Zafrilla et al., 2003).

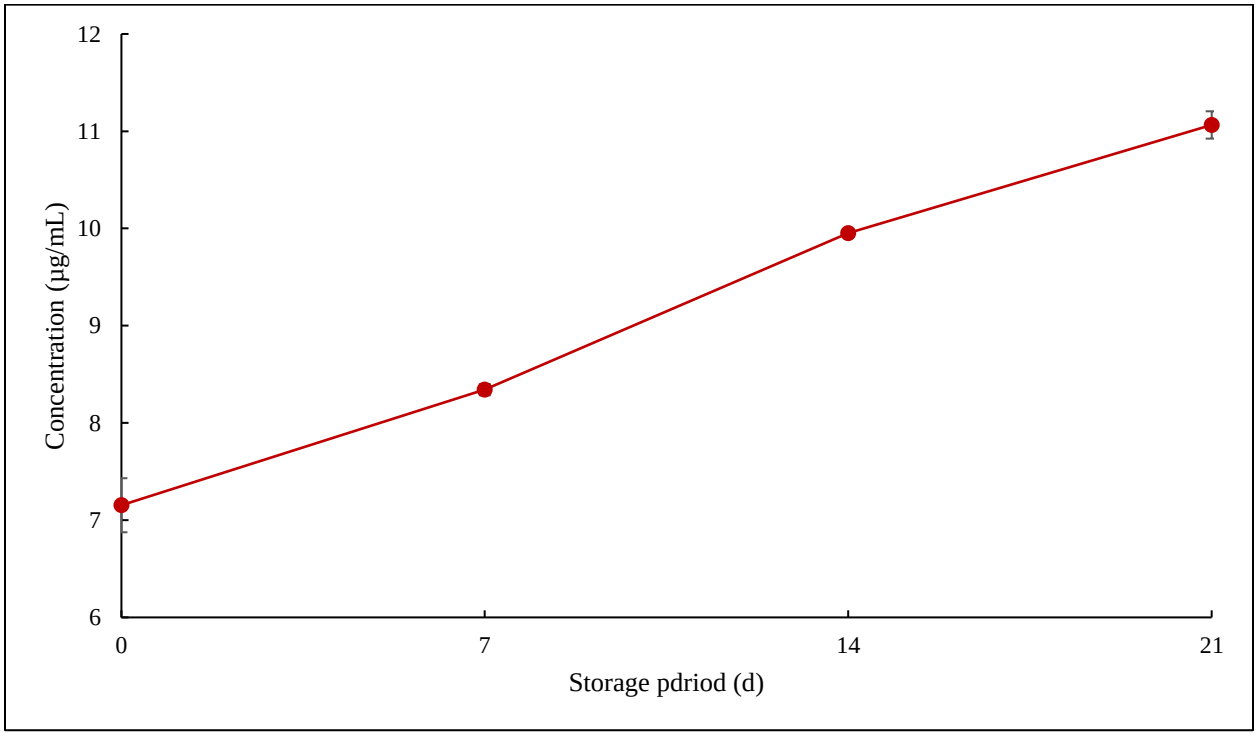

Figure 4.28 Mean concentration (µg/ml) of rutin in jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=4); experiments were replicated twice; d = day.

4.3.2.9 Sensory evaluation

Fermented jujube beverage was evaluated by consumer panellists during storage (Figure 4.29). Mean scores for overall acceptability and other sensory attributes (odour, flavour, sourness, and sweetness) steadily decreased from 0 d to 21 d. Meanwhile, the scores for appearance decreased from 7 d to 21 d. However, no differences ($p>0.05$) of the mean scores of the sensory attributes were observed during cold-storage of jujube beverage with the exception of flavour which decreased significantly ($p<0.05$) from 14 d to 21 d. Kilic, Uysal, Akbulut, Kavas, and Kesenkas (1999) reported significant decreases in the scores of all sensory attributes of kefir over a 5-d storage period. Decreasing sensory scores of overall acceptability of milk kefir during cold-storage for seven days have also been reported by Kök-Taş et al. (2013). The inconsistencies of the results discussed here may due to the differences in the formulations and microbial ecology (Hsieh et al., 2012). Overall, the results of consumer sensory evaluation suggested that the jujube water kefir beverage could be stored at 4°C for up to 3 weeks without significant changes in sensory characteristics.

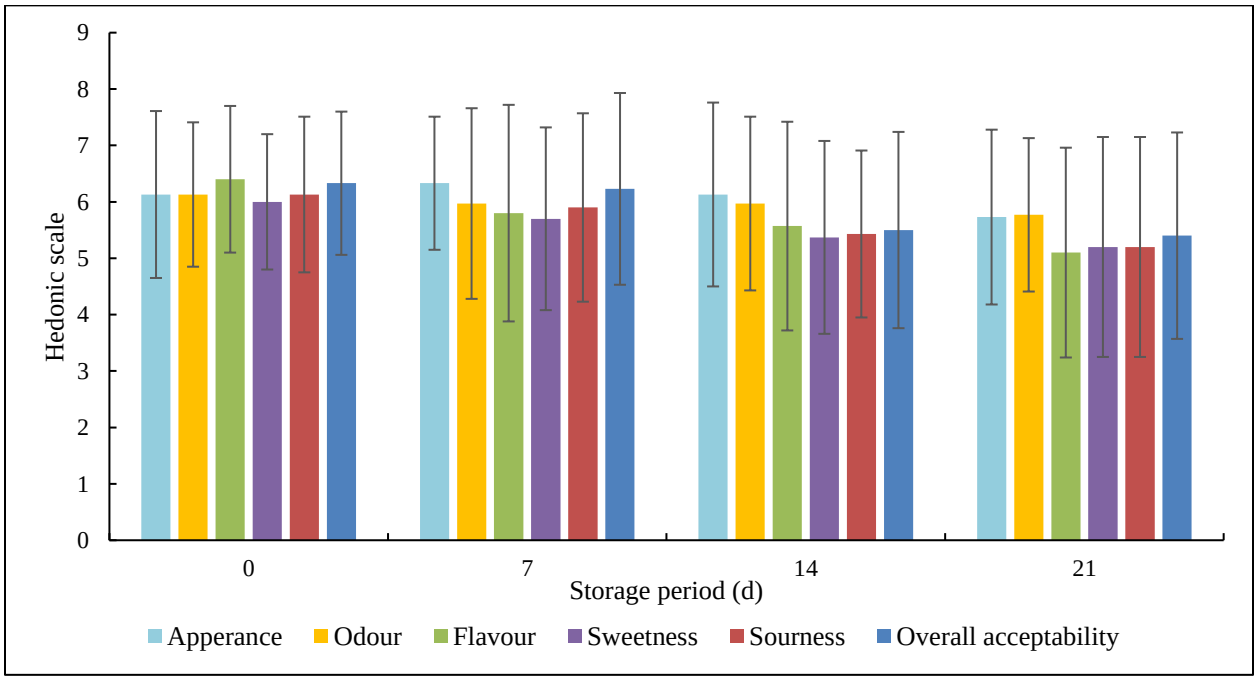

Figure 4.29 Mean consumer sensory evaluation scores of jujube water kefir beverage (K4) during storage (4°C)

Notes: Error bars = ± SD (n=30); d = day.

4.3.2.10 Summary – phase III part II

This section summarises the microbiological and physicochemical properties of the beverage K4 during storage at 4°C for 21 d.

Results in phase II (part II) showed that during the storage (4°C) of jujube water kefir beverage (K4), the mean total soluble solids, pH, viable cell counts, and sugar concentrations decreased while ethanol and rutin levels increased. The colour of the beverage were variable while the concentration of acetic acids was stable during the storage period. The results of the sensory evaluation suggested that the fermented jujube water kefir beverage was stable during storage for three weeks at 4°C.

Chapter 5. OVERALL CONCLUSIONS

Syrup extracted from *Ziziphus jujuba* Mill. (jujube) can be used as a fermentation substrate to develop water kefir beverage. For the extraction of jujube syrup, the water-bath method was the most efficient which consisted of 650 mL extraction water in the mixture at an extraction temperature of 70°C. The most promising jujube kefir beverage was fermented for 72 h at 27°C. The fermented beverage was well-liked by consumer sensory panellists, and it contained an appreciable amount of rutin which may be beneficial to consumer health. During storage of the beverage for three weeks at 4°C, the mean total soluble solids, pH, viable cell counts of LAB and *S. cerevisiae*, and sugars decreased while the levels of lactic acid, ethanol and rutin increased. The mean consumer sensory scores remained stable during storage with a slight decrease in overall acceptability.

Chapter 6. RECOMMENDATIONS

In the present study, the composition of dried jujube fruit and extracted syrup was not analysed. Information on the chemical composition of two materials would be useful for investigating the efficiency of the extraction method and the consistency of the extracted syrup. Moreover, the study on the nutritional composition of the extracted syrup would be worthwhile for developing the jujube water kefir beverage.

The yield of the syrup extracted in the current study was lower compared to the microwave-assisted processing and ultrasonic treatment (El-Nagga & El–Tawab, 2012; Li, Ding, et al., 2007). Further studies on jujube water kefir beverage using these two methods for syrup extraction.

Two levels of fermentation temperatures and jujube syrup concentrations were investigated for the optimum fermentation condition of the jujube water kefir beverage. Additional levels of fermentation temperatures and syrup concentrations may be considered in future studies of kefir beverage.

The mean viable cell counts of *S. cerevisiae* in the jujube water kefir beverage were low during storage at 4°C. Prebiotics may be added to support the survival of microorganisms (Bansal, Mangal, Sharma, & Gupta, 2016).

In this study, the profile of microflora of the water kefir grain was not identified. The microbial community of water kefir grains is complex, which may contain strains with probiotic characteristics (Koh et al., 2018; Leite et al., 2015; Stadie et al., 2013). Identification of the probiotic strains present in jujube water kefir beverage is recommended in future research. In the present study, rutin was present in an appreciable level. Therefore, it would be desirable to investigate the effect of the antioxidant in human health such as anti-inflammation. Such studies could commence with *in vitro* experiments.

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APPENDIX

A. Composition of agar media for microbiological analysis

Table A.1 List of ingredients of agar media

Product	Ingredients	Composition (g/L)
MRS agar (CM0361) Oxoid	Peptone	10.0
	'Lab-Lemco' Powder	8.0
	Yeast extract	4.0
	Glucose	20.0
	Di-potassium hydrogen phosphate	2.0
	Sodium acetate $3H_20$	5.0
	Tri-ammonium citrate	2.0
	Magnesium sulphate $7H_20$	0.2
	Manganese sulphate $4H_20$	0.05
	Agar	10.0
	Sorbitan mono-oleate	1.0 (mL)
YGC agar (1.16000.0500) Merck KGaA	Yeast extract	5.0
	D(+) glucose	20.0
	Chloramphenicol	0.1
	Agar	14.9

B. Gram-staining of water kefir grains starter culture

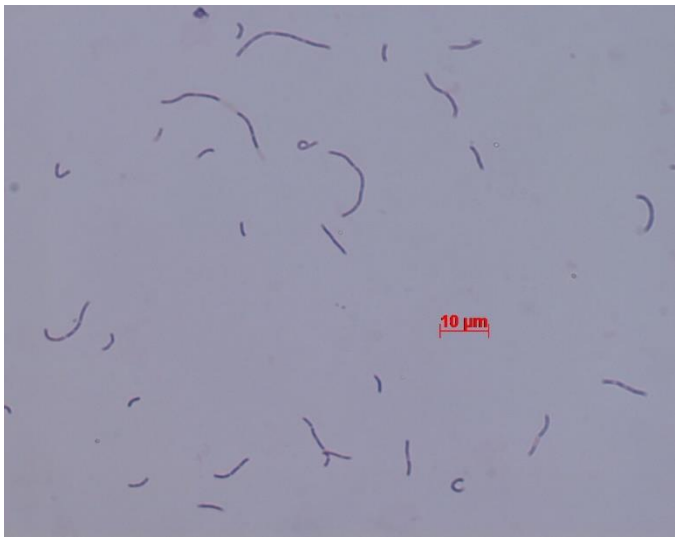


Figure B.1 Gram staining of LAB grown on MRS agar under oil immersion (100× magnifications) using Carl Zeis (model HBO 50/AC, Germany) transmission light microscope

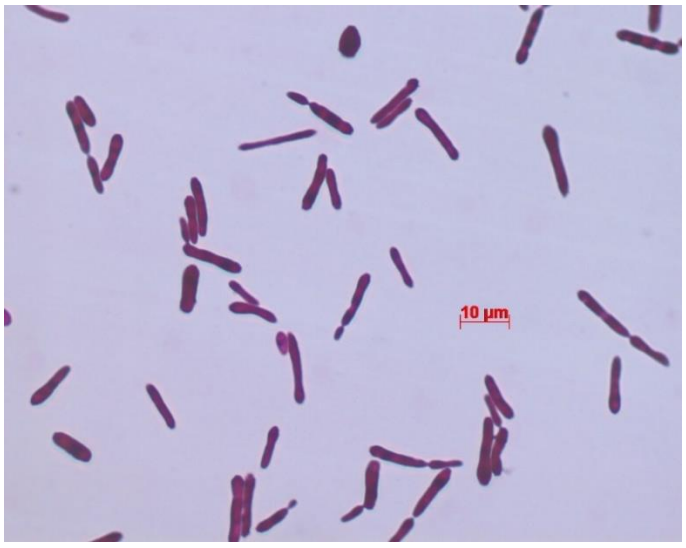


Figure B.2 Gram staining of yeast grown on YGC agar under oil immersion (100× magnifications) using Carl Zeis (model HBO 50/AC, Germany) transmission light microscope

C. Sensory evaluation questionaire

INFORMATION SHEET

Project Title: Kefir fermentation of *Ziziphus Jujuba* Mill. Beverage

Introduction

I am Xinyi Mu, a Master of Food Technology student at School of Food and Nutrition (SFN), Albany, Massey University. My research project is to develop a water kefir beverage using sugar and *Ziziphus Jujuba Mill.* (jujube) syrup. This study is part of my project and may contribute to the development of jujube water kefir beverage. You are therefore invited to take part in a study that evaluates the sensory characteristics of jujube water kefir beverage. The objective of this sensory evaluation is to evaluate the level of acceptance of jujube water kefir beverage by potential consumers.

Participant involvement

This study involves tasting and evaluating one jujube water kefir beverage, it may take 5 minutes. The jujube water kefir beverage that you will taste may contain all or some of following ingredients: **jujube (red dates) syrup, commercial starter cultures (water kefir grains), traces of alcohol** and **organic raw sugar**.

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

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

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

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

Ethics Notification 4000018836

Project Contacts

- Xinyi Mu (Researcher) – xinyi.mu@gmail.com
- Dr. Tony Mutukumira (Supervisor) – a.n.mutukumira@massey.ac.nz
- Dr. Emilia Nowak (Co-Supervisor) – e.nowak@massey.ac.nz

This project has been evaluated by peer review and judged to be low risk (Application No. 4000018836). 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 want to raise with someone other than the researcher(s), please contact Dr. Brian Finch, Director (Research Ethics), email humanethics@massey.ac.nz.

PARTICIPANT CONSENT FORM

Kefir fermentation of *Ziziphus Jujuba* Mill. Beverage

- 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, and I understand that I may ask further questions at any time.
- I understand that I have the right to withdraw from the study at any time and decline my answers.
- I agree to voluntarily participate in this study under the condition set out the Information Sheet.
- I have discussed and advised the researcher of any potentially relevant cultural, religious or ethical beliefs that may prevent me from consuming this product under consideration.

Signature: _____ **Date:** _____

Full Name (Printed): _____

Ethics Notification 4000018836

SENSORY ACCEPTANCE TEST

You will be given two 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 are welcome to provide additional comments regarding the sample. You may taste the sample more than once. Please rinse your mouth with water between samples.

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

Name: _____

Product: Jujube water kefir beverage **Sample Code:** _____

Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance/ Color	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									
Sweetness	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									
Sourness	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									
Overall acceptability	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments: _____									

D. Data analysis

Table D.1 Characteristics of jujube syrup extracted with different combinations of extraction method (raw data of phase I stage I)

Combinations of extraction method	Replicates	TSS (°Brix)	Volume before evaporation (mL liquid/300 g DJ)	Volume of final syrup	Volume of final syrup (mL/100 g DJ)	Mass (g)	Mass (g/100 g DJ)	Density (g/mL)
NR/E	1	39.7	235	75.0 (mL/100 mL liquid)	58.78	86.71	67.96	1.1561
	1	39.8						
	2	39.7	232	68.0 (mL/100 mL liquid)	52.69	78.58	60.88	1.1556
	2	39.8						
NR/NE	1	22.6	-	217 (mL/300 g DJ)	72.33	233.96	77.99	1.0782
	1	22.6						
	2	22.7	-	215 (mL/300 g DJ)	71.67	231.90	77.30	1.0786
	2	22.7						
R/NE	1	22.6	-	60.0 (mL/300 g DJ)	20.00	64.68	21.56	1.0780
	1	22.6						
	2	22.7	-	57.0 (mL/300 g DJ)	19.00	61.45	20.48	1.0781
	2	22.6						

Notes: NR/E = non-rehydrated, evaporated, NR/NE = non-rehydrated, non-evaporated, R/NE = rehydrated, non-evaporated. Liquid = syrup before evaporation.

Table D.2 Characteristics of jujube syrup extracted with different extraction conditions (raw data of phase I stage II)

Extraction conditions	Replicates	TSS (°Brix)	Volume (mL)	Volume (mL/100 g DJ)	Mass (g)	Mass (g/100 g DJ)	Density (g/mL)
600/70	1	22.6	220	73.33	237.16	79.05	1.0780
	1	22.6					
	2	22.6	225	75.00	242.66	80.88	1.0785
	2	22.7					
600/75	1	22.7	218	72.67	235.29	78.43	1.0793
	1	22.7					
	2	22.8	220	73.33	237.57	79.19	1.0799
	2	22.8					
650/70	1	22.0	316	105.33	338.65	112.88	1.0717
	1	21.9					
	2	21.9	313	104.33	335.45	111.82	1.0717
	2	22.0					
650/75	1	22.0	312	104.00	334.38	111.46	1.0717
	1	21.9					
	2	22.0	308	102.67	330.47	110.16	1.0730
	2	22.1					

Notes: 600/70 = 600 mL/70°C, 600/75 = 600 mL/75°C, 650/70 = 650 mL/70°C, 650/75 = 650 mL/75°C.

Table D.3 Microbial profile analysis of water kefir grains (starter culture) (raw data of phase II)

Parameter	Trial 1		Trial 2		Mean±SD
	Measurement 1 log cfu/g	Measurement 2 log cfu/g	Measurement 1 log cfu/g	Measurement 2 log cfu/g	
LAB on MRS agar	7.13	7.11	7.25	7.21	7.18±0.06
S. cerevisiae on YGC agar	6.16	6.23	6.32	6.32	6.26±0.08

Table D.4 Characteristic of extracted jujube syrup for jujube water kefir fermentation (raw data of phase II)

Parameter	Trial 1		Trial 2		Mean±SD
	Measurement 1	Measurement 2	Measurement 1	Measurement 2	
Colour L*	41.62	41.20	41.20	41.97	41.50±0.37
Colour a*	4.07	4.24	4.82	4.87	4.50±0.40
Colour b*	6.24	6.49	7.45	7.51	6.92±0.65
TSS (°Brix)	21.80	21.90	21.90	22.00	21.90±0.08

Table D.5 Characteristics of jujube water kefir beverages during fermentation for 72 h (raw data of phase II)

Sample code	Fermentation time	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	*S. cerevisae* log cfu/mL
K1	1	1	4.86	4.0	0.036	68.86	3.02	17.62	6.70	5.96
K1	1	1	4.87	4.0	0.036	68.86	3.02	17.63	6.65	5.96
K1	1	2	4.85	4.0	0.036	68.72	3.06	17.61	6.65	5.88
K1	1	2	4.84	4.0	0.038	68.73	3.06	17.34	6.62	5.86
K1	2	1	3.60	1.8	0.315	71.11	2.70	16.28	7.20	6.15
K1	2	1	3.60	1.8	0.324	71.16	2.71	16.27	7.08	6.15
K1	2	2	3.60	1.8	0.305	70.63	2.82	16.10	7.20	6.20
K1	2	2	3.60	1.9	0.320	70.65	2.81	16.15	7.00	6.43
K1	3	1	3.39	1.4	0.684	74.48	2.70	15.22	7.20	4.91
K1	3	1	3.39	1.4	0.683	74.48	2.72	15.25	7.00	4.96
K1	3	2	3.39	1.4	0.674	73.89	2.76	15.49	6.90	5.11
K1	3	2	3.40	1.4	0.678	73.89	2.76	15.49	6.90	5.08
K1	4	1	3.42	2.5	0.977	50.55	3.20	11.28	7.60	6.30
K1	4	1	3.42	2.6	0.974	50.55	3.20	11.26	7.76	6.52
K1	4	2	3.45	2.5	0.972	53.83	3.28	11.48	7.60	6.00
K1	4	2	3.43	2.5	0.986	53.51	3.28	11.49	7.68	6.08

Sample code	Fermentation time	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	*S. cerevisae* log cfu/mL
K2	1	1	4.86	4.0	0.036	68.49	3.10	16.96	6.62	5.97
K2	1	1	4.84	4.0	0.036	68.49	3.10	17.02	6.64	5.97
K2	1	2	4.84	4.0	0.036	68.95	3.03	17.36	6.63	5.88
K2	1	2	4.84	4.1	0.036	68.95	3.04	17.05	6.71	5.91
K2	2	1	3.60	1.8	0.342	70.63	2.82	16.20	7.15	6.11
K2	2	1	3.59	1.8	0.342	70.65	2.82	16.21	7.32	6.00
K2	2	2	3.60	1.8	0.323	70.51	2.79	16.25	7.08	6.28
K2	2	2	3.60	1.8	0.346	70.51	2.80	16.17	7.04	6.34
K2	3	1	3.39	1.4	0.723	73.57	2.81	15.47	6.95	4.96
K2	3	1	3.40	1.3	0.729	73.59	2.81	15.49	6.95	5.04
K2	3	2	3.39	1.4	0.719	73.32	2.79	15.32	7.11	4.84
K2	3	2	3.38	1.4	0.736	73.09	2.78	15.34	7.18	4.90
K2	4	1	3.42	2.4	1.095	48.78	3.15	11.28	7.68	6.30
K2	4	1	3.41	2.5	1.061	48.99	3.15	11.29	7.74	6.30
K2	4	2	3.43	2.5	0.996	52.66	3.20	11.18	7.70	6.00
K2	4	2	3.43	2.5	1.008	52.75	3.22	11.20	7.58	6.18

Sample code	Fermentation time	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	*S. cerevisae* log cfu/mL
K3	1	1	4.95	5.6	0.056	55.95	4.08	16.48	6.59	5.95
K3	1	1	4.94	5.6	0.054	55.95	4.10	16.48	6.76	5.88
K3	1	2	4.93	5.6	0.057	56.47	4.17	16.44	6.62	5.96
K3	1	2	4.93	5.5	0.059	56.50	4.17	16.46	6.60	5.96
K3	2	1	3.70	2.5	0.359	57.45	4.11	15.63	7.30	6.56
K3	2	1	3.70	2.5	0.345	57.45	4.13	15.63	7.42	6.56
K3	2	2	3.71	2.5	0.375	57.23	4.09	16.11	7.17	6.42
K3	2	2	3.71	2.6	0.357	57.23	4.06	16.1	7.09	6.43
K3	3	1	3.54	1.9	0.646	61.54	3.54	13.91	7.18	5.42
K3	3	1	3.54	1.9	0.642	61.60	3.52	13.94	7.23	5.34
K3	3	2	3.54	1.9	0.608	61.71	3.63	14.85	7.04	5.32
K3	3	2	3.54	1.9	0.603	61.52	3.65	14.87	6.95	5.04
K3	4	1	3.61	4.0	0.873	46.70	3.84	10.14	7.63	6.42
K3	4	1	3.63	4.0	0.879	46.69	3.85	10.14	7.77	6.40
K3	4	2	3.63	3.9	0.845	46.04	3.79	10.10	7.69	6.63
K3	4	2	3.62	3.9	0.844	46.06	3.78	10.11	7.67	6.65

Sample code	Fermentation time	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	*S. cerevisae* log cfu/mL
K4	1	1	4.96	5.6	0.056	56.25	4.19	16.47	6.60	5.97
K4	1	1	4.96	5.6	0.058	56.27	4.18	16.46	6.62	5.95
K4	1	2	4.95	5.6	0.057	56.33	4.17	16.17	6.63	5.84
K4	1	2	4.95	5.6	0.056	56.64	4.04	16.17	6.71	5.92
K4	2	1	3.72	2.3	0.387	57.50	4.00	15.61	7.26	6.51
K4	2	1	3.72	2.3	0.386	57.50	4.00	15.51	7.26	6.46
K4	2	2	3.71	2.2	0.377	58.12	4.11	15.28	7.32	6.36
K4	2	2	3.69	2.2	0.381	58.12	4.11	15.81	7.23	6.50
K4	3	1	3.53	1.8	0.720	62.03	3.55	14.53	7.00	5.26
K4	3	1	3.53	1.8	0.737	62.01	3.55	14.56	7.18	5.30
K4	3	2	3.53	1.9	0.738	62.62	3.20	13.07	7.23	5.18
K4	3	2	3.53	1.8	0.729	62.60	3.19	13.32	7.23	5.08
K4	4	1	3.62	3.8	0.962	46.11	3.80	10.02	7.79	6.46
K4	4	1	3.62	3.8	0.961	46.10	3.83	10.04	7.62	6.30
K4	4	2	3.60	3.9	0.966	45.67	3.79	10.00	7.71	6.54
K4	4	2	3.61	3.9	0.971	45.67	3.76	10.04	7.78	6.60

Notes: Sample code K1 = 10%/25°C, K2 = 10%/27°C, K3 = 20%/25°C, K4 = 20%/27°C; Fermentation time: 1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h.

Table D.6 Consumer sensory evaluation scores of jujube water kefir beverage at the end of fermentation (72 h) (raw data of phase II)

Panellist	Sample code	Apperance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	K3	6	5	6	6	5	6
2	K3	5	3	3	5	6	3
3	K3	6	4	6	6	6	6
4	K3	4	4	7	8	8	7
5	K3	8	5	6	5	6	6
6	K3	8	7	7	4	6	7
7	K3	5	5	4	4	4	4
8	K3	7	6	7	6	6	7
9	K3	7	8	8	5	8	7
10	K3	8	8	7	7	8	8
11	K3	7	8	8	9	9	8
12	K3	7	5	7	4	7	7
13	K3	4	5	6	6	6	7
14	K3	5	7	7	6	4	6
15	K3	9	9	9	9	8	8
16	K3	4	4	4	6	7	4
17	K3	6	5	4	6	7	7
18	K3	4	5	4	6	6	5
19	K3	8	7	6	4	7	6
20	K3	6	7	7	7	7	7
21	K3	6	4	7	6	8	8
22	K3	6	6	6	6	7	7
23	K3	8	8	8	5	5	7
24	K3	6	6	3	5	5	6
25	K3	4	5	7	7	6	6
26	K3	7	4	3	4	3	2
27	K3	7	8	8	6	7	8
28	K3	7	7	8	8	9	8
29	K3	7	4	4	7	4	5
30	K3	7	7	8	8	8	8

Panellist	Sample code	Apperance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	K4	6	6	5	4	4	4
2	K4	5	5	6	5	4	5
3	K4	5	5	5	5	5	5
4	K4	4	5	8	9	8	8
5	K4	7	4	6	6	6	6
6	K4	8	8	8	5	7	8
7	K4	5	5	3	3	2	2
8	K4	7	6	7	6	7	8
9	K4	7	8	8	6	9	8
10	K4	8	8	7	7	8	8
11	K4	7	8	8	9	7	8
12	K4	7	6	7	6	7	7
13	K4	5	5	5	5	4	5
14	K4	5	8	7	6	6	8
15	K4	9	8	6	4	8	6
16	K4	5	7	7	7	8	7
17	K4	6	6	7	7	7	7
18	K4	4	5	4	5	6	4
19	K4	8	7	6	7	7	7
20	K4	6	6	6	6	6	6
21	K4	6	7	6	6	7	7
22	K4	6	6	6	6	7	7
23	K4	8	8	8	5	5	8
24	K4	7	7	7	8	8	8
25	K4	7	5	8	8	6	7
26	K4	7	5	4	5	5	4
27	K4	7	6	6	6	6	6
28	K4	7	7	7	6	7	7
29	K4	5	6	7	7	7	8
30	K4	7	7	6	7	6	7

Notes: Sample code K3 = 20%/25°C, K4 = 20%/27°C.

Table D.7 Characteristics of jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III part II)

Sample code	Storage time	Replication	pH	TSS (°Brix)	T.A. (%)	Colour L*	Colour a*	Colour b*	LAB log cfu/mL	*S. cerevisae* log cfu/mL
K4	1	1	3.70	3.7	0.960	45.86	3.58	10.53	7.60	6.54
K4	1	1	3.69	3.7	0.970	45.90	3.59	10.53	7.591	6.48
K4	1	2	3.69	3.8	0.971	46.01	3.62	10.64	7.54	6.62
K4	1	2	3.70	3.9	0.968	45.99	3.63	10.55	7.54	6.65
K4	2	1	3.66	3.4	0.979	42.91	3.05	13.55	7.38	4.74
K4	2	1	3.66	3.4	0.971	42.89	3.08	13.68	7.40	4.80
K4	2	2	3.68	3.5	0.969	43.56	3.12	13.28	7.32	4.51
K4	2	2	3.68	3.5	0.989	43.58	3.16	13.26	7.32	4.58
K4	3	1	3.64	3.3	0.987	41.63	2.78	13.58	7.05	3.36
K4	3	1	3.65	3.2	0.989	41.64	2.79	13.59	7.01	3.48
K4	3	2	3.66	3.3	0.986	42.21	2.81	13.62	7.03	3.65
K4	3	2	3.66	3.3	0.969	42.23	2.82	13.63	6.98	3.60
K4	4	1	3.64	3.1	0.995	41.28	2.71	14.01	6.56	2.54
K4	4	1	3.65	3.1	1.005	41.29	2.68	14.09	6.54	2.54
K4	4	2	3.65	3.1	0.990	40.95	2.51	14.19	6.60	1.79
K4	4	2	3.65	3.2	0.986	41.00	2.52	14.20	6.54	2.81

Notes: Sample code K4 = 20%/27°C; 1 = 0 d, 2 = 7 d, 3 = 14 d, 4 = 21 d.

Table D.8 Consumer sensory evaluation scores for jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III, part II)

Panellist	Sample code	Apperance	Odour	Flavour	Sourness	Sweetness	Overall acceptability
1	K4	6	5	6	6	5	6
2	K4	5	3	3	5	6	3
3	K4	6	4	6	6	6	6
4	K4	4	4	7	8	8	7
5	K4	8	5	6	5	6	6
6	K4	8	7	7	4	6	7
7	K4	5	5	4	4	4	4
8	K4	7	6	7	6	6	7
9	K4	7	8	8	5	8	7
10	K4	8	8	7	7	8	8
11	K4	7	8	8	9	9	8
12	K4	7	5	7	4	7	7
13	K4	4	5	6	6	6	7
14	K4	5	7	7	6	4	6
15	K4	9	9	9	9	8	8
16	K4	4	4	4	6	7	4
17	K4	6	5	4	6	7	7
18	K4	4	5	4	6	6	5
19	K4	8	7	6	4	7	6
20	K4	6	7	7	7	7	7
21	K4	6	4	7	6	8	8
22	K4	6	6	6	6	7	7
23	K4	8	8	8	5	5	7
24	K4	6	6	3	5	5	6
25	K4	4	5	7	7	6	6
26	K4	7	4	3	4	3	2
27	K4	7	8	8	6	7	8
28	K4	7	7	8	8	9	8
29	K4	7	4	4	7	4	5
30	K4	7	7	8	8	8	8

Note: Sample code K4 = 20%/27°C.

E. GC data

E.1 GC standard curve of ethanol

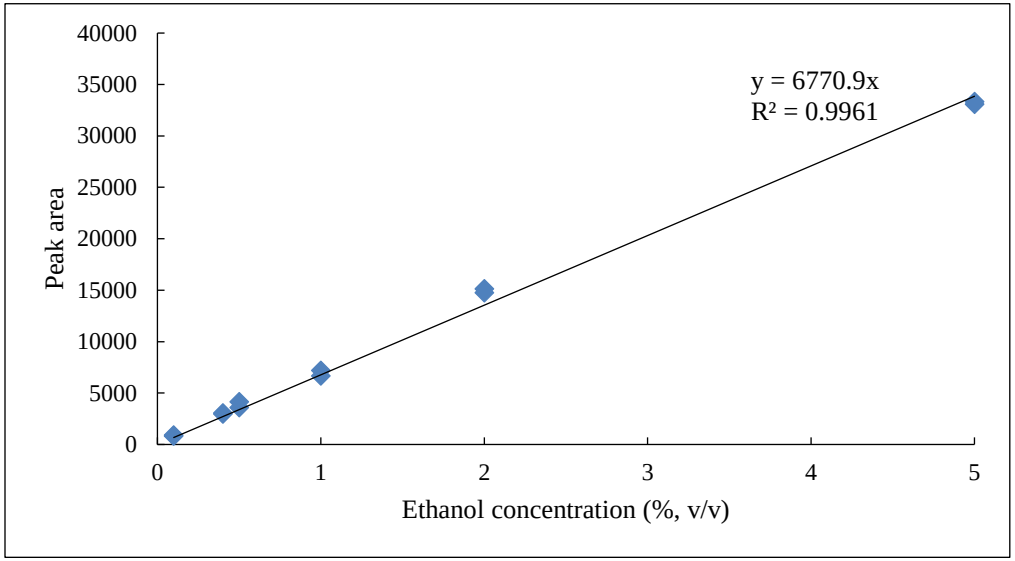


Table E.1 GC data for ethanol standards peak area and retention time

Standard	Concentration (%, v/v)	Mean peak area	Mean retention time (min)
Ethanol	0.1	860.85	0.776
	0.4	3004.95	
	0.5	3852.85	
	1.0	6922.00	
	2.0	14928.90	
	5.0	33195.25	

Table E.2 GC data of ethanol concentration in jujube water kefir beverage (K4) during 72 h fermentation (raw data of phase III part I)

Sample code	Fermentation time	Replication	Peak area	Ethanol concentration (%, v/v)
K4	1	1	nd	0
K4	1	1	nd	0
K4	1	2	nd	0
K4	1	2	nd	0
K4	2	1	16607.2	2.45
K4	2	1	16909.2	2.50
K4	2	2	16547.0	2.44
K4	2	2	16725.4	2.47
K4	3	1	14020.5	2.07
K4	3	1	15545.2	2.30
K4	3	2	15675.4	2.32
K4	3	2	15794.9	2.33
K4	4	1	23970.0	3.54
K4	4	1	22342.2	3.30
K4	4	2	22039.2	3.25
K4	4	2	22954.5	3.39

Notes: Sample code K4 = 20%/27°C; Fermentation time: 1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h; nd = not detected.

Table E.3 GC data of ethanol concentration in jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III part II)

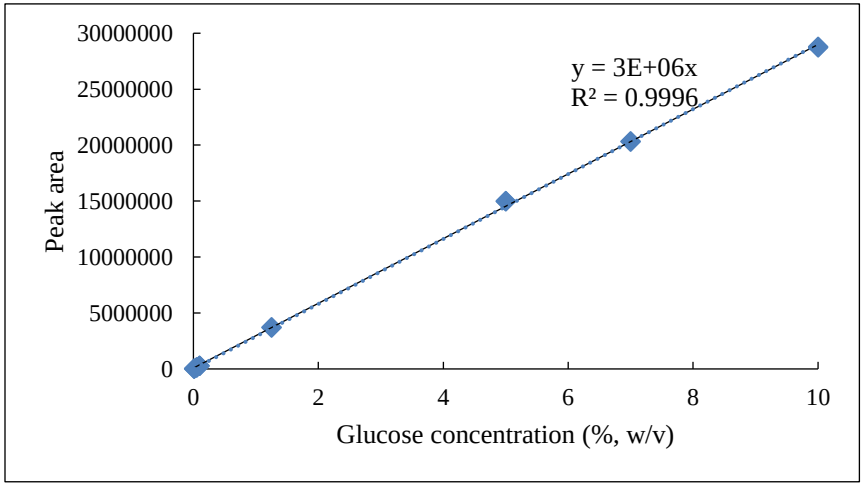
Sample code	Storage time	Replication	Peak area	Ethanol concentration (%, v/v)
K4	1	1	27092.6	4.00
K4	1	1	23211.4	3.42
K4	1	2	21860.8	3.23
K4	1	2	23388.8	3.45
K4	2	1	24703.0	3.65
K4	2	1	26795.5	3.96
K4	2	2	28417.3	4.20
K4	2	2	26638.1	3.93
K4	3	1	29578.1	4.37
K4	3	1	31882.2	4.71
K4	3	2	28675.0	4.24
K4	3	2	29244.1	4.32
K4	4	1	30142.7	4.45
K4	4	1	30709.5	4.54
K4	4	2	29517.2	4.36
K4	4	2	29672.7	4.38

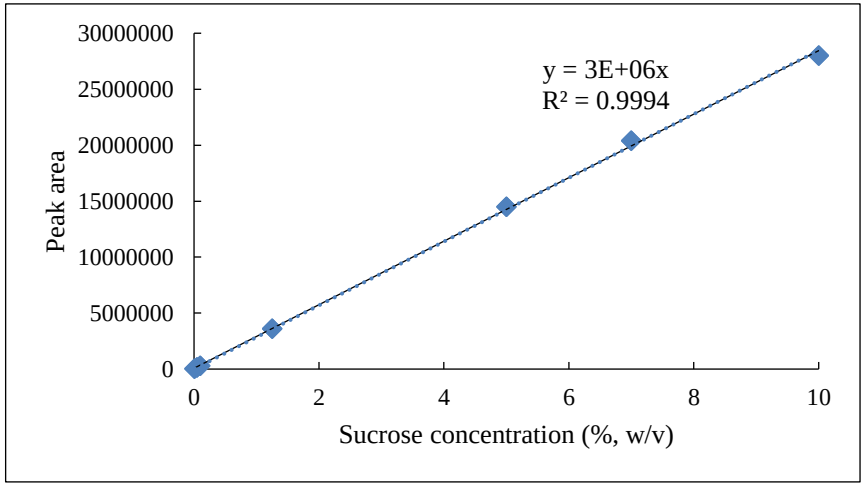
Notes: Sample code K4 = 20%/27°C; 1 = 0 d, 2 = 7 d, 3 = 14 d, 4 = 21 d.

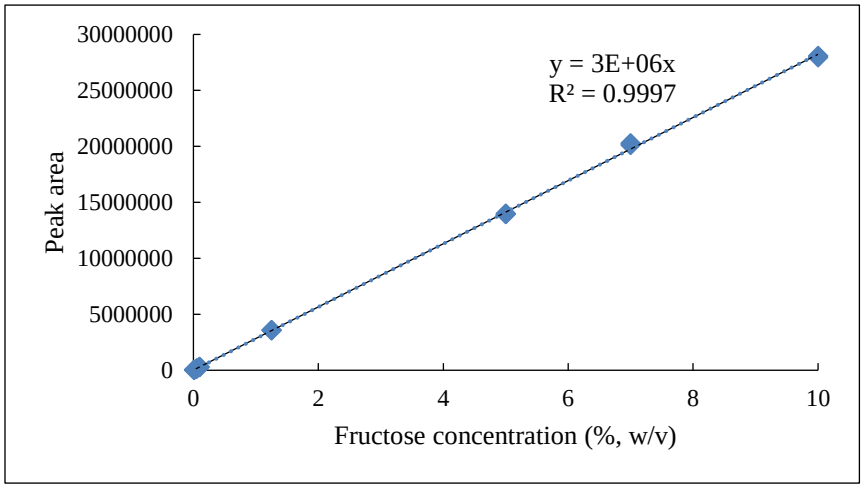
F. HPLC data

F.1 HPLC standard curve

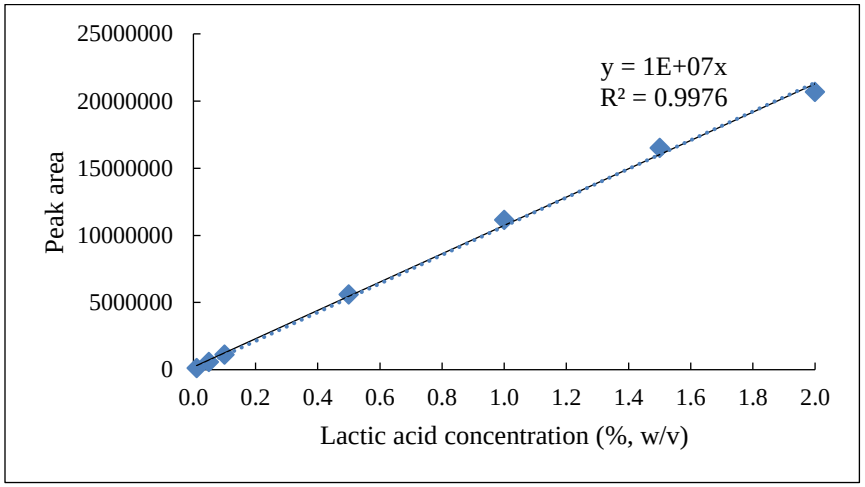
F.1.1 Sugars

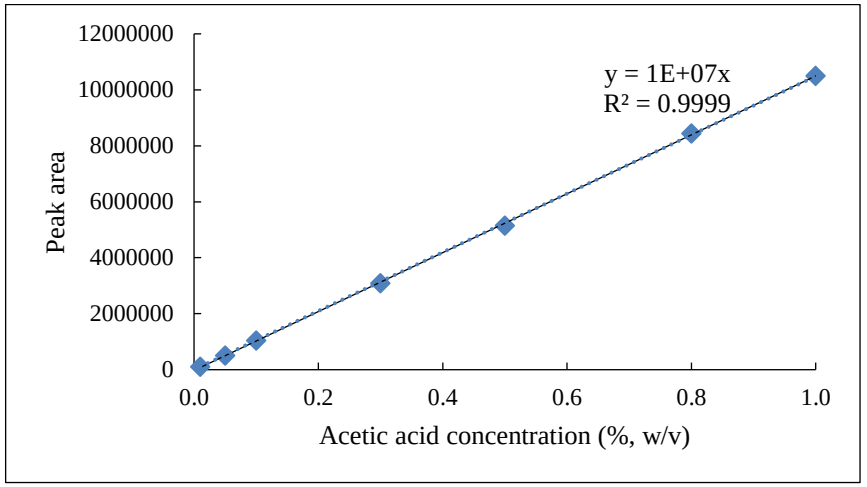




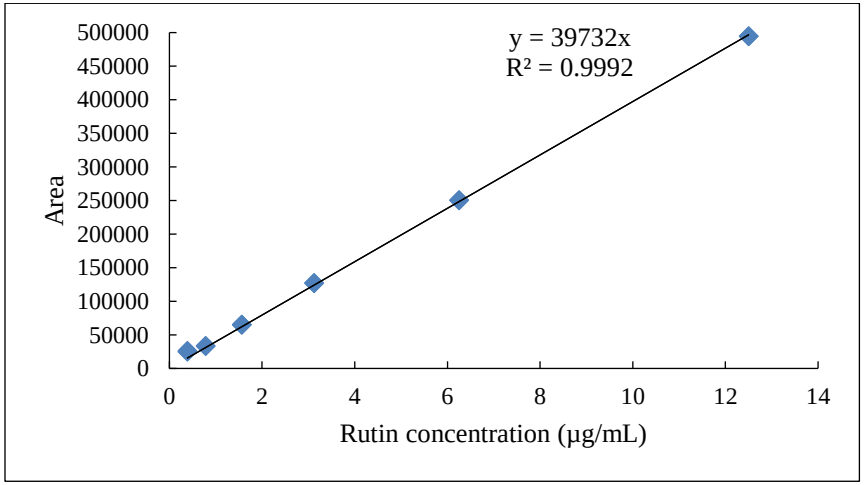


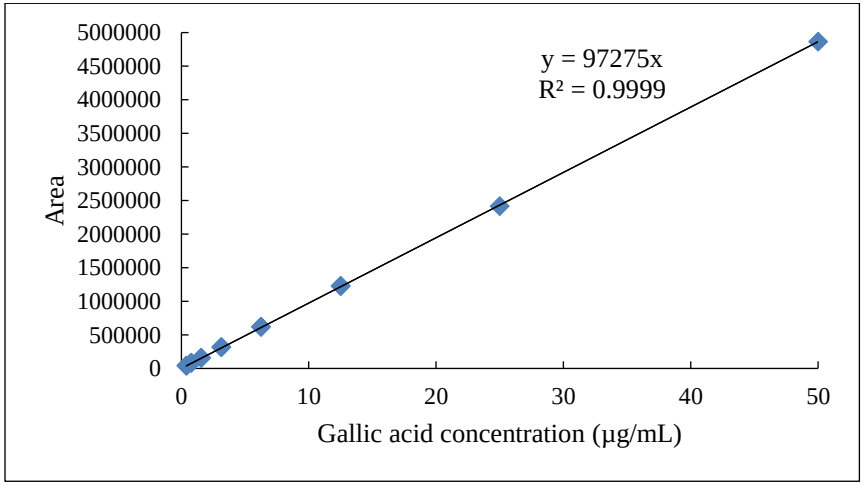
F.1.2 Organic acids

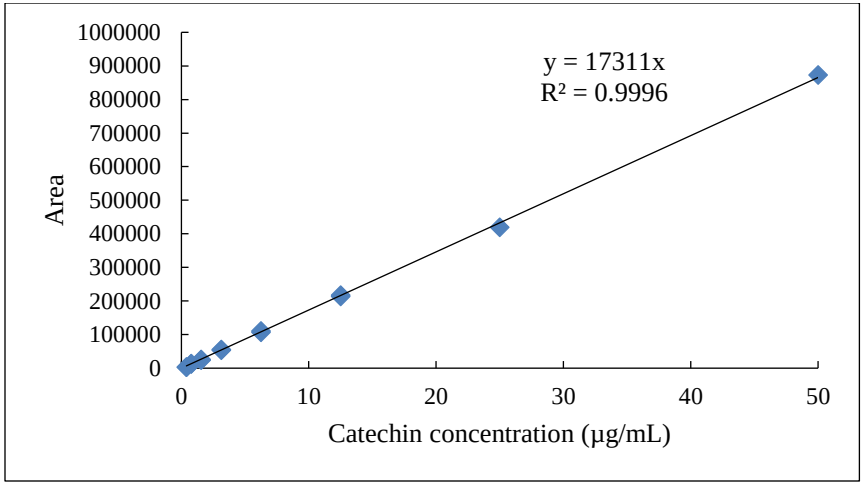




F.1.3 Antioxidants



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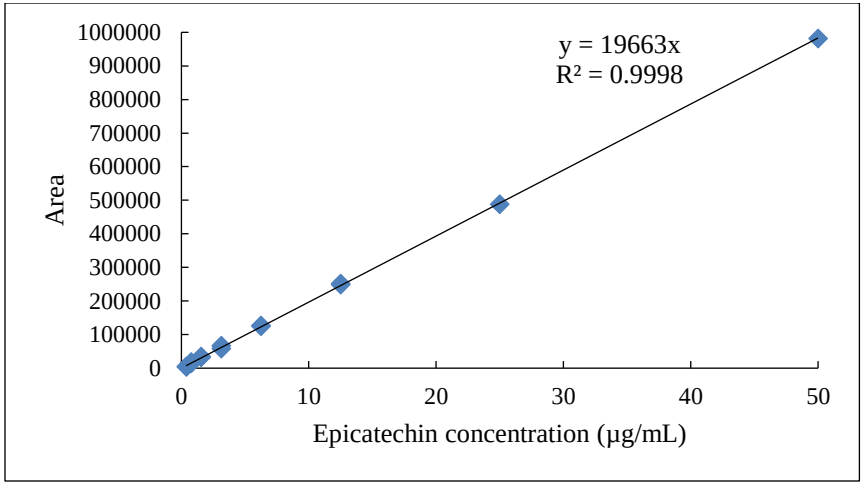
Area
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
Catechin concentration (µg/mL)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R² = 0.9998
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900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
Epicatechin concentration (µg/mL)

Table F.1 HPLC data for sugar, organic acids, and antioxidants standard peak area and retention time

Standards	Concentration (%, w/v)	Mean peak area	Mean retention time (min)
Sucrose	0.01	28389.0	9.160
	0.05	140827.5	
	0.10	284971.0	
	1.25	3598129.0	
	5.00	14494116.0	
	7.00	20403135.0	
	10.00	28007334.0	
Glucose	0.01	28090.5	11.012
	0.05	141538.0	
	0.10	282770.5	
	1.25	3721322.0	
	5.00	14985422.0	
	7.00	20328765.0	
	10.00	28763756.0	
Fructose	0.01	27119.5	13.598
	0.05	137766.0	
	0.10	275238.5	
	1.25	3559544.0	
	5.00	13966054.0	
	7.00	20182171.0	
	10.00	28008560.0	
Lactic acid	0.01	134362.0	13.924
	0.05	576669.0	
	0.10	1124219.5	
	0.50	5599564.0	
	1.00	11147584.5	
	1.50	16511947.0	
	2.00	20682533.5	
Acetic acid	0.01	106347.5	16.023
	0.05	511328.5	
	0.10	1040061.0	
	0.30	3094932.5	
	0.50	5153534.0	
	0.50	8446742.0	
	1.00	10499266.5	

Standards	Concentration (µg/mL)	Mean peak area	Mean retention time (min)
Rutin	0.7815	33149.5	46.934
	1.5625	65011.5	
	3.125	126841.5	
	6.25	250142.0	
	12.5	494259.5	
	25	971260.0	
	50	1952981.0	
Gallic acid	0.390625	39091.0	11.418
	0.7815	83037.0	
	1.5625	156742.0	
	3.125	316577.5	
	6.25	618968.5	
	12.5	1225789.0	
	25	2413803.0	
	50	4862995.0	
Catechin	0.390625	2703.5	25.487
	0.7815	13060.0	
	1.5625	24686.5	
	3.125	54693.0	
	6.25	108659.5	
	12.5	215583.5	
	25	418992.0	
	50	8782895.0	
Epicatechin	0.390625	4459.0	32.174
	0.7815	16428.5	
	1.5625	33392.0	
	3.125	62807.5	
	6.25	125898.0	
	12.5	197732.5	
	25	488129.0	
	50	981378.0	

Table F.2 HPLC data of sugar concentrations in jujube water kefir beverage (K4) during fermentation (72 h) (raw data of phase III part I)

Sample code	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
K4	1	1	8741544	2.91	3261604	1.09	2958942	0.99
K4	1	1	8740630	2.91	3242338	1.08	2935246	0.98
K4	1	2	8858670	2.95	3257875	1.09	2971382	0.99
K4	1	2	8847275	2.95	3269697	1.09	2971326	0.99
K4	2	1	682674	0.23	1205032	0.40	2276207	0.76
K4	2	1	674452	0.22	1207902	0.40	2270819	0.76
K4	2	2	1117124	0.37	944898	0.31	2072658	0.69
K4	2	2	1100031	0.37	925139	0.31	2032863	0.68
K4	3	1	nd	0.00	183557	0.06	719546	0.24
K4	3	1	nd	0.00	177668	0.06	720850	0.24
K4	3	2	nd	0.00	166123	0.06	720656	0.24
K4	3	2	nd	0.00	159246	0.05	713944	0.24
K4	4	1	590223	0.20	3095360	1.03	4504286	1.50
K4	4	1	605208	0.20	3123359	1.04	4523216	1.51
K4	4	2	420860	0.14	2381007	0.79	4096470	1.37
K4	4	2	418611	0.14	2398537	0.80	4109531	1.37

Notes: Sample code K4 = 20%/27°C; Fermentation time: 1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h; nd = not detected.

Table F.3 HPLC data of sugar concentrations in jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III part II)

Sample code	Fermentation time	Replication	Sucrose		Glucose		Fructose	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
K4	1	1	296861	0.10	1136408	0.38	4109531	1.37
K4	1	1	285862	0.10	1175778	0.39	4096470	1.37
K4	1	2	274088	0.09	1331164	0.44	4504586	1.50
K4	1	2	233407	0.08	1338560	0.45	4523206	1.51
K4	2	1	188103	0.06	395261	0.13	3090626	1.03
K4	2	1	128877	0.04	297129	0.10	2920951	0.97
K4	2	2	192529	0.06	642363	0.21	3367603	1.12
K4	2	2	194074	0.06	636636	0.21	3343722	1.11
K4	3	1	194336	0.06	184610	0.06	2418429	0.81
K4	3	1	198393	0.07	184259	0.06	2404010	0.80
K4	3	2	157865	0.05	268479	0.09	2660251	0.89
K4	3	2	124310	0.04	230911	0.08	2451976	0.82
K4	4	1	nd	0	84456	0.03	1856550	0.62
K4	4	1	nd	0	83289	0.03	1819584	0.61
K4	4	2	nd	0	98643	0.03	1935145	0.65
K4	4	2	nd	0	99581	0.03	1907409	0.64

Notes: Sample code K4 = 20%/27°C; 1 = 0 d, 2 = 7 d, 3 = 14 d, 4 = 21 d; nd = not detected.

Table F.4 HPLC data of organic acids concentrations in jujube water kefir beverage (K4) during fermentation (72 h) (raw data of phase III part I)

Sample code	Fermentation time	Replication	Lactic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
K4	1	1	113758	0.01	nd	0
K4	1	1	117891	0.01	nd	0
K4	1	2	99986	0.01	nd	0
K4	1	2	109802	0.01	nd	0
K4	2	1	844858	0.10	540701	0.05
K4	2	1	854763	0.10	544609	0.05
K4	2	2	848886	0.10	523455	0.05
K4	2	2	848008	0.10	530598	0.05
K4	3	1	904175	0.11	2486716	0.25
K4	3	1	908346	0.11	2496640	0.25
K4	3	2	906917	0.11	2490145	0.25
K4	3	2	907073	0.11	2493605	0.25
K4	4	1	1175628	0.14	3589830	0.36
K4	4	1	1166054	0.14	3546683	0.35
K4	4	2	1179364	0.14	3952808	0.40
K4	4	2	1189978	0.14	3690227	0.37

Notes: Sample code K4 = 20%/27°C; Fermentation time: 1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h.

Table F.5 HPLC data of organic acids concentrations in jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III part II)

Sample code	Fermentation time	Replication	Lactic acid		Acetic acid	
			Peak area	Concentration (%, w/v)	Peak area	Concentration (%, w/v)
K4	1	1	1179364	0.14	2506519	0.25
K4	1	1	1163054	0.14	2610231	0.26
K4	1	2	1189978	0.14	2496353	0.25
K4	1	2	1175928	0.14	2599118	0.26
K4	2	1	1654570	0.19	2797427	0.28
K4	2	1	1659694	0.20	2767046	0.28
K4	2	2	1423600	0.17	2230076	0.22
K4	2	2	1425356	0.17	2207750	0.22
K4	3	1	1866705	0.22	2523766	0.25
K4	3	1	1857158	0.22	2518505	0.25
K4	3	2	1697066	0.20	2440188	0.24
K4	3	2	1693149	0.20	2437639	0.24
K4	4	1	1918810	0.23	2430876	0.24
K4	4	1	1912962	0.23	2434289	0.24
K4	4	2	1789000	0.21	2443892	0.24
K4	4	2	1759659	0.21	2453368	0.25

Notes: Sample code K4 = 20%/27°C; 1 = 0 d, 2 = 7 d, 3 = 14 d, 4 = 21 d.

Table F.6 HPLC data of rutin concentration in jujube water kefir beverage (K4) during fermentation (72 h) (raw data of phase III part I)

Sample code	Fermentation time	Replication	Rutin	
			Peak area	Concentration (µg/mL)
K4	1	1	113669	2.86
K4	1	1	117735	2.96
K4	1	2	116430	2.93
K4	1	2	112382	2.83
K4	2	1	105594	2.66
K4	2	1	107510	2.71
K4	2	2	117446	2.96
K4	2	2	117763	2.96
K4	3	1	95469	2.40
K4	3	1	94510	2.38
K4	3	2	95050	2.39
K4	3	2	94989	2.39
K4	4	1	244433	6.15
K4	4	1	242412	6.10
K4	4	2	251496	6.33
K4	4	2	256227	6.45

Notes: Sample code K4 = 20%/27°C; Fermentation time: 1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h.

Table F.7 HPLC data of rutin concentration in jujube water kefir beverage (K4) during storage (4°C) (raw data of phase III part II)

Sample code	Fermentation time	Replication	Rutin	
			Peak area	Concentration (µg/mL)
K4	1	1	286169	7.20
K4	1	1	270504	6.81
K4	1	2	297375	7.48
K4	1	2	282856	7.12
K4	2	1	332087	8.36
K4	2	1	329621	8.30
K4	2	2	329643	8.30
K4	2	2	334359	8.42
K4	3	1	396829	9.99
K4	3	1	393565	9.91
K4	3	2	396784	9.99
K4	3	2	394656	9.93
K4	4	1	436059	10.98
K4	4	1	433902	10.92
K4	4	2	445871	11.22
K4	4	2	442626	11.14

Notes: Sample code K4 = 20%/27°C; 1 = 0 d, 2 = 7 d, 3 = 14 d, 4 = 21 d.

G. Statistic Output

G.1 Statistical analysis of total soluble solids (°Brix), volume, mass and density of extracted jujube syrup using different combinations of extraction method.

One-way ANOVA: °Brix versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05
Rows unused	4

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	3	S1 (NR/E), S3 (NR/NE), S4 (R/NE)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	2	780.902	390.451	127783.91	0.000
Error	9	0.027	0.003		
Total	11	780.929			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0552771	100.00%	100.00%	99.99%

Means

Sample code	N	Mean	StDev	95% CI
S1 (NR/E)	4	39.7500	0.0577	(39.6875, 39.8125)
S2 (NR/NE)	4	22.6500	0.0577	(22.5875, 22.7125)
S4 (R/NE)	4	22.6250	0.0500	(22.5625, 22.6875)

Pooled StDev = 0.0552771

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
S1 (NR/E)	4	39.7500	A
S2 (NR/NE)	4	22.6500	B
S4 (R/NE)	4	22.6250	B

Means that do not share a letter are significantly different.

Notes: S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated.

One-way ANOVA: Volume versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05
Rows unused	2

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	3	S1 (NR/E), S3 (NR/NE), S4 (R/NE)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	2	2889.17	1444.58	224.84	0.001
Error	3	19.27	6.42		
Total	5	2908.44			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.53472	99.34%	98.90%	97.35%

Means

Sample code	N	Mean	StDev	95% CI
S1 (NR/E)	2	55.73	4.31	(50.03, 61.44)
S2 (NR/NE)	2	72.000	0.471	(66.296, 77.704)
S4 (R/NE)	2	19.500	0.707	(13.796, 25.204)

Pooled StDev = 2.53472

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
S2 (NR/NE)	2	72.000	A
S1 (NR/E)	2	55.73	B
S4 (R/NE)	2	19.500	C

Means that do not share a letter are significantly different.

Notes: S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated.

One-way ANOVA: Mass versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05
Rows unused	2

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	3	S1 (NR/E), S3 (NR/NE), S4 (R/NE)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	2	3509.60	1754.80	203.92	0.001
Error	3	25.82	8.61		
Total	5	3535.42			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.93347	99.27%	98.78%	97.08%

Means

Sample code	N	Mean	StDev	95% CI
S1 (NR/E)	2	64.42	5.00	(57.82, 71.02)
S2 (NR/NE)	2	77.643	0.486	(71.042, 84.245)
S4 (R/NE)	2	21.022	0.761	(14.420, 27.623)

Pooled StDev = 2.93347

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
S2 (NR/NE)	2	77.643	A
S1 (NR/E)	2	64.42	B
S4 (R/NE)	2	21.022	C

Means that do not share a letter are significantly different.

Notes: S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated.

One-way ANOVA: Density versus Sample code

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05
Rows unused	2

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	3	S1 (NR/E), S3 (NR/NE), S4 (R/NE)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	2	0.008040	0.004020	47978.28	0.000
Error	3	0.000000	0.000000		
Total	5	0.008040			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0002895	100.00%	99.99%	99.99%

Means

Sample code	N	Mean	StDev	95% CI
S1 (NR/E)	2	1.15586	0.00039	(1.15521, 1.15651)
S2 (NR/NE)	2	1.07838	0.00032	(1.07773, 1.07903)
S4 (R/NE)	2	1.07804	0.00005	(1.07738, 1.07869)

Pooled StDev = 0.000289463

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
S1 (NR/E)	2	1.15586	A
S2 (NR/NE)	2	1.07838	B
S4 (R/NE)	2	1.07804	B

Means that do not share a letter are significantly different.

Notes: S1 (NR/E) = non-rehydrated, evaporated; S2 (NR/NE) = non-rehydrated, non-evaporated; S4 (R/NE) = rehydrated, non-evaporated.

G.2 Statistical analysis of total soluble solids (°Brix), volume, mass and density of extracted jujube syrup using different extraction conditions.

One-way ANOVA: °Brix versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	SA (600/70), SB (600/75), SC (650/70), SD (650/75)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	2.06688	0.688958	174.05	0.000
Error	12	0.04750	0.003958		
Total	15	2.11438			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0629153	97.75%	97.19%	96.01%

Means

Sample code	N	Mean	StDev	95% CI
SA (600/70)	4	22.6250	0.0500	(22.5565, 22.6935)
SB (600/75)	4	22.7500	0.0577	(22.6815, 22.8185)
SC (650/70)	4	21.9500	0.0577	(21.8815, 22.0185)
SD (650/75)	4	22.0000	0.0816	(21.9315, 22.0685)

Pooled StDev = 0.0629153

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
SB (600/75)	4	22.7500	A	
SA (600/70)	4	22.6250	A	
SD (650/75)	4	22.0000		B
SC (650/70)	4	21.9500		B

Means that do not share a letter are significantly different.

Notes: SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C.

One-way ANOVA: Volume versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	SA (600/70), SB (600/75), SC (650/70), SD (650/75)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	1864.11	621.370	828.49	0.000
Error	4	3.00	0.750		
Total	7	1867.11			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.866025	99.84%	99.72%	99.36%

Means

Sample code	N	Mean	StDev	95% CI
SA (600/70)	2	74.167	1.179	(72.466, 75.867)
SB (600/75)	2	73.000	0.471	(71.300, 74.700)
SC (650/70)	2	104.833	0.707	(103.133, 106.534)
SD (650/75)	2	103.333	0.943	(101.633, 105.034)

Pooled StDev = 0.866025

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
SC (650/70)	2	104.833	A
SD (650/75)	2	103.333	A
SA (600/70)	2	74.167	B
SB (600/75)	2	73.000	B

Means that do not share a letter are significantly different.

Notes: SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C.

One-way ANOVA: Mass versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	SA (600/70), SB (600/75), SC (650/70), SD (650/75)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	2076.01	692.002	817.10	0.000
Error	4	3.39	0.847		
Total	7	2079.39			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.920269	99.84%	99.71%	99.35%

Means

Sample code	N	Mean	StDev	95% CI
SA (600/70)	2	79.970	1.296	(78.163, 81.777)
SB (600/75)	2	78.810	0.537	(77.003, 80.617)
SC (650/70)	2	112.350	0.754	(110.543, 114.157)
SD (650/75)	2	110.808	0.922	(109.002, 112.615)

Pooled StDev = 0.920269

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
SC (650/70)	2	112.350	A
SD (650/75)	2	110.808	A
SA (600/70)	2	79.970	B
SB (600/75)	2	78.810	B

Means that do not share a letter are significantly different.

Notes: SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C.

One-way ANOVA: Density versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	SA (600/70), SB (600/75), SC (650/70), SD (650/75)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.000097	0.000032	126.95	0.000
Error	4	0.000001	0.000000		
Total	7	0.000098			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0005054	98.96%	98.18%	95.84%

Means

Sample code	N	Mean	StDev	95% CI
SA (600/70)	2	1.07824	0.00035	(1.07725, 1.07924)
SB (600/75)	2	1.07959	0.00039	(1.07860, 1.08058)
SC (650/70)	2	1.07170	0.00003	(1.07071, 1.07269)
SD (650/75)	2	1.07234	0.00087	(1.07135, 1.07333)

Pooled StDev = 0.000505387

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
SB (600/75)	2	1.07959	A	
SA (600/70)	2	1.07824	A	
SD (650/75)	2	1.07234		B
SC (650/70)	2	1.07170		B

Means that do not share a letter are significantly different.

Notes: SA (600/70) = 600 mL/70°C; SB (600/75) = 600 mL/75°C; SC (650/70) = 650 mL/70°C; SD (650/75) = 650 mL/75°C.

G.3 Statistical analysis of total soluble solids (°Brix) of jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: °Brix versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	15.6025	5.20083	4160.67	0.000
Error	12	0.0150	0.00125		
Total	15	15.6175			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0353553	99.90%	99.88%	99.83%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.000	0.000	(3.961, 4.039)
24 hrs	4	1.8250	0.0500	(1.7865, 1.8635)
48 hrs	4	1.400	0.000	(1.361, 1.439)
72 hrs	4	2.5250	0.0500	(2.4865, 2.5635)

Pooled StDev = 0.0353553

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.000	A
72 hrs	4	2.5250	B
24 hrs	4	1.8250	C
48 hrs	4	1.400	D

Means that do not share a letter are significantly different.

G.4 Statistical analysis of total soluble solids (°Brix) of jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: °Brix versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	16.2219	5.40729	2883.89	0.000
Error	12	0.0225	0.00187		
Total	15	16.2444			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0433013	99.86%	99.83%	99.75%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.0250	0.0500	(3.9778, 4.0722)
24 hrs	4	1.800	0.000	(1.753, 1.847)
48 hrs	4	1.3750	0.0500	(1.3278, 1.4222)
72 hrs	4	2.4750	0.0500	(2.4278, 2.5222)

Pooled StDev = 0.0433013

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.0250	A
72 hrs	4	2.4750	B
24 hrs	4	1.800	C
48 hrs	4	1.3750	D

Means that do not share a letter are significantly different.

G.5 Statistical analysis of total soluble solids (°Brix) of jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: °Brix versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	32.0725	10.6908	5131.60	0.000
Error	12	0.0250	0.0021		
Total	15	32.0975			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0456435	99.92%	99.90%	99.86%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.5750	0.0500	(5.5253, 5.6247)
24 hrs	4	2.5250	0.0500	(2.4753, 2.5747)
48 hrs	4	1.900	0.000	(1.850, 1.950)
72 hrs	4	3.9500	0.0577	(3.9003, 3.9997)

Pooled StDev = 0.0456435

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	5.5750	A
72 hrs	4	3.9500	B
24 hrs	4	2.5250	C
48 hrs	4	1.900	D

Means that do not share a letter are significantly different.

G.6 Statistical analysis of total soluble solids (°Brix) of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: °Brix versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	35.3769	11.7923	5145.73	0.000
Error	12	0.0275	0.0023		
Total	15	35.4044			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0478714	99.92%	99.90%	99.86%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.600	0.000	(5.548, 5.652)
24 hrs	4	2.2500	0.0577	(2.1978, 2.3022)
48 hrs	4	1.8250	0.0500	(1.7728, 1.8772)
72 hrs	4	3.8500	0.0577	(3.7978, 3.9022)

Pooled StDev = 0.0478714

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	5.600	A
72 hrs	4	3.8500	B
24 hrs	4	2.2500	C
48 hrs	4	1.8250	D

Means that do not share a letter are significantly different.

G.7 Statistical analysis of total soluble solids (°Brix) of jujube water kefir beverage samples at 72 h of fermentation.

One-way ANOVA: °Brix versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	7.86500	2.62167	898.86	0.000
Error	12	0.03500	0.00292		
Total	15	7.90000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0540062	99.56%	99.45%	99.21%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	2.5250	0.0500	(2.4662, 2.5838)
K2 (10/27)	4	2.4750	0.0500	(2.4162, 2.5338)
K3 (20/25)	4	3.9500	0.0577	(3.8912, 4.0088)
K4 (20/27)	4	3.8500	0.0577	(3.7912, 3.9088)

Pooled StDev = 0.0540062

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
K3 (20/25)	4	3.9500	A	
K4 (20/27)	4	3.8500	A	
K1 (10/25)	4	2.5250		B
K2 (10/27)	4	2.4750		B

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.8 Statistical analysis of pH of jujube water kefir beverage K1 (10/25) during 72 h of fermentation

One-way ANOVA: pH versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	5.81792	1.93931	19805.68	0.000
Error	12	0.00118	0.00010		
Total	15	5.81909			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0098953	99.98%	99.97%	99.96%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.85500	0.01291	(4.84422, 4.86578)
24 hrs	4	3.600	0.000	(3.589, 3.611)
48 hrs	4	3.39250	0.00500	(3.38172, 3.40328)
72 hrs	4	3.43000	0.01414	(3.41922, 3.44078)

Pooled StDev = 0.00989529

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.85500	A
24 hrs	4	3.600	B
72 hrs	4	3.43000	C
48 hrs	4	3.39250	D

Means that do not share a letter are significantly different.

G.9 Statistical analysis of pH of jujube water kefir beverage K2 (10/27) during 72 h of fermentation

One-way ANOVA: pH versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	5.77153	1.92384	27160.12	0.000
Error	12	0.00085	0.00007		
Total	15	5.77238			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0084163	99.99%	99.98%	99.97%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.84500	0.01000	(4.83583, 4.85417)
24 hrs	4	3.59750	0.00500	(3.58833, 3.60667)
48 hrs	4	3.39000	0.00816	(3.38083, 3.39917)
72 hrs	4	3.42250	0.00957	(3.41333, 3.43167)

Pooled StDev = 0.00841625

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.84500	A
24 hrs	4	3.59750	B
72 hrs	4	3.42250	C
48 hrs	4	3.39000	D

Means that do not share a letter are significantly different.

G.10 Statistical analysis of pH of jujube water kefir beverage K3 (20/25) during 72 h of fermentation

One-way ANOVA: pH versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	5.24212	1.74737	32259.23	0.000
Error	12	0.00065	0.00005		
Total	15	5.24278			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0073598	99.99%	99.98%	99.98%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.93750	0.00957	(4.92948, 4.94552)
24 hrs	4	3.70500	0.00577	(3.69698, 3.71302)
48 hrs	4	3.540	0.000	(3.532, 3.548)
72 hrs	4	3.62250	0.00957	(3.61448, 3.63052)

Pooled StDev = 0.00735980

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.93750	A
24 hrs	4	3.70500	B
72 hrs	4	3.62250	C
48 hrs	4	3.540	D

Means that do not share a letter are significantly different.

G.11 Statistical analysis of pH of jujube water kefir beverage K4 (20/27) during 72 h of fermentation

One-way ANOVA: pH versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	5.43167	1.81056	22283.77	0.000
Error	12	0.00097	0.00008		
Total	15	5.43264			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0090139	99.98%	99.98%	99.97%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.95500	0.00577	(4.94518, 4.96482)
24 hrs	4	3.71000	0.01414	(3.70018, 3.71982)
48 hrs	4	3.530	0.000	(3.520, 3.540)
72 hrs	4	3.61250	0.00957	(3.60268, 3.62232)

Pooled StDev = 0.00901388

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.95500	A
24 hrs	4	3.71000	B
72 hrs	4	3.61250	C
48 hrs	4	3.530	D

Means that do not share a letter are significantly different.

G.12 Statistical analysis of pH of jujube water kefir beverages (K1-K4) at 72 h of fermentation

One-way ANOVA: pH versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.146619	0.048873	411.56	0.000
Error	12	0.001425	0.000119		
Total	15	0.148044			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0108972	99.04%	98.80%	98.29%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	3.43000	0.01414	(3.41813, 3.44187)
K2 (10/27)	4	3.42250	0.00957	(3.41063, 3.43437)
K3 (20/25)	4	3.62250	0.00957	(3.61063, 3.63437)
K4 (20/27)	4	3.61250	0.00957	(3.60063, 3.62437)

Pooled StDev = 0.0108972

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K3 (20/25)	4	3.62250	A
K4 (20/27)	4	3.61250	A
K1 (10/25)	4	3.43000	B
K2 (10/27)	4	3.42250	B

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.13 Statistical analysis of titratable acid (T.A.) of jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: T.A. versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.03578	0.678592	21539.60	0.000
Error	12	0.00038	0.000032		
Total	15	2.03615			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0056129	99.98%	99.98%	99.97%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.036421	0.000885	(0.030306, 0.042535)
24 hrs	4	0.31604	0.00801	(0.30992, 0.32215)
48 hrs	4	0.67996	0.00450	(0.67384, 0.68607)
72 hrs	4	0.97732	0.00638	(0.97121, 0.98343)

Pooled StDev = 0.00561288

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	0.97732	A
48 hrs	4	0.67996	B
24 hrs	4	0.31604	C
0 hr	4	0.036421	D

Means that do not share a letter are significantly different.

G.14 Statistical analysis of titratable acid (T.A.) of jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: T.A. versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.31799	0.772662	1348.54	0.000
Error	12	0.00688	0.000573		
Total	15	2.32486			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0239366	99.70%	99.63%	99.47%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.035969	0.000031	(0.009893, 0.062046)
24 hrs	4	0.33824	0.00999	(0.31216, 0.36431)
48 hrs	4	0.72644	0.00742	(0.70036, 0.75252)
72 hrs	4	1.0401	0.0462	(1.0140, 1.0662)

Pooled StDev = 0.0239366

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	1.0401	A
48 hrs	4	0.72644	B
24 hrs	4	0.33824	C
0 hr	4	0.035969	D

Means that do not share a letter are significantly different.

G.15 Statistical analysis of titratable acid (T.A.) of jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: T.A. versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1.43779	0.479262	1879.10	0.000
Error	12	0.00306	0.000255		
Total	15	1.44085			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0159703	99.79%	99.73%	99.62%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.05655	0.00234	(0.03916, 0.07395)
24 hrs	4	0.35905	0.01239	(0.34165, 0.37644)
48 hrs	4	0.6249	0.0227	(0.6075, 0.6423)
72 hrs	4	0.86026	0.01857	(0.84286, 0.87766)

Pooled StDev = 0.0159703

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	0.86026	A
48 hrs	4	0.6249	B
24 hrs	4	0.35905	C
0 hr	4	0.05655	D

Means that do not share a letter are significantly different.

G.16 Statistical analysis of titratable acid (T.A.) of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: T.A. versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1.90184	0.633947	22560.27	0.000
Error	12	0.00034	0.000028		
Total	15	1.90218			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0053010	99.98%	99.98%	99.97%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.056635	0.001051	(0.050860, 0.062410)
24 hrs	4	0.38271	0.00433	(0.37694, 0.38849)
48 hrs	4	0.73081	0.00853	(0.72504, 0.73659)
72 hrs	4	0.96523	0.00446	(0.95946, 0.97101)

Pooled StDev = 0.00530096

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	0.96523	A
48 hrs	4	0.73081	B
24 hrs	4	0.38271	C
0 hr	4	0.056635	D

Means that do not share a letter are significantly different.

G.17 Statistical analysis of titratable acid (T.A.) of jujube water kefir beverages (K1-K4) at 72 h of fermentation.

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

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.066740	0.022247	35.00	0.000
Error	12	0.007627	0.000636		
Total	15	0.074368			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0252111	89.74%	87.18%	81.77%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	0.97732	0.00638	(0.94986, 1.00479)
K2 (10/27)	4	1.0401	0.0462	(1.0126, 1.0675)
K3 (20/25)	4	0.86026	0.01857	(0.83280, 0.88773)
K4 (20/27)	4	0.96523	0.00446	(0.93777, 0.99270)

Pooled StDev = 0.0252111

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K2 (10/27)	4	1.0401	A
K1 (10/25)	4	0.97732	B
K4 (20/27)	4	0.96523	B
K3 (20/25)	4	0.86026	C

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.18 Statistical analysis of colour (L*) of jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: L* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1162.55	387.516	447.20	0.000
Error	12	10.40	0.867		
Total	15	1172.95			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.930880	99.11%	98.89%	98.42%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	68.7925	0.0780	(67.7784, 69.8066)
24 hrs	4	70.887	0.287	(69.873, 71.902)
48 hrs	4	74.185	0.341	(73.171, 75.199)
72 hrs	4	52.110	1.806	(51.096, 53.124)

Pooled StDev = 0.930880

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
48 hrs	4	74.185	A
24 hrs	4	70.887	B
0 hr	4	68.7925	C
72 hrs	4	52.110	D

Means that do not share a letter are significantly different.

G.19 Statistical analysis of colour (L*) of jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: L* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1256.41	418.804	334.72	0.000
Error	12	15.01	1.251		
Total	15	1271.43			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.11857	98.82%	98.52%	97.90%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	68.720	0.266	(67.501, 69.939)
24 hrs	4	70.5750	0.0755	(69.3564, 71.7936)
48 hrs	4	73.392	0.236	(72.174, 74.611)
72 hrs	4	50.80	2.21	(49.58, 52.01)

Pooled StDev = 1.11857

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
48 hrs	4	73.392	A
24 hrs	4	70.5750	B
0 hr	4	68.720	B
72 hrs	4	50.80	C

Means that do not share a letter are significantly different.

G.20 Statistical analysis of colour (L*) of jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: L* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	497.093	165.698	2571.53	0.000
Error	12	0.773	0.064		
Total	15	497.866			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.253841	99.84%	99.81%	99.72%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	56.218	0.309	(55.941, 56.494)
24 hrs	4	57.3400	0.1270	(57.0635, 57.6165)
48 hrs	4	61.5925	0.0854	(61.3160, 61.8690)
72 hrs	4	46.373	0.373	(46.096, 46.649)

Pooled StDev = 0.253841

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
48 hrs	4	61.5925	A
24 hrs	4	57.3400	B
0 hr	4	56.218	C
72 hrs	4	46.373	D

Means that do not share a letter are significantly different.

G.21 Statistical analysis of colour (L*) of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: L* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	579.619	193.206	2270.68	0.000
Error	12	1.021	0.085		
Total	15	580.640			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.291698	99.82%	99.78%	99.69%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	56.3725	0.1815	(56.0547, 56.6903)
24 hrs	4	57.810	0.358	(57.492, 58.128)
48 hrs	4	62.315	0.341	(61.997, 62.633)
72 hrs	4	45.888	0.251	(45.570, 46.205)

Pooled StDev = 0.291698

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
48 hrs	4	62.315	A
24 hrs	4	57.810	B
0 hr	4	56.3725	C
72 hrs	4	45.888	D

Means that do not share a letter are significantly different.

G.22 Statistical analysis of colour (L*) of jujube water kefir beverage (K1-K4) at 72 h of fermentation.

One-way ANOVA: L* versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	117.24	39.082	18.75	0.000
Error	12	25.01	2.084		
Total	15	142.25			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.44365	82.42%	78.02%	68.75%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	52.110	1.806	(50.537, 53.683)
K2 (10/27)	4	50.80	2.21	(49.22, 52.37)
K3 (20/25)	4	46.373	0.373	(44.800, 47.945)
K4 (20/27)	4	45.888	0.251	(44.315, 47.460)

Pooled StDev = 1.44365

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K1 (10/25)	4	52.110	A
K2 (10/27)	4	50.80	A
K3 (20/25)	4	46.373	B
K4 (20/27)	4	45.888	B

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.23 Statistical analysis of colour (a*) of jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: a* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.69748	0.232492	121.83	0.000
Error	12	0.02290	0.001908		
Total	15	0.72037			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0436845	96.82%	96.03%	94.35%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	3.0400	0.0231	(2.9924, 3.0876)
24 hrs	4	2.7600	0.0638	(2.7124, 2.8076)
48 hrs	4	2.7350	0.0300	(2.6874, 2.7826)
72 hrs	4	3.2400	0.0462	(3.1924, 3.2876)

Pooled StDev = 0.0436845

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
72 hrs	4	3.2400	A		
0 hr	4	3.0400		B	
24 hrs	4	2.7600			C
48 hrs	4	2.7350			C

Means that do not share a letter are significantly different.

G.24 Statistical analysis of colour (a*) of jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: a* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.438319	0.146106	186.02	0.000
Error	12	0.009425	0.000785		
Total	15	0.447744			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0280253	97.90%	97.37%	96.26%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	3.0675	0.0377	(3.0370, 3.0980)
24 hrs	4	2.80750	0.01500	(2.77697, 2.83803)
48 hrs	4	2.79750	0.01500	(2.76697, 2.82803)
72 hrs	4	3.1800	0.0356	(3.1495, 3.2105)

Pooled StDev = 0.0280253

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
72 hrs	4	3.1800	A		
0 hr	4	3.0675		B	
24 hrs	4	2.80750			C
48 hrs	4	2.79750			C

Means that do not share a letter are significantly different.

G.25 Statistical analysis of colour (a*) of jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: a* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.79267	0.264223	124.46	0.000
Error	12	0.02548	0.002123		
Total	15	0.81814			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0460751	96.89%	96.11%	94.46%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.1300	0.0469	(4.0798, 4.1802)
24 hrs	4	4.0975	0.0299	(4.0473, 4.1477)
48 hrs	4	3.5850	0.0645	(3.5348, 3.6352)
72 hrs	4	3.8150	0.0351	(3.7648, 3.8652)

Pooled StDev = 0.0460751

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.1300	A
24 hrs	4	4.0975	A
72 hrs	4	3.8150	B
48 hrs	4	3.5850	C

Means that do not share a letter are significantly different.

G.26 Statistical analysis of colour (a*) of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: a* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1.4393	0.47976	37.01	0.000
Error	12	0.1556	0.01296		
Total	15	1.5948			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.113862	90.25%	87.81%	82.66%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	4.1450	0.0705	(4.0210, 4.2690)
24 hrs	4	4.0550	0.0635	(3.9310, 4.1790)
48 hrs	4	3.373	0.205	(3.248, 3.497)
72 hrs	4	3.7950	0.0289	(3.6710, 3.9190)

Pooled StDev = 0.113862

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	4.1450	A
24 hrs	4	4.0550	A
72 hrs	4	3.7950	B
48 hrs	4	3.373	C

Means that do not share a letter are significantly different.

G.27 Statistical analysis of colour (a*) of jujube water kefir beverage (K1-K4) at 72 h of fermentation.

One-way ANOVA: a* versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	1.42410	0.474700	347.34	0.000
Error	12	0.01640	0.001367		
Total	15	1.44050			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0369685	98.86%	98.58%	97.98%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	3.2400	0.0462	(3.1997, 3.2803)
K2 (10/27)	4	3.1800	0.0356	(3.1397, 3.2203)
K3 (20/25)	4	3.8150	0.0351	(3.7747, 3.8553)
K4 (20/27)	4	3.7950	0.0289	(3.7547, 3.8353)

Pooled StDev = 0.0369685

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
K3 (20/25)	4	3.8150	A	
K4 (20/27)	4	3.7950	A	
K1 (10/25)	4	3.2400		B
K2 (10/27)	4	3.1800		B

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.28 Statistical analysis of colour (b*) of jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: b* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	84.5455	28.1818	1736.49	0.000
Error	12	0.1948	0.0162		
Total	15	84.7403			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.127394	99.77%	99.71%	99.59%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	17.5500	0.1402	(17.4112, 17.6888)
24 hrs	4	16.2000	0.0891	(16.0612, 16.3388)
48 hrs	4	15.3625	0.1477	(15.2237, 15.5013)
72 hrs	4	11.3775	0.1245	(11.2387, 11.5163)

Pooled StDev = 0.127394

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	17.5500	A
24 hrs	4	16.2000	B
48 hrs	4	15.3625	C
72 hrs	4	11.3775	D

Means that do not share a letter are significantly different.

G.29 Statistical analysis of colour (b*) of jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: b* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	80.7092	26.9031	2454.57	0.000
Error	12	0.1315	0.0110		
Total	15	80.8407			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.104692	99.84%	99.80%	99.71%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	17.0975	0.1790	(16.9834, 17.2116)
24 hrs	4	16.2075	0.0330	(16.0934, 16.3216)
48 hrs	4	15.4050	0.0874	(15.2909, 15.5191)
72 hrs	4	11.2375	0.0556	(11.1234, 11.3516)

Pooled StDev = 0.104692

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	17.0975	A
24 hrs	4	16.2075	B
48 hrs	4	15.4050	C
72 hrs	4	11.2375	D

Means that do not share a letter are significantly different.

G.30 Statistical analysis of colour (b*) of jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: b* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	98.293	32.7644	356.48	0.000
Error	12	1.103	0.0919		
Total	15	99.396			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.303167	98.89%	98.61%	98.03%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	16.4650	0.0191	(16.1347, 16.7953)
24 hrs	4	15.868	0.274	(15.537, 16.198)
48 hrs	4	14.393	0.540	(14.062, 14.723)
72 hrs	4	10.1225	0.0206	(9.7922, 10.4528)

Pooled StDev = 0.303167

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	16.4650	A
24 hrs	4	15.868	A
48 hrs	4	14.393	B
72 hrs	4	10.1225	C

Means that do not share a letter are significantly different.

G.31 Statistical analysis of colour (b*) of jujube water kefir beverage K4 (20/27) during 72 hours of fermentation.

One-way ANOVA: b* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	94.339	31.4464	180.72	0.000
Error	12	2.088	0.1740		
Total	15	96.427			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.417138	97.83%	97.29%	96.15%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	16.3175	0.1704	(15.8631, 16.7719)
24 hrs	4	15.553	0.220	(15.098, 16.007)
48 hrs	4	13.870	0.786	(13.416, 14.324)
72 hrs	4	10.0250	0.0191	(9.5706, 10.4794)

Pooled StDev = 0.417138

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	16.3175	A
24 hrs	4	15.553	A
48 hrs	4	13.870	B
72 hrs	4	10.0250	C

Means that do not share a letter are significantly different.

G.32 Statistical analysis of colour (b*) of jujube water kefir beverages (K1-K4) at 72 h of fermentation.

One-way ANOVA: b* versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	6.14677	2.04892	423.00	0.000
Error	12	0.05813	0.00484		
Total	15	6.20489			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0695971	99.06%	98.83%	98.33%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	11.3775	0.1245	(11.3017, 11.4533)
K2 (10/27)	4	11.2375	0.0556	(11.1617, 11.3133)
K3 (20/25)	4	10.1225	0.0206	(10.0467, 10.1983)
K4 (20/27)	4	10.0250	0.0191	(9.9492, 10.1008)

Pooled StDev = 0.0695971

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping	
K1 (10/25)	4	11.3775	A	
K2 (10/27)	4	11.2375	A	
K3 (20/25)	4	10.1225		B
K4 (20/27)	4	10.0250		B

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.33 Statistical analysis of viable cell counts of LAB in jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: LAB versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.0866	0.695549	74.93	0.000
Error	12	0.1114	0.009283		
Total	15	2.1980			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0963495	94.93%	93.66%	90.99%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	6.6572	0.0312	(6.5522, 6.7621)
24 hrs	4	7.1219	0.1003	(7.0169, 7.2268)
48 hrs	4	7.0026	0.1419	(6.8976, 7.1075)
72 hrs	4	7.6622	0.0771	(7.5572, 7.7672)

Pooled StDev = 0.0963495

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	7.6622	A
24 hrs	4	7.1219	B
48 hrs	4	7.0026	B
0 hr	4	6.6572	C

Means that do not share a letter are significantly different.

G.34 Statistical analysis of viable cell counts of LAB in jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: LAB versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.1297	0.709895	82.65	0.000
Error	12	0.1031	0.008589		
Total	15	2.2327			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0926750	95.38%	94.23%	91.79%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	6.6519	0.0380	(6.5510, 6.7529)
24 hrs	4	7.1472	0.1244	(7.0463, 7.2482)
48 hrs	4	7.0496	0.1130	(6.9487, 7.1506)
72 hrs	4	7.6751	0.0682	(7.5741, 7.7761)

Pooled StDev = 0.0926750

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	7.6751	A
24 hrs	4	7.1472	B
48 hrs	4	7.0496	B
0 hr	4	6.6519	C

Means that do not share a letter are significantly different.

G.35 Statistical analysis of viable cell counts of LAB in jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: LAB versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.2319	0.74397	63.97	0.000
Error	12	0.1395	0.01163		
Total	15	2.3715			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.107838	94.12%	92.64%	89.54%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	6.6450	0.0801	(6.5275, 6.7624)
24 hrs	4	7.2432	0.1446	(7.1257, 7.3606)
48 hrs	4	7.1005	0.1258	(6.9831, 7.2180)
72 hrs	4	7.6917	0.0579	(7.5742, 7.8091)

Pooled StDev = 0.107838

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	7.6917	A
24 hrs	4	7.2432	B
48 hrs	4	7.1005	B
0 hr	4	6.6450	C

Means that do not share a letter are significantly different.

G.36 Statistical analysis of viable cell counts of LAB in jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: LAB versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.37513	0.791710	146.66	0.000
Error	12	0.06478	0.005398		
Total	15	2.43991			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0734727	97.35%	96.68%	95.28%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	6.6416	0.0459	(6.5615, 6.7216)
24 hrs	4	7.2658	0.0394	(7.1858, 7.3458)
48 hrs	4	7.1592	0.1092	(7.0792, 7.2393)
72 hrs	4	7.7253	0.0775	(7.6453, 7.8054)

Pooled StDev = 0.0734727

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	7.7253	A
24 hrs	4	7.2658	B
48 hrs	4	7.1592	B
0 hr	4	6.6416	C

Means that do not share a letter are significantly different.

G.37 Statistical analysis of viable cell counts of LAB in jujube water kefir beverages (K1-K4) at 72 h of fermentation.

One-way ANOVA: LAB versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.008955	0.002985	0.60	0.628
Error	12	0.059861	0.004988		
Total	15	0.068816			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0706287	13.01%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	7.6622	0.0771	(7.5853, 7.7391)
K2 (10/27)	4	7.6751	0.0682	(7.5981, 7.7520)
K3 (20/25)	4	7.6917	0.0579	(7.6147, 7.7686)
K4 (20/27)	4	7.7253	0.0775	(7.6484, 7.8023)

Pooled StDev = 0.0706287

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	4	7.7253	A
K3 (20/25)	4	7.6917	A
K2 (10/27)	4	7.6751	A
K1 (10/25)	4	7.6622	A

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.38 Statistical analysis of viable cell counts of *Saccharomyces cerevisiae* in jujube water kefir beverage K1 (10/25) during 72 h of fermentation.

One-way ANOVA: S. cerevisae versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	3.9338	1.31127	61.96	0.000
Error	12	0.2540	0.02116		
Total	15	4.1878			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.145480	93.94%	92.42%	89.22%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.9167	0.0521	(5.7583, 6.0752)
24 hrs	4	6.2319	0.1357	(6.0734, 6.3904)
48 hrs	4	5.0177	0.0944	(4.8592, 5.1762)
72 hrs	4	6.225	0.234	(6.066, 6.383)

Pooled StDev = 0.145480

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
24 hrs	4	6.2319	A
72 hrs	4	6.225	A
0 hr	4	5.9167	B
48 hrs	4	5.0177	C

Means that do not share a letter are significantly different.

G.39 Statistical analysis of viable cell counts of *Saccharomyces cerevisiae* in jujube water kefir beverage K2 (10/27) during 72 h of fermentation.

One-way ANOVA: S. cerevisae versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	4.2579	1.41931	105.87	0.000
Error	12	0.1609	0.01341		
Total	15	4.4188			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.115787	96.36%	95.45%	93.53%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.9327	0.0454	(5.8066, 6.0589)
24 hrs	4	6.1838	0.1558	(6.0576, 6.3099)
48 hrs	4	4.9372	0.0836	(4.8110, 5.0633)
72 hrs	4	6.1945	0.1424	(6.0684, 6.3207)

Pooled StDev = 0.115787

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	6.1945	A
24 hrs	4	6.1838	A
0 hr	4	5.9327	B
48 hrs	4	4.9372	C

Means that do not share a letter are significantly different.

G.40 Statistical analysis of viable cell counts of *Saccharomyces cerevisiae* in jujube water kefir beverage K3 (20/25) during 72 h of fermentation.

One-way ANOVA: S. cerevisae versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	4.1003	1.36678	102.76	0.000
Error	12	0.1596	0.01330		
Total	15	4.2600			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.115331	96.25%	95.32%	93.34%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.9380	0.0422	(5.8124, 6.0637)
24 hrs	4	6.4914	0.0753	(6.3657, 6.6170)
48 hrs	4	5.2803	0.1641	(5.1546, 5.4059)
72 hrs	4	6.5249	0.1372	(6.3993, 6.6505)

Pooled StDev = 0.115331

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	6.5249	A
24 hrs	4	6.4914	A
0 hr	4	5.9380	B
48 hrs	4	5.2803	C

Means that do not share a letter are significantly different.

G.41 Statistical analysis of viable cell counts of *Saccharomyces cerevisiae* in jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: S. cerevisae versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	4.3058	1.43526	167.81	0.000
Error	12	0.1026	0.00855		
Total	15	4.4084			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0924814	97.67%	97.09%	95.86%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	5.9305	0.0578	(5.8297, 6.0312)
24 hrs	4	6.4577	0.0655	(6.3570, 6.5585)
48 hrs	4	5.2029	0.0973	(5.1021, 5.3036)
72 hrs	4	6.4774	0.1308	(6.3766, 6.5781)

Pooled StDev = 0.0924814

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	6.4774	A
24 hrs	4	6.4577	A
0 hr	4	5.9305	B
48 hrs	4	5.2029	C

Means that do not share a letter are significantly different.

G.42 Statistical analysis of viable cell counts of *Saccharomyces cerevisiae* in jujube water kefir beverage (K1-K4) at 72 h of fermentation.

One-way ANOVA: S. cerevisae versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	4	K1 (10/25), K2 (10/27), K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	3	0.3463	0.11543	4.17	0.031
Error	12	0.3324	0.02770		
Total	15	0.6787			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.166446	51.02%	38.77%	12.92%

Means

Sample code	N	Mean	StDev	95% CI
K1 (10/25)	4	6.225	0.234	(6.043, 6.406)
K2 (10/27)	4	6.1945	0.1424	(6.0132, 6.3759)
K3 (20/25)	4	6.5249	0.1372	(6.3436, 6.7062)
K4 (20/27)	4	6.4774	0.1308	(6.2961, 6.6587)

Pooled StDev = 0.166446

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K3 (20/25)	4	6.5249	A
K4 (20/27)	4	6.4774	A
K1 (10/25)	4	6.225	A
K2 (10/27)	4	6.1945	A

Means that do not share a letter are significantly different.

Notes: Sample code K1 (10/25) = 10%/25°C; K2 (10/27) = 10%/27°C; K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.43 Statistical analysis of sensory evaluation of jujube water kefir beverage samples at 72 h of fermentation.

One-way ANOVA: Appearance versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	0.067	0.06667	0.04	0.849
Error	58	105.267	1.81494		
Total	59	105.333			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.34720	0.06%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	6.300	1.418	(5.808, 6.792)
K4 (20/27)	30	6.367	1.273	(5.874, 6.859)

Pooled StDev = 1.34720

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	30	6.367	A
K3 (20/25)	30	6.300	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

One-way ANOVA: Odour versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	3.267	3.267	1.60	0.210
Error	58	118.133	2.037		
Total	59	121.400			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.42716	2.69%	1.01%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	5.867	1.613	(5.345, 6.388)
K4 (20/27)	30	6.333	1.213	(5.812, 6.855)

Pooled StDev = 1.42716

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	30	6.333	A
K3 (20/25)	30	5.867	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

One-way ANOVA: Flavour versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	0.600	0.6000	0.25	0.616
Error	58	137.133	2.3644		
Total	59	137.733			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.53765	0.44%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	6.167	1.744	(5.605, 6.729)
K4 (20/27)	30	6.367	1.299	(5.805, 6.929)

Pooled StDev = 1.53765

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	30	6.367	A
K3 (20/25)	30	6.167	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

One-way ANOVA: Sweetness versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	0.017	0.01667	0.01	0.927
Error	58	114.833	1.97989		
Total	59	114.850			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.40708	0.01%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	6.033	1.426	(5.519, 6.548)
K4 (20/27)	30	6.067	1.388	(5.552, 6.581)

Pooled StDev = 1.40708

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	30	6.067	A
K3 (20/25)	30	6.033	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

One-way ANOVA: Sourness versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	0.150	0.1500	0.06	0.800
Error	58	134.033	2.3109		
Total	59	134.183			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.52017	0.11%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	6.433	1.524	(5.878, 6.989)
K4 (20/27)	30	6.333	1.516	(5.778, 6.889)

Pooled StDev = 1.52017

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K3 (20/25)	30	6.433	A
K4 (20/27)	30	6.333	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

One-way ANOVA: Overall acceptability versus Sample code

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample code	2	K3 (20/25), K4 (20/27)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample code	1	0.417	0.4167	0.17	0.680
Error	58	140.433	2.4213		
Total	59	140.850			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.55604	0.30%	0.00%	0.00%

Means

Sample code	N	Mean	StDev	95% CI
K3 (20/25)	30	6.367	1.542	(5.798, 6.935)
K4 (20/27)	30	6.533	1.570	(5.965, 7.102)

Pooled StDev = 1.55604

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample code	N	Mean	Grouping
K4 (20/27)	30	6.533	A
K3 (20/25)	30	6.367	A

Means that do not share a letter are significantly different.

Notes: Sample code K3 (20/25) = 20%/25°C; K4 (20/27) = 20%/27°C.

G.44 Statistical analysis of ethanol content of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: Ethanol versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	24.6392	8.21305	1043.22	0.000
Error	12	0.0945	0.00787		
Total	15	24.7336			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0887286	99.62%	99.52%	99.32%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.000000	0.000000	(-0.096662, 0.096662)
24 hrs	4	2.4660	0.0236	(2.3694, 2.5627)
48 hrs	4	2.2536	0.1229	(2.1570, 2.3503)
72 hrs	4	3.3713	0.1259	(3.2746, 3.4679)

Pooled StDev = 0.0887286

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	3.3713	A
24 hrs	4	2.4660	B
48 hrs	4	2.2536	C
0 hr	4	0.000000	D

Means that do not share a letter are significantly different.

G.45 Statistical analysis of sugars of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: Sucrose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	23.3060	7.76866	3656.35	0.000
Error	12	0.0255	0.00212		
Total	15	23.3315			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0460945	99.89%	99.86%	99.81%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	2.9323	0.0216	(2.8821, 2.9826)
24 hrs	4	0.2979	0.0828	(0.2476, 0.3481)
48 hrs	4	0.000000	0.000000	(-0.050216, 0.050216)
72 hrs	4	0.1696	0.0343	(0.1194, 0.2198)

Pooled StDev = 0.0460945

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	2.9323	A
24 hrs	4	0.2979	B
72 hrs	4	0.1696	C
48 hrs	4	0.000000	D

Means that do not share a letter are significantly different.

One-way ANOVA: Glucose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.75991	0.919972	167.55	0.000
Error	12	0.06589	0.005491		
Total	15	2.82580			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0740991	97.67%	97.09%	95.85%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	1.08596	0.00383	(1.00524, 1.16668)
24 hrs	4	0.3569	0.0523	(0.2762, 0.4376)
48 hrs	4	0.05722	0.00366	(-0.02351, 0.13794)
72 hrs	4	0.9165	0.1386	(0.8358, 0.9972)

Pooled StDev = 0.0740991

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0 hr	4	1.08596	A
72 hrs	4	0.9165	B
24 hrs	4	0.3569	C
48 hrs	4	0.05722	D

Means that do not share a letter are significantly different.

One-way ANOVA: Fructose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	3.00538	1.00179	492.84	0.000
Error	12	0.02439	0.00203		
Total	15	3.02978			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0450856	99.19%	98.99%	98.57%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.98641	0.00567	(0.93729, 1.03552)
24 hrs	4	0.7210	0.0428	(0.6719, 0.7702)
48 hrs	4	0.239583	0.001085	(0.190466, 0.288700)
72 hrs	4	1.4361	0.0791	(1.3870, 1.4853)

Pooled StDev = 0.0450856

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	1.4361	A
0 hr	4	0.98641	B
24 hrs	4	0.7210	C
48 hrs	4	0.239583	D

Means that do not share a letter are significantly different.

G.46 Statistical analysis of organic acids of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: Lactic acid versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.034626	0.011542	16502.84	0.000
Error	12	0.000008	0.000001		
Total	15	0.034634			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0008363	99.98%	99.97%	99.96%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.012983	0.000902	(0.012072, 0.013895)
24 hrs	4	0.099898	0.000486	(0.098986, 0.100809)
48 hrs	4	0.106662	0.000207	(0.105751, 0.107573)
72 hrs	4	0.138480	0.001306	(0.137569, 0.139391)

Pooled StDev = 0.000836297

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	0.138480	A
48 hrs	4	0.106662	B
24 hrs	4	0.099898	C
0 hr	4	0.012983	D

Means that do not share a letter are significantly different.

One-way ANOVA: Acetic acid versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.354102	0.118034	1418.12	0.000
Error	12	0.000999	0.000083		
Total	15	0.355100			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0091232	99.72%	99.65%	99.50%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	0.000000	0.000000	(-0.009939, 0.009939)
24 hrs	4	0.053484	0.000962	(0.043545, 0.063423)
48 hrs	4	0.249178	0.000429	(0.239239, 0.259117)
72 hrs	4	0.36949	0.01822	(0.35955, 0.37943)

Pooled StDev = 0.00912319

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	0.36949	A
48 hrs	4	0.249178	B
24 hrs	4	0.053484	C
0 hr	4	0.000000	D

Means that do not share a letter are significantly different.

G.47 Statistical analysis of rutin of jujube water kefir beverage K4 (20/27) during 72 h of fermentation.

One-way ANOVA: Rutin versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	0 hr, 24 hrs, 48 hrs, 72 hrs

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	38.5164	12.8388	918.29	0.000
Error	12	0.1678	0.0140		
Total	15	38.6842			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.118242	99.57%	99.46%	99.23%

Means

Time	N	Mean	StDev	95% CI
0 hr	4	2.8958	0.0619	(2.7670, 3.0246)
24 hrs	4	2.8209	0.1618	(2.6921, 2.9497)
48 hrs	4	2.39115	0.00988	(2.26234, 2.51997)
72 hrs	4	6.2580	0.1606	(6.1292, 6.3868)

Pooled StDev = 0.118242

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
72 hrs	4	6.2580	A
0 hr	4	2.8958	B
24 hrs	4	2.8209	B
48 hrs	4	2.39115	C

Means that do not share a letter are significantly different.

G.48 Statistical analysis total soluble solids (°Brix) of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: °Brix versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.93688	0.312292	71.38	0.000
Error	12	0.05250	0.004375		
Total	15	0.98937			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0661438	94.69%	93.37%	90.57%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	3.7750	0.0957	(3.7029, 3.8471)
Day 14	4	3.2750	0.0500	(3.2029, 3.3471)
Day 21	4	3.1250	0.0500	(3.0529, 3.1971)
Day 7	4	3.4500	0.0577	(3.3779, 3.5221)

Pooled StDev = 0.0661438

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping			
Day 0	4	3.7750	A			
Day 7	4	3.4500		B		
Day 14	4	3.2750			C	
Day 21	4	3.1250				D

Means that do not share a letter are significantly different.

G.49 Statistical analysis of pH and titratable acid (T.A.) of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: pH versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.005525	0.001842	26.00	0.000
Error	12	0.000850	0.000071		
Total	15	0.006375			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0084163	86.67%	83.33%	76.30%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	3.69500	0.00577	(3.68583, 3.70417)
Day 14	4	3.65250	0.00957	(3.64333, 3.66167)
Day 21	4	3.64750	0.00500	(3.63833, 3.65667)
Day 7	4	3.67000	0.01155	(3.66083, 3.67917)

Pooled StDev = 0.00841625

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
Day 0	4	3.69500	A		
Day 7	4	3.67000		B	
Day 14	4	3.65250		B	C
Day 21	4	3.64750			C

Means that do not share a letter are significantly different.

One-way ANOVA: T.A. versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.001514	0.000505	7.99	0.003
Error	12	0.000757	0.000063		
Total	15	0.002271			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0079443	66.65%	58.31%	40.71%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	0.96704	0.00509	(0.95839, 0.97570)
Day 14	4	0.98275	0.00891	(0.97409, 0.99140)
Day 21	4	0.99397	0.00817	(0.98531, 1.00262)
Day 7	4	0.97714	0.00897	(0.96849, 0.98580)

Pooled StDev = 0.00794433

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
Day 21	4	0.99397	A	
Day 14	4	0.98275	A	B
Day 7	4	0.97714		B
Day 0	4	0.96704		B

Means that do not share a letter are significantly different.

G.50 Statistical analysis of colour (L*, a*, b*) of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: L* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	53.3299	17.7766	235.82	0.000
Error	12	0.9046	0.0754		
Total	15	54.2344			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.274556	98.33%	97.92%	97.03%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	45.9400	0.0716	(45.6409, 46.2391)
Day 14	4	41.928	0.338	(41.628, 42.227)
Day 21	4	41.1300	0.1802	(40.8309, 41.4291)
Day 7	4	43.235	0.387	(42.936, 43.534)

Pooled StDev = 0.274556

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	45.9400	A
Day 7	4	43.235	B
Day 14	4	41.928	C
Day 21	4	41.1300	D

Means that do not share a letter are significantly different.

One-way ANOVA: a* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.27757	0.759190	214.49	0.000
Error	12	0.04248	0.003540		
Total	15	2.32004			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0594944	98.17%	97.71%	96.75%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	3.6050	0.0238	(3.5402, 3.6698)
Day 14	4	2.80000	0.01826	(2.73519, 2.86481)
Day 21	4	2.6050	0.1047	(2.5402, 2.6698)
Day 7	4	3.1025	0.0479	(3.0377, 3.1673)

Pooled StDev = 0.0594944

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	3.6050	A
Day 7	4	3.1025	B
Day 14	4	2.80000	C
Day 21	4	2.6050	D

Means that do not share a letter are significantly different.

One-way ANOVA: b* versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	30.9814	10.3271	765.33	0.000
Error	12	0.1619	0.0135		
Total	15	31.1433			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.116163	99.48%	99.35%	99.08%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	10.5625	0.0525	(10.4360, 10.6890)
Day 14	4	13.6050	0.0238	(13.4785, 13.7315)
Day 21	4	14.1225	0.0900	(13.9960, 14.2490)
Day 7	4	13.442	0.206	(13.316, 13.569)

Pooled StDev = 0.116163

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 21	4	14.1225	A
Day 14	4	13.6050	B
Day 7	4	13.442	B
Day 0	4	10.5625	C

Means that do not share a letter are significantly different.

G.51 Statistical analysis of microbiological growth of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: LAB versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.32201	0.774002	734.61	0.000
Error	12	0.01264	0.001054		
Total	15	2.33465			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0324596	99.46%	99.32%	99.04%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	7.5703	0.0306	(7.5350, 7.6057)
Day 14	4	7.0172	0.0312	(6.9819, 7.0526)
Day 21	4	6.5616	0.0276	(6.5263, 6.5970)
Day 7	4	7.3556	0.0393	(7.3203, 7.3910)

Pooled StDev = 0.0324596

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	7.5703	A
Day 7	4	7.3556	B
Day 14	4	7.0172	C
Day 21	4	6.5616	D

Means that do not share a letter are significantly different.

One-way ANOVA: S. cerevisae versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	37.7169	12.5723	211.38	0.000
Error	12	0.7137	0.0595		
Total	15	38.4306			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.243881	98.14%	97.68%	96.70%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	6.5744	0.0795	(6.3087, 6.8401)
Day 14	4	3.5235	0.1308	(3.2578, 3.7892)
Day 21	4	2.423	0.439	(2.158, 2.689)
Day 7	4	4.6628	0.1465	(4.3972, 4.9285)

Pooled StDev = 0.243881

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	6.5744	A
Day 7	4	4.6628	B
Day 14	4	3.5235	C
Day 21	4	2.423	D

Means that do not share a letter are significantly different.

G.52 Statistical analysis of ethanol content of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: Ethanol versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.2293	0.74309	14.18	0.000
Error	12	0.6290	0.05242		
Total	15	2.8583			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.228944	77.99%	72.49%	60.88%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	3.528	0.331	(3.279, 3.778)
Day 14	4	4.408	0.208	(4.158, 4.657)
Day 21	4	4.4323	0.0792	(4.1829, 4.6817)
Day 7	4	3.934	0.225	(3.685, 4.184)

Pooled StDev = 0.228944

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
Day 21	4	4.4323	A		
Day 14	4	4.408	A	B	
Day 7	4	3.934		B	C
Day 0	4	3.528			C

Means that do not share a letter are significantly different.

G.53 Statistical analysis of sugars of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: Sucrose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.017096	0.005699	69.18	0.000
Error	12	0.000988	0.000082		
Total	15	0.018085			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0090760	94.53%	93.17%	90.28%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	0.09085	0.00924	(0.08096, 0.10074)
Day 14	4	0.05624	0.01159	(0.04635, 0.06613)
Day 21	4	0.000000	0.000000	(-0.009887, 0.009887)
Day 7	4	0.05863	0.01048	(0.04874, 0.06852)

Pooled StDev = 0.00907602

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	0.09085	A
Day 7	4	0.05863	B
Day 14	4	0.05624	B
Day 21	4	0.000000	C

Means that do not share a letter are significantly different.

One-way ANOVA: Glucose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.35652	0.118840	99.63	0.000
Error	12	0.01431	0.001193		
Total	15	0.37083			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0345363	96.14%	95.18%	93.14%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	0.4152	0.0348	(0.3775, 0.4528)
Day 14	4	0.07235	0.01356	(0.03473, 0.10998)
Day 21	4	0.03050	0.00294	(-0.00713, 0.06812)
Day 7	4	0.1643	0.0580	(0.1267, 0.2019)

Pooled StDev = 0.0345363

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
Day 0	4	0.4152	A		
Day 7	4	0.1643		B	
Day 14	4	0.07235			C
Day 21	4	0.03050			C

Means that do not share a letter are significantly different.

One-way ANOVA: Fructose versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	1.44933	0.483110	146.17	0.000
Error	12	0.03966	0.003305		
Total	15	1.48899			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0574911	97.34%	96.67%	95.26%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	1.4361	0.0791	(1.3735, 1.4988)
Day 14	4	0.8279	0.0398	(0.7653, 0.8905)
Day 21	4	0.62656	0.01720	(0.56393, 0.68919)
Day 7	4	1.0602	0.0713	(0.9976, 1.1229)

Pooled StDev = 0.0574911

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
Day 0	4	1.4361	A		
Day 7	4	1.0602		B	
Day 14	4	0.8279			C
Day 21	4	0.62656			D

Means that do not share a letter are significantly different.

G.54 Statistical analysis of organic acids of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: Lactic acid versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.015139	0.005046	42.53	0.000
Error	12	0.001424	0.000119		
Total	15	0.016563			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0108933	91.40%	89.25%	84.72%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	0.138480	0.001306	(0.126613, 0.150347)
Day 14	4	0.20924	0.01134	(0.19737, 0.22110)
Day 21	4	0.21707	0.00972	(0.20520, 0.22894)
Day 7	4	0.18127	0.01580	(0.16940, 0.19314)

Pooled StDev = 0.0108933

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 21	4	0.21707	A
Day 14	4	0.20924	A
Day 7	4	0.18127	B
Day 0	4	0.138480	C

Means that do not share a letter are significantly different.

One-way ANOVA: Acetic acid versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0.000263	0.000088	0.31	0.816
Error	12	0.003359	0.000280		
Total	15	0.003622			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0167307	7.26%	0.00%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	0.25531	0.00599	(0.23708, 0.27353)
Day 14	4	0.24800	0.00475	(0.22978, 0.26623)
Day 21	4	0.244061	0.001014	(0.225834, 0.262287)
Day 7	4	0.2501	0.0326	(0.2318, 0.2683)

Pooled StDev = 0.0167307

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	4	0.25531	A
Day 7	4	0.2501	A
Day 14	4	0.24800	A
Day 21	4	0.244061	A

Means that do not share a letter are significantly different.

G.55 Statistical analysis of rutin of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: Rutin versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	35.7913	11.9304	467.26	0.000
Error	12	0.3064	0.0255		
Total	15	36.0977			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.159789	99.15%	98.94%	98.49%

Means

Time	N	Mean	StDev	95% CI
Day 0	4	7.154	0.278	(6.980, 7.328)
Day 14	4	9.9532	0.0408	(9.7792, 10.1273)
Day 21	4	11.0646	0.1405	(10.8905, 11.2387)
Day 7	4	8.3416	0.0572	(8.1676, 8.5157)

Pooled StDev = 0.159789

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 21	4	11.0646	A
Day 14	4	9.9532	B
Day 7	4	8.3416	C
Day 0	4	7.154	D

Means that do not share a letter are significantly different.

G.56 Statistical analysis of sensory evaluation of jujube water kefir beverage K4 (20/27) during storage (4°C) for three weeks.

One-way ANOVA: Appearance versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	5.700	1.900	0.88	0.456
Error	116	251.467	2.168		
Total	119	257.167			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47235	2.22%	0.00%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.133	1.479	(5.601, 6.666)
Day 14	30	6.133	1.634	(5.601, 6.666)
Day 21	30	5.733	1.552	(5.201, 6.266)
Day 7	30	6.333	1.184	(5.801, 6.866)

Pooled StDev = 1.47235

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 7	30	6.333	A
Day 14	30	6.133	A
Day 0	30	6.133	A
Day 21	30	5.733	A

Means that do not share a letter are significantly different.

One-way ANOVA: Odour versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	2.025	0.6750	0.31	0.818
Error	116	252.767	2.1790		
Total	119	254.792			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47615	0.79%	0.00%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.133	1.279	(5.600, 6.667)
Day 14	30	5.967	1.542	(5.433, 6.500)
Day 21	30	5.767	1.357	(5.233, 6.300)
Day 7	30	5.967	1.691	(5.433, 6.500)

Pooled StDev = 1.47615

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	30	6.133	A
Day 7	30	5.967	A
Day 14	30	5.967	A
Day 21	30	5.767	A

Means that do not share a letter are significantly different.

One-way ANOVA: Flavour versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	26.30	8.767	2.86	0.040
Error	116	356.07	3.070		
Total	119	382.37			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.75201	6.88%	4.47%	0.35%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.400	1.303	(5.766, 7.034)
Day 14	30	5.567	1.851	(4.933, 6.200)
Day 21	30	5.100	1.863	(4.466, 5.734)
Day 7	30	5.800	1.919	(5.166, 6.434)

Pooled StDev = 1.75201

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
Day 0	30	6.400	A	
Day 7	30	5.800	A	B
Day 14	30	5.567	A	B
Day 21	30	5.100		B

Means that do not share a letter are significantly different.

One-way ANOVA: Sweetness versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	11.40	3.800	1.40	0.245
Error	116	314.07	2.707		
Total	119	325.47			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.64544	3.50%	1.01%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.000	1.203	(5.405, 6.595)
Day 14	30	5.367	1.712	(4.772, 5.962)
Day 21	30	5.200	1.955	(4.605, 5.795)
Day 7	30	5.700	1.622	(5.105, 6.295)

Pooled StDev = 1.64544

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	30	6.000	A
Day 7	30	5.700	A
Day 14	30	5.367	A
Day 21	30	5.200	A

Means that do not share a letter are significantly different.

One-way ANOVA: Sourness versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	14.57	4.856	1.82	0.147
Error	116	309.40	2.667		
Total	119	323.97			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.63317	4.50%	2.03%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.133	1.383	(5.543, 6.724)
Day 14	30	5.433	1.478	(4.843, 6.024)
Day 21	30	5.267	1.946	(4.676, 5.857)
Day 7	30	5.900	1.668	(5.309, 6.491)

Pooled StDev = 1.63317

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	30	6.133	A
Day 7	30	5.900	A
Day 14	30	5.433	A
Day 21	30	5.267	A

Means that do not share a letter are significantly different.

One-way ANOVA: Overall acceptability versus Time

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time	4	Day 0, Day 14, Day 21, Day 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	21.13	7.044	2.60	0.056
Error	116	314.73	2.713		
Total	119	335.87			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.64718	6.29%	3.87%	0.00%

Means

Time	N	Mean	StDev	95% CI
Day 0	30	6.333	1.269	(5.738, 6.929)
Day 14	30	5.500	1.737	(4.904, 6.096)
Day 21	30	5.400	1.831	(4.804, 5.996)
Day 7	30	6.233	1.695	(5.638, 6.829)

Pooled StDev = 1.64718

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
Day 0	30	6.333	A
Day 7	30	6.233	A
Day 14	30	5.500	A
Day 21	30	5.400	A

Means that do not share a letter are significantly different.