Development of a probiotic beverage using breadfruit flour as a substrate

Yifeng Gao

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, "Development of a probiotic beverage using breadfruit flour as a substrate", contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Name: ……**yifeng gao**……..

Signed: …… …….

Date: ……23/01/2018…….

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List of abbreviations

BS	bacteria species
BF	breadfruit
BFF	breadfruit flour
CFU	colony forming unit
DMD	D-optimal mixture design
FT	fermentation time
GC-MS	gas chromatography–mass spectrometry
HHP	high-hydrostatic pressure
HPLC	high-performance liquid chromatography
H	hour
LA	lactic acid
L. acidophilus / LA	*Lactobacillus acidophilus*
L. casei / LC	*Lactobacillus casei*
L. plantarum / L.P	*Lactobacillus. plantarum*
LSD	least significant difference
L	litre
MCF	methyl chloroformate
De Man, Rogosa, Sharpe	MRS
ANOVA	one-way analysis of variance
PCA	principle components analysis
PM	projective mapping

RSM	response surface methodology
S	sugar
TA	titratable acidity
V	volume
Wt	weight

Abstract

The development of non-dairy probiotic beverages has been of great interest in recent years. The main driver for growth of lactose-free food is driven by the increased incidences of lactose intolerant individuals. The aim of this research was to develop a probiotic breadfruit substrate beverage and to examine how microbiological, physicochemical and sensory characteristics of the beverage changes with different fermentation conditions. In the preliminary study, the ability of *Lactobacillus plantarum DPC206, Lactobacillus acidophilus* and *Lactobacillus casei* and their mixed stains to grow in a breadfruit substrate media was investigated. Mixed strains of *Lactobacillus acidophilus* and *Lactobacillus plantarum DPC206* yielded satisfactory probiotic value of over 7 $\log_{10}$ CFU/mL after 24 h fermentation with 5% breadfruit flour and 10% sugar. Preliminary results further showed that beverages containing *Lactobacillus plantarum DPC206* or *Lactobacillus acidophilus* and *Lactobacillus plantarum DPC206* were positively described in terms of sensory characteristics. However, beverage containing *Lactobacillus casei* presented undesirable flavour. Based on the results, *Lactobacillus acidophilus and Lactobacillus plantarum DPC 206* were selected as starter culture for the optimization of fermented breadfruit substrate beverage.

In the optimization of fermented beverage using *Lactobacillus acidophilus* and *Lactobacillus plantarum DPC206*, four different levels of breadfruit (2 to 7%), sucrose (5 to 15%), fermentation temperature (30 to 37℃) and inoculum concentration (1 to 3%), were investigated by applying the D-optimal mixture design. The effects of fermentation

parameters on cell viability, pH, titratable acidity, sugar concentration and lactic acid in beverages were determined. Results using the D-optimal mixture design showed that sugar, inoculum concentration and proportion of breadfruit flour significantly influenced cell viability. The optimized values based on the contour plots generated were: 7% breadfruit flour, 1% inoculum, and 15% sugar after fermentation at 30℃ for 48 h. CFU of fermented beverage was positively correlated with sugar increase. Interactions between amount of sugar and proportion of breadfruit flour, as well as inoculum and proportion of breadfruit flour were negatively correlated with titratable acidity and lactic acid, respectively.

Sensory evaluation was further carried out on six different breadfruit substrate beverages using projective mapping and measuring sensory acceptance. Results showed that the fermented breadfruit substrate beverage was characterized by a pale-yellow appearance, fruity flavour, and sweet and sour taste. The hedonic test was carried out liking of appearance, odour, flavour, aftertaste and overall liking. Liking was not significantly different ($p > 0.05$) for almost all samples except for the formulation 4, which contained 7% breadfruit, 3% inoculum, and 5% sugar, and were described as bitter and had the least acceptance. The most obvious finding to come out from this research is the development of a novel fermented breadfruit-based beverage with acceptable sensory characteristics and cell viability using a mixture strain of *L. acidophilus* and *L. plantarum DPC 206.*

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Chapter 1. Introduction

Health-promoting food products have a direct positive effect on consumer food choice behaviour. The global market of probiotic foods in the twenty first century generated over 30 billion USD, and this quickly grew to over 73 billion USD in 2005, with an estimated market is estimated growth potential of 11.7% per year (de Souza Neves Ellendersen, Granato, Bigetti Guergoletto, & Wosiacki, 2012; Dongmo, Procopio, Sacher, & Becker, 2016; Gökmen, Acar, & TAYDAS, 2003). Some factors like lactose free, low cholesterol, non-alcoholic and medical effects have increased consumers interest in functional foods (Dongmo, Sacher, Kollmannsberger, & Becker, 2017). Food products that can offer specific active health components include prebiotics, probiotics and synbiotics (Shanahan, 2002).

The most common functional foods manufactured are probiotic foods and beverages. *Lactobacillus* and *Bifidobacterium* are two common probiotic bacteria are applied in functional food. Probiotics as a live microbial can balance and adjust host intestinal microorganism (Joint, 2001). Particularly, it has been recorded that probiotics can enhance immune-system function and improve overall health (Luckow & Delahunty, 2004). Probiotic microorganisms when present in the gastrointestinal tract help create a favorable microbial condition to enhance digestive function (Rivera-Espinoza & Gallardo-Navarro, 2010). Prebiotics are digestible to the probiotic bacteria and can further enhance the benefits of probiotic bacteria for maintaining a healthy immune system (Luckow & Delahunty, 2004). Tripathi and Giri (2014) estimated that probiotic

foods, particular from lactic acid bacteria, take up to 60 and 70% of the whole probiotic food market. For health benefits, the minimum dose of 10^7 colony forming per unit (CFU)/g or mL probiotic bacteria in food is recommended (Madureira, Amorim, Gomes, Pintado, & Malcata, 2011; Sanz & Dalmau, 2008).

For centuries, probiotics have been introduced to dairy products such as yoghurt (Rivera-Espinoza & Gallardo-Navarro, 2010). Dairy-based fermented products with lactic acid microorganisms can improve shelf life and nutritional quality (Hati, Mandal, & Prajapati, 2013). Commercial functional foods in food market are mainly dairy-based products, although consumers are increasingly requiring new functional products that are non-dairy (Flávera C Prado, Parada, Pandey, & Soccol, 2008). Fruits and vegetables due to essential nutrient composition may thus serve as the carriers of probiotic bacteria (Salmerón, Thomas, & Pandiella, 2015).

Fruits can be used to develop alternative non-dairy functional foods with increasing incidences of lactose intolerance and allergy (Flávera C Prado et al., 2008). Fruit-based beverages containing lactic acid bacteria also have an established market sector, and their functional properties have been scientifically demonstrated.

Breadfruit is a nutritious fruit cultivated by Pacific Islanders over 3000 years and is abundantly found in Polynesia, Jamaica, and the Caribbean Islands (H. A. Bakare, Osundahunsi, Adegunwa, & Olusanya, 2013). Breadfruit (*Artocarpus altilis*) belongs to

the *Moraceae* family and consists of more than 50 species. Amusa, Kehinde, and Ashaye (2002) reported that breadfruit can be propagated through stem-cuttings and the first fruiting average period of the crop is from 4 to 6 years. Every year, a single breadfruit tree produces fresh fruit 150-200 Kg or more, usually ovoid or oblong (Singh, 2009). It is now recognized as a staple food and can be consumed either cooked, roasted, fried, boiled, dried, or pickled (Turi, Liu, Ragone, & Murch, 2015). This abundant staple food has the potential to solve the problem of hunger that is rife in some developing countries (Adebowale, Ajayi, & Ibikunle, 2013; A Maxwell P Jones, Murch, Wiseman, & Ragone, 2013).

Technological processes have been introduced to produce various breadfruit derivative products including cookie, bread and flours because of poor storage properties of whole breadfruit (de Souza, Soares, Queiroz, dos Santos, & Ferreira, 2016). Breadfruit when processed into flour, can further increase shelf life and enhance its versatility when included in food products (Oladunjoye, Ologhobo, & Olaniyi, 2010). Ragone and Cavaletto (2006) have reported presence of abundant minerals in breadfruit like calcium, sodium, phosphorus, boron, magnesium, and copper.

Recently, there has been increasing interest in the utilized of probiotic strains for the development of non-dairy fermented beverages. Studies however have not investigated the incorporation of breadfruit flour as a substrate for lactic acid fermentation. Hence this study aims to investigate lactic acid fermentation using breadfruit as substrate and

monitor the changes in physiochemical properties (pH, organic acids, sugar concentration), and acceptability of selected fermented beverages. The contribution of this study is obvious as the resulting outcomes can be capitalized to provide a novel way of producing a novel breadfruit-based functional beverage.

Chapter 2. Literature Review

2.1 Current applications of breadfruit

Breadfruit is gaining recognition as a staple food and are found in Africa (Adekanmi Adeniran, Gbadamosi, & Omobuwajo, 2012). Breadfruit (*Artocarpus altilis*) is relatively cheap and nutritious. Breadfruit contains 68% starch, 4% protein, and 1% fat (Andrew Maxwell Phineas Jones, Ragone, Bernotas, & Murch, 2011). Akanbi, Saari, Adebowale, Farooq, and Olaoye (2011) have shown that breadfruit can prevent an onset of type II diabetes because of its high amylose content that can assist control of sugar levels in blood. In the last decade, breadfruit flour has found applications in bread, cake, biscuits, noodles, and fermented food as shown in Table 1.

Table1: Summary of breadfruit flour applications	
Applications	References
Noodles	Adebowale, Salaam, Komolafe, Adebiyi, and Ilesanmi (2017); Purwandari et al. (2014); Akanbi et al. (2011)
Cakes	H. A. Bakare et al. (2013); Eke-Ejiofor (2013)
Bread	A. H. Bakare, Osundahunsi, and Olusanya (2015); Malomo, Eleyinmi, and Fashakin (2011)
Biscuits	Omobuwajo (2003); A. H. Bakare, Adegunwa, Akinribido, and Obadina (2014); Adebowale et al. (2013)
Fermented food	Adekanmi Adeniran et al. (2012); Adeniran and Ajifolokun (2015)

Breadfruit flour has been widely used to make noodles products that are gluten-free. Purwandari et al. (2014) have studied the effect of physical and sensory properties of noodles made from breadfruit flour. They found that hardness, cooking loss and adhesiveness of breadfruit noodles were higher than wheat noodle necessitating the incorporation of a texturing agent. In another study, Akanbi et al. (2011) found that noodles made from blend 80% wheat flour and 20% breadfruit starch showed superior sensory attributes and culinary properties. Adebowale et al. (2017) further demonstrated that wheat flour can be mixed with up to around 30% breadfruit starch without

significantly affecting the overall acceptance of noodles.

A. H. Bakare et al. (2014) pointed that wheat flour could be partially substituted up to 20% with fermented breadfruit flour to make biscuits without significant changes in taste, appearance, texture and crispiness. The biscuit supplemented with breadfruit was found to improved dietary fiber and minerals content. Omobuwajo (2003) reported no obvious changes in flavour, crispness and overall acceptability between the biscuit made from 100% wheat flour and wheat flour mixed with breadfruit flour (67% wheat and 23% breadfruit). In another study, Adebowale et al. (2013) examined acceptability of biscuits made with a blend of African breadfruit flour and green plantain flour. The addition of green plantain flour of more than 15% had a significant negative effect on biscuit acceptability.

Malomo et al. (2011) investigated the acceptability of bread made from a mixture of wheat and breadfruit flour. The bread produced using a mixture of 5% breadfruit flour and 95% was the most acceptable, and had increased protein and minerals with reduced anti-nutrient content. Incorporation of breadfruit flour influences dough characteristics. A. H. Bakare et al. (2015) reported that dough characteristics changed when more than 5% breadfruit flour was added into wheat flour. However, acceptability of bread was similar to bread produced from wheat flour within 15% substitution levels of breadfruit flour.

Addition of breadfruit flour into wheat flour to make cake has also been investigated by

H. A. Bakare et al. (2013). There was little change in cake quality when reducing the proportion of wheat flour. Cake produced by incorporation of 30% breadfruit flour did not alter the baking qualities of cake. Cake made from up to a 40% of breadfruit flour were acceptable. Eke-Ejiofor (2013) further showed that acceptability cake did not change when wheat flour was supplemented with African breadfruit flour (10%) and sweet potato flour (10%).

Breadfruit has also been used in producing a *fufu* and *gari* analogue by fermentation. *Fufu* is made from cassava flour and can be further processed to *gari* that is a dehydrated coarse flour that is a local staple food from South of Nigerians (Obadina, Oyewole, Sanni, Tomlins, & Westby, 2008). Adekanmi Adeniran et al. (2012) further showed that *fufu* made from 10% breadfruit and 90% cassava did not influence physical and sensory properties. In another study, Adeniran and Ajifolokun (2015) further reported that 20% of breadfruit co-fermented with cassava had favourable acceptability compared to 100% cassava *gari.*

Overall, breadfruit is commonly processed into flour, which is more stable to store and can be incorporated into a variety of food products such as noodle (Adebowale et al., 2017; Akanbi et al., 2011; Purwandari et al., 2014), biscuit (A. H. Bakare et al., 2014; Omobuwajo, 2003), bread (A. H. Bakare et al., 2015; Malomo et al., 2011), cake (H. A. Bakare et al., 2013; Eke-Ejiofor, 2013) and fermented food (Adekanmi Adeniran et al., 2012; Adeniran & Ajifolokun, 2015). Breadfruit flour can also be incorporated with flour

such as wheat flour (Adebowale et al., 2013), cassava flour (Adekanmi Adeniran et al., 2012) and green plantain flour (Adebowale et al., 2013) to boost the nutrient composition of food products without affecting food products acceptability. However, commercial potential of breadfruit applied in food market is ignored. Utilization of breadfruit is suitable for gluten intolerant or health conscious individuals who want to be gluten-free. Priven, Baum, Vieira, Fung, and Herbold (2015) carried out a survey that reported 25.5% participants perceiving gluten–free products as healthier.

2.2 Current trends in non-dairy probiotic beverage

Recently, development of non-dairy probiotic substrate beverages as an alternative food choice to traditional functional beverages has been explored. Vegetables such as fermented red beet juice (Chwastek, Klewicka, Klewicki, & Sójka, 2015), and cereals juice such as oat-based probiotic drink (Angelov, Gotcheva, Kuncheva, & Hristozova, 2006) can be an ideal vehicle to deliver probiotics to customers (Kun, Rezessy-Szabó, Nguyen, & Hoschke, 2008). To ensure health benefits, it is important to maintain viability cells in the non-dairy beverage during production, storage and gastrointestinal digestion (Tannock et al., 2000).

2.3 The health benefits of probiotics

Probiotic bacteria has been associated with beneficial health effects in the host (Di Pierro, Adami, Rapacioli, Giardini, & Streitberger, 2013). The main health benefit seems to be on the maintenance and balance of the host intestinal microorganisms and inhibition of

gastrointestinal pathogens (Gibson, Rastall, & Fuller, 2003). Further, studies have demonstrated immunomodulation (Ouwehand, Lagström, Suomalainen, & Salminen, 2002), reduction of blood cholesterol (Ooi & Liong, 2010) and anti-cancer (Haghshenas et al., 2014) effects of probiotic bacteria. Some of the functionalities of probiotics are summarized in Table 2.

Table 2: Functionality of probiotics bacteria

Probiotics bacteria	Functionality	Reference
Lactobacillus acidophilus CHO-220	Cholesterol reducing ability in hypercholesterolemic rats	Ooi and Liong (2010)
Streptococcus salivarius K12	Production of bacteriocins to protect oral health	Di Pierro et al. (2013)
Lactobacillus acidophilus	Lactose absorption in lactose intolerance patient, alleviating symptoms of lactose intolerance.	Di Pierro et al. (2013)
Lactobacillus rhamnosus	Prevention or relief of infantile diarrhea.	Gibson et al. (2003)
Bifidobacterium longum BB536	Reduction of constipation and improvement in bowel movement frequency.	Ouwehand et al. (2002)

Lactococcus lactis	Anti-cancer effects on human cancer cell	Haghshenas et al. (2014)

2.4 Application of probiotic bacteria in fruit-based beverages

Recent studies have shown that fruit juice can be an ideal carrier for the incorporation of probiotics strains in non-dairy functional beverages (Costa, Fonteles, de Jesus, & Rodrigues, 2013). Fruit juice is an ideal food substrate for growth of probiotic strains because it is high in nutrients like carbohydrates, dietary fibers, antioxidant, minerals, and vitamins (Ding & Shah, 2008). Lactic acid bacteria require various essential nutrient for growth such as vitamins and amino acids (Rivera-Espinoza & Gallardo-Navarro, 2010). The findings of previous researches on the fermentation of different fruit juices using probiotic bacteria are summarized in Table 3.

Table 3: Probiotic bacteria in fermented fruit juice.			
Fruit juice	Probiotic bacteria	Viable cell (log CFU/ml)	Reference
Litchi juice	*Lactobacillus casei*	Above 8.0	Zheng et al. (2014); Yu, Xiao, Xu, Wu, and Wen (2014)
Apple juice	*Lactobacillus plantarum*	Above 6.0	Dimitrovski, Velickova, Langerholc, and Winkelhausen (2015); de Souza Neves Ellendersen et al. (2012)
Cashew apple juice	*Lactobacillus casei NRRL B*	Above 8.0	Ana Lúcia F Pereira, Maciel, and Rodrigues (2011)
Coconut water beverage	*Lactobacillus plantarum B-7*	8.7	Flávera Camargo Prado et al. (2015)
Sonicated pineapple juice	*Lactobacillus casei NRRL B442*	Non-sweetened 6.03 sweetened sample 4.77	Costa et al. (2013)

Pomegranate juice	*Lactobacillus acidophilus,* *Lactobacillus. plantarum,* *Lactobacillus. delbrueckii,* *Lactobacillus. paracasei*	8.0 (for all strains)	Z. Mousavi, Mousavi, Razavi, Emam-Djomeh, and Kiani (2011)

High hydrostatic pressure (HHP) was applied in litchi juice and this juice substrate further fermented to produce a probiotic beverage (Table 3) as reported by Zheng et al. (2014). The results revealed that *Lactobacillus casei* attained exponential growth when it reached 8.31 log CFU/mL after 18 h fermentation. After four weeks of storage at 4℃, the viable number remained at a concentration of more than 8.0 log CFU/mL. The fermented high hydrostatic pressure treated litchi juice was found to possess better sensory properties such as colour, flavour and overall acceptance. These results were confirmed in another study performed using a dimethyl dicarbonate-treated litchi juice that was fermented by *Lactobacillus casei* (Yu et al., 2014).

Dimitrovski et al. (2015) pointed the production of a probiotic pure apple juice fermented by *Lactobacillus plantarum PCS 26*, with whey supplementation as a growth enhancer. They found 5%v/v whey mixed with apple juice produced the best probiotics, with up to three times growth rate as high as in pure apple juice. After 11 h of fermentation, the highest cell concentration of 1.3 * 10^{10} CUF/mL in apple juice by *Lactobacillus*

plantarum PCS 26 was attained at an initial pH of 5.1. The number of viable bacteria remained at the recommended level of 10^6 CUF/mL after 30 days at refrigeration temperature. In a different study, the best fermentation conditions to produce fermented pure apple juice with *Lactobacillus casei* were found to be 10 h of fermentation at 37 ℃ (de Souza Neves Ellendersen et al., 2012). Furthermore, Pimentel, Madrona, and Prudencio (2015) showed that sugar substitutes such as sucralose and oligo-fructose can be used to improve the acceptance of a functional fermented apple juice that had similar sensory properties to a sucrose-containing formulation.

Developing a probiotic cashew apple juice with the incorporation of *L. casei* was attempted by Ana Lúcia F Pereira et al. (2011). The optimal fermentation conditions observed was at a fermentation temperature of 30℃, 16 h incubation and an inoculation level of 7.48 log CFU/mL. The colour of fermented cashew apple juice was enhanced after six weeks of refrigerated storage, with the viable count of *Lactobacillus casei* was still greater than 8 CFU/mL. Consistent with the findings of Ana Lúcia Fernandes Pereira, Almeida, de Jesus, da Costa, and Rodrigues (2013), the characteristic color (yellowness) of fermented cashew juice was maintained and was well accepted by consumers.

According to Costa et al. (2013), *Lactobacillus casei NRRL B442* grew well in pineapple juice that was pre-treated by sonication. The viable cell was fast growing between 8 and10 h, with the number of lactic acid bacteria over 8 log CFU/mL. The bacteria demonstrated rapid growth in sonicated pineapple juice within 8-10 h producing a high viable cell

concentration of 8 log CUF/mL. After six weeks of cold storage, non-sweetened sample showed higher microbial viability (6.03 log CFU/mL) than that of the sweetened sample (4.77 log CFU/mL). The colour of fermented beverage did not obviously change during the storage and no browning was observed. Another study by Shukla, Jha, and Admassu (2013) on pineapple juice developed a satisfactorily good quality fermentation pineapple juice using *Lactobacillus acidophilus* with whey supplementation. A blend of whey and pineapple juice produced a high number of lactic acid bacteria over 8 log CFU/mL after one day fermentation, with maintained viability of over 6 log CFU/mL after five days at 30℃. A more recent study, Sah, Vasiljevic, McKechnie, and Donkor (2015) showed that pineapple pulp, a by-product of juice production, could improve the growth and maintain the viability of the probiotic bacteria used in the fermentation (*Lactobacillus. Paracasei ATCC BAA52*). The bacteria remained viable at an acceptable concentration (7 log CFU/mL) after storage at 4 ℃ for 28 days.

Z. Mousavi et al. (2011) found that pomegranate juice as a raw fruit development a probiotic beverage using *Lactobacillus acidophilus, Lactobacillus delbruekii, Lactobacillus paracasei* and *Lactobacillus plantarum*. All the strains reached maximum viable number (10^8 CFU/mL) after two days of fermentation. After seven days of refrigerated storage, the number of *L. plantarum and L. delbruekii* remained at a recommended level of above 10^6 CFU/mL. The viability decreased to below the minimum acceptable level after two weeks of cold storage. All the lactic acid bacteria utilized monosaccharide such as fructose and glucose as the carbon energy source with

glucose utilization rate higher than that of fructose. The above results are in agreement with those obtained from the study by Z. E. Mousavi et al. (2013), who used *L. plantarum* and *L. acidophilus* used as probiotic starter that showed faster growth during fermentation of pomegranate juice. *L. plantarum* utilized more sugar in comparison to *L. acidophilus* and glucose was the preferred sugar.

Another functional fermented beverage in using green coconut water as a medium and fermented with *Lactobacillus plantarum* has been developed (Flávera Camargo Prado et al., 2015). The species demonstrated an ability to grow in coconut water (maximal viable cell 9.3 log CFU/mL) and survived in the matrix during storage. Viable cells were maintained at 8.7 log CFU/mL during four weeks of storage at 4℃. With the same objective, mature coconut water fermented with *Lactobacillus plantarum* DW12 produced an acceptable functional beverage using supplementation with monosodium glutamate (Kantachote, Ratanaburee, Hayisama-ae, Sukhoom, & Nunkaew, 2017). Adding honey produced a better taste for the beverage. More recently, Lee, Boo, and Liu (2013) showed that both *L.acidophilus L10* and *L. casei L26* grew well in coconut water to approximately 8 log CFU/mL and showed similar growth patterns. Most interestingly, mineral content in coconut water was not significantly changed during the fermentation.

In summary, fermentation can improve fruit juice nutritional, safety, and shelf-life. In fruit juice-based beverages, lactic acid bacteria were observed to grow well (Ana Lúcia F Pereira et al., 2011). The sensory quality of fermented fruit juice was shown to improve

because of organic acids and volatile compounds (de Souza Neves Ellendersen et al., 2012). However, some fermented fruit juices demonstrated lower concentration of viable bacteria than recommended after storage (Ana Lúcia Fernandes Pereira et al., 2013). High acidity and low pH mainly accounted for the reduction in number of viable bacteria (Z. Mousavi et al., 2011). From the studies reviewed, it can be seen that shelf life extension and sensory properties are also important factors to consider in the development of a probiotic fruit beverage.

2.5 Application of probiotic bacteria in vegetable-based beverages

Vegetable juice are suitable substrates for lactic acid bacteria fermentation due to their carbohydrate content (Crittenden, Martinez, & Playne, 2003). Plant tissue provides pores that favour microbial attachment and protection (Sapers, 2001). Steps in the preparation of vegetables, such as washing, peeling and cutting, can break up and release cellular nutrient content such as minerals, vitamins, and sugars, which creates the ideal condition for growth of probiotic bacteria (De Oliveira, De Souza, Bergamini, & De Martinis, 2011). Table 4 summarizes the studies carried out on the fermentation of vegetable juices using probiotic strains.

Table 4: Probiotic bacteria used in the fermentation of vegetable juices			
Vegetable based beverages	Probiotic bacteria	Viable cell (CUF/mL)	Reference
Beet juice	*Lactobacillus acidophilus,* *Lactobacillus plantarum,* *Lactobacillus delbrueckii,* *Lactobacillus casei*	10^6-10^8	Yoon, Woodams, and Hang (2005)
Cabbage juice	*Lactobacillus. PlantarumC3* *Lactobacillus.delbrueckiiD7* *Lactobacillus casei A4*	Nearly 10^8	Yoon, Woodams, and Hang (2006)

Carrot juice	*Lactobacillus rhamnosus Lactobacillus bulgaricus*	Nearly $5*10^9$	Nazzaro, Fratianni, Sada, and Orlando (2008)
Maple sap	*Lactobacillus rhamnosus Lactobacillus helveticus Bifidobacterium animals subsp.lactis*	10^8-10^{10}	Lupien-Meilleur, Roy, and Lagacé (2016)
Moringa leaves and beetroot	*Lactobacillus plantarum Enterococcus hirae*	Above 10^8	Vanajakshi, Vijayendra, Varadaraj, Venkateswaran, and Agrawal (2015)
Beetroot and carrot juice	*Lactobacillus acidophilus NCDO1748*	Above 2.95 * 10^7	Rakin, Vukasinovic, Siler-Marinkovic, and Maksimovic (2007)

Yoon et al. (2005) reported production of a probiotic beet juice fermented by *L. acidophilus, L. plantarum, L. delbrueckii* and *L. casei.* All the probiotic strains rapidly utilized beet juice for growth, reaching 10^9 CFU/mL after two days of fermentation at 30℃. *Lactobacillus acidophilus* and *Lactobacillus plantarum* quickly decreased the pH from 6.3 to 4.5 after 2 h of fermentation. Similarly, Klewicka, Motyl, and Libudzisz (2004) found that probiotic beet juice fermented by lactobacillus species (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus delbrueckii*) quickly reduced the pH to 4.5 and lower after 2 days of fermentation. The fermentation of beet juice resulted in maximum accumulation of lactic acid after six days of cultivation. Chwastek et al. (2015) further showed that fermented red beet juice mixed with highbush blueberry sucrose

osmotic syrup produced acid and had a low pH after 5 days of incubation without significant loss of recommended viable numbers of lactic acid bacteria.

Cabbage has also been used to produce a probiotic juice fermented by three lactic acid bacteria (*L. plantarum, L. casei, L. delbrueckii*) (Yoon et al., 2006). All the three species of lactic acid bacteria reached nearly 10^8 CFU/mL after two days of fermentation at 30℃. *Lactobacillus plantarum and Lactobacillus delbrueckii* were able to survive at low pH, and the number of viable cell maintaining acceptable level even after three weeks of cold storage. In contrast, *Lactobacillus casei* lost cell viability completely after two weeks of cold storage. Other studies by Jaiswal and Abu-Ghannam (2013), further showed that three *Lactobacillus* strains (*L. brevis, L. plantarum, L. rhamnosus*) had high viable cell counts (10^9-10^{10} CFU/mL) after fermentation, and maintained a high level of viable probiotics (above 10^8 CFU/mL) in cabbage juice after four weeks of storage.

Nazzaro et al. (2008) developed a functional carrot beverage and found that *Lactobacillus rhamnosus and Lactobacillus bulgaricus* grew well, reaching nearly $5 * 10^9$ CFU/mL. Both strains survived in the fermented carrot juice after 30 days of cool storage with minimal decrease in viability of $2.8 * 10^9$ CFU/mL. Similarly, Tamminen, Salminen, and Ouwehand (2013) showed that Lactobacilli levels in carrot juice remained almost unchanged after 12 weeks of cool storage. However, Bifidobacteria grew in the first two weeks, and became undetectable after 8 weeks storage. Rakin et al. (2007) further showed that brewer's yeast autolysate was added into carrot and beetroot juice and obtained a

satisfactory number of probiotic bacteria ranging from 10^7 to 10^8 CFU was obtained.

Moringa leaves in combination with beetroot juice have been fermented by *Lactobacillus plantarum* and *Enterococcus hirae* to yield a high quality probiotic beverage (Vanajakshi et al., 2015). After two days of fermentation at 37℃, the addition of beetroot juice at 1:2 ratio to *moringa* leaves produced maximum viable cell (9.98 log CFU/mL), and the viable counts of probiotic bacteria remained around 7 log CFU/mL after 30 days of storage at 4℃. Maple sap substrate also supported cell growth (10^8-10^{10} CFU/mL) of *L. rhamnosus R0011* and *Bifidobacterium animalis subsp.*(Lupien-Meilleur et al., 2016). *Lactis BB12* and maple sap concentrate sustained viability of the mixed strains at a high concentration of viability cell after prolonged storage of around 12 weeks at 4℃.

In general, vegetable based substrates are suitable to produce a probiotic beverage. Probiotic bacteria grow well in vegetable or plant substrate, and remain largely viable after cool storage. Functional vegetable-based juice usually can be stored up to four weeks at cool conditions because organic acid (mainly lactic acid) formation results in pH decrease and suppresses the growth of putrefying and pathogenic bacteria. The potential of developing fermented beverages from vegetable substrates using single lactic acid bacteria or mixed strains is becoming more attractive as consumer's demand for healthy food increases.

2.6 Application of probiotic bacteria in cereal-based beverages

Cereals have an enrich nutrient and are widely consumed as staple food worldwide. Cereals are considered healthy as they contain non-digestible carbohydrates, resistant starch, and oligosaccharides (Andersson et al., 2001).

Beverages made from cereals are important classes of fermented beverages. Probiotic strains have been used to ferment cereals grains such as oats, barley, wheat, maize or rice (Marsh, Hill, Ross, & Cotter, 2014). Cereal can provide functional components including vitamins, flavonoids, dietary fibers, phenolic compounds and antioxidants, which can inhibit oxidative stress, carcinogenesis, and hyperglycemia (T. Wang, He, & Chen, 2014). Current functional cereal-based beverages are presented in Table 5.

Table 5: Cereals-based probiotic beverages.

Cereal based beverage	Probiotic bacteria	Viable cell (CFU/mL)	Reference
Oat-based drink	*Lactobacillus plantarum B28*	$7.5*10^{10}$ - $9.3*10^{9}$	Angelov et al. (2006)
Emmer-based drink	*Lactobacillus plantarum 6E Lactobacillus rhamnosus SP1*	Around $5*10^{8}$	Coda, Rizzello, Trani, and Gobbetti (2011)
Malt-based	*Lactobacillus*	Around 10^{8}	Salmerón et al.

beverage	*plantarum*		(2015)
Mixed cereals beverage	*Lactobacillus plantarum Lactobacillus acidophilus*	Around 10^8	Rathore, Salmerón, and Pandiella (2012)

Angelov et al. (2006) reported that whole-grain oat substrate fermented by *Lactobacillus plantarum B28* had viable cell counts that reached 7.5 * 10^{10} CFU/mL, which remained high (10^6-10^7 CFU/mL) after three weeks of refrigerated storage. Interestingly, the β-glucan content in the fermented beverage remained similar throughout fermentation and storage. Gokavi, Zhang, Huang, Zhao, and Guo (2005) also found that the oat beverage fermented by *Lactobacillus plantarum* had good viable counts of between 10^7 to 10^8 CFU/mL throughout storage under refrigerated condition. Luana et al. (2014) further reported no significant alteration in the chemical compounds and sensory properties in oat beverage fermented by *Lactobacillus plantarum LP09* after 30 days of storage at 4℃.

Coda et al. (2011) reported the use of *L. plantarum 6E* and *L. rhamnosus SP1* in the preparation of emmer beverage (30% wt/wt gelatinized flour). Both probiotic bacteria remained at 5 * 10^8 CFU/mL after fermentation. After four weeks of storage at 4℃, the viable number of *Lactobacillus plantarum 6*E decreased from 2.9 to 1.3 *10^8 CFU/mL, and that of *Lactobacillus rhamnosus SP1* was reduced from 5.1 to 8.0 * 10^8 CFU/mL.

Salmerón et al. (2015) showed that a beverage formulated with a malt substrate and fermented with *Lactobacillus plantarum* had high viable cells concentration (10^8

CFU/mL) after 10 h of fermentation. Rozada-Sánchez, Sattur, Thomas, and Pandiella (2008) showed that *Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium breve and Bifidobacterium longue* grew well in a malt-based beverage (around 9log CFU/mL). Malt medium was shown to support the growth of *L. plantarum* better than barley and wheat media (Charalampopoulos, Pandiella, & Webb, 2003). Dongmo et al. (2017) further found that lactic acid bacteria strains impacted on the volatile aroma compounds in malt-based beverages. For example, *Lactobacillus plantarum* produced some key aroma compounds such as furaneol, 2-phenylethanol and ethyl 2-methybutanoate and was correlated to fruity flavour such as apple juice, strawberry, caramel and citrus.

Rathore et al. (2012) reported the production of fermented cereal substrates (malt, barley, and barley mixed with malt) with *L. plantarum (NCIMB 8826)* and *L. acidophilus (NCIMB8821*. Mixed-culture fermentations of barley and malt substrates produced similar number of viable cells, but organic acids formation was considerably lower. *Lactobacillus plantarum* grown in barley-malt mixed media had a comparable viable count to that of malt media (8.6 log CFU/mL). Similarly, Salari, Razavi, and Gharibzahedi (2014) reported better growth of *Lactobacillus delbrueckii and Lactobacillus paracasei* in mixed cereals media (barley-malt) than that in barley medium after fermentation for 15 h at 37℃. Both strains maintained the acceptable level of viability in mixed barley-malt functional beverage after two weeks of cold storage at 4℃, while *Lactobacillus paracasei* could not survive in the barley medium after two weeks of storage. Ai, Li, Su,

and Meng (2015a) used *Lactobacillus helveticus KLDS1.9204* to ferment multi-cereal substrates (a mixture of malt, rice and maize) to develop a novel beverage. The number of viable cell reached 8.43 log CFU/mL at the end of fermentation. The strains showed good proteolytic capability, but could not utilize starch, resulting in an insignificant change in the total reducing sugar concentration.

Overall, studies showed that single lactic acid bacteria cultures contributed to better flavour than mixed lactic acid bacteria in cereal drinks (Dongmo et al., 2017). The majority of lactobacilli species studies can utilize carbohydrates and produce lactic acid. *Lactobacillus helveticus* utilized simpler carbohydrates such as monosaccharides and disaccharides (Ai, Li, Su, & Meng, 2015b). Bifidobacteria grew well in the cereals substrates, but required additional growth promoters such as yeast extract to provide additional nitrogen and minerals (Rakin et al., 2007). Multi-cereal substrate beverages usually have suitable aroma, flavour and acceptability taste.

2.7 Application of sensory evaluation in non-dairy probiotic beverage

In the production of non-dairy functional beverage, lactic acid bacteria could alter products sensory properties, such as flavour, aroma, textural and taste (Tripathi & Giri, 2014). Probiotic strains produce different metabolic compounds was mainly caused sensory changes (Panghal et al., 2017). Selection of optimum probiotic microorganism is

the main challenge in food industry (Ventura & Perozzi, 2011). Safety consideration like toxicity and pathogenicity are also main factors that are important in the selection of probiotic strains (Anadón, Castellano, & Martínez-Larrañaga, 2013). In order to cater market requirement, main challenge for probiotic beverages is acceptability or unacceptability by consumer (Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011).

Purchase decision by consumers and consumption of beverages make sensory evaluation is an essential tool to understand consumers perception (King & Meiselman, 2010; Spinelli, Masi, Zoboli, Prescott, & Monteleone, 2015). Sensory evaluation involve screening, description, evaluation and final selection of food products (Meilgaard, Carr, & Civille, 2006). Application of preliminary sensory testing of food can help assess sensory properties of products. The projective mapping (PM) methodology is based on the identification of similarity and differences among products, in such a way that similarity samples are located close to each other, while different samples should be located further apart (Mielby, Hopfer, Jensen, Thybo, & Heymann, 2014).

2.8 Addition of ingredients to modify lactic bacteria fermentation

Recent studies investigated the addition of ingredients that can increase lactic acid bacteria number, maintain cell number during storage, and produce a highly acceptable non-dairy functional beverage. These ingredients added are presented in Table 6.

Table 6: Ingredients to enhance probiotic growth		
Ingredient	Function	Reference
Tea extract	Enhance stability of *Lactobacillus plantarum*	Zhao and Shah (2016)
Inulin	Fortification of viability of *Lactobacillus rhamnosus*	Gandomi, Abbaszadeh, Misaghi, Bokaie, and Noori (2016)
Brewer's yeast autolysate	Increase number of *Lactobacillus acidophilus NCDO1748*	Rakin et al. (2007)
Riboflavin	Vitamin fortification of oat-based foods	Russo et al. (2016)
Sucrose	Enhance *Lactobacillus casei* growth	Ana Lúcia Fernandes Pereira et al. (2013)

Rakin et al. (2007) found that brewer's yeast autolysate increased viable cell numbers, and resulted in a fermented vegetable beverage with acceptable pigment. In another study, Russo et al. (2016) indicated that riboflavin biofortification could be achieved in oat-based foods fermented with *Lactobacillus plantarum*. The amount of riboflavin further enhanced shelf life when products were stored at cold conditions suggesting a favourable effect on probiotic bacteria viability.

Zhao and Shah (2016) reported that lactic acid bacteria fermentation modified the phenolic composition of tea extract, enhancing the overall antioxidant capacity and increasing cellular uptake of the main tea flavonoids. Fruit juice combined with tea extract was found to improved the stability of lactic acid bacteria (Shah, Ding, Fallourd, & Leyer, 2010). Gandomi et al. (2016) further showed that apple juice fermentation fortified with

inulin, an oligosaccharide, resulted in enhanced bacteria growth and longer shelf-life of the product.

Ana Lúcia Fernandes Pereira et al. (2013) investigated that sucrose added into probiotic formulation generated a higher number of cell viability as well as acceptability sensory. However, Ana Lúcia Fernandes Pereira et al. (2013) further pointed that the addition of sucrose also could not mask the undesirable flavour. The composition of food substrates mainly influence probiotic sensory properties. It was reported that *L. casei* fermented with apple juice resulted in good sensory properties (de Souza Neves Ellendersen et al., 2012). During the fermentation, probiotic bacteria may consume sucrose mainly through chemical conversion such as malolactic conversion (Reuss et al., 2010). In previous studies, *L. plantarum* was responsible for malolactic conversion in vegetable and fruit substrates (Di Cagno et al., 2011). *L. plantarum* through the breakdown of sucrose and formation of malic acid can further degrade malic acid and produce energy for cell growth (Di Cagno et al., 2011). However, malolactic conversion did not involve all probiotic strains such as *L. acidophilus*. *L. acidophilus* strains may not involve in consume malic acid may due to it being homofermentative, and therefore did not degrade malic acid (Lee et al., 2013). *L. acidophilus* as an obligatory homofermentative strain that produces lactic acid through the glycolysis of carbohydrate, and reacts with pyruvate to produce energy (Leroy & De Vuyst, 2004).

2.9 D-optimal applied in functional food

Mixture design methodology is commonly applied in functional foods studies as it can provides valuable information on food property interactions (Afshari et al., 2015; Sarteshnizi, Hosseini, Bondarianzadeh, & Colmenero, 2015). The optimization experimental design is a common application (Bernaerts, Gysemans, Minh, & Van Impe, 2005). Statistical design tools, like response surface methodology (RSM), involve screening, selection and optimization of food formulation that have describable physicochemical and sensory characteristics (Castro, Barros, Marquez, Motizuki, & Sawada, 2005; Shiby, Radhakrishna, & Bawa, 2013). RSM can help to create models, regression equations and analyzing of the interrelations between input parameters and product properties (Garrido-Vidal, Pizarro, & González-Sáiz, 2003; Quanhong & Caili, 2005). The advantages of RSM include rational analysis, and further evaluate multiple factors and their interaction (Yin, Chen, Gu, & Han, 2009).

The D-optimal mixture design (DMD) has been applied in complex food processing, such as food fermentation (Yin et al., 2009). It is widely used in optimizing microbial growth (López, Quintana, & Fernández, 2006; Tsapatsaris & Kotzekidou, 2004), culture substrate (Didier, Etcheverrigaray, Kratje, & Goicoechea, 2007), and results are depicted in simplex coordinate systems (Arroyo-López, Bautista-Gallego, Chiesa, Durán-Quintana, & Garrido-Fernández, 2009). Each of the side points of the triangle represents a pure component, and each triangle edge represents a mixture of two factors (Arroyo-López et al., 2009). Inside the triangle, interior points are mixtures where all ingredients are present and correspond to the proportion of all ingredients (Myers, Montgomery, & Anderson-

Cook, 2016). The D-optimal design has been applied in the optimization of non-dairy functional beverage. The studies are summarized in Table 7.

Table 7. D-optimal applied in production of non-dairy functional beverage.

RSM	Functional foods	Reference
D-optimal	Functional olive juice	Tsapatsaris and Kotzekidou (2004)
	Functional grape and pomegranate	Shiby et al. (2013)
	Functional mixed fruit (carrot, pineapple, and orange) beverage	Ogundele, Awolu, Badejo, Nwachukwu, and Fagbemi (2016)
	Functional borojo beverage	Salamanca, Osorio, and Montoya (2010)

Chapter 3. Materials and methods

3.1 Breadfruit flour and experimental materials

Breadfruit flour was obtained from a local company (Maiden South Pacific Company,

New Zealand) located in Auckland and kept at 4℃ prior to use. White sugar, centrifuge tubes, MRS (De Man, Rogosa, Sharpe, Becton, Dickinson and Company) broth, and MRS (De Man, Rogosa, Sharpe, Becton, Dickinson and Company) agar, were purchased from local company (Fort Richard Laboratories, New Zealand).

3.2 Microorganisms

Three kinds of lactic acid bacteria were used in this research, namely, *Lactobacillus plantarum DPC206* (Bioactive Research, New Zealand), *Lactobacillus acidophilus* (De Winkel yoghurt, Fonterra Cooperative Group, New Zealand) and *Lactobacillus casei* (Yakult, Australia and New Zealand). Stock cultures of each species were maintained at -80℃ using a 48 hr grown-culture in MRS broth (De Man, Rogosa, Sharpe, Difco, Fort Richard Laboratories, New Zealand) containing sterile glycerol (10%) as a cryoprotectant. The stock cultures were laid down into 10 ml sterile screw bottles at 1 ml each bottle for freezing in order to activate culture next time.

3.3 Preparation of lactobacilli fermented breadfruit beverages

3.31 Preliminary studies on the selection of lactobacilli strains

Before the preparation of the breadfruit-based beverage, each strain from the stock cultures was activated by cultivation in MRS broth at 37 ℃ for 3 days in a CO_2 incubator. The steps in making the fermented breadfruit beverages are summarized in Figure 1. Before cooking, the breadfruit flour (BFF) was sterilized at 121℃ for 15 min. Sterilized

breadfruit flour (6.5%, weight/weight) was blended in 1litre (L) tap water in two 2L beakers that were boiled for 1 h. After cooling for 30 minutes, the cool slurry was centrifuged using a GYROZEN centrifuge, model 1580R (Bio-strategy, New Zealand) at 4000 x g for 30 minutes. The supernatant (300mL) was transferred into a screw cap bottle (500mL container PC Natural, Thermo Fisher Scientific Inc, New Zealand), and sterilized at 121℃ for 15 min.

In order to screen for the optimum strain, seven types of inoculum formula were prepared as shown in Table 8. Individual or mixed cultures (5%) were added into the sterilized supernatant fermented one day as starter culture. A starter culture from each type was prepared by inoculating 50 mL of breadfruit flour supernatant with a 3 days MRS broth culture of the appropriate species at 5% v/v (Table 8). The fermentation substrate consisted of sterile breadfruit flour supernatant (300mL) and white table sugar (10%) in 500 mL screw cap bottle. Each bottle was inoculated with start culture at 1% v/v. The inoculated breadfruit flour supernatants were incubated at 37℃ for 72 h. Samples (50mL) were taken at 0, 12, 24, 48, 72 h for chemical and microbiological analyses. Selection of the lactic acid strains to be used in probiotic beverage production was performed based on the viable cell number and preliminary sensory quality evaluation.

Table 8 The seven inoculums used in preliminary studies

Strains	Volume of MRS broth culture	Breadfruit extracts	Fermentation time
Lactobacillus plantarum DPC206 (L.P)	2.5 mL	50 mL	24 h
Lactobacillus casei (L.C)			
Lactobacillus acidophilus (L.A)			
L.A+L.C	(1.25 +1.25) mL		
L.A+L.P			
L.P+L.C			
L.A+L.P+L.C	(0.625+1.25+0.625) mL		

Breadfruit flour (BFF) sterilized at 121℃ for 15 min

↓

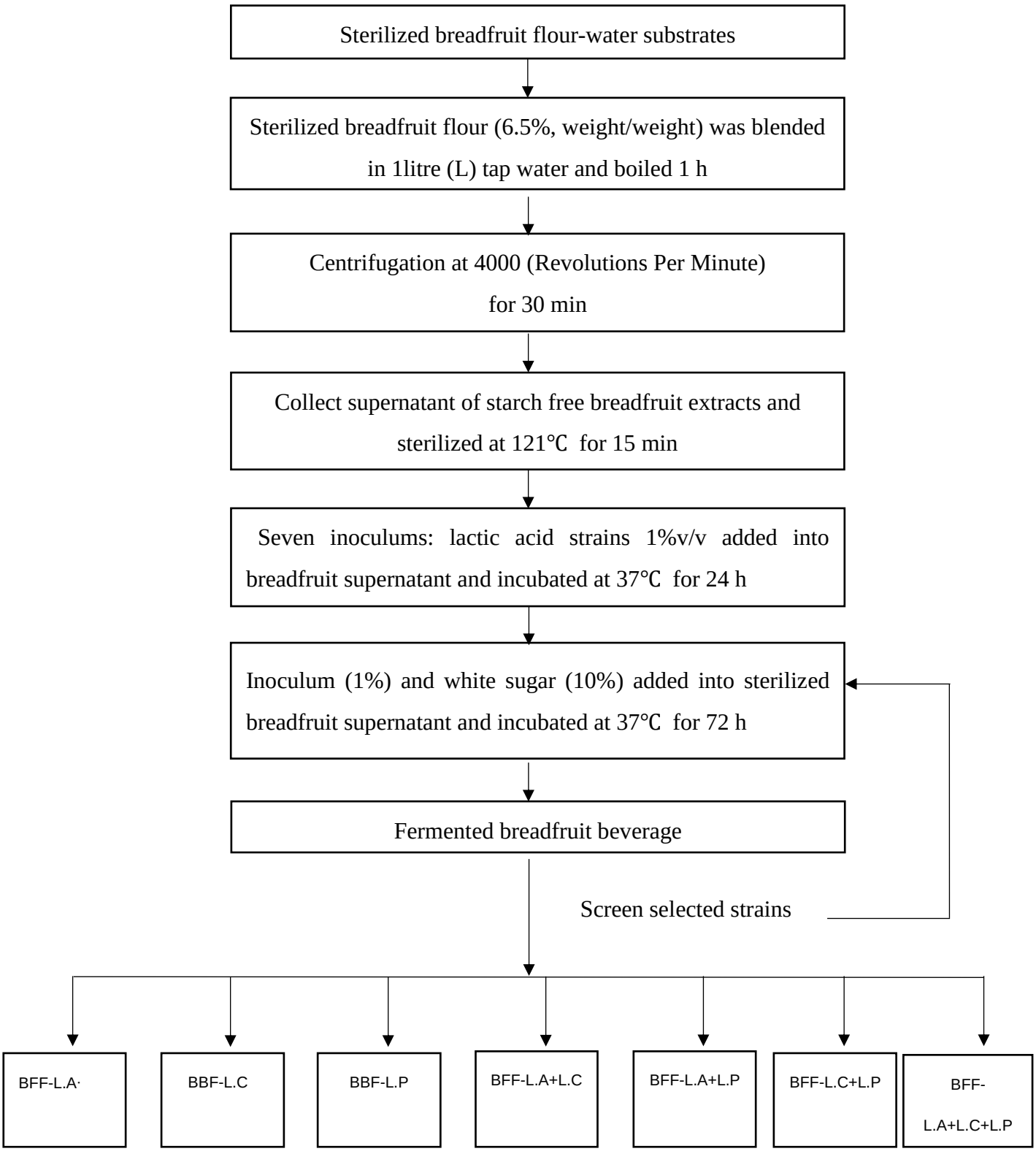

Figure 1. Production of seven lactobacilli fermented beverages formulations using breadfruit flour as a substrate.

3.3.2 Viable cell counts determination

Determination of the number of viable cell was carried out using the plate count technique. Suspensions of fermented beverage were decimally diluted in sterile peptone water up to 10^{-5} dilution. Aliquots of 0.1 mL of diluted fermented beverage were inoculated on MRS agar plates (spread plate method). The plates were incubated at 37 ℃ for 72 h in the CO_2 incubator. Plates containing 30 to 300 colonies were counted manually and recorded as colony forming units (CFU) per mL of culture. Viable cell count was obtained using triplicate plates for each beverage sampled periodically at 0, 12, 24, 48, and 72 h.

3.4 Preliminary sensory evaluation of samples

Preliminary sensory evaluation of the fermented breadfruit beverage was carried out in a sensory testing facility. Choosing seven beverage samples with maximum number of viable cell. Three-digital random numbers label on the plastic portion cups with serving beverage samples. Twelve panelists determined if there were differences in smell, colour and taste of the fermented beverages.

3.5 Determination of pH

The pH of beverages was detected whole fermentation procedures using a digital pH meter (Eutech pH 700 meter, Thermo Fisher Scientific Inc, New Zealand) with a glass electrode (Electrode ECFC7252101B, Thermo Fisher Scientific Inc, New Zealand). Before measurement, the pH meter was calibrated with buffers (Thermo Fisher Scientific Inc, New Zealand) at pH 4.0 and 7.0. pH determination was performed in triplicate for each fermented beverage sample (20ml) at 0, 12, 24, 48, and 72 h.

3.6 Experimental design for optimization of fermentation process for production of probiotic beverage.

The D-optimal design (value 0.95) was applied to investigate the influence of breadfruit, sugar, temperature and inoculum concentrations, on the growth of selected strains, and other physicochemical characteristics of the formulated beverages, using Unscrambler X v10.1 (CAMO ASA, Oslo, Norway) software. D-optimal design is meant to minimize the covariance of the parameter estimates for a specified model (Sarteshnizi et al., 2015). In this experimental design, breadfruit substrate concentration range used was 2 to 7%, sugar range was 5 to 15%, temperature range was 30 to 37℃, and inoculum concentrations from 1 to 3%. D-optimal design was utilized with the constraints for the following: Sugar (X_1) + Inoculum (X_2) + Breadfruit (X_3). Component ranges were $5 < X_1 < 15$, $1 < X_2 < 3$, and $3 < X_3 < 7$. Unscrambler designed 19 runs with duplicates (Table 9).

Fitting response was done using linear, quadratic, and cubic models. ANOVA was used to determine statistical significance of each model.

$$Y = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 \text{ (linear)}$$

$$Y = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \lambda_1 X_1 \lambda_2 X_2 + \lambda_1 X_1 \lambda_3 X_3 + \lambda_2 X_2 \lambda_3 X_3 \text{ (quadratic)}$$

$$Y = \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \lambda_1 X_1 \lambda_2 X_2 + \lambda_1 X_1 \lambda_3 X_3 + \lambda_2 X_2 \lambda_3 X_3 + \lambda_1 X_1 \lambda_2 X_2 \lambda_3 X_3 \text{ (special cubic)}$$

Where Y represents the responses of the experiment (CFU, pH, Titratable acidity, Lactic

Acid, and Sugar), λ is constant coefficients, and X is the proportions of the components.

The fermented breadfruit beverage was prepared according to the experimental design in Table 9 in different screw cap bottles (500mL, container PC Natural, Thermo Fisher Scientific Inc, New Zealand). Samples were withdrawn at fermentation times of 0, 12, 24, 48, 72 h for pH and viable cell count determinations. The breadfruit beverage fermented after 48 h was collected and put into cool storage for sensory evaluation. Samples (30 mL) for each fermentation time were transferred into tubes for chemical analysis and stored in a freezer (-80℃).

Table 9: Experimental design for formulation of probiotic beverages in this study				
Experiment number	Sugar concentration (%wt/wt)	Inoculum concentration (%wt/wt)	Breadfruit concentrations (%wt/wt)	Temperature (℃)
1	5	1	2	30
2	15	3	2	30
3	15	1	7	30
4	5	3	7	30
5	15	3	2	37
6	15	1	7	37
7	5	1	2	33.5
8	10	1	2	30
9	5	1	2	30
10	15	3	2	30
11	15	1	7	30
12	5	3	7	30
13	15	3	2	30
14	15	1	7	37
15	5	1	2	33.5
16	10	1	2	30
17	10	2	4.5	33.5
18	10	2	4.5	33.5
19	10	2	4.5	33.5

3.7 Determination of Titratable Acidity

Titratable Acidity (TA) of fermented beverages was carried out using the AOAC method (AOAC, 2002). 0.1 N NaOH solution was used as titration solution. The percentage of lactic acid as titratable acidity is determined using the following equation:

$$\%\ \textbf{Titratable Acidity, as lactic acid} = \frac{N * V * 90.08}{W * 10}$$

where: *N* = normality of titrant, 0.1 N NaOH; V = volume of titrant (mL); W = mass of breadfruit substrate beverage (g).

3.8 Determination of sugar concentration

High Performance Liquid Chromatography (HPLC, Agilent Technologies, Inc, USA) was used to analysis sugar concentration in the 48 h fermented beverages samples based on the AOAC method (AOAC, 1992). In each run, injected volume was 50 µL, quantified by a R401 refractive index detector, and separated on a Shodex Asahipak (250 * 4.6mm) column. The mobile phase was used 80% acetonitrile solution and flow rate at a 1.5 mL/min. Before injection, samples were centrifuged for 10 min at 2000 rpm and filtered through a 0.45 µm Swinney syringe filter. The refractive index detector was thermostated at 40℃ in order to analysis sugars concentration.

3.9 Determination of lactic acid concentration

The methyl chloroformate (MCF) method was used in this study to derivatize metabolites that can then be analyzed by GC-MS (Smart, Aggio, Van Houtte, & Villas-Bôas, 2010a). This method involves a fast alkylation reaction, where amino acid and non-amino organic acid are quickly reacted with MCF to form esters and carbamates formation (Smart, Aggio, Van Houtte, & Villas-Bôas, 2010b). The AMDIS (distributed by NIST) software was used to identify compounds in samples. D4-alanine was used as an internal standard

to provide a better quantification of metabolites.

Derivatized samples were analyzed by GC-MS (Model 5977B, Agilent Technologies Inc, 2013, USA) equipped with a column (Model122-5532G, length 30 m, diameter 0.250 mm, film 0.25 μm, Agilent Technologies Inc, USA). Beverage samples from different fermentation times were placed 40μL into silanized inert (NTSC4010-S629, Thermo Fisher Scientific Inc, New Zealand) and placed inside an autosampler vial (THC11090520, Thermo Fisher Scientific Inc, New Zealand) with a magnetic cap. Autosampler (MultiPurpose sampler, Gerstel, part no:013863-000-02, USA) was used to extract sample to derivatization. After MCF derivatization, GC-MS analysis was carried out using a temperature program that started with 4 min at 30℃, followed by a 10 ℃/min increase to 250 ℃, and maintained at 250 ℃ for 3 min. The mobile phase used was helium at a flow rate of 54.4 mL/min. The concentration of lactic acid was determined by preparation standard solution (concentration: 0.1, 0.05, 0.01, 0.005, 0.001 g/L).

3.10 Sensory evaluation

Sensory testing of fermented breadfruit beverage samples was performed using sensory projective mapping and consumer testing.

Projective mapping

Projective mapping is a descriptive technique to describe samples.(de Souza Neves Ellendersen et al., 2012). The panelists compared and described the product. This method

required panelists listed the samples own attributes (descriptor). Projective mapping was carried out by a semi-trained panel composed of 17 panelists (aged from 20 to 29, with equal males and females). Six different beverage samples fermented for 48 h were selected. Panelists were served 20 mL each sample in a 30 mL plastic portion cup that were coded with three-digital random numbers and served in a random order at room temperature. Panelists were also asked to described attributes that differentiated beverage samples.

Consumer testing

Consumer testing was carried out by 48 consumer panelists. Panelists evaluated liking of beverages in terms of overall liking, appearance, odour, flavour, texture and aftertaste. Furthermore, panelists were required to rate liking of selected attribute. Data was obtained using the FIZZ Acquisition system (Biosystemes, France). Panelists rated each term on an unstructured line scale, anchored "extremely dislike" on the left and "extremely like" on the right. After evaluation of samples for each term, participants were given a compulsory 45-second break.

3.11 Statistical analysis.

All experiments were carried out in triplicate and analysis was carried out at least twice. The results (CFU, pH, lactic acid) were presented as average ± standard deviation. The data were analyzed statistically using the XLSTAT software (version 2016.2). Two-way analysis of variance (ANOVA) analysis was carried out at a significance level of 95%. If

the results are significant, Fisher's Least Significant Difference (LSD) Post Hoc Test ($p < 0.05$) method was further carried out to determine any significant differences between fermentation times. Experiment design data were analyzed using Unscrambler X v10.1 (CAMO ASA, Oslo, Norway) software. A multivariate analysis of sample sensory characteristics was conducted using a principal components analysis (PCA) (de Souza Neves Ellendersen et al., 2012). PCA was further used to describe projective mapping data and hedonic data.

4. Results and Discussion

In this study, a non-dairy probiotic drink was developed using a breadfruit flour substrate. Preliminary studies were carried out to determine suitable starter cultures with acceptable sensory character. Then, a mixture experimental design was generated using the Unscrambler X v10.1 (CAMO ASA, Oslo, Norway) software for optimization of fermented beverage in terms of breadfruit (2 to 7%), inoculum (1 to 3%), and sugar (5 to 15%). The changes in lactic acid bacteria numbers, pH, titratable acidity, lactic acid, and sugar content were determined.

4.1 Growth of lactic acid bacteria

Three probiotic strains (*L. acidophilus, L. casei* and *L. plantarum DPC206)* were selected for fermentation of a probiotic drink using a water extract of breadfruit flour fermented at 37℃ from 0 to 72 h. The viability of cells during the fermentation are presented in Table 10. As shown in Table 10, *L. acidophilus, L. casei* and *L. plantarum DPC206* were capable of utilizing breadfruit substrate for growth and organic acid production. After fermentation from 48 to 72 h, the maximum number of the three lactobacilli were between 7.931 and 8.029 $\log_{10}$ CFU/mL with no significant difference ($p > 0.05$). The most rapid growth occurred with *L. acidophilus*, which started off with the lowest viable cell number and reached a maximum of 8.029 $\log_{10}$ CFU/mL after 72 h fermentation. *L. casei and L. plantarum DPC206* showed similar maximum cell viability in the fermented beverage. *L. plantarum DPC206* started with a relatively low viable cell number and reached a maximum after two days fermentation, while numbers of *L. casei* reached a maximum

after 72 h fermentation.

In the four groups of mixed strains fermentation (*L. acidophilus* + *L. casei*, *L. acidophilus* + *L. plantarum DPC206*, *L. casei* + *L. plantarum DPC206*, *L. acidophilus* + *L. casei* + *L. plantarum DPC206*), the maximum number of the mixed lactobacilli were between 7.962 and 8.238 $\log_{10}$ CFU/mL. The beverages fermented with a mixture of three lactobacilli presented the highest cell counts (8.238 $\log_{10}$ CFU/mL) as compared to those fermented with a mixture of two lactobacilli. *L. acidophilus* with *L. casei* and *L. acidophilus* with *L. plantarum DPC206*, showed similar characteristics in bacteria growth. During the fermentation, both groups reached maximum viable counts, with *L. acidophilus* and *L. casei* showing a higher growth at 8.014 Log_{10} CFU/mL than a mixture of *L. acidophilus* and *L. plantarum DPC206* (7.962 Log_{10} CFU/mL), but with no significantly difference ($p > 0.05$). After 24 h fermentation, the viability of both groups showed moderate decrease between 7.435 and 7.701 Log_{10} CFU/mL. The *L. casei* and *L. plantarum DPC206* group as well as the mixture strains group of three lactobacilli at 48 h fermentation, showed similar bacteria growth and achieved high number of viable cell counts of 8.22 Log_{10} CFU/mL and 8.238 Log_{10} CFU/mL, respectively.

For all seven groups of individual probiotic bacteria and their mixtures, the cell concentration of samples showed no significant difference ($p > 0.05$) when fermented for 72 h. A significantly ($p < 0.05$) low cell concentration of *L. acidophilus* was found at 24 h, compared to other groups. Significantly ($p < 0.05$) rapid growth occurred for most

strains for *L. acidophilus* and *L. plantarum DPC206* from 0 to 12 h. Cell viability increased in the early stage of fermentation and contained enough probiotics (7 log_{10}CFU/mL). This result was in agreement with similar previous studies (Angelov et al., 2006; Helland, Wicklund, & Narvhus, 2004). Z. Mousavi et al. (2011) reported that once lactic acid bacteria, such as *L. acidophilus* and *L. plantarum* have successfully grew under new conditions, they enter the exponential growing phase. For all seven groups, no significant difference ($p > 0.05$) were observed between 12 and 24 h. When probiotic grew up to maximum, the viability of probiotic bacteria experienced a slight loss because of the production of inhibition substances such as lactic acid (Gökmen et al., 2003). Usually, the growth of capacity of *L. acidophilus* and *L. plantarum* mainly depend on the nutrient content in the medium (Gokavi et al., 2005). Probiotic species and fermentation time were however significantly influenced in terms of cell concentration (F value 5.82^{**}, 96.7^{**}, $p < 0.01$ in Table 10, respectively).

For all seven groups of individual probiotic bacteria and their mixtures, the total number of viable cells were over 7 Log_{10} CFU/ml in the final product. Thus, the results demonstrated that the selected lactobacilli were able to grow in breadfruit substrate beverages successfully. The results also showed that mixed culture of lactic acid bacteria grew faster than single lactic acid bacteria in the breadfruit substrates beverages. Mixed cultures have been reported to contain more than the recommended probiotic cell level (7 Log_{10} CFU/mL) after fermentation (Madureira et al., 2011). For all seven groups, the cell concentration of samples showed significant ($p < 0.05$) increase between 0 and 12 h.

Breadfruit has been reported to fulfill the nutritional requirement of lactic acid bacteria (Meilleur, Jones, Titchenal, & Huang, 2004). However with fermentation, cell concentration increase can slow down due to the decrease in metabolite formation that may result in accumulation of toxic compounds that can retard growth (Gajewska & Blaszczyk, 2012). In general, mixed cultures are involved in the interaction mechanism that may stimulate or inhibit strains growth. (Angelov, Gotcheva, Hristozova, & Gargova, 2005). Mixed strains presented fast growth that could be due to the interaction mechanism that can produce different metabolites. For example, *L. acidophilus* is a homofermentative bacteria that produces lactic acid by glycolysis ((Rathore et al., 2012). *L. plantarum* is a heterofermentative bacteria that produces lactic acid and others end-products (Rathore et al., 2012). During fermentation, these metabolites can stimulate the growth of mixed cultures. In conclusion, the three lactobacilli strains and mixed strains used in this study exhibited good adaptation to the breadfruit substrates, and the viability of cells in the fermented beverages yielded a satisfactory probiotic value.

Table 10. The number of bacteria cells in fermented breadfruit beverage over 72 h of fermentation.

Bacteria Species (BS)	*Fermentation time (FT) /H (Log$_{10}$ CFU / mL)*					*F VALUE*		
	0	12	24	48	72	BS	FT	BS*FT
L. acidophilus	5.275 ± 0.052^{Cd}	6.761 ± 0.031^{Bc}	6.846 ± 0.031^{Bbc}	7.379 ± 0.338^{Ab}	8.029 ± 0.096^{Aa}	5.82*	96.7*	1.155
L. casei	6.055 ± 0.222^{Ab}	7.48 ± 0.474^{Aa}	7.597 ± 0.432^{Aa}	7.845 ± 0.239^{Aa}	7.952 ± 0.247^{Aa}			
L. plantarum DPC 206	5.555 ± 0.093^{BCb}	7.856 ± 0.157^{Aa}	7.888 ± 0.156^{Aa}	7.931 ± 0.118^{Aa}	7.764 ± 0.121^{Aa}			
L. acidophilus + L. casei	5.857 ± 0.091^{ABb}	7.994 ± 0.188^{Aa}	8.014 ±0.217^{Aa}	7.644 ± 0.571^{Aa}	7.435 ± 0.688^{Aa}			
L. acidophilus + L. plantarum DPC206	5.955 ± 0.231^{ABb}	7.826 ± 0.196^{Aa}	7.872 ± 0.103^{Aa}	7.962 ± 0.173^{Aa}	7.701 ± 0.297^{Aa}			
L. casei + L. plantarum DPC206	5.868 ± 0.031^{ABb}	7.957 ± 0.091^{Aa}	8.126 ± 0.122^{Aa}	8.220 ± 0.166^{Aa}	7.998 ± 0.229^{Aa}			
L. acidophilus+ L. casei + L. plantarum DPC206	5.847 ± 0.139^{ABb}	8.003 ± 0.142^{Aa}	8.098 ± 0.083^{Aa}	8.238 ± 0.112^{Aa}	8.106 ± 0.198^{Aa}			

The values given above are reported as means and standard deviations. Values with a different letter are significantly different ($p<0.05$) according to the Fisher's Least Significant Difference (LSD) Post Hoc Test. Uppercase superscript represent a statistically significant effect within column and lowercase superscripts across each row. * symbol represents p value (*$p < 0.01$,

** p< 0.001).

Table 11. The changes in pH value during the 72 h fermentation in breadfruit (5%) fermented beverages.

Bacteria Species (BS)	Fermentation time (Hour)					F value		
	0	12	24	48	72	BS	FT	BS*FT
L. acidophilus	5.41 ± 0.02^{ABa}	5.24 ± 0.01^{Ab}	4.94 ± 0.02^{Ac}	4.70 ± 0.05^{Ad}	4.62 ± 0.09^{Ad}	92.1^{***}	549.8^{***}	5.5^{***}
L. casei	5.38 ± 0.05^{ABa}	4.3 ± 0.07^{Bb}	4.06 ± 0.09^{Bbc}	3.84 ± 0.19^{Bc}	3.7 ± 0.10^{BCc}			
L. plantarum DPC 206	5.43 ± 0.03^{Aa}	4.18 ± 0.04^{BCb}	3.92 ± 0.03^{Bc}	3.68 ± 0.01^{Bd}	3.55 ± 0.05^{BCe}			
L. acidophilus + L. casei	5.40 ± 0.00^{ABa}	4.34 ± 0.11^{Bb}	4.05 ± 0.19^{Bbc}	3.84 ± 0.21^{Bbc}	3.72 ± 0.13^{Bc}			
L. acidophilus + L. plantarum DPC206	5.39 ± 0.02^{ABa}	4.13 ± 0.02^{Cb}	3.95 ± 0.06^{Bc}	3.69 ± 0.06^{Bd}	3.57 ± 0.01^{BCd}			
L. casei + L. plantarum DPC206	5.37 ± 0.02^{Ba}	4.06 ± 0.01^{Cb}	3.81 ± 0.05^{Bc}	3.53 ± 0.04^{Bd}	3.47 ± 0.00^{Cd}			
L. acidophilus+ L. casei + L. plantarum DPC206	5.38 ± 0.03^{ABa}	4.09 ± 0.02^{Cb}	3.82 ± 0.06^{Bc}	3.58 ± 0.04^{Bd}	3.49 ± 0.05^{BCd}			

The values given below are reported as means and standard deviations. Values with a different letter are significantly different ($p<0.05$) according to the Fisher's Least Significant Difference (LSD) Post Hoc Test.

Uppercase superscripts represent a statistically significant effect within column and lowercase superscripts across each row. *** symbol represents p value (*** $p< 0.0001$).

4.2 Acidification in breadfruit fermented beverage

The change in pH values during fermentation is presented in Table 11. For all the three strains and mixture of strains, fermentation started at a similar pH and dropped between 4.62 and 3.49 at the end of fermentation. *L. acidophilus* pH was significantly ($p < 0.05$) higher than other strains at all fermentation times of 12, 24, 48 and 72 hours. Interestingly, although a higher cell growth was found with *L. acidophilus* at all fermentation times, a lower acidification was produced. The pH of mixture strains containing *L. plantarum DPC206* except for a mixture of *L. acidophilus* and *L. plantarum DPC206* strains, were significant ($p < 0.05$) with the lowest at 48 and 72 h. The type of probiotic bacteria, fermentation time, and their interaction significantly influenced pH (F value 92.1^{***}, 549.8^{***}, and 5.5^{***}, $p < 0.0001$ in Table 11, respectively). Figure 2 shows the interaction plot between probiotic bacteria and fermentation time. Only *L. acidophilus* had the highest pH compared to other strains and strain mixtures. *L. acidophilus* could have produced less acidity due to requirement of some essential nutrients, which might be deficient in breadfruit.

After fermentation, almost all beverages samples had higher acid production with pH ranging from 3.72 to 3.47, except for *L. acidophilus*. Decrease in pH can be due to the lactic acid bacteria producing organic acids, which is mainly lactic acid. After 72 h of fermentation, pH values of all strains were below 4 except for *L. acidophilus* (4.62). The pH of mixed culture was lowered because of large amount of lactic acid produce (Ai et al., 2015b). The low pH was one of the main reason that results in probiotic loss. However,

Costa et al. (2013) indicated that initial pH was not a major factor influencing lactic acid bacteria growth and viability. Temperature can also significantly influence cell growth and viability.

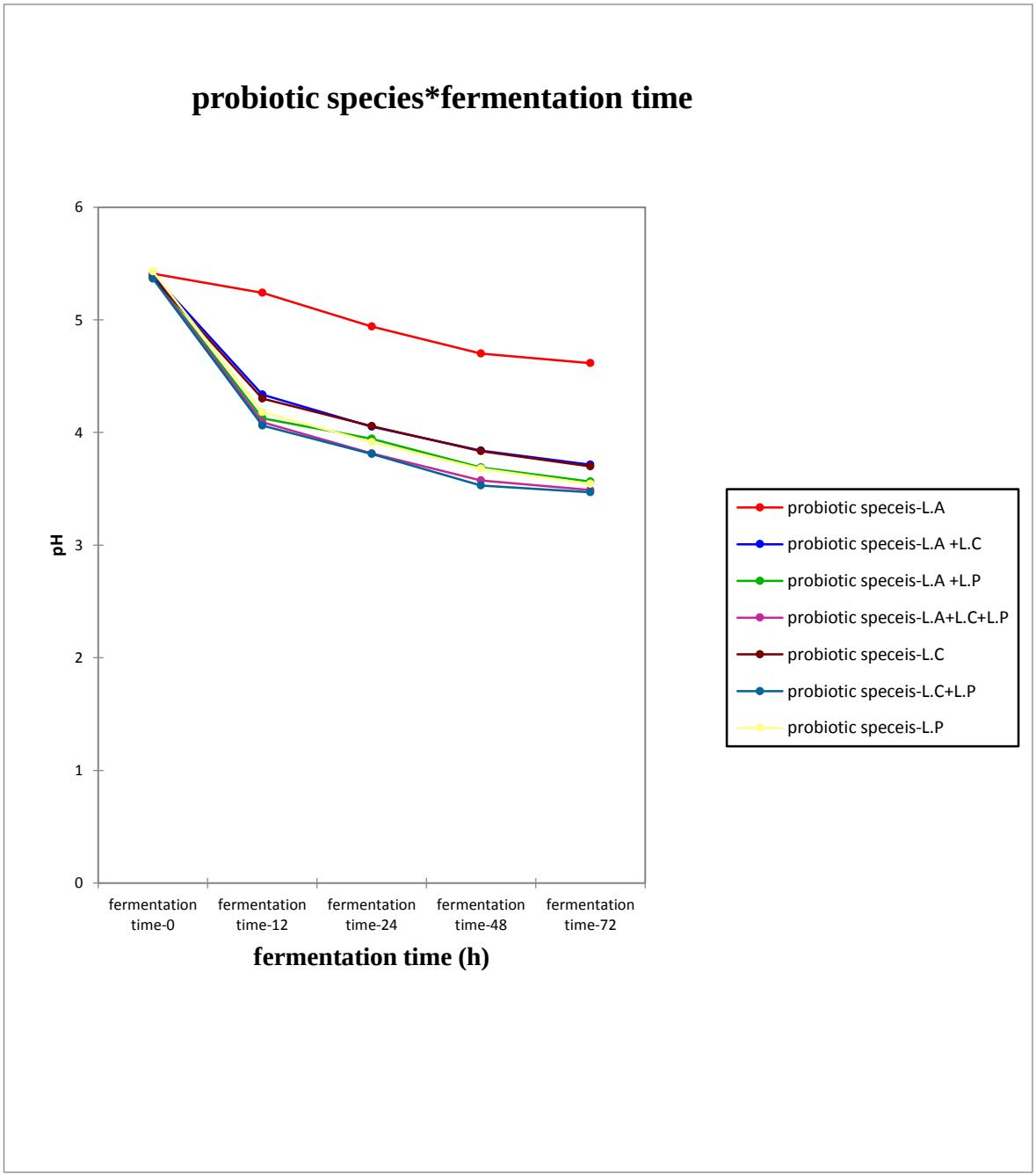

Figure 2. Probiotic stains and fermentation time showed significant effects on the value of pH.

L.A, L.P and L.C denote *Lactobacillus acidophilus*, *Lactobacillus plantarum DPC 206*, and *Lactobacillus casei* respectively.

4.3 Preliminary sensory evaluation

In order to select suitable lactobacilli, preliminary sensory testing was carried out screening for a suitable starter culture. Seven beverages made with different strains, and had maximum viable cells were evaluated. The fermented beverages fermented by *L. casei* had an undesirable smell and was unacceptable. The beverage obtained using a mix of *L. casei* and other strains after fermentation were also characterized by negative organoleptic properties in this study. The negative acceptance of *L. casei* fermented beverage might be due to probiotic off-flavour and higher lactic acid content that can decrease acceptability. *L. casei* fermented with litchi juice have been reported to result in unflavourable flavor amongst panelists (Zheng et al., 2014).

The beverage fermented with *L. acidophilus* was on the other hand acceptable. It was described as slightly sour, pleasant flavour and sweet. The beverage obtained using a mix of *L. acidophilus* and *L. plantarum DPC206* strains after fermentation also received good sensory evaluation, i.e. yellow colour, sour taste, sweet and a pleasant flavour. Both *L. plantarum DPC206* and a mix of *L. acidophilus* and *L. plantarum DPC206*, fermented beverages that were the most acceptable, with a good balance of sour and sweet. These beverages also had similar viable cell numbers (7.931 Log_{10} CFU/mL, 7.962 Log_{10} CFU/mL, respectively) and a low pH (3.9-3.7). However, since the *L. acidophilus* and *L. plantarum DPC206* mix strains containing beverage was fast growing and only needed 48 h fermentation to reach a maximum viable cell count, this inoculum was used for further work on the optimization of a fermented breadfruit substrate beverage. In

fermentation procedures, short fermentation periods can enhance output and avoid microbial contamination (do Amaral Santos, da Silva Libeck, & Schwan, 2014). The co-cultured organisms grow quickest under these conditions to gain ascendance and predominates because organisms must compete for nutrients or produce metabolites that stimulate each other's growth (Kedia, Wang, Patel, & Pandiella, 2007). In addition, *L. plantarum DPC206* has been reported to adapt well in various environments because of metabolic flexibility (Kleerebezem et al., 2003).

4.4 Optimization of fermented breadfruit beverage using a mixture design experiment

In the development of fermented beverages, the most important factors that need to be considered are cell viability and sensory quality. Substrate concentration, sucrose, concentration of starter culture, fermentation time and temperature are direct factors that influence bacteria growth. In this research, a mixture design experiment was used to determine the optimal fermentation parameters in terms of viability of lactic acid bacteria, as well as chemical properties that can influence sensory properties of the fermented beverage. According to Table 10, *L. acidophilus* and *L. plantarum DPC206* reached maximum cell viability with 48 h fermentation. This result was supported by Yoon et al. (2006) who found that *L. acidophilus* and *L. plantarum* had maximum growth at 30℃ after 48 h fermentation, and longer fermentation did not significantly change viable count.

Cell viability results in this study, decreased linearly with temperature increase, with no

significant ($p > 0.05$) effect. Hence optimization of the fermented beverage was carried out using CFU, pH, TA, LA and sugar concentration instead. Our results are in agreement with previous researches (Charernjiratrakul, Kantachote, & Vuddhakul, 2007; Ana Lúcia F Pereira et al., 2011) for vegetable juice and cashew apple juice (Anadón et al., 2013). In their study, mixed probiotic strains were observed at moderate fermentation temperature, with maximum cell viability at 30℃. Temperature higher than 30℃ can cause viability losses.

According to Table 10, cell viability (*L. acidophilus and L. plantarum*) significantly increased at 12 h, which then decreased with no significant changes between 12 and 72 h fermentation observed. This result was in agreement with similar previous studies (Angelov et al., 2006; Helland et al., 2004). Z. Mousavi et al. (2011) had reported that once probiotic strains such as *L. acidophilus* and *L. plantarum* have adapted to the new survival conditions and then they can enter the exponentially growing phase. When probiotics grew up to a maximum, the viability of probiotic bacteria experienced slight loss because of production of inhibition substances such as lactic acid (Gökmen et al., 2003). Furthermore, the viability of probiotic organisms also depended on pH, oxygen concentration, and temperature (Shah, 2000). Usually, the growth capacity of *L. acidophilus* and *L. plantarum*, is mainly influenced by nutrient content in the medium (Gokavi et al., 2005).

The D-optimal mixture experimental design is often applied in food fermentation as it is

an effective tool for optimization (Kamoun, Chaabouni, Sergent, & Phan-Tan-Luu, 2002). This design was employed in this research using a mixed culture grown at 30℃ after 48 hours fermentation. Seven percent breadfruit flour was used because proportions higher than that resulted in a more viscous product that cannot be fermented. Sugar added at 15% to the fermented beverage to give a balance sweet and sour taste as recommended by the focus group who carried out preliminary sensory testing and found that *L. acidophilus* and *L. plantarum DPC206* resulted in acceptable fermented beverage sensory attributes.

Experiments runs were generated using the Unscrambler X v10.1 (CAMO ASA, Oslo, Norway) software. The fitted models obtained for each response were fitted to a model based on SS and R^2. Table 13 presents the equations and adjusted coefficients of determination of models. Results showed that five responses (CFU, TA, pH, LA, and sugar concentration) belonged to the quadratic, quartic and special cubic models (Table 13). The polynomial models that explain the relationship between response and the variables are presented in Table 12.

Table 12. Cubic, quadratic, quartic models obtained from D-optimal design.

Response	Equation
CFU	CFU = 0.075692*A[a] + 0.076848*B[a] + 0.080568*C[a] + 1.01665E-004*AB +1.75774E-004*AC + 5.32542E-005*BC – 5.77027E-006*ABC +1.21489E-006*AB(A-B) +4.20509E-006*AC(A-C)[a] + 5.41514E-007*BC(C-B)
pH	pH = 0.036188*A + 0.036682*B + 0.035848*C + 2.24981E-005*AB +1.25414E-004*AC[a] + 2.76765E-005*BC
TA	TA = 1.12385E-003*A + 1.16534E-003*B + 1.85367E-003*C + 3.27095E-005*AB[a] – 2.09864E-006*AC + 5.00860E-006*BC
LA	LA = 0.53*A + 0.53*B + 0.48*C – 0.13*AB +1.23*AC – 0.30*BC – 0.55*AB(A-B) + 1.78*AC(A-C) – 0.57*BC(B-C) + 13.05*A^2BC – 18.68*AB^2C + 6.39*ABC^2 – 0.73*$AB(A-B)^2$ – 12.09*$AC(A-C)^{2a}$ + 3.39*$BC(B-C)^2$
S	S = 0.050304*A + 0.043473*B + 0.042798*C + 3.32747E-003*AB[a] +1.51232E-003*AC + 2.02706E-003*BC – 1.02840E-004*ABC

A = sugar, B = inoculum, C = breadfruit.

Lowercase superscript [a] represents a statistically significantly effect ($p < 0.05$).

Table 13. ANOVA of the regression models and regression coefficients for parameter used in the optimization of fermented breadfruit beverages. A = sugar, B = inoculum, C = breadfruit. $p^{**} < 0.01$; $0.01 <= p^{*} < 0.05$; $p >= 0.10$.

Response	Model	A	B	C	AB	AC	BC	ABC	AB(A-B)	AC(A-C)	BC(B-C)	A^2BC	AB^2C	ABC^2	AB(A-B)^2	AC(A-C)^2	BC(B-C)^2
CFU	Cubic	7.57*	7.68*	8.06*	1.02	1.76*	0.53	-5.77	1.21	4.21**	0.54	---	---	---	---	---	---
pH	Quadratic	3.62	3.67	3.58	0.22	1.25**	0.28	---	---	---	---	---	---	---	---	---	---
TA	Quadratic	0.11	0.12	0.19	0.33**	-0.02	0.05	---	---	---	---	---	---	---	---	---	---
LA	Quartic	0.53	0.53	0.48	-0.13	1.23	-0.30	---	-0.55	1.78	-0.57	13.05	-18.68	6.39	-0.73	-12.09*	3.39
S	Special Cubic	5.03	4.35	4.28	33.27**	15.12	20.27	-102.84	---	---	---	---	---	---	---	---	---

4.4.1 Optimization of five responses (CFU, pH, TA, LA and Sugar concentration) based on breadfruit substrate beverage on 48 h.

In Figure 3, the mixture contour plot presented a two-dimension view wherein all points located in the same shade regions are related to the cubic model. The effect of sugar, inoculum and breadfruit flour concentration and their interactions were investigated to understand the changes in growth of *L. acidophilus* and *L. plantarum DPC206* using a cubic model. Each side of triangle represents maximum values of fermentation parameters and the opposite side represented the minimum value.

As seen in Figure 3, the area with the highest CFU was located on the right-hand side of the triangle plot. The maximum value was located on the near the top region of this line. Decreasing breadfruit and inoculum contributed to significant ($p < 0.05$) increase in CFU of fermented beverage. Sugar content significantly ($p < 0.05$) increased CFU in fermented breadfruit beverage. Angelov et al. (2006) similar showed that increasing sugar concentration enhanced cell growth. The interaction between sugar and breadfruit proportion also significantly affected CFU (Table 13).

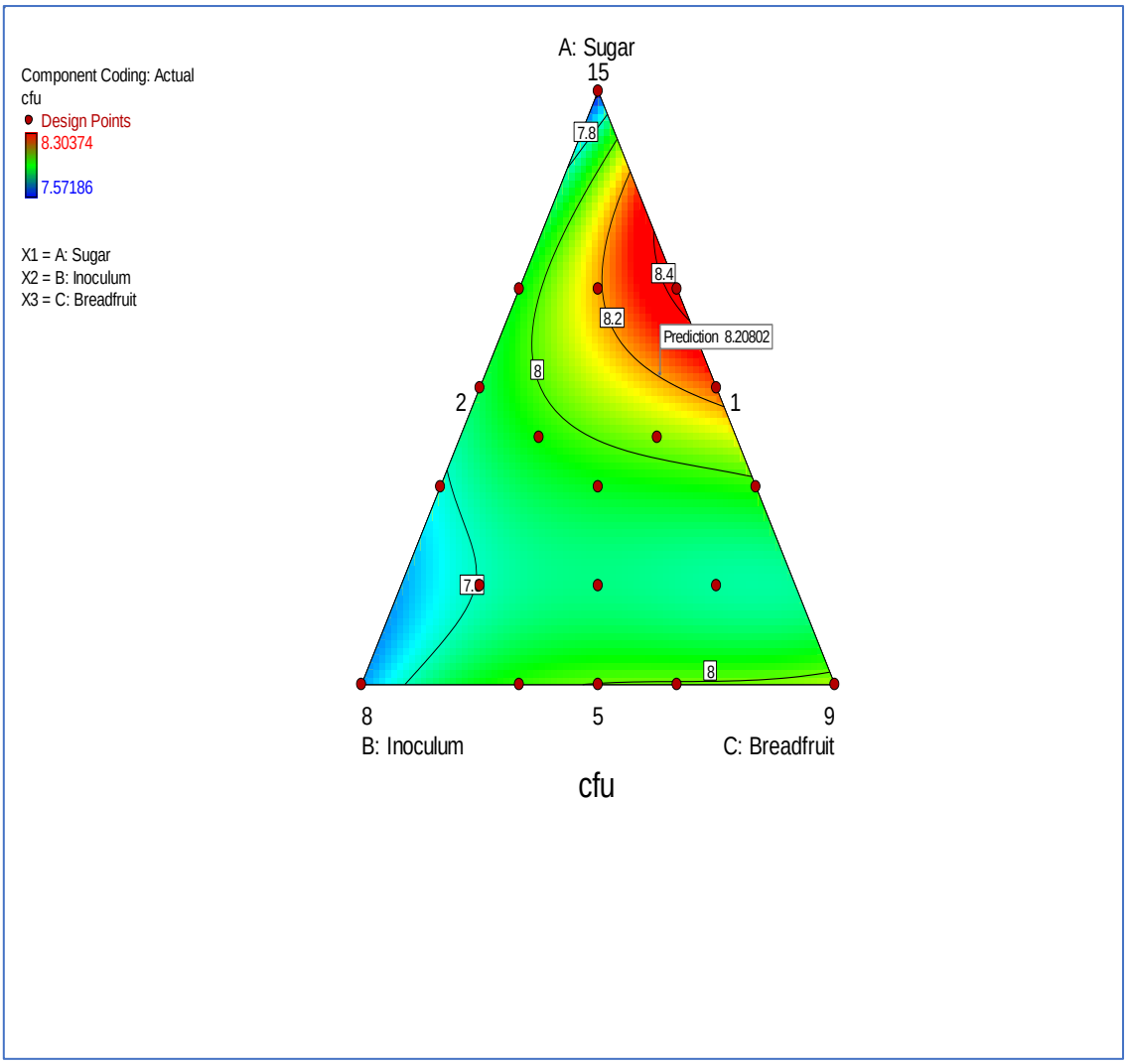

Figure 3. Contour plot showing the effect of sugar, inoculum and breadfruit flour concentration on CFU.

The contour plot for pH is presented in Figure 4. The effect of sugar, inoculum and breadfruit flour concentration and their interactions were investigated to understand the changes in pH using a quadratic model. The regression model equation is presented in Table 12. As seen in Figure 4, the area with the highest pH was located on the right-hand side of the triangle plot. The maximum pH region was located midway. pH was at a maximum with around 5.5% breadfruit and 13% sugar concentration. According to Table13, only both breadfruit proportion and sugar concentration significantly ($p<0.05$)

influenced pH values.

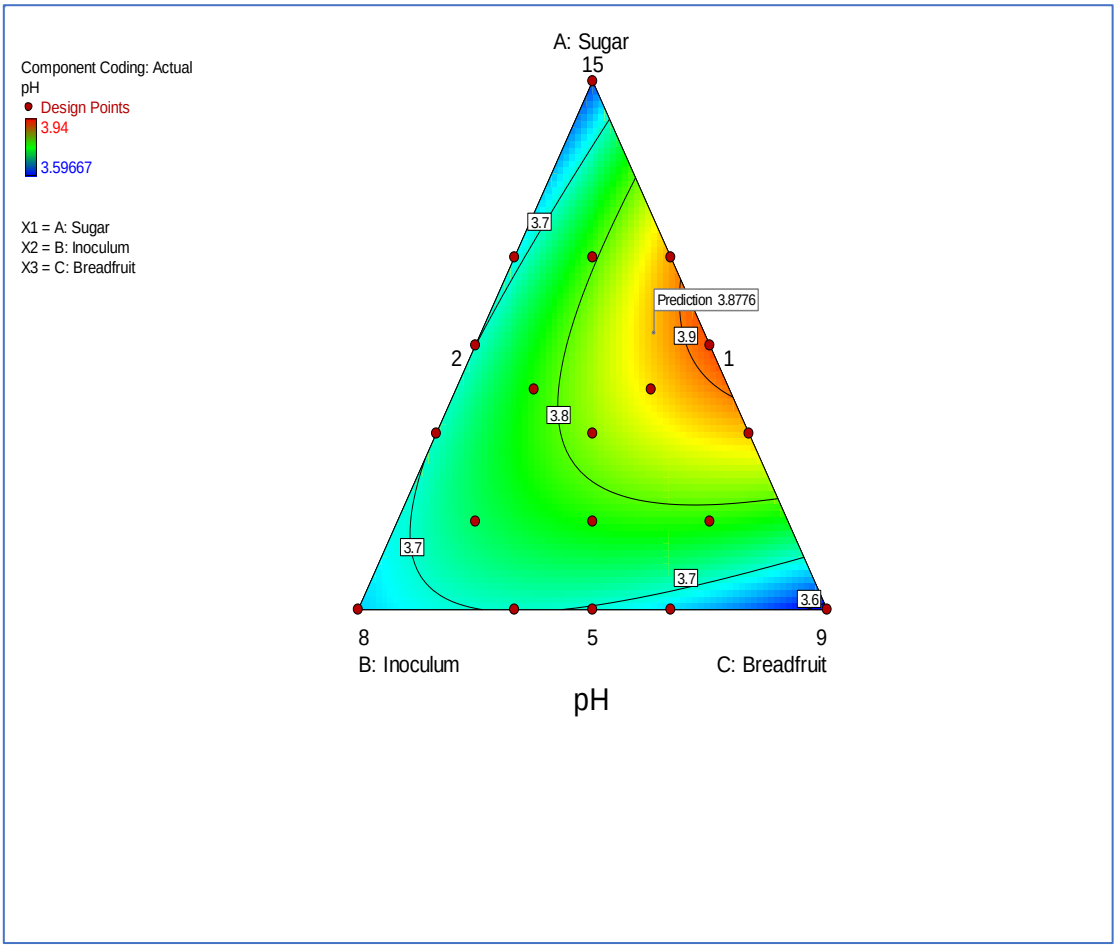

Figure 4. Contour plot showing the effect of sugar, inoculum and breadfruit flour concentration on the pH.

The optimum value of pH in contour plot was found at 3.88 (Figure 4). Kailasapathy and Chin (2000) pointed that pH value at 3.5 – 4.5 increased stability of probiotic in the gastrointestinal tract, which enhances survival of probiotic strains consumed. Although lower pH resulted in probiotic strains loss, *L. acidophilus* and *L. plantarum* were able to tolerate lower pH because a proton gradient existed in the cell in order to counteract the large amount of lactate in the food medium (Giraud, Champailler, Moulard, & Raimbault, 1998). Muyanja, Narvhus, Treimo, and Langsrud (2003) found that pH below 4.5 could

inhibit pathogen such as *Escherichia coli*. In addition, the optimum pH (3.88) value of breadfruit beverage which were lower than fermented dairy products (4.3 to 4.5) and similar to other non-dairy product (fermented cereals 3.5 to 4.0) (Farnworth et al., 2007).

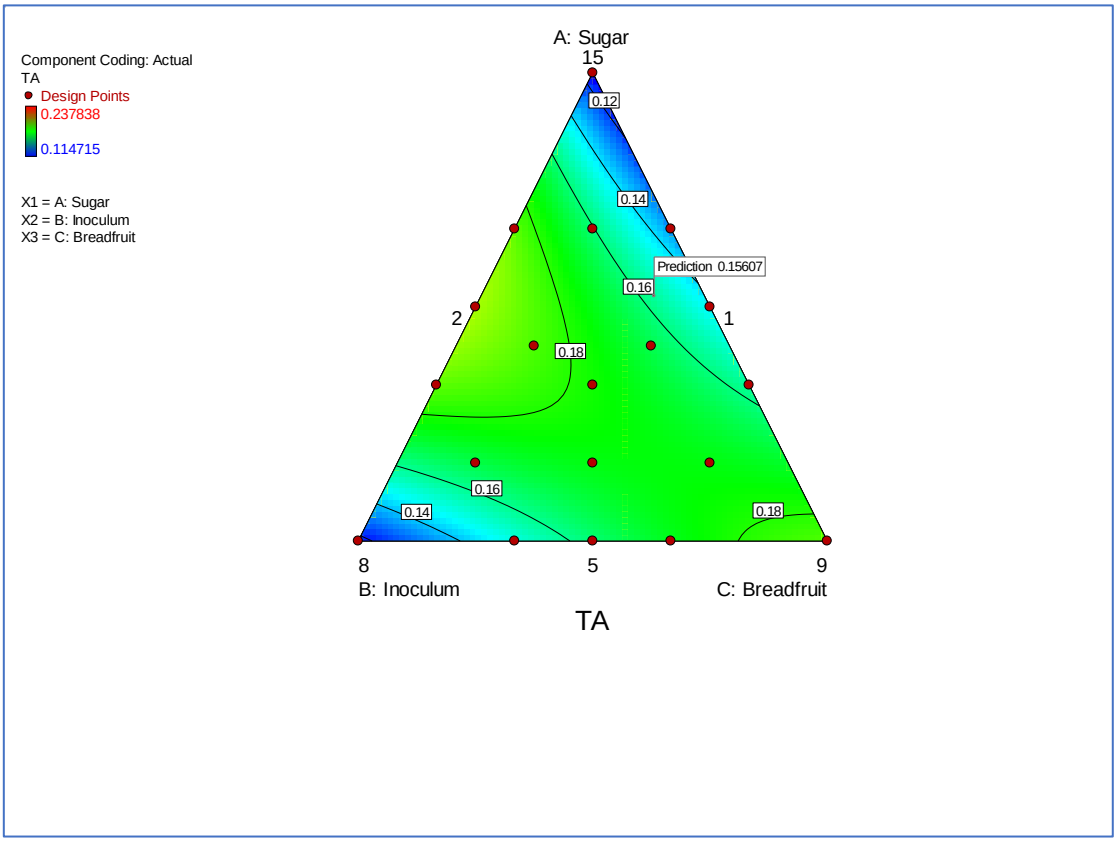

Figure 5. Contour plot showing the effect of sugar, inoculum and breadfruit flour concentration on the titratable acidity (TA).

The contour plot for TA is presented in Figure 5. The effect of sugar, inoculum and breadfruit flour concentration, and their interactions were investigated to understand the changes in TA using a quadratic model. The regression model equation is presented in Table 12. As seen in Figure 5, the area with the highest TA was located on the middle range of the triangle plot. The increase in TA was only significantly ($p < 0.05$) affected by sugar and inoculum interaction (Table 13). The maximum value of TA in the contour

plot was 0.2%. These TA values were similarly to those found in fermented soy-based products (0.08 – 0.19%) (Y.-C. Wang, Yu, Yang, & Chou, 2003). The differences in TA value may be due to different nutrient content as well as different fermentation parameters was studied in different probiotic strains (Angelov et al., 2005).

The contour plot for LA is presented in Figure 6. The effect of sugar, inoculum and breadfruit flour concentration, and their interactions were investigated to understand the changes in LA using a quartic model. The regression model equation is presented in Table 12. As seen in Figure 6, the area with the highest LA was located midway on the right-hand side of the triangle plot. LA was at a maximum with around 5.5% of breadfruit and 13% sugar concentration. The highest value of LA was 0.89 g/mL. the results showed that 5% of sugar was enough for lactic acid bacteria to grow and accumulate lactic acid. Significance was only observed in the quartic interaction (sugar*breadfruit*(sugar – breadfruit)^2). Sugar and breadfruit had the most significant effect on LA at a mid-range content (also known as a turning point in polynomial equations). On contrary, the lowest LA points were observed in the lowest and highest content of sugar and breadfruit respectively.

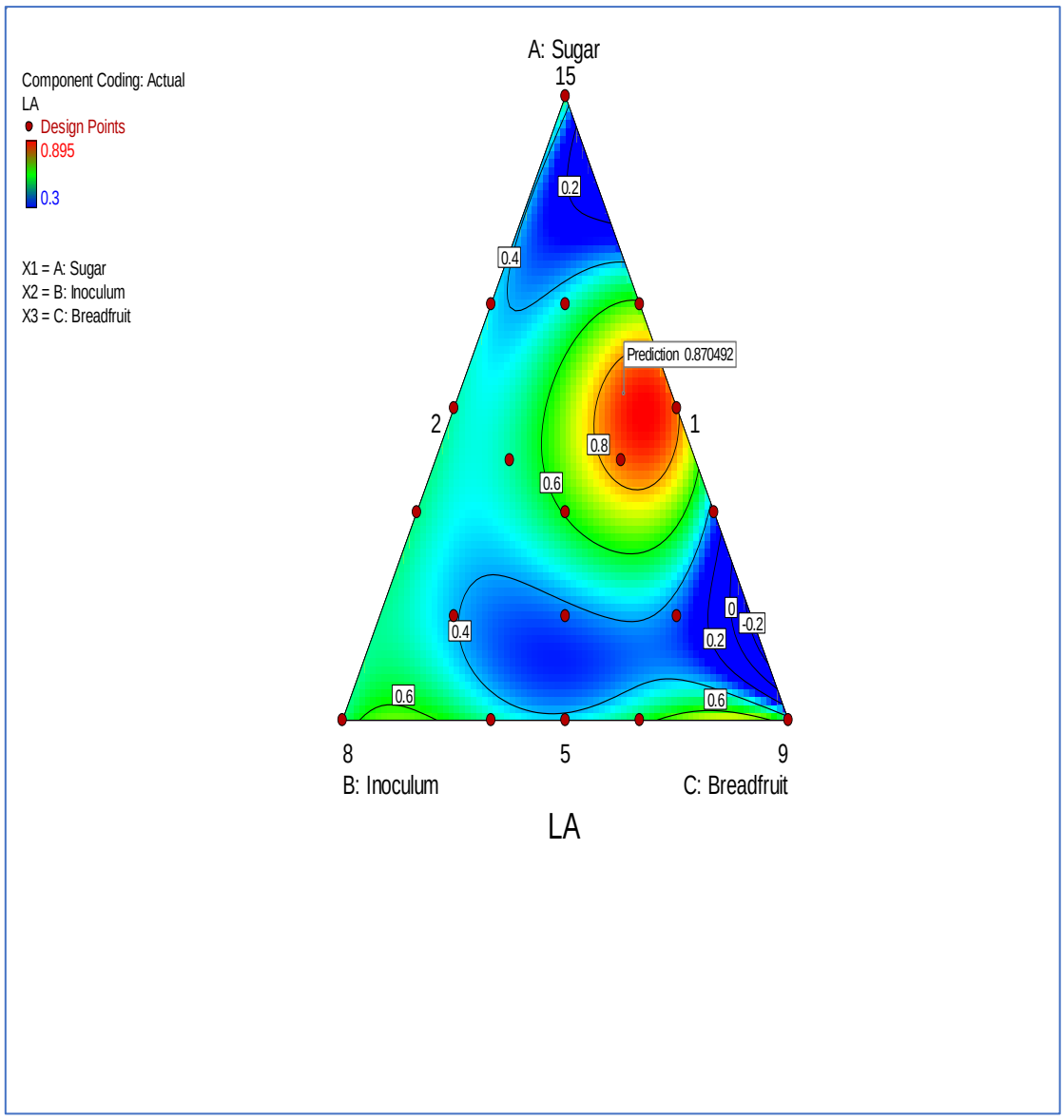

Figure 6. Contour plot showing the effect of sugar, inoculum and breadfruit flour concentration on the lactic acid (LA).

Sugar analysis showed that sucrose was the dominant sugar in fermented breadfruit substrate beverage. The contour plot for sucrose is presented in Figure 7. The effect of sugar, inoculum and breadfruit flour concentration, and their interactions were investigated to understand the changes in sucrose using a cubic model. The regression model equation is presented in Table 12. As seen in Figure 7, the area with the highest sucrose concentration was located midway on the left-hand side of the triangle plot. The

maximum sucrose concentration region was located in the middle range of sugar and inoculum. According to Table 13, it was the interaction between sugar and inoculum that significantly influenced sucrose concentration.

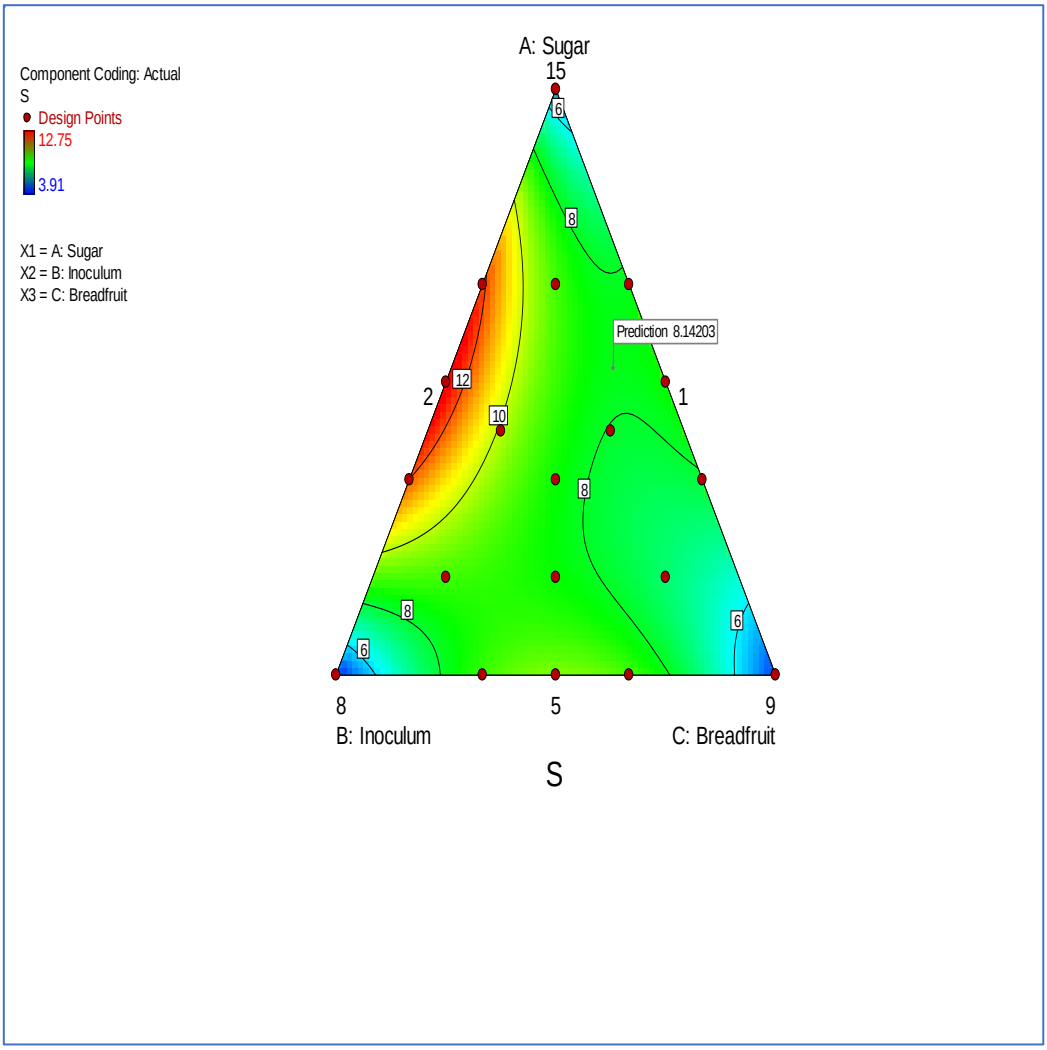

Figure 7. Contour plot showing the effect of sugar, inoculum and breadfruit flour concentration on the sucrose concentration.

Sucrose is consumed because microbial growth and production of organic acid can cause pH decrease. Sucrose was added into breadfruit substrate to increase fermentation rate (Angelov et al., 2006). The plot of maximum sucrose concentration occurred in formulations with mild level concentration of inoculum (1.5%) indicating that higher

starter culture resulted in low viable growth rate (Angelov et al., 2006).

Table 14. Mean of experimental value and prediction value in D-optimal mixture design experiment.

Response	Mean of experimental value	Prediction value
CFU	7.924 log CFU/mL	8.208 log CFU/mL
pH	3.82	3.877
TA	0.177%	0.156%
LA	0.70 g/mL	0.87 g/mL
S	8.373%	8.142%

The data shown in Table 14 compares the experimental value with the D-optimal prediction value. Predicted values were calculated for the optimized design based on CFU, pH, TA, LA, and sucrose concentration. In this study, CFU was set as the most important variable while TA, LA, and sucrose concentration results were set as second priority. The optimum experimental values of CFU, pH, and LA were slightly lower than the predicted values except for TA and sugar concentration. Overall, the optimum fermentation parameters for our breadfruit beverage were 7% breadfruit, 15% sugar and 1% sugar on 48 h fermentation at 30^{o}C based on the optimized results using the D optimal design.

5. Sensory quality evaluation

Six of the nineteen formulations (Formulations 1, 2, 3, 4, 6 and 18) in the experimental design that had high viable counts were subjected to sensory testing. The selected formulations were those fermented for 48 h. Results for sensory projective mapping and acceptance test are discussed in the section below.

5.1 Projective mapping

Figure 8 shows the results of descriptive sensory attributes of formulations 1, 2, 3, 4, 6 and 18 obtained from sensory projective mapping of appearance, aroma, taste and flavour attributes. As seen in Figure 8, a total of 58.64% of the variation between samples was explained. The first axis explained 31.35% of the total variation, and the second axis up to 27.29% variance. The first component (F1) separated bitter from sour, honey, fruity and sweet. As for the second axis, appearance characteristics of opaque were separated form pale yellow.

According to Figure 8, Formulation 1, 3 and 6 were characterized primarily by mint, sour, creamy appearance, honey, fruity flavour and sweet. Formulation 2 was mainly characterized by opaque. Formulation 4 was mainly separated by the appearance - pale yellow and bitter. Costa et al. (2013) reported that juices with added sugar that tasted sweet helped reduce the perception of sour. Formulation 4 presented a bitter taste that may have been caused by some metabolites. This could be due to long fermentation time

(48h). Bitter peptides (peptides aS1-CN) in the beverages have been reported to contribute to bitterness (Ong, Henriksson, & Shah, 2006). Lactic acid bacteria growth can lead to consumption or formation compounds that may change flavour or aroma (de Souza Neves Ellendersen et al., 2012).

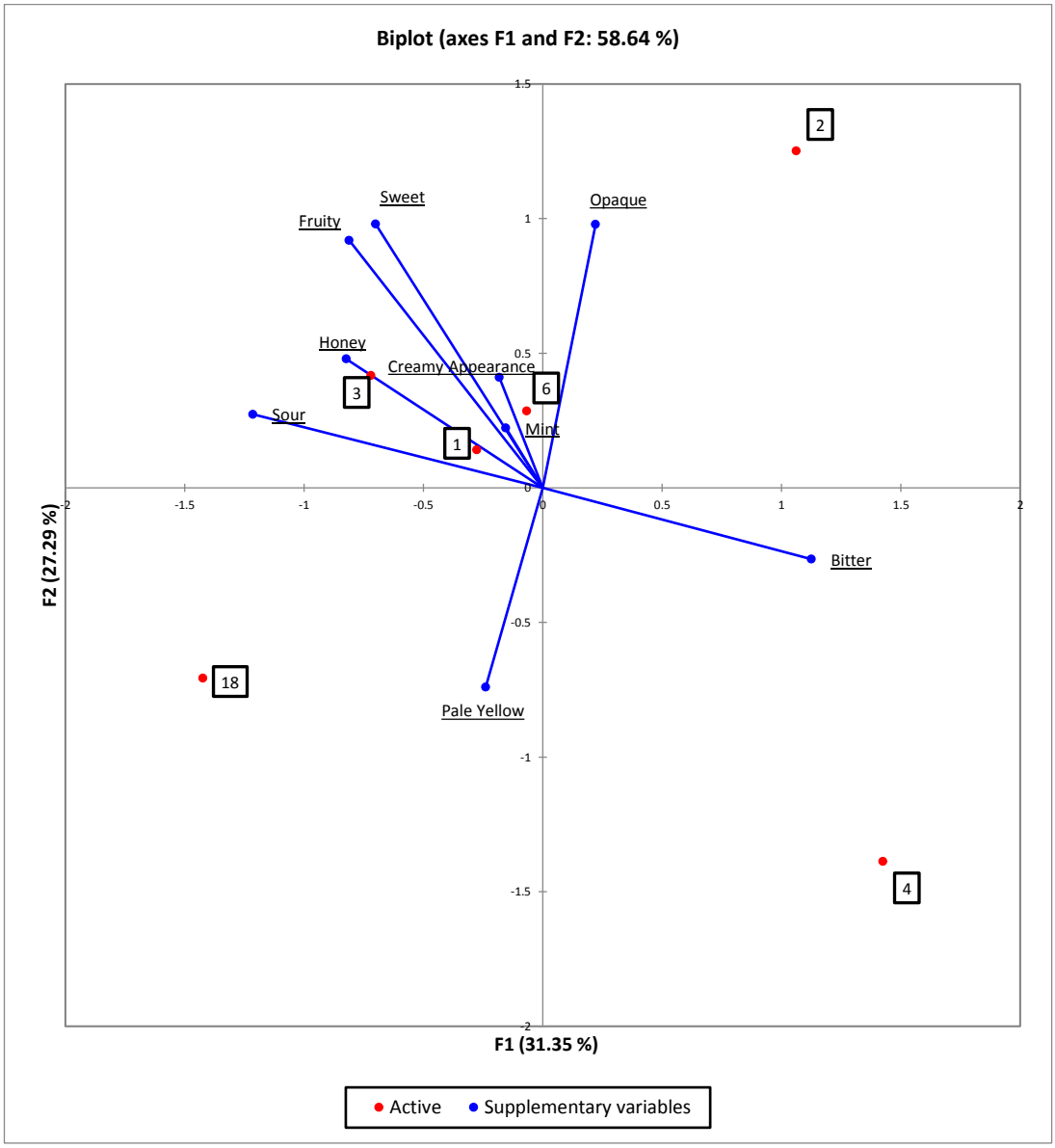

Figure 8. Sample configuration in the first and second dimensions of the Principal. Components Analysis performed on projective mapping data. The main sensory attributes were projected as supplementary variables in the analysis. Formulations 1, 2, 3, 4, 6, and 18 were analysis.

5.2 Sensory acceptance

Sensory evaluation in this study was effective in evaluating the hedonic qualities of fermented beverages. The acceptability of new functional breadfruit beverages does not only rely on enough probiotic cell, but also beverages should be acceptable by the consumers. Figure 9 summarizes the results of the hedonic test. Acceptability of the beverage was determined in terms of appearance, odour, flavour, aftertaste and overall liking. There was no significant difference in acceptance ($p > 0.05$) among the different formulations when evaluated for appearance and odour. This indicated that different fermentation conditions and sugar addition did not affect the appearance and odour of breadfruit beverage. Sensorially, Formulation 4 was consistently significantly lower than the other formulations based on liking of appearance, flavour and aftertaste, as well as overall liking. Formulation 4 happened to contain low sugar and the higher concentration of cultures (8.181 Log_{10} CFU/ml, significantly difference with formulation 6 and 18) may explain why it was least accepted ($p < 0.05$). Other studies on probiotic cashew apple juice also reported that increasing sucrose from 62.5% to 75% led to increased overall taste acceptance (Ana Lúcia Fernandes Pereira et al., 2013).

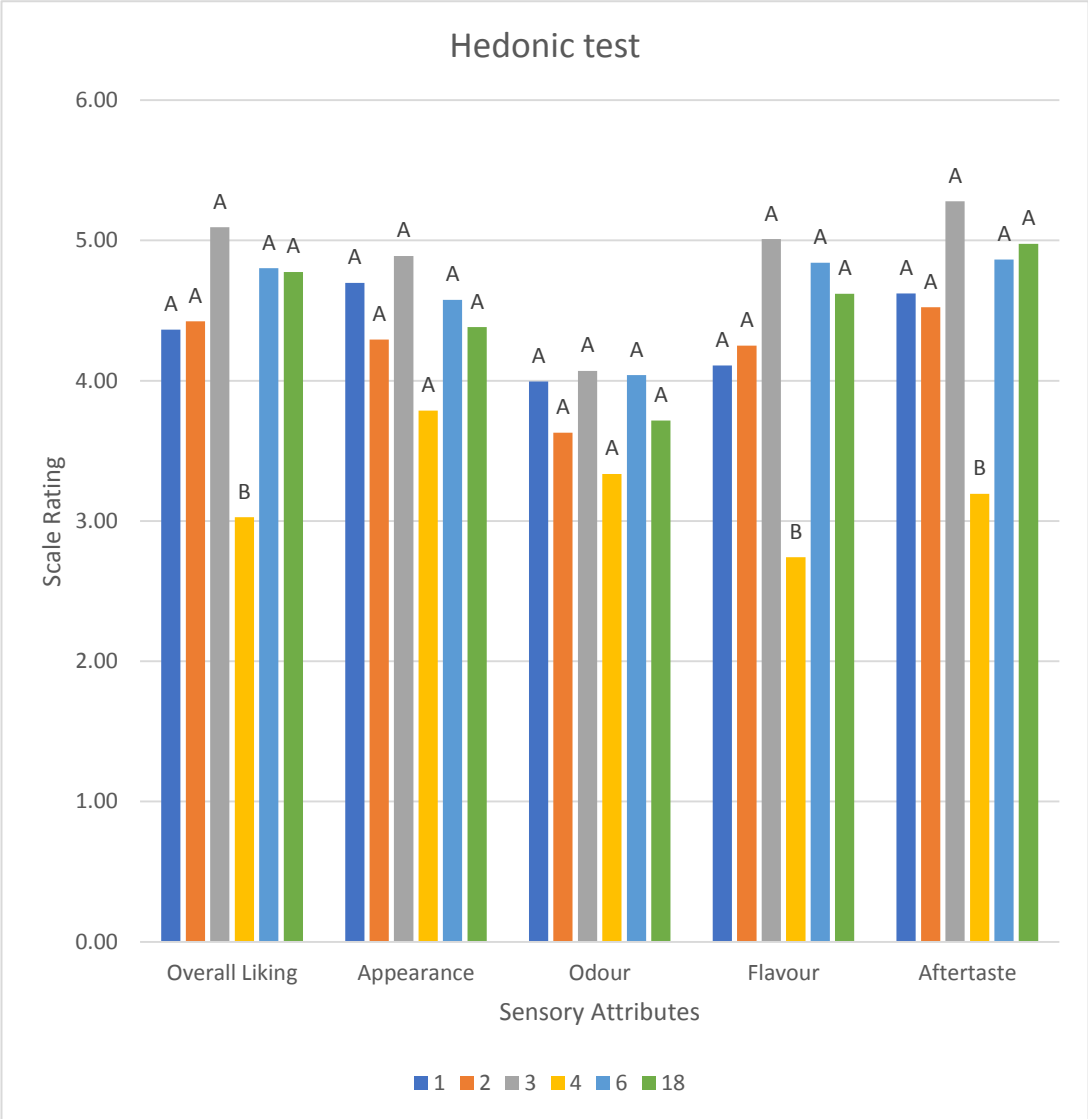

Figure 9. Hedonic testing carried out like of appearance, odour, flavour, aftertaste and overall liking. Values labelled with a different letter represent significant differences ($p<0.05$) according to the Tukey's multiple range comparison test.

Formulation 4 (7% breadfruit, 3% inoculum and 5% sugar) that was described bitter was the significantly ($p < 0.05$) least acceptable compared to other formulations. Marcellini, Chainho, and Bolini (2005) found that the intensity of taste, and absence or presence of aftertaste affected sensory perception. According to Cruz et al. (2010), metabolites from lactic acid bacteria can negatively contribute to the aroma, off-flavour and taste of a probiotic product. The results from this sensory test indicated that the beverage

formulated with a higher concentration of *L. acidophilus* and *L. plantarum DPC206* had better scores for liking. Hence, the culture types can also directly influence beverages taste and flavour.

Conclusion

There has been increased interest in the probiotic potential of cereal-based beverages in recent years due to lactose intolerance associated with dairy products. The preliminary study demonstrated the suitability of *L. acidophilus* and *L. plantarum DPC206* mixed strains as a starter culture that reached 7.963 log CFU/mL after 48 h fermentation with no negative sensory attributes associated with this formulation.

This is the first study reporting the use of a breadfruit substrate as a medium for probiotic growth. The optimum conditions for production of the fermented beverage using the D-optimal mixture design approach in terms of CFU, pH, TA, LA and sucrose concentration were found maximum cell viability could be at 7% breadfruit, 15% sugar, and 1% inoculum fermented at 30℃ after 48 h. Contour plot analysis revealed that breadfruit substrate beverage fermented with *L. acidophilus* and *L. plantarum DPC206* under optimal fermentation parameters presented optimal value of pH (3.88), sucrose concentration (8.14%), lactic acid (0.87g/mL), titratable acidity (0.156%), and CFU (8.208 log CFU/mL)。

Sensory projective mapping results showed that the fermented breadfruit substrate beverage formulation of 1, 3, and 6 were characterized by fruity flavour, with a balanced sweet and sour taste. Formulation 3 had the highest score in overall liking, and followed by formulation 6 and 1. However no significant ($p > 0.05$) differences between these three

formulations were found.

The present study was designed to determine the effect of lactic acid bacteria in fermented breadfruit substrate beverage. The most obvious finding from this research is the development of a novel fermented breadfruit-based beverage with acceptable sensory characteristic and cell viability using a mixture strain of *L. acidophilus* and *L. plantarum DPC 206*. It is recommended that further research be undertaken to investigate the possibility of the addition of natural flavours or fruit extracts to enhance acceptability and flavour of the fermented beverage, as well as to carry out further storage trials of the fermented product.

References

Adebowale, O. J., Ajayi, A., & Ibikunle, T. A. (2013). Quality Assessment of Biscuits from Green Plantain (Musa paradisiaca) and African Breadfruit (Treculia africana) Flours. *Journal of Culinary Science & Technology, 11*(4), 336-345.

Adebowale, O. J., Salaam, H. A., Komolafe, O. M., Adebiyi, T., & Ilesanmi, I. O. (2017). Quality characteristics of noodles produced from wheat flour and modified starch of African Breadfruit (Artocarpus altilis) blends. *Journal of Culinary Science & Technology, 15*(1), 75-88.

Adekanmi Adeniran, H., Gbadamosi, S. O., & Omobuwajo, T. O. (2012). Microbiological and physico-chemical characteristics of fufu analogue from breadfruit (Altocarpus altilis F). *International Journal of Food Science & Technology, 47*(2), 332-340.

Adeniran, H., & Ajifolokun, O. (2015). Microbiological studies and sensory evaluation of breadfruit and cassava co-fermented into gari analogue. *Nigerian Food Journal, 33*(1), 39-47.

Afshari, R., Hosseini, H., Khaksar, R., Mohammadifar, M. A., Amiri, Z., Komeili, R., & Khaneghah, A. M. (2015). Investigation of the Effects of Inulin and β-Glucan on the Physical and Sensory Properties of Low-Fat Beef Burgers Containing Vegetable Oils: Optimisation of the Formulation Using D-Optimal Mixture Design. *Food Technology and Biotechnology, 53*(4), 436.

Ai, J., Li, A. L., Su, B. X., & Meng, X. C. (2015a). Multi-Cereal Beverage Fermented by Lactobacillus Helveticus and Saccharomyces Cerevisiae. *Journal of food science, 80*(6).

Ai, J., Li, A. L., Su, B. X., & Meng, X. C. (2015b). Multi-Cereal Beverage Fermented by Lactobacillus Helveticus and Saccharomyces Cerevisiae. *Journal of food science, 80*(6), M1259-M1265.

Akanbi, T. O., Saari, N., Adebowale, A., Farooq, A., & Olaoye, A. (2011). Breadfruit starch-wheat flour noodles: preparation, proximate compositions and culinary properties. *International Food Research Journal, 18*(4), 1283-1287.

Amusa, N., Kehinde, I., & Ashaye, O. (2002). Bio-deterioration of breadfruit

(Artocarpus communis) in storage and its effects on the nutrient composition. *African Journal of Biotechnology, 1*(2), 57-60.

Anadón, A., Castellano, V., & Martínez-Larrañaga, M. (2013). Regulation and guidelines of probiotics and prebiotics. *Probiotics and Prebiotics in Food, Nutrition and Health*, 91.

Andersson, H., Asp, N.-G., Bruce, Å., Roos, S., Wadström, T., & Wold, A. E. (2001). Health effects of probiotics and prebiotics A literature review on human studies. *Näringsforskning, 45*(1), 58-75.

Angelov, A., Gotcheva, V., Hristozova, T., & Gargova, S. (2005). Application of pure and mixed probiotic lactic acid bacteria and yeast cultures for oat fermentation. *Journal of the Science of Food and Agriculture, 85*(12), 2134-2141.

Angelov, A., Gotcheva, V., Kuncheva, R., & Hristozova, T. (2006). Development of a new oat-based probiotic drink. *International journal of food microbiology, 112*(1), 75-80.

AOAC. (1992). Determination of mono- and disaccharides in foods by interlaboratory study: quantitation of bias components for liquid chromatography. 75:443.

AOAC. (2002). Official methods of analysis of the association of official analytical chemists (17th ed., vol. 2) p. 2200). Arlington, Virginia, USA: Association of Official Analytical Chemists, Inc.. ISBN 0-935584-67-6.

Arroyo-López, F., Bautista-Gallego, J., Chiesa, A., Durán-Quintana, M., & Garrido-Fernández, A. (2009). Use of a D-optimal mixture design to estimate the effects of diverse chloride salts on the growth parameters of Lactobacillus pentosus. *Food microbiology, 26*(4), 396-403.

Bakare, A. H., Adegunwa, M. O., Akinribido, O. M., & Obadina, O. A. (2014). Proximate, baking, and sensory qualities of biscuits from wheat and fermented breadfruit (Artocarpus communis Frost) flour. *Journal of Culinary Science & Technology, 12*(4), 316-325.

Bakare, A. H., Osundahunsi, O. F., & Olusanya, J. O. (2015). Rheological, baking, and sensory properties of composite bread dough with breadfruit (Artocarpus communis Forst) and wheat flours. *Food science & nutrition*.

Bakare, H. A., Osundahunsi, O. F., Adegunwa, M. O., & Olusanya, J. O. (2013). Batter rheology, baking, and sensory qualities of cake from blends of

breadfruit and wheat flours. *Journal of Culinary Science & Technology, 11*(3), 203-221.

Bernaerts, K., Gysemans, K. P., Minh, T. N., & Van Impe, J. (2005). Optimal experiment design for cardinal values estimation: guidelines for data collection. *International journal of food microbiology, 100*(1), 153-165.

Castro, I., Barros, S. M., Marquez, U. L., Motizuki, M., & Sawada, T. H. (2005). Optimization of the antioxidant capacity of a mixture of carotenoids and α-tocopherol in the development of a nutritional supplement. *Food Research International, 38*(8), 861-866.

Charalampopoulos, D., Pandiella, S., & Webb, C. (2003). Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. *International journal of food microbiology, 82*(2), 133-141.

Charernjiratrakul, W., Kantachote, D., & Vuddhakul, V. (2007). Probiotic lactic acid bacteria for applications in vegetarian food products. *Warasan Songkhla Nakharin (Sakha Witthayasat lae Technology)*.

Chwastek, A., Klewicka, E., Klewicki, R., & Sójka, M. (2015). Lactic Acid Fermentation of Red Beet Juice Supplemented with Waste Highbush Blueberry–Sucrose Osmotic Syrup as a Method of Probiotic Beverage Production. *Journal of Food Processing and Preservation*.

Coda, R., Rizzello, C. G., Trani, A., & Gobbetti, M. (2011). Manufacture and characterization of functional emmer beverages fermented by selected lactic acid bacteria. *Food microbiology, 28*(3), 526-536.

Costa, M. G. M., Fonteles, T. V., de Jesus, A. L. T., & Rodrigues, S. (2013). Sonicated pineapple juice as substrate for L. casei cultivation for probiotic beverage development: process optimisation and product stability. *Food chemistry, 139*(1), 261-266.

Crittenden, R., Martinez, N., & Playne, M. (2003). Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria. *International journal of food microbiology, 80*(3), 217-222.

Cruz, A. G., Cadena, R. S., Walter, E. H., Mortazavian, A. M., Granato, D., Faria, J. A., & Bolini, H. (2010). Sensory analysis: relevance for prebiotic, probiotic, and synbiotic product development. *Comprehensive Reviews in Food Science and Food Safety, 9*(4), 358-373.

De Oliveira, M. A., De Souza, V. M., Bergamini, A. M. M., & De Martinis, E. C. P. (2011). Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control, 22*(8), 1400-1403.

de Souza, C. T., Soares, S. A., Queiroz, A. F., dos Santos, A. M., & Ferreira, S. L. (2016). Determination and evaluation of the mineral composition of breadfruit (Artocarpus altilis) using multivariate analysis technique. *Microchemical Journal, 128*, 84-88.

de Souza Neves Ellendersen, L., Granato, D., Bigetti Guergoletto, K., & Wosiacki, G. (2012). Development and sensory profile of a probiotic beverage from apple fermented with Lactobacillus casei. *Engineering in Life Sciences, 12*(4), 475-485.

Di Cagno, R., Surico, R. F., Minervini, G., Rizzello, C. G., Lovino, R., Servili, M., . . . Gobbetti, M. (2011). Exploitation of sweet cherry (Prunus avium L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. *Food microbiology, 28*(5), 900-909.

Di Pierro, F., Adami, T., Rapacioli, G., Giardini, N., & Streitberger, C. (2013). Clinical evaluation of the oral probiotic Streptococcus salivarius K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes in adults. *Expert opinion on biological therapy, 13*(3), 339-343.

Didier, C., Etcheverrigaray, M., Kratje, R., & Goicoechea, H. C. (2007). Crossed mixture design and multiple response analysis for developing complex culture media used in recombinant protein production. *Chemometrics and Intelligent Laboratory Systems, 86*(1), 1-9.

Dimitrovski, D., Velickova, E., Langerholc, T., & Winkelhausen, E. (2015). Apple juice as a medium for fermentation by the probiotic Lactobacillus plantarum PCS 26 strain. *Annals of microbiology, 65*(4), 2161-2170.

Ding, W., & Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *Int Food Res J, 15*(2), 219-232.

do Amaral Santos, C. C. A., da Silva Libeck, B., & Schwan, R. F. (2014). Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. *International journal of food microbiology, 186*, 32-41.

Dongmo, S. N., Procopio, S., Sacher, B., & Becker, T. (2016). Flavor of lactic acid fermented malt based beverages: Current status and perspectives. *Trends*

in Food Science & Technology, 54, 37-51.

Dongmo, S. N., Sacher, B., Kollmannsberger, H., & Becker, T. (2017). Key volatile aroma compounds of lactic acid fermented malt based beverages–impact of lactic acid bacteria strains. *Food chemistry, 229*, 565-573.

Eke-Ejiofor, J. (2013). Proximate and sensory properties of African breadfruit and sweet potato-wheat composite flour in cakes and biscuits. *International Journal of Nutrition and food sciences, 2*(5), 232-236.

Farnworth, E., Mainville, I., Desjardins, M.-P., Gardner, N., Fliss, I., & Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International journal of food microbiology, 116*(1), 174-181.

Gajewska, J., & Blaszczyk, M. (2012). Probiotyczne bakterie fermentacji mlekowej (LAB). *Postępy Mikrobiologii, 1*(51).

Gandomi, H., Abbaszadeh, S., Misaghi, A., Bokaie, S., & Noori, N. (2016). Effect of chitosan-alginate encapsulation with inulin on survival of Lactobacillus rhamnosus GG during apple juice storage and under simulated gastrointestinal conditions. *LWT-Food Science and Technology, 69*, 365-371.

Garrido-Vidal, D., Pizarro, C., & González-Sáiz, J. M. (2003). Study of process variables in industrial acetic fermentation by a continuous pilot fermentor and response surfaces. *Biotechnology progress, 19*(5), 1468-1479.

Gibson, G., Rastall, R., & Fuller, R. (2003). The health benefits of probiotics and prebiotics. *Gut flora, nutrition, immunity and health*, 52-76.

Giraud, E., Champailler, A., Moulard, S., & Raimbault, M. (1998). Development of a miniaturized selective counting strategy of lactic acid bacteria for evaluation of mixed starter in a model cassava fermentation. *Journal of applied microbiology, 84*(3), 444-450.

Gokavi, S., Zhang, L., Huang, M. K., Zhao, X., & Guo, M. (2005). Oat-based Symbiotic Beverage Fermented by Lactobacillus plantarum, Lactobacillus paracasei ssp. casei, and Lactobacillus acidophilus. *Journal of food science, 70*(4).

Gökmen, V., Acar, J., & TAYDAS, E. (2003). Presence and formation of lactic acid in apple juices. *Fruit Processing. Mar/abr*, 114-117.

Haghshenas, B., Abdullah, N., Nami, Y., Radiah, D., Rosli, R., & Khosroushahi,

A. Y. (2014). Different effects of two newly-isolated probiotic Lactobacillus plantarum 15HN and Lactococcus lactis subsp. Lactis 44Lac strains from traditional dairy products on cancer cell lines. *Anaerobe, 30*, 51-59.

Hati, S., Mandal, S., & Prajapati, J. (2013). Novel starters for value added fermented dairy products. *Current Research in Nutrition and Food Science Journal, 1*(1), 83-91.

Helland, M. H., Wicklund, T., & Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. *International journal of food microbiology, 91*(3), 305-313.

Jaiswal, A. K., & Abu-Ghannam, N. (2013). Kinetic studies for the preparation of probiotic cabbage juice: Impact on phytochemicals and bioactivity. *Industrial Crops and Products, 50*, 212-218.

Joint, F. (2001). WHO Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Córdoba, Argentina. October*, 1-4.

Jones, A. M. P., Murch, S. J., Wiseman, J., & Ragone, D. (2013). Morphological diversity in breadfruit (Artocarpus, Moraceae): insights into domestication, conservation, and cultivar identification. *Genetic resources and crop evolution, 60*(1), 175-192.

Jones, A. M. P., Ragone, D., Bernotas, D. W., & Murch, S. J. (2011). Beyond the bounty: Breadfruit (Artocarpus altilis) for food security and novel foods in the 21st century. *Ethnobotany Research and Applications, 9*, 129-149.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. *Immunology and cell Biology, 78*(1), 80.

Kamoun, A., Chaabouni, M., Sergent, M., & Phan-Tan-Luu, R. (2002). Mixture design applied to the formulation of hydrotropes for liquid detergents. *Chemometrics and Intelligent Laboratory Systems, 63*(1), 69-79.

Kantachote, D., Ratanaburee, A., Hayisama-ae, W., Sukhoom, A., & Nunkaew, T. (2017). The use of potential probiotic Lactobacillus plantarum DW12 for producing a novel functional beverage from mature coconut water. *Journal of Functional Foods, 32*, 401-408.

Kedia, G., Wang, R., Patel, H., & Pandiella, S. S. (2007). Use of mixed cultures for the fermentation of cereal-based substrates with potential probiotic

properties. *Process Biochemistry, 42*(1), 65-70.

King, S. C., & Meiselman, H. L. (2010). Development of a method to measure consumer emotions associated with foods. *Food Quality and Preference, 21*(2), 168-177.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., . . . Fiers, M. W. (2003). Complete genome sequence of Lactobacillus plantarum WCFS1. *Proceedings of the National Academy of Sciences, 100*(4), 1990-1995.

Klewicka, E., Motyl, I., & Libudzisz, Z. (2004). Fermentation of beet juice by bacteria of genus Lactobacillus sp. *European Food Research and Technology, 218*(2), 178-183.

Kun, S., Rezessy-Szabó, J. M., Nguyen, Q. D., & Hoschke, Á. (2008). Changes of microbial population and some components in carrot juice during fermentation with selected Bifidobacterium strains. *Process Biochemistry, 43*(8), 816-821.

Lee, P.-R., Boo, C. X., & Liu, S.-Q. (2013). Fermentation of coconut water by probiotic strains Lactobacillus acidophilus L10 and Lactobacillus casei L26. *Annals of microbiology, 63*(4), 1441-1450.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology, 15*(2), 67-78.

López, F., Quintana, M. D., & Fernández, A. (2006). The use of a D-optimal design to model the effects of temperature, NaCl, type and acid concentration on Lactobacillus pentosus IGLAC01. *Journal of applied microbiology, 101*(4), 913-926.

Luana, N., Rossana, C., Curiel, J. A., Kaisa, P., Marco, G., & Rizzello, C. G. (2014). Manufacture and characterization of a yogurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. *International journal of food microbiology, 185*, 17-26.

Luckow, T., & Delahunty, C. (2004). Consumer acceptance of orange juice containing functional ingredients. *Food Research International, 37*(8), 805-814.

Lupien-Meilleur, J., Roy, D., & Lagacé, L. (2016). Viability of probiotic bacteria in a maple sap beverage during refrigerated storage. *LWT-Food Science and*

Technology, 74, 160-167.

Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. *Food Research International, 44*(1), 465-470.

Malomo, S., Eleyinmi, A., & Fashakin, J. (2011). Chemical composition, rheological properties and bread making potentials of composite flours from breadfruit, breadnut and wheat. *African Journal of Food Science, 5*(7), 400-410.

Marcellini, P., Chainho, T., & Bolini, H. (2005). Ideal sweetness and acceptance analysis of pineapple juice concentrate reconstituted sweetened with sucrose and different sweeteners. *Food Nutrition, 16*, 177-182.

Marsh, A. J., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Fermented beverages with health-promoting potential: past and future perspectives. *Trends in Food Science & Technology, 38*(2), 113-124.

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (2006). *Sensory evaluation techniques*: CRC press.

Meilleur, B. A., Jones, R. R., Titchenal, C. A., & Huang, A. S. (2004). Hawaiian breadfruit: Ethnobotany, nutrition, and human ecology: University of Hawaii.

Mielby, L. H., Hopfer, H., Jensen, S., Thybo, A. K., & Heymann, H. (2014). Comparison of descriptive analysis, projective mapping and sorting performed on pictures of fruit and vegetable mixes. *Food Quality and Preference, 35*, 86-94.

Mohammadi, R., Mortazavian, A. M., Khosrokhavar, R., & da Cruz, A. G. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Annals of microbiology, 61*(3), 411-424.

Mousavi, Z., Mousavi, S., Razavi, S., Emam-Djomeh, Z., & Kiani, H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Microbiology and Biotechnology, 27*(1), 123-128.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Hadinejad, M., Emam-Djomeh, Z., & Mirzapour, M. (2013). Effect of fermentation of pomegranate juice by Lactobacillus plantarum and Lactobacillus acidophilus on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds.

Food Biotechnology, 27(1), 1-13.

Muyanja, C., Narvhus, J., Treimo, J., & Langsrud, T. (2003). Isolation, characterisation and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. *International journal of food microbiology, 80*(3), 201-210.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2016). *Response surface methodology: process and product optimization using designed experiments*: John Wiley & Sons.

Nazzaro, F., Fratianni, F., Sada, A., & Orlando, P. (2008). Synbiotic potential of carrot juice supplemented with Lactobacillus spp. and inulin or fructooligosaccharides. *Journal of the Science of Food and Agriculture, 88*(13), 2271-2276.

Obadina, A., Oyewole, O., Sanni, L., Tomlins, K., & Westby, A. (2008). Identification of hazards and critical control points (CCP) for cassava fufu processing in South-West Nigeria. *Food Control, 19*(1), 22-26.

Ogundele, O., Awolu, O. O., Badejo, A. A., Nwachukwu, I. D., & Fagbemi, T. N. (2016). Development of functional beverages from blends of Hibiscus sabdariffa extract and selected fruit juices for optimal antioxidant properties. *Food science & nutrition, 4*(5), 679-685.

Oladunjoye, I., Ologhobo, A., & Olaniyi, C. (2010). Nutrient composition, energy value and residual antinutritional factors in differently processed breadfruit (Artocarpus altilis) meal. *African Journal of Biotechnology, 9*(27), 4259-4263.

Omobuwajo, T. (2003). Compositional characteristics and sensory quality of biscuits, prawn crackers and fried chips produced from beadfruit. *Innovative Food Science & Emerging Technologies, 4*(2), 219-225.

Ong, L., Henriksson, A., & Shah, N. (2006). Development of probiotic Cheddar cheese containing. *Lb. acidophilus, Lb. paracasei, Lb. casei*, 446-456.

Ooi, L.-G., & Liong, M.-T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. *International journal of molecular sciences, 11*(6), 2499-2522.

Ouwehand, A. C., Lagström, H., Suomalainen, T., & Salminen, S. (2002). Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly. *Annals of nutrition and metabolism, 46*(3-4), 159-

162.

Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., & Chhikara, N. (2017). Potential Non-Dairy Probiotic Products–A Healthy Approach. *Food Bioscience*.

Pereira, A. L. F., Almeida, F. D. L., de Jesus, A. L. T., da Costa, J. M. C., & Rodrigues, S. (2013). Storage stability and acceptance of probiotic beverage from cashew apple juice. *Food and Bioprocess Technology, 6*(11), 3155-3165.

Pereira, A. L. F., Maciel, T. C., & Rodrigues, S. (2011). Probiotic beverage from cashew apple juice fermented with Lactobacillus casei. *Food Research International, 44*(5), 1276-1283.

Pimentel, T. C., Madrona, G. S., & Prudencio, S. H. (2015). Probiotic clarified apple juice with oligofructose or sucralose as sugar substitutes: Sensory profile and acceptability. *LWT-Food Science and Technology, 62*(1), 838-846.

Prado, F. C., Lindner, J. D. D., Inaba, J., Thomaz-Soccol, V., Brar, S. K., & Soccol, C. R. (2015). Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of Functional Foods, 12*, 489-497.

Prado, F. C., Parada, J. L., Pandey, A., & Soccol, C. R. (2008). Trends in non-dairy probiotic beverages. *Food Research International, 41*(2), 111-123.

Priven, M., Baum, J., Vieira, E., Fung, T., & Herbold, N. (2015). The influence of a factitious free-from food product label on consumer perceptions of healthfulness. *Journal of the Academy of Nutrition and Dietetics, 115*(11), 1808-1814.

Purwandari, U., Khoiri, A., Muchlis, M., Noriandita, B., Zeni, N., Lisdayana, N., & Fauziyah, E. (2014). Textural, cooking quality, and sensory evaluation of gluten-free noodle made from breadfruit, konjac, or pumpkin flour. *International Food Research Journal, 21*(4).

Quanhong, L., & Caili, F. (2005). Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. *Food chemistry, 92*(4), 701-706.

Ragone, D., & Cavaletto, C. G. (2006). Sensory evaluation of fruit quality and nutritional composition of 20 breadfruit (Artocarpus, Moraceae) cultivars.

Economic botany, 60(4), 335-346.

Rakin, M., Vukasinovic, M., Siler-Marinkovic, S., & Maksimovic, M. (2007). Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. *Food chemistry, 100*(2), 599-602.

Rathore, S., Salmerón, I., & Pandiella, S. S. (2012). Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food microbiology, 30*(1), 239-244.

Reuss, R., Stratton, J., Smith, D., Read, P., Cuppett, S., & Parkhurst, A. (2010). Malolactic fermentation as a technique for the deacidification of hard apple cider. *Journal of food science, 75*(1).

Rivera-Espinoza, Y., & Gallardo-Navarro, Y. (2010). Non-dairy probiotic products. *Food microbiology, 27*(1), 1-11.

Rozada-Sánchez, R., Sattur, A. P., Thomas, K., & Pandiella, S. S. (2008). Evaluation of Bifidobacterium spp. for the production of a potentially probiotic malt-based beverage. *Process Biochemistry, 43*(8), 848-854.

Russo, P., de Chiara, M. L. V., Capozzi, V., Arena, M. P., Amodio, M. L., Rascón, A., . . . Spano, G. (2016). Lactobacillus plantarum strains for multifunctional oat-based foods. *LWT-Food Science and Technology, 68*, 288-294.

Sah, B., Vasiljevic, T., McKechnie, S., & Donkor, O. (2015). Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *Journal of dairy science, 98*(9), 5905-5916.

Salamanca, G., Osorio, M. P., & Montoya, L. M. (2010). Elaboración de una bebida funcional de alto valor biológico a base de borojó (Borojoa patinoi Cuatrec). *Revista chilena de nutrición, 37*(1), 87-96.

Salari, M., Razavi, S., & Gharibzahedi, S. (2014). Characterising the synbiotic beverages based on barley and malt flours fermented by Lactobacillus delbrueckii and paracasei strains. *Quality Assurance and Safety of Crops & Foods, 7*(3), 355-361.

Salmerón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *Journal of Functional Foods, 15*, 106-115.

Sanz, Y., & Dalmau, J. (2008). Los probióticos en el marco de la nueva normativa europea que regula los alimentos funcionales. *Acta Pediatr Esp, 66*(1), 27-31.

Sapers, G. M. (2001). Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technology and Biotechnology, 39*(4), 305-312.

Sarteshnizi, R. A., Hosseini, H., Bondarianzadeh, D., & Colmenero, F. J. (2015). Optimization of prebiotic sausage formulation: effect of using β-glucan and resistant starch by D-optimal mixture design approach. *LWT-Food Science and Technology, 62*(1), 704-710.

Shah, N. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of dairy science, 83*(4), 894-907.

Shah, N., Ding, W., Fallourd, M., & Leyer, G. (2010). Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *Journal of food science, 75*(5), M278-M282.

Shanahan, F. (2002). Probiotics and inflammatory bowel disease: from fads and fantasy to facts and future. *British Journal of Nutrition, 88*(S1), s5-s9.

Shiby, V. K., Radhakrishna, K., & Bawa, A. S. (2013). Development of whey-fruit-based energy drink mixes using D-optimal mixture design. *International Journal of Food Science & Technology, 48*(4), 742-748.

Shukla, M., Jha, Y., & Admassu, S. (2013). Development of probiotic beverage from whey and pineapple juice. *J Food Process Technol, 4*(2), 1-4.

Singh, H. (2009). Tapping into breadfruit's bounty.

Smart, K. F., Aggio, R. B., Van Houtte, J. R., & Villas-Bôas, S. G. (2010a). Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. *Nature Protocols, 5*(10), 1709.

Smart, K. F., Aggio, R. B., Van Houtte, J. R., & Villas-Bôas, S. G. (2010b). Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. *Nature Protocols, 5*(10), 1709-1729.

Spinelli, S., Masi, C., Zoboli, G., Prescott, J., & Monteleone, E. (2015). Emotional responses to branded and unbranded foods. *Food Quality and Preference, 42*, 1-11.

Tamminen, M., Salminen, S., & Ouwehand, A. C. (2013). Fermentation of carrot juice by probiotics: Viability and preservation of adhesion. *International Journal of Biotechnology for Wellness Industries, 2*(1), 10-15.

Tannock, G., Munro, K., Harmsen, H., Welling, G., Smart, J., & Gopal, P. (2000). Analysis of the fecal microflora of human subjects consuming a probiotic product containing Lactobacillus rhamnosusDR20. *Applied and environmental microbiology, 66*(6), 2578-2588.

Tripathi, M., & Giri, S. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods, 9*, 225-241.

Tsapatsaris, S., & Kotzekidou, P. (2004). Application of central composite design and response surface methodology to the fermentation of olive juice by Lactobacillus plantarum and Debaryomyces hansenii. *International journal of food microbiology, 95*(2), 157-168.

Turi, C. E., Liu, Y., Ragone, D., & Murch, S. J. (2015). Breadfruit (Artocarpus altilis and hybrids): A traditional crop with the potential to prevent hunger and mitigate diabetes in Oceania. *Trends in Food Science & Technology, 45*(2), 264-272.

Vanajakshi, V., Vijayendra, S., Varadaraj, M., Venkateswaran, G., & Agrawal, R. (2015). Optimization of a probiotic beverage based on Moringa leaves and beetroot. *LWT-Food Science and Technology, 63*(2), 1268-1273.

Ventura, M., & Perozzi, G. (2011). Introduction to the special issue “Probiotic bacteria and human gut microbiota”. *Genes & nutrition, 6*(3), 203.

Wang, T., He, F., & Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods, 7*, 101-111.

Wang, Y.-C., Yu, R.-C., Yang, H.-Y., & Chou, C.-C. (2003). Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food microbiology, 20*(3), 333-338.

Yin, H., Chen, Z., Gu, Z., & Han, Y. (2009). Optimization of natural fermentative medium for selenium-enriched yeast by D-optimal mixture design. *LWT-Food Science and Technology, 42*(1), 327-331.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *LWT-Food Science and Technology, 38*(1), 73-75.

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2006). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource technology, 97*(12), 1427-1430.

Yu, Y., Xiao, G., Xu, Y., Wu, J., & Wen, J. (2014). Effects of dimethyl dicarbonate (DMDC) on the fermentation of litchi juice by Lactobacillus casei as an alternative of heat treatment. *Journal of food science, 79*(5).

Zhao, D., & Shah, N. P. (2016). Lactic acid bacterial fermentation modified phenolic composition in tea extracts and enhanced their antioxidant activity and cellular uptake of phenolic compounds following in vitro digestion. *Journal of Functional Foods, 20*, 182-194.

Zheng, X., Yu, Y., Xiao, G., Xu, Y., Wu, J., Tang, D., & Zhang, Y. (2014). Comparing product stability of probiotic beverages using litchi juice treated by high hydrostatic pressure and heat as substrates. *Innovative Food Science & Emerging Technologies, 23*, 61-67.