DOMINANT LACTIC ACID BACTERIA AND YEASTS IN RICE SOURDOUGH PRODUCED IN NEW ZEALAND

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Massey University

Albany, New Zealand

Qiwei Yang

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ABSTRACT

Most gluten free (GF) products on the market are described as bland with poor mouth feel and are considered low quality in terms of texture due to lack of gluten, which has positive effects on the texture and appearance of cereal bakery products. The application of sourdough is a recent development in improving the quality of GF bread due to its efficiency and low-cost. This study aims to understand the fermentation of GF rice flour mix used to improve the quality of rice sourdough bread. Rice sourdough samples from three stages of fermentation mother sourdough (MSD), dough before proofing (DBP) and dough after proofing (DAP) and sourdough bread were characterised for their acidity, soluble sugars and organic acids content and total free amino acid content. Sourdough breads were also tested for their texture and colour. Yeasts and LAB colonies were enumerated from sourdough samples and isolates of LAB and yeasts were identified using API test kits (API 50 CHL for LAB and API 32 C for yeasts) and sequenced using 16S metagenetics for LAB and ITS region for yeasts. Due to the metabolic activities of sourdough lactic acid bacteria (LAB) and yeasts, dough acidity increased significantly ($p>0.05$) and total free amino acid content decreased during fermentation. Compared to unleavened rice bread, the final rice sourdough bread had a softer, more elastic, less crumbly and chewier crumb and its crust colour was more similar to unleavened wheat bread. Mean LAB counts in MSD, DBP and DAP were 8.6 log CFU/g, 7.9 log CFU/g and 8.5 log CFU/g, respectively; while yeast counts were 5.4 log CFU/g, 6.4 log CFU/g, and 6.7 log CFU/g, respectively. LAB counts increased significantly ($p<0.05$) during proofing but yeasts did not exhibit significant growth ($p>0.05$). Dominant LAB and yeasts responsible for the fermentation of rice sourdough were of the genus *Lactobacillus* and *S. cerevisiae*. LAB isolates were identified as *Lactobacillus plantarum* CIP 102980 and *Lactobacillus fermentarum* DSM 10667 and yeast colonies as *S. cerevisiae* CBS 1171.

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LIST OF SYMBOLS AND ABBREVIATIONS

a* = Redness-greenness

AFLP = Amplified fragment length polymorphism

ANOVA= Analysis of variance

AOAC = Association of Official Analytical Chemist

APC= Aerobic plate count

AP-PCR = Arbitrarily primed-polymerase chain reaction

ATP = Adenosine triphosphate

b* = yellowness-blueness

CFU = Colony forming per Unit

DBP = Dough before proofing

DAP = Dough after proofing

DGGE = Denaturing gradient gel electrophoresis

DNA = Deoxyribonucleic acid

EMP = Emden-Meyerhoff-Parnas

EPS = Exopolysaccharides

FAO = Food and Agriculture Organization

FISH = Fluorescence in situ hybridization

FQ = Fermentation quotient

g = Gram

GF = Gluten free

GRAS = General regarded as safe

HPLC = High performance liquid chromatography

ITS = Internal transcribed spacer

L = Litre

L* = Lightness

LAB = Lactic acid bacteria

LH-PCR = Length heterogeneity-polymerase chain reaction

mL = milliliter

NaCl = Sodium chloride

NAD = Nicotinamide adenine dinucleotide

NADH = Nicotinamide adenine dinucleotide hydride

PCR = Polymerase chain reaction

PFGE = Pulsed-field gel electrophoresis

RISA = Ribosomal spacer analysis

ARISA-PCR = Automated ribosomal spacer analysis polymerase chain reaction

RFLP = Restriction fragment length polymorphism

SD = Standard deviation

SDB = Sourdough bread

SSCP = Single strand conformation polymorphism

TGGE = Temperature gradient gel electrophoresis

TTA = Total titratable acidity

MRS = de Man, Rogosa and Sharpe

MSD = Mother sourdough

WHO = World Health Organization

YGC = Yeast Glucose Chloramphenicol

CHAPTER 1 INTRODUCTION

1.1 Background of gluten free products and sourdough technology

The need for gluten free products is increasing due to the special dietary needs of celiac patients and non-celiac consumers (Miranda, Lasa, Bustamante, Churruca, & Simon, 2014). Celiac disease is a common immunological food intolerance disease, with about 1 % of the world's population suffering from this disease (Green & Cellier, 2007; Sapone et al., 2012). Celiac disease is triggered by the consumption of gluten present in wheat, rye and barley (Preedy, Watson, & Patel, 2011; Gobbetti & Gänzle, 2012). It is therefore recommended that celiac patients consume a gluten-free (GF) diet throughout their lifetime (Preedy et al., 2011). Consumption of GF products has also been suggested to help control non-celiac disorders such as autism and schizophrenia (Kalaydjian, Eaton, Cascella, & Fasano, 2006; Jackson, Eaton, Cascella, Fasano, & Kelly, 2012). As a result, the GF product market is expected to be worth over US$6 billion by 2018, growing at a compounded average growth rate of about 10 % (Miranda et al., 2014).

Recent research in the GF field has aimed at improving the sensory quality of GF breads (Gobbetti & Gänzle, 2012; Witczak, Ziobro, Juszczak, & Korus, 2016). Most GF products on the market are described as bland with poor mouth feel and are considered to be of low quality due to lack of gluten, which has positive effects on texture and the appearance of cereal bakery products (Gobbetti, De Angelis, Di Cagno, & Rizzello, 2008; Gobbetti & Gänzle, 2012; Witczak et al., 2016). To improve the overall quality of GF bread, different formulations containing various additives such as hydrocolloids, non-gluten proteins, starches and enzymes are used (Moroni, Dal Bello, & Arendt, 2009; Gobbetti & Gänzle, 2012; Witczak et al., 2016). However, improvements face challenges such as high cost and variable ingredient matrices (Gobbetti et al., 2008; Gobbetti & Gänzle, 2012; Witczak et al., 2016). The application of the sourdough technique is a recent development in improving the sensory quality of GF bread due to its efficiency and low-cost (Moroni et al., 2009; Samaroo et al., 2010).

1.2 Significance of sourdough starter culture on sourdough quality

Sourdough is made by mixing flour and water followed by fermentation using lactic acid bacteria (LAB) and yeasts (Salim, Paterson, & Piggott, 2006; Hui & Evranuz, 2012). Previous research from the few available reports on GF products, indicate that fermentation of sourdough increases volume and improves texture, flavour and the nutrient content of bakery products (Arendt, Ryan, & Dal Bello, 2007; Gobbetti et al., 2008; Gobbetti & Gänzle, 2012). Compared to unleavened cereal flour, higher levels of free amino acids, vitamins and bioactive minerals are released during fermentation (Arendt et al., 2007; Moroni et al., 2009).

The sensory and textural quality of sourdough and final bakery products are affected by the inherent LAB and yeasts responsible for fermentation (Moore, Juga, Schober, & Arendt, 2007). During fermentation, the activities of LAB and yeasts produce metabolites such as organic acids, carbon dioxide, and exopolysaccharides (EPS) which increase dough stickiness and extendibility compared with non-fermented bread dough, resulting in increased bread volume (De Vuyst & Neysens, 2005). LAB are mainly responsible for the synthesis of aroma compounds, enzymes and exopolysaccharides which are related to textural properties and the nutritional value of the bread (Gobbetti & Gänzle, 2012). Whereas, yeasts contribute to the leavening of the bread which relates to bread volume (Moore et al., 2007).

Since the quality and characteristics of sourdough are correlated to activities of the starter cultures used, understanding the composition of the cultures and their metabolic activities is important. With this knowledge, artisans and industry can find better ways to control the fermentation factors (pH, fermentation time) to produce wholesome and consistent high quality products (Bamforth, 2005; De Vuyst & Neysens, 2005; Ehrmann & Vogel, 2005). In addition, defined starter cultures with predictable metabolic characteristics can be developed to produce fermented food with desirable properties (Catzeddu, Ehrmann & Vogel, 2005; Hui & Evranuz, 2012). Also, although microorganisms in bread are inactivated during baking, recent studies have reported that some strains of inactivated probiotics can still confer health benefits to the consumer and may even be safer for the

host to consume (Kataria, Li, Wynn, & Neu, 2009; Adams, 2010; De Almada, Almada, Martinez, & Sant'Ana, 2016). Therefore, identification of sourdough starter cultures may help reveal potential probiotic properties of sourdough bread, which have previously been under-estimated (Ehrmann & Vogel, 2005).

1.3 Motivation for exploring sourdough starter culture composition

Research on wheat and rye sourdoughs have been conducted to better understand the composition and metabolic characteristics of the starter cultures used (De Vuyst & Neysens, 2005; Ehrmann & Vogel, 2005; Gobbetti & Gänzle, 2012). Currently, over 80 species of LAB and more than 20 species of yeasts have been isolated from sourdough (De Vuyst & Neysens, 2005). In mature sourdoughs, which have a stable performance, more than 8 log CFU/g LAB have been reported (Ehrmann & Vogel, 2005; Ercolini et al., 2013). The number of co-existing yeasts are usually one or two logarithmic magnitudes lower than LAB, possibly due to yeast growth being inhibited at low pH (Ehrmann & Vogel, 2005; Ercolini et al., 2013; Minervini, De Angelis, Di Cagno, & Gobbetti, 2014).

There are however, limited reports on GF sourdoughs which frequently contain novel strains that have the potential to produce high quality GF bread (De Vuyst & Neysens, 2005; Gobbetti et al., 2008; Gobbetti & Gänzle, 2012; Foschia, Horstmann, Arendt, & Zannini, 2016). Among the published reports on GF sourdoughs (De Vuyst & Neysens, 2005; Foschia, Horstmann, Arendt, & Zannini, 2016), there are only two reports available on rice sourdough starter culture composition (Meroth, Hammes, & Hertel, 2004; Lim et al., 2018). Therefore, more research on rice sourdough starter culture composition may provide useful information for the potential development of defined cultures with predictable fermentation characteristics. This information may allow artisans and producers to have better control of fermentation processes to develop novel rice sourdough GF products for a diverse consumer market.

1.4 Objectives of this research

Main objective

The overall aim of the project was to determine the composition of LAB and yeasts in a GF rice sourdough starter culture.

Specific objectives

1. To determine the acidity (pH and total titratable acidity) of mother sourdough (MSD), bread dough before proofing (DBP), bread dough after proofing (DAP) and sourdough bread (SDB);
2. To analyse sugar, organic acid and free amino acid contents of DBP, DAP and SDB samples;
3. To analyse colour and texture of SDB;
4. To enumerate and isolate LAB and yeasts from sourdough samples;
5. To identify yeast and LAB isolates obtained from sourdough using API tests and genome sequencing;

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The literature review below will primarily discuss sourdough starter cultures from four aspects: how production parameters and technology affect sourdough starter culture composition, common sourdough LAB and yeasts and their metabolic characteristics, probiotic potential of sourdough starter cultures and methods used in determining starter culture composition.

2.2 Fermented foods and fermentation microorganisms

Fermentation biotechnology is the oldest method for preserving and preparing food (Nair & Prajapati, 2003; Giraffa & Carminati, 2012; Mehta, Kamal-Eldin, & Iwanski, 2012). During fermentation, food substrates (cooked or raw) are transformed by enzymes and living microorganisms through metabolism and biochemical reactions (Bamforth, 2005; Liu & Han, 2014). As a result, foods are cooked and preserved in a cost-effective way, aroma and flavour are enriched and texture is improved, and availability of essential nutrient amino acids and vitamins are enhanced and anti-nutritional factors inhibited. In addition, probiotics which may be involved in the fermentation process can be delivered to the consumer (Farnworth, 2008; Sanders, & Marco, 2010; Robinson, 2014).

With over 500 types of fermented beverages and foods available, fermented products comprise one-third of the total food consumed around the world (Liu & Han, 2014). The features of fermented foods are closely correlated to the responsible fermenting microorganisms (Giraffa & Carminati, 2012). In fermentation ecosystems, bacteria such as LAB and acetic bacteria are responsible for the low pH of foods such as sourdough, pickles and cheese, whereas *Bacillus* species play key roles in alkaline fermentation. Yeasts (eg. *Saccharomyces* sp, *Candida* sp) are used for alcohol production and dough leavening, while moulds such as *Penicillum* species can be used in cheese production to enhance the flavour (Coeuret, Dubernet, Bernardeau, Gueguen, & Vernoux, 2003; Giraffa & Carminati, 2012; Ray & Joshi, 2014; Robinson, 2014). Fermenting microorganisms, which may pre-

exist in the food or be purposely added, are involved in fermenting a wide range of food substrates such as dairy, meat, fish, fruits, vegetables, and cereals (Poutanen, Flander, & Katina, 2009; Guyot, 2010; Kohajdová, 2014). Fermented cereal products represent the greatest volume of all fermented products (Poutanen, Flander, & Katina, 2009; Guyot, 2010; Brandt, 2014; Kohajdová, 2014).

2.3 Fermented cereal foods

Cereal foods have been consumed as a staple food providing people with essential proteins, carbohydrates and minerals for thousands of years (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Usually, cereals are cooked before consumption (Peyer, Zannini, & Arendt, 2016). However, ground cereals can be mixed with water and microbes allowed to ferment the uncooked cereal to produce fermented products which may be categorised as porridge, gruel, beverage or leavened bread (Guyot, 2010; Brandt, 2014).

In Asian countries, rice is fermented into beverages, while in Europe, Australia and America, cereals such as wheat and rye are commonly fermented into batter, dough bread or loaves (Tamang & Kailasapathy, 2010). These foods can be fermented using moulds, yeasts and/or LAB (Kamal-Eldin, 2012; Kohajdová, 2014). Species of *Leuconostoc*, *Lactobacillus* and *Pediococcus* are the predominant lactic starter cultures used in fermented cereal foods and beverages, while most yeasts isolated from fermented cereal foods belong to the genera *Saccharomyces* (Kohajdová, 2014).

2.3.1 Fermented bread

Bread is a staple food in many countries (Hutkins, 2006; Zhou & Therdthai, 2012) and fermentation renders fermented bread more palatable than the raw cereal materials and improves their nutritional properties (Hutkins, 2006). Depending on the leavening starter cultures used, fermented bread can be divided into either yeast or sourdough bread (Zhou & Therdthai, 2012).

2.3.1.1 Baker's yeast bread

Yeast bread is fermented using a single microorganism, baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) which converts sucrose to glucose and fructose (Kulp, 2003; Bamforth, 2008). Bread fermented by baker's yeast is usually made with flours high in fermentable sugars (Bamforth, 2008). During fermentation, the fermentable sugars are converted into carbon dioxide which can be trapped in the dough to increase the bread volume (Liu & Han, 2014). After baking, yeast bread usually has a relatively thin crust, uniform grain and soft crumb (Kulp, 2003).

2.3.1.2 Sourdough bread

Sourdough bread is characterised by its sour flavour, which occurs due to the presence of organic acids, primarily lactic acid (1.2-1.7 %) and acetic acid (0.1-0.4 %) (Hui & Evranuz, 2012). It is made by mixing water and flour followed by fermentation using LAB and yeast cultures (Catzeddu, 2011; Zhou & Therdthai, 2012). During fermentation, the dough pH drops below 4.6 and the volume increases to 4-5 times its original size (Zhou & Therdthai, 2012; Corsetti, 2013; Todorov & Holzapfel, 2014). Compared to yeast bread, sourdough bread has a richer flavour and aroma, slower staling rate, longer shelf life and higher level of free amino acids (Kulp, 2003; Corsetti, 2013).

2.4 Cereals used for fermentation

The most important cereals used for fermentation are wheat, rice and maize. Other grains such as rye, sorghum, and millet are also used, but to a lesser extent (Kamal-Eldin, 2012; Kohajdová, 2014). In addition, pseudocereals such as buckwheat, quinoa and amaranth may also be incorporated into gluten free products (Arendt & Zannini, 2013; Witczak, Ziobro, Juszczak, & Korus, 2016). Cereals contain different fermentable carbohydrates and endogenous enzymes (amylases, peptidases and xylanases), resulting in variations in available amino acids, monosaccharides and disaccharides which are essential for the growth of the starter microorganisms (Hui & Evranuz, 2012). Some cereals also contain the storage protein gluten, while others do not. Cereals can therefore be divided into two groups, gluten cereals and GF cereals (Arendt & Dal Bello, 2011). GF cereals, as

previously mentioned (Section 1.1), are important for individuals with celiac disease. GF cereals include rice, maize, millet and pseudo-cereals, while gluten can be found in wheat, rye and barley (Arendt & Dal Bello, 2011).

2.4.1 Cereal gluten and celiac disease

Celiac disease is one of most common immunological food allergen induced diseases, which is triggered by consumption of gluten. Approximately 1 % of the worlds' population suffers from this disease (Green & Cellier, 2007; Lohi et al., 2007). Clinical symptoms of celiac disease include diarrhoea, nervous depression, and nausea (Gobbetti, Rizzello, Di Cagno, & De Angelis, 2007). To control the symptoms, celiac patients rely on a life-long GF diet and there is thus an increasing market for GF products with improved sensory and nutritional features (Kalaydjian, Eaton, Cascella, & Fasano, 2006; Preedy, Watson, & Patel, 2011; Jackson, Eaton, Cascella, Fasano, & Kelly, 2012).

2.4.2 Gluten free rice products

Of all the GF cereals, rice is regarded as the most appropriate flour for GF products due to its zero-cholesterol content, white colour, mild taste, hypoallergenic features, low sodium, nutritious protein which contains the highest lysine content among cereals and good digestibility (Arendt & Zannini, 2013; Gómez & Sciarini, 2015). There are two basic forms of rice, brown and white (Haard, 1999). Compared to white rice, brown rice has higher levels of vitamins and fibre, which can contribute to the unique sensory properties of bakery products (Haard, 1999; McKevith, 2004).

From a nutritional perspective, although rice has a low protein content (6 % to 8 %) compared to other flours (8 % to 15 % for wheat), it has a high concentration of glutelin, which is rich in the essential amino acid lysine (Arendt & Dal Bello, 2001; Heinemann, Fagundes, Pinto, Penteado, & Lanfer-Marquez, 2005). In addition, rice is rich in complex carbohydrates which are available for fermentation. Compared to wheat, rye and maize, brown rice has higher available levels of carbohydrate (Charalampopoulos et al., 2002; Arendt & Zannini, 2013). In terms of vitamin comntents, brown rice also has the highest

riboflavin and niacin contents compared to wheat, maize, barley, sorghum, oat, pearl millet and rye (Haard, 1999).

Rice is commonly used in GF breakfast cereals and snacks such as energy bars (Arendt & Dal Bello, 2011). Its application in baking is increasing as it is a suitable replacement for wheat flour in the production of GF products for celiac patients. However, GF bread made using plain rice flour has a compact crumb and lower specific volume compared to wheat bread, and this negatively affects the consumer's acceptance of GF products (Arendt & Dal Bello, 2011; Gómez & Sciarini, 2015). The compact crumb and lower specific volume of GF rice bread may result from the insolubility of rice proteins, which cannot hold the carbon dioxide produced during baking (Catzeddu, 2011; Corsetti, 2013).

2.4.3 Sourdough technique: a novel method to improve gluten-free products

Most GF breads, including rice bread are perceived as having a poor mouth feel, dry crumb, and bland flavour (Gobbetti & Gänzle, 2012; Miranda, Lasa, Bustamante, Churruca, & Simon, 2014). The main reason for this poor perception of the products is the lack of the structure-forming cereal protein gluten, which has positive effects on texture, appearance and flavour (Gobbetti, De Angelis, Di Cagno, & Rizzello, 2008; Gobbetti & Gänzle, 2012; Witczak et al., 2016). Compared to bread-containing gluten, the nutritional quality of GF breads is lower, as the levels of essential nutrients such as protein and vitamins are lower than wheat bread (Gobbetti et al., 2008; Gobbetti & Gänzle, 2012; Witczak et al., 2016).

To improve the nutritional and sensory aspects of GF bread, different formulations incorporating various additives such as hydrocolloids, non-gluten proteins, starches and enzymes have been developed (Gobbetti et al., 2008; Mandala & Kapsokefalou, 2011). However, these improvements are associated with the high cost of multiple ingredients and procedures, as well as batch to batch variability of the ingredients (Gobbetti & Gänzle, 2012).

Application of the sourdough fermentation method on wheat and rye bread results in a loaf with improved nutritional value, texture, flavour and shelf life (Moroni, Dal Bello, & Arendt, 2009). Although available information on the utilisation of the sourdough

technique in GF sourdough bread improvement is limited, studies indicate that GF sourdough bread is characterised by a softer crumb, higher specific volume and improved nutritional content compared to unleavened GF bread (Moroni et al., 2009; Arendt & Dal Bello, 2011).

2.5 Sourdough bread

2.5.1 Technology and production

Generally, sourdough is prepared by mixing all the ingredients (water, flour, salt and starter cultures) to form a bread dough, which is then fermented by sourdough microflora LAB and yeasts to produce a loaf with its own unique sour taste and increased bread volume (Hansen, 2004; Catzeddu, 2011). After proofing, the leavened bread dough is baked (Hansen, 2004). An overview of the production of sourdough bread is outlined in Figure 2.1.

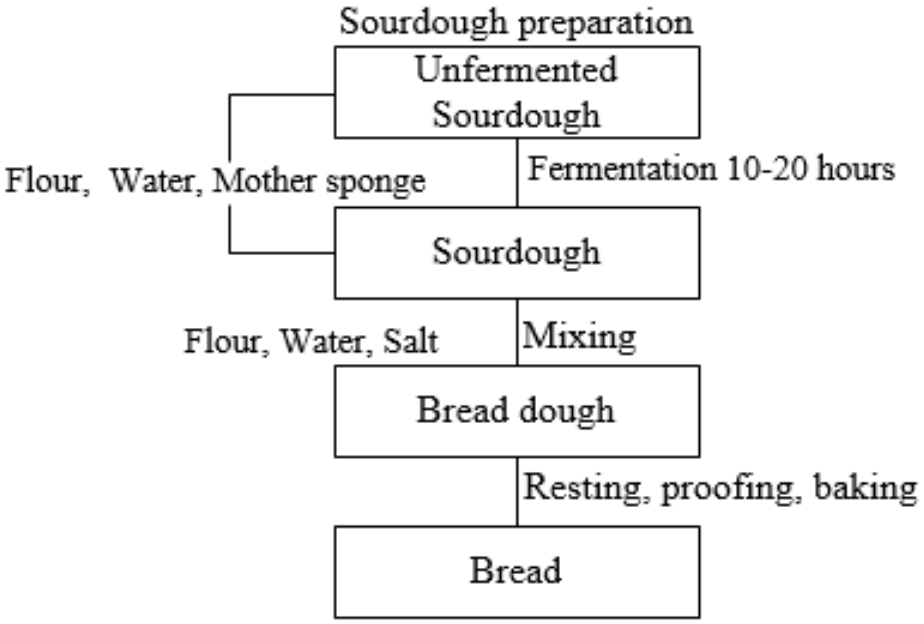

Figure 2.1 Overview of sourdough bread production (Hansen, 2004)

Sourdough breads produced around the world are variable in terms of sourdough composition due to a number of factors including regional differences, technological parameters (e.g. temperature) and recipes (e.g. flour type) (Corsetti & Settanni, 2007; Zhou & Therdthai, 2012). Based on technological parameters, sourdough production can be grouped into four types: type 0, type 1, type 2 and type 3, as described in the subsequent sections (Corsetti & Settanni, 2007; Zhou & Therdthai, 2012).

2.5.2 Type 0 sourdough

Type 0 sourdough (also known as spontaneous/natural fermented sourdough) is made in a traditional way, by mixing flour and water, and then leaving the mixture at ambient temperature until it becomes sour, which can take from a few hours to a few days depending on the temperature applied and the inoculated starter cultures (Aplevicz, Ogliari, & Sant'Anna, 2013; Corsetti, 2013). Due to differences in environmental conditions such as temperature, humidity and atmospheric air quality, the sourdough microflora can differ from place to place. For example, *L. plantarum* RTa12 and *P. pentosaceus* RTa11 were found in spontaneous fermented sourdough and bread in Germany, while *L. graminis* and *L. rossiae* were reported in Canadian sourdough (Sterr, Weiss, & Schmidt, 2009; Ripari, Gänzle, & Berardi, 2016). The dominant LAB isolated from type 0 sourdough are mainly the homofermentative type, which produce lactic acid as the main end product (Zhou & Therdthai, 2012).

2.5.3 Type 1 sourdough

Type 1 sourdough is also prepared in a traditional way, by regular back-slopping (previous mother sourdough is used to initiate fermentation), which will be discussed in detail in Section 2.6.3.1. The inoculated fresh dough is allowed to ferment at room temperature (20-30 °C) until the pH decreases to around pH 4.0 (Garofalo, Silvestri, Aquilanti, & Clementi, 2008; Zhou & Therdthai, 2012). Fermentation times range from 3-48 hours in wheat and rye sourdough manufacturing (De Vuyst & Neysens, 2005). In this type of sourdough, *L. sanfranciscensis* predominates and the maltose-negative yeasts *C. humilis* and *S. exiguus* co-exist (Corsetti, 2013). Other identified LAB and yeast species include *L. pontis*, *L.*

brevis, *L. fermentum*, *L. fructivorans*, *L. rossiaeand* and *S. cerevisiae* (Zhou & Therdthai, 2012; Corsetti, 2013).

2.5.4 Type 2 sourdough

Features of type 2 sourdough include high incubation temperature (>30 °C), high dough yield (ca. 200, e.g. 200 kg of dough obtained from 100 kg flour) and long fermentation time (15 hours to 5 days) (Zhou & Therdthai, 2012; Corsetti, 2013). This type of sourdough is applied in the bakery industry to enhance bread acidity and aroma (Zhou & Therdthai, 2012; Corsetti, 2013). The final pH can be lower than 3.5 after fermenting for 24 hours (De Vuyst & Neysens, 2005), therefore, starter cultures used in this type of sourdough must be able to tolerate higher temperatures and increased acidity (Zhou & Therdthai, 2012). Due to the poor survival of wild yeast, additional baker's yeast is usually used for leavening (Gobbetti, 1998; Zhou & Therdthai, 2012). The majority of isolated LAB from type 2 sourdough are *L. fermentum*, *L. pontis* and *L. reuteri* (Zhou & Therdthai, 2012). In type 2 sourdough fermentation, *L. sanfranciscensis* is not competitive enough to become the dominant LAB under type 2 fermentation conditions (De Vuyst & Neysens, 2005).

2.5.5 Type 3 sourdough

Type 3 sourdough is prepared using dried starter cultures to produce sourdough with a more stable performance and as flavour promoters (Zhou & Therdthai, 2012; Corsetti, 2013). Therefore, LAB used in this type of sourdough must be able to survive the freeze-drying process (De Vuyst & Neysens, 2005; Zhou & Therdthai, 2012). *L. plantarum*, *L. brevis* and *P. pentosaceus* are typical freeze-dried starter cultures resistant to the drying process and therefore can be used with this type of sourdough (Corsetti, 2013).

2.6 Starter cultures used in the production of fermented foods

2.6.1 General aspects of starter cultures

Agricultural products such as dairy, meat and cereal products can be fermented by indigenous microflora or defined starter cultures to produce fermented foods with desirable properties such as a longer shelf-life and improved sensory properties (Ammor & Mayo,

2007). A starter culture is composed of a large number of desirable microbes which are used to initiate fermentation (Caplice & Fitzgerald, 1999; Leroy & De Vuyst, 2004). The composition of a starter culture may have one or more strains of the same or different species of microorganism which can promote a more rapid start of the fermentation compared to spontaneous fermentation (Axelsson & Ahrné, 2000; Romano, Fiore, Paraggio, Caruso, & Capece, 2003).

Using a starter culture with defined composition for food fermentation has several advantages compared to spontaneous fermentation such as standardisation and better control of the fermentation process (Giraffa, 2004; Marsilio et al., 2005). Therefore, development of a starter culture is important for upscaling a traditional homemade fermented food to industry level (Holzapfel, 2002; Giraffa, 2004). Back-slopping can be used as a source of starter cultures (Leroy & De Vuyst, 2004), particularly for small-scale traditional and artisan products. Commercial starter cultures in frozen or dried form are commonly used in large scale sourdough production to reduce the cost associated with bulk volumes of back-slopping and this also decreases the risk of bacteriophage infection, which can affect bacterial reproduction and therefore inhibit fermentation (Holzapfel, 2002; Speranza, 2017). Use of commercial cultures with known traits and composition is economical as it promotes the production of final products with predictable quality and characteristics (Palavecino Prpich et al., 2015). However, back-slopping and spontaneous fermentation of foods are likely to continue as these products have specialised characteristics and appeal to a certain segment of society (Sieuwerts, De Bok, Hugenholtz, & Van Hylckama Vlieg, 2008). Traditional fermentation of food is also likely to continue or even increase due to demand for naturally processed products (Holzapfel, 2002; Speranza, 2017).

2.6.2 Sourdough starter cultures

Sourdough starter cultures are mainly composed of LAB and yeasts (Gobbetti & Gänzle, 2012). During fermentation, dough acidity decreases and certain strains grow at higher rates than others (Charalampopoulos et al., 2002; Todorov & Holzapfel, 2014). As a result, prokaryotic LAB and eukaryotic yeasts outcompete other microorganisms from the flour,

and become the dominant microflora of sourdough, which can then be used as the starter culture for another batch of sourdough bread production (Minervini, De Angelis, Di Cagno, & Gobbetti, 2014). Other microorganisms such as acetic acid bacteria may be present but they are not considered as key bacteria in starter cultures (Hutkins, 2008).

In mature sourdoughs which have stable performance, the predominant sourdough LAB and yeasts are present in significant numbers, with more than 8 log CFU/g of LAB and the number of co-existing yeasts usually being one or two logarithmic magnitudes lower (Ehrmann & Vogel, 2005; Ercolini et al., 2013; Minervini et al., 2014). The higher numbers of LAB than yeasts may result from a higher growth rate of LAB during fermentation and antagonistic interactions between LAB and yeasts, which benefits the growth of LAB (De Vuyst & Neysens, 2005; Venturi, Guerrini, & Vincenzini, 2012). As well as competing for nitrogen and carbon sources, LAB produce bacterial enzymes and organic acids which may accelerate yeast lysis and hence hinder yeast growth (De Vuyst & Neysens, 2005; Corsetti, 2013; Minervini et al., 2014).

2.6.3 Application of sourdough starter cultures in production

Sourdough starter culture is used to inoculate fresh flour mix and water to initiate a new fermentation (Cauvain & Young, 2007; Corsetti & Settanni, 2007; Catzeddu, 2011). As described in Section 2.5, fermentation of sourdough can be initiated in one of three ways: spontaneous fermentation (discussed in Section 2.5.2), back-slopping, or by adding a commercial/purified starter culture (Zhou & Therdthai, 2012; Minervini et al., 2014; Todorov & Holzapfel, 2014).

2.6.3.1 Back-slopping

Most artisan bakery shops and some manufacturers initiate a new batch of sourdough by back-slopping (De Vuyst & Neysens, 2005; Guyot, 2010; Minervini et al., 2014). Sourdoughs prepared by back-slopping contain dominant and sub-dominant microflora from the mother sourdough (Valjakka, Kerojoki, & Katina, 2003; Zhou & Therdthai, 2012; Todorov & Holzapfel, 2014).

When production parameters such as water content, pH, and temperature are consistent and the propagation continues, sourdough microflora becomes stable (Meroth, Walter, Hertel, Brandt, & Hammes, 2003). For example, some LAB such as *L. reuteri* dominate because they can produce bacteriocins which inhibit contaminant microbes. Some LAB such as *L. plantarum* RTa12 can remain stable due to its ability to adjust to a wide range of temperatures (Zhou & Therdthai, 2012).

After repeated use, back-slopping sourdough starter cultures become stable irrespective of changes in raw materials, contamination and temperature (De Vuyst & Neysens, 2005). According to Meroth et al. (2004), predominate LAB became stable after 10-14 days of propagation after the initial inoculation. However, previous studies have shown that dominant microflora and microflora ratios may change over years of propagation (Gobbetti & Gänzle, 2012; Todorov & Holzapfel, 2014). Thus, standardised procedures do not always guarantee the consistency of the microflora in sourdough and the final product quality (Todorov & Holzapfel, 2014). The growth and survival of LAB species can be affected by four key factors: adaption to type of carbohydrates (carbohydrate metabolism), temperature, pH and stress response. Changes in any of these factors can influence the sensory properties of the final sourdough bread (Gobbetti & Gänzle, 2012).

2.6.3.2 Defined starter cultures

For industrial scale production of sourdough which requires reproducibility, defined starter cultures containing certain selected pure species of LAB and yeasts with desired characteristics are utilised (Zhou & Therdthai, 2012; Altieri, Soro Yao, Brou, Amani, Thonart, & Djè, 2014; Ciuffreda, Di Maggio, & Sinigaglia, 2016;). Defined cultures are usually supplied in freeze dried or frozen dried forms and therefore must be able to survive the freezing process (Brandt, 2014). An overview of selected species of starter culture used for commercial sourdough production is summarised in Table 2.1 (Brandt, 2014).

Table 2.1 Starter culture composition in commercial starter preparations.

Preparation	Lactic acid bacteria	Yeasts
Frozen/freeze-dried/spray-dried	*L. brevis, L. plantarum* *L. sanfranciscensis, L. casei* *L. delbrueckii, L. fermentum* *P. pentosaceus, P. acidilactici*	*S. cerevisiae* *S. cerevisiae var. chevalieri* *T. delbrueckii*
Cereal-based	*L. sanfranciscensis, L. pontis* *L. crispatus, L. brevis, L. casei* *L. plantarum, L. fermentum* *L. paracasei, L. helveticus* *L. paralimentarius* *Leuconostoc lactis*	*C. milleri* *S. cerevisiae,* *S. pastorianus*

Source: Brandt, (2014).

Although dominant species such as *L. fermentum*, *L. plantarum* and *S. cerevisiae* are commonly found in both GF fermented products and wheat and rye sourdough, starter cultures developed for wheat and rye sourdough bread may not always be suitable for fermentation of GF flours in terms of adaptability and product sensory properties. This may be attributed to variable nutrient levels, as well as the presence of antimicrobial substances in different flours (Moroni et al., 2009). For example, *L. paralimenrarius* was a dominant species in buckwheat and amaranth sourdough but not in wheat or rice sourdough, probably due to the higher levels of free amino acids in pseudocereals, particularly lysine and threonine (Vogelmann, Seitter, Singer, Brandt, & Hertel, 2009). Also, in order for LAB species to survive in some GF flours, they need a high tolerance against substances such as tannins which have antimicrobial properties (Vogelmann et al., 2009). Apart from the adaptability of LAB species, undefined starter cultures used for GF sourdough bread production may produce undesirable aromas such as the mouldy odour associated with buckwheat and quinoa sourdough bread (Settanni, 2017).

More research on defined GF sourdough starter cultures is required for the manufacture of high quality sourdough products (Moroni, Dal Bello, & Arendt, 2009). Novel strains of competitive starter cultures have been isolated and identified from gluten free sourdough and more research on their characterisations is needed (Meroth, Hammes, & Hertel, 2004;

Moroni et al., 2009). Several bacterial strains, such as *Pediococcus pentosaceus* RTa11 and *Lactobacillus plantarum* RTa12 have been recommended for used in GF sourdough bread starter cultures because of their adaptability to growth at various temperatures and their ability to rapidly acidify the dough (Zhou & Therdthai, 2012).

2.7 Important sourdough parameters

The physico-chemical parameters of pH, total titratable acidity (TTA), ratio of lactic and acetic acid and microbial composition, such as number of LAB and yeasts are important for successful sourdough production (Valjakka et al., 2003; Gobbetti & Gänzle, 2012). The number of LAB and yeasts and their ratio influence sourdough performance, which can be evaluated through dough acidity (pH and TTA) and the fermentation quotient (ratio of lactic acid: acetic acid) as shown in Table 2.2 (Gobbetti & Gänzle, 2012).

Table 2.2 Sourdough production parameters

Production parameters	Range
pH	3.5-4.3
Fermentation time	8-24 hours
Fermentation temperature	25-35 °C
LAB	8-10 log CFU/g
Yeasts	5-7 log CFU/g
Amount of mother sponge	10-20 % flour (long fermentation time)
	25-35 % flour (short fermentation time)
Total titratable acid (TTA)	
-- Whole meal flour	15-26*
-- Straight grade flour	8-11*

Source: Hui & Evranuz (2012); Gobbetti & Gänzle (2012).
Note: * Volume (mL) of 0.1 M NaOH used to titrate 10 g sourdough sample

2.7.1 pH

pH is used to evaluate the development level of sourdough during fermentation, with a final pH of 3.5 to 4.3 expected for well-developed sourdough (Gobbetti & Gänzle, 2012). For most rye and wheat sourdoughs, the final pH ranges from 3.5-3.8 (Valjakka et al., 2003), while for rice mother sourdoughs, the pH ranges from 3.8-3.9 (Meroth et al., 2003).

2.7.2 Total titratable acidity

Total titratable organic acids produced during sourdough fermentation are measured using TTA which can be expressed as percentage of lactic acid in dough samples or required volume of NaOH to titrate 10 g of sourdough sample (Gobbetti & Gänzle, 2012). Organic acids produced during sourdough fermentation includes lactic acid, acetic acid, caproic acid, formic acid and phenyllactic acid (Valjakka et al., 2003; Leroy & De Vuyst, 2004; Gobbetti & Gänzle, 2012). The values of TTA generally vary from 8-26 mL (of 0.1 mol/L NaOH used to titrate 10 g of sourdough sample), depending on the fermentation temperature, dough yield and flour types used (Valjakka et al., 2003; Gobbetti & Gänzle, 2012).

2.7.3 Fermentation quotient

The fermentation quotient (FQ) is the molar ratio of lactic acid and acetic acid produced during fermentation (Valjakka et al., 2003; Gobbetti & Gänzle, 2012). In whole meal rye sourdoughs, the content of lactic and acetic acid were shown to be 1.2-1.7 % and 0.3-0.4 %, respectively (Valjakka et al., 2003). This ratio directly impacts on the taste and flavour of sourdough bread (Valjakka et al., 2003; Gobbetti & Gänzle, 2012). Lactic acid is not volatile and its aroma is not as strong as acetic acid which has a pickling smell (Corsetti, 2013). For a mild balanced flavour and aroma, a quotient value of between 4 and 9 is favoured (Gobbetti & Gänzle, 2012). The FQ value also reflects the ratio of homofermentative and heterofermentative *Lactobacillus* leavening the dough due to their different predominating metabolic pathways (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002; Gobbetti & Gänzle, 2012).

2.8 Important sourdough parameters

Flour type and flour extraction rate, dough yield (DY), fermentation time, temperature, and concentration of starter cultures are key factors affecting the composition of sourdough microflora (Figure 2.2) (Valjakka et al., 2003; De Vuyst, Van Kerrebroeck, & Leroy, 2017). Different types of flours and their extraction rates result in different levels of available carbohydrates, proteins, mineral and enzymes such as amylases and proteases (De Vuyst et al., 2017). Due to differences in flour and process conditions, sourdough microflora has a wide diversity. More than 20 species of yeast and 80 species of LAB have been isolated from mature sourdoughs (Arendt et al., 2007; Lattanzi et al., 2013; Gobbetti, Minervini, Pontonio, Di Cagno, & De Angelis, 2016b; Nionelli & Rizzello, 2016).

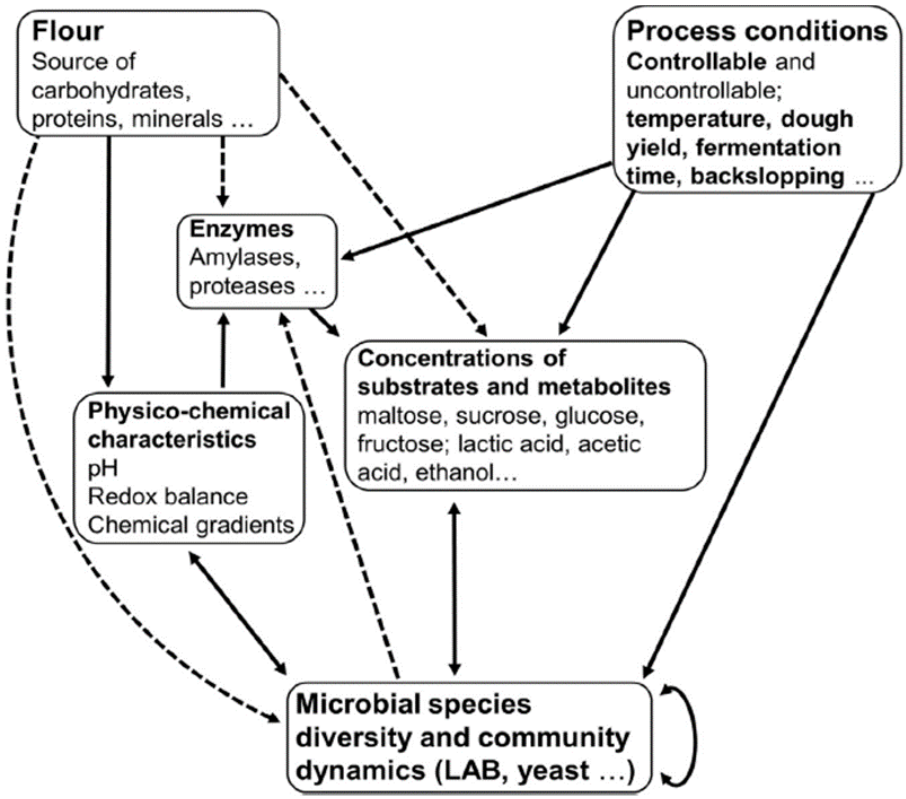

Figure 2.2 Overview of key factors affecting sourdough microflora.

Source: De Vuyst et al. (2017).
Note: Discontinuous lines with arrows indicate sources of respective item; continuous lines with arrows indicate inducing factors.

2.8.1 Flour

Flours, even of the same type of flour, can vary from brand to brand in terms of flour microorganisms and available nutrients supporting their growth (Ehrmann & Vogel, 2005; Gobbetti et al., 2016b). In addition, GF flours may harbour different species of LAB from wheat and rye flours (De Vuyst & Vancanneyt, 2007).

The place of origin, farming practices and milling system which will likely contain different microbes will add to the diversity of sourdough microflora (De Vuyst & Neysens, 2005; Gobbetti et al., 2016b). From non-sterile flour, total aerobic cell counts can reach as high as 7 log CFU/g, while yeast and fungi count can range between 4-7 log CFU/g and coliform bacteria 3-7 log CFU/g (Brandt, 2014). Yeasts belonging to genera *Candida* and *Saccharomyces* have been detected in flour, with counts of up to 3 log CFU/g (De Vuyst & Neysens, 2005). However, *S. cerevisiae* has not been isolated from flour (De Vuyst & Neysens, 2005). Gram-positive LAB and many types of Gram-negative bacteria such as *Enterobacteriaceae, Bacillus cereus* and *Staphylococcus sp*. have also been isolated from non-sterile flours (De Vuyst & Neysens, 2005; Gobbetti, Minervini, Pontonio, Di Cagno, & De Angelis, 2016a). However, with the exception of *Enterobacteriaceae*, the growth of contaminant microflora is almost completely inhibited after one day fermentation (Gobbetti et al., 2016a).

Different flour types also have different levels of available carbon and nitrogen resources for microorganisms, which will impact on the growth of individual species (Gobbetti et al., 2016a). Flours with higher levels of fermentable carbon and nitrogen sources result in a lower cell density of yeasts and higher levels of heterofermentative LAB (De Vuyst & Vancanneyt, 2007; Gobbetti et al., 2016a). Also, the availability of fermentable carbon sources such as maltose, fructose, and glucose and nitrogen sourced from free amino acids are correlated with the LAB and yeast cell density (De Vuyst & Vancanneyt, 2007; Gobbetti et al., 2016a, 2016b).

2.8.2 Dough yield

The ratio between dough weight and flour weight is defined as DY which affects the composition of sourdough microbiota (Minervini et al., 2014). In sourdough, DY can range from firmer: 150, to 225 which is more liquid (Minervini et al., 2014). Water content in sourdough, which is related to the amount of available fermentable carbohydrates, amino acids and nutrients such as vitamin B is related to DY (Valjakka et al., 2003; Minervini et al., 2014). These nutrients are important substrates for microorganisms and affect the growth ratio between LAB and yeasts, and the ratio between homofermentative and heterofermentative LAB (Minervini et al., 2014). In sourdough with a high DY, LAB can grow faster than yeasts, whereas a lower ratio and firmer sourdough supports the growth of yeasts (Di Cagno et al., 2014; Minervini et al., 2014).

The dominant LAB in sourdough is also affected by DY because it can alter the pH of the dough (Valjakka et al., 2003). For example, rye sourdoughs made with higher water content result in higher acidity per gram of dry mass than those made with lower water content (Valjakka et al., 2003). With higher DY, higher levels of organic acids can be produced during sourdough fermentation and acid-tolerant *L. plantarum*, *L. reuteri* and *L. fermentum* can be found. However, *L. sanfranciscensis* is not found as it cannot grow below pH 3.8 (Valjakka et al., 2003; Gobbetti & Gänzle, 2012).

2.9 Lactic acid bacteria sourdough starter culture

Lactic acid bacteria are one of the main microbial groups that affect the quality of sourdough (Gobbetti & Gänzle, 2012; Gobbetti et al., 2016b). They are characterised by having lactic acid as the main end-product metabolite (Konings & Kuipers, 2013). LAB include Gram-positive, non-spore forming, catalase-negative, aerotolerant or non-aerobic, and acid-tolerant bacteria (Robinson & Batt, 2014). LAB are composed of different genera of microorganisms with variable phenotypic and chemotaxonomic features (Temmerman, Huys, & Swings, 2004). Their classification is based on their morphological features (cocci, tetrad, rod), phenotypical features (e.g. fermentation modes, configuration of lactic acid) and genetic features (e.g. DNA, RNA) (Holzapfel & Wood, 2012).

Isolated LAB from sourdough commonly belong to the genera *Enterococcus*, *Pediococcus*, *Leuconostoc*, *Weissela* and *Lactobacillus* (Gobbetti & Gänzle, 2012; Gobbetti et al., 2016b; Speranza, Bevilacqua, Corbo, & Sinigaglia, 2016). Of these, the majority of isolated LAB species belong to *Lactobacillus*, which are competitive in the sourdough environment and are therefore regarded as typical sourdough LAB (Luc De Vuyst & Neysens, 2005; Speranza et al., 2016).

2.9.1 Sourdough Lactobacillus

Lactobacillus used in fermented foods have several advantages: (1) they improve the nutritional value of the food, (2) stimulate vitamin synthesis, (3) inhibit pathogens by producing antimicrobial substances and also compete for available nutrients, (4) reduce cholesterol levels, and (5) decrease risk of colon cancer (Tamang, Shin, Jung, & Chae, 2016).

Lactobacillus is the largest genus of LAB with over 170 species and subspecies (Luc De Vuyst & Neysens, 2005; Gobbetti et al., 2016a). They are rod-shaped, gram-positive, catalase-negative, non-spore-forming and most are non-motile (Gobbetti & Gänzle, 2012; Holzapfel & Wood, 2012). The cells are usually arranged in chains and can grow in temperatures ranging from 2-53 ℃, although for most Lactobacillus, the optimum growth temperature ranges from 30-40 ℃ (Batt, 2000). *Lactobacillus* grow at pHs ranging from 3-8, preferentially under anaerobic conditions (Batt, 2000), with some species being strictly anaerobic while others are aero tolerant (Hammes & Vogel, 1995; Batt, 2000). For growth, *Lactobacillus* species require various nutrients (amino acids, peptides, carbohydrates, vitamins, nucleic acid derivatives, salts, etc) (De Vuyst, Vrancken, Ravyts, Rimaux, & Weckx, 2009; Holzapfel & Wood, 2012).

2.9.2 Dominant Lactobacillus in sourdough

Irrespective of the type of flours used for making sourdough, the most common *Lactobacillus* species are obligate heterofermentatives belonging to *L. brevis*, *L. sanfranciscensis* (especially type 1 sourdough), *L. reuteri*, *L. fermentum* and *L. rossiae*; facultative heterofermentative *L. alimentarius*, *L. plantarum* and *L. paralimentarius*; and

from the obligate homofermentative group: *L. amylovorus* and *L. delbrueckii* (De Vuyst & Neysens, 2005; Van der Meulen et al., 2007; Kamal-Eldin, 2012; Venturi, Guerrini, & Vincenzini, 2012; Lattanzi et al., 2013; Giraffa, 2014a; Minervini et al., 2014; Gobbetti et al., 2016).

Sourdough made with GF flours can support growth of different types of LAB species including some which are similar to those reported in wheat sourdoughs (Gobbetti et al., 2008). Of the little research carried out on GF sourdough, *L. paracasei*, *L. paralimentarius*, *L. perolens* and *L. spicheri* have been shown to be the dominant LAB species in rice sourdough (Meroth et al., 2003).

2.10 Lactic acid bacteria sourdough starter culture

Yeasts are single celled fungi, which grow by budding or fission (De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016a). Sourdough yeasts can tolerate stress conditions such as low acidity, osmotic stress and low carbon source concentrations. Therefore, they are able to produce important metabolites such as carbon dioxide during dough fermentation which are important for final bread quality (Gobbetti & Gänzle, 2012). Yeasts isolated from sourdough include *S. cerevisiae*, *C. humilis* (syn. *C. milleri*), *P. kudriavzevii*, *T. delbrueckii*, *P. anomala*, *H. anomala* and *K. exigua* (Reed & Nagodawithana, 1991; Stolz, 2003; Catzeddu, 2011; Lattanzi et al., 2013; Minervini et al., 2014). In a single sourdough, one or two yeast species may be present (Gobbetti & Gänzle, 2012; De Vuyst et al., 2016).

Key functions of yeasts in bread-making include leavening and forming flavour compounds. The metabolic activities of yeasts increase the nutritional value of sourdough, in addition to increasing the inherent antioxidant capacity of cereal products (Boekhout & Robert, 2003; Maloney & Foy, 2003; De Vuyst et al., 2016). Several yeast strains also have probiotic potential and can dephosphorylate phytic acid, which can bind important minerals such as iron and zinc and lower their availability for consumers (Czerucka, Piche, & Rampal, 2007; De Vuyst et al., 2016a).

2.11 Metabolic characteristics of sourdough starter cultures

Metabolic characteristics of sourdough starter cultures are key to the final properties of the fermented products (Gobbetti & Gänzle, 2012). The ability of starter cultures to utilise nutrients such as sugars and amino acids from the substrate determines their competitiveness and adaptability. Meanwhile, their metabolites affect the final quality of the sourdough bread, including parameters such as texture, sensory properties, nutritional value, and shelf life (Gobbetti & Gänzle, 2012; Hui & Evranuz, 2012). Therefore, knowledge of the metabolic characteristics of starter culture strains is important for improvement of a sourdough product.

Organic acids released during carbohydrate metabolism have positive effects on texture, shelf life and flavour (Arendt, Ryan, & Dal Bello, 2007; Moore, Juga, Schober, & Arendt, 2007; Arendt, Moroni, & Zannini, 2011; Zhou & Therdthai, 2012). In addition, the acids decrease the pH of the dough which increases the activities of cereal proteases and amylases to release structure-forming compounds and increase protein solubility (Arendt et al., 2007; Catzeddu, 2011). Organic acids also delay the spoilage of bread products by related microorganisms and contribute to the sensory properties of the bread (Valjakka, Kerojoki, & Katina, 2003; De Vuyst & Vancanneyt, 2007; Catzeddu, 2011).

LAB carbohydrate metabolism also generates EPS which can act as gelling and stabilising agents, which increase the softness and water absorption ability of the dough (Arendt et al., 2007; Arendt et al., 2011; Galle et al., 2012). In addition, EPS can act as prebiotics, which have positive effects on human health as discussed in Section 2.12.6 (Cho & Finocchiaro, 2009; Lee & Salminen, 2009).

Free amino acids are used by LAB and yeasts as nitrogen sources and are also produced by LAB and yeasts through nitrogen metabolism which contribute to flavour and aroma compounds of the sourdough bread (Hui & Evranuz, 2012; Corsetti, 2013). Some amino acids are reactants in the Maillard reaction, which impact on bread colour, flavour and aroma (Yilmaz, 2005). Therefore, understanding the metabolic pathways and the activities of sourdough microorganisms LAB and yeasts are important for improving sourdough bread quality.

2.11.1 Carbohydrate metabolism

2.11.1.1 Carbohydrate metabolism of *Lactobacillus*

Sugar fermentation by LAB can be divided into homofermentative and heterofermentative types (Kandler, 1983; Holzapfel & Wood, 2012). Obligate homofermentative LAB such as *L. delbrueckii* metabolise hexoses to lactic acid as the main end-product through the Embden–Meyerhof–Parnas (EMP or glycolysis) pathway supported by aldolase, but they cannot utilise gluconate nor pentoses because they do not possess the enzyme phosphoketolase (Holzapfel & Wood, 2012; Giraffa, 2014). Facultative heterofermentative LAB, including *L. plantarum* and *L. casei*, have both aldolase and phosphoketolase and therefore can ferment hexoses, pentose and gluconate into lactate, acetate, CO_2, ethanol and formate (De Vuyst, 2009; Holzapfel, 2012). Although obligate heterofermentative LAB can use both pentoses and hexoses, their sugar metabolism proceeds via the phosphoketolase pathway due to their lack of aldolase (Fugelsang & Edwards, 2006).

The homofermentative pathway carried out by LAB is shown in Figure 2.3 (Fugelsang & Edwards, 2006). During glycolysis, homofermentative LAB convert one mole of glucose into two moles of lactic acid and release two moles of ATP (Fugelsang & Edwards, 2006). One mole of fructose-1,6-bisphosphate is converted into two moles of glyceraldehyde-3-phosphate in a reaction catalysed by aldolase. Lactate is formed from pyruvate when glyceraldehyde-3-phosphate is oxidised by lactate dehydrogenase (LDH) to 1,3-bisphosphoglycerate, while NADH is oxidised to NAD^+ (Kandler, 1983; Fugelsang & Edwards, 2006). Homofermentative and facultative heterofermentative LAB also utilise glucose via this pathway (Fugelsang & Edwards, 2006).

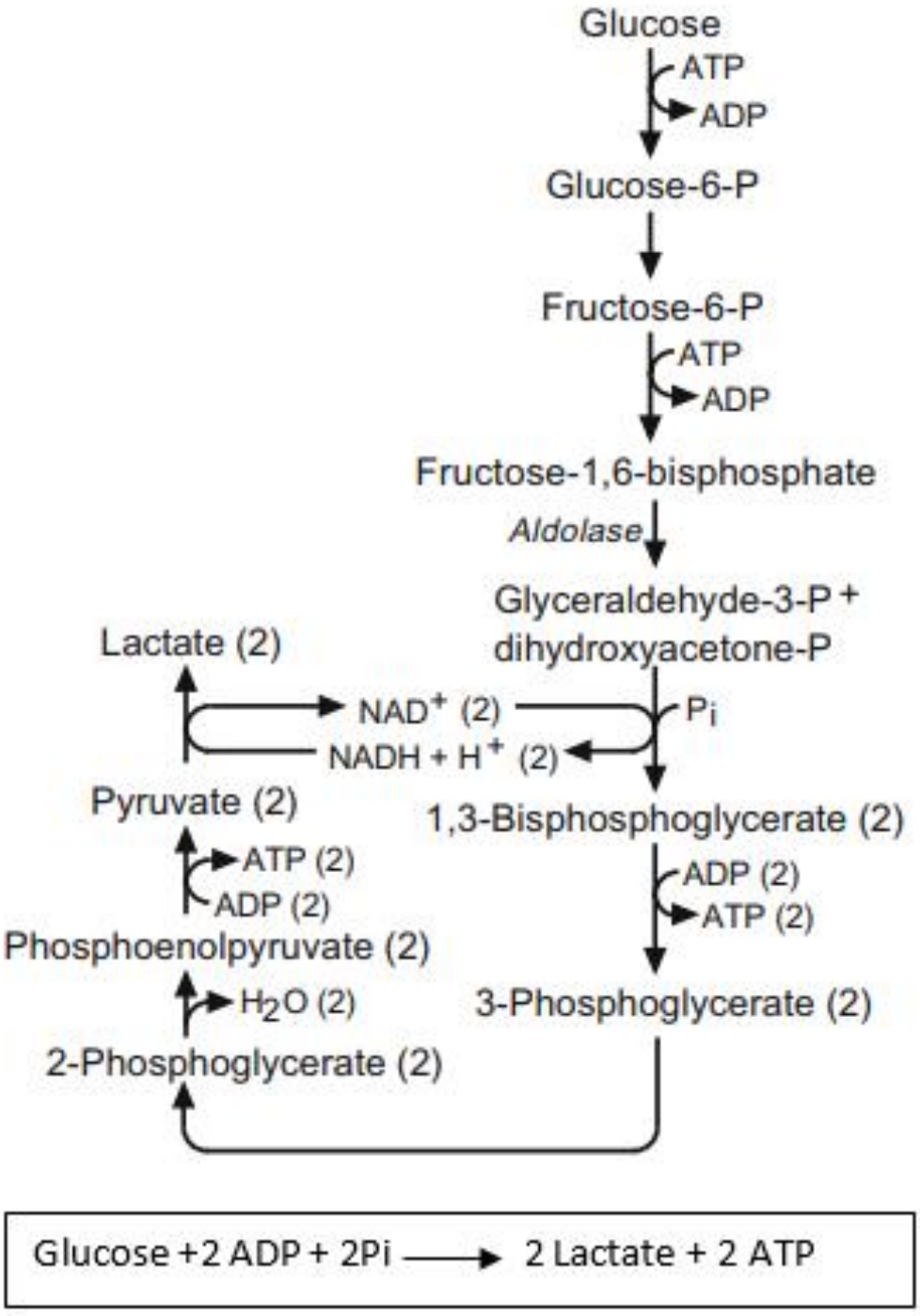

Figure 2.3 Homofermentation pathway of lactic acid bacteria.

Source: Fugelsang & Edwards (2006).

The heterofermentation pathway of LAB is shown in Figure 2.4 (Fugelsang & Edwards, 2006). One mole of glucose is converted to one mole each of CO_2, lactate, and ethanol or acetic acid depending on the fermenting microorganism (Kandler, 1983; Fugelsang & Edwards, 2006). When LAB cells are short of NAD^+, acetyl phosphate is converted to ethanol which produces only one mole of ATP, while conversion to acetate can produce two moles of ATP when electron acceptors such as fructose are available (Kandler, 1983; Fugelsang & Edwards, 2006). In the presence of phosphoketolase, xylulose-5-phosphate is cleaved into glyceraldehyde-3-phosphate (GAP) and acetyl phosphate. GAP is further converted to pyruvate and two ATP and one $NADH/H^+$ are released at the same time. Pyruvate, assisted by LDH, is reduced to lactate. Acetyl phosphate, can be dephosphorylated by phosphotransacetylase and aldehyde dehydrogenase to acetaldehyde or be converted to acetate (Fugelsang & Edwards, 2006). Acetaldehyde can be further reduced to ethanol by alcohol dehydrogenase (Schaechter, 2009).

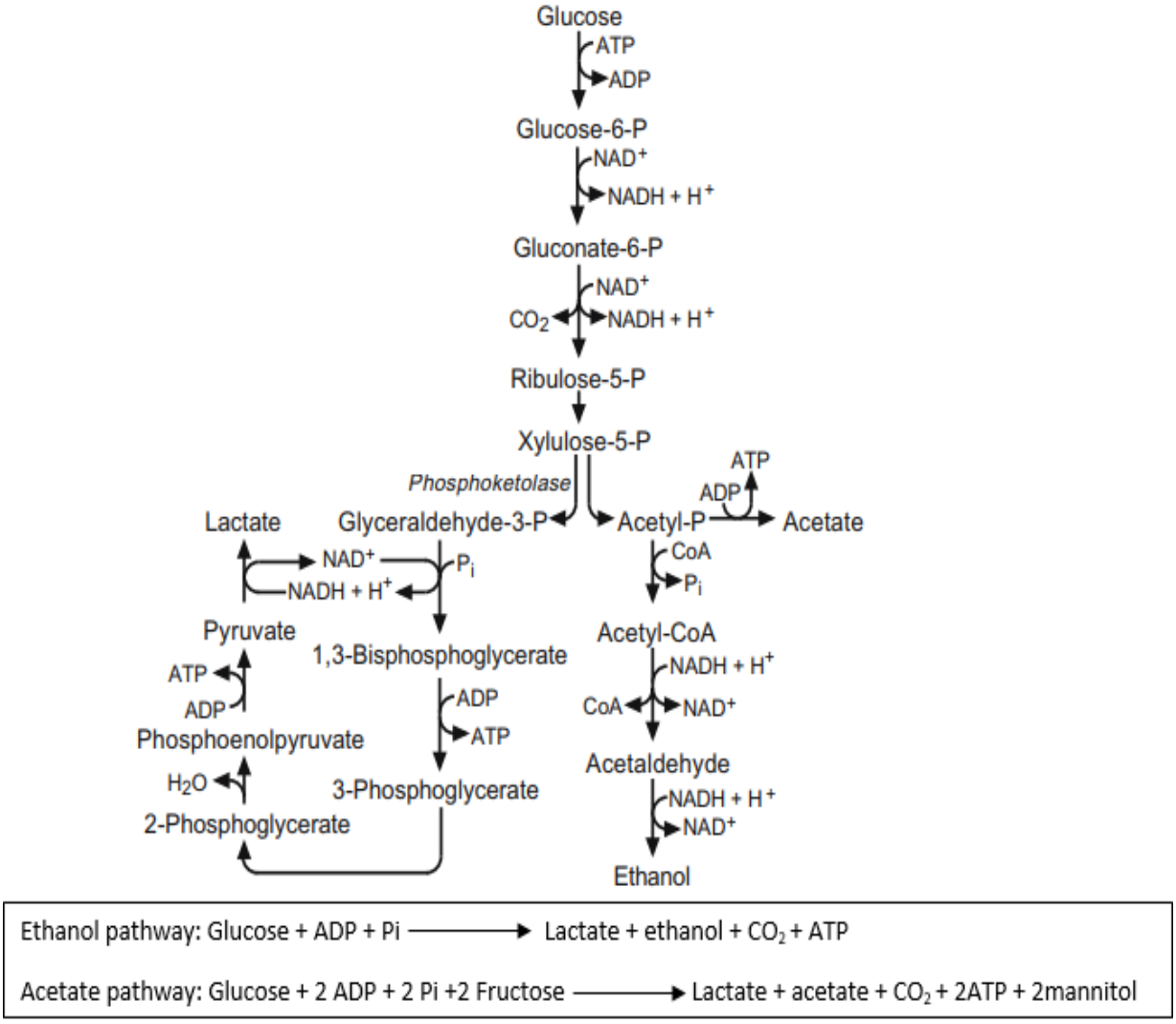

Figure 2.4 Heterofermentation pathway of lactic acid bacteria.

Source: Fugelsang & Edwards (2006).

2.11.1.2 Yeast carbohydrate metabolism

Compared to the carbohydrate metabolism of LAB, yeast carbohydrate metabolism produces fewer metabolites as shown in Figure 2.5 (De Vuyst, 2016). Based on their use of maltose, yeasts can be divided into maltose-negative or maltose-positive types (Hammes & Vogel, 1995; De Vuyst et al., 2009). Maltose-negative yeasts use glucose preferentially to other carbohydrates whereas maltose-positive yeasts are capable of using all types of flour carbohydrates (De Vuyst et al., 2009).

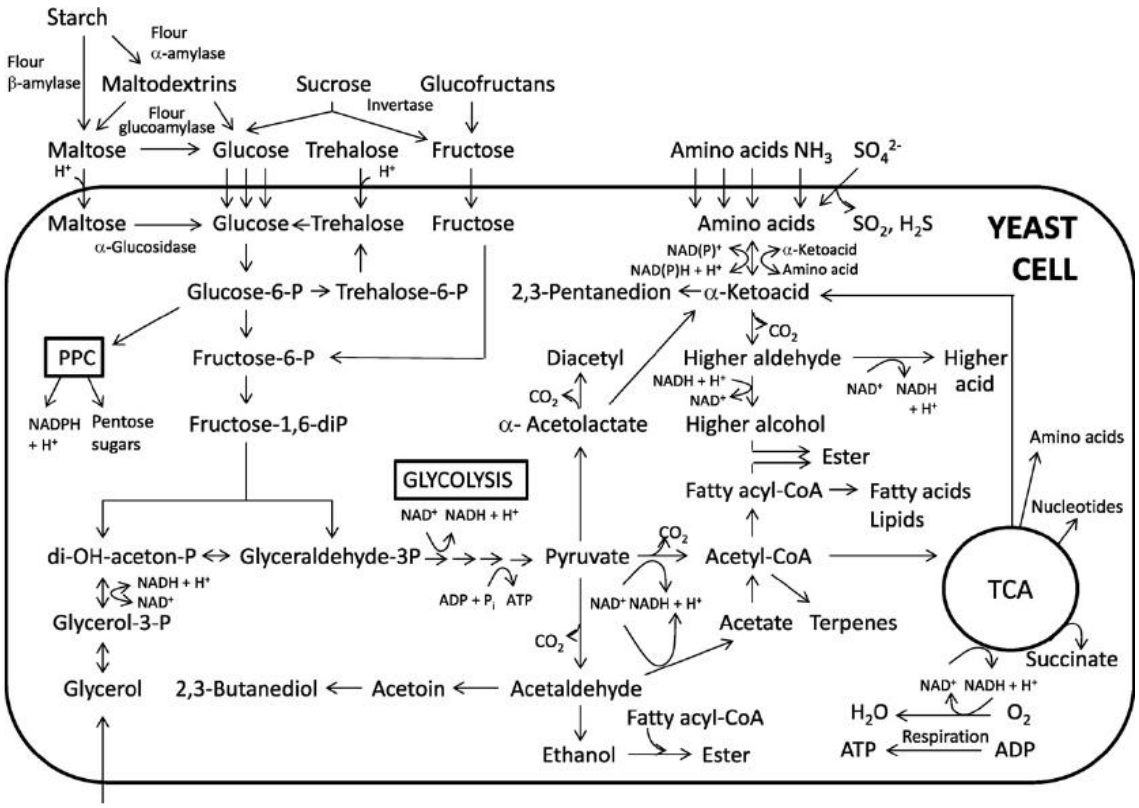

Figure 2.5 Yeast metabolism of important substrates.

Source: De Vuyst et al. (2016b).

Yeasts can hydrolyse glucofructans and sucrose using invertase to convert glucose into fructose, which can then be used as an electron acceptor by LAB (De Vuyst & Neysens, 2005; De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016b). After disaccharides are broken into monosaccharides, the resultant sugars are then fermented through the glycolytic pathway to produce ethanol and carbon dioxide (Boekhout, 2003; De Vuyst, 2016). The carbon dioxide contributes to the bread volume and the ethanol contributes to stiffness, resulting in a firm and less extensible dough (De Vuyst et al., 2009; Jayaram et al., 2014). Ethanol, which is produced at low levels, evaporates during baking due to the high temperatures used (Hui, 2006; De Vuyst et al., 2009; De Vuyst et al., 2016b).

The low availability of fermentable sugars can limit yeast sourdough fermentation by switching active metabolic pathways from fermentation to respiration (Gobbetti & Gänzle,

2012; De Vuyst et al., 2016b). When sugar concentrations are low due to the metabolism of starter cultures, the metabolic pathway switches to acetyl-CoA and more ATP is produced (Bamforth, 2005).

2.11.2 Nitrogen metabolism

2.11.2.1 Nitrogen sources in sourdough

In sourdough, amino acids function as the main nitrogen sources for LAB and yeasts (Gobbetti & Gänzle, 2012). Amino acids may have been released during proteolysis of flour proteins by proteases, or synthesised by LAB and other flour inherent microflora (Gobbetti, 1998; De Vuyst et al., 2009). In addition, lysis of LAB and yeast cells can result in the release of various amino acids (Nollet, Benjakul, Paliyath, & Hui, 2012).

Proteins can be degraded into peptides and then into amino acids through primary and secondary proteolysis (Gänzle, Vermeulen, and Vogel 2007). Primary proteolysis of cereal proteins during fermentation is mainly carried out by flour enzymes (Gobbetti & Gänzle, 2012). During fermentation, the pH of the dough can decrease to below 4.5, the pH at which aspartate protease has optimum activity (Gobbetti & Gänzle, 2012). Secondary proteolysis is carried out by microbial enzymes and amino acid metabolism by sourdough microbes. In sourdough, amino acids function as the main nitrogen sources for LAB and yeasts (Gobbetti & Gänzle, 2012).

Amino acids can also be released following the lysis of microbial cells or degradation induced by mechanical mixing or by bacterial enzymes (Gobbetti, 1998). Key amino acids released from *S. cerevisiae* cells include proline, glycine, alanine, isoleucine, valine and γ-aminobutyric acid, while glycine and alanine are released from LAB cell degradation (Gobbetti, 1998).

2.11.2.2 LAB nitrogen metabolism

LAB use amino acids for energy, protein synthesis and intracellular pH regulation (De Vuyst et al., 2009). Amino acids can be metabolised through the arginine deiminase (ADI) pathway or be catabolised for the production of flavour precursors (De Vuyst et al., 2009). ATP formed through the ADI pathway contributes to microbial growth (Laskin, Bennett, & Gadd, 2003). *L. brevis*, *L. pontis* and *L. fermentum* can convert ornithine and arginine to ammonia, which enhances the aroma of the sourdough bread (De Vuyst et al., 2009). The metabolite 2- acetylpyrroline, which is derived from ornithine is responsible for the roasted aroma of baked bread (De Vuyst & Neysens, 2005). In some LAB such as *L. fermentum*, *L. reuteri*, arginine is converted through the ADI pathway to ornithine which also enhances the survival ability of LAB because it improves their acid tolerance (De Vuyst et al., 2009).

Amino acids, including branched-chain amino acids (leucine, valine, isoleucine), aromatic amino acids (tyrosine, phenylalanine, tryptophan) and the sulphur-containing amino acid methionine are converted to α-keto acids through transamination reactions. The α-keto acids are then converted to aldehydes through decarboxylation. Branched-chain amino acids can also undergo oxidative decarboxylation to yield carboxylic acid (De Vuyst & Neysens, 2005). The resultant aldehydes can then be converted to alcohols and carboxylic acids. End-product esters and/or thioesters are synthesised by condensation of carboxylic acids and alcohols. When methionine is present, methanethiol can be produced as the end-product. These end-products derived from amino acids are important flavour compounds for breads (De Vuyst et al., 2009).

2.11.2.3 Nitrogen metabolism of yeasts

Yeasts can use various nitrogen sources such as free amino acids and NH_4^+ for growth. When amino acids and NH_4^+ co-exist, yeasts prefer to use NH_4^+ (Gobbetti, 1998), however, of the amino acids yeasts preferentially utilise asparagine (Gobbetti & Gänzle, 2012). Similar to the nitrogen metabolism of LAB, amino acids or ammonia are first converted to α-keto acids (Gobbetti & Gänzle, 2012). During decarboxylation, amino acids are transferred to higher aldehydes, then reduced into higher alcohols. The resulting ethanol can react with carboxylic acids such as lactic acid and acetic acid through a condensation

reaction yielding esters (Gobbetti & Gänzle, 2012). Yeasts can also excrete the amino acids leucine and valine, as well as nucleotides and succinate through the tricarboxylic acid cycle (Gobbetti & Gänzle, 2012).

2.11.3 Interactions between LAB and yeasts during sourdough fermentation

The stable associations between sourdough LAB and yeasts are important for the consistent industrial fermentation process under non-sterile conditions (De Vuyst & Neysens, 2005). Except for ecological factors such as temperature and pH, stable interactions between LAB and yeasts mainly depend on their metabolism of carbohydrate sources and amino acids (De Vuyst & Neysens, 2005; Venturi, Guerrini, & Vincenzini, 2012; De Vuyst et al., 2014). When sourdough LAB and yeasts compete for available sources, an antagonistic interaction occurs (De Vuyst & Neysens, 2005). For example, when maltose-positive yeasts *S. cerevisiae* coexist with maltose-positive *L. sanfranciscensis*, the metabolism of *L. sanfranciscensis* decreases (De Vuyst & Neysens, 2005).

In contrast, when LAB and yeasts are not competing for the major carbohydrate source, a synergistic interaction can be formed (Venturi et al., 2012). Maltose positive *L. sanfrancisensis* and maltose negative yeast species *C. humilis* or *K. exigua* can form a stable symbiosis in sourdough because they do not compete for their main carbon source (Venturi et al., 2012). *L. sanfrancisensis* preferentially uses maltose and releases glucose in a molar ratio of 1:1, while *C. humilis* and *K. exigua* use sucrose or glucose as carbon sources (De Vuyst & Neysens, 2005; Venturi et al., 2012).

Synergistic interactions also form between yeasts and LAB when yeasts provide LAB with fructose as an electron acceptor during yeast carbohydrate metabolism (De Vuyst & Neysens, 2005; Venturi et al., 2012). Some yeasts such as *S. cerevisiae* can hydrolyse sucrose into glucose and fructose whereas other yeast species such as *C. humilis* can degrade gluco-fructosans to provide more fructose (De Vuyst & Neysens, 2005). With more fructose available as an electron acceptor for heterofermentative LAB, more acetic acid can be released through the phosphoketolase pathway (De Vuyst & Neysens, 2005; Gobbetti & Gänzle, 2012). In addition, although yeasts can partially compete with LAB for nitrogen sources, yeasts excrete and synthesise essential amino acids such as leucine

and valine which can stimulate the growth of LAB, especially when essential amino acids are initially deficient (Hui & Evranuz, 2012). Thus, as glucose is metabolised by yeasts, they can release essential amino acids which assist the growth of LAB such as *L. sanfranciscensis* (Hui & Evranuz, 2012).

2.12 Probiotic potential of sourdough bread

It is well known that consumption of live probiotics is associated with health benefits (FAO/WHO, 2002). However, recent studies have reported that certain strains of inactivated probiotics may still confer health benefits to consumers. For example, several strains belonging to the genus *Lactobacillus* can still confer similar probiotic benefits in their inactivated form (Kataria et al., 2009; Komesu et al., 2017), and consumption of inactivated probiotics may be safer (Kataria, Li, Wynn, & Neu, 2009; Adams, 2010; De Almada, Almada, Martinez, & Sant'Ana, 2016).

Many species of *Lactobacillus* used for commercial probiotics are also found in sourdough, and given that some strains may confer health benefits following inactivation; this suggests that sourdough may have probiotic properties (Corsetti & Settanni, 2007; Vinderola, Binetti, Burns, & Reinheimer, 2011). Therefore the probiotic potential of sourdough bread may have been underestimated (Ouwehand & Röytiö, 2014). To prove the probiotic potential of sourdough bread, isolated strains from sourdough need to be identified to see whether these strains have been reported as being probiotic and also whether they can still confer health benefits after being inactivated (Cho & Finocchiaro, 2010; Ouwehand & Röytiö, 2014).

2.12.1 Probiotic and their health benefits

The health benefits of probiotics include mitigation of lactose intolerance symptoms, enhancement of the immune system, anti-tumour effects, alleviation of diarrhoea and anti-inflammation effects (Naidu, Bidlack, & Clemens, 1999; Saxelin, Tynkkynen, Mattila-Sandholm, & De Vos, 2005; Shah, 2007; Tamang, Shin, Jung, & Chae, 2016). Consumption of probiotics can improve gut health by inducing inherent beneficial bacteria and inhibit growth of harmful bacteria in the gastrointestinal tract (Cho & Finocchiaro,

2010), hence promoting a more balanced microflora in the gut (Lee & Seppo Salminen, 2009). In order to confer health benefits to consumers, recommended dosage levels of consumed probiotics range from 6-10 log CFU per day (Saavedra, 2001; Rutherfurd & Gill, 2004). For therapeutic purposes, the dosage for probiotics is 8-9 log CFU per day (Power, Toole, Stanton, Ross, & Fitzgerald, 2014).

Some fermented foods such as sourdough contain high amounts of LAB and yeasts which also possess probiotic properties (summarised in Table 2.3) (Van Der Aa Kühle et al. 2005; Parvez, Malik, Kang, & Kim, 2006). For example, *B. bifidus* Bb-11 and *L. plantarum* 299v are utilised in fermented dairy and vegetable products, respectively (Shah, 2007; Tamang et al., 2016). Certain yeast species have also demonstrated probiotic activity (Poutanen et al., 2009), with species of the genus *Saccharomyces* such as *S. cerevisiae* var. boulardii having been commercialised as probiotics (Martins et al., 2007; Etienne-Mesmin et al., 2011). *S. cerevisiae* var. boulardii was reported to be effective in treating gastroenteritis and has antimicrobial activities (Van Der Aa Kühle, Skovgaard, & Jespersen, 2005; Hatoum et al., 2012). However, the mechanisms behind the probiotic functions of yeasts, either in their live or inactivated form require further investigation (Van Der Aa Kühle et al., 2005; Poutanen et al., 2009). Therefore, fermented foods may also be a good source of probiotics.

Table 2.3 Common species of lactic acid bacteria and yeasts with probiotic properties

Lactobacillus sp.	Bifidobacterium sp.	Streptococcus sp.	Saccharomyces sp.
L. acidophilus	*B. bifidum*	*S. cremoris*	*S. cerevisiae* ssp. var. boulardii
L. casei	*B. adolescentis*	*S. salivarius*	
L. delbrueckii ssp. bulgaricus	*B. animalis*	*S. diacelylactis*	
L. cellobiosus	*B. infants*	*S. intermedius*	
L. curvatus	*B. thermophilum*		
L. fermentum	*B. longum*		
L. lactis			
L. plantarum			
L. reuteri			
L. brevis			

Source: Parvez, Malik, Kang, & Kim (2006); Van Der Aa Kühle et al. (2005).

2.12.2 Selection criteria for probiotic microorganisms

Even though microbes involved into fermentation are consumable, there are strict criteria surrounding the ability to make a claim that a certain microorganism has probiotic potential. In order to be safely used, and have beneficial effects on human health, probiotics must (1) be non-pathogenic and preferably of human origin; (2) adhere to epithelial surfaces and colonise (at least transiently) the human gastrointestinal tract; (3) tolerate bile salts and gastric acid; (4) remain viable during storage; (5) stimulate and regulate immune response; and (6) have clinically proven effects in humans (Saarela, Mogensen, Fonden, Mättö, & Mattila-Sandholm, 2000).

2.12.3 Safety of Lactobacillus as probiotics

As is shown in Table 2.3, many species of genus *Lactobacillus* have proven health benefits and *Lactobacillus* are generally regarded as safe (GRAS) (Ahrne et al., 1998; Saarela et al., 2000). However, they can still be of potential risk to consumers. Probiotics have been reported to modulate over-sensitive inflammatory feedback (Belkaid & Hand, 2014). Also, some viable probiotics may induce inflammation in vulnerable groups, such as premature infants and immunocompromised patients, thereby worsening symptoms of inflammation (Morisset, Aubert-Jacquin, Soulaines, Moneret-Vautrin, & Dupont, 2011).

2.12.4 Application of inactivated probiotics and probiotic potential of sourdough bread

An alternative way to mitigate safety concerns surrounding consumption of probiotics is to use inactivated probiotics, which still have the same health promoting effects as the live cells (Cho & Finocchiaro, 2010). As previously discussed, in some instances, inactivated probiotics may be as efficient at conferring health benefits as the live cells and therefore their use is safer (Kataria, Li, Wynn, & Neu, 2009; Adams, 2010). Several strains of heat-killed *L. acidophilus* have been reported to have anti-diarrhoea effects and to alleviate allergic reactions in children (Lin, Yu, Lin, Hwang, & Tsen, 2007; Moal, 2016). Previous studies on *L. acidophilus*, *L. brevis*, *L. gasserii*, *L. rhamnosus* GG and *L. casei* strain Shirota have shown that consumption of non-viable cells could still modulate immune reactions and enhance the proliferation of murine splenocytes (Kataria et al., 2009; Adams,

2010; Power et al., 2014). These LAB species have also been isolated from sourdough bread, indicating its probiotic potential (Saxelin et al., 2005; Pintado, Gomes, & Freitasb, 2014).

However, not all killed probiotics have the same function as the live cells. Studies on *L. johnsonii* La1 showed that after heat-treatment, anti-H-pylori compounds which can normally control the gastric pathogen *H. pylori* had lost their function (Makinen, Berger, Bel-Rhlid, & Ananta, 2012). Therefore, health benefits from killed probiotics are both strain and mechanism of action dependant (Barrangou et al., 2012; Lahtinen, 2012; De Almada et al., 2016).

Different probiotic strains elicit their effects by different mechanisms (De Almada et al., 2016). Some are via antagonistic effects including competitive adhesion and aggregation to the intestinal mucus and epithelium or via production of antimicrobial substances (De Almada et al., 2016). Adhesion of probiotics has been considered as one of the most important criterion when selecting probiotic microorganisms due to host-microbial interactions and related health benefits (Cho & Finocchiaro, 2010; Lahtinen, 2012). The adhesion properties of probiotics are affected by factors such as types of adhesins (cell components that are involved in the adhesion), bile resistance and digestive enzymes which can change the structure of surface protein(s) used by LAB as adhesins (Farnworth, 2008; Cho & Finocchiaro, 2010).

Whether the adhesion of probiotics is affected or not during the baking process depends on the particular strains and their mechanism of action (Farnworth, 2008; Lahtinen, 2012). Some probiotics attach to the gut epithelial cells via cell proteins, while other probiotics rely on their cell-wall polysaccharides, which can remain intact even after heating (Lee & Seppo Salminen, 2009). For example, heat-killed *L. acidophilus* LB is reported to still effectively adhere to epithelial cell structures and inhibit pathogen adhesion, while *S. thermophilus* loses its adhesive ability after heating (Lee & Salminen, 2009). The loss of function or adhesive ability by a probiotic may be caused by structural changes in the surface protein(s) normally used as adhesion sites, after heating at high temperature (Lahtinen, 2012).

Another mechanism of probiotic function is via production of bioactive metabolites or antimicrobial compounds (Stanton, Ross, Fitzgerald, & Van Sinderen, 2005). The antimicrobial functions of some probiotics are related to their specific metabolic activities through the production of organic acids, short chain fatty acids and diacetyl which can act as antimicrobial compounds (Soomro, Masud, & Anwaar, 2002; Parvez, Malik, Ah Kang, & Kim, 2006). In addition, studies have shown that short chain fatty acids such as acetic acid which remain in sourdough bread after baking (Hui & Evranuz, 2012) can improve epithelial integrity (Lahtinen, 2012). Thus implying that consumption of sourdough bread may be beneficial to epithelial integrity if sufficient is consumed. The immunogenic cell wall components released by *L. plantarum* help enhance immune responses in the human gut after heat treatment at 85 °C (Van Baarlen et al., 2009). However, whether these components remain intact or not following baking (bread loaf normally reaches a core temperature of 92-95 °C) remains to be determined (Cauvain, 2015).

2.12.5 Prebiotic potential of sourdough bread

Prebiotics are food ingredients such as non-digestible oligosaccharides (e.g. resistant starch and cereal fibres) that can promote the growth and/or activity of certain bacteria that have already colonised the human gastrointestinal tract (Cho & Finocchiaro, 2010). Significant proliferation of colonic microflora has been observed following consumption of prebiotics at a daily dose of 5-20 g/day (Lee & Salminen, 2009). To be called a prebiotic, the compound must satisfy at least three criteria: (1) non-digestible by the digestive system; (2) digestible by colonised intestinal microflora; and (3) selectively stimulate the growth and/or activity of beneficial intestinal microflora (Farnworth, 2008; Lee & Salminen, 2009; Cho & Finocchiaro, 2010).

Fermented foods, especially plant-based products such as sourdough bread, possess prebiotic functions (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Cho & Finocchiaro, 2010). Sourdough LAB can produce prebiotic EPS through sucrose metabolism and secrete it from the cells, where it can support the growth of intestinal probiotics such as *Bifidobacteria*, which can promote health to the host by modulating the immune system, lowering blood cholesterol levels and having anti-tumour effects

(Roberfroid, 2000; Cho & Finocchiaro, 2010; Gobbetti, Rizzello, Di Cagno, & De Angelis, 2014; Zhou & Therdthai, 2012). For example, *L. sanfranciscensis*, *L. frumenti*, *L. pontis*, *L. acidophilus*, *L. reutei* and *W. cibaria* can synthesise prebiotic EPS such as fructo-oligosaccharides and fructan during cereal fermentation, which supports the growth of intestinal *Bifidobacteria* (Zhou & Therdthai, 2012).

2.13 Microbiological characterisation of sourdough

The quality of sourdough bread, in terms of texture, flavour, nutritional value, and shelf life, is linked to the composition of the starter culture (Paramithiotis, Chouliaras, Tsakalidou, & Kalantzopoulos, 2005; Gobbetti & Gänzle, 2012). Knowledge of starter culture composition and their metabolic activities assists artisans and industrial fermentation companies to find better ways to control the fermentation process, and therefore produce high quality products. In addition, a desirable starter culture may add value by conferring potential probiotic properties to the sourdough (Temmerman, Huys, & Swings, 2004; De Vuyst & Neysens, 2005; Ehrmann & Vogel, 2005).

Sourdough starter cultures can be characterised through quantification and taxonomic identification namely, the LAB and yeasts (Gobbetti & Gänzle, 2012; Hui & Evranuz, 2012; Tamang, 2010; Tamang et al., 2016). Characterisation of the starter culture can be achieved by culture-dependent methods, which requires culturing of microbiota on different agar media under different incubation conditions. Knowledge of the culture can also be determined using culture-independent methods which rely on DNA/RNA extraction directly from the substrate (Jany & Barbier, 2008).

Quantification of probiotic strains will help to determine the amount of the fermented food needing to be consumed to generate health benefits, in addition, quantification of LAB and yeasts can provide information on sourdough maturity (Ehrmann & Vogel, 2005; Ercolini et al., 2013; Minervini, De Angelis, Di Cagno, & Gobbetti, 2014).). As previously discussed, the level of LAB in sourdough is expected to reach about 8 log CFU/g with yeasts usually 1-2 logarithmic lower (Ehrmann & Vogel, 2005; Corsetti, 2013; Ercolini et al., 2013).

Identification of starter cultures is necessary for selecting desirable cultures and managing the growth and metabolic activities of starter cultures as this provides information to optimise fermentation parameters (Vélez et al., 2007). For example, identification and gene sequence information of lactococci which is used in dairy fermentations helped select better strains according to their plasmid gene sequence (Mills, Sullivan, Hill, Fitzgerald, & Ross, 2010). In addition, identification of strains and species present in the starter culture will allow comparisons to information in the literature to determine if the strains have already been shown to have probiotic potential (Klingberg, Axelsson, Naterstad, Elsser, & Budde, 2005; Sornplang & Piyadeatsoontorn, 2016).

To obtain a comprehensive record of the native microorganisms present in a fermented food, both culture-dependent and culture-independent methods should be adopted (Temmerman et al., 2004; Tamang, 2010; Tamang et al., 2016). Culture-dependent methods based on culturable microorganisms can underestimate the total number of microorganisms present, whereas culture-independent methods such as q-PCR can be used to count both non-culturable and culturable microorganisms (Furet, Quénée, & Tailliez, 2004; De Vuyst et al., 2009). For better differentiation of species present in sample, culture-dependent methods are recommended (Temmerman et al., 2004) as these can also provide preliminary taxonomic and metabolic information of unknown microorganisms according to results from morphological, physiological and biochemical tests (Tamang, 2010). A limitation of the culture-dependent method is that non-culturable microorganisms may be excluded and the analysis is time-consuming (Tamang, 2010; Kralik, Beran, & Pavlik, 2012; Tamang et al., 2016).

Culture-independent methods involve extraction of DNA directly from the substrate for evaluation, which can identify non-culturable microorganisms (Tamang et al., 2016). However, using culture-independent methods such as denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) may induce bias because their detection limit is 2 log CFU/mL or 3 log CFU/g (Tamang, 2010, Kurtzman, Fell, & Boekhout, 2011). Therefore, functional microorganisms not present at sufficient levels may not be detected using DGGE or TGGE methods.

2.13.1 Quantification of LAB and yeasts

The cell density of yeasts and LAB can be estimated through standard plate count methods using selective medium or relevant genetic methods such as fluorescence in situ hybridization (FISH), microarray-based rRNA detection, fluorescence hybridisation, q-PCR or c-PCR (Corsetti, 2013). Other semi-methods used for this purpose include DGGE, rRNA quantitative hybridisation, and microarray-based rRNA detection by which only predominant species can be detected (Giraffa, 2004b; Gobbetti & Gänzle, 2012; Davis, 2014).

2.13.1.1 Quantification of sourdough starter cultures by the traditional culture-dependent methods

Standard plating methods are commonly applied for the enumeration of viable microbiota (Davis, 2014). However, as previously mentioned culture-dependent methods will not provide information on non-viable populations and may underestimate the total microflora population (Davis, 2014). This is because not all bacterial cells can grow on agar; a phenomenon explained by two reasons (Stewart, 2012): firstly, incubation conditions and preparation of media may deviate from the recommended original environment of target microbes which can affect the growth of certain species (Davis, 2014). Secondly, target cells can be viable but non-cultivable (Ramamurthy, Ghosh, Pazhani, & Shinoda, 2014). This may happen due to adverse conditions such as starvation, cold or other stresses (Heim, Del Mar Leo, Bonato, Guzman, & Canepari, 2002), hence the cells can be physically alive and still have metabolic activity, but they are unable to form colonies on the corresponding media (Heim et al., 2002). For example, microorganisms growing on the surface of cheese, fruits and vegetables can enter into a non-cultivable state because of nutrient limitations (Egli & Zinn, 2003).

However, plating methodology is still needed for the following two reasons. Firstly, isolated colonies grown on agar plates can provide metabolic information helpful for industrial fermentation companies to improve fermentation processes and commercialised starter culture development (Hansen, 2002). In addition, some cultures can still grow on agar plates and be further identified by culture dependent methods but may not be detected

by genomic methods because of the detect limitation of some genomic methods (Temmerman et al., 2004; De Vuyst & Vancanneyt, 2007).

2.13.2 Identification of sourdough starter cultures

Identification and classification of sourdough starter cultures can also be achieved through culture-dependent methods combining phenotypic (morphological, physiological and biochemical characterisation) and/or genotypic characterisation (Table 2.4) or directly through culture-independent methods, which involve primarily genotypic methods (Gobbetti & Gänzle, 2012; Pot et al., 2014).

Table 2.4 Summary of phenotypic and genotypic methods for sourdough starter culture identification

Technique	Principle	Work Load	Discriminatory Power	Reproducibility
Phenotypic method				
Morphological analysis	Microscopic analysis	L	Genus level or less	M
Physiological analysis	Growth characteristics simple tests	M	Genus level or less	L
Biochemical characterisation	Assimilation and fermentation patterns (API, BIOLOG)	L	Genus or species level	M
Protein profiling Sodium Dodecyl Sulphate	Polyacrylamide Gel Electrophoresis of cellular proteins	H	Species level	H
Genotypic method				
Specific primers	PCR with group-specific	L	Depending on primer	H
Sequencing	Determination of gene sequences (16S rDNA)	H	Genus or species level	H
RFLP	Restriction Enzyme Analysis (REA) of DNA or PCR amplicons	M	Species to strain level	H
AFLP	Combination of REA and PCR amplification	H	Species to strain level	H
RAPD-PCR	Randomly primed PCR	L	Species to strain level	L
Rep-PCR	PCR targeting repetitive interspersed sequences	L	Species to strain level	L
PFGE	REA and pulsed-field gel electrophoresis	H	Strain level	H
Ribotyping	REA and oligonucleotide probe detection	H	Species to strain level	H
Hybridisation probes	DNA–DNA hybridisation using labelled probes	H	Genus or species level	H

Notes: H = high, M = moderate, L = low. Source: Temmerman, Huys, & Swings (2004).

Although the biochemical and physiological features of LAB have been explored extensively and LAB can still be identified through phenotypic methods, molecular methods can provide rapid, reliable identification (Ehrmann & Vogel, 2005). Molecular methods are particularly useful when dealing with different species which have similar fermentation patterns, which can make differentiation difficult using phenotypic methods such as API tests (De Vuyst & Vancanneyt, 2007; Gobbetti & Gänzle, 2012). However, culture-dependent methods, including phenotypic characterisation are still necessary when

describing new species and for industry to optimise their processing parameters (Ehrmann & Vogel, 2005; Gobbetti & Gänzle, 2012). Therefore, both phenotypic and genotypic characterisation should be used for identification of microorganisms in sourdough starter cultures (Ehrmann & Vogel, 2005).

2.13.2.1 Morphological observations

Morphological studies on distinct colonies include microscopic observation of purity, shape and size following Gram-staining (Balkwill & Ghiorse, 1985; Müller, Ehrmann, & Vogel, 2000; Pot et al., 2014). For LAB, recorded cell lengths of different groups of *Lactobacillus* can provide a preliminary evaluation of species (Gobbetti & Gänzle, 2012).

2.13.2.2 Physiological and biochemical analysis of isolates

Physiological and biochemical analysis of isolates should be conducted following morphological evaluation (Valjakka et al., 2003; Gobbetti & Gänzle, 2012). After isolation and purification of colonies, distinct colonies undergo biochemical tests to assess their growth characteristics using methods described in Table 2.4. However, these analyses are time-consuming and do not differentiate sub-species such as *L. acidophilus* group (Temmerman et al., 2004).

Phenotypic methods suffer from poor reproducibility and discrimination powers, which may be affected by the complex growth conditions of microbes and the variability of some phenotypic characteristics (Temmerman et al., 2004; Gobbetti & Gänzle, 2012). Previous identification of sourdough LAB isolates based on phenotypic identification showed that only 38 % of 317 tested LAB isolates were identified to species level (Temmerman et al., 2004). The results of phenotypic identification may also be limited by the taxonomy of the corresponding database (Temmerman et al., 2004). Therefore, for accurate identification of LAB to species level, genotypic analysis is needed.

2.13.2.3 Genotypic analysis of isolates

The application of molecular methods can eliminate the effect of changing the growth conditions on microorganisms without the cultivation step (Temmerman et al., 2004). The

most widely used culture-independent techniques are shown in Table 2.5. DNA-based identification and detection methods have largely been developed over the past two decades and many of them are based on the polymerase chain reaction (PCR) (Temmerman et al., 2004). PCR selectively amplifies specific DNA fragments using oligonucleotide primers (Temmerman et al., 2004). By analysing PCR products, discriminatory power to species and strain levels can be achieved (Temmerman et al., 2004).

Table 2.5 Summary of culture-independent methods for evaluating microbial community

Method	Discriminatory Power	Application
(a) Genetic fingerprinting of microbial communities		
DGGE/TGGE [a]	Genus/species level	Dynamics between microbial populations in different natural environments
SSCP [b]	Genus/species level	Mutation analysis; dynamics between microbial populations in different natural environments
Other PCR-based methods		
T-RFLP [c]	Genus, species, strain level	Strain identification; dynamics between and within microbial populations in soils, activated sludge, aquifer sand, termite gut
LH-PCR [d]	Genus/species level	Dynamics between microbial populations in aquatic and soil microbial environments
PCR-ARDRA [e]	Species level	Automated assessment of microbial diversity within communities of isolated microorganisms
RISA/ARISA-PCR [f]	Species level	Estimation of microbial diversity and community composition in freshwater environments
AP-PCR [g]	Strain level	Automated estimation of microbial diversity (typing) within lactic acid bacteria populations
AFLP [h]	Genus, species, strain level	Automated estimation of microbial diversity within communities (species composition) and populations (typing) of various Gram positive and Gram negative bacteria
(b) Competitive PCR	Species level	Detection of microbial cells into the viable but unculturable state in freshwater samples
(c) Fluorescence in situ techniques		
Fluorescence in situ hybridisation （FISH）	Species level	Detection of viable cells within bacterial communities from environmental samples or food ecosystems
Fluorescence in situ PCR	Species level	Detection of viable, slow growing cells within bacterial communities, particularly pathogens in clinical specimens

Source: Giraffa and Neviani (2001).

Note: a: Denaturing gradient gel electrophoresis/thermal gradient gel electrophoresis; b: Single strand conformation polymorphism; c: Terminal-restriction fragment length polymorphism; d: Length heterogeneity-polymerase chain reaction; e: Polymerase chain reaction-amplified ribosomal DNA restriction analysis; f: Ribosomal spacer analysis/automated ribosomal spacer analysis-polymerase chain reaction; g: Arbitrarily primed-polymerase chain reaction; h: Adaptor fragment length polymorphism.

Genotypic methods used in the study of LAB and yeasts comprise species-specific PCR, repetitive extra-genic palindromic sequence-based PCR (rep-PCR), random amplification of polymorphic DNA (RAPD), DNA-DNA hybridization, restriction fragment length polymorphism(RFLP), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) (Temmerman et al., 2004; Tamang, 2010). Of these methods, RFLP and PFGE are mainly utilised for typing rather than for species identification and so will not be discussed in detail (Temmerman et al., 2004).

Sequencing and Multilocus Sequencing (MLS) of housekeeping genes

To identify unknown *Lactobacillus* and yeast isolates, 16S rRNA or 23S rDNA and large subunit (LSU) ribosomal ribonucleic acid gene positions (D1/D2) are used respectively, due to their high discriminatory power (Temmerman et al., 2004; Ehrmann & Vogel, 2005; Gobbetti & Gänzle, 2012). Other regions such as Internal Transcribed Spacer (ITS) regions have also been applied for LAB and yeast sequencing (Temmerman et al., 2004; Gobbetti & Gänzle, 2012).

Applying protein-encoding genes or housekeeping genes for sequencing has advantages for 16S rRNA and taxonomic resolution of fingerprinting techniques (Gobbetti & Gänzle, 2012). For LAB species identification, applying combinations of the housekeeping genes *atpA*, *rpoA* and *pheS* gives the processes high discriminatory power for identifying strains *Lactobacillus*, *Enterococcus*, and *Leuconotoc* (Gobbetti & Gänzle, 2012; Tamang et al., 2016).

Sequences can be compared to that from databases such as Genbank, EMBL, BLAST or FASTA to obtain information of corresponding microorganisms and their phylogenetic position (Temmerman et al., 2004; Gobbetti & Gänzle, 2012).

RAPD-PCR

RAPD-PCR is a relatively fast and less expensive DNA fingerprinting technique than the adaptor fragment length polymorphism (AFLP) method (Temmerman et al., 2004; Ehrmann & Vogel, 2005). For RAPD-PCR, segments of DNA are randomly amplified (Temmerman et al., 2004; Tamang et al., 2016), with short arbitrary and low-stringency

primers applied for the PCR reactions (Temmerman et al., 2004; Tamang et al., 2016). This method (RAPD-PCR) has successfully been applied to identify LAB and yeast isolates from sourdough (Ehrmann & Vogel, 2005; Gobbetti & Gänzle, 2012). The main issue with RAPD-PCR is that the inter laboratory reproducibility can be low because of variable conditions during PCR reactions (Temmerman et al., 2004).

Rep-PCR

By using repetitive sequences, rep-PCR produces various length of amplicons which can be further separated by electrophoresis to provide identification information of gene bands (Tamang et al., 2016). Repetitive primers which have been used to differentiate *Lactobacillus* and *Bifidobacteria* include ERIC, BOX or $(GTG)_5$ (Temmerman et al., 2004).

2.13.3 Recent studies on sourdough starter culture composition

Recent studies on sourdough starter culture composition have combined the use of both phenotypic and genotypic methods (Bessmeltseva, Viiard, Simm, Paalme, & Sarand, 2014; Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014; Lhomme et al., 2016). For LAB, after phenotypic screening tests such as Gram-stain, catalase test and API™ 50 CHL tests, 16S gene sequencing was carried out (Vélez et al., 2007; Lhomme et al., 2015; Liu et al., 2016). Also, culture-dependent analysis by DGGE was conducted on extracted total DNA (Lhomme et al., 2015; Zhang et al., 2015). For isolated yeast colonies, phenotypic methods and/or molecular methods such as sequencing and RAPD-PCR have been applied (Lu, Peng, Cao, Tatsumi, & Li, 2008; Alfonzo et al., 2013; Zhang et al., 2015).

2.14 Conclusion

Sourdough is fermented by LAB and yeasts, which can affect the final sourdough bread quality including sensory and texture. Due to sourdough characteristics such as acidity, and production parameters such as fermentation time and temperature, the composition of sourdough starter culture varies among different types of sourdoughs made with different flours. Research on sourdough starter cultures (species identification and quantification) helps to understand their metabolic activities, growth conditions, and adaptation to the food

system. Using this information, bakery artisans and companies are able to adjust their production parameters to have better control of the fermentation process.

To date, the majority of research on sourdough starter culture composition has been carried out on wheat and rye sourdough, with little having been done on GF sourdough, which may contain novel starter culture strains (Meroth, Hammes, & Hertel, 2004; Lim et al., 2018).

In New Zealand, rice sourdough bread is readily available however its starter culture composition is unknown. Therefore, a question was raised: what is the starter culture composition of rice sourdough produced in New Zealand? This research aims to answer this question by determining the composition of LAB and yeasts in a rice sourdough starter culture and evaluating the physico-chemical characteristics of MSD, DBP, DAP and SDB.

CHAPTER 3 MATERIALS AND METHODS

3.1 Overview of rice sourdough production

This section provides a brief description of the preparation of sourdough culture and production of sourdough bread. As the main aim of the study was not production of sourdough bread, this section only serves to provide an insight into the main steps involved in the process and production parameters. Information on process and production aids in better understanding of the role and significance of sourdough starter cultures in sourdough bread (Hui & Evranuz, 2012).

Sourdough and sourdough bread samples analysed in this study were produced and supplied by Venerdi Ltd, Auckland, New Zealand. The sourdough used in this study was prepared following the generalised procedure shown in Figure 3.1.

The initial MSD was prepared by mixing water, rice flour and sourdough starter culture, this mixture was stored and designated as MSD. A portion of the stored MSD containing starter culture was used in sourdough bread making, while the remainder was refreshed by mixing the remained MSD with water, gum and brown rice flour in order to maintain the viability of sourdough starter culture. Refreshed MSD was stored at 4 ℃ for two days and then be used for bread making.

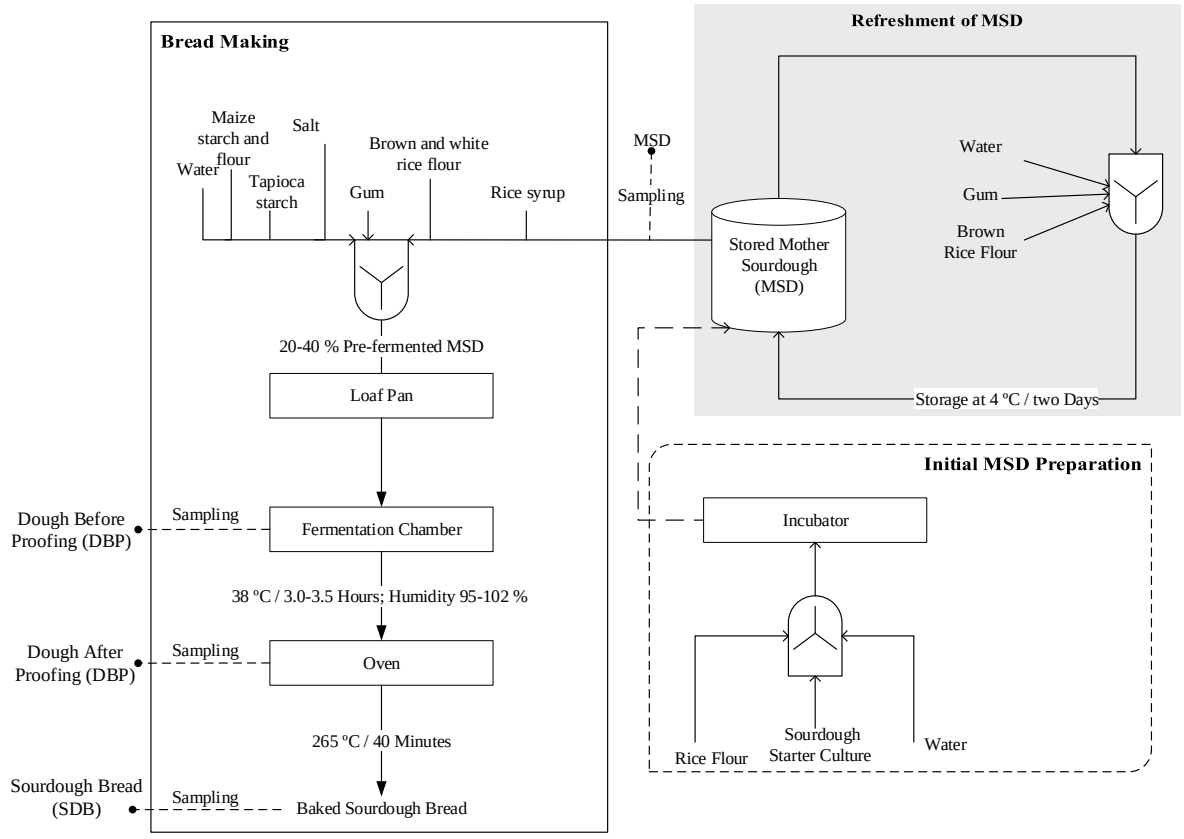

Figure 3.1 Generalised production of whole meal rice sourdough bread

Four batches of sourdough bread (90 loaves in each batch) were produced daily, by mixing a portion of the two-day old stored MSD with other ingredients (water, brown rice flour, white rice flour, tapioca starch, maize starch and flour, guar gum, rice syrup, salt) to produce bread dough, designated as DBP. The DBP was mechanically divided into equal portions (around 700 g) and placed into individual bread pans and then allowed to ferment at 38 ℃/ 3-3.5 hours in an incubator (under 95-102 % relative humidity to produce bread dough after proofing (DAP) . Following fermentation, the fermented and leavened bread dough was baked in a steam oven at 265 ℃/ 40 minutes to obtain SDB.

3.2 Sampling

In this study, the main objective was to determine the composition of rice sourdough starter culture (LAB and yeasts) during dough fermentation. The chemical characteristics of sourdough bread and dough samples were also determined. To fulfil these objectives,

samples were obtained at different stages of production as follows: MSD, DBP, DAP and SDB.

Samples were collected weekly for 10 weeks from April 2017 to July 2017. Approximately 300 g of each type of dough sample were collected into sterile stomacher bags (Global Science, NZ) and immediately frozen, before transportation to the Food Microbiology Laboratory (Massey University, Auckland Campus, Auckland). For the SDB samples, six freshly made loaves baked from the same batch as the dough samples, were randomly selected from the top, middle and bottom of the storage rack and all samples were placed in a cooler box with ice packs. All samples were transported under chilled conditions in an ice-packed bin to Massey University for analysis.

3.3 Experimental design

The study was conducted in three-integrated phases: Phase one involved the characterisation of physico-chemical properties of MSD, DBP, DAP and SDB by conducting the measurements and analyses listed in Table 3.1.

Table 3.1 Characterisation of MSD, DBP, DAP and SDB samples

Parameter	Samples
pH Total titratable acidity	MSD, DBP, DAP, SDB
Soluble sugars Organic acids Free amino acids	DBP, DAP, SDB
Colour Texture	SDB

Notes: MSD =Mother sourdough, DBP = Dough before proofing, DAP = Dough after proofing, SDB = Sourdough bread

In phase one, the following research questions will be answered:

- How did pH and TTA change during rice sourdough production?
- How did soluble sugar, organic acid and free amino acid contents change during fermentation?
- What was the fermentation quotient of SDB?

Phase two involved microbiological characterisation of MSD, DBP and DAP samples. In this phase, isolates of microbial cultures (LAB and yeasts) of sourdough were obtained, purified and their morphology examined.

In phase two, the following research questions will be answered:

- What were the total viable counts of total aerobic bacteria, LAB and yeasts?
- How did the number of LAB and yeasts change during rice sourdough fermentation?
- How many types of LAB and yeasts does the rice sourdough contain?

Phase 3: LAB and yeasts isolated from MSD, DBP and DAP were identified by API test kits and gene sequencing.

In phase three, the following research questions will be answered:

- What species of LAB and yeasts exist in rice sourdough starter culture?
- What the fermentation profiles were of isolated the LAB and yeasts?
- Can dominant LAB and yeasts in rice sourdough confer health benefits via the effects of probiotics based on identifying dominant LAB and information from exist literature?

3.4 Methods

Phase 1: Physicochemical characterisation MSD, DBP, DAP and SDB

In order to relate the final bread quality to its starter culture composition and understand the metabolic activities of LAB and yeasts, MSD, DBP, DAP, SDB were analysed for their total titratable acidity and pH. DBP, DAP, SDB were also analysed for their soluble sugars,

organic acids and free amino acid contents. For SDB, crumb texture and crust colour were also measured.

3.4.1 Total titratable acidity determination

Total titratable acidity (TTA) was determined following the AOAC standard method (AOAC, 1965). Sodium hydroxide (NaOH) (0.1 mol/L) (Univar, Ajax Finechem Pty Ltd, NZ) was standardised by titrating against standard potassium hydrogen phthalate (KHP) (Univar, Ajax Finechem Pty Ltd, NZ). To standardise NaOH, about 20 g KHP was dried and dissolved in 50 mL distilled water. Few drops of 1 % phenolphthalein solution were added to the KHP solution and mixed. The NaOH solution was then titrated against the KHP solution until the appearance of the first persistent pink colour was observed (pH 8.5). The volume of titre NaOH (mL) was recorded. The titrations were repeated until concordant quadruplicate results were achieved. The concentration of the prepared NaOH solution was calculated using Equation 1.

$$C_{NaOH} = \frac{m_{KHP}}{MW_{KHP}\,(204.23\ g/mol)} \times \frac{1}{V_{NaOH}} \qquad \text{Equation 1}$$

Where ^{C}NaOH is concentration of NaOH (mol/L); ^{m}KHP is mass of KHP (g); ^{V}NaOH is volume (L) used to titrate against the KHP solution; ^{MW}KHP is KHP molecular weight (204.23 g/mol).

Analysis of acidity of MSD, DBP, DAP and SDB samples were conducted according to Lhomme et al. (2015). Ten (10) g of sample was weighed into a stomacher bag (Global Science, NZ), and while still on the scale, 90 mL of water was added to the sample in the bag. The mixture was homogenised for four to five minutes using a stomacher lab paddle blender (Masticator 400 mL, IUL, Spain). Of the homogenised sample, 10-20 g was weighed into a clean Erlenmeyer flask and three to four drops of 1 % phenolphthalein solution was added to the solution and mixed. Standardised 0.1 mol/L NaOH was then used to titrate the test solution to a faint persistent light pink. The volume of NaOH used was recorded and the concentration of lactic acid was calculated using Equation 2. The results

were expressed as percentage of grams of lactic acid per g of sample. TTA measurements were conducted in duplicate.

$$\%\ Lactic\ Acid\ = \frac{C_{\ NaOH} \times V_{\ NaOH} \times MW_{\ Lactic\ acid}}{sample\ weight} \times 100\ \% \qquad \text{Equation 2}$$

Where ^{C}NaOH is concentration of NaOH solution (0.1 M), ^{V}NaOH is volume of NaOH (L) used in the sample titration, ^{MW}lactic acid is molecular weight of lactic acid (90.09 g/mol).

3.4.2 Measurement of pH

A standardised glass electrode pH meter (HI 2221, Hanna Instruments, UK) equipped with a glass electrode was used to measure the pH of dough suspensions prepared as described in Section 3.4.1. Prior to pH measurement, the equipment was calibrated using standard buffers at pH 7.0, 4.0 and 10.0 (LabServ, Thermo Fisher, NZ). pH measurements were conducted in duplicate.

3.4.3 Analysis of free amino acids

Free amino acids in the DBP, DAP and SDB were analysed by the Nutritional Laboratory Massey University, Palmerston North, following the AOAC Standard Method 994.12 (AOAC, 1997).

3.4.4 Analysis of sugars and organic acids in DBP, DAP and SDB

3.4.4.1 Sample preparation

Samples were prepared according to the method described by Lefebvre, Gabriel, Vayssier, and Fontagne-Faucher (2002). Ten (10) g of either sourdough bread or dough sample was homogenised in 60 mL distilled water in a laboratory scale stomacher blender for two minutes. After the sample was homogenised, the volume was adjusted to 100 mL with distilled water using a volumetric flask. The sample was centrifuged at 4000 × g (Heraeus Multifuge × 1R; Thermo Fisher, Germany) at 15 °C for 15 minutes and the supernatant was filtered through a 0.22 μm syringe filter (Terumo, Australia). A 10-mL aliquot of

filtrate was mixed with 60 mL distilled water, 5 mL of Carrez I solution (0.085 mol/ L potassium II hexaferrocyanate) (Thermo Fisher, NZ) and 5 mL of Carrez II solution (0.25 mol/L zinc sulphate) (Thermo Fisher, NZ). The mixture was adjusted to pH 8.0 $\pm$ 0.5 with 0.1 M NaOH and the volume adjusted to 100 mL with distilled water. Samples were then filtered through 0.22 μm syringe filters (Terumo, Australia) and stored in 2.0 mL vials (Shimadzu Prominence, NZ) prior to analysis by high performance liquid chromatography (HPLC).

3.4.4.2 HPLC mobile phase preparation

Calcium nitrate (200 mg/L) (Thermo Fisher, NZ) solution was used as the mobile phase for sugar analysis while 0.005 N H_2SO_4 (Fisher Scientific, UK) was the mobile phase for acid analysis. The mobile phase was degassed using an ultrasonic bath (Bandelin Sonorex Super RK510, Germany) until all air was removed.

3.4.4.3 Analysis of sugars and organic acids by HPLC

The concentrations of maltose, glucose, fructose, sucrose, and organic acids (lactic acid and acetic acid) in dough samples were analysed by HPLC). The HPLC system used was a Shimadzu model LC-10AT (Shimadzu Corporation, Japan), together with auto injector (SIL-10A, Shimadzu Corp, Japan), system controller (SCL-10A, Shimadzu Corp, Japan), refractive index (RI) detector (RID-10A, Shimadzu Corp, Japan) and ultra violet (UV) detection (SPD-10A, Shimadzu Corp, Japan) and column oven.

For sugar analysis, a Rezex RCM-Monosaccharide RCM Ca^{2+} (8 % cross-linked resin) column (300 × 7.8 mm) (Phenomenex, USA) was used. For analysis of lactic acid and acetic acid, a Rezex ROA-Organic Acid column (8 % cross-linked resin) (Phenomenex, USA) was used. HPLC separation conditions used for sugar and organic acid analysis are listed in Table 3.2. The injection volume of sugars or organic acids was 20 μL and the samples were analysed in duplicate.

Table 3.2 HPLC Column Conditions

Test sample	Column	Mobile Phase	Flow Rate	Detection	Temperature
Organic acids	ROA-Organic Acid	0.005 N H_2SO_4	0.5 mL/min	UV@210 nm	40 °C
Sugars	RCM-Monosaccharide	200 mg/L $CaNO_3$	0.6 mL/min	RI@40 °C	80 °C

Peak areas were determined by integration using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). Quantification and identification of sugars were obtained by comparing peak areas and retention time of relevant standards. Retention time of standard sugars (Sigma Aldrich, NZ) and organic acids was obtained after measuring HPLC standards of the respective single sugars and organic acids (Fisher Scientific, UK). Calibration curves of sugars and organic acids were made using different concentrations of standard sugars and organic acids listed in Table 3.3.

Table 3.3 Standard concentrations used for making calibration curves

Sugar/ Organic acid standard	Company information	Concentration series (mg/100 mL)
Maltose (≥99.0 %)	Sigma Aldrich, NZ	40, 60, 80, 100, 150, 200
Glucose (≥99.5 %)	Sigma Aldrich, NZ	5, 10, 20, 30, 50
Fructose (≥99.0 %)	Sigma Aldrich, NZ	5, 10, 20, 30, 50
Acetic acid (≥85.0 %)	Fisher scientific, UK	5, 10, 20, 50, 75, 100
Lactic acid (≥85.0 %)	Fisher scientific, UK	6, 15, 30, 60, 100, 120

3.4.5 Texture profile analysis (TPA)

Bread crumb samples were cut using a one-inch (25-mm) spherical mould with two pieces of each sample stacked for testing (Figure 3.2).

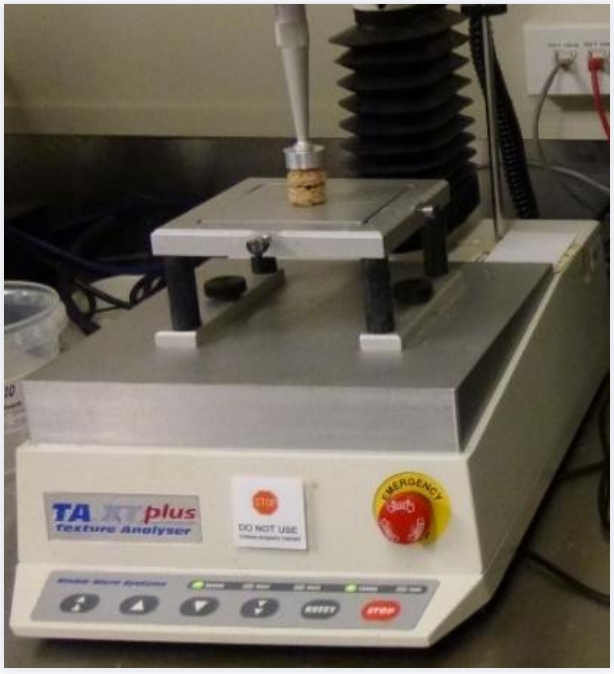

Figure 3.2 Texture analysis on bread crumbs

Hardness, cohesiveness, fracturability, adhesiveness, springiness (elasticity), chewiness and gumminess of SDB were measured using the Texture Analyser (TA.XT Plus, Stable Micro Systems, UK) equipped with a 31-mm diameter probe. The texture analyser settings were adjusted according to the AACC International Method 74-09.01 as shown in Table 3.4.

Table 3.4 TA.XT plus Texture Analyser TPA settings

Mode	Setting	Value
Measurement	Pre-Test Speed	10.00 mm per second
	Test Speed	1.70 mm per second
	Post-Test Speed	1.70 mm per second
	Target Mode	Strain
	Strain	50 %
	Time	30.00 second
Trigger	Type	Auto (Force)
	Force	20.0 g

3.4.6 Measurement of colour

To measure colour, a Minolta CR-300 model Chroma Meter (Japan) was used (CIE L*a*b* colour space). Colour was expressed by three coordinates: L*, a*, and b*. L* value indicates lightness, a* coordinates indicates red (+) and green (-) and b* coordinates indicate blue (-) and yellow (+) (Pérez-Quirce, Collar, & Ronda, 2014). CIE reflects all the colours visible to the human eye and can be used as a comparison (Kawamura-Konishi, Shoda, Koga, & Honda, 2013; Pongjaruvat, Methacanon, Seetapan, Fuongfuchat, & Gamonpilas, 2014). For each loaf, three positions on each whole crust piece were randomly chosen for measurement. A total of 12 crust pieces from 6 loaves were measured for each sampling time. Prior to analysis, the instrument was standardised using a white porcelain plate (L*= 97.10 a*=-0.07, b* = 1.97).

Phase 2: Microbiological analysis of dough samples and purification of LAB and yeast colonies

An overview of procedures for microbiological analysis of dough samples is shown in Figure 3.3. Enumeration of LAB, yeasts, and aerobic bacteria were achieved by plating serial dilutions of samples on selective media as described in Section 3.4.7. For identification of isolated LAB and yeasts, phenotypic methods (microscopy observation and API tests) and genotypic methods (sequencing) were applied.

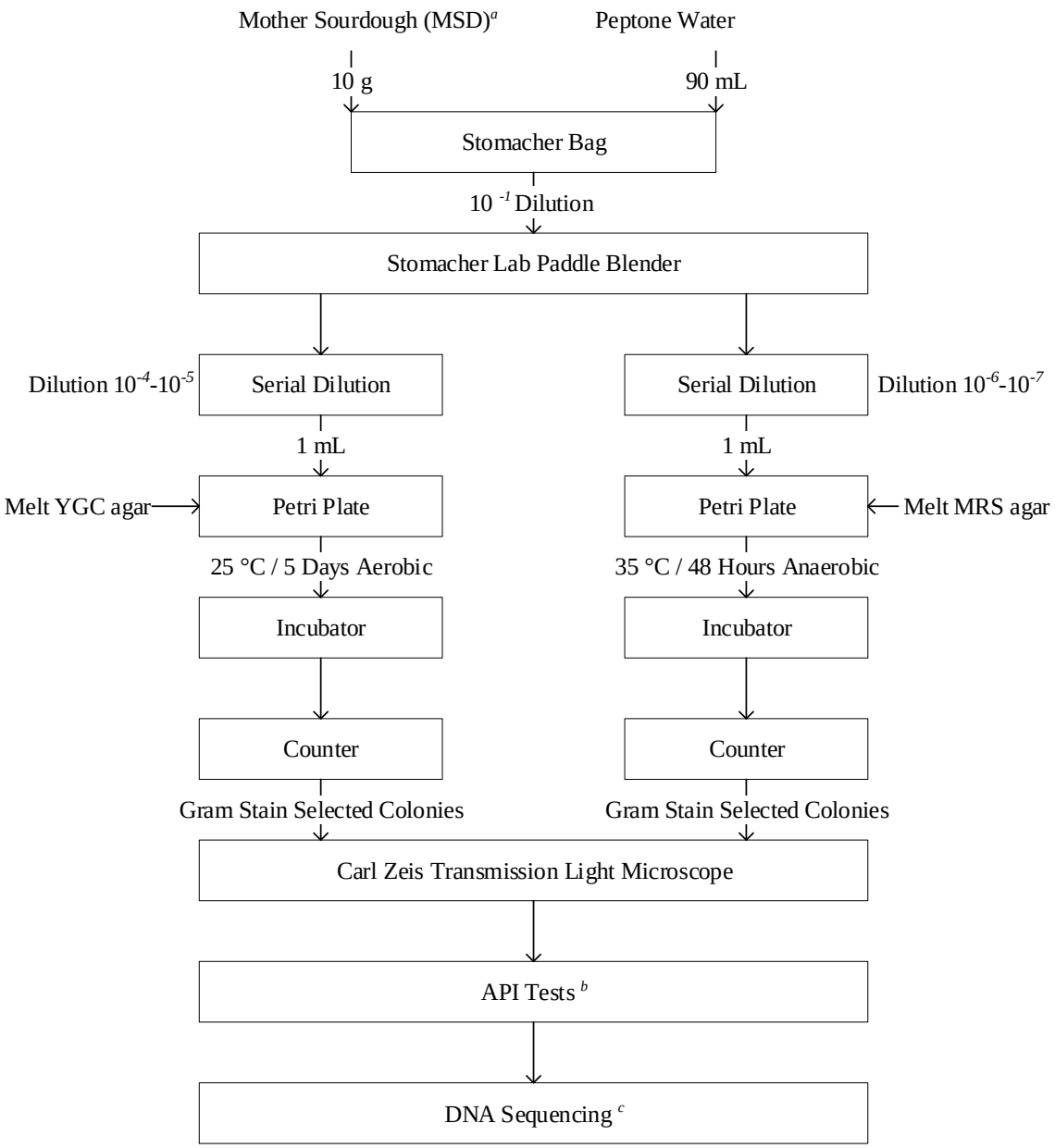

Figure 3.3 Microbiological analysis of LAB and yeasts

Note: The procedures used for total aerobic plate count (APC) were similar to that used for the LAB count except that plate count agar was used. a. Preparation for microbial analysis of dough before proofing (DBP) and dough after proofing (DAP) was similar to the that of mother sourdough (MSD); b. API 32 C kit was used for yeast identification and API 50 CHL kit was used for LAB identification; c. DNA extracted from selected purified yeast and LAB colonies and total LAB DNA extracted from MSD, DBP and DAP were subjected to sequencing. MRS = de Man, Rogosa, and Sharpe agar; YGC = yeast extract-glucose-chloramphenicol agar.

3.4.7 Total aerobic plate counts, LAB counts and yeast counts in MSD, DBP and DAP

In phase 2, total aerobic plate counts (APC), LAB counts and yeast counts were conducted. Total aerobic plate counts of the samples were performed on plate count agar (BD Diagnostics, Sparks, MD, USA). Enumerations of LAB and yeasts in MSD, DBP and DAP were carried out by plating serial dilutions on MRS agar (Oxoid, UK) or YGC agar (Merck, Germany), respectively (Lee & Lee, 2008; Gobbetti & Gänzle, 2012; Lhomme et al., 2016b). All media were prepared according to the manufacturers' instructions.

To prepare serial dilutions, 10 g of sourdough was measured aseptically into a sterile stomacher bag and 90 g peptone water (Merck, Germany) added to achieve a ten-fold dilution (AOAC, 1987). Sourdough samples were mixed for two minutes using a stomacher lab paddle blender (Masticator 400 ml, IUL, Spain). Ten-fold serial dilutions from 10^{-1} to 10^{-7} were prepared and 1 mL of each suitable dilution was plated in duplicate on appropriate molten agar described in Figure 3.3. After gentle swirling and cooling, the plated samples were incubated according to the conditions shown in Table 3.5. After incubation, developed colonies were counted using a colony counter (Bibbyscientific, UK).

Table 3.5 Incubation conditions for propagation of yeasts, LAB and total aerobic plate counts on agar plates

Microbial type	Incubation Temperature (°C)	Incubation time (h)	Incubation environment
LAB	35 ± 1	48 ± 2	Anaerobic *
Yeast	25 ± 1	120 ± 2	Aerobic
Anaerobic Bacteria	35 ± 1	48 ± 2	Aerobic

Note: Anaerogen pack (AN0035A) (Mitsubishi Gas Chemical Company Inc., Japan) was used to generate anaerobic conditions for growth of LAB. Source: Lee & Lee (2008).

3.4.8 Selection and purification of LAB and yeast colonies

The selection and purification LAB and yeast colonies were conducted according to the method described by Vieira-Dalodé et al. (2007) and Liu et al. (2016a). Morphologically distinct (such as size, shape, color) LAB and yeast colonies were selected and purified following procedures described in Figure 3.3. To select colonies for further purification, developed isolated colonies from MSD, DBP and DAP samples collected over 10 weeks were first examined for their colony morphology and recorded for their morphological

types. A portion of six to eight distinct colonies of each type of microorganism (LAB and yeasts) were Gram stained and examined under oil immersion using a Carl Zeis transmission light microscope (Model HBO 50/AC, Germany). Representative colonies with distinct cell morphology were chosen for further purification. Purification of cells was achieved by successive streaking on suitable solid agar as described in Section 3.4.7 and incubated according to conditions described in Table 3.5. Purity of the colonies was confirmed by uniformity of cells observed under a high magnification microscope (x 1000) after Gram staining; the size of cells were measured using the scale bar of AxioVision microscope software. Purified colonies were streaked on agar slants for long-term storage at 4 °C.

Phase 3: Identification of isolated LAB and yeast colonies

Purified colonies from Phase two on agar slants were subjected to phenotypic tests using API tests (bioMe´rieux, Inc., Marcy l'Etoile, France) as described in Section 3.4.9 (Lu, Peng, Cao, Tatsumi, & Li, 2008) and genotype methods using sequencing as described in Section 3.4.10.

3.4.9 API tests for LAB and yeasts

3.4.9.1 API tests for LAB and yeasts

An API ID 32C kit (bioMe´rieux, Inc., Marcy l'Etoile, France) was used to examine the fermentation patterns of isolated yeast colonies according to the manufacturer's instructions. Potato dextrose agar (PDA) (Oxoid, UK), a non-selective medium was used for the growth of isolated yeast colonies (Kozlinskis, Skudra, Klava, & Kunkulberga, 2008). Each purified colony was streaked on pre-prepared solidified PDA and incubated at 30 ℃ for 24-48 hours. Young yeast cells were then transferred to API ® Suspension Medium (2 mL) until a turbidity equivalent to 2 McFarland (McFarland standards were used as turbidity references of microorganism suspension) was obtained. Two (2) McFarland is equivalent to an absorbance of 0.45 at 600 nm (Kralik, Beran, & Pavlik, 2012). Turbidity was measured using a spectrometer (Novaspec III, Amersham Bioscience, UK).

Distinct purified yeast colonies were tested using API 32 C kit with 25 sterile ampoules. Of the prepared cell suspension, 135 µL was carefully transferred into each ampoule of the API ID 32 C strip and then the strip was incubated at 30 ℃ ± 1 ℃ for 24-48 hours. Growth of samples was confirmed by development of turbidity of the incubated samples. Interpretation of results was aided by apiweb^{TM} identification software database V 5.1. (https://apiweb.biomerieux.com/).

3.4.9.2 API 50 CHL system for LAB identification

Catalase tests were conducted on the Gram positive purified colonies as an initial screening of LAB prior to API 50 CHL tests (Tajabadi et al., 2013). Screened Gram-positive and catalase-negative pure colonies were cultivated on MRS agar and incubated at 30 ℃ for 24 hours. Formed LAB colonies were suspended in API Suspension Medium (2 mL) until a turbidity of 2 McFarland was obtained. Fresh cell suspension (200 µL) was inoculated into each API 50 CHL ampoule which contained a different test medium. After inoculation of 50 ampoules, two to three drops of sterile mineral oil were added to the top of each ampoule to generate an anaerobic environment. Inoculated test strips were incubated at 37 ℃ ± 1 ℃ for 48 hours, and then examined for changes in colour of the medium in the ampoule. Development of a yellow colour was indicative of a positive result as this change indicates acidification of the bromocresol purple indicator. For the Esculin test, a colour change from purple to black was recorded as positive. Interpretation of results was aided by the use of apiweb^{TM} identification software database V 5.1.

3.4.10 Genotype identification of yeasts and LAB colonies

Following the presumptive identification of the isolates by the API system, further identification of LAB and yeasts were done by partial genome sequencing. Due to the high microbial diversity of LAB, high-throughput sequencing analysis (Illumina sequencing) was first applied on total LAB DNA isolated from MSD, DBP, DAP. For identification of four purified LAB colonies, 16S rRNA gene was sequenced; and for identification of yeast colonies, ITS 1 DNA analysis was conducted (Chen et al., 2001; Liu et al., 2016b).

3.4.10.1 LAB DNA extraction from isolated purified LAB colonies and dough samples

DNA extraction from pure LAB colonies and dough LAB cultures was performed using a DNeasy® Blood & Tissue Kit (Qiagen, USA), according to the manufacturer's instructions. All centrifugation steps were conducted at room temperature (22 °C). To obtain fresh cells for DNA extraction, several identical purified LAB colonies or 1 mL of 10^{-1} dilution of dough samples (total LAB DNA extraction) described in Section 3.4.7 were inoculated using a sterile loop into 10 mL of MRS broth (Merck, Germany) in a 15 mL falcon tube and incubated anaerobically at 37 ℃ for 24 hours. The fresh incubated LAB cells were then used in the preparation of the LAB lysate for DNA extraction.

Preparation of LAB lysate for DNA extraction

The DNeasy® Blood & Tissue Kit can be used to extract DNA from a maximum of 2 x 10^9 bacteria cells. One (1) mL MRS broth suspension prepared as described in Section 3.4.10.1, with an absorbance reading of between 0.20-0.30 at OD_{600} (8-9 log cells/mL), was transferred into a 1.5 mL micro-centrifuge tube (LP Italian Spa, Italy) (Kralik, Beran, & Pavlik, 2012). Solutions with readings above 0.3 were diluted with sterile MRS broth until the absorbance of the solution falls into the range of 0.20-0.30 at OD_{600}. To harvest LAB cells, the 1.5 mL micro-centrifuge tube containing 1 mL suspension was centrifuged for 10 minutes at 5000 ×g (Heraeus Multifuge ×1R, Thermo Fisher, Germany). The supernatant was discarded and the bacteria pellet was re-suspended in 180 µL lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) containing lysozyme (20 mg/mL) (Thermo Fisher, NZ). The suspension was mixed by votexing (VM-96B JEIOTECH, Korea) for 15 seconds and incubated at 37 ℃ for 30 minutes.

After incubation, 25 µL of proteinase K (Qiagen, Germany) was added to the suspension and the sample mixture was vortexed for 15 seconds. DNeasy® Buffer AL (200 µL) was then added to the mixture and vortexed for 15 seconds, then incubated at 56 ℃ for 30 minutes. Following incubation, 200 µL 96-100 % ethanol was added to the sample and mixed by vortexing. The prepared lysate was used for DNA extraction.

DNA extraction of LAB lysate

Prepared LAB lysate was loaded onto a DNeasy ® Mini Spin Column and centrifuged at 6,000 × g for one minute. Centrifugation was repeated until all the liquid had passed through the silica gel membrane. The collection tube was discarded and replaced with a new DNeasy® Collection Tube.

Wash Buffer AW1 (500 µL) was then added to the spin column and the column centrifuged at 6,000 ×g for one minute. The collection tube was again discarded and replaced with a clean collection tube prior to the addition of 500 µL of Wash Buffer AW2, followed by centrifugation at 17,000 × g for three minutes. After emptying and replacing the collection tube, the Mini Spin column was centrifuged at 17,000 × g for another minute to avoid any carry-over of ethanol.

The collection tube was discarded and the spin column was placed in a sterile 1.5 mL micro-centrifuge tube. To elute the DNA from the column, 100-200 µL of DNeasy® Elution Buffer was added, and the column was incubated at room temperature for one minute prior to centrifugation at 6,000 ×g for one minute. The obtained DNA was evaluated for DNA yield and purity as described below.

Determination of DNA yield and purity

The yield and purity of the purified DNA was determined by conducting absorbance readings of the isolate at 260 nm and 280 nm using a GENOVA NANO spectrophotometer (JENWAY, UK) using DNeasy® elution buffer as the blank for calibration. A ratio of absorbance at 260 nm and absorbance at 280 nm (A260/A280) between 1.80 and 2.00 indicates that the extracted DNA was free from proteins that might interfere with downstream applications. Prepared DNA samples were stored at -20 ℃ until required for PCR reactions and sequencing analysis.

3.4.10.2 Yeast DNA extraction from isolated purified yeast colonies and dough samples

To obtain fresh cells from pure yeast colonies or yeast cells from MSD, DBP and DAP for DNA extraction, several identical pure yeast isolates or 1 mL of 10^{-1} dilution described in

Section 3.4.7 were inoculated using a sterile loop into 10 mL of YPD broth (Merck, Germany) in a 15 mL falcon tube and incubated at 30 ℃ for 24 hours.

One (1) mL YPD broth suspension containing yeast cells with absorbance values of between 0.30-1.00 at OD_{600} was transferred into a 1.5 mL micro-centrifuge tube. Yeast cells (maximum 5 x 10^7 cells) were harvested following the same procedure as LAB cells described in Section 3.4.10.1. Harvested cells were resuspended in 600 µL sorbitol buffer and 200 units of lyticase was added to the suspension. After mixing by vortexing for 15 seconds, the suspension was incubated at 30 ℃ for 30 minutes. After incubation, the suspension was placed at -80 ℃ for one minute and immediately heated at 90 ℃ for one minute. This step was repeated once more, and then the suspension was centrifuged at 300 ×g for 10 minutes to obtain the spheroblasts. The spheroblasts were resuspended in 180 µL ATL buffer. Proteinase K (20 µL) was added into the suspension and vortexed for 15 seconds. The suspension was then left overnight for lysis at 56 ℃. After incubation at 56 ℃, 200 µL 96-100 % ethanol was added to the sample, then the solution was mixed by vortexing to obtain a homogeneous solution. The remainder of the DNA extraction procedure for the yeast lysate and determination of DNA yield and purity procedures were the same as for DNA extraction of LAB lysate described in Section 3.4.10.1.

3.4.10.3 LAB PCR reactions prepared for pyrosequencing analysis

For total DNA extracted from MSD, DBP and DAP, V3 and V4 regions of the 16S rRNA genes were amplified using the primers listed in Table 3.6. Primers used to identify LAB were chosen based on the study by Klindworth *et al*. (2013) with minor modification. Illumina overhang adapter sequences which are shown in bold in Table 3.6 were attached to locus-specific sequences according to the Illumina MiSeq system instructions (Illumina,n.d.).

Table 3.6 Primers used for LAB identification

Primer name	Sequence (5' -3')
Forward primer	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AGACAGCCTACGGGNGGCWGCAG
Reverse primer	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATCC

Note: Bold = Illumina overhang adapter sequences.
Source: Klindworth et al. (2013)

All the primers were supplied by Integrated DNA technologies (IDT®, New Zealand). Prior to PCR reactions, all primer stock solutions were standardised to a concentration of 50 μM in TE buffer (10 mM Tris HCl, 1mM EDTA, pH 8.0). PCR amplification was carried out in 50 μL volumes in 0.2 mL PCR tubes (Merck, Germany). Prior to the PCR reactions, harvested DNA was diluted to a concentration of 5 μg/μL in 10 mM Tris-HCl pH 8.5 (Merck, Germany). The PCR mixture contained 5.0 μL template DNA, 5 μL forward primer (1 μM), 5 μL reverse primer (1 μM), and 25 μL TaqMan Real-Time PCR Master Mixes (Thermo Fisher, Germany).

The PCR reactions were carried out in a PTC 1148 Thermal Cycler (BIO-RAD, Mexico) under the following conditions: (1) denaturation at 95 ℃ for three minutes, (2) 25 cycles: 95 ℃ for 30 seconds + 55 ℃ for 30 seconds + 72 ℃ for 30 seconds; (3) final extension at 72 ℃ for five minutes and hold at 4 ℃. After PCR purification, 25 μl of each purified LAB PCR product was transferred to sterile PCR tubes and transported to Illumina Inc. for sequencing (Palmerston North, New Zealand).

3.4.10.4 Agarose gel electrophoresis of PCR products

A 2 % agarose gel (AppliChen, GmbH, Germany) was prepared by adding 2 g agarose to 100 mL 1 ×TAE buffer, and heating the solution in a domestic microwave (Inverter Sensor 1100W, Panasonic, New Zealand) until all the agarose was dissolved. SYBR® Safe DNA gel stain (1 ×) (Invitrogen, USA) was added after the agarose solution had cooled slightly, then the gel was poured into a supplied tray and a plastic comb was inserted into the gel to create wells for loading the PCR products. After solidification of the gel, the comb was

removed and sufficient 1 × TAE buffer was added to the electrophoresis chamber to cover the gel.

Five (5) µL of 100 bp ladder (Biolabs®, Lithuania) was loaded into the first and last sample wells. To prepare loaded amplified samples, 2 µL of 6 × loading dye (Thermo Fisher, Lihuania) was added to 10 µL PCR mixture. After mixing by gentle pipetting up and down several times, 10 µL of each amplified sample was loaded into the sample wells. Electrophoresis separation was conducted at 60 V for 60 minutes for the small gel apparatus, and at 100 V for 60 minutes for the large apparatus using a PowerPac™ Basic Power Supply (BIO-RAD, USA). A Gel Doc™ EZ Imager (BIO-RAD, USA) was used to view and record images. After PCR reactions, the products were purified as described in Section 3.4.10.5.

3.4.10.5 Purification of amplified PCR products

The QIAquick® PCR Purification Kit (Qiagen, Germany) was used to purify amplified PCR products according to the manufacturer's instructions. Wash buffer was prepared by adding 24 mL of 100 % ethanol to the PE buffer and the binding buffer prepared by adding a volume of 1:250 pH indicator I to Phosphate Buffer (PB) (pH ≤7.5). The pH was adjusted by adding 10 µL aliquots of 3 M sodium acetate to the solution until the colour was adjusted from violet to yellow.

PB reagent (500 µL) was added to the PCR products and mixed by pipetting up and down. The solution was transferred onto a QIAquick column and centrifuged for one minute at 13,000 × g. The liquid in the collection tube was discarded and the empty tube was added back to the column. PE buffer (750 µL, pH 7.5, 25 ℃) was added onto the column and the mixture was centrifuged at 13,000 × g for one minute. After centrifugation, the liquid in the collection tube was discarded and emptied tube was added back to the column. Residual wash buffer was removed by centrifuging the column under the same conditions.

Before eluting the DNA, the spin column was placed in a sterile 1.5 mL micro-centrifuge tube and 30 µL elution buffer was added onto the spin column and incubated for one minute. DNA was eluted by centrifugation at 17,000 ×g for one minute. The concentration

of each PCR product was determined using a GENOVA NANO spectrometer (JENWAY, UK) and PCR concentrations were recorded following procedures described in Section 3.4.11.1.3.

3.4.10.6 LAB PCR reactions prepared for 16S rRNA sequence

DNA extracted from four purified LAB colonies were sent to Macrogen Inc. (Seoul, Korea) in 1.5 mL micro-centrifuge tube at room temperature by FedEx Courier Company at room temperature for further PCR amplification, PCR purification and sequencing. Universal primers 27F (5`-AGAGTTTGATCMTGGCTCAG-3`) and 1492R (5`-TACGGYTACCTTGTTACGACTT-3`) were used to amplify 16S rRNA genes. PCR reactions were conducted as described by Liu et al. (2016a): (1) denaturation at 94 ℃ for five minutes, (2) 30 cycles: 94 ℃ for 30 seconds + 56 ℃ for 30 seconds + 72 ℃ for one minute; (3) final extension at 72 ℃ for 10 minutes and hold at 4 ℃.

3.4.10.7 PCR reaction of yeast DNA and sequencing

PCR reactions and sequencing were conducted by Macrogen Inc. (Seoul, Korea). Extracted yeast DNA (30-50 μg, over 50 μL) samples including total DNA from MSD, DBP, DAP and one yeast colony which had the lowest identity percentage through API 32 C tests were sealed tightly in 1.5 mL micro-centrifuge tube (LP Italian Spa, Italy) and shipped by FedEx Courier Company at room temperature. Universal primers ITS1 (5`-TCCGTAGGTGAACCTGCGG-3`) and ITS 4 (5`-TCCTCCGCTTATTGATATGC-3`) were selected according to Chen *et al*. (2001) and PCR reaction conditions were 95 °C for 6 minutes, followed by 25 cycles at 95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, followed by one final extension at 72 °C for 10 minutes. After sequencing, the results were downloaded from the Macrogen Inc. website:

(https://dna.macrogen.com/eng/index.jsp).

3.5 Data analysis

Data obtained from phase one and phase two were analysed by descriptive statistics. Graphs were generated using Microsoft Excel version 2016 (Santa, CA, USA). Acidity (pH

and total acidity) and microbiological plate counts (LAB and yeast) data were analysed by SPSS Version 25 (IBMTM Company, USA). Biochemical and microbiological data were analysed using univariate analysis of variance, descriptive and Tukey's multi-comparison tests (95 % confidence interval). Data for total DNA of LAB pyrosequencing were analysed by Massey Genome Centre to obtain taxa count information. 16S rRNA sequences of single LAB colonies and ITS region sequences were compared with Targeted Loci Nucleotide Blast Database of National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/blast). For LAB 16S rRNA gene BLAST, the LAB species was positively identified when a sequence similarity of more than 97.6 % was found. For yeasts, the species was aligned with existing database species when at least 80 % of sequence length was covered and 99 % of sequence similarity was achieved.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Phase 1 Physico-chemical characterisation of sourdough and sourdough bread

4.1.1 Introduction

In phase one, fermentation of sourdough by LAB and yeasts were investigated by analysing the acidity of MSD, DBP, DAP and SDB samples (TTA and pH using methods described in Section 3.4.1 and 3.4.2, respectively) collected over a 10-week period. Soluble fermentable sugars and levels of specific organic acids (lactic acid and acetic acid) of DBP, DAP and SDB samples collected from sampling week 8, 9, and 10 were also analysed (as described in Section 3.4.4). The free fatty acids compositions of DBP, DAP and SDB samples from sampling week 6 were analysed to determine nitrogen metabolism and the proteolytic abilities of the microorganisms during fermentation (as described in Section 3.4.3). The effect of fermentation on the texture and colour of SDB samples were also determined (as described in Sections 3.4.5 and 3.4.6, respectively).

4.1.2 Acidity

The acidity (pH and TTA) of MSD, DBP, DAP and SDB are shown in Figure 4.1. The increase in acidity of sourdough has been attributed to the production of organic acids from carbohydrate metabolism during fermentation (Arendt, Moroni, & Zannini, 2011; Zhou & Therdthai, 2012). High acidity is reflected by a high TTA and low pH (Perrin, 1972; Gobbetti & Gänzle, 2012).

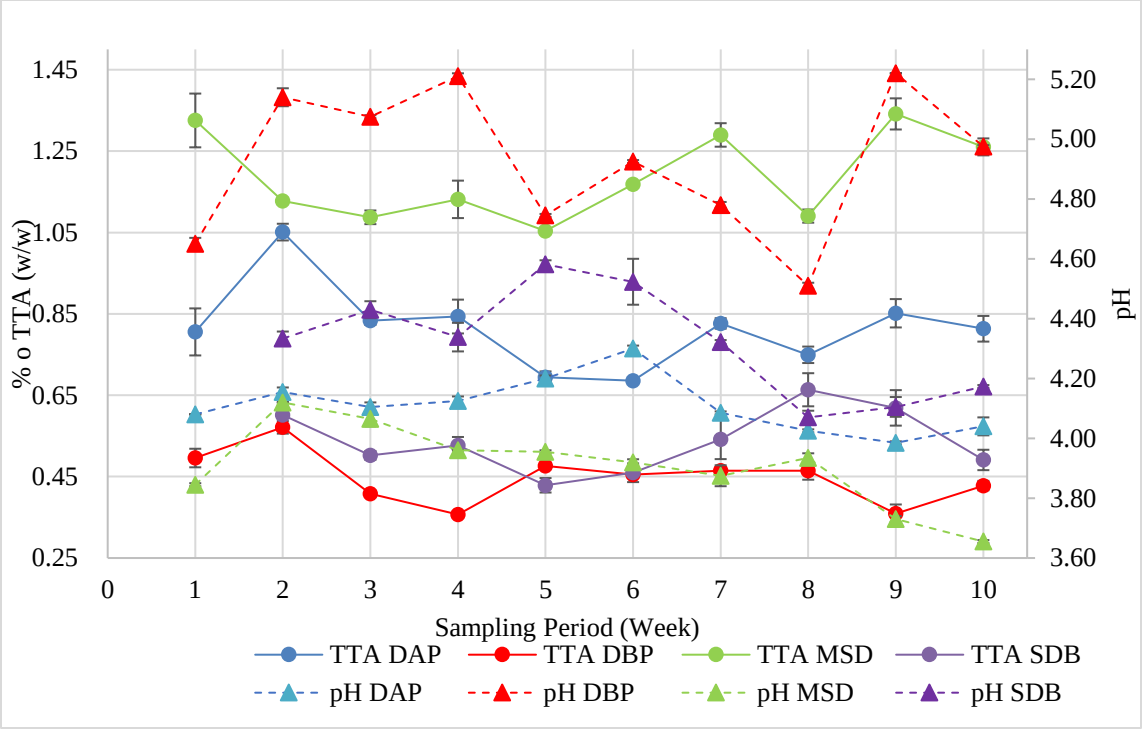

Figure 4.1 pH and TTA of MSD, DBP and DAP of different batches collected over a period of 10 weeks.

4.1.2.1 Acidity of mother sourdough

MSD had the highest TTA (1.05-1.34 %) and the lowest pH (3.66-4.12) when compared to DBP, DAP and SDB. These values are similar to those reported for German rice MSD, which had a TTA of 1.3-1.8 % and pH of 3.8-3.9 (Meroth et al., 2003) In New Zealand (NZ) and German sourdough production, MSD is used as a starter culture in sourdough bread production, a process commonly known as back-slopping (Todorov & Holzapfel, 2014). To maintain the starter culture in MSD, additional fresh water and flour must be added to the existing MSD and the refreshed MSD stored at 4 ℃ for two days to allow LAB and yeasts to grow (Valjakka, Kerojoki, & Katina, 2003; Zhou & Therdthai, 2012). During storage, organic acids were produced by the LAB and yeasts through carbohydrate metabolism and these organic acids accumulated (Figure 3.1) a result which is in agreement with previous studies (Valjakka, Kerojoki, & Katina, 2003; Zhou & Therdthai, 2012; Todorov & Holzapfel, 2014). Using a significant amount of MSD (>25 %) with a high

acidity for sourdough production can shorten the fermentation time (Gobbetti & Gänzle, 2012; Hui & Evranuz, 2012).

4.1.2.2 Acidity of dough before proofing

To produce a new batch of sourdough bread, MSD was mixed with fresh ingredients to obtain DBP. The TTA of DBP ranged from 0.36-0.57 % and the pH ranged from 4.51-5.22 (Figure 4.1). The pH of NZ rice sourdough was slightly higher than Korean rice DBP (pH 5.5) (Park et al., 2017). This difference in acidity may be because NZ rice sourdough used 40 % MSD which contained high levels of organic acids for the DBP production, whereas the Korean rice sourdough used 30 mL of rice wine as the starter culture which made little contribution to the acidity of the Korean DBP. The acidity of DBP is highly depended on the amount of MSD used for the inoculation, the acidity of the MSD and the ash content of the flour (Decock & Cappelle, 2005). Therefore, a DBP with high acidity can be obtained when using a MSD which has high amounts of organic acids (Gobbetti & Gänzle, 2012; Hui &Evranuz, 2012).

Flour with a higher ash content also results in a higher TTA due to its buffering capacity (Decock & Cappelle, 2005). For example, wheat flour with an ash content of 1.5 % had a higher TTA of 0.23 % and accounted for 25 % of the TTA of the mixed dough (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002; Phothiset and Charoenrein, 2007), whereas rice flour with an ash content of 0.39 % and a TTA of 0.05 % (Rani, Prasada Rao, Leelavathi, & Haridas Rao, 2001; Hagenimana, Ding, & Fang, 2006). Due to the low contribution of rice flour to TTA, the main contributor to TTA in rice DBP is expected to come from the fermentation of the MSD by starter cultures.

4.1.2.3 Acidity of dough after proofing

DAP was obtained after DBP was proved at 38 ℃ for 3-3.5 hours. The TTA of DAP ranged from 0.69 % (week 6) to 1.05 % (week 2) and its pH ranged from 3.99 (week 9) to 4.30 (week 6). The acidity of NZ DAP was lower than that of German rice DAP (pH 3.7-3.8), which may be attributed to the longer proofing time (one day) of German rice DAP, which is carried also out at a lower temperature (>28 ℃) (Meroth et al., 2003).

The acidity of rice sourdough increased significantly during proofing ($p<0.05$) (Figure 4.1). The increased acidity is closely related to the level of carbohydrate metabolism of the LAB, which produce lactic acid and acetic acid through the phosphoketolase pathway (heterofermentative LAB) or the EMP pathway (homofermentative LAB) (De Vuyst & Vancanneyt, 2007; Holzapfel & Wood, 2012). In addition, the metabolic activities of yeasts can contribute to increased TTA by producing carbonic acid (Stauffer, 1990).

When comparing the TTA between DAP and DBP, the TTA in sampling weeks 4 and 9 (increased by 0.49 % during proofing) increased the most, while the TTA in week 5 (increased by 0.22 % during proofing) increased the least. Generally, an increase in organic acid levels is closely related to the activity of LAB in the dough (De Vuyst & Vancanneyt, 2007).) However, the LAB counts in week 5 (8.41 log CFU/g) were actually the highest among the three batches (Section 4.2), (7.85 log CFU/g, 8.33 log CFU/g, for weeks 4 and 9 respectively) (Figure 4.12), yet the levels of organic acids produced during proofing were the lowest, which suggests that the activity of LAB in week 5 was weaker than that in weeks 4 and 9.

The fermentation behaviour and growth of sourdough LAB are affected by pH, temperature and salt concentration (Gobbetti & Gänzle, 2012). In the production of the NZ rice sourdough bread the temperature and salt concentration used were standardised. However, pH values were different during the sampling weeks, which may explain differences in LAB performance during this period. The optimum pH for sourdough LAB is between 5.0 and 6.0 (Gobbetti& Gänzle, 2012). The pH of DBP in weeks 4, 5 and 9 were 5.21, 4.74 and 5.22, respectively. Thus, the pH of DBP in weeks 4 and 9 fell within the optimum pH range for LAB at the beginning of proofing, whereas the week 5 DBP had a pH below the optimum range. This potentially explains why although the LAB counts in week 5 were higher than that of weeks 4 and 9, the levels or organic acids produced during proofing were the lowest.

4.1.2.4 Acidity of sourdough bread

SDB was obtained by baking DAP at 265 ℃ for 40 minutes. The TTA of SDB ranged from 0.43-0.66 % and its pH ranged from 4.07-4.58. The pH values of NZ SDB are similar to

that of rye and wheat SDB (pH 3.8-4.6) (Corsetti, 2013). Similar to other reported results (Corsetti et al., 2008; Tamani, Goh, and Brennan, 2013), the acidity of SDB decreased significantly ($p<0.05$) following baking (Corsetti et al., 2008). The decreased acidity is related to the decomposition and evaporation of organic acids. For example, carbonic acid can decompose into carbon dioxide and water (Loerting et al., 2000) and volatile organic acids can evaporate at the baking temperatures used (Bisutti, Hilke, & Raessler, 2004).

4.1.3 HPLC analysis of organic acids levels in sourdough and sourdough bread

4.1.3.1 Lactic acid

The lactic acid levels (measured as described in Section 3.4.4) in three batches (batch 8, 9, 10) of DBP, DAP and SDB are shown in Figure 4.2. Lactic acid levels in DBP ranged from 0.22-0.24 g/100 g (batches 9 and 8 respectively), while after proofing, the lactic acid content in DAP significantly increased ($p<0.05$) to 0.31 g /100 g. After baking, the lactic acid in SDB significantly decreased ($p<0.05$) to 0.22-0.28 / 100 g (batches 3 and 8 respectively). The increase in lactic acid produced during fermentation is related to the metabolic activities of the LAB sourdough starter culture. Lactic acid can be produced by homofermentative LAB through the EMP, or the glycolytic and phosphoketolase pathways by heterofermentative LAB (Kandler, 1983; Fugelsang & Edwards, 2006).

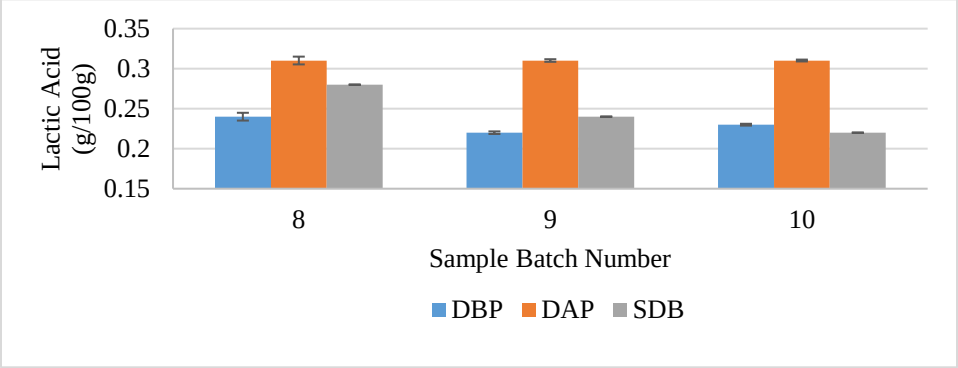

Figure 4.2 Lactic acid concentrations in DBP, DAP and SDB from three production batches.

Notes: DBP = dough before proofing; DAP = dough after proofing; SDB = sourdough bread. Error bars = standard deviation; n = 2.

Lactic acid levels in NZ DAP were lower than those reported for wheat sourdough inoculated with a single strain of *L. mesenteroides* BELLI7, which peaked at 0.5 g/100 g after fermentation for 20 hours at 28 °C (Lefebvre et al., 2002). This higher concentration of lactic acid may be attributed to the longer fermentation time used in wheat sourdough production (Valjakka et al., 2003; De Vuyst, Van Kerrebroeck, & Leroy, 2017). As has been previously reported (Clément et al., 2018), after baking at 220 °C for 40 minutes, the lactic acid content in SDB (0.66-0.73 g/100 g) decreased compared to DAP (0.99-1.26 g/100 g). A decrease in lactic acid was also observed in this study, which is possibly due to evaporation of volatile acids at the high baking temperature (Blake & Jackson, 1968; Komesu et al., 2017).

4.1.3.2 Acetic acid

The levels of acetic acid determined as described in Section 3.4.4 in three batches of DBP, DAP and SDB are shown in Figure 4.3. Acetic acid levels in DBP ranged from 0.09- 0.11 g/100 g (batches 9 and 8 respectively). After proofing, the acetic acid levels in DAP increased significantly ($p<0.05$), ranging from 0.13-0.17 g/100 g (batches 8 and 10 respectively). The increase in acetic acid levels indicates the existence of heterofermentative LAB and the presence of suitable electron acceptors in NZ sourdough because acetic acid is produced by heterofermentative LAB when electron acceptors such as fructose are available (Kandler, 1983; Fugelsang & Edwards, 2006). The acetic acid levels of NZ DAP (Figure 4.3) were similar to that reported for wheat sourdough (0.1 g/100 g) suggesting that rice flour can also provide LAB with the necessary electron acceptors (Lefebvre et al., 2002).

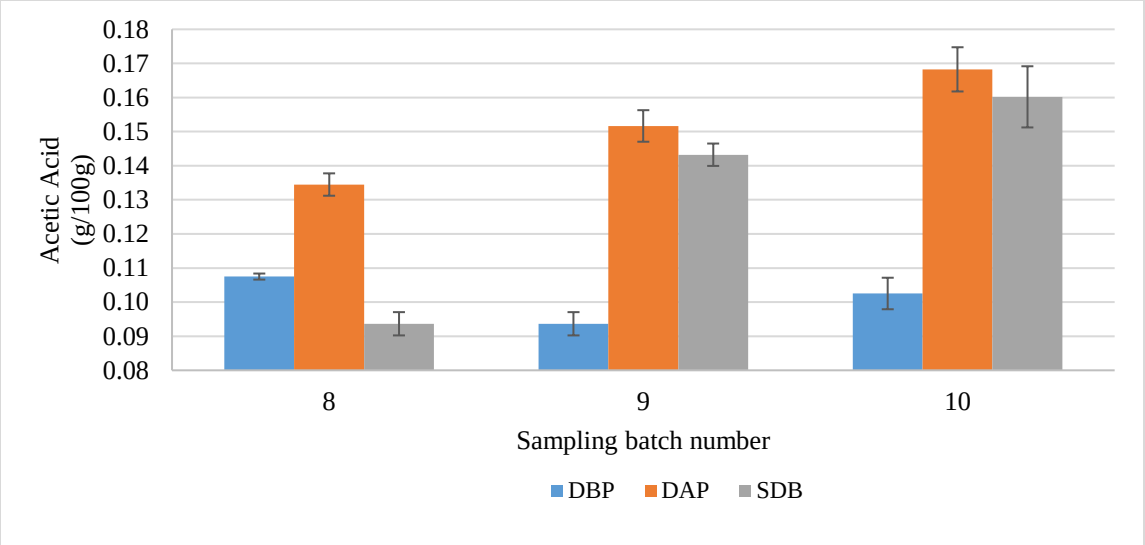

Figure 4.3 Acetic acid concentrations in DBP, DAP and SDB from three production batches.

Notes: DBP = dough before proofing; DAP = dough after proofing; SDB = sourdough bread. Error bars = standard deviation; n = 2.

No significant change (P>0.05) in acetic acid content was observed (except for batch 8) after baking with levels ranging from 0.09-0.16 g /100 g (batches 8 and 10 respectively). An acetic acid content higher than 0.09 g/100 g is desirable due to its effects on enhancing the palatability of sourdough bread, as well as extending shelf life and increasing nutritional value (Clément et al., 2018). According to Clément et al. (2018), the acetic acid content of SDB ranges from 0.07-0.09 g/100 g, which is lower than the levels found in this study. The higher acetic acid content in NZ SDB may be due to the presence of high numbers of heterofermentative LAB (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002; Gobbetti & Gänzle, 2012). Additional research is required to gain a better understanding of the correlation between the acetic acid levels and heterofermentative LAB numbers in sourdough. This could be done by carrying out a quantitative analysis of the ratio of homofermentative to heterofermentative LAB using q-PCR (Furet, Quénée, & Tailliez, 2004).

4.1.3.3 Fermentation quotient

The fermentation quotient (FQ) is the molar ratio between lactic acid and acetic acid produced during fermentation. This ratio is considered to have a direct impact on the sour taste, odour and shelf life (staling and antifungal properties) of sourdough bread (Valjakka et al., 2003; Gobbetti & Gänzle, 2012).

The FQ values calculated for three batches of SDB ranged from 1.19 to 2.67 (Table 4.1). For a mild balanced flavour and aroma, a FQ value of 4-9 is favoured (Gobbetti & Gänzle, 2012), which is higher than the values obtained here. However, according to Clément et al. (2018), there is no significant relationship between sour taste and the FQ. Rather, sour taste correlates to the acetic acid and carbon dioxide contents, as well as sourdough TTA and pH. In order to ascertain if a relationship does exist between the FQ and sensory scores, further sensory evaluations should be conducted. The lower FQ values of NZ SDB reflect the higher proportion of acetic acid, which helps prevent mould spoilage of the product (Gerez, Torino, Rollán, & Font de Valdez, 2009).

Table 4.1 Fermentation quotient of sourdough bread from batch 8, 9, 10 samples

Sample Batch	8	9	10
Fermentation quotient	2.67	1.43	1.19

4.1.4 HPLC analysis of soluble sugar levels in sourdough and sourdough bread

4.1.4.1 Maltose and sucrose

Maltose and sucrose, which are important fermentable disaccharides (De Vuyst & Vancanneyt, 2007) are produced from the hydrolysis of flour starch by enzymes such as amylases and cellulases (Ji, Liu, Li, Sun, & Xiong, 2018). During sourdough fermentation, the soluble sugars maltose, glucose and maltotriose can be obtained through the hydrolysis of α-1,4-glucosidic bonds by α-amylase and β-amylase (Chang, Lee, & Brown, 1986; Smyth & Prescott, 1989) and sucrose, glucose and fructose which are stored in the rice endosperm and can be used by microorganisms directly (Smyth & Prescott, 1989). Maltose can then be hydrolysed by maltose positive yeasts and LAB into the monosaccharide

glucose (De Vuyst & Neysens, 2005; De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016b). In this study, maltose and sucrose were measured together, and the maltose and sucrose concentrations of DBP, DAP and SDB from batches 8, 9 and 10 are shown in Figure 4.4. Overall, the maltose and sucrose levels of DBP, DAP and SDB were not significantly different ($p>0.05$).

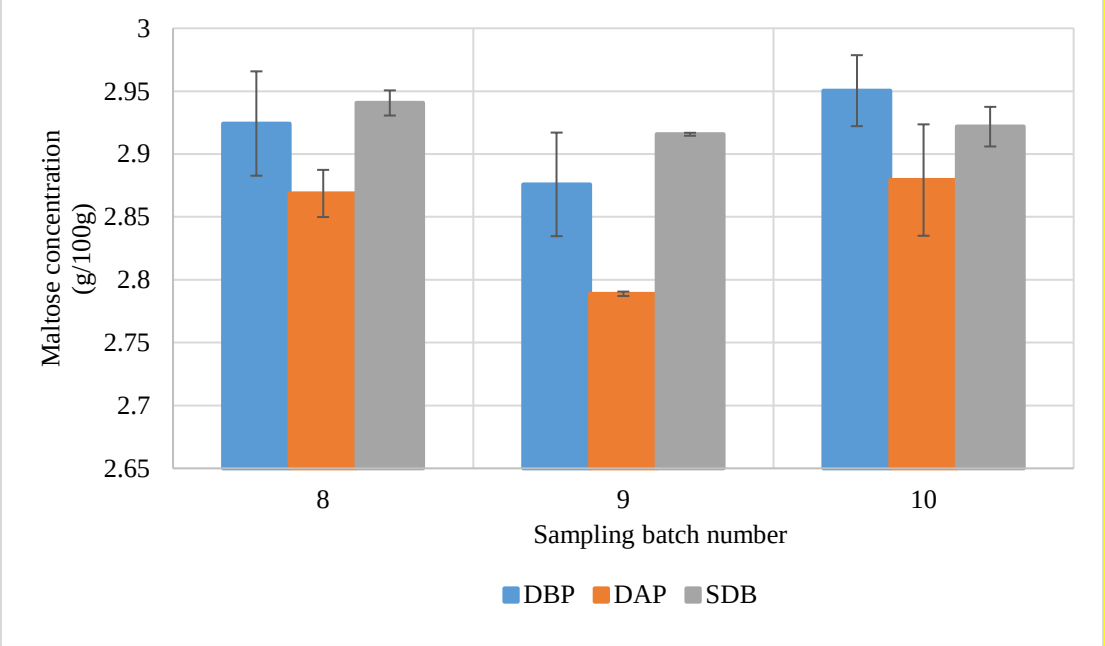

Figure 4.4 Maltose and sucrose concentrations in DBP, DAP and SDB of three batches of production.

Notes: = dough before proofing; DAP = dough after proofing; SDB = sourdough bread. Error bars = standard deviation; n = 2.

Maltose and sucrose concentrations in DBP ranged from 2.88-2.95 g/100 g (weeks 9 and 10 respectively). These values are higher than that reported for wheat sourdough (1.5 g/100 g), most likely due to higher maltose and sucrose concentrations in rice flour (Lefebvre, Gabriel, Vayssier, & Fontagné-Faucher, 2002).

After proofing, maltose and sucrose concentrations ranged from 2.79-2.88 g/100 g (weeks 9 and 10 respectively).Since these concentrations are not significantly different to the DBP concentrations this suggests that the amount of disaccharides produced during hydrolysis

was nearly equal to that being consumed by LAB and yeasts. Previous studies have shown that maltose-negative yeasts which preferentially consume glucose or fructose to maltose when all these soluble sugars are available, hence maltose normally increases during proofing (Hammes & Vogel, 1995; De Vuyst et al., 2009). However, in NZ rice sourdough, the yeast species recovered was a maltose-positive *S. cerevisiae* (Section 4.3) which is able to use all types of flour carbohydrates and can thus metabolise maltose and glucose simultaneously (Verstrepen et al., 2004; De Vuyst & Neysens, 2005; Gänzle, Vermeulen, & Vogel, 2007). Therefore, it is likely that maltose was also being consumed by the yeast during fermentation and therefore its levels did not change significantly.

After baking, sucrose and maltose concentrations in the SDB ranged from 2.90-2.92 g/100 g (batches 9 and 8 respectively) which is not significant different to the levels found in DAP. Similar results were found in research conducted by Langemeier and Rogers (1995) showing that baking did not have significant effect on maltose concentrations in white pan breads.

4.1.4.2 Glucose and fructose

In this study, fructose was not detected in any dough or bread samples. This is not surprising since rice contains only trace amounts of fructose and glucose (Biesiekierski et al., 2011). Any fructose that is present (either stored in the rice endorsperm or produced by hydrolysis of sucrose) (Smyth & Prescott, 1989) can be used as an electron acceptor to produce acetic acid by heterofermentative LAB or as carbohydrate source for metabolism by both LAB and yeasts (Fugelsang & Edwards, 2006, Gänzle et al., 2007, De Vuyst et al., 2009).

Glucose can be obtained through enzymatic hydrolysis of starch (Chang et al., 1986), as well as through carbohydrate metabolism, with one mole of glucose being obtained from hydrolysis of one mole of sucrose and one mole of glucose being released after phosphorylation of maltose by LAB (De Vuyst & Neysens, 2005; Yazar & Tavman, 2012). During carbohydrate metabolism, glucose can be used directly by LAB and yeasts to produce metabolites such as organic acids, which contribute to the increased TTA and decreased pH (Figure 4.1) (Holzapfel & Wood, 2012; De Vuyst, 2016).

Glucose in dough before proofing

The glucose levels detected in three batches of DBP, DAP and SDB are shown in Figure 4.5. The glucose levels of DBP ranged from 0.15-0.21 g/100 g (batches 10 and 8 respectively), with that in batch 8 being significantly higher than that of batch 10 ($p<0.05$). Glucose levels in DBP was similar to that reported for wheat sourdough (0.17 g/100 g) prior to proofing (Lefebvre et al., 2002).

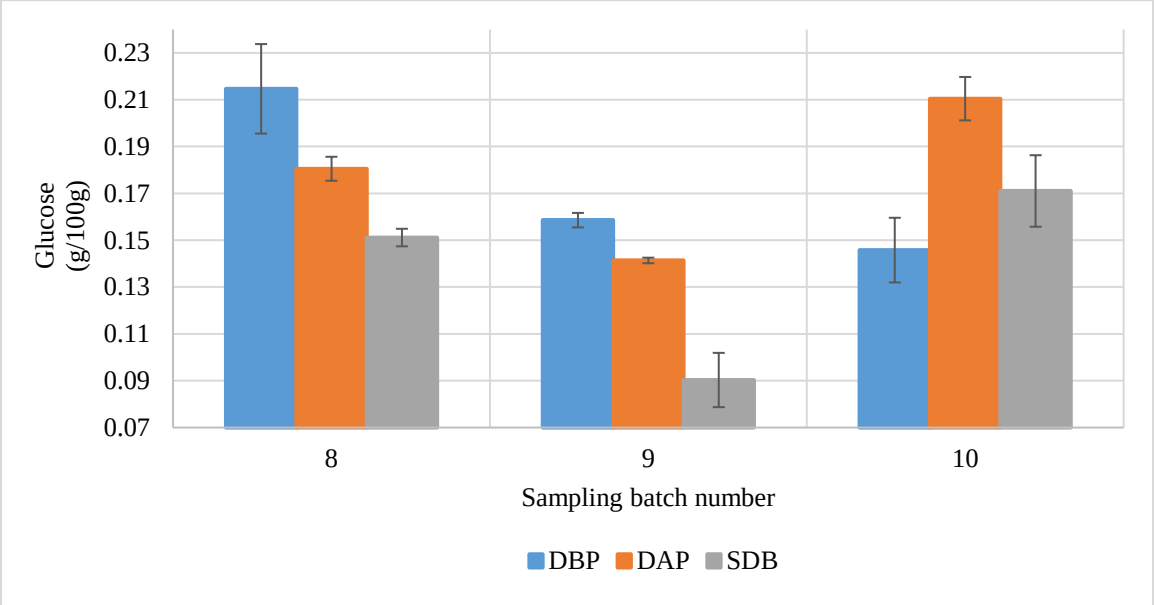

Figure 4.5 Glucose concentrations in DBP, DAP and SDB from three production batches

Notes: DBP = dough before proofing; DAP = dough after proofing; SDB = sourdough bread. Error bars = standard deviation; n = 2.

Glucose in dough after proofing

Glucose levels in DAP ranged from 0.14-0.21 g/100 g (batches 9 and 10 respectively), which is not significantly different to DBP. Similar glucose levels (0.17 g/100 g) were also found in wheat sourdough before and after fermentation (Lefebvre et al., 2002). It has been proposed that glucose released from the hydrolysis of maltose by LAB is then metabolised by LAB and yeasts, hence glucose levels are in dynamic balance and therefore do not

change significantly during fermentation (Arendt, Moroni, & Zannini, 2011; Zhou & Therdthai, 2012).

Glucose in sourdough bread

Glucose levels in SDB ranged from 0.09 g/100 g (week 9) to 0.17 g/100 g (week 10) which are significantly lower than that in DAP ($p<0.05$). Glucose is a reducing sugar and can take part in Maillard reactions and therefore significantly lower glucose levels ($p<0.05$) are normally observed in SDB compared to DAP (Langemeier & Rogers, 1995; Mundt & Wedzicha, 2005).

4.1.5 HPLC analysis of free amino acids in sourdough and sourdough bread

During sourdough fermentation, cereal proteins are completely degraded to release amino acids through primary proteolysis by cereal enzymes and secondary proteolysis by microbial enzymes and nitrogen metabolism by sourdough LAB and yeasts (Rizzello, Montemurro, & Gobbetti, 2016). From a nutritional standpoint, amino acids are the elementary units of proteins and for humans some amino acids are indispensable and must be obtained from the diet (Reeds, 2000). In addition, some amino acids have special functions such as stimulating hormonal release and acting as neurotransmitters (Van de Poll, Luiking, Dejong, & Soeters, 2005). In rice sourdough, FAA are released from the main rice proteins: albumin, globulin, glutelin and prolamin (Juliano, 1993). The concentrations of free amino acids detected (as described in Section 3.4.3) in DBP, DAP and SDB are shown in Figure 4.6 (Diana, Rafecas, & Quílez, 2014).

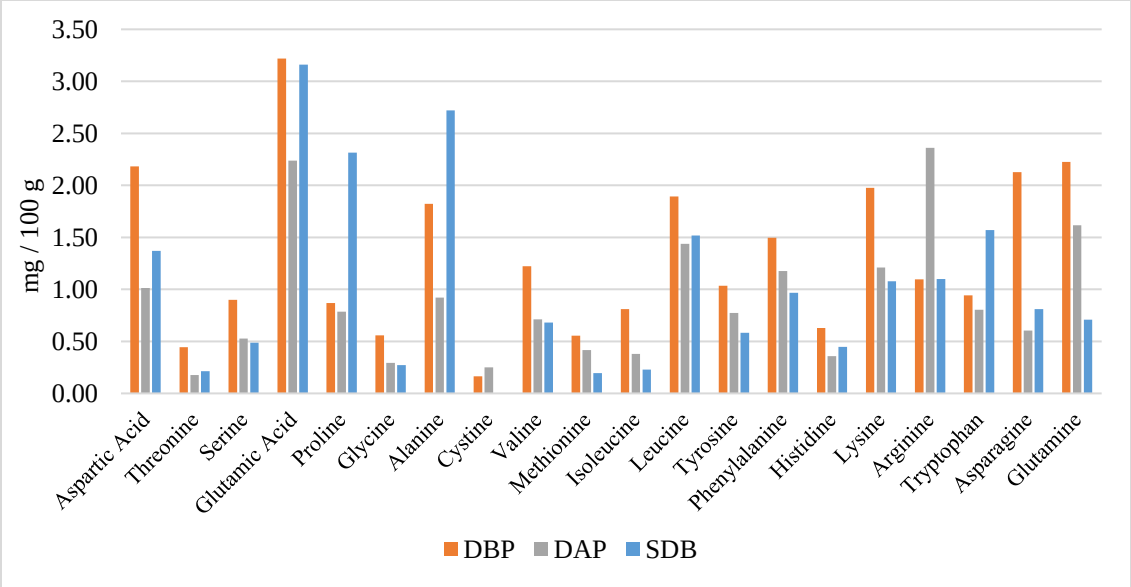

Figure 4.6 Concentration of free amino acids (mg/100 g) in DBP, DAP and SDB

Notes: DBP = dough before proofing; DAP = dough after proofing; SDB = sourdough bread. Error bars = standard deviation; n = 1

The total FAA levels in SDB was 20.43 mg/100 g, which is lower than the levels found in commercially available gluten free bread, which range from 21.9 - 60.6 mg/100 g (Rizzello et al., 2016). The release of FAA during fermentation is highly dependent on the raw materials used (Thiele, Gänzle, & Vogel, 2002; Pacyński, Wojtasiak, & Mildner-Szkudlarz, 2015), therefore the amount of FAA released from wheat proteins is usually higher than from the proteins found in rice and maize flour. The ingredients used in NZ SDB production: brown rice, white milled rice and maize have average protein levels of 7.1-8.3 %, 6.3-7.1 % and 6.9 % respectively (Eckhoff & Paulsen, 1996), which are at the low end of the range reported for wheat protein (6.1-18.8 %) (Caporaso, Whitworth, & Fisk, 2018). Therefore, GF breads made of rice and maize flour will generally have a lower FAA content than wheat breads. However, GF bread can still provide a good nitrogen source due to its high content of branched-chain amino acids, as 18% of the amino acids in brown rice protein (w/w),have been reported to be branched-chain amino acids (Kalman, 2014).

The total FAA content (18.05 mg/100 g) of DAP was lower than DBP (26.16 mg/100 g), which is in contrast to other reports that showed the total FAA level increased during sourdough fermentation (El-Dash & Johnson, 1970; Thiele et al., 2002). One possible reason for the difference in results may be that in the NZ GF sourdough bread fewer FAA were released through secondary proteolysis than were consumed by yeasts for growth (Thiele et al., 2002; Yazar & Tavman, 2012). The proteolytic activity of LAB is highly species dependant (Corsetti et al., 1998), for example, the proteinase activity of *L. sanfrancisco* CB1 is nearly three time higher than that of *L. plantarum* DC 400 (33.5 units vs 12.4 units) (Corsetti et al., 1998). Hence, it is possible that the LAB species in NZ rice sourdough are different to those used in other studies and do not have high proteolytic activity. In addition, sourdough contains high levels of yeast which can utilise large amounts of FAA for their growth (Yazar & Tavman, 2012), which can lead to a decrease in total FAA after fermentation. Compared to DBP, the total FAA of SDB decreased by nearly 22 %, which is similar to previous reports where the total FAA of baked sourdough bread reduced by 10-20 % compared to DBP due to Maillard reactions occurring between some FAA and reducing sugars (Thiele et al., 2002).

Certain free amino acids such as cystine and arginine increased during fermentation while others such as threonine and leucine decreased (Figure 4.6). The increased cystine and arginine may be a result of excretion by yeasts during fermentation (Engineers, 2011), while the decreased levels of other FAA may be due to their metabolism by yeasts and LAB (Gänzle et al., 2007). Following the baking process, levels of aspartic acid, glutamic acid, proline, alanine, leucine, histidine, tryptophan and asparagine increased, while other FAA decreased. It has been reported that lysis of LAB and yeast cells results in the release of certain free amino acids, for example: glycine and alanine from LAB cells (Gobbetti, 1998); proline, glycine, alanine, isoleucine, valine from *S. cerevisiae* cells (Diana et al., 2014) and aspartic acid, glycine, glutamic acid and lysine from the lysis of *C. milleri* (Engineers, 2011). Therefore, the increase in amino acids observed after baking could be from the lysis of LAB and yeast in the sourdough starter culture.

4.1.6 Crumb texture of sourdough bread

Sourdough technology has been reported to improve sourdough texture (Moroni et al., 2009; Samaroo et al., 2010). Bread texture can be evaluated based on crumb texture parameters such as hardness, cohesiveness, springiness, chewiness, cohesiveness and resilience (measured as described in Section 3.4.5) using a texture analyser (Matos & Rosell, 2012; De La Hera, Rosell, & Gomez, 2014). The measured texture parameters of fresh baked SDB collected over a 10-week sampling period are shown in Table 4.2. Variation in the measured attributes ($p<0.05$) can be observed both within the same batch and between different batches. Within batch variation could be at least partially explained by unevenly distributed hot steam during baking. While differences between batches may result from the variation in flours used or different technological parameters such as pH (Arendt, Ryan, & Dal Bello, 2007).

Table 4.2 Physical characteristics of the crumb of rice sourdough bread

	Sampling Period (Weeks)					
Parameters	5	6	7	8	9	10
Hardness (N)	13.12±2.84	16.73±2.66	7.58±2.58	12.74±3.88	9.56±2.14	12.07±3.14
Springiness	1.10±0.42	1.51±1.33	4.55±1.77	2.29±1.75	3.82±2.15	2.35±0.07
Cohesiveness	0.44±0.03	0.45±0.08	0.71±0.06	0.59±0.03	0.69±0.04	0.59±0.03
Gumminess	5.80±1.08	7.45±1.16	5.53±1.42	7.53±2.33	6.58±1.31	7.11±1.29
Chewiness	6.14±1.38	11.75±12.75	25.84±8.93	15.91±11.09	23.81±13.35	15.14±1.14
Resilience	0.23±0.02	0.23±0.04	0.40±0.04	0.33±0.02	0.39±0.03	0.34±0.01

Note: N = Newton; n= 18.

4.1.6.1 Hardness

The hardness of bread is related to the chewing force required to compress the food sample (Matos & Rosell, 2012). There was a high variation in the hardness of the SDB ranging from 7.58 ± 2.58 (week 7) to 16.73 ± 2.66 (week 6). This variation in SDB hardness values may be due to variation in the flours used for production of the different batches.

Compared to the hardness levels reported for unleavened rice bread (hardness above 17) in previous studies (Wolter 2013; Różyło et al.; 2016), the NZ SDB were softer. According to Różyło et al. (2016), there is a negative correlation between pH and bread hardness. Therefore, the application of sourdough bread technology and low pH achieved during fermentation may provide a softer bread crumb.

4.1.6.2 Springiness

Springiness indicates the freshness and elasticity of the bread, with a low springiness value being associated with brittleness (Matos & Rosell, 2012). The springiness values of SDB ranged from 1.10 ± 0.42 (week 5) to 4.55 ± 1.77 (week 7), which are higher than that reported for unleavened rice bread: 0.95 ± 0.02 (Matos & Rosell, 2012). A high springiness value indicates a stronger crumb cell wall network; therefore, that of NZ rice SDB may be stronger than that of unleavened rice bread (Cauvain, 2004).

4.1.6.3 Cohesiveness

The cohesiveness reflects the deformation ability of the bread and the higher the value, the better the extensibility of the bread (Matos & Rosell, 2012). The cohesiveness of SDB ranged from 0.44 ± 0.03 (week1) to 0.71 ± 0.06 (week 7), which is higher than reported for GF rice bread (0.37) and other unleavened GF bread (0.15) (Matos & Rosell, 2012).

4.1.6.4 Chewiness

The chewiness of bread describes how easily the bread can be broken down in the mouth, with bread having a high chewiness taking longer to chew and swallow (Matos & Rosell, 2012). The chewiness of SDB ranged from 6.14 ± 1.38 (week 5) to 25.84 ± 8.93 (week 7) which is higher than that reported for the majority of GF breads which ranged from 2.33 to

5.77 (Matos & Rosell, 2012). However, the majority of NZ SDB products were chewier than unleavened rice bread which has a chewiness value of 11 Newton (Wolter, 2013). The difference between fermented and unfermented rice bread is that acidification of the dough and protease activity of LAB can affect final dough structure (Różyło et al., 2016).

4.1.6.5 Resilience

The resilience of bread is associated with its elasticity. Reduced resilience or springiness indicates that the bread has decreased elasticity (Matos & Rosell, 2012). The resilience of SDB ranged from 0.23 ± 0.02 (week 5) to 0.40 ± 0.04 (week 7) which is within the range reported for other GF breads of 0.06 to 0.84 (Matos & Rosell, 2012).

4.1.7 Crust colour of sourdough bread

The colour of bread crusts (measured as described in Section 3.4.6) are determined by the presence of Maillard reaction products, which in turn are affected by the presence of reactants (amino acids and reducing sugars), pH, temperature and water activity (Yilmaz & Toledo, 2005). GF bakery products have generally been reported to have poor colour (Torbica, Hadnađev, & Dapčević, 2010). However, a previous study has shown that fermentation can improve the colour of GF cereal products (Phimolsiripol et al., 2012).

L* indicates lightness (the higher, the brighter) and the L* values of SDB from 10 sampling batches are shown in Figure 4.7. L* of SDB ranged from 41.27 ± 5.30 (week 8) to 59.29 ± 2.60 (week 10) which is lower than that of unleavened rice bread (L* from 61 to 76) and closer to L* of wheat bread (51.27) (Phimolsiripol, Mukprasirt, & Schoenlechner, 2012). The lower L* of fermented rice bread compared to unleavened rice bread may relate to the higher levels of Maillard reactants such as reducing sugars and free amino acids which are released during baking in the fermented rice bread (Fois, Piu, Sanna, Roggio, & Catzeddu, 2018).

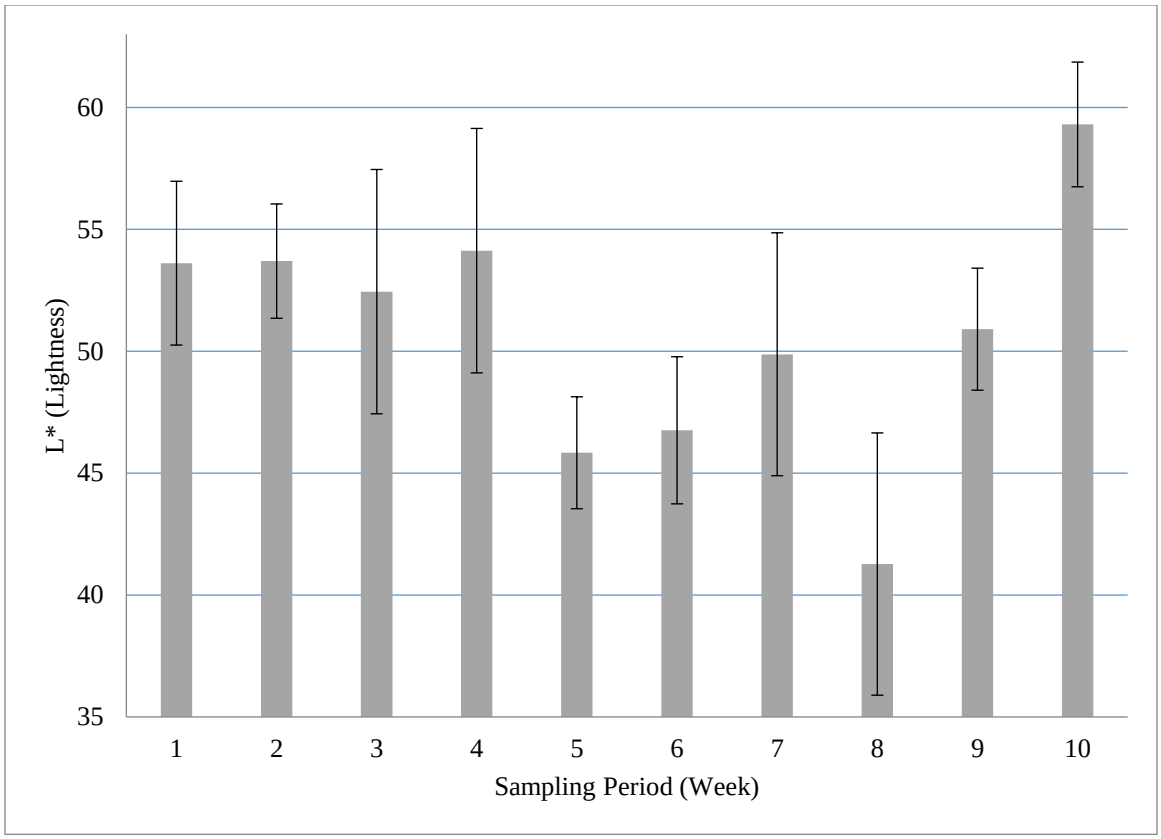

Figure 4.7 L* (Lightness) of the SDB crust

Notes: SDB = sourdough bread. Error bars = standard deviation; n = 12.

The a* indicates redness (positive red, negative green), and the a* values of SDB measured from 10 batches are shown in Figure 4.8. In this study, a* values for SDB were positive, ranging from 3.93 ± 1.21 (week 10) to 8.36 ± 1.56 (week 8). In contrast, in jasmine rice bread (JRB), a negative a* was reported (Pongjaruvat et al., 2014), which indicates that the Maillard reaction was not be well-developed in the JRB (Yilmaz & Toledo, 2005). Therefore, fermentation has a positive effect on Maillard reactions and on the a* of baked products.

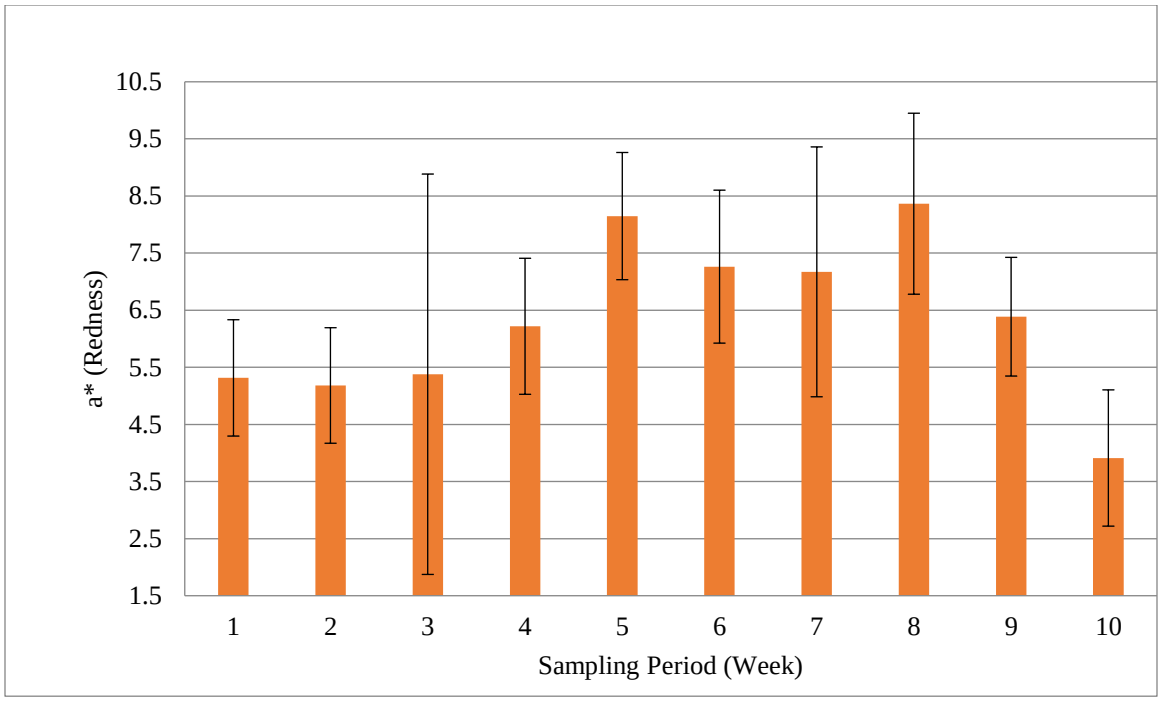

Figure 4.8 a* (redness) of the crust of SDB

Notes: SDB = sourdough bread. Error bars = standard deviation; n = 12

The b* values for SDB reflects the yellowness (positive yellow, negative blue) of the samples, which are shown in Figure 4.9. The b* for SDB ranged from 12.85 ± 2.31 to 16.45 ± 0.84 which are lower than that reported for both JRB (b* 19.93) and wheat bread (b* 31.36). Higher b* values are related to carotenoid pigments s in wheat (Fois et al., 2018) and NZ rice flour may have a lower content of natural pigment carotenoids than wheat thus leading to the lower b* value.

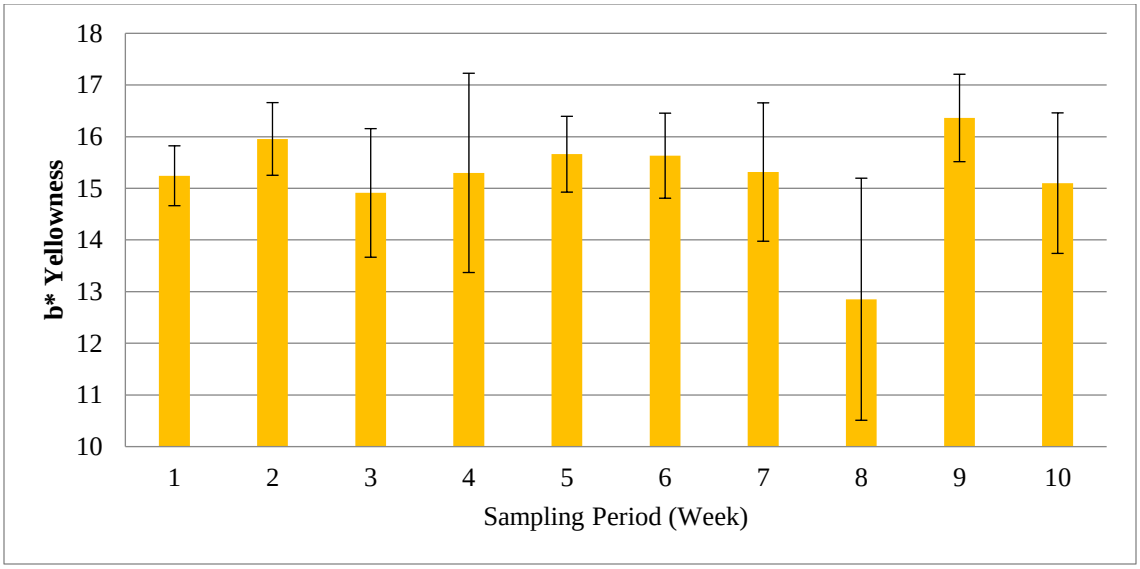

Figure 4.9 b* (yellowness) of the crust of SDB

Notes: SDB = sourdough bread. Error bars = standard deviation; n = 12

The L*, a* and b* values of wheat bread, JRB and SDB are shown in Table 4.3. The brightness (L* values) of SDB is similar to that reported for wheat bread; while the a* of SDB is positive and higher than unleavened rice bread but lower than wheat bread; and its b* is lower than both types of bread.

Table 4.3 The L*, a* and b* values of wheat bread, jasmine rice bread and New Zealand sourdough bread

	L*	a*	b*
Wheat bread	51.27	16.61	31.36
JRB	80.14	-0.15	19.93
SDB	41.27-59.29	3.93-8.36	12.85-16.45

Note: JRB: jasmine rice bread; SDB: New Zealand sourdough bread. L*, a* and b* of wheat bread and JRB are sourced from Phimolsiripol et al. (2012) and Pongjaruvat et al. (2014).

GF bread is usually reported as having poor colour (Phimolsiripol et al., 2012). However, from this study, it appears that fermentation can improve the colour of GF bread with the exception of the lower b* value which may be due to the lack of natural pigments in rice flour (Fois et al., 2018). The b* of NZ SDB may be improved by adding ingredients such as carrot and sweet potato that are high in natural pigments (Fois et al., 2018).

4.1.8 Summary of phase 1

As a fermentable substrate rice flour, has high levels of maltose and sucrose but low amounts of glucose and fructose. Fructose was not detected in any of the samples in this study, which was expected, given the low level found in rice flour and that any fructose released from the hydrolysis of sucrose was most likely used as an electron acceptor to produce acetic acid or consumed by LAB and yeasts. The ability of LAB and yeasts to metabolise carbohydrate and nitrogen contributes to the unique sour taste and flavour of sourdough (Hansen, 2004; Catzeddu, 2011)), through the production of metabolites such as lactic acid, acetic acid and carbon dioxide. In NZ sourdough, lactic acid is lower than reported for wheat sourdough possibly because the fermentation period for NZ sourdough is much shorter than that used for wheat sourdough. However, the acetic acid content of NZ sourdough was similar to that of wheat sourdough which had been fermented for a similar time (4 hours) (Lefebvre et al., 2002). The high concentrations of acetic acid detected in SDB likely result from the high carbohydrate metabolic activity of heterofermentative LAB in NZ sourdough. The FQ of NZ sourdough ranged between 1.37 and 2.98 which may correlate to a sharper sour taste and high antifungal activity compared to wheat sourdough which has a higher FQ value of 4-9.

NZ sourdough was produced by back-slopping using MSD. MSD had the highest acidity compared to DBP and DAP, possibly due to the accumulation of metabolised acids during its refreshment and storage time. The acidity of DBP was mainly derived from MSD because the ash content of rice flour is very low and therefore contributes little to DBP acidity. After proofing (DAP), the acidity increased significantly compared to DBP probably as a result of the carbohydrate activity of LAB and yeasts resulting in the formation of produced organic acids such as carbonic acid, lactic acid and acetic acid.

In contrast to other published findings (El-Dash and Johnson, 1970; Thiele et al., 2002), fermentation resulted in a decrease in total FAA (DAP) compared with DBP. This is likely due to the release of FAA through secondary proteolysis being less than that the amount of FAA consumed by yeasts for their growth. Though total FAA decreased during

fermentation, certain single FAA such as cystine and arginine increased, possibly due to excretion of FAA by yeasts during the fermentation process.

The results presented here indicate that the application of sourdough fermentation technology can improve the colour and texture of bread. Compared with unleavened rice bread, SDB was softer, more elastic, less crumbly, and exhibited a chewier crumb. SDB was also darker and more reddish than unleavened rice bread, possibly due to the production of more Maillard products which result in the darker colour of bread crusts.

4.2 Phase 1 Microbiological characterisation of sourdough samples

4.2.1 Introduction

In phase 2, total aerobic plate counts (APC), LAB counts and yeast counts of MSD, DBP and DAP were conducted over a 10-week sampling period using methods described in Section 3.4.7. The microbiological characterisation of MSD, DBP and DAP provides information on the sourdough starter culture: quantity of LAB and yeasts, LAB to yeast ratio, sourdough maturity, and competition between microorganisms, particularly Gram-positive and Gram-negative bacteria (Ercolini et al., 2013). In addition, the relationship between the sourdough microbial community and technological parameters such as temperature and important sourdough parameters such as acidity can be also explored. LAB counts on MRS agar and yeast counts on YGC agar obtained during the 10-week sampling period are shown in Figure 4.10. Batch to batch variations in the microbial community isolated from MSD, DBP and DAP can also be observed in Figure 4.10. Viable LAB cell counts (ranging from 7 to 9 log CFU/g) and APC were significantly higher than yeast cell counts (ranging from 4 to 7 log CFU/g) ($p<0.05$), however, total aerobic bacteria and LAB counts were not significantly different ($P>0.05$).

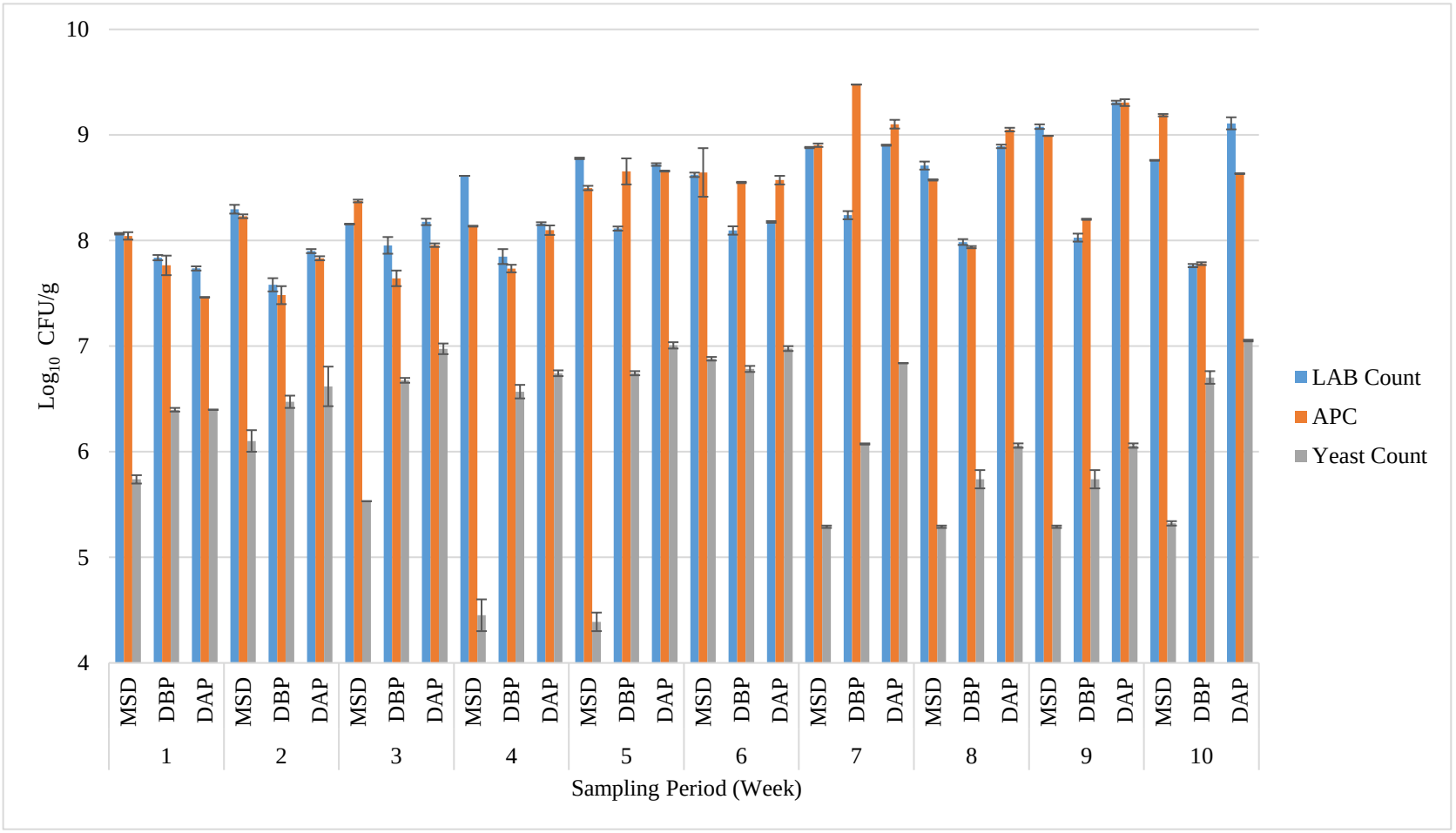

Figure 4.10 Aerobic plates counts of microorganisms in sourdough. Error bar =± SD (n=2)

Note: MSD = mother sourdough; DBP = dough before proofing; DAP = dough after proofing; LAB = lactic acid bacteria; APC = aerobic plate count

4.2.2 Ratio of LAB/yeasts

The ratio of LAB to yeasts in 10 batches of DAP are shown in Table 4.4. In week 1 to week 6 samples, the number of co-existing yeasts were less than two logarithmic magnitudes lower than LAB, whereas the ratios of LAB to yeast from week 7 to week 10 samples were over 2 logs CFU/g higher. In mature fermented sourdough, yeast numbers are usually one or two logs CFU/g lower than LAB (Ehrmann & Vogel, 2005; Ercolini et al., 2013; Minervini, De Angelis, Di Cagno, & Gobbetti, 2014). The reason is that the growth rate of LAB was higher than yeasts (Meignen et al., 2001).

Table 4.4 Ratio between lactic acid bacteria and yeast cell numbers in 10 DAP samples

Sampling Period (Week)	1	2	3	4	5	6	7	8	9	10
Ratio LAB/Yeasts (log 10)	1.34	1.24	1.23	1.40	1.71	1.50	2.18	2.60	2.91	2.02

4.2.3 Aerobic plate counts

For sourdough fermentation, un-sterile flour and water are used, which can introduce Gram-positive bacteria such as LAB and many types of Gram-negative bacteria such as *Bacillus cereus* and *Staphylococcus* into the bread dough (De Vuyst & Neysens, 2005; Gobbetti, Minervini, Pontonio, Di Cagno, & De Angelis, 2016a). The APC of MSD, DBP and DAP from 10 sampling weeks are shown in Figure 4.11.

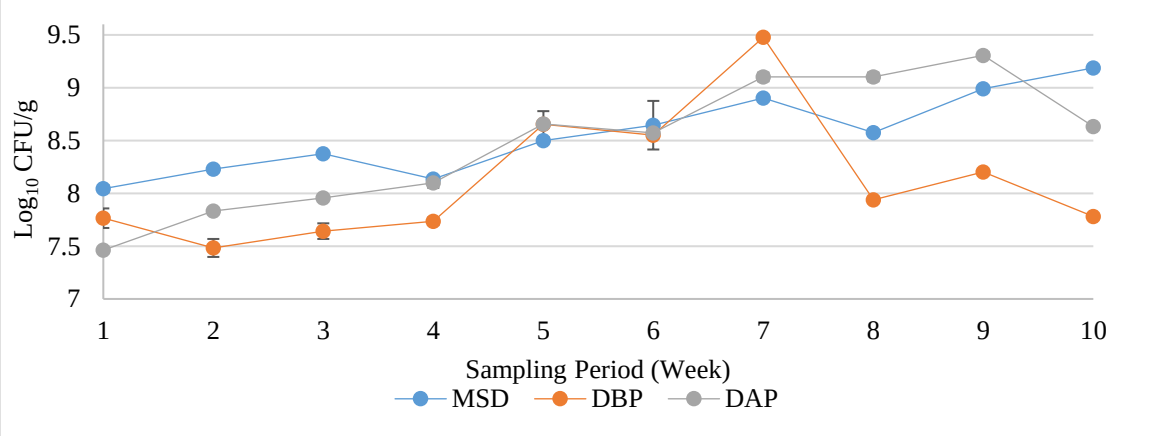

Figure 4.11 Aerobic plate counts (APC) of MSD, DBP and dough after proofing (DAP) for 10 different sampling weeks.

Notes: MSD = mother sourdough; DBP = dough before proofing; DAP = dough after proofing. Error bars = standard deviation; n = 2.

The APC of MSD varied from 8.04 to 9.19 log CFU/g (weeks 1 and 10 respectively). APC counts in later sampling weeks are higher than the earlier ones: with those in weeks 9 and 10 being significantly higher ($p<0.05$) than all other sampling weeks. The APC counts of sampling weeks 5-8 were also significantly higher ($p<0.05$) than that of sampling week 1.

The APC of NZ rice sourdough were one log or two logs higher than has been reported for flour (Brandt, 2014). Therefore, the higher level of APC in NZ rice sourdough may be related to higher level of LAB which can grow in the presence of oxygen than the level of aerobic bacteria in flour (Jackson, 2000; Sun, Yu, Dan, Zhang, & Zhang, 2014).

The APC counts of DBP varied more than both MSD and DAP, ranging from 7.49 log CFU/g (week 2) to 9.48 log CFU/g (week 7). The APC of week 7 DBP was significantly higher than the APC values for all other weeks, which may indicate that there were high amounts of other aerobic bacteria in the DBP possibly coming from non-sterile flour which has been reported to have a high APC (Brandt, 2014).

The APC of DAP ranged from 7.46 log CFU/g (week 1) to 9.31 log CFU/g (week 9). With the exception of the APC in weeks 1 and 7, all APC for DAP were higher than that of DBP. The APC of DAP in week 7 decreased compared to that of DBP, which may indicate that the growth of some bacteria was inhibited during fermentation. The decrease in aerobic bacteria is related to antimicrobial substances produced by LAB and the competition on available nutrients between LAB and other aerobic bacteria during fermentation (Tamang, Shin, Jung, & Chae, 2016).

4.2.4 Lactic acid bacteria counts

Lactic acid bacteria are mainly responsible for acidification, which affects the sensory and textural quality of sourdough (Gobbetti & Gänzle, 2012; Gobbetti et al., 2016b). The LAB counts of MSD, DBP and DAP over 10 sampling weeks are shown in Figure 4.12.

LAB counts of MSD ranged from 8.06 log CFU/g (week 1) to 9.08 log CFU/g (week 9). LAB counts in MSD in week 1 and week 3 were significantly lower than other weeks ($p<0.05$), while the LAB counts in week 9 were significantly higher than other LAB counts ($p<0.05$). LAB counts of NZ MSD were similar to LAB counts of German rice MSD (ranged from 8.1-9.2 log CFU/g) and were higher than reported for Korean rice MSD (LAB counts ranged from 6.53-7.87 log CFU/g) (Meroth et al., 2004; Lim et al., 2018). Therefore, these results indicate that

NZ MSD contains high amounts of LAB in order to initiate a new batch of fermentation (Valjakka, Kerojoki, & Katina, 2003; Zhou & Therdthai, 2012; Todorov & Holzapfel, 2014).

LAB counts of DBP ranged from 7.58 log CFU/g (week 2) to 8.24 log CFU/g (week 7), and were lower than MSD ($p<0.05$) because fresh ingredients were added to the DBP, which effectively dilutes the concentration of LAB, resulting in a lower CFU/g. Although LAB also exist in non-sterile flour, their numbers have been reported to be far lower than those in MSD, and therefore do not significantly contribute to the total LAB count (De Vuyst & Neysens, 2005).

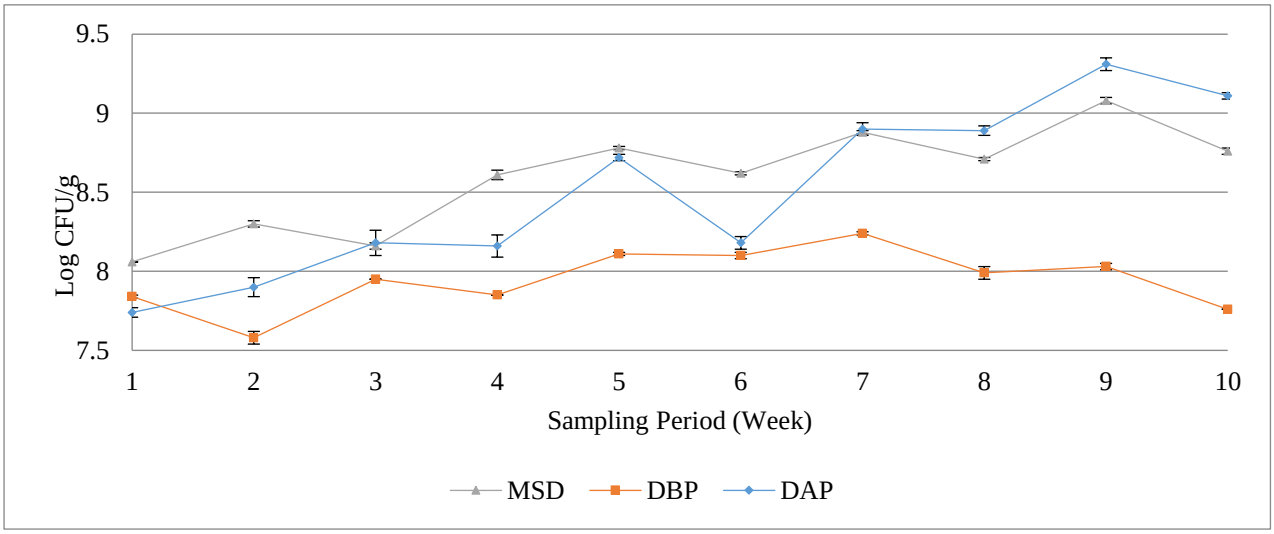

Figure 4.12 Lactic acid bacteria (LAB) counts from MSD, DBP, and DAP for 10 different sampling weeks.

Notes: MSD = mother sourdough; DBP = dough before proofing; DAP = dough after proofing. Error bars = standard deviation; n = 2.

LAB counts from DAP ranged from 7.74 to 9.31 log CFU/g (weeks 1 to 9 respectively). The LAB count of German rice DAP ranged from 8.9-9.2 log CFU/g (Meroth et al., 2004) which is similar to theDAP in weeks 7 and 8 from this study, but higher than other sampling weeks. However, 8 out of 10 the NZ DAP sampling batches had LAB counts over 8 log CFU/g, a count indicative of the maturity of sourdoughs which have a stable performance (Ehrmann & Vogel, 2005; Ercolini et al., 2013).

The different growth rates of LAB in the different sampling weeks may relate to differences in available nutrients in the sourdough and competition among microorganisms (Meignen et al.,

2001). In addition, the growth rate of LAB is affected by pH, temperature and salt concentration (Gobbetti & Gänzle, 2012). In NZ SDB production, the temperature and salt concentration were standardised, however, pH values varied significantly ($p<0.05$) (Figure 4.1) from batch to batch which can affect LAB growth in sourdough as their optimum pH is between 5.0 and 6.0 (Gobbetti& Gänzle, 2012). LAB levels in the week 9 sample batch increased the most (increased 1.0 log CFU/g), and the pH of this batch of DBP (5.22) was within the optimum growth pH range for LAB. Also, pH of DBP in week 9 was higher than pH of other sampling batches, possibly giving LAB in the week 9 batch a better growing environment.

4.2.5 Yeast counts

Using yeasts in sourdough fermentation increase the bread volume and flavour. The metabolic activities of yeasts also increase the nutritional value of sourdough, and increase the inherent antioxidant capacity of cereal products (Boekhout & Robert, 2003; Maloney & Foy, 2003; De Vuyst et al., 2016). The yeast counts of MSD, DBP and DAP from 10 sampling weeks are shown in Figure 4.13. Yeast counts of MSD varied from 4.39 log CFU/g (week 5) to 6.88 log CFU/g (week 6). The data were not normally distributed, with yeast counts being below log 4.50 CFU/g in sampling weeks 4 and 5.

For German MSD, yeast counts of 7.7 log CFU/g were found in type one MSD where *S. cerevisiae* dominated and yeast counts of 5.2 log CFU/g were found in German type two sourdough whose composition included *S. cerevisiae* and *P. membranifaciens* (Meroth et al., 2004). Results from phase 3 of this study showed that *S. cerevisiae* also dominated in NZ MSD although the yeast counts of NZ MSD were lower than the German sourdough. Perhaps German yeasts formed a stronger association with existing LAB and had better tolerance to environmental stresses and were therefore able to surviv and grow more effectively that those in NZ MSD (De Vuyst & Neysens, 2005; Venturi, Guerrini, & Vincenzini, 2012; De Vuyst et al., 2014).

Non-dissociated forms of acids, especially acetic acid, can affect the viability of yeasts, resulting in the yeast being starved of nutrients (Kitanovic et al., 2012). Yeasts in NZ MSD were exposed to organic acids during the two-day storage period and this along with a high acidity and nutritient deficient environment may have affected their viability (Marco Gobbetti & Gänzle, 2012). The stress tolerance of yeast towards acidity is also species and strain dependent (Attfield, 1997). The German MSD had a higher TTA than NZ MSD, yet the yeast

cell counts (7.7 log CFU/g) were higher than that of NZ MSD. Perhaps, the strains of *S. cerevisiae* in German MSD are able to tolerate higher levels of acidity than NZ MSD (Tanaka, Ishii, Ogawa, & Shima, 2012).

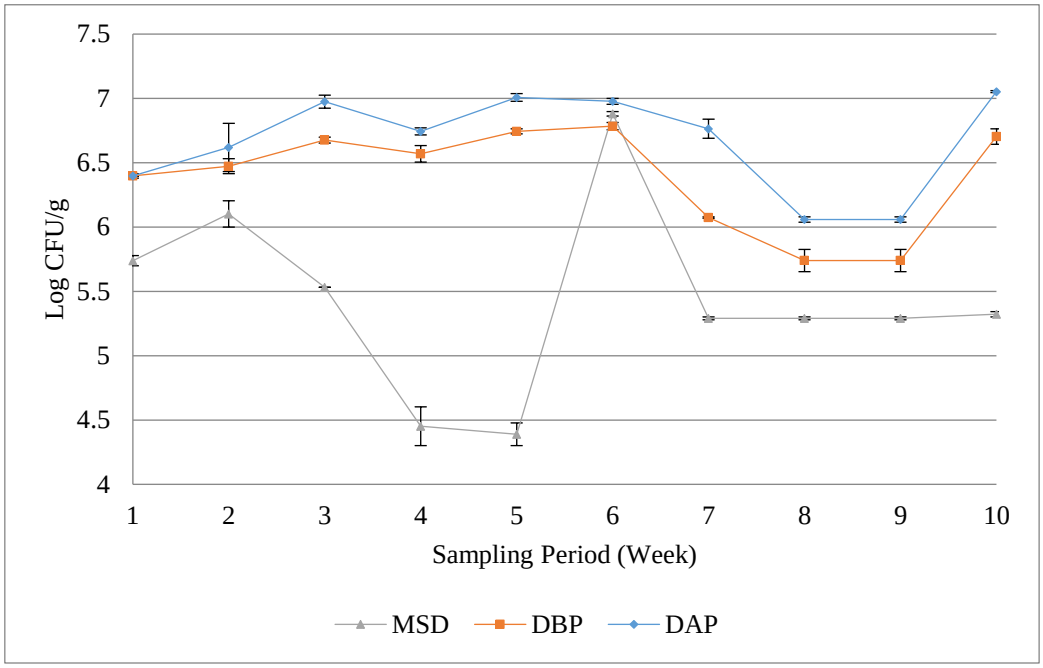

Figure 4.13 Yeast counts of MSD, DBP, and DAP for 10 different sampling weeks.

Notes: MSD = mother sourdough; DBP = dough before proofing; DAP = dough after proofing. Error bars = standard deviation; n = 2.

Yeast counts from DBP ranged from 6.40 to 6.70 log CFU/g (weeks 1 and 10 respectively), which are higher than the yeast counts from MSD. The LAB counts of DBP were lower than that of MSD. Potentially, after adding non-acidic ingredients such as flour and water to the MSD, more nutrients become available for the yeasts and the acidity of the DBP decreased due to dilution effects, resulting in lowered stress levels of the yeast leading to increased yeast cell viability (Marco Gobbetti & Gänzle, 2012). It is also possible that the higher yeast counts in DBP than MSD are related to the addition of non-sterile flour which has been reported to contain yeast and fungi counts ranging from 4-7 logs CFU/g (De Vuyst & Neysens, 2005; Brandt, 2014). In order to have a better understanding of the increased yeast counts in DBP compared to that of MSD, further investigations into the yeast counts in the raw ingredients utilised need to be conducted.

The yeast counts of DAP ranged from 6.06 log CFU/g (week 8) to 7.05 log CFU/g (week 10), which are lower than those reported from German DAP (7.6-7.7 log CFU/g) (Meroth et al., 2004). In this study the yeast counts in the DBP and DAP are similar ($p>0.05$), which indicates that the yeasts did not grow significantly during proofing. This was expected as the growth of yeast is dependent on proofing temperature and acidity. The proofing temperature used in NZ sourdough fermentation is 38 °C, which is much higher than the optimum growth temperature of *S. cerevisiae* (30-35 °C).

4.2.6 Morphology of LAB and Yeast cells

After enumeration of LAB and yeasts, colonies enumerated from incubated agar plates were observed from the top and bottom of agar plates for their size and shape (Figure 4.14). In this study, all observed yeast colonies growing on the surface were white and circular, ranging from 3-8 mm in diameter. All observed LAB colonies growing on the surface were milky and circular with a maximum diameter of 3 mm. The morphology types of LAB colonies are shown in Table 4.5 and that of yeast colonies are shown in Table 4.6.

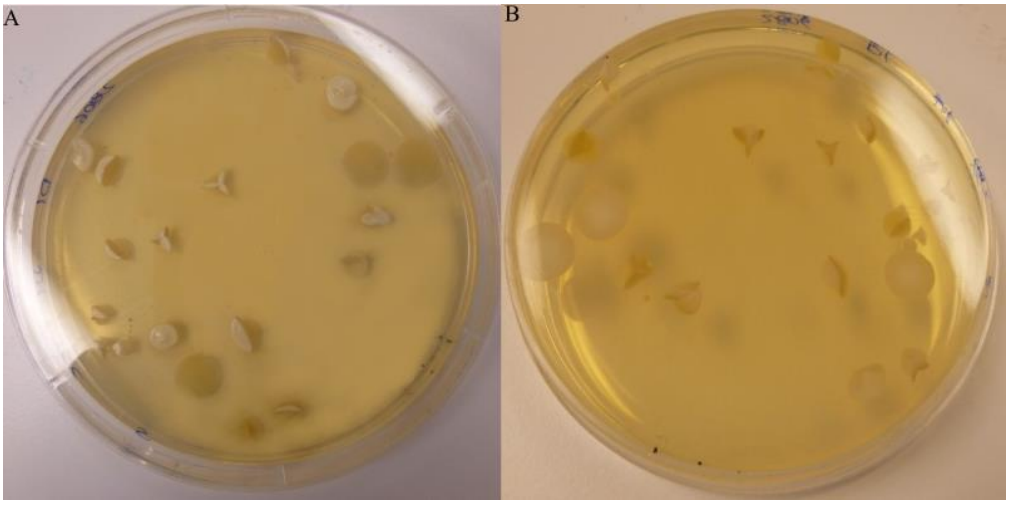

Figure 4.14 Typical yeast colonies enumerated using yeast extract-glucose-chloramphenicol agar. (A) Front view of agar plate; (B) Back view of agar plate.

For LAB colonies, the majority of observed colonies were flat and circular colonies. Some of the LAB colonies were circular and umbonate, and a small amount being fan-shaped.

Table 4.5 Morphology of lactic acid bacteria (LAB) colonies (n=1386 colonies)

Colony type	Shape	Percentage (%)
1	Circular and umbonate colony	36.03
2	Circular and flat colony	49.89
3	Spindle (top view) and circular (side view)	10.40
4	Fan-shape colony	3.68

All observed yeast colonies were white, with the majority of colonies observed being flat and circular colonies growing at the bottom and on the surface of the agar plate. Other observed yeast colony shapes include circular and umbonate colonies and fan-shaped colonies.

Table 4.6 Morphology of yeast colonies (n=924)

Colony type	Shape	Percentage (%)
1	Fan-shape colony	15.58
2	Circular and flat colony	56.49
3	Circular and umbonate colony	27.92

4.2.7 Summary of phase 2

In phase 2, APC, LAB and yeast counts from MSD, DBP and DAP were determined to obtain the numerical changes of sourdough microbiota during bread production. In MSD, all LAB counts were above 8 log CFU/g which indicates the maturity of MSD. APC were not significantly different to the LAB counts and APC were one or two logs higher than the reported APC of raw flour (Brandt, 2014).

In DBP, yeast counts were significantly higher than that of MSD. The addition of fresh ingredients may have reduced the stress on yeasts, increasing their growth. In sampling week 7, APC of DBP were much higher than other sampling weeks, indicating possible contamination. However, after fermentation, APC of DAP in week 7 decreased suggesting that the growth of some bacteria were inhibited.

In DAP, the majority of LAB counts were over 8 log CFU/g, indicating the maturity of the sourdough. LAB counts were 1-2 logs higher than yeast counts, which is in agreement with other studies (Ehrmann & Vogel, 2005; Ercolini et al., 2013; Minervini, De Angelis, Di Cagno, & Gobbetti, 2014).

After examining 1386 LAB colonies and 924 yeast colonies, four types of LAB and three types of yeast colonies were categorised for further purification and identification in phase 3.

4.3 Phase 3 Purification and identification of isolated LAB and yeasts

4.3.1 Introduction

In phase 3, representative LAB and yeast colonies were purified and examined for their fermentation profiles and metabolic reactions to carbohydrates using API test kits (method described in Section 3.4.9). In addition, DNA sequencing tests on total DNA extracted from MSD, DBP and DAP samples and purified LAB and yeast colonies were conducted.

In phase 3, six to eight colonies were selected from each colony type described in Section 4.2.6 and Gram-stained to further select distinct colonies for purification and identification. After examining the cell morphology (shape and length) under the microscope, colonies with distinct morphologies were chosen for further purification. Purification steps for LAB and yeast cells are shown in Figure 4.15.

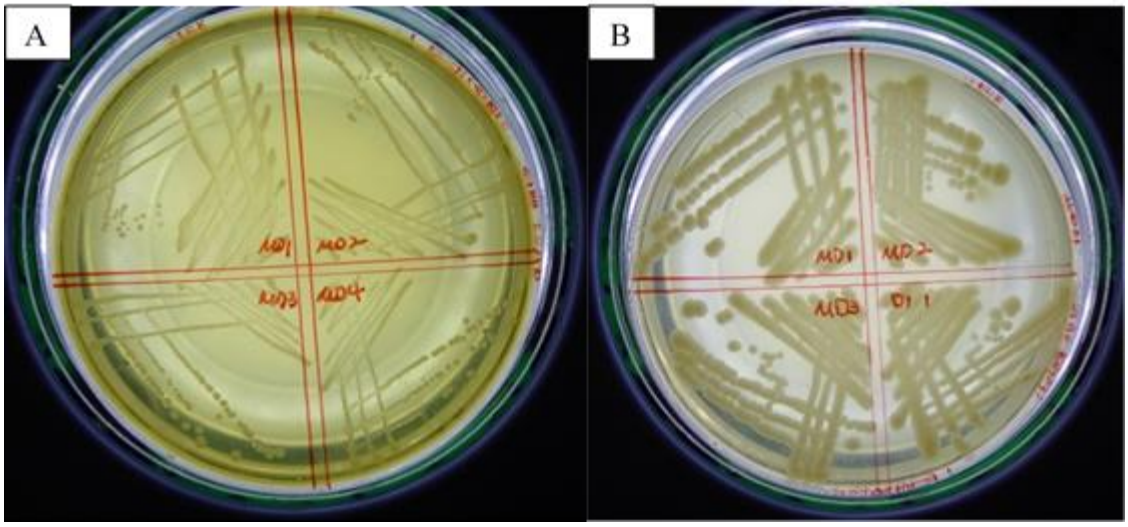

Figure 4.15 Purification of isolated sourdough microbes.

Note: A is purification of lactic acid bacteria colonies and B is purification of yeasts
Image captured by Carl Zeis transmission light microscope (Model HBO 50/AC, Germany)

4.3.2 Purification of selected distinct yeast colonies

Three distinct yeast groups isolated from MSD, DBP, DAP are shown in Table 4.7 and the cell features of these three groups are described in Figure 4.16. The majority of yeast cells in this study were 2-5 μm in diameter.

Table 4.7 Yeast cell types

Group	Shape
1	Oval and circular
2	Irregular and multilateral
3	Oval and multilateral cells (thin film compound observed)

After purification, the morphology of group one cells remained oval and conidiation was observed. This multilateral budding feature can be found in yeast of the genus *Saccharomyces* and *Candida* which have previously been isolated from sourdough (Reed & Nagodawithana, 1991). Purified group two colonies were multilateral shape with sizes ranging from 1-5 μm. In addition, the thin film compound produced in Figure 4.16C might be extracellular polysaccharides which may be a useful characteristic to aid in identification (Kurtzman, Fell, Boekhout, & Robert, 2010). Based on the morphology of the yeast cells in this study, it is possible that they belong to the species *S. cerevisiae* which are also described as "oval cells and multilateral budding" (Koehler, Chu, Houang, & Cheng, 1999). After purification, the three groups of yeasts described in Table 4.7 were subjected to API 32C tests and the results are discussed in Section 4.3.4.

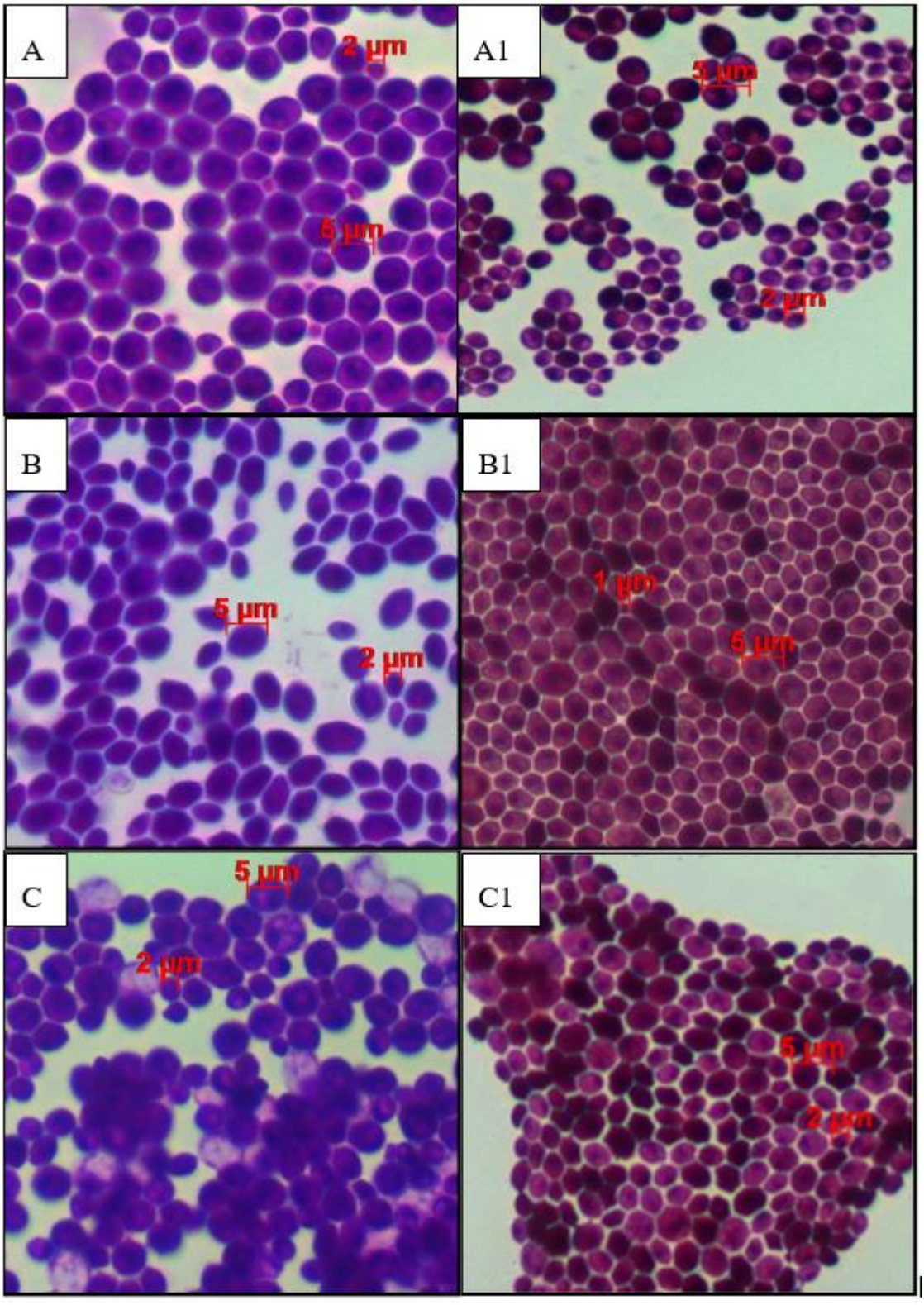

Figure 4.16 Selected distinct yeast colonies (left column) and purified yeast colonies (right column). (A) Group one yeast colony; (A1) Purified group one yeast colony; (B) group two yeast colony; (B1) purified group two yeast colony; (C) group three yeast colony; (C1) purified group three yeast colony.

Image captured by Carl Zeis transmission light microscope (Model HBO 50/AC, Germany)

4.3.3 Purification of representative LAB colonies

Isolated LAB colony cells were all rod shaped and were therefore grouped according to their cell length, which can provide a preliminary evaluation of species (Gobbetti & Gänzle, 2012). Four distinct groups of LAB colonies isolated from sourdough samples are shown in Figure 4.17. The length of group one colonies ranged from 1.50 to 2.00 μm; while the cell length of group two ranged from 1.40 to 3.65 μm, and in group three, the length of the majority of cells was over 2 μm, although a long cell of 3.2 μm was observed; the cell lengths of group four ranged from 1.15 to 10.00 μm.

By comparing cell length, growth temperature and the pH that the isolated LAB colonies in NZ sourdough can survive at and comparing with information from other studies, a preliminary identification of possible species of LAB isolated from NZ sourdough was made. These included: *L. delbrueckii* whose length ranges from 1.1-3.4 μm; *L. brevis* with a cell length ranging from 2-20 μm; *L. reuteri* (including *L. fermentum*) with cell length ranging from 1.2 to 2 μm and *L. plantarum* which is approximately 10.0 μm long (Axelsson & Ahrné, 2014).

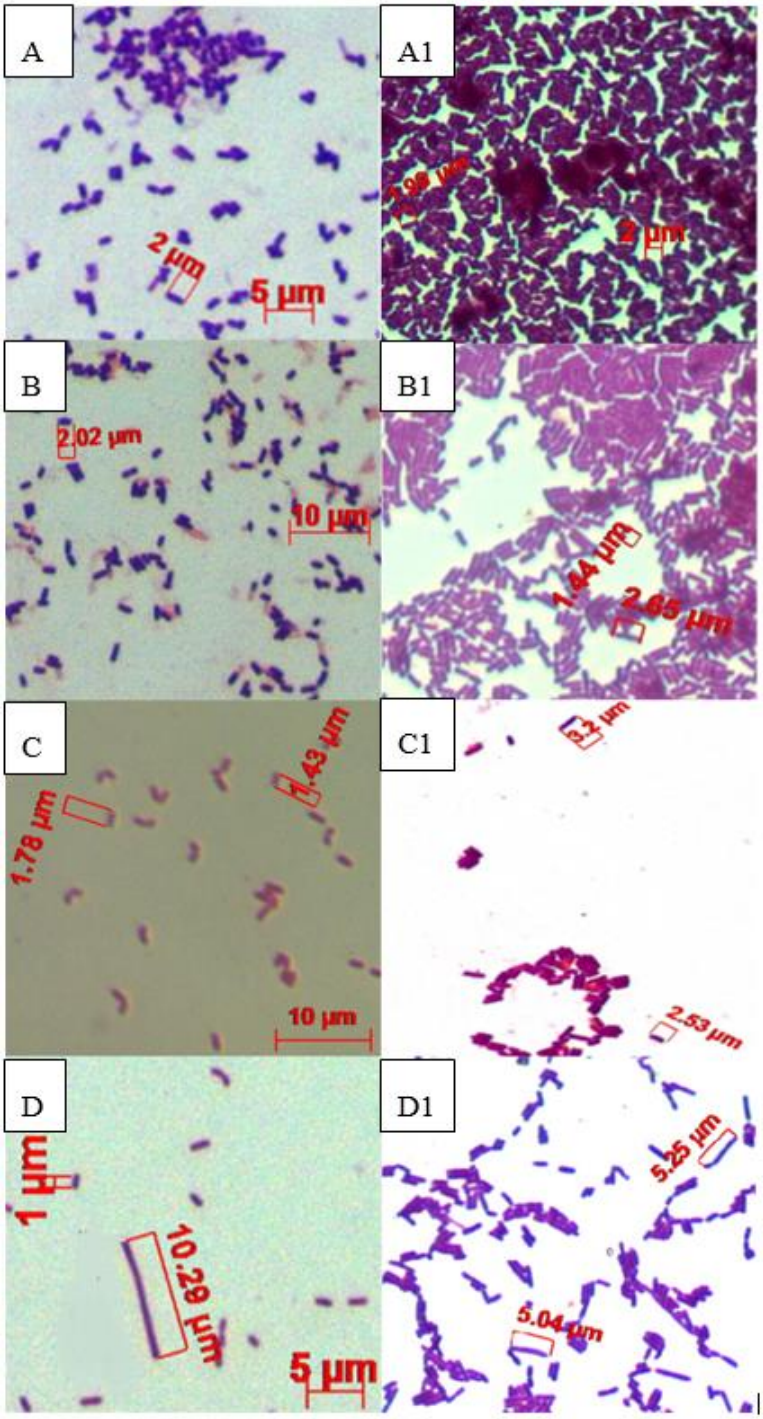

Figure 4.17 Selected distinct lactic acid bacteria (LAB) colonies (left column) and purified yeast colonies (right column). (A) Group one LAB colony; (A1) Purified group one LAB colony; (B) group two LAB colony; (B1) purified group two LAB colony; (C) group three LAB colony; (C1) purified group three LAB colony ; (D) group four LAB colony; (D1) purified group four LAB colony.

Image captured by Carl Zeis transmission light microscope (Model HBO 50/AC, Germany)

4.3.4 API tests of purified yeast cells

Phenotypic identification of purified yeast cells was achieved using API 32 C tests. A capsule with greater turbidity than the control capsule was recorded as positive. For each group described in Table 4.7, a carbohydrate fermentation profile was obtained and the details are shown in Table 4.8. The carbohydrate fermentation profiles of the three groups of yeasts were similar to each other. Each of the three yeast groups were able to ferment galactose, sucrose, raffinose, maltose, trehalose, and glucose. In contrast to the group two and group three yeast colonies, group one yeast did not react with lactic acid and could ferment sodium glucoronate. Only yeasts from group two were able to metabolise with xylose. Though the API identification results of three yeast groups indicated that all three groups of yeast were species of *S. cerevisiae*, but the fact that they had different fermentation profiles suggests that three groups of yeasts were different strains of *S. cerevisiae* (Van der Aa Kühle, Jesperen, Glover, Diawara, & Jakobsen, 2001).

Table 4.8 Carbohydrate fermentation profiles of purified yeast cells isolated from New Zealand rice sourdough using API 32 C test kits

Capsules	Test code	Substrate	Yeast colony groups		
			1	2	3
1	GAL	D-Galactose	+	+	+
1.1	ACT	Cycloheximide (Actidione)	-	-	-
1.2	SAC	D-Saccharose (Sucrose)	+	+	+
1.3	NAG	N-Acetyl-Glucosamine	-	-	-
1.4	LAT	Lactic Acid	-	+	+
1.5	ARA	L-Arabinose	-	-	-
1.6	CEL	D-Cellobiose	-	-	-
1.7	RAF	D-Raffinose	+	+	+
1.8	MAL	D-Maltose	+	+	+
1.9	TRE	D-Trehalose	+	+	+
1.A	2KG	Potassium 2-Ketogluconate	-	-	-
1.B	MDG	Methyl-Ad-Glucopyranoside	-	-	-
1.C	MAN	D-Mannitol	-	-	-
1.D	LAC	D-Lactose (Bovine Origin)	-	-	-
1.E	INO	Inositol	-	-	-
1.F	0	No Substrate	/	/	/
0	SOR	D-Sorbitol	-	-	-
0.1	XYL	D-Xylose	-	+	-
0.2	RIB	D-Ribose	-	-	-
0.3	GLY	Glycerol	-	-	-
0.4	RHA	L-Rhamnose	-	-	-
0.5	PLE	Palatinose	-	-	-
0.6	ERY	Erythritol	-	-	-
0.7	MEL	D-Melibiose	-	-	-
0.8	GRT	Sodium Glucuronate	+	-	-
0.9	MLZ	D-Melezitose	-	-	-
0.A	GNT	Potassium Gluconate	-	-	-
0.B	LVT	Levulinic Acid (Levulinate)	-	-	-
0.C	GLU	D-Glucose	+	+	+
0.D	SBE	L-Sorbose	-	-	-
0.E	GLN	Glucosamine	-	-	-

Notes: Group 1, 2, 3 yeast colonies are morphologically different yeast colonies

The fermentation profiles of the three groups of yeast were then compared with the API database to determine the most related species (https://apiweb.biomerieux.com/). API test results with a percentage of similarity over 90 % are considered acceptable at the species level (Bağder Elmacı, Tokatlı, Dursun, Özçelik, & Şanlıbaba, 2015). The results identified all three groups of yeast colonies as *S. cerevisiae* with high percentages of identity (Table 4.9).

Table 4.9 API 32 C identification results of purified yeasts

Group	Identified species	% Identity
1	*S. cerevisiae*	99.5
2	*S. cerevisiae*	96.2
3	*S. cerevisiae*	99.9

In a previous study carried out on sourdough, *S. cerevisiae* was also the most commonly identified yeast species (De Vuyst, Harth, Van Kerrebroeck, & Leroy, 2016). From a laboratory experiment on rice sourdough conducted in Germany, only two types of yeasts were reported: *S. cerevisiae* and *P. kudriavzevii* (Meroth et al., 2004). Similar to this study, in Korean rice sourdough, *S. cerevisiae* was also the only isolated and identified yeast species (Park et al., 2017). The prevalence of *S. cerevisiae* in sourdough is related to their better adaptability to higher temperatures (maximum 45.4 ℃) than other yeast species such as *C. milleri* (maximum 35 ℃) (Salvadó et al., 2011) and low pH (pH for growth ranges from 2.35 to 8.6) (Beales, 2004).

4.3.2 API tests of selected LAB colonies

Phenotypic identification of purified LAB cells was achieved using API 50 CHL test kits. The fermentation profile results for the purified LAB colonies are shown in Table 4.10. Group three LAB had a wider fermentation profile compared to the other LAB groups, while the fermentation profiles of groups one, two and four were similar. All groups of LAB were able to ferment galactose, glucose, fructose, ribose, maltose, lactose, melibiose, sucrose and raffinose. However, group three LAB cells was able to ferment compounds such as D-mannitol and sorbitol, but not L-arabinose, which the other groups were able to ferment. Based on the colony fermentation profiles, LAB colonies were identified and the results are shown in Table 4.11.

Table 4.10 Carbohydrate fermentation profiles of purified distinct lactic acid bacteria (LAB) colonies isolated from New Zealand rice sourdough using API 50 CHL test kits

Capsule	Test Code	Compositions	Distinct LAB colony groups			
			1	2	3	4
1	GLY	Glycerol	-	-	+	-
2	ERY	Erythritol	-	-	-	-
3	DARA	D-Arabinose	-	-	-	-
4	LARA	L-Arabinose	+	+	-	+
5	RIB	D-Ribose	+	+	+	+

6	DXYL	D-Xylose	-	-	-	-
7	LXYL	L-Xylose	-	-	-	-
8	ADO	D-Adonitol	-	-	-	-
9	MDX	Methyl-ßd-Xylopyranoside	-	-	-	-
10	GAL	D-Galactose	+	+	+	+
11	GLU	D-Glucose	+	+	+	+
12	FRU	D-Fructose	+	+	+	+
13	MNE	D-Mannose	+	+	+	-
14	SBE	L-Sorbose	-	-	-	-
15	RHA	L-Rhamnose	-	-	-	-
16	DUL	Dulcitol	-	-	-	-
17	INO	Inositol	-	-	-	-
18	MAN	D-Mannitol	-	-	+	-
19	SOR	D-Sorbitol	-	-	+	-
20	MDM	Methyl-Ad-Mannopyranoside	-	+	+	-
21	MDG	Methyl-Ad-Glucopyranoside	-	-	-	-
22	NAG	N-Acetylglucosamine	-	-	+	-
23	AMY	Amygdaline	-	-	+	-
24	ARB	Arbutine	-	-	+	-
25	ESC	Esculine	-	-	+	-
		Ferriccitrate	-	-	+	-
26	SAL	Salicine	-	-	+	-
27	CEL	D-Cellobiose	-	-	+	-
28	MAL	D-Maltose	+	+	+	+
29	LAC	D-Lactose(Bovine Origine)	+	+	+	+
30	MEL	D-Melibiose	+	+	+	+
31	SAC	D-Saccharose(Sucrose)	+	+	+	+
32	TRE	D-Trehalose	-	-	+	-
33	INU	Inuline	-	-	-	-
34	MLZ	D-Melezitose	-	-	+	-
35	RAF	D-Raffinose	+	+	+	+
36	AMD	Amidon(Starch)	-	-	-	-
37	GLYG	Glycogene	-	-	-	-
38	XLT	Xylitol	-	-	-	-
39	GEN	Gentiobiose	-	-	+	-
40	TUR	D-Turanose	+	-	-	-
41	LYX	D-Lyxose	-	-	-	-
42	TAG	D-Tagatose	-	-	-	-
43	DFUC	D-Fucose	-	-	-	-
44	LFUC	L-Fucose	-	-	-	-
45	DARL	D-Arabitol	-	-	+	-
46	LARL	L-Arabitol	-	-	-	-

47	GNT	Potassiumgluconate	+	+	+	+
48	2KG	Potassium 2-Ketogluconate	-	-	-	-
49	5KG	Potassium 5-Ketogluconate	-	-	-	-

With the exception of the group four LAB colony, all API 50 CHL LAB test results had percentages of identity of over 90 %. Of the four colonies, three were identified as *L. fermentum* 1, with the remaining identified as *L. plantarum* 1. Neither of these two species was found in German rice sourdough which contained *L. paracasei*, *L. paralimentarius*, and *L. spicheri* (Meroth et al., 2004) nor in Korean rice sourdough where *L. casei*, *L. brevis* and *Le. Pseudomenteroides* were identified (Park et al., 2017). Different species of LAB have been recovered from sourdough made using the same type of flour (Meroth et al., 2004; Lim et al., 2018) but from different locations. Therefore, the diversity of LAB recovered from sourdough may be related to the different places of origin, farming practices and milling systems used in flour production (De Vuyst & Neysens, 2005; Gobbetti et al., 2016b).

Table 4.11 API 50 CHL identification results of lactic acid bacteria

Group	Identified species	% Identity
1	*L. fermentum* 1	98.8
2	*L. fermentum* 1	98.8
3	*L. plantarum* 1	99.9
4	*L. fermentum* 1	89.7

L. plantarum and *L. fermentum* are able to tolerate low acidity (pH<3.8) and high temperature (Valjakka et al., 2003; Gobbetti & Gänzle, 2012), thus enabling them to survive at the high fermentation temperature used for NZ sourdough bread.

L. plantarum belongs to the group of facultative heterofermentative LAB which have the enzymes aldolase and phosphoketolase, and therefore can ferment hexoses, pentose and gluconate, generating lactic acid as the main product (De Vuyst, 2009; Holzapfel, 2012). *L. fermentum* is an obligate heterofermentative LAB, and can metabolise both pentose and hexoses through the phosphogluconate pathway to produce lactate, carbon dioxide and acetic acid/ethanol (Holzapfel & Wood, 2012). None of the isolated LAB from NZ sourdough are homofermentative LAB, therefore, the FQ in NZ rice sourdough (1.19-2.67; Section 4.1.3.3)

may be lower than those of other sourdoughs (4-9) which may contain considerable numbers of homofermentative LAB (Gobbetti & Gänzle, 2012).

L. plantarum and *L. fermentum* species have also been reported to possess potential probiotic properties (Parvez, Malik, Kang, & Kim, 2006; Van Der Aa Kühle et al., 2005). Normally to confer a probiotic effect the bacteria must be alive, and during baking the bacteria would be killed. However, heat inactivated *L. plantarum* may still be beneficial because it has been shown that heat inactivated *L. plantarum* bacterial cells may enhance immune responses in the human gut due to the release of immunogenic cell wall components teichoid acids and proteins (Van Baarlen et al., 2009). After consuming dead *L. plantarum*, increased gene expression of TNF-∝, as well as genes involved in T cell activation, and antigen presentation and processing were observed (Van Baarlen et al., 2009). Therefore, it is possible that inactivated rice sourdough LAB may have the potential to confer health benefits via the effects of probiotics.

4.3.5 Pyrosequencing analysis of LAB isolated from MSD, DBP and DAP

After PCR amplification of the V3 and V4 regions of LAB DNA, PCR products from MSD, DBP and DAP were electrophoresed on a 2 % gel containing SYBR® Safe DNA gel stain (Section 3.4.10.4). The gel image is shown in Figure 4.18. PCR products were transported to the Massey Genome Centre and pyrosequencing was conducted to characterise the bacterial ecosystem. The number of reads of each sample was around 1,000 and the length of the amplicons for the V3 and V4 region were about 460 bp. The taxonomic information for each operational taxonomic unit (OUT) was obtained by comparing with the 97 OUT database (Hildebrand, Tadeo, Voigt, Bork, & Raes, 2014). The counts of each taxa obtained from pyrosequencing are shown in Appendix 6.

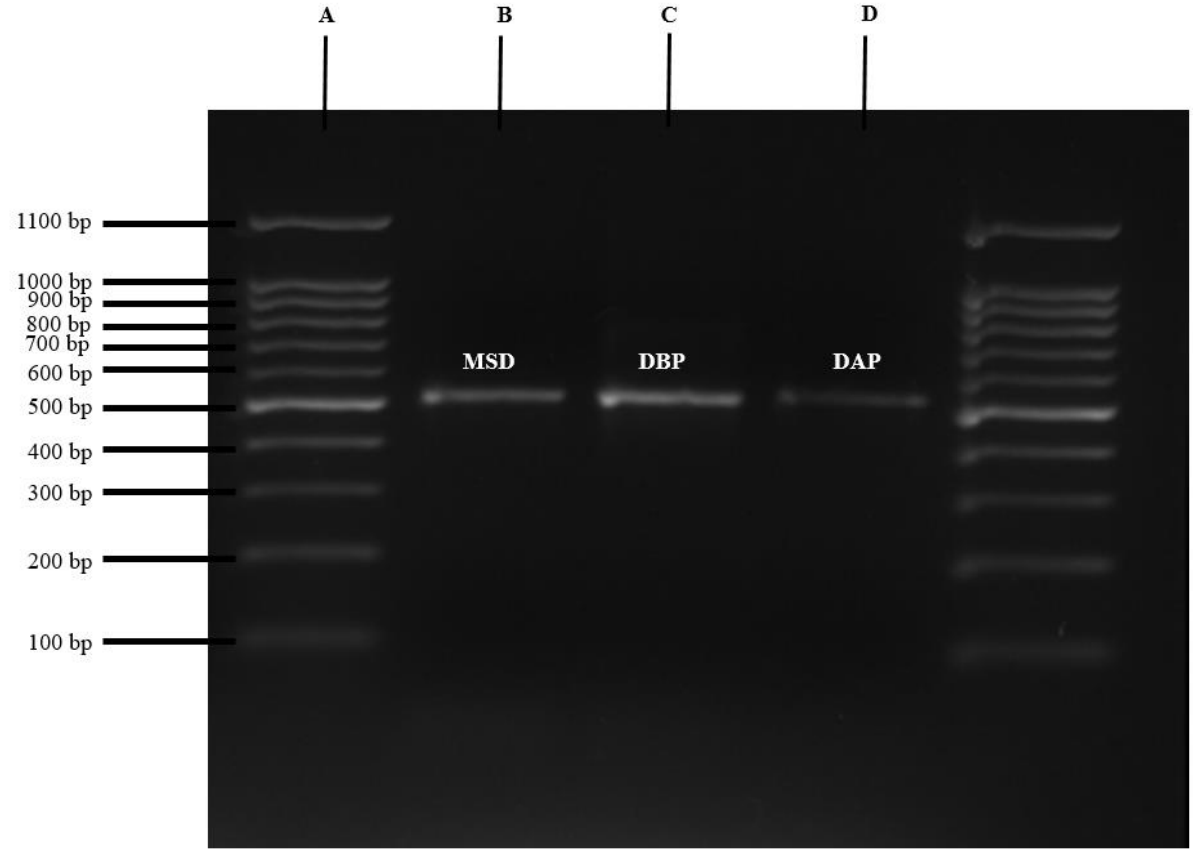

Figure 4.18 Agarose gel electrophoresis of polymerase chain reaction (PCR) products of total DNA extracted from mother sourdough (MSD), dough before proofing (DBP), dough after proofing (DAP). Lane A: 100 bp DNA ladder; Lane B: MSD amplicon; Lane C: DBP amplicon; Lane D: DAP amplicon.
Image captured by Gel Doc™ EZ Imager (BIO-RAD, USA).

The pyrosequencing analysis results are shown in Table 4.12. LAB genera *Lactobacillus* (>99.9 % taxonomy count in MSD, DBP and DAP samples) predominated rice sourdough fermentation, which is in agreement to previous studies (Luc De Vuyst & Neysens, 2005; Speranza et al., 2016). LAB of the genus *Pediococcus* were present in low amounts (<0.01 % taxonomy count in MSD, DBP and DAP samples). In other studies, LAB of the genus *Pediococcus* were also found as a subdominant LAB group (Corsetti, Settanni, Valmorri, Mastrangelo, & Suzzi, 2007). In DAP, other LAB of the genera *Leuconostoc* and *Fructobacillus* were also recovered. These LAB genera are inherent in flour or cereal kernels and probably grew during proofing (Corsetti et al., 2007).

Table 4.12 Pyrosequencing analysis of lactic acid bacteria (LAB) and LAB taxonomy count

Sample	Pyrosequencing analysis on lactic acid bacteria economy
Mother sourdough	*Lactobacillus* (>99.9 % taxonomy count), *Pediococcus*
Before proofing	*Lactobacillus* (>99.9 % taxonomy count), *Pediococcus*
After proofing	*Lactobacillus*(>99.9 % taxonomy count), *Pediococcus, Leuconostoc, Fructobacillus*

4.3.6 ITS sequencing analysis of yeast cells

Using ITS1 and ITS4 primers, PCR products of the ITS1 and ITS2 regions of yeasts can be obtained (Chen et al., 2001). Four yeast DNA samples were amplified: total DNA extracted from MSD, DBP, DAP and one purified colony from group 2 yeasts. For each DNA sample, two PCR amplicons were obtained: one from the ITS1 primer and another from the ITS4 primer. For each PCR product, a total length of more than 830 bp sequence raw data can normally be obtained (https://dna.macrogen.com/eng/). Poor quality reads at the beginning and the end, which had jagged, broad lines or overlapped peaks were trimmed. Trimmed sequence results were compared with the National Centre for Biotechnology Information Database (NCBI database) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the ISHAM Barcoding database (http://its.mycologylab.org/). The best match of a database taxon with greater than 99 % similarity and 90 % of length coverage was used as the criteria for identification (Yarza et al., 2014; Lhomme et al., 2016). The ITS region sequence results of yeasts are presented in Table 4.13.

All recovered yeast species were identified as *S. cerevisiae*, which matches the results obtained from the API 32 C tests. Using molecular identification methods can provide more accurate and rapid identification than API tests and DNA sequencing for yeast strain identification has also been used for brewing yeasts identification (Lodato, De Huergo, & Buera, 1999; Pincus, Orenga, & Chatellier, 2007; Spencer et al., 2011).

Table 4.13 Sequence analysis results of yeasts

Sample Name	Primer Used	TOP HIT Organism NCBI	Accession Number	Cover (%)	Identity (%)	E-value	ISHAM Barcoding Database	ID (%)	Overlap (%)
YC	ITS 1	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	99.0	100
	ITS 4	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	100	100
MSD	ITS 1	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	99.7	100
		S. cariocanus NRRL 27337	NR_144772.1	100	99	0			
		S. paradoxus 432 ITS region	NR_138272.1	100	99	0			
	ITS 4	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	97	99	0	*S. cerevisiae*	99.9	99.9
		S. cariocanus NRRL 27337	NR_144772.1	97	99	0			
DBP	ITS 1	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	99.3	100
	ITS 4	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	100	99.0
DAP	ITS 1	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	100	99	0	*S. cerevisiae*	99.4	99.3
	ITS 4	*S. cerevisiae* CBS 1171 ITS region	NR_111007.1	94	99	0	*S. cerevisiae*	99.8	97.79

Note: MSD = Mother sourdough; DBP = Dough before proofing; DAP = Dough after proofing; YC = Purified yeast colony.

4.3.7 16S rRNA sequencing analysis of four LAB colonies

Bacterial 16S rRNA genes were amplified using universal primers 27F and 1492R. For each DNA sample, two PCR amplicons were obtained: one amplicon from the 27F primer, which has a length of over 700 bp and another from the 1492R primer which provides a sequence length of over 900 bp. For each PCR product, poor quality reads at the beginning and the end which had jagged, broad lines or overlapped peaks were trimmed. Obtained trimmed sequences were aligned with the NCBI database and identified at a species level with over 99 % identity and 99 % coverage. BLAST results of the sequences are shown in Table 4.14.

Table 4.14 Sequence analysis results of pure lactic acid bacteria colonies

Colony Group	Primer Used	TOP HIT Organism NCBI	Accession Number	Cover (%)	Identity (%)	E-value
1	27F	*L. fermentum* strain CIP 102980	NR_104927.1	99	100	0
	1492R	*L. fermentum* strain CIP 102980	NR_104927.1	100	99	0
2	27F	*L. fermentum* strain CIP 102980	NR_104927.1	100	100	0
	1492R	*L. fermentum* strain CIP 102980	NR_104927.1	100	99	0
3	27F	*L. paraplantarum* strain DSM 10667	NR_025447.1	100	100	0
	1492R	*L. paraplantarum* strain DSM 10667	NR_025447.1	100	100	0
4	27F	*L. fermentum* strain CIP 102980	NR_104927.1	100	100	0
	1492R	*L. fermentum* strain CIP 102980	NR_104927.1	100	99	0

Sequence results for colony groups 1, 2, and 4 identified all three as being *L. fermentum* strain CIP 102980. The presence of *L. fermentum* strain CIP 102980 in the sourdough may have originated from the use of non-sterile flours and this strain has been previously isolated from maize, maize sourdough and fermented sorghum products (Ogodo, Ugbogu, Onyeagba, Okereke, & Agwaranze, 2016). *L. fermentum* strain CIP 102980 has been used to increase the body weight of poultry however, its probiotic function in humans requires investigation (Ramakrishna, 2014).

Colony three was identified as *L. papraplantarum* strain DSM 10667M by 16S rRNA gene sequencing, whereas it was identified as *L. plantarum* using the API 50 CHL test. Colony 3 should be aligned to *L. papraplantarum* as molecular methods are more accurate for identification than using phenotypic methods such as API tests (De Vuyst & Vancanneyt, 2007; Gobbetti & Gänzle, 2012).

4.3.8 Summary of phase 3

In phase 3, selected and purified LAB and yeast colonies were identified using API tests and gene sequencing methods. For LAB colonies, species *L. plantarum* and *L. fermentum* were identified using API 50 CHL kits. Identification of *L. fermentum* was confirmed by 16S rRNA gene sequencing. Colony 3 was identified as *L. plantarum* using the API 50 CHL tests but it was identified as *L. papraplantarum* by 16S rRNA gene sequencing. Colony 3 should be aligned to *L. papraplantarum* due to the higher accuracy of the molecular method. Through API 32 C test and ITS sequencing analysis on DNA extracted from MSD, DBP, DAP and selected yeast colony, it can be concluded that the dominant yeasts in NZ rice sourdough are *S. cerevisiae*.

To obtain a comprehensive microbiota composition of LAB, pyrosequencing analysis (Section 3.4.10) of 16 rRNA genes of LAB was conducted for MSD, DBP, and DAP samples. Throughout sourdough fermentation, the genus *Lactobacillus* predominated with a relative abundance of over 99.9 % in MSD, DBP and DAP. Other LAB genus *Pediococcus, Leuconostoc*, *Fructobacillus* were found in small amounts and the genus *Leuconostoc* and *Fructobacillus* grew during proofing.

It is possible that NZ SDB has the potential to become a health-promoting food because of its indwelling dominant LAB genus *Lactobacillus* and yeast genus *Saccharomyces* whose species are frequently related to human health benefits through their probiotic activities even in their inactivated form (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013). However, further *in vitro* work comparing the health promoting properties of live and heat inactivated LAB and yeasts strains isolated from baked NZ SDB is needed. In addition, if the *in vitro* work proved positive then *in vivo* work would be required to confirm any health benefits of this fermented GF product (Cho & Finocchiaro, 2010; Ouwehand & Röytiö, 2014).

4.4 Overall conclusion

Application of the sourdough technique had a positive effect on GF bread texture and colour. The acidity of the dough increased and total free amino acids decreased possibly due to the growth requirements of the sourdough yeasts and LAB. Due to the metabolic activities of the inherent yeasts and LAB, rice sourdough bread had a softer, more elastic and chewier crumb

than unleavened rice bread reported from other research and its crust colour was similar to wheat bread which was more appealing to consumers.

The acidity of dough samples reflects the carbohydrate metabolism of sourdough LAB and yeasts. During fermentation, the acidity of the dough increased significantly. Due to the effect of baking, the TTA of SDB decreased to 0.43-0.66 % and the pH to 4.07-4.58. The FQ of SDB ranged from 1.37-2.98 which is lower than the FQ of 4 recommended for a mild sour taste in sourdough. However, the presence of the higher ratio of acetic acid produced by heterofermentative LAB through carbohydrate metabolism may provide better antifungal properties.

Contrary to reported research, the total free amino acids (FAA) decreased after fermentation. A possible reason for this is that the FAA released through secondary proteolysis were less than that consumed by yeasts for their growth. However certain single FAA such as cystine and arginine increased. High levels of LAB and yeasts were found during different stages of rice sourdough fermentation. Based on the information obtained, the fermentation quotient and the sourness of the rice sourdough bread can be improved by changing the fermentation temperature and dough yield.

Brown rice can provide starter cultures with abundant carbohydrate sources such as maltose and sucrose and can support the growth of more than 7.9 log CFU/g LAB counts and 6.4 log CFU/g yeasts during fermentation. LAB counts increased significantly ($p<0.05$) during proofing, however, yeasts did not exhibit significant growth ($p>0.05$). LAB of the genus *Lactobacillus* and the yeast *S. cerevisiae* dominated fermentation. LAB isolates were identified as *Lactobacillus paraplantarum* CIP 102980 and *Lactobacillus fermentarum* DSM 10667 and yeast colonies as *S. cerevisiae* CBS 1171. It is quite possible that rice sourdough LAB may confer health benefits to the consumer in their heat-inactivated form. However, to understand the health properties of NZ SDB, further research needs to be conducted.

4.5 Recommendations

The following recommendations are suggested for future research:

1. In this study, the FQ of the NZ sourdough bread was lower than is normally recommended optimal FQ for rye and wheat sourdough. However, it is not understood how FQ affects the final sensory profile and further investigation is

needed to determine the optimum pH and TTA that would confer the highest sensory scores for NZ rice SDB. Thus further research may provide a better understanding of the correlation between pH, TTA, FQ and sensory evaluation data. This information could assist industry to have a better control of the fermentation process and provide a product with improved sensory scores;

2. In this study, the yeast counts of some batches of MSD were found to be at a low level (non-normally distributed data obtained) which indicated the viability and function of the yeast may be affected during sourdough fermentation. Therefore, further research investigating the stress tolerance of isolated yeast strain *S. cerevisiae* CBS 1171 needs to be conducted to optimise fermentation conditions which impacts on the leavening function which is closely related to bread volume;

3. In this study, LAB strains *Lactobacillus paraplantarum* CIP 102980 and *Lactobacillus fermentarum* DSM 10667 and the yeast strain *S. cerevisiae* CBS 1171 were identified. However, their probiotic properties and potential for conferring health benefits to the consumer have not been explored. Further research on the probiotic potential of these isolated strains, especially in their inactivated form, needs to be further explored in order to determine the potential of NZ SDB as a health promoting function food.

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APPENDIX

A. Composition of agar media used in this study

Table A. Composition of agar media used

Product name and brand	Ingredients	Composition (g)
MRS agar (CM0361),Oxoid	Peptone	10.0
	Lab-Lemco powder	8.0
	Yeast extract	4.0
	Hydrogen phosphate	2.0
	Sodium acetate 3H20	5.0
	Tri-ammonium citrate	2.0
	Magnesium sulphate 7H20	0.2
	Manganese sulphate 4H20	0.1
	Agar	10.0
YGC agar	Yeast extract	5.0
(1.16000.0500), Merck	D(+) glucose	20.0
KGaA	Chloramphenicol	0.1
	Agar	14.9
Plate count agar	Pancreatic digest of Casein	5.0
(DF0479-15-5), BD Diagnostics	Yeast extract	2.5
Sparks	Dextrose	1.0
	Agar	15.0
YPD broth	Bacteriological peptone	20.0
(Y1375), Merck	Yeast extract	10.0
	Glucose	20.0
MRS broth	Dipotassium hydrogen phosphate	2.0
(69966), Merck	Glucose	20.0
	Magnesium sulfate heptahydrate	0.2
	Manganous sulfate tetrahydrate	0.05
	Meat extract	8.0

B. API test kits (API CH50 and API 32 C)

Figure B.1 API 32 C tests on group one , two and three purified yeast colonies (from top to bottom).

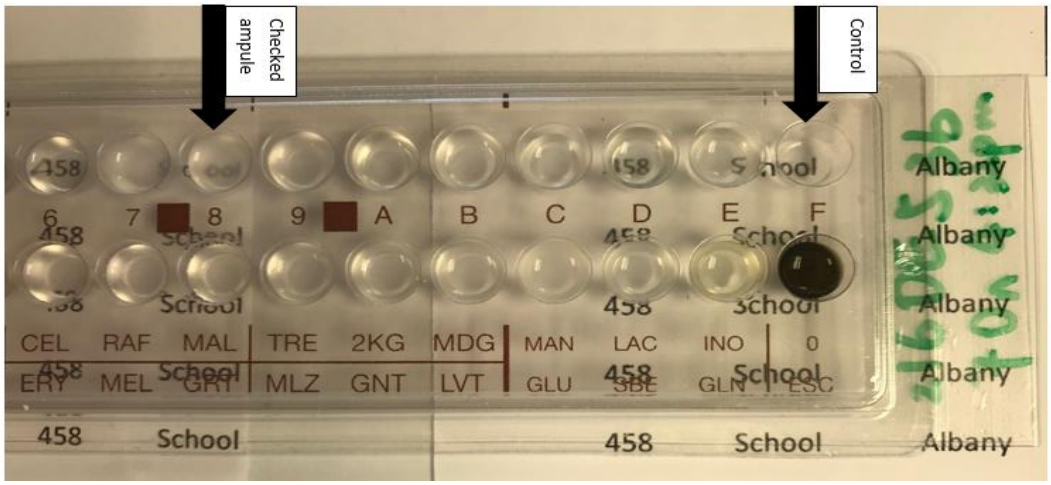

Figure B.2 Yeast API 32 C results observation method demonstration.

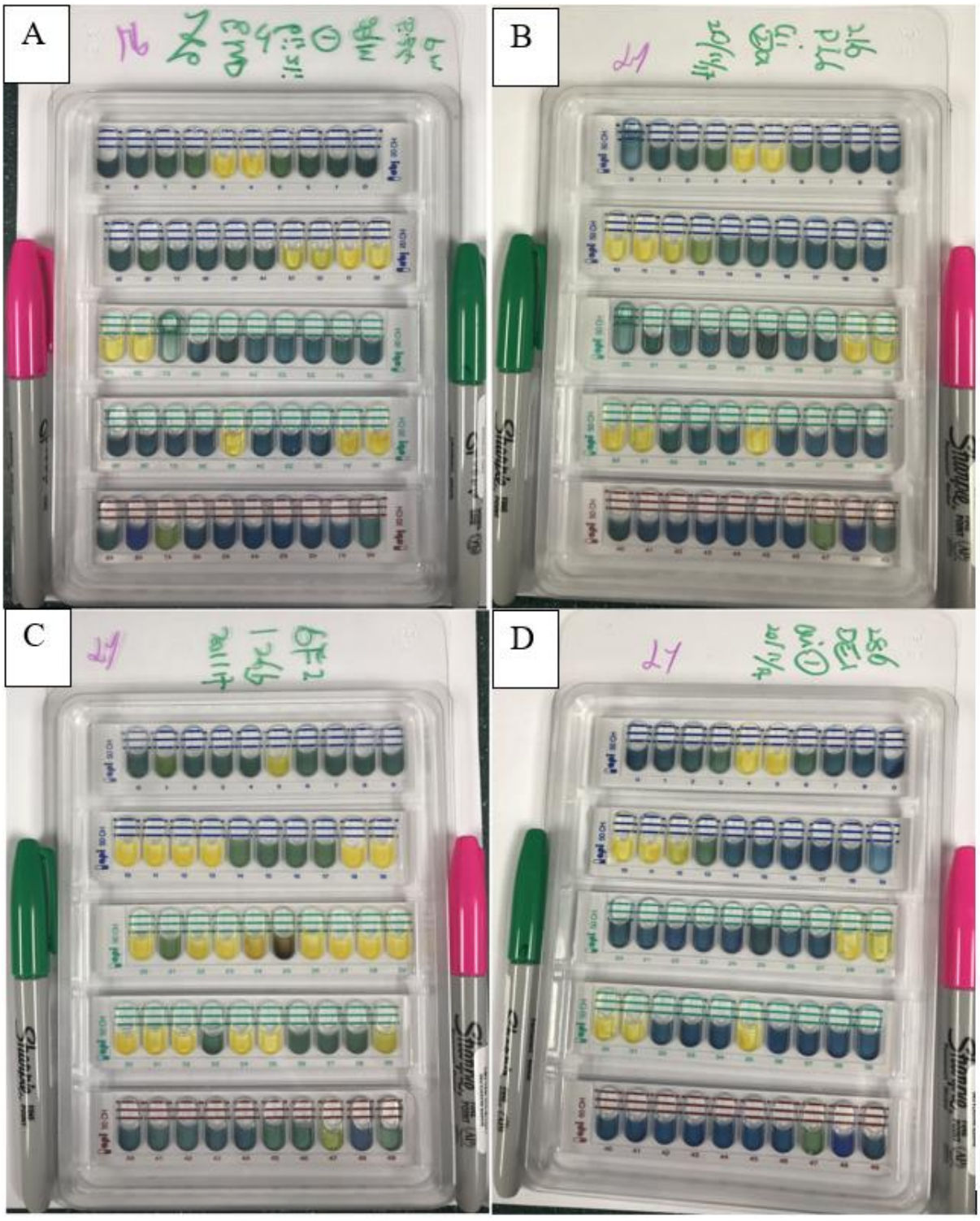

Figure B.3 API 50 CHL tests on (A) group one LAB colony, (B) group two LAB colony; (C) group three LAB colony and (D) group four LAB colony

C. Raw Data Record

Table C.1.a Acidity record of mother sourdough (MSD), dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB) during sampling period (week 1 to week 5)

Sampling period (weeks)	Samples	pH Value				Total Titratable Acidity (% of Lactic Acid)			
		Duplicate 1	Duplicate 2	Mean	SD	Duplicate 1	Duplicate 2	Mean	SD
1	MSD	3.85	3.84	3.85	0.01	1.26	1.39	1.33	0.07
	DBP	4.63	4.67	4.65	0.02	0.52	0.47	0.50	0.02
	DAP	4.08	4.08	4.08	0.00	0.86	0.75	0.81	0.06
	SDB	/	/	/	/	/	/	/	/
2	MSD	4.12	4.12	4.12	0.00	1.12	1.13	1.13	0.00
	DBP	5.17	5.11	5.14	0.03	0.59	0.56	0.57	0.02
	DAP	4.17	4.14	4.16	0.02	1.03	1.07	1.05	0.02
	SDB1	4.38	4.33	4.36	0.02	0.60	0.59	0.60	0.01
	SDB2	4.33	4.31	4.32	0.01	0.56	0.61	0.59	0.03
	SDB3	4.34	4.31	4.33	0.02	0.60	0.64	0.62	0.02
3	MSD	4.09	4.04	4.07	0.02	1.10	1.07	1.09	0.02
	DBP	5.07	5.08	5.08	0.00	0.41	0.40	0.41	0.01
	DAP	4.12	4.09	4.11	0.02	0.84	0.82	0.83	0.01
	SDB1	4.48	4.44	4.46	0.02	0.49	0.51	0.50	0.01
	SDB2	4.44	4.41	4.43	0.02	0.49	0.50	0.50	0.01
	SDB3	4.39	4.42	4.41	0.02	0.51	0.51	0.51	0.00
4	MSD	3.96	3.96	3.96	0.00	1.09	1.18	1.13	0.05
	DBP	5.20	5.22	5.21	0.01	0.35	0.36	0.36	0.01
	DAP	4.11	4.14	4.13	0.01	0.80	0.89	0.84	0.04
	SDB1	4.4	4.33	4.37	0.04	0.57	0.50	0.54	0.03
	SDB2	4.38	4.36	4.37	0.01	0.51	0.51	0.51	0.00
	SDB3	4.26	4.30	4.28	0.02	0.52	0.54	0.53	0.01
5	MSD	3.96	3.95	3.96	0.00	1.05	1.06	1.05	0.00
	DBP	4.74	4.75	4.75	0.00	0.48	0.47	0.48	0.01
	DAP	4.19	4.21	4.20	0.01	0.68	0.71	0.69	0.01
	SDB1	4.59	4.6	4.60	0.00	0.43	0.40	0.41	0.02
	SDB2	4.56	4.58	4.57	0.01	0.42	0.45	0.44	0.02
	SDB3	4.57	4.59	4.58	0.01	0.44	0.43	0.44	0.01

Table C.1.b. Acidity record of mother sourdough (MSD), dough before proofing (DBP), and dough after proofing (DAP) and sourdough bread (SDB) during sampling period (week 6 to week 10)

Sampling period (weeks)	Samples	pH Value				Total Titratable Acidity (% of Lactic Acid)			
		Duplicate 1	Duplicate 2	Mean	SD	Duplicate 1	Duplicate 2	Mean	SD
6	MSD	3.93	3.91	3.92	0.01	1.17	1.17	1.17	0.00
	DBP	4.93	4.92	4.93	0.00	0.46	0.45	0.45	0.00
	DAP	4.29	4.31	4.30	0.01	0.69	0.68	0.69	0.00
	SDB1	4.45	4.42	4.44	0.02	0.47	0.45	0.46	0.01
	SDB2	4.53	4.5	4.52	0.02	0.49	0.48	0.48	0.00
	SDB3	4.62	4.62	4.62	0.00	0.42	0.45	0.44	0.02
7	MSD	3.84	3.91	3.88	0.04	1.26	1.32	1.29	0.03
	DBP	4.79	4.77	4.78	0.01	0.81	0.84	0.46	0.01
	DAP	4.09	4.08	4.09	0.00	0.47	0.46	0.83	0.01
	SDB	4.32	4.32	4.32	0.00	0.51	0.48	0.49	0.02
	SDB	4.32	4.31	4.32	0.01	0.57	0.50	0.53	0.04
	SDB	4.33	4.33	4.33	0.00	0.62	0.57	0.59	0.02
8	MSD	3.95	3.92	3.94	0.02	1.11	1.07	1.09	0.02
	DBP	4.5	4.52	4.51	0.01	0.49	0.44	0.46	0.02
	DAP	4.03	4.02	4.03	0.01	0.77	0.73	0.75	0.02
	SDB1	4.04	4.04	4.04	0.00	0.63	0.71	0.67	0.04
	SDB2	4.08	4.08	4.08	0.00	0.62	0.62	0.62	0.00
	SDB3	4.08	4.1	4.09	0.01	0.69	0.71	0.70	0.01
9	MSD	3.72	3.74	3.73	0.01	1.38	1.30	1.34	0.04
	DBP	5.22	5.22	5.22	0.00	0.38	0.34	0.36	0.02
	DAP	3.99	3.98	3.99	0.01	0.82	0.89	0.85	0.03
	SDB1	4.16	4.13	4.15	0.02	0.64	0.62	0.63	0.01
	SDB2	4.11	4.09	4.10	0.01	0.54	0.59	0.57	0.02
	SDB3	4.06	4.08	4.07	0.01	0.68	0.64	0.66	0.02
10	MSD	3.65	3.66	3.66	0.01	1.28	1.24	1.26	0.02
	DBP	4.97	4.98	4.98	0.01	0.42	0.44	0.43	0.01
	DAP	4.07	4.01	4.04	0.03	0.84	0.78	0.81	0.03
	SDB1	4.18	4.18	4.18	0.00	0.52	0.50	0.51	0.01
	SDB2	4.17	4.17	4.17	0.00	0.45	0.47	0.46	0.01
	SDB3	4.17	4.17	4.17	0.00	0.51	0.49	0.50	0.01

Table C.2 Sugar standards used for HPLC standard curves

Standards	Concentration (%) w/v	Area	Average Retention Time
Maltose	0.04	1020188	9.272
	0.06	1505019	
	0.08	1988554	
	0.10	2526119	
	0.15	3784940	
	0.20	5203137	
Glucose	0.005	122794	10.739
	0.01	268521	
	0.02	514737	
	0.03	768444	
	0.05	1292685	
Fructose	0.005	66336	14.725
	0.01	219846	
	0.02	443753	
	0.03	673693	
	0.05	1210243	

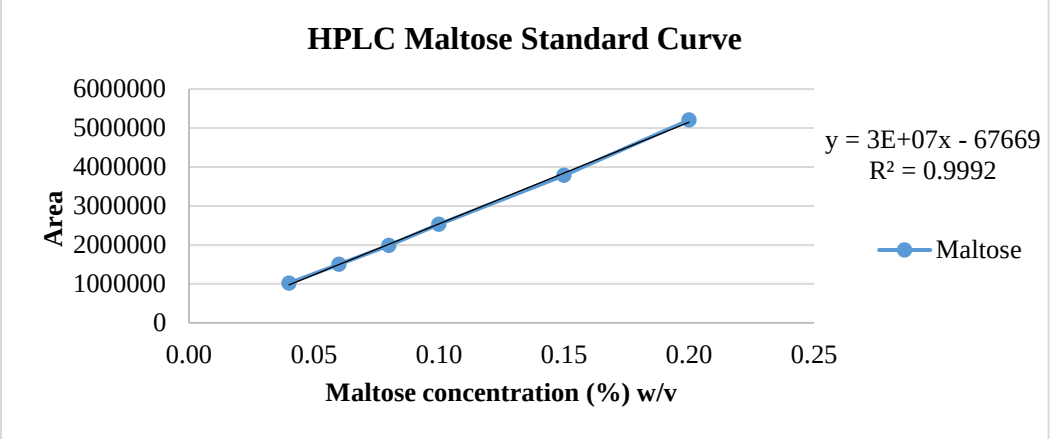

Figure C.1 Maltose standard curve

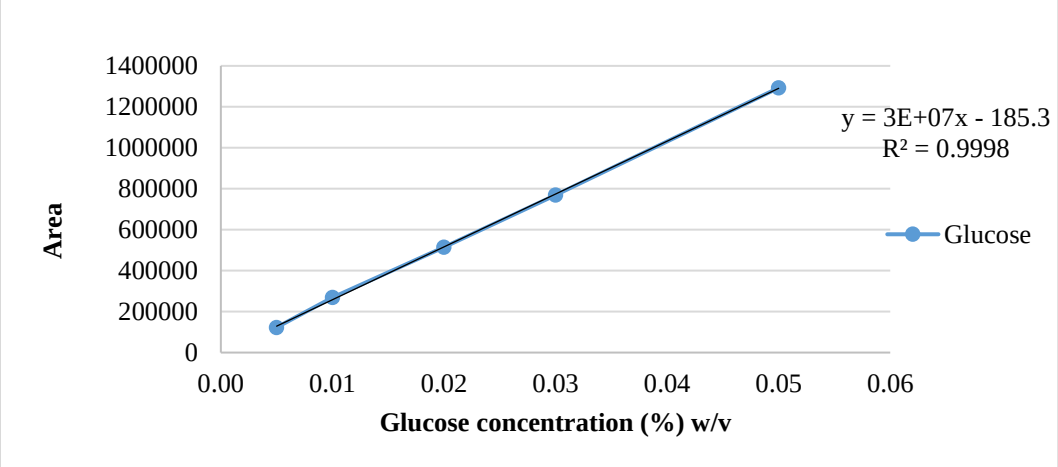

Figure C.2 Glucose standard curve

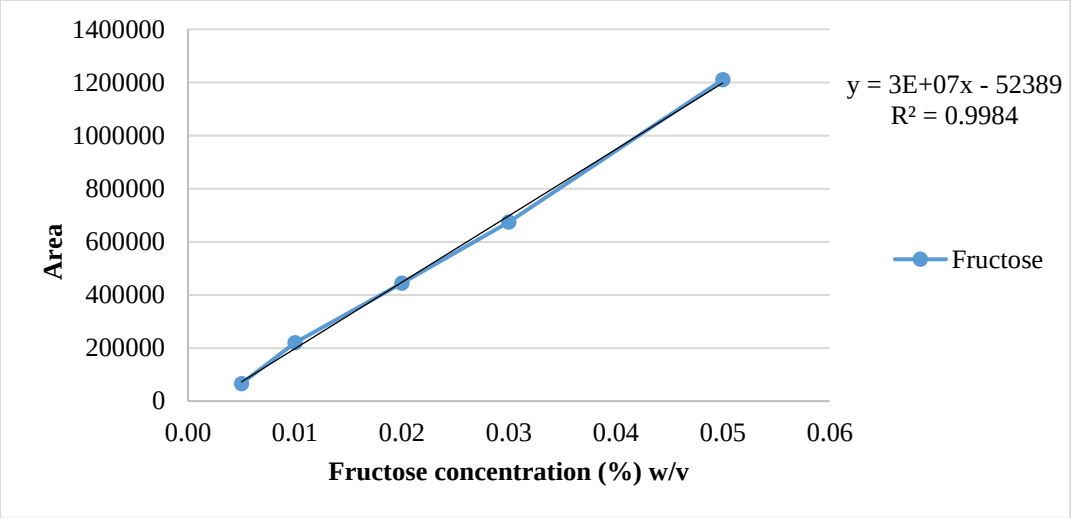

Figure C.3 Fructose standard curve

Table C.3 HPLC raw data of dough before proofing (DBP), and dough after proofing (DAP) and sourdough bread (SDB) for sugars from three sampling batches

Sampling Batch	Sample	Maltose					Glucose			
		Replication	Peak Area	Concentration (g/100 g)	Mean	SD	Peak Area	Concentration (g/100 g)	Mean	SD
1	DBP	1	7449101				519073			
		2	7600933	2.89	2.92	0.03	588883	0.20	0.21	0.01
	DAP	1	7346825				456511			
		2	7415493	2.86	2.87	0.01	475189	0.18	0.18	0.00
	SDB	1	7392236				396838			
		2	7742432	2.87	2.94	0.07	383171	0.15	0.15	0.00
2	DBP	1	7475140				414840			
		2	7324455	2.90	2.88	0.03	403534	0.16	0.16	0.00
	DAP	1	7092245				362603			
		2	7257299	2.76	2.79	0.03	366935	0.14	0.14	0.00
	SDB	1	7615382				254121			
		2	7390468	2.96	2.92	0.04	211790	0.10	0.09	0.01
3	DBP	1	7644255				401377			
		2	7541004	2.97	2.95	0.02	350917	0.16	0.15	0.01
	DAP	1	7489757				526215			
		2	7327728	2.91	2.88	0.03	560050	0.20	0.21	0.01
	SDB	1	7489757				413483			
		2	7547371	2.91	2.92	0.01	469243	0.16	0.17	0.01

Table C.4 Organic acid standards used for HPLC standard curves

Standards	Concentration (%) w/v	Area	Average Retention Time
Lactic Acid	0.006	161558	17.004
	0.015	468234	
	0.030	853438	
	0.060	1693163	
	0.100	3006329	
	0.120	3606433	
	0.150	4536769	
Acetic Acid	0.001	9996	19.797
	0.003	29434	
	0.005	51077	
	0.007	71602	
	0.010	103374	
	0.015	155352	
	0.020	218007	
	0.050	547330	

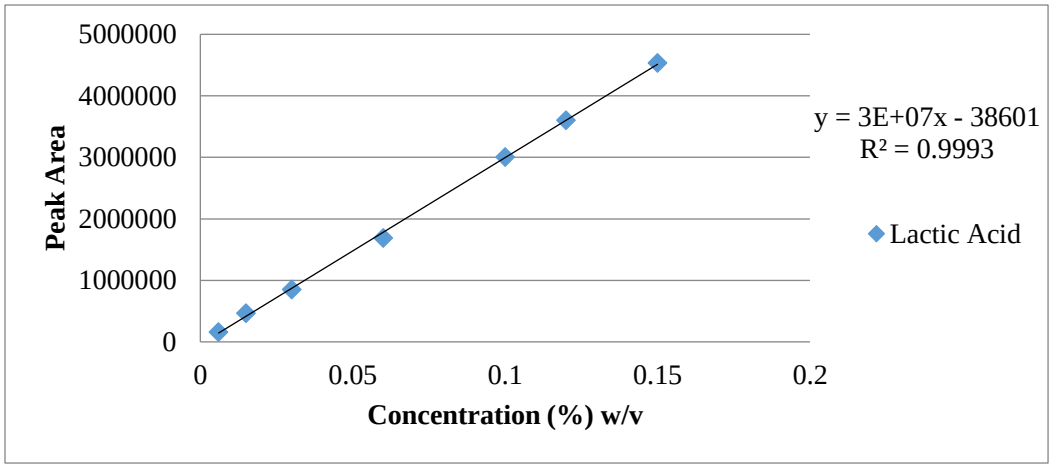

Figure C.4 Lactic acid standard curve

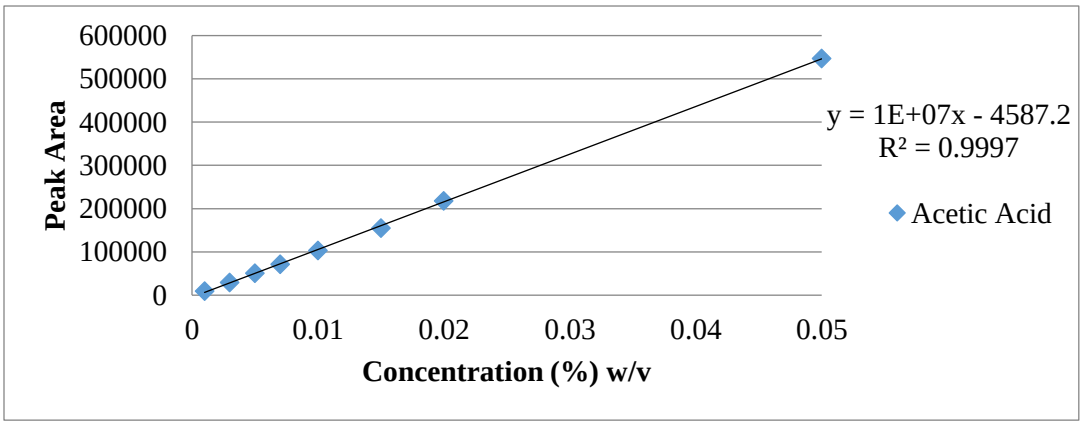

Figure C.5 Acetic acid standard curve

Table C.5 HPLC raw data of dough before proofing (DBP), and dough after proofing (DAP) and sourdough bread (SDB) for organic acids

Sampling Batch	Sample	Replication	Lactic Acid				Acetic Acid			
			Peak Area	Concentration (g/100 g)	Mean	SD	Peak Area	Concentration (g/100 g)	Mean	SD
1	DBP	1	22005	0.20	0.20	0.01	7353	0.11	0.11	0.00
		2	25059	0.21			7157	0.11		
	DAP	1	42658	0.27	0.26	0.00	10392	0.14	0.13	0.00
		2	40152	0.26			10065	0.13		
	SDB	1	32656	0.23	0.24	0.00	5354	0.09	0.09	0.00
		2	32866	0.24			6106	0.10		
2	DBP	1	16644	0.18	0.18	0.00	12578	0.09	0.09	0.00
		2	17286	0.18			11664	0.10		
	DAP	1	39897	0.26	0.26	0.00	10830	0.16	0.15	0.00
		2	40733	0.26			11553	0.15		
	SDB	1	22951	0.20	0.20	0.00	6199	0.14	0.14	0.00
		2	22738	0.20			7218	0.15		
3	DBP	1	20112	0.19	0.19	0.00	14664	0.10	0.10	0.00
		2	20951	0.20			13236	0.11		
	DAP	1	40749	0.26	0.26	0.00	12073	0.17	0.17	0.01
		2	40152	0.26			14050	0.16		
	SDB	1	18461	0.19	0.19	0.00	12799	0.15	0.16	0.01
		2	18604	0.19			12289	0.17		

Table C.6 HPLC raw data of dough before proofing (DBP), and dough after proofing (DAP) and sourdough bread (SDB) for free amino acids

Free Amino Acids	DBP (mg/100g)	DAP (mg/100g)	SDB (mg/100g)
Aspartic Acid	2.18	1.01	1.37
Threonine	0.44	0.18	0.21
Serine	0.90	0.53	0.49
Glutamic Acid	3.22	2.24	3.16
Proline	0.87	0.79	2.31
Glycine	0.56	0.29	0.27
Alanine	1.82	0.92	2.72
Cystine	0.16	0.25	ND
Valine	1.22	0.71	0.68
Methionine	0.55	0.42	0.19
Isoleucine	0.81	0.38	0.23
Leucine	1.89	1.44	1.52
Tyrosine	1.04	0.77	0.58
Phenylalanine	1.49	1.17	0.97
Histidine	0.63	0.36	0.45
Lysine	1.98	1.21	1.08
Arginine	1.10	2.36	1.10
Tryptophan	0.94	0.80	1.57
Asparagine	2.13	0.61	0.81
Glutamine	2.22	1.62	0.71

Table C.7 Aerobic plate count (APC) of mother sourdough (MSD), dough before proofing (DBP), and dough after proofing (DAP) samples in 10 sampling weeks

Sampling Period (Weeks)	Samples	APC (CFU/g)			APC (Log 10 CFU/g)			
		Duplicate 1	Duplicate 2	Mean	Duplicate 1	Duplicate 2	Mean	SD
1	MSD	1.02E+08	1.20E+08	1.11E+08	8.01	8.08	8.04	0.04
	DBP	4.70E+07	7.20E+07	5.95E+07	7.67	7.86	7.76	0.09
	DAP	2.90E+07	2.90E+07	2.90E+07	7.46	7.46	7.46	0.00
2	MSD	1.63E+08	1.77E+08	1.70E+08	8.21	8.25	8.23	0.02
	DBP	3.70E+07	2.50E+07	3.10E+07	7.57	7.40	7.48	0.09
	DAP	7.10E+07	6.50E+07	6.80E+07	7.85	7.81	7.83	0.02
3	MSD	2.30E+08	2.44E+08	2.37E+08	8.36	8.39	8.37	0.01
	DBP	3.70E+07	5.20E+07	4.45E+07	7.57	7.72	7.64	0.07
	DAP	8.70E+07	9.40E+07	9.05E+07	7.94	7.97	7.96	0.02
4	MSD	1.38E+08	1.35E+08	1.37E+08	8.14	8.13	8.14	0.00
	DBP	5.90E+07	5.00E+07	5.45E+07	7.77	7.70	7.73	0.04
	DAP	1.13E+08	1.39E+08	1.26E+08	8.05	8.14	8.10	0.04
5	MSD	3.00E+08	3.30E+08	3.15E+08	8.48	8.52	8.50	0.02
	DBP	3.40E+08	6.00E+08	4.70E+08	8.53	8.78	8.65	0.12
	DAP	4.50E+08	4.60E+08	4.55E+08	8.65	8.66	8.66	0.00
6	MSD	7.50E+08	2.60E+08	5.05E+08	8.88	8.41	8.65	0.23
	DBP	3.60E+08	3.50E+08	3.55E+08	8.56	8.54	8.55	0.01
	DAP	3.40E+08	4.10E+08	3.75E+08	8.53	8.61	8.57	0.04
7	MSD	7.70E+08	8.30E+08	8.00E+08	8.89	8.92	8.90	0.02
	DBP	3.00E+09	3.00E+09	3.00E+09	9.48	9.48	9.48	0.00
	DAP	1.39E+09	1.15E+09	1.27E+09	9.14	9.06	9.10	0.04
8	MSD	3.70E+08	3.80E+08	3.75E+08	8.57	8.58	8.57	0.01
	DBP	8.45E+07	8.86E+07	8.66E+07	7.93	7.95	7.94	0.01
	DAP	1.08E+09	1.17E+09	1.13E+09	9.03	9.07	9.05	0.02
9	MSD	9.80E+08	9.80E+08	9.80E+08	8.99	8.99	8.99	0.00
	DBP	1.61E+08	1.57E+08	1.59E+08	8.21	8.20	8.20	0.01
	DAP	2.18E+09	1.88E+09	2.03E+09	9.34	9.27	9.31	0.03
10	MSD	1.50E+09	1.58E+09	1.54E+09	9.18	9.20	9.19	0.01
	DBP	5.86E+07	6.23E+07	6.05E+07	7.77	7.79	7.78	0.01
	DAP	4.30E+08	4.30E+08	4.30E+08	8.63	8.63	8.63	0.00

Table C.8 Lactic acid bacteria (LAB) count of mother sourdough (MSD), dough before proofing (DBP), and dough after proofing (DAP) samples from 10 sampling weeks

Sampling Period (Week)	Samples	LAB (CFU/g)			LAB (Log 10 CFU/g)			
		Duplicate 1	Duplicate 2	Mean	Duplicate 1	Duplicate 2	Mean	SD
1	MSD	1.18E+08	1.14E+08	1.16E+08	8.07	8.06	8.06	0.01
	DBP	7.30E+07	6.50E+07	6.90E+07	7.86	7.81	7.84	0.03
	DAP	5.20E+07	5.70E+07	5.45E+07	7.72	7.76	7.74	0.02
2	MSD	1.80E+08	2.18E+08	1.99E+08	8.26	8.34	8.30	0.04
	DBP	3.30E+07	4.40E+07	3.85E+07	7.52	7.64	7.58	0.06
	DAP	7.60E+07	8.30E+07	7.95E+07	7.88	7.92	7.90	0.02
3	MSD	1.44E+08	1.42E+08	1.43E+08	8.16	8.15	8.16	0.00
	DBP	1.08E+08	7.50E+07	9.15E+07	8.03	7.88	7.95	0.08
	DAP	1.61E+08	1.40E+08	1.51E+08	8.21	8.15	8.18	0.03
4	MSD	4.10E+08	4.10E+08	4.10E+08	8.61	8.61	8.61	0.00
	DBP	6.00E+07	8.30E+07	7.15E+07	7.78	7.92	7.85	0.07
	DAP	1.40E+08	1.49E+08	1.45E+08	8.15	8.17	8.16	0.01
5	MSD	6.10E+08	5.90E+08	6.00E+08	8.79	8.77	8.78	0.01
	DBP	1.24E+08	1.36E+08	1.30E+08	8.09	8.13	8.11	0.02
	DAP	5.10E+08	5.40E+08	5.25E+08	8.71	8.73	8.72	0.01
6	MSD	4.00E+08	4.40E+08	4.20E+08	8.60	8.64	8.62	0.02
	DBP	1.14E+08	1.36E+08	1.25E+08	8.06	8.13	8.10	0.04
	DAP	1.53E+08	1.47E+08	1.50E+08	8.18	8.17	8.18	0.01
7	MSD	7.70E+08	7.50E+08	7.60E+08	8.89	8.88	8.88	0.01
	DBP	1.90E+08	1.59E+08	1.75E+08	8.28	8.20	8.24	0.04
	DAP	8.10E+08	7.90E+08	8.00E+08	8.91	8.90	8.90	0.01
8	MSD	4.70E+08	5.60E+08	5.15E+08	8.67	8.75	8.71	0.04
	DBP	1.03E+08	9.09E+07	9.70E+07	8.01	7.96	7.99	0.03
	DAP	8.10E+08	7.50E+08	7.80E+08	8.91	8.88	8.89	0.02
9	MSD	1.14E+09	1.26E+09	1.20E+09	9.06	9.10	9.08	0.02
	DBP	9.77E+07	1.16E+08	1.07E+08	7.99	8.07	8.03	0.04
	DAP	1.96E+09	2.11E+09	2.04E+09	9.29	9.32	9.31	0.02
10	MSD	5.70E+08	5.80E+08	5.75E+08	8.76	8.76	8.76	0.00
	DBP	5.59E+07	6.00E+07	5.80E+07	7.75	7.78	7.76	0.02
	DAP	1.47E+09	1.13E+09	1.30E+09	9.17	9.05	9.11	0.06

Table C.9 Yeast count of mother sourdough (MSD), dough before proofing (DBP), and dough after proofing (DAP) samples from 10 sampling weeks

Sampling Period (Weeks)	Samples	YGC (CFU/g)			YGC (Log 10 CFU/g)			
		Duplicate 1	Duplicate 2	Mean	Duplicate 1	Duplicate 2	Mean	SD
1	MSD	5.00E+05	6.00E+05	5.50E+05	5.70	5.78	5.74	0.04
	DBP	2.60E+06	2.40E+06	2.50E+06	6.41	6.38	6.40	0.02
	DAP	2.50E+06	2.50E+06	2.50E+06	6.40	6.40	6.40	0.00
2	MSD	1.60E+06	1.00E+06	1.30E+06	6.20	6.00	6.10	0.10
	DBP	2.60E+06	3.40E+06	3.00E+06	6.41	6.53	6.47	0.06
	DAP	6.40E+06	2.70E+06	4.55E+06	6.81	6.43	6.62	0.19
3	MSD	3.40E+05	3.40E+05	3.40E+05	5.53	5.53	5.53	0.00
	DBP	5.00E+06	4.50E+06	4.75E+06	6.70	6.65	6.68	0.02
	DAP	1.06E+07	8.40E+06	9.50E+06	7.03	6.92	6.97	0.05
4	MSD	2.00E+04	4.00E+04	3.00E+04	4.30	4.60	4.45	0.15
	DBP	3.20E+06	4.30E+06	3.75E+06	6.51	6.63	6.57	0.06
	DAP	5.20E+06	5.90E+06	5.55E+06	6.72	6.77	6.74	0.03
5	MSD	2.00E+04	3.00E+04	2.50E+04	4.30	4.48	4.39	0.09
	DBP	5.80E+06	5.30E+06	5.55E+06	6.76	6.72	6.74	0.02
	DAP	9.50E+06	1.09E+07	1.02E+07	6.98	7.04	7.01	0.03
6	MSD	7.30E+06	7.90E+06	7.60E+06	6.86	6.90	6.88	0.02
	DBP	5.70E+06	6.50E+06	6.10E+06	6.76	6.81	6.78	0.03
	DAP	1.00E+07	9.00E+06	9.50E+06	7.00	6.95	6.98	0.02
7	MSD	2.00E+05	1.90E+05	1.95E+05	5.30	5.28	5.29	0.01
	DBP	1.20E+06	1.17E+06	1.19E+06	6.08	6.07	6.07	0.01
	DAP	6.90E+06	/	6.90E+06	6.84	/	6.84	0.00
8	MSD	2.00E+05	1.90E+05	1.95E+05	5.30	5.28	5.29	0.01
	DBP	6.70E+05	4.50E+05	5.60E+05	5.83	5.65	5.74	0.09
	DAP	1.09E+06	1.20E+06	1.15E+06	6.04	6.08	6.06	0.02
9	MSD	2.00E+05	1.90E+05	1.95E+05	5.30	5.28	5.29	0.01
	DBP	6.70E+05	4.50E+05	5.60E+05	5.83	5.65	5.74	0.09
	DAP	1.09E+06	1.20E+06	1.15E+06	6.04	6.08	6.06	0.02
10	MSD	2.00E+05	2.20E+05	2.10E+05	5.30	5.34	5.32	0.02
	DBP	4.40E+06	5.80E+06	5.10E+06	6.64	6.76	6.70	0.06
	DAP	1.15E+07	1.11E+07	1.13E+07	7.06	7.05	7.05	0.01

Table C.10 Sequence results of whole lactic acid bacteria pure colonies and yeast DNA (extracted from mother sourdough, dough before proofing and dough after proofing)

Sequenced Microorganisms	Whole Sequence	
	Primer 785F	Primer 907R

LAB C1	AATCAAGGGTTCGATGAGTGCTAGGTGTT GGAGGGTTTCCGCCCTTCAGTGCCGGAGC TAACGCATTAAGCACTCCGCCTGGGGAGT ACGACCGCAAGGTTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGTGGAG CATGTGGTTTAATTCGAAGCTACGCGAAG AACCTTACCAGGTCTTGACATCTTGCGCC AACCCTAGAGATAGGGCGTTTCCTTCGGG AACGCAATGACAGGTGGTGCATGGTCGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTA AGTCCCGCAACGAGCGCAACCCTTGTTAC TAGTTGCCAGCATTAAGTTGGGCACTCTA GTGAGACTGCCGGTGACAAACCGGAGGA AGGTGGGGACGACGTCAGATCATCATGCC CCTTATGACCTGGGCTACACACGTGCTAC AATGGACGGTACAACGAGTCGCGAACTC GCGAGGGCAAGCAAATCTCTTAAAACCGT TCTCAGTTCGGACTGCAGGCTGCAACTCG CCTGCACGAAGTCGGAATCGCTAGTAATC GCGGATCAGCATGCCGCGGTGAATACGTT CCCGGGCCTTGTACACACCGCCCGTCACA CCATGAGAGTTTGTAACACCCAAAGTCGG TGGGGTAACCTTTTAGGAGCCAGCCGCCT AAGGTGGGACAGATGATTAGGGTGAAGT CGTAACAGGGAAACCCGTAAAAATCAAG GGTTCGATGAGTGCTAGGTGTTGGAGGGT TTCCGCCCTTCAGTGCCGGAGCTAACGCA TTAAGCACTCCGCCTGGGGAGTACGACCG CAAGGTTGAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCTACGCGAAGAACCTTA CCAGGTCTTGACATCTTGCGCCAACCCTA GAGATAGGGCGTTTCCTTCGGGAACGCAA TGACAGGTGGTGCATGGTCGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCTTGTTACTAGTTGC CAGCATTAAGTTGGGCACTCTAGTGAGAC TGCCGGTGACAAACCGGAGGAAGGTGGG GACGACGTCAGATCATCATGCCCCTTATG ACCTGGGCTACACACGTGCTACAATGGAC GGTACAACGAGTCGCGAACTCGCGAGGG CAAGCAAATCTCTTAAAACCGTTCTCAGT TCGGACTGCAGGCTGCAACTCGCCTGCAC GAAGTCGGAATCGCTAGTAATCGCGGATC AGCATGCCGCGGTGAATACGTTCCCGGGC CTTGTACACACCGCCCGTCACACCATGAG AGTTTGTAACACCCAAAGTCGGTGGGGTA ACCTTTTAGGAGCCAGCCGCCTAAGGTGG GACAGATGATTAGGGTGAAGTCGTAACA GGGAAACCCGTAAA	CAATGGCGGGAATCCCAGGCGGAGTGCTTAATGC GTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCA ACACCTAGCACTCATCGTTTACGGCATGGACTACC AGGGTATCTAATCCTGTTCGCTACCCATGCTTTCG AGTCTCAGCGTCAGTTGCAGACCAGGTAGCCGCCT TCGCCACTGGTGTTCTTCCATATATCTACGCATTCC ACCGCTACACATGGAGTTCCACTACCCTCTTCTGC ACTCAAGTTATCCAGTTTCCGATGCACTTCTCCGG TTAAGCCGAAGGCTTTCACATCAGACTTAGAAAAC CGCCTGCACTCTCTTTACGCCCAATAAATCCGGAT AACGCTTGCCACCTACGTATTACCGCGGCTGCTGG CACGTAGTTAGCCGTGACTTTCTGGTTAAATACCG TCAACGTATGAACAGTTACTCTCATACGTGTTCTT CTTTAACAACAGAGCTTTACGAGCCGAAACCCTTC TTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGC CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA GGAGTATGGGCCGTGTCTCAGTCCCATTGTGGCCG ATCAGTCTCTCAACTCGGCTATGCATCATCGCCTT GGTAGGCCGTTACCCCACCAACAAGCTAATGCAC CGCAGGTCCATCCAGAAGTGATAGCGAGAAGCCA TCTTTTAAGCGTTGTTCATGCGAACAACGCTGTTA TGCGGTATTAGCATCTGTTTCCAAATGTTGTCCCCC GCTTCTGGGCAGGTTACCTACGTGTTACTCACCCG TCCGCCACTCGTTGGCGACCAAAATCAATCAGGTG CAAGCACCATCAATCAATTGGGACCAACGCGTTC GACTTGCATGTATTAGCACACCGCCGGCGTTCATC CTGAGCAGATATCAAAAACTACTCATGA

LAB C2	ACGCAGTAGATGATGCTAAGTGTTGGAGG GTTTCCGCCCTTCAGTGCTGCAGCTAACG CATTAAGCATTCCGCCTGGGGAGTACGGC CGCAAGGCTGAAACTCAAAGGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGT GGTTTAATTCGAAGCTACGCGAAGAACCT TACCAGGTCTTGACATACTATGCAAATCT AAGAGATTAGACGTTCCCTTCGGGGACAT GGATACAGGTGGTGCATGGTTGTCGTCAG CTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTATTATCAGTT GCCAGCATTAAGTTGGGCACTCTGGTGAG ACTGCCGGTGACAAACCGGAGGAAGGTG GGGATGACGTCAAATCATCATGCCCCTTA TGACCTGGGCTACACACGTGCTACAATGG ATGGTACAACGAGTTGCGAACTCGCGAGA GTAAGCTAATCTCTTAAAGCCATTCTCAG TTCGGATTGTAGGCTGCAACTCGCCTACA TGAAGTCGGAATCGCTAGTAATCGCGGAT CAGCATGCCGCGGTGAATACGTTCCCGGG CCTTGTACACACCGCCCGTCACACCATGA GAGTTTGTAACACCCAAAGTCGGTGGGGT AACCTTTTAGGAACCAGCCGCCTAAGGTG GGACAGATGATTAGGGTGAAGTCGTAAC AGGGAAAACCCGGTAA	ACGTGGGCGTCTCCAGGCGGATGCTTAATGCGTTA GCTGCAGCACTGAAGGGCGGAAACCCTCCAACAC TTAGCATTCATCGTTTACGGTATGGACTACCAGGG TATCTAATCCTGTTTGCTACCCATACTTTCGAGCCT CAGCGTCAGTTACAGACCAGACAGCCGCCTTCGCC ACTGGTGTTCTTCCATATATCTACGCATTTCACCGC TACACATGGAGTTCCACTGTCCTCTTCTGCACTCA AGTTTCCCAGTTTCCGATGCACTTCTTCGGTTGAGC CGAAGGCTTTCACATCAGACTTAAAAAACCGCCTG CGCTCGCTTTACGCCCAATAAATCCGGACAACGCT TGCCACCTACGTATTACCGCGGCTGCTGGCACGTA GTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATA CCTGAACAGTTACTCTCAGATATGTTCTTCTTTAAC AACAGAGTTTTACGAGCCGAAACCCTTCTTCACTC ACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGT GGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTT GGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCC TCTCAGGTCGGCTACGTATCATTGCCATGGTGAGC CGTTACCTCACCATCTAGCTAATACGCCGCGGGAC CATCCAAAAGTGATAGCCGAAGCCATCTTTCAAAC TCGGACCATGCGGTCCAAGTTGTTATGCGGTATTA GCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGCA GGTTTCCCACGTGTTACTCACCAGTTCGCCACTCA CTCAAATGTAAATCATGATGCAAGCACCAATCAAT ACCAGAGTTCGTTCGACTTGCATGTATTAGCACGC CGCCAGCGTTCGTCTGAGTGATAAAAAAAAATAC ATAAAA
LAB C3	ACTAGAGCGTCGATGAGTGCTAGGTGTTG GAGGGTTTCCGCCCTTCAGTGCCGGAGCT AACGCATTAAGCACTCCGCCTGGGGAGTA CGACCGCAAGGTTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAGCGGTGGAGCA TGTGGTTTAATTCGAAGCTACGCGAAGAA CCTTACCAGGTCTTGACATCTTGCGCCAA CCCTAGAGATAGGGCGTTTCCTTCGGGAA CGCAATGACAGGTGGTGCATGGTCGTCGT CAGCTCGTGTCGTGAGATGTTGGGTTAAG TCCCGCAACGAGCGCAACCCTTGTTACTA GTTGCCAGCATTAAGTTGGGCACTCTAGT GAGACTGCCGGTGACAAACCGGAGGAAG GTGGGGACGACGTCAGATCATCATGCCCC TTATGACCTGGGCTACACACGTGCTACAA TGGACGGTACAACGAGTCGCGAACTCGCG AGGGCAAGCAAATCTCTTAAAACCGTTCT CAGTTCGGACTGCAGGCTGCAACTCGCCT GCACGAAGTCGGAATCGCTAGTAATCGCG GATCAGCATGCCGCGGTGAATACGTTCCC GGGCCTTGTACACACCGCCCGTCACACCA TGAGAGTTTGTAACACCCAAAGTCGGTGG GGTAACCTTTTAGGAGCCAGCCGCCTAAG GTGGGACAGATGATTAGGGTGAAGTCGTA ACAGGGAAAACCGTTAAA	CCCCGGCGGCGTCTCCCAGGCGGAGTGCTTAATGC GTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCA ACACCTAGCACTCATCGTTTACGGCATGGACTACC AGGGTATCTAATCCTGTTCGCTACCCATGCTTTCG AGTCTCAGCGTCAGTTGCAGACCAGGTAGCCGCCT TCGCCACTGGTGTTCTTCCATATATCTACGCATTCC ACCGCTACACATGGAGTTCCACTACCCTCTTCTGC ACTCAAGTTATCCAGTTTCCGATGCACTTCTCCGG TTAAGCCGAAGGCTTTCACATCAGACTTAGAAAAC CGCCTGCACTCTCTTTACGCCCAATAAATCCGGAT AACGCTTGCCACCTACGTATTACCGCGGCTGCTGG CACGTAGTTAGCCGTGACTTTCTGGTTAAATACCG TCAACGTATGAACAGTTACTCTCATACGTGTTCTT CTTTAACAACAGAGCTTTACGAGCCGAAACCCTTC TTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGC CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA GGAGTATGGGCCGTGTCTCAGTCCCATTGTGGCCG ATCAGTCTCTCAACTCGGCTATGCATCATCGCCTT GGTAGGCCGTTACCCCACCAACAAGCTAATGCAC CGCAGGTCCATCCAGAAGTGATAGCGAGAAGCCA TCTTTTAAGCGTTGTTCATGCGAACAACGCTGTTA TGCGGTATTAGCATCTGTTTCCAAATGTTGTCCCCC GCTTCTGGGCAGGTTACCTACGTGTTACTCACCCG TCCGCCACTCGTTGGCGACCAAAATCAATCAGGTG CAAGCACCATCAATCAATTGGGCCAACGCGTTCG ACTTGCATGTATTATGCACACCGCCGGCGTTCATC CTGAGCAGAAAAAAAAAAATCTTAAA

LAB C4	AATCAATGGTACGATGAGGTGCTAGGTGT TGGAGGGTTTCCGCCCTTCAGTGCCGGAG CTAACGCATTAAGCACTCCGCCTGGGGAG TACGACCGCAAGGTTGAAACTCAAAGGA ATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGAAGCTACGCGAA GAACCTTACCAGGTCTTGACATCTTGCGC CAACCCTAGAGATAGGGCGTTTCCTTCGG GAACGCAATGACAGGTGGTGCATGGTCGT CGTCAGCTCGTGTCGTGAGATGTTGGGTT AAGTCCCGCAACGAGCGCAACCCTTGTTA CTAGTTGCCAGCATTAAGTTGGGCACTCT AGTGAGACTGCCGGTGACAAACCGGAGG AAGGTGGGGACGACGTCAGATCATCATGC CCCTTATGACCTGGGCTACACACGTGCTA CAATGGACGGTACAACGAGTCGCGAACTC GCGAGGGCAAGCAAATCTCTTAAAACCGT TCTCAGTTCGGACTGCAGGCTGCAACTCG CCTGCACGAAGTCGGAATCGCTAGTAATC GCGGATCAGCATGCCGCGGTGAATACGTT CCCGGGCCTTGTACACACCGCCCGTCACA CCATGAGAGTTTGTAACACCCAAAGTCGG TGGGGTAACCTTTTAGGAGCCAGCCGCCT AAGGTGGGACAGATGATTAGGGTGAAGT CGTAACAAGGTAACCCGTAAA	CAGGTGGGGGTCTTCCCAGGCGGAGTGCTTAATGC GTTAGCTCCGGCACTGAAGGGCGGAAACCCTCCA ACACCTAGCACTCATCGTTTACGGCATGGACTACC AGGGTATCTAATCCTGTTCGCTACCCATGCTTTCG AGTCTCAGCGTCAGTTGCAGACCAGGTAGCCGCCT TCGCCACTGGTGTTCTTCCATATATCTACGCATTCC ACCGCTACACATGGAGTTCCACTACCCTCTTCTGC ACTCAAGTTATCCAGTTTCCGATGCACTTCTCCGG TTAAGCCGAAGGCTTTCACATCAGACTTAGAAAAC CGCCTGCACTCTCTTTACGCCCAATAAATCCGGAT AACGCTTGCCACCTACGTATTACCGCGGCTGCTGG CACGTAGTTAGCCGTGACTTTCTGGTTAAATACCG TCAACGTATGAACAGTTACTCTCATACGTGTTCTT CTTTAACAACAGAGCTTTACGAGCCGAAACCCTTC TTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGC CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA GGAGTATGGGCCGTGTCTCAGTCCCATTGTGGCCG ATCAGTCTCTCAACTCGGCTATGCATCATCGCCTT GGTAGGCCGTTACCCCACCAACAAGCTAATGCAC CGCAGGTCCATCCAGAAGTGATAGCGAGAAGCCA TCTTTTAAGCGTTGTTCATGCGAACAACGCTGTTA TGCGGTATTAGCATCTGTTTCCAAATGTTGTCCCCC GCTTCTGGGCAGGTTACCTACGTGTTACTCACCCG TCCGCCACTCGTTGGCGACCAAAATCAATCAGTGC AAGCACCATCAATCAATTGGGCCAACGCGTTCGA CTTGCATGTATTAGCACACCGCCGGCGTTCATCCT GAGCAGTATCACAAAATTCTAGA
	Primer ITS1	Primer ITS 4
Yeast MSD	TGCTTGGGCGTTGGTTTCCTTCTGGTTTGG GTTTTTTTGGTTTGGAAAAGACAGATAGC TTTTGGGGGGGCAGAAGACAAGAGAAGG AGGGTTCTGCCCGGCCTGCGCTGAGTGGC GGTCTTGCTTGGCCTGTGCTTTCTTTCCTG CTCTTCCAAACGGTGGGAGGTTTCTGTGC TTTTTTTATACGAAAATTAAAACCGTTTCA ATGGGACGCACTGGGGAGTTTTCGTGTCT TTGCAACTTTTTCTTGGGGGATTCCAGCA ATCGGGGCCCACAAGTAACAAACGCAAA CCTGTTTTTTTTATTCATTTAATTTTTGTCAA AAACAACATTTTCCGGACCGGAAAAATTT AAACAATTTAAAACTTTCCACAACGGATC TCTTGGGTCTCCCATCGGTGAGACACGCC TCCCATGCGATACTGATGTGAATTTCACA ATTCCGGGAAGCTCCAATCTTTGAACGCA AATTGCGGCCCTTGGAATTCCCGGGGGGG GGCCGTCTGAGCGGCATTTCCTTCTCAAA CATTCTGATTGGGAGGGGGTGATTCTCCT TGGAATTAACTTGAAAATTCTGGCCGTTT CATTGGATTTTTTTTTTGCAAAAAAAGGTT TCTCTGCGTGCTTGTAGTATAATGCAAGT ACCGCCGTGTTACGTTGTACTCACTGCGG CTAAACAATTTTTTTACTGAGCGTATTGG AACGTTTTTCGATAAAAAAGAGAGCGTCT AGGCGAACAAAGGTTCTTATAGTTTGACC TCCAAATCAGGAAGGAGTACCCGCTGAAC TTAATCCTATCATAAGCGGAGGAAGAGAA	AATAAATTGGGTTTCATTTCCGGATTTTGAGGTCA ACTTGAAGAATTTGTTCGCCTAGACGCGCTCGTCT TGTCGATAACGTTCGATACGCTCAGTATAAAAAAG ATTAGCCGCGGTTGGTAAAACCTAAGACCACCGTA CTTGCATTATACCTCAAACACGCAGAGAAACCTCT CTTTGGAGAAAAAACCTCCAATGAAAAGGCGATC GATTTCTAGTTAACTCCAAAGAGTATCACTCACTA CCAAACAGAATGTGGGAGAAGGAAATGACTCTCA AAAAGGAATGCCCCCTGGAATACCAAGGGGCGCA ATGTGCGTTCAAAGATTCGATGATTCACGGAATTC TGGAACTCACATTACGTATCGCATTTCTCTGCGTTC TTCATCGATGCGAGAACCAACAGATCCGTGGTTGA AAGGTTTTAATATTTTTAAATTTCCAGTTACGAAG GTTCTTGTTTTTGACAAAAATTTAATGAATAAAAT AAAATTGTTTGTGTTTTGTTACCTCTGGGCCCCCAT TGCTCGGATGCCACAAGAGAAAGTTGCAAAGATA TGAGAAACTCCACAGCGTGTTGTATTGAAACTGAT TTTATTGTCCTATCACAAAAGCGCAGAAATCTCTC ACCGTCTGGAATAGCGAGAAAGAAACTTACAAGC CTAGCGAGACCTCTCACATATGCGCATGTCCAGCT GGACTCTCCATCTCTTGTCTTCTTGCCCAGTAAAA GCTCTCATGCTCTTGCCAAAACAAAAAATCCATTT TCAAATTATTAAATTTCTTTAATGATCCTTCCGCAG GTTCACCTACGGAAGGAGGAT

Yeast DBP	TCCGTGGGCGGTTCACCCCTGTTCTGCGG TCTCCTGTTGATTATGATGAGATCTGCGA GGCCTGGGCGGCCCGTCGTGGAAGAGCTG GTTTGTTCCCATTCACCTACGAAGCCGGA ATCGCGGCCTGGCCACTGGTTTTCGGGCG CGTCCCGCACCCCCCAAGACGAGGGCGCC GGACGGTCGTCCATCACACAAGCCGGGCT TGATGGGCTGAACTGACGCTCGAACAGGC ATGTCCCCCGGAATGCCAGGGGGGCAATG TGCGTTCAAAGATTCAATGATTCACGGAA TTCTGCATTCTATTACTTATGCCATTTCAC TGCGTGCCTCAACGATGCCGGAACCAAGA AATCCTCTGTTTGAAACTTAATTGATTTTA GCTTTTGATCCATCGGACAGACAGTCTTC TGAAAATTTTTGTTGGTTCGCTCTTTGCGG ACTCGTCAATCAGTTTTCCTTTAAAAAAG GTCCCCCCAAGGCTTTTCTGGGGGAAACT CCGTTTGCAAGGCGTTCAAGTCAGGAAAA TAAAGAGGGGGAAGGTTCCGCAGGGCTT CTCTCTTCTCCCTCAATAGGAGTTTTTCCG TACAAGGGGGGGGTACCCCCAGACACTG CACGCTACGAGACCCTCCGCGTGTTCCCA TACGAACTCTTGTACATTTTTCCTCTCAAA A	GAAAGGGCTGGGAATCCTACTTGATTTGAGGTCAA CTTTAAGAACATTGTTCGCCTAGACGCTCTCTTCTT ATCGATAACGTTCCAATACGCTCAGTATAAAAAAG ATTAGCCGCAGTTGGTAAAACCTAAAACGACCGT ACTTGCATTATACCTCAAGCACGCAGAGAAACCTC TCTTTGGAAAAAAAACATCCAATGAAAAGGCCAG CAATTTCAAGTTAACTCCAAAGAGTATCACTCACT ACCAAACAGAATGTTTGAGAAGGAAATGACGCTC AAACAGGCATGCCCCCTGGAATACCAAGGGGCGC AATGTGCGTTCAAAGATTCGATGATTCACGGAATT CTGCAATTCACATTACGTATCGCATTTCGCTGCGTT CTTCATCGATGCGAGAACCAAGAGATCCGTTGTTG AAAGTTTTTAATATTTTAAAATTTCCAGTTACGAA AATTCTTGTTTTTGACAAAAATTTAATGAATAAAT AAAATTGTTTGTGTTTGTTACCTCTGGGCCCCGATT GCTCGAATGCCCAAAGAAAAAGTTGCAAAGATAT GAAAACTCCACAGTGTGTTGTATTGAAACGGTTTT AATTGTCCTATAACAAAAAGCACAGAAATCTCTCA CCGTTTGGAATAGCAAGAAAGAAACTTACAAGCC TAGCAAGACCGCGCACTTAAGCGCAGGCCCGGCT GGACTCTCCATCTCTTGTCTTCTTGCCCAGTAAAA GCTCTCATGCTCTTGCCAAAACAAAAAAATCCATT TTCAAAATTATTAAATTTCTTTAATGATCCTTCCGC AGGTTCACCTACGGAAAGATGATT
Yeast DAP	GCCTGGGTTTTTTATCCTTTTGGACTGGAT TTTTTTGTTTTGGCAAGAGCATGAGAGCTT TTACTGGGCAAGAAGACAAGAGATGGAG AGTCCAGCCGGGCCTGCGCTTAAGTGCGC GGTCTTGCTAGGCCTGTAAGTTTCTTTCTT GCTATTCCAAACGGTGAGAGATTTCTGTG CTTTTGTTATAGGACAATTAAAACCGTTTC AATACAACACACTGTGGAGTTTTCATATC TTTGCAACTTTTTCTTTGGGCATTCGAGCA ATCGGGGCCCAAAGGTAACAAACACAAA CAATTTTATTTATTCATTAAATTTTTGTCA AAAACAAGAATTTTCGTAACTGGAAATTT TAAAATATTAAAAACTTTCAACAACGGAT CTCTTGGTTCTCGCATCGATGAAGAACGC AGCGAAATGCGATACGTAATGTGAATTGC AGAATTCCGTGAATCATCGAATCTTTGAA CGCACATTGCGCCCCTTGGTATTCCAGGG GGCATGCCTGTTTGAGCGTCATTTCCTTCT CAAACATTCTGTTTGGTAGTGAGTGATAC TCTTTGGAGTTAACTTGAAATTGCTGGCCT TTTCATTGGATGTTTTTTTTCCAAAGAGAG GTTTCTCTGCGTGCTTGAGGTATAATGCA AAGTACGGTCGTTTTAAGTTTTACCAACT GCGGCTAATCTTTTTTTATACTGAGCGTATT GGAACGTTATCGATAAGAAGAGAGCGTCT AGGCGAACAATGTTCTTAAAGTTTGACCT CAAATCAGGTAGGAGTACCCCGCTGAACT TAAGCATATCAATAAAGCGGAGGAAAAA GATC	GGGAACCTGGGGACTCTACCTGATTTGAGGTCAAA CTTTAAGAACATTGTTCGCCTAGACGCTCTCTTCTT ATCGATAACGTTCCAATACGCTCAGTATAAAAAAG ATTAGCCGCAGTTGGTAAAACCTAAAACGACCGT ACTTGCATTATACCTCAAGCACGCAGAGAAACCTC TCTTTGGAAAAAAAACATCCAATGAAAAGGCCAG CAATTTCAAGTTAACTCCAAAGAGTATCACTCACT ACCAAACAGAATGTTTGAGAAGGAAATGACGCTC AAACAGGCATGCCCCCTGGAATACCAAGGGGCGC AATGTGCGTTCAAAGATTCGATGATTCACGGAATT CTGCAATTCACATTACGTATCGCATTTCGCTGCGTT CTTCATCGATGCGAGAACCAAGAGATCCGTTGTTG AAAGTTTTTAATATTTTAAAATTTCCAGTTACGAA AATTCTTGTTTTTGACAAAAATTTAATGAATAAAT AAAATTGTTTGTGTTTGTTACCTCTGGGCCCCGATT GCTCGAATGCCCAAAGAAAAAGTTGCAAAGATAT GAAAACTCCACAGTGTGTTGTATTGAAACGGTTTT AATTGTCCTATAACAAAAGCACAGAAATCTCTCAC CGTTTGGAATAGCAAGAAAGAAACTTACAAGCCT AGCAAGACCGCGCACTTAAGCGCAGGCCCGGCTG GACTCTCCATCTCTTGTCTTCTTGCCCAGTAAAAGC TCTCATGCTCTTGCCAAAACAAAAAAATCCATTTT CAAAATTATTAAATTTCTTTAATGATCCTTCCGCA GGTTCACCCTACCGGAAGGAGGATT

Yeast Colony	CGGCGTGGGTTTCATCTTTTGAATGGATTT TTTTGTTTTGGCAAGAGCATGAAAGCTTTT ACTGGGCAAAAAGACCAGAGATGGAGAG TCCAGCCGGGCCTGCGCTTAAGTGCGCGG TCTTGCTAAGCTTGTAAGTTTCTTTCTTGC TATTCCAAACGGTGAGAGATTTCTGTGCT TTTGTTATAGGACAATTAAAACCGTTTCA ATACCACACACTGTGGAGTTTTCATATCTT TGCAACTTTTTCTTTGGGCATTCCAGCAAT CGGGGCCCAAAGGTAACAAACACAAACA ATTTTATTTATTCATTAAATTTTTGTCAAA AACAAGAATTTTCGTAACTGGAAATTTTA AAATATTAAAAACTTTCAACAACGGATCT CTTGGTTCTCGCATCCATGAAGAACGCAG CGAAATGCGATACGTAATGTGAATTGCAG AATTCCGTGAATCATCGAATCTTTGAACG CACATTGCGCCCCTTGGTATTCCAGGGGG CATGCCTGTTTGAGCGTCATTTCCTTCTCA AACATTCTGTTTGGTAGTGAGTGATACTC TTTGGAGTTAACTTGAAATTGCTGGCCTTT TCATTGGATGTTTTTTTTCCAAAGAGAGGT TTCTCTGCGTGCTTGAGGTATAATGCAAG TACTGTCGTTTTATGTTTTACCAACTGCGG CTAATCTTTTTTATACTGAGCGTATTGGAA CGTTATCGATAAGAAGAGAGCGTCTAGCG AACAATGTTCTTAAAGTTTGACCTCAAAT CAGTAGGAGTACCCGCTGAACTTAAGCAT ATCAATAACGGAGGAAAAAA	AGGAACTTGGGTCTCTACTGATTTGAGGTCAACTT TAAGAACATTGTTCGCCTAGACGCTCTCTTCTTATC GATAACGTTCCAATACGCTCAGTATAAAAAAGATT AGCCGCAGTTGGTAAAACCTAAAACGACCGTACTT GCATTATACCTCAAGCACGCAGAGAAACCTCTCTT TGGAAAAAAAACATCCAATGAAAAGGCCAGCAAT TTCAAGTTAACTCCAAAGAGTATCACTCACTACCA AACAGAATGTTTGAGAAGGAAATGACGCTCAAAC AGGCATGCCCCCTGGAATACCAAGGGGCGCAATG TGCGTTCAAAGATTCGATGATTCACGGAATTCTGC AATTCACATTACGTATCGCATTTCGCTGCGTTCTTC ATCGATGCGAGAACCAAGAGATCCGTTGTTGAAA GTTTTTAATATTTTAAAATTTCCAGTTACGAAAATT CTTGTTTTTGACAAAAATTTAATGAATAAATAAAA TTGTTTGTGTTTGTTACCTCTGGGCCCCGATTGCTC GAATGCCCAAAGAAAAAGTTGCAAAGATATGAAA ACTCCACAGTGTGTTGTATTGAAACGGTTTTAATT GTCCTATAACAAAAGCACAGAAATCTCTCACCGTT TGGAATAGCAAGAAAGAAACTTACAAGCCTAGCA AGACCGCGCACTTAAGCGCACGCCCGGCTGGACT CTCCATCTCTTGTCTTCTTGCCCAGTAAAAGCTCTC ATGCTCTTGCCAAAACAAAAAAATCCATTTTCAAA ATTATTAAATTTCTTTAATGATCCTTCCGCAGTCCC CTACGGAAGGAG

Table C.10 Sequence results of trimmed lactic acid bacteria pure colonies and yeast DNA (extracted from mother sourdough, dough before proofing and dough after proofing) sequence results used in BLAST database for identification

Sequenced Microorganisms	Trimmed Sequence	
	Primer 785F	Primer 907R

LAB C1	GCCCTTCAGTGCCGGAGCTAACGCATTAA GCACTCCGCCTGGGGAGTACGACCGCAAG GTTGAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGTGGAGCATGTGGTTTAA TTCGAAGCTACGCGAAGAACCTTACCAGG TCTTGACATCTTGCGCCAACCCTAGAGAT AGGGCGTTTCCTTCGGGAACGCAATGACA GGTGGTGCATGGTCGTCGTCAGCTCGTGT CGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGTTACTAGTTGCCAGCA TTAAGTTGGGCACTCTAGTGAGACTGCCG GTGACAAACCGGAGGAAGGTGGGGACGA CGTCAGATCATCATGCCCCTTATGACCTG GGCTACACACGTGCTACAATGGACGGTAC AACGAGTCGCGAACTCGCGAGGGCAAGC AAATCTCTTAAAACCGTTCTCAGTTCGGA CTGCAGGCTGCAACTCGCCTGCACGAAGT CGGAATCGCTAGTAATCGCGGATCAGCAT GCCGCGGTGAATACGTTCCCGGGCCTTGT ACACACCGCCCGTCACACCATGAGAGTTT GTAACACCCAAAGTCGGTGGGGTAACCTT TTAG GAGCCAGCCGCCTAAGGTGGGACAGATG ATTAGGGTGAAGTCGTAACAGGG	CCGGCACTGAAGGGCGGAAACCCTCC AACACCTAGCACTCATCGTTTACGGC ATGGACTACCAGGGTATCTAATCCTG TTCGCTACCCATGCTTTCGAGTCTCAG CGTCAGTTGCAGACCAGGTAGCCGCC TTCGCCACTGGTGTTCTTCCATATATC TACGCATTCCACCGCTACACATGGAG TTCCACTACCCTCTTCTGCACTCAAGT TATCCAGTTTCCGATGCACTTCTCCGG TTAAGCCGAAGGCTTTCACAT CAGACTTAGAAAACCGCCTGCACTCT CTTTACGCCCAATAAATCCGGATAAC GCTTGCCACCTACGTATTACCGCGGCT GCTGGCACGTAGTTAGCCGTGACTTT CTGGTTAAATACCGTCAACGTATGAA CAGTTACTCTCATACGTGT TCTTCTTTAACAACAGAGCTTTACGAG CCGAAACCCTTCTTCACTCACGCGGT GTTGCTCCATCAGGCTTGCGCCCATTG TGGAAGATTCCCTACTGCTGCCTCCCG TAGGAGTATGGGCCGTGTCTCAGTCC CATTGTGGCCGATCAGT CTCTCAACTCGGCTATGCATCATCGCC TTGGTAGGCCGTTACCCCACCAACAA GCTAATGCACCGCAGGTCCATCCAGA AGTGATAGCGAGAAGCCATCTTTTAA GCGTTGTTCATGCGAACAACGCTGTT ATGCGGTATTAGCATCTGT TTCCAAATGTTGTCCCCCGCTTCTGGG CAGGTTACCTACGTGTTACTCACCCGT CCGCCACTCGTTGGCGACC

LAB C2	CCTTCAGTGCTGCAGCTAACGCATTAAGC ATTCCGCCTGGGGAGTACGGCCGCAAGGC TGAAACTCAAAGGAATTGACGGGGGCCC GCACAAGCGGTGGAGCATGTGGTTTAATT CGAAGCTACGCGAAGAACCTTACCAGGTC TTGACATACTATGCAAATC TAAGAGATTAGACGTTCCCTTCGGGGACA TGGATACAGGTGGTGCATGGTTGTCGTCA GCTCGTGTCGTGAGATGTTGGGTTAAGTC CCGCAACGAGCGCAACCCTTATTATCAGT TGCCAGCATTAAGTTGGGCACTCTGGTGA GACTGCCGGTGACAAACCGGAGGAAGGT GGGGATGACGTCAAATCATCATGCCCCTT ATGACCTGGGCTACACACGTGCTACAATG GATGGTACAACGAGTTGCGAACTCGCGAG AGTAAGCTAATCTCTTAAAGCCATTCTCA GTTCGGATTGTAGGCTGCAACTCGCCTAC ATGAAGTCGGAATCGCTAGTAATCGCGGA TCAGCATGCCGCGGTGAATACGTTCCCGG GCCTTGTACACACCGCCCGTCACACCATG AGAGTTTGTAACACCCAAAGTCGGTGGGG TAACCTTTTAGGAACC AGCCGCCTAAGGTGGGACAGATGATT	GTTAGCTGCAGCACTGAAG GGCGGAAACCCTCCAACACTTAGCAT TCATCGTTTACGGTATGGACTACCAG GGTATCTAATCCTGTTTGCTACCCATA CTTTCGAGCCTCAGCGTCAGTTACAG ACCAGACAGCCGCCTTCGCCACTGGT GTTCTTCCATATATCTACG CATTTCACCGCTACACATGGAGTTCC ACTGTCCTCTTCTGCACTCAAGTTTCC CAGTTTCCGATGCACTTCTTCGGTTGA GCCGAAGGCTTTCACATCAGACTTAA AAAACCGCCTGCGCTCGCTTTACGCC CAATAAATCCGGACAACG CTTGCCACCTACGTATTACCGCGGCTG CTGGCACGTAGTTAGCCGTGGCTTTCT GGTTAAATACCGTCAATACCTGAACA GTTACTCTCAGATATGTTCTTCTTTAA CAACAGAGTTTTACGAGCCGAAACCC TTCTTCACTCACGCGGC GTTGCTCCATCAGACTTTCGTCCATTG TGGAAGATTCCCTACTGCTGCCTCCCG TAGGAGTTTGGGCCGTGTCTCAGTCC CAATGTGGCCGATTACCCTCTCAGGT CGGCTACGTATCATTGCCATGGTGAG CCGTTACCTCACCATCTAGCTAATACG CCGCGGGACCATCCAAAAGTGATAGC CGAAGCCATCTTTCAAACTCGGACCA TGCGGTCCAAGTTGTTATGCGGTATTA GCATCTGTTTCCAGGTGTTATCCCCCG CTTCTG

LAB C3	GCCGGAGCTAACGCATTAAGCACTCCGCC TGGGGAGTACGACCGCAAGGTTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGC GGTGGAGCATGTGGTTTAATTCGAAGCTA CGCGAAGAACCTTACCAGGTCTTGACATC TTGCGCCAACCCTAGAGATAGGGCGTTTC CTTCGGGAACGCAATGACAGGTGGTGCAT GGTCGTCGTCAGCTCGTGTCGTGAGATGT TGGGTTAAGTCCCGCAACGAGCGCAACCC TTGTTACTAGTTGCCAGCATTAAGTTGGG CACTCTAGTGAGACTGCCGGTGACAAACC GGAGGAAGGTGGGGACGACGTCAGATCA TCATGCCCCTTATGACCTGGGCTACACAC GTGCTACAATGGACGGTACAACGAGTCGC GAACTCGCGAGGGCAAGCAAATCTCTTAA AACCGTTCTCAGTTCGGACTGCAGGCTGC AACTCGCCTGCACGAAGTCGGAATCGCTA GTAATCGCGGATCAGCATGCCGCGGTGAA TACGTTCCCGGGCCTTGTACACACCGCCC GTCACACCATGAGAGTTTGTAACACCCAA AGTCGGTGGGGTAACCTTTTAGGAGCCAG CCGCCTAAGGTGGGACAGATGATT	TGCGTTAGCTCCGGCACTGAAGGGCG GAAACCCTCCAACACCTAGCACTCAT CGTTTACGGCATGGACTACCAGGGTA TCTAATCCTGTTCGCTACCCATGCTTT CGAGTCTCAGCGTCAGTTGCAGACCA GGTAGCCGCCTTCGCCACTGGTGTTCT TCCATATATCTACGCATTCCACCGCTA CACATGGAGTTCCACTACCCTCTTCTG CACTCAAGTTATCCAGTTTCCGATGCA CTTCTCCGGTTAAGCCGAAGGCTTTCA CATCAGACTTAGAAAACCGCCTGCAC TCTCTTTACGCCCAATAAATCCGGATA ACGCTTGCCACCTACGTATTACCGCG GCTGCTGGCACGTAGTTAGCCGTGAC TTTCTGGTTAAATACCGTCAACGTATG AACAGTTACTCTCATACGTGTTCTTCT TTAACAACAGAGCTTTACGAGCCGAA ACCCTTCTTCACTCACGCGGTGTTGCT CCATCAGGCTTGCGCCCATTGTGGAA GATTCCCTACTGCTGCCTCCCGTAGGA GTATGGGCCGTGTCTCAGTCCCATTGT GGCCGATCAGTCTCTCAACTCGGCTA TGCATCATCGCCTTGGTAGGCCGTTAC CCCACCAACAAGCTAATGCACCGCAG GTCCATCCAGAAGTGATAGCGAGAAG CCATCTTTTAAGCGTTGTTCATGCGAA CAACGCTGTTATGCGGTATTAGCATCT GTTTCCAAATGTTGTCCCCCGCTTCTG GGCAGGTTACCTACGTGTTACTCACC CGTCCGCCACTCGTTGGCGACCAAAA TCAATCAGGTGCAAGCACCATCAATC AATTGGGCCAACGCGTTCGACTTGCA TGTATTA

LAB C4	CCGCCCTTCAGTGCCGGAGCTAACGCATT AAGCACTCCGCCTGGGGAGTACGACCGCA AGGTTGAAACTCAAAGGAATTGACGGGG GCCCGCACAAGCGGTGGAGCATGTGGTTT AATTCGAAGCTACGCGAAGAACCTTACCA GGTCTTGACATCTTGCGCCAACCCTAGAG ATAGGGCGTTTCCTTCGGGAACGCAATGA CAGGTGGTGCATGGTCGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTTACTAGTTGCCAG CATTAAGTTGGGCACTCTAGTGAGACTGC CGGTGACAAACCGGAGGAAGGTGGGGAC GACGTCAGATCATCATGCCCCTTATGACC TGGGCTACACACGTGCTACAATGGACGGT ACAACGAGTCGCGAACTCGCGAGGGCAA GCAAATCTCTTAAAACCGTTCTCAGTTCG GACTGCAGGCTGCAACTCGCCTGCACGAA GTCGGAATCGCTAGTAATCGCGGATCAGC ATGCCGCGGTGAATACGTTCCCGGGCCTT GTACACACCGCCCGTCACACCATGAGAGT TTGTAACACCCAAAGTCGGTGGGGTAACC TTTTAGGAGCCAGCCGCCTAAGGTGGGAC AGATGA	GCTCCGGCACTGAAGGGCGGAAACCC TCCAACACCTAGCACTCATCGTTTACG GCATGGACTACCAGGGTATCTAATCC TGTTCGCTACCCATGCTTTCGAGTCTC AGCGTCAGTTGCAGACCAGGTAGCCG CCTTCGCCACTGGTGTTCTTCCATATA TCTACGCATTCCACCGCTACACATGG AGTTCCACTACCCTCTTCTGCACTCAA GTTATCCAGTTTCCGATGCACTTCTCC GGTTAAGCCGAAGGCTTTCACATCAG ACTTAGAAAACCGCCTGCACTCTCTTT ACGCCCAATAAATCCGGATAACGCTT GCCACCTACGTATTACCGCGGCTGCT GGCACGTAGTTAGCCGTGACTTTCTG GTTAAATACCGTCAACGTATGAACAG TTACTCTCATACGTGTTCTTCTTTAAC AACAGAGCTTTACGAGCCGAAACCCT TCTTCACTCACGCGGTGTTGCTCCATC AGGCTTGCGCCCATTGTGGAAGATTC CCTACTGCTGCCTCCCGTAGGAGTAT GGGCCGTGTCTCAGTCCCATTGTGGC CGATCAGTCTCTCAACTCGGCTATGC ATCATCGCCTTGGTAGGCCGTTACCCC ACCAACAAGCTAATGCACCGCAGGTC CATCCAGAAGTGATAGCGAGAAGCCA TCTTTTAAGCGTTGTTCATGCGAACAA CGCTGTTATGCGGTATTAGCATCTG TTTCCAAATGTTGTCCCCCGCTTCTGG GCAGGTTACCTACGTGTTACTCACCC GTCCGCCACTCGTTGGCGACCAAAAT CAATCAGTGC
	Primer ITS1	Primer ITS 4
Yeast MSD	TGAAAGCTTTTACTGGGCAAGAAGACAAG AGATGGAGAGTCCAGCCGGGCCTGCGCTT AAGTGCGCGGTCTTGCTAGGCTTGTAAGT TTCTTTCTTGCTATTCCAAACGGTGAGAG ATTTCTGTGCTTTTGTTATAGGACAATTAA AACCGTTTCAATACAACACACTGTGGAGT TTTCATATCTTTGCAACTTTTTCTTTGGGC ATTCGAGCAATCGGGGCCCAGAGGTAAC AAACACAAACAATTTTATTTATTCATTAA ATTTTTGTCAAAAACAAGAATTTTCGTAA CTGGAAATTTTAAAATATTAAAAACTTTC AACAACGGATCTCTTGGTTCTCGCATCGA TGAAGAACGCAGCGAAATGCGATACGTA ATGTGAATTGCAGAATTCCGTGAATCATC GAATCTTTGAACGCACATTGCGCCCCTTG GTATTCCAGGGGGCATGCCTGTTTGAGCG TCATTTCCTTCTCAAACATTCTGTTTGGTA GTGAGTGATACTCTTTGGAGTTAACTTGA AATTGCTGGCCTTTTCATTGGATGTTTTTT	CTAGACGCTCTCTTCTTATCGATAACG TTCCAATACGCTCAGTATAAAAAAGA TTAGCCGCAGTTGGTAAAACCTAAAA CGACCGTACTTGCATTATACCTCAAG CACGCAGAGAAACCTCTCTTTGGAAA AAAAACATCCAATGAAAAGGCCAGC AATTTCAAGTTAACTCCAAAGAGTAT CACTCACTACCAAACAGAATGTTTGA GAAGGAAATGACGCTCAAACAGGCAT GCCCCCTGGAATACCAAGGGGCGCAA TGTGCGTTCAAAGATTCGATGATTCA CGGAATTCTGCAATTCACATTACGTAT CGCATTTCGCTGCGTTCTTCATCGATG CGAGAACCAAGAGATCCGTTGTTGAA AGTTTTTAATATTTTAAAATTTCCAGT TACGAAAATTCTTGTTTTTGACAAAA ATTTAATGAATAAATAAAATTGTTTGT GTTTGTTACCTCTGGGCCCCGATTGCT CGAATGCCCAAAGAAAAAGTTGCAAA

	TTCCAAAGAGAGGTTTCTCTGCGTGCTTG AGGTATAATGCAAGTACGGTCGTTTTAGG TTTTACCAACTGCGGCTAATCTTTTTTATA CTGAGCGTATTGGAACGTTATCGATAAGA AGAGAGCGTCTAGTGAAAGCTTTTACTGG GCAAGAAGACAAGAGATGGAGAGTCCAG CCGGGCCTGCGCTTAAGTGCGCGGTCTTG CTAGGCTTGTAAGTTTCTTTCTTGCTATTC CAAACGGTGAGAGATTTCTGTGCTTTTGT TATAGGACAATTAAAACCGTTTCAATACA ACACACTGTGGAGTTTTCATATCTTTGCA ACTTTTTCTTTGGGCATTCGAGCAATCGG GGCCCAGAGGTAACAAACACAAACAATT TTATTTATTCATTAAATTTTTGTCAAAAAC AAGAATTTTCGTAACTGGAAATTTTAAAA TATTAAAAACTTTCAACAACGGATCTCTT GGTTCTCGCATCGATGAAGAACGCAGCGA AATGCGATACGTAATGTGAATTGCAGAAT TCCGTGAATCATCGAATCTTTGAACGCAC ATTGCGCCCCTTGGTATTCCAGGGGGCAT GCCTGTTTGAGCGTCATTTCCTTCTCAAAC ATTCTGTTTGGTAGTGAGTGATACTCTTTG GAGTTAACTTGAAATTGCTGGCCTTTTCAT TGGATGTTTTTTTTCCAAAGAGAGGTTTCT CTGCGTGCTTGAGGTATAATGCAAGTACG GTCGTTTTAGGTTTTACCAACTGCGGCTA ATCTTTTTTATACTGAGCGTATTGGAACGT TATCGATAAGAAGAGAGCGTCTAGTGAA AGCTTTTACTGGGCAAGAAGACAAGAGAT GGAGAGTCCAGCCGGGCCTGCGCTTAAGT GCGCGGTCTTGCTAGGCTTGTAAGTTTCTT TCTTGCTATTCCAAACGGTGAGAGATTTC TGTGCTTTTGTTATAGGACAATTAAAACC GTTTCAATACAACACACTGTGGAGTTTTC ATATCTTTGCAACTTTTTCTTTGGGCATTC GAGCAATCGGGGCCCAGAGGTAACAAAC ACAAACAATTTTATTTATTCATTAAATTTT TGTCAAAAACAAGAATTTTCGTAACTGGA AATTTTAAAATATTAAAAACTTTCAACAA CGGATCTCTTGGTTCTCGCATCGATGAAG AACGCAGCGAAATGCGATACGTAATGTG AATTGCAGAATTCCGTGAATCATCGAATC TTTGAACGCACATTGCGCCCCTTGGTATTC CAGGGGGCATGCCTGTTTGAGCGTCATTT CCTTCTCAAACATTCTGTTTGGTAGTGAGT GATACTCTTTGGAGTTAACTTGAAATTGC TGGCCTTTTCATTGGATGTTTTTTTTCCAA AGAGAGGTTTCTCTGCGTGCTTGAGGTAT AATGCAAGTACGGTCGTTTTAGGTTTTAC CAACTGCGGCTAATCTTTTTTATACTGAGC GTATTGGAACGTTATCGATAAGAAGAGAG CGTCTAG	GATATGAAAACTCCACAGTGTGTTGT ATTGAAACGGTTTTAATTGTCCTATAA CAAAAGCACAGAAATCTCTCACCGTT TGGAATAGCAAGAAAGAAACTTACAA GCCTAGCAAGACCGCGCACTTAAGCG CAGGCCCGGCTGGACTCTCCATCTCTT GTCTTCTTGCCCAGTAAAAGCTCTCAT GCTCTTGCCAAAACAAAAAAATCCAT TTTCAAAATTATTAAATTTCTTTAATG ATCCTTCCGCAGC

Yeast DBP	GAGTCCAGCCGGGCCTGCGCTTAGGTGCG CGGTCCTGCTAGGCTTGTAAGTTTCTTTCT TGCTATTCCAAACGGTGAGAGATTTCTGT GCTTTTGTTATAGGACAATTAAAACCGTT TCAATACAACACACTGTGGAGTTTTCATA TCTTTGCAACTTTTTCTTTGGGCATTCGAG CAATCGGGGCCCAGAGGTAACAAACACA AACAATTTTATTTATTCATTAAATTTTTGT CAAAAACAAGAATTTTCGTAACTGGAAAT TTTAAAATATTAAAAACTTTCAACAACGG ATCTCTTGGTTCTCGCATCGATGAAGAAC GCAGCGAAATGCGATACGTAATGTGAATT GCAGAATTCCGTGAATCATCGAATCTTTG AACGCACATTGCGCCCCTTGGTATTCCAG GGGGCATGCCTGGTTGAGCGTCATTTCCT TCTCAAACATTCTGTTTGGTAGTGAGTGA TACTCTTTGGAGTTAACTTGAAATTGCTG GCCTTTTCATTGGATGTTTTTTTTCCAAAG AGAGGTTTCTCTGCGTGCTTGAGGTATAA TGCAAGTACGGTCGTTTTAGGTTTTACCA ACTGCGGCTAATCTTTTTTATACTGAGCGT ATTGG	CTAGACGCTCTCTTCTTATCGATAACG TTCCAATACGCTCAGTATAAAAAAGA TTAGCCGCAGTTGGTAAAACCTAAAA CGACCGTACTTGCATTATACCTCAAG CACGCAGAGAAACCTCTCTTTGGAAA AAAAACATCCAATGAAAAGGCCAGC AATTTCAAGTTAACTCCAAAGAGTAT CACTCACTACCAAACAGAATGTTTGA GAAGGAAATGACGCTCAAACAGGCAT GCCCCCTGGAATACCAAGGGGCGCAA TGTGCGTTCAAAGATTCGATGATTCA CGGAATTCTGCAATTCACATTACGTAT CGCATTTCGCTGCGTTCTTCATCGATG CGAGAACCAAGAGATCCGTTGTTGAA AGTTTTTAATATTTTAAAATTTCCAGT TACGAAAATTCTTGTTTTTGACAAAA ATTTAATGAATAAATAAAATTGTTTGT GTTTGTTACCTCTGGGCCCCGATTGCT CGAATGCCCAAAGAAAAAGTTGCAAA GATATGAAAACTCCACAGTGTGTTGT ATTGAAACGGTTTTAATTGTCCTATAA CAAAAGCACAGAAATCTCTCACCGTT TGGAATAGCAAGAAAGAAACTTACAA GCCTAGCAAGACCGCGCACTTAAGCG CAGGCCCGGCTGGACTCTCCATCTCTT GTCTTCTTGCCCAGTAAAAGCTCTCAT GCTCTTGCCAAAACAA

Yeast DAP	TTTTGAATGGATTTTTTTGTTTTGGCAAGA GCATGAAAGCTTTTACTGGGCAAGAAGAC CAGAGATGGAGAGTCCAGCCGGGCCTGC GCTTAAGTGCGCGGGCTTGCTAGGCTTGT AAGTTTCTTTCTTGCTATTCCAAACGGTGA GAGATTTCTGTGCTTTTGTTATAGGACAAT TAAAACCGTTTCAATACAACACACTGTGG AGTTTTCATATCTTTGCAACTTTTTCTTTG GGCATTCGAGCAATCGGGGCCCAGAGGT AACAAACACAAACAATTTTATTTATTCAT TAAATTTTTGTCAAAAACAAGAATTTTCG TAACTGGAAATTTTAAAATATTAAAAACT TTCAACAACGGATCTCTTGGTTCTCGCATC GATGAAGAACGCAGCGAAATGCGATACG TAATGTGAATTGCAGAATTCCGTGAATCA TCGAATCTTTGAACGCACATTGCGCCCCT TGGTATTCCAGGGGGCATGCCTGTTTGAG CGTCATTTCCTTCTCAAACATTCTGTTTGG TAGTGAGTGATACTCTTTGGAGTTAACTT GAAATTGCTGGCCTTTTCATTGGATGTTTT TTTTCCAAAGAGAGGTTTCTCTGCGTGCTT GAGGTATAATGCAAGTACGGTCGTTTTAG GTTTTACCAACTGCGGCTAATCTTTTTTAT ACTGAGCGTATTGGAACGTTATCGATAAG AAGAGAGCGTCTAGGC	GACGCTCTCTTCTTATCGATAACGTTC CAATACGCTCAGTATAAAAAAGATTA GCCGCAGTTGGTAAAACCTAAAACGA CCGTACTTGCATTATACCTCAAGCAC GCAGAGAAACCTCTCTTTGGAAAAAA AACATCCAATGAAAAGGCCAGCAATT TCAAGTTAACTCCAAAGAGTATCACT CACTACCAAACAGAATGTTTGAGAAG GAAATGACGCTCAAACAGGCATGCCC CCTGGAATACCAAGGGGCGCAATGTG CGTTCAAAGATTCGATGATTCACGGA ATTCTGCAATTCACATTACGTATCGCA TTTCGCTGCGTTCTTCATCGATGCGAG AACCAAGAGATCCGTTGTTGAAAGTT TTTAATATTTTAAAATTTCCAGTTACG AAAATTCTTGTTTTTGACAAAAATTTA ATGAATAAATAAAATTGTTTGTGTTTG TTACCTCTGGGCCCCGATTGCTCGAAT GCCCAAAGAAAAAGTTGCAAAGATAT GAAAACTCCACAGTGTGTTGTATTGA AACGGTTTTAATTGTCCTATAACAAA AGCACAGAAATCTCTCACCGTTTGGA ATAGCAAGAAAGAAACTTACAAGCCT AGCAAGACCGCGCACTTAAGCGCAGG CCCGGCTGGACTCTCCATCTCTTGTCT TCTTGCCCAGTAAAAGCTCTCATGCTC TTGCCAAAACAAAAAAATCCATTTTC AAAATTATTAAATTTCTTTAATGATCC TTCCGCAGGTTCACCCTACCGGAAGG AGGATT
Yeast Colony	GAGATGGAGAGTCCAGCCGGGCCTGCGCT TAAGTGCGCGGTCTTGCTAGGCCTGTAAG TTTCTTTCTTGCTATTCCAAACGGTGAGAG ATTTCTGTGCTTTTGTTATAGGACAATTAA AACCGTTTCAATACAACACACTGGGGAGT TTTCATATCTTTGCAACTTTTTCTTTGGGC ATTCGAGCAATCGGGGCCCAGAGGTAAC AAACACAAACAATTTTATTTATTCATTAA ATTTTTGTCAAAAACAAGAATTTTCGTAA CTGGAAATTTTAAAATATTAAAAACTTTC AACAACGGATCTCTTGGTTCTCGCATCGA TGAAGAACGCAGCGAAATGCGATACGTA ATGTGAATTGCAGAATTCCGTGAATCATC CAATCTTTGAACGCACATTGCGCCCCTTG GTATTCCAGGGGGCATGCCTGTTTGAGCG TCATTTCCTTCTCAAACATTCTGTTTGGTA GTGAGTGATACTCTTTGGAGTTAACTTGA AATTGCTGGCCTTTTCATTGGATGTTTTTT TTCCAAAGAGAAGTTTCTCTGCGTGCTTG AGGTATAATGCAAATACGGTCGTTTTAGG TTTTACCAACTGCGGCTAATCTTTTTTATA	GCCTAGACGCTCTCTTCTTATCGATAA CGTTCCAATACGCTCAGTATAAAAAA GATTAGCCGCAGTTGGTAAAACCTAA AACGACCGTACTTGCATTATACCTCA AGCACGCAGAGAAACCTCTCTTTGGA AAAAAAACATCCAATGAAAAGGCCA GCAATTTCAAGTTAACTCCAAAGAGT ATCACTCACTACCAAACAGAATGTTT GAGAAGGAAATGACGCTCAAACAGG CATGCCCCCTGGAATACCAAGGGGCG CAATGTGCGTTCAAAGATTCGATGAT TCACGGAATTCTGCAATTCACATTAC GTATCGCATTTCGCTGCGTTCTTCATC GATGCGAGAACCAAGAGATCCGTTGT TGAAAGTTTTTAATATTTTAAAATTTC CAGTTACGAAAATTCTTGTTTTTGACA AAAATTTAATGAATAAATAAAATTGT TTGTGTTTGTTACCTCTGGGCCCCGAT TGCTCGAATGCCCAAAGAAAAAGTTG CAAAGATATGAAAACTCCACAGTGTG TTGTATTGAAACGGTTTTAATTGTCCT ATAACAAAAGCACAGAAATCTCTCAC CGTTTGGAATAGCAAGAAAGAAACTT

	CTGAGCGTATTGGAACGTTATCGATAAGA AGAGAGCGTCTAGG	ACAAGCCTAGCAAGACCGCGCACTTA AGCGCACGCCCGGCTGGACTCTCCAT CTCTTGTCTTCTTGCCCAGTAAAAGCT CTCATGCTCTTGCCAAAACA

D. Statistical Outputs

pH Analysis

a) Statistical analysis of pH of mother sourdough (MSD), dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB)

Descriptives

	Sample			Statistic	Std. Error
pH	DAP	Mean		4.1100	.02021
		95% Confidence Interval for Mean	Lower Bound	4.0677	
			Upper Bound	4.1523	
		5% Trimmed Mean		4.1061	
		Median		4.0900	
		Variance		.008	
		Std. Deviation		.09038	
		Minimum		3.98	
		Maximum		4.31	
		Range		.33	
		Interquartile Range		.12	
		Skewness		.736	.512
		Kurtosis		.261	.992
	DBP	Mean		4.9230	.05343
		95% Confidence Interval for Mean	Lower Bound	4.8112	
			Upper Bound	5.0348	
		5% Trimmed Mean		4.9300	
		Median		4.9500	
		Variance		.057	
		Std. Deviation		.23895	
		Minimum		4.50	
		Maximum		5.22	
		Range		.72	
		Interquartile Range		.41	
		Skewness		-.304	.512
		Kurtosis		-1.146	.992
	MSD	Mean		3.9060	.03065
		95% Confidence Interval for Mean	Lower Bound	3.8419	
			Upper Bound	3.9701	
		5% Trimmed Mean		3.9083	
		Median		3.9250	
		Variance		.019	
		Std. Deviation		.13705	
		Minimum		3.65	
		Maximum		4.12	
		Range		.47	
		Interquartile Range		.12	
		Skewness		-.330	.512
		Kurtosis		-.333	.992

	SDB	Mean	4.3196	.02353
		95% Confidence Interval for Mean — Lower Bound	4.2724	
		95% Confidence Interval for Mean — Upper Bound	4.3668	
		5% Trimmed Mean	4.3185	
		Median	4.3300	
		Variance	.030	
		Std. Deviation	.17289	
		Minimum	4.04	
		Maximum	4.62	
		Range	.58	
		Interquartile Range	.27	
		Skewness	.053	.325
		Kurtosis	-1.014	.639

b) Multiple Comparisons between mother sourdough (MSD), dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB)

Dependent Variable: pH
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.8120*	.07707	.000	-1.0198	-.6042
	MSD	.2040	.07707	.056	-.0038	.4118
	SDB	-.2059	.07918	.062	-.4194	.0077
DBP	DAP	.8120*	.07707	.000	.6042	1.0198
	MSD	1.0160*	.07707	.000	.8082	1.2238
	SDB	.6061*	.07918	.000	.3926	.8197
MSD	DAP	-.2040	.07707	.056	-.4118	.0038
	DBP	-1.0160*	.07707	.000	-1.2238	-.8082
	SDB	-.4099*	.07918	.000	-.6234	-.1963
SDB	DAP	.2059	.07918	.062	-.0077	.4194
	DBP	-.6061*	.07918	.000	-.8197	-.3926
	MSD	.4099*	.07918	.000	.1963	.6234

Based on observed means.
The error term is Mean Square (Error) = .030.
*. The mean difference is significant at the .05 level

c) Statistical analysis of pH of mother sourdough (MSD)

Descriptives analysis

		Statistic	Std. Error
pH	Mean	3.9090	.04411
	95% Confidence Interval for Mean — Lower Bound	3.8092	
	95% Confidence Interval for Mean — Upper Bound	4.0088	
	5% Trimmed Mean	3.9111	
	Median	3.9300	
	Variance	.019	
	Std. Deviation	.13948	
	Minimum	3.66	
	Maximum	4.12	
	Range	.46	
	Interquartile Range	.17	
	Skewness	-.386	.687
	Kurtosis	.016	1.334

a. Sample = MSD

Tests of Normality analysis

	Kolmogorov-Smirnovb			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
pH	.157	10	.200*	.960	10	.785

*. This is a lower bound of the true significance.
a. Sample = MSD
b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of MSD (pH)

Dependent Variable: pH
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1	2	-.2750*	.02168	.000	-.3608	-.1892
	3	-.2200*	.02168	.000	-.3058	-.1342
	4	-.1150*	.02168	.008	-.2008	-.0292
	5	-.1100*	.02168	.010	-.1958	-.0242
	6	-.0750	.02168	.102	-.1608	.0108
	7	-.0300	.02168	.908	-.1158	.0558
	8	-.0900*	.02168	.038	-.1758	-.0042
	9	.1150*	.02168	.008	.0292	.2008
	10	.1900*	.02168	.000	.1042	.2758
2	1	.2750*	.02168	.000	.1892	.3608
	3	.0550	.02168	.350	-.0308	.1408
	4	.1600*	.02168	.001	.0742	.2458
	5	.1650*	.02168	.000	.0792	.2508
	6	.2000*	.02168	.000	.1142	.2858
	7	.2450*	.02168	.000	.1592	.3308
	8	.1850*	.02168	.000	.0992	.2708
	9	.3900*	.02168	.000	.3042	.4758
	10	.4650*	.02168	.000	.3792	.5508
3	1	.2200*	.02168	.000	.1342	.3058
	2	-.0550	.02168	.350	-.1408	.0308
	4	.1050*	.02168	.014	.0192	.1908
	5	.1100*	.02168	.010	.0242	.1958
	6	.1450*	.02168	.001	.0592	.2308
	7	.1900*	.02168	.000	.1042	.2758
	8	.1300*	.02168	.003	.0442	.2158
	9	.3350*	.02168	.000	.2492	.4208
	10	.4100*	.02168	.000	.3242	.4958
4	1	.1150*	.02168	.008	.0292	.2008
	2	-.1600*	.02168	.001	-.2458	-.0742
	3	-.1050*	.02168	.014	-.1908	-.0192
	5	.0050	.02168	1.000	-.0808	.0908
	6	.0400	.02168	.700	-.0458	.1258
	7	.0850	.02168	.053	-.0008	.1708
	8	.0250	.02168	.965	-.0608	.1108
	9	.2300*	.02168	.000	.1442	.3158
	10	.3050*	.02168	.000	.2192	.3908
5	1	.1100*	.02168	.010	.0242	.1958
	2	-.1650*	.02168	.000	-.2508	-.0792
	3	-.1100*	.02168	.010	-.1958	-.0242

	4	-.0050	.02168	1.000	-.0908	.0808
	6	.0350	.02168	.816	-.0508	.1208
	7	.0800	.02168	.073	-.0058	.1658
	8	.0200	.02168	.991	-.0658	.1058
	9	.2250*	.02168	.000	.1392	.3108
	10	.3000*	.02168	.000	.2142	.3858
6	1	.0750	.02168	.102	-.0108	.1608
	2	-.2000*	.02168	.000	-.2858	-.1142
	3	-.1450*	.02168	.001	-.2308	-.0592
	4	-.0400	.02168	.700	-.1258	.0458
	5	-.0350	.02168	.816	-.1208	.0508
	7	.0450	.02168	.575	-.0408	.1308
	8	-.0150	.02168	.999	-.1008	.0708
	9	.1900*	.02168	.000	.1042	.2758
	10	.2650*	.02168	.000	.1792	.3508
7	1	.0300	.02168	.908	-.0558	.1158
	2	-.2450*	.02168	.000	-.3308	-.1592
	3	-.1900*	.02168	.000	-.2758	-.1042
	4	-.0850	.02168	.053	-.1708	.0008
	5	-.0800	.02168	.073	-.1658	.0058
	6	-.0450	.02168	.575	-.1308	.0408
	8	-.0600	.02168	.263	-.1458	.0258
	9	.1450*	.02168	.001	.0592	.2308
	10	.2200*	.02168	.000	.1342	.3058
8	1	.0900*	.02168	.038	.0042	.1758
	2	-.1850*	.02168	.000	-.2708	-.0992
	3	-.1300*	.02168	.003	-.2158	-.0442
	4	-.0250	.02168	.965	-.1108	.0608
	5	-.0200	.02168	.991	-.1058	.0658
	6	.0150	.02168	.999	-.0708	.1008
	7	.0600	.02168	.263	-.0258	.1458
	9	.2050*	.02168	.000	.1192	.2908
	10	.2800*	.02168	.000	.1942	.3658
9	1	-.1150*	.02168	.008	-.2008	-.0292
	2	-.3900*	.02168	.000	-.4758	-.3042
	3	-.3350*	.02168	.000	-.4208	-.2492
	4	-.2300*	.02168	.000	-.3158	-.1442
	5	-.2250*	.02168	.000	-.3108	-.1392
	6	-.1900*	.02168	.000	-.2758	-.1042
	7	-.1450*	.02168	.001	-.2308	-.0592
	8	-.2050*	.02168	.000	-.2908	-.1192
	10	.0750	.02168	.102	-.0108	.1608
10	1	-.1900*	.02168	.000	-.2758	-.1042
	2	-.4650*	.02168	.000	-.5508	-.3792
	3	-.4100*	.02168	.000	-.4958	-.3242
	4	-.3050*	.02168	.000	-.3908	-.2192
	5	-.3000*	.02168	.000	-.3858	-.2142
	6	-.2650*	.02168	.000	-.3508	-.1792
	7	-.2200*	.02168	.000	-.3058	-.1342
	8	-.2800*	.02168	.000	-.3658	-.1942
	9	-.0750	.02168	.102	-.1608	.0108

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

d) Statistical analysis of pH of dough before proofing (DBP)

Descriptives analysis

			Statistic	Std. Error
pH	Mean		4.9250	.07753
	95% Confidence Interval for Mean	Lower Bound	4.7496	
		Upper Bound	5.1004	
	5% Trimmed Mean		4.9317	
	Median		4.9550	
	Variance		.060	
	Std. Deviation		.24519	
	Minimum		4.51	
	Maximum		5.22	
	Range		.71	
	Interquartile Range		.43	
	Skewness		-.358	.687
	Kurtosis		-1.099	1.334

a. Sample = DBP

Tests of Normality a

	Kolmogorov-Smirnovb			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
pH	.136	10	.200*	.942	10	.578

*. This is a lower bound of the true significance.

a. Sample = DBP

b. Lilliefors Significance Correction

Multiple Comparisons between weeks of DBP (pH)

Dependent Variable: pH

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.4900*	.01844	.000	-.5630	-.4170
	3	-.4250*	.01844	.000	-.4980	-.3520
	4	-.5600*	.01844	.000	-.6330	-.4870
	5	-.0950*	.01844	.009	-.1680	-.0220
	6	-.2750*	.01844	.000	-.3480	-.2020
	7	-.1300*	.01844	.001	-.2030	-.0570
	8	.1400*	.01844	.000	.0670	.2130
	9	-.5700*	.01844	.000	-.6430	-.4970
	10	-.3250*	.01844	.000	-.3980	-.2520
2	1	.4900*	.01844	.000	.4170	.5630
	3	.0650	.01844	.093	-.0080	.1380
	4	-.0700	.01844	.063	-.1430	.0030
	5	.3950*	.01844	.000	.3220	.4680
	6	.2150*	.01844	.000	.1420	.2880
	7	.3600*	.01844	.000	.2870	.4330
	8	.6300*	.01844	.000	.5570	.7030
	9	-.0800*	.01844	.029	-.1530	-.0070
	10	.1650*	.01844	.000	.0920	.2380
3	1	.4250*	.01844	.000	.3520	.4980
	2	-.0650	.01844	.093	-.1380	.0080
	4	-.1350*	.01844	.001	-.2080	-.0620
	5	.3300*	.01844	.000	.2570	.4030
	6	.1500*	.01844	.000	.0770	.2230

	7	.2950*	.01844	.000	.2220	.3680
	8	.5650*	.01844	.000	.4920	.6380
	9	-.1450*	.01844	.000	-.2180	-.0720
	10	.1000*	.01844	.006	.0270	.1730
4	1	.5600*	.01844	.000	.4870	.6330
	2	.0700	.01844	.063	-.0030	.1430
	3	.1350*	.01844	.001	.0620	.2080
	5	.4650*	.01844	.000	.3920	.5380
	6	.2850*	.01844	.000	.2120	.3580
	7	.4300*	.01844	.000	.3570	.5030
	8	.7000*	.01844	.000	.6270	.7730
	9	-.0100	.01844	1.000	-.0830	.0630
	10	.2350*	.01844	.000	.1620	.3080
5	1	.0950*	.01844	.009	.0220	.1680
	2	-.3950*	.01844	.000	-.4680	-.3220
	3	-.3300*	.01844	.000	-.4030	-.2570
	4	-.4650*	.01844	.000	-.5380	-.3920
	6	-.1800*	.01844	.000	-.2530	-.1070
	7	-.0350	.01844	.672	-.1080	.0380
	8	.2350*	.01844	.000	.1620	.3080
	9	-.4750*	.01844	.000	-.5480	-.4020
	10	-.2300*	.01844	.000	-.3030	-.1570
6	1	.2750*	.01844	.000	.2020	.3480
	2	-.2150*	.01844	.000	-.2880	-.1420
	3	-.1500*	.01844	.000	-.2230	-.0770
	4	-.2850*	.01844	.000	-.3580	-.2120
	5	.1800*	.01844	.000	.1070	.2530
	7	.1450*	.01844	.000	.0720	.2180
	8	.4150*	.01844	.000	.3420	.4880
	9	-.2950*	.01844	.000	-.3680	-.2220
	10	-.0500	.01844	.282	-.1230	.0230
7	1	.1300*	.01844	.001	.0570	.2030
	2	-.3600*	.01844	.000	-.4330	-.2870
	3	-.2950*	.01844	.000	-.3680	-.2220
	4	-.4300*	.01844	.000	-.5030	-.3570
	5	.0350	.01844	.672	-.0380	.1080
	6	-.1450*	.01844	.000	-.2180	-.0720
	8	.2700*	.01844	.000	.1970	.3430
	9	-.4400*	.01844	.000	-.5130	-.3670
	10	-.1950*	.01844	.000	-.2680	-.1220
8	1	-.1400*	.01844	.000	-.2130	-.0670
	2	-.6300*	.01844	.000	-.7030	-.5570
	3	-.5650*	.01844	.000	-.6380	-.4920
	4	-.7000*	.01844	.000	-.7730	-.6270
	5	-.2350*	.01844	.000	-.3080	-.1620
	6	-.4150*	.01844	.000	-.4880	-.3420
	7	-.2700*	.01844	.000	-.3430	-.1970
	9	-.7100*	.01844	.000	-.7830	-.6370
	10	-.4650*	.01844	.000	-.5380	-.3920
9	1	.5700*	.01844	.000	.4970	.6430
	2	.0800*	.01844	.029	.0070	.1530
	3	.1450*	.01844	.000	.0720	.2180
	4	.0100	.01844	1.000	-.0630	.0830
	5	.4750*	.01844	.000	.4020	.5480
	6	.2950*	.01844	.000	.2220	.3680
	7	.4400*	.01844	.000	.3670	.5130
	8	.7100*	.01844	.000	.6370	.7830

	10	.2450*	.01844	.000	.1720	.3180
10	1	.3250*	.01844	.000	.2520	.3980
	2	-.1650*	.01844	.000	-.2380	-.0920
	3	-.1000*	.01844	.006	-.1730	-.0270
	4	-.2350*	.01844	.000	-.3080	-.1620
	5	.2300*	.01844	.000	.1570	.3030
	6	.0500	.01844	.282	-.0230	.1230
	7	.1950*	.01844	.000	.1220	.2680
	8	.4650*	.01844	.000	.3920	.5380
	9	-.2450*	.01844	.000	-.3180	-.1720

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

e) Statistical analysis of pH of dough after proofing (DAP)

Descriptives analysis

			Statistic	Std. Error
pH	Mean		4.1130	.02868
	95% Confidence Interval for Mean	Lower Bound	4.0481	
		Upper Bound	4.1779	
	5% Trimmed Mean		4.1094	
	Median		4.1000	
	Variance		.008	
	Std. Deviation		.09068	
	Minimum		3.99	
	Maximum		4.30	
	Range		.31	
	Interquartile Range		.13	
	Skewness		.826	.687
	Kurtosis		.797	1.334

a. Sample = DAP

Tests of Normality analysis

	Kolmogorov-Smirnovb			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
pH	.126	10	.200*	.959	10	.777

*. This is a lower bound of the true significance.
a. Sample = DAP
b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of DAP (pH)

Dependent Variable: pH

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1	2	-.0750	.01924	.054	-.1511	.0011
	3	-.0250	.01924	.933	-.1011	.0511
	4	-.0450	.01924	.439	-.1211	.0311
	5	-.1200*	.01924	.002	-.1961	-.0439

	6	-.2200*	.01924	.000	-.2961	-.1439
	7	-.0050	.01924	1.000	-.0811	.0711
	8	.0550	.01924	.233	-.0211	.1311
	9	.0950*	.01924	.012	.0189	.1711
	10	.0400	.01924	.573	-.0361	.1161
2	1	.0750	.01924	.054	-.0011	.1511
	3	.0500	.01924	.324	-.0261	.1261
	4	.0300	.01924	.841	-.0461	.1061
	5	-.0450	.01924	.439	-.1211	.0311
	6	-.1450*	.01924	.000	-.2211	-.0689
	7	.0700	.01924	.079	-.0061	.1461
	8	.1300*	.01924	.001	.0539	.2061
	9	.1700*	.01924	.000	.0939	.2461
	10	.1150*	.01924	.003	.0389	.1911
3	1	.0250	.01924	.933	-.0511	.1011
	2	-.0500	.01924	.324	-.1261	.0261
	4	-.0200	.01924	.981	-.0961	.0561
	5	-.0950*	.01924	.012	-.1711	-.0189
	6	-.1950*	.01924	.000	-.2711	-.1189
	7	.0200	.01924	.981	-.0561	.0961
	8	.0800*	.01924	.038	.0039	.1561
	9	.1200*	.01924	.002	.0439	.1961
	10	.0650	.01924	.114	-.0111	.1411
4	1	.0450	.01924	.439	-.0311	.1211
	2	-.0300	.01924	.841	-.1061	.0461
	3	.0200	.01924	.981	-.0561	.0961
	5	-.0750	.01924	.054	-.1511	.0011
	6	-.1750*	.01924	.000	-.2511	-.0989
	7	.0400	.01924	.573	-.0361	.1161
	8	.1000*	.01924	.009	.0239	.1761
	9	.1400*	.01924	.001	.0639	.2161
	10	.0850*	.01924	.026	.0089	.1611
5	1	.1200*	.01924	.002	.0439	.1961
	2	.0450	.01924	.439	-.0311	.1211
	3	.0950*	.01924	.012	.0189	.1711
	4	.0750	.01924	.054	-.0011	.1511
	6	-.1000*	.01924	.009	-.1761	-.0239
	7	.1150*	.01924	.003	.0389	.1911
	8	.1750*	.01924	.000	.0989	.2511
	9	.2150*	.01924	.000	.1389	.2911
	10	.1600*	.01924	.000	.0839	.2361
6	1	.2200*	.01924	.000	.1439	.2961
	2	.1450*	.01924	.000	.0689	.2211
	3	.1950*	.01924	.000	.1189	.2711
	4	.1750*	.01924	.000	.0989	.2511
	5	.1000*	.01924	.009	.0239	.1761
	7	.2150*	.01924	.000	.1389	.2911
	8	.2750*	.01924	.000	.1989	.3511
	9	.3150*	.01924	.000	.2389	.3911
	10	.2600*	.01924	.000	.1839	.3361
7	1	.0050	.01924	1.000	-.0711	.0811
	2	-.0700	.01924	.079	-.1461	.0061
	3	-.0200	.01924	.981	-.0961	.0561
	4	-.0400	.01924	.573	-.1161	.0361
	5	-.1150*	.01924	.003	-.1911	-.0389
	6	-.2150*	.01924	.000	-.2911	-.1389
	8	.0600	.01924	.164	-.0161	.1361

	9	.1000*	.01924	.009	.0239	.1761
	10	.0450	.01924	.439	-.0311	.1211
8	1	-.0550	.01924	.233	-.1311	.0211
	2	-.1300*	.01924	.001	-.2061	-.0539
	3	-.0800*	.01924	.038	-.1561	-.0039
	4	-.1000*	.01924	.009	-.1761	-.0239
	5	-.1750*	.01924	.000	-.2511	-.0989
	6	-.2750*	.01924	.000	-.3511	-.1989
	7	-.0600	.01924	.164	-.1361	.0161
	9	.0400	.01924	.573	-.0361	.1161
	10	-.0150	.01924	.997	-.0911	.0611
9	1	-.0950*	.01924	.012	-.1711	-.0189
	2	-.1700*	.01924	.000	-.2461	-.0939
	3	-.1200*	.01924	.002	-.1961	-.0439
	4	-.1400*	.01924	.001	-.2161	-.0639
	5	-.2150*	.01924	.000	-.2911	-.1389
	6	-.3150*	.01924	.000	-.3911	-.2389
	7	-.1000*	.01924	.009	-.1761	-.0239
	8	-.0400	.01924	.573	-.1161	.0361
	10	-.0550	.01924	.233	-.1311	.0211
10	1	-.0400	.01924	.573	-.1161	.0361
	2	-.1150*	.01924	.003	-.1911	-.0389
	3	-.0650	.01924	.114	-.1411	.0111
	4	-.0850*	.01924	.026	-.1611	-.0089
	5	-.1600*	.01924	.000	-.2361	-.0839
	6	-.2600*	.01924	.000	-.3361	-.1839
	7	-.0450	.01924	.439	-.1211	.0311
	8	.0150	.01924	.997	-.0611	.0911
	9	.0550	.01924	.233	-.0211	.1311

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

f) Statistical analysis of pH of sourdough bread (SDB)

Sample = SDB

Descriptives [a]

			Statistic	Std. Error
pH	Mean		4.3189	.05884
	95% Confidence Interval for Mean	Lower Bound	4.1832	
		Upper Bound	4.4546	
	5% Trimmed Mean		4.3182	
	Median		4.3300	
	Variance		.031	
	Std. Deviation		.17653	
	Minimum		4.07	
	Maximum		4.58	
	Range		.51	
	Interquartile Range		.33	
	Skewness		-.015	.717
	Kurtosis		-1.077	1.400

a. Sample = SDB

Tests of Normality [a]

	Kolmogorov-Smirnov[b]	Shapiro-Wilk

	Statistic	df	Sig.	Statistic	df	Sig.
pH	.169	9	.200*	.950	9	.688

*. This is a lower bound of the true significance.

a. Sample = SDB

b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of SDB (pH)

Dependent Variable: pH

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
2	3	-.0967*	.02248	.003	-.1699	-.0235
	4	-.0050	.02248	1.000	-.0782	.0682
	5	-.2483*	.02248	.000	-.3215	-.1751
	6	-.1900*	.02248	.000	-.2632	-.1168
	7	.0117	.02248	1.000	-.0615	.0849
	8	.2633*	.02248	.000	.1901	.3365
	9	.2283*	.02248	.000	.1551	.3015
	10	.1600*	.02248	.000	.0868	.2332
3	2	.0967*	.02248	.003	.0235	.1699
	4	.0917*	.02248	.005	.0185	.1649
	5	-.1517*	.02248	.000	-.2249	-.0785
	6	-.0933*	.02248	.004	-.1665	-.0201
	7	.1083*	.02248	.001	.0351	.1815
	8	.3600*	.02248	.000	.2868	.4332
	9	.3250*	.02248	.000	.2518	.3982
	10	.2567*	.02248	.000	.1835	.3299
4	2	.0050	.02248	1.000	-.0682	.0782
	3	-.0917*	.02248	.005	-.1649	-.0185
	5	-.2433*	.02248	.000	-.3165	-.1701
	6	-.1850*	.02248	.000	-.2582	-.1118
	7	.0167	.02248	.998	-.0565	.0899
	8	.2683*	.02248	.000	.1951	.3415
	9	.2333*	.02248	.000	.1601	.3065
	10	.1650*	.02248	.000	.0918	.2382
5	2	.2483*	.02248	.000	.1751	.3215
	3	.1517*	.02248	.000	.0785	.2249
	4	.2433*	.02248	.000	.1701	.3165
	6	.0583	.02248	.217	-.0149	.1315
	7	.2600*	.02248	.000	.1868	.3332
	8	.5117*	.02248	.000	.4385	.5849
	9	.4767*	.02248	.000	.4035	.5499
	10	.4083*	.02248	.000	.3351	.4815
6	2	.1900*	.02248	.000	.1168	.2632
	3	.0933*	.02248	.004	.0201	.1665
	4	.1850*	.02248	.000	.1118	.2582
	5	-.0583	.02248	.217	-.1315	.0149
	7	.2017*	.02248	.000	.1285	.2749
	8	.4533*	.02248	.000	.3801	.5265
	9	.4183*	.02248	.000	.3451	.4915
	10	.3500*	.02248	.000	.2768	.4232
7	2	-.0117	.02248	1.000	-.0849	.0615
	3	-.1083*	.02248	.001	-.1815	-.0351
	4	-.0167	.02248	.998	-.0899	.0565
	5	-.2600*	.02248	.000	-.3332	-.1868
	6	-.2017*	.02248	.000	-.2749	-.1285

	8	.2517*	.02248	.000	.1785	.3249
	9	.2167*	.02248	.000	.1435	.2899
	10	.1483*	.02248	.000	.0751	.2215
8	2	-.2633*	.02248	.000	-.3365	-.1901
	3	-.3600*	.02248	.000	-.4332	-.2868
	4	-.2683*	.02248	.000	-.3415	-.1951
	5	-.5117*	.02248	.000	-.5849	-.4385
	6	-.4533*	.02248	.000	-.5265	-.3801
	7	-.2517*	.02248	.000	-.3249	-.1785
	9	-.0350	.02248	.822	-.1082	.0382
	10	-.1033*	.02248	.001	-.1765	-.0301
9	2	-.2283*	.02248	.000	-.3015	-.1551
	3	-.3250*	.02248	.000	-.3982	-.2518
	4	-.2333*	.02248	.000	-.3065	-.1601
	5	-.4767*	.02248	.000	-.5499	-.4035
	6	-.4183*	.02248	.000	-.4915	-.3451
	7	-.2167*	.02248	.000	-.2899	-.1435
	8	.0350	.02248	.822	-.0382	.1082
	10	-.0683	.02248	.084	-.1415	.0049
10	2	-.1600*	.02248	.000	-.2332	-.0868
	3	-.2567*	.02248	.000	-.3299	-.1835
	4	-.1650*	.02248	.000	-.2382	-.0918
	5	-.4083*	.02248	.000	-.4815	-.3351
	6	-.3500*	.02248	.000	-.4232	-.2768
	7	-.1483*	.02248	.000	-.2215	-.0751
	8	.1033*	.02248	.001	.0301	.1765
	9	.0683	.02248	.084	-.0049	.1415

Based on observed means.
The error term is Mean Square(Error) = .002.
*. The mean difference is significant at the .05 level.

TTA Analysis

Statistical analysis of TTA of MSD, DBP, DAP and SDB

Descriptives

	Sample			Statistic	Std. Error
TTA	DAP	Mean		.8150	.03215
		95% Confidence Interval for Mean	Lower Bound	.7423	
			Upper Bound	.8877	
		5% Trimmed Mean		.8089	
		Median		.8200	
		Variance		.010	
		Std. Deviation		.10168	
		Minimum		.69	
		Maximum		1.05	
		Range		.36	
		Interquartile Range		.11	
		Skewness		1.171	.687
		Kurtosis		2.925	1.334
	DBP	Mean		.4480	.02004
		95% Confidence Interval for Mean	Lower Bound	.4027	
			Upper Bound	.4933	
		5% Trimmed Mean		.4461	
		Median		.4550	
		Variance		.004	
		Std. Deviation		.06339	

		Minimum	.36	
		Maximum	.57	
		Range	.21	
		Interquartile Range	.09	
		Skewness	.310	.687
		Kurtosis	.421	1.334
	MSD	Mean	1.1880	.03402
		95% Confidence Interval for Mean — Lower Bound	1.1110	
		95% Confidence Interval for Mean — Upper Bound	1.2650	
		5% Trimmed Mean	1.1872	
		Median	1.1500	
		Variance	.012	
		Std. Deviation	.10758	
		Minimum	1.05	
		Maximum	1.34	
		Range	.29	
		Interquartile Range	.21	
		Skewness	.324	.687
		Kurtosis	-1.658	1.334
	SDB	Mean	.5370	.01512
		95% Confidence Interval for Mean — Lower Bound	.5060	
		95% Confidence Interval for Mean — Upper Bound	.5681	
		5% Trimmed Mean	.5350	
		Median	.5100	
		Variance	.006	
		Std. Deviation	.07858	
		Minimum	.41	
		Maximum	.70	
		Range	.29	
		Interquartile Range	.12	
		Skewness	.416	.448
		Kurtosis	-.752	.872

Tests of Normality

		Kolmogorov-Smirnova			Shapiro-Wilk		
	Sample	Statistic	df	Sig.	Statistic	df	Sig.
TTA	DAP	.265	10	.044	.853	10	.063
	DBP	.125	10	.200*	.953	10	.703
	MSD	.205	10	.200*	.898	10	.206
	SDB	.153	27	.105	.956	27	.292

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

Multiple Comparisons Between MSD, DBP, DAP and SDB

Dependent Variable: TTA
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	.3670*	.03856	.000	.2647	.4693
	MSD	-.3730*	.03856	.000	-.4753	-.2707
	SDB	.2780*	.03191	.000	.1933	.3626
DBP	DAP	-.3670*	.03856	.000	-.4693	-.2647
	MSD	-.7400*	.03856	.000	-.8423	-.6377
	SDB	-.0890*	.03191	.036	-.1737	-.0044
MSD	DAP	.3730*	.03856	.000	.2707	.4753
	DBP	.7400*	.03856	.000	.6377	.8423

	SDB	.6510*	.03191	.000	.5663	.7356
SDB	DAP	-.2780*	.03191	.000	-.3626	-.1933
	DBP	.0890*	.03191	.036	.0044	.1737
	MSD	-.6510*	.03191	.000	-.7356	-.5663

Based on observed means.
The error term is Mean Square (Error) = .007.
*. The mean difference is significant at the .05 level.

a) Statistical analysis of TTA of MSD

Descriptives analysis

	Sample			Statistic	Std. Error
TTA	MSD	Mean		1.1880	.03402
		95% Confidence Interval for Mean	Lower Bound	1.1110	
			Upper Bound	1.2650	
		5% Trimmed Mean		1.1872	
		Median		1.1500	
		Variance		.012	
		Std. Deviation		.10758	
		Minimum		1.05	
		Maximum		1.34	
		Range		.29	
		Interquartile Range		.21	
		Skewness		.324	.687
		Kurtosis		-1.658	1.334

a. Sample = MSD

Tests of Normality

		Kolmogorov-Smirnovb			Shapiro-Wilk		
	Sample	Statistic	df	Sig.	Statistic	df	Sig.
TTA	MSD	.205	10	.200*	.898	10	.206

*. This is a lower bound of the true significance.
a. Sample = MSD
b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of MSD (TTA)

Dependent Variable: TTA
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.2000*	.04433	.023	.0245	.3755
	3	.2400*	.04433	.007	.0645	.4155
	4	.1900*	.04433	.031	.0145	.3655
	5	.2700*	.04433	.003	.0945	.4455
	6	.1550	.04433	.097	-.0205	.3305
	7	.0350	.04433	.997	-.1405	.2105
	8	.2350*	.04433	.008	.0595	.4105
	9	-.0150	.04433	1.000	-.1905	.1605
	10	.0650	.04433	.879	-.1105	.2405
2	1	-.2000*	.04433	.023	-.3755	-.0245
	3	.0400	.04433	.993	-.1355	.2155
	4	-.0100	.04433	1.000	-.1855	.1655
	5	.0700	.04433	.832	-.1055	.2455
	6	-.0450	.04433	.984	-.2205	.1305

	7	-.1650	.04433	.070	-.3405	.0105
	8	.0350	.04433	.997	-.1405	.2105
	9	-.2150*	.04433	.014	-.3905	-.0395
	10	-.1350	.04433	.182	-.3105	.0405
3	1	-.2400*	.04433	.007	-.4155	-.0645
	2	-.0400	.04433	.993	-.2155	.1355
	4	-.0500	.04433	.969	-.2255	.1255
	5	.0300	.04433	.999	-.1455	.2055
	6	-.0850	.04433	.661	-.2605	.0905
	7	-.2050*	.04433	.019	-.3805	-.0295
	8	-.0050	.04433	1.000	-.1805	.1705
	9	-.2550*	.04433	.004	-.4305	-.0795
	10	-.1750	.04433	.051	-.3505	.0005
4	1	-.1900*	.04433	.031	-.3655	-.0145
	2	.0100	.04433	1.000	-.1655	.1855
	3	.0500	.04433	.969	-.1255	.2255
	5	.0800	.04433	.722	-.0955	.2555
	6	-.0350	.04433	.997	-.2105	.1405
	7	-.1550	.04433	.097	-.3305	.0205
	8	.0450	.04433	.984	-.1305	.2205
	9	-.2050*	.04433	.019	-.3805	-.0295
	10	-.1250	.04433	.245	-.3005	.0505
5	1	-.2700*	.04433	.003	-.4455	-.0945
	2	-.0700	.04433	.832	-.2455	.1055
	3	-.0300	.04433	.999	-.2055	.1455
	4	-.0800	.04433	.722	-.2555	.0955
	6	-.1150	.04433	.326	-.2905	.0605
	7	-.2350*	.04433	.008	-.4105	-.0595
	8	-.0350	.04433	.997	-.2105	.1405
	9	-.2850*	.04433	.002	-.4605	-.1095
	10	-.2050*	.04433	.019	-.3805	-.0295
6	1	-.1550	.04433	.097	-.3305	.0205
	2	.0450	.04433	.984	-.1305	.2205
	3	.0850	.04433	.661	-.0905	.2605
	4	.0350	.04433	.997	-.1405	.2105
	5	.1150	.04433	.326	-.0605	.2905
	7	-.1200	.04433	.284	-.2955	.0555
	8	.0800	.04433	.722	-.0955	.2555
	9	-.1700	.04433	.060	-.3455	.0055
	10	-.0900	.04433	.600	-.2655	.0855
7	1	-.0350	.04433	.997	-.2105	.1405
	2	.1650	.04433	.070	-.0105	.3405
	3	.2050*	.04433	.019	.0295	.3805
	4	.1550	.04433	.097	-.0205	.3305
	5	.2350*	.04433	.008	.0595	.4105
	6	.1200	.04433	.284	-.0555	.2955
	8	.2000*	.04433	.023	.0245	.3755
	9	-.0500	.04433	.969	-.2255	.1255
	10	.0300	.04433	.999	-.1455	.2055
8	1	-.2350*	.04433	.008	-.4105	-.0595
	2	-.0350	.04433	.997	-.2105	.1405
	3	.0050	.04433	1.000	-.1705	.1805
	4	-.0450	.04433	.984	-.2205	.1305
	5	.0350	.04433	.997	-.1405	.2105
	6	-.0800	.04433	.722	-.2555	.0955
	7	-.2000*	.04433	.023	-.3755	-.0245
	9	-.2500*	.04433	.005	-.4255	-.0745

	10	-.1700	.04433	.060	-.3455	.0055
9	1	.0150	.04433	1.000	-.1605	.1905
	2	.2150*	.04433	.014	.0395	.3905
	3	.2550*	.04433	.004	.0795	.4305
	4	.2050*	.04433	.019	.0295	.3805
	5	.2850*	.04433	.002	.1095	.4605
	6	.1700	.04433	.060	-.0055	.3455
	7	.0500	.04433	.969	-.1255	.2255
	8	.2500*	.04433	.005	.0745	.4255
	10	.0800	.04433	.722	-.0955	.2555
10	1	-.0650	.04433	.879	-.2405	.1105
	2	.1350	.04433	.182	-.0405	.3105
	3	.1750	.04433	.051	-.0005	.3505
	4	.1250	.04433	.245	-.0505	.3005
	5	.2050*	.04433	.019	.0295	.3805
	6	.0900	.04433	.600	-.0855	.2655
	7	-.0300	.04433	.999	-.2055	.1455
	8	.1700	.04433	.060	-.0055	.3455
	9	-.0800	.04433	.722	-.2555	.0955

Based on observed means.
The error term is Mean Square (Error) = .002.
*. The mean difference is significant at the .05 level.

b) Statistical analysis of TTA of DBP

Descriptives a

	Sample			Statistic	Std. Error
TTA	DBP	Mean		.4480	.02004
		95% Confidence Interval for Mean	Lower Bound	.4027	
			Upper Bound	.4933	
		5% Trimmed Mean		.4461	
		Median		.4550	
		Variance		.004	
		Std. Deviation		.06339	
		Minimum		.36	
		Maximum		.57	
		Range		.21	
		Interquartile Range		.09	
		Skewness		.310	.687
		Kurtosis		.421	1.334

a. Sample = DBP

Tests of Normality a

		Kolmogorov-Smirnovb			Shapiro-Wilk		
	Sample	Statistic	df	Sig.	Statistic	df	Sig.
TTA	DBP	.125	10	.200*	.953	10	.703

*. This is a lower bound of the true significance.
a. Sample = DBP
b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of DBP (TTA)

Dependent Variable: TTA
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.0800	.02145	.069	-.1649	.0049
	3	.0900*	.02145	.036	.0051	.1749
	4	.1400*	.02145	.002	.0551	.2249
	5	.0200	.02145	.991	-.0649	.1049
	6	.0400	.02145	.690	-.0449	.1249
	7	-.3300*	.02145	.000	-.4149	-.2451
	8	.0300	.02145	.903	-.0549	.1149
	9	.1350*	.02145	.002	.0501	.2199
	10	.0650	.02145	.185	-.0199	.1499
2	1	.0800	.02145	.069	-.0049	.1649
	3	.1700*	.02145	.000	.0851	.2549
	4	.2200*	.02145	.000	.1351	.3049
	5	.1000*	.02145	.018	.0151	.1849
	6	.1200*	.02145	.005	.0351	.2049
	7	-.2500*	.02145	.000	-.3349	-.1651
	8	.1100*	.02145	.010	.0251	.1949
	9	.2150*	.02145	.000	.1301	.2999
	10	.1450*	.02145	.001	.0601	.2299
3	1	-.0900*	.02145	.036	-.1749	-.0051
	2	-.1700*	.02145	.000	-.2549	-.0851
	4	.0500	.02145	.443	-.0349	.1349
	5	-.0700	.02145	.134	-.1549	.0149
	6	-.0500	.02145	.443	-.1349	.0349
	7	-.4200*	.02145	.000	-.5049	-.3351
	8	-.0600	.02145	.253	-.1449	.0249
	9	.0450	.02145	.563	-.0399	.1299
	10	-.0250	.02145	.963	-.1099	.0599
4	1	-.1400*	.02145	.002	-.2249	-.0551
	2	-.2200*	.02145	.000	-.3049	-.1351
	3	-.0500	.02145	.443	-.1349	.0349
	5	-.1200*	.02145	.005	-.2049	-.0351
	6	-.1000*	.02145	.018	-.1849	-.0151
	7	-.4700*	.02145	.000	-.5549	-.3851
	8	-.1100*	.02145	.010	-.1949	-.0251
	9	-.0050	.02145	1.000	-.0899	.0799
	10	-.0750	.02145	.097	-.1599	.0099
5	1	-.0200	.02145	.991	-.1049	.0649
	2	-.1000*	.02145	.018	-.1849	-.0151
	3	.0700	.02145	.134	-.0149	.1549
	4	.1200*	.02145	.005	.0351	.2049
	6	.0200	.02145	.991	-.0649	.1049
	7	-.3500*	.02145	.000	-.4349	-.2651
	8	.0100	.02145	1.000	-.0749	.0949
	9	.1150*	.02145	.007	.0301	.1999
	10	.0450	.02145	.563	-.0399	.1299
6	1	-.0400	.02145	.690	-.1249	.0449
	2	-.1200*	.02145	.005	-.2049	-.0351
	3	.0500	.02145	.443	-.0349	.1349
	4	.1000*	.02145	.018	.0151	.1849
	5	-.0200	.02145	.991	-.1049	.0649
	7	-.3700*	.02145	.000	-.4549	-.2851
	8	-.0100	.02145	1.000	-.0949	.0749
	9	.0950*	.02145	.026	.0101	.1799
	10	.0250	.02145	.963	-.0599	.1099
7	1	.3300*	.02145	.000	.2451	.4149

	2	.2500*	.02145	.000	.1651	.3349
	3	.4200*	.02145	.000	.3351	.5049
	4	.4700*	.02145	.000	.3851	.5549
	5	.3500*	.02145	.000	.2651	.4349
	6	.3700*	.02145	.000	.2851	.4549
	8	.3600*	.02145	.000	.2751	.4449
	9	.4650*	.02145	.000	.3801	.5499
	10	.3950*	.02145	.000	.3101	.4799
8	1	-.0300	.02145	.903	-.1149	.0549
	2	-.1100*	.02145	.010	-.1949	-.0251
	3	.0600	.02145	.253	-.0249	.1449
	4	.1100*	.02145	.010	.0251	.1949
	5	-.0100	.02145	1.000	-.0949	.0749
	6	.0100	.02145	1.000	-.0749	.0949
	7	-.3600*	.02145	.000	-.4449	-.2751
	9	.1050*	.02145	.013	.0201	.1899
	10	.0350	.02145	.808	-.0499	.1199
9	1	-.1350*	.02145	.002	-.2199	-.0501
	2	-.2150*	.02145	.000	-.2999	-.1301
	3	-.0450	.02145	.563	-.1299	.0399
	4	.0050	.02145	1.000	-.0799	.0899
	5	-.1150*	.02145	.007	-.1999	-.0301
	6	-.0950*	.02145	.026	-.1799	-.0101
	7	-.4650*	.02145	.000	-.5499	-.3801
	8	-.1050*	.02145	.013	-.1899	-.0201
	10	-.0700	.02145	.134	-.1549	.0149
10	1	-.0650	.02145	.185	-.1499	.0199
	2	-.1450*	.02145	.001	-.2299	-.0601
	3	.0250	.02145	.963	-.0599	.1099
	4	.0750	.02145	.097	-.0099	.1599
	5	-.0450	.02145	.563	-.1299	.0399
	6	-.0250	.02145	.963	-.1099	.0599
	7	-.3950*	.02145	.000	-.4799	-.3101
	8	-.0350	.02145	.808	-.1199	.0499
	9	.0700	.02145	.134	-.0149	.1549

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

c) Statistical analysis of TTA of DAP

Descriptives a

		Sample		Statistic	Std. Error
TTA	DAP	Mean		.8150	.03215
		95% Confidence Interval for Mean	Lower Bound	.7423	
			Upper Bound	.8877	
		5% Trimmed Mean		.8089	
		Median		.8200	
		Variance		.010	
		Std. Deviation		.10168	
		Minimum		.69	
		Maximum		1.05	
		Range		.36	
		Interquartile Range		.11	
		Skewness		1.171	.687

	Kurtosis	2.925	1.334

a. Sample = DAP

Tests of Normality a

		Kolmogorov-Smirnovb			Shapiro-Wilk		
	Sample	Statistic	df	Sig.	Statistic	df	Sig.
TTA	DAP	.265	10	.044	.853	10	.063

a. Sample = DAP

b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks of DAP (TTA)

Dependent Variable: TTA

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.2450*	.04087	.003	-.4068	-.0832
	3	-.0250	.04087	1.000	-.1868	.1368
	4	-.0400	.04087	.987	-.2018	.1218
	5	.1100	.04087	.289	-.0518	.2718
	6	.1200	.04087	.210	-.0418	.2818
	7	.3400*	.04087	.000	.1782	.5018
	8	.0550	.04087	.919	-.1068	.2168
	9	-.0500	.04087	.951	-.2118	.1118
	10	-.0050	.04087	1.000	-.1668	.1568
2	1	.2450*	.04087	.003	.0832	.4068
	3	.2200*	.04087	.007	.0582	.3818
	4	.2050*	.04087	.011	.0432	.3668
	5	.3550*	.04087	.000	.1932	.5168
	6	.3650*	.04087	.000	.2032	.5268
	7	.5850*	.04087	.000	.4232	.7468
	8	.3000*	.04087	.001	.1382	.4618
	9	.1950*	.04087	.016	.0332	.3568
	10	.2400*	.04087	.004	.0782	.4018
3	1	.0250	.04087	1.000	-.1368	.1868
	2	-.2200*	.04087	.007	-.3818	-.0582
	4	-.0150	.04087	1.000	-.1768	.1468
	5	.1350	.04087	.127	-.0268	.2968
	6	.1450	.04087	.090	-.0168	.3068
	7	.3650*	.04087	.000	.2032	.5268
	8	.0800	.04087	.640	-.0818	.2418
	9	-.0250	.04087	1.000	-.1868	.1368
	10	.0200	.04087	1.000	-.1418	.1818
4	1	.0400	.04087	.987	-.1218	.2018
	2	-.2050*	.04087	.011	-.3668	-.0432
	3	.0150	.04087	1.000	-.1468	.1768
	5	.1500	.04087	.076	-.0118	.3118
	6	.1600	.04087	.053	-.0018	.3218
	7	.3800*	.04087	.000	.2182	.5418
	8	.0950	.04087	.446	-.0668	.2568
	9	-.0100	.04087	1.000	-.1718	.1518
	10	.0350	.04087	.995	-.1268	.1968
5	1	-.1100	.04087	.289	-.2718	.0518
	2	-.3550*	.04087	.000	-.5168	-.1932
	3	-.1350	.04087	.127	-.2968	.0268

	4	-.1500	.04087	.076	-.3118	.0118
	6	.0100	.04087	1.000	-.1518	.1718
	7	.2300*	.04087	.005	.0682	.3918
	8	-.0550	.04087	.919	-.2168	.1068
	9	-.1600	.04087	.053	-.3218	.0018
	10	-.1150	.04087	.247	-.2768	.0468
6	1	-.1200	.04087	.210	-.2818	.0418
	2	-.3650*	.04087	.000	-.5268	-.2032
	3	-.1450	.04087	.090	-.3068	.0168
	4	-.1600	.04087	.053	-.3218	.0018
	5	-.0100	.04087	1.000	-.1718	.1518
	7	.2200*	.04087	.007	.0582	.3818
	8	-.0650	.04087	.827	-.2268	.0968
	9	-.1700*	.04087	.037	-.3318	-.0082
	10	-.1250	.04087	.178	-.2868	.0368
7	1	-.3400*	.04087	.000	-.5018	-.1782
	2	-.5850*	.04087	.000	-.7468	-.4232
	3	-.3650*	.04087	.000	-.5268	-.2032
	4	-.3800*	.04087	.000	-.5418	-.2182
	5	-.2300*	.04087	.005	-.3918	-.0682
	6	-.2200*	.04087	.007	-.3818	-.0582
	8	-.2850*	.04087	.001	-.4468	-.1232
	9	-.3900*	.04087	.000	-.5518	-.2282
	10	-.3450*	.04087	.000	-.5068	-.1832
8	1	-.0550	.04087	.919	-.2168	.1068
	2	-.3000*	.04087	.001	-.4618	-.1382
	3	-.0800	.04087	.640	-.2418	.0818
	4	-.0950	.04087	.446	-.2568	.0668
	5	.0550	.04087	.919	-.1068	.2168
	6	.0650	.04087	.827	-.0968	.2268
	7	.2850*	.04087	.001	.1232	.4468
	9	-.1050	.04087	.336	-.2668	.0568
	10	-.0600	.04087	.878	-.2218	.1018
9	1	.0500	.04087	.951	-.1118	.2118
	2	-.1950*	.04087	.016	-.3568	-.0332
	3	.0250	.04087	1.000	-.1368	.1868
	4	.0100	.04087	1.000	-.1518	.1718
	5	.1600	.04087	.053	-.0018	.3218
	6	.1700*	.04087	.037	.0082	.3318
	7	.3900*	.04087	.000	.2282	.5518
	8	.1050	.04087	.336	-.0568	.2668
	10	.0450	.04087	.973	-.1168	.2068
10	1	.0050	.04087	1.000	-.1568	.1668
	2	-.2400*	.04087	.004	-.4018	-.0782
	3	-.0200	.04087	1.000	-.1818	.1418
	4	-.0350	.04087	.995	-.1968	.1268
	5	.1150	.04087	.247	-.0468	.2768
	6	.1250	.04087	.178	-.0368	.2868
	7	.3450*	.04087	.000	.1832	.5068
	8	.0600	.04087	.878	-.1018	.2218
	9	-.0450	.04087	.973	-.2068	.1168

Based on observed means.
The error term is Mean Square (Error) = .002.
*. The mean difference is significant at the .05 level.

d) Statistical analysis of TTA of SDB

Descriptive Statistics

Dependent Variable: pH

Week	Mean	Std. Deviation	N
2	4.3333	.02582	6
3	4.4300	.03098	6
4	4.3383	.05231	6
5	4.5817	.01472	6
6	4.5233	.08406	6
7	4.3217	.00753	6
8	4.0700	.02449	6
9	4.1050	.03619	6
10	4.1733	.00516	6
Total	4.3196	.17289	54

Tests of Normalitya

		Kolmogorov-Smirnovb			Shapiro-Wilk		
	Sample	Statistic	df	Sig.	Statistic	df	Sig.
TTA	SDB	.193	21	.039	.945	21	.271

a. There are no valid cases for TTA when Sample = .000.
Statistics cannot be computed for this level.
b. Lilliefors Significance Correction

Multiple Comparisons Between Weeks for SDB (TTA)

Dependent Variable: pH

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
2	3	-.0967*	.02248	.003	-.1699	-.0235
	4	-.0050	.02248	1.000	-.0782	.0682
	5	-.2483*	.02248	.000	-.3215	-.1751
	6	-.1900*	.02248	.000	-.2632	-.1168
	7	.0117	.02248	1.000	-.0615	.0849
	8	.2633*	.02248	.000	.1901	.3365
	9	.2283*	.02248	.000	.1551	.3015
	10	.1600*	.02248	.000	.0868	.2332
3	2	.0967*	.02248	.003	.0235	.1699
	4	.0917*	.02248	.005	.0185	.1649
	5	-.1517*	.02248	.000	-.2249	-.0785
	6	-.0933*	.02248	.004	-.1665	-.0201
	7	.1083*	.02248	.001	.0351	.1815
	8	.3600*	.02248	.000	.2868	.4332
	9	.3250*	.02248	.000	.2518	.3982
	10	.2567*	.02248	.000	.1835	.3299
4	2	.0050	.02248	1.000	-.0682	.0782
	3	-.0917*	.02248	.005	-.1649	-.0185
	5	-.2433*	.02248	.000	-.3165	-.1701
	6	-.1850*	.02248	.000	-.2582	-.1118
	7	.0167	.02248	.998	-.0565	.0899
	8	.2683*	.02248	.000	.1951	.3415
	9	.2333*	.02248	.000	.1601	.3065
	10	.1650*	.02248	.000	.0918	.2382
5	2	.2483*	.02248	.000	.1751	.3215

	3	.1517*	.02248	.000	.0785	.2249
	4	.2433*	.02248	.000	.1701	.3165
	6	.0583	.02248	.217	-.0149	.1315
	7	.2600*	.02248	.000	.1868	.3332
	8	.5117*	.02248	.000	.4385	.5849
	9	.4767*	.02248	.000	.4035	.5499
	10	.4083*	.02248	.000	.3351	.4815
6	2	.1900*	.02248	.000	.1168	.2632
	3	.0933*	.02248	.004	.0201	.1665
	4	.1850*	.02248	.000	.1118	.2582
	5	-.0583	.02248	.217	-.1315	.0149
	7	.2017*	.02248	.000	.1285	.2749
	8	.4533*	.02248	.000	.3801	.5265
	9	.4183*	.02248	.000	.3451	.4915
	10	.3500*	.02248	.000	.2768	.4232
7	2	-.0117	.02248	1.000	-.0849	.0615
	3	-.1083*	.02248	.001	-.1815	-.0351
	4	-.0167	.02248	.998	-.0899	.0565
	5	-.2600*	.02248	.000	-.3332	-.1868
	6	-.2017*	.02248	.000	-.2749	-.1285
	8	.2517*	.02248	.000	.1785	.3249
	9	.2167*	.02248	.000	.1435	.2899
	10	.1483*	.02248	.000	.0751	.2215
8	2	-.2633*	.02248	.000	-.3365	-.1901
	3	-.3600*	.02248	.000	-.4332	-.2868
	4	-.2683*	.02248	.000	-.3415	-.1951
	5	-.5117*	.02248	.000	-.5849	-.4385
	6	-.4533*	.02248	.000	-.5265	-.3801
	7	-.2517*	.02248	.000	-.3249	-.1785
	9	-.0350	.02248	.822	-.1082	.0382
	10	-.1033*	.02248	.001	-.1765	-.0301
9	2	-.2283*	.02248	.000	-.3015	-.1551
	3	-.3250*	.02248	.000	-.3982	-.2518
	4	-.2333*	.02248	.000	-.3065	-.1601
	5	-.4767*	.02248	.000	-.5499	-.4035
	6	-.4183*	.02248	.000	-.4915	-.3451
	7	-.2167*	.02248	.000	-.2899	-.1435
	8	.0350	.02248	.822	-.0382	.1082
	10	-.0683	.02248	.084	-.1415	.0049
10	2	-.1600*	.02248	.000	-.2332	-.0868
	3	-.2567*	.02248	.000	-.3299	-.1835
	4	-.1650*	.02248	.000	-.2382	-.0918
	5	-.4083*	.02248	.000	-.4815	-.3351
	6	-.3500*	.02248	.000	-.4232	-.2768
	7	-.1483*	.02248	.000	-.2215	-.0751
	8	.1033*	.02248	.001	.0301	.1765
	9	.0683	.02248	.084	-.0049	.1415

Based on observed means.
The error term is Mean Square(Error) = .002.
*. The mean difference is significant at the .05 level.

HPLC Analysis of Soluble Sugars and Organic Acids

a) Soluble Sugars and Organic Acids in DBP

Maltose of DBP

Descriptive Statistics

Dependent Variable: Maltose

Week	Mean	Std. Deviation	N
1	2.9100	.04243	2
2	2.8600	.04243	2
3	2.9400	.02828	2
Total	2.9033	.04676	6

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.[a,b]

a. Dependent variable: Maltose

b. Design: Intercept + Week

Multiple Comparisons of DBP (Maltose)

Dependent Variable: Maltose

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0500	.03830	.483	-.1100	.2100
	3	-.0300	.03830	.737	-.1900	.1300
2	1	-.0500	.03830	.483	-.2100	.1100
	3	-.0800	.03830	.239	-.2400	.0800
3	1	.0300	.03830	.737	-.1300	.1900
	2	.0800	.03830	.239	-.0800	.2400

Based on observed means.

The error term is Mean Square(Error) = .001.

Glucose content of DBP

Descriptive Statistics

Dependent Variable: Glucose

Week	Mean	Std. Deviation	N
1	.2150	.02121	2
2	.1600	.00000	2
3	.1500	.01414	2
Total	.1750	.03332	6

Multiple Comparisons of DBP (Glucose)

Dependent Variable: Glucose

Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0550	.01472	.066	-.0065	.1165
	3	.0650*	.01472	.043	.0035	.1265
2	1	-.0550	.01472	.066	-.1165	.0065
	3	.0100	.01472	.791	-.0515	.0715
3	1	-.0650*	.01472	.043	-.1265	-.0035
	2	-.0100	.01472	.791	-.0715	.0515

Based on observed means.

The error term is Mean Square(Error) = .000.

*. The mean difference is significant at the .05 level.

Lactic Acid content of DBP

Descriptive Statistics

Dependent Variable: Lacticacid

Week	Mean	Std. Deviation	N

1	.2409	.00837	2
2	.2154	.00176	2
3	.2293	.00230	2
Total	.2285	.01207	6

Multiple Comparisons of DBP (Lactic Acid)

Dependent Variable: Lacticacid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0255*	.00511	.031	.0041	.0468
	3	.0116	.00511	.204	-.0097	.0330
2	1	-.0255*	.00511	.031	-.0468	-.0041
	3	-.0138	.00511	.142	-.0352	.0075
3	1	-.0116	.00511	.204	-.0330	.0097
	2	.0138	.00511	.142	-.0075	.0352

Based on observed means.
The error term is Mean Square (Error) = 2.62E-005.
*. The mean difference is significant at the .05 level.

Acetic Acid content of DBP

Descriptive Statistics
Dependent Variable: Acetic acid

Week	Mean	Std. Deviation	N
1	.1100	.00000	2
2	.0950	.00707	2
3	.1050	.00707	2
Total	.1033	.00816	6

Multiple Comparisons of DBP (Acetic Acid)

Dependent Variable: Acetic acid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0150	.00577	.155	-.0091	.0391
	3	.0050	.00577	.695	-.0191	.0291
2	1	-.0150	.00577	.155	-.0391	.0091
	3	-.0100	.00577	.329	-.0341	.0141
3	1	-.0050	.00577	.695	-.0291	.0191
	2	.0100	.00577	.329	-.0141	.0341

Based on observed means.
The error term is Mean Square(Error) = 3.33E-005.

b) Soluble Sugars and Organic Acids in DAP

Maltose content of DAP

Descriptive Statistics

Dependent Variable: Maltose

Week	Mean	Std. Deviation	N

1	2.8550	.02121	2
2	2.7750	.04950	2
3	2.8700	.04243	2
Total	2.8333	.05502	6

Multiple Comparisons of DAP (Maltose)

Dependent Variable: Maltose
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0800	.03958	.254	-.0854	.2454
	3	-.0150	.03958	.926	-.1804	.1504
2	1	-.0800	.03958	.254	-.2454	.0854
	3	-.0950	.03958	.183	-.2604	.0704
3	1	.0150	.03958	.926	-.1504	.1804
	2	.0950	.03958	.183	-.0704	.2604

Based on observed means.
The error term is Mean Square (Error) = .002.

Glucose content of DAP

Descriptive Statistics

Dependent Variable: Glucose

Week	Mean	Std. Deviation	N
1	.1800	.00000	2
2	.1400	.00000	2
3	.2100	.01414	2
Total	.1767	.03204	6

Multiple Comparisons of DAP (Glucose)

Dependent Variable: Glucose
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0400*	.00816	.033	.0059	.0741
	3	-.0300	.00816	.069	-.0641	.0041
2	1	-.0400*	.00816	.033	-.0741	-.0059
	3	-.0700*	.00816	.007	-.1041	-.0359
3	1	.0300	.00816	.069	-.0041	.0641
	2	.0700*	.00816	.007	.0359	.1041

Based on observed means.
The error term is Mean Square(Error) = 6.667E-5.
*. The mean difference is significant at the .05 level.

Lactic Acid content of DAP

Descriptive Statistics

Dependent Variable: Lacticacid

Week	Mean	Std. Deviation	N
1	.2842	.00348	2
2	.2663	.00891	2

3	.2474	.00577	2
Total	.2659	.01723	6

Multiple Comparisons of DAP (Lactic Acid)

Dependent Variable: Lacticacid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0180	.00645	.133	-.0090	.0449
	3	.0369*	.00645	.022	.0099	.0638
2	1	-.0180	.00645	.133	-.0449	.0090
	3	.0189	.00645	.119	-.0080	.0459
3	1	-.0369*	.00645	.022	-.0638	-.0099
	2	-.0189	.00645	.119	-.0459	.0080

Based on observed means.
The error term is Mean Square (Error) = 4.158E-5.
*. The mean difference is significant at the .05 level.

Acetic Acid content of DAP

Descriptive Statistics
Dependent Variable: Aceticacid

Week	Mean	Std. Deviation	N
1	.1350	.00707	2
2	.1550	.00707	2
3	.1650	.00707	2
Total	.1517	.01472	6

Multiple Comparisons of DAP (Acetic Acid)

Dependent Variable: Aceticacid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.0200	.00707	.129	-.0495	.0095
	3	-.0300*	.00707	.048	-.0595	-.0005
2	1	.0200	.00707	.129	-.0095	.0495
	3	-.0100	.00707	.439	-.0395	.0195
3	1	.0300*	.00707	.048	.0005	.0595
	2	.0100	.00707	.439	-.0195	.0395

Based on observed means.
The error term is Mean Square(Error) = 5.000E-5.
*. The mean difference is significant at the .05 level.

a) Soluble Sugars and Organic Acids in SDB

Maltose content of SDB

Descriptive Statistics
Dependent Variable: Maltose

Week	Mean	Std. Deviation	N
1	2.9250	.09192	2
2	2.9050	.06364	2
3	2.9100	.01414	2

Total	2.9133	.05125	6

Multiple Comparisons of SDB (Maltose)

Dependent Variable: Maltose
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0200	.06506	.950	-.2519	.2919
	3	.0150	.06506	.971	-.2569	.2869
2	1	-.0200	.06506	.950	-.2919	.2519
	3	-.0050	.06506	.997	-.2769	.2669
3	1	-.0150	.06506	.971	-.2869	.2569
	2	.0050	.06506	.997	-.2669	.2769

Based on observed means.
The error term is Mean Square(Error) = .004.

Glucose content of SDB

Descriptive Statistics

Dependent Variable: Glucose

Week	Mean	Std. Deviation	N
1	.1500	.00000	2
2	.0900	.01414	2
3	.1700	.01414	2
Total	.1367	.03830	6

Multiple Comparisons of SDB (Glucose)

Dependent Variable: Glucose
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0600*	.01155	.028	.0117	.1083
	3	-.0200	.01155	.329	-.0683	.0283
2	1	-.0600*	.01155	.028	-.1083	-.0117
	3	-.0800*	.01155	.013	-.1283	-.0317
3	1	.0200	.01155	.329	-.0283	.0683
	2	.0800*	.01155	.013	.0317	.1283

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

Lactic Acid content of SDB

Descriptive Statistics

Dependent Variable: Lacticacid

Week	Mean	Std. Deviation	N
1	.2767	.00058	2
2	.2382	.00058	2
3	.2215	.00039	2
Total	.2455	.02530	6

Multiple Comparisons of SDB (Lactic Acid)

Dependent Variable: Lacticacid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.0384*	.00052	.000	.0363	.0406
	3	.0552*	.00052	.000	.0530	.0574
2	1	-.0384*	.00052	.000	-.0406	-.0363
	3	.0167*	.00052	.000	.0145	.0189
3	1	-.0552*	.00052	.000	-.0574	-.0530
	2	-.0167*	.00052	.000	-.0189	-.0145

Based on observed means.
The error term is Mean Square(Error) = 2.754E-7.
*. The mean difference is significant at the .05 level.

Acetic Acid content of SDB

Descriptive Statistics
Dependent Variable: Acetic acid

Week	Mean	Std. Deviation	N
1	.0950	.00707	2
2	.1450	.00707	2
3	.1600	.01414	2
Total	.1333	.03141	6

Multiple Comparisons of SDB (Acetic Acid)

Dependent Variable: Acetic acid
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.0500*	.01000	.031	-.0918	-.0082
	3	-.0650*	.01000	.015	-.1068	-.0232
2	1	.0500*	.01000	.031	.0082	.0918
	3	-.0150	.01000	.406	-.0568	.0268
3	1	.0650*	.01000	.015	.0232	.1068
	2	.0150	.01000	.406	-.0268	.0568

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

Multiple Comparisons of Maltose contents in sample batches 8, 9, and 10

Dependent Variable: Maltose week 8
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.0550	.05972	.666	-.3046	.1946
	SDB	-.0700	.05972	.543	-.3196	.1796
DBP	DAP	.0550	.05972	.666	-.1946	.3046
	SDB	-.0150	.05972	.966	-.2646	.2346
SDB	DAP	.0700	.05972	.543	-.1796	.3196
	DBP	.0150	.05972	.966	-.2346	.2646

Dependent Variable: Maltose week 9

Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.0850	.05260	.366	-.3048	.1348
	SDB	-.1300	.05260	.172	-.3498	.0898
DBP	DAP	.0850	.05260	.366	-.1348	.3048
	SDB	-.0450	.05260	.700	-.2648	.1748
SDB	DAP	.1300	.05260	.172	-.0898	.3498
	DBP	.0450	.05260	.700	-.1748	.2648

Dependent Variable: Maltose week 10

Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.0700	.03055	.201	-.1977	.0577
	SDB	-.0400	.03055	.481	-.1677	.0877
DBP	DAP	.0700	.03055	.201	-.0577	.1977
	SDB	.0300	.03055	.635	-.0977	.1577
SDB	DAP	.0400	.03055	.481	-.0877	.1677
	DBP	-.0300	.03055	.635	-.1577	.0977

Based on observed means.
The error term is Mean Square (Error) = .001.

Multiple Comparisons of glucose contents in sample batches 8, 9, and 10

Dependent Variable: Glucose week 8

Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.0350	.01225	.126	-.0862	.0162
	SDB	.0300	.01225	.175	-.0212	.0812
DBP	DAP	.0350	.01225	.126	-.0162	.0862
	SDB	.0650*	.01225	.026	.0138	.1162
SDB	DAP	-.0300	.01225	.175	-.0812	.0212
	DBP	-.0650*	.01225	.026	-.1162	-.0138

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

Dependent Variable: Glucose week 9

Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	-.0200	.00816	.175	-.0541	.0141
	SDB	.0500*	.00816	.018	.0159	.0841
DBP	DAP	.0200	.00816	.175	-.0141	.0541
	SDB	.0700*	.00816	.007	.0359	.1041
SDB	DAP	-.0500*	.00816	.018	-.0841	-.0159
	DBP	-.0700*	.00816	.007	-.1041	-.0359

Based on observed means.
The error term is Mean Square(Error) = 6.667E-5.
*. The mean difference is significant at the .05 level.
Dependent Variable: Glucose week 10
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
DAP	DBP	.0600*	.01414	.048	.0009	.1191
	SDB	.0400	.01414	.129	-.0191	.0991
DBP	DAP	-.0600*	.01414	.048	-.1191	-.0009
	SDB	-.0200	.01414	.439	-.0791	.0391
SDB	DAP	-.0400	.01414	.129	-.0991	.0191
	DBP	.0200	.01414	.439	-.0391	.0791

Based on observed means.
The error term is Mean Square (Error) = .000.
*. The mean difference is significant at the .05 level.

Multiple Comparisons of lactic acid content in sample batches 8, 9, and 10

Dependent Variable: Lactic acid week 8
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
DAP	DBP	.0450*	.00913	.032	.0069	.0831
	SDB	.0050	.00913	.855	-.0331	.0431
DBP	DAP	-.0450*	.00913	.032	-.0831	-.0069
	SDB	-.0400*	.00913	.044	-.0781	-.0019
SDB	DAP	-.0050	.00913	.855	-.0431	.0331
	DBP	.0400*	.00913	.044	.0019	.0781

Based on observed means.
The error term is Mean Square (Error) = 8.333E-5.
*. The mean difference is significant at the .05 level.

Dependent Variable: Lactic acid week 9
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
DAP	DBP	.0500*	.00577	.007	.0259	.0741
	SDB	.0250*	.00577	.046	.0009	.0491
DBP	DAP	-.0500*	.00577	.007	-.0741	-.0259
	SDB	-.0250*	.00577	.046	-.0491	-.0009
SDB	DAP	-.0250*	.00577	.046	-.0491	-.0009
	DBP	.0250*	.00577	.046	.0009	.0491

Dependent Variable: Lactic acid week 10
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
DAP	DBP	.0150	.00408	.069	-.0021	.0321
	SDB	.0250*	.00408	.018	.0079	.0421
DBP	DAP	-.0150	.00408	.069	-.0321	.0021
	SDB	.0100	.00408	.175	-.0071	.0271
SDB	DAP	-.0250*	.00408	.018	-.0421	-.0079
	DBP	-.0100	.00408	.175	-.0271	.0071

Based on observed means.
The error term is Mean Square (Error) = 1.667E-5.
*. The mean difference is significant at the .05 level.

Multiple Comparisons of acetic acid content in sample batches 8, 9, and 10

Dependent Variable: Acetic acid week 8
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound

DAP	DBP	.0250*	.00577	.046	.0009	.0491
	SDB	.0400*	.00577	.013	.0159	.0641
DBP	DAP	-.0250*	.00577	.046	-.0491	-.0009
	SDB	.0150	.00577	.155	-.0091	.0391
SDB	DAP	-.0400*	.00577	.013	-.0641	-.0159
	DBP	-.0150	.00577	.155	-.0391	.0091

Based on observed means.
The error term is Mean Square(Error) = 3.333E-5.
*. The mean difference is significant at the .05 level.

Dependent Variable: Acetic acid week 9
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	.0600*	.00707	.007	.0305	.0895
	SDB	.0100	.00707	.439	-.0195	.0395
DBP	DAP	-.0600*	.00707	.007	-.0895	-.0305
	SDB	-.0500*	.00707	.012	-.0795	-.0205
SDB	DAP	-.0100	.00707	.439	-.0395	.0195
	DBP	.0500*	.00707	.012	.0205	.0795

Based on observed means.
The error term is Mean Square(Error) = 5.000E-5.
*. The mean difference is significant at the .05 level.

Dependent Variable: Acetic acid week 10
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	.0600*	.01000	.019	.0182	.1018
	SDB	.0050	.01000	.877	-.0368	.0468
DBP	DAP	-.0600*	.01000	.019	-.1018	-.0182
	SDB	-.0550*	.01000	.024	-.0968	-.0132
SDB	DAP	-.0050	.01000	.877	-.0468	.0368
	DBP	.0550*	.01000	.024	.0132	.0968

Based on observed means.
The error term is Mean Square(Error) = .000.
*. The mean difference is significant at the .05 level.

Microbiological Analysis

Statistical analysis of anaerobic plate count (APC), lactic acid bacteria count on de Man, Rogosa and Sharpe (MRS) agar and yeast count on Yeast Extract Glucose Chloramphenicol Agar (YGC) of MSD, DBP and DAP

Descriptives

	Sample			Statistic	Std. Error
YGC	DAP	Mean		6.6728	.08420
		95% Confidence Interval for Mean	Lower Bound	6.4966	
			Upper Bound	6.8491	
		5% Trimmed Mean		6.6866	
		Median		6.8225	
		Variance		.142	
		Std. Deviation		.37655	
		Minimum		6.04	
		Maximum		7.06	

		Range		1.02	
		Interquartile Range		.60	
		Skewness		-.734	.512
		Kurtosis		-1.039	.992
	DBP	Mean		6.3901	.08808
		95% Confidence Interval for Mean	Lower Bound	6.2057	
			Upper Bound	6.5744	
		5% Trimmed Mean		6.4075	
		Median		6.5183	
		Variance		.155	
		Std. Deviation		.39391	
		Minimum		5.65	
		Maximum		6.81	
		Range		1.16	
		Interquartile Range		.65	
		Skewness		-.836	.512
		Kurtosis		-.733	.992
	MSD	Mean		5.4285	.15921
		95% Confidence Interval for Mean	Lower Bound	5.0952	
			Upper Bound	5.7617	
		5% Trimmed Mean		5.4095	
		Median		5.3010	
		Variance		.507	
		Std. Deviation		.71200	
		Minimum		4.30	
		Maximum		6.90	
		Range		2.60	
		Interquartile Range		.48	
		Skewness		.410	.512
		Kurtosis		.370	.992
LAB	DAP	Mean		8.5081	.11842
		95% Confidence Interval for Mean	Lower Bound	8.2603	
			Upper Bound	8.7560	
		5% Trimmed Mean		8.5068	
		Median		8.4572	
		Variance		.280	
		Std. Deviation		.52960	
		Minimum		7.72	
		Maximum		9.32	
		Range		1.61	
		Interquartile Range		.76	
		Skewness		.048	.512
		Kurtosis		-1.460	.992
	DBP	Mean		7.9447	.04321
		95% Confidence Interval for Mean	Lower Bound	7.8543	
			Upper Bound	8.0352	
		5% Trimmed Mean		7.9499	
		Median		7.9743	
		Variance		.037	
		Std. Deviation		.19323	
		Minimum		7.52	
		Maximum		8.28	
		Range		.76	
		Interquartile Range		.30	
		Skewness		-.402	.512
		Kurtosis		-.173	.992
	MSD	Mean		8.5960	.07101

		95% Confidence Interval for Mean	Lower Bound	8.4474	
			Upper Bound	8.7446	
		5% Trimmed Mean		8.5979	
		Median		8.6578	
		Variance		.101	
		Std. Deviation		.31756	
		Minimum		8.06	
		Maximum		9.10	
		Range		1.04	
		Interquartile Range		.51	
		Skewness		-.421	.512
		Kurtosis		-.839	.992
APC	DAP	Mean		8.4671	.13259
		95% Confidence Interval for Mean	Lower Bound	8.1896	
			Upper Bound	8.7447	
		5% Trimmed Mean		8.4746	
		Median		8.6231	
		Variance		.352	
		Std. Deviation		.59296	
		Minimum		7.46	
		Maximum		9.34	
		Range		1.88	
		Interquartile Range		1.11	
		Skewness		-.220	.512
		Kurtosis		-1.135	.992
	DBP	Mean		8.1227	.13405
		95% Confidence Interval for Mean	Lower Bound	7.8421	
			Upper Bound	8.4033	
		5% Trimmed Mean		8.0877	
		Median		7.8922	
		Variance		.359	
		Std. Deviation		.59950	
		Minimum		7.40	
		Maximum		9.48	
		Range		2.08	
		Interquartile Range		.84	
		Skewness		1.185	.512
		Kurtosis		.726	.992
	MSD	Mean		8.5583	.08439
		95% Confidence Interval for Mean	Lower Bound	8.3817	
			Upper Bound	8.7349	
		5% Trimmed Mean		8.5532	
		Median		8.4978	
		Variance		.142	
		Std. Deviation		.37740	
		Minimum		8.01	
		Maximum		9.20	
		Range		1.19	
		Interquartile Range		.69	
		Skewness		.284	.512
		Kurtosis		-1.201	.992

APC, MRS and YGC counts from MSD

Dependent Variable: Count

MSD	Mean	Std. Deviation	N

APC	8.5580	.38020	10
MRS	8.5960	.32565	10
YGC	5.4285	.72820	10
Total	7.5275	1.58793	30

Multiple Comparisons of MSD (APC MRS and YGC counts)

Dependent Variable: Count
Tukey HSD

(I) MSD	(J) MSD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
APC	MRS	-.0380	.22816	.985	-.6037	.5277
	YGC	3.1295*	.22816	.000	2.5638	3.6953
MRS	APC	.0380	.22816	.985	-.5277	.6037
	YGC	3.1675*	.22816	.000	2.6018	3.7333
YGC	APC	-3.1295*	.22816	.000	-3.6953	-2.5638
	MRS	-3.1675*	.22816	.000	-3.7333	-2.6018

Based on observed means.
The error term is Mean Square (Error) = .260.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for MSD (APC)

Dependent Variable: APC
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	10	-1.1435*	.10538	.000	-1.5606	-.7263
	2	-.1862	.10538	.742	-.6033	.2310
	3	-.3307	.10538	.160	-.7478	.0865
	4	-.0922	.10538	.994	-.5094	.3249
	5	-.4539*	.10538	.030	-.8711	-.0368
	6	-.6011*	.10538	.004	-1.0183	-.1840
	7	-.8589*	.10538	.000	-1.2761	-.4417
	8	-.5301*	.10538	.011	-.9473	-.1129
	9	-.9473*	.10538	.000	-1.3645	-.5302
10	1	1.1435*	.10538	.000	.7263	1.5606
	2	.9573*	.10538	.000	.5401	1.3745
	3	.8128*	.10538	.000	.3957	1.2300
	4	1.0513*	.10538	.000	.6341	1.4684
	5	.6896*	.10538	.002	.2724	1.1067
	6	.5424*	.10538	.009	.1252	.9595
	7	.2846	.10538	.286	-.1326	.7017
	8	.6134*	.10538	.004	.1962	1.0305
	9	.1961	.10538	.692	-.2210	.6133
2	1	.1862	.10538	.742	-.2310	.6033
	10	-.9573*	.10538	.000	-1.3745	-.5401
	3	-.1445	.10538	.912	-.5616	.2727
	4	.0940	.10538	.993	-.3232	.5111
	5	-.2677	.10538	.348	-.6849	.1494
	6	-.4149	.10538	.052	-.8321	.0022
	7	-.6727*	.10538	.002	-1.0899	-.2555
	8	-.3439	.10538	.135	-.7611	.0732
	9	-.7611*	.10538	.001	-1.1783	-.3440
3	1	.3307	.10538	.160	-.0865	.7478
	10	-.8128*	.10538	.000	-1.2300	-.3957
	2	.1445	.10538	.912	-.2727	.5616

	4	.2384	.10538	.477	-.1787	.6556
	5	-.1233	.10538	.962	-.5404	.2939
	6	-.2705	.10538	.338	-.6876	.1467
	7	-.5282*	.10538	.011	-.9454	-.1111
	8	-.1994	.10538	.675	-.6166	.2177
	9	-.6167*	.10538	.004	-1.0338	-.1995
4	1	.0922	.10538	.994	-.3249	.5094
	10	-1.0513*	.10538	.000	-1.4684	-.6341
	2	-.0940	.10538	.993	-.5111	.3232
	3	-.2384	.10538	.477	-.6556	.1787
	5	-.3617	.10538	.106	-.7789	.0555
	6	-.5089*	.10538	.015	-.9261	-.0917
	7	-.7667*	.10538	.001	-1.1838	-.3495
	8	-.4379*	.10538	.038	-.8550	-.0207
	9	-.8551*	.10538	.000	-1.2723	-.4379
5	1	.4539*	.10538	.030	.0368	.8711
	10	-.6896*	.10538	.002	-1.1067	-.2724
	2	.2677	.10538	.348	-.1494	.6849
	3	.1233	.10538	.962	-.2939	.5404
	4	.3617	.10538	.106	-.0555	.7789
	6	-.1472	.10538	.903	-.5644	.2700
	7	-.4050	.10538	.059	-.8221	.0122
	8	-.0762	.10538	.998	-.4933	.3410
	9	-.4934*	.10538	.018	-.9106	-.0762
6	1	.6011*	.10538	.004	.1840	1.0183
	10	-.5424*	.10538	.009	-.9595	-.1252
	2	.4149	.10538	.052	-.0022	.8321
	3	.2705	.10538	.338	-.1467	.6876
	4	.5089*	.10538	.015	.0917	.9261
	5	.1472	.10538	.903	-.2700	.5644
	7	-.2578	.10538	.389	-.6749	.1594
	8	.0710	.10538	.999	-.3461	.4882
	9	-.3462	.10538	.130	-.7634	.0710
7	1	.8589*	.10538	.000	.4417	1.2761
	10	-.2846	.10538	.286	-.7017	.1326
	2	.6727*	.10538	.002	.2555	1.0899
	3	.5282*	.10538	.011	.1111	.9454
	4	.7667*	.10538	.001	.3495	1.1838
	5	.4050	.10538	.059	-.0122	.8221
	6	.2578	.10538	.389	-.1594	.6749
	8	.3288	.10538	.164	-.0884	.7460
	9	-.0884	.10538	.995	-.5056	.3287
8	1	.5301*	.10538	.011	.1129	.9473
	10	-.6134*	.10538	.004	-1.0305	-.1962
	2	.3439	.10538	.135	-.0732	.7611
	3	.1994	.10538	.675	-.2177	.6166
	4	.4379*	.10538	.038	.0207	.8550
	5	.0762	.10538	.998	-.3410	.4933
	6	-.0710	.10538	.999	-.4882	.3461
	7	-.3288	.10538	.164	-.7460	.0884
	9	-.4172*	.10538	.050	-.8344	-.0001
9	1	.9473*	.10538	.000	.5302	1.3645
	10	-.1961	.10538	.692	-.6133	.2210
	2	.7611*	.10538	.001	.3440	1.1783
	3	.6167*	.10538	.004	.1995	1.0338
	4	.8551*	.10538	.000	.4379	1.2723
	5	.4934*	.10538	.018	.0762	.9106

	6	.3462	.10538	.130	-.0710	.7634
	7	.0884	.10538	.995	-.3287	.5056
	8	.4172*	.10538	.050	.0001	.8344

Based on observed means.
The error term is Mean Square (Error) = .011.
*. The mean difference is significant at the 0.05 level.

Multiple Comparisons Between Weeks for MSD (LAB Count)

Dependent Variable: LAB
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	10	-.6953*	.02912	.000	-.8105	-.5800
	2	-.2325*	.02912	.000	-.3478	-.1172
	3	-.0915	.02912	.159	-.2068	.0238
	4	-.5484*	.02912	.000	-.6637	-.4331
	5	-.7137*	.02912	.000	-.8290	-.5984
	6	-.5584*	.02912	.000	-.6737	-.4431
	7	-.8164*	.02912	.000	-.9317	-.7011
	8	-.6457*	.02912	.000	-.7610	-.5305
	9	-1.0142*	.02912	.000	-1.1295	-.8990
10	1	.6953*	.02912	.000	.5800	.8105
	2	.4628*	.02912	.000	.3475	.5781
	3	.6038*	.02912	.000	.4885	.7191
	4	.1469*	.02912	.011	.0316	.2622
	5	-.0184	.02912	.999	-.1337	.0969
	6	.1369*	.02912	.017	.0216	.2522
	7	-.1211*	.02912	.038	-.2364	-.0058
	8	.0495	.02912	.775	-.0658	.1648
	9	-.3190*	.02912	.000	-.4343	-.2037
2	1	.2325*	.02912	.000	.1172	.3478
	10	-.4628*	.02912	.000	-.5781	-.3475
	3	.1410*	.02912	.014	.0257	.2563
	4	-.3159*	.02912	.000	-.4312	-.2006
	5	-.4812*	.02912	.000	-.5965	-.3659
	6	-.3259*	.02912	.000	-.4412	-.2106
	7	-.5839*	.02912	.000	-.6992	-.4686
	8	-.4133*	.02912	.000	-.5286	-.2980
	9	-.7818*	.02912	.000	-.8971	-.6665
3	1	.0915	.02912	.159	-.0238	.2068
	10	-.6038*	.02912	.000	-.7191	-.4885
	2	-.1410*	.02912	.014	-.2563	-.0257
	4	-.4569*	.02912	.000	-.5722	-.3416
	5	-.6222*	.02912	.000	-.7375	-.5069
	6	-.4669*	.02912	.000	-.5822	-.3516
	7	-.7249*	.02912	.000	-.8402	-.6096
	8	-.5543*	.02912	.000	-.6696	-.4390
	9	-.9228*	.02912	.000	-1.0380	-.8075
4	1	.5484*	.02912	.000	.4331	.6637
	10	-.1469*	.02912	.011	-.2622	-.0316
	2	.3159*	.02912	.000	.2006	.4312
	3	.4569*	.02912	.000	.3416	.5722
	5	-.1653*	.02912	.005	-.2806	-.0500
	6	-.0100	.02912	1.000	-.1253	.1053
	7	-.2680*	.02912	.000	-.3833	-.1527
	8	-.0974	.02912	.120	-.2127	.0179

	9	-.4659*	.02912	.000	-.5811	-.3506
5	1	.7137*	.02912	.000	.5984	.8290
	10	.0184	.02912	.999	-.0969	.1337
	2	.4812*	.02912	.000	.3659	.5965
	3	.6222*	.02912	.000	.5069	.7375
	4	.1653*	.02912	.005	.0500	.2806
	6	.1553*	.02912	.007	.0400	.2706
	7	-.1027	.02912	.093	-.2180	.0126
	8	.0679	.02912	.442	-.0473	.1832
	9	-.3005*	.02912	.000	-.4158	-.1853
6	1	.5584*	.02912	.000	.4431	.6737
	10	-.1369*	.02912	.017	-.2522	-.0216
	2	.3259*	.02912	.000	.2106	.4412
	3	.4669*	.02912	.000	.3516	.5822
	4	.0100	.02912	1.000	-.1053	.1253
	5	-.1553*	.02912	.007	-.2706	-.0400
	7	-.2580*	.02912	.000	-.3733	-.1427
	8	-.0874	.02912	.193	-.2027	.0279
	9	-.4559*	.02912	.000	-.5712	-.3406
7	1	.8164*	.02912	.000	.7011	.9317
	10	.1211*	.02912	.038	.0058	.2364
	2	.5839*	.02912	.000	.4686	.6992
	3	.7249*	.02912	.000	.6096	.8402
	4	.2680*	.02912	.000	.1527	.3833
	5	.1027	.02912	.093	-.0126	.2180
	6	.2580*	.02912	.000	.1427	.3733
	8	.1706*	.02912	.004	.0553	.2859
	9	-.1979*	.02912	.001	-.3132	-.0826
8	1	.6457*	.02912	.000	.5305	.7610
	10	-.0495	.02912	.775	-.1648	.0658
	2	.4133*	.02912	.000	.2980	.5286
	3	.5543*	.02912	.000	.4390	.6696
	4	.0974	.02912	.120	-.0179	.2127
	5	-.0679	.02912	.442	-.1832	.0473
	6	.0874	.02912	.193	-.0279	.2027
	7	-.1706*	.02912	.004	-.2859	-.0553
	9	-.3685*	.02912	.000	-.4838	-.2532
9	1	1.0142*	.02912	.000	.8990	1.1295
	10	.3190*	.02912	.000	.2037	.4343
	2	.7818*	.02912	.000	.6665	.8971
	3	.9228*	.02912	.000	.8075	1.0380
	4	.4659*	.02912	.000	.3506	.5811
	5	.3005*	.02912	.000	.1853	.4158
	6	.4559*	.02912	.000	.3406	.5712
	7	.1979*	.02912	.001	.0826	.3132
	8	.3685*	.02912	.000	.2532	.4838

Based on observed means.
The error term is Mean Square (Error) = .001.
*. The mean difference is significant at the 0.05 level.

Multiple Comparisons Between Weeks for MSD (YGC Count)

Dependent Variable: YGC
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.3635	.09326	.055	-.7327	.0057

	3	.2071	.09326	.499	-.1621	.5763
	4	1.2870*	.09326	.000	.9178	1.6562
	5	1.3495*	.09326	.000	.9803	1.7187
	6	-1.1419*	.09326	.000	-1.5111	-.7727
	7	.4487*	.09326	.015	.0795	.8178
	8	.4487*	.09326	.015	.0795	.8178
	9	.4487*	.09326	.015	.0795	.8178
	10	.4168*	.09326	.024	.0477	.7860
2	1	.3635	.09326	.055	-.0057	.7327
	3	.5706*	.09326	.003	.2014	.9398
	4	1.6505*	.09326	.000	1.2813	2.0197
	5	1.7130*	.09326	.000	1.3438	2.0822
	6	-.7784*	.09326	.000	-1.1476	-.4092
	7	.8122*	.09326	.000	.4430	1.1813
	8	.8122*	.09326	.000	.4430	1.1813
	9	.8122*	.09326	.000	.4430	1.1813
	10	.7803*	.09326	.000	.4112	1.1495
3	1	-.2071	.09326	.499	-.5763	.1621
	2	-.5706*	.09326	.003	-.9398	-.2014
	4	1.0799*	.09326	.000	.7108	1.4491
	5	1.1424*	.09326	.000	.7732	1.5116
	6	-1.3490*	.09326	.000	-1.7182	-.9798
	7	.2416	.09326	.328	-.1276	.6108
	8	.2416	.09326	.328	-.1276	.6108
	9	.2416	.09326	.328	-.1276	.6108
	10	.2098	.09326	.484	-.1594	.5789
4	1	-1.2870*	.09326	.000	-1.6562	-.9178
	2	-1.6505*	.09326	.000	-2.0197	-1.2813
	3	-1.0799*	.09326	.000	-1.4491	-.7108
	5	.0625	.09326	.999	-.3067	.4316
	6	-2.4289*	.09326	.000	-2.7981	-2.0598
	7	-.8383*	.09326	.000	-1.2075	-.4692
	8	-.8383*	.09326	.000	-1.2075	-.4692
	9	-.8383*	.09326	.000	-1.2075	-.4692
	10	-.8702*	.09326	.000	-1.2394	-.5010
5	1	-1.3495*	.09326	.000	-1.7187	-.9803
	2	-1.7130*	.09326	.000	-2.0822	-1.3438
	3	-1.1424*	.09326	.000	-1.5116	-.7732
	4	-.0625	.09326	.999	-.4316	.3067
	6	-2.4914*	.09326	.000	-2.8606	-2.1222
	7	-.9008*	.09326	.000	-1.2700	-.5316
	8	-.9008*	.09326	.000	-1.2700	-.5316
	9	-.9008*	.09326	.000	-1.2700	-.5316
	10	-.9327*	.09326	.000	-1.3018	-.5635
6	1	1.1419*	.09326	.000	.7727	1.5111
	2	.7784*	.09326	.000	.4092	1.1476
	3	1.3490*	.09326	.000	.9798	1.7182
	4	2.4289*	.09326	.000	2.0598	2.7981
	5	2.4914*	.09326	.000	2.1222	2.8606
	7	1.5906*	.09326	.000	1.2214	1.9598
	8	1.5906*	.09326	.000	1.2214	1.9598
	9	1.5906*	.09326	.000	1.2214	1.9598
	10	1.5587*	.09326	.000	1.1896	1.9279
7	1	-.4487*	.09326	.015	-.8178	-.0795
	2	-.8122*	.09326	.000	-1.1813	-.4430
	3	-.2416	.09326	.328	-.6108	.1276
	4	.8383*	.09326	.000	.4692	1.2075

	5	.9008*	.09326	.000	.5316	1.2700
	6	-1.5906*	.09326	.000	-1.9598	-1.2214
	8	.0000	.09326	1.000	-.3692	.3692
	9	.0000	.09326	1.000	-.3692	.3692
	10	-.0318	.09326	1.000	-.4010	.3373
8	1	-.4487*	.09326	.015	-.8178	-.0795
	2	-.8122*	.09326	.000	-1.1813	-.4430
	3	-.2416	.09326	.328	-.6108	.1276
	4	.8383*	.09326	.000	.4692	1.2075
	5	.9008*	.09326	.000	.5316	1.2700
	6	-1.5906*	.09326	.000	-1.9598	-1.2214
	7	.0000	.09326	1.000	-.3692	.3692
	9	.0000	.09326	1.000	-.3692	.3692
	10	-.0318	.09326	1.000	-.4010	.3373
9	1	-.4487*	.09326	.015	-.8178	-.0795
	2	-.8122*	.09326	.000	-1.1813	-.4430
	3	-.2416	.09326	.328	-.6108	.1276
	4	.8383*	.09326	.000	.4692	1.2075
	5	.9008*	.09326	.000	.5316	1.2700
	6	-1.5906*	.09326	.000	-1.9598	-1.2214
	7	.0000	.09326	1.000	-.3692	.3692
	8	.0000	.09326	1.000	-.3692	.3692
	10	-.0318	.09326	1.000	-.4010	.3373
10	1	-.4168*	.09326	.024	-.7860	-.0477
	2	-.7803*	.09326	.000	-1.1495	-.4112
	3	-.2098	.09326	.484	-.5789	.1594
	4	.8702*	.09326	.000	.5010	1.2394
	5	.9327*	.09326	.000	.5635	1.3018
	6	-1.5587*	.09326	.000	-1.9279	-1.1896
	7	.0318	.09326	1.000	-.3373	.4010
	8	.0318	.09326	1.000	-.3373	.4010
	9	.0318	.09326	1.000	-.3373	.4010

Based on observed means.
The error term is Mean Square (Error) = .009.
*. The mean difference is significant at the .05 level.

a) APC, MRS and YGC counts for DBP

Descriptive Statistics

Dependent Variable: Count

DBP	Mean	Std. Deviation	N
APC	8.1210	.61387	10
MRS	7.9450	.19295	10
YGC	6.3901	.40083	10
Total	7.4854	.89677	30

Multiple Comparisons of DBP (APC, LAB and YGC Counts)

Dependent Variable: Count
Tukey HSD

(I) DBP	(J) DBP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
APC	MRS	.1760	.19574	.645	-.3093	.6613
	YGC	1.7309*	.19574	.000	1.2456	2.2162
MRS	APC	-.1760	.19574	.645	-.6613	.3093
	YGC	1.5549*	.19574	.000	1.0696	2.0402

YGC	APC	-1.7309*	.19574	.000	-2.2162	-1.2456
	MRS	-1.5549*	.19574	.000	-2.0402	-1.0696

Based on observed means.
The error term is Mean Square (Error) = .192.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DBP (APC)

Dependent Variable: APC
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.2816	.08733	.142	-.0641	.6274
	3	.1226	.08733	.901	-.2231	.4683
	4	.0298	.08733	1.000	-.3159	.3755
	5	-.8901*	.08733	.000	-1.2358	-.5444
	6	-.7855*	.08733	.000	-1.1312	-.4398
	7	-1.7124*	.08733	.000	-2.0581	-1.3667
	8	-.1726	.08733	.629	-.5183	.1731
	9	-.4366*	.08733	.011	-.7824	-.0909
	10	-.0165	.08733	1.000	-.3622	.3292
2	1	-.2816	.08733	.142	-.6274	.0641
	3	-.1590	.08733	.713	-.5047	.1867
	4	-.2518	.08733	.226	-.5976	.0939
	5	-1.1717*	.08733	.000	-1.5175	-.8260
	6	-1.0671*	.08733	.000	-1.4128	-.7214
	7	-1.9941*	.08733	.000	-2.3398	-1.6483
	8	-.4543*	.08733	.009	-.8000	-.1086
	9	-.7183*	.08733	.000	-1.0640	-.3726
	10	-.2982	.08733	.109	-.6439	.0476
3	1	-.1226	.08733	.901	-.4683	.2231
	2	.1590	.08733	.713	-.1867	.5047
	4	-.0928	.08733	.978	-.4385	.2529
	5	-1.0127*	.08733	.000	-1.3584	-.6670
	6	-.9081*	.08733	.000	-1.2538	-.5624
	7	-1.8350*	.08733	.000	-2.1807	-1.4893
	8	-.2952	.08733	.114	-.6410	.0505
	9	-.5593*	.08733	.002	-.9050	-.2135
	10	-.1391	.08733	.826	-.4848	.2066
4	1	-.0298	.08733	1.000	-.3755	.3159
	2	.2518	.08733	.226	-.0939	.5976
	3	.0928	.08733	.978	-.2529	.4385
	5	-.9199*	.08733	.000	-1.2656	-.5742
	6	-.8153*	.08733	.000	-1.1610	-.4696
	7	-1.7422*	.08733	.000	-2.0879	-1.3965
	8	-.2024	.08733	.450	-.5482	.1433
	9	-.4665*	.08733	.007	-.8122	-.1207
	10	-.0463	.08733	1.000	-.3920	.2994
5	1	.8901*	.08733	.000	.5444	1.2358
	2	1.1717*	.08733	.000	.8260	1.5175
	3	1.0127*	.08733	.000	.6670	1.3584
	4	.9199*	.08733	.000	.5742	1.2656
	6	.1046	.08733	.957	-.2411	.4503
	7	-.8223*	.08733	.000	-1.1680	-.4766
	8	.7175*	.08733	.000	.3718	1.0632
	9	.4535*	.08733	.009	.1077	.7992
	10	.8736*	.08733	.000	.5279	1.2193

6	1	.7855*	.08733	.000	.4398	1.1312
	2	1.0671*	.08733	.000	.7214	1.4128
	3	.9081*	.08733	.000	.5624	1.2538
	4	.8153*	.08733	.000	.4696	1.1610
	5	-.1046	.08733	.957	-.4503	.2411
	7	-.9269*	.08733	.000	-1.2726	-.5812
	8	.6128*	.08733	.001	.2671	.9585
	9	.3488*	.08733	.048	.0031	.6945
	10	.7690*	.08733	.000	.4232	1.1147
7	1	1.7124*	.08733	.000	1.3667	2.0581
	2	1.9941*	.08733	.000	1.6483	2.3398
	3	1.8350*	.08733	.000	1.4893	2.1807
	4	1.7422*	.08733	.000	1.3965	2.0879
	5	.8223*	.08733	.000	.4766	1.1680
	6	.9269*	.08733	.000	.5812	1.2726
	8	1.5398*	.08733	.000	1.1941	1.8855
	9	1.2758*	.08733	.000	.9300	1.6215
	10	1.6959*	.08733	.000	1.3502	2.0416
8	1	.1726	.08733	.629	-.1731	.5183
	2	.4543*	.08733	.009	.1086	.8000
	3	.2952	.08733	.114	-.0505	.6410
	4	.2024	.08733	.450	-.1433	.5482
	5	-.7175*	.08733	.000	-1.0632	-.3718
	6	-.6128*	.08733	.001	-.9585	-.2671
	7	-1.5398*	.08733	.000	-1.8855	-1.1941
	9	-.2640	.08733	.187	-.6097	.0817
	10	.1561	.08733	.731	-.1896	.5018
9	1	.4366*	.08733	.011	.0909	.7824
	2	.7183*	.08733	.000	.3726	1.0640
	3	.5593*	.08733	.002	.2135	.9050
	4	.4665*	.08733	.007	.1207	.8122
	5	-.4535*	.08733	.009	-.7992	-.1077
	6	-.3488*	.08733	.048	-.6945	-.0031
	7	-1.2758*	.08733	.000	-1.6215	-.9300
	8	.2640	.08733	.187	-.0817	.6097
	10	.4201*	.08733	.015	.0744	.7658
10	1	.0165	.08733	1.000	-.3292	.3622
	2	.2982	.08733	.109	-.0476	.6439
	3	.1391	.08733	.826	-.2066	.4848
	4	.0463	.08733	1.000	-.2994	.3920
	5	-.8736*	.08733	.000	-1.2193	-.5279
	6	-.7690*	.08733	.000	-1.1147	-.4232
	7	-1.6959*	.08733	.000	-2.0416	-1.3502
	8	-.1561	.08733	.731	-.5018	.1896
	9	-.4201*	.08733	.015	-.7658	-.0744

Based on observed means.
The error term is Mean Square(Error) = .008.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DBP (LAB Count)

Dependent Variable: LAB
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.2571	.06581	.054	-.0034	.5176
	3	-.1161	.06581	.743	-.3766	.1444

	4	-.0105	.06581	1.000	-.2710	.2500
	5	-.2754*	.06581	.036	-.5359	-.0149
	6	-.2570	.06581	.054	-.5175	.0035
	7	-.4020*	.06581	.003	-.6625	-.1414
	8	-.1480	.06581	.484	-.4085	.1125
	9	-.1898	.06581	.226	-.4503	.0707
	10	.0753	.06581	.967	-.1852	.3358
2	1	-.2571	.06581	.054	-.5176	.0034
	3	-.3733*	.06581	.005	-.6338	-.1127
	4	-.2676*	.06581	.043	-.5281	-.0071
	5	-.5325*	.06581	.000	-.7930	-.2720
	6	-.5141*	.06581	.000	-.7746	-.2536
	7	-.6591*	.06581	.000	-.9196	-.3986
	8	-.4051*	.06581	.002	-.6656	-.1446
	9	-.4469*	.06581	.001	-.7074	-.1864
	10	-.1818	.06581	.264	-.4423	.0787
3	1	.1161	.06581	.743	-.1444	.3766
	2	.3733*	.06581	.005	.1127	.6338
	4	.1056	.06581	.821	-.1549	.3661
	5	-.1593	.06581	.401	-.4198	.1013
	6	-.1409	.06581	.541	-.4014	.1196
	7	-.2858*	.06581	.029	-.5463	-.0253
	8	-.0319	.06581	1.000	-.2924	.2287
	9	-.0737	.06581	.971	-.3342	.1868
	10	.1914	.06581	.218	-.0691	.4519
4	1	.0105	.06581	1.000	-.2500	.2710
	2	.2676*	.06581	.043	.0071	.5281
	3	-.1056	.06581	.821	-.3661	.1549
	5	-.2649*	.06581	.045	-.5254	-.0044
	6	-.2465	.06581	.068	-.5070	.0140
	7	-.3915*	.06581	.003	-.6520	-.1309
	8	-.1375	.06581	.568	-.3980	.1230
	9	-.1793	.06581	.277	-.4398	.0812
	10	.0858	.06581	.931	-.1747	.3463
5	1	.2754*	.06581	.036	.0149	.5359
	2	.5325*	.06581	.000	.2720	.7930
	3	.1593	.06581	.401	-.1013	.4198
	4	.2649*	.06581	.045	.0044	.5254
	6	.0184	.06581	1.000	-.2421	.2789
	7	-.1266	.06581	.658	-.3871	.1339
	8	.1274	.06581	.651	-.1331	.3879
	9	.0856	.06581	.932	-.1749	.3461
	10	.3507*	.06581	.007	.0902	.6112
6	1	.2570	.06581	.054	-.0035	.5175
	2	.5141*	.06581	.000	.2536	.7746
	3	.1409	.06581	.541	-.1196	.4014
	4	.2465	.06581	.068	-.0140	.5070
	5	-.0184	.06581	1.000	-.2789	.2421
	7	-.1450	.06581	.508	-.4055	.1155
	8	.1090	.06581	.797	-.1515	.3695
	9	.0672	.06581	.983	-.1933	.3277
	10	.3323*	.06581	.011	.0718	.5928
7	1	.4020*	.06581	.003	.1414	.6625
	2	.6591*	.06581	.000	.3986	.9196
	3	.2858*	.06581	.029	.0253	.5463
	4	.3915*	.06581	.003	.1309	.6520
	5	.1266	.06581	.658	-.1339	.3871

	6	.1450	.06581	.508	-.1155	.4055
	8	.2540	.06581	.058	-.0065	.5145
	9	.2122	.06581	.142	-.0484	.4727
	10	.4773*	.06581	.001	.2167	.7378
8	1	.1480	.06581	.484	-.1125	.4085
	2	.4051*	.06581	.002	.1446	.6656
	3	.0319	.06581	1.000	-.2287	.2924
	4	.1375	.06581	.568	-.1230	.3980
	5	-.1274	.06581	.651	-.3879	.1331
	6	-.1090	.06581	.797	-.3695	.1515
	7	-.2540	.06581	.058	-.5145	.0065
	9	-.0418	.06581	.999	-.3023	.2187
	10	.2233	.06581	.112	-.0372	.4838
9	1	.1898	.06581	.226	-.0707	.4503
	2	.4469*	.06581	.001	.1864	.7074
	3	.0737	.06581	.971	-.1868	.3342
	4	.1793	.06581	.277	-.0812	.4398
	5	-.0856	.06581	.932	-.3461	.1749
	6	-.0672	.06581	.983	-.3277	.1933
	7	-.2122	.06581	.142	-.4727	.0484
	8	.0418	.06581	.999	-.2187	.3023
	10	.2651*	.06581	.045	.0046	.5256
10	1	-.0753	.06581	.967	-.3358	.1852
	2	.1818	.06581	.264	-.0787	.4423
	3	-.1914	.06581	.218	-.4519	.0691
	4	-.0858	.06581	.931	-.3463	.1747
	5	-.3507*	.06581	.007	-.6112	-.0902
	6	-.3323*	.06581	.011	-.5928	-.0718
	7	-.4773*	.06581	.001	-.7378	-.2167
	8	-.2233	.06581	.112	-.4838	.0372
	9	-.2651*	.06581	.045	-.5256	-.0046

Based on observed means.
The error term is Mean Square(Error) = .004.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DBP (YGC Count)

Dependent Variable: YGC
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.0756	.07497	.984	-.3724	.2211
	3	-.2785	.07497	.071	-.5753	.0183
	4	-.1717	.07497	.463	-.4685	.1251
	5	-.3463*	.07497	.020	-.6430	-.0495
	6	-.3868*	.07497	.009	-.6836	-.0900
	7	.3239*	.07497	.030	.0271	.6207
	8	.6579*	.07497	.000	.3612	.9547
	9	.6579*	.07497	.000	.3612	.9547
	10	-.3058*	.07497	.042	-.6026	-.0091
2	1	.0756	.07497	.984	-.2211	.3724
	3	-.2029	.07497	.284	-.4996	.0939
	4	-.0961	.07497	.937	-.3929	.2007
	5	-.2706	.07497	.082	-.5674	.0261
	6	-.3112*	.07497	.038	-.6079	-.0144
	7	.3995*	.07497	.007	.1028	.6963
	8	.7336*	.07497	.000	.4368	1.0304

	9	.7336*	.07497	.000	.4368	1.0304
	10	-.2302	.07497	.176	-.5270	.0666
3	1	.2785	.07497	.071	-.0183	.5753
	2	.2029	.07497	.284	-.0939	.4996
	4	.1068	.07497	.894	-.1900	.4036
	5	-.0678	.07497	.992	-.3645	.2290
	6	-.1083	.07497	.887	-.4051	.1885
	7	.6024*	.07497	.000	.3056	.8992
	8	.9364*	.07497	.000	.6397	1.2332
	9	.9364*	.07497	.000	.6397	1.2332
	10	-.0273	.07497	1.000	-.3241	.2694
4	1	.1717	.07497	.463	-.1251	.4685
	2	.0961	.07497	.937	-.2007	.3929
	3	-.1068	.07497	.894	-.4036	.1900
	5	-.1745	.07497	.445	-.4713	.1222
	6	-.2151	.07497	.230	-.5119	.0817
	7	.4956*	.07497	.001	.1989	.7924
	8	.8297*	.07497	.000	.5329	1.1264
	9	.8297*	.07497	.000	.5329	1.1264
	10	-.1341	.07497	.730	-.4309	.1626
5	1	.3463*	.07497	.020	.0495	.6430
	2	.2706	.07497	.082	-.0261	.5674
	3	.0678	.07497	.992	-.2290	.3645
	4	.1745	.07497	.445	-.1222	.4713
	6	-.0405	.07497	1.000	-.3373	.2562
	7	.6702*	.07497	.000	.3734	.9669
	8	1.0042*	.07497	.000	.7074	1.3010
	9	1.0042*	.07497	.000	.7074	1.3010
	10	.0404	.07497	1.000	-.2564	.3372
6	1	.3868*	.07497	.009	.0900	.6836
	2	.3112*	.07497	.038	.0144	.6079
	3	.1083	.07497	.887	-.1885	.4051
	4	.2151	.07497	.230	-.0817	.5119
	5	.0405	.07497	1.000	-.2562	.3373
	7	.7107*	.07497	.000	.4139	1.0075
	8	1.0448*	.07497	.000	.7480	1.3415
	9	1.0448*	.07497	.000	.7480	1.3415
	10	.0810	.07497	.976	-.2158	.3777
7	1	-.3239*	.07497	.030	-.6207	-.0271
	2	-.3995*	.07497	.007	-.6963	-.1028
	3	-.6024*	.07497	.000	-.8992	-.3056
	4	-.4956*	.07497	.001	-.7924	-.1989
	5	-.6702*	.07497	.000	-.9669	-.3734
	6	-.7107*	.07497	.000	-1.0075	-.4139
	8	.3340*	.07497	.025	.0373	.6308
	9	.3340*	.07497	.025	.0373	.6308
	10	-.6298*	.07497	.000	-.9265	-.3330
8	1	-.6579*	.07497	.000	-.9547	-.3612
	2	-.7336*	.07497	.000	-1.0304	-.4368
	3	-.9364*	.07497	.000	-1.2332	-.6397
	4	-.8297*	.07497	.000	-1.1264	-.5329
	5	-1.0042*	.07497	.000	-1.3010	-.7074
	6	-1.0448*	.07497	.000	-1.3415	-.7480
	7	-.3340*	.07497	.025	-.6308	-.0373
	9	.0000	.07497	1.000	-.2968	.2968
	10	-.9638*	.07497	.000	-1.2606	-.6670
9	1	-.6579*	.07497	.000	-.9547	-.3612

	2	-.7336*	.07497	.000	-1.0304	-.4368
	3	-.9364*	.07497	.000	-1.2332	-.6397
	4	-.8297*	.07497	.000	-1.1264	-.5329
	5	-1.0042*	.07497	.000	-1.3010	-.7074
	6	-1.0448*	.07497	.000	-1.3415	-.7480
	7	-.3340*	.07497	.025	-.6308	-.0373
	8	.0000	.07497	1.000	-.2968	.2968
	10	-.9638*	.07497	.000	-1.2606	-.6670
10	1	.3058*	.07497	.042	.0091	.6026
	2	.2302	.07497	.176	-.0666	.5270
	3	.0273	.07497	1.000	-.2694	.3241
	4	.1341	.07497	.730	-.1626	.4309
	5	-.0404	.07497	1.000	-.3372	.2564
	6	-.0810	.07497	.976	-.3777	.2158
	7	.6298*	.07497	.000	.3330	.9265
	8	.9638*	.07497	.000	.6670	1.2606
	9	.9638*	.07497	.000	.6670	1.2606

Based on observed means.
The error term is Mean Square(Error) = .006.
*. The mean difference is significant at the .05 level.

b) APC, MRS and YGC counts from DAP

Descriptive Statistics

Dependent Variable: Count

DAP	Mean	Std. Deviation	N
APC	8.4670	.60893	10
MRS	8.5090	.54221	10
YGC	6.6654	.37808	10
Total	7.8805	1.00728	30

Multiple Comparisons of DAP (APC, LAB and YGC Count)

Dependent Variable: Count
Tukey HSD

		Mean Difference			95% Confidence Interval	
(I) DAP	(J) DAP	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
APC	MRS	-.0420	.23205	.982	-.6174	.5334
	YGC	1.8016*	.23205	.000	1.2262	2.3769
MRS	APC	.0420	.23205	.982	-.5334	.6174
	YGC	1.8436*	.23205	.000	1.2682	2.4189
YGC	APC	-1.8016*	.23205	.000	-2.3769	-1.2262
	MRS	-1.8436*	.23205	.000	-2.4189	-1.2682

Based on observed means.
The error term is Mean Square (Error) = .269.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DAP (APC)

Dependent Variable: APC
Tukey HSD

		Mean Difference (I-			95% Confidence Interval	
(I) Week	(J) Week	J)	Std. Error	Sig.	Lower Bound	Upper Bound
1	2	-.3697*	.03841	.000	-.5217	-.2176
	3	-.4939*	.03841	.000	-.6460	-.3419
	4	-.6356*	.03841	.000	-.7877	-.4836

	5	-1.1956*	.03841	.000	-1.3476	-1.0435
	6	-1.1097*	.03841	.000	-1.2618	-.9577
	7	-1.6395*	.03841	.000	-1.7915	-1.4874
	8	-1.5884*	.03841	.000	-1.7405	-1.4363
	9	-1.8439*	.03841	.000	-1.9960	-1.6919
	10	-1.1711*	.03841	.000	-1.3231	-1.0190
2	1	.3697*	.03841	.000	.2176	.5217
	3	-.1242	.03841	.140	-.2763	.0278
	4	-.2660*	.03841	.001	-.4180	-.1139
	5	-.8259*	.03841	.000	-.9780	-.6738
	6	-.7400*	.03841	.000	-.8921	-.5880
	7	-1.2698*	.03841	.000	-1.4218	-1.1177
	8	-1.2187*	.03841	.000	-1.3708	-1.0667
	9	-1.4742*	.03841	.000	-1.6263	-1.3222
	10	-.8014*	.03841	.000	-.9534	-.6493
3	1	.4939*	.03841	.000	.3419	.6460
	2	.1242	.03841	.140	-.0278	.2763
	4	-.1417	.03841	.074	-.2938	.0103
	5	-.7017*	.03841	.000	-.8537	-.5496
	6	-.6158*	.03841	.000	-.7679	-.4637
	7	-1.1455*	.03841	.000	-1.2976	-.9935
	8	-1.0945*	.03841	.000	-1.2465	-.9424
	9	-1.3500*	.03841	.000	-1.5020	-1.1979
	10	-.6771*	.03841	.000	-.8292	-.5251
4	1	.6356*	.03841	.000	.4836	.7877
	2	.2660*	.03841	.001	.1139	.4180
	3	.1417	.03841	.074	-.0103	.2938
	5	-.5599*	.03841	.000	-.7120	-.4079
	6	-.4741*	.03841	.000	-.6261	-.3220
	7	-1.0038*	.03841	.000	-1.1559	-.8518
	8	-.9528*	.03841	.000	-1.1048	-.8007
	9	-1.2083*	.03841	.000	-1.3603	-1.0562
	10	-.5354*	.03841	.000	-.6875	-.3834
5	1	1.1956*	.03841	.000	1.0435	1.3476
	2	.8259*	.03841	.000	.6738	.9780
	3	.7017*	.03841	.000	.5496	.8537
	4	.5599*	.03841	.000	.4079	.7120
	6	.0859	.03841	.491	-.0662	.2379
	7	-.4439*	.03841	.000	-.5959	-.2918
	8	-.3928*	.03841	.000	-.5449	-.2408
	9	-.6483*	.03841	.000	-.8004	-.4963
	10	.0245	.03841	.999	-.1275	.1766
6	1	1.1097*	.03841	.000	.9577	1.2618
	2	.7400*	.03841	.000	.5880	.8921
	3	.6158*	.03841	.000	.4637	.7679
	4	.4741*	.03841	.000	.3220	.6261
	5	-.0859	.03841	.491	-.2379	.0662
	7	-.5297*	.03841	.000	-.6818	-.3777
	8	-.4787*	.03841	.000	-.6307	-.3266
	9	-.7342*	.03841	.000	-.8862	-.5821
	10	-.0613	.03841	.824	-.2134	.0907
7	1	1.6395*	.03841	.000	1.4874	1.7915
	2	1.2698*	.03841	.000	1.1177	1.4218
	3	1.1455*	.03841	.000	.9935	1.2976
	4	1.0038*	.03841	.000	.8518	1.1559
	5	.4439*	.03841	.000	.2918	.5959
	6	.5297*	.03841	.000	.3777	.6818

	8	.0511	.03841	.924	-.1010	.2031
	9	-.2045*	.03841	.007	-.3565	-.0524
	10	.4684*	.03841	.000	.3163	.6204
8	1	1.5884*	.03841	.000	1.4363	1.7405
	2	1.2187*	.03841	.000	1.0667	1.3708
	3	1.0945*	.03841	.000	.9424	1.2465
	4	.9528*	.03841	.000	.8007	1.1048
	5	.3928*	.03841	.000	.2408	.5449
	6	.4787*	.03841	.000	.3266	.6307
	7	-.0511	.03841	.924	-.2031	.1010
	9	-.2555*	.03841	.001	-.4076	-.1034
	10	.4173*	.03841	.000	.2653	.5694
9	1	1.8439*	.03841	.000	1.6919	1.9960
	2	1.4742*	.03841	.000	1.3222	1.6263
	3	1.3500*	.03841	.000	1.1979	1.5020
	4	1.2083*	.03841	.000	1.0562	1.3603
	5	.6483*	.03841	.000	.4963	.8004
	6	.7342*	.03841	.000	.5821	.8862
	7	.2045*	.03841	.007	.0524	.3565
	8	.2555*	.03841	.001	.1034	.4076
	10	.6728*	.03841	.000	.5208	.8249
10	1	1.1711*	.03841	.000	1.0190	1.3231
	2	.8014*	.03841	.000	.6493	.9534
	3	.6771*	.03841	.000	.5251	.8292
	4	.5354*	.03841	.000	.3834	.6875
	5	-.0245	.03841	.999	-.1766	.1275
	6	.0613	.03841	.824	-.0907	.2134
	7	-.4684*	.03841	.000	-.6204	-.3163
	8	-.4173*	.03841	.000	-.5694	-.2653
	9	-.6728*	.03841	.000	-.8249	-.5208

Based on observed means.
The error term is Mean Square(Error) = .001.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DAP (LAB Count)

Dependent Variable: LAB
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	-.1640*	.03439	.016	-.3001	-.0279
	3	-.4405*	.03439	.000	-.5767	-.3044
	4	-.4237*	.03439	.000	-.5598	-.2876
	5	-.9840*	.03439	.000	-1.1202	-.8479
	6	-.4401*	.03439	.000	-.5762	-.3040
	7	-1.1671*	.03439	.000	-1.3032	-1.0310
	8	-1.1558*	.03439	.000	-1.2920	-1.0197
	9	-1.5723*	.03439	.000	-1.7085	-1.4362
	10	-1.3743*	.03439	.000	-1.5104	-1.2381
2	1	.1640*	.03439	.016	.0279	.3001
	3	-.2765*	.03439	.000	-.4127	-.1404
	4	-.2597*	.03439	.000	-.3958	-.1236
	5	-.8200*	.03439	.000	-.9562	-.6839
	6	-.2761*	.03439	.000	-.4122	-.1400
	7	-1.0031*	.03439	.000	-1.1392	-.8670
	8	-.9918*	.03439	.000	-1.1279	-.8557
	9	-1.4083*	.03439	.000	-1.5444	-1.2722
	10	-1.2103*	.03439	.000	-1.3464	-1.0741

3	1	.4405*	.03439	.000	.3044	.5767
	2	.2765*	.03439	.000	.1404	.4127
	4	.0168	.03439	1.000	-.1193	.1529
	5	-.5435*	.03439	.000	-.6796	-.4074
	6	.0005	.03439	1.000	-.1357	.1366
	7	-.7266*	.03439	.000	-.8627	-.5905
	8	-.7153*	.03439	.000	-.8514	-.5792
	9	-1.1318*	.03439	.000	-1.2679	-.9957
	10	-.9337*	.03439	.000	-1.0698	-.7976
4	1	.4237*	.03439	.000	.2876	.5598
	2	.2597*	.03439	.000	.1236	.3958
	3	-.0168	.03439	1.000	-.1529	.1193
	5	-.5603*	.03439	.000	-.6964	-.4242
	6	-.0164	.03439	1.000	-.1525	.1198
	7	-.7434*	.03439	.000	-.8795	-.6073
	8	-.7321*	.03439	.000	-.8682	-.5960
	9	-1.1486*	.03439	.000	-1.2847	-1.0125
	10	-.9505*	.03439	.000	-1.0867	-.8144
5	1	.9840*	.03439	.000	.8479	1.1202
	2	.8200*	.03439	.000	.6839	.9562
	3	.5435*	.03439	.000	.4074	.6796
	4	.5603*	.03439	.000	.4242	.6964
	6	.5440*	.03439	.000	.4078	.6801
	7	-.1831*	.03439	.007	-.3192	-.0470
	8	-.1718*	.03439	.012	-.3079	-.0357
	9	-.5883*	.03439	.000	-.7244	-.4522
	10	-.3902*	.03439	.000	-.5263	-.2541
6	1	.4401*	.03439	.000	.3040	.5762
	2	.2761*	.03439	.000	.1400	.4122
	3	-.0005	.03439	1.000	-.1366	.1357
	4	.0164	.03439	1.000	-.1198	.1525
	5	-.5440*	.03439	.000	-.6801	-.4078
	7	-.7270*	.03439	.000	-.8632	-.5909
	8	-.7158*	.03439	.000	-.8519	-.5796
	9	-1.1322*	.03439	.000	-1.2684	-.9961
	10	-.9342*	.03439	.000	-1.0703	-.7981
7	1	1.1671*	.03439	.000	1.0310	1.3032
	2	1.0031*	.03439	.000	.8670	1.1392
	3	.7266*	.03439	.000	.5905	.8627
	4	.7434*	.03439	.000	.6073	.8795
	5	.1831*	.03439	.007	.0470	.3192
	6	.7270*	.03439	.000	.5909	.8632
	8	.0113	.03439	1.000	-.1248	.1474
	9	-.4052*	.03439	.000	-.5413	-.2691
	10	-.2071*	.03439	.003	-.3433	-.0710
8	1	1.1558*	.03439	.000	1.0197	1.2920
	2	.9918*	.03439	.000	.8557	1.1279
	3	.7153*	.03439	.000	.5792	.8514
	4	.7321*	.03439	.000	.5960	.8682
	5	.1718*	.03439	.012	.0357	.3079
	6	.7158*	.03439	.000	.5796	.8519
	7	-.0113	.03439	1.000	-.1474	.1248
	9	-.4165*	.03439	.000	-.5526	-.2804
	10	-.2184*	.03439	.002	-.3545	-.0823
9	1	1.5723*	.03439	.000	1.4362	1.7085
	2	1.4083*	.03439	.000	1.2722	1.5444
	3	1.1318*	.03439	.000	.9957	1.2679

	4	1.1486*	.03439	.000	1.0125	1.2847
	5	.5883*	.03439	.000	.4522	.7244
	6	1.1322*	.03439	.000	.9961	1.2684
	7	.4052*	.03439	.000	.2691	.5413
	8	.4165*	.03439	.000	.2804	.5526
	10	.1981*	.03439	.004	.0620	.3342
10	1	1.3743*	.03439	.000	1.2381	1.5104
	2	1.2103*	.03439	.000	1.0741	1.3464
	3	.9337*	.03439	.000	.7976	1.0698
	4	.9505*	.03439	.000	.8144	1.0867
	5	.3902*	.03439	.000	.2541	.5263
	6	.9342*	.03439	.000	.7981	1.0703
	7	.2071*	.03439	.003	.0710	.3433
	8	.2184*	.03439	.002	.0823	.3545
	9	-.1981*	.03439	.004	-.3342	-.0620

Based on observed means.
The error term is Mean Square(Error) = .001.
*. The mean difference is significant at the .05 level.

Multiple Comparisons Between Weeks for DAP (YGC Count)

Dependent Variable: YGC
Tukey HSD

(I) Week	(J) Week	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	10	-.6551*	.09028	.001	-1.0125	-.2977
	2	-.2208	.09028	.389	-.5782	.1366
	3	-.5769*	.09028	.002	-.9343	-.2194
	4	-.3455	.09028	.060	-.7029	.0119
	5	-.6096*	.09028	.001	-.9670	-.2522
	6	-.5792*	.09028	.002	-.9366	-.2218
	7	-.4415*	.09028	.013	-.7989	-.0841
	8	.3395	.09028	.067	-.0179	.6969
	9	.3395	.09028	.067	-.0179	.6969
10	1	.6551*	.09028	.001	.2977	1.0125
	2	.4342*	.09028	.015	.0768	.7916
	3	.0782	.09028	.994	-.2792	.4356
	4	.3096	.09028	.107	-.0478	.6670
	5	.0454	.09028	1.000	-.3120	.4028
	6	.0759	.09028	.995	-.2815	.4333
	7	.2136	.09028	.427	-.1438	.5710
	8	.9945*	.09028	.000	.6371	1.3519
	9	.9945*	.09028	.000	.6371	1.3519
2	1	.2208	.09028	.389	-.1366	.5782
	10	-.4342*	.09028	.015	-.7916	-.0768
	3	-.3560	.09028	.051	-.7134	.0014
	4	-.1247	.09028	.909	-.4821	.2327
	5	-.3888*	.09028	.030	-.7462	-.0314
	6	-.3583*	.09028	.049	-.7158	-.0009
	7	-.2207	.09028	.390	-.5781	.1368
	8	.5603*	.09028	.002	.2029	.9177
	9	.5603*	.09028	.002	.2029	.9177
3	1	.5769*	.09028	.002	.2194	.9343
	10	-.0782	.09028	.994	-.4356	.2792
	2	.3560	.09028	.051	-.0014	.7134
	4	.2314	.09028	.339	-.1260	.5888
	5	-.0328	.09028	1.000	-.3902	.3246

	6	-.0023	.09028	1.000	-.3597	.3551
	7	.1354	.09028	.866	-.2220	.4928
	8	.9163*	.09028	.000	.5589	1.2737
	9	.9163*	.09028	.000	.5589	1.2737
4	1	.3455	.09028	.060	-.0119	.7029
	10	-.3096	.09028	.107	-.6670	.0478
	2	.1247	.09028	.909	-.2327	.4821
	3	-.2314	.09028	.339	-.5888	.1260
	5	-.2641	.09028	.213	-.6216	.0933
	6	-.2337	.09028	.329	-.5911	.1237
	7	-.0960	.09028	.978	-.4534	.2614
	8	.6849*	.09028	.000	.3275	1.0423
	9	.6849*	.09028	.000	.3275	1.0423
5	1	.6096*	.09028	.001	.2522	.9670
	10	-.0454	.09028	1.000	-.4028	.3120
	2	.3888*	.09028	.030	.0314	.7462
	3	.0328	.09028	1.000	-.3246	.3902
	4	.2641	.09028	.213	-.0933	.6216
	6	.0305	.09028	1.000	-.3270	.3879
	7	.1682	.09028	.691	-.1893	.5256
	8	.9491*	.09028	.000	.5917	1.3065
	9	.9491*	.09028	.000	.5917	1.3065
6	1	.5792*	.09028	.002	.2218	.9366
	10	-.0759	.09028	.995	-.4333	.2815
	2	.3583*	.09028	.049	.0009	.7158
	3	.0023	.09028	1.000	-.3551	.3597
	4	.2337	.09028	.329	-.1237	.5911
	5	-.0305	.09028	1.000	-.3879	.3270
	7	.1377	.09028	.855	-.2197	.4951
	8	.9186*	.09028	.000	.5612	1.2760
	9	.9186*	.09028	.000	.5612	1.2760
7	1	.4415*	.09028	.013	.0841	.7989
	10	-.2136	.09028	.427	-.5710	.1438
	2	.2207	.09028	.390	-.1368	.5781
	3	-.1354	.09028	.866	-.4928	.2220
	4	.0960	.09028	.978	-.2614	.4534
	5	-.1682	.09028	.691	-.5256	.1893
	6	-.1377	.09028	.855	-.4951	.2197
	8	.7809*	.09028	.000	.4235	1.1383
	9	.7809*	.09028	.000	.4235	1.1383
8	1	-.3395	.09028	.067	-.6969	.0179
	10	-.9945*	.09028	.000	-1.3519	-.6371
	2	-.5603*	.09028	.002	-.9177	-.2029
	3	-.9163*	.09028	.000	-1.2737	-.5589
	4	-.6849*	.09028	.000	-1.0423	-.3275
	5	-.9491*	.09028	.000	-1.3065	-.5917
	6	-.9186*	.09028	.000	-1.2760	-.5612
	7	-.7809*	.09028	.000	-1.1383	-.4235
	9	.0000	.09028	1.000	-.3574	.3574
9	1	-.3395	.09028	.067	-.6969	.0179
	10	-.9945*	.09028	.000	-1.3519	-.6371
	2	-.5603*	.09028	.002	-.9177	-.2029
	3	-.9163*	.09028	.000	-1.2737	-.5589
	4	-.6849*	.09028	.000	-1.0423	-.3275
	5	-.9491*	.09028	.000	-1.3065	-.5917
	6	-.9186*	.09028	.000	-1.2760	-.5612
	7	-.7809*	.09028	.000	-1.1383	-.4235

	8	.0000	.09028	1.000	-.3574	.3574

Based on observed means.
The error term is Mean Square(Error) = .008.
*. The mean difference is significant at the 0.05 level.

Microbiological differences between MSD, DBP and DAP

a) Lactic acid bacteria plate count on MRS of MSD, DBP and DAP

Descriptive Statistics
Dependent Variable: Count

Sample	Mean	Std. Deviation	N
DAP	8.5090	.54221	10
DBP	7.9450	.19295	10
MSD	8.5960	.32565	10
Total	8.3500	.47101	30

Multiple Comparisons of LAB between MSD, DBP and DAP

Dependent Variable: Count
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	.5640*	.17074	.007	.1407	.9873
	MSD	-.0870	.17074	.867	-.5103	.3363
DBP	DAP	-.5640*	.17074	.007	-.9873	-.1407
	MSD	-.6510*	.17074	.002	-1.0743	-.2277
MSD	DAP	.0870	.17074	.867	-.3363	.5103
	DBP	.6510*	.17074	.002	.2277	1.0743

Based on observed means.
The error term is Mean Square(Error) = .146.
*. The mean difference is significant at the .05 level.

b) Anaerobic plate count of MSD, DBP and DAP

Descriptive Statistics

Dependent Variable: Anaerobic Plate Count

Sample	Mean	Std. Deviation	N
DAP	8.4670	.60893	10
DBP	8.1210	.61387	10
MSD	8.5580	.38020	10
Total	8.3820	.55995	30

Multiple Comparisons of APC Between MSD, DBP and DAP

Dependent Variable: Count
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DAP	DBP	.3460	.24388	.346	-.2587	.9507
	MSD	-.0910	.24388	.926	-.6957	.5137
DBP	DAP	-.3460	.24388	.346	-.9507	.2587
	MSD	-.4370	.24388	.191	-1.0417	.1677
MSD	DAP	.0910	.24388	.926	-.5137	.6957
	DBP	.4370	.24388	.191	-.1677	1.0417

Based on observed means.
The error term is Mean Square(Error) = .297.

c) Yeast plate count of MSD, DBP and DAP

Descriptive Statistics

Dependent Variable: Count

Sample	Mean	Std. Deviation	N
DAP	6.6654	.37808	10
DBP	6.3901	.40083	10
MSD	5.4285	.72820	10
Total	6.1613	.74139	30

Multiple Comparisons of YGC Count Between MSD, DBP and DAP

Dependent Variable: Count
Tukey HSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
DAP	DBP	.2753	.23578	.482	-.3093	.8599
	MSD	1.2370*	.23578	.000	.6524	1.8216
DBP	DAP	-.2753	.23578	.482	-.8599	.3093
	MSD	.9616*	.23578	.001	.3770	1.5462
MSD	DAP	-1.2370*	.23578	.000	-1.8216	-.6524
	DBP	-.9616*	.23578	.001	-1.5462	-.3770

Based on observed means.
The error term is Mean Square(Error) = .278.
*. The mean difference is significant at the .05 level.

Principle Component Analysis among pH, TTA, APC, LAB and yeast Counts

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
pH	4.3130	.47257	60
TTA	.8168	.31832	60
Yeast count	6.1638	.73985	60
LAB count	8.3496	.46849	60
APC	8.3827	.55733	60

Correlation Matrix

		pH	TTA	YGC	LAB	APC
Correlation	pH	1.000	-.887	.371	-.674	-.435
	TTA	-.887	1.000	-.500	.526	.297
	Yeast Count	.371	-.500	1.000	-.307	-.188
	LAB Count	-.674	.526	-.307	1.000	.789
	APC	-.435	.297	-.188	.789	1.000

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.635
Bartlett's Test of Sphericity	Approx. Chi-Square	202.216
	df	10

	Sig.	.000

Communalities

	Initial	Extraction
pH	1.000	.831
TTA	1.000	.874
Yeast Count	1.000	.596
LAB Count	1.000	.902
APC	1.000	.887

Extraction Method: Principal Component Analysis.

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings	
Component	Total	% of Variance	Cumulative %	Total	% of Variance
1	3.052	61.048	61.048	3.052	61.048
2	1.038	20.756	81.805	1.038	20.756
3	.662	13.246	95.051		
4	.169	3.372	98.422		
5	.079	1.578	100.000		