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The potential of wheat and rye sourdough starter cultures to produce functional and nutritional components during sourdough fermentation

A thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology

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ABSTRACT

Production of sourdough bread has been part of human culture for over 5000 years worldwide. The traditional sourdough technology involves the use of different flours mixed with water to make dough which is then allowed to ferment at ambient temperature using inherent microorganisms. It is common practice to use previously fermented sourdough as a starter culture to produce consistent products. The demand for sourdough bread has increased due to the unique sensory properties of the products and their perceived health properties. The aim of the study was to investigate the potential of sourdough fermentation by wheat and rye sourdough starters to increase the concentration of folate and resistant starch in sourdough and sourdough bread. The specific objectives were to: 1) analyse the composition of wheat and rye sourdough starter cultures; 2) optimise the fermentation conditions of the sourdough fermentation process, 3) analyse sugars; organic acids and the content of folate and resistant starch in the sourdough and sourdough bread.

In phase 1, lactic acid bacteria (LAB) and yeast were isolated from the original wheat sourdough starter and rye sourdough starter, obtained from a local artisan producer. Representative LAB and yeast strains were subjected to sequence analysis of 16S rRNA gene the intergenic transcribed spacer (ITS), respectively. Total titratable acid (TTA), pH and rheology of sourdough and sourdough bread were also determined. Colour of bread crumb and crust, loaf weight and volume of sourdough bread were measured. Eight isolates were identified from the original wheat and rye sourdough starters of which, two LAB and two yeast were each isolated from the wheat sourdough starter and from the rye sourdough starter. *Fructilactobacillus* (*F.*) *sanfranciscensis* was the dominant LAB in the wheat sourdough starter, whereas *Saccharomyces* (*S.*) *paradoxus* and *Torulaspora* (*T.*) *delbrueckii* were the dominant yeast. In the rye sourdough starter, *F. sanfranciscensis* and *Latilactobacillus* (*L.*) *curvatus* were dominant, while the main yeast was *Saccharomyces* (*S.*) *paradoxus* and *Saccharomyces* (*S.*) *kudriavzevii*.

Phase 2, aimed to select optimised fermentation conditions by fermenting sourdoughs. Three temperatures – (27°C, 29°C, 31°C) were used to the ferment sourdoughs using back-slopping culture of wheat sourdough starter and rye sourdough starter. Sensory evaluation of the bread was conducted by focus groups. Physico-chemical properties, colour measurement and loaf characteristics were conducted to sourdoughs and sourdough bread as phase 1. pH of final sourdough bread fermented at 27°C, 29°C, 31°C were 3.75 ± 0.01, 3.86 ± 0.01 and 3.91 ± 0.01.

Sourdough bread fermented at 27 °C/8.5 h had more gentle sourness, flavour and aroma compared to the other two treatments fermented at 29°C and 31°C. This is probably caused by more flavour and aroma synthesised by yeast at lower temperatures fermentation (25–28°C), and the more flavouring-enhancing volatile compounds accumulated by lower pH in sourdough bread.

In phase 3, the optimised fermentation conditions (27°C/8.5 h) were used to produce sourdough and sourdough bread. Physico-chemical properties, colour measurement and loaf characteristics were conducted to sourdoughs and sourdough bread as phase 1. Additional analysis of sugars (glucose, sucrose, fructose, maltose) and organic acids (lactic acid, acetic acid), folate and starch (dry matter basis, DM) were performed for sourdough and sourdough bread during fermentation. Total folate reduced from 6.00 µg/100 g in DBP to < 3.00 µg/100 g in DAP (DM), during fermentation of sourdough using the optimum process conditions (27°C for 8.5 h). The vitamin increased to 5.48 µg/100 g in sourdough bread (SDB) after baking. Resistant starch increased in SDB after baking. The decrease of folate in the dough samples during fermentation possibly due to: 1) LAB may be metabolising the vitamin during fermentation resulting in a reduction; or 2) the low pH of the dough may be suppressing the synthesis of folate and increasing degradation of labile folates during fermentation. The resistant starch in sourdough bread may be mainly (resistant) starch type 3 as baking at high temperatures (>120°C) catalyse the gelatinization of starch and the formation of retrograded amylose.

F. sanfranciscensis, *S. paradoxus* and *Torulaspora* (*T.*) *delbrueckii* were the first time found in wheat sourdough starter, while *F. sanfranciscensis, Lactobacillus* (*L.*) *curvatus Saccharomyces* (*S.*) *paradoxus* and *Saccharomyces* (*S.*) *kudriavzevii* were dominant in rye sourdough starter. These findings can assist artisanal and industrial bakery to develop better sourdough fermentation process for controlling the quality of sourdough bread. Moreover, LAB and yeast species isolated from two spontaneous sourdough starters can be used in sourdough-based baking products such as biscuits, cookies, crackers, pastry, pizza, and pasta to obtain final products with better sensory quality. They may also be used in other industrial sectors including cosmetics and pharmaceuticals in New Zealand. Furthermore, the optimum fermentation process (27°C/8.5 h) can be applied to industry as a standard operation procedure for producing sourdough bread with consistent and high overall quality. Additionally, total folate and resistant starch decreased during sourdough fermentation using both the wheat and rye sourdough starters. However, the content of these two nutrients apparently increased in the final baked sourdough bread. The mechanisms surrounding the formation of folate and resistant starch in sourdough bread are not clearly understood, hence more research is required. Current findings of nutritional compounds provide a possibility to bakery industry to develop a sourdough fermentation process which focus on increase of the nutritional and functional compounds in the future.

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LIST OF SYMBOLS AND ABBREVIATIONS

a *	=	Redness to greenness
AACC	=	American Association of Cereal Chemists
Abbreviation	=	Definition
ACE	=	Angiotensin I-converting enzyme
ADI	=	Arginine deaminase
ADP	=	Adenosine diphosphate
AFLP	=	Amplified fragment length polymorphism
AMG	=	Amyloglucosidase
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemist
ATP	=	Adenosine triphosphate
b*	=	Yellowness to blueness
CD	=	Celiac disease
cf	=	Compare
CFU g−1	=	Colony forming unit per gram
CK	=	Carbamate kinase
cm^3 /g	=	Cubic centimeter per gram
CoA	=	Coenzyme A
CVD	=	Control high blood pressure and cardiovascular disease
DAP	=	Dough-after-proofing
DBP	=	Dough-before-proofing
DNA	=	Deoxyribonucleic acid
DTT	=	0.1% dithiothreitol
DY	=	Dough yield
EMP	=	Emden-Meyerhoff-Parnas
EPS	=	Exopolysaccharides
EU	=	European Union
FAA	=	Free amino acid
FQ	=	Fermentation quotient
g	=	Gram
GABA	=	γ-amino butyric acid
GI	=	Glycaemic index
GOPOD	=	Glucose determination
h	=	Hour
H2O	=	dihydrogen oxide
H_2O_2	=	Hydrogen peroxide
H2SO4	=	Sulfuric acid
HPLC	=	High performance liquid chromatography
II	=	Insulin index
IS	=	Internal standard
ITS	=	Intergenic transcribed spacer

KHP	=	Potassium hydrogen phthalate
KOH	=	Potassium hydroxide
L	=	Litres
L*	=	Lightness
LAB	=	Lactic acid bacteria
LC	=	Liquid chromograph
m/ v	=	Weight/volume
mg/ml	=	Milligrams per milliliter
min	=	Minute
ml	=	Milliliter
mm	=	Millimeters
mm/s	=	Millimeters per second
mol/L	=	Mole per litre
MRS	=	De Man, Rogosa and Sharpe
MW	=	Molecular weight
N	=	Newton
NAD	=	Nicotinamide adenine dinucleotide
NADH	=	Nicotinamide adenine dinucleotide hydride
NaOH	=	Sodium hydroxide
NCBI	=	National Center for Biotechnology Information
OTC	=	Ornithine carbamoyl transferase
PCR	=	Polymerase chain reaction
PFGE	=	Pulsed-field gel electrophoresis
PG/PK	=	Phosphogluconate/phosphoketolase
PPC	=	Pentose phosphate cycle
REA	=	Restriction Enzyme Analysis
RFLP	=	Restriction fragment length polymorphism
RI	=	Refractive index
rpm	=	Revolutions per minute
rRNA	=	Ribosomal ribonucleic acid
RSS	=	Rye sourdough starter
RT	=	Room temperature
s	=	Seconds
SAX	=	Strong anion exchange
SDB	=	Sourdough bread
TCA	=	Tricarboxylic acid cycle
TTA	=	Total titratable acidity
UHPLC-MS/MS	=	Ultra-high performance liquid chromatography
UV	=	Ultraviolet
µg/ml	=	Microgram per milliliter
µm	=	Micrometer
µl	=	Microliter
v/ v	=	Volume/volume
WHO	=	World Health Organization

WSS	=	Wheat sourdough starter
YGC	=	Yeast Glucose Chloramphenicol
YPD	=	Yeast Peptone Dextrose

CHAPTER 1 INTRODUCTION

Cereals have been used as fundamental sources of nutrients in bread products for more than 10,000 years (Catzeddu, 2019), and spontaneous fermentation of sourdough for leavening bread was utilised in ancient Egypt (Feuillet et al., 2008). In recent years, the sourdough market has been increasing at a compounded annual growth rate (CAGR) of 5.7% and it is estimated to reach USD 3.5 billion by 2025 (Grand View Research, 2019). This increase has been driven primarily by increased consumer demand for natural and preservative-free baked products (Diowksz & Ambroziak, 2007).

The sourdough starter culture is generated during fermentation of a mixture of water and flour by the indigenous microorganisms present in the environment (Samuel, 1996). The sourdough microbial community is thus complex, with more than 50 species of lactic acid bacteria (LAB) and 20 species of yeast having been identified in sourdough worldwide (Lau et al., 2021)(. The majority of the isolated LAB species in sourdough cultures are the genus *Lactobacillus* (Catzeddu, 2019). The symbiotic metabolism of LAB and yeast during sourdough fermentation leads to the production of sourdough bread with better texture and a longer shelf-life, compared to bread leavened by yeast only (Cauvain, 2015). Furthermore, the metabolic activities of sourdough cultures during fermentation affect the formation of nutritional and functional compounds (Pérez-Alvarado et al., 2022). such as exopolysaccharides (EPS), folate and resistant starch in the sourdough bread, which are reported to be beneficial to human health (Atkinson et al., 2008).

The majority of sourdough research has focused on the sensory properties, shelf-life of sourdough bread, and the identification of the microbial community in the sourdough starter cultures (Baye et al., 2013; Cavallo et al., 2017; Fujimoto et al., 2019; Koistinen et al., 2018; Lhomme et al., 2015; Liu et al., 2016; Scheirlinck et al., 2007; Ua-Arak et al., 2017). In New Zealand, only three studies have been conducted on the physico-chemical characteristics of sourdough, and the diversity of the sourdough starter microbiota (Landis et al., 2021; Limbad et al., 2020; Tamani et al., 2013). Therefore, there is limited published information on the microbiota of New Zealand sourdough cultures and the formation of functional compounds such as folate and resistant starch. Furthermore, there is scanty information on the fermentation characteristics of wheat sourdough and sourdough bread produced by combinations of wheat sourdough starter and rye sourdough starter. Such knowledge would provide New Zealand sourdough artisans with a better understanding of the fermentation characteristics of the wheat-rye sourdough starter. This may assist the bakery to find better processes to control the sourdough fermentation, producing sourdough bread of a more consistent high quality. Information on whether certain wheat-rye sourdough starter can produce potential functional and nutritional compounds may assist the bakery to select the sourdough starter for produce sourdough bread with specific nutrients.

The current project aims to determine the microbial composition of two New Zealand sourdough starter cultures and investigate the fermentation characteristics, the functional and nutritional compounds (total folate content and resistant starch content) of the resultant sourdough and sourdough bread.

The specific objectives:

1. To isolate LAB and yeast from wheat sourdough starter and rye sourdough starter;
2. To identify the species and strains of the isolated sourdough LAB and yeast by ribosomal genotyping;
3. To optimise the fermentation time and temperature of the wheat sourdough bread process;
4. To determine the effect of fermentation temperatures and time on the physico-chemical properties (acidity and texture) of sourdough;
5. To determine the effect of fermentation temperatures and time on the physicochemical properties (acidity, specific volume, colour and texture) and sensory attributes of resultant sourdough bread;
6. To determine levels of lactic acid, acetic acid, fructose, sucrose, maltose and glucose in sourdough/sourdough bread produced under optimised fermentation conditions, and,
7. To analyse the levels of folate and resistant starch in sourdough and sourdough bread.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

Traditional sourdough is made from a mixture of different flours and water, spontaneously fermented by lactic acid bacteria (LAB) and yeast (Hammes & Gänzle, 1998a). The spontaneous sourdough fermentation gives an aromatic and pleasing flavour to the sourdough bread. It also improves the overall quality, structure, shelf-life and the nutritional composition of the final product (Table 2.1) (Chavan & Chavan, 2011; Gobbetti & Gänzle, 2013).

Table 2.1 Comparison of sourdough bread and baker's yeast bread

Characteristic	Sourdough bread	Baker's yeast bread
pH	3.8-4.6	5.3-5.8
Lactic acid	0.4-0.8%	0.005-0.04%
Acetic acid	0.10-0.40%	0.005-0.04%
Bread volume	0.22-0.30	≤0.20
Flavour	Complex aroma and flavour	
Staling	Slow	Rapid
Shelf-life	Good protection against microbial contamination	High sensibility to bacteria and mould spoilage
Nutritional composition	Optimal phytase activity and hydrolysis of phytic acid responsible for ion (Ca^{2+}, Fe^{2+}, Mg^{2+} etc.) binding	Low phytase, decalcifying effect
	Free amino acid concentrations (FAA) increase	Free amino acid concentrations (FAA) similar to that of flour
	Reduced glycaemic index compared to yeast leavened bread	

Adapted from Handbook on Sourdough Biotechnology, in Corsetti (2013), Technology of Sourdough Fermentation and Sourdough Applications, 85–104, *reproduced with permission from Springer Nature.*

Compared with the rheology and the sensory properties of grain leavened product, the potential of sourdough fermentation to enhance functional and nutritional characteristics of cereal leavened bread has received much less attention (De Vuyst et al., 2016; Gobbetti et al., 2014; Zannini & Arendt, 2018; Zhao et al., 2016). This literature review discusses the diversity of the sourdough ecosystem, metabolic activities of sourdough microbiota, the content of functional and nutritional compounds during fermentation and the health benefits of sourdough consumption.

2.2 The physico-chemical properties of sourdough

The physico-chemical properties of sourdough are influenced by the composition of the sourdough microorganisms. The evaluation of dough yield (DY), dough acidity (pH and total titratable acidity, TTA), and fermentation quotient (FQ) can be used to determine the physico-chemical properties of sourdough which are directly associated with sourdough quality (Corsetti, 2013; Martín-Garcia et al., 2021).

2.2.1 Dough yield (DY)

Dough yield refers to the ratio of flour to water in the dough, which is normally used to maintain dough consistency. Generally, the DY of a firm wheat flour sourdough is 150 to 160, which is considered a low DY, while a liquid sourdough is close to 200; considered a high DY (Corsetti, 2013). Sourdough with intermediate DY values can be considered as a soft dough (Decock & Cappelle, 2005).

2.2.2 Acetic acid, pH and total titratable acidity

Organic acids such as acetic acid naturally present in sourdough can influence the characteristics of sourdough bread such as shelf-life, flavour and antifungal properties (Corsetti, 2013). The interaction between LAB and yeast affects the concentration of acetic acid in the sourdough (Brandt, 2007; Gobbetti et al., 2005). At low temperatures (< 32°C), the yeast hydrolyse ketose and other fructo-oligosaccharides, releasing fructose which is metabolised by the LAB to produce acetic acid. However, at high temperatures (>32°C), yeast growth is inhibited, leaving residual fructo-oligosaccharides unhydrolysed. These intact fructo-oligosaccharides limit the availability of fructose for LAB, thus limiting acetic acid production by LAB (Gobbetti et al., 2005).

Sourdough with a final pH of 3.5 - 4.3 is considered a well-developed sourdough. The pH of the sourdough is influenced by the amount of sourdough starter inoculum used to initiate fermentation.

With a standard inoculum of 20% , the final pH of the dough can range from 4.7 to 5.4 (Esteve et al., 1994).

Total titratable acidity (TTA) is a measure of the total organic acids produced during sourdough fermentation. The TTA for liquid sourdough can range from 30 to 150 mL NaOH 0.1 N/10 g, whereas for firm sourdoughs, they range from 40–220 NaOH 0.1 N/10 g. Generally, sourdoughs with a high TTA are preferred for bread made with rye flour (Brandt, 2007).

2.2.3 Fermentation quotient (FQ)

The molar ratio between lactic and acetic acids during sourdough fermentation is defined as the fermentation quotient (FQ). The FQ is associated with not only the type of LAB dominating during fermentation but also the balance between homo-and hetero-fermentative lactobacilli. The ratio between homo- and hetero-fermentative lactobacilli is influenced by exogenous and endogenous factors including type of flour, fermentable sugar, DY, fermentation temperature and time (Corsetti, 2013).

2.3 Classification of sourdough

2.3.1 Type 1 sourdough

Type 1 sourdough is produced by using the previous mature sourdough (5–20%, m/v) to mix with fresh flour and water. Fermentation is carried out at 20-30 °C for 5 to 7 days. Type 1 sourdough is commonly used to achieve dough-leavening without adding baker's yeast, leading to firm sourdough with a low DY (< 200) (De Vuyst et al., 2017). The process of type 1 sourdough involves daily back-slopping, which maintains the metabolic activity of the sourdough LAB and yeast (De Vuyst et al., 2014). Type 1 sourdough is commonly used in household and artisan bakeries to made sourdough products (Venturi et al., 2012). Most type 1 sourdoughs produced by bakeries have been found to contain *Fructilactobacillus* (*F.*) *sanfranciscensis, Lactobacillus* (*L.*) *brevis* and *L. plantarum* as LAB, and *Kazachstania* (*K.) humilis* as yeast (Gänzle & Zheng, 2019). These microbes require a well-controlled maintenance and production environment, which can be achieved by artisan bakeries (Van Kerrebroeck et al., 2018; Venturi et al., 2012).

2.3.2 Type 2 sourdough

Type 2 sourdough is produced by using an industrial baking-process. This sourdough is prepared by temperature-controlled fermentation of fresh flour-water mixture with addition of specific acid-tolerant LAB (Siepmann et al., 2018). The process is a one-stage fermentation at high temperature (30-37°C) with a short fermentation duration (~24 h). The addition of baker's yeast usually occurs at the end of fermentation (De Vuyst et al., 2017). Type 2 sourdough can be used as a flavour or functional ingredient (dough acidifier and leavening agent) in commercial bakeries (De Vuyst et al., 2014).

2.3.3 Type 3 sourdough

Type 3 sourdoughs are initially produced by fermenting a fresh flour-water mixture, followed by daily refreshment with flour and water (Minervini et al., 2010; Siragusa et al., 2009). The type 3 sourdough process is a combination of type 1 and 2 sourdough techniques, which are commonly used in both artisan and industrial bakeries (Coda et al., 2018; Moroni et al., 2009).

2.4 Sourdough microorganisms

LAB and yeast are dominant in sourdough fermentation (Leroy & De Vuyst, 2004). *Lactobacillus* species are the most frequently found in sourdough, with more than 60 *Lactobacillus* species having been identified (Carbonetto et al., 2020). Other species include those of the *Leuconostoc, Weissella, Pediococcus, Lactococcus, Enterococcus and Streptococcus* genera (Table 2.2) (Huys et al., 2013).

Apart from LAB, yeast are also important in the sourdough ecosystem, with over 20 species reported (Table 2.2) (Leroy & De Vuyst, 2004). *Saccaromyces* (*S.*) *cerevisiae* is the most dominant in sourdough. *S. exiguous, K. humilis* and *Candida* (*C.*) *krusei* are other typical yeast found in sourdough (Corsetti et al., 2001; Foschino et al., 1999; Gobbetti et al., 1994; Succi et al., 2003).

The number and classification of species isolated from sourdough may vary due to the changes of ingredients and fermentation conditions, such as type of flour, leavening, and sourdough maintenance temperature. Different ingredients and fermentation conditions used during sourdough fermentation can lead to complex biological ecosystems in sourdoughs (Gobbetti et al., 1994; Gobbetti et al., 1999)

Table 2.2 Different species of LAB and yeast isolated from sourdough

Obligate heterofermentative LAB	Facultative heterofermentative LAB	Homofermentative LAB	Yeasts
• *Lactobacillus sanfranciscensis* • *Lactobacillus brevis* • *Lactobacillus fermentum* • *Lactobacillus reuteri* • *Lactobacillus panis* • *Lactobacillus pontis* • *Lactobacillus fructivorans* • *Lactobacillus rossiae* • *Weissella confuse* • *Weissella cibaria* • *Leuconostoc citreum* • *Leuconostoc mesenteroides*	• *Lactobacillus plantarum* • *Lactobacillus casei* • *Lactobacillus rhamnosus* • *Lactobacillus alimentarius*	• *Lactobacillus amylovorus* • *Lactobacillus acidophilus* • *Lactobacillus farciminis* • *Lactobacillus delbrueckii*	• *Saccharomyces cerevisiae* • *Candida humilis* [synonym *C. milleri*] • *Wickerhamomyces anomalus* (synonym *Pichia anomala* and *Hansenula anomala*); *anamorph Candida pelliculosa* • *Torulaspora delbrueckii (anamorph Candida colliculosa)* • *Kazachstania exigua* [synonym *Saccharomyces exiguus*); *anamorph Candida (Torulopsis) holmii]* • *Pichia kudriavzevii* (synonym *Issatchenkia orientalis*); *anamorph Candida krusei)*

Source: Catzeddu (2019).

2.4.1 Diversity of sourdough LAB

LAB is a Gram-positive, catalase-negative, anaerobic and fermentative bacteria (Axelsson, 2004). LAB is responsible for the acidification and the formation of volatile and metabolic compounds of sourdough breads (Yu et al., 2019). Its rapid acidifying ability is advantageous in improving the texture, sensory properties and microbial safety, and extending the shelf-life of sourdough bread (Ravyts & De Vuyst, 2011). LAB can be categorised into homofermentative and heterofermentative LAB based on their fermentation products (Di Cagno et al., 2014). These two types of LAB species complete redox balancing in two ways (De Vuyst et al., 2017): homofermentative LAB species produce lactic acid via the EMP pathway from flour saccharides during glucose fermentation, causing low pH and high TTA (Corsetti et al., 2008)., whereas heterofermentative LAB produce lactic acid, ethanol, acetic acid and CO_2 by the phosphogluconate pathway from flour saccharides (Ripari et al., 2016).

Over 60 species of LAB, mainly heterofermentative LAB (*Lactobacillus* strains) have been reported to be specifically well-adapted to the sourdough ecosystem (De Vuyst & Neysens, 2005b; Gobbetti & Gänzle, 2013). The most dominant LAB species in sourdough are *F. sanfranciscensis, L. plantarum, L. brevis, Pediococcus* (*P.*) *pentosaceus, L. paralimentarius* and *L. fermentum* (Table

2.3) (De Vuyst et al., 2017). Other LAB species, such as *Pediococcus, Enterococcus, Lactococcus* and *Weissella* have also been isolated from sourdough (Fujimoto et al., 2019; Li et al., 2016; Yan et al., 2019).

Table 2.3 LAB species found in sourdough made with different flours

Flour type	LAB species
Wheat	*L. plantarum/pentosus/ Lactobacillus* (*L.*) *paraplantarum, F. sanfranciscensis, L. fermentum, W. cibaria, W. confusa, L. citreum, F. sanfranciscensis, L. mesenteroides, Lactobacillus* (*L.*) *sakei, P. pentosaceus, L. paralimentarius, Lactobacillus* (*L.*) *gallinarum, Lactobacillus* (*L.*) *lactis, L. brevis, Pediococcus* (*P.*) *inopinatus, L. casei, Pediococcus* (*P.*) *argentinicus, L. rossiae, Weissella* (*W.*) *paramesenteroides, Lactobacillus* (*L.*) *spicheri, Lactobacillus* (*L.*) *namurensis, Enterococcus* (*E.*) *durans, Lactobacillus* (*L.*) *curvatus, Lactobacillus* (*L.*) *hammesii, Lactobacillus* (*L.*)*lindneri*
Barley	*L. fermentum, L. plantarum, L. brevis, W. confusa, P. pentosaceus*
Rye	*L. fermentum, L. plantarum, L. brevis, P. pentosaceus, L. sakei L. amylovorus, L. panis, L. reuteri Lactobacillus* (*L.*) *helveticus, L. pontis, Lactobacillus* (*L.*) *zymae*
Rye-wheat	*L. brevis, L. plantarum, L. zymae, L. pentosus, F. sanfranciscensis L. alimentarius, L. sakei*
Oat	*Lactobacillus* (*L.*) *coryniformis, Lactobacillus* (*L.*) *argentinum, P. pentosaceus, W. cibaria*
Buckwheat	*L. plantarum, L. graminis, L. sakei, W. cibaria, P. pentosaceus, Lactobacillus* (*L.*) *holzapfelii*
Chestnut	*L. plantarum, P. pentosaceus, W. cibaria, Pediococcus* (*P.*) *lolii/stilesii, W. paramesenteroides, L. farciminis*

Source: Martín-Garcia et al. (2021)

Sourdough LAB generally originate from the flour itself or the environment (Martín-Garcia et al., 2021). They can be distinguished based on their living hosts. *F. sanfranciscensis* and *L. fructivorans* are insect-adapted (Gänzle & Zheng, 2019; Vasilica et al., 2022). Whereas *L. fermentum*, *L. plantarum*, and *L. brevis* are environmentally-adapted or nomadic LAB (Minervini et al., 2015).

The optimal fermentation temperature for the growth of sourdough LAB depends on the type of LAB species. Mesophilic LAB grow optimally between 30 and 35 °C, while thermophilic LAB grow optimally between 40 and 45 °C (Gänzle & Gobbetti, 2013). Fermentation temperatures for traditional sourdough in Europe are generally between 25 to 35 °C, and hence mesophilic LAB dominates in European sourdough (Valjakka et al., 2003). Industrial processes and cereal fermentations in tropical climates are carried out at higher temperatures. Therefore, thermophilic LAB are preferred as the starter culture (De Vuyst & Neysens, 2005b; Meroth et al., 2003; Vogel et al., 1999). The optimal pH of sourdough LAB ranges from 5.0 to 6.0, which is similar to the pH of sourdough after inoculation with 5–20% of a previous batch of sourdough (Sterr et al., 2009).

2.4.2 Diversity of sourdough yeast

Yeast are unicellular fungi with characteristic growth by budding and fission. Sourdough yeast are well-adapted to stressful environments with low pH, high carbohydrate concentrations and high cell densities (Arici et al., 2017; Banu et al., 2011). Yeast in sourdough mainly contribute to the leavening and the aroma compound of sourdough breads (Yu et al., 2019). Classic yeast in sourdough is *Saccharomyces* (*S.*) *cerevisiae*, which ferments carbohydrates (glucose, fructose, maltose, and sucrose) during the fermentation period. Ethanol and CO_2 are synthesised via glycolysis during fermentation. Production of CO_2 contributes to the leavening of bread dough, while the effects of ethanol on the dough are minor due to its evaporation during baking (Guerzoni et al., 2013).

Over 30 different yeast species have been isolated from sourdough (Table 2.4) (Carbonetto et al., 2018). Of these the six most dominant yeast species worldwide in sourdough are *S. cerevisiae, K. humilis, T. delbrueckii, W. anomalus, Kazachstania* (*K.*) *exigua*, and *Pichia* (*P.*) *kudriavzevii* (De Vuyst et al., 2016; Sakandar et al., 2018). Normally only one or two yeast species are present in any one sourdough at the same time (De Vuyst et al., 2016). *K. humilis* and/or *S. cerevisiae* are commonly found in Italian sourdoughs, whereas the presence of *Kazachstania* (*K.*) *bulderi* and *K. humilis* are often observed in French sourdoughs (Lhomme et al., 2016; Vrancken et al., 2010).

Table 2.4 LAB species found in sourdough made with different flours

Flour type	Yeast species
Wheat	*S. cerevisiae, K. humilis, W. anomalus, K. bulderi, Kazachstania* (*K.*) *barnettii, Kazachstania* (*K.*) *saulgeensis, Kazachstania* (*K.*) *unispora, Candida* (*C.*) *carpophila, T. delbrueckii, Hyphopichia* (*H.*) *pseudoburtonii, Rhodotorula* (*R.*) *mucilaginosa, W. anomalus, Meyerozyma* (*M.*) *guilliermondii, Candida* (*C.*) *parapsilosis, Candida* (*C.*) *pararugosa, K. exigua*
Barley	*S. cerevisiae*

Rye	*S. cerevisiae, W. anomalus, Candida* (*C.*) *glabrata, K. humilis, Kazachstania* (*K.*) *telluris*
Rye-wheat	*S. cerevisiae, T. delbrueckii, W. anomalus, K. humilis*
Spelt	*W. anomalus, C. glabrata, S. cerevisiae*
Buckwheat	*K. barnettii*
Teff	*C. glabrata, S. cerevisiae*
Wheat-Rye-Spelt	*S. cerevisiae*

Source: Martín-Garcia et al. (2021)

2.4.3 Interactions between sourdough LAB and yeast during fermentation

The sourdough microbial community is composed of a stable combination of LAB and yeast (De Vuyst & Neysens, 2005b). Stability is important for the consistent quality of the sourdough product during industrial processes (Minervini et al., 2014). Apart from technological parameters such as DY, the stability of the sourdough ecosystem also relies on metabolic interactions between the LAB and the yeast (De Vuyst & Neysens, 2005b). These interactions can be: 1) negative (antagonistic), when the population of one bacteria reduces in the presence of another microorganism; 2) positive (synergistic), when the quantity of one bacteria increases in the presence of another microorganism; 3) neutral, when the presence of both microorganisms has no influence (Carbonetto et al., 2020). These interactions (Figure 2.1) are classified based on the relationships between the LAB and yeast, including competition, cross-feeding and inhibition (Galli et al., 2019; Seth & Taga, 2014; Sieuwerts et al., 2018).

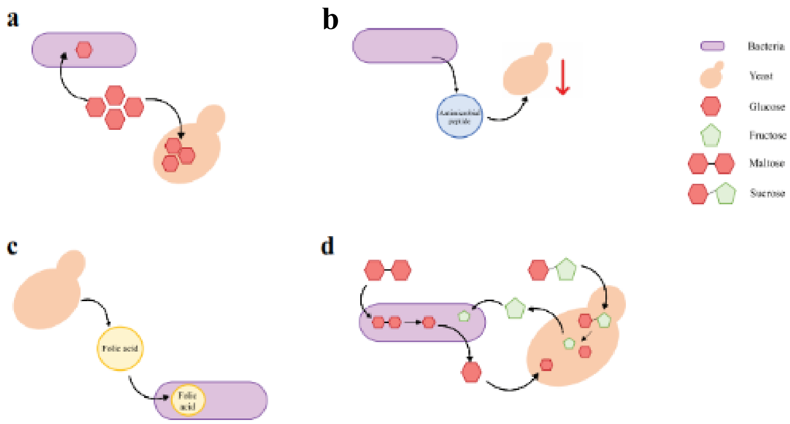


Figure 2.1 Interactions between LAB and yeast in the sourdough environment

Competition for nutrients (glucose) (a); LAB produce antimicrobial peptides that inhibit yeast growth (b); Commensalism (c); Cross-feeding (d).

Source: Martín-Garcia et al. (2021)

Some metabolites synthesised by LAB or yeast during sourdough fermentation can change the sourdough environment and influence the growth of another species (Figure 2.1b) (Martín-Garcia et al., 2021). Some products of organic acids produced by LAB have effective antimicrobial activity, inhibiting the growth of yeast during sourdough fermentation (Mantzourani et al., 2019). Stimulating interactions (cross-feeding) (Figure 2.1d), however, occur when one microorganism produces essential nutrients which can support the growth of another specie (Seth & Taga, 2014). (Venturi et al., 2012). Typically, yeast hydrolyses sucrose, providing a carbon source to support the growth of LAB during sourdough fermentation. Hence, cross-feeding decreases competition between species and improves their growth rate in the sourdough environment (Sieuwerts et al., 2018).

2.4.4 Microbiological characterisation of sourdough starter culture

The composition of sourdough starter culture is related to the overall quality of sourdough products, including sensory quality, nutritional value and shelf-life (Paramithiotis et al., 2005; Plessas, 2021). Understanding the composition of sourdough starter culture and its metabolism during sourdough fermentation provide valuable information to industrial and artisanal sourdough producers. The sourdough producers can develop better processes to control sourdough fermentation using the information, thereby producing sourdough products with consistent high quality (Corsetti, 2013). Additionally, the use of desirable starter cultures during sourdough fermentation can produce potentially functional nutrients beneficial to consumers (Catzeddu, 2019).

The sourdough starter culture mainly consists of LAB and yeast (De Vuyst & Neysens, 2005b). These sourdough microorganisms can be characterised and quantified by culture-dependent method, which cultures microorganisms on particular agar media under specific incubation condition such as incubation of LAB on MRS agar at 30 °C / incubation of yeast on YGC agar at 25 °C (Jany & Barbier, 2008). The enumeration of sourdough LAB and yeast can be used to monitor the maturity of sourdough starter, thereby determining their activities. Generally, mature sourdough has over 10^8 CFU g^{-1} of LAB with 10^6 CFU g^{-1} of yeast, which indicates that the ratio of LAB and yeast is 100:1 (Ercolini et al., 2013).

Apart from quantification, identification of sourdough LAB and yeast is necessary for selecting the desirable species of LAB and yeast to improve overall quality of sourdough product. The identification of sourdough LAB and yeast is also important for controlling the fermentation parameters to produce high-quality sourdough product (Siepmann et al., 2018). Kariluoto et al. (2006) reported the increase of folate content of sourdough bread fermented by *S. cerevisiae* and *L. curvatus*. Meanwhile, Venturi et al. (2013) found that the sensory qualities of wheat sourdough bread were improved by adjusting the fermentation temperature based on the composition of starter culture (*S. cereviasiae, F. sanfranciscensis, L. brevis, L. curvatus, and L. plantarum*).

Sourdough LAB and yeast can be identified by culture-independent method such as direct genotypic method or culture-dependent method combined with genotypic and/or phenotypic characterisation, including morphological, physiological and biochemical characterisation (Table 2.5) (Temmerman et al., 2004).

Table 2.5 Techniques used for the identification of sourdough LAB and yeast

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

Source: Temmerman et al. (2004)

2.5 Effect of environmental factors on sourdough fermentation

Sourdough fermentation can be affected by both exogenous and endogenous factors (Figure 2.2). These factors directly determine the microbial species, balance and the number of LAB and yeast (Catzeddu, 2019). The balance between LAB and yeast species may change due to the ratio of water to flour applied at each propagation, the fermentation time and/or temperature, the storage temperature and the frequency of back-slopping (Bamforth & Cook, 2019).

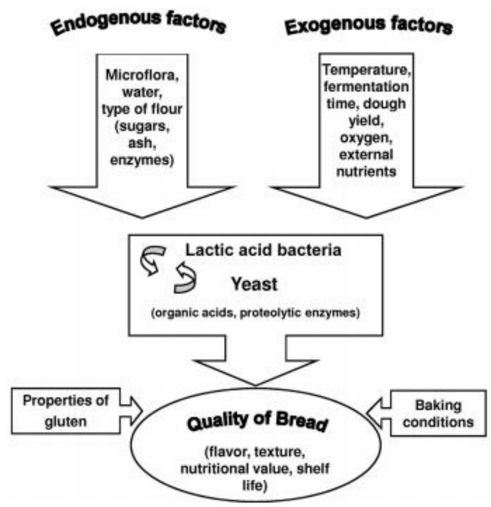


Figure 2.2 Factors affecting the quality and fermentation of sourdough

Source: Catzeddu (2019)

2.5.1 Effects of processing factors on sourdough fermentation

Different process parameters influence the growth of microorganisms (Figure 2.3) (Vrancken et al., 2011). The combinations of these parameters can affect the stability of the sourdough ecosystem, thereby affecting the overall quality of sourdough and sourdough breads. Therefore, process parameters are crucial for maintaining the consistent quality of the sourdough and sourdough breads (Martín-Garcia et al., 2021).

Fermentation temperature

The effects of fermentation temperature are dependent on the composition of the sourdough microorganisms. A low temperature (23 °C) is suitable for the growth of *Leuconostoc citreum* (Vrancken et al., 2011), whereas high temperatures (30 °C – 37 °C) favour the growth of *L. fermentum* or *L. plantarum*, and these species are dominant during sourdough fermentation at high temperatures. Similarly, the optimal fermentation temperatures for yeast are diverse. *K. humilis* and *S. cerevisiae* prefer growing between 27–28 °C, while *P. kudriavzevii* or *T. delbrueckii* are normally isolated during sourdough fermentation at 35 °C or higher (De Vuyst et al., 2016; Vrancken et al., 2011).

Fermentation temperature affects the growth of LAB and yeast, which in turn influences the synthesis of organic acids and flavour compounds (Banu et al., 2011; Birch et al., 2013; Fujimoto et al., 2019). Low temperatures (25–28 °C) support the growth of yeast during sourdough fermentation, leading to increased amounts of carbon dioxide, ethanol, and aroma compounds (Fujimoto et al., 2019; Siepmann et al., 2019). High temperature (≥ 30 °C) increases the metabolism of LAB, which influences the FQ, increasing the acidification of the sourdough (De Vuyst et al., 2017), and consequently increasing the synthesis of organic acids (Banu et al., 2011).

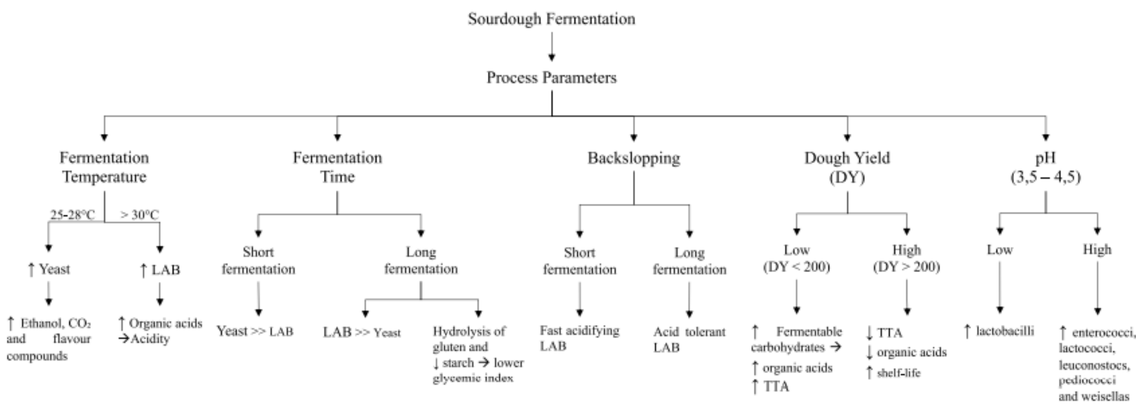


Figure 2.3 Effect of process parameters on sourdough microorganisms and sourdough

Source: Martín-Garcia et al. (2021)

Fermentation time

The length of fermentation is another factor that affects sourdough ecosystems (Minervini et al., 2014). Type I sourdough is typically produced using a prolonged fermentation at low temperatures (Vrancken et al., 2011). Therefore, *F. sanfranciscensis* is often predominant in type I sourdough (Yan et al., 2019). In contrast, *S. cerevisiae* dominates in sourdough with a short fermentation time, as the yeast growth rate is faster than LAB during the short fermentation (Fujimoto et al., 2019). However, longer fermentation times increase the production of lactic and acetic acids, which can reduce digestion of starch to produce bread with a lower glycaemic index (GI) (Longoria-García et al., 2018).

Back-slopping

Back-slopping is carried out by adding a small amount of fermented sourdough starter into a fresh mix of water and flour (Wirawati et al., 2019). This method is considered a key factor affecting the quality of the sourdough (Minervini et al., 2014). Increasing the back-slopping frequency causes certain species (heterofermentative LAB, *F. sanfranciscensis*) to dominate in sourdough (Lattanzi et al., 2013). Furthermore, the acidification and the growth rate of the microorganisms can be modified by the frequency of refreshment of the starter culture (De Vuyst et al., 2017). Long period between back-slopping is advantageous to acid-tolerant LAB species, whereas short back-slopping times benefit fast-acidifying LAB species (De Vuyst et al., 2017).

2.6 Effect of flour type on sourdough microorganisms

Flour is the major source of microorganisms in spontaneous sourdough fermentation. The inherent LAB and yeast are essential for establishing stable and diverse microbial consortia within a short period (De Vuyst et al., 2014). The type of flour and the concentration of nutrients are important for the sourdough LAB (Minervini et al., 2012). Triticum durum (T. durum) flour contains more maltose, glucose, fructose and free amino acid (FAA) than Triticum aestivum (T. aestivum) flour. Therefore, T. durum-based sourdoughs have more obligate heterofermentative LAB (mainly *F. sanfranciscensis, Leuconostoc spp., Weissella cibaria* and *L. brevis*). Whereas the T. durum flour has less facultative heterofermentative LAB, and lower yeast than T. aestivum-based sourdough (De Vuyst et al., 2009; Robert et al., 2009; Scheirlinck et al., 2007).

2.7 Sourdough fermentation mediated by LAB and yeast

The nutrition, texture, shelf-life and sensory qualities of the final sourdough bread are determined by the metabolic activities of microorganisms during sourdough fermentation (Gobbetti, 1998; Katina et al., 2005). These metabolic activities include the formation of organic acids, proteolysis, synthesis of volatile compounds, antifungal and anti-ropiness compounds (Corsetti & Settanni, 2007; Gobbetti et al., 1999; Hammes & Gänzle, 1998a).

The synthesis of organic acids during fermentation can contribute to texture, aroma and shelf-life of sourdough products. The formation of organic acids impart antimicrobial activity to the sourdough, thus delaying the staling of bread (Catzeddu, 2019). Acidification of the sourdough increases the activity of proteases and amylases in the cereal, which increases the protein solubility, and softens the sourdough bread (Catzeddu, 2019). An acidic environment also increases phytase activity in the flour, decreasing the chelating capacity of phytic acid, thereby increasing the bioavailability of minerals (Poutanen et al., 2009).

Organic acids and free amino acids synthesised during sourdough fermentation effectively improve bread flavour (Arora et al., 2021). Additionally, production of exopolysaccharides (EPS) during fermentation increases the softness and water absorption ability of the dough (Chavan & Chavan, 2011). Therefore, it is important to analyse the metabolic activity of sourdough fermentation due to its potential contribution to the nutritional, sensory and textural characteristics of the sourdough products.

2.7.1 Carbohydrate metabolism during sourdough LAB fermentation

Sourdough LAB can be classified into three metabolic categories: (1) obligately homofermentative microorganisms (*L. delbrueckii, L. acidophilus, L. farciminis, L. amylovorus*, and *L. mindensis*), which ferment hexoses through the Embden-Meyerh of Parnas (EMP) pathway, with lactate as the major final product of carbohydrate metabolism (Figure 2.4). Obligate heterofermentative microorganisms (*F. sanfranciscensis, L. rossiae, L. brevis, L. pontis,* and *L. fermentum*), ferment hexoses and pentoses through the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway and synthesise equimolecular amounts of lactate and ethanol or acetate. Carbon dioxide is additionally produced from hexoses (Figure 2.5).Facultative heterofermentative microorganisms (*L. plantarum, L. alimentarius, L. paralimentarius*, and *L. curvatus*), ferment hexoses through the EMP pathway, and pentoses and gluconate through the 6-PG/PK pathway (Gobbetti & Gänzle, 2013).

Obligate heterofermentative LAB dominate sourdough fermentation (De Vuyst & Neysens, 2005a). Their predominance depend on (1) the metabolism of maltose by the enzyme maltose phosphorylase, simultaneous fermentation of hexoses and pentoses through the 6-PG/PK pathway, and the use of fructose and other substrates as external acceptors of electrons; (2) optimal pH and temperatures; (3) capacity to adapt to various environmental stresses; (4) the synthesis of a large spectrum of antimicrobial compounds (De Vuyst & Neysens, 2005a; Gobbetti & Gänzle, 2013).

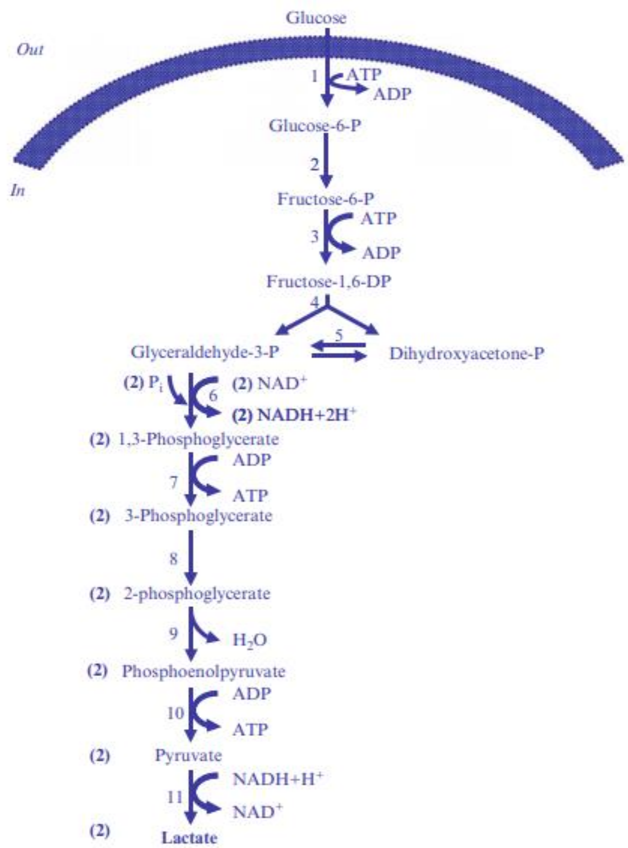


Figure 2.4 Embden-Meyerhof-Parnas (EMP) pathway

(2) indicates the formation of two moles of each compound; 1 Glucokinase, 2 glucose-6-phosphate isomerase, 3 phosphofructokinase, 4 fructose 1,6-bisphosphate aldolase, 5 triosephosphate isomerase, 6 glyceraldehyde 3-phosphate dehydrogenase, 7 3-phosphoglycerate kinase, 8 phosphoglycerate mutase, 9 enolase, 10 pyruvate kinase, 11 lactate dehydrogenase.

Adapted from Handbook on sourdough biotechnology in Gobbetti and Gänzle (2013), *reproduced with permission from Springer Nature.*

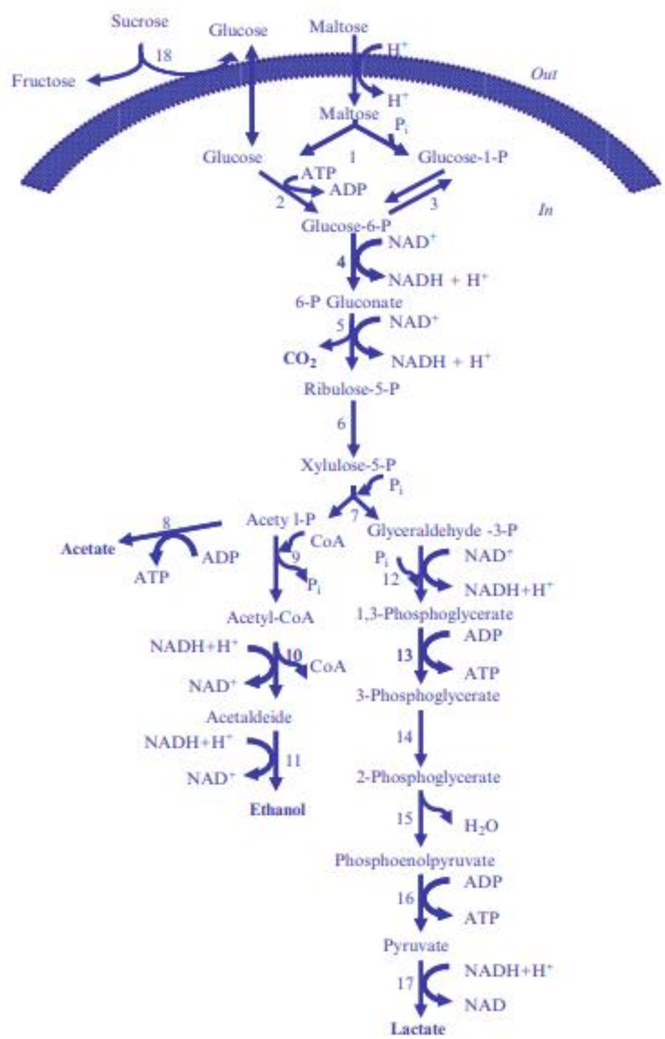


Figure 2.5 6-Phosphogluconate/phosphoketolase pathway (6-PG/PK)

Maltose phosphorylase (1), hexokinase (2), phosphoglucomutase (3), glucose-6-phosphate dehydrogenase (4), 6-phosphogluconate decarboxylase (5), epimerase (6), phosphoketolase (7), acetate kinase (8), phosphotransacetylase (9), aldehyde dehydrogenase (10), alcohol dehydrogenase (11), glyceraldehyde 3-phosphate dehydrogenase (12), 3-phosphoglycerate kinase (13), phosphoglycerate mutase (14), enolase (15), pyruvate kinase (16), lactate dehydrogenase (17), levansucrase (18).

Adapted from Handbook on sourdough biotechnology in Gobbetti and Gänzle (2013), *reproduced with permission from Springer Nature.*

2.7.2 Carbohydrate metabolism during sourdough yeast fermentation

Fewer metabolites are produced during yeast carbohydrate metabolism compared to LAB carbohydrate metabolism (De Vuyst et al., 2016). Sourdough yeast can be categorised into maltose-negative and maltose-positive species based on their capability of using maltose (De Vuyst et al., 2009; Hammes & Gänzle, 1998b). Maltose-negative yeast prefer glucose over other carbohydrates, whereas maltose-positive yeast can ferment all four flour carbohydrates (maltose, sucrose, fructose, and glucose) (De Vuyst et al., 2009).

Yeast convert disaccharides and glucofructans into monosaccharides, which are then fermented by yeast through glycolytic pathway, producing ethanol and carbon dioxide (De Vuyst et al., 2016). Carbon dioxide contributes to the leavening of sourdough products, while ethanol contributes to the flavour of the sourdough (Hansen & Schieberle, 2005). When the fermentable sugars are limited in the sourdough, the yeast use respiration as their preferred metabolic pathway (Bamforth & Cook, 2019).

2.7.3 Metabolism of nitrogen compounds by LAB

Sourdough fermentation with LAB increases the level of amino acid in sourdough due to the increase of proteolysis (Gobbetti et al., 2005). Proteolysis during sourdough fermentation is a result of the proteolytic activity of sourdough LAB or an activation of proteolysis by cereal enzymes under the acidic sourdough environment (Gobbetti, 1998; Thiele et al., 2002a). Generally, proteolytic enzymes (proteases) include peptidases and proteinases. Proteinases catalyse protein degradation, breaking proteins down into smaller peptide fractions. Meanwhile, peptidases completely hydrolyse peptide bonds and break down peptides into amino acids (Chavan & Chavan, 2011). The degradation of wheat or rye protein during sourdough fermentation is important for the flavour, volume and texture of the sourdough products (Chavan & Chavan, 2011).

Of the amino acids, ornithine as a precursor compound responsible for the synthesis of 2-acetyl-1-pyrroline during baking which is an essential flavour compound in the wheat bread crust. Ornithine in wheat dough is derived from the yeast biomass or arginine metabolism by *Lactobacillus* (Gänzle et al., 2007). Three enzymes, arginine deaminase (ADI), ornithine carbamoyl transferase (OTC), and carbamate kinase (CK), are involved in the catabolism of arginine (Zúñiga et al., 2002). A fourth protein, located at the cell membrane acts as transporter, allowing the antiporter exchange between arginine and ornithine (Wipf et al., 2002). Generally, arginine is quantitatively converted to ornithine by ADI-positive lactic acid bacteria during sourdough fermentation (Gänzle & Gobbetti, 2013).

During sourdough fermentation, the ADI pathway of amino acid metabolism favours: (1) microbial growth and survival by the formation of ATP; (2) enhanced acidic tolerance of LAB by contributing to the homeostasis of the intracellular pH; and (3) increased formation of ornithine, which is converted into 2-acetyl-1-pyrroline during baking - which contributes to the typical flavour of the bread crust (Gänzle et al., 2007).

2.8 Rheology, sensory and shelf-life of sourdough and sourdough bread

Sourdough fermentation can positively affect the sensory quality of the final bread (including aroma, taste, texture and visual properties such as bubbles, height of a bread loaf, crumb texture and structure of bread) (Fujimoto et al., 2019; Lawless & Heymann, 2010), making the use of sourdough popular (Ma et al., 2021).

2.8.1 Rheology of sourdough and sourdough bread

The rheology of baked products can be improved by sourdough fermentation (Arora et al., 2021). Specifically, improvements in texture (hardness, adhesiveness, resilience, cohesiveness, chewiness, springiness and gumminess), shape, specific volume, crust and crumb colour, moisture retention, and crumb structure can be observed (Martín-Garcia et al., 2021).

Increasing the application of sourdough (5 to 40 %) during fermentation decreases firmness and elasticity of the final bread dough (Clarke et al., 2004). These rheological changes in the bread dough are mainly attributed to the acids produced and enzymatic activity (protease activity) during fermentation (Arendt et al., 2007). Acidification during fermentation increases protein solubility due to the increased net positive charges under the acidified conditions (Retailleau et al., 2002). Therefore, bread dough acidified by sourdough has increased elasticity, softness and gluten extensibility (Schober, 2003). Moreover, enzymes from cereal and microorganisms can affect the dough texture during progressive sourdough fermentations (Thiele et al., 2002b). Protease activity in the acidic environment weakens the gluten during sourdough fermentation, resulting in increased solubility of pentosans and the formation of EPS. These factors contribute to textural changes in the sourdough and sourdough bread (Clarke et al., 2004; Thiele et al., 2002b).

Sourdough fermentation also influences the rheology of the final sourdough bread. The specific volume of the bread can be increased by sourdough fermentation, resulting in decreased firmness in the bread crumb (Corsetti et al., 2008; Crowley et al., 2002). Wheat bread made with sourdough has a higher specific volume, lower resilience, cohesiveness, hardness, gumminess and chewiness compared to wheat bread made with baker's yeast (Pontonio et al., 2020). Furthermore, sourdough bread has more smaller holes in the crumbs compared to yeast-leavened bread (Arora et al., 2021) The smaller holes increase the expansion of the dough, which contributes to the increase in bread specific volume and decrease of crumb firmness (Clarke et al., 2002).

2.8.2 Sensory characteristics of sourdough bread

Bread flavour is produced during the baking process and fermentation (Hansen & Schieberle, 2005). Sourdough can impact bread flavour in three ways; 1) providing acidity, 2) synthesising flavour precursors (amino acids), 3) synthesising volatile compounds including alcohols, aldehydes, ketones, esters, and sulphur (Heiniö, 2014).

Sourdough acidification increases the overall aroma and taste intensity of the bread (Arora et al., 2021; Katina et al., 2006). However, high lactic acid and acetic acid can cause an unpleasant and pungent flavour in the bread, thus organic acid concentrations of 0.35% of flour weight with a final pH of around 4.9 in bread has been suggested (Salovaara & Valjakka, 2007). The liberation of free amino acids (FAA) via Erlich's mechanism during yeast fermentation contribute the most to bread aroma (Hazelwood et al., 2008; Thiele et al., 2002b). The synthesis of ornithine is considered a key factor for improving the roasted flavour in the final bread product, while proline is a main precursor for 2-acetyl-1-pyrroline which contributes to a roasted flavour in the bread crust (Yoshihashi et al., 2002). During yeast fermentation, leucine and phenylalanine increase the formation of 3-methylbuthanol and 2-phenylethanol via the Ehrlich pathway, which are responsible for crumb flavour (Gassenmeier & Schieberle, 1995). Additionally, thermal reactions (Maillard reaction and caramelisation) during baking also contribute to crust flavour and colour (Purlis, 2010).

2.8.3 Shelf-life of sourdough product

Shelf-life affects consumer expectations of food products. Shortened shelf-life is primarily attributed to fungal contamination and staling (Corsetti et al., 2008). Microbial spoilage of yeast-leavened bread is caused by *Bacillus* microorganisms and mould. Flours may contain *Bacillus* spores which could sprout after baking, whereas mould growth can occur due to contamination after baking (Catzeddu, 2019). Sourdough fermentation can be used as a natural preservative, extending the shelf-life of the final product (Moroni et al., 2009; Ur-Rehman et al., 2007). The production of organic acids such as lactic and acetic acids during sourdough fermentation contribute to prolonging the shelf-life of the sourdough product due to their antimicrobial function (Mantzourani et al., 2019).

2.8.4 Antifungal activity of sourdough microorganisms

Sourdough microorganisms are considered essential for the preservation and microbial safety of a number of foods. The production of organic acids during sourdough fermentation contributes to antimicrobial activity. Sourdough LAB also produce antimicrobial compounds such as cyclic dipeptides, hydroxy fatty acids, antifungal peptides or phenyl and substituted phenyl derivates. These compounds work synergically with organic acids as natural preservatives in sourdough products (Catzeddu, 2019; Lavermicocca et al., 2000; Ryan et al., 2008). Additionally, sourdough products fermented by LAB with antifungal activity are effective in controlling the growth of contaminant moulds such as *Aspergillus, Fusarium,* and *Penicillium* (Axel et al., 2016; Axel et al., 2017; Mantzourani et al., 2019).

2.9 Nutritional and functional properties of sourdough bread

Sourdough fermentation not only improves the overall quality of the bread dough and final bread, but also positively affects the functional and nutritional qualities of the baked goods (Katina & Poutanen, 2013). The microorganisms and chemical composition of the fermented products are responsible for the functional and nutritional compounds of the final products (Katina et al., 2005; Lorenz & Kulp, 2003; Poutanen et al., 2009).

2.9.1 Effects of functional compounds produced by sourdough fermentation on starch digestibility

The main carbohydrate sources in the western diet such as bread contain high levels of rapidly digestible starches. Consuming foods with a high glycaemic index (GI) and high insulin index (II) may result in periodic spikes in plasma glucose and insulin concentrations, which can eventually cause health issues such as type 2 diabetes mellitus (Barclay et al., 2008; Katina & Poutanen, 2013).

Sourdough fermentation has been considered as an efficient technology to reduce the GI of bread (De Angelis et al., 2006; De Angelis et al., 2009; Maioli et al., 2008). Generally, reducing the pH of sourdough bread to 3.8-5.1 results in a bread with low GI/II (Poutanen et al., 2009). The decrease of GI is mainly due to the synthesis of organic acids, especially lactic acid during sourdough fermentation (Galle et al., 2010). Organic acids reduce starch digestibility by decreasing the degree of starch gelatinisation (Ačkar et al., 2015; Östman, 2003). Lactic acid reduces the starch digestion rate of the bread (Liljeberg et al., 1995), while propionic and acetic acids slow the rate of gastric empty (Liljeberg & Björck, 1998). Moreover, the resistant starch formed by sourdough fermentation slows the digestion rate (Scazzina et al., 2009). Additionally, peptides, amino acids and free phenolic compounds produced during sourdough fermentation also reduce the GI/II of the sourdough products (Gänzle et al., 2008; Katina, Laitila, et al., 2007).

2.9.2 Effects of functional compounds produced by sourdough fermentation on controlling hypertension

Controlling dietary sodium intake is an efficient way to control high blood pressure and cardiovascular disease (CVD) (He & Macgregor, 2007). Therefore, the World Health Organization (WHO) and European Union (EU) have suggested that food industries reduce sodium in products in order to decrease consumer intake to < 2 g Na/ day (Kloss et al., 2015). However, salt is added to cereal-baked products because it improves their texture and flavour (Poutanen et al., 2009). Therefore, reduction of salt intake is challenging in western countries where around 30% of daily sodium intake is from bread and grained-baked products.

Sourdough fermentation can be used to reduce the use of salt in the formulation without losing the sensory qualities of the final product (Valerio et al., 2017). For example, the sodium chloride content of bread produced by sourdough fermentation can be reduced from 1.5 to 1%, without loss of taste and other sensory quality properties in the final bread (Zhao et al., 2015). Moreover, sourdough bread made with reduced salt may exhibit a higher perception of saltiness compared to the bread made with original recipe (Rizzello et al., 2010). This phenomenon can be caused by the formation of free amino acids and amino acid derivatives during sourdough fermentation, which contribute to the saltiness of the sourdough product (De Bellis et al., 2020).

Sourdough fermentation can also increase the levels of functional antihypertensive compounds in sourdough products (Gobbetti et al., 2019). Based on clinical trials, sourdough *Lactobacilli* fermentation can convert glutamate to γ-amino butyric acid (GABA), which is known for its potential to reduce moderately high blood pressure (Diana et al., 2014; Inoue et al., 2003). Additionally, the synthesis of Angiotensin I-converting enzyme (ACE) inhibitory and antioxidant peptides by sourdough LAB during fermentation is also effective in lowering blood pressure (Gobbetti et al., 2019).

2.9.3 Protein digestion

Gluten protein contained in cereal can cause celiac disease (CD) in gluten-sensitive individuals (Gänzle et al., 2008). During sourdough fermentation, proteolysis degrades the gluten protein, reducing the disulphide bonds in the gluten network and increasing the protein digestibility of the sourdough product (De Angelis et al., 2007). Therefore, sourdough fermented products can often be consumed by gluten-sensitive individuals (Di Cagno et al., 2004; El-Ghaish et al., 2011). For example, wheat products produced using sourdough fermentation have been proven to be non-allergenic to celiac disease patients (Greco et al., 2011; Wehrle & Arendt, 1998).

2.9.4 Mineral bioavailability

The phytic acid present in cereal grains acts as an anti-nutritional compound for humans, due to its strong cation (Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}) chelating capacity, resulting in decreased mineral bioavailability (Gobbetti et al., 2005; Lopez et al., 2002). Sourdough fermentation can reduce phytate concentrations in bread products by increasing acidification of the dough, which increase endogenous phytase activity, thereby increasing mineral solubility and bioavailability (La´sztity & La´sztity, 1990; Lopez et al., 2001; Poutanen et al., 2009). Leenhardt et al. (2005) and Türk et al. (1996) reported that phytate content of wholemeal sourdough can be reduced by 70% due to the phytase activity of flour and yeast in acidic environment (3.5 – 5.0).

2.9.5 Effects of sourdough fermentation on content of vitamin and bioactive compounds in sourdough bread

The antioxidant activity of cereal products can be increased by sourdough processing (Banu et al., 2010). Wheat and rye products fermented by sourdough LAB have a higher antioxidant activity than yeast fermented products, attributed to the increased content of phenolic compounds (Liukkonen et al., 2003; Rizzello et al., 2012). Furthermore, sourdough LAB fermentation increases the formation of glutathione (a major free-radical scavenger and non-enzymatic antioxidant) and the synthesis of exopolysaccharides, which beneficially influence the texture and shelf-life of the sourdough product (Galle & Arendt, 2014; Laurent-Babot & Guyot, 2017).

The effects of sourdough fermentation on levels of bioactive compounds vary depending on the sourdough microorganisms and the nature of the ingredient compounds (Banu et al., 2010; Leblanc et al., 2017). For example, sourdough fermentation by *L. plantarum* was shown to increase the content of folate, flavonoids, total phenols, lignans and benzoxazinoids, but decrease tocopherol and tocotrienol in the final sourdough product containing quinoa, buckwheat and wholemeal flours (Chiş et al., 2020; Katina et al., 2005). Thiamine and folate in rye and wheat sourdough breads are increased by sourdough fermentation by *Candida milleri* yeast and *L. plantarum* (Kariluoto et al., 2004; Katina, Liukkonen, et al., 2007).

2.10 Conclusion and future trends

The beneficial effects of sourdough fermentation on the overall quality of the final bread products are well known, but information is limited regarding the microbial composition and content of nutritional and functional compounds in sourdough bread produced in New Zealand. This research aims to 1) identify the composition of sourdough LAB and yeast from wheat sourdough starter and rye sourdough starter; 2) determine the levels of potential nutritional and functional compounds in sourdoughs and sourdough bread. This research can help fill research gaps in sourdough fermentation in New Zealand and provide helpful information about fermentation parameters and nutrition to local bakeries. The results obtained may be useful in assisting artisanal and industrial bakeries to find better ways to control the sourdough fermentation process to better maintain the production of sourdough breads with consistent high-quality. Moreover, knowledge of the potential nutritional and functional compounds produced during sourdough fermentation in sourdough bread may assist bakeries to improve their recipes.

CHAPTER 3 MATERIALS AND METHODS

3.1 Overview of sourdough product

The sourdough starter and other ingredients used in this project were supplied by commercial bakery in Auckland, New Zealand (Table 3.1). The sourdough and sourdough breads analysed in phase I of this study were prepared at a commercial bakery, while the sourdough and sourdough bread samples used in phase II and III were prepared in the Food Processing Laboratory at Massey University (Albany, Auckland).

Table 3.1 Ingredients used

Ingredient	**Supplier**
Wheat/ rye mother sourdough	Bread and Butter Ltd, Auckland New Zealand
Sea salt	BioGro Ltd, Wellington New Zealand
Malt flour	Davis Food Ingredients Ltd, Auckland New Zealand
Organic New Zealand wheat flour (100%)	Chantal Organics Ltd, Napier New Zealand
Organic rye wholemeal flour (100%)	Davis Food Ingredients Ltd, Auckland New Zealand

3.2 Preparation of sourdough and sourdough breads

3.2.1 Preparation of sourdough and sourdough bread for phase I

Sourdough and sourdough bread samples were prepared at commercial bakery (Figure 3.1). Sourdough breads were prepared using organic wheat flour, malt flour, sea salt, gluten, water, rye sourdough starter and wheat sourdough starter, bulk-fermented for 3.5 h and the mixing was proofed for 2 h at room temperature (27 ± 0.5 °C), then cold-fermented at 6 ± 1 °C in the chiller for 19 ± 1 h. After cold-fermentation (proofing), sourdough loaves were baked at 220 °C / 45 min by rack oven.

(1) Sampling in phase I

Samples were collected from three different batches at follows: wheat sourdough starter (WSS), rye sourdough starter (RSS), dough-before-proofing (DBP), dough-after-proofing (DAP) and sourdough bread (SDB) (Figure 3.1).

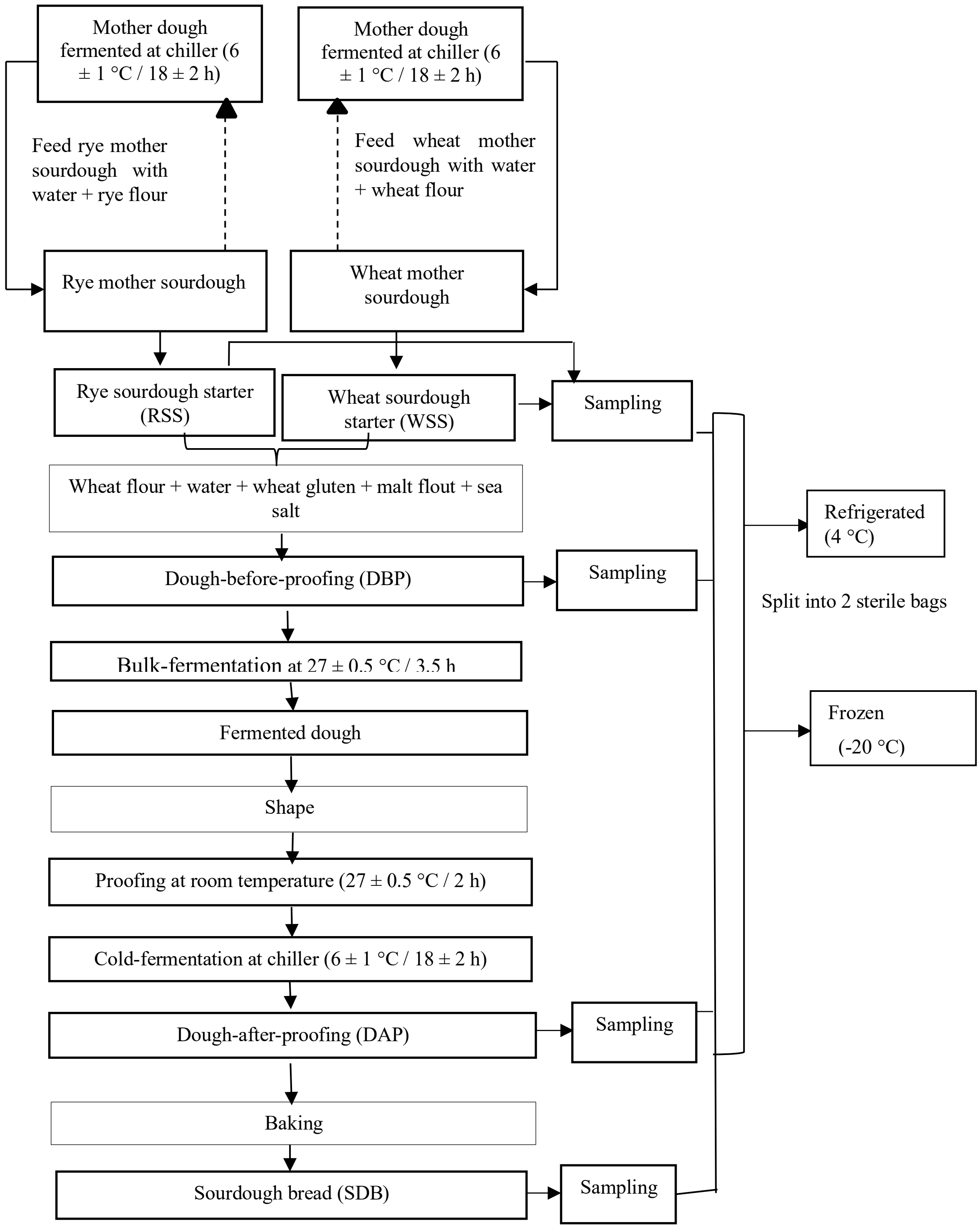


Figure 3.1 Preparation of sourdough and sourdough bread at commercial bakery

Sourdough samples collected from the bakery were split into two sterile stomacher bags (Global Science, NZ), one sample was refrigerated at 4 °C and the other frozen at -20 °C before transporting on ice to the Food and Microbiology Laboratory (Massey University, Auckland Campus, Auckland). Two sourdough bread samples from each batch were also collected, and transported at room temperature to Massey University, Auckland.

Textural analysis of the dough samples was performed immediately after transport to Massey University. The frozen samples were stored in the freezer for chemical and microbiological analyses.

3.2.2 Preparation of sourdough starters, sourdoughs and sourdough bread for phases II and III

(1) Preparation of sourdough starters, sourdoughs and sourdough bread for phase II

Sourdoughs and sourdough breads for phase II were prepared as shown in Figure 3.2. Briefly, sourdough breads were prepared using organic wheat flour, malt flour, sea salt, gluten, potable water, rye sourdough starter and wheat sourdough starter. Bread dough was fermented at three different fermentation conditions: (1) bulk-fermentation at 27 °C / 3.5 h and proofing at 27 °C / 5 h, (2) bulk-fermentation at 29 °C / 3 h and proofing at 29 °C / 4 h, (3) bulk-fermentation at 31 °C / 2.5 h and proofing at 31 °C / 3 h. After proofing, sourdough was baked at 200 °C for 30 min. Samples were collected follows the productional sourdough process: WSS, RSS, DBP, DAP (27/29/31) and SDB (27/29/31) as shown in Figure 3.2.

The sourdough and sourdough bread samples used for the analysis as shown in Table 3.2. Sourdough samples for microbiological and chemistry tests were frozen. They were fully thawed before tests. Microbiological and chemistry tests were performed after 2 days of sourdough samples produced. Textural analysis for sourdough samples was carried out at room temperature as soon as they were ready. Sourdough bread samples for textural analysis, colour measurement, loaf volume and weight were kept at room temperature overnight and tests were performed on the following day. The sensory evaluation (focus group) of sourdough bread was carried out to determine the preferrable sourdough bread to carry on to phase III. Method of sensory evaluation (focus group) referred to Section 5.

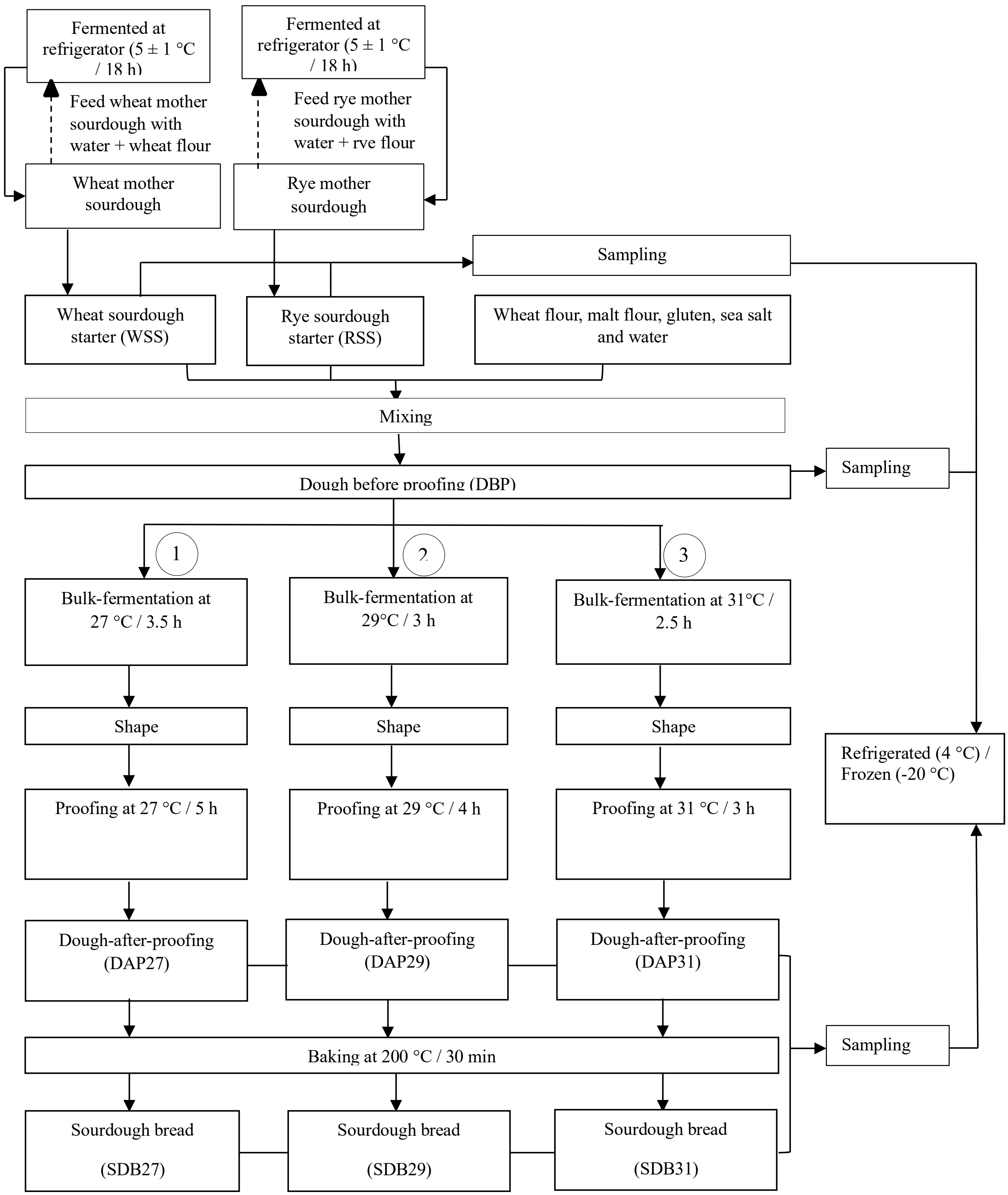


Figure 3.2 Preparation of sourdough and sourdough bread in the Food Processing Laboratory at Massey University Auckland

Table 3.2 Characterisation of sourdough starter, sourdough and sourdough bread

Sample (Code)	Test
Wheat sourdough starter (WSS)	pH, TTA, microbiology
Rye sourdough starter (RSS)	pH, TTA, microbiology
Dough-before-proofing (DBP)	pH, TTA, texture, microbiology
Dough-after-proofing (DAP 27/29/31)	pH, TTA, texture, microbiology
Sourdough bread (SDB 27/29/31)	pH, TTA, texture, colour, loaf volume, loaf weight

(2) Preparation of sourdough starters, sourdoughs and sourdough bread for Phase III

Based on results from the sensory evaluation (focus group) of the sourdough breads produced in Section 2.3.1, the (sourdough) bread produced under the condition 1 (bulk-fermentation at 27 °C / 3.5 h and proofing at 27 °C/ 5 h) was selected for Phase 3 studies. Therefore, sourdough and sourdough breads were prepared with bulk-fermentation at 27 °C / 3.5 h and proofing at 27 °C/ 5 h (Figure 3.3). Samples were collected for analysis (Table 3.3) at the follows: WSS, RSS, DBP, DAP and SDB (Figure 3.3). Physico-chemical and microbiological characteristics of sourdough and sourdough bread samples were performed. The analysis of resistant starch and folate was carried out by the Nutrition Laboratory (Massey University Palmerston North, New Zealand).

Table 3.3 Characterisation of sourdough starter, sourdoughs before baking and sourdough bread in Phase 3

Sample	Test
Wheat sourdough starter (WSS)	pH, TTA, microbiology
Rye sourdough starter (RSS)	pH, TTA, microbiology
Dough before proofing (DBP)	pH, TTA, texture, microbiology, folate, resistant starch
Dough after proofing (DAP)	pH, TTA, texture, microbiology, folate, resistant starch
Sourdough bread (SDB)	pH, TTA, texture, colour, loaf volume, loaf weight, folate, resistant starch

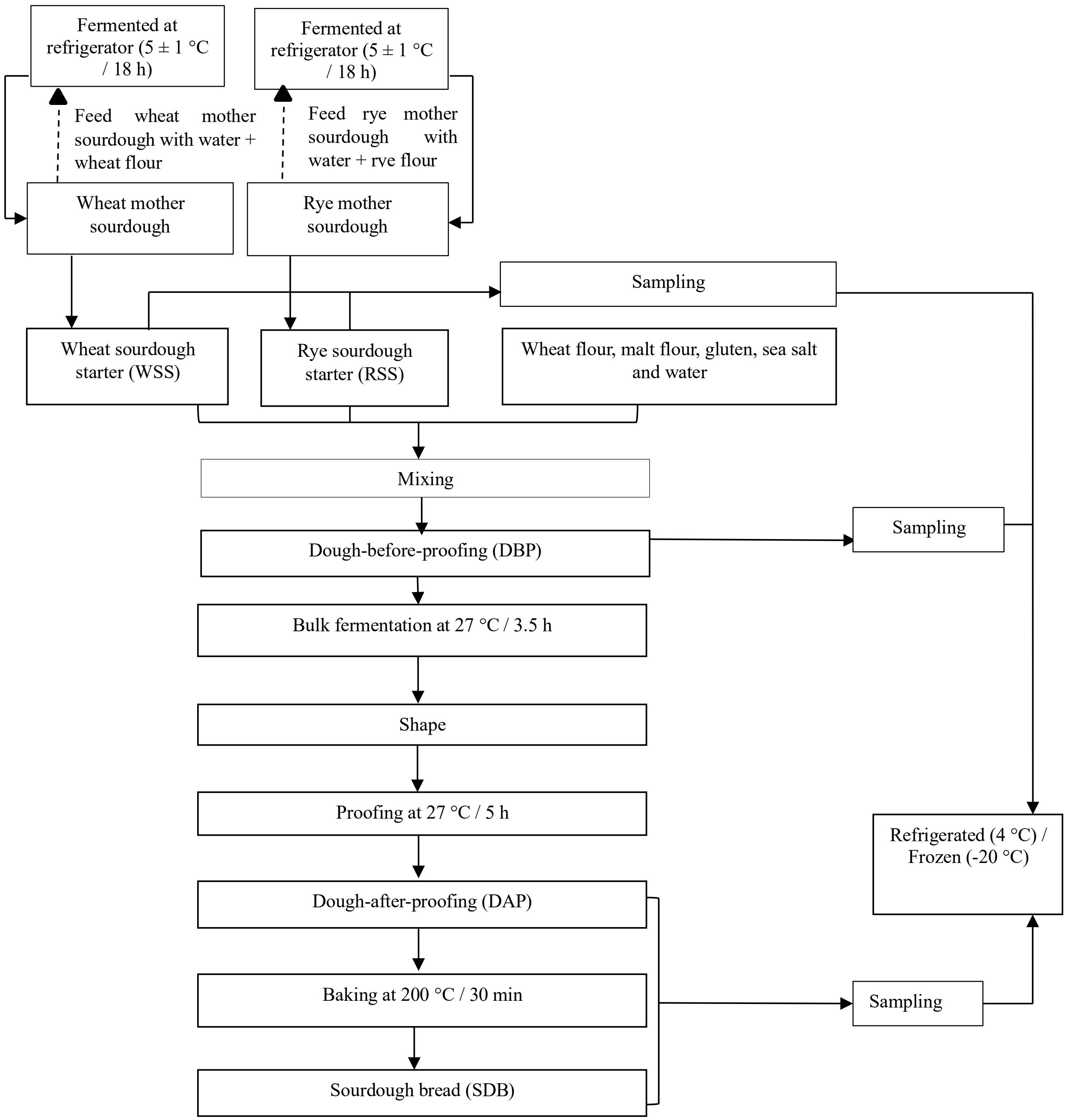


Figure 3.3 Laboratory Preparation of sourdough and sourdough bread

3.3 Microbiology of sourdough starters

3.3.1 Sample preparation

Ten (10) g of sourdough were homogenised with 90 g of peptone water (Merck, Germany) in a stomacher bag (Global Science, NZ) for 2 min at 260 rpm using the 400 ml Masticator (IUL, Spain).

3.3.2 Enumeration and isolation of LAB and yeast

Ten (10) g of homogenised samples were used to prepare tenfold serial dilutions (10^{-1} to 10^{-7}) in 0.1% peptone solution. One (1) ml was plated in duplicate on suitable solidified agar (Figure 3.4).

Enumeration and isolation of LAB were carried out by plating 1 ml of serial diluted samples (10^{-5}– 10^{-7}) on solidified MRS agar (Oxoid, UK). The plated LAB plates were incubated under anaerobic conditions at 37 °C / 2 days (Minervini et al., 2012). Developed colonies were counted after incubation.

Enumeration and isolation of yeast were carried out by plating 1 ml of serial diluted samples (10^{-1}– 10^{-3}) on solidified YGC agar (Merck, Germany). The plated yeast plates incubated at 25 °C / 3 days under aerobic conditions (Sterr et al., 2009). Developed colonies were counted after incubation.

3.3.3 Selection and purification of LAB and yeast colonies

Selection of LAB and yeast colonies

The selection and purification of LAB and yeast colonies were conducted based on method described by Vieira - Dalodé et al. (2007)and Yang et al. (2021) with slight modification. LAB and yeast colonies were visually examined and measured by vernier scale on MRS agar and YGC agar. Their morphological type including appearance, shape, colour and diameter were recorded. Two-five distinct colonies of each morphological type were selected for further purification (Figure 3.4).

Purification of LAB colonies

Purification of selected colonies was achieved by re-streaking cultures on suitable agar and plates were incubated under suitable condition (Figure 3.4). Selected LAB colonies were re-streaked onto solidified MRS agar and plates were incubated under anaerobic conditions at 37 °C / 2 days.

Purified LAB colonies were examined for Gram reactions under oil immersion using the Carl Zeis transmission light microscope (Model HBO 50/AC, Germany). Thirteen representative pure LAB colonies (six colonies isolated from WSS and seven colonies isolated from RSS) were Gram stained. For Gram reaction, one drop of distilled water was added onto a clean glass slide. A fresh bacterial colony was transferred to the water droplet by sterilised loop, then mixing the colony and water to produce a smear. The smear was dried in an incubator at 35 °C and heat-fixed for 3 seconds. Crystal violet was added over the fixed culture for 1 min. After 1 min, the stain was poured off and rinsed with running potable water. The smear was cover by Iodine solution for 1 min and rinsed with running potable water. Around 3 drops of decolouriser (ethanol) were added to the slide, then the slide was rinsed after 5 seconds. Safranin was added to the slide and left for 30 seconds, then gently rinsed off with running water followed by air-drying. The Gram-stained cell were examined under oil immersion (x1000) with a Carl Zeiss Transmission light microscope (Model HBO 50/AC, Germany). The cell sizes were measured with the AxioVision microscope software version 4.8.1 and cell shapes were recorded. The Gram-positive cells with rod-shaped and cell length of 1 – 4 µm were presumptively considered to be *Lactobacillus* spp. when examined under the microscope (Aplevicz et al., 2014; Calasso & Gobbetti, 2011; Corsetti et al., 2005; Gül et al., 2005).

The catalase activity test was performed on the purified LAB colonies according to the method described by Reiner (2010). A small portion of bacterial colony was smeared onto a clean, dried glass slide by sterilised inoculating loop. A drop of H_2O_2 was added on the top of the smear. The formation of bubbles was immediately observed on the slide against a dark background. No bubble formation represents a catalase-negative reaction. The Catalase-negative and Gram-positive cells were presumptively considered to be *Lactobacillus* spp. (Aplevicz et al., 2014; Calasso & Gobbetti, 2011; Corsetti et al., 2005; Gül et al., 2005). Four Catalase-negative and Gram-positive colonies were selected as representative LAB colonies (two colonies from WSS and two colonies from RSS). They were purified again by re-streaking on MRS agar. The purified representative colonies were stored in MRS broth containing 30% (v/v) sterilised glycerol at -80 °C for further identification.

Purification of yeast colonies

Selected yeast colonies were re-streaked onto solidified YGC agar and plates were incubated under anaerobic conditions at 25 °C / 3 days under aerobic conditions. Purified yeast colonies were examined for methylene blue staining under the Carl Zeis transmission light microscope (Model HBO 50/AC, Germany) (Sami et al., 1994; Smart et al., 1999). Fifteen representative pure yeast colonies (eight colonies isolated from WSS and seven colonies isolated from RSS) were subjected to methylene blue staining.

A drop of 1% methylene blue was pipetted on a clean microscope slide. A small portion of fresh pure colony was transferred to the microscope slide and gently mixed well with microscope slide methylene blue drop by a sterile inoculating loop. A cover slip was gently placed on top of the slide to avoid trapping air bubbles. The methylene blue stained culture was examined x1000 under oil immersion with a Carl Zeiss transmission light microscope (Model HBO 50/AC, Germany). The cell sizes were measured using the AxioVision microscope software version 4.8.1 and cell shape was recorded. The methylene blue stained cells with spherical and egg-shape, partially budding and cell diameters between 1.00 to 10.00 µm were presumptively considered to be yeast when examined under the microscope (Tofalo & Suzzi, 2016). Four representative yeast colonies (two colonies from WSS and two colonies from RSS) were purified again by re-streaking on YGC agar. The purified representative colonies were stored in YPD broth (Merck, Germany) containing 30% (v/v) sterilised glycerol at -80 °C for further identification (Yang et al., 2021; Yuma, 2020).

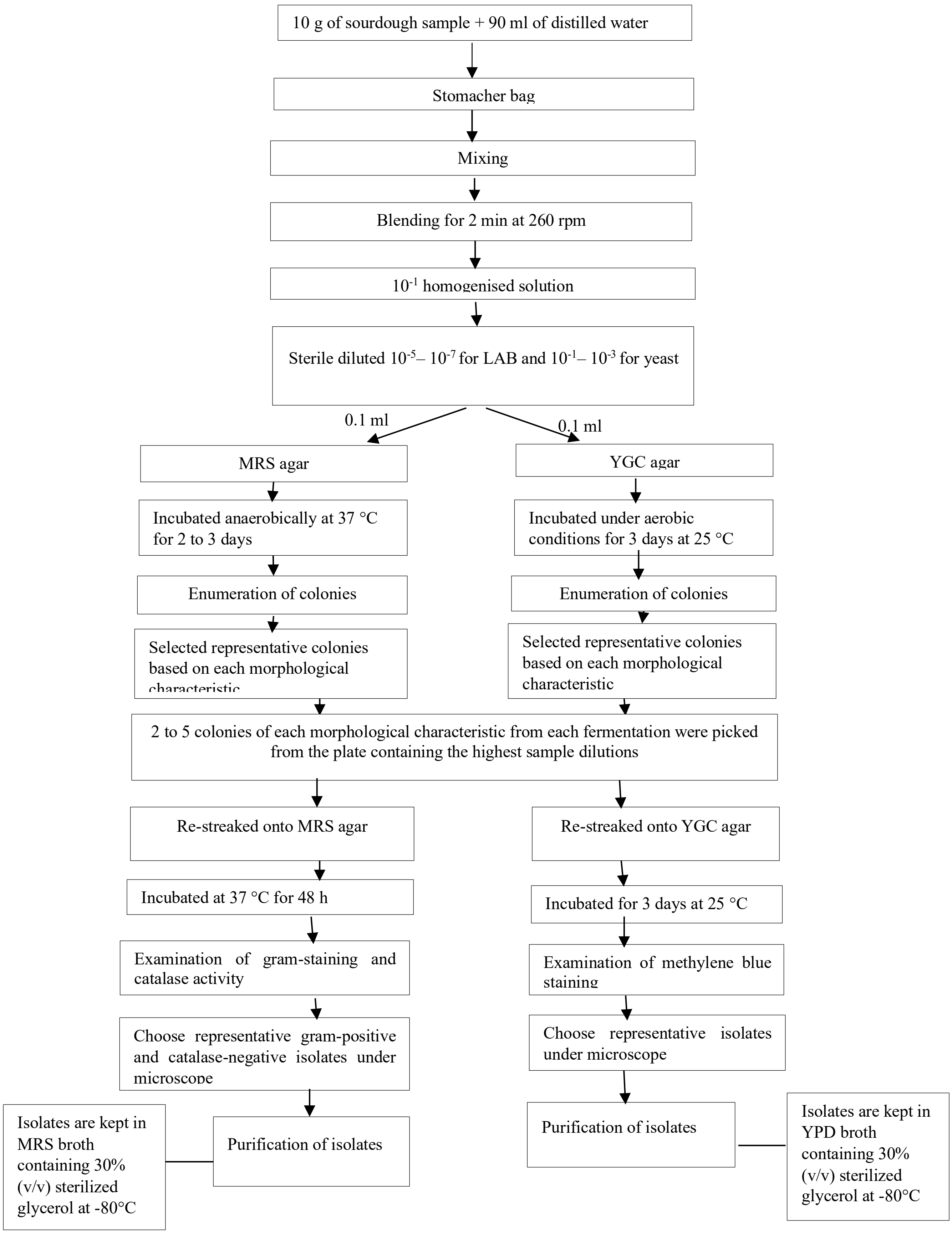


Figure 3.4 Isolation and purification of LAB and yeast

3.3.4 Identification of LAB and yeast

Identification of LAB and yeast were carried out by the Molecular Laboratory (Auckland Campus, Massey University) and Massey Genome Service (Palmerston North, New Zealand). The methods are briefly described in section 3.3.7.

3.3.5 16S rRNA gene sequencing analysis of LAB

Total DNA for each isolate was prepared using the Promega Wizard Genomic DNA Purification kit (Thermo Fisher Scientific, Auckland) according to the manufacturer's instructions. The purified DNAs were then used as templates to amplify the full-length 16S rRNA genes by polymerase chain reaction (PCR) with one pair of general primers 27F (Lane, 1991) and 1492R (TURNER et al., 1999). The PCR reactions were performed using Taq DNA polymerase from Invitrogen (Auckland, New Zealand). The PCR products were then purified using the E.Z.N.A. ® Cycle Pure Kit (Omega Bio-Tek Inc., Norcross). DNA sequencing was performed by the Massey Genome Service (Palmerston North, New Zealand) using three primers 27F and 1492R plus an internal primer 533R (Weisburg et al., 1991). Sequences of the primers are shown in the 5'-to-3' direction: 27F, AGAGTTTGATCMTGGCTCAG; 1492R, CGGTTACCTTGTTACGACTT; 533F, GTGCCAGCMGCCGCGGTAA.

The obtained DNA sequences were analysed by Geneious 9.1.8 (Biomatters Ltd, Auckland). The closest homologues (or best hits) were identified by searching the EzBioCloud 16S rRNA gene database (Yoon et al., 2017).

3.3.6 Sequence analysis of the intergenic transcribed spacer (ITS) regions of yeast rRNA gene locus

Primers ITS1 and ITS4 were used to amplify a 450-800 bp DNA fragment containing two ITS regions flanking the 5.8S rRNA gene (Raja et al., 2017; White et al., 1990). PCR was performed using Taq DNA polymerase (Invitrogen) directly from cells in a single colony on agar plate. The PCR products were then purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek InC, Norcross). DNA sequencing was performed by the Massey Genome Service (Palmerston North, New Zealand) using primers ITS1 and ITS4. Sequences of the primers are shown in the 5'-to-3' direction: ITS1, TCCGTAGGTGAACCTGCGG; ITS4, TCCTCCGCTTATTGATATGC.

The obtained DNA sequences were analysed y Geneious 9.1.8 (Biomatters Ltd, Auckland). The closest homologues (or best hits) were identified by searching the rRNA/ITS database of NCBI using Basic Local Alignment Search Tool (BLAST).

3.4 Determination of the physical properties of sourdough and sourdough baked bread

3.4.1 Texture analysis

(1) Texture analysis of sourdough bread

Bread crumb samples (25-mm thickness) were cut from a bread loaf. Hardness, cohesiveness, adhesiveness, springiness (elasticity), chewiness and gumminess of the sourdough bread (SDB) were measured using the Texture Analyser (TA.XT Plus, Stable Micro Systems, UK) with a 31-mm diameter probe. Tests were conducted in triplicate. The settings for the texture analyser are shown in Table 3.4. The AACC International Method 74-09.01 (AACC International, 2013) was used for the texture analysis.

Table 3.4 Texture analyser settings for assessing bread crumb

	Measurement	**Setup**	**Trigger**	**Setup**
Parameter	Pre-Test Speed	10 mm/s	Type	Auto (Force)
	Test Speed	1.70 mm/s	Force	20.0g
	Post-Test Speed	1.70 mm/s		
	Target Mode	Strain		
	Strain	50 %		
	Time	30s		

(2) Dough texture analysis

Texture parameters (hardness, adhesiveness, cohesiveness, springiness, gumminess, resilience, and chewiness) were determined with a Texture Analyser (Stable Micro Systems, UK). Analysis included a double compression test (Texture Profile Analysis (TPA)) (Table 4.2). A 75 mm diameter cylindrical probe was used at 20°C. The pre-test speed was set at 10 mm/s, the trial speed at 0.5 mm/s and the post-test speed at 10 mm/s, with a double cycle test to 60% compression level, at

0.98 N and for thirty-five seconds. The test was conducted in triplicate. Results were processed using Texture Expert software package (Stable Micro Systems, UK) (Casado et al., 2017).

Table 3.5 Texture analyser settings for bread dough

	Measurement	**Setup**	**Trigger**	**Setup**
Parameter	Pre-Test Speed	10 mm/s	Type	Auto(Force)
	Test Speed	0.5 mm/s	Force	98 N
	Post-Test Speed	10 mm/s		
	Target Mode	Strain		
	Strain	60%		
	Time	35s		

3.4.2 Loaf weight, loaf volume and specific volume

Bread samples were weighed using a top-pan digital balance (UW6200H, Shimadzu, Japan). Then the loaf volume was measured using the rapeseed displacement method (Al-Saleh & Brennan, 2012).

Briefly, 5 L of rapeseeds was measured in a graduated cylinder, then the loaf was placed into a rectangular plastic container (5 L) with flat bottom. The container was filled with rapeseeds to the top. The extra rapeseeds, which equal the loaf volume, were measured in a graduated cylinder. The specific volume of the loaf was calculated using the following equation 1.

$$\text{Specific volume } (cm^3/g) = \text{loaf weight} / \text{loaf volume} \qquad \text{Equation 1}$$

3.4.3 Colour analysis of bread crust and crumb

The colour parameters L* (lightness), a * (redness to greenness), b* (yellowness to blueness) of the baked loaves crumb and crust were determined using the Minolta CR-300 Chroma meter (Konica Minolta, Osaka, Japan). The equipment was used following the manufacturer's instructions. To determine the colour of the bread crust, the top crust was divided equally into three selected positions, and colour parameters L*, a*, b* were determined (Figure 3.5). Crumb colour measurements were taken in the centre of three chosen slices for each sample loaf. Colour measurements were performed in triplicate (Shittu et al., 2007).

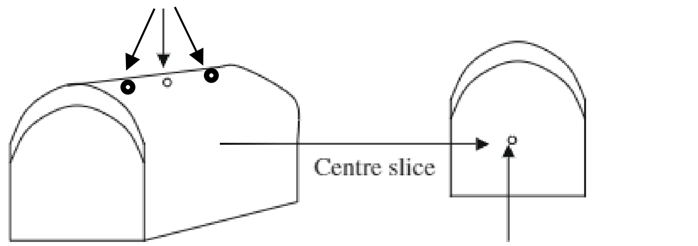


Figure 3.5 Locations outer crust and inner crumb (Mohd Jusoh et al., 2009).

3.5 Determination of the chemical properties of sourdough and sourdough bread

3.5.1 Standardization of NaOH

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). To standardise NaOH, about 20 g KHP (Univar, Ajax Finechem Pty Ltd, NZ) were dried and then dissolved in 500 ml distilled water. A 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 was observed. The volume of NaOH used was recorded. The titrations were repeated until concordant quadruplicate results were achieved.

The concentration of the prepared NaOH solution was calculated using equation 2.

$$C_{NaOH} = (m_{KHP} / MW_{KHP}) \times 1/ V_{NaOH} \qquad \text{Equation 2}$$

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 molecular weight of KHP (204.23 g/mol).

3.5.2 Sample preparation of sourdough for pH and total titratable acidity

Ten (10 g) of sample were weighed into a stomacher bag (Global Science, NZ), then 90 ml of distilled water were added to the sample in the bag. The mixture was homogenised for 3 min using a stomacher lab paddle blender (Masticator 400 ml, IUL, Spain).

3.5.3 Determination of total titratable acidity

Analysis of acidity of collected samples (WSS, RSS, DBP, DAP and SDB) were conducted (Ercolini et al., 2013; Lhomme et al., 2015) (Figure 3.6). Ten (10 g) of homogenised sample were placed into an Erlenmeyer flask and three - 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 were recorded. Titrations were conducted in triplicate.

The results were expressed as percentage of grams of lactic acid per g of sample (equation 3).

$$\% \text{ Lactic acid} = (C_{NaOH} \times V_{NaOH} \times MW_{\text{Lactic acid}}) / \text{Sample weight} \times 100\% \qquad \text{Equation 3}$$

3.5.4 pH measurement

A standardised glass electrode pH meter (HI 2221, Hanna Instruments, UK) equipped with a glass electrode was used to measure the pH of sourdough and sourdough bread suspensions prepared as described in Section 2.1.1. The pH meter was calibrated using standard buffers at pH 7.0, 4.0 and 10.0. pH were conducted in triplicate (Fujimoto et al., 2019; Yang et al., 2021).

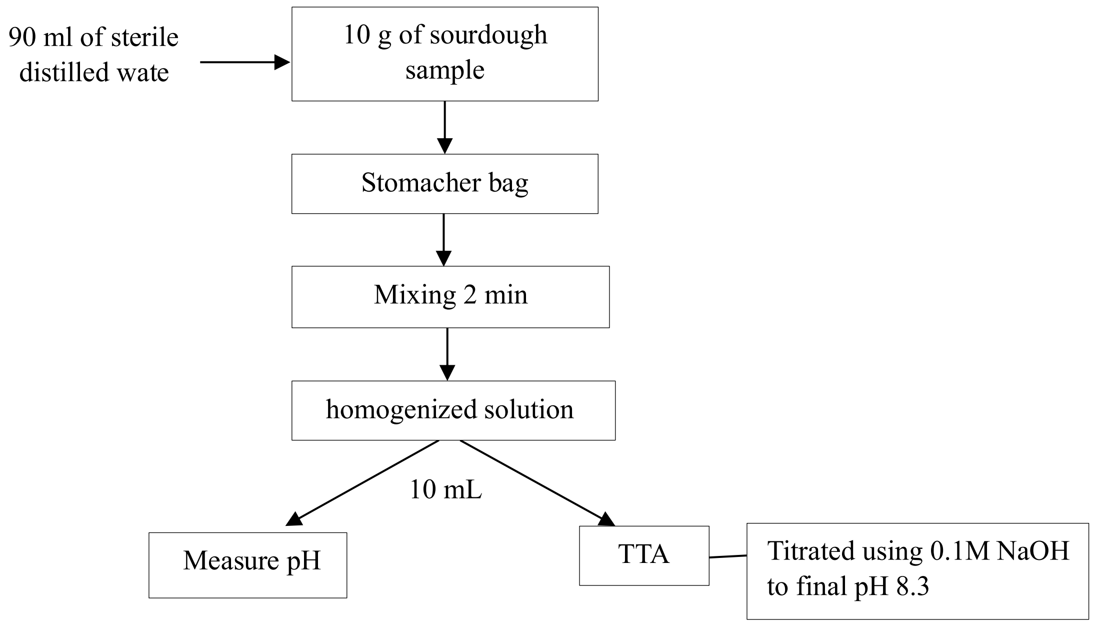


Figure 3.6 Determination of pH and TTA (Yang et al., 2021)

3.6 Determination of sugars and organic acids

Organic acids (lactic and acetic acids) and sugars (maltose, sucrose, glutose and fructose) were determined by HPLC according to the methods described by Buksa (2020)and Fujimoto et al. (2019) (Figures 3.7.1 and 3.7.2), respectively.

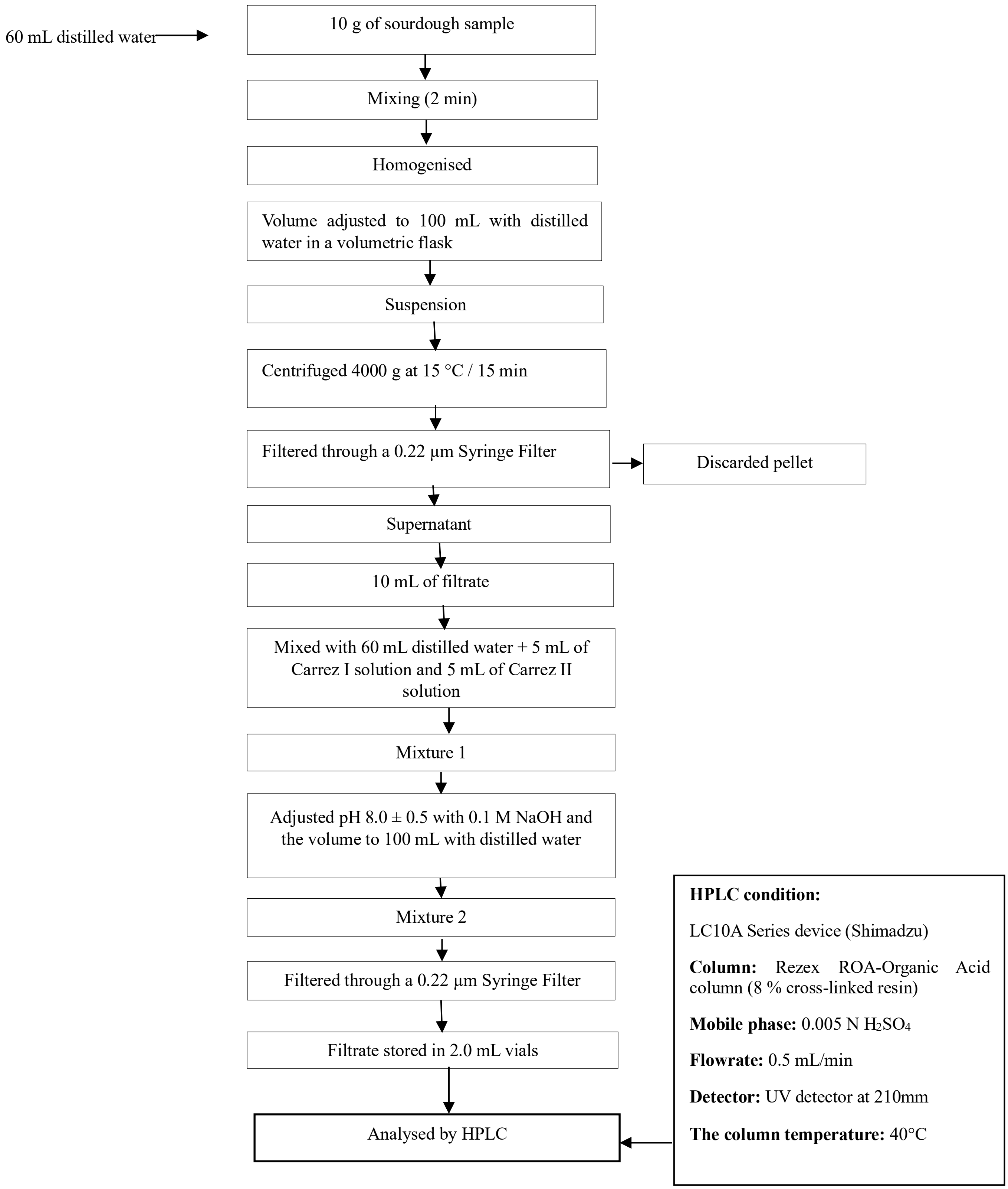


Figure 3.7.1 Determination of organic acids by HPLC (Buksa, 2020)

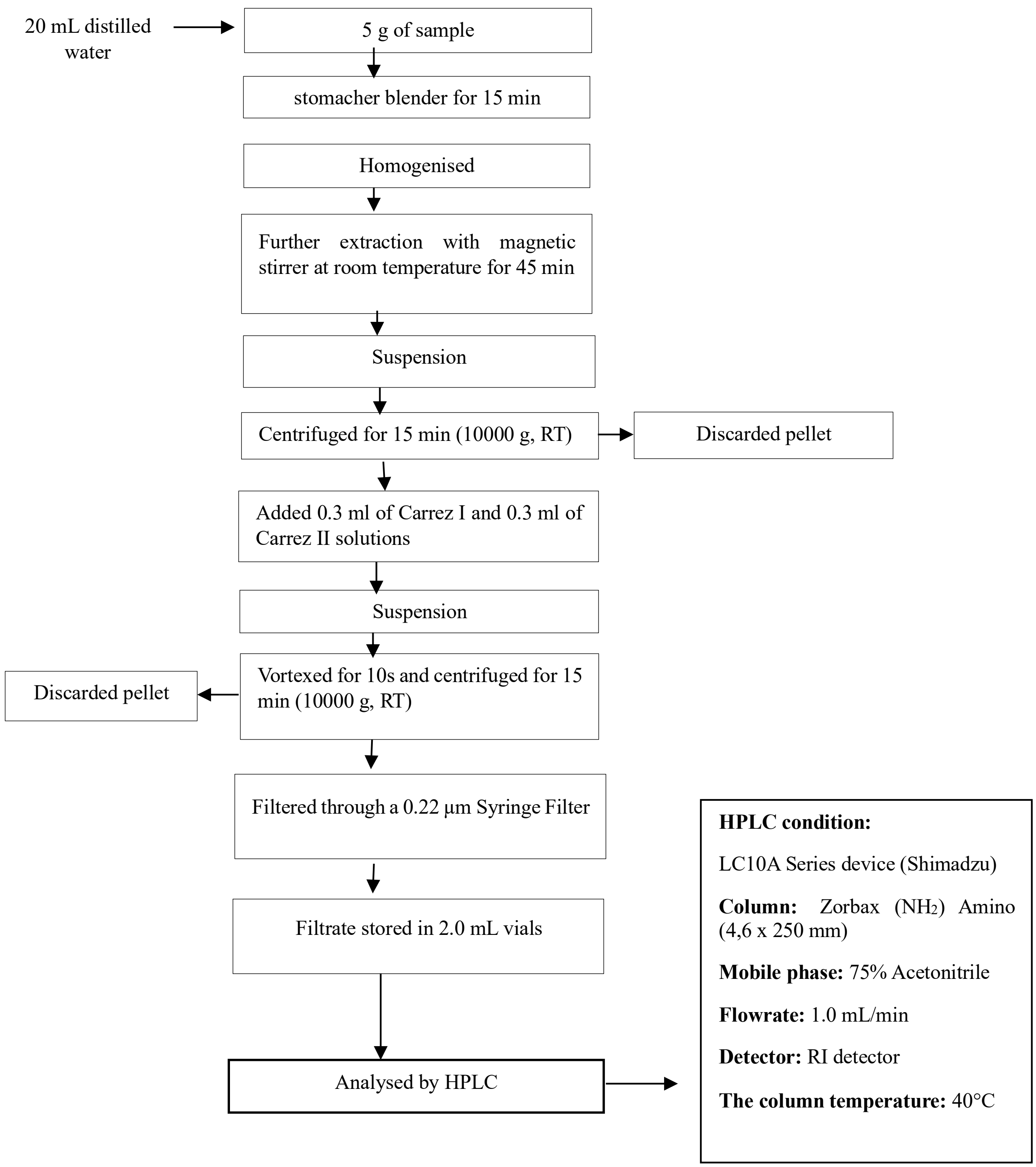


Figure 3.7.2 Determination of sugars by HPLC (Fujimoto et al., 2019)

3.6.1 Sample preparation for organic acid analysis

Organic acids were extracted from the sourdough and sourdough bread samples according to the method of Lefebvre et al. (2002). Ten g of sample was homogenised with 60 ml distilled water in a sterile stomacher bag (Global Science, NZ) using a stomacher lab paddle blender (Masticator 400 ml, IUL, Spain) for 2 min. The volume of the homogenised sample was adjusted to 100 ml with distilled water in a volumetric flask. The homogenised sample was centrifuged at 4000 g (Heraeus Multifuge × 1R; Thermo Fisher, Germany) at 15 °C for 15 min and the supernatant was filtered through a 0.22 µm syringe filter (Terumo, Australia). Ten ml aliquots of the filtrate were mixed with 60 ml distilled water, and 5 ml of Carrez I solution (0.085 mol/ L potassium II hexaferrocyanate) and 5 ml of Carrez II solution (0.25 mol/L zinc sulphate). The pH of the mixture was adjusted to pH 8.0 ± 0.5 with 0.1 mol/L NaOH and the volume made up to 100 ml with distilled water. The samples were filtered through 0.22 µm syringe filters and stored in 2.0 ml vials at 4 °C.

3.6.2 Sample preparation for sugar analysis

Sugars were extracted from the sourdough and sourdough bread samples (Buksa, 2020) with slight modification. Five g of sample were homogenised with 20 ml distilled water in a sterile stomacher bag (Global Science, NZ) using a stomacher lab paddle blender (Masticator 400 ml, IUL, Spain) for 15 min. Homogenised solution was covered by aluminium foil in a baker and stirred on a magnetic stirrer (IKA) at room temperature (RT, 22 ℃) for 45 min. After stirring, the sample solution was evenly distributed to 1.5 ml microtubes and centrifuged in a micro-centrifuge at RT (22 ℃) for 15 min at 10000 g (Heraeus Multifuge ×1R, Thermo Fisher, Germany). Carrez I (0.3 ml) and Carrez II (0.3 ml) solutions were added to 10 ml of supernatant; samples were mixed using a vortex mixer for 10 seconds and centrifuged for 15 min (10000 g, RT). The supernatant was filtered through a 0.22 µm syringe (Terumo, Australia), and stored in HPLC vials at 4°C. The filtrate (20 µl) was used for analysis of sugars.

3.6.3 Preparation of standard solutions

HPLC-grade standard sugars (fructose (Sigma Aldrich, NZ), glucose (Sigma Aldrich, NZ), sucrose (Sigma Aldrich, NZ) and maltose (Sigma Aldrich, NZ)) and organic acids (lactic acid (Fisher Scientific, UK) and acetic acid (Fisher Scientific, UK)) were dissolved in distilled water for use as standards. Retention times were determined for sugars and organic acids were recorded and read to construct standard curves produced from solutions diluted as shown in Tables 3.6 and 3.7 presented. HPLC conditions and the mobile phase used are shown in Table 3.8.

Table 3.6 Serial dilutions of organic acids

Organic acid	**Supplier**	**Serial dilution (mg/100 ml)**
L-(+)-Lactic acid (≥98.0%)	Sigma-Aldrich, NZ	0.5, 1, 2, 3, 5, 10, 15, 25
Acetic acid (≥99.7%)	Fisher Scientific, UK	0.5, 1, 2, 3, 5, 10, 15, 25

Table 3.7 Serial dilutions of sugars

Sugar	**Supplier**	**Serial dilution (mg/ml)**
D-(-)-Fructose (≥99.0 %)	Sigma-Aldrich, NZ	0.1, 0.2, 0.4, 0.8, 1.0
D-(+)-Glucose (≥99.5 %)	Sigma-Aldrich, NZ	1.5, 2.0, 4.0, 8.0, 15
Sucrose (≥99.5 %)	Sigma-Aldrich, NZ	0.1, 0.2, 0.4, 0.8, 1.0
D-(+)-Maltose-monohydrate (≥99.0 %)	Sigma-Aldrich, NZ	1.5, 2.0, 4.0, 8.0, 15

Table 3.8 HPLC conditions used for analysis of organic acids and sugars

	Organic acid	**Sugar**
Column	Rezex ROA-Organic Acid column (8 % cross-linked resin)	Zorbax (NH_2) Amino (4,6 x 250 mm)
Manufacturer	Phenomenex	Agilent Technologies
Mobile Phase	0.005 N H_2SO_4	75% Acetonitrile
Flowrate	0.5 ml/min	1.0 ml/min
Detector	UV detector at 210 mm	RI detector
Column Temperature	40°C	40°C

3.7 Analysis of nutritional and functional compounds in sourdough and sourdough bread

Analysis of resistant starch and total folate in sourdough and sourdough bread were performed by the Nutrition Laboratory (Massey University, Palmerston North, NZ).

3.7.1 Analysis of resistant starch in sourdough and sourdough bread

(1) Hydrolysis and solubilisation of non-resistant starch

Resistant starch was determined using the Megazyme (2019) method (Figure 3.8). Ground sample was weighed (100 ± 5 mg) into screw capped tubes and the tubes gently tapped to ensure the sample was at the bottom Pancreatic α-amylase (4.0 ml; 10 mg/ml) containing amyloglucosidase (AMG) (3 U/ml) was added to the tubes. The tubes were tightly capped, mixed on a vortex mixer and attached horizontally in a shaking water bath, aligned in the direction of motion. The tubes were incubated at 37°C in incubator with continuous shaking (200 strokes /min) for 16 h. After incubation, 4.0 ml of ethanol (99% v/v) were added into each tube with stirring on a vortex mixer Mixed samples were then centrifuged at 1500 g for 10 min. The supernatant was discarded, and the pellet resuspended in 2 ml of 50% ethanol with stirring on a vortex mixer, followed by centrifugation at 1500 g for 10 min. The supernatant was discarded, and the suspension and centrifugation were repeated.

(2) Analysis of Resistant Starch

After the third centrifugation, the supernatant was decanted, and the pellet resuspended in 2 ml of 2 M KOH and stirred for 20 min in an ice/water bath. Eight (8) ml of 1.2 M sodium acetate buffer (pH 3.8) were added to the tube with stirring with a magnetic stirrer, and 1 ml of amyloglucosidase (AMG) (solution 1; 3,300 U/ml) added to the tube and mixed well. The tubes were then incubated in a water bath at 50°C for 30 min with intermittent mixing on a vortex mixer. The incubated tubes were finally centrifuged at 1,500×g for 10 min. An aliquot (0.1 ml) of the supernatants were transferred into glass test tubes, then 3.0 ml of glucose determination reagent (GOPOD reagent) were added, and the tubes were mixed and incubated at 50°C for 20 min. The absorbance of the solutions was then measured at 510 nm against the reagent blank of water.

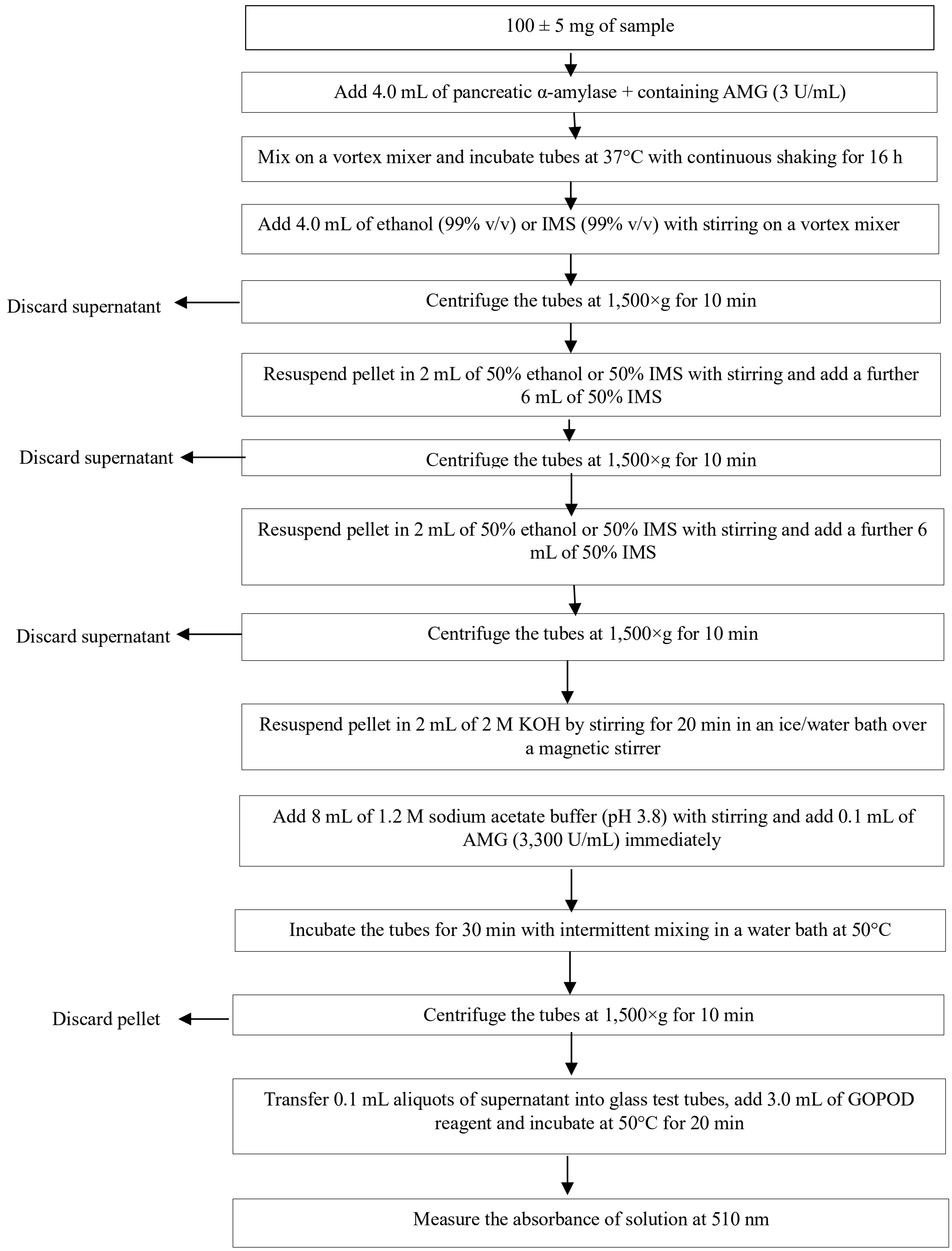


Figure 3.8 Determination of resistant starch by the Megazyme method (2019)

3.7.2 Analysis of total folate in sourdough and sourdough bread

(1)Sample extraction

Total folate in the sourdough and sourdough breads were analysed as described by the AOAC Official Method 2013.13 (Meisser-Redeuil et al., 2019) (Figure 3.9). Ground samples (25g) were dissolved with 50 mg α-amylase in 200 g of warm water (40°C) for 15 min. An aliquot of 15 g of the reconstituted sample was then weighed into a 100 ml amber volumetric flask, then 40 ml extraction buffer (100 mmol/L phosphate buffer; 2% ascorbic acid; 0.1% dithiothreitol (DTT); pH 4.5) were added. The flask was heated at 90°C for 30 min while stirring. Protease solution (2 ml) (4 mg/ml) was added into the flask after cooling to room temperature (RT, 22 °C) and the flask containing the protease solution was then incubated in a water bath at 37°C for 30 min. After cooling the solution to RT, the volume of the solution was made up to 100 ml mark with distilled water. The solution was filtered through folded paper filter (90 mm) and 10 ml of filtrate was then transferred to a 10-ml amber glass volumetric flask followed by the addition of 50 µL of 5 µg/ml internal standard (IS) solution. The solution (3 ml), containing the IS was loaded on a strong anion exchange (SAX) cartridge (previously conditioned with 4 ml acetonitrile and equilibrated with 10 ml extraction buffer). The cartridge then washed with 6 ml extraction buffer, and analytes were eluted with 4 ml of SPE eluting solution (Acetonitrile–extraction buffer– acetic acid - 6 : 3 : 1) into amber glass tubes. The eluate was then evaporated in a water bath (55 °C) under nitrogen. Finally, the extracts were reconstituted with 1.5 ml deionised H_2O, 1% ascorbic acid, 0.5% DTT and filtered through a 0.22 µm membrane filter into an amber liquid chromograph (LC) vial.

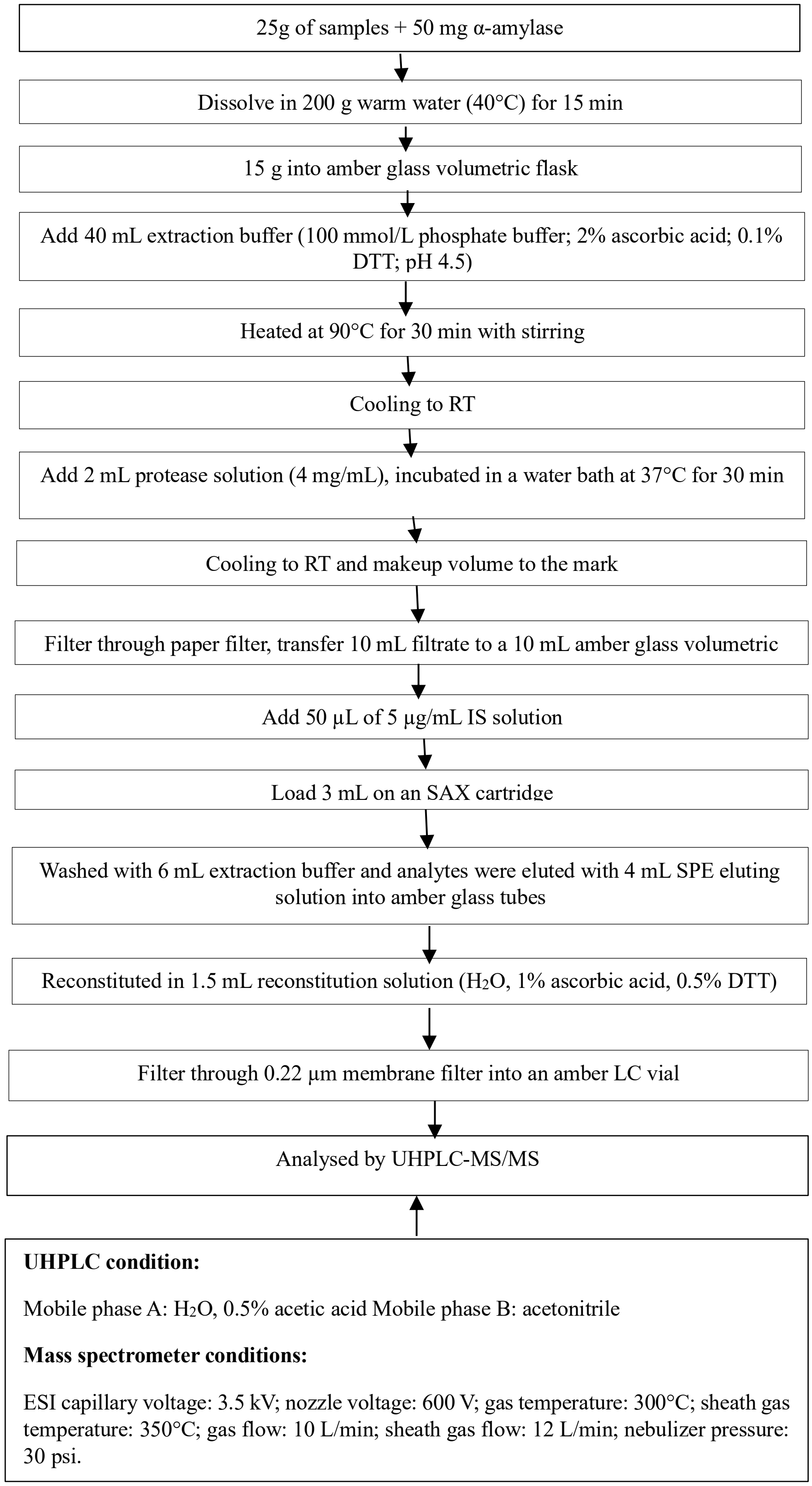


Figure 3.9 Determination of total folate by UHPLC-MS/MS method (Meisser-Redeuil et al., 2019)

3.8 Sensory evaluation of sourdough bread

Sensory evaluation of sourdough breads was carried out one day after baking using a focus group (Svensson, 2012). Six panellists who were familiar with the characteristics of sourdough bread were chosen at a commercial bakery. The sensory evaluation was carried out as advised by Resurreccion (1998). The focus group evaluated the bread samples for colour, sourness, taste, texture, saltiness, aroma and overall acceptability.

3.9 Data Analysis

Data were analysed by statistical tests using procedure of Minitab 16 Statistical Software (Minitab Inc., State College, PA, USA). Significancy were determined through one-way ANOVA, paired t-test and two-sample t-test in this work.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Phase I

In phase I, wheat sourdough fermentation was standardised at a commercial bakery. Wheat sourdough starter and rye sourdough starter were characterised for acidity (pH and total titratable acidity, TTA) and LAB and yeast content. Sourdough fermentation using wheat sourdough starter and rye sourdough starter was investigated by determining the acidity of DBP, DAP and SDB samples. LAB and yeast in DBP and DAP were enumerated by using solidified MSR and YGC agars. Texture analysis was performed on DBP, DAP and SDB to determine the impact of sourdough fermentation on bread dough texture and the texture of sourdough bread. Various other characteristics (loaf weight, loaf volume, specific volume, bread colour) were determined for the SDB.

4.1.1 Acidity of sourdough and sourdough bread

The acidity of rye sourdough starter and wheat sourdough starter are shown in Figure 4.1. The pH of WSS and RSS were similar ($p > 0.05$) whereas the TTA of RSS was higher than the WSS ($p < 0.01$). These findings were similar to a previous study (Salovaara & Valjakka, 2007), which reported the TTA of rye sourdough was higher than wheat sourdough, although the pH of the two starters were similar. The slight differences in the acidity of the two starter cultures may be due to differences in the ash content and buffering capacity of the flours (Decock & Cappelle, 2005). Rye flour has a higher ash content and buffering capacity than wheat flour, resulting in higher biochemical activities of the sourdough microflora, and hence more acids are formed in the rye sourdough starter than the wheat sourdough starter (Banu et al., 2011; Salovaara & Valjakka, 1987).

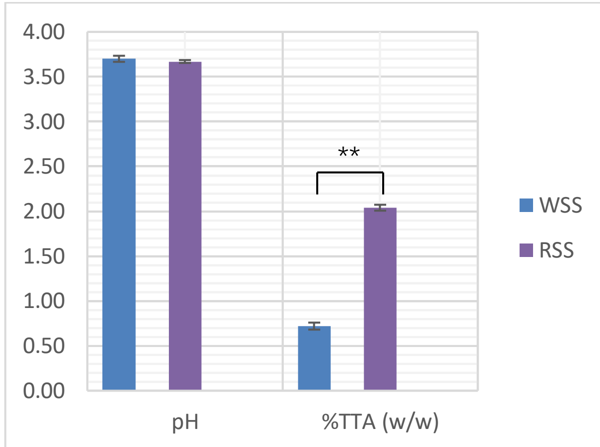


Figure 4.1 Acidity of sourdough starters

RSS = rye sourdough starter, WSS = wheat sourdough starter; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; TTA = total titratable acid; error bars indicate SEM; TTA values between WSS and RSS were significantly different (** indicates that values were significantly different at $p < 0.01$).

The acidity of both WSS and RSS dough-before-proofing increased after sourdough fermentation at 27 ± 0.5 °C/5.5 h followed by cold-fermentation at 6 ± 1 °C / 19 ± 1 h (Figure 4.2). The pH of DBP (4.06 ± 0.03) was higher than DAP (3.56 ± 0.00) (($p < 0.01$), whereas DBP (0.68 ± 0.03) had a lower TTA than DAP (1.17 ± 0.01) ($p < 0.01$). The ambient and cold-fermentation temperatures for the sourdough process also caused a reduction in acidity of the dough in a study by Wang et al. (2020), who reported a pH decrease from 4.70 to 3.80 while TTA of the dough increased from 3.42 to 9.96 after fermentation. The increase in acidity was attributed to the organic acids produced by LAB accumulating in the sourdough during ambient fermentation and cold-fermentation (Welch & Ullman, 1999).

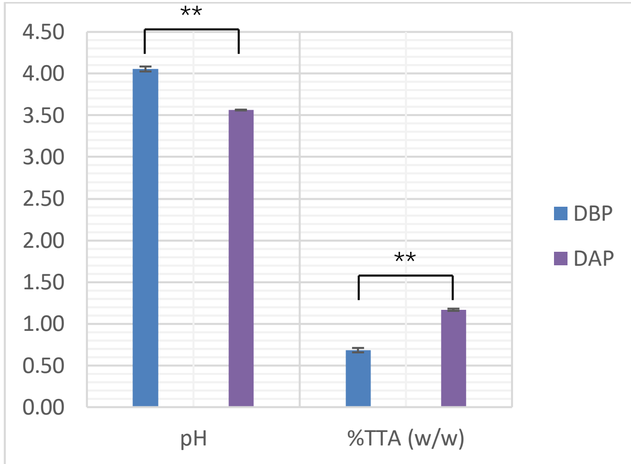


Figure 4.2 Acidity of sourdough before and after proofing (fermentation)

DBP = dough-before-proofing, DAP = dough-after-proofing; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; TTA = total titratable acid; error bars indicate SEM; TTA and pH values between DBP and DAP were significantly different (** indicates that values were significantly different at $p < 0.01$).

The acidity of dough-after-proofing and sourdough bread is shown in Figure 4.3. Sourdough bread had a higher pH than DAP ($p < 0.01$), whereas the TTA of the DAP was higher than the SDB ($p < 0.01$). Baking (220°C/20 min) reduced the acidity of the baked bread, likely due to the volatility of acetic acid (bp118 °C) and other fatty acids such as propionic acid (b.p.141°C) and butyric acid (b.p.163°C) at high temperatures (> 200 °C) (Brandt, 2019; Dimian et al., 2019; Schönfeld & Wojtczak, 2016; Umeta & Faulks, 1989). Another study also reported a reduction in acidity of sourdough bread following baking (230°C for 17 min) (Novotni et al., 2013).

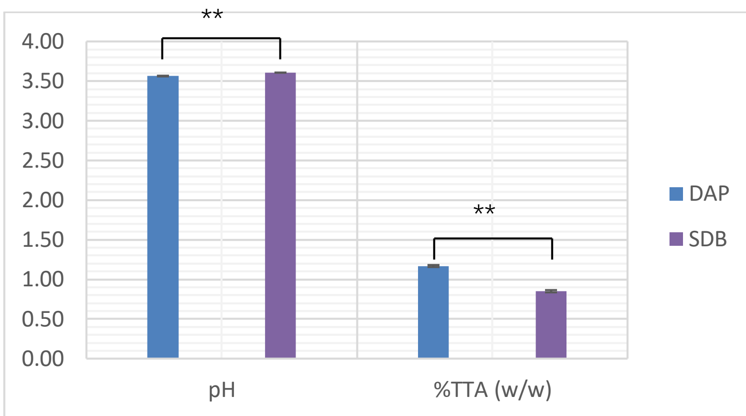


Figure 4.3 Acidity of sourdough-after-proofing and sourdough bread

DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; TTA = total titratable acid; error bars indicate SEM; TTA and pH values between DAP and SDB were significantly different (** indicates that values were significantly different at $p < 0.01$).

4.1.2 Texture of sourdough and sourdough bread

Hardness of sourdough and sourdough bread

The hardness of the dough-before-proofing (1007.40 ± 63.90 g) did not differ from dough-after-proofing (920.94 ± 20.80 g) following sourdough fermentation at 27 ± 0.5 °C/2 h followed by cold-fermentation for about 20 h ($p > 0.05$) (Figure 4.4). This finding is different to a previous study (Tomić et al., 2023), which reported a softer dough after sourdough fermentation at 25°C / 24 h. The different findings are probably due to different fermentation temperatures (Bernklau et al., 2017). Cold-fermentation used in this study can cause water crystallisation in the dough, which results in the loss of polymer cross-linking through breakage of interchain disulphide (S–S) bonds (Kontogiorgos et al., 2007; Wang et al., 2015). Consequently, the gluten structure weakens (Ribotta et al., 2001; Ribotta et al., 2004; Wang et al., 2015), thereby resulting in the reduction of hardness.

As expected, the hardness of the sourdough bread increased to nearly 1200 *g* after baking at 220°C/20 min (Figure 4.4), which was similar to a study by İçöz et al. (2004), who reported an increase in hardness of sourdough bread baked at 225 °C/12 min. The firming of the bread crumb is caused by gelatinisation during baking, retrogradation during cooling and crystallisation during storage (Bou-Orm et al., 2023). Baking disrupts starch granules, leading to the reorganisation of amylose and amylopectin molecules to form orderly structures during cooling, which firms the bread crumb. The hardening of bread can also be attributed to the loss of water in the bread crumb during storage (Bárcenas & Rosell, 2006). Additionally, protein denaturation and coagulation that occur during baking and sourdough acidification respectively also contribute to the hardening of the bread crumb (Singh, 2005).

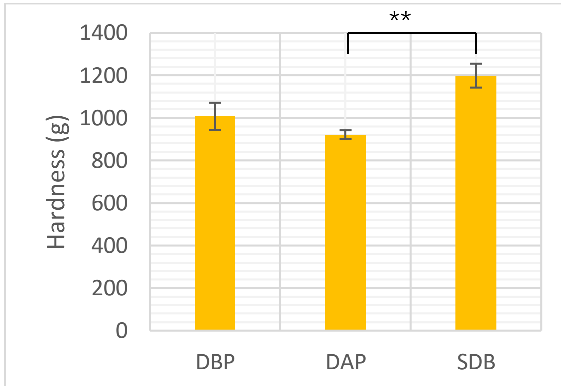


Figure 4.4 Hardness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were measured in triplicate; error bars indicate SEM; Hardness (g) were significantly different between DAP and SDB (** indicates that values were significantly different at $p < 0.01$).

Adhesiveness of sourdough and sourdough bread

Adhesiveness/stickiness is one of the most important textural parameters of bread. It is defined as the force required to overcome the attractive forces between the dough/bread surface and the material surface in contact with the dough/bread (Üçok & Sert, 2020).

The adhesiveness of dough increased after sourdough fermentation ($p < 0.01$) (Figure 4.5). This finding is similar to others (Clarke et al., 2002), who also reported an increase in adhesiveness of sourdough fermented at 30 °C/20 h. Adhesiveness of DAP (- 468.26 ± 14.30 g s^{-1}) was higher than DBP (-705.83 ± 64.20 g s^{-1}). The increase of adhesiveness of the DAP may be attributed to the increase in moisture content of the dough and the gluten structure which is weakened by acids during sourdough fermentation (Fu et al., 2018; Suwannarong et al., 2020). Starch gelatinisation caused by α-amylase in wheat and malt flours can also contribute to increased adhesiveness of bread (Tebben et al., 2018). High-molecular starch polymers are broken down into low-molecular dextrin by α-amylase during fermentation, causing increased stickiness in the dough (Chamberlain et al., 1981; Chen et al., 2013).

The adhesiveness of SDB (-0.90 ± 0.30 g s^{-1}) was higher than DAP ($p < 0.01$) (Figure 4.5) which indicated that the bread crumb had lower stickiness than the dough. This may be the result of starch gelatinisation during baking (Singh, 2005), where the starch granules swell due to the absorption of free water in the dough, thereby forming a viscous bread crumb (Chen, 1993). Similar findings were reported by Sidari et al. (2020) when they baked bread at 250 °C/15 min.

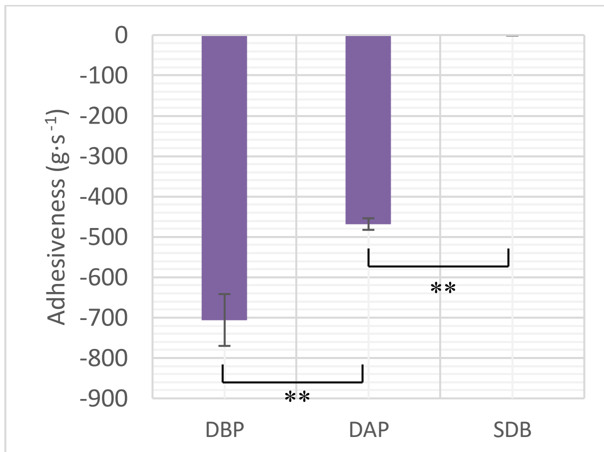


Figure 4.5 Adhesiveness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Adhesiveness were significantly different between DBP and DAP, DAP and SDB, respectively (** indicates that values were significantly different at $p < 0.01$).

Springiness of sourdough and sourdough bread

Springiness/elasticity in bread/dough is the ability of the bread/dough reverting to its non-deformed state after the compressive force is removed (Singh et al., 2014). The springiness of the sourdoughs during fermentation is shown in Figure 4.6. The springiness of the sourdough increased from 0.97 ± 0.00 (DBP) to 0.98 ± 0.00 (DAP) after cold-fermentation at 6 ± 1 °C/19 ± 1 h ($p < 0.05$). The increased springiness is caused by the formation of a more stable cross-linking of gluten proteins at a lower fermentation temperature (< 30°C), hence the dough becomes more elastic (Casado et al., 2017). A study by Wang et al. (2023) also reported an increase in elasticity of sourdough after cold-fermentation (6 °C/24 h).

Springiness of SDB increased from 0.98 ± 0.00 (DAP) to 1.48 ± 0.26 after baking ($p < 0.01$) (Figure 4.6). The increase in springiness is caused by the protein denaturation during baking and coagulation during fermentation (Singh, 2005). Denaturation of gluten protein occurs during sourdough acidification and baking process, resulting in the unfolding of proteins with the polypeptide chains becoming disordered. The unfolded proteins form an insoluble network during baking, which solidify to form a firm crumb; increasing the springiness of the bread crumb (Croxford, 2020; Ortolan & Steel, 2017).

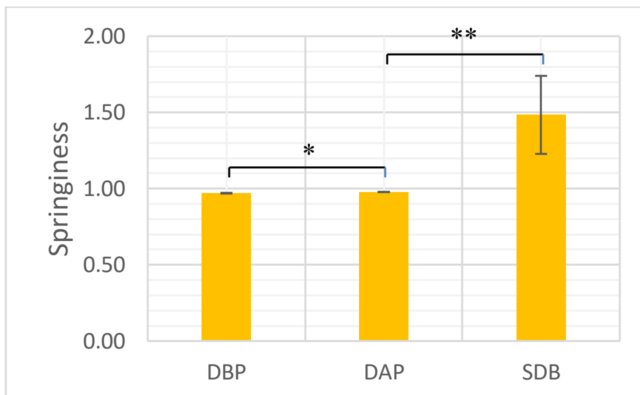


Figure 4.6 Springiness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Springiness between DBP and DAP were significantly different (* indicates that values were significantly different at $p < 0.05$); Springiness between DAP and SDB were significantly different (** indicates that values were significantly different at $p < 0.01$)

Cohesiveness of sourdough and sourdough bread

Cohesiveness is the strength of the internal bonds making up the body of the product, which is determined as the ratio of the positive area between the second bite and the first bite in the texture profile analysis (Figure 4.7) (Correa et al., 2017).

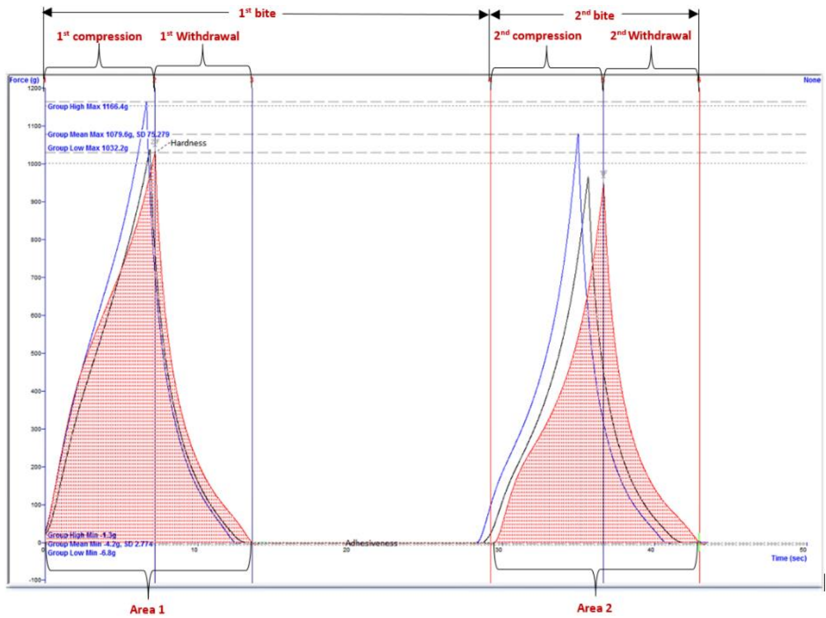


Figure 4.7 Image of texture analysis of sourdough bread crumb

Image captured by TA.XT Plus, Stable Micro Systems, UK

In this study, the cohesiveness of the dough reduced after sourdough fermentation (Figure 4.8), from 0.77 ± 0.01 to 0.71 ± 0.01 ($p < 0.01$). The reduction of cohesiveness is possibly due to the sea salt (1.10 %) added into the formulation (Simsek & Martinez, 2016). A study by (Král et al., 2018), also reported the cohesiveness of sourdough made with 2.0 % sea salt in the formulation decreased after fermentation. Sea salt contains $MgCl_2$ and KCl which can cause retention of more moisture in the dough after fermentation (Voinea et al., 2020). Therefore, the water-protein interaction is enhanced by the reduction in protein-protein interactions, which in turn increases the gluten solubility, and decreases dough strength/cohesiveness (Kłosok et al., 2021).

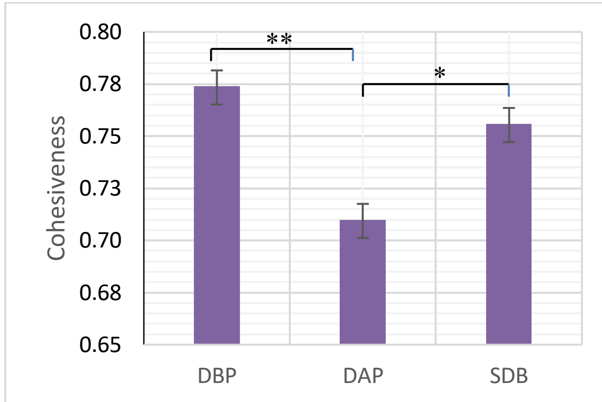


Figure 4.8 Cohesiveness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Cohesiveness between DBP and DAP were significantly different (** indicates that values were significantly different at $p < 0.01$); Cohesiveness between DAP and SDB were significantly different (* indicates that values were significantly different at $p < 0.05$).

Gumminess of sourdough and sourdough bread

Gumminess is proportional to hardness and cohesiveness (Huang et al., 2016), and represents the energy needed to disintegrate semi-solid state of food to a ready-to-swallow state (Correa et al., 2017). Gumminess depends more on the hardness of a product than cohesiveness (Armero & Collar, 1997).

The gumminess of the dough decreased from 779.00 ± 48.40 to 653.70 ± 15.90 after sourdough fermentation ($p < 0.05$) (Figure 4.9). This reduction can be linked to the reduced hardness and cohesiveness caused by the weakened gluten network during sourdough acidification (Armero & Collar, 1997). For the baked bread, gumminess increased from 653.70 ± 15.90 to 903.40 ± 36.70 ($p < 0.01$), which may be related to the increase of hardness and cohesiveness after baking (Casado et al., 2017).

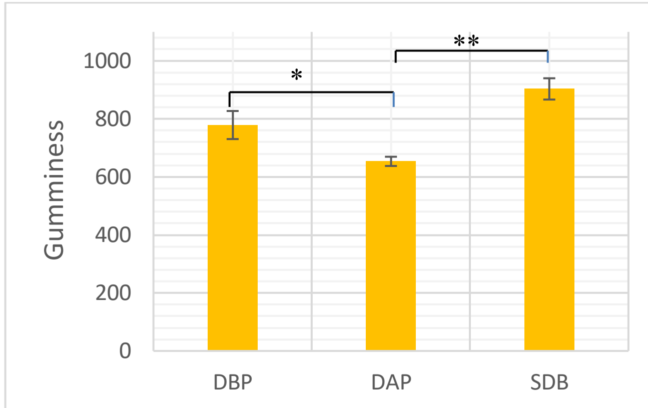


Figure 4.9 Gumminess of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Gumminess between DBP and DAP were significantly different (* indicates that values were significantly different at $p < 0.05$); Gumminess between DAP and SDB were significantly different (** indicates that values were significantly different at $p < 0.01$).

Chewiness of sourdough and sourdough bread

Chewiness is the combined product of hardness, gumminess and springiness (Bourne & Szczesniak, 2003). It is a secondary parameter in the texture profile, representing the force of chewing solid food into a state that can be swallowed (Singh et al., 2014).

The chewiness of sourdoughs and sourdough bread is shown in Figure 4.10. The chewiness of DAP (639.00 ± 15.20 g) was lower than DBP (756.41 ± 48.30 g) ($p < 0.05$). The reduction in chewiness of the dough after fermentation was expected due to the decrease in hardness (Armero & Collar, 1997). This is likely caused by the increase in protein solubility during fermentation, which decreases the chewiness of the dough (Demirkesen-Bicak et al., 2021).

Chewiness increased ($p < 0.05$) in SDB after baking due to the increased hardness in SDB (Armero & Collar, 1997). Several factors contribute to increased chewiness including loss of moisture, starch gelatinisation, protein denaturation during baking, gelation/amylose retrogradation of the starch during cooling and amylopectin retrogradation during storage (Bou-Orm et al., 2023; Purhagen et al., 2011).

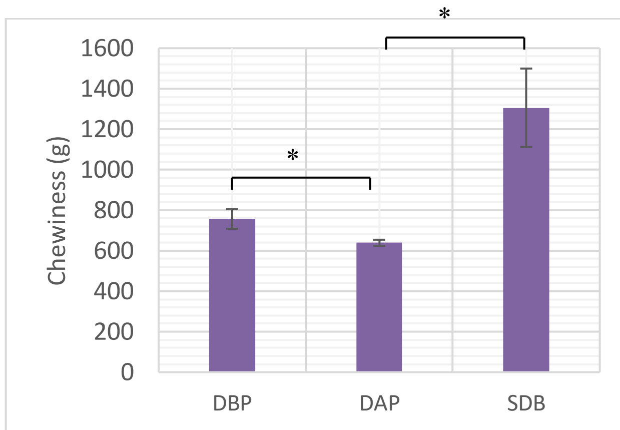


Figure 4.10 Chewiness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Chewiness between DBP and DAP were significantly different (* indicates that values were significantly different at $p < 0.05$); Chewiness between DAP and SDB were significantly different (* indicates that values were significantly different at $p < 0.05$).

Resilience of sourdough and sourdough bread

The ability of the bread/dough to spring back or return to its original shape when treated with a compressive force is described as resilience (Cauvain, 2015; Sahi et al., 2014). Resilience is an important textural parameter used to evaluate the freshness, crumb, and mechanical strength of leavened-baking products (Sahi et al., 2014).

Resilience of the dough did not change ($p > 0.05$) after sourdough fermentation (27 ± 0.5°C/2 h) followed by cold-fermentation (~20 h) (Figure 4.11). However, Demirkesen-Bicak et al. (2021), reported a reduction in the resilience of the dough fermented at 25 °C/24 h. These differences in results may be due to the different fermentation temperatures (Tang et al., 2019). The cold-fermentation process in the current study can result in moisture loss from the dough (Ding et al., 2015), which leads to the loss of polymer cross-linking and gluten structural deterioration (Kontogiorgos et al., 2007). As a consequence, the firmness of the dough changes very little after cold-fermentation, resulting in no significant changes in the resilience of the dough (Phimolsiripol et al., 2008).

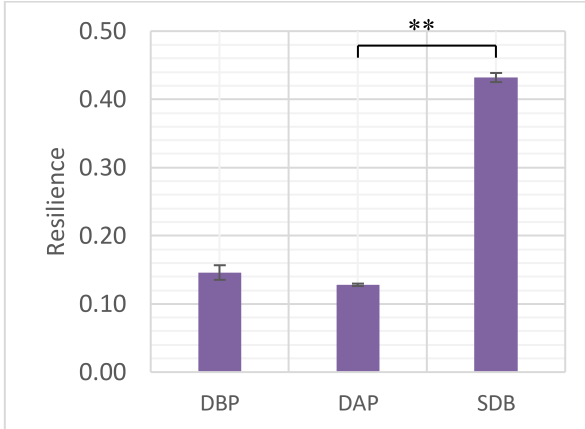


Figure 4.11 Resilience of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in triplicate; error bars indicate SEM; Resilience between DAP and SDB were significantly different (* indicates that values were significantly different at $p < 0.05$).

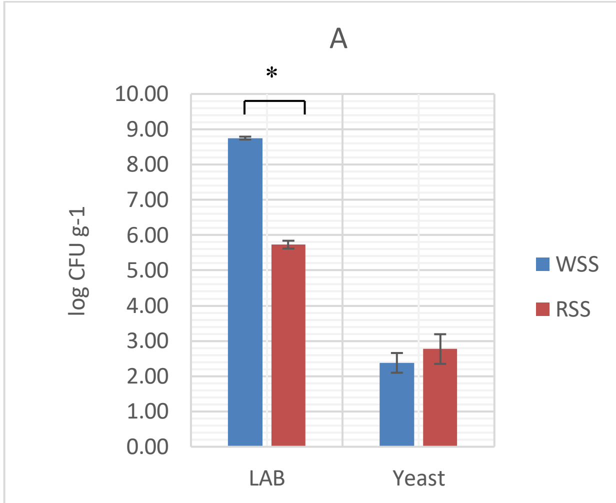
4.1.3 Microbiology of sourdough

Concentrations of lactic acid bacteria and yeast

Figure 4.12A shows that the LAB counts in WSS (8.75 ± 0.04 log CFU g^{-1}) were higher than those in RSS (5.73 ± 0.11 log CFU g^{-1}) ($p < 0.05$). These results are similar to previous studies (Bartkiene et al., 2019; Gül et al., 2005), which reported that LAB were about 8.14 ± 0.97 log CFU g^{-1} in wheat sourdough, and about 7.48 ± 0.11 log CFU g^{-1} in rye sourdough. The yeast counts in the WSS (2.38 ± 0.28 log CFU g^{-1}) did not differ from RSS (2.77 ± 0.42 log CFU g^{-1}) ($p > 0.05$). Similar yeast counts in rye sourdough (2.50 ± 0.40 log CFU g^{-1}) and wheat sourdough (2.85 ± 1.20 log CFU g^{-1}) have been reported (Katsi et al., 2021; Weckx et al., 2010).

In the current study, LAB grew much faster than the yeast, possibly due to competition for nutrients (nitrogen sources and fermentable carbohydrates) (Martín-Garcia et al., 2021). Another factor contributing to the differences in cell counts of LAB is the dough yield, which is the ratio of flour and water. RSS is a more viscous liquid starter culture as it contains less water and has more flour than the WSS. The firmer (more viscous) the sourdough (lower DY) the less LAB grow (Chavan & Chavan, 2011). Variations in the nutritional composition of the two types of starters may also have contributed to differences in cell counts (Posner, 2000). Rye flour is considered more nutrient and amylase dense than wheat flour (Sluková et al., 2021), therefore the growth of LAB may differ between the rye sourdough starter and wheat sourdough starter (Cauvain, 2015).

After sourdough fermentation (27 ± 0.5 °C/5.5 h followed by cold-fermentation at 6.5 ± 0.5 °C/19 ± 1 h), the DAP (8.54 ± 0.03 log CFU g^{-1}) contained higher LAB counts than DBP (8.32 ± 0.01 log CFU g^{-1}) ($p < 0.05$) (Figure 4.12B). Similar findings were observed in another study (Wang et al., 2020b), which reported high LAB counts in the wheat-dough after sourdough fermentation (25 °C/9 h) followed by cold-fermentation (6 °C/15 h). The significant increase of LAB in DAP is likely caused by the dominance of LAB after sourdough fermentation because LAB grow better than the yeast in the acidified sourdough environment (Vrancken et al., 2011). Yeast counts in the DBP (3.88 ± 0.14 CFU g^{-1}) were similar to that in DAP (3.74 ± 0.26 CFU g^{-1}) ($p > 0.05$). The acidification by LAB during sourdough fermentation causes the inhibition of yeast activity and growth (Casado et al., 2017), and hence the decrease in yeast counts in the dough after proofing.



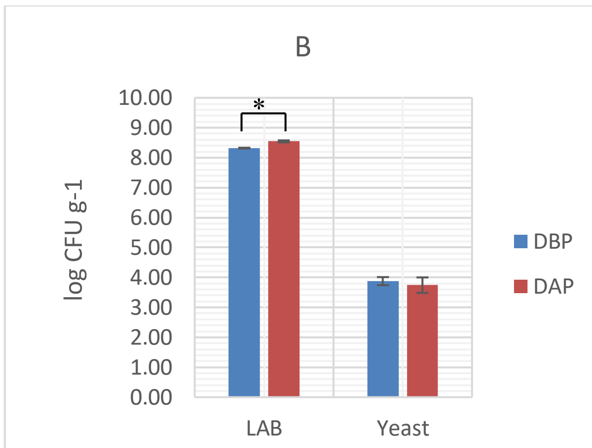


Figure 4.12 LAB and yeast counts of sourdough starters (A) and before and after proofing (fermentation) of sourdough (B)

RSS = rye sourdough starter; WSS = wheat sourdough starter, DBP = dough before proofing, DAP=dough after proofing; SDB = sourdough bread; mean values are results of three independent replications (n = 3); samples were analysed in duplicate; error bars indicate SEM; LAB cell counts between WSS and RSS were significantly different (* indicates that values were significantly different at $p < 0.05$); LAB cell counts between DBP and DAP were significantly different (* indicates that values were significantly different at $p < 0.05$).

4.1.4 Isolation and identification of LAB and yeast in sourdough starters

Morphology of isolated LAB colonies on agar plates

LAB colonies isolated from wheat sourdough starter and rye sourdough starters were small, creamy, white and circular with three different diameters (Table 4.1). Creamy and circular LAB colonies (diameter < 3 mm) isolated from sourdough starters were also observed in previous studies (Savic et al., 2006; Sun et al., 2022; Yang et al., 2021).

Table 4.1 Morphology of LAB colonies grown on MRS agar

Sourdough starter	Morphology of colonies	Diameter (Ø) mm
Wheat sourdough starter	Small, creamy, white, circular	1
	Small, creamy, white, circular	1.9
Rye sourdough starter	Small, creamy, white, circular	1
	Small, creamy, white, circular	1.9
	Small, creamy, white, circular	< 1

Morphology of isolated yeast colonies on agar plates

The yeast colonies isolated from wheat sourdough and rye sourdough starters were smooth, circular, flat-conical, creamy and white (Table 4.2). Yeast colonies isolated from rye sourdough starter had three different diameters (1, 2 and 3 mm), whereas the yeast colonies isolated from wheat sourdough starter also had three different diameters (1, 3 and 3.5 mm). In previous studies, yeast colonies isolated from sourdough starters were white, flat and circular with diameters ranging from 1 – 9 mm (Sun et al., 2022; Yang et al., 2021).

Table 4.2 Morphology of yeast colonies grown on YGC agar

Sourdough starters	Morphology of colonies	Diameter (Ø) mm
Wheat sourdough starter	Smooth, circular, flat-conical, creamy, white	1
	Smooth, circular, flat-conical, creamy, white	3
	Smooth, circular, flat-conical, creamy, white	3.5
Rye sourdough starter	Smooth, circular, flat-conical, creamy, white	1
	Smooth, circular, flat-conical, creamy, white	2
	Smooth, circular, flat-conical, creamy, white	3

Cellular morphology of isolated LAB

Gram-positive and catalase-negative LAB isolates were selected and divided into two groups: LAB originating from wheat sourdough starter (LWSS) (Figure 4.13) and those originating from rye sourdough starter (Figure 4.14). The LAB colonies from wheat sourdough (LWSS) appeared as long/short rods. The cell length of LWSS ranged from 1.52 µm to 3.02 µm, whereas the LAB from rye sourdough were short/long rods with cell length ranging from 1.48 µm to 4.03 µm. The LAB isolates of the two starter cultures may be presumptively identified as species of *Lactobacillus* based on their cellular morphology (Gram-positive and catalase-negative short/long rods with cell length between 1.00 µm to 10.00 µm) (Gänzle & Gobbetti, 2013).

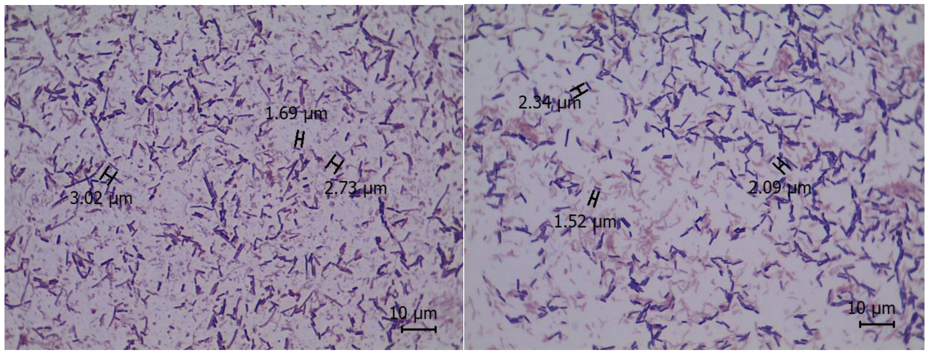


Figure 4.13 Gram-positive and catalase-negative LAB isolated from wheat sourdough starter

Magnification x1000 under Carl Zeis transmission light microscope (Model HBO 50/AC, Germany).

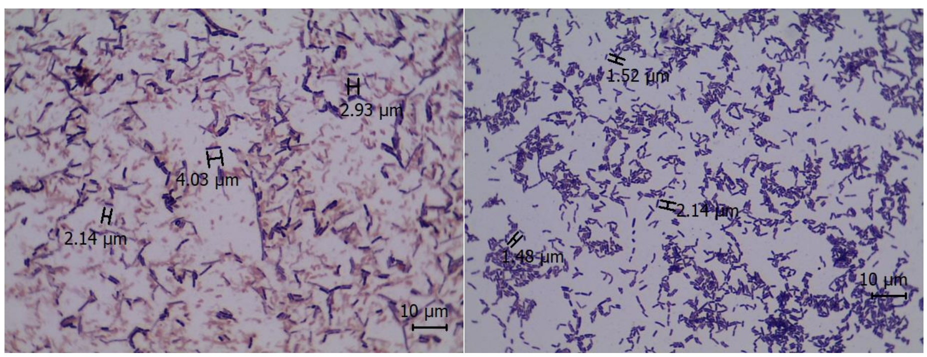


Figure 4.14 Gram-positive and catalase-negative LAB originated from rye sourdough starter

Magnification x1000 under Carl Zeis transmission light microscope (Model HBO 50/AC, Germany).

Cellular morphology of isolated yeast

Methylene-blue-stained yeast colonies were divided into two groups: yeast originating from wheat sourdough starter (YWSS) (Figure 4.15) and yeast originating from rye sourdough starter (YRSS) (Figure 4.16). The YWSS were circular and ovoid with cell diameters between 4.08 µm to 6.34 µm. The YRSS appeared spherical and egg-shaped with cell diameters between 3.86 µm to 6.79 µm. The YWSS and YRSS may be presumptively identified as species of yeast based on their cellular morphology (spherical and egg-shaped cell, partially budding and cell diameters between 1.00 µm to 10.00 µm) (Tofalo & Suzzi, 2016).

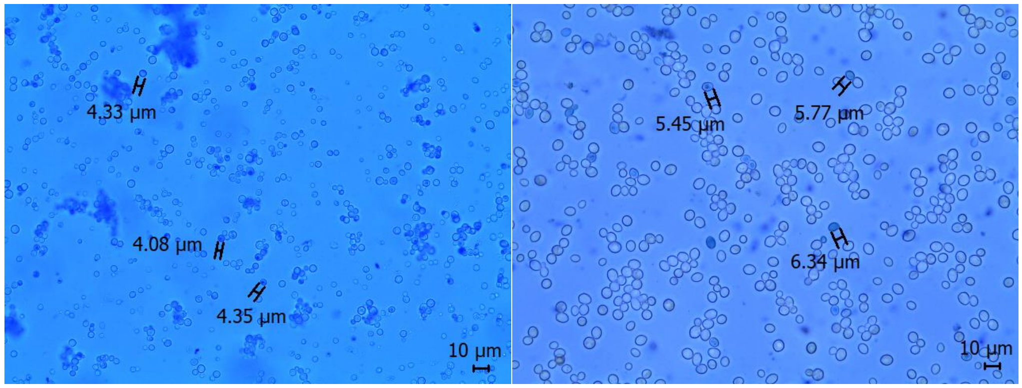


Figure 4.15 Methylene-blue-stained yeast originated from wheat sourdough starter

Magnification x1000; Carl Zeis transmission light microscope (Model HBO 50/AC, Germany).

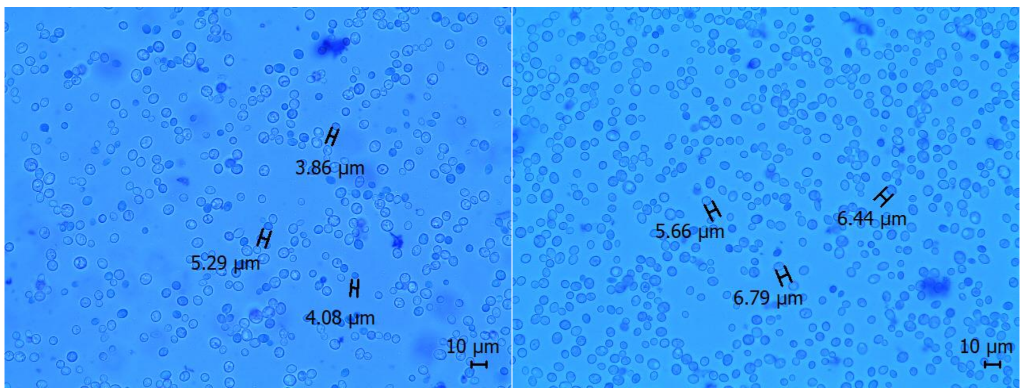


Figure 4.16 Methylene-blue-stained yeast originated from rye sourdough starter

Magnification x1000 under Carl Zeis transmission light microscope (Model HBO 50/AC, Germany).

4.1.5 Identification of isolated LAB and yeast

Genetic identification of the LAB and yeast isolates was carried out in the molecular microbiology laboratory. Briefly, ribosomal RNA genes were amplified by polymerase chain reaction (PCR), and resulting PCR products were subjected to Sanger's DNA sequencing at Massey Genome Service (Palmerston North, New Zealand). This led to the identification of two species of LAB and three species of yeast for strains isolated from wheat sourdough starter and rye sourdough starter (Table 4.3).

Table 4.3 LAB and yeast isolates from rye and wheat sourdough starters

Sourdough starter	LAB	Yeast
Rye sourdough starter (RSS)	*Fructilactobacillus* (*F.*) *sanfranciscensis* ATCC 27651	*Saccharomyces* (*S.*) *paradoxus* CBS 432
	Latilactobacillus (*L.*) *curvatus* JCM 1096	*Saccharomyces* (*S.*) *kudriavzevii* ATCC MYA 4449
Wheat sourdough starter (WSS)	*Fructilactobacillus* (*F.*) *sanfranciscensis* ATCC 27651	*Torulaspora* (*T.*) *delbrueckii* CBS 1146 *Saccharomyces* (*S.*) *paradoxus* CBS 432

Genetic identification of LAB isolates

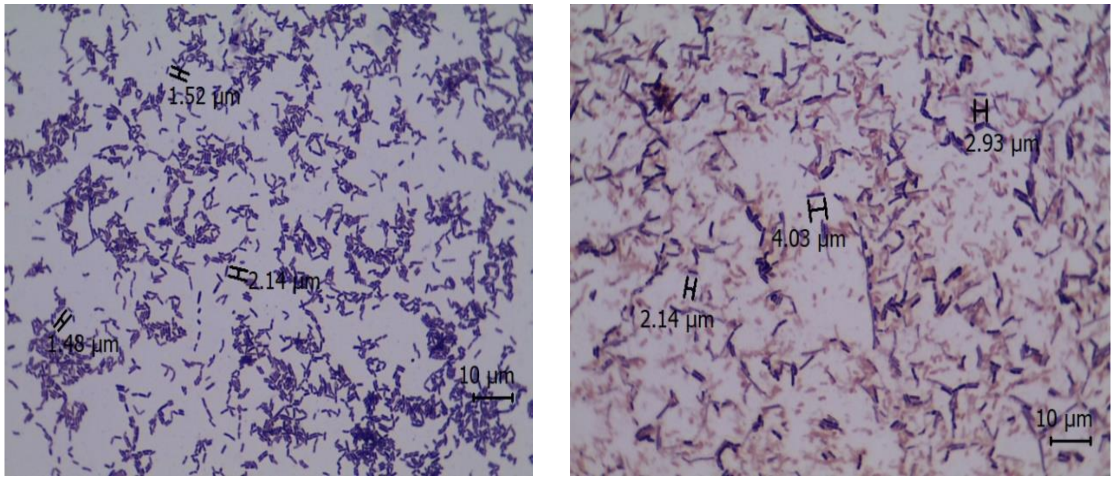
Results of the LAB genetic identification are summarised in Table 4.4. The four LAB isolates belong to two species of *F. sanfranciscensis* and *L. curvatus* (Figure 4.17).

Table 4.4 Genetic identification of four LAB isolates

Isolate	Best hit in the EzBioCloud 16S rRNA gene database				
	Taxon	Type strain	% Similarity	Variation	GeneBank accession no.
1	*Fructilactobacillus (F.) sanfranciscensis*	ATCC 27651	99.65	5/1425	X76327
2	*Latilactobacillus (L.) curvatus*	JCM 1096	99.86	2/1419	BBBQ01000060
3	*Fructilactobacillus (F.) sanfranciscensis*	ATCC 27651	99.65	5/1433	X76327
4	*Fructilactobacillus (F.) sanfranciscensis*	ATCC 27651	99.58	6/1435	X76327

Identified LAB species isolated from rye and wheat sourdough starters

F. sanfranciscensis ATCC 27651 was isolated from rye sourdough starter and wheat sourdough starter culture spontaneously fermented at low temperature (6 ± 1 °C / 18 ± 2 h). Results indicated that *F. sanfranciscensis* was the dominant bacterium in sourdough fermented at low temperature with long fermentation time. This is likely due to the tolerance of the bacterium to acidic conditions and low temperature (M Gobbetti et al., 1995; Zhang & Gänzle, 2010). Another contributory factor is the adaptability of *F. sanfranciscensis* in wheat/rye flour (Zhang et al., 2019). Similar results have been reported by others during wheat and rye sourdough fermentation at low temperatures for prolonged time (De Vuyst & Neysens, 2005; Vrancken et al., 2011).



(A) *L. curvatus* (B) *F. sanfranciscensis*

Figure 4.17 *L. curvatus* (A) identified in rye sourdough starter and *F. sanfranciscensis* (B) identified in rye sourdough starter and wheat sourdough starter

Magnification x1000 under Carl Zeis transmission light microscope (Model HBO 50/AC, Germany)

L. curvatus JCM 1096 was isolated from rye sourdough with a pH of 3.67 ± 0.02. The LAB found in the investigated rye sourdough was similar with a previous study (Lhomme et al., 2015). In the previous study, pH of rye sourdough was 3.23 ± 0.02. The dominance of *L. curvatus* in highly acidified rye sourdough is possibly due to the high acid tolerance of the species (Hong et al., 2018; Zommiti et al., 2017). Another reason may be that the *L. curvatus* has a strong ability to ferment carbohydrates in highly acidic environments (Papagianni & Anastasiadou, 2009; Stella et al., 2016).

Genetic identification of yeast isolates

Results of yeast genetic identification are summarised in Table 4.5. The four yeast isolates belonged to three species, namely, *S. paradoxus*, *T. delbrueckii* and *S. kudriavzevii* (Figure 4.18).

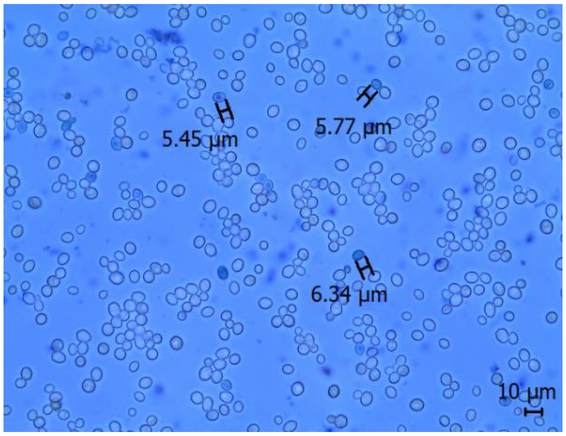
Table 4.5 Genetic identification of four yeast isolates

Isolate	Best hit in the rRNA/ITS database of NCBI				
	Taxon	Type strain	% Identity	Variation	GeneBank accession no.
5	*Saccharomyces (S.) paradoxus*	CBS 432	98.96	5/479	NR_138272.1
6	*Torulaspora (T.) delbrueckii*	CBS 1146	100.00	0/693	NR_111257.1
7	*Saccharomyces (S.) kudriavzevii*	ATCC MYA-4449	98.75	6/479	NR_111355.1
8	*Saccharomyces (S.) paradoxus*	CBS 432	98.96	5/479	NR_138272.1

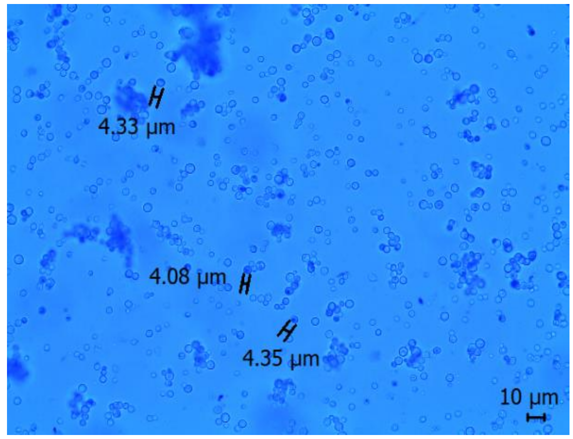
Identified yeast isolates in wheat and rye sourdough starter cultures

Yeast belonging to the genus *Saccharomyces* were dominant in both sourdough starters. *S. paradoxus* and *S. kudriavzevii* were identified in rye sourdough starter, while *S. paradoxus* was identified in wheat sourdough starter. The presence of two dominant *Saccharomyces* yeasts in the sourdough starters may be due to the strong capability of the *genus* in fermenting carbohydrates (Sicard & Legras, 2011). Another reason may be that both *S. paradoxus* and *S. kudriavzevii* have a higher growth rate and perform better at lower temperatures (< 30 °C) (Salvadó et al., 2011; Tronchoni et al., 2012). Therefore, these two yeast dominated in rye and wheat sourdough starters fermented at lower temperatures (Vrancken et al., 2010). Yeast species have also been isolated from fermented wheat and rye sourdough starters at 28 ℃ and 26 ℃, respectively (HittingerLab, 2018; Vrancken et al., 2010).

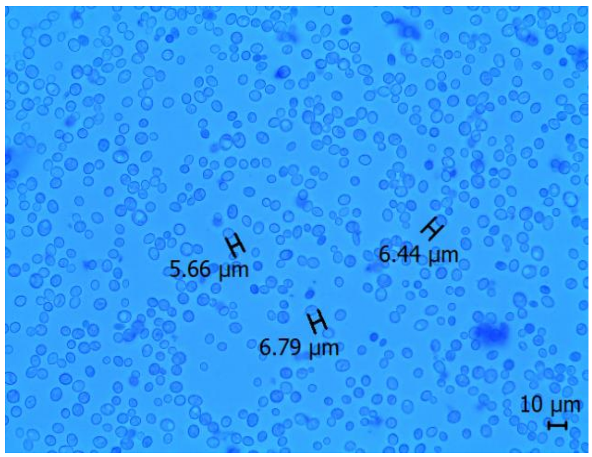
Another yeast isolated from the wheat sourdough starter was *T. delbrueckii*, which has also been reported (Paramithiotis et al., 2010). The presence of *T. delbrueckii* in the rye sourdough starter produced by the cold-fermentation process may be due to the freeze-tolerance of *T. delbrueckii*. This fungi is well-adapted in frozen/cold fermented doughs (Alves-Araújo et al., 2004; Paramithiotis et al., 2010). *T. delbrueckii* has a mutualistic relationship with *F. sanfranciscensis*, which may help explain its dominance in wheat sourdough starters (Raimondi et al., 2017).



(A) *S. paradoxus*



(B) *T. delbrueckii*



(C) *S. kudriavzevii*

Figure 4.18 *S. paradoxus* (A) identified in wheat sourdough starter and rye sourdough starter, *T. delbrueckii* (B) identified in wheat sourdough starter, *S. kudriavzevii* (C) identified in rye sourdough starter

Magnification x1000 under Carl Zeis transmission light microscope (Model HBO 50/AC, Germany).

4.1.6 Colour of sourdough bread

Crust colour of sourdough bread

Table 4.6 shows the crust colour of the sourdough bread. The brightness (L* value) of wheat sourdough bread crust was higher (42.75 ± 0.66) than the L* value of sourdough bread (26.34 ± 2.14) made by Bartkiene et al. (2017) using wheat flour and similar baking condition (210°C/25 min). The high brightness of the sourdough bread in the current study was possibly due to the variations in the formulations used resulting in different levels of Maillard reactions (Erbas et al., 2012). The formulation used in the present study contained wheat flour and malt flour. Malt flour contains more fermentable sugars including β-glucan than wheat flour, which increases Maillard reactions during baking, resulting in the higher L* values (brightness) of the bread crust (Belcar et al., 2022; Rögner et al., 2021). Another reason may be the high temperature (> 190 °C) used in baking bread with malt flour (Yang et al., 2020), which causes intense caramelisation, leading to a brighter bread crust (Fu et al., 2018).

The redness/greenness (a* value) of the bread crust was 7.66 ± 0.11, whereas the yellowness/blueness (b* value) of the sourdough bread crust was 9.03 ± 0.59. This reflects that the crust of the sourdough bread had more redness and yellowness. Another study (Bartkiene et al., 2017) also reported high redness and yellowness of the bread crust of baked wheat sourdough bread (210 °C/25 min) in formulations with added malt flour. Wholemeal wheat flour also has a high lutein and carotene content which may contribute to the yellowness and redness of the final product (Colasuonno et al., 2019; Rodriguez-Amaya & Kimura, 2004).

Crumb colour of sourdough bread

Table 4.6 shows the crumb colour of the sourdough bread. The brightness (54.66 ± 0.29), redness (1.02 ± 0.10) and yellowness (10.05 ± 0.19) of sourdough bread were lower than those reported in a previous study which used similar fermentation and baking conditions (García-Segovia et al., 2017). The dark colour of the bread crumb produced in this study was probably caused by the flours used (wholemeal wheat flour) (Paciulli et al., 2016). Flour containing finely ground bran can lead to a darker crumb than flour with coarsely ground bran (García-Segovia et al., 2017). However, the coarseness of the flour in the present study was not determined. It has been also reported that homogenisation of bran and flour can contribute to the darkness of the bread crumb (Bucsella et al., 2016).

Table 4.6 Crumb and crust colour of sourdough bread

Colour Parameter	L*	a*	b*
Crumb	54.66 ± 0.29	1.02 ± 0.10	10.05 ± 0.19
Crust	42.75 ± 0.66	7.66 ± 0.11	9.03 ± 0.59

L* crumb = lightness of bread crumb; a* crumb = yellowness/blueness of bread crumb; b* crumb = redness/greenness of bread crumb; L* crust = lightness of bread crust; a* crust = yellowness/blueness of bread crust; b* crust = redness/greenness of bread crust; data expressed as mean ± SEM; mean values are results of three independent replications (n = 3); samples were measured in triplicate.

4.1.7 Loaf specific volume of sourdough breads

The specific volume of the sourdough bread was 2.71 ± 0.02 cm^3/g, which was higher than the specific volume reported by Tomić et al. (2023). Bread specific volume may be affected by the levels of salt and sourdough (mother) added to the bread dough. The bread dough used in this study contained 40% sourdough (mother) and 1.10 % sea salt (Axel et al., 2015). Whereas Tomić et al. (2023) used 25 % sourdough (mother) with no sea salt added to their wheat sourdough bread. The use of high concentrations of sourdough (mother) in the dough increases the level of acidification and solubility of protein during fermentation (Abedfar & Sadeghi, 2019), leading to better gas retention in the dough, resulting in a higher specific volume in the final sourdough bread (Abedfar & Sadeghi, 2019; Corsetti et al., 2000). In addition, the use of salt increases the extensibility of the dough, causing better gas cell expansion, thereby the specific volume of the loaf increases (Arena et al., 2020; Simsek & Martinez, 2016). Hence the higher specific volume of the sourdough bread in this study compared to that of Tomić et al. (2023) is likely due to the use of a higher amount of mother dough and the addition of sea salt.

4.2 Phase II

In phase II, sourdough fermentation conditions were optimised by fermenting the wheat sourdough at different temperatures (27 °C, 29° C and 31 °C). The optimisation process was conducted by determining the acidity of samples of DBP, DAP and SDB. LAB and yeast in DBP and DAP were enumerated. Texture characteristics were measured on DBP, DAP and SDB to determine the effect of different fermentation temperatures on the texture of sourdoughs and sourdough bread. Loaf volume, loaf weight, specific volume and sourdough bread colour were also measured on the SDB to monitor the optimisation process. Sensory evaluation of the sourdough breads was performed using focus groups to select the optimum sourdough fermentation.

4.2.1 Acidity of sourdough and sourdough bread

Acidity of sourdoughs (dough-before-proofing and dough-after-proofing)

The pH of sourdough before and after proofing are shown on Table 4.7. During the fermentation period (DBP compared to DAP), the pH of sourdough fermented at 31 °C/5.5 h decreased the least (8.57%), while the pH of sourdough fermented at 29° C/7 h and 27 °C/8.5 h had the highest decrease, 19.70% and 19.96% respectively. The pH of all the sourdoughs after-proofing were within 3.50 – 4.30 range, which means they are well-developed (Corsetti, 2012). The results indicate that a longer fermentation time with lower fermentation temperature caused a higher acidification level in the sourdough. Similar results have been reported (Casado et al., 2017), for sourdough fermented at 25 °C and 35 °C. The higher level of acidification is attributed to the higher amounts of organic acids which accumulate in the dough after a long fermentation period at a lower fermentation temperature (Corsetti & Settanni, 2007). Heterofermentative LAB dominate in sourdough systems at low temperatures (< 30°C) during a long fermentation time. Heterofermentative LAB acidify the dough by producing lactic acid and acetic acid resulting in a higher acidification of the dough. With a short fermentation time, at a high temperature (> 30°C), homofermentative and facultative heterofermentative LAB are abundant in the sourdough system, acidifying the dough by producing mainly lactic acid, which results in a lower acidification of the dough (De Vuyst et al., 2014).

Table 4.7 Acidity of sourdough before- and after-proofing (fermentation) at 27° C, 29 °C and 31 °C, and the average change in acidity between sourdough before- and after-proofing (fermentation) at each temperature

Fermentation Temperature (°C)	Sample	pH	Average Change in pH (%)	TTA	Average Change in TTA (%)
27	DBP	4.55 ± 0.00	19.96[a]	0.56 ± 0.02	95.83[a]
	DAP	3.64 ± 0.00		1.10 ± 0.01	
29	DBP	4.65 ± 0.01	19.70[a]	0.44 ± 0.00	125.00[a]
	DAP	3.73 ± 0.00		0.99 ± 0.01	
31	DBP	4.16 ± 0.01	8.57[b]	0.76 ± 0.02	42.73[b]
	DAP	3.81 ± 0.00		1.08 ± 0.02	

DBP = dough-before-proofing, DAP = dough-after-proofing; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; pH values between DBP and DAP at each temperature are significantly different at $p < 0.01$; TTA values between DBP and DAP at each temperature are significantly different at $p < 0.01$; Values within columns with identical subscript letters are not different ($p > 0.05$).

During fermentation (DBP compared to DAP) the total titratable acidity of sourdough fermented at 31 °C increased the least (42.73%), while the TTA of sourdough fermented at 27 °C and 29° C had the greatest increase, 95.83% and 125.00%, respectively (Table 4.7). The higher decrease in TTA in sourdough fermented at 27 °C and 29 °C was likely due to more fermentable sugars being converted to organic acids through microbial metabolism during the longer fermentation period at a lower temperature (Martín-Garcia et al., 2023). A higher increase of TTA was reported in dough fermented at low temperature (25 °C) compared to the dough fermented at high temperature (35 °C) (Casado et al., 2017).

Acidity of sourdough bread

The pH of all sourdough breads increased after baking ($p < 0.01$) irrespective of the fermentation temperature (Table 4.8), whereas the TTA of the bread samples decreased ($p < 0.01$). The average change in acidity was significantly different between fermentation conditions of 27 °C/8.5 h and 31 °C/5.5 h. These changes may be due to volatilisation (loss) of organic acids such as lactic acid (b.p. 122.0 °C), acetic acid (b.p. 117.9 °C), formic acid (b.p. 100.8 °C) propionic acid (b.p. 141.2 °C) and butyric acids (b.p. 163.5 °C) during baking at 200 °C (Akamine et al., 2023; Brandt, 2007).

The fermentation process at 27 °C/8.5 h led to the lowest pH for the sourdough bread (3.75 ± 0.00), whereas fermentation conditions of 31 °C/5.5 h resulted in the highest pH of the sourdough bread (3.91 ± 0.01). This was mainly due to the different concentrations of organic acids produced during the different fermentation times and temperatures (Menezes et al., 2019). Fermentation at a higher temperature over a short period results in lower accumulation of organic acids, resulting in higher pH of the bread, whereas fermentation at a lower temperature for a longer period leads to higher concentrations of organic acids, resulting in a lower pH of the bread (De Vuyst et al., 2014). The pH of sourdough bread produced at 29 °C and 31 °C reached the ideal pH range of sourdough bread (3.8 - 4.6) (Gänzle & Gobbetti, 2013), 3.86 ± 0.00 and 3.91 ± 0.01, respectively, while the pH of the sourdough bread fermented at 27 °C (3.75 ± 0.00) was slightly lower than the ideal pH.

Table 4.8 Acidity of sourdough after-proofing and sourdough bread produced at 27° C, 29 °C and 31 °C, and the average change in acidity between sourdough after-proofing and sourdough bread at each temperature

Fermentation Temperature (°C)	Sample	pH	Average Change in pH (%)	TTA	Average Change in TTA (%)
27	DAP	3.64 ± 0.00	3.02^{a}	1.10 ± 0.01	26.00^{c}
	SDB	3.75 ± 0.00		0.82 ± 0.01	
29	DAP	3.73 ± 0.00	3.35^{ab}	0.99 ± 0.01	22.96^{cd}
	SDB	3.86 ± 0.00		0.76 ± 0.01	
31	DAP	3.81 ± 0.00	2.58^{b}	1.08 ± 0.02	14.28^{d}
	SDB	3.91 ± 0.01		0.93 ± 0.01	

DAP = dough-after-proofing, SDB = sourdough bread; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; pH values between DAP and SDB produced at each temperature were significantly different at $p < 0.01$; TTA values between DAP and SDB produced at each temperature were significantly different at $p < 0.01$; Values within columns with identical subscript letters are not different ($p > 0.05$).

4.2.2 Texture analysis of sourdough and sourdough bread

(1) Texture changes of dough-before-proofing and dough-after-proofing

Sourdough fermented for a shorter time at a higher temperature (31 °C/5.5 h) resulted in a dough with a higher decrease in hardness, gumminess, and chewiness compared to that fermented at lower temperatures for a longer time (27 °C/8.5 h and 29 °C/7 h). Higher fermentation temperature (31 °C/5.5 h) resulted in a higher increased adhesiveness of the dough, compared to the lower fermentation temperature (27 °C/8.5 h and 29 °C/7 h). Textural changes in cohesiveness, springiness, and resilience of the doughs fermented under the different fermentation conditions did not differ ($p > 0.05$) [Table B4, Appendix A].

Hardness of dough-before proofing and dough-after-proofing

The reduction in hardness was different ($p < 0.05$) between sourdoughs during fermentation at the three temperatures used in this study (27 °C/8.5 h, 29 °C/7 h, 31 °C/5.5 h) (Table 4.9) The dough fermented at 31 °C/5.5 h had the highest decrease in hardness (63.45%; $p < 0.01$), while the dough fermented at 27 °C/8.5 h had the lowest reduction in hardness (19.36%; $p < 0.01$).

Gumminess of dough-before proofing and dough-after-proofing

The gumminess of all three sourdoughs reduced ($p < 0.05$) after fermentation as shown in Table 4.9. The highest reduction in gumminess (DBP compared to DAP) was recorded from the sample after fermentation at 31 °C/5.5 h (reduced by 63.52%; $p < 0.01$). The dough proofed at 27 °C/8.5 h had the lowest reduction in gumminess (decreased by 23.64%; $p < 0.01$).

Chewiness of dough-before proofing and dough-after-proofing

The reduction in chewiness of the three types of doughs fermented under different conditions varied ($p < 0.05$) (Table 4.9). The highest reduction in chewiness was obtained in the dough fermented at 31°C/5.5 h (decreased by 63.15%; $p < 0.01$). Meanwhile, the dough fermented at 27 °C/8.5 h had the lowest reduction in chewiness (23.46%; $p < 0.01$).

Adhesiveness of dough-before proofing and dough-after-proofing

The sourdough fermented at 31 °C/5.5 h had the highest increase in adhesiveness ($p < 0.05$) compared to the two doughs prepared at 27 °C/8.5 h and 29 °C/7 h (Table 4.9). The adhesiveness of the dough significantly increased by 61.38% after fermentation at 31 °C/5.5 h. The observed level of change in the adhesiveness of the doughs fermented at the two temperatures were not different ($p > 0.05$)

Table 4.9 Texture properties of sourdoughs before- and after-proofing (fermentation) at 27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h, and the average change in texture properties between sourdoughs before- and after-proofing at each temperature

Fermentation Temperature (°C)	Sample	Hardness (g)	Average Change in Hardness (%)	Gumminess	Average Change in Gumminess (%)	Chewiness	Average Change in Chewiness (%)	Adhesiveness (g s^{-1})	Average Change in Adhesiveness (%)
27	DBP27	452.05 ± 6.48	19.36[a]	323.96 ± 6.10	23.64[d]	317.86 ± 5.38	23.46[g]	-575.00 ± 47.10	25.88[j]
	DAP27	364.53 ± 7.63		247.38 ± 4.26		243.29 ± 4.14		-426.20 ± 15.20	
29	DBP29	774.5 ± 18.2	23.28[b]	540.10 ± 12.30	25.68[e]	526.90 ± 11.40	25.68[h]	-874.20 ± 36.80	23.66[j]
	DAP29	594.2 ± 21.5		401.4 ± 11.80		391.60 ± 10.80		-667.50 ± 16.40	
31	DBP31	724.42 ± 5.91	63.45[c]	532.12 ± 8.44	63.52[f]	518.75 ± 9.29	63.15[i]	-897.30 ± 37.40	61.38[k]
	DAP31	264.78 ± 8.22		194.08 ± 7.24		191.18 ± 7.00		-346.50 ± 23.80	

DBP27 = dough-before-proofing at 27 °C/8.5 h, DAP27 = dough-after-proofing at 27 °C/8.5 h, DBP29 = dough-before-proofing at 29 °C/7 h, DAP29 = dough-after-proofing at 29 °C/7 h, DBP31 = dough-before-proofing at 31 °C/5.5 h, DAP31 = dough-after-proofing at 31 °C/5.5 h; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; hardness, gumminess, chewiness between DBP and DAP produced at each temperature were significantly different at $p < 0.01$; adhesiveness between DBP27 and DAP27 were significantly different at $p < 0.05$; adhesiveness between DBP29 and DAP29, DBP31 and DAP31 were significantly different at $p < 0.01$, respectively; values within columns with identical subscript letters are not different ($p > 0.05$)

(2) Discussion of texture changes of dough-before-proofing and dough-after-proofing

The current study indicated that fermentation at a higher temperature for a shorter time (31 °C/5.5 h) resulted in higher changes in hardness, gumminess, chewiness and adhesiveness of the dough, compared to prolonged fermentation at lower temperatures (27 °C/8.5 h and 29 °C/7 h). According to others (Casado et al., 2017) fermentation at a higher temperature (35°C) can lead to higher changes in hardness, gumminess, chewiness and adhesiveness of the dough than at a lower temperature (25°C). The rheological changes in sourdough during fermentation are mainly due to the proteolytic activities of the starter cultures. Greater proteolytic activity and degradation of gluten protein can occur during sourdough fermentation at high temperatures for a short period due to the high acidification rate (Arendt et al., 2007). As a result, the gluten structure is weakened (Angioloni et al., 2006), hence the dough becomes softer and less elastic (Decock & Cappelle, 2005; Yazar & Tavman, 2012). The softer dough with less elasticity is desirable due to its potential to increase loaf volume and produce a better quality loaf (Clarke et al., 2004).

(3) Changes in texture of dough-after-proofing and sourdough bread

The changes of hardness, gumminess, springiness and chewiness during the baking process (DAP compared to SDB) did not differ between the sourdoughs fermented at 27 °C/8.5 h, 29 °C/7 h and 31 °C/5.5 h ($p > 0.05$) [Table B5, Appendix A]. Significant changes in adhesiveness and resilience were induced by baking compared to the dough-after-proofing at 27 °C/8.5 h, 29 °C/7 h and 31 °C/5.5 h ($p < 0.05$).

Adhesiveness of dough-after-proofing and sourdough bread

Table 4.10 shows the adhesiveness of dough-after-proofing and sourdough bread. The baking process increased ($p < 0.05$) the adhesiveness of the bread compared to dough-after-proofing at 27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h. Dough proofed at 29 °C/7 h showed the highest increase in adhesiveness after baking (99.21%; $p < 0.01$). The lowest increase in adhesiveness was obtained in the baked bread made from the dough proofed at 31°C/5.5 h (98.47%; $p < 0.01$).

Resilience of dough-after-proofing and sourdough bread

The baking process increased ($p < 0.05$) the resilience of all three proofed doughs (27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h) (Table 4.10). Compared to the other fermentation conditions, proofing at 31°C/5.5 h led to the highest increase in resilience (84.31%; $p < 0.01$). The changes in the resilience (DAP compared to SDB) between fermentation at 27 °C/8.5 h and 29 °C/7 h were not significant ($p > 0.05$).

Table 4.10 Texture properties of sourdoughs-after-proofing (fermentation) and sourdough bread produced at 27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h, and the average change in texture properties between sourdough-after-proofing and sourdough bread at each temperature

Fermentation Temperature (°C)	Sample	Adhesiveness (g s^{-1})	Average Change in Adhesiveness (%)	Resilience	Average Change in Resilience (%)
27	DAP27	-426.20 ± 15.20	98.87^{a}	0.07 ± 0.00	81.96^{d}
	SDB27	-4.80 ± 1.00		0.4 ± 0.01	
29	DAP29	-667.50 ± 16.40	99.21^{b}	0.07 ± 0.00	81.29^{d}
	SDB29	-5.30 ± 0.70		0.39 ± 0.01	
31	DAP31	-346.50 ± 23.80	98.47^{c}	0.07 ± 0.00	84.31^{e}
	SDB31	-5.40 ± 0.50		0.43 ± 0.01	

DAP27 = dough-after-proofing at 27 °C/8.5 h, SDB27 = sourdough bread produced at 27 °C/8.5 h, DAP29 = dough-after-proofing at 29 °C/7 h, SDB29 = sourdough bread produced at 29 °C/7 h, DAP31 = dough-after-proofing at 31 °C/5.5 h, SDB31 = sourdough bread produced at 31 °C/5.5 h; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; adhesiveness and resilience between DAP and SDB produced at each temperature were significantly different at $p < 0.01$; values within columns with identical subscript letters are not different ($p > 0.05$).

(4) Discussion of texture changes of dough-after-proofing and sourdough bread

The significant changes in adhesiveness and resilience of the proofed doughs after baking observed in the current study are similar to a previous study (Casado et al., 2017), which compared the rheological changes of the sourdoughs and sourdough bread prepared at 25°C and 35°C. The change in adhesiveness is possibly due to starch gelatinisation during baking (Singh, 2005). The starch granules swell due to the absorption of free water in the dough, forming a viscous texture of the bread crumb (Chen et al., 2013). The change of resilience observed after baking may be caused by the denaturation of gluten protein (Singh, 2005), which occurs during the sourdough acidification and baking process, resulting in the unfolding of proteins. The unfolded proteins form an insoluble network during baking, which solidify to form a firm crumb; increasing the resilience of the bread crumb (Croxford, 2020; Ortolan & Steel, 2017).

4.2.3 Microbiology of sourdough

Concentration of lactic acid bacteria and yeast

The concentrations of LAB and yeast in the DBP and DAP samples are shown in Table 4.11. Fermentation at 27 °C/8.5 h resulted in the highest growth of LAB in the dough, compared to fermentation at 29 °C/7 h and 31 °C/5.5 h ($p < 0.05$). The lowest growth of LAB cells was observed in the dough fermented at 31°C/5.5 h with only a small but significant increase (2.88%).

The highest reduction ($p < 0.05$) in yeast cell counts resulted from fermentation at 27 °C/8.5 h (23.55%). Compared with fermentation at 31 °C/5.5 h, fermentation at 29 °C/7 h caused a higher reduction of yeast cell counts ($p < 0.05$). The lowest decrease in the yeast population in the dough was caused by fermentation at 31 °C/5.5 h (1.22%). These findings are similar to another study (Casado et al., 2017), which reported high increases in LAB and large decreases in yeast cell numbers in sourdough subjected to prolonged fermentation (25°C) compared to sourdough fermented at 35°C for a short time.

Table 4.11 LAB and yeast counts in sourdough during fermentation, and the average change of LAB and yeast counts in sourdough during fermentation

Fermentation Temperature (°C)	Sample	LAB (log CFU g^{-1})	Average Change in LAB (%)	Yeast (log CFU g^{-1})	Average Change in Yeast (%)
27	DBP	8.49 ± 0.04	6.42^{a}	4.60 ± 0.03	23.55^{c}
	DAP	9.03 ± 0.02		3.51 ± 0.02	
29	DBP	8.64 ± 0.04	4.14^{b}	4.34 ± 0.04	7.83^{d}
	DAP	9.00 ± 0.02		4.00 ± 0.03	
31	DBP	8.69 ± 0.04	2.88^{b}	4.71 ± 0.00	1.22^{e}
	DAP	8.93 ± 0.02		4.66 ± 0.01	

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were analysed in duplicate data expressed as mean ± SEM; LAB cell counts between DBP and DAP produced at 27°C and 29°C were significantly different at $p < 0.01$, respectively; LAB cell counts between DBP and DAP produced at 31°C were significantly different at $p < 0.05$; Yeast cell counts between DBP and DAP produced at 27°C and 29°C were significantly different at $p < 0.01$, respectively; values within columns with identical subscript letters are not different ($p > 0.05$).

In the current study, the differences in LAB and yeast cell counts in the samples fermented at different temperatures for different times are likely due to the different levels of organic acids produced by the LAB (Martín-Garcia et al., 2023). At low fermentation temperatures (<30 °C) with prolonged fermentation, carbohydrate metabolism by heterofermentative LAB leads to a higher content of lactic and acetic acids in the sourdough, which inhibits the growth of yeast (De Vuyst et al., 2014; Yildirim & Arici, 2019). At high fermentation temperatures (>30 °C) with a short fermentation time, homofermentative and facultative heterofermentative LAB rapidly acidify the dough by producing lactic acid, resulting in less acid accumulating in the dough, and hence less inhibition of yeast growth (Gänzle et al., 2007; Yildirim & Arici, 2019).

The ratio of LAB to yeast in current study was greater than 10000:1 (without adding commercial yeast) in three dough-after-proofing samples, which was higher than the ratio of LAB to yeast (100: 1) reported by others (Katina et al., 2002; Ottogalli et al., 1996), who used 20% mother sourdough in their formulations. A higher amount of mother sourdough (40% of the dough weight) was used in the current formulation, which caused a highly acidic environment in the dough-before-proofing (Brandt et al., 2004), leading to greater inhibition of yeast growth during fermentation (Narendranath et al., 2001), and a higher LAB to yeast ratio in the dough-after-proofing.

4.2.4 Loaf volume, loaf weight and specific volume of sourdough bread

Specific volume of loaf did not differ ($p > 0.05$) between sourdough bread produced at different fermentation temperatures (27°C, 29°C and 31°C) (Table 4.12). Sourdough prepared at 31°C reached the expected height of standard dough (Figure 4.19) within the shortest period (3 h), while sourdough fermented at 27°C took the longest period (5 h). Loaf volume is attributed to the amount of gas produced and the gas holding capacity of the dough gluten (Clarke et al., 2003; M. Gobbetti et al., 1995). At a high fermentation temperature (> 30°C), homofermentative and heterofermentative LAB dominate, producing high amounts of lactic acid (De Vuyst et al., 2017; M. Gobbetti et al., 1995), leading to a softer and more elastic dough with greater expansion and better gas holding capacity (Kawai et al., 2006). The dominance of homofermentative LAB also favours yeast fermentation in sourdough at high fermentation temperatures, resulting in higher production of CO_2 in the dough (Clarke et al., 2003; De Vuyst et al., 2014). Therefore, the dough fermented at the high temperature (> 30°C) reached the standard volume within a shorter time. At low fermentation temperatures (< 30°C), heterofermentative LAB synthesise high concentrations of acetic acid (De Vuyst et al., 2014; M. Gobbetti et al., 1995), resulting in a firmer and less elastic dough with less expansion capacity and poor gas holding capacity (M. Gobbetti et al., 1995; Kawai et al., 2006), hence the dough fermented at low temperature (< 30°C) takes a relatively longer time to reach the standard volume.

Table 4.12 Mean loaf volume, loaf weight and specific volume of sourdough bread

Sample	Loaf volume (cm^3)	Loaf weight (g)	Specific volume (cm^3/g)
SDB27	2237.50 ± 103.08^a	749.90 ± 7.37^a	2.98 ± 0.11^a
SDB29	2175.00 ± 28.87^a	753.15 ± 7.37^a	2.89 ± 0.05^a
SDB31	2262.50 ± 62.92^a	747.85 ± 3.14^a	3.03 ± 0.09^a

SDB27 = sourdough bread fermented at 27°C, SDB29 = sourdough bread fermented at 29°C, SDB31 = sourdough bread fermented at 31°C; mean loaf volume, loaf weight and specific volume are results of two independent replications (n = 2); data expressed as mean ± SEM; measurements of samples were conducted in duplicate; Values within columns with identical subscript letters are not different ($p > 0.05$).

Figure 4.19 Image of measuring the standard height of dough

Image captured by Samsung Galaxy A05

4.2.5 Colour of sourdough bread

Table 4.13 shows the crust and crumb colour of the bread samples: L* (lightness), a* (redness to greenness), and b* (yellowness to blueness) values. Different fermentation times and temperatures did not affect the crust colour of the fermented sourdough bread ($p > 0.05$).

Table 4.13 Mean colour of crumb and crust of sourdough bread

Sample	Crumb			Crust		
	L*	a*	b*	L*	a*	b*
SDB27	58.94 ± 1.15^{a}	1.07 ± 0.13^{a}	10.62 ± 0.40^{a}	31.93 ± 2.98^{a}	7.88 ± 1.27^{a}	7.44 ± 2.21^{a}
SDB29	62.86 ± 0.84^{b}	1.27 ± 0.12^{a}	11.25 ± 0.14^{b}	29.27 ± 1.22^{a}	7.51 ± 0.62^{a}	5.98 ± 0.88^{a}
SDB31	60.40 ± 0.76^{c}	1.37 ± 0.14^{b}	10.86 ± 0.14^{b}	31.71 ± 1.23^{a}	7.70 ± 0.67^{a}	6.94 ± 0.91^{a}

SDB27 = sourdough bread fermented at 27°C, SDB29 = sourdough bread fermented at 29°C, SDB31 = sourdough bread fermented at 31°C; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; Values within columns with different subscript letters are different ($p < 0.05$).

Crumb colour did differ ($p < 0.05$) among the sourdough bread samples. Sourdough bread fermented at 29°C had higher L* (62.86 ± 0.84) and b* (11.25 ± 0.14) values for the crumb than the sourdough bread prepared at 27°C and 31°C. The L* and b* values of sourdough bread prepared at 27°C were the lowest ($p < 0.05$), 58.94 ± 1.15 and 10.62 ± 0.40, respectively. The current findings differed from another study (Yildirim & Arici, 2019), which reported no significant differences in crumb colour between sourdough breads prepared at 25°C, 30°C and 35°C. The different crumb colour of the breads from the current study may be due to the uneven and varying sizes of the crumb pores as can be seen in Figure 4.20, which were caused by the handmade process (Rinaldi et al., 2015). Lighter crumb colour has been reported for finer crumb grain structures with smaller pores, while darker crumb colour is caused by larger crumb pores (García-Segovia et al., 2017).

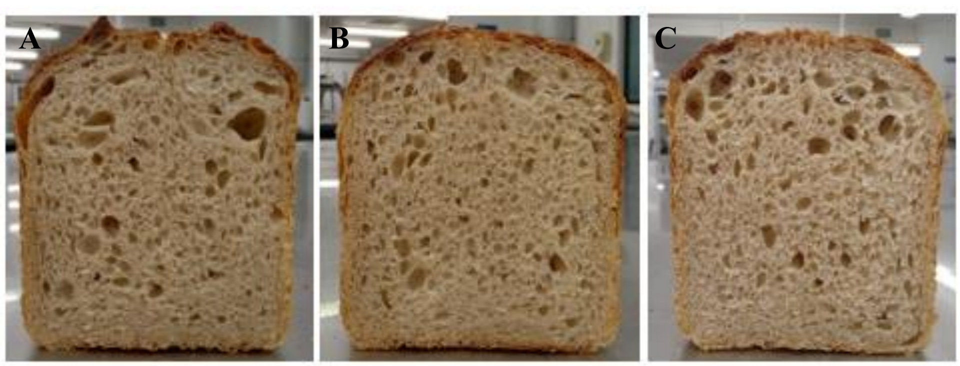


Figure 4.20 Cross-section of sourdough bread fermented at 27 °C (A), 29 °C (B), 31 °C (C)

4.2.6 Selection of the best performing sourdough breads

The overall acceptability, taste, sourness and aroma of the three sourdough breads (SDB27, SDB29, SDB31) were well-accepted by the focus group participants. Sourdough bread (SDB27) fermented at 27°C/8.5 h, was characterised by a mild acidity, well-balanced sourness and having more flavour than the other two samples (SDB29 and SDB31). Hence, SDB27 was the most liked by the focus group panellists. The latter two samples were described as having an unpleasant sharp sourness, as well as having less aroma and flavour than SDB27. A previous study also reported that sourdough bread fermented at a lower temperature (28 °C) was more aromatic than sourdough bread produced at a higher temperature (35°C) (Siepmann et al., 2019).

The different sensory characteristics between sourdough breads is probably due to the different levels of organic acids and volatile compounds synthesised by yeast and LAB under the different fermentation conditions (Gobbetti, Corsetti, & Rossi, 1995). Prolonged fermentation at low temperatures (25–28°C) favours yeast metabolism, which increases the degradation of branched-chain amino acids, thereby increasing the aroma and flavour components in the sourdough bread (Fujimoto et al., 2019; Pico et al., 2015; Siepmann et al., 2018). Prolonged fermentation also increases the amount of acetic acid, contributing to the typical sourness of sourdough bread (De Vuyst et al., 2017; Gobbetti et al., 2000; Osimani et al., 2009; Pérez-Alvarado et al., 2022). Fermentation at high temperatures (>30°C) for a short time can reduce yeast activity due to the rapid acidification by LAB (Häggman & Salovaara, 2008). This may lead to low amounts of flavour compounds and high levels of lactic and acetic acids in the sourdough bread (Siepmann et al., 2019), resulting in the sourdough bread with less flavour and aroma, but with stronger sourness.

Overall, the results of the focus group indicated that sourdough bread produced at 27°C/8.5 h had better sensory characteristics compared to sourdough bread produced at higher temperatures with a shorter fermentation time. Therefore, the formulation of sourdough bread fermented at 27°C/8.5 h was chosen for further experiments.

4.3 Phase III

In phase III, sourdoughs and sourdough bread prepared using the optimum fermentation temperature and time (27 °C/8.5 h) were investigated. Acidity, texture, soluble sugars (fructose, glucose and maltose), total folate and resistant starch of DBP, DAP and SDB were determined. To monitor the fermentation process, LAB and yeast in the DBP and DAP were enumerated; and loaf volume, loaf weight, specific volume and colour of the sourdough bread were measured.

The baking process in phase III was performed at 200 °C/30 min at the Food Processing Laboratory Massey University (Auckland Campus), therefore the baking temperature and time were different from phase I which was performed at a commercial bakery (220 °C/45 min).

4.3.1 Acidity of sourdough and sourdough bread

The acidity of sourdoughs and sourdough bread is shown in Figure 4.21. Acidity of the dough-before-proofing increased after sourdough fermentation at 27°C/8.5 h (DAP). The pH of the dough reduced after fermentation (DBP: 4.75 ± 0.00; DAP: 3.73 ± 0.01) ($p < 0.01$), whereas TTA increased (DBP: 0.39 ± 0.02; DAP: 1.04 ± 0.03) ($p < 0.01$). Similar acidity increases were reported in another study (Sha et al., 2023), in which the pH of the dough reduced from 5.50 to 4.20, while TTA increased from 2.00 to 4.00 after sourdough fermentation (30 °C/8 h). Such increases in acidity are likely due to the accumulation of organic acids produced by LAB during fermentation (Clarke et al., 2002) as previously discussed (Phase I, Section 4.1.1.).

As expected, sourdough bread baked at 200 °C/30 min had a lower acidity compared to dough-after-proofing (Figure 4.30). The pH of sourdough bread was slightly higher than that of dough-after-proofing (SDB: 3.83 ± 0.01; DAP: 3.73 ± 0.01) ($p < 0.01$), whereas the TTA of baked bread (0.79 ± 0.02) was lower than the dough-after-proofing (1.04 ± 0.03) ($p < 0.01$). Baking reduces the acidity of bread, possibly due to the volatility of some organic acids and fatty acids at high temperatures (> 200 °C) (Brandt, 2019; Schönfeld & Wojtczak, 2016b; Umeta & Faulks, 1989).

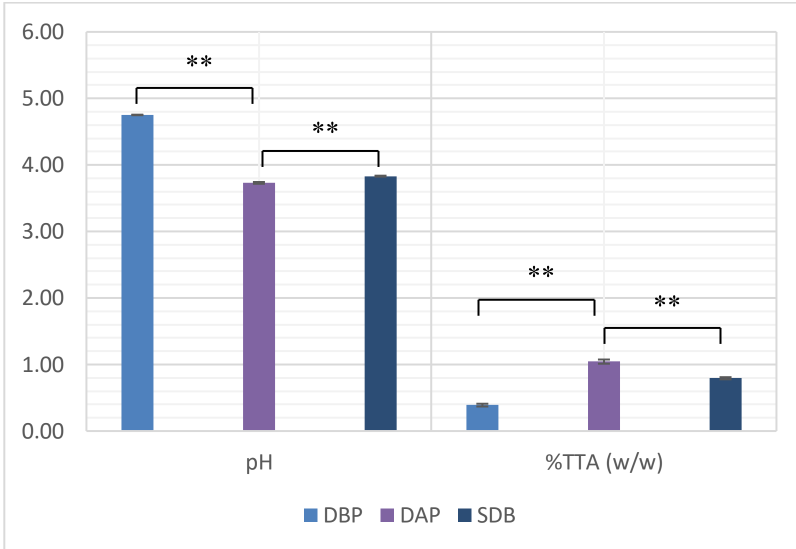


Figure 4.21 Acidity of sourdough before- and after-proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing; SDB = sourdough bread; TTA =total titratable acidity; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; pH values between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively; TTA values between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

4.3.2 Texture analysis of sourdough and sourdough bread

Hardness of sourdoughs and sourdough bread

The hardness of sourdough is affected by both fermentation and the baking process (Figure 4.22). The hardness of the dough was reduced (DBP: 762.40 ± 15.20 g; DAP: 622.79 ± 18.70 g; $p < 0.01$) after sourdough fermentation (27°C/8.5 h), resulting in a softer bread dough. The reduction in hardness is likely due to the degradation of the protein structure of the dough caused by LAB and yeast metabolism during fermentation (Bleukx et al., 1997; Kobrehel et al., 1992). Acidification during sourdough fermentation also increases protein solubility, thereby weakening the protein structure as previously reported (Tomić et al., 2023) during fermentation (25°C/24 h) (Tomić et al., 2023)

After baking at 200°C/30 min, the hardness of the sourdough bread increased to 1138.80 ± 13.40 g ($p < 0.01$) (Figure 4.22), which agrees with İçöz et al. (2004), who used a similar baking process. The reasons for the changes in hardness of baked bread have been previously discussed (Phase I, Section 4.1.2.).

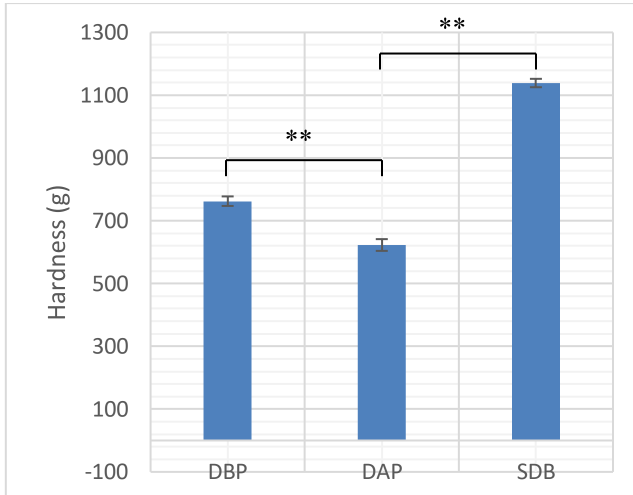


Figure 4.22 Hardness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Hardness between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

Adhesiveness of sourdoughs and sourdough bread

Figure 4.23 shows the increase in dough adhesiveness ($p < 0.01$) after sourdough fermentation. Another study (Clarke et al., 2002) also reported an increase in adhesiveness following sourdough fermentation (30 °C/20 h). Adhesiveness of DAP (-588.97 ± 20.80 g s^{-1}) was higher than DBP (-922.13 ± 55.20 g s^{-1}), while the adhesiveness of SDB (-1.20 ± 0.10 g s^{-1}) was higher than DAP ($p < 0.01$). This indicates that the bread crumb in the baked bread was less sticky than the dough; a finding similar to a previous study using bread baked at 250 °C/15 min (Sidari et al., 2020). The reasons for the changes in adhesiveness during sourdough fermentation and the baking process were discussed previously (Phase I, Section 4.1.2.).

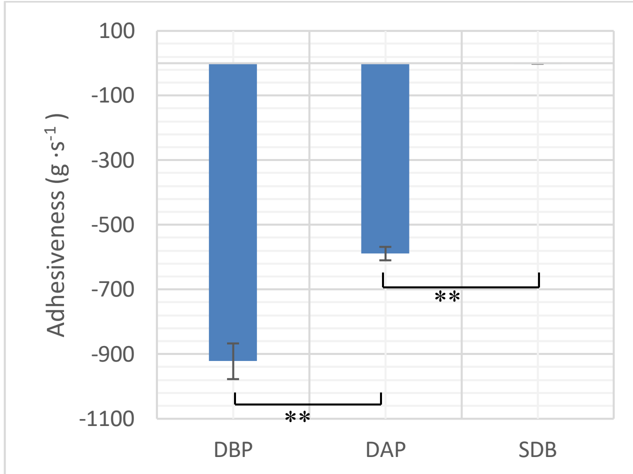


Figure 4.23 Adhesiveness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Adhesiveness between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

Cohesiveness of sourdoughs and sourdough bread

Figure 4.24 shows that the cohesiveness of the dough reduced after sourdough fermentation (DBP: 0.76 ± 0.01; DAP: 0.69 ± 0.01; $p < 0.01$). The reduction of cohesiveness is possibly due to the sea salt (1.10 %) added into the formulation (Simsek & Martinez, 2016), a finding similar to a previous study (Král et al., 2018), which reported decreased cohesiveness of sourdough with 2.0 % sea salt after fermentation. The cohesiveness of sourdough bread increased to 0.74 ± 0.00 after baking ($p < 0.01$), as previously reported (Král et al., 2018). The causes of changes in cohesiveness during sourdough fermentation and baking have been discussed previously (Phase I, Section 4.1.2.).

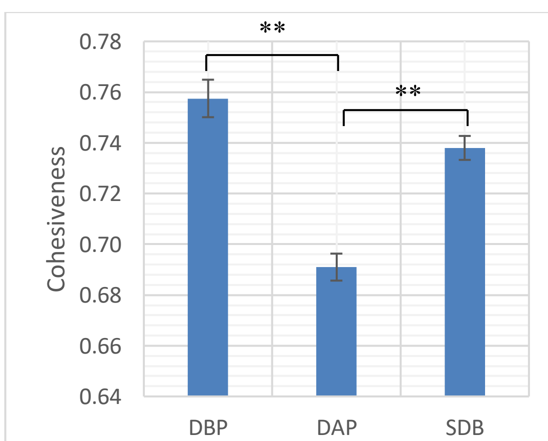


Figure 4.24 Cohesiveness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Cohesiveness between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

Gumminess of sourdoughs and sourdough bread

The gumminess of the dough decreased after sourdough fermentation (DBP: 577.80 ± 15.90; DAP: 430.10 ± 12.60; $p < 0.01$), and increased after baking (SDB: 840.50 ± 12.40; $p < 0.01$) (Figure 4.25). Decrease in gumminess of the dough after fermentation (25°C/6 h) and an increase in gumminess of the dough after baking (210°C/40 min) have also been reported previously (Casado et al., 2017). The causes of changes in gumminess during sourdough fermentation and baking have been discussed previously (Phase I, Section 4.1.2.).

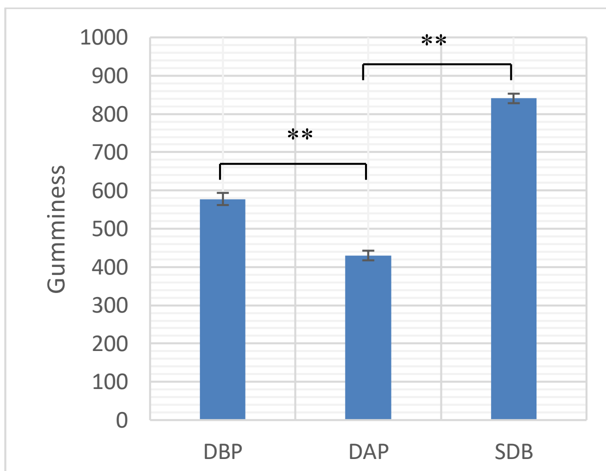


Figure 4.25 Gumminess of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Gumminess between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

Chewiness of sourdoughs and sourdough bread

The chewiness (Figure 4.26) of the dough reduced after fermentation (DBP: 563.90 ± 15.50 g; DAP: 422.70 ± 12.70 g) ($p < 0.01$). The reduction in chewiness of the dough after fermentation is likely due to the increase in protein solubility during fermentation (Demirkesen-Bicak et al., 2021).

Chewiness increased in the baked bread (SDB; 1287.00 ± 254.00 g) ($p < 0.01$) due to the increased hardness of the SDB (Armero & Collar, 1997). Several factors contribute to the increased chewiness including loss of moisture, starch gelatinisation, protein denaturation during baking, gelation/amylose retrogradation during cooling and amylopectin retrogradation of starch during storage (Bou-Orm et al., 2023; Purhagen et al., 2011).

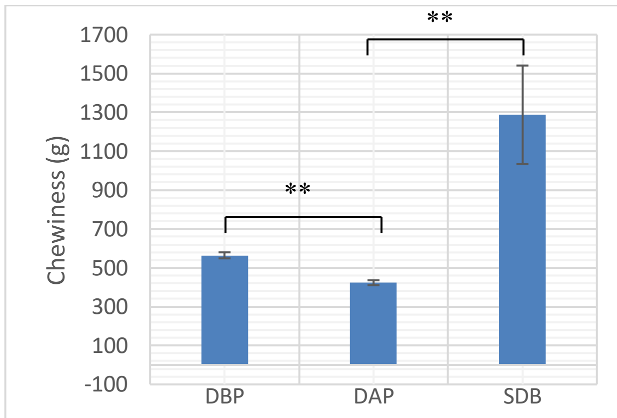


Figure 4.26 Chewiness of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Chewiness between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

Resilience of sourdoughs and sourdough bread

Figure 4.27 shows the decrease in dough resilience after sourdough fermentation (DBP: 0.103 ± 0.00; DAP: 0.098 ± 0.00; $p < 0.01$). These findings are similar to another study (Demirkesen-Bicak et al., 2021), which also showed a reduction in resilience of dough fermented at 25 °C/24 h. The reduction in resilience may be caused by the organic acids produced during sourdough fermentation (Clarke et al., 2004a), which break down the large protein aggregates into small protein aggregates, weakening the gluten network. As a result, the firmness and elasticity of the dough are reduced, causing a decrease in resilience (Thiele et al., 2002).

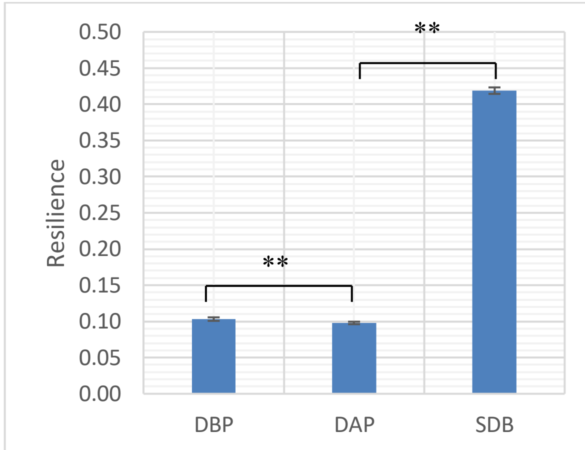


Figure 4.27 Resilience of sourdough before and after proofing (fermentation) and sourdough bread

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB= sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Resilience between DBP and DAP, DAP and SDB were significantly different at $p < 0.01$, respectively.

4.3.3 Microbiology of sourdough

Determination of LAB and yeast abundance

Figure 4.28 shows the changes in LAB and yeast counts in wheat sourdough during fermentation under optimal fermentation conditions (27°C/8.5 h). LAB counts in the DBP (8.42 ± 0.04 log CFU $^{-1}$) were lower than those in the DAP (8.82 ± 0.02 log CFU $^{-1}$) ($p < 0.01$). Similar findings were reported by Vogelmann and Hertel (2011), from fermented wheat sourdough at 30°C/12 h. The significantly higher LAB counts in the DAP can be attributed to their growth during fermentation (Brandt et al., 2004). In contrast, yeast counts in the dough decreased after fermentation (DBP: 4.40 ± 0.03 log CFU $^{-1}$; DAP: 2.75 ± 0.05 log CFU $^{-1}$; $p < 0.01$). The growth of yeast is inhibited during sourdough fermentation due to the acidification by LAB (Casado et al., 2017). Another study also observed lower yeast counts in wheat sourdough after fermentation (30°C/20 h) (Banu et al., 2011).

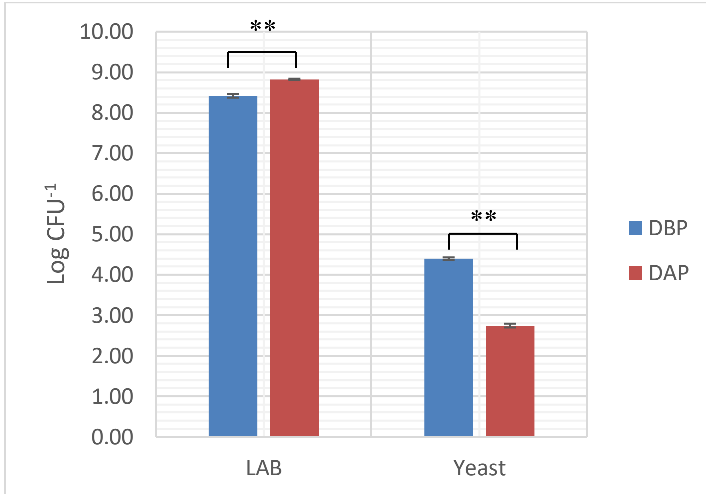


Figure 4.28 LAB and yeast counts of sourdoughs

DBP = dough-before-proofing, DAP = dough-after-proofing; mean values are results of two independent replications (n = 2); samples were measured in duplicate; error bars indicate SEM; LAB cell counts between DBP, and DAP were significantly different at $p < 0.01$; Yeast cell counts between DBP and DAP were significantly different at $p < 0.01$.

4.3.4 Determination of the organic acids in sourdough and sourdough bread prepared using optimal fermentation conditions

Th concentration of lactic acid and acetic acid in sourdoughs and sourdough bread are shown in Figure 4.29. The lactic acid levels in sourdough increased ($p < 0.01$) after fermentation (27°C/8.5 h) (Figure 4.29A), increasing from 0.13 ± 0.01 mg/g (DBP) to 0.41 ± 0.05 mg/g (DAP), while the acetic acid content decreased (DBP: 0.79 ± 0.05 mg/g; DAP: 0.21 ± 0.03 mg/g; $p < 0.01$). These results are similar to another study (Purhagen et al., 2011), which reported that wheat sourdough fermentation (28 °C/4 h) increased the amount of lactic acid but decreased the content of acetic acid in the dough. The changes in levels of lactic and acetic acids in the sourdoughs are likely due to the carbohydrate metabolism by heterofermentative LAB at a lower fermentation temperature (< 30 °C), which leads to increased lactic acid levels in the sourdough (Menezes et al., 2019; Weckx et al., 2010), and inhibit the accumulation of acetic acid (Gianotti et al., 1997; Röcken & Voysey, 1995) The baking process did not affect the lactic acid and acetic acid content in the sourdough bread ($p > 0.05$) (Figure 4.29B).

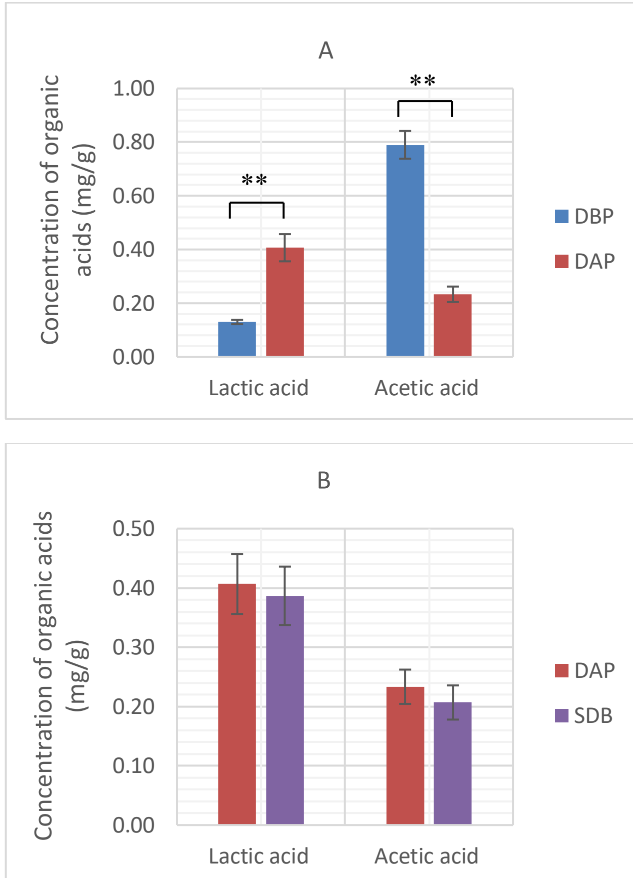
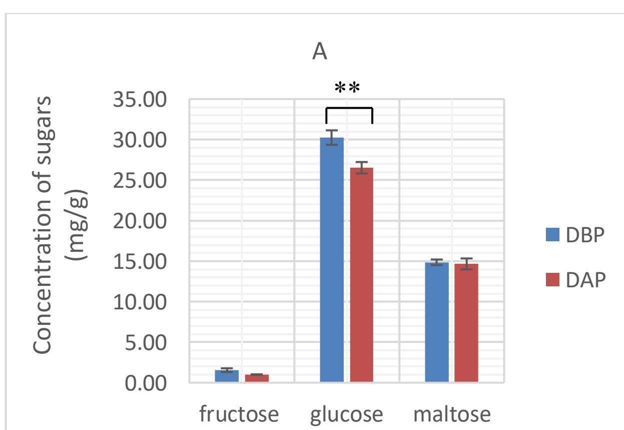


Figure 4.29 Concentration of organic acids in sourdough before and after proofing (A), concentration of organic acids in sourdough-after-proofing and sourdough bread (B)

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Concentration of lactic acid between DBP and DAP were significantly different at $p < 0.01$; Concentration of acetic acid between DBP and DAP were significantly different at $p < 0.01$.

4.3.5 Determination of sugars in sourdough and sourdough bread prepared using optimal fermentation conditions

Changes in concentrations of fructose, glucose and maltose in sourdough during sourdough fermentation and after baking are shown in Figure 4.30. Glucose concentrations in the dough reduced after fermentation (DBP: 30.26 ± 0.89 mg/g; DAP: 26.53 ± 0.71 mg/g; $p < 0.01$). Sourdough fermentation did not change ($p > 0.05$) fructose and maltose concentrations in DAP (Figure 4.30A). These results are similar to a study by Lefebvre et al. (2002) on wheat sourdough fermented at 28 °C/22 h. The observed results are likely due to the order in which different carbohydrates are metabolised during sourdough fermentation (Struyf et al., 2017). Glucose is the first fermentable sugar to be metabolised during sourdough fermentation and can be utilised faster in the presence of fructose. The second fermentable sugar is fructose, followed by the metabolism of maltose (Struyf et al., 2017; Timmermans et al., 2022). The baking process did not impact the content of fructose and maltose in the dough ($p > 0.05$) (Figure 4.30B), while glucose in the dough increased ($p < 0.05$) with baking, likely due to the thermal liberation of glucose from starch during baking (Arya et al., 2015; Lindenmeier & Hofmann, 2004).



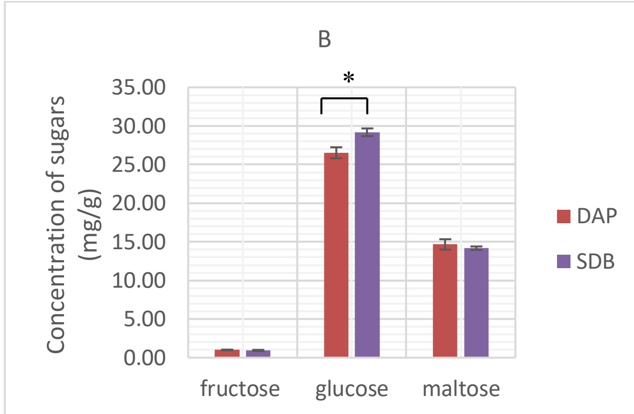


Figure 4.30 Concentrations of sugars in sourdough before and after proofing (A), sourdough-after-proofing and sourdough bread (B)

DBP = dough-before-proofing, DAP = dough-after-proofing, SDB = sourdough bread; mean values are results of two independent replications (n = 2); samples were measured in triplicate; error bars indicate SEM; Concentration of glucose between DBP and DAP were significantly different at $p < 0.01$; Concentration of glucose between DAP and SDB were significantly different at $p < 0.05$.

4.3.6 Determination of folate and resistant starch in sourdough and sourdough bread prepared using optimal fermentation conditions

The levels of resistant starch (RS) and folate in the DBP, DAP and SDB are shown in Table 4.14. Resistant starch decreased slightly after sourdough fermentation. A similar reduction in RS content in wholemeal sourdough after fermentation (23 °C/7 h) was also observed in another study (Buddrick et al., 2015). The reduction may be caused by the addition of vegetable oil to the sourdough during fermentation (Buddrick et al., 2015; Escarpa et al., 1997), as the presence of lipids can partially cover the starch granules, which decreases their capacity to absorb water. Therefore, the inhibition of swelling of the starch granules and their subsequent happens, which limit the formation of RS during fermentation (Buddrick et al., 2015). Resistant starch increased to 1.2% (SDB) after baking (200 °C/30 min). The baking process increases the gelatinisation of starch and the formation of retrograded amylose, thereby increasing the amount of RS in sourdough bread (Hallström et al., 2011; Sajilata et al., 2006). The presence of lactic acid during baking may also increase RS in sourdough bread due to the increase of starch retrogradation (Hallström et al., 2011). The current findings agree with others (Liljeberg et al., 1996), who also reported an increase in RS in sourdough bread baked at 200 °C/40 min.

Table 4.14 Folate and resistant starch content of sourdough and sourdough bread

Sample	Resistant Starch (RS) %	Total Folate µg/100g
Dough-before-proofing (DBP)	0.4	6.00
Dough-after-proofing (DAP)	0.2	<3.00
Sourdough bread (SDB)	1.5	5.48

Total folate decreased during fermentation (DBP: 6.00 µg/100g; DAP: <3.00 µg/100g) (Table 4.14). A similar reduction in folate during sourdough fermentation (30°C/16 h) has been previously reported (Kariluoto et al., 2004). The decrease of folate is possibly caused by the low pH of the dough, which may suppress folate formation or increase destruction of labile folates during sourdough fermentation (Kariluoto et al., 2004). Another potential reason may be the utilisation of folate by LAB strains in the sourdough starter (*F. sanfranciscensis* and *T. delbrueckii*) during fermentation (Kariluoto et al., 2006; Lin & Young, 2000). Total folate increased to 5.48 µg/100 g (SDB) after the baking process, as has been reported by others (Gujska & Majewska, 2005; Osseyi et al., 2001), who observed more than 50% increase in total folate in wheat sourdough bread baked at 230 °C/30 min. The increase in folate during baking may be related to an increase in 5-methyltetrahydrofolate (5-CH3-THF) (Gujska & Majewska, 2005; Osseyi et al., 2001) due to the enzymatic interconversions that occur at high baking temperatures (Gujska & Majewska, 2005; Vahteristo et al., 1998).

4.3.7 Loaf volume, loaf weight and specific volume of sourdough bread

The specific volume of sourdough bread (Table 4.15) ranged from 3.12 ± 0.06 to 3.22 ± 0.01 cm^3/g, which is higher than the bread fermented with yeast only (2.67 cm^3/g) (Wu et al., 2012). The higher specific volume of sourdough bread produced with LAB is attributed to the acidification by sourdough LAB during fermentation (Katina et al., 2006). The acids produced by LAB increase the solubility of the glutenin fraction (extracted from wheat flour). Therefore, the swelling power and gluten and gas holding capability of the dough increase, leading to a higher loaf volume in the final sourdough bread (Chavan & Chavan, 2011).

Table 4.15 Loaf volume, loaf weight and specific volume of sourdough bread produced with sourdough fermented at 27°C

SDB	Loaf volume (cm^3)	Loaf weight (g)	Specific volume (cm^3/g)
Batch 1	2400.00 ± 0.00	746.24 ± 1.63	3.22 ± 0.01
Batch 2	2336.67 ± 55.08	749.04 ± 2.90	3.12 ± 0.06

SDB = sourdough bread; mean loaf volume, loaf weight and specific volume are results of two independent batches (n=2); data expressed as mean ± SEM; measurement of samples were conducted in duplicate.

4.3.8 Colour of sourdough bread prepared using optimal fermentation conditions

Colour analysis of the sourdough bread crumb and crust are shown in Table 4.16. Sourdough bread crust was darker and less yellow/blue (lower L* and b* value) but more red/green (higher a* value) than the crumb. This is possibly due to Maillard and caramelisation reactions (Rizzello et al., 2016), which reduce sugars and amino acids, proteins, and other nitrogen-containing compounds during baking (Purlis, 2010), thereby causing the brownness on the bread crust. Additionally, the different lightness (L* value) between the bread crumb and crust is likely caused by the evaporation of moisture during the baking process (Mohd Jusoh et al., 2009). Moisture from the bread crust is removed faster than from the bread crumb, providing more favourable conditions for Maillard reaction to form the brown colour, and hence a bread crust with a darker colour (lower L* value) than the bread crumb (Capuano et al., 2008). Similar findings were also reported in another study (Olojede et al., 2020), on sourdough bread using a similar formulation and baking conditions (210°C/20 min).

Table 4.16 Crumb and crust colour of sourdough bread

Colour Parameter	L*	a*	b*
Crumb	59.64 ± 0.56	0.63 ± 0.03	9.93 ± 0.12
Crust	27.75 ± 0.48	6.18 ± 0.31	5.25 ± 0.36

L* crumb = lightness of bread crumb; a* crumb = yellowness/blueness of bread crumb; b* crumb = redness/greenness of bread crumb; L* crust = lightness of bread crust; a* crust = yellowness/blueness of bread crust; b* crust = redness/greenness of bread crust; data expressed as mean ± SEM; results are means of two independent batches (n=2); samples were measured in triplicate; error bars indicate SEM.

Chapter 5 Conclusion and Future Research

In phase I, the LAB and yeast present in a rye sourdough starter and wheat sourdough starter were identified, and the sourdough fermentation process was optimised. In the rye sourdough starter *F. sanfranciscensis, L. curvatus, S. paradoxus* and *S. kudriavzevii* were present, while in the wheat sourdough starter *F. sanfranciscensis, S. paradoxus* and *T. delbrueckii* dominated. The optimised sourdough fermentation for wheat sourdough was determined as fermentation at 27 °C / 8.5 h. According to the focus group sensory evaluation, sourdough bread fermented at 27 °C/8.5 h had a more gentle, well-balanced sourness and better aroma than sourdough bread fermented at higher temperatures (29° C/7 h and 31 °C/5.5h). Therefore, the most preferred sourdough bread by sensory group panellists was that fermented at 27 °C/8.5 h. Consequently, sourdough fermentation conditions of 27 °C/8.5 h were chosen as the optimum process for further experiments. In phase III, optimum sourdough fermentation (27 °C/8.5 h) was conducted, and the concentrations of total folate and resistant starch in the sourdoughs and sourdough bread were determined. Optimum fermentation conditions (27 °C/8.5 h) resulted in an increase of LAB cell counts but reduced the yeast cell count. Acidity of the dough increased after sourdough fermentation, leading to sourdough bread with an ideal pH (3.83 ± 0.01). The reduction of total folate and resistant starch in dough-after-proofing indicated that these two nutrients were not increased by using the optimum sourdough fermentation conditions.

The optimum sourdough fermentation determined from the current study can be applied in commercial bakeries to obtain sourdough bread with acceptable sensory characteristics and of consistent quality. The current findings of resistant starch and folate provide an opportunity for the bakery industry to develop a sourdough fermentation process focusing on increasing these nutritional and functional compounds in the future, by determining the levels of these nutritional compounds during fermentation under different conditions. Moreover, the identification of the sourdough LAB and yeast species in the wheat sourdough starter and rye sourdough starter are meaningful due to the shortage of information on sourdough starters available in New Zealand. These LAB and yeast species can be used in other sourdough-based baking products such as biscuits, cookies, crackers, pastry, pizza and pasta to improve their sensory characteristics. Further studies should focus on the following areas:

- Identification of flavour compounds produced by the combination of rye and wheat sourdough starters due to different flavours being produced during sourdough fermentation. This information may help bakeries to have a better understanding of the flavour profiles of their products.
- Application of rye-wheat sourdough starters in different sourdough products such as pastry and pizza to improve the product texture and shelf-life.
- Investigation of whether rye-wheat sourdough starters are able to generate bioactive compounds, such as γ-amino butyric acid (GABA) and angiotensin I-converting enzyme

(ACE) inhibitors. If present in adequate amounts these functional compounds may be effective in lowering blood pressure, which could then be determined in human trials.

- Application of rye-wheat sourdough starters in sourdough fermentation to degrade gluten protein, which can increase the protein digestibility of the sourdough product. Wheat products produced using rye-wheat sourdough starters may be non-allergenic to celiac disease patients.

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APPENDIX

A. Raw data of phase I

Table A1. Acidity record of rye sourdough starter (RSS), wheat sourdough starter (WSS), dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB)for 3 batches of sourdough process.

Replication	Sample	Triplication	pH	TTA
1	WSS	1	3.75	0.62
		2	3.75	0.62
		3	3.75	0.62
	RSS	1	3.66	2.03
		2	3.66	2.03
		3	3.66	2.03
	DBP	1	4.17	0.62
		2	4.17	0.62
		3	4.18	0.53
	DAP	1	3.56	1.23
		2	3.57	1.15
		3	3.57	1.15
	SDB	1	3.61	0.79
		2	3.61	0.79
		3	3.61	0.79
2	WSS	1	3.56	0.79
		2	3.57	0.88
		3	3.57	0.88
	RSS	1	3.73	1.94
		2	3.74	1.94
		3	3.74	1.94
	DBP	1	3.99	0.71
		2	3.99	0.79
		3	4.01	0.71
	DAP	1	3.57	1.15
		2	3.58	1.15
		3	3.58	1.15
	SDB	1	3.61	0.88
		2	3.61	0.88
		3	3.61	0.88
3	WSS	1	3.79	0.62
		2	3.79	0.71
		3	3.79	0.62
	RSS	1	3.63	2.12
		2	3.63	2.16
		3	3.62	2.20
	DBP	1	3.99	0.71
		2	4.00	0.75
		3	4.00	0.71

	DAP	1	3.55	1.23
		2	3.55	1.15
		3	3.54	1.15
	SDB	1	3.60	0.88
		2	3.60	0.88
		3	3.60	0.88

Table A2. LAB and yeast count of rye sourdough starter (RSS), wheat sourdough starter (WSS), dough before proofing (DBP) and dough after proofing (DAP) for 3 batches of sourdough process.

Replication	Samples	Duplication	LAB (Log CFU/g)	Yeast (Log CFU/g)
1	RSS	1	6.15	3.63
		2	5.90	3.71
	WSS	1	8.77	2.95
		2	8.74	3.15
	DBP	1	8.35	4.30
		2	8.35	4.00
	DAP	1	8.52	3.85
		2	8.50	3.60
2	RSS	1	5.61	1.30
		2	5.69	1.70
	WSS	1	8.89	1.48
		2	8.82	1.60
	DBP	1	8.33	3.48
		2	8.28	3.48
	DAP	1	8.49	3.00
		2	8.47	3.30
3	RSS	1	5.32	3.16
		2	5.72	3.15
	WSS	1	8.69	2.57
		2	8.61	2.52
	DBP	1	8.32	4.00
		2	8.28	4.00
	DAP	1	8.65	4.85
		2	8.64	3.85

Table A3. Sequence results of whole lactic acid bacteria pure colonies and yeast DNA (extracted from wheat sourdough starter-WSS and rye sourdough starter-RSS)

Sequenced Microorganisms	Isolate code	Sequences obtained
LAB	**1**	GCAGTCGAACGAAGTCGCCCAATTGATTCTTAGTGCTTGCACTAA GATGATTTTGGATCCGACTGAGTGGCGAACTGGTGAGTAACACGT GGGTAACCTGCCCAGAAGAAGGGGATAACACCTGGAAACAGATG

		CTAATACCGTATAACAACAAGAACCACATGGTTCTTGTTTGAAAGC TGGCCTTTGTGCTAGTGCTTCTGGATGGACCCGCGGCGTATTAGCT AGTTGGTGAGATAATAGCTCACCAAGGCAATGATACGTAGCAGAC CTGAGAGGGTAATCTGCCACAATGGGACTGAGACACGGCCCATAC TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAA GTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTC GTAAAACTCTGTTGTTAGAGAAGAACAGCCGTGAGAGCAACTGC TCACGGTATGACGGTATCTAACCAGAAAGTCACGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGTGGCAAACGTTGTCCGGATT TATTGGGCGTAAAGGGAGCGCAGGCGGTTTATTAAGTCTGATGTG AAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGATAAAC TTGAGTGCAGAAAAGGATAGTGGAACTTCATGTGTAGCGGTGAAA TGCGTAGATATATGAAGGAACACCAGTGGCGAAGGCGGCTATCTG GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAG GATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGT GTTGGAAGGTTTCCGCCTTTCAGTGCCGCAGCTAACGCATTAAGC ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAA TTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA TGCTACGCGAAGAACCTTACCAGGACTTGACATCTTCTGCCAATCT AAGAGATTAGACGTTCCCTTCGGGGACAGAATGACAGGTGGTGC ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCTTGTCTTTAGTTGCCAGCATTAAGTTGGGC ACTCTAGAGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGAT GACGTCAAATCATCATGCCCCTTATGTCCTGGGCTACACACGTGCT ACAATGGGCGATACAACGAGTTGCGAAACCGCGAGGTCAAGCTA ATCTCTTAAAGTCGTTCTCAGTTCGGATTGCAGTCTGCAACTCGAC TGCATGAAGTTGGAATCGCTAGTAATCGTGGATCAGCATGCCACG GTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATG AGAGTTTGTAACACCCAAAGTCGGTTGGATAACC
LAB	**2**	GCAGTCGAACGCACTCTCGTTAGATTGAAGAAGCTTGCTTCTGAT TGATAACATTTGAGTGAGTGGCGGACGGGTGAGTAACACGTGGGT AACCTGCCCTAAAGTGGGGGATAACATTTGGAAACAGATGCTAAT ACCGCATAAAACCTAGCACCGCATGGTGCAAGGTTGAAAGATGGT TTCGGCTATCACTTTAGGATGGACCCGCGGTGCATTAGTTAGTTGG TGAGGTAAAGGCTCACCAAGACCGTGATGCATAGCCGACCTGAGA GGGTAATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGA TGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAA CTCTGTTGTTGGAGAAGAACGTATTTGATAGTAACTGATCAGGTAG TGACGGTATCCAACCAGAAAGCCACGGCTAACTACGTGCCAGCAG CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCG TAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCTTC GGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGC AGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAG ATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTA ACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGGATTAGAT ACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAG GGTTTCCGCCCTTCAGTGCCGCAGCTAACGCATTAAGCACTCCGC CTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC GCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAG ATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGCATGGTT GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA GCGCAACCCTTATTACTAGTTGCCAGCATTTAGTTGGGCACTCTAG TGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCA AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGG ATGGTACAACGAGTCGCGAGACCGCGAGGTTTAGCTAATCTCTTA AAACCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAA GCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTG TAACACCCAAAGCCGGTGAGGTAACC

LAB	**3**	GCAGTCGACGAAGTCGCCCAATTGATTCTTAGTGCTTGCACTAAG ATGATTTTGGATCCGACTGAGTGGCGAACTGGTGAGTAACACGTG GGTAACCTGCCCAGAAGAAGGGGATAACACCTGGAAACAGATGC TAATACCGTATAACAACAAGAACCACATGGTTCTTGTTTGAAAGCT GGCCTTTGTGCTAGTGCTTCTGGATGGACCCGCGGCGTATTAGCTA GTTGGTGAGATAATAGCTCACCAAGGCAATGATACGTAGCAGACC TGAGAGGGTAATCTGCCACAATGGGACTGAGACACGGCCCATACT CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAG TCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCG TAAAACTCTGTTGTTAGAGAAGAACAGCCGTGAGAGCAACTGCTC ACGGTATGACGGTATCTAACCAGAAAGTCACGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGTGGCAAACGTTGTCCGGATTTA TTGGGCGTAAAGGGAGCGCAGGCGGTTTATTAAGTCTGATGTGAA AGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGATAAACTT GAGTGCAGAAAAGGATAGTGGAACTTCATGTGTAGCGGTGAAATG CGTAGATATATGAAGGAACACCAGTGGCGAAGGCGGCTATCTGGT CTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAGGAT TAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGTGTT GGAAGGTTTCCGCCTTTCAGTGCCGCAGCTAACGCATTAAGCATT CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTG ACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC TACGCGAAGAACCTTACCAGGACTTGACATCTTCTGCCAATCTAA GAGATTAGACGTTCCCTTCGGGGACAGAATGACAGGTGGTGCATG GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCTTTAGTTGCCAGCATTAAGTTGGGCACT CTAGAGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGAC GTCAAATCATCATGCCCCTTATGTCCTGGGCTACACACGTGCTACA ATGGGCGATACAACGAGTTGCGAAACCGCGAGGTCAAGCTAATCT CTTAAAGTCGTTCTCAGTTCGGATTGCAGTCTGCAACTCGACTGC ATGAAGTTGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTG AATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGAGA GTTTGTAACACCCAAAGTCGGTTGGATAACCTTTTTAGGA
LAB	**4**	TGCAGTCGAACGAAGTCGCCCAATTGATTCTTAGTGCTTGCACTA AGATGATTTTGGATCCGACTGAGTGGCGAACTGGTGAGTAACACG TGGGTAACCTGCCCAGAAGAAGGGGATAACACCTGGAAACAGAT GCTAATACCGTATAACAACAAGAACCACATGGTTCTTGTTTGAAAG CTGGCCTTTGTGCTAGTGCTTCTGGATGGACCCGCGGCGTATTAGC TAGTTGGTGAGATAATAGCTCACCAAGGCAATGATACGTAGCAGA CCTGAGAGGGTAATCTGCCACAATGGGACTGAGACACGGCCCATA CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAA GTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTC GTAAAACTCTGTTGTTAGAGAAGAACAGCCGTGAGAGCAACTGC TCACGGTATGACGGTATCTAACCAGAAAGTCACGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGTGGCAAACGTTGTCCGGATT TATTGGGCGTAAAGGGAGCGCAGGCGGTTTATTAAGTCTGATGTG AAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGATAAAC TTGAGTGCAGAAAAGGATAGTGGAACTTCATGTGTAGCGGTGAAA TGCGTAGATATATGAAGGAACACCAGTGGCGAAGGCGGCTATCTG GTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCAAACAG GATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAATGCTAGGT GTTGGAAGGTTTCCGCCTTTCAGTGCCGCAGCTAACGCATTAAGC ATTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAA TTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA TGCTACGCGAAGAACCTTACCAGGACTTGACATCTTCTGCCAATCT AAGAGATTAGACGTTCCCTTCGGGGACAGAATGACAGGTGGTGC ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCTTGTCTTTAGTTGCCAGCATTAAGTTGGGC ACTCTAGAGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGAT GACGTCAAATCATCATGCCCCTTATGTCCTGGGCTACACACGTGCT ACAATGGGCGATACAACGAGTTGCGAAACCGCGAGGTCAAGCTA ATCTCTTAAAGTCGTTCTCAGTTCGGATTGCAGTCTGCAACTCGAC

		TGCATGAAGTTGGAATCGCTAGTAATCGTGGATCAGCATGCCACG GTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATG AGAGTTTGTAACACCCAAAGTCGGTTGGATAACCTTTTTAGGA
Yeast	**11**	TAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAA CAAGAATTTTCGTAACTGGAAATTTTAAAATATTAAAAACTTTCAA CAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT GCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTT TGAACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGCCTGTTT GAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTAGTGAGTGATAC TCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTT TTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAG TACGGTCGTTTTAGGTTTTACCAACTGCGGCTAATCTTTTTTATACT GAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGAA CAATGTTCTTAAAGTT
Yeast	**12**	TGAAGTTAGAGGACGTCTAAAGATACTGTAAGAGAGGATCAGGTT CAAGACCAGCGCTTAATTGCGCGGTTGCGGCTTGGTTCGCCTTTT GCGGAACATGTCTTTTCTCGTTGTTAACTCTACTTCAACTTCTACA ACACTGTGGAGTTTTCTACACAACTTTTCTTCTTTGGGAAGATACG TCTTGTGCGTGCTTCCCAGAGGTGACAAACACAAACAACTTTTTA TTATTATAAACCAGTCAAAACCAATTTCGTTATGAAATTAAAAATAT TTAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA ACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCCGTGA ATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCAGGG GGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACAATCATGTTTG GTAGTGAGTGATACTCTGTCAAGGGTTAACTTGAAATTGCTAGCCT GTTATTTGGTTGTGATTTTGCTGGCTTGGATGACTTTGTCCAGTCTA GCTAATACCGAATTGTCGTATTAGGTTTTACCAACTTCGGCAGACT GTGTGTTGGCTCGGGCGCTTTAAAGACTTTGTCGTAAACGATTTAT CGTTTGTTTGAGCTTTTCGCATACGCAATCCGGCGAACAATACTCT CAAAG
Yeast	**13**	TAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAA CAAGAATTTTCGTAACTGGAAATTTTAAAATATTAAAAACTTTCAA CAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT GCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTT TGAACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGCCTGTTT GAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTAGTGAGTGATAC TCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTT TTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAG TACGGTCGTTTTAGGTTTTACCAACTGCGGCTAATCTTTTTTATACT GAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGAA CCAATGTTCTTAAAGTTG
Yeast	**14**	TAACAAACACAAACAATTTTATTTATTCATTAAATTTTTGTCAAAAA CAAGAATTTTCGTAACTGGAAATTTTAAAATATTAAAAACTTTCAA CAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAT GCGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTT TGAACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGCCTGTTT GAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTAGTGAGTGATAC TCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTT TTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAG TACGGTCGTTTTAGGTTTTACCAACTGCGGCTAATCTTTTTTATACT GAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGAA CAATGTTCTTAAAGTTG

Table A4. Texture analysis of dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB) for 3 batches of sourdough process.

Replication	Sample	Triplication	Parameter of texture analysis						
			Hardness (g)	Adhesiveness (g/s)	Springiness	Cohesiveness	Gumminess	Chewiness (g)	Resilience
1	DBP	1	786.99	-897.89	0.962	0.786	618.866	595.39	0.111
		2	778.39	-1064.85	0.956	0.777	605.029	578.504	0.105
		3	706.4	-879.32	0.969	0.781	551.934	534.809	0.097
2		1	1111.2	-587.89	0.977	0.779	865.765	845.734	0.167
		2	1123.36	-664.56	0.973	0.821	922.494	897.562	0.167
		3	1083.53	-584.39	0.972	0.77	834.68	811.611	0.158
3		1	1109.72	-500.46	0.975	0.732	812.131	791.674	0.163
		2	1181.36	-592	0.971	0.76	898.224	871.806	0.164
		3	1185.43	-581.07	0.977	0.76	901.483	880.627	0.182
1	DAP	1	921.22	-426.954	0.98	0.707	651.611	638.803	0.126
		2	1005.31	-506.89	0.978	0.704	707.266	691.703	0.137
		3	888.62	-462.382	0.977	0.669	594.849	581.465	0.128
2		1	993.41	-498.176	0.973	0.692	687.011	668.443	0.128
		2	958.3	-529.845	0.978	0.745	713.72	698.129	0.132
		3	943.09	-476.466	0.974	0.704	663.495	646.076	0.132
3		1	824.12	-425.735	0.982	0.714	588.46	577.87	0.122
		2	841.73	-400.952	0.98	0.719	604.851	593.024	0.119
		3	912.67	-486.944	0.976	0.736	671.832	655.49	0.129
1	SDB	1	1140.58	*	1.755	0.771	878.9	1542.85	0.448
		2	1183.82	*	1.106	0.773	915.03	1011.63	0.447
		3	948.49	-0.031	3.092	0.766	726.24	2245.58	0.427
2		1	1177.05	-1.287	0.998	0.737	867.72	865.9	0.418
		2	1569.97	-0.898	0.988	0.724	1137.04	1123.65	0.413
		3	1129.56	-2.411	0.992	0.809	914.22	907.06	0.474
3		1	1208.68	-1.119	1.001	0.745	900.91	901.52	0.428
		2	1119.97	*	1.058	0.74	828.97	876.97	0.419
		3	1309.11	*	2.366	0.735	961.81	2275.97	0.417

Table A5. Loaf volume, loaf weight and specific volume of sourdough breads

Replication	Sample	Duplication	Loaf volume (ml)	Loaf weight (g)	Specific volume (ml/g)
1	SDB	1	2000	757.4	2.64
		2	2100	756.3	2.78
2	SDB	1	2100	762.03	2.76
		2	2100	772.44	2.72
3	SDB	1	2000	755.45	2.65
		2	2050	753.53	2.72

Table A6. Colour of sourdough bread

Replication	Sample	Triplication	L*crumb	a*crumb	b*crumb	L*crust	a*crust	b*crust
1	SDB	1	54.50	1.13	9.85	45.51	8.03	11.30
		2	54.48	1.31	10.38	42.19	7.24	8.70
		3	55.05	1.47	10.59	42.52	7.95	9.54
2	SDB	1	52.63	0.79	8.76	43.41	8.07	9.75
		2	55.50	0.78	10.25	39.83	7.29	6.74
		3	54.67	1.14	10.21	45.87	7.54	11.83
3	SDB	1	54.45	1.18	10.37	43.04	7.44	8.67
		2	55.52	0.76	10.43	41.30	7.56	7.43
		3	55.11	0.63	9.59	41.04	7.84	7.33

B. Raw data of phase II

Table B1. Acidity record of rye sourdough starter (RSS), wheat sourdough starter (WSS), dough before proofing (DBP), dough after proofing (DAP) and sourdough bread (SDB) for sourdough and sourdough bread produced at 27°C, 29°C and 31°C.

Fermentation temperature (°C)	Replication	Sample	Duplication	pH	TTA%
27	1	RSS	1	4.34	1.32
			2	4.34	1.37
			3	4.35	1.37
	2		1	4.34	1.32
			2	4.34	1.37
			3	4.35	1.37
	1	WSS	1	3.84	0.66
			2	3.85	0.62
			3	3.86	0.71
	2		1	3.86	0.66
			2	3.88	0.62
			3	3.88	0.71
	1	DBP	1	4.55	0.57
			2	4.56	0.60
			3	4.54	0.60
	2		1	4.55	0.46
			2	4.55	0.57
			3	4.56	0.57
	1	DAP	1	3.63	1.08
			2	3.64	1.13
			3	3.65	1.06
	2		1	3.65	1.13
			2	3.65	1.15
			3	3.64	1.08
	1	SDB	1	3.76	0.80
			2	3.74	0.85
			3	3.77	0.81
	2		1	3.74	0.80
			2	3.75	0.79
			3	3.76	0.84
29	1	RSS	1	4.32	1.32
			2	4.33	1.41
			3	4.30	1.41
	2		1	4.32	1.32
			2	4.31	1.41
			3	4.29	1.41
	1	WSS	1	3.91	0.62
			2	3.92	0.62
			3	3.93	0.62
	2		1	3.91	0.62
			2	3.92	0.62

			3	3.93	0.62
	1	DBP	1	4.64	0.44
			2	4.65	0.45
			3	4.65	0.43
	2		1	4.67	0.44
			2	4.63	0.44
			3	4.63	0.44
	1	DAP	1	3.72	0.97
			2	3.73	1.01
			3	3.73	0.97
	2		1	3.74	1.01
			2	3.73	0.97
			3	3.73	1.01
	1	SDB	1	3.86	0.79
			2	3.86	0.71
			3	3.85	0.79
	2		1	3.86	0.79
			2	3.85	0.75
			3	3.85	0.75
31	1	RSS	1	3.95	2.03
			2	3.96	2.12
			3	3.96	2.03
	2		1	3.95	2.03
			2	3.96	2.12
			3	3.96	2.03
	1	WSS	1	3.68	0.97
			2	3.68	0.97
			3	3.69	1.01
	2		1	3.68	0.97
			2	3.68	0.97
			3	3.69	1.01
	1	DBP	1	4.14	0.71
			2	4.15	0.79
			3	4.15	0.71
	2		1	4.18	0.79
			2	4.18	0.75
			3	4.18	0.79
	1	DAP	1	3.81	1.01
			2	3.82	1.15
			3	3.82	1.10
	2		1	3.80	1.10
			2	3.80	1.10
			3	3.79	1.01
	1	SDB	1	3.91	0.93
			2	3.92	0.97
			3	3.92	0.88

	2			1	3.89	0.93
				2	3.89	0.93
				3	3.90	0.93

Table B2. LAB and yeast count of rye sourdough starter (RSS), wheat sourdough starter (WSS), dough before proofing (DBP) and dough after proofing (DAP) for sourdough produced at 27°C, 29°C and 31°C.

Fermentation temperature (°C)	Replication	Sample	Duplication	LAB (Log CFU/g)	Yeast (Log CFU/g)
27	1	RSS	1	6.75	4.18
			2	6.86	4.04
	2	RSS	1	7.00	4.26
			2	7.33	4.20
	1	WSS	1	9.07	3.76
			2	9.12	3.69
	2	WSS	1	9.05	3.72
			2	9.12	3.72
	1	DBP	1	8.51	4.66
			2	8.58	4.53
	2	DBP	1	8.43	4.60
			2	8.43	4.59
	1	DAP	1	8.97	3.56
			2	9.03	3.48
	2	DAP	1	9.04	3.48
			2	9.09	3.54
29	1	RSS	1	7.62	3.68
			2	7.63	3.72
	2	RSS	1	7.65	3.46
			2	7.66	3.43
	1	WSS	1	9.10	4.08
			2	9.10	4.19
	2	WSS	1	9.18	4.13
			2	9.06	4.10
	1	DBP	1	8.74	4.34
			2	8.59	4.30
	2	DBP	1	8.62	4.28
			2	8.59	4.45
	1	DAP	1	9.03	4.01
			2	9.02	3.93
	2	DAP	1	8.92	4.02
			2	9.00	4.05
31	1	RSS	1	7.08	3.91
			2	7.41	3.94
	2	RSS	1	7.53	4.09
			2	7.65	3.92
	1	WSS	1	9.38	3.59
			2	9.32	3.60
	2	WSS	1	9.18	3.72

			2	9.23	3.63
	1	DBP	1	8.66	4.71
			2	8.79	4.72
	2	DBP	1	8.62	4.72
			2	8.66	4.70
	1	DAP	1	8.91	4.66
			2	8.93	4.65
	2	DAP	1	8.98	4.65
			2	8.91	4.68

Table B3. Texture analysis of dough-before-proofing (DBP), dough-after-proofing (DAP) and sourdough bread produced at 27°C, 29°C and 31°C.

Fermentation temperature (°C)	Sample	Replication	Triplication	Hardness (g)	Adhesiveness (g/s)	Springiness	Cohesiveness	Gumminess	Chewiness (g)	Resilience
27	DBP	1	1	438.49	-510.79	0.99	0.73	318.37	313.50	0.09
			2	454.27	-449.84	0.99	0.67	304.18	300.62	0.10
			3	460.54	-494.41	0.99	0.69	316.29	311.68	0.10
		2	1	442.36	-728.57	0.97	0.79	347.16	338.24	0.08
			2	478.65	-562.67	0.98	0.70	333.80	327.54	0.10
			3	437.99	-703.92	0.97	0.74	323.99	315.57	0.09
	DAP	1	1	342.49	-464.37	0.98	0.69	237.75	232.17	0.07
			2	347.57	-387.48	0.99	0.68	234.82	231.69	0.07
			3	389.56	-473.65	0.98	0.68	263.68	258.02	0.08
		2	1	381.86	-409.93	0.99	0.66	251.87	248.33	0.08
			2	358.43	-388.35	0.99	0.70	250.01	246.86	0.07
			3	367.28	-433.52	0.99	0.67	246.17	242.68	0.07
	SDB	1	1	1270.56	-7.05	0.98	0.73	924.53	908.67	0.40
			2	1078.96	-5.05	0.98	0.73	784.19	771.98	0.40
			3	1164.56	-5.79	2.07	0.73	845.17	1749.29	0.40
		2	1	868.85	-2.36	1.01	0.75	650.28	653.82	0.42
			2	1132.14	-7.05	0.99	0.73	825.02	815.39	0.40
			3	1157.40	-1.50	1.15	0.72	828.33	950.92	0.38
29	DBP	1	1	853.58	-882.66	0.98	0.68	576.35	562.12	0.11
			2	772.82	-731.62	0.98	0.66	505.97	496.91	0.11
			3	727.48	-809.09	0.98	0.69	500.14	489.26	0.10
		2	1	786.95	-937.10	0.97	0.71	555.57	540.88	0.10
			2	767.92	-979.61	0.97	0.72	550.51	534.28	0.10
			3	738.16	-905.32	0.97	0.75	552.01	537.72	0.10
	DAP	1	1	572.76	-694.49	0.98	0.72	410.28	400.39	0.07
			2	532.28	-614.80	0.98	0.68	361.52	354.16	0.07
			3	667.90	-667.98	0.98	0.64	425.51	416.80	0.08
		2	1	642.27	-726.06	0.96	0.68	438.90	422.72	0.07
			2	597.86	-666.96	0.98	0.66	391.83	382.71	0.07
			3	552.19	-634.47	0.98	0.69	380.55	372.56	0.07
	SDB	1	1	1192.16	-3.30	1.00	0.72	854.55	853.37	0.40
			2	1195.29	-6.84	2.86	0.73	875.26	2500.65	0.41
			3	1349.00	-7.21	0.99	0.70	941.33	930.05	0.38
		2	1	1295.86	-3.53	1.03	0.69	895.67	923.16	0.37
			2	1340.95	-6.02	1.01	0.71	955.44	960.24	0.39
			3	1419.67	-4.74	1.13	0.71	1005.03	1138.86	0.38
31	DBP	1	1	734.10	-780.38	0.98	0.75	547.67	538.92	0.10
			2	724.36	-966.74	0.98	0.76	549.02	536.82	0.09
			3	711.55	-928.36	0.97	0.74	528.43	513.57	0.09

		2	1	736.54	-788.03	0.98	0.72	528.09	515.95	0.10
			2	702.61	-923.03	0.97	0.70	494.49	477.50	0.10
			3	737.36	-997.37	0.97	0.74	544.99	529.74	0.09
	DAP	1	1	250.45	-406.58	0.98	0.81	203.47	199.58	0.06
			2	264.53	-327.66	0.99	0.70	185.24	182.39	0.08
			3	268.95	-399.37	0.98	0.76	203.01	199.33	0.07
		2	1	302.56	-381.61	0.99	0.73	220.29	217.27	0.07
			2	249.80	-301.14	0.99	0.71	176.40	174.31	0.07
			3	252.41	-262.88	0.99	0.70	176.05	174.20	0.07
	SDB	1	1	1116.28	-3.34	1.70	0.74	827.75	1405.23	0.42
			2	1043.00	-4.64	2.17	0.74	774.13	1679.62	0.42
			3	1039.31	-7.11	1.72	0.75	783.25	1345.34	0.44
		2	1	1079.56	-5.64	2.25	0.76	815.58	1836.88	0.43
			2	847.38	-5.57	1.03	0.78	660.17	682.40	0.44
			3	1046.86	-6.00	2.02	0.77	805.55	1626.62	0.45

Table B4. Texture properties of sourdough-before-proofing and sourdough-before-proofing (fermentation) at 27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h, and the average change in texture properties between sourdough-before-proofing and sourdough-before-proofing at each temperature.

Fermentation Temperature (°C)	Sample	Springiness	Average Change in Springiness (%)	Cohesiveness	Average Change in Cohesiveness (%)	Resilience	Average Change in Resilience (%)
27	DBP27	0.98 ± 0.00	0.25%^{a}	0.72 ± 0.02	5.34%^{b}	0.09 ± 0.00	23.22%^{c}
	DAP27	0.98 ± 0.00		0.68 ± 0.01		0.07 ± 0.00	
29	DBP29	0.98 ± 0.00	0.02%^{a}	0.70 ± 0.01	3.08%^{b}	0.10 ± 0.00	29.79%^{c}
	DAP29	0.98 ± 0.00		0.68 ± 0.01		0.07 ± 0.00	
31	DBP31	0.97 ± 0.00	1.06%^{a}	0.73 ± 0.01	0.20%^{b}	0.10 ± 0.00	28.42%^{c}
	DAP31	0.99 ± 0.00		0.73 ± 0.02		0.07 ± 0.00	

DAP27 = dough-after-proofing at 27 °C/8.5 h, SDB27 = sourdough bread produced at 27 °C/8.5 h, DAP29 = dough-after-proofing at 29 °C/7 h, SDB29 = sourdough bread produced at 29 °C/7 h, DAP31 = dough-after-proofing at 31 °C/5.5 h, SDB31 = sourdough bread produced at 31 °C/5.5 h; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; values within columns with identical subscript letters are not different ($p > 0.05$).

Table B5. Texture properties of sourdoughs-after-proofing (fermentation) and sourdough bread produced at 27 °C/8.5 h, 29 °C/7 h and 31°C/5.5 h, and the average change in texture properties between sourdough-after-proofing and sourdough bread at each temperature.

Fermentation Temperature (°C)	Sample	Hardness (g)	Average Change in Hardnes (%)	Gumminess	Average Change in Gumminess (%)	Springiness	Average Change in Springiness (%)	Chewiness	Average Change in Chewiness (%)
27	DAP27	364.53 ± 7.60	205.08%[a]	247.38 ± 4.30	227.24%[b]	0.98 ± 0.00	21.65%[c]	243.29 ± 4.00	301.23%[d]
	SDB27	1112.10 ± 55.00		809.60 ± 37.00		1.20 ± 0.18		975.00 ± 161.00	
29	DAP29	594.20 ± 21.50	118.58%[a]	401.40 ± 11.80	129.50%[b]	0.98 ± 0.00	36.89%[c]	391.60 ± 11.00	210.71%[d]
	SDB29	1298.8 ± 37.00		921.20 ± 23.00		1.34 ± 0.31		1218.00 ± 260.00	
31	DAP31	264.78 ± 8.20	288.48%[a]	194.08 ± 7.20	300.72%[b]	0.99 ± 0.00	84.26%[c]	191.18 ± 7.00	648.17%[d]
	SDB31	1028.70 ± 38.20		777.70 ± 24.90		1.82 ± 0.18		1429.00 ± 167.00	

DAP27 = dough-after-proofing at 27 °C/8.5 h, SDB27 = sourdough bread produced at 27 °C/8.5 h, DAP29 = dough-after-proofing at 29 °C/7 h, SDB29 = sourdough bread produced at 29 °C/7 h, DAP31 = dough-after-proofing at 31 °C/5.5 h, SDB31 = sourdough bread produced at 31 °C/5.5 h; data expressed as mean ± SEM; mean values are results of two independent replications (n = 2); samples were measured in triplicate; values within columns with identical subscript letters are not different ($p > 0.05$).

Table B6. Loaf volume, loaf weight and specific volume of sourdough breads.

Fermentation temperature	Sample	Replication	Duplication	Loaf volume (ml)	Loaf weight (g)	Specific volume (ml/g)
27°C	SDB	1	1	2150.00	742.80	2.89
			2	2150.00	744.28	2.89
		2	1	2350.00	756.73	3.11
			2	2300.00	755.77	3.04
29°C	SDB	1	1	2200.00	749.19	2.94
			2	2150.00	744.83	2.89
		2	1	2200.00	758.08	2.90
			2	2150.00	760.49	2.83
31°C	SDB	1	1	2200.00	751.36	2.93
			2	2250.00	749.60	3.00
		2	1	2350.00	745.64	3.15
			2	2250.00	744.81	3.02

Table B7. Colour of sourdough bread produced at 27°C, 29°C and 31°C.

Fermentation temperature	Sample	Replication	Triplication	L*crumb	a*crumb	b*crumb	L*crust	a*crust	b*crust
27°C	SDB	1	1	60.17	1.05	10.98	37.36	8.89	11.38
			2	59.70	1.08	10.69	32.10	8.44	7.67
			3	59.79	1.11	11.15	32.27	9.07	7.95
		2	1	57.98	0.85	10.29	30.38	6.54	6.09
			2	57.24	1.04	10.10	28.57	6.06	4.97
			3	58.76	1.26	10.51	30.89	8.26	6.60
29°C	SDB	1	1	61.32	1.27	11.25	29.35	8.35	6.19
			2	62.67	1.25	11.20	28.15	7.42	5.32
			3	62.79	1.13	11.05	27.84	6.81	4.83
		2	1	63.26	1.15	11.19	30.44	6.95	6.79
			2	63.55	1.39	11.37	30.91	8.12	7.12
			3	63.54	1.41	11.45	28.90	7.42	5.63
31°C	SDB	1	1	60.60	1.11	11.02	31.84	6.88	6.91
			2	60.48	1.38	10.88	31.92	7.04	7.31
			3	61.53	1.44	10.79	30.13	7.52	5.64
		2	1	59.80	1.45	10.77	31.69	7.89	7.20
			2	60.64	1.48	10.66	30.86	8.46	6.28
			3	59.32	1.34	11.01	33.79	8.41	8.29

Table B8. Questionnaire of focus group sensory evaluation

(1) How would you like the **COLOR** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(2) How would you like the **SOUR SMELL** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(3) How would you like the **SOUR TASTE** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(4) How would you like the **Saltiness** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(5) How would you like the **TEXTURE** of the sample

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(6) How would you like the **AROMA** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

(7) How would you like the **OVERALL ACCEPTABILITY** of the sample?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Common:

(8) Which sample you like the most?

S31	S29	S27

C. Raw data of phase III

Table C1. Acidity of wheat sourdough starter (WSS), rye sourdough starter (RSS), dough-before-proofing (DBP), dough-after-proofing (DAP) and sourdough bread (SDB)

Sample	Replication	Duplication	pH	TTA
WSS	1	1	3.99	0.53
		2	3.98	0.62
		3	3.99	0.62
	2	1	3.95	0.62
		2	3.93	0.62
		3	3.93	0.62
RSS	1	1	4.32	1.19
		2	4.32	1.06
		3	4.32	1.06
	2	1	4.30	1.28
		2	4.30	1.19
		3	4.29	1.19
DBP	1	1	4.76	0.44
		2	4.76	0.44
		3	4.76	0.40
	2	1	4.74	0.35
		2	4.74	0.31
		3	4.75	0.40
DAP	1	1	3.76	0.93
		2	3.76	1.01
		3	3.76	1.06
	2	1	3.70	1.10
		2	3.70	1.15
		3	3.70	1.01
SDB	1	1	3.85	0.79
		2	3.85	0.75
		3	3.84	0.75
	2	1	3.82	0.84
		2	3.82	0.79
		3	3.81	0.84

Table C2. LAB and yeast count of wheat sourdough starter (WSS), rye sourdough starter (RSS), dough-before-proofing (DBP), dough-after-proofing (DAP).

Sample	Replication	Duplication	LAB (Log CFU/g)	Yeast (Log CFU/g)
WSS	1	1	8.79	2.93
		2	8.84	2.95
	2	1	8.69	3.57
		2	8.70	3.61

RSS	1	1	7.45	3.66
		2	7.45	3.59
	2	1	7.27	3.89
		2	7.28	4.10
DBP	1	1	8.51	4.47
		2	8.48	4.34
	2	1	8.34	4.34
		2	8.34	4.43
DAP	1	1	8.83	2.86
		2	8.87	2.78
	2	1	8.79	2.63
		2	8.81	2.72

Table C3. Texture analysis of dough-before-proofing (DBP), dough-after-proofing (DAP) and sourdough bread.

Sample	Replication	Triplication	Hardness (g)	Adhesiveness (g/s)	Springiness	Cohesiveness	Gumminess	Chewiness (g)	Resilience
DBP	1	1	784.72	-1110.15	0.97	0.77	606.40	586.61	0.10
		2	753.67	-958.46	0.98	0.75	568.15	553.71	0.10
		3	735.97	-1018.01	0.97	0.76	556.24	539.19	0.10
	2	1	816.31	-902.19	0.98	0.76	621.76	608.44	0.11
		2	772.50	-779.76	0.99	0.78	598.66	589.48	0.11
		3	711.21	-764.19	0.98	0.73	515.77	506.05	0.10
DAP	1	1	683.48	-587.69	0.98	0.69	474.07	466.11	0.10
		2	658.07	-572.55	0.99	0.69	450.58	445.14	0.10
		3	638.36	-608.01	0.98	0.67	428.47	420.68	0.09
	2	1	610.86	-679.11	0.98	0.71	434.36	426.20	0.11
		2	561.77	-550.15	0.98	0.69	388.48	379.56	0.10
		3	584.23	-536.33	0.98	0.69	404.82	398.28	0.10
SDB	1	1	1154.66	-1.30	1.00	0.74	853.31	852.72	0.42
		2	1123.35	-0.64	1.00	0.73	818.97	819.51	0.41
		3	1180.65	-1.31	1.00	0.73	859.83	858.71	0.42
	2	1	1162.22	-1.43	1.24	0.76	878.85	1089.98	0.43
		2	1121.35	-1.24	2.77	0.75	837.19	2315.27	0.42
		3	1090.80	-1.55	2.25	0.73	794.71	1784.64	0.40

Table C4. Load volume, loaf weight and specific volume of sourdough bread.

Sample	Replication	Triplication	loaf volume (ml)	Loaf weight (g)	Specific volume (ml/g)
SDB	1	1	2400.00	748.07	3.21
		2	2400.00	744.96	3.22
		3	2400.00	745.69	3.22
	2	1	2300.00	747.56	3.08
		2	2310.00	747.17	3.09
		3	2400.00	752.38	3.19

Table C5. Colour of sourdough bread.

Sample	Replication	Triplication	L*crumb	a*crumb	b*crumb	L*crust	a*crust	b*crust
SDB	1	1	59.94	0.65	9.42	28.57	7.51	6.15
		2	61.63	0.51	9.89	28.53	6.17	5.39
		3	60.75	0.60	9.97	28.59	6.48	6.07
	2	1	58.21	0.72	10.16	26.41	5.91	4.62
		2	58.25	0.69	10.23	28.34	5.60	5.39
		3	59.08	0.62	9.92	26.05	5.42	3.87

Table C6. Serial dilution of organic acid standard solution

Standard	Serial dilution (mg/100 ml)	Area	
Lactic acid	0.5	7781	7796
	1.0	15682	16066
	2.0	33488	35039
	3.0	51824	53149
	5.0	86917	86669
	10.0	173669	171742
	15.0	262126	263817
	25.0	439624	438908
Acetic acid	0.5	5776	5943
	1.0	11482	11657
	2.0	24218	24732
	3.0	36754	37053
	5.0	61753	61469
	10.0	125552	125536
	15.0	188756	189352
	25.0	317291	316959
Fructose	0.1	20933	19039
	0.2	38209	38537
	0.4	65472	64045
	0.8	124689	124148

	1.0	154388	155219
Sucrose	0.1	20482	23869
	0.2	32876	31853
	0.4	70047	69930
	0.8	132368	136741
	1.0	164081	161735
Glucose	1.5	137796	131670
	2.0	175538	175229
	4.0	359226	349115
	8.0	697920	681502
	15.0	1371656	1378003
Maltose	1.5	99043	108276
	2.0	175538	175229
	4.0	359226	349115
	8.0	697920	681502
	15.0	1371656	1378003

Table C7. Concentration of organic acid and sugar in dough-before-proofing (DBP), dough-after-proofing (DAP) and sourdough bread.

Organic acid/sugar	Sample	Replication	Triplication	Peak area	Analyte concentration (mg/g)
Lactic acid	DBP	1	1	25895	0.15
			2	27415	0.16
			3	20335	0.12
		2	1	19427	0.12
			2	19753	0.12
			3	18642	0.11
	DAP	1	1	104168	0.6
			2	78319	0.45
			3	82743	0.48
		2	1	54111	0.31
			2	52756	0.31
			3	50025	0.29
	SDB	1	1	94422	0.54
			2	92018	0.53
			3	65526	0.38
		2	1	53241	0.31
			2	48748	0.28
			3	47630	0.28
Acetic acid	DBP	1	1	122400	0.97
			2	112501	0.89
			3	104462	0.83
		2	1	90093	0.72

			2	85698	0.68
			3	81862	0.65
	DAP	1	1	43874	0.35
			2	31061	0.25
			3	33153	0.27
		2	1	23321	0.19
			2	20505	0.17
			3	20002	0.17
	SDB	1	1	35734	0.29
			2	35241	0.29
			3	26160	0.22
		2	1	18311	0.15
			2	17845	0.15
			3	16474	0.14
Fructose	DBP	1	1	51920	1.22
			2	44369	1.02
			3	45665	1.05
		2	1	95697	2.41
			2	73467	1.81
			3	73814	1.82
	DAP	1	1	49558	1.15
			2	44232	1.01
			3	39899	0.89
		2	1	41173	0.93
			2	46091	1.06
			3	43420	0.99
	SDB	1	1	53913	1.28
			2	36318	0.80
			3	35649	0.78
		2	1	42356	0.97
			2	38900	0.88
			3	40036	0.91
Glucose	DBP	1	1	622908	27.57
			2	623137	27.58
			3	706817	31.21
		2	1	724908	32.23
			2	687268	30.58
			3	728013	32.36
	DAP	1	1	558284	24.60
			2	547131	24.12
			3	619486	27.23
		2	1	614128	27.19
			2	636807	28.17
			3	629865	27.87
	SDB	1	1	666807	29.56
			2	623468	27.68

			3	663302	29.41
		2	1	691471	30.75
			2	622682	27.75
			3	671956	29.90
Maltose	DBP	1	1	327459	15.14
			2	291565	13.60
			3	310791	14.43
		2	1	323897	15.10
			2	315786	14.75
			3	348395	16.15
	DAP	1	1	289054	13.40
			2	277083	12.89
			3	360978	16.46
		2	1	282937	13.23
			2	347880	16.01
			3	346735	15.97
	SDB	1	1	289027	13.53
			2	314150	14.61
			3	288546	13.51
		2	1	305552	14.30
			2	304466	14.25
			3	318358	14.85

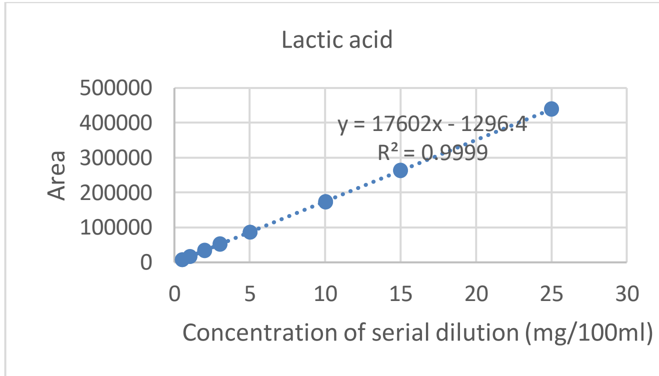


Figure C1 Standard curve of lactic acid

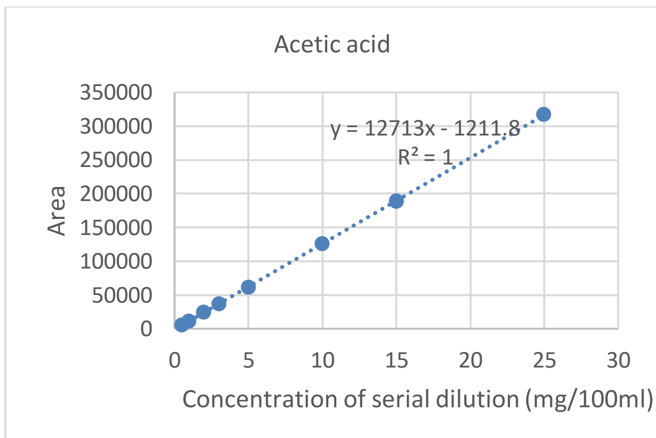


Figure C2 Standard curve of acetic acid

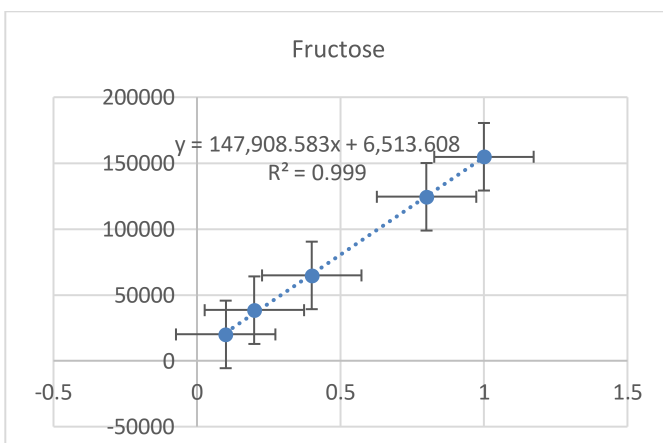


Figure C3 Standard curve of fructose

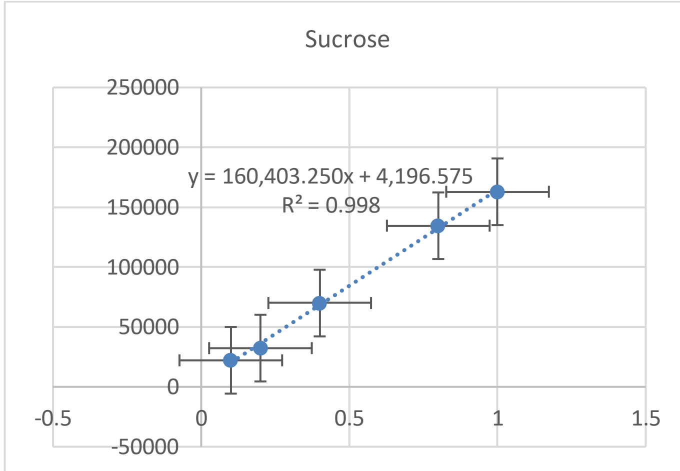


Figure C4 Standard curve of sucrose

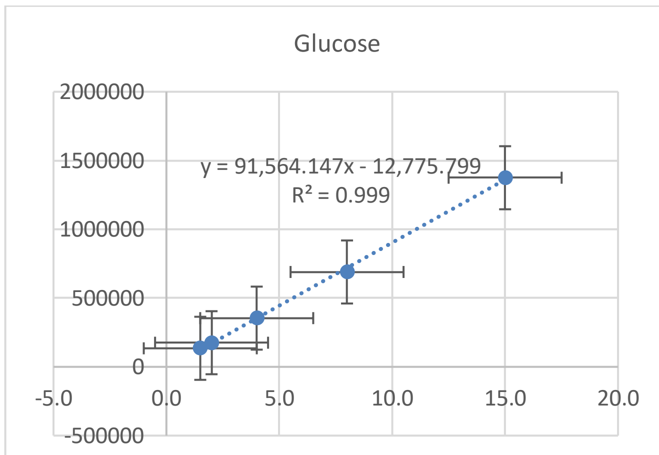


Figure C5 Standard curve of glucose

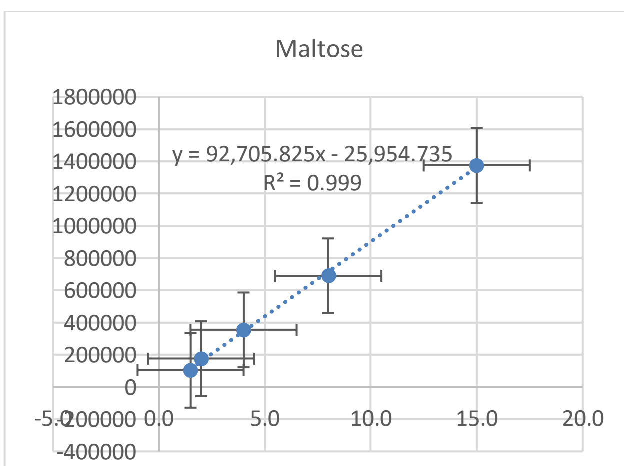


Figure C6 Standard curve of maltose

D. Data Analysis

Phase I

1. **Acidity**

1.1. **WSS vs RSS**

TTA: Two-Sample T-Test and CI: WSS, RSS

Two-Sample T-Test and CI: WSS-%TTA, RSS-%TTA

Method

μ_1: population mean of WSS-%TTA
μ_2: population mean of RSS-%TTA
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
WSS-%TTA	9	0.707	0.114	0.038
RSS-%TTA	9	2.0433	0.0979	0.033

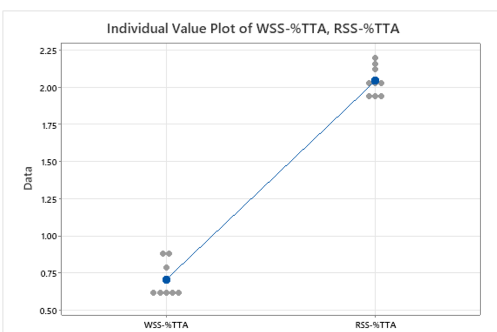
Estimation for Difference

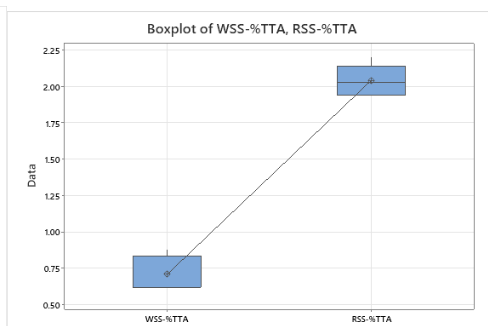
Difference	95% CI for Difference
-1.3367	(-1.4436, -1.2297)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-26.64	15	0.000





Two-Sample T-Test and CI: WSS-%TTA, RSS-%TTA

Method

μ_1: population mean of WSS-%TTA
μ_2: population mean of RSS-%TTA
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
WSS-%TTA	9	0.707	0.114	0.038
RSS-%TTA	9	2.0433	0.0979	0.033

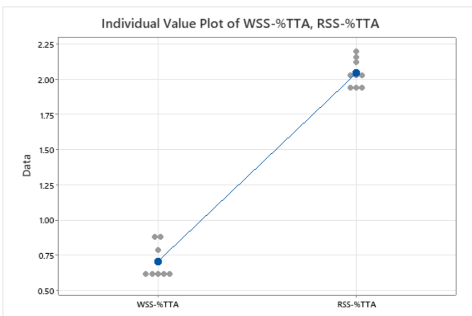
Estimation for Difference

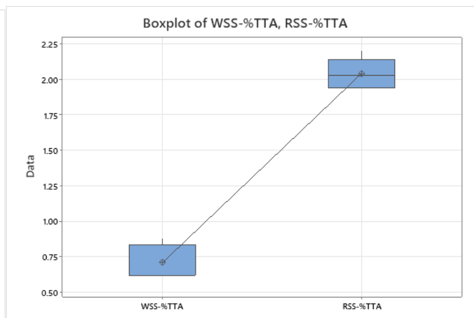
Difference	95% CI for Difference
-1.3367	(-1.4436, -1.2297)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-26.64	15	0.000





1.2. DBP vs DAP

Paired T-Test and CI: DBP-%TTA, DAP-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DBP-%TTA	9	0.6833	0.0792	0.0264
DAP-%TTA	9	1.1678	0.0353	0.0118

Estimation for Paired Difference

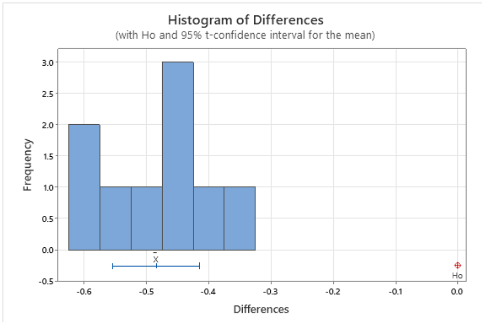
Mean	StDev	SE Mean	95% CI for μ_difference
-0.4844	0.0908	0.0303	(-0.5543, -0.4146)

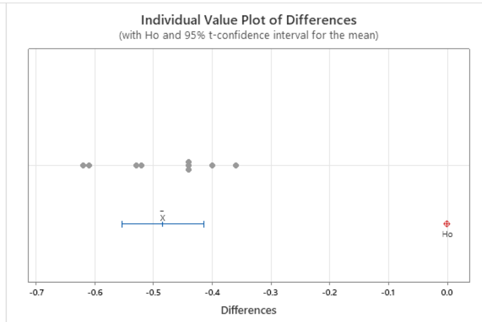
μ_difference: population mean of (DBP-%TTA - DAP-%TTA)

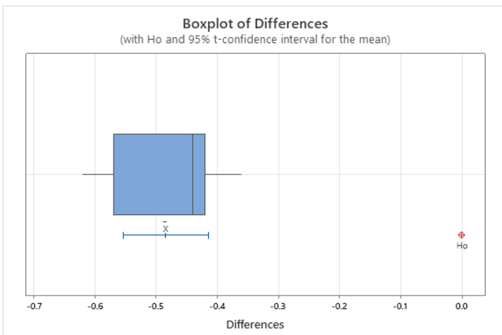
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-16.00	0.000







Paired T-Test and CI: DBP-pH, DAP-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DBP-pH	9	4.0556	0.0886	0.0295
DAP-pH	9	3.5633	0.0141	0.0047

Estimation for Paired Difference

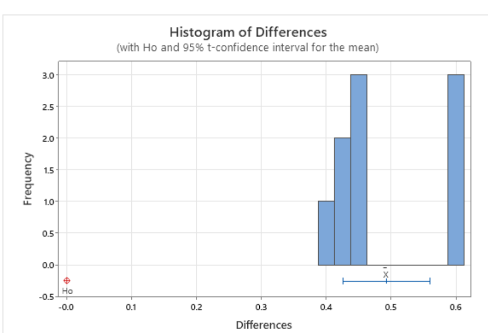
Mean	StDev	SE Mean	95% CI for μ_difference
0.4922	0.0871	0.0290	(0.4252, 0.5592)

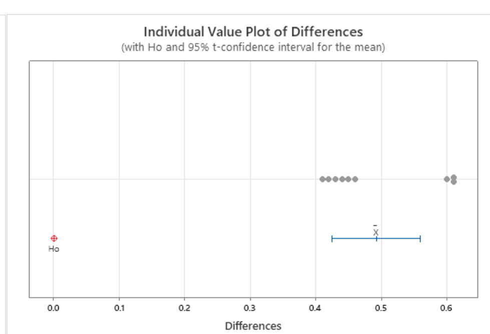
μ_difference: population mean of (DBP-pH - DAP-pH)

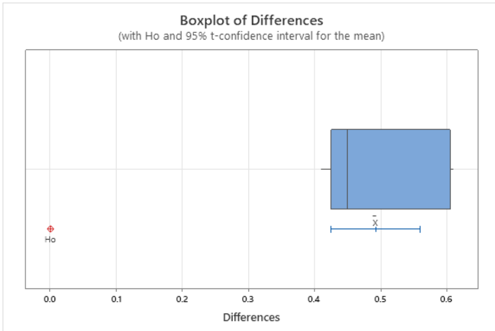
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
16.94	0.000







1.3. DAP vs SDB

Paired T-Test and CI: DAP-%TTA, SDB-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DAP-%TTA	9	1.1678	0.0353	0.0118
SDB-%TTA	9	0.8500	0.0450	0.0150

Estimation for Paired Difference

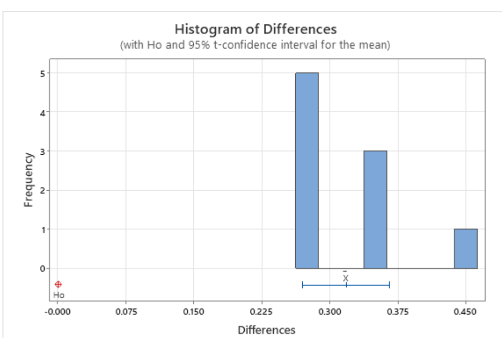
Mean	StDev	SE Mean	95% CI for μ_difference
0.3178	0.0622	0.0207	(0.2700, 0.3656)

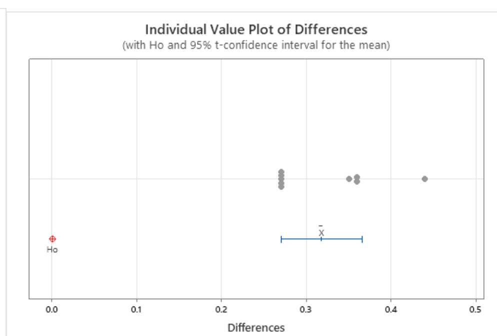
μ_difference: population mean of (DAP-%TTA - SDB-%TTA)

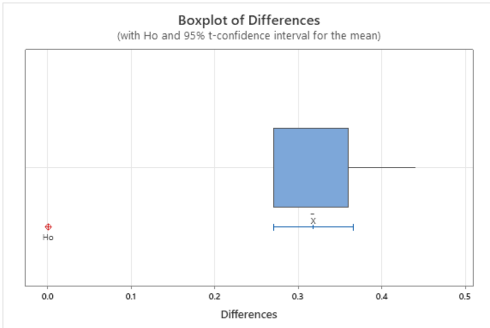
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
15.33	0.000







Paired T-Test and CI: DAP-pH, SDB-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DAP-pH	9	3.56333	0.01414	0.00471
SDB-pH	9	3.60667	0.00500	0.00167

Estimation for Paired Difference

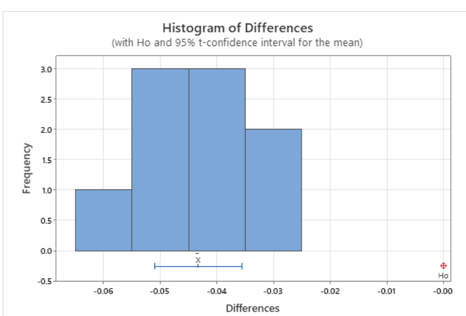
Mean	StDev	SE Mean	95% CI for μ_difference
-0.04333	0.01000	0.00333	(-0.05102, -0.03565)

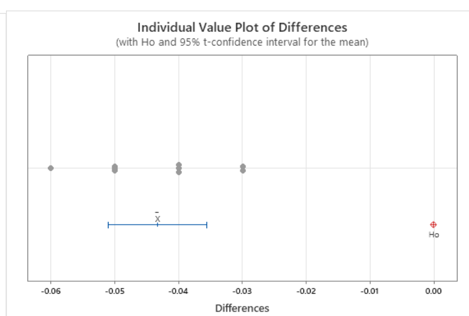
μ_difference: population mean of (DAP-pH - SDB-pH)

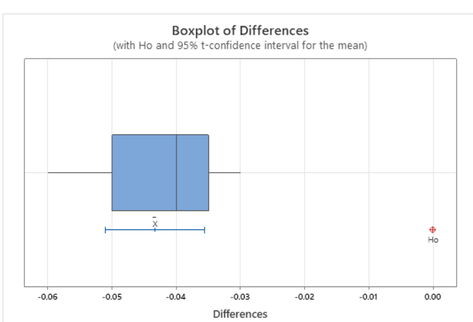
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-13.00	0.000







1. **Texture analysis**
1.1. **DBP vs DAP**

Paired T-Test and CI: Hardness-DBP, Hardness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Hardness-DBP	9	1007.4	191.7	63.9
Hardness-DAP	9	920.9	62.3	20.8

Estimation for Paired Difference

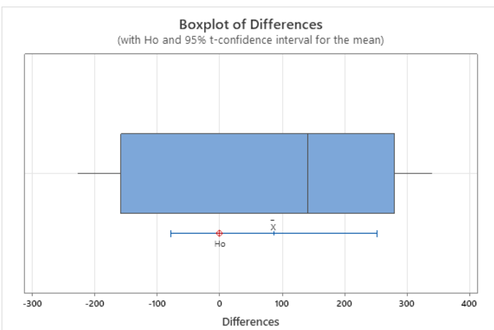
Mean	StDev	SE Mean	95% CI for μ_difference
86.4	214.4	71.5	(-78.3, 251.2)

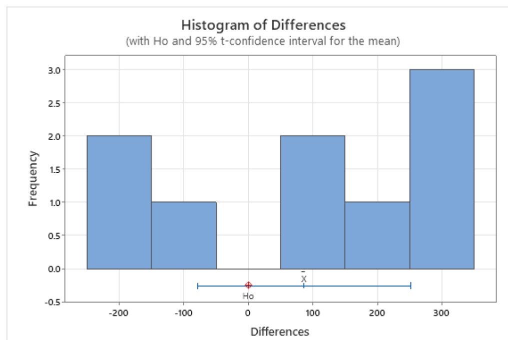
μ_difference: population mean of (Hardness-DBP - Hardness-DAP)

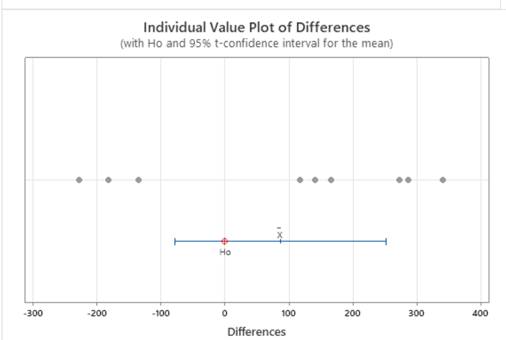
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
1.21	0.261







Paired T-Test and CI: Adhesiveness-DBP, Adhesiveness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Adhesiveness-DBP	9	-705.8	192.7	64.2
Adhesiveness-DAP	9	-468.3	42.8	14.3

Estimation for Paired Difference

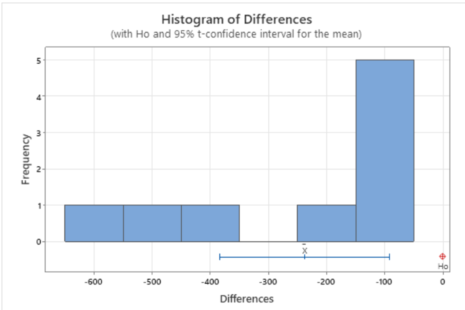
Mean	StDev	SE Mean	95% CI for μ_difference
-237.6	189.7	63.2	(-383.4, -91.8)

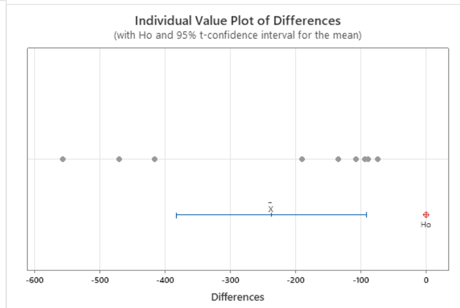
μ_difference: population mean of (Adhesiveness-DBP - Adhesiveness-DAP)

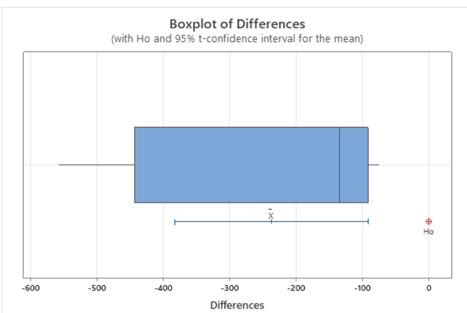
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-3.76	0.006







Paired T-Test and CI: Springiness-DBP, Springiness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Springiness-DBP	9	0.97022	0.00705	0.00235
Springiness-DAP	9	0.97756	0.00292	0.00097

Estimation for Paired Difference

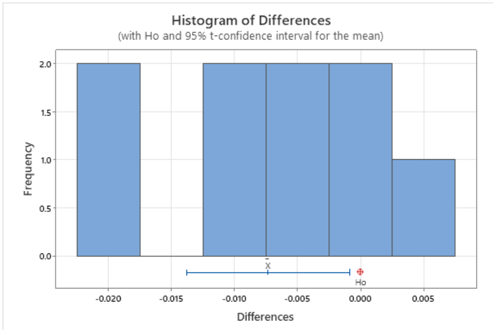
Mean	StDev	SE Mean	95% CI for μ_difference
-0.00733	0.00840	0.00280	(-0.01379, -0.00088)

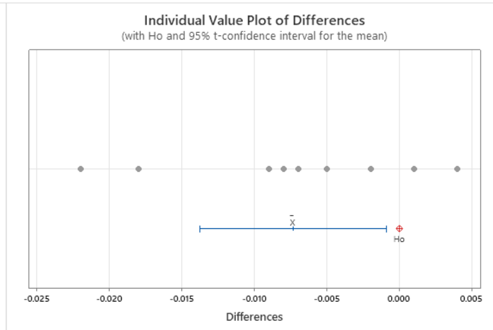
μ_difference: population mean of (Springiness-DBP - Springiness-DAP)

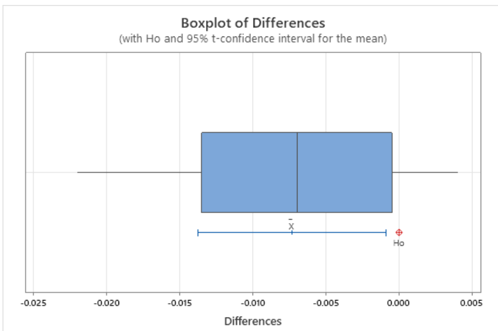
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-2.62	0.031







Paired T-Test and CI: Cohesiveness-DBP, Cohesiveness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Cohesiveness-DBP	9	0.77400	0.02400	0.00800
Cohesiveness-DAP	9	0.71000	0.02259	0.00753

Estimation for Paired Difference

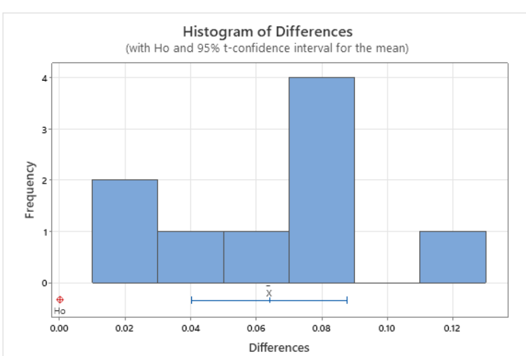
Mean	StDev	SE Mean	95% CI for μ_difference
0.0640	0.0307	0.0102	(0.0404, 0.0876)

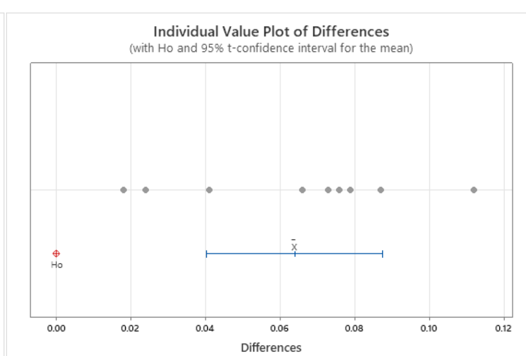
μ_difference: population mean of (Cohesiveness-DBP - Cohesiveness-DAP)

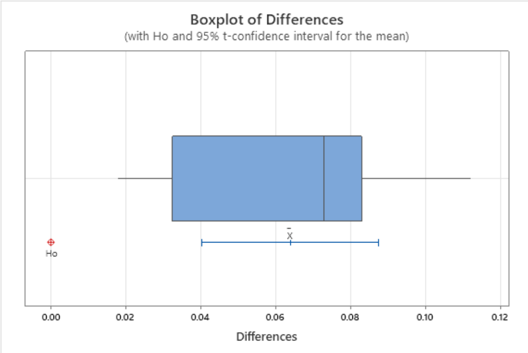
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
6.26	0.000







Paired T-Test and CI: Gumminess-DBP, Gumminess-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Gumminess-DBP	9	779.0	145.3	48.4
Gumminess-DAP	9	653.7	47.6	15.9

Estimation for Paired Difference

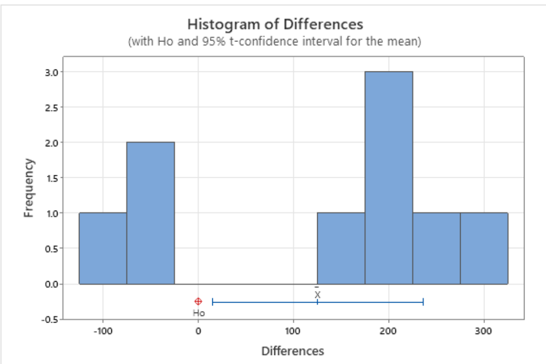
Mean	StDev	SE Mean	95% CI for μ_difference
125.3	144.0	48.0	(14.6, 235.9)

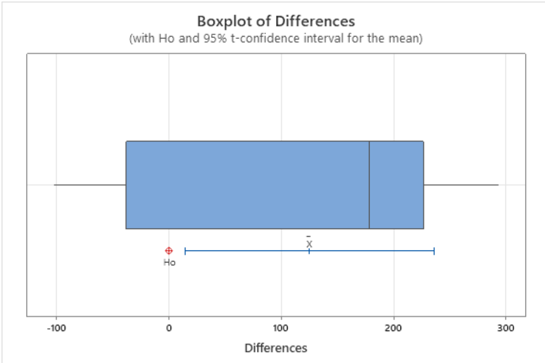
μ_difference: population mean of (Gumminess-DBP - Gumminess-DAP)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
2.61	0.031





Paired T-Test and CI: Chewiness-DBP, Chewiness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Chewiness-DBP	9	756.4	144.8	48.3
Chewiness-DAP	9	639.0	45.6	15.2

Estimation for Paired Difference

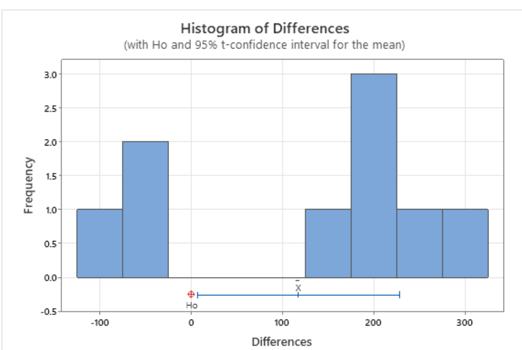
Mean	StDev	SE Mean	95% CI for μ_difference
117.4	143.8	47.9	(6.8, 228.0)

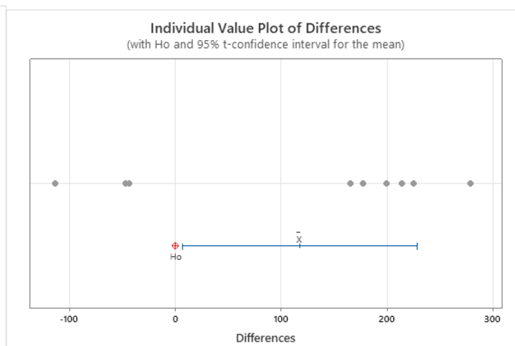
μ_difference: population mean of (Chewiness-DBP - Chewiness-DAP)

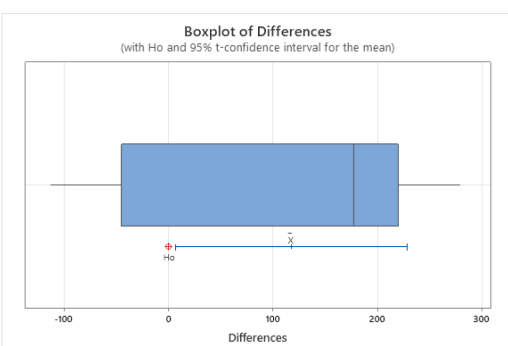
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
2.45	0.040







Paired T-Test and CI: Resilience-DBP, Resilience-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Resilience-DBP	9	0.1460	0.0321	0.0107
Resilience-DAP	9	0.1281	0.0054	0.0018

Estimation for Paired Difference

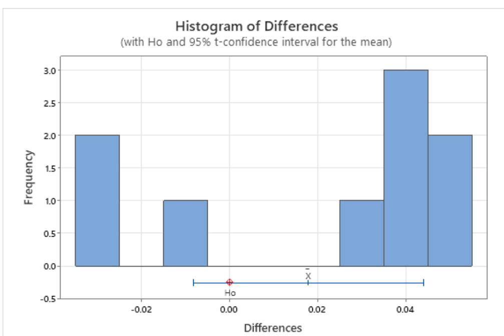
Mean	StDev	SE Mean	95% CI for μ_difference
0.0179	0.0340	0.0113	(-0.0083, 0.0441)

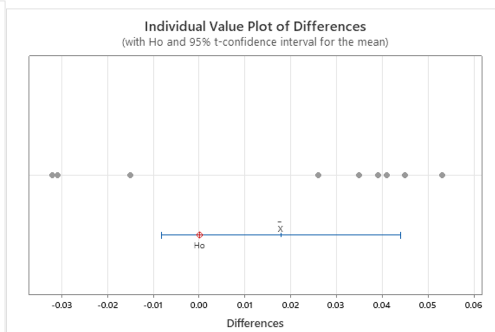
μ_difference: population mean of (Resilience-DBP - Resilience-DAP)

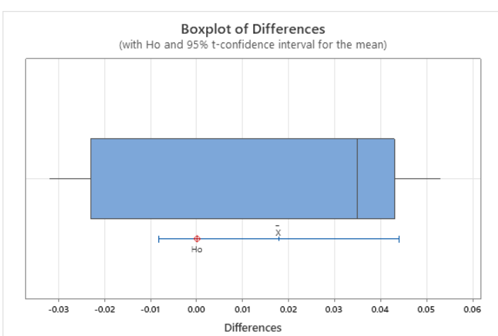
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
1.58	0.153







1.1. DAP vs SDB

Paired T-Test and CI: Hardness-DAP, Hardness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Hardness-DAP	9	920.9	62.3	20.8
Hardness-SDB	9	1198.6	168.7	56.2

Estimation for Paired Difference

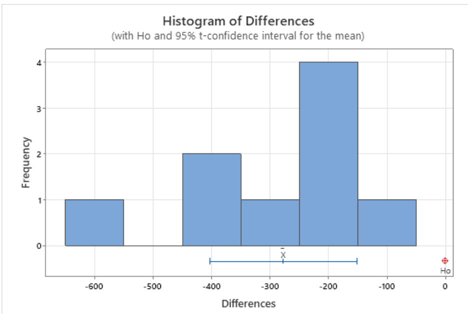
Mean	StDev	SE Mean	95% CI for μ_difference
-277.6	163.9	54.6	(-403.6, -151.7)

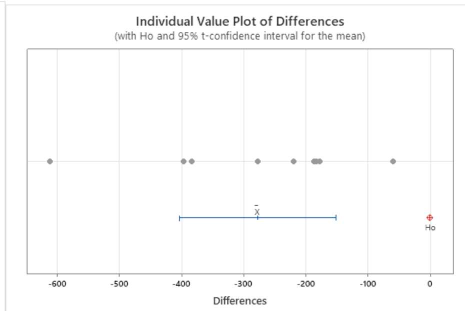
μ_difference: population mean of (Hardness-DAP - Hardness-SDB)

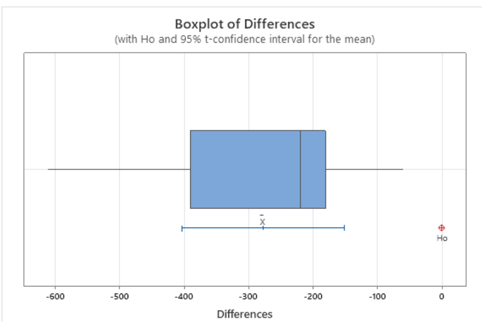
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-5.08	0.001







Paired T-Test and CI: Adhesiveness-DAP, Adhesiveness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Adhesiveness-DAP	9	-468.3	42.8	14.3
Adhesiveness-SDB	9	-0.9	0.8	0.3

Estimation for Paired Difference

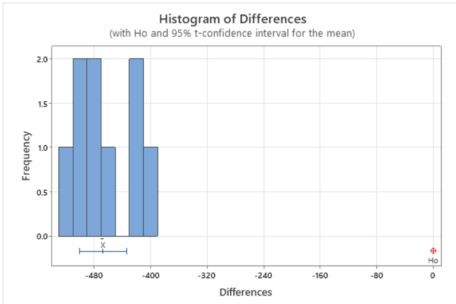
Mean	StDev	SE Mean	95% CI for μ_difference
-467.4	42.8	14.3	(-500.3, -434.5)

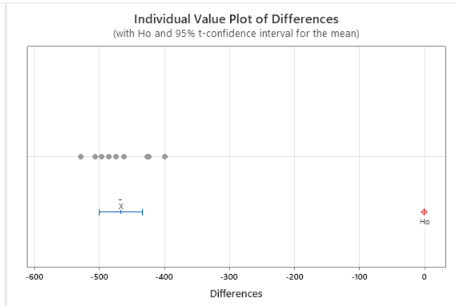
μ_difference: population mean of (Adhesiveness-DAP - Adhesiveness-SDB)

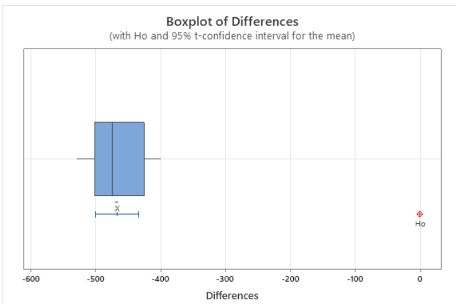
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-32.76	0.000







Paired T-Test and CI: Springiness-DAP, Springiness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Springiness-DAP	9	0.978	0.003	0.001
Springiness-SDB	9	1.484	0.768	0.256

Estimation for Paired Difference

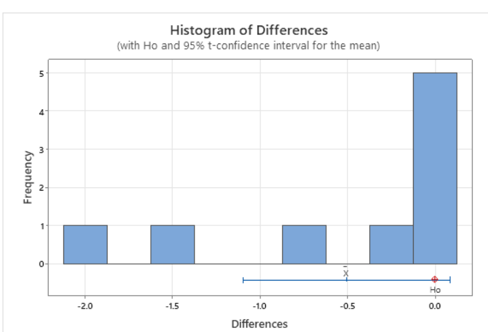
Mean	StDev	SE Mean	95% CI for μ_difference
-0.506	0.768	0.256	(-1.097, 0.084)

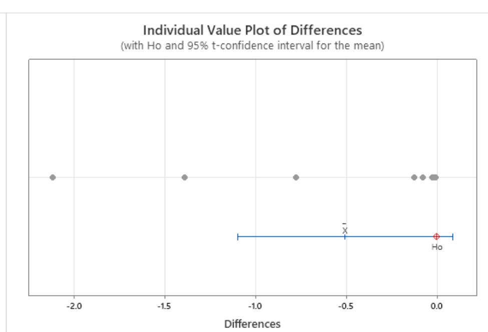
μ_difference: population mean of (cohesive-DAP - Springiness-SDB)

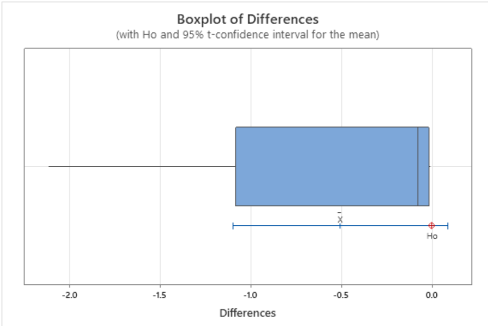
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-1.98	0.083







Paired T-Test and CI: Cohesiveness-DAP, Cohesiveness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Cohesiveness-DAP	9	0.71000	0.02259	0.00753
Cohesiveness-SDB	9	0.75556	0.02651	0.00884

Estimation for Paired Difference

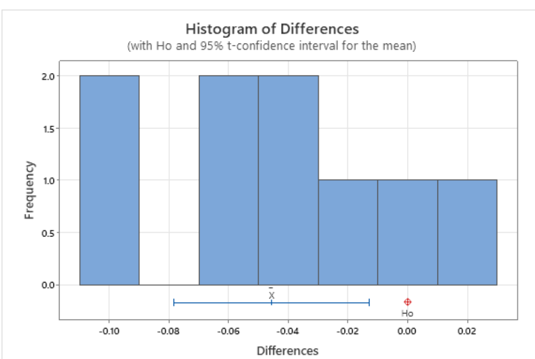
Mean	StDev	SE Mean	95% CI for μ_difference
-0.0456	0.0425	0.0142	(-0.0783, -0.0129)

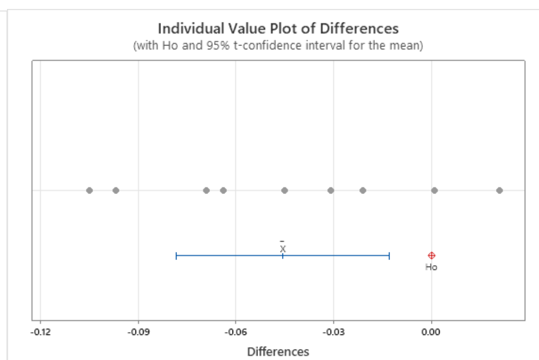
μ_difference: population mean of (Cohesiveness-DAP - Cohesiveness-SDB)

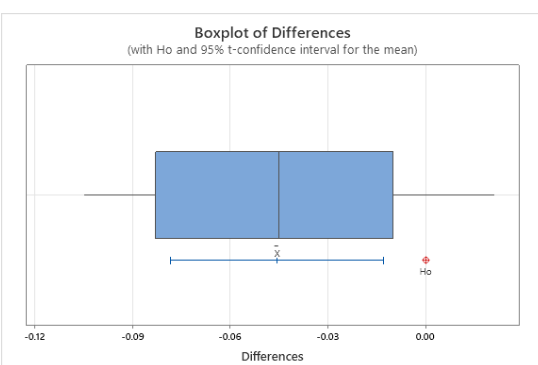
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-3.21	0.012







Paired T-Test and CI: Gumminess-DAP, Gumminess-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Gumminess-DAP	9	653.7	47.6	15.9
Gumminess-SDB	9	903.4	110.1	36.7

Estimation for Paired Difference

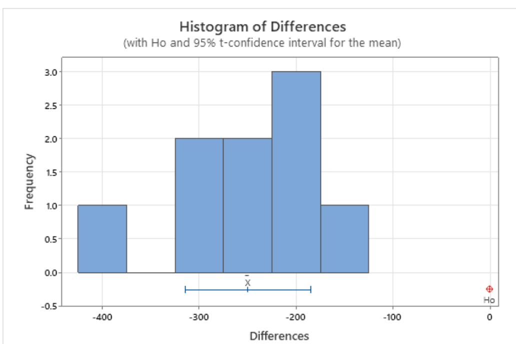
Mean	StDev	SE Mean	95% CI for μ_difference
-249.7	84.7	28.2	(-314.8, -184.7)

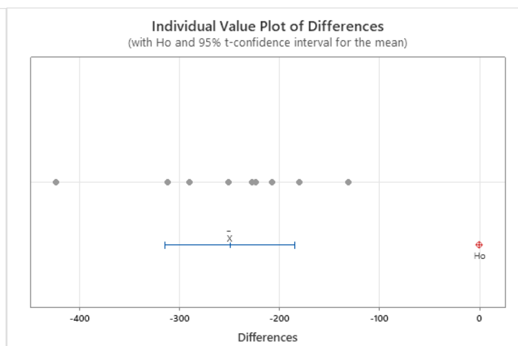
μ_difference: population mean of (Gumminess-DAP - Gumminess-SDB)

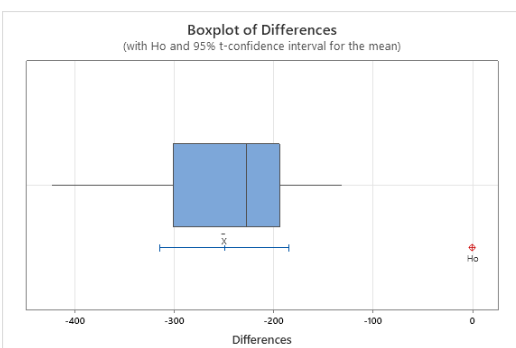
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-8.85	0.000







Paired T-Test and CI: Chewiness-DAP, Chewiness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Chewiness-DAP	9	639	46	15
Chewiness-SDB	9	1306	581	194

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference

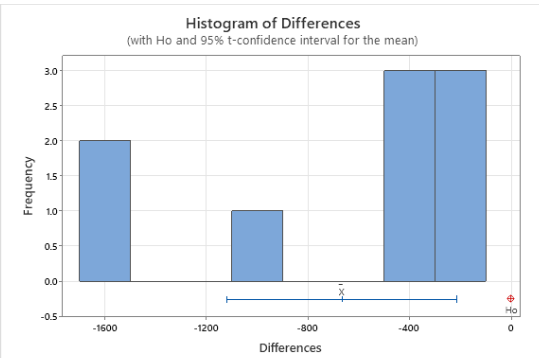
-667	590	197	(-1120, -213)

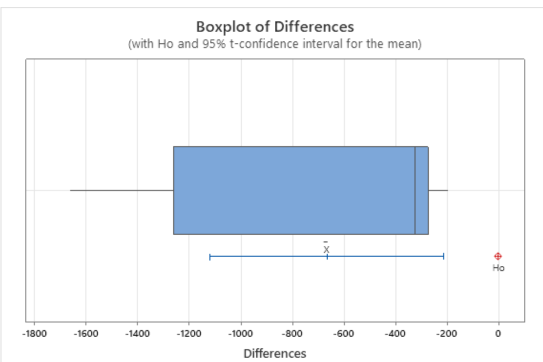
μ_difference: population mean of (Chewiness-DAP - Chewiness-SDB)

Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-3.39	0.010





Paired T-Test and CI: Resilience-DAP, Resilience-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Resilience-DAP	9	0.12811	0.00542	0.00181
Resilience-SDB	9	0.43233	0.02011	0.00670

Estimation for Paired Difference

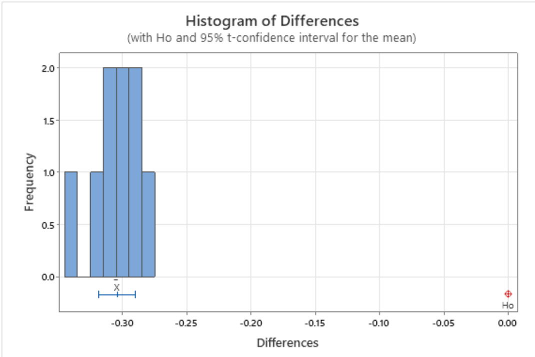
Mean	StDev	SE Mean	95% CI for μ_difference
-0.30422	0.01881	0.00627	(-0.31868, -0.28977)

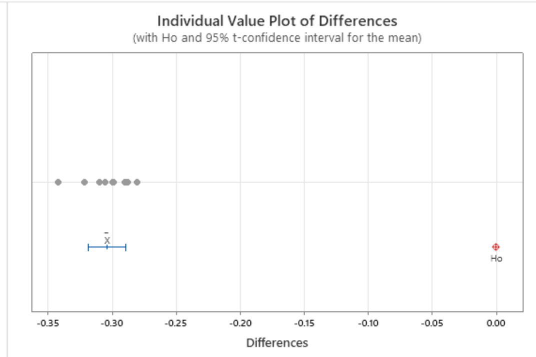
μ_difference: population mean of (Resilience-DAP - Resilience-SDB)

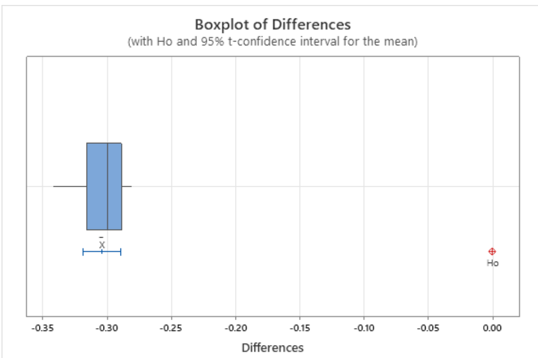
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-48.53	0.000







2. Microbiology
2.1. LAB (Log CFU g^{-1}): WSS vs RSS

Two-Sample T-Test and CI: LAB-WSS, LAB-RSS

Method

μ_1: population mean of LAB-WSS
μ_2: population mean of LAB-RSS
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-WSS	6	8.7533	0.0981	0.040
LAB-RSS	6	5.732	0.279	0.11

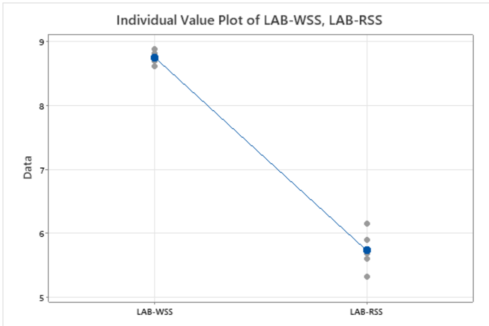
Estimation for Difference

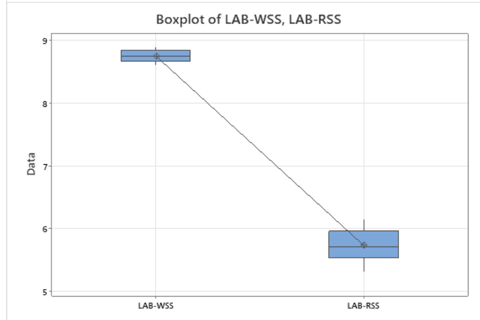
Difference	95% CI for Difference
3.022	(2.726, 3.317)

Test

Null hypothesis	H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis	H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
25.02	6	0.000





2.2. LAB (Log CFU g^{-1}): DBP vs DAP

Paired T-Test and CI: LAB-DBP, LAB-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-DBP	6	8.3183	0.0319	0.0130
LAB-DAP	6	8.5450	0.0792	0.0323

Estimation for Paired Difference

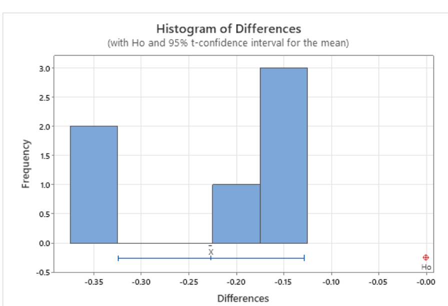
Mean	StDev	SE Mean	95% CI for μ_difference
-0.2267	0.0931	0.0380	(-0.3244, -0.1290)

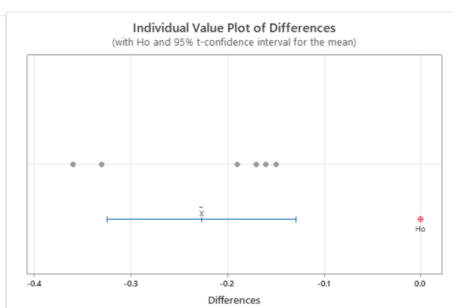
μ_difference: population mean of (LAB-DBP - LAB-DAP)

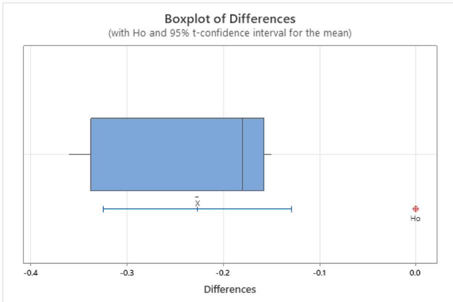
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-5.96	0.002







2.3. Yeast (Log CFU g^{-1}): WSS vs RSS

Two-Sample T-Test and CI: Yeast-WSS, Yeast-RSS

Method

μ_1: population mean of Yeast-WSS
μ_2: population mean of Yeast-RSS
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-WSS	6	2.378	0.692	0.28
Yeast-RSS	6	2.77	1.02	0.42

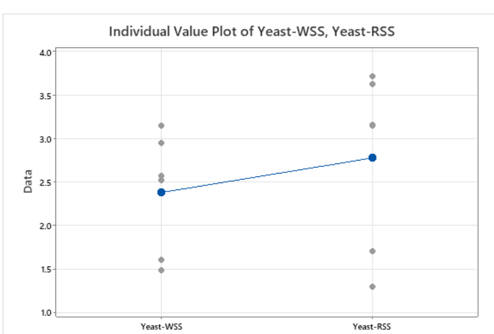
Estimation for Difference

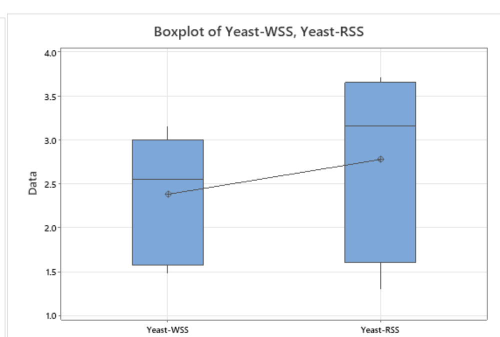
Difference	95% CI for Difference
-0.397	(-1.559, 0.765)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-0.79	8	0.454





2.4. Yeast (Log CFU g^{-1}): DBP vs DAP

Paired T-Test and CI: Yeast-DBP, Yeast-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-DBP	6	3.877	0.328	0.134
Yeast-DAP	6	3.742	0.635	0.259

Estimation for Paired Difference

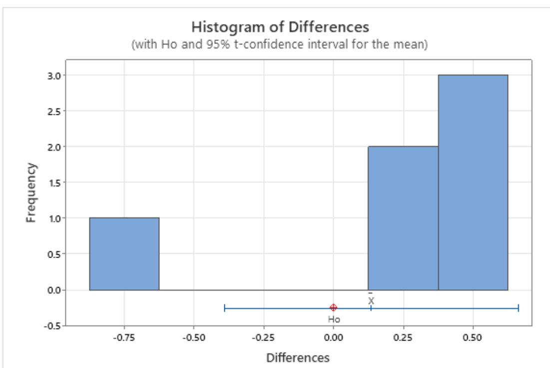
Mean	StDev	SE Mean	95% CI for μ_difference
0.135	0.502	0.205	(-0.392, 0.662)

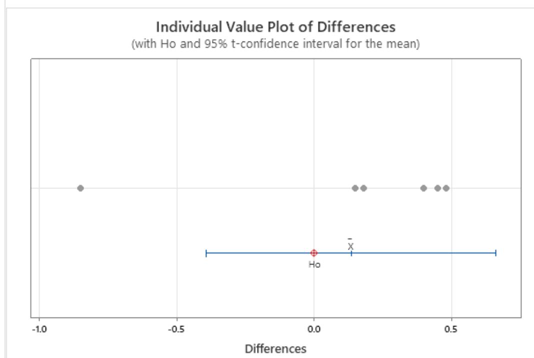
μ_difference: population mean of (Yeast-DBP - Yeast-DAP)

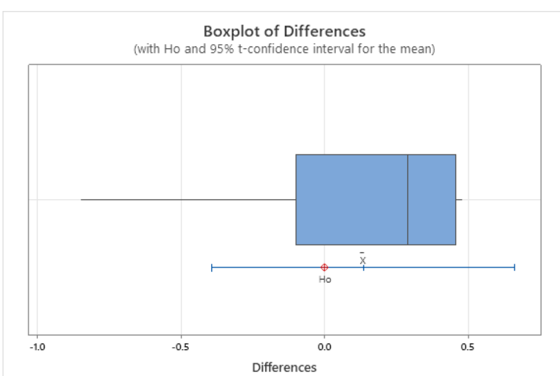
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
0.66	0.539







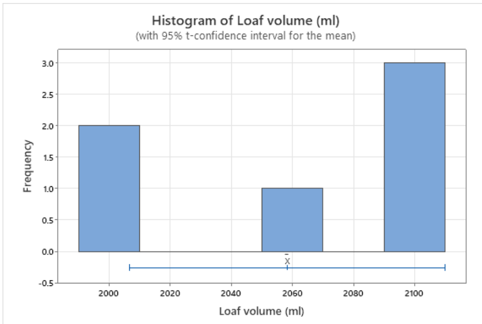
3. **SDB: Loaf volume, loaf weight and specific volume**

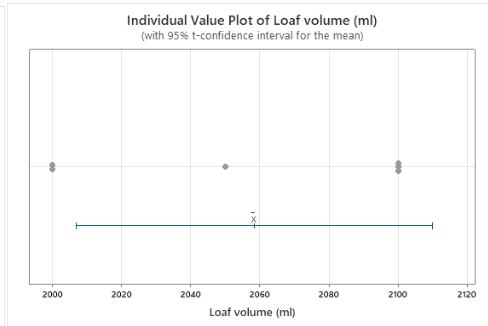
One-Sample T: Loaf volume (ml)

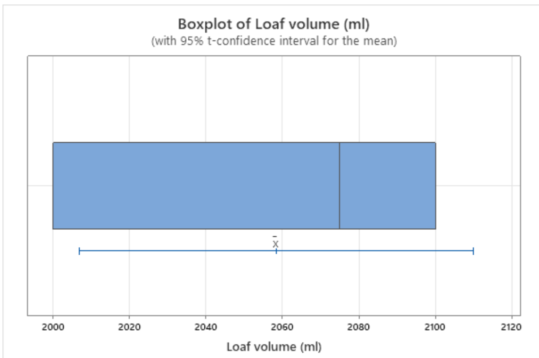
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	2058.3	49.2	20.1	(2006.7, 2109.9)

μ: population mean of Loaf volume (ml)





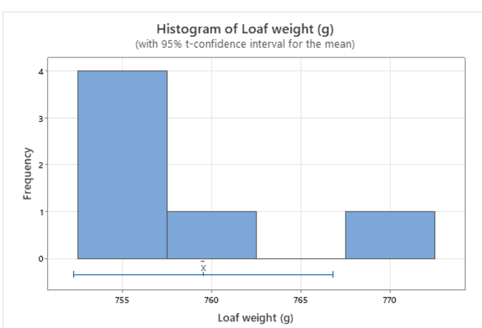


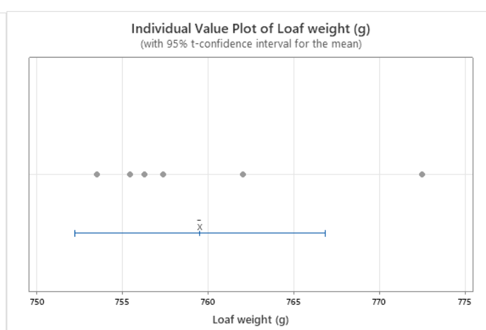
One-Sample T: Loaf weight (g)

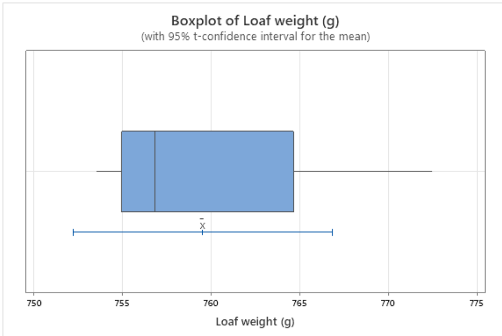
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	759.52	6.94	2.83	(752.25, 766.80)

μ: population mean of Loaf weight (g)





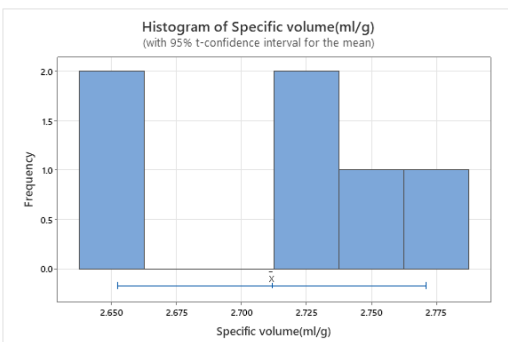


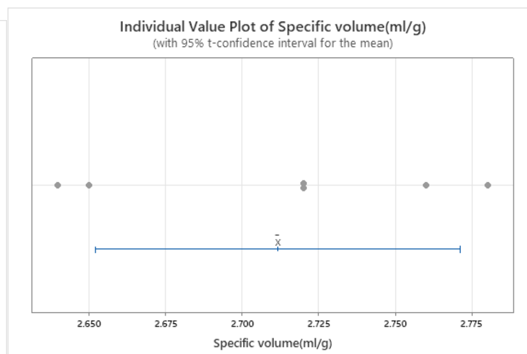
One-Sample T: Specific volume(ml/g)

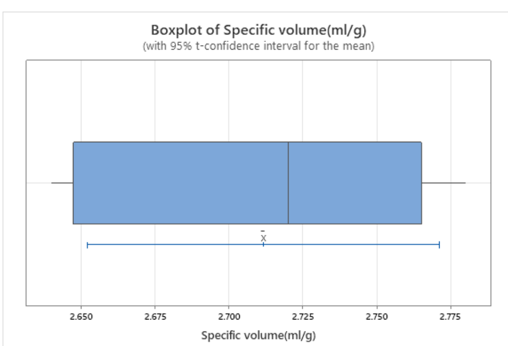
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	2.7117	0.0567	0.0232	(2.6521, 2.7712)

μ: population mean of Specific volume(ml/g)







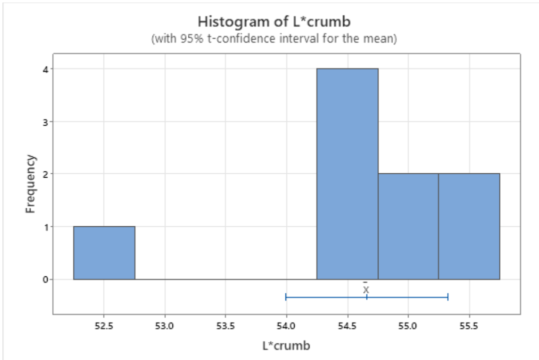
4. **Colour of sourdough bread**

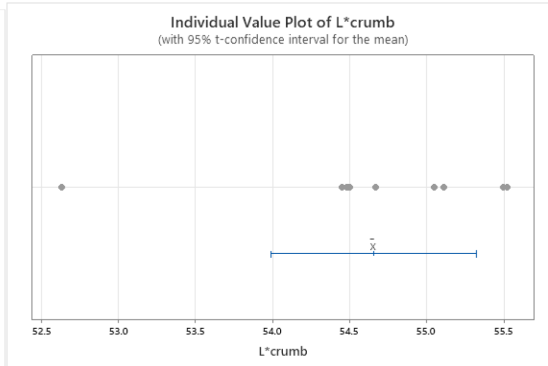
One-Sample T: L*crumb

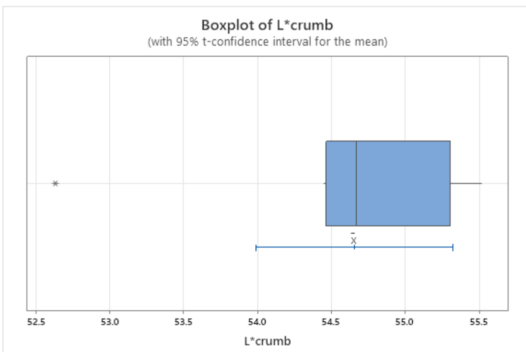
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	54.657	0.868	0.289	(53.990, 55.324)

*μ: population mean of L*crumb*





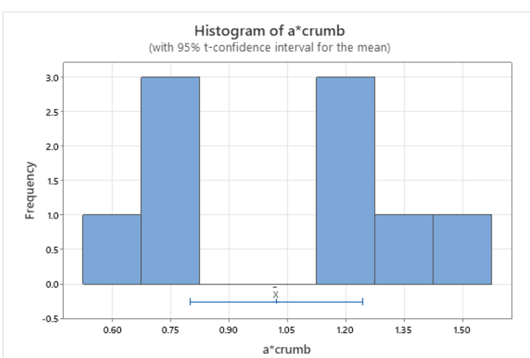


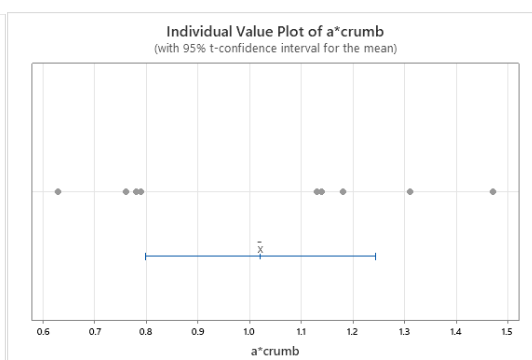
One-Sample T: a*crumb

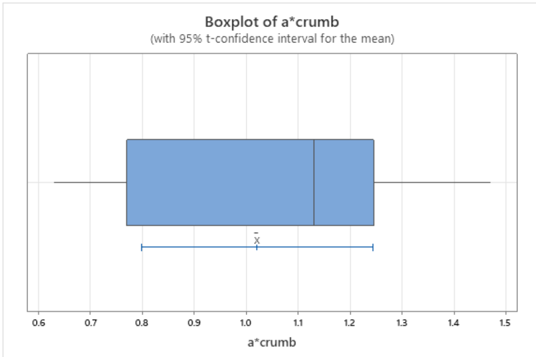
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	1.0211	0.2892	0.0964	(0.7988, 1.2434)

*μ: population mean of a*crumb*





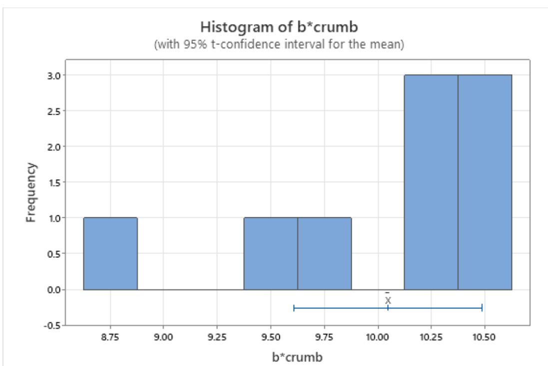


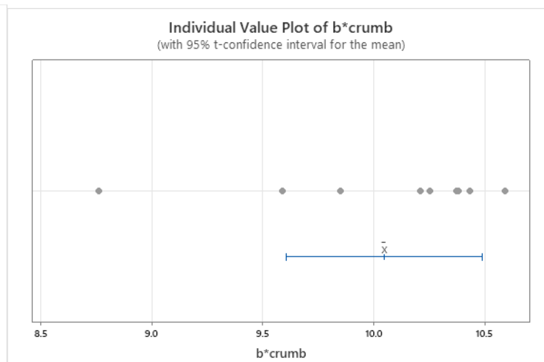
One-Sample T: b*crumb

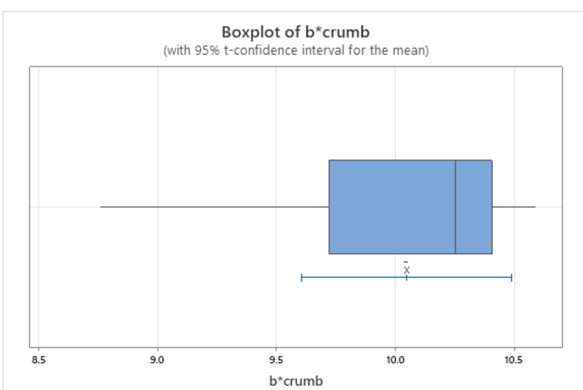
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	10.048	0.573	0.191	(9.607, 10.488)

*μ: population mean of b*crumb*





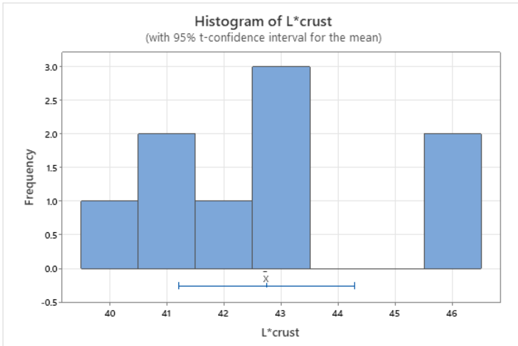


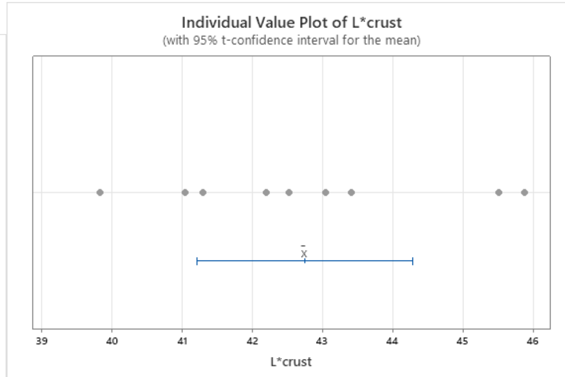
One-Sample T: L*crust

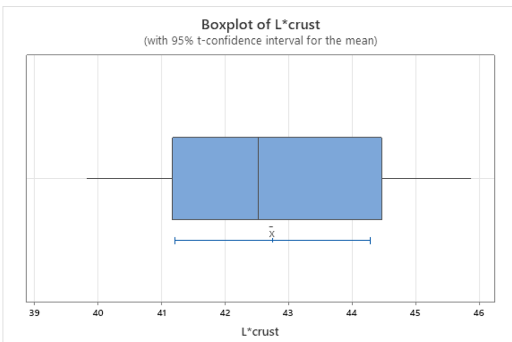
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	42.746	1.993	0.664	(41.213, 44.278)

*μ: population mean of L*crust*





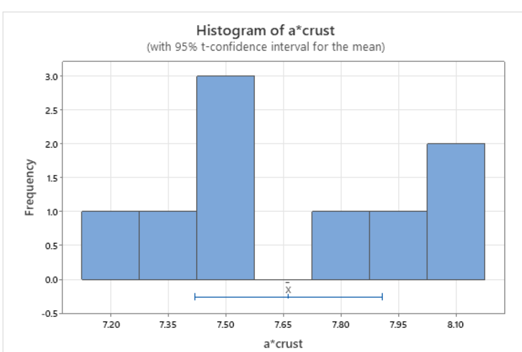


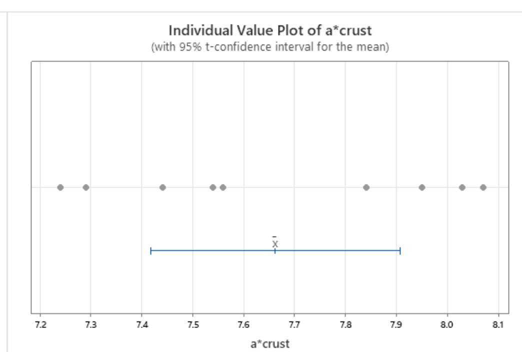
One-Sample T: a*crust

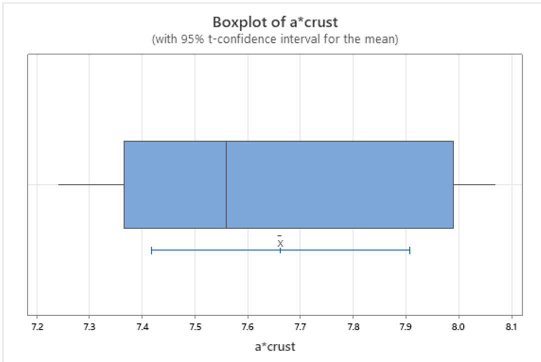
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	7.662	0.318	0.106	(7.418, 7.906)

*μ: population mean of a*crust*





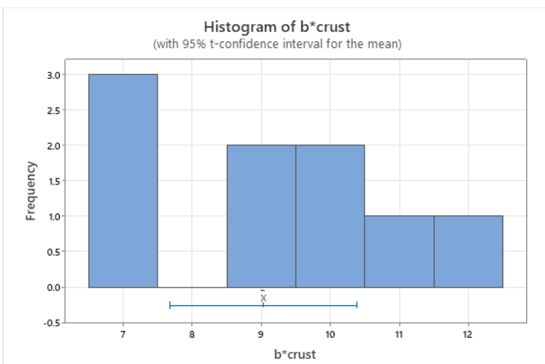


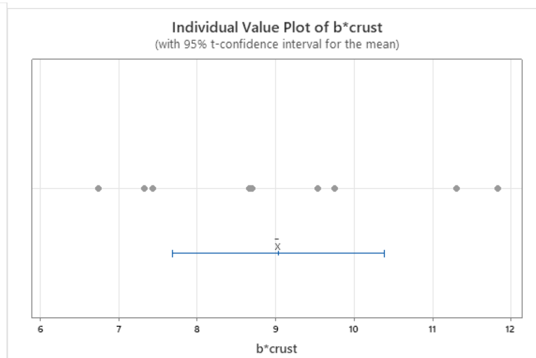
One-Sample T: b*crust

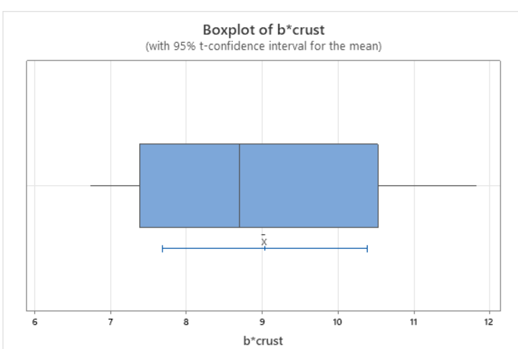
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
9	9.032	1.757	0.586	(7.681, 10.383)

*μ: population mean of b*crust*







Phase II

1. **Acidity**

1.1. **DBP vs DAP**

Paired T-Test and CI: 27DBP-%TTA, 27DAP-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-%TTA	6	0.5629	0.0527	0.0215
27DAP-%TTA	6	1.1024	0.0366	0.0149

Estimation for Paired Difference

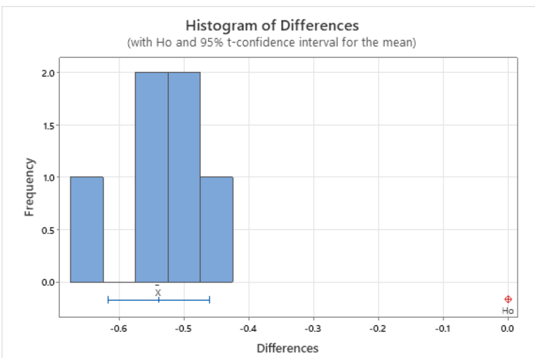
Mean	StDev	SE Mean	95% CI for μ_difference
-0.5394	0.0743	0.0303	(-0.6174, -0.4615)

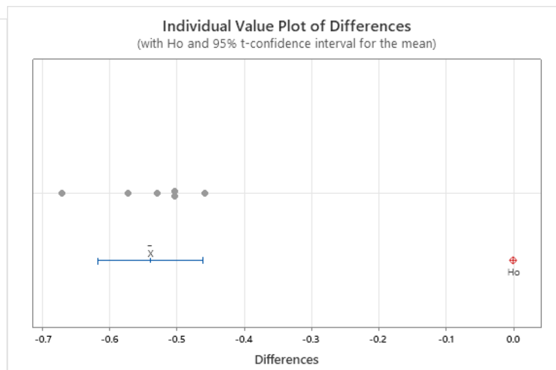
μ_difference: population mean of (27DBP-%TTA - 27DAP-%TTA)

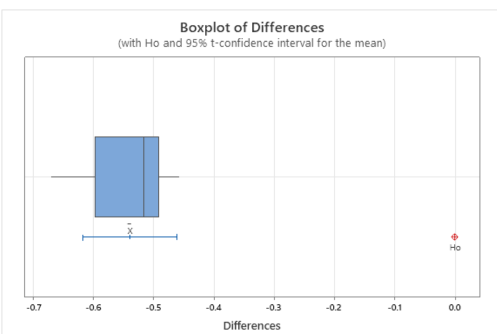
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-17.79	0.000







Paired T-Test and CI: 29DBP-%TTA, 29DAP-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-%TTA	6	0.44094	0.00558	0.00228
29DAP-%TTA	6	0.99212	0.02415	0.00986

Estimation for Paired Difference

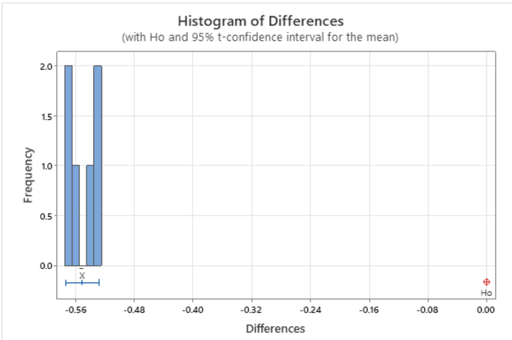
Mean	StDev	SE Mean	95% CI for μ_difference
-0.55118	0.02142	0.00875	(-0.57366, -0.52870)

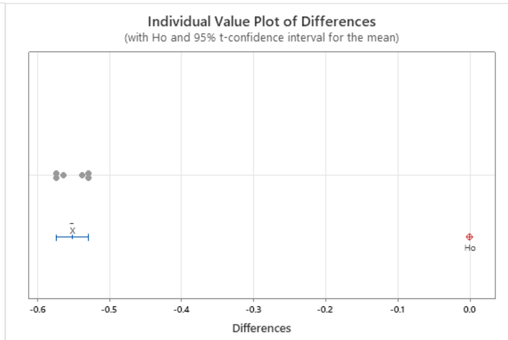
μ_difference: population mean of (29DBP-%TTA - 29DAP-%TTA)

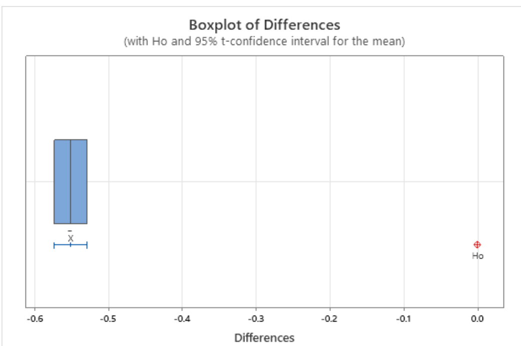
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-63.03	0.000







Paired T-Test and CI: 31DBP-%TTA, 31DAP-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-%TTA	6	0.7569	0.0434	0.0177
31DAP-%TTA	6	1.0803	0.0540	0.0220

Estimation for Paired Difference

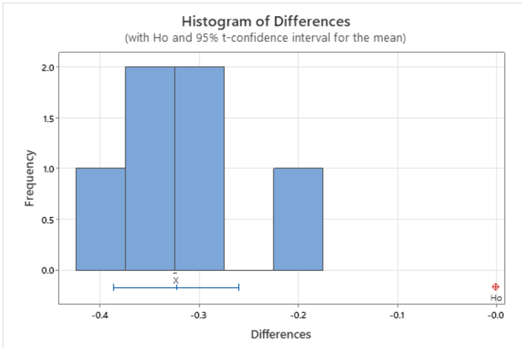
Mean	StDev	SE Mean	95% CI for μ_difference
-0.3234	0.0602	0.0246	(-0.3866, -0.2601)

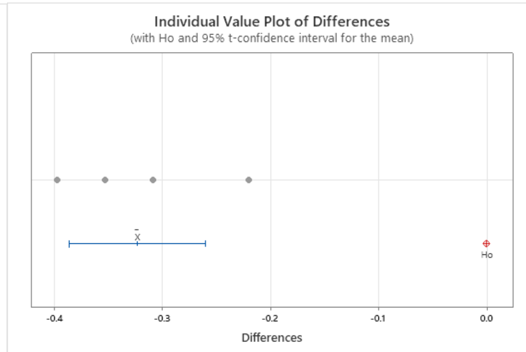
μ_difference: population mean of (31DBP-%TTA - 31DAP-%TTA)

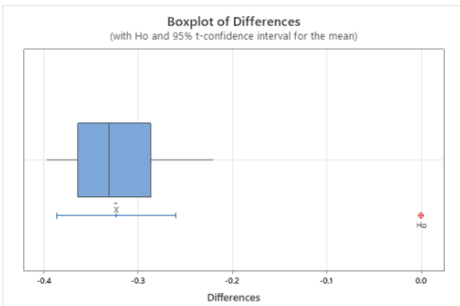
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-13.15	0.000







Paired T-Test and CI: 27DBP-pH, 27DAP-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-pH	6	4.55167	0.00753	0.00307
27DAP-pH	6	3.64333	0.00816	0.00333

Estimation for Paired Difference

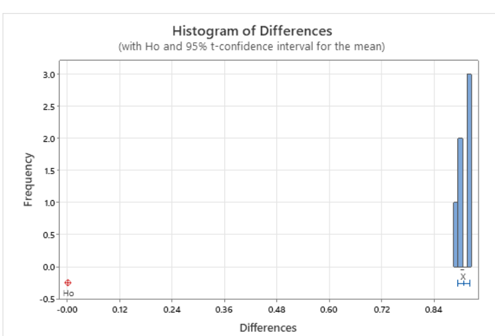
Mean	StDev	SE Mean	95% CI for μ_difference
0.90833	0.01329	0.00543	(0.89438, 0.92228)

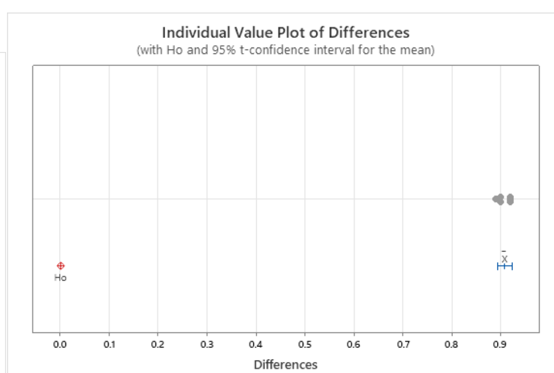
μ_difference: population mean of (27DBP-pH - 27DAP-pH)

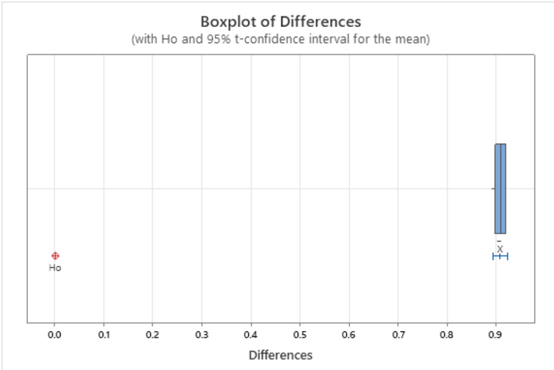
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
167.40	0.000







Paired T-Test and CI: 29DBP-pH, 29DAP-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-pH	6	4.64500	0.01517	0.00619
29DAP-pH	6	3.73000	0.00632	0.00258

Estimation for Paired Difference

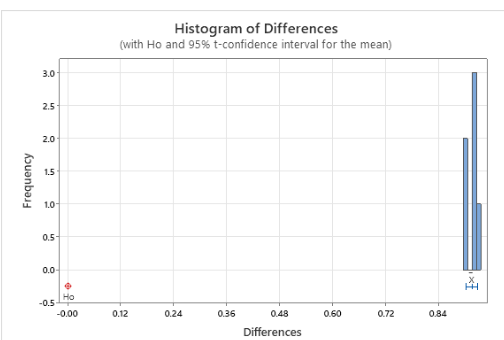
Mean	StDev	SE Mean	95% CI for μ_difference
0.91500	0.01225	0.00500	(0.90215, 0.92785)

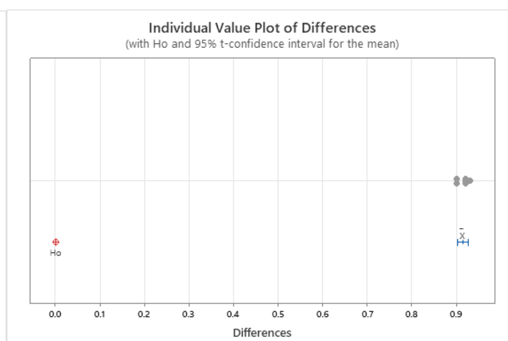
μ_difference: population mean of (29DBP-pH - 29DAP-pH)

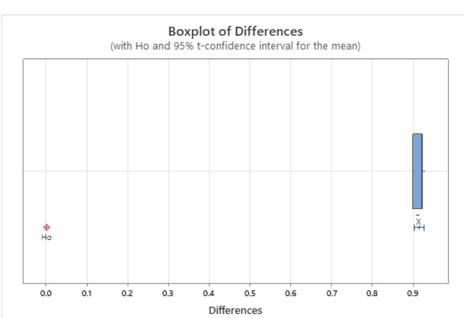
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
183.00	0.000







Paired T-Test and CI: 31DBP-pH, 31DAP-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-pH	6	4.16333	0.01862	0.00760
31DAP-pH	6	3.80667	0.01211	0.00494

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.3567	0.0294	0.0120	(0.3258, 0.3876)

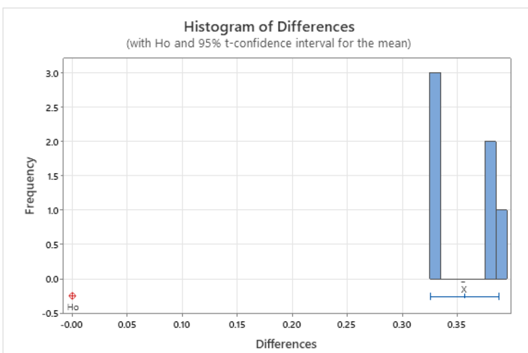
μ_difference: population mean of (31DBP-pH - 31DAP-pH)

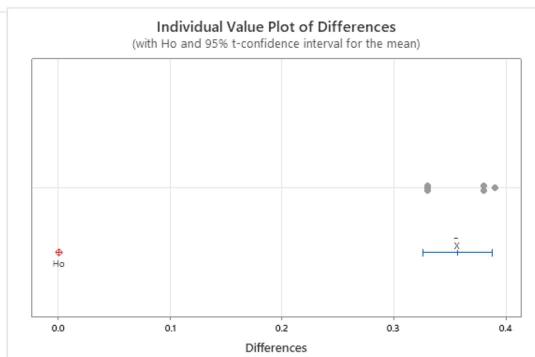
Test

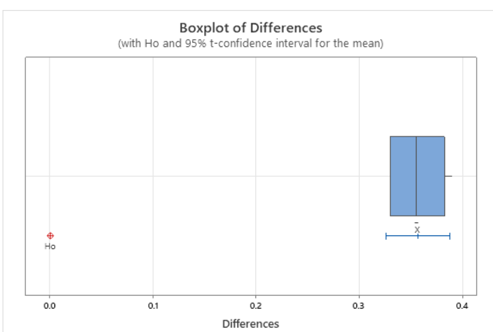
Null hypothesis H₀: μ_difference = 0

Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
29.68	0.000







1.1. DAP vs SDB

Paired T-Test and CI: 27DAP-%TTA, 27SDB-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-%TTA	6	1.1024	0.0366	0.0149
27SDB-%TTA	6	0.8157	0.0214	0.0087

Estimation for Paired Difference

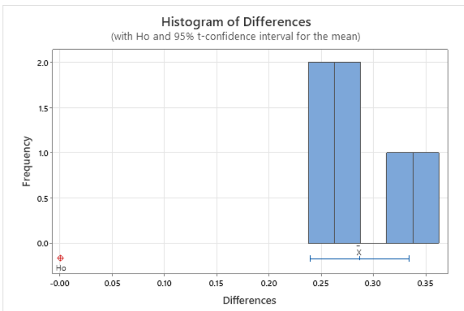
Mean	StDev	SE Mean	95% CI for μ_difference
0.2866	0.0449	0.0183	(0.2395, 0.3337)

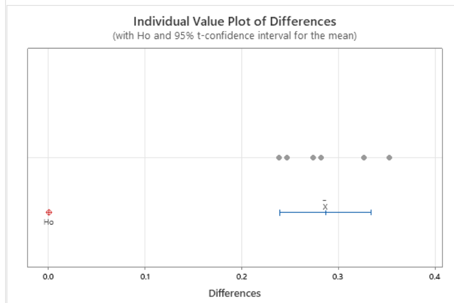
μ_difference: population mean of (27DAP-%TTA - 27SDB-%TTA)

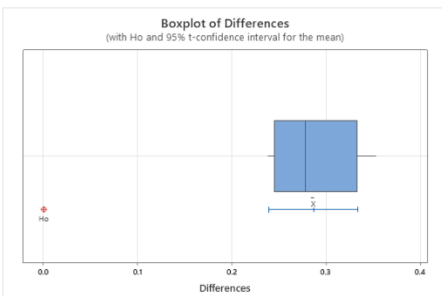
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
15.64	0.000







Paired T-Test and CI: 29DAP-%TTA, 29SDB-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-%TTA	6	0.9921	0.0242	0.0099
29SDB-%TTA	6	0.7643	0.0360	0.0147

Estimation for Paired Difference

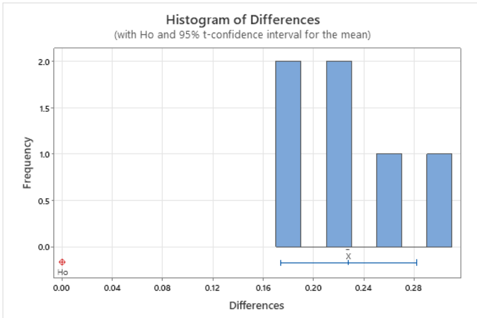
Mean	StDev	SE Mean	95% CI for μ_difference
0.2278	0.0515	0.0210	(0.1737, 0.2819)

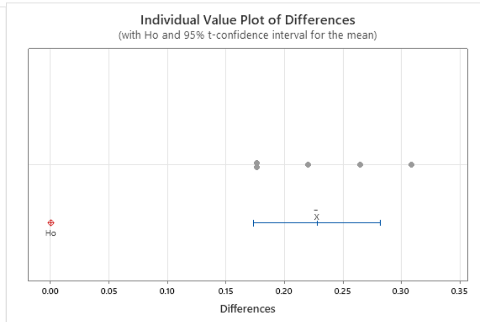
μ_difference: population mean of (29DAP-%TTA - 29SDB-%TTA)

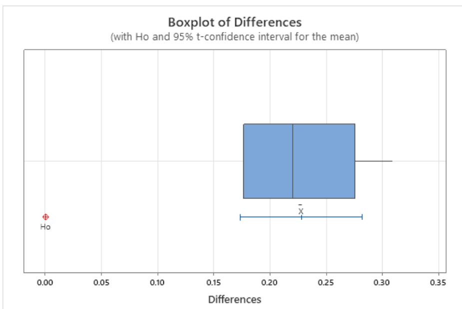
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
10.83	0.000







Paired T-Test and CI: 31DAP-%TTA, 31SDB-%TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-%TTA	6	1.0803	0.0540	0.0220
31SDB-%TTA	6	0.9260	0.0279	0.0114

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.1543	0.0540	0.0220	(0.0977, 0.2110)

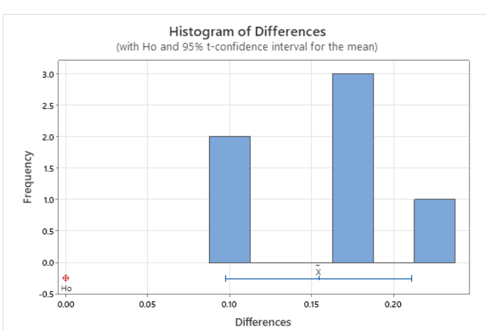
μ_difference: population mean of (31DAP-%TTA - 31SDB-%TTA)

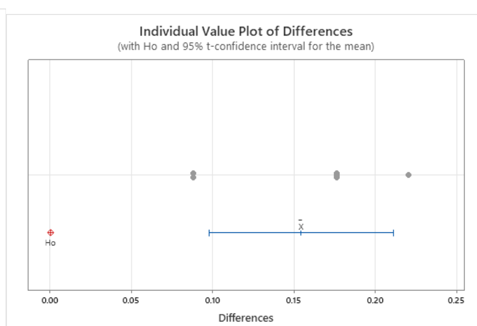
Test

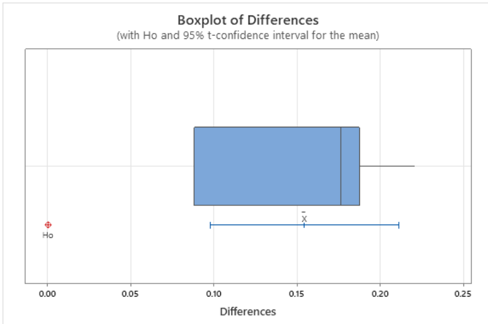
Null hypothesis H₀: μ_difference = 0

Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
7.00	0.001







Paired T-Test and CI: 27DAP-pH, 27SDB-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-pH	6	3.64333	0.00816	0.00333
27SDB-pH	6	3.75333	0.01211	0.00494

Estimation for Paired Difference

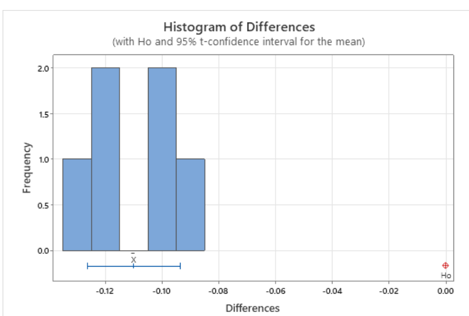
Mean	StDev	SE Mean	95% CI for μ_difference
-0.11000	0.01549	0.00632	(-0.12626, -0.09374)

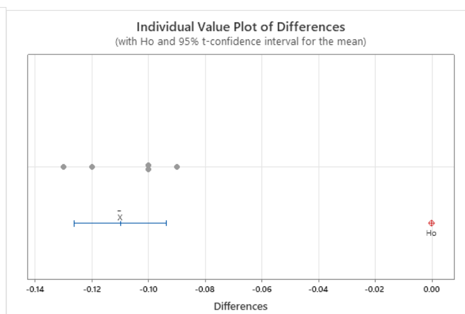
μ_difference: population mean of (27DAP-pH - 27SDB-pH)

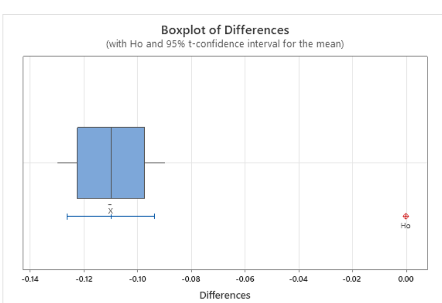
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-17.39	0.000







Paired T-Test and CI: 29DAP-pH, 29SDB-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-pH	6	3.73000	0.00632	0.00258
29SDB-pH	6	3.85500	0.00548	0.00224

Estimation for Paired Difference

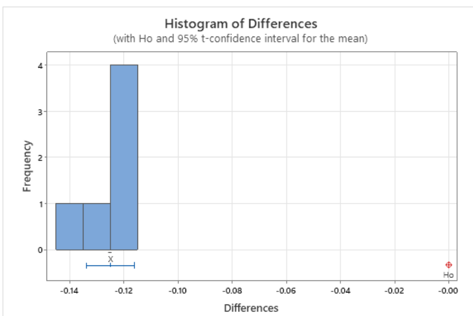
Mean	StDev	SE Mean	95% CI for μ_difference
-0.12500	0.00837	0.00342	(-0.13378, -0.11622)

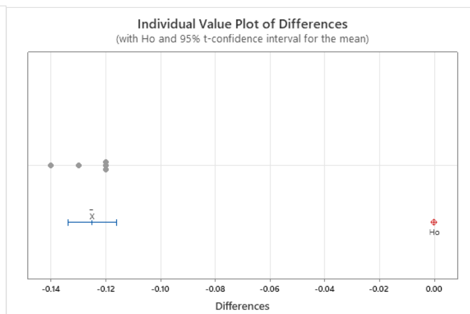
μ_difference: population mean of (29DAP-pH - 29SDB-pH)

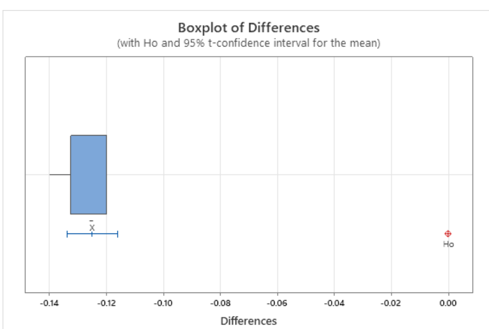
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-36.60	0.000







Paired T-Test and CI: 31DAP-pH, 31SDB-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-pH	6	3.80667	0.01211	0.00494
31SDB-pH	6	3.90500	0.01378	0.00563

Estimation for Paired Difference

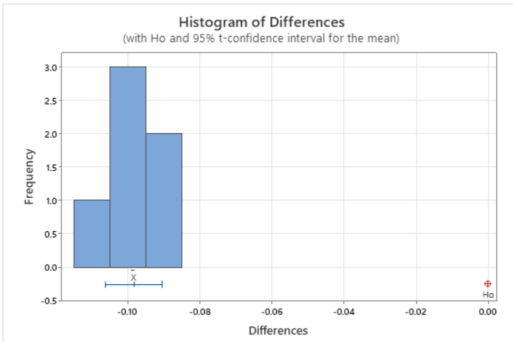
Mean	StDev	SE Mean	95% CI for μ_difference
-0.09833	0.00753	0.00307	(-0.10623, -0.09043)

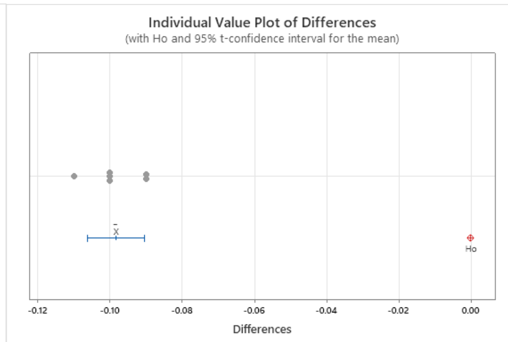
μ_difference: population mean of (31DAP-pH - 31SDB-pH)

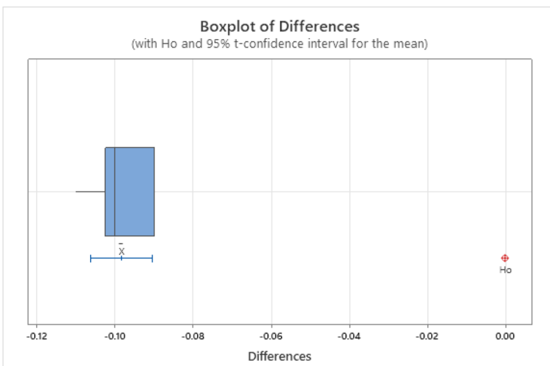
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-32.00	0.000







Acidity: 27DAP-DBP, 29DAP-DBP, 31DAP-DBP

One-way ANOVA: 27DAP-DBP-pH, 29DAP-DBP-pH, 31DAP-DBP-pH

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-pH, 29DAP-DBP-pH, 31DAP-DBP-pH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1.23223	0.616117	1548.90	0.000
Error	15	0.00597	0.000398		
Total	17	1.23820			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)

0.0199444	99.52%	99.45%	99.31%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-pH	6	0.90833	0.01329	(0.89098, 0.92569)
29DAP-DBP-pH	6	0.91500	0.01225	(0.89765, 0.93235)
31DAP-DBP-pH	6	0.3567	0.0294	(0.3393, 0.3740)

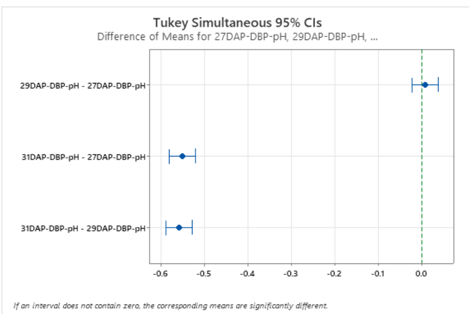
Pooled StDev = 0.0199444

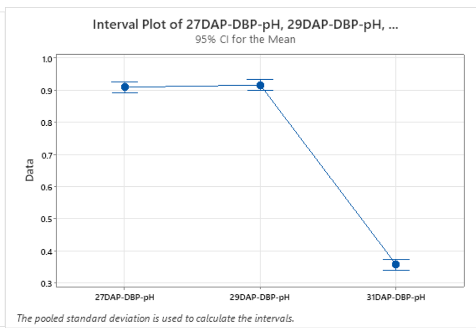
Tukey Pairwise Comparisons

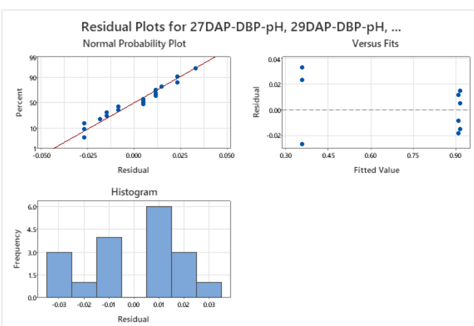
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
29DAP-DBP-pH	6	0.91500	A	
27DAP-DBP-pH	6	0.90833	A	
31DAP-DBP-pH	6	0.3567		B

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-%TTA, 29DAP-DBP-%TTA, 31DAP-DBP-%TTA

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-%TTA, 29DAP-DBP-%TTA, 31DAP-DBP-%TTA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.19745	0.098723	30.83	0.000
Error	15	0.04804	0.003202		
Total	17	0.24548			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0565904	80.43%	77.82%	71.82%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-%TTA	6	0.5394	0.0743	(0.4902, 0.5887)
29DAP-DBP-%TTA	6	0.55118	0.02142	(0.50193, 0.60042)
31DAP-DBP-%TTA	6	0.3234	0.0602	(0.2741, 0.3726)

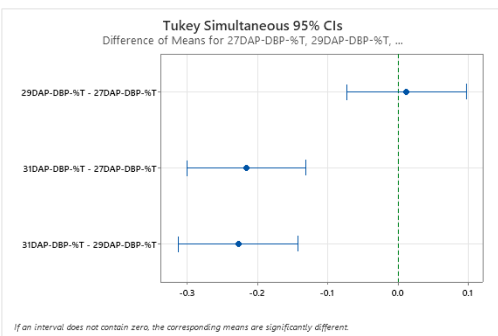
Pooled StDev = 0.0565904

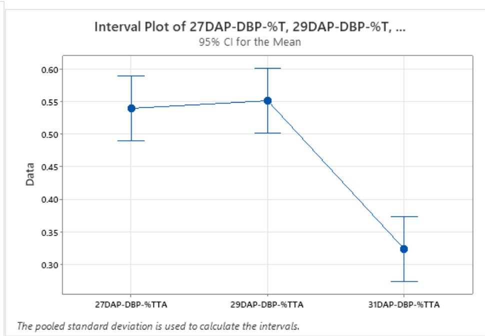
Tukey Pairwise Comparisons

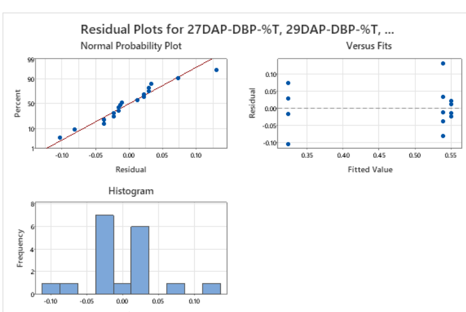
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
29DAP-DBP-%TTA	6	0.55118	A	
27DAP-DBP-%TTA	6	0.5394	A	
31DAP-DBP-%TTA	6	0.3234		B

Means that do not share a letter are significantly different.







Acidity: 27SDB-DAP, 29 SDB-DAP, 31 SDB-DAP

One-way ANOVA: 27SDB-DAP-pH, 29SDB-DAP-pH, 31SDB-DAP-pH

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-pH, 29SDB-DAP-pH, 31SDB-DAP-pH

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.002144	0.001072	8.77	0.003
Error	15	0.001833	0.000122		
Total	17	0.003978			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0110554	53.91%	47.77%	33.63%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-pH	6	0.11000	0.01549	(0.10038, 0.11962)
29SDB-DAP-pH	6	0.12500	0.00837	(0.11538, 0.13462)
31SDB-DAP-pH	6	0.09833	0.00753	(0.08871, 0.10795)

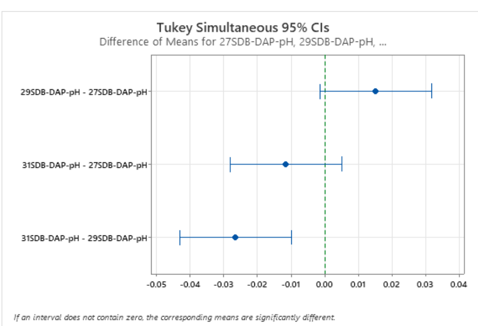
Pooled StDev = 0.0110554

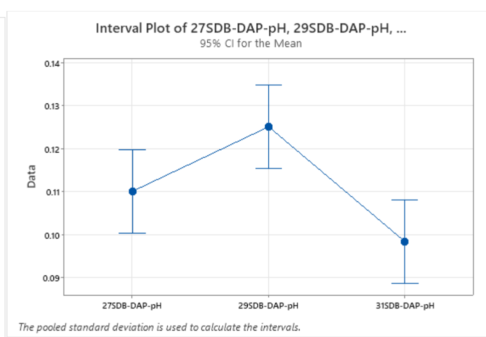
Tukey Pairwise Comparisons

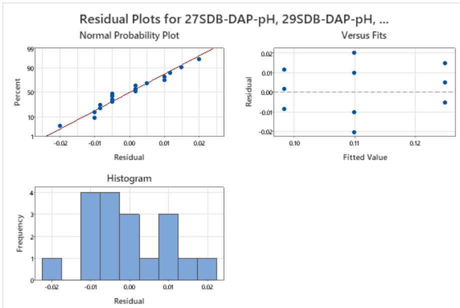
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
29SDB-DAP-pH	6	0.12500	A	
27SDB-DAP-pH	6	0.11000	A	B
31SDB-DAP-pH	6	0.09833		B

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-%TTA, 29SDB-DAP-%TTA, 31SDB-DAP-%TTA

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-%TTA, 29SDB-DAP-%TTA, 31SDB-DAP-%TTA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.05271	0.026356	10.42	0.001
Error	15	0.03794	0.002529		
Total	17	0.09065			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0502923	58.15%	52.57%	39.73%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-%TTA	6	-0.2866	0.0449	(-0.3304, -0.2428)
29SDB-DAP-%TTA	6	-0.2278	0.0515	(-0.2716, -0.1841)
31SDB-DAP-%TTA	6	-0.1543	0.0540	(-0.1981, -0.1106)

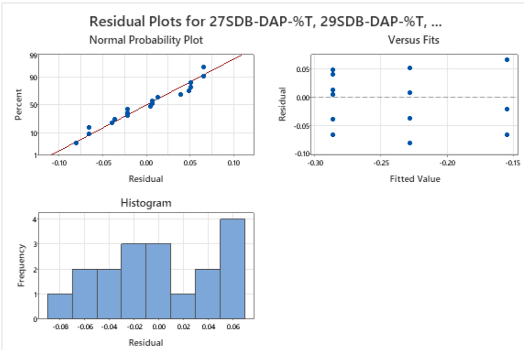
Pooled StDev = 0.0502923

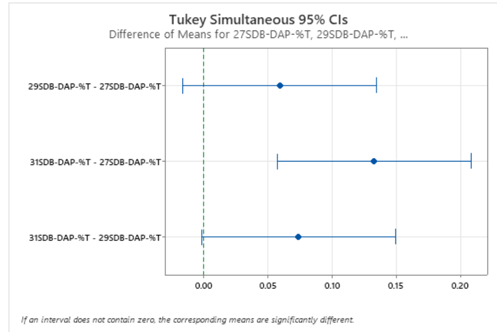
Tukey Pairwise Comparisons

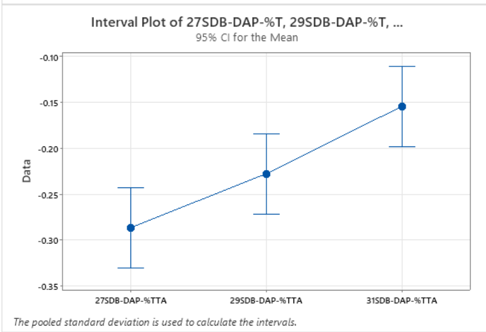
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
31SDB-DAP-%TTA	6	-0.1543	A	
29SDB-DAP-%TTA	6	-0.2278	A	B
27SDB-DAP-%TTA	6	-0.2866		B

Means that do not share a letter are significantly different.







1. Texture

1.1. 27DBP vs 27DAP

Paired T-Test and CI: 27DBP-Hardness, 27DAP-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Hardness	6	452.05	15.87	6.48
27DAP-Hardness	6	364.53	18.68	7.63

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
87.52	23.64	9.65	(62.71, 112.33)

μ_difference: population mean of (27DBP-Hardness - 27DAP-Hardness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
9.07	0.000

Paired T-Test and CI: 27DBP-Adhesiveness, 27DAP-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Adhesiveness	6	-575.0	115.4	47.1
27DAP-Adhesiveness	6	-426.2	37.3	15.2

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-148.8	125.4	51.2	(-280.4, -17.2)

μ_difference: population mean of (27DBP-Adhesiveness - 27DAP-Adhesiveness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-2.91	0.034

Paired T-Test and CI: 27DBP-Springiness, 27DAP-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Springiness	6	0.98117	0.00598	0.00244
27DAP-Springiness	6	0.98367	0.00446	0.00182

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.00250	0.00880	0.00359	(-0.01174, 0.00674)

μ_difference: population mean of (27DBP-Springiness - 27DAP-Springiness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-0.70	0.518

Paired T-Test and CI: 27DBP-Cohesiveness, 27DAP-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Cohesiveness	6	0.7175	0.0418	0.0171
27DAP-Cohesiveness	6	0.6792	0.0144	0.0059

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.0383	0.0507	0.0207	(-0.0149, 0.0916)

μ_difference: population mean of (27DBP-Cohesiveness - 27DAP-Cohesiveness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
1.85	0.123

Paired T-Test and CI: 27DBP-Gumminess, 27DAP-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Gumminess	6	323.96	14.94	6.10
27DAP-Gumminess	6	247.38	10.44	4.26

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
76.58	14.47	5.91	(61.40, 91.77)

μ_difference: population mean of (27DBP-Gumminess - 27DAP-Gumminess)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
12.96	0.000

Paired T-Test and CI: 27DBP-Chewiness, 27DAP-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Chewiness	6	317.86	13.18	5.38
27DAP-Chewiness	6	243.29	10.13	4.14

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
74.57	12.57	5.13	(61.38, 87.76)

μ_difference: population mean of (27DBP-Chewiness - 27DAP-Chewiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
14.53	0.000

Paired T-Test and CI: 27DBP-Resilience, 27DAP-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DBP-Resilience	6	0.09400	0.00867	0.00354
27DAP-Resilience	6	0.07217	0.00585	0.00239

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.02183	0.01105	0.00451	(0.01023, 0.03343)

μ_difference: population mean of (27DBP-Resilience - 27DAP-Resilience)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
4.84	0.005

1.2. 27DAP vs 27 SDB

Paired T-Test and CI: 27DAP-Hardness, 27SDB-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Hardness	6	364.5	18.7	7.6
27SDB-Hardness	6	1112.1	134.6	55.0

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-747.5	144.2	58.9	(-898.9, -596.2)

μ_difference: population mean of (27DAP-Hardness - 27SDB-Hardness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-12.70	0.000

Paired T-Test and CI: 27DAP-Adhesiveness, 27SDB-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Adhesiveness	6	-426.2	37.3	15.2
27SDB-Adhesiveness	6	-4.8	2.4	1.0

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-421.4	37.1	15.1	(-460.3, -382.5)

μ_difference: population mean of (27DAP-Adhesiveness - 27SDB-Adhesiveness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-27.83	0.000

Paired T-Test and CI: 27DAP-Springiness, 27SDB-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Springiness	6	0.984	0.004	0.002
27SDB-Springiness	6	1.196	0.433	0.177

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.213	0.435	0.178	(-0.669, 0.244)

μ_difference: population mean of (27DAP-Springiness - 27SDB-Springiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-1.20	0.285

Paired T-Test and CI: 27DAP-Cohesiveness, 27SDB-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Cohesiveness	6	0.67917	0.01443	0.00589
27SDB-Cohesiveness	6	0.72900	0.01043	0.00426

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.04983	0.02039	0.00832	(-0.07123, -0.02843)

μ_difference: population mean of (27DAP-Cohesiveness - 27SDB-Cohesiveness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-5.99	0.002

Paired T-Test and CI: 27DAP-Gumminess, 27SDB-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Gumminess	6	247.4	10.4	4.3
27SDB-Gumminess	6	809.6	90.7	37.0

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-562.2	93.2	38.1	(-660.0, -464.4)

μ_difference: population mean of (27DAP-Gumminess - 27SDB-Gumminess)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-14.77	0.000

Paired T-Test and CI: 27DAP-Chewiness, 27SDB-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Chewiness	6	243	10	4
27SDB-Chewiness	6	975	394	161

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-732	387	158	(-1138, -325)

μ_difference: population mean of (27DAP-Chewiness - 27SDB-Chewiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-4.63	0.006

Paired T-Test and CI: 27DAP-Resilience, 27SDB-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
27DAP-Resilience	6	0.07217	0.00585	0.00239
27SDB-Resilience	6	0.40000	0.01324	0.00540

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.32783	0.01055	0.00431	(-0.33891, -0.31676)

μ_difference: population mean of (27DAP-Resilience - 27SDB-Resilience)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-76.09	0.000

1.3. 29DBP vs 29DAP

Paired T-Test and CI: 29DBP-Hardness, 29DAP-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Hardness	6	774.5	44.7	18.2
29DAP-Hardness	6	594.2	52.6	21.5

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
180.3	77.1	31.5	(99.4, 261.2)

μ_difference: population mean of (29DBP-Hardness - 29DAP-Hardness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
5.73	0.002

Paired T-Test and CI: 29DBP-Adhesiveness, 29DAP-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Adhesiveness	6	-874.2	90.2	36.8
29DAP-Adhesiveness	6	-667.5	40.1	16.4

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-206.8	74.9	30.6	(-285.4, -128.1)

μ_difference: population mean of (29DBP-Adhesiveness - 29DAP-Adhesiveness)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-6.76	0.001

Paired T-Test and CI: 29DBP-Springiness, 29DAP-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Springiness	6	0.97567	0.00383	0.00156
29DAP-Springiness	6	0.97583	0.00649	0.00265

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.00017	0.00618	0.00252	(-0.00665, 0.00632)

μ_difference: population mean of (29DBP-Springiness - 29DAP-Springiness)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-0.07	0.950

Paired T-Test and CI: 29DBP-Cohesiveness, 29DAP-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Cohesiveness	6	0.6980	0.0329	0.0134
29DAP-Cohesiveness	6	0.6765	0.0275	0.0112

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.0215	0.0444	0.0181	(-0.0250, 0.0680)

μ_difference: population mean of (29DBP-Cohesiveness - 29DAP-Cohesiveness)

Test

Null hypothesis H_0: μ_difference = 0

Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
1.19	0.288

Paired T-Test and CI: 29DBP-Gumminess, 29DAP-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Gumminess	6	540.1	30.2	12.3
29DAP-Gumminess	6	401.4	28.9	11.8

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
138.7	37.0	15.1	(99.8, 177.5)

μ_difference: population mean of (29DBP-Gumminess - 29DAP-Gumminess)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
9.18	0.000

Paired T-Test and CI: 29DBP-Chewiness, 29DAP-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Chewiness	6	526.9	28.0	11.4
29DAP-Chewiness	6	391.6	26.5	10.8

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
135.3	35.1	14.3	(98.5, 172.1)

μ_difference: population mean of (29DBP-Chewiness - 29DAP-Chewiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
9.45	0.000

Paired T-Test and CI: 29DBP-Resilience, 29DAP-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DBP-Resilience	6	0.10350	0.00650	0.00266
29DAP-Resilience	6	0.07267	0.00398	0.00163

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.03083	0.00889	0.00363	(0.02151, 0.04016)

μ_difference: population mean of (29DBP-Resilience - 29DAP-Resilience)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
8.50	0.000

1.4. 29DAP vs 29SDB

Paired T-Test and CI: 29DAP-Hardness, 29SDB-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Hardness	6	594.2	52.6	21.5
29SDB-Hardness	6	1298.8	90.6	37.0

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-704.6	89.6	36.6	(-798.7, -610.6)

μ_difference: population mean of (29DAP-Hardness - 29SDB-Hardness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-19.26	0.000

Paired T-Test and CI: 29DAP-Adhesiveness, 29SDB-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Adhesiveness	6	-667.5	40.1	16.4
29SDB-Adhesiveness	6	-5.3	1.7	0.7

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-662.2	41.2	16.8	(-705.4, -619.0)

μ_difference: population mean of (29DAP-Adhesiveness - 29SDB-Adhesiveness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-39.38	0.000

Paired T-Test and CI: 29DAP-Springiness, 29SDB-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Springiness	6	0.976	0.006	0.003

29SDB-Springiness	6	1.335	0.747	0.305

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.360	0.745	0.304	(-1.142, 0.422)

μ_difference: population mean of (29DAP-Springiness - 29SDB-Springiness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-1.18	0.290

Paired T-Test and CI: 29DAP-Cohesiveness, 29SDB-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Cohesiveness	6	0.6765	0.0275	0.0112
29SDB-Cohesiveness	6	0.7098	0.0145	0.0059

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.0333	0.0270	0.0110	(-0.0617, -0.0050)

μ_difference: population mean of (29DAP-Cohesiveness - 29SDB-Cohesiveness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-3.02	0.029

Paired T-Test and CI: 29DAP-Gumminess, 29SDB-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Gumminess	6	401.4	28.9	11.8
29SDB-Gumminess	6	921.2	56.2	23.0

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-519.8	67.2	27.5	(-590.3, -449.2)

μ_difference: population mean of (29DAP-Gumminess - 29SDB-Gumminess)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-18.93	0.000

Paired T-Test and CI: 29DAP-Chewiness, 29SDB-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Chewiness	6	392	27	11
29SDB-Chewiness	6	1218	636	260

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-826	656	268	(-1515, -138)

μ_difference: population mean of (29DAP-Chewiness - 29SDB-Chewiness)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-3.08	0.027

Paired T-Test and CI: 29DAP-Resilience, 29SDB-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
29DAP-Resilience	6	0.07267	0.00398	0.00163
29SDB-Resilience	6	0.38833	0.01252	0.00511

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.31567	0.01556	0.00635	(-0.33200, -0.29933)

μ_difference: population mean of (29DAP-Resilience - 29SDB-Resilience)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-49.68	0.000

1.5. 31DBP vs 31DAP

Paired T-Test and CI: 31DBP-Hardness, 31DAP-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Hardness	6	724.42	14.49	5.91
31DAP-Hardness	6	264.78	20.14	8.22

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
459.64	21.04	8.59	(437.56, 481.71)

μ_difference: population mean of (31DBP-Hardness - 31DAP-Hardness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
53.52	0.000

Paired T-Test and CI: 31DBP-Adhesiveness, 31DAP-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Adhesiveness	6	-897.3	91.7	37.4
31DAP-Adhesiveness	6	-346.5	58.4	23.8

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-550.8	140.9	57.5	(-698.6, -402.9)

μ_difference: population mean of (31DBP-Adhesiveness - 31DAP-Adhesiveness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-9.58	0.000

Paired T-Test and CI: 31DBP-Springiness, 31DAP-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Springiness	6	0.97483	0.00621	0.00254
31DAP-Springiness	6	0.98517	0.00319	0.00130

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.01033	0.00862	0.00352	(-0.01938, -0.00129)

μ_difference: population mean of (31DBP-Springiness - 31DAP-Springiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-2.94	0.032

Paired T-Test and CI: 31DBP-Cohesiveness, 31DAP-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Cohesiveness	6	0.7345	0.0201	0.0082
31DAP-Cohesiveness	6	0.7330	0.0444	0.0181

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference

0.0015	0.0441	0.0180	(-0.0448, 0.0478)

μ_difference: population mean of (31DBP-Cohesiveness - 31DAP-Cohesiveness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
0.08	0.937

Paired T-Test and CI: 31DBP-Gumminess, 31DAP-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Gumminess	6	532.12	20.68	8.44
31DAP-Gumminess	6	194.08	17.72	7.24

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
338.0	25.0	10.2	(311.8, 364.3)

μ_difference: population mean of (31DBP-Gumminess - 31DAP-Gumminess)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
33.11	0.000

Paired T-Test and CI: 31DBP-Chewiness, 31DAP-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Chewiness	6	518.75	22.76	9.29
31DAP-Chewiness	6	191.18	17.13	7.00

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
327.6	25.5	10.4	(300.8, 354.3)

μ_difference: population mean of (31DBP-Chewiness - 31DAP-Chewiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
31.48	0.000

Paired T-Test and CI: 31DBP-Resilience, 31DAP-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DBP-Resilience	6	0.09500	0.00303	0.00124

31DAP-Resilience	6	0.06800	0.00443	0.00181

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.02700	0.00626	0.00256	(0.02043, 0.03357)

μ_difference: population mean of (31DBP-Resilience - 31DAP-Resilience)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
10.56	0.000

1.6. 31DAP vs 31SDB

Paired T-Test and CI: 31DAP-Hardness, 31SDB-Hardness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Hardness	6	264.8	20.1	8.2
31SDB-Hardness	6	1028.7	93.6	38.2

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-763.9	88.8	36.2	(-857.1, -670.8)

μ_difference: population mean of (31DAP-Hardness - 31SDB-Hardness)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-21.08	0.000

Paired T-Test and CI: 31DAP-Adhesiveness, 31SDB-Adhesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Adhesiveness	6	-346.5	58.4	23.8
31SDB-Adhesiveness	6	-5.4	1.3	0.5

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-341.2	58.6	23.9	(-402.7, -279.6)

μ_difference: population mean of (31DAP-Adhesiveness - 31SDB-Adhesiveness)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-14.25	0.000

Paired T-Test and CI: 31DAP-Springiness, 31SDB-Springiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Springiness	6	0.985	0.003	0.001
31SDB-Springiness	6	1.815	0.445	0.182

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.830	0.446	0.182	(-1.298, -0.362)

μ_difference: population mean of (31DAP-Springiness - 31SDB-Springiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-4.56	0.006

Paired T-Test and CI: 31DAP-Cohesiveness, 31SDB-Cohesiveness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Cohesiveness	6	0.7330	0.0444	0.0181
31SDB-Cohesiveness	6	0.7568	0.0148	0.0060

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.0238	0.0538	0.0220	(-0.0803, 0.0327)

μ_difference: population mean of (31DAP-Cohesiveness - 31SDB-Cohesiveness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-1.08	0.328

Paired T-Test and CI: 31DAP-Gumminess, 31SDB-Gumminess

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Gumminess	6	194.1	17.7	7.2
31SDB-Gumminess	6	777.7	60.9	24.9

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-583.7	52.7	21.5	(-639.0, -528.3)

μ_difference: population mean of (31DAP-Gumminess - 31SDB-Gumminess)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-27.11	0.000

Paired T-Test and CI: 31DAP-Chewiness, 31SDB-Chewiness

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Chewiness	6	191	17	7
31SDB-Chewiness	6	1429	408	167

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-1238	400	163	(-1658, -818)

μ_difference: population mean of (31DAP-Chewiness - 31SDB-Chewiness)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-7.58	0.001

Paired T-Test and CI: 31DAP-Resilience, 31SDB-Resilience

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
31DAP-Resilience	6	0.06800	0.00443	0.00181
31SDB-Resilience	6	0.43333	0.01236	0.00504

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-0.36533	0.01421	0.00580	(-0.38024, -0.35042)

μ_difference: population mean of (31DAP-Resilience - 31SDB-Resilience)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-62.98	0.000

1.1. DBP vs DAP

One-way ANOVA: 27DAP-DBP-Hardness, 29DAP-DBP-Hardness, 31DAP-DBP-Hardness

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Hardness, 29DAP-DBP-Hardness, 31DAP-DBP-Hardness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	450239	225120	97.26	0.000
Error	15	34718	2315		
Total	17	484957			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
48.1093	92.84%	91.89%	89.69%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Hardness	6	-87.52	23.64	(-129.38, -45.66)
29DAP-DBP-Hardness	6	-180.3	77.1	(-222.1, -138.4)
31DAP-DBP-Hardness	6	-459.64	21.04	(-501.50, -417.77)

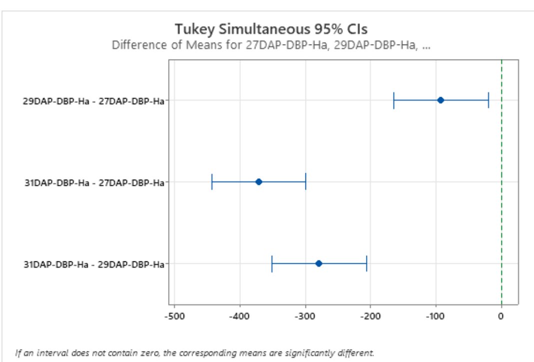
Pooled StDev = 48.1093

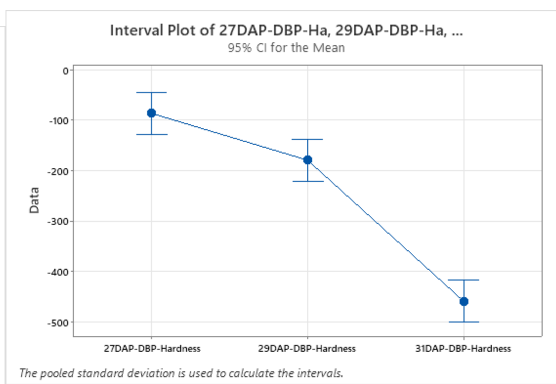
Tukey Pairwise Comparisons

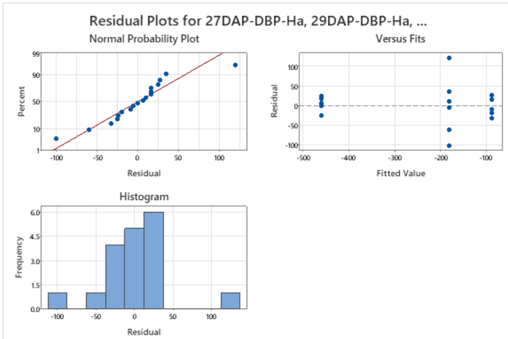
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
27DAP-DBP-Hardness	6	-87.52	A		
29DAP-DBP-Hardness	6	-180.3		B	
31DAP-DBP-Hardness	6	-459.64			C

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Adhesiveness, 29DAP-DBP-Adhesiveness, 31DAP-DBP-Adhesiveness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Adhesiveness, 29DAP-DBP-Adhesiveness, 31DAP-DBP-Adhesiveness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	566545	283272	20.63	0.000
Error	15	205960	13731		
Total	17	772505			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
117.178	73.34%	69.78%	61.61%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Adhesiveness	6	148.8	125.4	(46.9, 250.8)
29DAP-DBP-Adhesiveness	6	206.8	74.9	(104.8, 308.7)
31DAP-DBP-Adhesiveness	6	550.8	140.9	(448.8, 652.7)

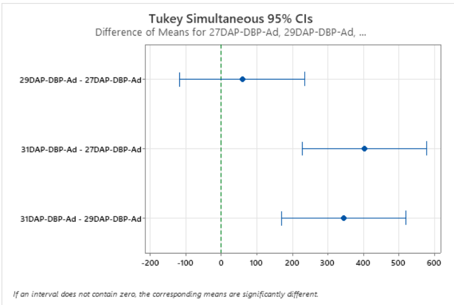
Pooled StDev = 117.178

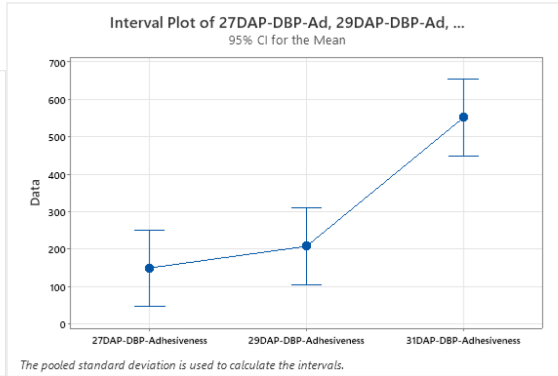
Tukey Pairwise Comparisons

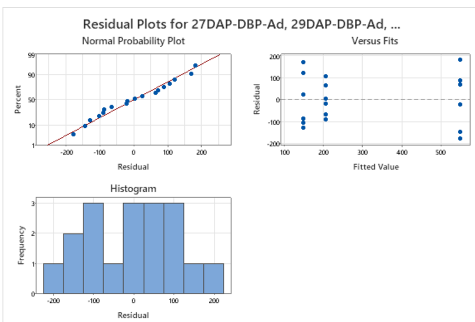
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
31DAP-DBP-Adhesiveness	6	550.8	A	
29DAP-DBP-Adhesiveness	6	206.8		B
27DAP-DBP-Adhesiveness	6	148.8		B

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Springiness, 29DAP-DBP-Springiness, 31DAP-DBP-Springiness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Springiness, 29DAP-DBP-Springiness, 31DAP-DBP-Springiness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.000340	0.000170	2.69	0.101
Error	15	0.000950	0.000063		
Total	17	0.001290			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0079568	26.38%	16.57%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Springiness	6	0.00250	0.00880	(-0.00442, 0.00942)
29DAP-DBP-Springiness	6	0.00017	0.00618	(-0.00676, 0.00709)
31DAP-DBP-Springiness	6	0.01033	0.00862	(0.00341, 0.01726)

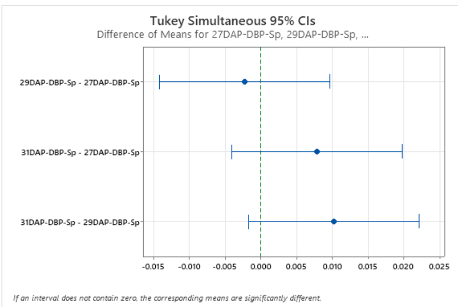
Pooled StDev = 0.00795683

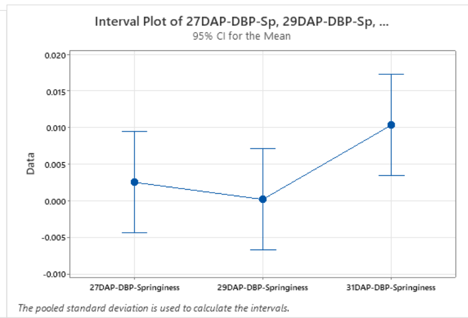
Tukey Pairwise Comparisons

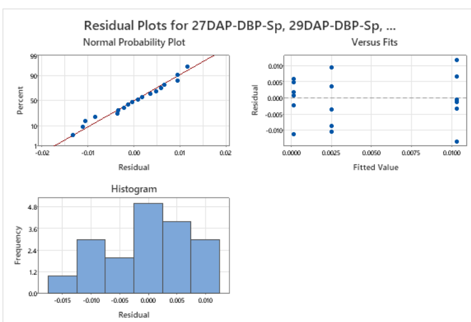
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31DAP-DBP-Springiness	6	0.01033	A
27DAP-DBP-Springiness	6	0.00250	A
29DAP-DBP-Springiness	6	0.00017	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Cohesiveness, 29DAP-DBP-Cohesiveness, 31DAP-DBP-Cohesiveness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Cohesiveness, 29DAP-DBP-Cohesiveness, 31DAP-DBP-Cohesiveness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.004080	0.002040	0.94	0.411
Error	15	0.032446	0.002163		
Total	17	0.036526			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0465090	11.17%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Cohesiveness	6	-0.0383	0.0507	(-0.0788, 0.0021)
29DAP-DBP-Cohesiveness	6	-0.0215	0.0444	(-0.0620, 0.0190)
31DAP-DBP-Cohesiveness	6	-0.0015	0.0441	(-0.0420, 0.0390)

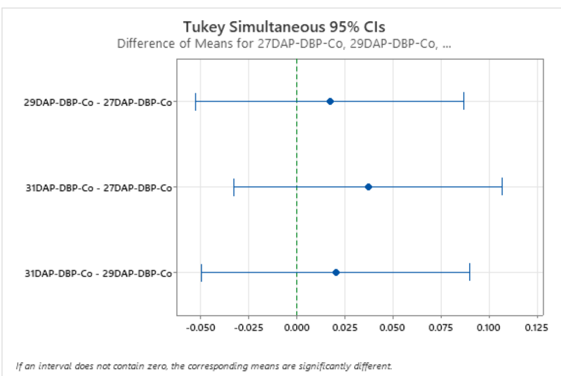
Pooled StDev = 0.0465090

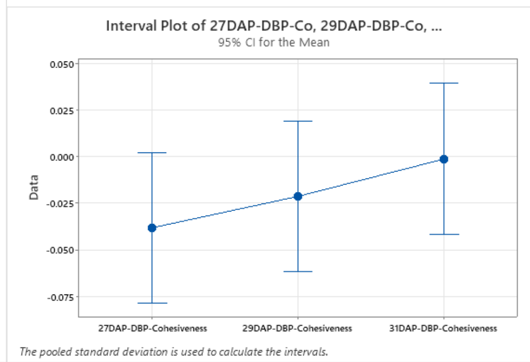
Tukey Pairwise Comparisons

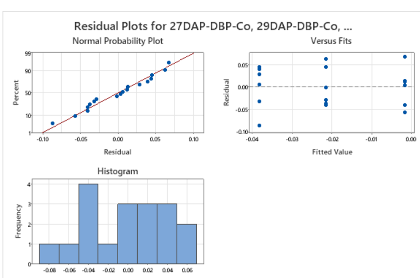
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31DAP-DBP-Cohesiveness	6	-0.0015	A
29DAP-DBP-Cohesiveness	6	-0.0215	A
27DAP-DBP-Cohesiveness	6	-0.0383	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Gumminess, 29DAP-DBP-Gumminess, 31DAP-DBP-Gumminess

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Gumminess, 29DAP-DBP-Gumminess, 31DAP-DBP-Gumminess

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	223931	111966	152.44	0.000
Error	15	11018	735		
Total	17	234949			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
27.1017	95.31%	94.69%	93.25%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Gumminess	6	-76.58	14.47	(-100.16, -53.00)
29DAP-DBP-Gumminess	6	-138.7	37.0	(-162.2, -115.1)
31DAP-DBP-Gumminess	6	-338.0	25.0	(-361.6, -314.5)

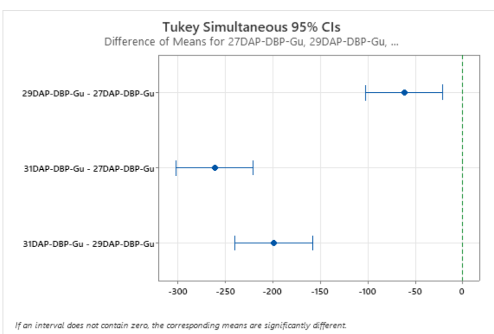
Pooled StDev = 27.1017

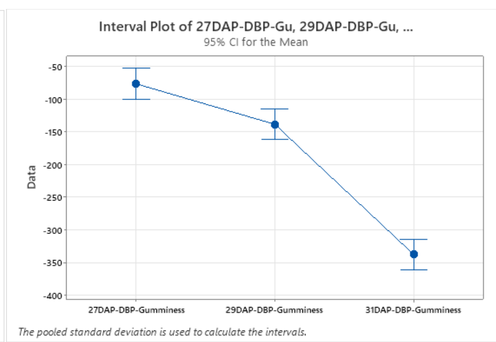
Tukey Pairwise Comparisons

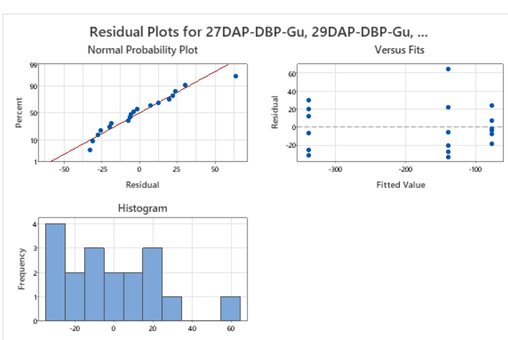
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
27DAP-DBP-Gumminess	6	-76.58	A		
29DAP-DBP-Gumminess	6	-138.7		B	
31DAP-DBP-Gumminess	6	-338.0			C

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Chewiness, 29DAP-DBP-Chewiness, 31DAP-DBP-Chewiness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Chewiness, 29DAP-DBP-Chewiness, 31DAP-DBP-Chewiness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	209331	104665	154.04	0.000
Error	15	10192	679		
Total	17	219522			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
26.0664	95.36%	94.74%	93.31%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Chewiness	6	-74.57	12.57	(-97.25, -51.88)
29DAP-DBP-Chewiness	6	-135.3	35.1	(-158.0, -112.6)
31DAP-DBP-Chewiness	6	-327.6	25.5	(-350.3, -304.9)

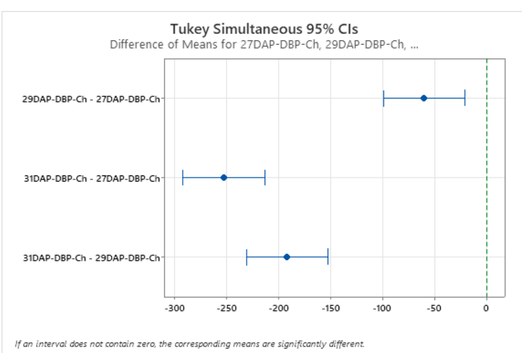
Pooled StDev = 26.0664

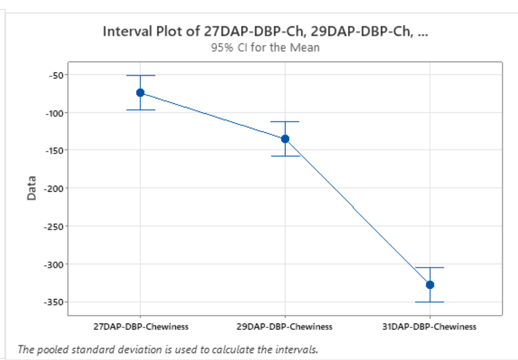
Tukey Pairwise Comparisons

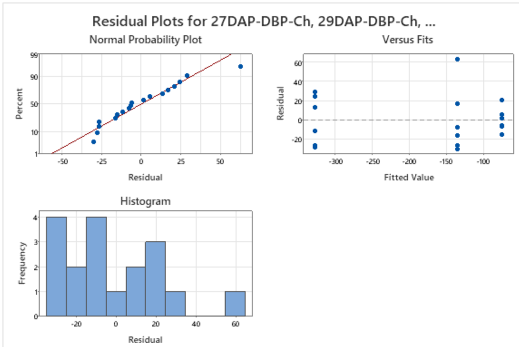
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
27DAP-DBP-Chewiness	6	-74.57	A		
29DAP-DBP-Chewiness	6	-135.3		B	
31DAP-DBP-Chewiness	6	-327.6			C

Means that do not share a letter are significantly different.







One-way ANOVA: 27DAP-DBP-Resilience, 29DAP-DBP-Resilience, 31DAP-DBP-Resilience

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27DAP-DBP-Resilience, 29DAP-DBP-Resilience, 31DAP-DBP-Resilience

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.000245	0.000122	1.53	0.249
Error	15	0.001202	0.000080		
Total	17	0.001446			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0089505	16.92%	5.85%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27DAP-DBP-Resilience	6	-0.02183	0.01105	(-0.02962, -0.01404)
29DAP-DBP-Resilience	6	-0.03083	0.00889	(-0.03862, -0.02304)
31DAP-DBP-Resilience	6	-0.02700	0.00626	(-0.03479, -0.01921)

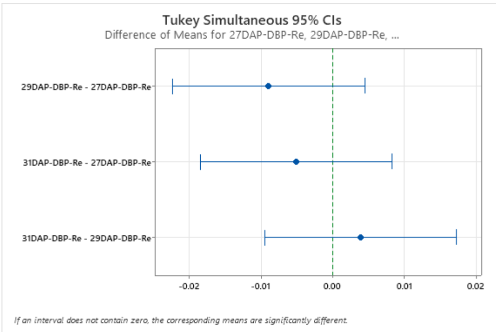
Pooled StDev = 0.00895048

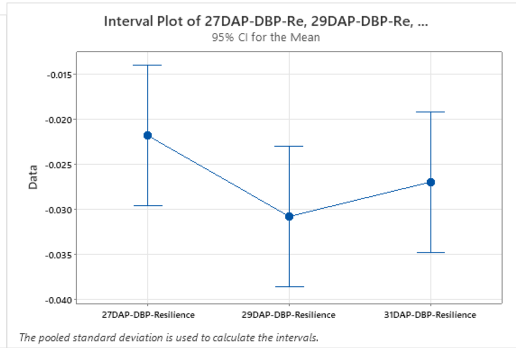
Tukey Pairwise Comparisons

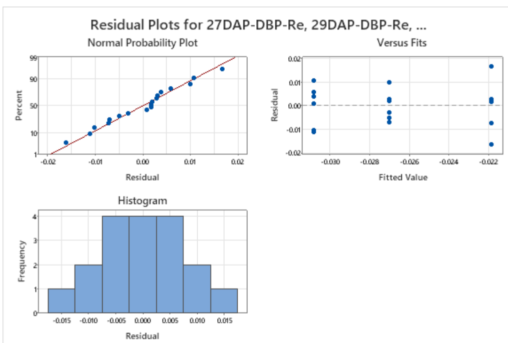
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
27DAP-DBP-Resilience	6	-0.02183	A
31DAP-DBP-Resilience	6	-0.02700	A
29DAP-DBP-Resilience	6	-0.03083	A

Means that do not share a letter are significantly different.







1.1. SDB vs DAP

One-way ANOVA: 27SDB-DAP-Hardness, 29SDB-DAP-Hardness, 31SDB-DAP-Hardness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Hardness, 29SDB-DAP-Hardness, 31SDB-DAP-Hardness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	11266	5633	0.46	0.640
Error	15	183559	12237		
Total	17	194825			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
110.622	5.78%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI

27SDB-DAP-Hardness	6	747.5	144.2	(651.3, 843.8)
29SDB-DAP-Hardness	6	704.6	89.6	(608.4, 800.9)
31SDB-DAP-Hardness	6	763.9	88.8	(667.7, 860.2)

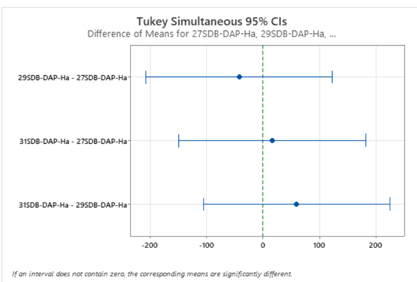
Pooled StDev = 110.622

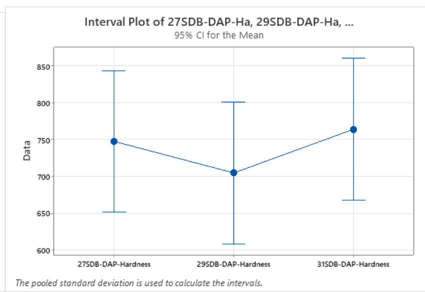
Tukey Pairwise Comparisons

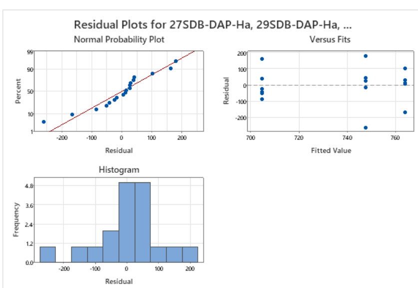
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-DAP-Hardness	6	763.9	A
27SDB-DAP-Hardness	6	747.5	A
29SDB-DAP-Hardness	6	704.6	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-Adhesiveness, 29SDB-DAP-Adhesiveness, 31SDB-DAP-Adhesiveness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Adhesiveness, 29SDB-DAP-Adhesiveness, 31SDB-DAP-Adhesiveness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	334941	167470	77.18	0.000
Error	15	32549	2170		

Total	17	367489

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
46.5824	91.14%	89.96%	87.25%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Adhesiveness	6	421.4	37.1	(380.9, 462.0)
29SDB-DAP-Adhesiveness	6	662.2	41.2	(621.7, 702.7)
31SDB-DAP-Adhesiveness	6	341.2	58.6	(300.6, 381.7)

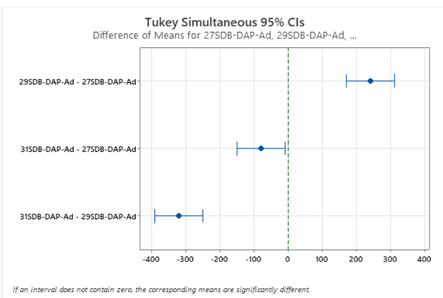
Pooled StDev = 46.5824

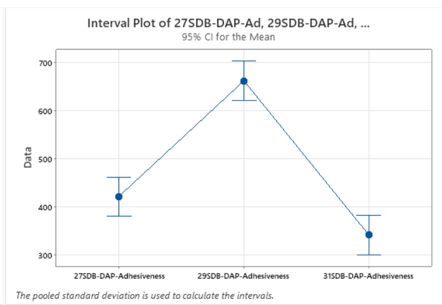
Tukey Pairwise Comparisons

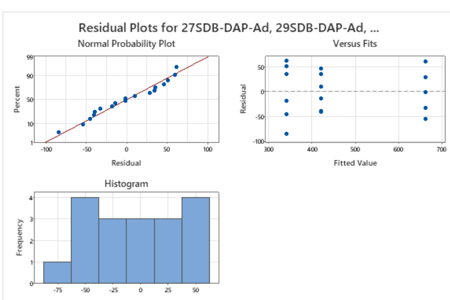
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
29SDB-DAP-Adhesiveness	6	662.2	A		
27SDB-DAP-Adhesiveness	6	421.4		B	
31SDB-DAP-Adhesiveness	6	341.2			C

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-Springiness, 29SDB-DAP-Springiness, 31SDB-DAP-Springiness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Springiness, 29SDB-DAP-Springiness, 31SDB-DAP-Springiness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1.248	0.6239	1.99	0.172
Error	15	4.715	0.3143		
Total	17	5.962			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.560633	20.93%	10.39%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Springiness	6	0.213	0.435	(-0.275, 0.701)
29SDB-DAP-Springiness	6	0.360	0.745	(-0.128, 0.848)
31SDB-DAP-Springiness	6	0.830	0.446	(0.342, 1.318)

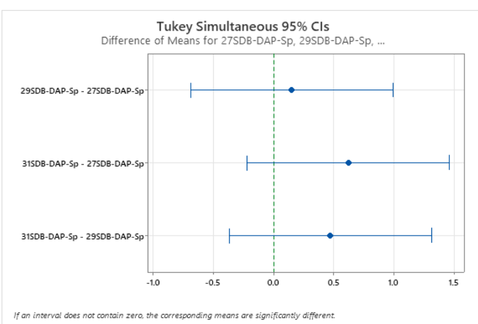
Pooled StDev = 0.560633

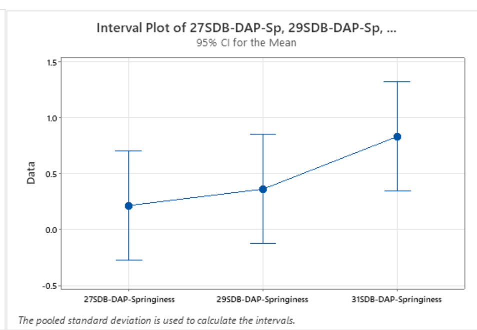
Tukey Pairwise Comparisons

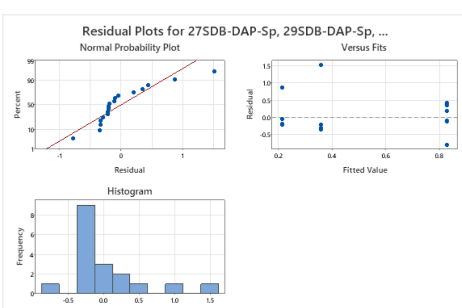
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-DAP-Springiness	6	0.830	A
29SDB-DAP-Springiness	6	0.360	A
27SDB-DAP-Springiness	6	0.213	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-Cohesiveness, 29SDB-DAP-Cohesiveness, 31SDB-DAP-Cohesiveness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Cohesiveness, 29SDB-DAP-Cohesiveness, 31SDB-DAP-Cohesiveness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.002077	0.001038	0.77	0.480
Error	15	0.020231	0.001349		
Total	17	0.022308			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0367251	9.31%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Cohesiveness	6	0.04983	0.02039	(0.01788, 0.08179)
29SDB-DAP-Cohesiveness	6	0.0333	0.0270	(0.0014, 0.0653)
31SDB-DAP-Cohesiveness	6	0.0238	0.0538	(-0.0081, 0.0558)

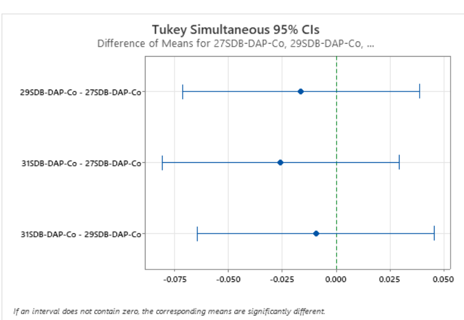
Pooled StDev = 0.0367251

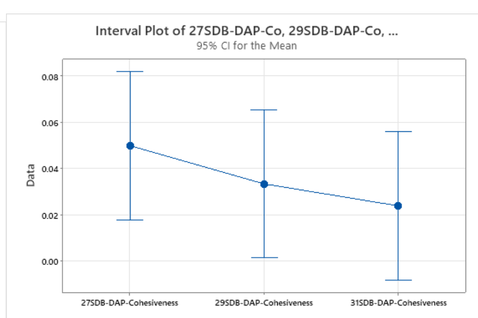
Tukey Pairwise Comparisons

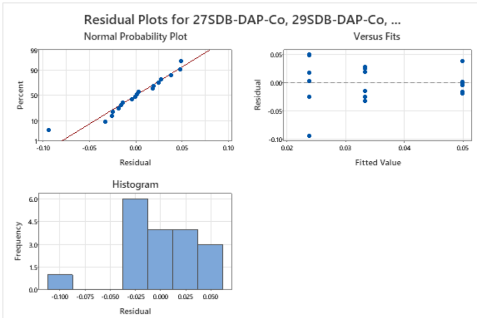
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
27SDB-DAP-Cohesiveness	6	0.04983	A
29SDB-DAP-Cohesiveness	6	0.0333	A
31SDB-DAP-Cohesiveness	6	0.0238	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-Gumminess, 29SDB-DAP-Gumminess, 31SDB-DAP-Gumminess

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Gumminess, 29SDB-DAP-Gumminess, 31SDB-DAP-Gumminess

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	12683	6341	1.19	0.331
Error	15	79958	5331		
Total	17	92641			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
73.0106	13.69%	2.18%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Gumminess	6	562.2	93.2	(498.7, 625.7)
29SDB-DAP-Gumminess	6	519.8	67.2	(456.2, 583.3)
31SDB-DAP-Gumminess	6	583.7	52.7	(520.1, 647.2)

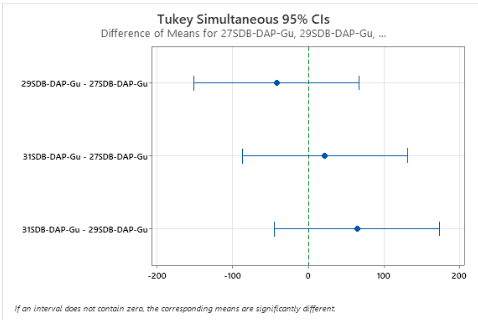
Pooled StDev = 73.0106

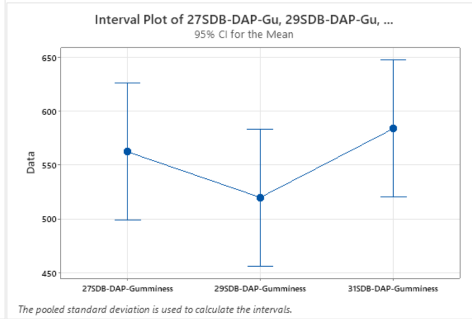
Tukey Pairwise Comparisons

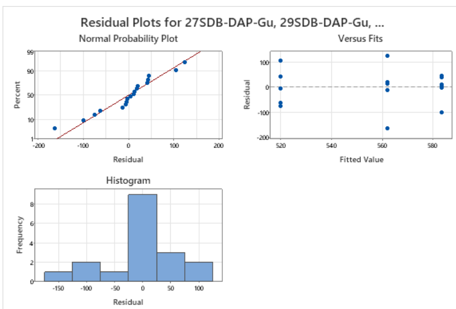
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-DAP-Gumminess	6	583.7	A
27SDB-DAP-Gumminess	6	562.2	A
29SDB-DAP-Gumminess	6	519.8	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-DAP-Chewiness, 29SDB-DAP-Chewiness, 31SDB-DAP-Chewiness

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Chewiness, 29SDB-DAP-Chewiness, 31SDB-DAP-Chewiness

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	870310	435155	1.76	0.205
Error	15	3703248	246883		
Total	17	4573557			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
496.873	19.03%	8.23%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Chewiness	6	732	387	(299, 1164)
29SDB-DAP-Chewiness	6	826	656	(394, 1259)
31SDB-DAP-Chewiness	6	1238	400	(806, 1671)

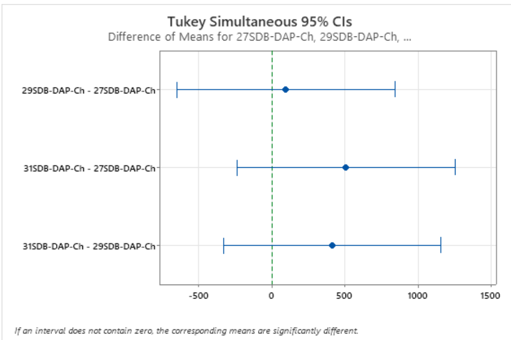
Pooled StDev = 496.873

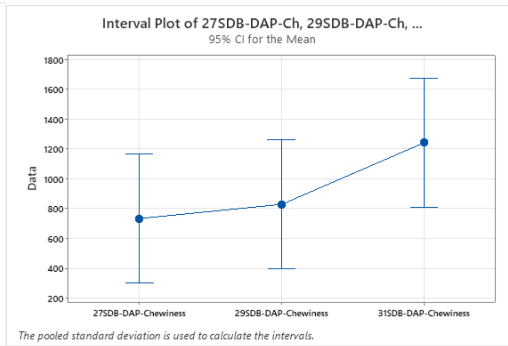
Tukey Pairwise Comparisons

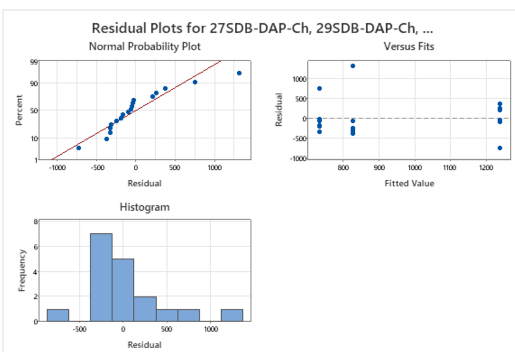
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-DAP-Chewiness	6	1238	A
29SDB-DAP-Chewiness	6	826	A
27SDB-DAP-Chewiness	6	732	A

Means that do not share a letter are significantly different.







PHASE 2-TEXTURE B

One-way ANOVA: 27SDB-DAP-Resilience, 29SDB-DAP-Resilience, 31SDB-DAP-Resilience

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-DAP-Resilience, 29SDB-DAP-Resilience, 31SDB-DAP-Resilience

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.008042	0.004021	21.72	0.000
Error	15	0.002778	0.000185		
Total	17	0.010820			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0136076	74.33%	70.91%	63.03%

Means

Factor	N	Mean	StDev	95% CI
27SDB-DAP-Resilience	6	0.32783	0.01055	(0.31599, 0.33967)
29SDB-DAP-Resilience	6	0.31567	0.01556	(0.30383, 0.32751)
31SDB-DAP-Resilience	6	0.36533	0.01421	(0.35349, 0.37717)

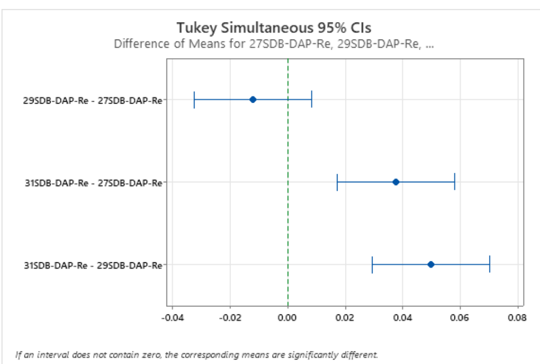
Pooled StDev = 0.0136076

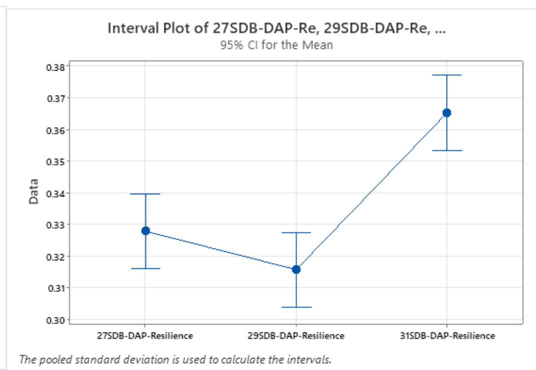
Tukey Pairwise Comparisons

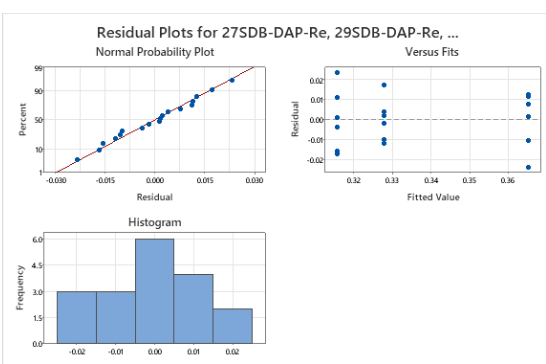
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
31SDB-DAP-Resilience	6	0.36533	A	
27SDB-DAP-Resilience	6	0.32783		B
29SDB-DAP-Resilience	6	0.31567		B

Means that do not share a letter are significantly different.







2. Microbiology

Paired T-Test and CI: LAB-27DBP, LAB-27DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-27DBP	4	8.4869	0.0710	0.0355
LAB-27DAP	4	9.0313	0.0497	0.0249

Estimation for Paired Difference

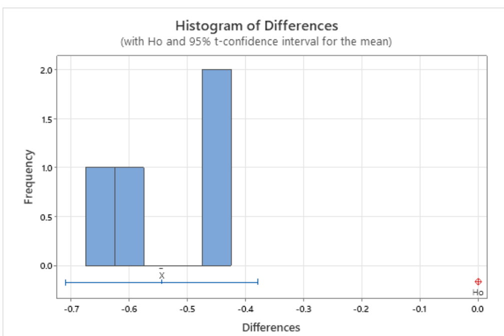
Mean	StDev	SE Mean	95% CI for μ_difference
-0.5444	0.1039	0.0520	(-0.7097, -0.3790)

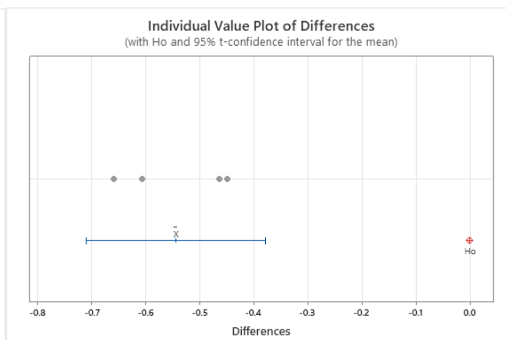
μ_difference: population mean of (LAB-27DBP - LAB-27DAP)

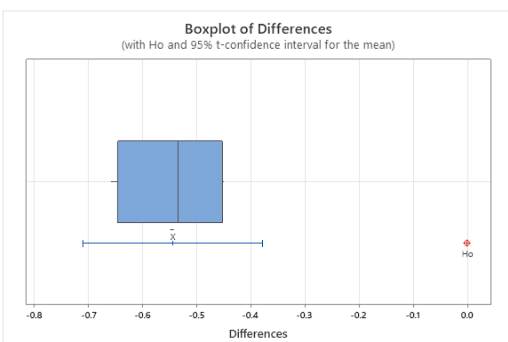
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-10.48	0.002







Paired T-Test and CI: LAB-29DBP, LAB-29DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-29DBP	4	8.6364	0.0709	0.0355
LAB-29DAP	4	8.9948	0.0485	0.0242

Estimation for Paired Difference

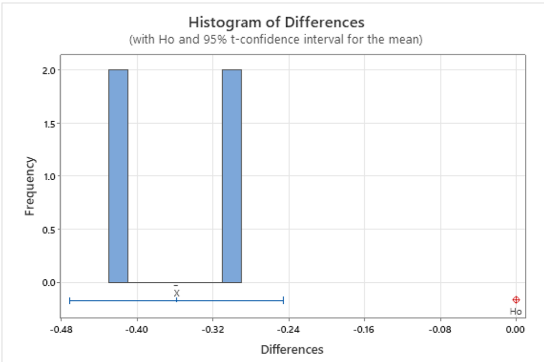
Mean	StDev	SE Mean	95% CI for μ_difference
-0.3583	0.0710	0.0355	(-0.4714, -0.2453)

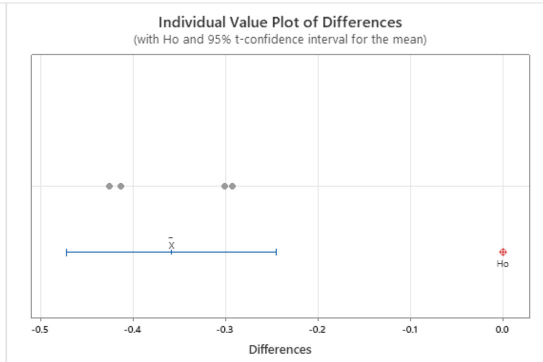
μ_difference: population mean of (LAB-29DBP - LAB-29DAP)

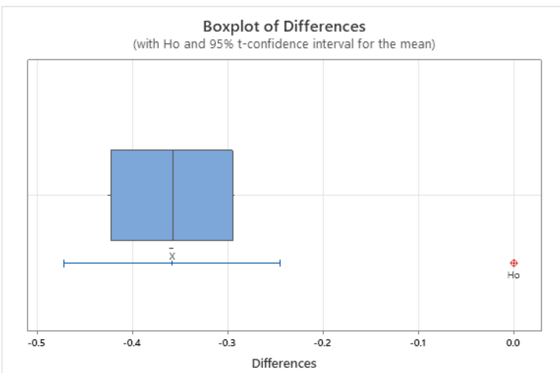
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-10.09	0.002







Paired T-Test and CI: LAB-31DBP, LAB-31DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-31DBP	4	8.6853	0.0738	0.0369
LAB-31DAP	4	8.9334	0.0348	0.0174

Estimation for Paired Difference

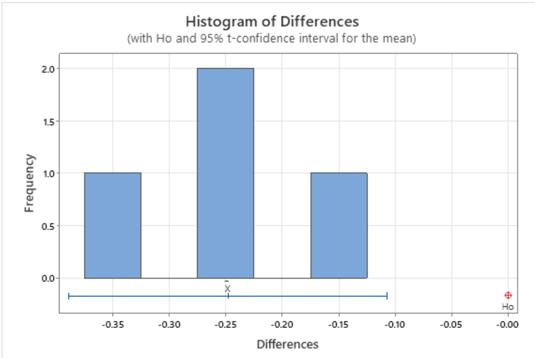
Mean	StDev	SE Mean	95% CI for μ_difference
-0.2481	0.0886	0.0443	(-0.3891, -0.1072)

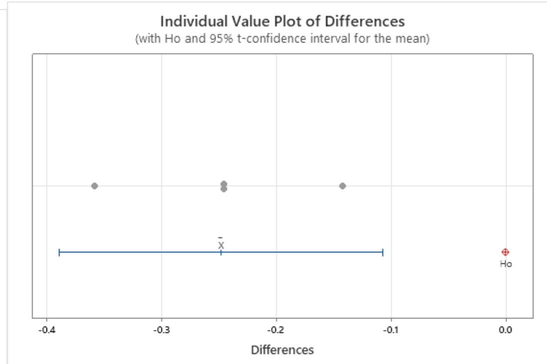
μ_difference: population mean of (LAB-31DBP - LAB-31DAP)

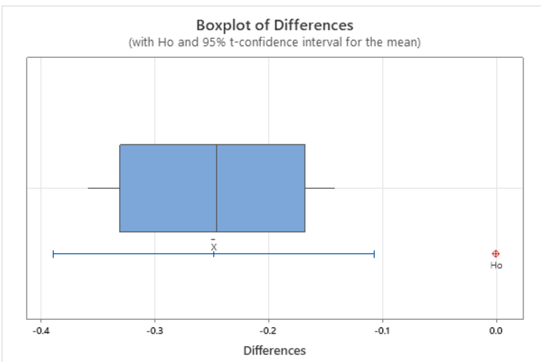
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-5.60	0.011







Paired T-Test and CI: Yeast-27DBP, Yeast-27DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-27DBP	4	4.5968	0.0538	0.0269
Yeast-27DAP	4	3.5137	0.0425	0.0212

Estimation for Paired Difference

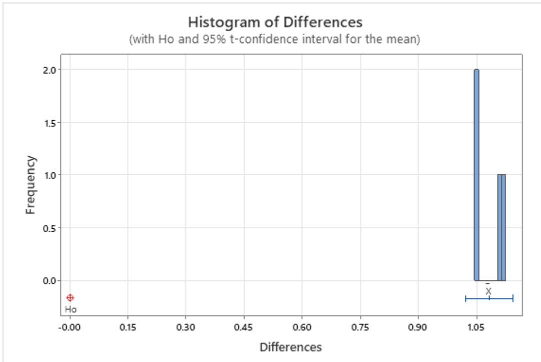
Mean	StDev	SE Mean	95% CI for μ_difference
1.0832	0.0384	0.0192	(1.0221, 1.1443)

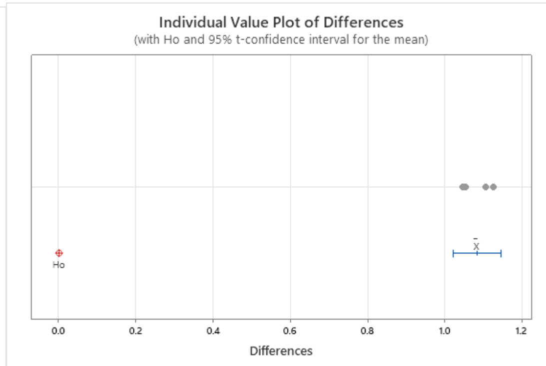
μ_difference: population mean of (Yeast-27DBP - Yeast-27DAP)

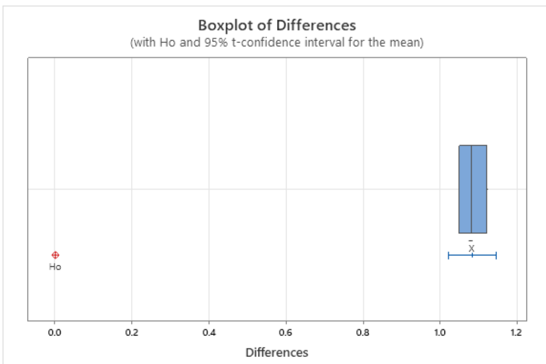
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
56.40	0.000







Paired T-Test and CI: Yeast-29DBP, Yeast-29DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-29DBP	4	4.3423	0.0747	0.0373
Yeast-29DAP	4	4.0022	0.0504	0.0252

Estimation for Paired Difference

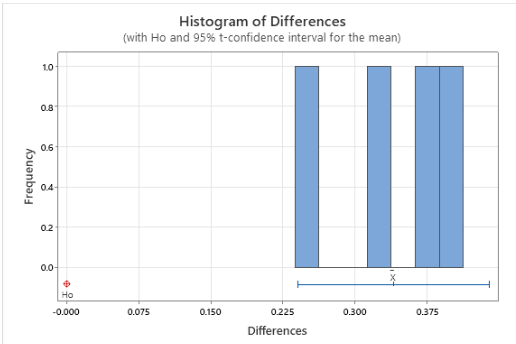
Mean	StDev	SE Mean	95% CI for μ_difference
0.3401	0.0625	0.0313	(0.2407, 0.4396)

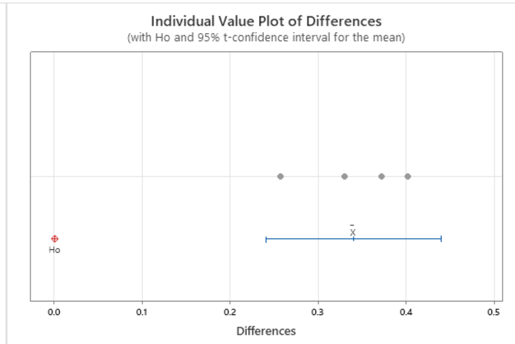
μ_difference: population mean of (Yeast-29DBP - Yeast-29DAP)

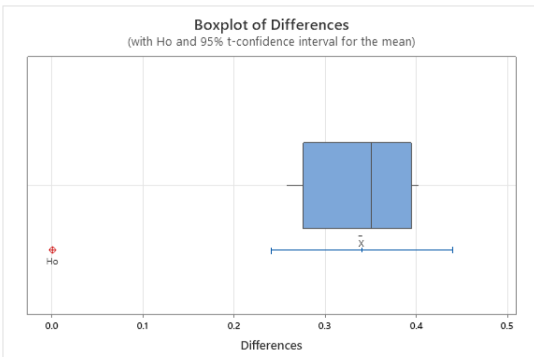
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
10.88	0.002







Paired T-Test and CI: Yeast-31DBP, Yeast-31DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-31DBP	4	4.71426	0.00891	0.00446
Yeast-31DAP	4	4.66004	0.01125	0.00562

Estimation for Paired Difference

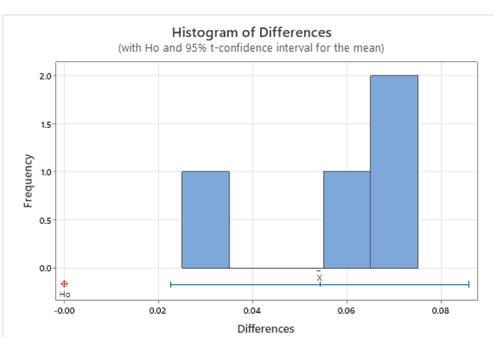
Mean	StDev	SE Mean	95% CI for μ_difference
0.05422	0.01988	0.00994	(0.02258, 0.08585)

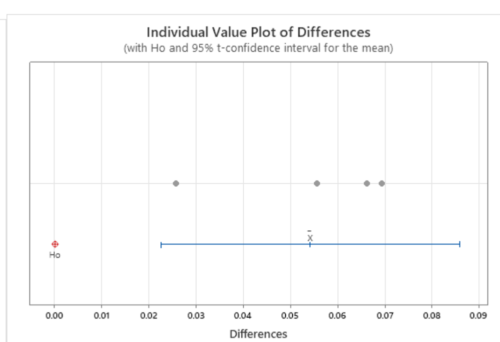
μ_difference: population mean of (Yeast-31DBP - Yeast-31DAP)

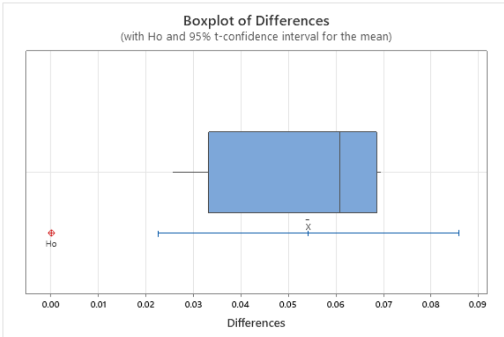
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
5.45	0.012







One-way ANOVA: LAB-27DAP-DBP, LAB-29DAP-DBP, LAB-31DAP-DBP

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	LAB-27DAP-DBP, LAB-29DAP-DBP, LAB-31DAP-DBP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.17832	0.089158	10.88	0.004
Error	9	0.07377	0.008197		
Total	11	0.25209			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0905385	70.73%	64.23%	47.97%

Means

Factor	N	Mean	StDev	95% CI
LAB-27DAP-DBP	4	0.5450	0.1060	(0.4426, 0.6474)
LAB-29DAP-DBP	4	0.3575	0.0727	(0.2551, 0.4599)
LAB-31DAP-DBP	4	0.2500	0.0898	(0.1476, 0.3524)

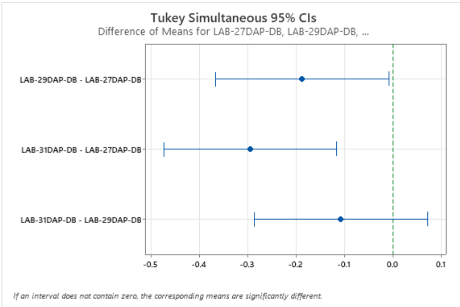
Pooled StDev = 0.0905385

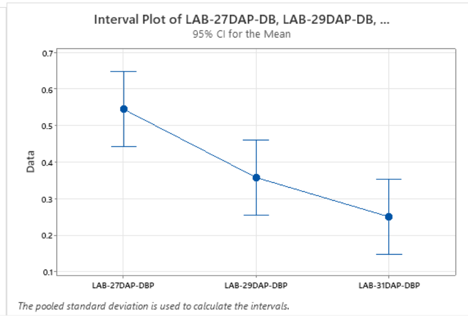
Tukey Pairwise Comparisons

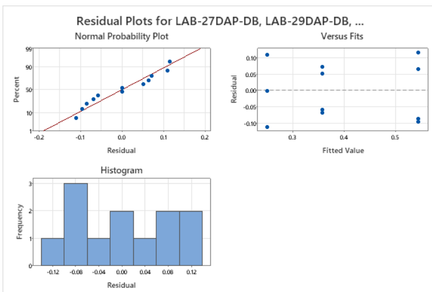
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
LAB-27DAP-DBP	4	0.5450	A	
LAB-29DAP-DBP	4	0.3575		B
LAB-31DAP-DBP	4	0.2500		B

Means that do not share a letter are significantly different.







One-way ANOVA: Yeast-27DAP-DBP, Yeast-29DAP-DBP, Yeast-31DAP-DBP

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	Yeast-27DAP-DBP, Yeast-29DAP-DBP, Yeast-31DAP-DBP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	2.24232	1.12116	617.15	0.000
Error	9	0.01635	0.00182		
Total	11	2.25867			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0426224	99.28%	99.12%	98.71%

Means

Factor	N	Mean	StDev	95% CI
Yeast-27DAP-DBP	4	1.0825	0.0377	(1.0343, 1.1307)
Yeast-29DAP-DBP	4	0.3400	0.0606	(0.2918, 0.3882)
Yeast-31DAP-DBP	4	0.05750	0.01893	(0.00929, 0.10571)

Pooled StDev = 0.0426224

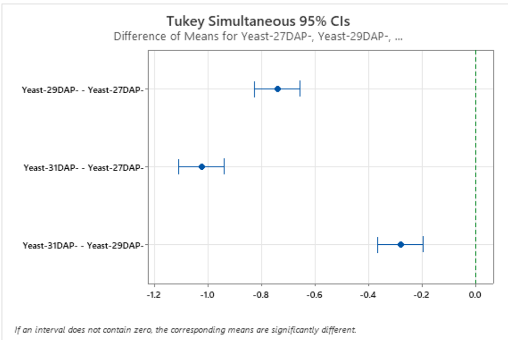
Tukey Pairwise Comparisons

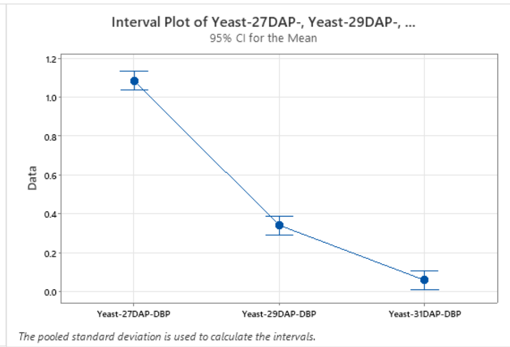
Grouping Information Using the Tukey Method and 95% Confidence

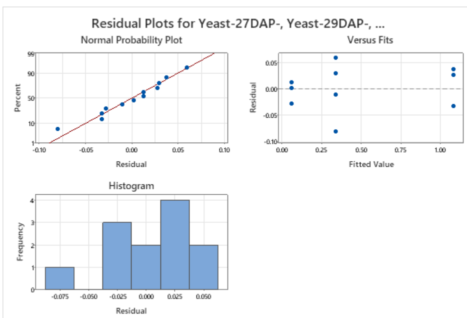
Factor	N	Mean	Grouping

Yeast-27DAP-DBP	4	1.0825	A		
Yeast-29DAP-DBP	4	0.3400		B	
Yeast-31DAP-DBP	4	0.05750			C

Means that do not share a letter are significantly different.







1. **Loaf volume, loaf weight, specific volume**

One-way ANOVA: 27SDB-Loaf volume, 29SDB-Loaf volume, 31SDB-Loaf volume

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-Loaf volume, 29SDB-Loaf volume, 31SDB-Loaf volume

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	16250	8125	1.58	0.258
Error	9	46250	5139		
Total	11	62500			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
71.6860	26.00%	9.56%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-Loaf volume	4	2237.5	103.1	(2156.4, 2318.6)
29SDB-Loaf volume	4	2175.0	28.9	(2093.9, 2256.1)
31SDB-Loaf volume	4	2262.5	62.9	(2181.4, 2343.6)

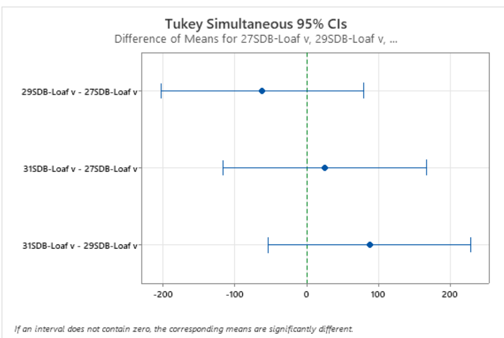
Pooled StDev = 71.6860

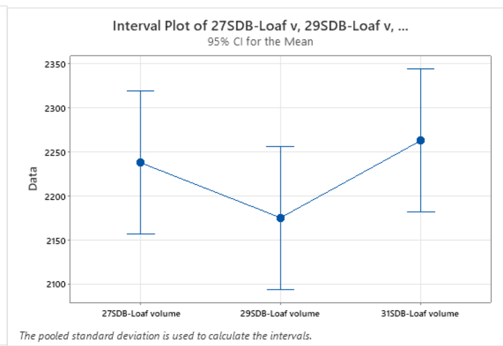
Tukey Pairwise Comparisons

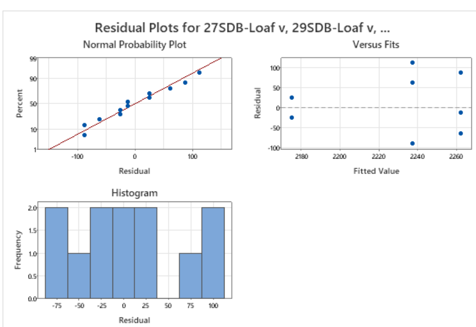
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-Loaf volume	4	2262.5	A
27SDB-Loaf volume	4	2237.5	A
29SDB-Loaf volume	4	2175.0	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-Loaf weight, 29SDB-Loaf weight, 31SDB-Loaf weight

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-Loaf weight, 29SDB-Loaf weight, 31SDB-Loaf weight

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	57.05	28.53	0.72	0.512
Error	9	355.69	39.52		
Total	11	412.74			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6.28661	13.82%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-Loaf weight	4	749.89	7.37	(742.78, 757.01)
29SDB-Loaf weight	4	753.15	7.37	(746.04, 760.26)
31SDB-Loaf weight	4	747.85	3.14	(740.74, 754.96)

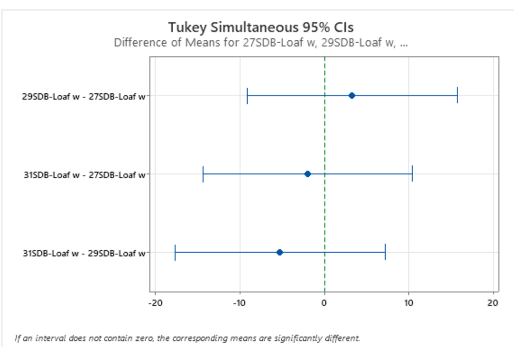
Pooled StDev = 6.28661

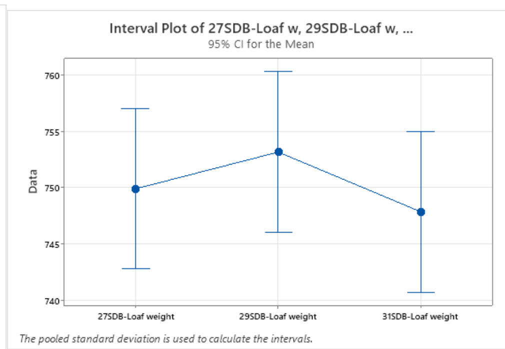
Tukey Pairwise Comparisons

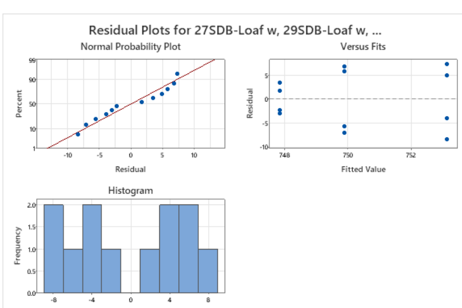
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
29SDB-Loaf weight	4	753.15	A
27SDB-Loaf weight	4	749.89	A
31SDB-Loaf weight	4	747.85	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-Specific volume, 29SDB-Specific volume, 31SDB-Specific volume

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-Specific volume, 29SDB-Specific volume, 31SDB-Specific volume

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.03963	0.019813	2.64	0.126
Error	9	0.06763	0.007514		
Total	11	0.10726			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0866856	36.95%	22.93%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-Specific volume	4	2.9830	0.1086	(2.8849, 3.0810)
29SDB-Specific volume	4	2.8881	0.0457	(2.7900, 2.9861)
31SDB-Specific volume	4	3.0255	0.0931	(2.9275, 3.1236)

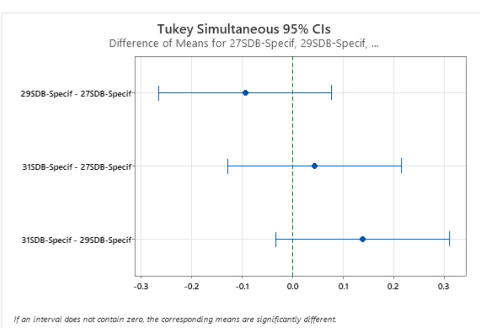
Pooled StDev = 0.0866856

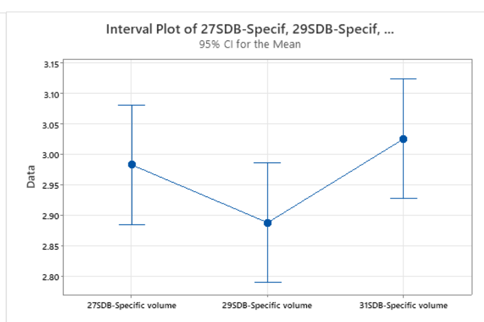
Tukey Pairwise Comparisons

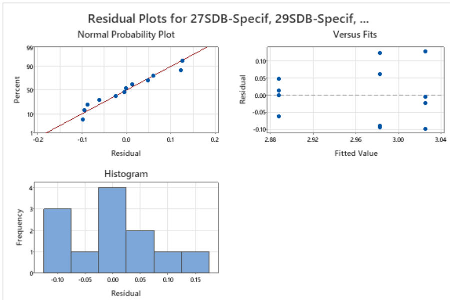
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
31SDB-Specific volume	4	3.0255	A
27SDB-Specific volume	4	2.9830	A
29SDB-Specific volume	4	2.8881	A

Means that do not share a letter are significantly different.







2. **Colour of sourdough bread**

One-way ANOVA: 27SDB-L*crumb, 29SDB-L*crumb, 31SDB-L*crumb

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-L*crumb, 29SDB-L*crumb, 31SDB-L*crumb

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	46.99	23.4958	26.95	0.000
Error	15	13.08	0.8717		
Total	17	60.07			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.933634	78.23%	75.33%	68.65%

Means

Factor	N	Mean	StDev	95% CI
27SDB-L*crumb	6	58.940	1.154	(58.128, 59.752)
29SDB-L*crumb	6	62.855	0.838	(62.043, 63.667)
31SDB-L*crumb	6	60.395	0.763	(59.583, 61.207)

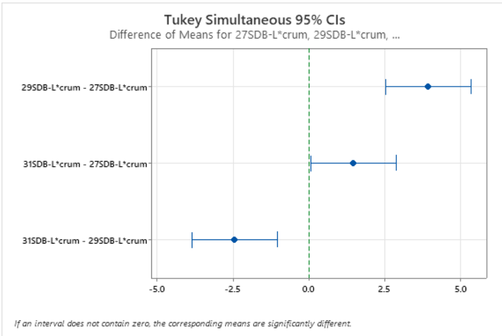
Pooled StDev = 0.933634

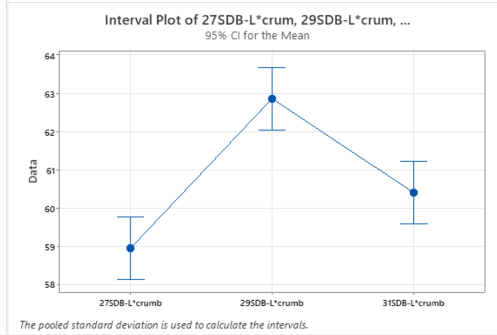
Tukey Pairwise Comparisons

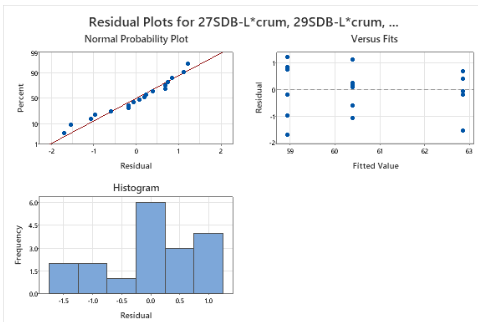
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
29SDB-L*crumb	6	62.855	A		
31SDB-L*crumb	6	60.395		B	
27SDB-L*crumb	6	58.940			C

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-a*crumb, 29SDB-a*crumb, 31SDB-a*crumb

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-a*crumb, 29SDB-a*crumb, 31SDB-a*crumb

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.2833	0.14167	8.58	0.003
Error	15	0.2476	0.01651		
Total	17	0.5310			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.128483	53.36%	47.15%	32.84%

Means

Factor	N	Mean	StDev	95% CI
27SDB-a*crumb	6	1.0650	0.1322	(0.9532, 1.1768)
29SDB-a*crumb	6	1.2667	0.1169	(1.1549, 1.3785)
31SDB-a*crumb	6	1.3667	0.1356	(1.2549, 1.4785)

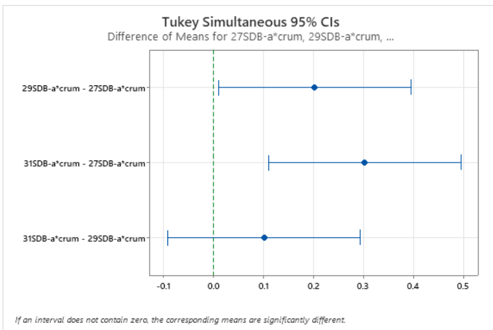
Pooled StDev = 0.128483

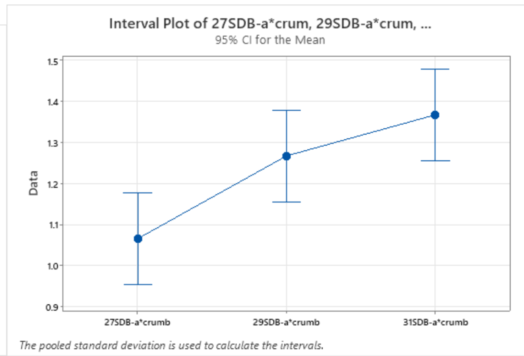
Tukey Pairwise Comparisons

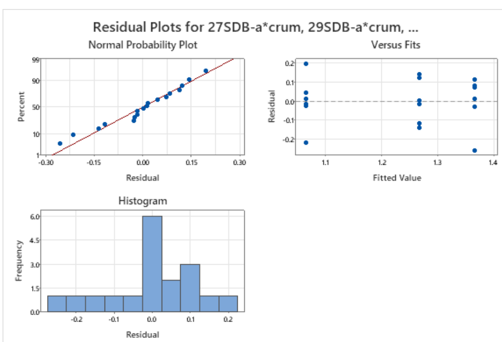
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
31SDB-a*crumb	6	1.3667	A	
29SDB-a*crumb	6	1.2667	A	
27SDB-a*crumb	6	1.0650		B

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-b*crumb, 29SDB-b*crumb, 31SDB-b*crumb

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-b*crumb, 29SDB-b*crumb, 31SDB-b*crumb

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	1.223	0.61157	9.10	0.003
Error	15	1.009	0.06724		
Total	17	2.232			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.259311	54.81%	48.78%	34.92%

Means

Factor	N	Mean	StDev	95% CI
27SDB-b*crumb	6	10.620	0.402	(10.394, 10.846)
29SDB-b*crumb	6	11.2517	0.1418	(11.0260, 11.4773)
31SDB-b*crumb	6	10.8550	0.1424	(10.6294, 11.0806)

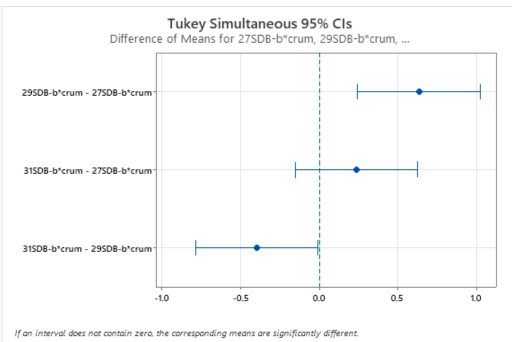
Pooled StDev = 0.259311

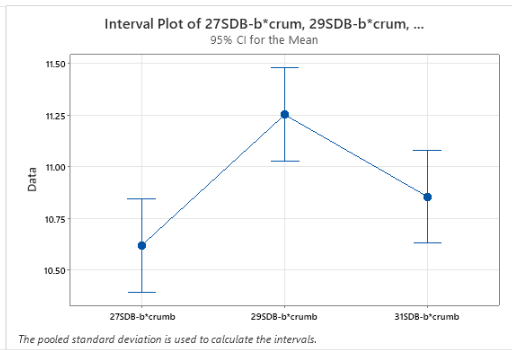
Tukey Pairwise Comparisons

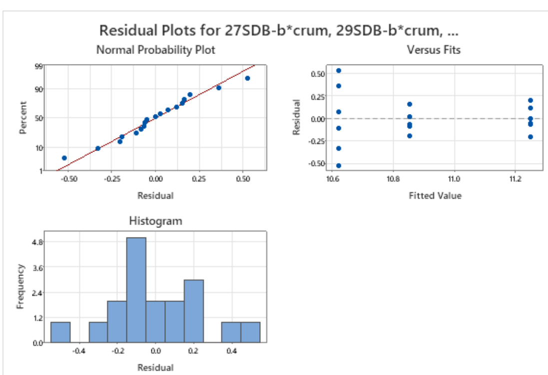
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
29SDB-b*crumb	6	11.2517	A	
31SDB-b*crumb	6	10.8550		B
27SDB-b*crumb	6	10.620		B

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-L*crust, 29SDB-L*crust, 31SDB-L*crust

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-L*crust, 29SDB-L*crust, 31SDB-L*crust

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	26.19	13.097	3.30	0.065
Error	15	59.51	3.967		
Total	17	85.70			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.99183	30.56%	21.30%	0.01%

Means

Factor	N	Mean	StDev	95% CI
27SDB-L*crust	6	31.93	2.98	(30.20, 33.66)
29SDB-L*crust	6	29.265	1.225	(27.532, 30.998)
31SDB-L*crust	6	31.705	1.233	(29.972, 33.438)

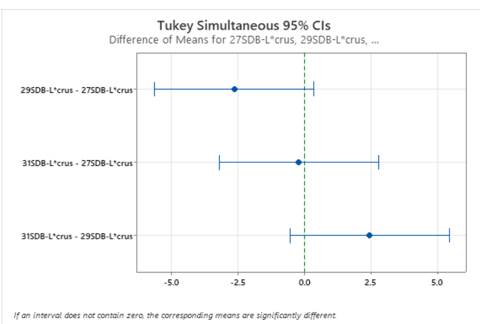
Pooled StDev = 1.99183

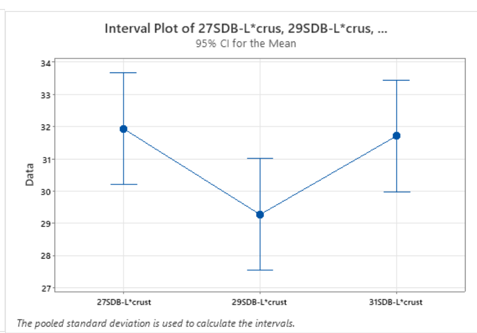
Tukey Pairwise Comparisons

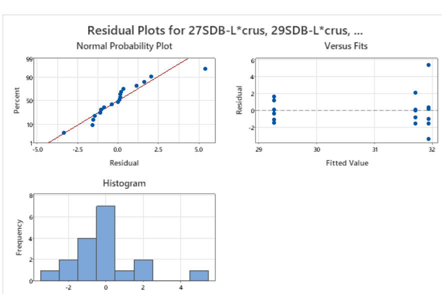
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
27SDB-L*crust	6	31.93	A
31SDB-L*crust	6	31.705	A
29SDB-L*crust	6	29.265	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-a*crust, 29SDB-a*crust, 31SDB-a*crust

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-a*crust, 29SDB-a*crust, 31SDB-a*crust

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.3998	0.1999	0.25	0.785
Error	15	12.1578	0.8105		
Total	17	12.5576			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.900289	3.18%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-a*crust	6	7.877	1.265	(7.093, 8.660)
29SDB-a*crust	6	7.512	0.616	(6.728, 8.295)
31SDB-a*crust	6	7.700	0.672	(6.917, 8.483)

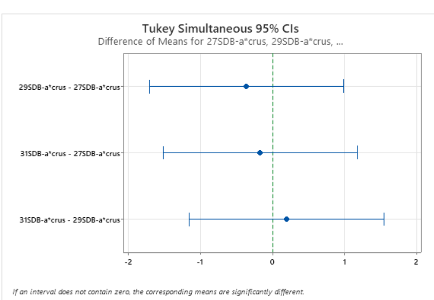
Pooled StDev = 0.900289

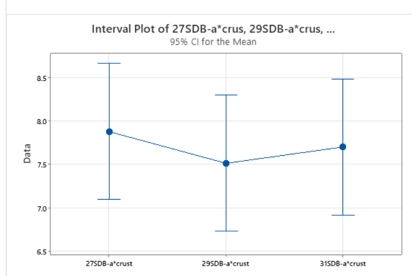
Tukey Pairwise Comparisons

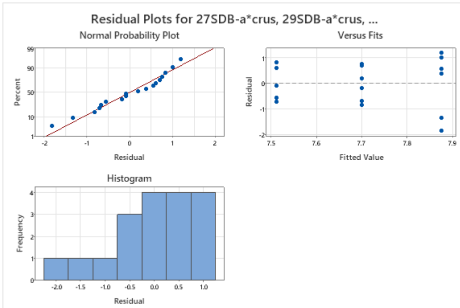
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
27SDB-a*crust	6	7.877	A
31SDB-a*crust	6	7.700	A
29SDB-a*crust	6	7.512	A

Means that do not share a letter are significantly different.







One-way ANOVA: 27SDB-b*crust, 29SDB-b*crust, 31SDB-b*crust

Method

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

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	27SDB-b*crust, 29SDB-b*crust, 31SDB-b*crust

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	6.630	3.315	1.53	0.249
Error	15	32.499	2.167		
Total	17	39.129			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47195	16.94%	5.87%	0.00%

Means

Factor	N	Mean	StDev	95% CI
27SDB-b*crust	6	7.443	2.212	(6.163, 8.724)
29SDB-b*crust	6	5.980	0.881	(4.699, 7.261)
31SDB-b*crust	6	6.938	0.911	(5.658, 8.219)

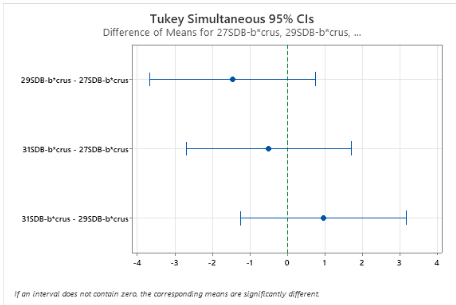
Pooled StDev = 1.47195

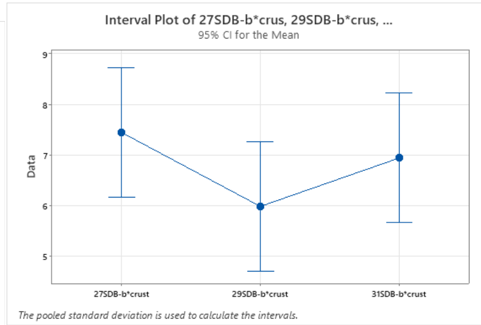
Tukey Pairwise Comparisons

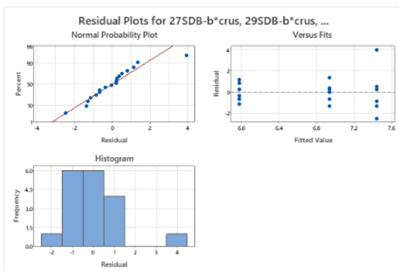
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
27SDB-b*crust	6	7.443	A
31SDB-b*crust	6	6.938	A
29SDB-b*crust	6	5.980	A

Means that do not share a letter are significantly different.







Phase III

1. **Acidity**

Paired T-Test and CI: DBP-pH, DAP-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DBP-pH	6	4.7517	0.0098	0.0040
DAP-pH	6	3.7300	0.0329	0.0134

Estimation for Paired Difference

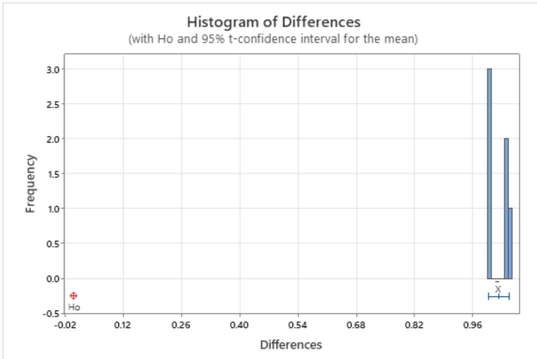
Mean	StDev	SE Mean	95% CI for μ_difference
1.02167	0.02401	0.00980	(0.99647, 1.04687)

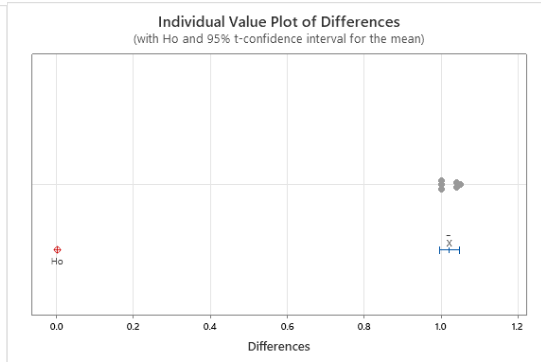
μ_difference: population mean of (DBP-pH - DAP-pH)

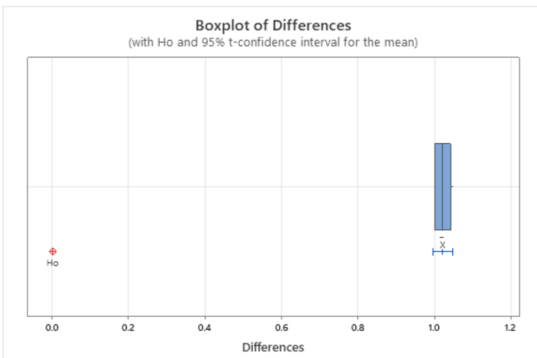
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
104.21	0.000







Paired T-Test and CI: DAP-pH, SDB-pH

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DAP-pH	6	3.7300	0.0329	0.0134
SDB-pH	6	3.8317	0.0172	0.0070

Estimation for Paired Difference

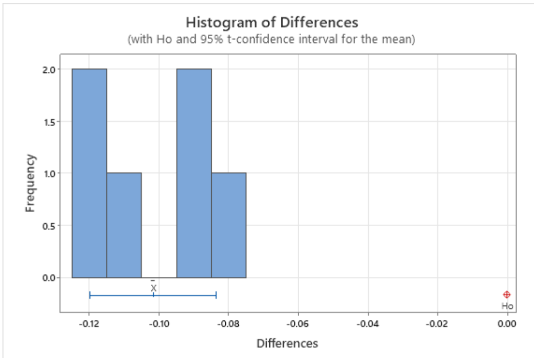
Mean	StDev	SE Mean	95% CI for μ_difference
-0.10167	0.01722	0.00703	(-0.11974, -0.08359)

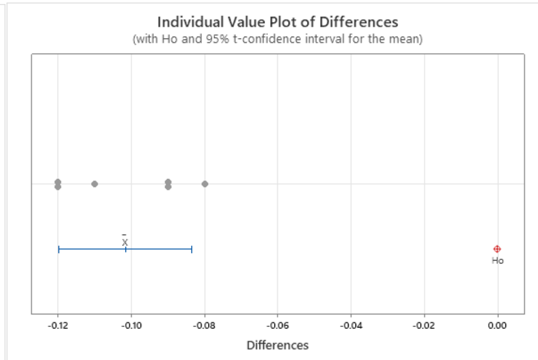
μ_difference: population mean of (DAP-pH - SDB-pH)

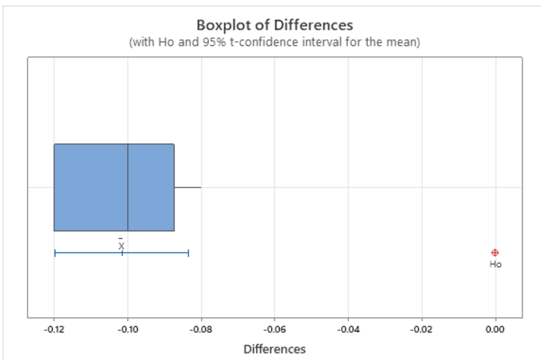
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-14.46	0.000







Paired T-Test and CI: DBP-TTA, DAP-TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DBP-TTA	6	0.3895	0.0515	0.0210
DAP-TTA	6	1.0436	0.0772	0.0315

Estimation for Paired Difference

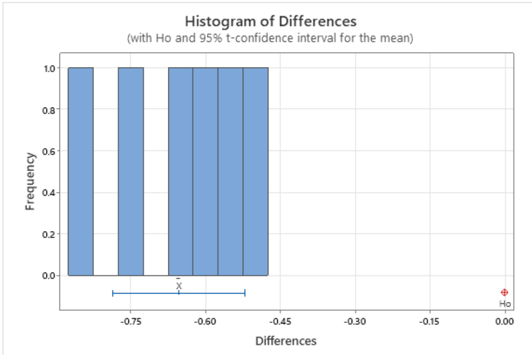
Mean	StDev	SE Mean	95% CI for μ_difference
-0.6541	0.1260	0.0514	(-0.7863, -0.5218)

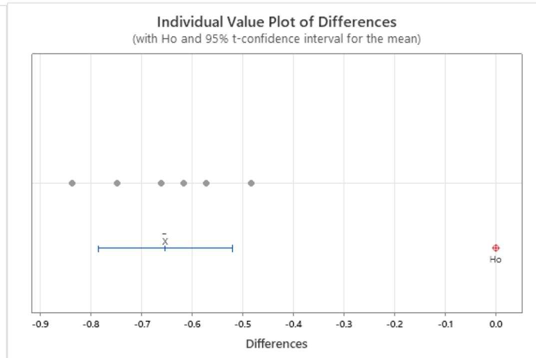
μ_difference: population mean of (DBP-TTA - DAP-TTA)

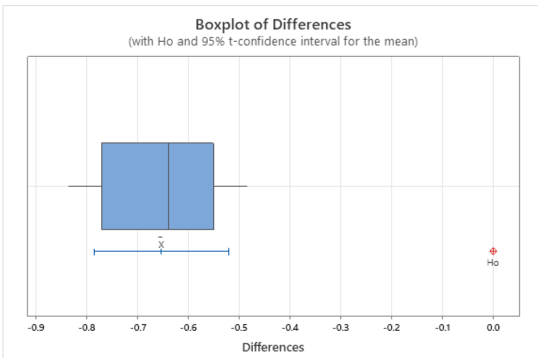
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-12.71	0.000







Paired T-Test and CI: DAP-TTA, SDB-TTA

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
DAP-TTA	6	1.0436	0.0772	0.0315
SDB-TTA	6	0.7937	0.0394	0.0161

Estimation for Paired Difference

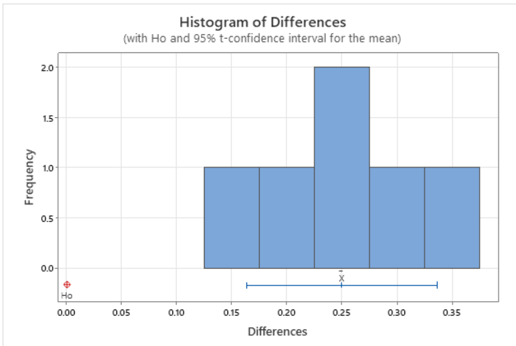
Mean	StDev	SE Mean	95% CI for μ_difference
0.2499	0.0821	0.0335	(0.1637, 0.3360)

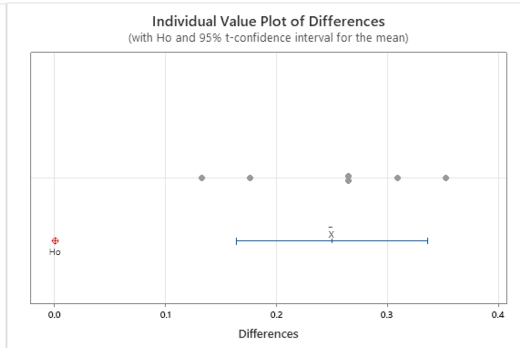
μ_difference: population mean of (DAP-TTA - SDB-TTA)

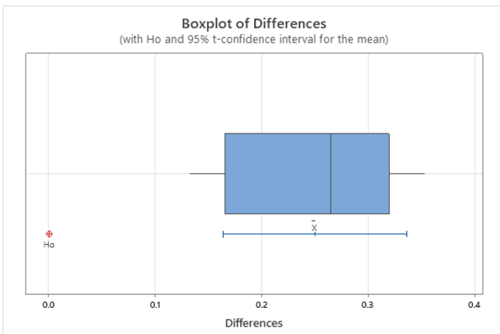
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
7.45	0.001







2. Texture

2.1. DBP vs DAP

Paired T-Test and CI: Hardness-DBP, Hardness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Hardness-DBP	6	762.4	37.2	15.2
Hardness-DAP	6	622.8	45.9	18.7

Estimation for Paired Difference

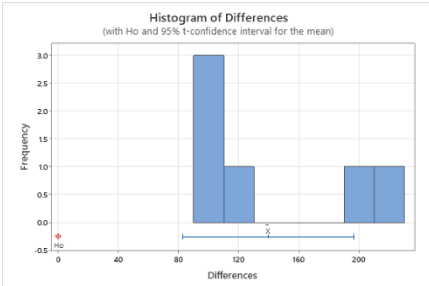
Mean	StDev	SE Mean	95% CI for μ_difference
139.6	54.3	22.2	(82.6, 196.6)

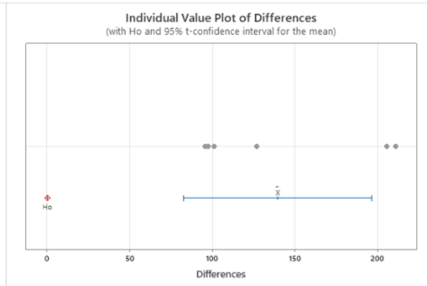
μ_difference: population mean of (Hardness-DBP - Hardness-DAP)

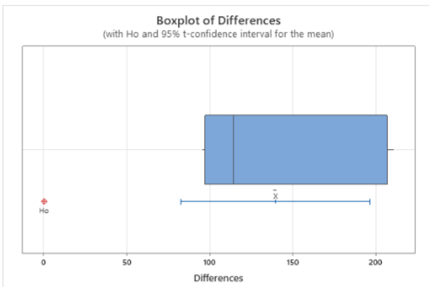
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
6.30	0.001







Paired T-Test and CI: Adhesiveness-DBP, Adhesiveness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Adhesiveness-DBP	6	-922.1	135.3	55.2
Adhesiveness-DAP	6	-589.0	51.1	20.8

Estimation for Paired Difference

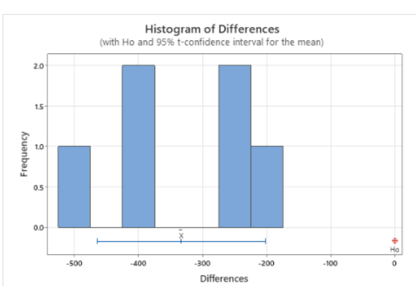
Mean	StDev	SE Mean	95% CI for μ_difference
-333.2	125.3	51.1	(-464.6, -201.7)

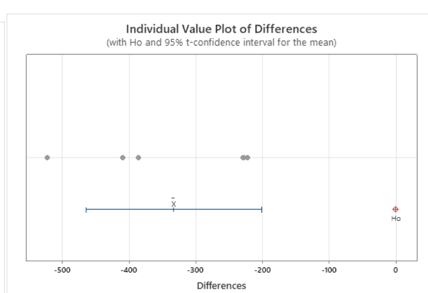
μ_difference: population mean of (Adhesiveness-DBP - Adhesiveness-DAP)

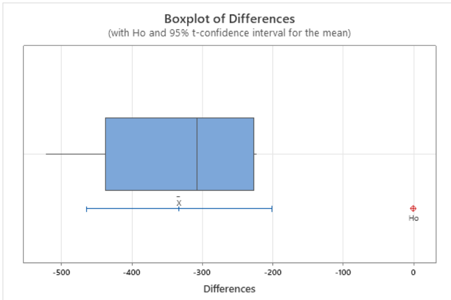
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-6.51	0.001







Paired T-Test and CI: Springiness-DBP, Springiness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Springiness-DBP	6	0.97600	0.00701	0.00286
Springiness-DAP	6	0.98250	0.00362	0.00148

Estimation for Paired Difference

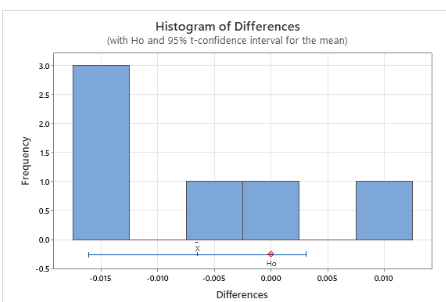
Mean	StDev	SE Mean	95% CI for μ_difference
-0.00650	0.00914	0.00373	(-0.01609, 0.00309)

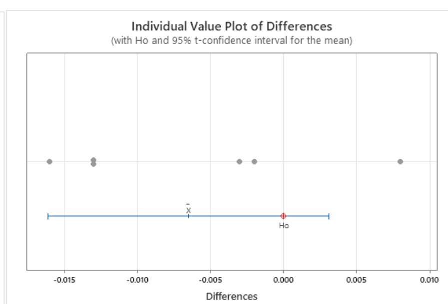
μ_difference: population mean of (Springiness-DBP - Springiness-DAP)

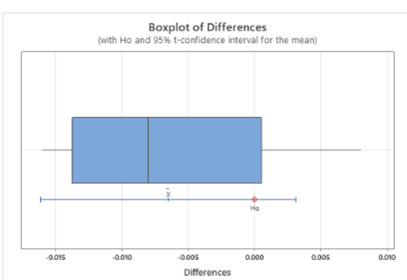
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-1.74	0.142







Paired T-Test and CI: Cohesiveness-DBP, Cohesiveness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Cohesiveness-DBP	6	0.75750	0.01810	0.00739
Cohesiveness-DAP	6	0.69100	0.01304	0.00532

Estimation for Paired Difference

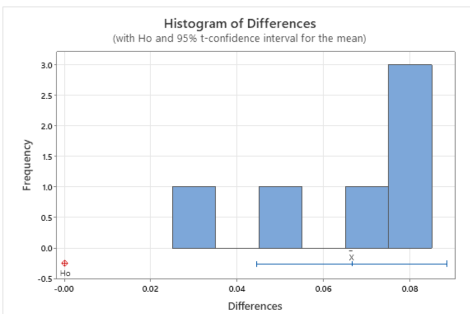
Mean	StDev	SE Mean	95% CI for μ_difference
0.06650	0.02101	0.00858	(0.04445, 0.08855)

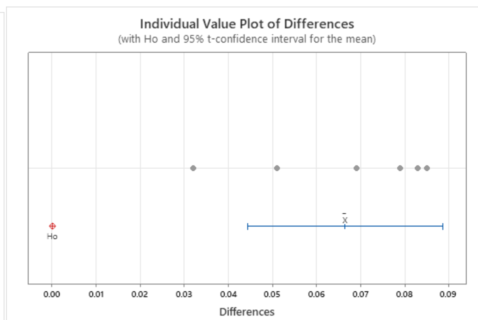
μ_difference: population mean of (Cohesiveness-DBP - Cohesiveness-DAP)

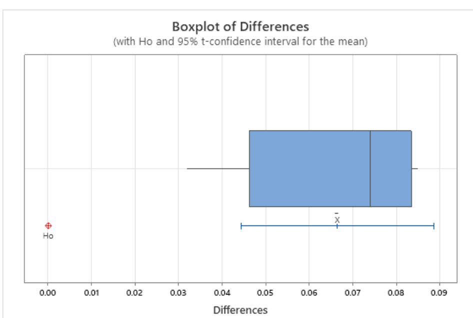
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
7.75	0.001







Paired T-Test and CI: Gumminess-DBP, Gumminess-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Gumminess-DBP	6	577.8	39.0	15.9
Gumminess-DAP	6	430.1	30.8	12.6

Estimation for Paired Difference

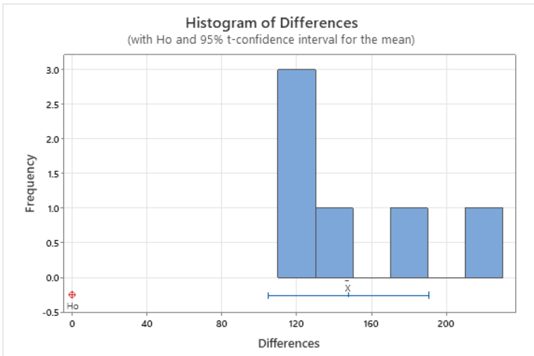
Mean	StDev	SE Mean	95% CI for μ_difference
147.7	40.9	16.7	(104.8, 190.6)

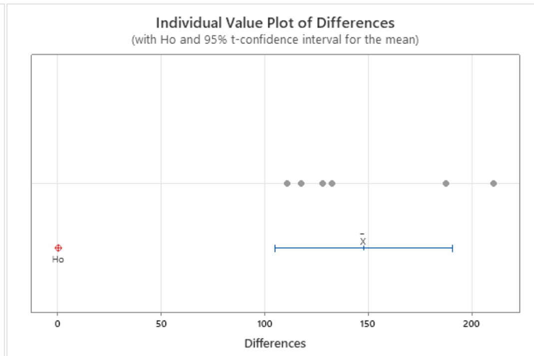
μ_difference: population mean of (Gumminess-DBP - Gumminess-DAP)

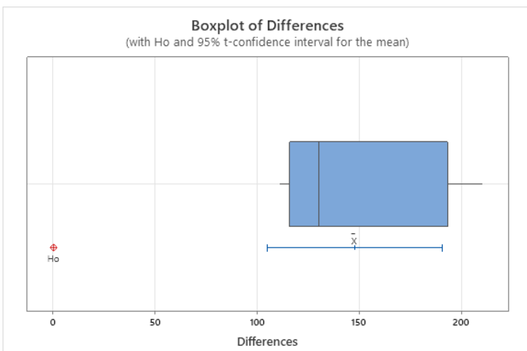
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
8.84	0.000







Paired T-Test and CI: Chewiness-DBP, Chewiness-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Chewiness-DBP	6	563.9	38.0	15.5
Chewiness-DAP	6	422.7	31.2	12.7

Estimation for Paired Difference

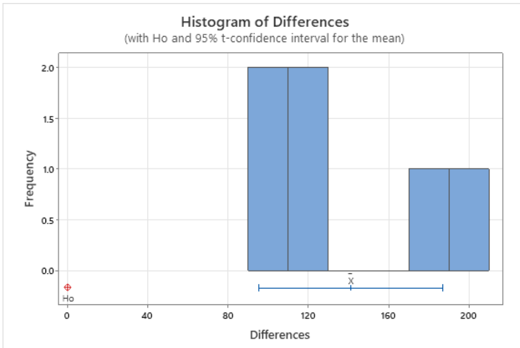
Mean	StDev	SE Mean	95% CI for μ_difference
141.3	43.7	17.8	(95.4, 187.1)

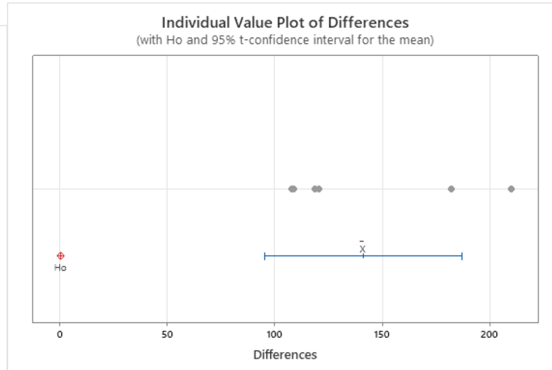
μ_difference: population mean of (Chewiness-DBP - Chewiness-DAP)

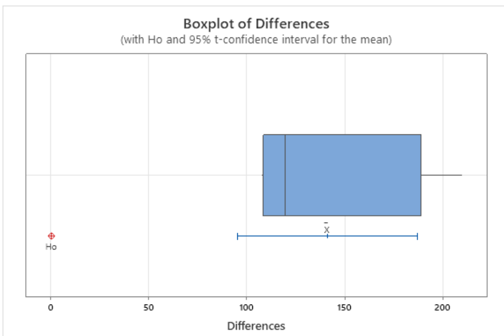
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
7.92	0.001







Paired T-Test and CI: Resilience-DBP, Resilience-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Resilience-DBP	6	0.10317	0.00598	0.00244
Resilience-DAP	6	0.09783	0.00426	0.00174

Estimation for Paired Difference

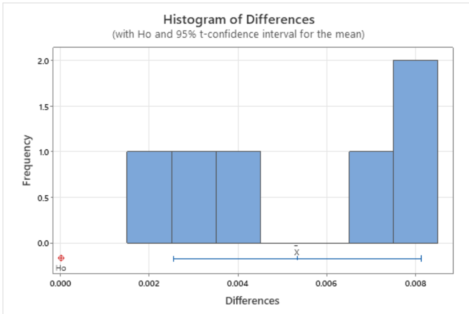
Mean	StDev	SE Mean	95% CI for μ_difference
0.00533	0.00266	0.00109	(0.00254, 0.00812)

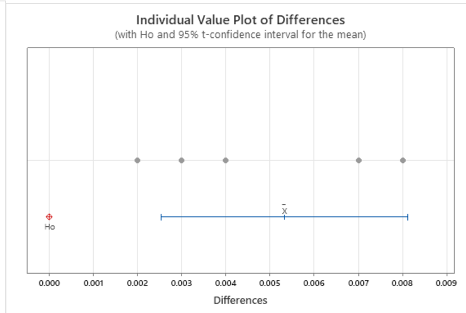
μ_difference: population mean of (Resilience-DBP - Resilience-DAP)

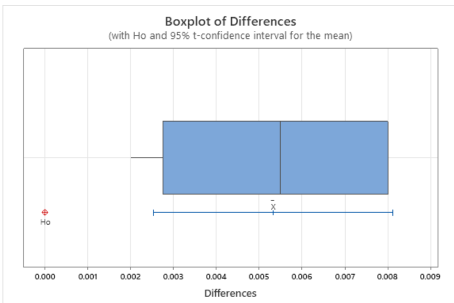
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
4.91	0.004







Paired T-Test and CI: Hardness-DAP, Hardness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Hardness-DAP	6	622.8	45.9	18.7
Hardness-SDB	6	1138.8	32.9	13.4

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
-516.0	41.3	16.8	(-559.3, -472.7)

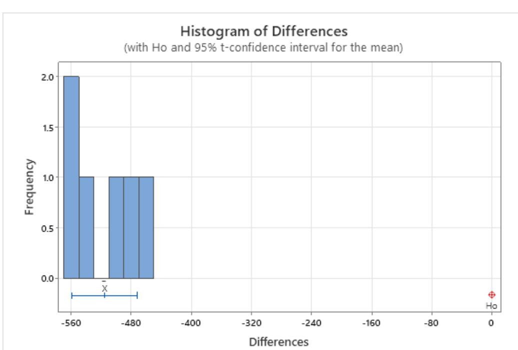
μ_difference: population mean of (Hardness-DAP - Hardness-SDB)

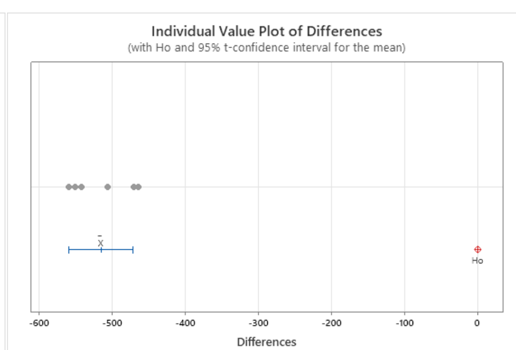
Test

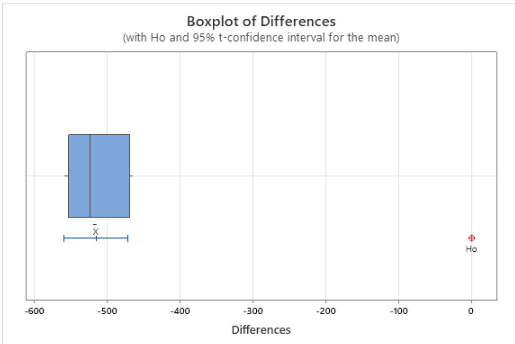
Null hypothesis H₀: μ_difference = 0

Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-30.64	0.000







Paired T-Test and CI: Adhesiveness-DAP, Adhesiveness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Adhesiveness-DAP	6	-589.0	51.1	20.8
Adhesiveness-SDB	6	-1.2	0.3	0.1

Estimation for Paired Difference

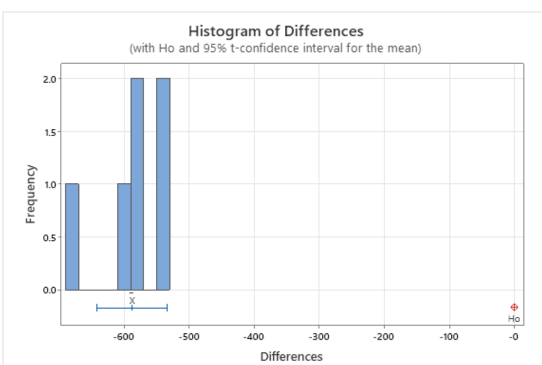
Mean	StDev	SE Mean	95% CI for μ_difference
-587.7	51.0	20.8	(-641.3, -534.2)

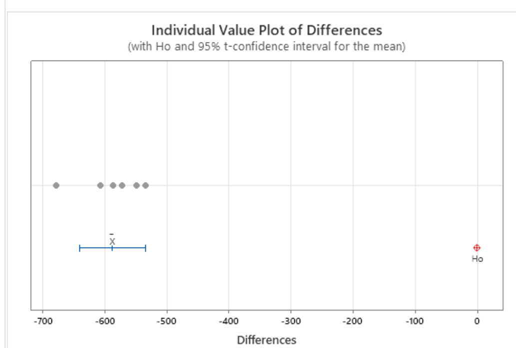
μ_difference: population mean of (Adhesiveness-DAP - Adhesiveness-SDB)

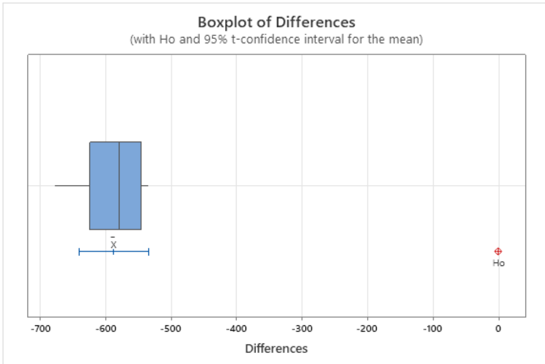
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-28.22	0.000







Paired T-Test and CI: Springiness-DAP, Springiness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Springiness-DAP	6	0.983	0.004	0.001
Springiness-SDB	6	1.542	0.770	0.315

Estimation for Paired Difference

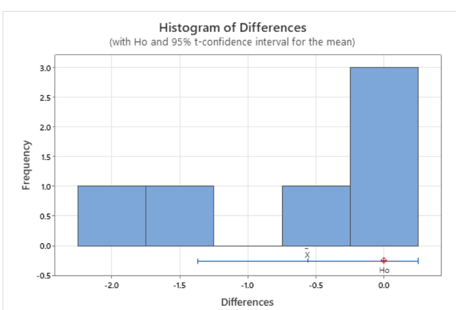
Mean	StDev	SE Mean	95% CI for μ_difference
-0.559	0.773	0.315	(-1.370, 0.251)

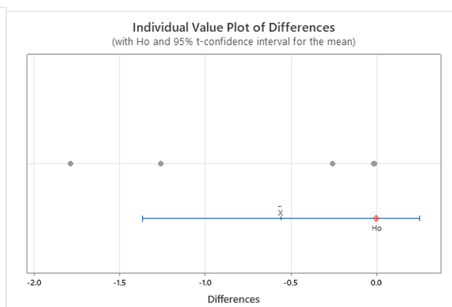
μ_difference: population mean of (Springiness-DAP - Springiness-SDB)

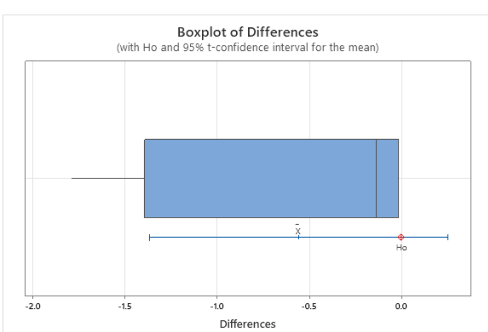
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-1.77	0.136







Paired T-Test and CI: Cohesiveness-DAP, Cohesiveness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Cohesiveness-DAP	6	0.69100	0.01304	0.00532
Cohesiveness-SDB	6	0.73800	0.01156	0.00472

Estimation for Paired Difference

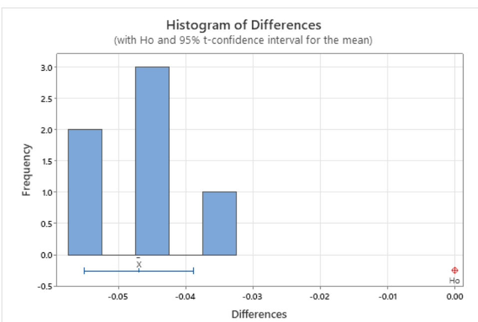
Mean	StDev	SE Mean	95% CI for μ_difference
-0.04700	0.00777	0.00317	(-0.05516, -0.03884)

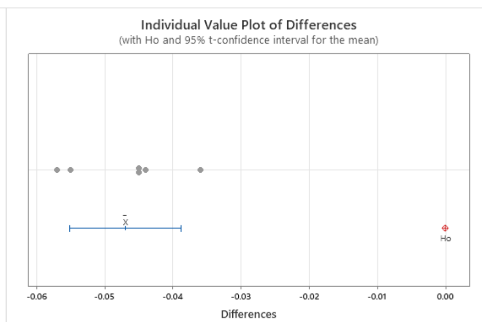
μ_difference: population mean of (Cohesiveness-DAP - Cohesiveness-SDB)

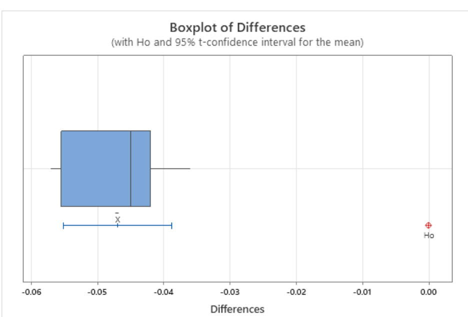
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-14.81	0.000







Paired T-Test and CI: Gumminess-DAP, Gumminess-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Gumminess-DAP	6	430.1	30.8	12.6
Gumminess-SDB	6	840.5	30.3	12.4

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference

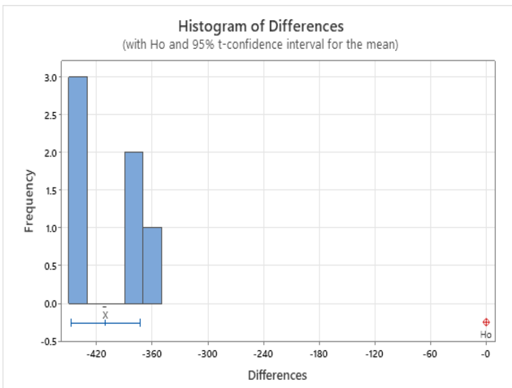
-410.3	35.3	14.4	(-447.4, -373.3)

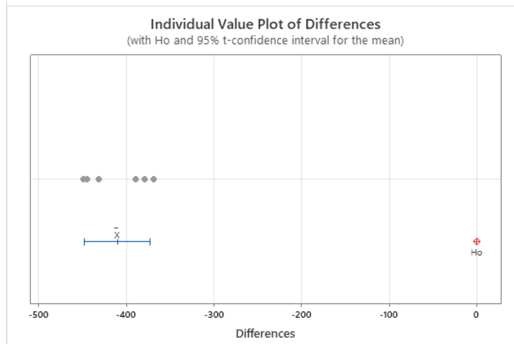
μ_difference: population mean of (Gumminess-DAP - Gumminess-SDB)

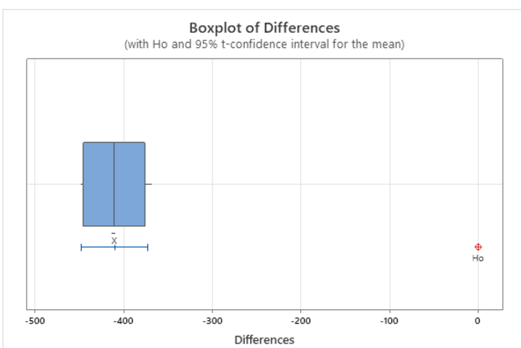
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-28.49	0.000







Paired T-Test and CI: Chewiness-DAP, Chewiness-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Chewiness-DAP	6	423	31	13
Chewiness-SDB	6	1287	622	254

Estimation for Paired Difference

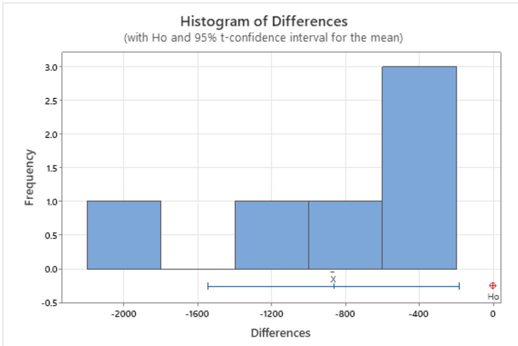
Mean	StDev	SE Mean	95% CI for μ_difference
-864	650	265	(-1546, -182)

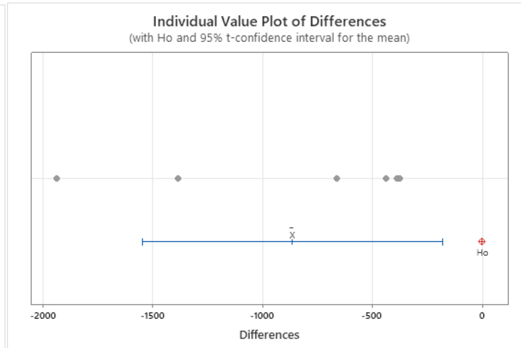
μ_difference: population mean of (Chewiness-DAP - Chewiness-SDB)

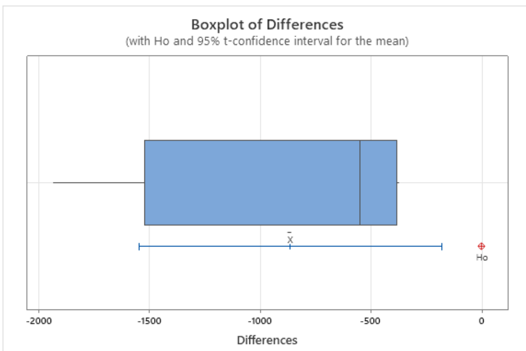
Test

Null hypothesis	H₀: μ_difference = 0
Alternative hypothesis	H₁: μ_difference ≠ 0

T-Value	P-Value
-3.26	0.022







Paired T-Test and CI: Resilience-DAP, Resilience-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Resilience-DAP	6	0.09783	0.00426	0.00174
Resilience-SDB	6	0.41883	0.01100	0.00449

Estimation for Paired Difference

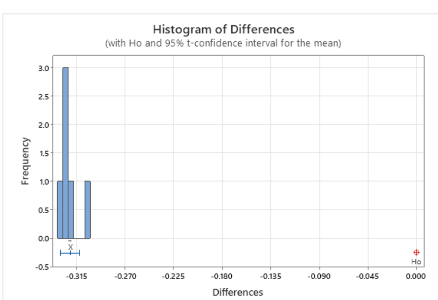
Mean	StDev	SE Mean	95% CI for μ_difference
-0.32100	0.00865	0.00353	(-0.33008, -0.31192)

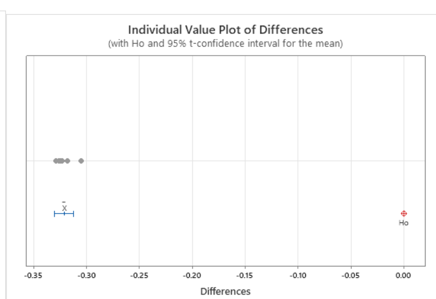
μ_difference: population mean of (Resilience-DAP - Resilience-SDB)

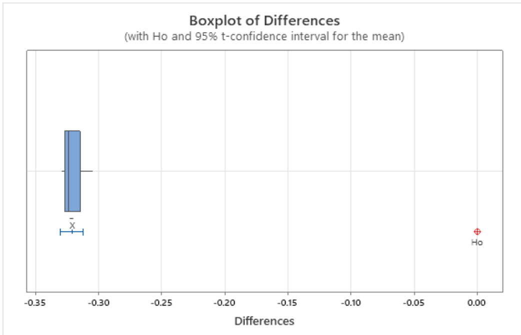
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-90.91	0.000







1. **Microbiology**

Paired T-Test and CI: LAB-DBP, LAB-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
LAB-DBP	4	8.4153	0.0883	0.0442
LAB-DAP	4	8.8235	0.0335	0.0167

Estimation for Paired Difference

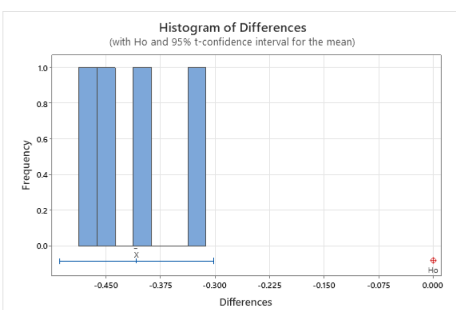
Mean	StDev	SE Mean	95% CI for μ_difference
-0.4082	0.0665	0.0333	(-0.5140, -0.3023)

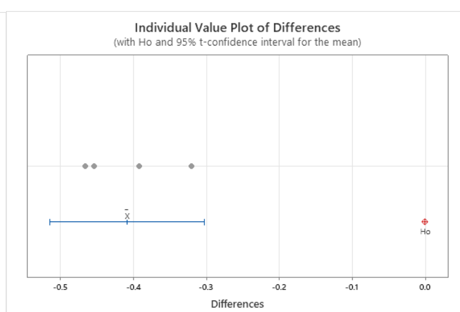
μ_difference: population mean of (LAB-DBP - LAB-DAP)

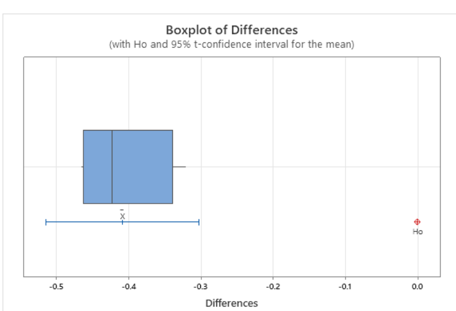
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
-12.27	0.001







Paired T-Test and CI: Yeast-DBP, Yeast-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Yeast-DBP	4	4.3969	0.0647	0.0324
Yeast-DAP	4	2.7462	0.0949	0.0474

Estimation for Paired Difference

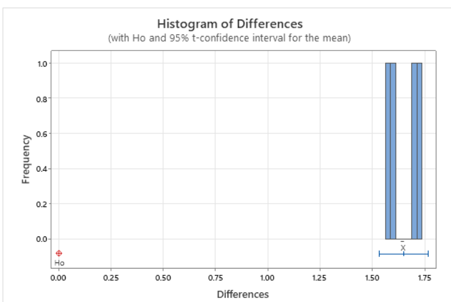
Mean	StDev	SE Mean	95% CI for μ_difference
1.6507	0.0734	0.0367	(1.5339, 1.7674)

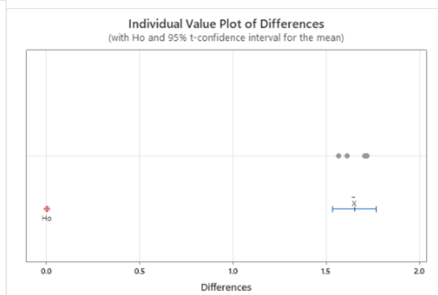
μ_difference: population mean of (Yeast-DBP - Yeast-DAP)

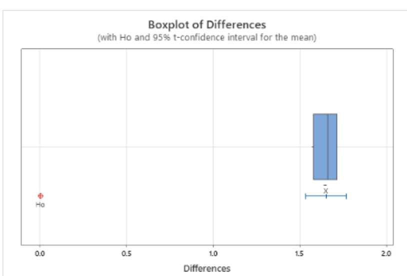
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
44.99	0.000







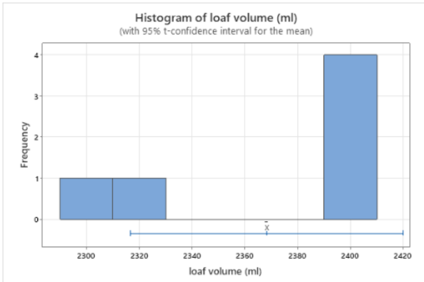
1. **Loaf volume, loaf weight, specific volume**

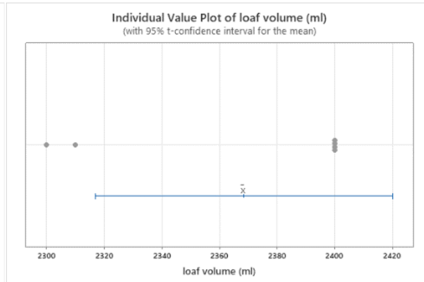
One-Sample T: loaf volume (ml)

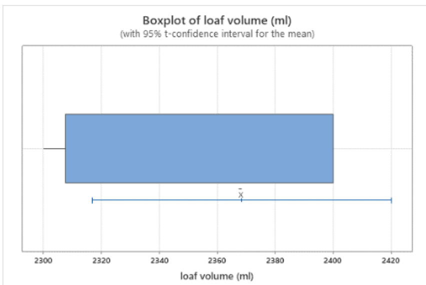
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	2368.3	49.2	20.1	(2316.7, 2419.9)

μ: population mean of loaf volume (ml)





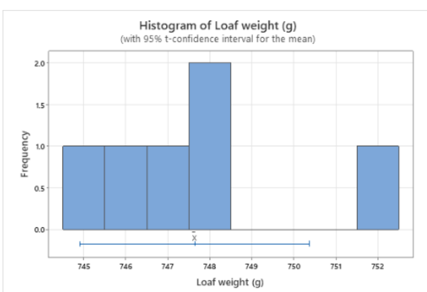


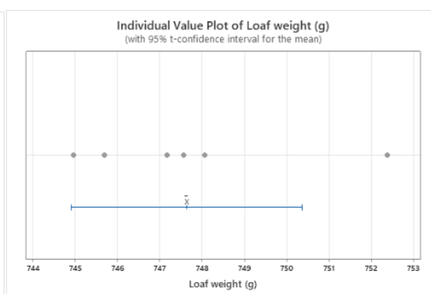
One-Sample T: Loaf weight (g)

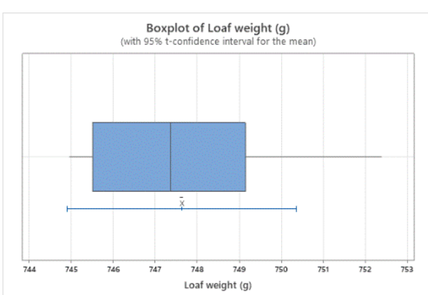
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	747.64	2.60	1.06	(744.91, 750.37)

μ: population mean of Loaf weight (g)





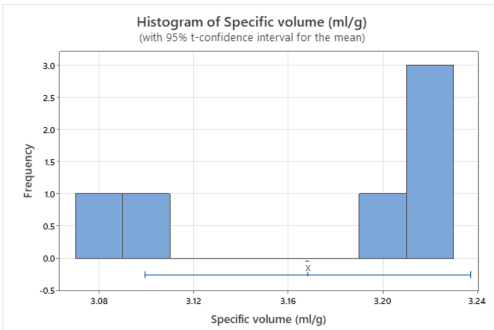


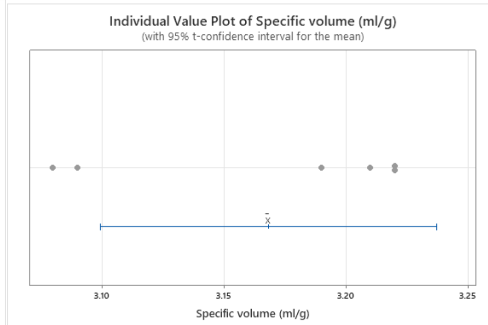
One-Sample T: Specific volume (ml/g)

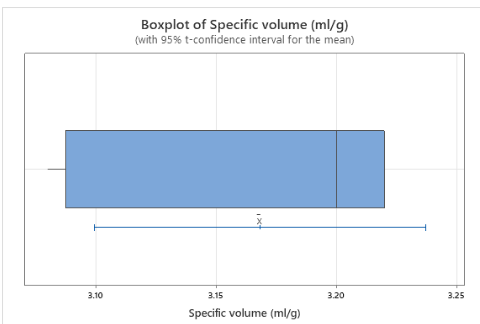
Descriptive Statistics

N	Mean	StDev	SE Mean	95% CI for μ
6	3.1683	0.0655	0.0268	(3.0995, 3.2371)

μ: population mean of Specific volume (ml/g)







2. Colour of sourdough bread

Two-Sample T-Test and CI: L*crumb, L*crust

Method

μ_1: population mean of L*crumb
μ_2: population mean of L*crust
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
L*crumb	6	59.64	1.38	0.56
L*crust	6	27.75	1.18	0.48

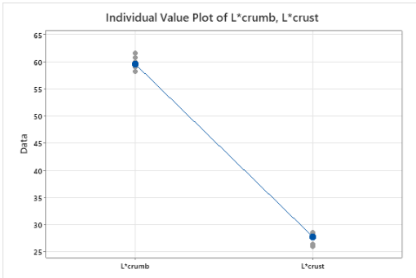
Estimation for Difference

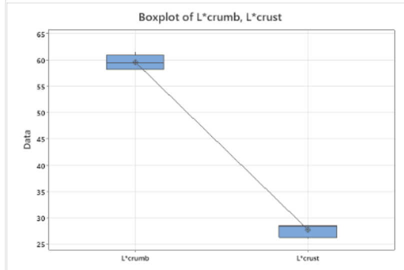
Difference	95% CI for Difference
31.895	(30.213, 33.577)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
42.89	9	0.000





Two-Sample T-Test and CI: a*crumb, a*crust

Method

μ_1: population mean of a*crumb
μ_2: population mean of a*crust
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
a*crumb	6	0.6317	0.0741	0.030
a*crust	6	6.182	0.754	0.31

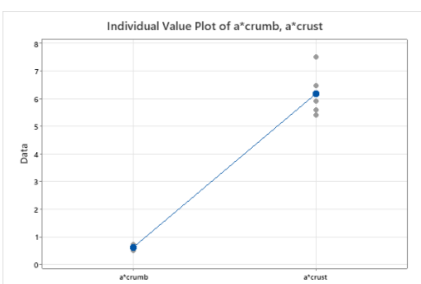
Estimation for Difference

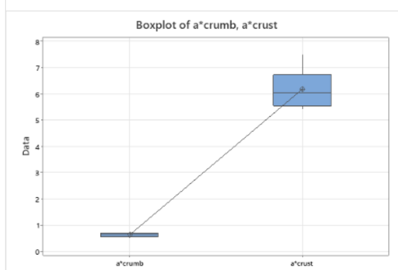
Difference	95% CI for Difference
-5.550	(-6.346, -4.754)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-17.93	5	0.000





Two-Sample T-Test and CI: b*crumb, b*crust

Method

μ_1: population mean of b*crumb
μ_2: population mean of b*crust
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
b*crumb	6	9.932	0.285	0.12
b*crust	6	5.248	0.874	0.36

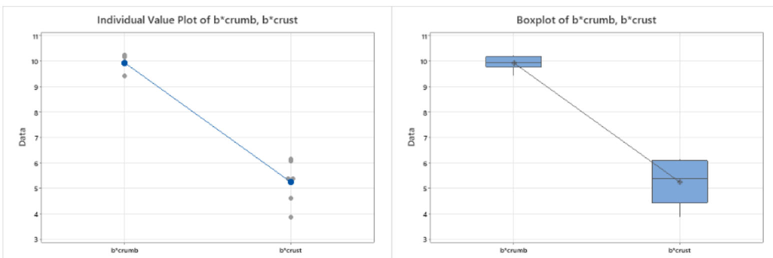
Estimation for Difference

Difference	95% CI for Difference
4.683	(3.765, 5.602)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
12.47	6	0.000



3. **Organic acids**

Paired T-Test and CI: lactic acid-DBP, lactic acid-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
lactic acid-DBP	6	0.1300	0.0200	0.0082
lactic acid-DAP	6	0.4067	0.1240	0.0506

Estimation for Paired Difference

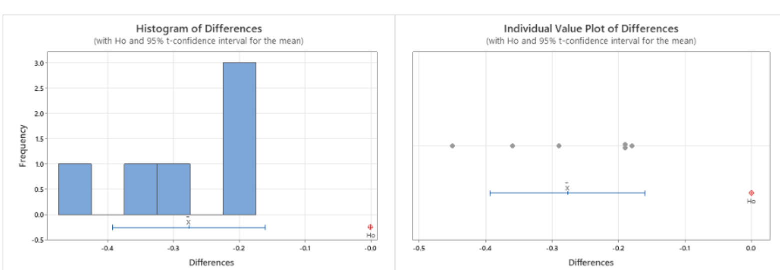
Mean	StDev	SE Mean	95% CI for μ_difference
-0.2767	0.1109	0.0453	(-0.3931, -0.1602)

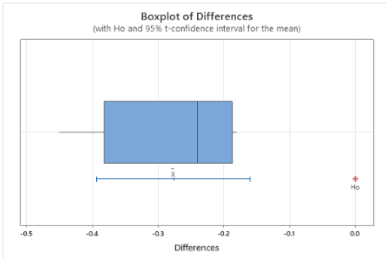
μ_difference: population mean of (lactic acid-DBP - lactic acid-DAP)

Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
-6.11	0.002





Paired T-Test and CI: lactic acid-DAP, lactic acid-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
lactic acid-DAP	6	0.4067	0.1240	0.0506
lactic acid-SDB	6	0.3867	0.1206	0.0492

Estimation for Paired Difference

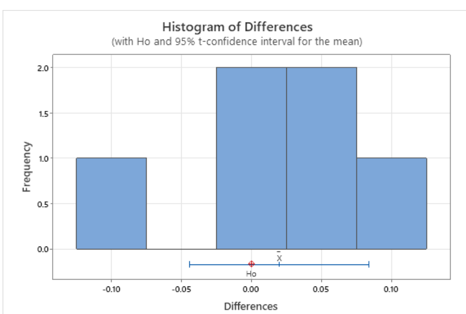
Mean	StDev	SE Mean	95% CI for μ_difference
0.0200	0.0610	0.0249	(-0.0440, 0.0840)

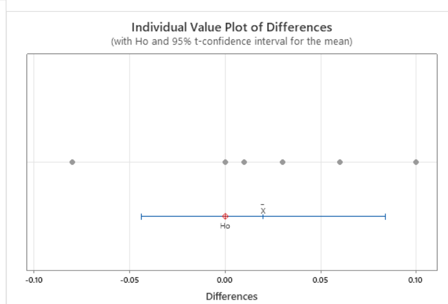
μ_difference: population mean of (lactic acid-DAP - lactic acid-SDB)

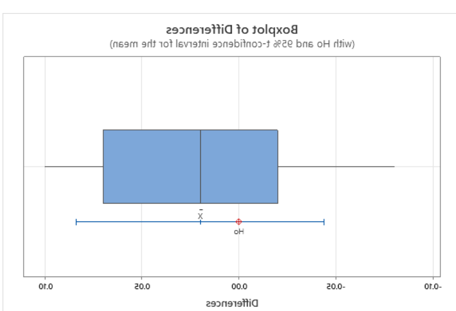
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
0.80	0.458







Paired T-Test and CI: acetic acid-DBP, acetic acid-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
acetic acid-DBP	6	0.7900	0.1270	0.0518
acetic acid-DAP	6	0.2333	0.0709	0.0289

Estimation for Paired Difference

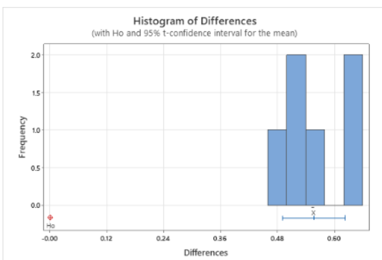
Mean	StDev	SE Mean	95% CI for μ_difference
0.5567	0.0628	0.0256	(0.4907, 0.6226)

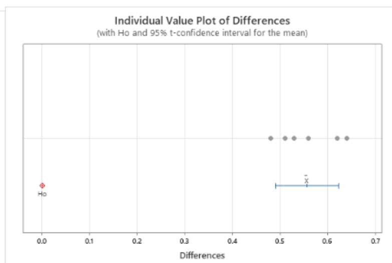
μ_difference: population mean of (acetic acid-DBP - acetic acid-DAP)

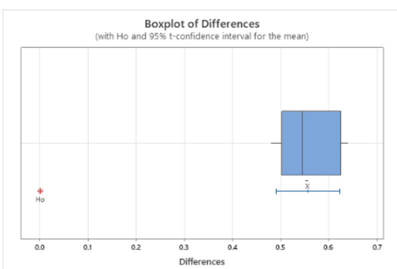
Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
21.70	0.000







Paired T-Test and CI: acetic acid-DAP, acetic acid-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
acetic acid-DAP	6	0.2333	0.0709	0.0289
acetic acid-SDB	6	0.2067	0.0706	0.0288

Estimation for Paired Difference

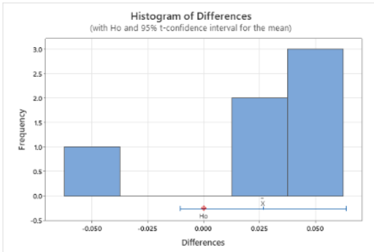
Mean	StDev	SE Mean	95% CI for μ_difference
0.0267	0.0356	0.0145	(-0.0107, 0.0640)

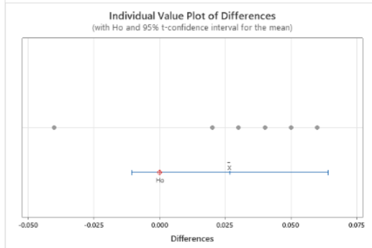
μ_difference: population mean of (acetic acid-DAP - acetic acid-SDB)

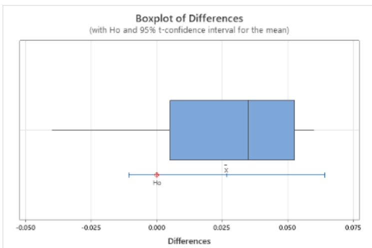
Test

Null hypothesis H_0: μ_difference = 0
Alternative hypothesis H_1: μ_difference ≠ 0

T-Value	P-Value
1.84	0.126







4. **Sugar**

Paired T-Test and CI: fructose-DBP, fructose-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
fructose-DBP	6	1.555	0.553	0.226
fructose-DAP	6	1.005	0.092	0.038

Estimation for Paired Difference

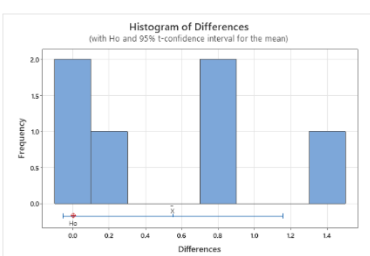
Mean	StDev	SE Mean	95% CI for μ_difference
0.550	0.575	0.235	(-0.054, 1.154)

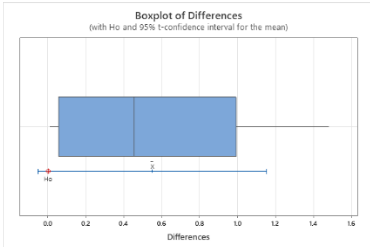
μ_difference: population mean of (fructose-DBP - fructose-DAP)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
2.34	0.066





Paired T-Test and CI: fructose-DAP, fructose-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
fructose-DAP	6	1.0047	0.0923	0.0377
fructose-SDB	6	0.9357	0.1801	0.0735

Estimation for Paired Difference

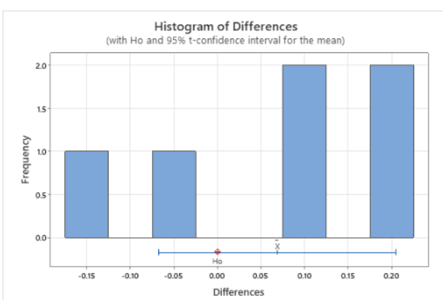
Mean	StDev	SE Mean	95% CI for μ_difference
0.0689	0.1297	0.0530	(-0.0672, 0.2051)

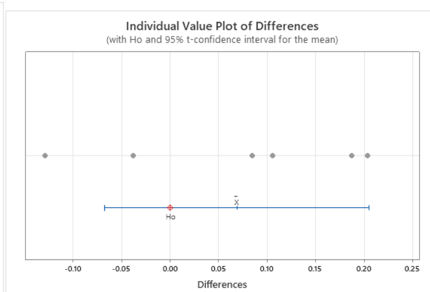
μ_difference: population mean of (fructose-DAP - fructose-SDB)

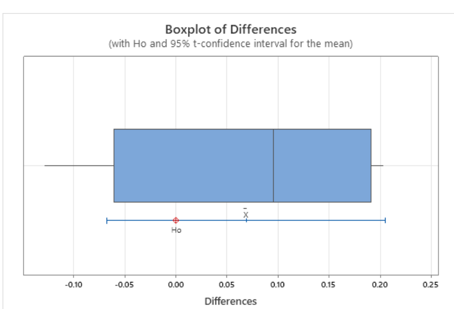
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
1.30	0.250







Paired T-Test and CI: glucose-DBP, glucose-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
glucose-DBP	6	30.255	2.177	0.889
glucose-DAP	6	26.531	1.732	0.707

Estimation for Paired Difference

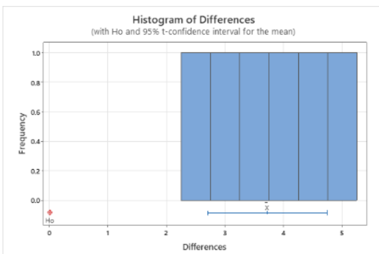
Mean	StDev	SE Mean	95% CI for μ_difference
3.724	0.973	0.397	(2.703, 4.745)

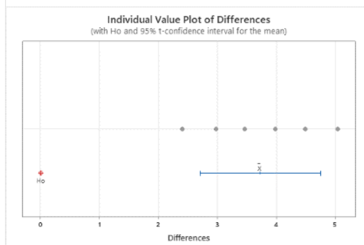
μ_difference: population mean of (glucose-DBP - glucose-DAP)

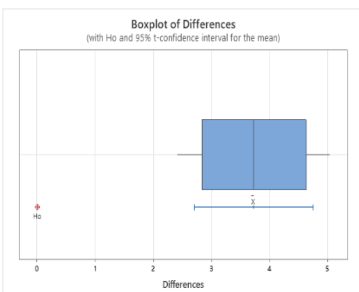
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
9.38	0.000







Paired T-Test and CI: glucose-DAP, glucose-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
glucose-DAP	6	26.531	1.732	0.707
glucose-SDB	6	29.176	1.224	0.500

Estimation for Paired Difference

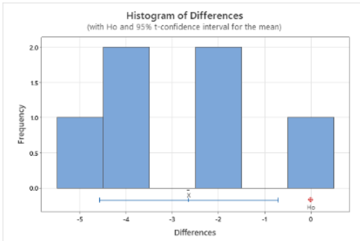
Mean	StDev	SE Mean	95% CI for μ_difference
-2.645	1.849	0.755	(-4.585, -0.704)

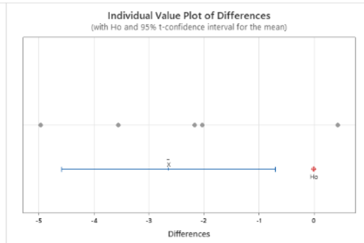
μ_difference: population mean of (glucose-DAP - glucose-SDB)

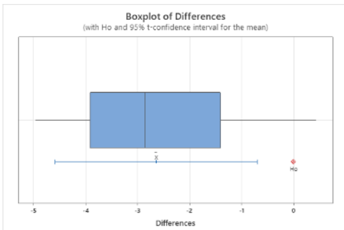
Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
-3.50	0.017







Paired T-Test and CI: maltose-DBP, maltose-DAP

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
maltose-DBP	6	14.860	0.847	0.346
maltose-DAP	6	14.661	1.645	0.672

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.199	1.584	0.647	(-1.464, 1.861)

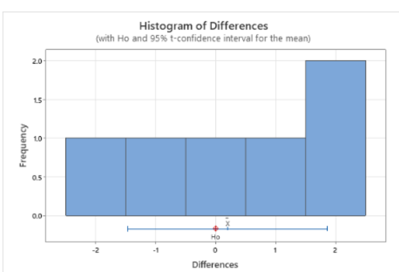
μ_difference: population mean of (maltose-DBP - maltose-DAP)

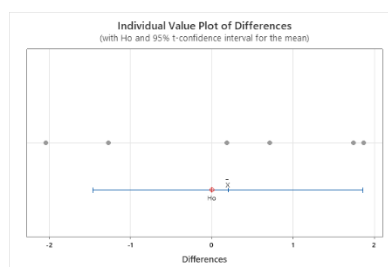
Test

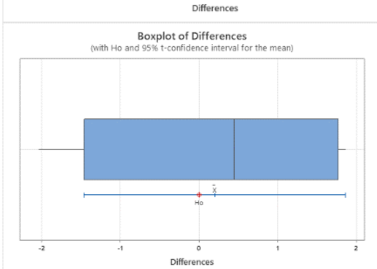
Null hypothesis H₀: μ_difference = 0

Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
0.31	0.771







Paired T-Test and CI: maltose-DAP, maltose-SDB

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
maltose-DAP	6	14.661	1.645	0.672
maltose-SDB	6	14.177	0.551	0.225

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0.485	1.775	0.725	(-1.378, 2.347)

μ_difference: population mean of (maltose-DAP - maltose-SDB)

Test

Null hypothesis H₀: μ_difference = 0
Alternative hypothesis H₁: μ_difference ≠ 0

T-Value	P-Value
0.67	0.533