APPLICATION OF PUMPKIN AND MUNG BEAN FLOURS IN WHEAT SOURDOUGH STARTERS FOR LEAVENING AND STEAMED BUN QUALITY

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Sourdough fermented using lactic acid bacteria (LAB) and yeast, functions mainly as a leavening agent for breads with limited nutrients. Nutritious non-cereal flours from pumpkin (vegetable) and mung bean (legume) were thus targeted to be used along with the typical ingredient, wheat (cereal) as composite flours to improve sourdough starter and bread. Sourdough starters with up to 20% pumpkin or mung bean flour were developed for the first time by mixing equal amount of composite flour and distilled water, fermented for 14 days at room temperature with daily pH and total titratable acidity (TTA) measurement, followed by backslopping at 1:1:1 mass ratio. As starters developed and gained maturity, a sour aroma developed as pH decreased in overall by > 30.0% and TTA increased more than twofold. Pumpkin and mung bean addition slightly raised the final sourdough pH and TTA to satisfactory pH < 4.0.

Based on the stable pH and most excellent leavening, matured composite starters with pumpkin and mung bean flours at 15% were selected together with control for three-stage activation study where leavening performances were modelled as a new attempt

to improve the challenging and time-consuming sourdough fermentation processes. The re-parameterised Gompertz equation was used for modelling the hourly changes in volume expansion ratio measured by its height in a beaker against fermentation time. Backslopping was conducted at maximum height separately for each sourdough starter to initiate the second stage, followed by the same process repetitively until the end of third stage. The addition of pumpkin and mung bean markedly increased the maximum volume expansion ratio from 0.40 to 1.72 and 2.03 cm^3/cm^3 respectively and reduced lag time from 6.39 to 2.13 and 2.35 h following the activation system. The leavening model was predicted accurately with most $R^2 > 0.97$.

To tackle the uncertain acceptance of local consumers, the new potential of all three activated starters were explored to make steamed sourdough buns (SSBs). Doughs were initially formed, proofed for 4 hours and mixed until fully developed using pumpkin flour fortification at 0, 5, 10, 15 and 20% for the 1-hour second proofing. The doughs were steamed for 30 minutes. SSB specific volume was significantly raised from 1.05 to 1.88-2.22 cm^3/g, whereas spread ratio and hardness significantly dropped from 1.86 to 1.41-1.59 and from 7353 to 2605-2991 gram-force respectively by involving pumpkin and mung bean composite starters. The pumpkin fortification of SSBs was optimum within 5-10% for attractive colour, soft texture and satisfactory sensory scores.

Apart from the usual studies on sourdough starters, microbiological analyses were also conducted on sourdough products, SSBs with each composite sourdough starter at 5% pumpkin fortification and control SSB to detect differences of microbiological profiles from starters. Possibly probiotic LAB under *Lactobacillus* genus was the major bacteria in composite sourdough starters at 94.7-97.1% while the predominance

remained at lower abundances of 51.8-53.9% in SSBs. Composite sourdough starters had fungal communities dominated by the *Saccharomyces* genus at 72.9-82.6% indicating non-cereal addition has encouraged yeast growth that was absent in control, thus improving leavening capability.

Keywords: Leavening, Mung Bean, Pumpkin, Sourdough, Steamed Bun

SDG: GOAL 3: Good Health and Well-being, GOAL 12: Responsible Consumption and Production

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

APLIKASI TEPUNG LABU DAN KACANG HIJAU DALAM PEMULA DOH MASAM GANDUM BAGI PENAIKAN DAN KUALITI ROTI KUKUS

Oleh

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Doh masam yang ditapai menggunakan bakteria asid laktik (LAB) dan yis, berfungsi terutamanya sebagai agen penaik bagi roti dengan nutrien yang terhad. Tepung bukan bijirin yang berkhasiat daripada labu (sayur-sayuran) dan kacang hijau (kekacang) disasarkan untuk digunakan bersama-sama dengan ramuan lazim, gandum (bijirin) sebagai tepung komposit untuk menambah baik pemula doh dan roti. Pemula doh masam dengan sehingga 20% tepung labu atau kacang hijau telah dikembangkan buat kali pertama dengan mencampurkan tepung komposit dan air suling yang berjumlah sama, ditapai selama 14 hari pada suhu bilik dengan pengukuran harian pH dan keasidan boleh dititrasi (TTA), berikutan dengan backslopping pada nisbah jirim 1:1:1. Seiring dengan perkembangan dan pematangan setiap pemula, aroma masam menyerbak apabila keseluruhannya, pH menurun sebanyak > 30.0% dan TTA meningkat lebih daripada dua kali ganda. Penambahan labu dan kacang hijau menaikkan pH dan TTA akhir sedikit kepada pH < 4.0 yang memuaskan.

Berdasarkan pH yang stabil dan penaikan yang paling cemerlang, pemula komposit dengan tepung labu dan kacang hijau pada 15% yang matang telah dipilih bersama-sama dengan kawalan untuk kajian pengaktifan tiga peringkat di mana prestasi penaikan dimodelkan sebagai percubaan baru untuk menambah baik proses penapaian doh masam yang mencabar dan mengambil masa. Persamaan Gompertz yang diparameterkan semula telah digunakan untuk memodelkan perubahaan nisbah pengembangan isipadu setiap jam yang diukur dengan ketinggiannya dalam bikar terhadap masa penapaian. Backslopping dilakukan pada ketinggian maksimum secara berasingan bagi setiap pemula doh masam untuk memulakan peringkat kedua, diikuti dengan proses yang sama berulang-ulang sehingga peringkat ketiga tamat. Penambahan labu dan kacang hijau meningkatkan nisbah pengembangan isipadu maksimum dengan ketara daripada 0.40 kepada 1.72 dan 2.03 cm^3/cm^3 masing-masing serta mengurangkan masa susulan daripada 6.39 kepada 2.13 dan 2.35 jam selepas sistem pengaktifan. Model penaikan tersebut telah diramalkan dengan tepat dengan kebanyakan $R^2 > 0.97$.

Bagi menangani ketidakpastian penerimaan pengguna tempatan, potensi baharu ketiga-tiga pemula yang diaktifkan telah diterokai untuk membuat roti doh masam kukus (SSB). Doh dibentuk pada mulanya, dinaik selama 4 jam dan digaul sehingga dikembangkan sepenuhnya dengan menggunakan pengayaan tepung labu pada 0, 5, 10, 15 dan 20% bagi penaikan kedua sepanjang 1 jam. Doh dikukus selama 30 minit. Isipadu khusus SSB telah dinaikkan dengan ketara daripada 1.05 kepada 1.88-2.22 cm^3/g, manakala nisbah hamparan dan kekerasan menurun dengan ketara daripada 1.86 kepada 1.41-1.59 dan daripada 7353 kepada 2605-2991 gram-daya masing-masing dengan melibatkan pemula komposit labu dan kacang hijau. Pengayaan labu

SSB adalah optimum dalam lingkungan 5-10% demi warna yang menarik, tekstur yang lembut dan skor deria yang memuaskan.

Selain daripada kajian lazim mengenai pemula doh masam, analisis mikrobiologi juga dijalankan atas produk doh masam, SSB dengan setiap pemula doh masam komposit pada 5% pengayaan labu serta SSB kawalan untuk mengesan perbezaan profil mikrobiologi daripada pemula doh masam. LAB yang berkemungkinan probiotik bawah genus Lactobacillus merupakan bakteria utama dalam pemula komposit pada 94.7-97.1% manakala dominasi tersebut dikekalkan pada kelimpahan yang lebih rendah iaitu 51.8-53.9% dalam SSB. Pemula komposit mempunyai komuniti kulat yang didominasi oleh genus Saccharomyces pada 72.9-82.6%, menunjukkan bahawa penambahan bukan bijiran telah menggalakkan pertumbuhan yis yang tidak wujud dalam kawalan, maka meningkatkan keupayaan penaik.

Kata Kunci: Doh Masam, Kacang Hijau, Labu, Penaikan, Roti Kukus

SDG: MATLAMAT 3: Kesihatan Baik dan Kesejahteraan, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab

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LAB	Lactic acid bacteria
SSB	Steamed sourdough bun
TTA	Total titratable acidity
DY	Dough yield
GI	Glycaemic index
TPA	Texture Profile Analysis
CFU	Colony-forming unit
OTU	Operational taxonomic units
ESC	Estimated sample coverage
PCoA	Principal Coordinate Analysis
NGS	Next-Generation Sequencing
PCR/DGGE	Polymerase chain reaction/denaturing gradient gel electrophoresis
PCR	Polymerase chain reaction
gDNA	Genomic deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SSE	Sum of squared errors
SST	Total corrected sum of squares
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
AAB	Acetic acid bacteria

CHAPTER 1

INTRODUCTION

1.1 Overview

In recent years, the surging popularity of fermented food products, shown by a growth rate of 4% on sales in 2020 (Nielson-Stowell, 2020), acts as the springboard for revival of attentions and interests towards some forgotten fermented foods, such as kimchi, *tempeh* and particularly the sourdough (Roesler, 2019). Sourdough has gained its outstanding market value which has been estimated by Grand View Research (2019) to record an annual growth rate of 5.9% from 2019 to 2025. Sourdough has also been utilised more commonly in bakery products since consumer demand is now developing towards healthier, tastier and more natural bakery products without additives and chemical residues (Albagli et al., 2023). The physical and online engagement with sourdough was claimed to have peaked of late, especially when many people took their first baby steps into the journey of sourdough baking during the COVID-19 pandemic (Albagli et al., 2023). Some regional sourdough products have also been widely publicised, including steamed buns from China, panettone from Italy and *injera* from East Africa.

Sourdough is a dough, fermented by lactic acid bacteria (LAB) and/or yeast, for leavening, flavour enhancement and shelf-life extension of bakery products (Chavan & Chavan, 2011; Hammes & Gänzle, 1998; Siepmann et al., 2017). These wonderful characteristics of sourdough products are contributed by biochemical activities and interactions of different LAB during fermentation process. For example, perishable bread can be kept for a longer period when added with sourdough because LAB in

sourdough possess antimicrobial properties and release organic acids as by-products that create unfavourable environment for growth of foodborne pathogens (Hammes & Gänzle, 1998; Siepmann et al., 2017). Both Siepmann et al. (2017) and Gobbetti (1998) claimed that microbial communities within sourdough are different from one to another by adjusting the process parameters like water content and fermentation temperature, ingredient parameters such as naturally present microorganisms, nutrition and enzymatic reactions, and types of inoculated microorganisms. Hence, the physical, sensory and nutritional qualities of sourdough products are also affected.

To further develop the market of sourdough, the ingredients for preparing sourdough are usually altered. Most bakers prefer utilisation of cereal flours such as wheat (Capurso & Capurso, 2020; Coda, Kianjam, et al., 2017; Hendek Ertop & Şeker, 2018; Minervini et al., 2016; Shchekoldina & Aider, 2014) or rye flours (Ercolini et al., 2013; Fujimoto et al., 2018), yet, usage of non-cereal flours like legumes, vegetables, fruits and herbs in sourdough starters have also been fruitfully tried by some bakers and researchers. Piasecka-Jóźwiak et al. (2018) chose to use mixture of lupin and wheat flours, Lebedenko et al. (2019) made traditional Caucasian pea-anise sourdough whereas Minervini et al. (2016) added fruits like macerated pears and grape must into wheat-based sourdough. Among the non-cereals, pumpkin is anticipated to assist consumer acceptance of sourdough products with its natural sweet taste and attractive colour (Kamarubahrin et al., 2018). Mung bean is also a potential candidate for excellent sourdough products as it contains hydrocolloids and protein that have been claimed to aid leavening by forming viscous gel to trap gases like gluten (Houben et al., 2012).

These interesting combinations of ingredients for sourdough products are eye-catching when they are introduced to the market as they may give distinctive flavour and enhanced nutritional values. Despite that, studies about their systematic preparation processes and various properties are still limited (Hendek Ertop & Şeker, 2018; Kefalas et al., 2009). The focus on improving the leavening capabilities of sourdough with the presence of non-cereals was less discussed too. Making sourdough products could easily failed without adequate understanding of the ingredients and relevant processing steps. The long fermentation time of sourdough is also challenging the management of processing schedule and cost effectiveness of sourdough products. Therefore, engineering techniques such as computation of mass balance and modelling of processes can be applied to ensure the sourdough fermentation and breadmaking processes are guaranteed with precise parameters and consistent qualities.

1.2 Problem Statement

The latest focus in food industry is slowly shifting towards functional food, food safety and food security since the COVID-19 pandemic as health awareness has heightened. Using cereals that are dominantly comprised of carbohydrates as the main ingredients, white bread loaves which have always been a staple food are now often being related to the many downsides including high glycaemic index (GI), low protein quality, low dietary fibre (Gaglio et al., 2020; Rizzello et al., 2014), relatively bland taste and the usage of chemical additives for longer shelf-life. The overall sales and market value of white loaves could deteriorate if this issue is continuously attacked and widely spread. Sourdough is then speculated to be the natural saviour for the bread products with some claimed benefits such as aiding mineral uptake and decreasing GI (Siepmann et al., 2017). The concept of incorporating non-cereal flours like legumes

could also help to enhance its nutritional values in terms of the dietary fibre level from 15.38 up to 16.88 g/100 g and overall acceptability scores from less than 5 up to 7.36, to a more wholesome food besides making its colour more attractive (Olojede et al., 2019).

However, it is challenging and time-consuming to make satisfactory food products using sourdough (Albagli et al., 2023). Unlike baker's yeast that is carefully cultivated and selected for solely dough leavening, the naturally fermented sourdough is a relatively weaker leavening agent without proper activation. Apart from the long fermentation time, the dough formed using weak sourdough may not contain adequately stable supporting structure, leading to chewy, densed baked sourdough breads with low volume. The attention received by the beneficial sourdough could be faded if its application on food products is not improved. Three-stage activation has been a regular practice to make sourdough bread (Corsetti, 2013; De Vuyst et al., 2017), yet the contribution and influences especially on leavening remains unclear. Hence, this study was targeted to reveal more on the effects of activation practices upon the sourdough leavening performances.

Although the sourdough products are slowly gaining attention and getting more popular amongst consumers regionally in Malaysia, they are still not as well-known as they are in the European countries, especially Italy that covered 40% of sourdough publications shown in the study of Albagli et al. (2023). The local consumers could be unfamiliar with the slightly sour taste in sourdough breads. The involvement of non-cereals like legumes in bakery products is also still new since Piasecka-Jóźwiak et al. (2018) reported that the scores for overall organoleptic evaluation of sourdough breads containing composite flour were only comparable with 10% lupin as compared to the

one made using wheat flour alone. Nielson-Stowell (2020) explained that the taste is always emphasised before any other functional benefits of food products when it comes to consumer acceptability. Thus, fortifying bread-based products naturally through the sourdough methods needs to be carefully studied for its quality and control.

1.3 Objectives

The general research objective was to investigate the potential of non-cereal flours to improve sourdough products conventionally based on wheat solely. The process of fermentation is monitored up to the production of final product. To achieve this, the following specific objectives were made:

1. to develop mature sourdough starters made from wheat (cereal), pumpkin (vegetable) and mung bean (legume) composite flours with evaluations of their pH and total titratable acidity (TTA),
2. to model leavening performances of developed sourdough starters during the three-stage activation process using re-parameterised Gompertz equation,
3. to apply the activated sourdough starters to make a product, *i.e.* steamed sourdough bun (SSB) with pumpkin fortification and analyse its physical properties, sensory properties and proximate composition, and
4. to evaluate and compare the wholesome microbiota of sourdough starters and SSBs.

1.4 Scope of Work

This research focused on the development and comparison of sourdough starter fermentation by using composite flours, *i.e.* wheat-pumpkin and wheat-mung bean at different flour compositions. A small portion of each starter was extracted for analysis at 24-hour intervals before backslopping by adding their respective flours and water throughout the 14-day fermentation period at room temperature. The changes of pH and total titratable acidity (TTA) with time were measured and plotted in graphs. The developed composite sourdough starters were then selected for the three-stage activation process whereby their leavening performances were analysed using re-parameterised Gompertz model. The activated starters which acted as leavening agents were used to make SSBs with different levels of pumpkin flour fortification. The effects of different sourdough starters and percentage of pumpkin flour fortification on the physical properties which include SSB volume, spread ratio, colour and texture, sensory properties and proximate composition were studied. Lastly, microbiological test was conducted for three activated sourdough starters, three selected steamed buns and some outsourced fermented foods to ascertain the microbial diversities and profiles with respect to bacterial and fungal populations.

CHAPTER 2

LITERATURE REVIEW

Sourdough, the ancient and beneficial biotechnology for bakery products, has been gaining a lot of attention from the public, especially the researchers. Sourdough is so famous that apart from wheat flour as the typical ingredient, the potential of atypical ingredients such as vegetables and legumes has also been explored. The suitability of these innovations has been evaluated with basic qualities of sourdough, *i.e.*, pH and total titratable acidity (TTA). Another key feature of sourdough is the leavening capability, which can be continuously maintained and even activated through backslopping. For further understanding and precise control of leavening, some mathematical models have been developed and applied for more satisfying sourdough products. Sourdough technology is consistently applied among breads, although scientific studies of steamed sourdough breads are fewer than baked ones. Thus, the characterisation of steamed sourdough breads is important since the ideal qualities in terms of volume, spread ratio, colour, texture, sensory qualities and proximate composition deviate from baked sourdough breads. Besides, sourdough qualities are also greatly influenced by LAB and yeasts, which can be analysed as sourdough microbiological profiles. For more efficient and thorough microbiological studies, Next-Generation Sequencing (NGS) appears as the emerging technology that reveals the potentially health-promoting microbiota of sourdough and sourdough products.

2.1 Sourdough

Dated back to the second millennium B.C., sourdough had already been produced as leavening agent and consumed in the form of sourdough bread (Cappelle et al., 2013; Lhomme et al., 2015). It is one of the most ancient biotechnological processes for cereal food products (Chavan & Chavan, 2011; Siepmann et al., 2017). By mixing flour and water, sourdough is spontaneously formed through fermentation as time goes by. The innate yeast and lactic acid bacteria (LAB) found in sourdough are vital for releasing carbon dioxide and organic acids, that respectively lead to leavening and formation of signature sour taste (Chavan & Chavan, 2011; Hammes & Gänzle, 1998; Lönner et al., 1986; Siepmann et al., 2017). Sourdough can be produced by different inoculation means that classify them into three main types. Type I sourdough involves backslopping, *i.e.*, cyclic reinoculation by using part of the previous sourdough during fermentation; type II sourdough requires the addition of starter culture; whereas type III combines backslopping and addition of starter culture (De Vuyst et al., 2017; Hammes & Gänzle, 1998).

The studies on type I sourdough have been substantial in laboratories (De Vuyst et al., 2017), especially for wheat (Fujimoto et al., 2018; Lebedenko et al., 2019; Minervini et al., 2016; Taccari et al., 2016; Van Kerrebroeck et al., 2016; Yu et al., 2018) and rye sourdough (Ercolini et al., 2013; Fujimoto et al., 2018; Lönner et al., 1986; Siepmann et al., 2017). Sourdough, especially the one used as type I sourdough, is also named *Anstellgut* in Germany, mother sponge in the San Francisco area, *le chef* (the boss) in France, *masa madre* in Spain, *madre* or *capolievito* in Italy and *isco* (bait) or *crescente* (crescent) in Northern Portugal (Hammes & Gänzle, 1998; Hansen, 2006; Novotni et al., 2021). This indirectly shows the popularity of type I sourdough across

the countries. The practice of backslopping is also beneficial since it helps to decrease sourness with stable acidification in sourdough products (Cappelle et al., 2013; Taccari et al., 2016). During backslopping, the dough yield (DY) of sourdough should be maintained properly too. Dough yield, expressed in Equation 2.1, is equivalent to the ratio between the dough mass and the mass of flour used.

$$DY = \frac{mass\ of\ flour + mass\ of\ water}{mass\ of\ flour} \times\ 100\% \qquad (2.1)$$

The microbiological profiles of type I sourdough starters, associated with properties of sourdough products, may vary to a more limited range of LAB and yeast species when DY is increased with extra water (De Vuyst et al., 2017; Di Cagno et al., 2014).

Regardless of the sourdough types, sourdough is advantageous overall, both for the product quality and consumer health. The more detailed benefits of sourdough have been published in the paper entitled "Sourdough microbiome comparison and benefits" as shown in list of publications of the thesis. Sourdough bread has been proven to have lengthened shelf life due to the higher resistance against mold and rope spoilage (Plessas, 2021). After addition of sourdough, the drop in moisture content for steamed bread crumb was slower, together with a lower increase in hardness during storage (Chen et al., 2018). The flavour, aroma and texture of sourdough bread are also improved as compared to yeasted bread. Bread with LAB as preferments had an enhanced flavour, associated with the rise of free amino acids concentration in dough (Calvert et al., 2021; Thiele et al., 2002). From the aspects of health, sourdough addition in bread relieves gastrointestinal problems. Postprandial symptoms such as bloating and fullness were less severe by 36% after consumption of sourdough

croissants as compared to yeasted ones, in relation to the improved gastric emptying rate (Polese et al., 2018). The reduced GI of sourdough bread was also deduced as starch has mostly been consumed by sourdough microbes and converted into organic acids, ethanol and carbon dioxide during fermentation (Siepmann et al., 2017). Phytic acid, that hinders nutrient assimilation in body by binding minerals, is more likely to be hydrolysed by phytase enzyme under acidic conditions during sourdough fermentation (Chiş et al., 2020; Siepmann et al., 2017). Arising from that, consumers gain a greater concentration of nutrients from sourdough bread than yeasted bread. For further improvement on nutritional values of bread, sourdough bread can be added with more nutritious ingredients, *e.g.*, legumes, fruits and vegetables.

2.2 Sourdough Ingredients

Most of the time, sourdough is only made of flour and water (De Angelis et al., 2018; Mariotti et al., 2014; Stefanello et al., 2018). This further amplifies the attractiveness of sourdough as it is simple and that the two types of ingredients are easily available. Although most sourdough uses wheat flour, the chosen type of flour is not certainly the same, probably due to different geographical regions and nutritional profiles, involving protein, carbohydrates, ash, and fibres (Hansen, 2006; Landis et al., 2021). Innovative bakers and manufacturers switched or fortified wheat flour with other cereal flours, for instance, rye, sorghum, rice and millet or non-cereal flours such as pseudocereals, legumes, nuts, fruits and vegetables. Meanwhile, the attempt on involving both cereals and non-cereals for composite sourdough starters is very limited. Most food ingredients are potential candidates for sourdough ingredients, provided that they possess life-supporting components for LAB and yeasts, especially fermentable sugars.

2.2.1 Wheat as the Typical Ingredient

Cereals are usually chosen as the main ingredient for sourdough. Cereals refer to grasses of which their grains are used to be consumed as food, either cultivated or wild (Brink, 2006; De Vuyst et al., 2017). Cereal grains usually contain three main edible parts, namely germ, bran and endosperm, as shown in Figure 2.1 (Orchardson, 2020). Examples of cereals flour are common wheat, durum wheat, einkorn wheat, spelt wheat, rye, sorghum, maize, rice, millet, barley and oat. Besides being consumed by humans, cereals are also supplying for animal feed, industrial uses and seed corns (Lásztity, 2017). As cereals are the major source of carbohydrates, which are also foods for microorganisms in fermenting cereals into sourdough, the studies of sourdough using cereal flours have been continuously flourished. For example, there are researches involving sourdough made from wheat (Capurso & Capurso, 2020; Charoenthaikij et al., 2012; Coda, Varis, et al., 2017; Galle et al., 2010; Hendek Ertop & Şeker, 2018; Kefalas et al., 2009; Minervini et al., 2016; Rizzello et al., 2014; Shchekoldina & Aider, 2014), rye (Bondia-Pons et al., 2011; Ercolini et al., 2013; Fujimoto et al., 2018; Siepmann et al., 2017), sorghum (Galle et al., 2010, 2012; Karrar et al., 2020; Mezgebe et al., 2020; Ogunsakin et al., 2015; Olojede et al., 2019; Seyoum et al., 2016), maize (Edema & Sanni, 2008; Hashemi et al., 2021), rice (Charoenthaikij et al., 2012; Hanis-Syazwani et al., 2018; Sadeghi et al., 2019) and millet (Akinola & Osundahunsi, 2017).

Referring to Figure 2.2, common wheat or *Triticum aestivum*, is the most generally consumed, seen and used cereal (Chavan & Chavan, 2011; Sakandar et al., 2019). It is grown in almost every corner of the world and converted into various food products like breads, crackers, noodles, baby foods, food supplements *etc*. Before being served

as food, this grain is mostly milled into flour, which is differentiated into a few types. White wheat flour is refined by milling until it contains minimum wheat bran or germ for finer texture, while whole wheat flour retains the bran and germ with various nutrients (Capurso & Capurso, 2020; De Angelis et al., 2018; Orchardson, 2020). Sometimes, wheat flour is also bleached. Both bleached and refined wheat flour contain lower amounts of fibres, essential amino acids, phytochemicals, vitamins and minerals (Sakandar et al., 2019; Shchekoldina & Aider, 2014), which are mostly stored naturally in the bran and germ (Orchardson, 2020).

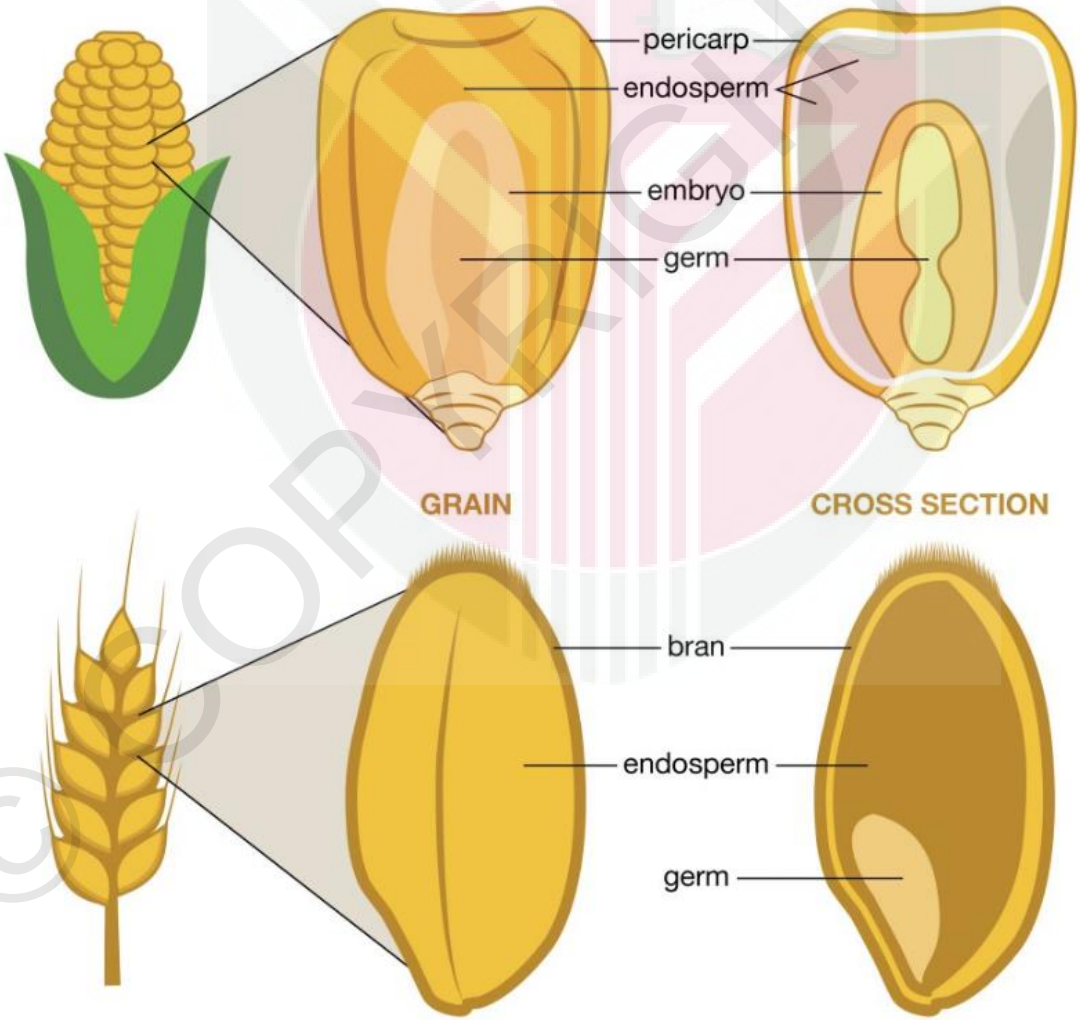

Figure 2.1: Bran, germ and endosperm as the main parts of cereal grains
(Source: Orchardson, 2020)

Figure 2.2: Wheat (*Triticum aestivum*) grain (Source: Lyddon, 2018)

Since cereals are usually the bank of carbohydrates, plain flour or white wheat flour also has a high percentage of carbohydrates at around 74.6% by weight (United States Department of Agriculture, 2020). Lactic acid bacteria (LAB), which is the key factor in sourdough fermentation, consume the fermentable sugars found in wheat. Hence, if wheat flour is refined, the sourdough microbiota and thus the characteristics of sourdough products vary too since the amount and composition of sugars are influenced (Chavan & Chavan, 2011; Siepmann et al., 2017). *Weissella* spp. was observed to be sensitive towards the carbohydrates available in sourdough, whereby its abundance was clearly distinctive in whole wheat flour and white wheat flour (Taccari et al., 2016).

Typically, there are 3.0% of total dietary fibre, 12.0% of protein and 0.6% of ash in plain flour (United States Department of Agriculture, 2020). White wheat flour, containing less hydrophilic dietary fibre, absorbs less water than whole wheat flour (Bae et al., 2014; F. Ma et al., 2017; Taccari et al., 2016). Hence, the water absorption ability of plain flour is not as high as whole wheat flour in making sourdough. The

fibre in wheat plays the role as prebiotics for gut microbiota (Capurso & Capurso, 2020), therefore enhancing gastrointestinal health for consumers of wheat sourdough products. Nevertheless, dough development in white wheat sourdough can be slightly stronger as compared to whole wheat sourdough due to the lower total fibre content (Bae et al., 2014; Taccari et al., 2016).

Dough development of wheat sourdough is associated with its protein content. Wheat contains the highest abundance of proteins among the cereals (Lásztity, 2017). Gliadin and glutenin are two basic types of protein in wheat, collectively known as gluten, which support the dough for its elastic structure (Chavan & Chavan, 2011; X.-Y. Wang et al., 2016) when wheat flour is wetted. The gluten is responsible for supporting the framework of sourdough products, followed by improving their volume and elasticity (Bae et al., 2014; X.-Y. Wang et al., 2016). Therefore, the elastic and stable wheat sourdough can be easily kneaded and shaped.

Capurso and Capurso (2020) investigated the benefits brought by wheat sourdough bread, such as decreasing of GI by slowing the digestion and absorption of starch. Apart from that, the drop of pH in wheat dough due to sourdough fermentation till 5.5 was proven to significantly reduce phytate content that existed naturally in wheat flour (Leenhardt et al., 2005; Taccari et al., 2016). A drop by 62% was detected for phytate content in whole wheat sourdough bread, which is more significant than commercial yeast fermented bread (38%) (Sakandar et al., 2019). By achieving this, the mineral absorption in gastrointestinal tract, which are initially disturbed by phytate, can be enhanced (Chavan & Chavan, 2011; Siepmann et al., 2017). Sourdough incorporation in wheat products also generates greater amount of the initially deficient amino acids, which enrich nutrition and flavour (Sakandar et al., 2019; Thiele et al., 2002).

2.2.2 Pumpkins and Mung Beans, the Atypical Non-cereals

Besides using cereal flours, there are also many sourdough products derived from other ingredients, for instance, pseudocereals (Carbó et al., 2020; Carrizo et al., 2017; Chiş et al., 2020; Houben et al., 2010; Rizzello, Lorusso, et al., 2015; Sterr et al., 2009), legumes (Coda, Varis, et al., 2017; Curiel et al., 2015; Hendek Ertop & Şeker, 2018; Kefalas et al., 2009; Mohammadi-Kouchesfahani et al., 2019; Olojede et al., 2019; Rizzello et al., 2014), fruits (Karrar et al., 2020; Minervini et al., 2016; Yu et al., 2018) and nuts (Chinma et al., 2016; Gaglio et al., 2020).

Pseudocereals refer to those cereals which do not belong to grasses but produce fruits or seeds used as flour (Brink, 2006; Chiş et al., 2020; Fletcher, 2016; Gobbetti et al., 2020). Amaranth, one of the pseudocereals, consists of a few cultivated species, such as Inca wheat (*Amaranthus caudatus*), purple amaranth (*Amaranthus cruentus*) and Prince's feather (*Amaranthus hypochondriacus*) (Brink, 2006; Fletcher, 2016). It contains great amount of protein (up to 16.5%), vitamins and minerals (Gobbetti et al., 2020; Sterr et al., 2009). It is usually sprouted as a type of vegetable, toasted, ground into flour, made into confectioneries and balls (Brink, 2006; Fletcher, 2016). Quinoa (*Chenopodium quinoa*) is another famous pseudocereal, that mostly originated from Peru and Bolivia (Fletcher, 2016). Its protein content is higher (up to 22.8%) than many other cereals due to the larger proportion of its embryo in the grain (Fletcher, 2016). Adding on the list, buckwheat (*Fagopyrum esculentum*), cultivated in China, possesses high level of vitamins (Petrova & Petrov, 2020) and phenolic compounds, leading to higher antioxidant activity than some cereals, such as wheat, barley and oats (Holasova et al., 2002; Selimović et al., 2017).

Pseudocereals are usually not as popular as cereals but the attentions of food scientists and consumers towards them are rising these days. In wheat (cereal) doughs, nutrients including free amino acids and total phenols increase through incorporation of quinoa flour (Rizzello, Lorusso, et al., 2015). The protein content of amaranth is higher than wheat (Badiu et al., 2014). Amaranth and quinoa can substitute cereals for preparing gluten-free meals and for providing new flavours (Carbó et al., 2020; Sterr et al., 2009). Despite being gluten-free, the rheological properties of amaranth and quinoa (pseudocereals) doughs can be enhanced to the level almost similar with plain wheat dough (Chiş et al., 2020; Houben et al., 2010). Volume, organoleptic and aroma qualities of pseudocereal products could be enhanced through sourdough fermentation too (Petrova & Petrov, 2020). In addition, LAB isolated from amaranth sourdough demonstrated the release of vitamins B_2 and B_9 and high phytase activities that boosts mineral bioavailability (Carrizo et al., 2017).

The incorporation or substitution of non-cereal flours as main ingredients in sourdough are still not as developed as the sourdough made of cereal flours. The textural properties and consumer acceptance are still lacking (Gobbetti et al., 2020). Among the non-cereals, fruits and vegetables are studied for their potentials in sourdough fermentation. Fruits are defined as the fleshy or dry ripened ovaries of plants, with embedded seed or seeds (Sidhu & Al-Zenki, 2006; Stevens & Ware, 2018). It is common for fruits to be classified into simple fruits, aggregate fruits and composite or multiple fruits. For example, apples, coconuts and peaches are simple fruits, strawberries and raspberries are aggregate fruits, while pineapple and fig are multiple fruits. Fruits are grown in different climates, hence they are originated from many places around the world (Stevens & Ware, 2018). Vegetables are plants or parts of them, that are not fruits and seeds and are consumed together with staples either in the

cooked or raw forms (Radovich, 2018; Tsao & Lo, 2006). Warm-season vegetables, such as cucumber and pumpkins grow optimally within temperature range of 12-35 ºC, while cool-season vegetables like radish, garlic, *etc*. have optimum temperatures lower than the optimum range for warm-season vegetables (Radovich, 2018).

Fruits like pears, navel oranges, doum (*Hyphaene thebaica*), nabag (*Zizyphus spina-christi*) and vegetables like sweet potatoes and pumpkins are usually high in fibre, minerals and vitamins, which enhance the nutrients in sourdough (Aboshora et al., 2016; Aparecida Pereira et al., 2019; Karrar et al., 2020; Sadeghi et al., 2019; Yu et al., 2018). The presence of some components like phenols, flavonoids and their derivatives in fruits and vegetables also helps in retarding microbial growth as they possess antioxidant and antibacterial characteristics (Aboshora et al., 2016; Comasio et al., 2021). Similar to other non-cereal ingredients, fruits and vegetables supply auxiliary microorganisms that may not be naturally found in sourdough (Comasio et al., 2021). The carbohydrates, such as glucose, fructose and sucrose, and organic acids in fruits and vegetables are also fermentable substrates for sourdough (Aparecida Pereira et al., 2019; Comasio et al., 2021; Karrar et al., 2020; Minervini et al., 2016). By adding fruits and vegetables, acidification kinetics during sourdough fermentation are affected too. Yu et al. (2018) reported that pH was lower and TTA was higher for sourdough with pears and oranges. With high concentration of ascorbic acid, nabag has been found to be a dough conditioner that also boosts volume and crumb structure of sourdough products (Grosch & Wieser, 1999; Karrar et al., 2020). Unique flavours can also be generated for sourdough products due to incorporation of fruits and vegetables (Aparecida Pereira et al., 2019; Yu et al., 2018).

As portrayed in Figure 2.3, pumpkins (*Cucurbita pepo*) are vegetables whereby their edible parts are the young or mature fruits (Radovich, 2018). Pumpkins are mainly water, which comprises 91.6% by weight (United States Department of Agriculture, 2018). They contain 6.5% of carbohydrates, 0.8% of ash and 0.5% of dietary fibre. They possess low calories but rich vitamins and minerals. The amount of potassium is outstandingly high among minerals available in pumpkins. They are also significantly high in beta-carotene, a strong antioxidant that can be converted into vitamin A after being consumed (Tsao & Lo, 2006; Ware, 2019), thus giving pumpkins their attractive orange or yellow colour (Pongjanta et al., 2006; Ware, 2019). They are usually consumed in the forms of boiled vegetables, soups, desserts, salads, preserves, soft alcoholic drinks and sometimes butter substitute (Ware, 2019; Yadav et al., 2010).

Figure 2.3: Pumpkins (Source: Ware, 2019)

With the 2.8% of total sugars in pumpkins by weight (United States Department of Agriculture, 2018), they give consumers a natural sweet taste, both when they are consumed by their own or incorporated into other food products (Kamarubahrin et al., 2018). Pumpkins are easily sourced in Malaysia since 25,475 metric tons of pumpkins

were harvested in the year of 2018 (Ministry of Agriculture and AgroBased Industry, 2018). Pumpkins are also widely accepted and favoured due to its identity as one of the prophetic crops (Kamarubahrin et al., 2018).

Sadeghi et al. (2019) added pumpkin puree into sourdough bread rolls and deduced that this addition contributed positively from the aspects of hardness, overall acceptability and antioxidant capacity. The almost similar contributions of pumpkins towards bakery products were also observed in the study of Różyło et al. (2014) from the aspects of overall acceptability and antioxidant capacity. Loaf volume for breads with weak or low gluten was also enhanced with the presence of small amount of 5-10 % pumpkin powder (Hoxha et al., 2023; Ptitchkina et al., 1998). Pumpkin has been popular for bread fortification but its role in developing composite sourdough starters has never been analysed.

As one of the efforts in creating more diverse types of sourdough, legumes were also selected to be investigated in this study since they are high in proteins which can make sourdough products more wholesome (Olojede et al., 2019). As one of the commonly found legumes, mung beans (*Vigna radiata*) as shown in Figure 2.4, are originated from India, which the cultivation has been widespread across Africa, America, Asia, Australia and West Indies. Hence, this leads to diversified types of food products, such as soups, porridge, snacks, breads, noodles, ice cream, sauces and bean sprouts as vegetables. Mung beans, which are cheap and easily available in many regions especially Asian countries, are highly recommended to be consumed due to their rich nutritional composition. By referring to United States Department of Agriculture (2019), ash, dietary fibre and protein constitutes 3.3%, 16.3% and 23.9% respectively by weight in raw mung beans. The protein of raw mung beans is also easy to digest at

an *in vitro* protein digestibility rate of 80.2% (D. Hou et al., 2023; Mubarak, 2005) as compared to 37.9-70.0% for highland barley, buckwheat and millet flours (Fu et al., 2023). The major component of mung beans is carbohydrates at 62.6% which include 6.6% of sugar (United States Department of Agriculture, 2019).

Figure 2.4: Mung beans

Since its abundance in protein, particularly lysine (1.7%) (Singh et al., 2015), mung beans can play the role in fortifying cereal flours, which are lacking in lysine and other sulphur-containing amino acids (Rizzello et al., 2014; Singh et al., 2015). The cereal-mung bean composite flour is complementary from the aspect of proteins (Curiel et al., 2015; Olojede et al., 2019). Following the boosting demand of sourdough products, the incorporation of composite flours, made from cereal and non-cereal flours are getting more attentions. Cereals possess phytic acid naturally which may hinder mineral absorption and affect enzymatic activities after being consumed (Singh et al., 2015). To overcome this, phytase is required to degrade phytic acid. Mohammadi-Kouchesfahani et al. (2019) mixed mung bean flour with wheat flour, wheat bran and red lentil flour at different proportions for sourdough and found that sourdough

containing mung bean flour showed higher phytase activities. Apart from this, the studies on composite sourdough starters involving legumes or mung beans are very uncommon. Sourdough incorporated with mung bean flour should be also low in GI, due to high contents of indigestible carbohydrates, including resistant starch and total dietary fibre (Hefni et al., 2021). In addition, mung beans are believed to further enhance the prolonged shelf life of sourdough products as they contain some antibacterial and antioxidant properties naturally (D. Hou et al., 2023). The distinct taste of mung bean is also speculated to aid in expanding the market of bakery products by bringing consumers a special nutty flavour.

2.3 Sourdough pH and Total Titratable Acidity (TTA)

Regardless of the different ingredients used, sourdough products are easily identified by their tangy, sour taste due to the production of organic acids by sourdough microbiota. Lactic acid is usually preferable and is released at higher amount than acetic acid (Ognean, 2015). The amount of acid can be generally quantified by scientific units of pH and total titratable acidity. Sour food products have lower pH. The pH can be expressed as the negative log of the hydrogen ion concentration. Acids dissociate into ions at different extents. In the case of organic acids from sourdough fermentation, they are weaker in dissociation than strong acids such as hydrochloric acid and sulphuric acid, hence the pH is comparatively higher among acids (Bartkiene et al., 2019; Institute of Food Technologists, 2003).

For total titratable acidity (TTA), it is the measure of amount of standard alkali (usually 0.1 M NaOH) required to neutralise the acid with the endpoint at pH of 8.5 for weak organic acids from sourdough (Harth et al., 2016; Institute of Food Technologists,

2003). TTA values, which are always the microbiological stability indicator of food products, can be affected by fermentation temperature, extraction rate of flour, and water content (De Vuyst et al., 2017; Hansen, 2006). As buffering capacities of food products are different, TTA is often used together with pH to show the total acidity of food products (De Vuyst et al., 2017; Institute of Food Technologists, 2003).

As sourdough fermentation proceeds, pH drops and TTA rises (Ogunsakin et al., 2015). Organic acids, particularly acetic acids and lactic acids, which are pulling down the pH, are released as one of the antimicrobial substances (Chavan & Chavan, 2011; Siepmann et al., 2017). In addition, pH declination of a food increases the effectiveness of organic acids as natural preservatives in the undissociated form, contributing to extension of shelf life for sourdough products (Calasso et al., 2023; Guizani & Mothershaw, 2007). The pH and TTA values, manipulated by mass ratio between lactic acid and acetic acid, are also associated with the iconic taste, aroma and flavour of sourdough products (Ognean, 2015).

With reference to the study of Van Kerrebroeck et al. (2016), the pH of mature wheat sourdough was ranged between 3.4 and 3.5 whereas the TTA for 0.1M sodium hydroxide recorded a value of 11.5 ml. Millet sourdough had an almost similar pH of 3.5 for the pearl millet and 3.3 for the finger millet (Akinola & Osundahunsi, 2017). TTA values were slightly different, at 3.4 and 3.6 ml of 1.0 N sodium hydroxide for pearl and finger millet respectively, as sodium hydroxide with different concentrations were used.

As the basic parameters that determine sourdough qualities, the dynamic changes of pH throughout sourdough fermentation may require further analysis. Vera et al. (2012)

stated that the daily pH study was less common on sourdough starters as compared to dairy products. The dynamic studies have mostly stopped after 10 days (Rizzello et al., 2014; Vera et al., 2012), while those monitoring sourdough fermentation period of 14 days (Coda, Kianjam, et al., 2017; Stefanello et al., 2018) only conducted pH measurement on selective days.

2.4 Sourdough Leavening

The other iconic byproduct of sourdough fermentation which contributes to leavening is the carbon dioxide. The wheat gluten in sourdough starters retains the carbon dioxide from escaping (De Vuyst et al., 2017), hence allowing the expansion of gluten network in sourdough starter or eventually in dough. Sourdough is the ancient leavening agent for bakery products (Chavan & Chavan, 2011; Siepmann et al., 2017), but its role has been gradually replaced by baker's yeast as time goes, especially for industrial purposes that are demanding from the aspect of production speed (De Vuyst et al., 2017). Dough leavening can also occur without any agent through vigorous beating that introduces air pockets inside (Lai & Lin, 2006; Sahin, Zannini, et al., 2019), or alternatively occur chemically by adding sodium bicarbonate or better known as baking soda for rapid release of carbon dioxide (Bellido et al., 2009; Sahin, Rice, et al., 2019). The importance of this natural leavening agent has been growing in recent years, associated with the flourishing popularity of fermented foods and the consumer demand over healthier, tastier and more natural bakery products (Grand View Research, 2019).

2.4.1 Backslopping for Continuous, Activated Leavening

During type I sourdough fermentation, the backslopping process made from the cyclic leavening ensures the maturity and sustainability of sourdough starters. When leavening peaks, a portion of the sourdough starter is usually extracted for reinoculation of the freshly prepared flour and water (De Vuyst et al., 2017). The leavening starts all over again since the gas pockets in sourdough starters are dispersed and redistributed during backslopping. The number of daily backslopping is not fixed and is typically 7 times (Akinola & Osundahunsi, 2017), 10 times (Ercolini et al., 2013; Harth et al., 2016; Mohammadi-Kouchesfahani et al., 2019; Oshiro et al., 2020; Van Kerrebroeck et al., 2016) or 14 times (Coda, Kianjam, et al., 2017; Stefanello et al., 2018). The proportion of the reinoculated sourdough starter is also flexible, including 10% (Harth et al., 2016; Van der Meulen et al., 2007; Van Kerrebroeck et al., 2016), 15% (Mohammadi-Kouchesfahani et al., 2019) and 25% (Coda, Kianjam, et al., 2017; Ercolini et al., 2013; Oshiro et al., 2020).

Backslopping also gives rise to the three-phase evolution of sourdough microbiota in the formation of stable and mature sourdough starters. During the first 3 days of daily backslopping, the diverse microbiological profiles of sourdough starters are usually dominated by microbes naturally found in flour and water, such as those under *Enterococcus*, *Enterobacter* and *Pantoea* genera (Ercolini et al., 2013; Novotni et al., 2021; Van der Meulen et al., 2007). Those sourdough-associated microbes, *e.g.*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella* genera start to grow and get active in the acidic environment as backslopping progresses (De Vuyst et al., 2017). Along the periodic backslopping, sourdough starters become more stable from the aspects of microbiological profiles and related properties including pH and TTA

(Ognean, 2015; Van Kerrebroeck et al., 2016), since only well-adapted sourdough microbes dominate the microbiota after 5-10 days of backslopping (De Vuyst et al., 2017).

Mature sourdough starters are activated through precise backslopping to give better leavening performances. During activation, backslopping is carried out when microbial population and activities are at climax as the sourdough microbes release carbon dioxide at the maximum amount followed by impressive leavening of sourdough starters. The activation especially for Type II sourdough is achievable through one-stage system that involves only one backslopping (Corsetti, 2013; De Vuyst et al., 2017; Hansen, 2006), while three-stage activation system is a more traditional practice for Type I sourdough (Corsetti, 2013; De Vuyst et al., 2017). By going through three-stage activation system, longer time is spared for antimicrobial activities (Elsanhoty et al., 2017) and the amount of sourdough starter is increased. The effect of three-stage activation system was not significant on acidification of sourdough inoculated by sourdough yeast, *Saccharomyces cerevisiae* and LAB such as *Lactobacillus sanfranciscensis* (Paramithiotis et al., 2005), however pH drop was significant in sourdough studies of Elsanhoty et al. (2017) involving inoculation of *Lb. acidophilus* or *Bifidobacterium lactis* and Reidzane et al. (2021) through spontaneous fermentation. Although this concept is widely practised by sourdough bakers, there are limited studies, especially in terms of leavening on the three-stage activation system of sourdough starter.

2.4.2 Leavening Precisely Described through Modelling

Leavening of sourdough starters can be described using existing mathematical models for easier interpretation and application. Sourdough starters expand due to the activities of sourdough microbes, especially from the production of carbon dioxide (Chavan & Chavan, 2011; De Vuyst et al., 2017). Therefore, each backslopping cycle and leavening corresponds to the growth of living things (sourdough LAB and yeast) in a sigmoidal curve (Romano et al., 2007; Tjørve & Tjørve, 2017). Three distinct phases are observed in both leavening and growth processes, *i.e.* the lag, growth and stationary phases (Chevallier et al., 2012; Romano et al., 2007; Tsatsaragkou et al., 2023). During lag phase, the nutrients for microbes in the newly inhabited environment are plentiful, so they are active but growth in population has not begun yet (Leite, 2018). Carbon dioxide released by the microbes also travels across dough matrix to join the initially formed air nuclei in this phase (Baker & Mize, 1941; Chevallier et al., 2012; Meerts et al., 2018; Romano et al., 2007). Microbes start to divide exponentially in growth phase (Chevallier et al., 2012; Meerts et al., 2018; Romano et al., 2007). The microbiological activities reach plateau in the following stationary phase shown by the minimal changes in dough volume at its maximum (Chevallier et al., 2012; Meerts et al., 2018; Romano et al., 2007).

The description of dough leavening using growth models has been highly accurate, mostly referring to those leavened by yeast (Chevallier et al., 2012; Gally et al., 2017; Meerts et al., 2018; Romano et al., 2007). Among the growth models, Gompertz, Logistic and von Bertalanffy models consist of three parameters, while Weibull, Morgan and Richards have four parameters (Maruyama et al., 2001; Romano et al., 2007; Tjørve & Tjørve, 2017). Gompertz model is broadly used for studies involving

microbial growth in food (Tjørve & Tjørve, 2017). It was initially proposed by Gompertz (1825) for mortality-related studies. The model is expressed in Equation 2.2 as follows:

$$w(t) = a \exp[-\exp(b - ct)] \quad (2.2)$$

whereby $w(t)$ is Gompertz function, a is the dependent variable when t approaches infinity, b is the slope parameter and c is the point of inflection (Romano et al., 2007; Tjørve & Tjørve, 2017). The Gompertz model has been commonly re-parameterised by Zwietering et al. (1990) as follows:

$$L(t) = A \exp\{-\exp\left[\frac{\mu e}{A}\left(t_{lag} - t\right) + 1\right]\} \quad (2.3)$$

whereby $L(t)$ refers to the growth or leavening function of time, A is the maximum volume expansion ratio, μ is the maximum volume expansion rate, e is Euler's number and t_{lag} is equivalent to the lag time of the process (Romano et al., 2007; Tsatsaragkou et al., 2023).

Gompertz model has been easy and accurate in describing dough leavening (Meerts et al., 2018; Romano et al., 2007). One of the advantages in using Zwietering's re-parameterised Gompertz model is that the μ value, *i.e.*, absolute growth rate at inflection point, is free from the influence of the α value, the upper asymptote (Tjørve & Tjørve, 2017). The data collection is also simpler as only dough volume is required as leavening progresses for constructing this model. Thus, it is anticipated that the outcome is also satisfactory for leavening of sourdough starters that has not been studied before. Such application of Gompertz model is essential to predict the time

required for sourdough microbes to be active enough as leavening agent in order to proceed making sourdough products.

2.5 Sourdough Products

Since sourdough is advantageous in various aspects as mentioned (Section 2.1), especially in terms of leavening, it is incorporated in many food products. Most of the time, sourdough is associated with bakery products, such as breads, soda crackers, biscuits, Panettone and Pandoro cakes. Sourdough has positively affected the bakery products from the aspects of final volume, crumb elasticity, shelf life and sensory qualities (De Vuyst et al., 2017; Hammes & Gänzle, 1998). The addition of sourdough in crackers has improved the antioxidant activity and sensory qualities significantly, including its taste and texture (Selimović et al., 2017). Healthier sourdough biscuits with lower sugar content are produced, with minimum negative effects on the texture and spreading (Sahin, Rice, et al., 2019). Besides, sourdough which has antimicrobial features is also suitable to be added in panettone as potential food additive replacement for longer shelf life up to a few months (Aparecida Pereira et al., 2019) as panettone is usually baked earlier to meet the huge demand during Christmas. Similar to panettone, the large amount of sugar in pandoro cakes limits the gluten development in doughs, hence they are highly dependent on sourdough incorporation for leavening (Sahin, Zannini, et al., 2019).

Sourdough also could be added when preparing pancakes, which is a staple food with different names and different tastes in various countries. With incorporation of sourdough, starch gelatinisation was reduced in Shandong pancakes, *Jianbing*, that is traditionally prepared using coarse cereals like millet, buckwheat and sorghum in

northern China (W. Liu et al., 2023; Tang et al., 2022). Addition of sourdough in pancake products has given benefits to health, for example, the potential possesses hypoglycaemic effects with decreasing fasting blood glucose levels detected on diabetic mice (Tang et al., 2022). The nutrient bioavailability of *injera*, a type of sourdough pancake in East African countries that involve fermentation of tef flour or sometimes sorghum, wheat and barley flours has been proven to be boosted (Baye et al., 2013; Mezgebe et al., 2020; Seyoum et al., 2016). By undergoing sourdough fermentation in *injera*, the contents of phytate and iron-binding polyphenols, that blocks iron absorption was reduced (Baye et al., 2013; Seyoum et al., 2016).

The presence of sourdough improves the qualities of pizza and pasta which are largely comprised of durum wheat (*Triticum durum*) as the main ingredient (Fois et al., 2018; Montemurro et al., 2019). The shelf life of fresh pizza base was extended by 10 days within modified atmosphere packaging after including sourdough (Calasso et al., 2023). Such improvement by sourdough addition has been observed for fresh pasta too (Fois et al., 2018). Traditionally, no fermentation is involved during pasta making, but the addition of beneficial sourdough in pasta has great potentials in raising level of inaccessible digestible starch (34.5 to 40.2 g/100g) and vitamin B (riboflavin) fortification that contributed to 19.2% of the Reference Daily Intake (RDI) for men and 22.7% of the RDI for women (Fois et al., 2018; Montemurro et al., 2019).

There are also some atypical food products that are linked with sourdough, such as Turkish *tarhana* powder that is obtained through traditional wheat fermentation to be cooked as soup (İçen et al., 2021) and some African traditional cereal-based beverages including *ting*, fermented sorghum that exists as intermediate product of porridge from Botswana, highly viscous *boza* from Balkan countries and *mageu* which is maize-

based (Gänzle, 2014; Hammes & Gänzle, 1998; Madilo et al., 2024; Nyanzi & Jooste, 2012). After fermentation, both *boza* and *mageu* have been proven to be antimicrobial as they contain beneficial LAB such as *Lb. fermentum*, *Lb. paracasei*, *Lb. rhamnosus*, *Lb. delbrueckii*, *Lb. plantarum*, *Ec. faecium* ST62BZ and *Ec. faecium* BFE 900 (Madilo et al., 2024; Nyanzi & Jooste, 2012). Despite the diverse varieties of sourdough products, sourdough bread is still the most developed and studied sourdough product among all.

2.5.1 Sourdough Bread

The structure of dough and eventually bread is supported by baker's yeast typically, which is used to aid in leavening though it can also be replaced by sourdough. Sourdough is a potential candidate to promote leavening in most types of breads, *e.g.*, croissants (Di Monaco et al., 2014; Polese et al., 2018), French baguette (Catană et al., 2022; Sahin, Zannini, et al., 2019), German rye bread (Decock & Cappelle, 2005; Gänzle, 2014; Sahin, Zannini, et al., 2019), San Franciso sourdough bread (De Vuyst et al., 2017; Decock & Cappelle, 2005; Gänzle, 2014) and bread loaves (Coda, Varis, et al., 2017; Katina et al., 2006; Lebedenko et al., 2019; Rizzello et al., 2014; Rizzello, Lorusso, et al., 2015). Sourdough, as a leavening agent, brings along improvement in terms of flavour, texture, shelf life and nutritional value too (De Vuyst et al., 2017; Hammes & Gänzle, 1998).

The mixture of sourdough and other ingredients of bread doughs, such as flour, water and salt (optional), undergoes a few stages of fermentation and makeup before being baked (Lai & Lin, 2006; Putseys & Schooneveld-Bergmans, 2019). Apart from proteolytic enzymes naturally found in wheat, the sourdough LAB also release some

enzymes such as amylase and protease, hence flour proteins including gluten are broken down and converted into flavour precursor compounds along the fermentation process (Arendt et al., 2007; Carrizo et al., 2017). Further leavening of doughs usually happens during proofing, the final fermentation stage. Proofing temperature and time are critical to ensure qualities of baked bread, as overproofing may cause collapsing of gluten network that gives coarse texture in bread (Clarke et al., 2003; Huang & Miskelly, 2019; Lai & Lin, 2006). The study of Gally et al. (2017) depicted that the proofing time at 35 °C was reduced from 122 minutes to 65-70 minutes with ohmic heating of 1-10 °C/min for bread dough leavened by baker's yeast.

Owing to sourdough fermentation, aroma volatiles such as diacetyl, acetaldehyde, hexanal and ethyl acetate are released in dough during baking (Aparecida Pereira et al., 2019; Bolourian et al., 2010) via Maillard reaction (Sahin, Zannini, et al., 2019). Such reaction which involves both reducing sugars and free amino acids contributes to browning of sourdough bread too (Sahin, Zannini, et al., 2019). In order to optimise the crust colour of bread, baking temperature (typically 160-250 °C) is always controlled within suitable range following to the type of bread, *i.e.*, 205 to 220 °C for lean bread with low sugar and fat and lower range of 175 to 205 °C for rich bread with more sugar and fat to avoid excessive caramelisation (Bredariol et al., 2019; Lai & Lin, 2006; Putseys & Schooneveld-Bergmans, 2019). The firm structure of bread is formed during baking. The dough protein is denatured by heat, leading to crosslinking that forms a structural network together with the partially gelatinised starch granules (Bredariol et al., 2019; Lai & Lin, 2006). During heating, the bread is also risen when the steam is released from the water content of dough or sometimes is injected from the oven (Bredariol et al., 2019).

The benefits of sourdough in breads have been continuously studied. A remarkable improvement in crumb elasticity by 30.5–37.9% and slower staling were demonstrated in sourdough breads from straight doughs, whereby all ingredients were mixed in a single operation and fermented in (Lebedenko et al., 2019). Sourdough croissants were found to be chewier, less hard and having larger number of big bubbles in the crumb than those prepared using straight doughs (Di Monaco et al., 2014). The glycaemic impact on human body was proposed to be lesser, contributed by the reduced amount of available carbohydrates, in sourdough baguettes fortified with Jerusalem artichoke flour (Catană et al., 2022). Similar results were also deduced by Coda, Varis, et al. (2017) with the decreased GI in wheat breads leavened by faba bean sourdough. The postprandial gastrointestinal function was also improved, indicated by 30% lower hydrogen production and the reduced bloating sensation in sourdough croissants as compared to croissants leavened by brewer's yeast (Polese et al., 2018). From the aspects of taste and appearance, sourdough reduced the intensities of dough and yeast flavours, together with the colours of brown crust and yellow crumb (Di Monaco et al., 2014).

2.5.2 Steamed Sourdough Bread Instead of Baked Ones

Apart from the common baked bread, sourdough can also be incorporated in steamed bread where the main difference between them is in the method of heating (Gänzle, 2014). Steamed bread is a common staple food in China for almost two thousand years, particularly the northern provinces (Huang & Miskelly, 2019; Nip, 2006). The popularity can be reflected by the wheat consumption for steamed bread at approximately 40% in China (Kim et al., 2009; F. Ma et al., 2017). As sharing of food recipes and food making skills through social media is prevalent nowadays (Albagli et

al., 2023), the culture of steaming bread is widespread to other countries, including Korea, Japan and some southeast Asian countries too (Huang & Miskelly, 2019; Su et al., 2005). The steamed bread usually possesses smooth, white and thin skin (S. Ma et al., 2014; Nip, 2006; X.-Y. Wang et al., 2016) along with soft, moist and uniform crumb (X.-Y. Wang et al., 2016). Sometimes, steamed bread contains fillings or is deep-fried too (Huang & Miskelly, 2019; Nip, 2006). Being constantly held in steamer, the steamed bread is served hot with its optimum quality. Otherwise, the steamed bread hardens with firmer structure when it is cool (Zhu, 2014). Steamed bread is classified geographically in China; northern ones are usually chewy and dense while southern ones have more open and softer texture (Zhu et al., 2016).

Although baker's yeast is more common in steamed breads, the incorporation of sourdough in steamed bread has been practiced continuously since the time before baker's yeast was commercially available (Huang et al., 1993; Kim et al., 2009; G. Zhang et al., 2016). However, the studies on SSBs are not as extensively analysed as for baked sourdough breads. The fermentation mechanisms in doughs for both steamed and baked sourdough breads do not deviate much from each other. Similarly, sourdough LAB provide the sour flavour while sourdough yeasts supply carbon dioxide for leavening of SSB (Zhu, 2014). The pH of sourdough starters for steamed breads is below 4.0 and at 3.7 on average (De Vuyst et al., 2017). Chen et al. (2018) proposed that the hardness of the steamed bread reduces from 232.5 g to 170.7-225.9 g as the baker's yeast used was replaced by 10-40% sourdough. One of the significant differences between baked sourdough bread and steamed sourdough bread is the choice of flour. The protein content of 10-11% is preferred rather than high protein flour for the northern style SSB to ensure the smooth bread surface and satisfying eating quality (Zhu, 2014). Meanwhile, G. Zhang et al. (2016) claimed that by using

wheat flour with 7.56% protein content, unique aromatic profiles were formed when 10% sourdough was incorporated in SSB.

Steaming is usually carried out at temperature of 100 °C (Zhu, 2014). During steaming, the starch in dough gelatinises with the presence of water, whereby it loses its crystalline structure and turns amorphous (Zhu, 2014). Such condition is known as thermal transition (X.-Y. Wang et al., 2016). Gluten plays its role by further associating and retaining the carbon dioxide released, hence aids the soft crumb formation with open gas cells in the dough (Zhu, 2014). By the time when the steamed bread is cooled, the rubbery starch influences the quality by interacting with water, leading to retrogradation that hardens the structure (Zhu, 2014).

Since steaming involves comparatively lower processing temperature and higher moisture content, both the natural and fortified nutrients of steamed bread could be retained better without experiencing the Maillard reactions (P. Wang et al., 2015; X.-Y. Wang et al., 2016). The lower temperature of steaming inhibited the formation of some potentially carcinogenic toxins, such as acrylamide (Becalski et al., 2003; Zhu, 2014) and furan (Zhu, 2014) that are occasionally found in baked bread. Crust formation does not occur in steamed bread, ascribed to the lower heating temperature (S. Ma et al., 2014; Nip, 2006). Thus, this may satisfy the demand of some consumers, such as children and elders who enjoy breads but find the texture of bread crust unfavourable.

The market of breads is also widened by popularisation of steamed bread. Consumers from all over the world are introduced to a new series of sensation in steamed bread, including those with dense, cohesive and elastic texture (X.-Y. Wang et al., 2016),

fresh aroma of wheat (S. Ma et al., 2014) or other ingredients, and the natural taste. As the market, *e.g.* in China (S. Ma et al., 2014) demands for fresh, nutritional and healthy food products these days (Grand View Research, 2019), steamed bread can be said to have great potential in fulfilling these demands. The ingredient list for steamed bread is usually simpler, *i.e.*, mainly wheat flour, leavening agent and water than that for baked bread, which also includes sucrose and salt (X.-Y. Wang et al., 2016). Hence, steamed bread is believed to be healthier as it contains less oil and sodium (Zhu, 2014).

2.6 Characterisation of Steamed Sourdough Bread

Contributed by the metabolic activities of LAB and yeast, sourdough bread always portrays unique characteristics that stands out among the commercial breads. There are four key factors that describe the quality of food products, *i.e.* appearance including colour, shape and size, flavour that comprises taste and odour, texture and nutrition (Bourne, 2014). Sourdough breads, in the form of baked bread or steamed bread, excite consumers with new, interesting flavours. At the same time, they support consumers' health with the presence of prebiotics and probiotics, together with some beneficial biochemical components (Petrova & Petrov, 2020). Despite their similarities, the expected qualities of baked pan bread and steamed bread are different from some aspects, *e.g.* colour, hardness, elasticity, cohesiveness and smoothness (S. Ma et al., 2014). Hence, the characterisation of SSB is gradually built up and highlighted by their beneficial properties, fulfilling the current consumer demands in healthier, tastier and more natural bakery products (Grand View Research, 2019).

2.6.1 Volume

Volume of bread is initiated by carbon dioxide produced from yeast and LAB activities and sustained by gluten network (Chavan & Chavan, 2011; De Vuyst et al., 2017). Hence, volume is an indicator of fluffiness, which is influenced by gas production and gas holding capacities in steamed bread (Li et al., 2021). Specific volume, the ratio of volume to weight (Huang & Miskelly, 2019; Su et al., 2005) is usually analysed and compared among researchers. The specific volume of steamed bread is usually smaller than baked pan bread (S. Ma et al., 2014). Aiming for making healthier breads, such as those which are gluten-free or high fibre, the mission in achieving acceptable specific volume may be even more difficult as the usage of non-typical flour may lack in gas holding capability. As portrayed by the specific volume drop of baked bread by 7-27%, the addition of green bean, pea and mesquite whole pod flours decreased the strength of gluten in supporting the bread structure (González-Montemayor et al., 2021). Similarly, the usage of 10-50% wheat-foxtail millet composite flours in making steamed breads affected the specific volume negatively, from 2.25 cm^3/g to a range of 1.38-2.10 cm^3/g (Li et al., 2021).

By incorporating sourdough at adequate amount, the organic acids boost the gas retention capacity by swelling the gluten, thus enhance the loaf volume (Chavan & Chavan, 2011; Olojede et al., 2019). The specific volume of legume-fortified sourdough sorghum bread had been raised dramatically to 3.63 cm^3/g (Olojede et al., 2019). High fibre sourdough starters using durum wheat flour also showed bigger volume increase than the control starter using soft wheat flour (De Angelis et al., 2018). In SSB, the specific volume increased by 26.7% with 30% sourdough (Chen et al., 2018). Conversely, with 40% sourdough, the specific volume dropped from 2.56 to

2.32 ml/g, as compared to the one with 30% sourdough (Chen et al., 2018). When the dough pH is too low, the high acidity reduces the bread volume (Chen et al., 2018).

2.6.2 Spread Ratio

An ideal steamed bread should be round and symmetrical. Therefore, spread ratio is usually used to determine the shape of steamed bread quantitatively (Li et al., 2021). Spread ratio refers to the ratio of width to height (F. Ma et al., 2017; Zhu, 2014) which shows the spreading of dough during steaming. The desired value of spread ratio for Chinese steamed bread may deviate from one study to another, for example, 1.40-1.44 from a quality grading system (Huang et al., 1998; Zhu, 2014) and 1.5 as a quick guide for steamed buns (Huang & Miskelly, 2019; Kruger et al., 1992). The bigger the spread ratio, the poorer the quality of steamed bread (Huang et al., 1998; Zhu, 2014) as the shape is flattened.

The spread ratio of steamed bread is affected by the dough composition, for example, added non-starch polysaccharides, wheat protein and sourdough. The dough that was added with 0.2% non-starch polysaccharides, *i.e.*, carboxymethylcellulose, locust bean gum and psyllium husk powder respectively showed smaller spread ratio of 1.61, 1.65 and 1.56 than the control at 1.69 after being steamed (Sim et al., 2015). The protein content of the flour is negatively correlated to the spread ratio, potentially because of the higher moisture content in steamed bread using high protein flour (F. Ma et al., 2017). This is supported by the study of F. Ma et al. (2017) that used high protein flour in making steamed bread, resulting in a lower average spread ratio of 1.4 and softer texture. The spread ratio of steamed bread can also be markedly reduced by sourdough, *i.e.*, from 1.52 to 1.38 with 30% sourdough addition (Chen et al., 2018).

2.6.3 Colour

Usually, consumers focus more on the colour rather than other attributes for steamed bread (S. Ma et al., 2014) and they expect that it is bright white or milky white in colour (Zhu, 2014). Colour is highly subjective, hence researchers use colorimeter (Kruger et al., 1992; Loong & Wong, 2018) or colour meter (He et al., 2003; Huang & Miskelly, 2019; Su et al., 2005) to objectively determine the colour of food products including steamed bread. By doing so, colour can be identified as numerical values in the form of lightness (L*), a* (+red, -green) and b* (+yellow, -blue) (Loong & Wong, 2018). Consequently, if new ingredients are added in steamed bread, more attention should be paid on the colour of steamed bread to maintain its quality.

The hard type wheat is an example ingredient that may influence the colour of steamed bread. This wheat is rich in gluten that boosts volume and texture in bread, yet its nature colour is dark, especially when the gluten is too stiff, thus it is not desirable for steamed bread production (He et al., 2003; Shchekoldina & Aider, 2014). Wheat germ addition at low level increased the L* value, possibly on account of bleaching effect of lipoxygenase. However, with wheat germ more than 4%, the steamed bread appeared darker as the L* value dropped eventually (S. Ma et al., 2014).

The processing methods also can influence the colour of food products. The baked bread is always darker in colour as browning occurs due to Maillard reaction (Sahin, Zannini, et al., 2019). The crumb and crust colours were negatively influenced by adding legumes, since the greater amount of protein supported the reaction for longer time during baking (Olojede et al., 2019). Conversely, under controlled conditions,

Maillard reaction brought attractive, bright yellow colour to sourdough Panettones through addition of orange-fleshed sweet potato flour (Aparecida Pereira et al., 2019).

2.6.4 Texture

In general, consumers expect bread crumb, whether baked or steamed, to be soft while bread manufacturers pay attention on crumb uniformity and distribution in size of gas bubbles (Bourne, 2014). Fresh bread usually deforms easily but this does not happen to stale bread due to starch retrogradation (Bourne, 2014). For more impressive bread texture even after some days of storage, sourdough has been proven to play a vital role. Chavan and Chavan (2011) and Gobbetti et al. (2020) reported that sourdough bread is softer and more chewy than commercial bread. This is attributed by the boosted ability of gluten network in trapping carbon dioxide, as the protein molecules have been modified due to the acidic environment during sourdough fermentation (Katina et al., 2006; X. Wang et al., 2020). The release of exopolysaccharides during sourdough fermentation is also great in amount to retain carbon dioxide better by adjusting dough viscosity, thus enhancing the soft texture of bread (Gänzle, 2014).

The texture of steamed bread is quantified by using various scientific instruments, such as baker compressimeter, penetrometer and texture analyser (Bourne, 2014). The instruments apply the principle of deformation, *i.e.*, the distance whereby the food is compressed when some external force is applied (Bourne, 2014). By applying Texture Profile Analysis (TPA) as in Figure 2.5, the equipment mimics the chewing of mouth on food and objectively determines the various types of textural parameters (Olojede et al., 2019). Among the parameters, hardness refers to the height of the force peak on the first compression cycle or first bite, whereas the definition for springiness is the

distance which food recovered its height during the period between the end of the first bite and the start of the second bite (Bourne, 2014). Cohesiveness, that describes the ability of withstanding a second deformation after the second compression cycle, is also studied for steamed bread (Rizzello et al., 2014). By conducting TPA, chewiness, as the product of hardness, cohesiveness and springiness (Bourne, 2014), and resilience, the ability of crumb to recover from compression (Chen et al., 2018) are also determined.

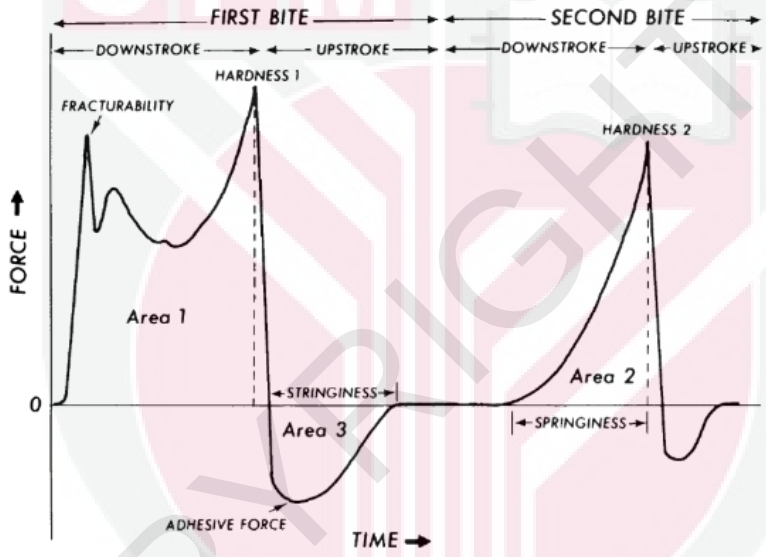

Figure 2.5: A Texture Profile Analysis (TPA) curve (Source: Bourne, 2014)

The parameters can be studied selectively following the nature of food products. For example, the texture of kefir produced by kefir grains or starter culture has been studied by measuring firmness, consistency, cohesiveness and viscosity index (Cais-Sokolińska et al., 2016) while researchers can also describe texture of yogurt samples with different percentages of inulin by focusing on hardness, adhesiveness, cohesiveness and springiness (Helal et al., 2018). The level of staleness in bread is measured by compressing bread and measuring its hardness and chewiness (Chen et

al., 2018). The addition of atypical bread ingredients, such as foxtail millet, had pronouncedly raised the hardness, gumminess and chewiness in steamed bread (Li et al., 2021). Within three days of storage, the hardness and chewiness of SSB increased significantly, whereas the values of cohesiveness, springiness and resilience decreased significantly (Chen et al., 2018). In short, the palatability of SSB is affected by the texture and hence portrayed by the values of relevant textural parameters.

2.6.5 Sensory Qualities

Consumers pay high attention towards the sensory qualities of food products they consume. They appreciate and consume more food products with better taste and smell. Nevertheless, machinery equipment is still incompetent to measure and analyse consumer acceptance and preference completely and accurately. The sensory quality test that involves panellists is therefore an important and scientific technique in detecting food products' sensorial attributes. Some methods that are applied for sensory quality tests include the (a) ordinal methods that focus on preferences, (b) interval methods that consider absolute levels of liking using rating scales and (c) ratio methods that judge the liking based on ratio (Cardello & Schutz, 2006). Fois et al. (2018) conducted sensory analysis using ordinal methods and found that addition of beneficial sourdough in pasta is highly potential as there was no significant difference in preference of pasta, including control, pasteurised or sourdough pasta.

By using interval methods, the 9-point hedonic scale is one of the popular sensory evaluating methods for sourdough (Chinma et al., 2016; Hanis-Syazwani et al., 2018; Karrar et al., 2020; Kefalas et al., 2009; Mariotti et al., 2014; Olojede et al., 2019). It can measure the degree of acceptance for consumers towards the qualities like

appearance, taste, texture and aroma (Olojede et al., 2019). The aroma of sourdough bread crust is usually sourced from the baking parameters while the flavour of crumb is originated from sourdough fermentation (Aparecida Pereira et al., 2019; Bredariol et al., 2019; Corsetti & Settanni, 2007). In sourdough products, amino acids and peptides, which are naturally present, affect the taste and initiate the volatile flavour compounds, *e.g.* the alcohols, aldehydes, ketones, esters, and sulphur for the distinctive taste and flavour (Aparecida Pereira et al., 2019; Chavan & Chavan, 2011). Trained or untrained panellists are invited to test the food samples so that they can rate the qualities objectively and quantitatively for scientific analysis. A scale of 1 (extremely dislike) to 9 (extremely like) is available for panellists to rate each of the qualities in each food sample (Cardello & Schutz, 2006; Olojede et al., 2019).

Ogunsakin et al. (2015) deduced that the scores of overall acceptability given by panellists for sourdough breads are at least 1.75 higher than the bread baked with baker's yeast. The replacement of baker's yeast by wholemeal sourdough in wholemeal wheat bread was also successful since the colour, aroma and overall liking scores were significantly higher, with respective values roughly from 2.8 to 4.9, from 2.0 to 3.8 and from 3.0 to 3.5 (De Angelis et al., 2018). Similar results were obtained in another study involving sorghum bread with sorghum sourdough. However, when less sourdough was added, the differences between scores for bread with 10% sourdough addition and control bread are all insignificant, *e.g.*, overall liking scores are respectively 7.9 and 8.1 (Karrar et al., 2020). The global liking scores for sourdough breads consisting of wheat, wheat-barley composite and barley flours were insignificantly different from one another, ranging from 4.44 to 5.33 (Mariotti et al., 2014).

2.6.6 Proximate Composition

Proximate composition, mainly involving crude protein, carbohydrates, fats and energy (Kok & Mohamed Radzi, 2017) of food products is commonly analysed. Such composition portrays the basic nutritional content in food products. Being one of the key factors in determining qualities of food products (Bourne, 2014), nutritional content attracts a lot of attention from researchers, food manufacturers and even consumers. The proximate composition of food products is determined by researchers and are controlled during food manufacturing where food manufacturers will print the values on nutrition label as what the consumers look into today. The nutrition label is a communication tool that passes important nutritional information such as values of calories, fats, sugars and sodium to the consumers (Kok & Mohamed Radzi, 2017). The presence of nutrition label on food products including bread, is also essential to abide by the Food Act 1983 (Act 281) & Regulations (Kok & Mohamed Radzi, 2017).

Proximate analysis is conducted from time to time to find the proximate composition of breads, regardless of whether it is leavened by baker's yeast (Mudau et al., 2021; Noor Aziah et al., 2012) or sourdough (Catană et al., 2022; Chinma et al., 2016; González-Montemayor et al., 2021; Olojede et al., 2019) along with the ingredients used, *e.g.* flour (Chinma et al., 2016; Coda, Kianjam, et al., 2017; González-Montemayor et al., 2021; Kim et al., 2009; Rizzello, Lorusso, et al., 2015; Yang, 2016) and wheat bran (F. Ma et al., 2017; Noort et al., 2010). Sourdough bread with low amount of carbohydrate and great amount of protein, ash and fibre is more advantageous for consumers (Catană et al., 2022) especially those with health problems. By adding quinoa sourdough in bread, the levels of soluble fibre and sugar were respectively increased from 0.3 to 2.4% and decreased from 1.0 to 0.7%, as

compared to wheat bread with baker's yeast (Rizzello, Lorusso, et al., 2015). The abundance of dietary fibre in sorghum bread was raised by sourdough fermentation, which was then further boosted by fortification using cowpea or chickpea (Olojede et al., 2019).

Proximate composition is also important to manipulate the quality of sourdough bread. The levels of protein and starch have negative and positive correlations respectively with spread ratio score of steamed bread (F. Ma et al., 2017). Loaf volume is a good indicator of dough leavening that is positively correlated to the protein content that represents strength and quality of gluten (protein) (Aparecida Pereira et al., 2019; Kim et al., 2009) and negatively correlated to the dietary fibre content which disturbs the formation of gluten network due to the fibre-protein interactions (F. Ma et al., 2017; Noort et al., 2010). Although the incorporation of legumes, *i.e.* pea, green bean or mesquite have reduced the carbohydrate content and boosted the protein content, the loaf volume of wheat bread was reduced as the additional protein was not for structural purposes (González-Montemayor et al., 2021). This finding contradicted with another study of sorghum breads that demonstrated improvement on loaf volume after adding cowpea or chickpea flours (Olojede et al., 2019). By adding legumes, the escalation of protein content may lead to adverse effects on crumb and crust colour of bread as Maillard reaction carried on longer with more available protein (Olojede et al., 2019).

2.7 Microbiological Profiles in Sourdough

The success of sourdough starters in contributing to impressive sourdough products has been acknowledged widely. Excellent qualities of sourdough products, such as satisfactory volume, textural properties and signature sour flavour were due to the

activities of microorganisms inhabiting the starters. Within the starters, the microorganisms, mainly made up of more than 60 species of lactic acid bacteria (LAB) and around 40 species of yeast (Calvert et al., 2021) that affect and interact among one another, forming ecological communities known as microbiota (De Vuyst et al., 2017). The sourdough microbiota gets more mature and stable as time goes, releasing organic acids which pull down the sourdough pH (Oshiro et al., 2020) and carbon dioxide which aids leavening. The wholesome microbes were also claimed to have probiotic potential by improving health or wellness especially for gastrointestinal tract (De Angelis & Gobbetti, 2011; Montemurro et al., 2019).

The mature microbiota of sourdough varies with one another and could be altered by adjusting the types and proportion of ingredients and even some of the process parameters like frequency and number of backslopping (Chavan & Chavan, 2011; Montemurro et al., 2020; Ogunsakin et al., 2015; Plessas, 2021; Reese et al., 2020; Sekwati-Monang et al., 2012). By involving different ingredients, innate microorganisms from raw cereals or non-cereals that remained in flours after milling act as pioneers to initiate the spontaneous fermentation of sourdough starters. This was supported by the findings of Reese et al. (2020) that reported the highest similarity was between microbiota in flour and sourdough starters as compared to the external factors like water source, bakers' hands and dust. The usage of different batches or types of flours also harboured different sourdough microbiota, since the abundances of carbohydrates, proteins, minerals, lipids and enzymes do vary (Plessas, 2021).

Despite under different circumstances, *e.g.* temperature (Reidzane et al., 2021; Vrancken et al., 2011) and pH (Van Kerrebroeck et al., 2016), sourdough microorganisms are still able to adapt accordingly. Akinola and Osundahunsi (2017)

found out that during fermentation of millet sourdough, microbial population had undergone typical growth curve, including lag phase and exponential phase, which showed their adaptations towards the conditions. Apart from the aspect of microbial population, the microbiological profile also changed following the conditions before achieving maturation of sourdough (Ben Omar & Ampe, 2000; Oshiro et al., 2020; Vrancken et al., 2011). This adapting phenomenon is usually known as succession, whereby the species which fails to survive, successively making way for the growth of another species that favours the conditions (Oshiro et al., 2020). The three-phase microbial succession is usually initiated by the domination of natural microbes from wheat flour, especially *Enterobacter* and *Enterococcus* (Ercolini et al., 2013; Ispirli et al., 2018). Following that, sourdough bacteria such as *Weissella* and *Lactobacillus* are the majority during the second phase from day 2 to day 5 (Ercolini et al., 2013; Ispirli et al., 2018; Van der Meulen et al., 2007) while the final phase is only dominated by those adapting well in the acidic condition, *e.g., Leuconostoc* spp., *Lb. sakei, Lb. brevis, Lb. plantarum* and *Lb. fermentum* (Ercolini et al., 2013; Siepmann et al., 2017; Van der Meulen et al., 2007). The relevant details have been published in the paper entitled "Sourdough microbiome comparison and benefits" as shown in list of publications of the thesis.

Thus, the microbiological profile of each sourdough starter is found to be distinctive from the others with their signature aroma, flavour and level of sourness (Novotni et al., 2021; Salim-ur-Rehman et al., 2006). The sourdough microbiota has been mostly studied through total viable count, *i.e.* a quantitative estimation on microbial population. The averaged LAB count of 9.60 $\pm$ 0.03 log CFU/g was consistent in ripe sourdough after spontaneous fermentation of 256 hours using the mixture of amaranth, buckwheat and quinoa flours, while the yeast count were raised from 4.64 $\pm$ 0.18 to

8.21 ± 0.24 log CFU/g (Carbó et al., 2020). Taxonomic profiles were also demonstrated in bar plots (De Angelis et al., 2018; Ercolini et al., 2013; Gaglio et al., 2020; Menezes et al., 2020; Oshiro et al., 2020; Van Kerrebroeck et al., 2016) or heat maps (Coda, Kianjam, et al., 2017; Minervini et al., 2016) after undergoing 16S rRNA sequencing through Ilumina MiSeq platform. Alpha diversity, in terms of number of operational taxonomic units (OTU), Good's estimated sample coverage (ESC), rarefaction and indices such as Chao1 richness and Shannon diversity were used to analyse the diversities of microbiota within each sourdough starter by Minervini et al. (2016) and Ercolini et al. (2013). Shannon and Simpson indices were used instead in the study of Urien et al. (2019) to investigate the yeast communities in wheat sourdoughs. The microbial dissimilarities among each sourdough starters were compared and displayed in previous studies through phylogenetic tree (Urien et al., 2019) and Principal Coordinate Analysis (PCoA) plots based on unweighted (Minervini et al., 2016; Oshiro et al., 2020) and weighted (Coda, Kianjam, et al., 2017; Ercolini et al., 2013) UniFrac distance matrices.

2.7.1 Next-Generation Sequencing for Microbiological Study

In determining sourdough microbiota for deeper understanding and analysis, various approaches such as the culture-dependent methods and culture-independent ones including the Next-Generation Sequencing (NGS) (Coda, Kianjam, et al., 2017; De Angelis et al., 2018; Ercolini et al., 2013; Gaglio et al., 2020; Menezes et al., 2020; X. Wang et al., 2020), polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/DGGE) (Di Cagno et al., 2014; Ercolini et al., 2013; Harth et al., 2016; Taccari et al., 2016) and real-time quantitative PCR (Lhomme et al., 2015; Oshiro et al., 2020) were conducted.

Among them, NGS emerges as a popular approach in association with its increasing throughput, reliability and affordability (Hess et al., 2020). The presence of subpopulations in microbiota can also be detected by applying NGS while it may be biased and challenging for the culture-dependent approach (De Vuyst et al., 2017; Ercolini et al., 2013). The main protocol of NGS involves DNA extraction from samples, library preparation with defined lengths and adapters, sequencing and analysis by bioinformatics (Hess et al., 2020). Quality control measures throughout the protocol are also vital in NGS for its recognised reliability. De Angelis et al. (2018) eliminated merged reads below minimum quality of 30, while Coda, Kianjam, et al. (2017) conducted agarose gel electrophoresis using the extracted RNA to ensure accuracy of final microbiological profiles.

For investigation of sourdough bacterial communities, the profiles are usually obtained by amplifying the hypervariable V1-V3 (Coda, Kianjam, et al., 2017; De Angelis et al., 2018; Ercolini et al., 2013), V3-V4 (Gaglio et al., 2020; Menezes et al., 2020) or the V4 (X. Wang et al., 2020) regions of 16S ribosomal RNA (rRNA) gene sequences. Urien et al. (2019) claimed that amplification of internal transcribed spacers (ITS1 and ITS2) rRNA gene sequences (Coda, Kianjam, et al., 2017; Landis et al., 2021; Urien et al., 2019; Van Kerrebroeck et al., 2016; X. Wang et al., 2020) from fungal gDNA described the fungal communities better than the D1-D2 region of the 26S rRNA gene amplified by studies of Ercolini et al. (2013) and Van Kerrebroeck et al. (2016).

NGS offers a more thorough description of microbiological profiles in sourdough starters. X. Wang et al. (2020) monitored the microbial dynamics and detected increasing abundances of *Lactobacillus* including *Lb. sanfranciscensis* and *Lb. pontis* during the 24-hour sourdough maturation and sponge dough retarding. Menezes et al.

(2020) suggested the suitable fermentation temperature of 21 °C after observing the gradual decrease in abundances of *Bacillus*, *Clostridium*, *Pseudomonas* and *Enterobacteriaceae* which are non-beneficial in sourdough starters. By applying NGS technology, the microbial diversity was found to be significantly decreased after 5 days of backslopping and by using dehulled faba bean flour for sourdough fermentation in the study of Coda, Kianjam, et al. (2017).

2.7.2 Microbiota of Sourdough and Its Products

The NGS and advanced technology are capable of detecting microbiological profiles in a more complete manner. LAB, which are frequently isolated from sourdough, are gram-positive and non-motile cocci or rods (Hansen, 2006; Rizzello, Cavoski, et al., 2015). Sourdough LAB can be represented by *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* while *Saccharomyces*, *Kazachstania* and *Kluyveromyces* are some common sourdough yeasts (Akinola & Osundahunsi, 2017; Ercolini et al., 2013; Van Kerrebroeck et al., 2016). Other subdominant yet beneficiary microbes, such as acetic acid bacteria (AAB) (Di Cagno et al., 2014; X. Wang et al., 2020) and novel or atypical LAB and yeast (De Angelis et al., 2018; Rizzello et al., 2014) are also identifiable. From Table 2.1, some isolated LAB and yeasts from sourdough are listed following the types of ingredients.

Table 2.1: Summary of LAB and yeasts isolated from sourdough using different types of flours

Flour	LAB	Yeasts	References
Cereal			
Wheat	*Lb. brevis* *Lb. plantarum* *Lb. sanfranciscensis* *Lc. lactis* *L. citreum* *W. cibaria*	*C. humilis* *K. barnettii* *R. glacialis* *S. bayanus* *S. cerevisiae*	(Rizzello, Cavoski, et al., 2015)
	Lb. sanfranciscensis *Lb. pontis*	*K. humilis* *S. cerevisiae*	(X. Wang et al., 2020)

Table 2.1: Continued

	Ec. Cecorum *Ec. faecium* *Lb. crustorum* *Lb. delbrueckii* *Lb. plantarum* *Lb. sanfranciscensis* *Lc. garvieae*	*C. humilis* *C. tropicalis* *Cyberlindnera jadinii* *S. cerevisiae* *T. delbrueckii* *W. anomalus*	(G. Zhang et al., 2019)
	Lb. plantarum *Lb. sakei* *Lc. lactis* *Leuconostoc* spp. *P. pentosaceus* *Weissella* spp.	*C. humilis* *K. barnettii* *S. bayanus* *S. cerevisiae* *Wi. anomalus*	(Ercolini et al., 2013)
	Lb. brevis *Lb. plantarum* *Lb. sanfranciscensis* *L. lactis* *L. citreum* *L. mesenteroides*	*C. humilis* *T. delbrueckii* *S. cerevisiae* *S. bayanus-Kazachstania* sp.	(Di Cagno et al., 2014)
	Lb. brevis *Lb. sanfranciscensis* *P. pentosaceus* *W. cibaria* *W. confusa*		(Oshiro et al., 2020)
	Lb. fermentum *Lb. plantarum* group	*K. exigua/bulderi/barnettii*	(Van Kerrebroeck et al., 2016)
	Lb. brantae *Lb. lindneri* *Lb. nodensis* *Lb. plantarum* *L. carnosum* *L. kimchi* *L. mesenteroides* *P. argentinicus* *P. pentosaceus* *W. cibaria* *W. confusa* *W. salipiscis*		(De Angelis et al., 2018)
Sorghum	*P. pentosaceus*	*S. cerevisiae*	(Ogunsakin et al., 2015)
Rye	*Lb. graminis* *Lb. plantarum* *Lc. lactis* subsp. *cremoris* *L. citreum* *W. cibaria* *W. confusa*		(Ispirli et al., 2018)
Rice	*Lb. brevis* *Lb. crustorum* *Lb. harbinensis* *Lb. pentosus* *Lb. plantarum* *L. pseudomesenteroides*	*S. cerevisiae*	(Lim et al., 2017)
Maize	*Lb. acidophilus* *Lb. brevis* *Lb. fermentum* *Lb. plantarum* *L. mesenteroides* *L. dextranicum* *P. acidilactici*	*S. cerevisiae*	(Edema & Sanni, 2008)
	Ec. saccharolyticus *Lb. casei* *Lb. delbrueckii* *Lb. fermentum* *Lb. plantarum* *Strep. bovis*		(Ben Omar & Ampe, 2000)

Table 2.1: Continued

Millet	*Lb. plantarum* *P. pentosaseus* *Lb. pentosus*	*S. cerevisiae* *C. milleri*	(Akinola & Osundahunsi, 2017)
Non-cereal			
Faba bean	*Lb. sakei* *Lc. lactis* *L. mesenteroides* *P. pentosaceus* *W. cibaria* *W. koreensis*		(Coda, Kianjam, et al., 2017)
Composite			
Apple Honey Wheat	*Lb. plantarum* *Lb. sakei* *P. pentosaceus*		(Gordún et al., 2014)
White grape Wheat	*Lb. brevis* *Lb. plantarum* *Lb. sakei* *P. pentosaceus* *W. cibaria*		
Yogurt Wheat	*P. pentosaceus*		
Wheat Chickpea Lentil bean	*Lb. brevis* *Lb. coryneformis* *Lb. fermentum* *Lb. parabuchneri* *Lb. paraplantarum* *Lb. plantarum* *Lb. pentosus* *Lb. rossiae* *Lb. sanfranciscensis* *L. mesenteroides* *W. cibaria*		(Rizzello et al., 2014)

Abbreviations: *C.*, *Candida*; *Ec*., *Enterococcus*; *K., Kazachstania*; *L., Leuconostoc*; *Lb*., *Lactobacillus*; *Lc*., *Lactococcus*; *P., Pediococcus; R., Rhodotorula; S., Saccharomyces; Strep., Streptococcus; T., Torulaspora; W., Weissella; Wi., Wickerhamomyces.*

Among the common sourdough LAB, *Lb. sanfranciscensis* has been isolated in traditional sourdoughs made of wheat (Di Cagno et al., 2014; Ercolini et al., 2013; Lhomme et al., 2015; Oshiro et al., 2020), wheat-legume composite flour (Rizzello et al., 2014) and rye (Lhomme et al., 2015). The growth of this LAB is inhibited below pH 3.8, therefore its presence is unlikely in highly acidic sourdough starters (De Vuyst et al., 2014; Van Kerrebroeck et al., 2016). Conversely, *Lb. fermentum, Lb. plantarum, Lb. reuteri, Lb. rossiae, and Lb. pontis* can still survive in sourdough starter with pH below 3.8 (De Vuyst et al., 2017; Gänzle & Gobbetti, 2013). Hence, they adapt well as acid-tolerant LAB and are usually predominant in type II sourdough where fermentation time is longer (De Vuyst et al., 2017; Gänzle & Gobbetti, 2013). Oshiro

et al. (2020) observed that *Lb. brevis* is the majority in sourdough microbiota under low pH at 4.5 and 5.5. It is often discussed together with *Lb. fermentum* (Minervini et al., 2016; Van der Meulen et al., 2007), which is possibly related to their similar range of favourable growth conditions and carbohydrate fermentation pattern. Harth et al. (2016) proposed that *Lb. fermentum* and *Lb. brevis* dominated microbiota of barley sourdough that showed excellent performances in breadmaking.

Many LAB, especially from the *Lactobacillus* genus such as *Lb. fermentum, Lb. alimentarius, Lb. brevis, Lb. sanfranciscensis* and *Lb. hilgardii* can be claimed as probiotics that reduce gluten content of wheat products, develop gut health by adjusting the microecology of gastrointestinal tract or synthesise antibacterial substances (De Angelis & Gobbetti, 2011; Montemurro et al., 2019). The mentioned lactobacilli were all obligately heterofermentative, releasing mainly lactic acid at around 50%, ethanol, acetic acid, carbon dioxide and acetaldehyde as by-products of metabolism (Chavan & Chavan, 2011; Hansen, 2006; Siepmann et al., 2017). The obligately heterofermentative lactobacilli are the only group that produce carbon dioxide through glucose fermentation, although there are two more groups of lactobacilli, *i.e.* obligately homofermentative and facultatively heterofermentative (Chavan & Chavan, 2011; Siepmann et al., 2017).

Yeasts play the role of leavening food products without the presence of obligately fermentative lactobacilli in sourdough starters. *S. cerevisiae* is one of the most common sourdough yeasts (Hansen, 2006; Lhomme et al., 2015; Urien et al., 2019). It possesses strong gassing power as compared to other yeasts for rapid fermentation (De Vuyst et al., 2016; Gobbetti, 1998), therefore is also cultured as baker's and brewer's yeast for industrial purposes. However, some strains of *S. cerevisiae* are susceptible to

acetic acid, especially the undissociated ones in sourdough starters at pH ranging between 4.0 and 4.5 (Gobbetti, 1998; Van Kerrebroeck et al., 2016). Another example of dominating sourdough yeast is *Kazachstania humilis* (De Vuyst et al., 2014; Urien et al., 2019). It is acid-tolerant, cold-tolerant and excellent in leavening too (Calvert et al., 2021; T. Liu et al., 2018). *K. humilis* has a phylogenetically close relation with *K. exigua*, which originally detected from sourdough (De Vuyst et al., 2014). *K. exigua* usually dominates the type I sourdough microbiota of the traditional San Franciso bread together with *Lb. sanfranciscensis* (Corsetti, 2013; De Vuyst et al., 2016).

The metabolism of sourdough bacteria and yeasts generates carbon dioxide and organic acids that provide leavening and iconic sour taste respectively as specialties of sourdough products. The microbiota of sourdough starters has been extensively studied but for sourdough products, most studies only focused on the qualities influenced by microbiota of sourdough starters (Albagli et al., 2023). The microbiota of sourdough products was not sufficiently studied and understood. The sourdough microbiota was deduced to be stable during the sponge dough retardation up to 24 hours and proofing process up to 60 minutes for SSB (X. Wang et al., 2020).

2.8 Summary

Sourdough, the most ancient method in making bread, was claimed beneficial in terms of product quality and consumer health. Apart from cereal flours, the nutrition of sourdough has been boosted by adding other nutritious yet atypical ingredients, *e.g.* pseudocereals, legumes, nuts, fruits and vegetables as sourdough preferment or bread fortification. The involvement of non-cereals as composite sourdough starter is very rare instead. These ingredients contain essential components, especially fermentable

sugars for microbiological activities during sourdough fermentation, thus could partially replace the role of cereal flours. Among the ingredients, pumpkin provided positive outcomes on hardness, overall acceptability and antioxidant capacity of sourdough bread, while mung beans complement the cereal proteins and have decreased GI in sourdough. Typically, the mixing of cereal flour and water leads to spontaneous fermentation of type I sourdough. The fermentation usually requires backslopping that replenishes the available substrates to guarantee the optimum microbiological activities. Fermentable sugars in flours are metabolised while organic acids and carbon dioxide are released. The metabolic by-products contributed to pH drop, TTA rise and leavening in sourdough. Sourdough starters that qualify to be used in making food products are to be assessed precisely and quantitatively through its leavening performances in bread dough as described by various mathematical models. In present, modelling is only conducted on dough leavened by baker's yeast but none for sourdough starter.

Sourdough is extensively applied in food products such as pizza, pasta and bakery products, such as breads, soda crackers and biscuits due to its wide range of benefits. Baked sourdough bread has been always investigated, and contributed significantly in terms of crumb texture, shelf life, GI and so on. Sourdough is also beneficial in steamed bread but this application is less studied by researchers. The qualities of SSB are usually studied from main aspects of appearance, flavour, texture and nutrition. Some of the popular parameters measured in SSB are the volume, spread ratio, colour, texture, sensory qualities and proximate composition. These parameters cover the major qualities of SSB, thus are useful in checking the suitability of non-cereal fortifications. In spontaneous sourdough, the microbiological profiles are usually diverse since microbes are mostly sourced from the ingredients and surrounding

environment. The sourdough LAB coexist with wild yeasts that adapt well in the acidic sourdough starters. *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* are the common sourdough LAB while *Saccharomyces*, *Kazachstania* and *Kluyveromyces* are typical sourdough yeasts. The microbiological profiles change consistently to adapt the surrounding conditions, *e.g.* pH and temperature. Therefore, with limited microbiological studies on sourdough products, it is speculated that the microbiological profiles in steamed sourdough breads may differ from sourdough starters. Recently, NGS has been applied in studying the microbial communities with its increasing throughput, reliability and affordability. The description of sourdough microbiota is also more complete by using NGS that displays all identified microbes irrespective of the abundances.

CHAPTER 3

METHODOLOGY

3.1 Overall Research Framework

This chapter presents materials, equipment and methods used in this research. The overall research framework is summarised in Figure 3.1. Two types of composite sourdough starters using pumpkin or mung bean flours that stabilise dough gas cells were each prepared at 5-20% and compared with control sourdough starter made from solely wheat flour, the typical ingredient. Equal amounts of flour and distilled water were mixed to allow 14-day fermentation with daily backslopping and analyses of pH and total titratable acidity (TTA). Composite sourdough starters containing 15% pumpkin or mung bean flours with the greatest rise were chosen together with control to proceed with the three-stage activation study, where the ongoing fermentation gave rise to the dough height that was measured hourly. The three sourdough starters were activated through three cycles, each ended by performing backslopping when the leavening height reached plateau. The leavening performances of sourdough starters were reported as specific volume and volume expansion ratio, that were described using sigmoidal, re-parameterised Gompertz model. Steamed sourdough buns (SSBs) were made by using activated sourdough starters at 10% and pumpkin fortification at 0, 5, 10, 15 or 20% to aid consumer acceptance with sweet taste of pumpkin. Analyses of SSBs covered their physical properties *i.e.* specific volume, spread ratio, colour and texture, sensorial properties and proximate composition. The results of microbiological analysis using Next-Generation Sequencing (NGS) method were reported by the rarefaction curves, alpha and beta diversities and taxonomic profiles for three activated sourdough starters and three selected SSBs.

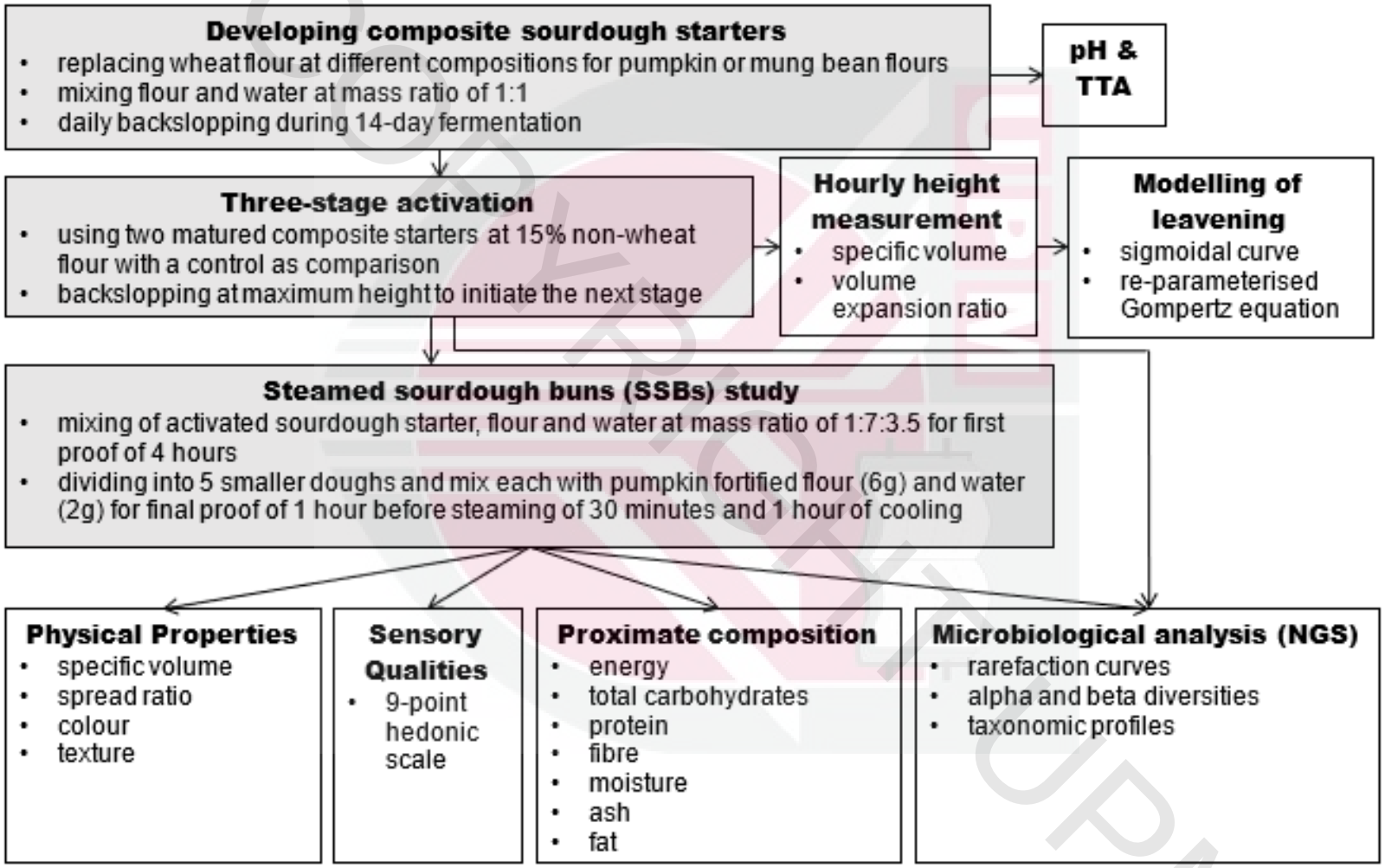

Figure 3.1: Summary of research framework

3.2 Developing Sourdough Starters using Composite Flours

The research was initiated by preparing composite flours, which were mainly wheat flour with 5, 10, 15 or 20% of pumpkin or mung bean flours. Pumpkin was involved with its rich starch and pectin that helps gas cells stabilisation in dough (Houben et al., 2012; Ptitchkina et al., 1998) and beta-carotene that provides attractive orangish colour (Pongjanta et al., 2006). Meanwhile, mung bean flour was used due to ability of its protein and hydrocolloids to form viscous gel that helps leavening (Houben et al., 2012). Mung bean also supplies complementary proteins that are lacking in wheat flour (Curiel et al., 2015; Olojede et al., 2019). The composite flours were used as dry ingredients for 14-day-long sourdough fermentation. The effects of non-cereal flour addition were determined by pH and TTA measurements as these two chemical properties are common and basic for sourdough starters. Each sourdough starter regardless plainly wheat or composite ones, was extracted for daily analysis before backslopping.

3.2.1 Backslopping of Composite Sourdough Starters

The ingredients including plain wheat flour as the cereal-based flour, and non-cereal flours, represented by the pumpkin and mung bean flours were used in this study. Figure C.1 (Appendix C) shows the packaging of the commercially available flours used. They are non-bleached and organic. The plain wheat flour (Health Paradise, Malaysia) with 11% protein was milled by Aichermühle in Germany. The pumpkin flour (Green Bio Tech, Malaysia) was freeze-dried by Xinghua Hongsheng Foods Co. Ltd in China, while the mung bean flour (Health Paradise, Malaysia) with 23.33% protein was made of hulled mung beans from India. The moisture content of wheat, pumpkin and mung bean flours are respectively 13.93 $\pm$ 1.00, 10.58 $\pm$ 0.75 and 12.09

± 0.30%. The particle size was not measured in the present study and assumed to be consistent for each type of flour since their sources and manufacturers are also consistent with continuous control of product qualities. The particle size should remain constant as this parameter may influence the hydration capacity of flours particularly the bran or fibre, and eventually the gas retention and leavening of sourdough starters (Noort et al., 2010).

Adapted from Mohammadi-Kouchesfahani et al. (2019) and Rizzello et al. (2014) that involved legumes at 10 and 15% respectively, two types of composite sourdough starters were prepared using either pumpkin or mung bean flours at four levels each, 5%, 10%, 15% or 20% by weight. The additional level at 5 and 20% was anticipated to show whether the impacts on sourdough properties were concentration dependent. The control sourdough starter was made using wheat flour solely and labelled 00W. Table 3.1 summarises the composition of dry ingredients used for developing the sourdough starters. There was a total of nine sourdough starter samples undergoing fermentation process. Each sample was triplicated to ensure precision of results thus 27 samples underwent fermentation simultaneously.

Table 3.1: Composition of dry ingredients in sourdough starters

Sample name	Composition (%)		
	Wheat flour	**Pumpkin flour**	**Mung bean flour**
00W (control)	100	0	0
05WP	95	5	0
10WP	90	10	0
15WP	85	15	0
20WP	80	20	0
05WM	95	0	5
10WM	90	0	10
15WM	85	0	15
20WM	80	0	20

Adopting the procedures described by Lönner et al. (1986) and Akinola and Osundahunsi (2017), each dry ingredient following compositions listed in Table 3.1 was mixed with 30 g of distilled water at mass ratio of 1:1 to make a 60 g dough in a transparent beaker with 250 ml volume using a manual stirring method with a spoon. The mixture was stirred for around 20-30 s or until a homogenous, viscous state was achieved. The ambient conditions were monitored and determined with a mini data logger (174H, Testo SMI Sdn Bhd, Malaysia). The beakers were covered slightly and left at room temperature of 29.7 $\pm$ 0.4 °C and humidity of 85.0 $\pm$ 3.1% for uncontrolled and spontaneous fermentation for 14 days with a backslopping process at every 24 hours. The backslopping of sourdough starter was a process to 'refresh' the starter where two-thirds of fermenting starter was discarded or used for analyses (pH and TTA). The leftover of one-third was retained and added with new amount of water and flour (dry ingredients) at mass ratio of 1:1:1 to make a dough-water-flour mixture with the same initial weight of 60 g. The discard of some starter was necessary during the backslopping process to manage the increasing mass of starter from the repeated cycles of refreshed fermentation process over the entire period of 14 days.

3.2.2 Analysis of Composite Sourdough Starters

pH and total titratable acidity (TTA) tests were conducted on the sourdough discard samples of the backslopping process during the 14-day long development. For pH measurement, 1 gram of sourdough starter was suspended in 9 ml of sterile distilled water and homogenised for about 1 minute at room temperature. Figure 3.2 shows the magnetic hotplate stirrer (HS-5 basic 2, Labfirst Scientific Instruments (Shanghai) Co., Ltd., China) used for homogenisation. pH was measured by dipping the pH meter's (Mi 805, Miwaukee, U.S.A.) sensor tip into the homogenated mixture (Ogunsakin et

al., 2015). The pH meter shown in Figure 3.3 was calibrated with buffer solutions at two pH levels of 4 and 7. The meter was also rinsed in distilled water before every measurement.

Figure 3.2: A magnetic hotplate stirrer for homogenisation of sample solution

Figure 3.3: A pH meter used for pH measurement

For total titratable acidity (TTA), 10 g of sample was suspended in 90 ml of distilled water and homogenised following the method for pH measurement. The homogenate was titrated against 0.1 N sodium hydroxide (NaOH) with 2-3 drops of phenolphthalein indicator until the endpoint was reached. TTA value is defined as the amount of 0.1 N NaOH used to neutralise 10 g of sample (Hanis-Syazwani et al., 2018; Lefebvre et al., 2002).

3.3 Activation of Composite Sourdough Starters

At the end of the 14-day fermentation, the developed sourdough starters in scaled beakers were measured for their leavening heights before backslopping. The starter that attained the greatest height was selected from respective wheat-pumpkin and wheat-mung bean composites for sourdough starters activation. The chosen starters containing 15% pumpkin (15WP) and mung bean (15WM) flours had leavened markedly by 48% and 45% at the end of day 14. Each starter had also recorded stability in terms of acidification, indicating a mature microbiota within each of them (Ercolini et al., 2013). The average pH values were 3.37 ± 0.06, 3.46 ± 0.04 and 3.56 ± 0.07 respectively for 00W (control), 15WP and 15WM whereas the fluctuation of TTA values were reduced with the averages of 13.0 ± 0.8, 14.7 ± 0.3 and 18.5 ± 2.0 ml respectively since day 12 until the end of fermentation. Both the selected composite starters underwent the three-stage activation system along with the wheat starter.

3.3.1 Three-Stage Activation System

To achieve more efficient leavening, microbes in sourdough starters are needed to be activated to yield a strong starter. The three-stage activation system has been traditionally practised (Corsetti, 2013) and involves backslopping at the time when the number and activities of sourdough microbes reach its peak as opposed to the time-based backslopping process during the development stage (Section 3.2). The activated sourdough starters are more guaranteed to show better rise in sourdough products (Gänzle, 2014). The heights of 00W, 15WP and 15WM in transparent beakers were determined every hour. For each sourdough starter, backslopping that marked the end of first fermentation cycle was carried out at the point when the height of dough peaked. This cycle of fermentation is labelled as the first stage of activation and the similar was followed for the second and third stages. The peak height achieved in each stage was measured and recorded. The surrounding temperature and humidity were measured using a mini data logger (174H, Testo SMI Sdn Bhd, Malaysia).

3.3.2 Modelling the Leavening of Sourdough Starters

The volume of sourdough starters as cylindrical solids, V, was calculated using values of height recorded hourly as:

$$V = \frac{\pi}{4} d^2 h \qquad (3.1)$$

where d is the interior diameter of beaker and h is the height of sourdough starter (Romano et al., 2007). All volumes calculated were then respectively plotted against

time in terms of specific volume and volume expansion ratio ($\frac{\Delta V}{V_o}$) to analyse the leavening performances of different sourdough starters in every activation stage.

$$\text{Specific volume} = \frac{V_t}{m} \quad (3.2)$$

$$\text{Volume expansion ratio} = \frac{V_t - V_o}{V_o} \quad (3.3)$$

where V_t refers to sourdough starter volume at the specific time, m is mass of sourdough starter and V_o is the initial sourdough starter volume when $t = 0$ (Romano et al., 2007).

The Gompertz equation describes leavening as a sigmoidal growth curve in a more direct and accurate manner. The determined values of volume expansion ratio were involved to fit in the re-parameterised Gompertz model (Equation 2.3).

$$L(t) = A \exp\{-\exp\left[\frac{\mu e}{A}(t_{lag} - t) + 1\right]\} \quad (2.3)$$

where $L(t)$ is the leavening or volume expansion ratio as a function of time, A is the maximum volume expansion ratio, μ is the maximum volume expansion rate at inflection, e is Euler's number and t_{lag} is the lag time of the leavening process (Romano et al., 2007).

The non-linear data was fitted using Solver, an add-in function of Microsoft Excel (version 2308, Microsoft Corporation, US), through the iteration method to minimise the sum of squared errors (SSE) between the experimental data and predicted data

(Chin et al., 2009; Tripathi, 2022). SSE is the measure of model fitness and the only model selected has the greatest similarities with the experimental data, shown by the lowest SSE (Tripathi, 2022). The involved formula to obtain SSE is as follows:

$$SSE = \sum_{i=1}^{n}(\hat{y}_i - y_i)^2 \quad (3.4)$$

where $\hat{y}_i$ refers to the predicted data and y_i refers to the experimental data (Tripathi, 2022). 100 iterations in total were done at 0.000001 precision level. The parameters in the model, *i.e.* A, μ and t_{lag} were then acquired for each stage and each sourdough starter.

The data predicted by the models was statistically assessed using coefficient of determination (R^2) and adjusted coefficient of determination ($adj\text{-}R^2$) values that also involve SSE obtained from equation 3.4 using Microsoft Excel (Hashemi et al., 2021).

$$R^2 = 1 - \frac{SSE}{SST} = \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (3.5)$$

$$adj\text{-}R^2 = 1 - \left[\frac{(n-x)(1-R^2)}{n-p}\right] \quad (3.6)$$

where SST represents the total corrected sum of squares, n refers to the number of experimental data, $\bar{y}$ is the observed mean, x is either 1 for model with intercept or 0 otherwise and p is the number of parameters.

3.4 Preparation of Steamed Sourdough Buns (SSBs)

The activated sourdough starters, *i.e.*, wheat starter, 15% wheat-pumpkin starter and 15% wheat-mung bean starter were made into steamed buns to assess their capabilities in enhancing qualities of sourdough products. 10 g of each activated sourdough starter was mixed with 70 g of wheat flour and 35 g of distilled water (Table 3.2) using an electric hand mixer (ISM-6, Imarflex, Thailand) in Figure 3.4 at low speed for 5 to 10 minutes, as adapted from methods used by Zhang et al. (2016) and X.-Y. Wang et al. (2016). High mixing speed exceeding 60 rpm has been shown to bring negative effects on specific volume of steamed breads (Huang et al., 1998). After the first mixing, the dough was left to proof for around 4 hours in an enclosed container until it doubled in size. The proofing conditions, *i.e.*, temperature of 29.5 $\pm$ 0.1 °C and humidity of 72.6 $\pm$ 3.5% were monitored and determined with a mini data logger (174H, Testo SMI Sdn Bhd, Malaysia).

Table 3.2: Ingredients for SSBs in two-stage mixing

Ingredients		Composition (g)				
Mixing 1						
Activated sourdough starter		10				
Wheat flour		70				
Distilled water		35				
Total (dough)		115				
Mixing 2						
Dough		23	23	23	23	23
Distilled water		2	2	2	2	2
Pumpkin fortified flour	Wheat	6	5	4	3	2
	Pumpkin	0	1	2	3	4
Total		31	31	31	31	31

The dough was divided into five smaller doughs where each smaller dough of 23 g was mixed with distilled water and pumpkin-fortified flours at 0, 5, 10, 15 or 20%. For example, 10% pumpkin fortification was achieved by adding 2 g of pumpkin flour in the smaller dough that contained 20 g flour in total, including wheat flour added at one

fifth of 70 g , *i.e.* 14 g during mixing 1 and 4 g during mixing 2. This was conducted to mask the slightly sour taste in SSBs with sweetness from pumpkin, the common ingredient for bread fortification (El-Demery, 2011; Hoxha et al., 2023; Pongjanta et al., 2006; Ptitchkina et al., 1998; Różyło et al., 2014; Sadeghi et al., 2019). The final doughs at about 31 g each were placed in an enclosed container for 1-hour proofing under similar conditions as in during first proofing. They were shifted into a stainless steel 3-tier steamer (Lotus's, Thailand) as shown in Figure 3.5, steamed for 30 minutes under medium heat and lastly taken out after 5 minutes to avoid bun shrinkage due to sudden temperature drop (Huang & Miskelly, 2019). The steamed sourdough buns were made in triplicates. The control steamed bun was made using wheat sourdough starter without pumpkin fortification.

Figure 3.4: An electric hand mixer for dough mixing

Figure 3.5: A stainless steel 3-tier steamer to steam SSB

3.5 Analysis of Steamed Sourdough Buns (SSBs)

Fresh SSBs which were left to cool at ambient for 1 hour before analysis (Yang, 2016). The qualities of steamed breads are primarily determined by the similar methods as baked breads despite the different expectations on them (S. Ma et al., 2014). In the present study, physical properties of volume, spread ratio, colour and texture were measured. These properties quantitatively showed the effectiveness of composite sourdough starters and pumpkin fortification in improving SSB. Sensory qualities were also determined by inviting 15 untrained panellists who rated the steamed bun samples from the aspects of texture, colour, taste, aroma and overall liking using the 9-point hedonic scale. Proximate analysis was conducted to compare the difference in compositions of the studied samples.

3.5.1 Physical Properties of Buns

The rapeseed displacement method was used to determine the SSB volume following AACC approved method 10-05.01 (AACC, 2009). Each bun was also weighed using a digital balance (LYC® Precision Portable Lab Electronic Balance XY-2C, China). The volume was subsequently presented in the form of specific volume, *i.e.*, ratio of volume to weight for each bun (Charoenthaikij et al., 2012; Yang, 2016). The evaluation of bread dimensions has been practised with the usage of Vernier callipers (Loong & Wong, 2018). For spread ratio, the dimensions of each bun sample were measured using a ruler (Sim et al., 2015), due to limited access to selective laboratory apparatus during Covid-19 pandemic. Figure 3.6 demonstrated the diameter measurement from three different angles whereby the averaged values were reported as width. Spread ratio was evaluated by determining the ratio of width to height (Sim et al., 2015; Su et al., 2005).

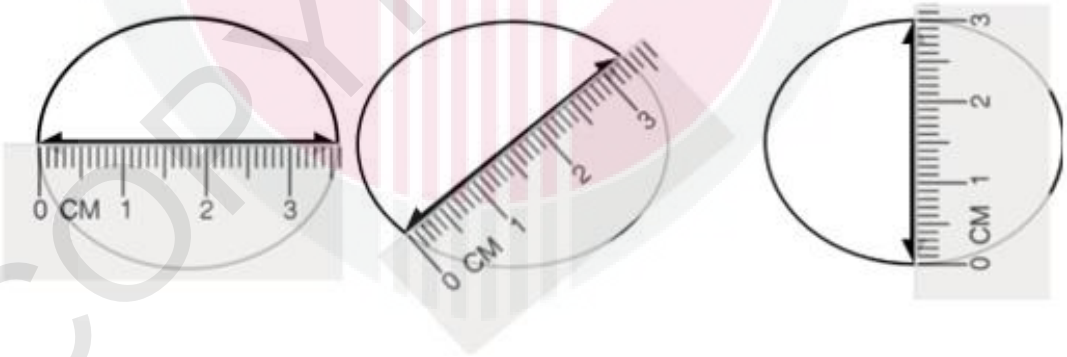

Figure 3.6: The diameter measurement of steamed sourdough buns from different angles (top view)

The procedures for colour measurement were done following adaptions to Dmytrów et al.'s (2021) and Charoenthaikij et al.'s (2012) work. A colorimeter (WR-18, FRU, Shenzhen, China) shown in Figure 3.7, which has been calibrated using a white reference plate (L^* = +88.3; a^* = +0.37; b^* = +1.50) was used. The outcomes were

recorded as L* (lightness), a* (redness) and b* (yellowness) values, representing the specific colour. The colour difference (ΔE) with control SSB was calculated according to the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (3.7)$$

where ΔL, Δa, and Δb respectively represent the differences of each sample's L^*, a^*, and b^* values from the control SSB's.

Figure 3.7: A colourimeter used for colour measurement

Each steamed bun sample was sliced at 25 mm thickness for Texture Profile Analysis. Figure 3.8 shows the texture analyser (TA.XTplus, Stable Micro Systems, Godalming, UK) used in the present study. Each bun slice was compressed to 50% deformation (pre-test speed = 3 mms^{-1}, test speed = 1 mms^{-1}, post-test speed = 5 mms^{-1}, trigger force = 5 g, probe diameter = 25 mm) (Wu et al., 2012). The parameters evaluated included hardness, springiness, cohesiveness, chewiness, and resilience (Li et al., 2021).

Figure 3.8: A texture analyser used for Texture Profile Analysis

3.5.2 Sensory Properties of Buns

SSBs for sensory tests were made a day before the sensory evaluation and were stored in refrigerator following methods of Laohaprasit & Sricharoenpong (2018). The refrigerated samples were steamed for 7 minutes and cut into bite size before serving. The SSBs were served warm in identical sampling containers that were labelled by random 3-digit codes. There was a total of 15 untrained panellists (7 males and 8 females) who are students of Department of Food and Process Engineering, Universiti Putra Malaysia with sufficient knowledge and some experiences in sensory evaluation. To enhance the accuracy, the panelists attended a briefing on the meaning of sensory attributes (De Angelis et al., 2018; Haglund et al., 1998) in terms of texture, colour, taste, aroma and overall liking, as described in Table B.3 (Appendix B), from 1 (dislike extremely) to 9 (like extremely). They followed the instructions by cleansing their palate with water between samples and by rating the samples using a 9-point hedonic scale (Hanis-Syazwani et al., 2018; Yang, 2016) under day-light balanced lighting (İçen et al., 2021).

3.5.3 Proximate Composition of Buns

Proximate analysis was conducted on selected SSBs. The steamed bun samples with the most satisfying physical properties at 5% pumpkin fortification containing the wheat-pumpkin or wheat-mung bean composite sourdough starters were selected along with control SSB. About 50 g of steamed bun samples were taken, put in sealed in plastic bags and sent for proximate analysis at ALS Technichem Sdn. Bhd. (Shah Alam, Malaysia). The measured parameters were total carbohydrates, energy, protein, total dietary fibre, moisture, ash, and fat contents. Table 3.3 lists the respective methods and principles used. The method references are provided in Table B.2 of Appendix B.

Table 3.3: Methods and principles of proximate analysis on SSB

Nutrient	Method	Principle
Total carbohydrates	OF/17-035	Determination through calculation, *i.e.*, 100% - (fat + protein + moisture + ash)
Energy	OF/17-036	Determination through calculation by summing up the measured protein, fat and carbohydrate contents which are multiplied with factors at 16.7 kJ/g, 37.4 kJ/g and 16.7 kJ/g respectively.
Protein	OF/17-119 (AOAC 972.43)	Microchemical determination of carbon, hydrogen and nitrogen through combustion
Total dietary fibre	OF/17-014 (AOAC 985.29)	Determination of fibre residue after enzymatic digestion with α-amylase, protease and amyloglucosidase using enzymatic-gravimetric method
Moisture content	OF/17-038	Determination of weight loss due to vaporisation using moisture analyser
Ash content	OF/17-002	Determination of inorganic residue after incineration of organic matter at between 500 and 550 °C
Fat	OF/17-010	Determination of free lipids through extraction using organic solvents

3.6 Microbiological Analysis

Microbiological analysis was outsourced and conducted on selected samples of sourdough starters and buns *i.e.*, solely wheat, 15% wheat-pumpkin and 15% wheat-mung bean sourdough starters, steamed sourdough buns at 5% pumpkin fortification made using both types of composite starters and control SSB by Apical Scientific Sdn. Bhd. (Seri Kembangan, Malaysia). Next-Generation Sequencing (NGS) which consists of a series of steps, *i.e.*, DNA extraction, amplification, amplicon sequencing and lastly bioinformatics data analysis was conducted. For extraction of genomic DNA, 250 mg of each sourdough starter and SSB sample was eluted into 80 µl DNA with the commercial kit, FastDNA™ Spin Kit for Soil (MP Biomedicals, USA), by following the manufacturer's instructions. 20 µl of the purified DNA was used to check its quality and integrity on 1% TAE agarose gel through electrophoresis. The concentration of DNA was validated through spectrometric quantification (UVA_{260nm}/A_{280nm}) using NanoPhotometer® spectrophotometer (Implen N60/N50, Germany) and fluorometric quantification using iQuant™ Broad Range dsDNA Quantification Kit (ABP Biosciences, USA) with a fluorescence plate reader. The similar steps were repeated once as two sets of gDNA were required for 16S and ITS rRNA gene sequencing separately.

After passing the quality check, purified gDNA played the role as the template for amplification of the 16S or ITS rRNA gene sequences. A quality-check for PCR amplicons were conducted using locus-specific sequence primer pairs: (a) forward (5'-CCTACGGGNGGCWGCAG-3') and reverse (5'-GACTACHVGGGTATCTAATCC-3') for bacterial 16S V3-V4 region and (b) forward (5'-GCATCGATGAAGAACGCAGC-3') and reverse (5'-

TCCTCCGCTTATTGATATGC-3') for fungal ITS ITS2 region. The REDiant 2X PCR Master Mix (1st BASE, Singapore) including reaction buffer, 0.06 U/µl of Taq DNA polymerase, 3 mM $MgCl_2$ and 400 µM of each dNTPs, was used for this specific polymerase chain reaction (PCR) reaction. 1 µL DNA template was added with both forward and reverse primers at 1 µL each, 12.5 µL REDiant 2X PCR Master Mix and finally nuclease-free water to make up the 25 µL reaction volume.

With the success of quality check, the 16S and ITS rRNA gene sequences were respectively amplified using primer pairs attached to the Illumina overhang adapters: (a) forward overhang (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and (b) reverse overhang 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'), as adopted from Illumina's 16S metagenomic library preparation manual (Illumina, 2013). The PCR process was conducted after preparing a 25 µl mixture with similar compositions as the mixture for PCR amplicon QC, whereby REDiant 2X PCR Master Mix was replaced by KOD -Multi & Epi-® (Toyobo, Japan).

The first stage of PCR was started by initial denaturation at 95 °C for 1 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 50 s, with final extension at 72 °C for 5 min. The amplification was augmented by attaching dual indices using the Illumina Nextera XT Index Kit v2, according to the manufacturer's guidelines in the second stage of PCR. After each stage of PCR, clean-up was done using AMPure XP beads to purify the libraries. The quality of the libraries was assessed using Agilent Bioanalyzer 2100 System by Agilent DNA 1000 Kit and for fluorometric quantification, by Helixyte Green™ Quantifying Reagent (AAT Bioquest, USA).

The libraries were proceeded to normalisation and pooling according to the protocol recommended by Illumina before being introduced to sequencing on MiSeq platform (Ilumina, USA) using 300 paired ends. Lastly, the raw data generated, in the form of FASTQ files, was processed and analysed according to the bioinformatics pipeline. Removal of primers, sequence adapters and low-quality reads from the paired end reads was conducted by Cutadapt 3.5, followed by the extraction of first 60 000 raw reads. To secure the accuracy of data, qualities of raw reads were assessed using FastQC (Andrews, 2010). Forward and reverse reads were merged, error reads were removed and/or corrected, while low-quality regions and chimeric errors were removed in the linux-based DADA2 V1.18 pipeline (Callahan et al., 2016). The outcomes existed in the form of amplicon sequence variant (ASV), which formed the taxonomic profiles using Naive Bayes classifier with SILVA (release 132) database and UNITE database for 16S and ITS rRNA sequences respectively.

Through the QIIME2 pipeline, ASVs were assigned to respective taxonomic identities, existed in the form of rarefaction curves, alpha diversities, beta diversities and taxonomic abundance bar plots. The abundances of 10 major classes and genera for both bacterial and fungal communities were plotted into bar charts as normalised percentages for easier comparison with uniform total abundance of 100%. In the present study, alpha diversities, which compare microbes within the same community (Urien et al., 2019), were represented by Chao1 richness and Shannon diversity indices (Ercolini et al., 2013; Minervini et al., 2016). Meanwhile, beta diversities were demonstrated using Principal Coordinate Analysis (PCoA) plots to identify the similarities and differences between microbial communities (Urien et al., 2019).

3.7 Data Analysis

For the data of sourdough starters, the evolution of chemical properties and leavening capabilities influenced by different types or proportions of non-cereals addition can be presented in line graphs against the fermentation time. Meanwhile, each measured physical property of SSBs was differentiated using bar chart. The radar charts were constructed for clearer comparison among SSBs in terms of textural parameters and sensory properties. Under the microbiological section, rarefaction curves showed the relationship between species richness and number of sequences in line graphs, whereas alpha and beta diversities were displayed in the forms of box and scatter plots respectively. The normalised taxonomical profiles were summarised in bar charts to have an easier understanding.

All results obtained including the pH, TTA and leavening performances of sourdough starters, physical properties, sensory qualities and proximate composition of SSBs were presented as average $\pm$ standard deviation from all triplicated experiments. Error bars indicating standard errors are values from standard deviation of mean. One way analysis of variance (ANOVA) was conducted for all results except proximate and microbiological analysis using Minitab software (version 20.2, Minitab Inc., US) to measure statistical difference and significances of response data. The outcomes of ANOVA focused on the p-value, which infers significant difference at values lesser than 0.05. When comparing value differences between two samples, such as parameters between two Gompertz models or physical properties between two steamed buns, the effects of changes in recipe were measured with pair-wise comparison of treatment means using Tukey's test with significance level of 0.05. For

example in Section 4.3, the bar columns in the same graph with different letters are significantly different ($p \leqslant 0.05$).

3.8 Summary

Composite sourdough starters involving wheat flour along with pumpkin or mung bean flours were developed following a 14-day fermentation process with daily backslopping that helped maturation of sourdough microbes. The matured microbes were activated through a 3-stage process of backslopping at peak activities, indicated by the peak leavening height of sourdough starters. The activated starters were then applied in making steamed sourdough buns with pumpkin flour fortification that conformed the focus of this research on studying potentials in non-cereal flours for sourdough and bread products. The influences of non-cereal flours on composition of sourdough microbiota, mainly LAB and yeasts were also examined to compare and determine the key beneficial microbes for leavening and SSB qualities.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Chemical Properties in Sourdough Starters during Development

Sourdough starters are usually characterised by their chemical properties, *i.e.*, pH and TTA (Corsetti, 2013; Hansen, 2006). In this study, sourdough starters which were made of wheat-pumpkin and wheat-mung bean composite flours were developed through a 14-day fermentation at ambient temperature of 29.7 $\pm$ 0.4 °C and humidity of 85.0 $\pm$ 3.1% (Table A.7 in Appendix A). Sourdough samples were taken daily before the backslopping process to monitor the pH and TTA changes. In general, pH of sourdough starters decreased with fermentation time. With addition of non-cereals, *i.e.*, pumpkin and mung bean, pH increased. TTA of all sourdough starters increased with a fluctuating manner with three peaks during fermentation. Similarly, in starters with non-cereal flours *i.e.*, pumpkin and mung bean, TTA increased.

4.1.1 pH

Figure 4.1 shows that pH decreased during the development of sourdough starter for the continuous 14-day fermentation. The initial pH of wheat-pumpkin and wheat-mung bean composite sourdough starters were 5.32-5.38 and 5.15-5.37 respectively. The pH of wheat sourdough starter at 5.30 on day 0 was insignificantly different ($p > 0.05$) from the values of composite sourdough starters. On day 14, the pH of wheat starter was at 3.31, while the addition of pumpkin and mung bean showed significantly higher values for most final pH of 3.38-3.50 and 3.35-3.50 respectively (Table A.1 in Appendix A). During fermentation, organic acids *e.g.* lactic acid and acetic acid, are released and accumulated due to LAB activities, mainly metabolism of fermentable

sugars (Komlenić et al., 2012). As the result, the amount of free hydrogen ions raises and pH decreases in sourdough starters. The low pH condition is not conducive for foodborne pathogens, leading to the antimicrobial feature in sourdough starters and their food products (Hammes & Gänzle, 1998; Siepmann et al., 2017).

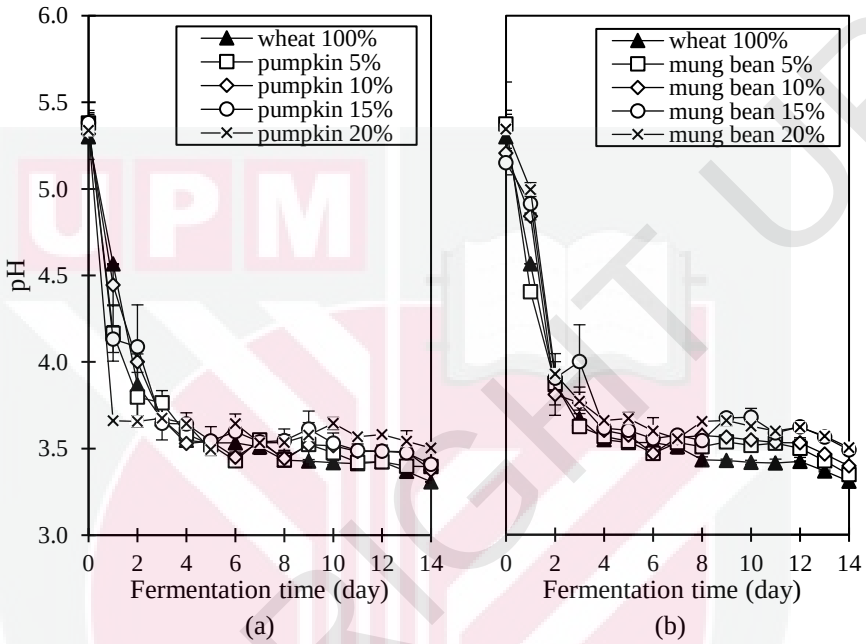

Figure 4.1: The pH changes for (a) wheat-pumpkin and (b) wheat-mung bean composite sourdough starters during the 14-day development

The pH of sourdough starters decreased markedly in the first 3 days and converged towards the end of fermentation. On day 3, pH of wheat starter dropped by 30.8%, from 5.30 on day 0 to 3.67. The pH of starter with 15% pumpkin recorded the greatest pH drop from 5.38 to 3.65 (32.3%) among the wheat-pumpkin composite sourdough starters (Figure 4.1a). The pH reduced the most by 32.5% for 5% wheat-mung bean composite sourdough starter from 5.37 to 3.63, as observed in Figure 4.1(b). Since day 4, minimal pH fluctuation was observed in the sourdough starters. The sourdough starters were said to be mature since the pH was low and stable for a period (Ognean, 2015; Van Kerrebroeck et al., 2016). From this study, pH for all sourdough starters

had dropped for more than 30.0% at the end of fermentation, suggesting presence of active LAB.

The final pH which ranged from 3.31 to 3.50, agreed with Mohammadi-Kouchesfahani et al. (2019) who reported pH ranged 3.14-3.97 for spontaneous sourdough starters using composite flours of wheat, red lentil, and mung bean. Nevertheless, the pH values in the present study were slightly lower than pH values of 3.6-3.7 for sourdough studies with the similar period of fermentation, *i.e.*, 14 days (Stefanello et al., 2018; C. Zhang et al., 2010). The studies of Rizzello et al. (2014) and Vera et al. (2012) that took 10 days for sourdough fermentation depicted higher values of pH, *i.e.*, 3.80-4.19 and 3.41-3.70 respectively. This may be attributed to usage of different types, sources and species of ingredients for sourdough fermentation. The plain wheat flour used in current study has lower gluten of 11% when compared to usual wheat flour at 12.7-15.0% (Chen et al., 2018; De Angelis et al., 2018) thus possessing a lower hydration capacity (Boz & Karaoglu, 2013). Therefore, sourdough pH is lower when more free hydrogen ions are released with excess water (Ognean, 2015). The addition of non-cereals may provide some hydrocolloids which raise water absorption capacity (Mastromatteo et al., 2012). As a result, higher final pH values were observed in sourdough starters with greater percentage of non-cereals. The highest final pH of 3.50 was attained by both wheat-pumpkin and wheat-mung bean composite sourdough starters with highest non-cereal percentage of 20%. Therefore with pumpkin or mung bean flours, the pH values of 3.35-3.50 were more comparable with values of 3.41-4.19 in the mentioned literatures (Rizzello et al., 2014; Stefanello et al., 2018; Vera et al., 2012; C. Zhang et al., 2010) than the wheat starter at 3.31.

4.1.2 Total Titratable Acidity (TTA)

Figure 4.2 shows a significant increment of TTA along the 14-day development. In the present study, TTA was expressed as the volume of 0.1 N sodium hydroxide (NaOH) required to neutralise 10 g of sourdough starter. Initially, 3.2 ml of 0.1 N NaOH was utilised to neutralise 10 g of wheat starter. This amount was lower than the others, which ranged from 4.0 to 5.8 ml and from 4.7 to 5.3 ml respectively for wheat-pumpkin and wheat-mung bean composite sourdough starters. Table A.2 in Appendix A shows that the final TTA of wheat-pumpkin and wheat-mung bean composite sourdough starters exceeded the value for wheat starter at 14.0 ml, *i.e.* from 15.1 to 18.4 ml and from 17.7 to 20.1 ml respectively. There were slight deviations of the TTA values in other studies, possibly due to the longer fermentation period in this study. For example, TTA values of 13.9-22.4 ml, 24.64 ml and 10.7-16.9 ml were detected in respective studies by Vera et al. (2012), Rizzello et al. (2014) and Mohammadi-Kouchesfahani et al. (2019). The values for pH and TTA are negatively correlated, yet they are both the indicators for the concentration of organic acids released during sourdough fermentation (Ogunsakin et al., 2015).

The TTA values showed three-phase evolution that indirectly presented the microbial succession during sourdough fermentation. The first peak on day 2 or 3 indicated that the microbiological activity was at optimum in the first phase, mostly constituted by the innate microbiota of water and flour. Significant acidification occurred in phase 2 from day 2 to day 5. LAB such as *Lactobacillus*, *Pediococcus*, and *Weissella*, that prefer acidic environment survived and dominated the microbiota. The third peak on day 11 was caused by the activated sourdough strains, *e.g.*, *Lb. fermentum* and *Lb. plantarum* (Van der Meulen et al., 2007). After the third peak, the sourdough starters

achieved the maturity gradually with lesser fluctuation of TTA values (Ognean, 2015; Van Kerrebroeck et al., 2016).

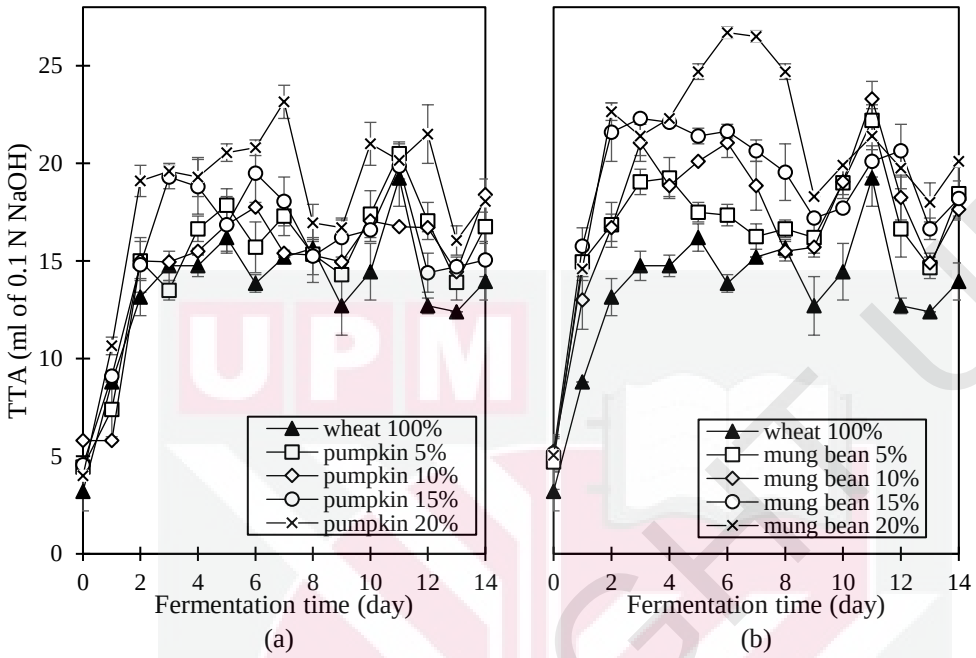

Figure 4.2: The Total Titratable Acidity (TTA) changes for (a) wheat-pumpkin and (b) wheat-mung bean composite sourdough starters during the 14-day development

The addition of non-cereals raised the TTA values of wheat sourdough starters. After the succession, all composite sourdough starters achieved higher TTA values than wheat starter, despite its great TTA increase by over 330%. 20% addition of mung bean gave rise to the greatest increase of 15.0 ml from 5.1 ml to 20.1 ml, which was also the highest final TTA value. This result is consistent with findings of Rizzello et al. (2014) and Coda, Varis, et al. (2017) who determined the highest TTA values of 24.64 mL for wheat-legume sourdough starter and 7.4 mL for wheat bread leavened by faba bean sourdough respectively. The higher ash and protein contents at 3.5-3.76% (Mubarak, 2005; Singh et al., 2015) and 23.33% respectively in mung bean are probably the justification of the high TTA values in wheat-mung bean composite sourdough starters. They both affect the buffering capacity in sourdough, hence TTA

values are possibly augmented with the presence of ash (Chavan & Chavan, 2011; Stefanello et al., 2018) and protein (Gaglio et al., 2020).

4.1.3 Summary

In developing composite sourdough starters through a 14-day fermentation process, the pH decreased and TTA increased in general, indicating the accumulation of organic acids released by sourdough LAB. The changes of chemical properties in the first 3 days were substantial. The greatest pH drop by 32.5% was observed in starter with 5% mung bean, whereas for TTA, the greatest boost by 17.1 ml was detected at 15% mung bean. The sourdough LAB may be considered active in all starters since each of them experienced pH drop exceeding 30.0%. The pH convergence since day 4 suggested the maturation process of sourdough starters. For fluctuating TTA in reducing trend, the maturation became visible only after the two peaks which appeared on day 5 and day 11. A total of three peaks indicated the adaptation of microbes within the acidic condition. The greater the proportion of non-cereals, the higher the final pH and TTA values which led the values nearing to starters in other literatures. The final pH of wheat sourdough starter was at 3.31, while the addition of pumpkin and mung bean showed values of 3.38-3.50 and 3.35-3.50 respectively. The final TTA of starters containing pumpkin and mung bean exceed the value of solely wheat at 14.0 ml, with values of 15.1-18.4 ml and 17.7-20.1 ml respectively.

4.2 Modelling Leavening of Sourdough Starters during Activation

This subsection has been published in the paper entitled "Modelling the three-stage activation process of composite sourdough starters developed using pumpkin and mung bean flours" as shown in list of publications of the thesis. The sourdough structure is supposedly similar to structure of wheat flour dough, whereby the gluten network traps the carbon dioxide released through microbial metabolism (Chavan & Chavan, 2011; Hammes & Gänzle, 1998; G. G. Hou & Hsu, 2013). The acidification process, however, brings some impacts on the sourdough structure, for example, acetic acid is associated with short and hard gluten while lactic acid provides more elastic gluten structure (Komlenić et al., 2012). Thus, such condition influences leavening performances of sourdough starters. Leavening or rising of dough is often quantified in term of volume expansion. The low pH was claimed to form more viscous sourdough with increased phase angle (δ) (Komlenić et al., 2012), allowing stronger gas-trapping abilities. As mentioned in Section 4.1, the incorporation of non-cereals contributed to higher pH and TTA values. Therefore, their impacts on leavening, which are associated with acidification, are included in this section. The effort in maximising leavening abilities through the three-stage activation was evaluated in the present study. The volume expansion ratio was fitted into Gompertz model that describes the evolution with time accurately.

4.2.1 Leavening in Terms of Specific Volume

Leavening occurs naturally in sourdough starters by raising their volume throughout fermentation. Similarly in doughs containing baker's yeast, leavening of sourdough starters is initiated by carbon dioxide production from the wild yeasts and some LAB within them (Hansen, 2006). The involved sourdough microbes are usually mesophilic

(De Vuyst et al., 2017). Hence, especially for type I sourdough, leavening is believed to be strengthened under conducive temperatures ranged between 20 and 30 °C (De Vuyst et al., 2017; Hammes & Gänzle, 1998). During activation in the present study, the surrounding temperature was within 29.4 $\pm$ 0.4 °C with humidity of 80.9 $\pm$ 1.2%. Figure 4.3 shows the dynamics of specific volume with time for solely wheat and composite sourdough starters calculated from values of measured height and mass in Equations 3.1 and 3.2. The incorporation of non-cereals has raised the leavening of sourdough starters pronouncedly.

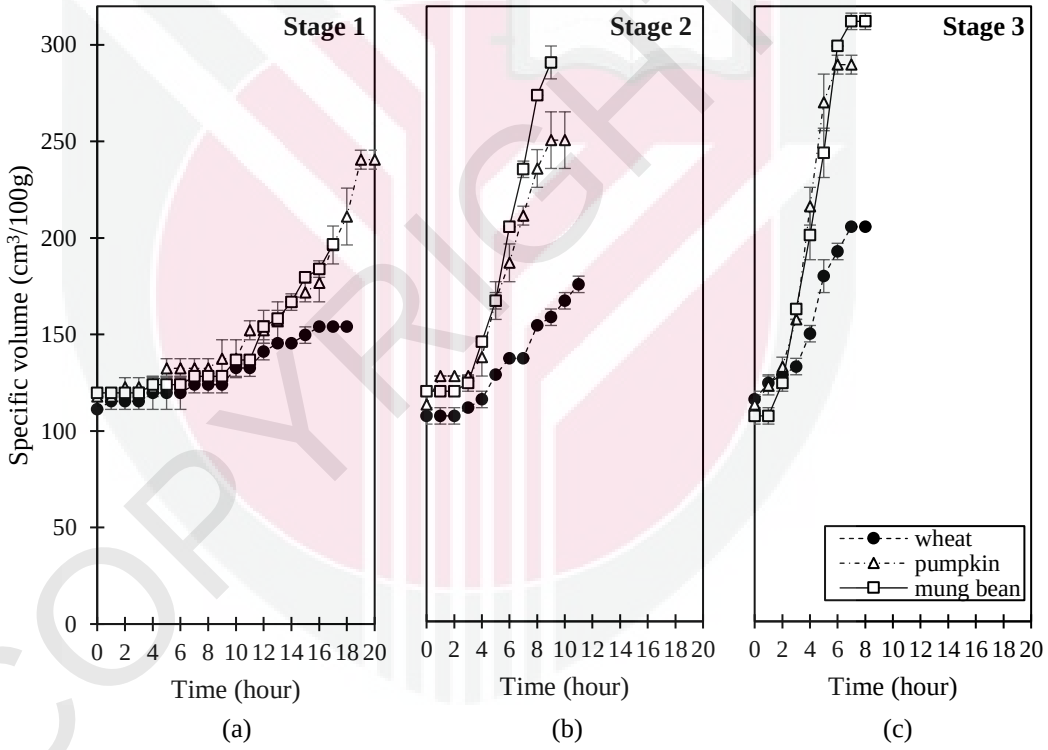

Figure 4.3: Specific volume increase of sourdough starters at (a) stage 1, (b) stage 2 and (c) stage 3 for composite flours involving pumpkin and mung bean and solely wheat flour (control)

The specific volume was the highest at 240.5 cm^3/100g for wheat-pumpkin composite sourdough starter, followed by 196.7 cm^3/100g for starter with mung bean and lastly 153.9 cm^3/100g for wheat during stage 1. The significant improvement ($p \leqslant 0.05$) of

leavening by non-cereal incorporation was also observed in the following stages. The wheat starter demonstrated weaker leavening performance than other wheat sourdough starters, potentially resulted from the usage of plain wheat flour at relative lower protein content of 11% instead of 12.7-15.0% (Chen et al., 2018; De Angelis et al., 2018). Low concentration of wheat protein, which is constituted by gluten could not develop strong interconnected network leading to weaker gas-holding capability and leavening (Le Vu Lan et al., 2021). The gas-trapping structure in sourdough is compromised in situations when flour has poor water absorption (Boz & Karaoglu, 2013) and also when water is in excess as gluten tends to form undesired water-protein hydrogen bonds (Schopf & Scherf, 2021). Despite having no gluten contents, the pumpkin and mung bean flours have contributed to better leavening possibly by the presence of hydrocolloids which facilitate water absorption (Mastromatteo et al., 2012) and form non-gluten network that traps the carbon dioxide produced during fermentation. The improvement on gluten elasticity and eventually the leavening also may be associated with greater amount of lactic acid released (Komlenić et al., 2012), as observed by higher TTA values in composite starters.

The non-cereals also accelerated the leavening of sourdough starters. The time taken to achieve 200 cm^3/100 g was shortened from 7 hours in wheat starter to 4 hours in composite starters during stage 3, as shown in Table A.3 in Appendix A. Apart from improved gas-holding capabilities (Houben et al., 2012), there are supplementary nutrients from the non-cereals in composite starters which may further activate the sourdough microbes. In accordance with this, Rizzello et al. (2014) found that wheat-legume composite sourdough achieved the fastest acidification and greater number of presumptive LAB whereas Portman et al. (2018) deduced the 12.2% reduction of time

consumed for wheat dough development after adding 5% dehulled lentil (legume) flour.

The continuous activation in stages has uplifted the leavening performances of sourdough starters. The specific volume crested at 205.3, 289.6 and 312.2 cm^3/100g in stage 3 after taking the shortest time of 8, 7 and 8 hours respectively in wheat, wheat-pumpkin and wheat-mung bean composite sourdough starters. Activation of microbial metabolism through backslopping in three stages (Corsetti, 2013) is therefore usually accompanied by raised amount of carbon dioxide released as fermentable sugars and water are reloaded when sourdough LAB are the most active. Studies discussing the relationship between three-stage activation and sourdough leavening are not available. The findings of Elsanhoty et al. (2017) supported the present study with gradual pH drop and TTA increase in sourdough cultured by *Lb. acidophilus* or *Bifidobacterium lactis* after being activated in three stages. As deduced by Reidzane et al. (2021), three-stage activation after optimisation was also significant in terms of pH drop in spontaneously fermented barley sourdough.

It is uncommon to find research about development of specific volume in sourdough starters. The most relatable studies are on doughs leavened using other chemical and biological leavening agents. Sodium bicarbonate with neutralising acidulants expanded doughs up to 157 cm^3/100g at 27 °C (Bellido et al., 2009) within one hour while the combined efforts of baker's yeast and salt in doughs led to specific volume of 200 and 275 cm^3/100g at 25 and 30 °C respectively (Chevallier et al., 2012). Despite speedy leavening by other leavening agents, sourdough leavening remains unique with the distinguishing sensorial, textural and nutritional advantages (Hansen, 2006). Loaf volume could also be improved through involvement of legume in wheat sourdough

starter, from 3.42 to 3.87 cm^3/g, which was comparable to 3.96 cm^3/g in yeasted bread (Rizzello et al., 2014).

The development of specific volume in Figure 4.3 also corresponded to the sigmoidal growth curves, describing metabolism of living things (Romano et al., 2007), particularly sourdough LAB and yeast in the present study. Each of the curves could be differentiated into three distinct phases of lag, growth and stationary (Chevallier et al., 2012; Romano et al., 2007). The lag phase considered the hydrolysis and metabolism of fermentable sugars (Gally et al., 2017) in cereals or non-cereals by sourdough microbes. The time in lag phase was also consumed by carbon dioxide diffusion across dough matrix to form bigger air bubbles with those initially formed during mixing (Baker & Mize, 1941; Chevallier et al., 2012; Meerts et al., 2018; Romano et al., 2007). The dramatic leavening of sourdough starters in the growth phase was in relation to carbon dioxide accumulation. When the carbon dioxide released during fermentation was offset by carbon dioxide escaped from starter (Chevallier et al., 2012; Meerts et al., 2018; Romano et al., 2007), this implied the beginning of the stationary phase and values of maximum volume were obtained. The depletion of nutrients at this phase also made it competitive for further expansion of microbial population. Therefore, the evolution of specific volume in three phases fits to be described by Gompertz model, that has frequently described growth of plants, animals and population of microbes (Tjørve & Tjørve, 2017). The death phase of typical growth curves is not included here since the major target of sourdough starter activation is to maximise growth among sourdough microbes for satisfactory applications in bakery products.

4.2.2 Leavening Models in Three-stage Activation System

Dough leavening has been modelled using the Gompertz equation (Chevallier et al., 2012; Gally et al., 2017; Meerts et al., 2018; Romano et al., 2007), associated with the success of the equation in describing microbial growth, particularly in food (Tjørve & Tjørve, 2017). The Gompertz model was re-parameterised, referring to Equation 2.3, to fit and describe the dynamics of leavening for different types of sourdough starters throughout the three-stage activation in Figure 4.4. The leavening performances were compared easily by expressing them as volume expansion ratio ($\frac{\Delta V}{V_o}$) with Equation 3.3 and making the starting point of curves identical at 0 cm^3/cm^3.

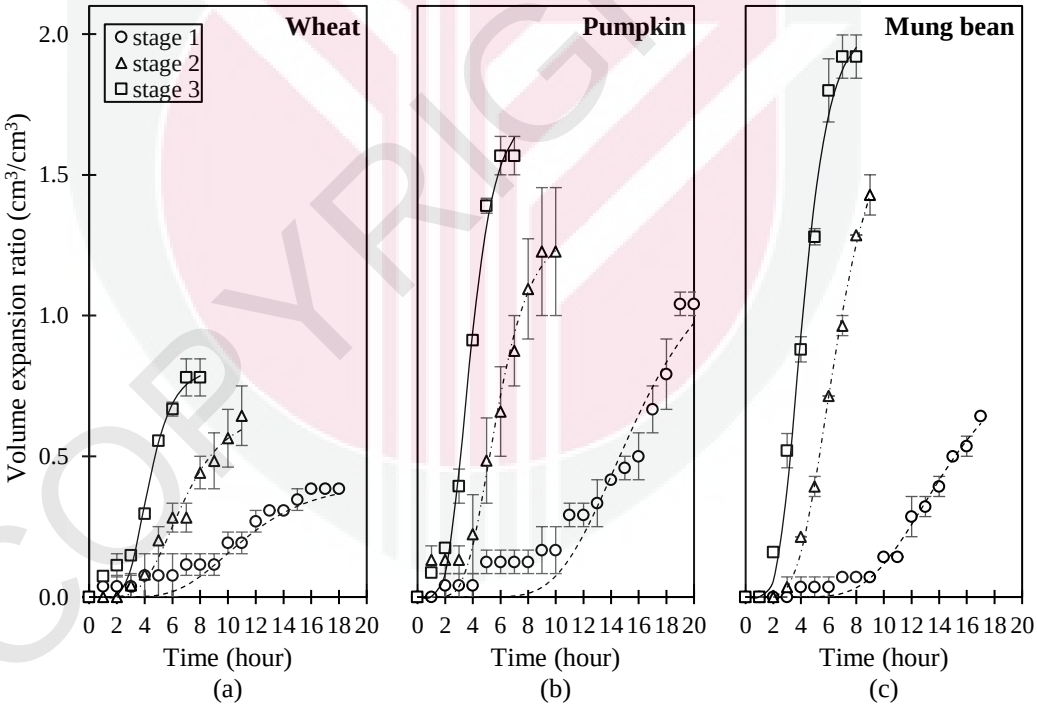

Figure 4.4: Volume expansion ratio of three sourdough starter types using (a) wheat flour as control and composite flours incorporating (b) pumpkin and (c) mung bean at three activation stages (data: markers; model: lines)

Table 4.1 shows the parameters in the fitted Gompertz models, *i.e.*, maximum volume expansion ratio (A), maximum volume expansion rate (μ) and lag time of leavening

process (t_{lag}) for different sourdough starters at different activation stages. The overall fermentation time (t_{∞}) and model accuracies (R^2 and *adj*-R^2) were also included for comparison. Along the activation from stages 1 to 3, the leavening capabilities were ameliorated pronouncedly in wheat starter, in terms of increasing maximum volume expansion ratio by 0.41 cm^3/cm^3, maximum volume expansion rate by 0.21 h^{-1}, decreasing lag time by 3.70 h and overall fermentation time by 10 h. The addition of pumpkin and mung bean in wheat sourdough starters also contributed to the improvement of leavening capabilities. The parameters of activated wheat starter were enhanced, in terms of increased values of maximum volume expansion ratio at 1.72 and 2.03 cm^3/cm^3, maximum volume expansion rate at 0.51 and 0.57 h^{-1}, leavening lag time at 2.13 and 2.35 h and overall fermentation time at 7 and 8 h respectively in the wheat-pumpkin and wheat-mung bean composite sourdough starters in stage 3. By applying equations 3.5 and 3.6, the models are fitted well with R^2 and *adj*-R^2 values exceeding 0.97, except the starter containing pumpkin in stage 1. The accuracy of models also increased with the activation stage, conveyed by R^2 at 0.99 in stage 3 for all sourdough starters.

Table 4.1: Values of parameters in re-parameterised Gompertz model for three sourdough starter types at three activation stages and the respective accuracy

Sourdough starter	Stage	Maximum volume expansion ratio, A (cm^3/cm^3)	Maximum volume expansion rate, μ (h^{-1})	Lag time of leavening process, t_{lag} (h)	Overall fermentation time, t_{∞} (h)	R^2	*adj*-R^2
00W (control)	1	0.403^{e}	0.0430^{f}	6.39^{b}	18	0.975	0.973
	2	0.652^{de}	0.113^{e}	3.79^{c}	11	0.976	0.974
	3	0.810^{d}	0.252^{d}	2.69^{c}	8	0.986	0.984
15WP	1	1.25^{c}	0.111^{e}	10.1^{a}	20	0.946	0.943
	2	1.30^{c}	0.284^{cd}	3.45^{c}	10	0.988	0.986
	3	1.72^{b}	0.514^{b}	2.13^{c}	7	0.992	0.991
15WM	1	0.886^{d}	0.0799^{ef}	8.78^{a}	17	0.987	0.986
	2	1.77^{ab}	0.316^{c}	3.75^{c}	9	0.998	0.998
	3	2.03^{a}	0.568^{a}	2.35^{c}	8	0.990	0.989

Note: Values having different letters within a column are significantly different ($p \leqslant 0.05$).

Significant differences ($p \leqslant 0.05$) among the A values for mung bean starter (15WM) and all μ values were detected in each activation stage. Hence, it can be assumed that activation played a relatively minor role in amplifying maximum volume expansion ratio of wheat and pumpkin starters than when adding pumpkin or mung bean flour. Activation boosts metabolic activities, especially the carbon dioxide production of sourdough microbes by refilling fermentation substrates during the stationary phase where they are the most active. Meanwhile, the stability of wheat starter in retaining the released carbon dioxide was negatively influenced by its lowest pH (Komlenić et al., 2012). By adding pumpkin and mung bean, the deficiencies of sole wheat flour in terms of leavening and stability were reinforced with their components, for example, starch, hydrocolloids and proteins, that create viscous gel in hydrated form to strengthen the gas holding capability (Bai et al., 2020; Houben et al., 2012; Monteiro et al., 2021). Therefore, the volume expansion was greater and faster in the composite sourdough starters than the wheat starter containing only wheat.

The synergy of non-cereal addition and activation stages prominently affected lag time of leavening process (t_{lag}) only during stage 1 of activation. The lag time decreased and converged to approximately 2-3 hours regardless of the sourdough types in stages 2 and 3. Any pronounced reduction of lag time during these stages is challenging since the time is allocated for commencing sugar metabolism and carbon dioxide diffusion during lag phase of each activation stage (Baker & Mize, 1941; Chevallier et al., 2012; Gally et al., 2017; Meerts et al., 2018; Romano et al., 2007). The addition of pumpkin and mung bean respectively extended the t_{lag} of wheat sourdough starter from 6.39 h to 10.1 and 8.78 h in the first stage of activation. In pumpkin and mung bean, the nutrients including protein, starch, fibre and ash were more abundant (González-

Montemayor et al., 2021; Nigam, 2015), possibly taking more time for complete breakdown in composite sourdough starters.

By using the same parameters in Gompertz equation, the modelling of leavening capabilities facilitates the more precise and direct comparison among sourdough starters in the present study and others (Meerts et al., 2018; Romano et al., 2007). The doughs in the study of Chevallier et al. (2012) achieved values of A, μ and t_{lag} at 2.43-4.50, 1.90-3.26 h^{-1}, 0.25-0.39 h respectively, meanwhile in Romano et al.'s (2007) research, the values were 2.44-2.97, 1.14-4.29 h^{-1} and 0.14-0.70 h accordingly. By adding sugar and/or salt, the leavening capabilities in doughs could be improved, as shown by the greater values of μ at 4.68-6.84 h^{-1} and lower values of t_{lag} at 0.13-0.16 h (Meerts et al., 2018). The lower t_{lag} and higher A and μ values as compared to values in the present study were expected due to the usage of different leavening agents. These studies utilised baker's yeast that constructs different growth curves from sourdough microbes, hence showing markedly different values in each parameter. Still, Gompertz equation is reassuring in describing leavening of sourdough starters with only three unknowns. This equation presents the μ value as absolute volume expansion rate without the interference of A values (Tjørve & Tjørve, 2017).

The models are foreseen to be helpful for more effective sourdough activation control. The time-consuming sourdough fermentation always challenges home and artisan bakers with the inconsistent bread-making schedule. By applying or adapting the models to their own sourdough starters, bakers can activate sourdough starters on time as they are clear about the time required for each activation cycle. Similar to industrial scale baking, the application of models potentially allows prediction about volume

expansion ratio of sourdough starters and timing to use them for baking. Hence, batch manufacturing of sourdough products could be optimised for bigger production capacity within minimal time.

4.2.3 Summary

The incorporation of pumpkin and mung bean substantially aided leavening improvement in sourdough starters. The maximum specific volume was enhanced significantly to 240.5 and 196.7 cm^3/100g in starters containing pumpkin and mung bean respectively as compared to wheat starter at 153.9 cm^3/100g during stage 1. The greater pH and TTA values of composite sourdough starters possibly aided by improving gluten elasticity and dough stability of wheat starter. For wheat, the three-stage activation had also boosted the specific volume up to 175.3 cm^3/100g and subsequently 205.3 cm^3/100g. The sigmoidal curves of specific volume development resemble the typical growth curves, possibly for the sourdough microbes in this case. Hence, the leavening performances were expressed by volume expansion ratio and were fitted using Gompertz model for easier control of sourdough activation. After activation, sourdough starters displayed more excellent leavening performances through pumpkin or mung bean addition, as shown by the raised maximum volume expansion ratio from 0.40 to 1.72 and 2.03 cm^3/cm^3, maximum volume expansion rate from 0.04 to 0.51 and 0.57 h^{-1}, reduced leavening lag time from 6.39 h to 2.13 and 2.35 h and overall fermentation time from 18 to 7 and 8 h respectively.

4.3 Effects of Non-Cereals in Steamed Buns

Sourdough starters are natural leavening agents that are important in making bakery products. Activated sourdough starters are usually incorporated into doughs at their optimum state. The leavening performances of composite sourdough starters with 15% pumpkin and mung bean respectively were tested by using them to make steamed sourdough buns (SSBs). At dough forming stage, the involvement of non-cereals was also tested through pumpkin flour fortification. Pumpkin has been a popular choice to fortify baked bread (El-Demery, 2011; Hoxha et al., 2023; Pongjanta et al., 2006; Ptitchkina et al., 1998; Różyło et al., 2014; Sadeghi et al., 2019). Through fortification, it was then anticipated that the naturally sweet and orangish pumpkin could improve the consumer acceptance of slightly sour SSBs more easily than mung bean from the aspects of appearance and taste. Hence in this study, SSBs were made using each activated sourdough starter and respectively fortified with pumpkin flours at 5, 10, 15 and 20%. For comparison, SSBs were also prepared without pumpkin fortification using each type of sourdough starter, in which control SSB was referred to the one containing wheat starter. The collective and respective effects of composite sourdough starters and pumpkin fortification on the properties of steamed buns were investigated in terms of volume, spread ratio, colour, texture, sensory qualities and proximate composition. These parameters covered both the objective and subjective qualities measurement of SSBs in evaluating potentials of composite sourdough starters in the bakery products.

4.3.1 Volume

Dough, with the help of leavening agent, yields greater volume after being steamed. The gluten network in dough retains the gases produced, including carbon dioxide that is released during microbiological activities (Chavan & Chavan, 2011) and evaporated steam during heating (Lai & Lin, 2006). Volume is associated with the fluffiness of SSB (Li et al., 2021). Hence, volume is always measured and discussed particularly in the form of specific volume *i.e.*, ratio of volume to weight (Su et al., 2005). Figure 4.5 shows the significantly different values ($p \leqslant 0.05$) in specific volume of SSBs by different letters above the bars under the influences of different types of sourdough starters and composition of fortified pumpkin.

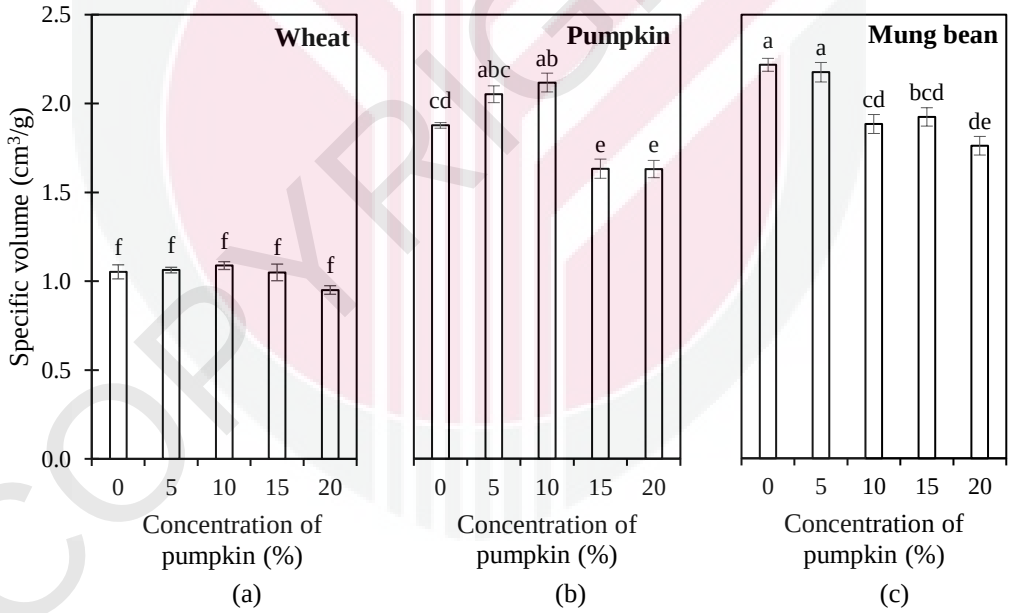

Figure 4.5: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on specific volume of SSBs with 0 to 20% pumpkin fortification

The specific volume of SSB was enhanced, in relation with the improved leavening performances of composite sourdough starters. From Figure 4.5(a), the specific volume at 1.05 cm^3/g was observed for control SSB. This value is considered to be poor as the values are typically greater than 1.7 cm^3/g for commercially available steamed bread (Li et al., 2021). Such criterion was fulfilled after incorporating wheat-pumpkin and wheat-mung bean composite sourdough starters in SSBs, giving specific volume of 1.88 and 2.22 cm^3/g respectively, as shown in Figures 4.5(b) and 4.5(c). This is comparable with 2.12 cm^3/g from the finding of Chen et al. (2018) with same concentration of sourdough starter at 10% and 2.03 cm^3/g from the study of Zhu et al. (2016) that used all-purpose flour. Conversely, the specific volume in this study was slightly lower as compared to the values ranged between 2.23 and 2.38 cm^3/g in most studies that used baker's yeast and/or high-protein bread flour (Laohaprasit & Sricharoenpong, 2018; Li et al., 2021; S. Ma et al., 2014; Su et al., 2005). The deviation in specific volume values is probably due to the different degree of leavening, in accordance with the leavening agents and gluten content of flour (Le Vu Lan et al., 2021).

The adequate fortification of pumpkin increased or maintained the specific volume in SSBs. Figure 4.5(a) shows that by using wheat sourdough starter, the specific volume was maintained with relatively lower range of values at 0.95-1.09 cm^3/g through 0-20% pumpkin fortification. Significant drop in specific volume was detected at pumpkin fortification greater than the optimum percentage of 10% and 5%, respectively for SSBs using wheat-pumpkin and wheat-mung bean sourdough starters. Pumpkin pectin was possibly one of the contributors that stabilise the air pockets within dough gluten network (Ptitchkina et al., 1998). The surplus pumpkin conversely diluted the gluten content, thus weakening the gas holding capacity of doughs (Li et

al., 2021; Yang, 2016). Only pumpkin fortification of 5 and 10% were satisfying at 2.05 and 2.12 cm^3/g respectively for specific volume of SSBs with wheat-pumpkin sourdough starter. Similar outcomes were depicted by the study of Ptitchkina et al. (1998), whereby the specific volume of baked bread was raised from 3.20 to 4.20 cm^3/g after fortification of 10% pumpkin, but with 25% pumpkin, the value was dropped to 3.50 cm^3/g. Meanwhile by referring to Figure 4.5(c), with pumpkin fortification up to 20%, wheat-mung bean sourdough starter was still able to maintain adequate specific volume between 1.76 and 2.18 cm^3/g in SSBs. This further supported the strong leavening capability of wheat-mung bean sourdough starter that overpowered the negative influence of excessive pumpkin fortification.

In comparing with baked bread, specific volume is usually lower for SSB. As compared to 0.95-2.22 cm^3/g from the present study, specific volume values of baked breads were 3.1 cm^3/g with faba bean sourdough (Coda, Varis, et al., 2017) and 3.87 cm^3/g with wheat-legume sourdough (Rizzello et al., 2014). The values at 2.23-2.38 cm^3/g were still lower even when baker's yeast and/or high-protein bread flour were used in other steamed bread studies (Laohaprasit & Sricharoenpong, 2018; Li et al., 2021; S. Ma et al., 2014; Su et al., 2005). During steaming, the heat supplied is not as high as during baking, thus possibly lead to smaller amount of steam formation from the moisture content of dough and smaller degree of leavening (Lai & Lin, 2006).

4.3.2 Spread Ratio

Similar to volume, spread ratio is also an indicator of gas holding ability for steamed bread (Laohaprasit & Sricharoenpong, 2018). It is equivalent to the ratio of width to height (F. Ma et al., 2017; Zhu, 2014), as illustrated in Figure 4.6. Figures 4.7(a), 4.7(b) and 4.7(c) showed the respective incorporation of wheat, wheat-pumpkin and wheat-mung bean sourdough starters that gave rise to different spread ratio with different degrees of pumpkin fortification in SSBs. The control SSB achieved the greatest spread ratio of 1.86, that was significantly higher than 1.59 and 1.41 with wheat-pumpkin and wheat-mung bean sourdough starters respectively. The high spread ratio explains that the SSB spreads more during steaming (Su et al., 2005), ends up with less satisfactory flat shape (Huang et al., 1998; Zhu, 2014) instead of the ideal round shape (Kruger et al., 1992). This may have occurred due to the weaker leavening ability of wheat sourdough starter. The gluten content was also lower in plain wheat flour, thus building gluten network that is weaker in trapping gases released (Le Vu Lan et al., 2021). Values with composite starters in this study (1.41-1.59) was still comparable with the spread ratio at 1.47 using high protein wheat flour in SSB (Chen et al., 2018).

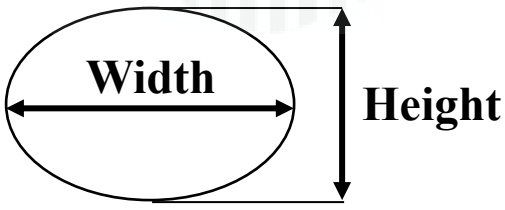

Figure 4.6: The width and height of SSBs (side view)

As degree of pumpkin fortification increased to 20%, the spread ratio shown in Figure 4.7(a) markedly decreased from 1.86 to 1.41 for SSBs containing wheat sourdough starter. The gluten network in dough was gradually strengthened, possibly due to the contribution of pumpkin pectin that improved the gas-cell stability with its surface activity (Ptitchkina et al., 1998). The pumpkin fibre may also aid water absorption of dough (Sadeghi et al., 2019), thus reducing the collapse of dough matrix associated with irregular distribution of bubbles within gluten network (Mastromatteo et al., 2012). However, the impacts of increasing pumpkin fortification were less significant for SSBs with composite starters, except the one with wheat-pumpkin sourdough starter at 20% pumpkin fortification. Similar outcome was observed in another report that studied the effect of foxtail millet addition instead of pumpkin (Li et al., 2021).

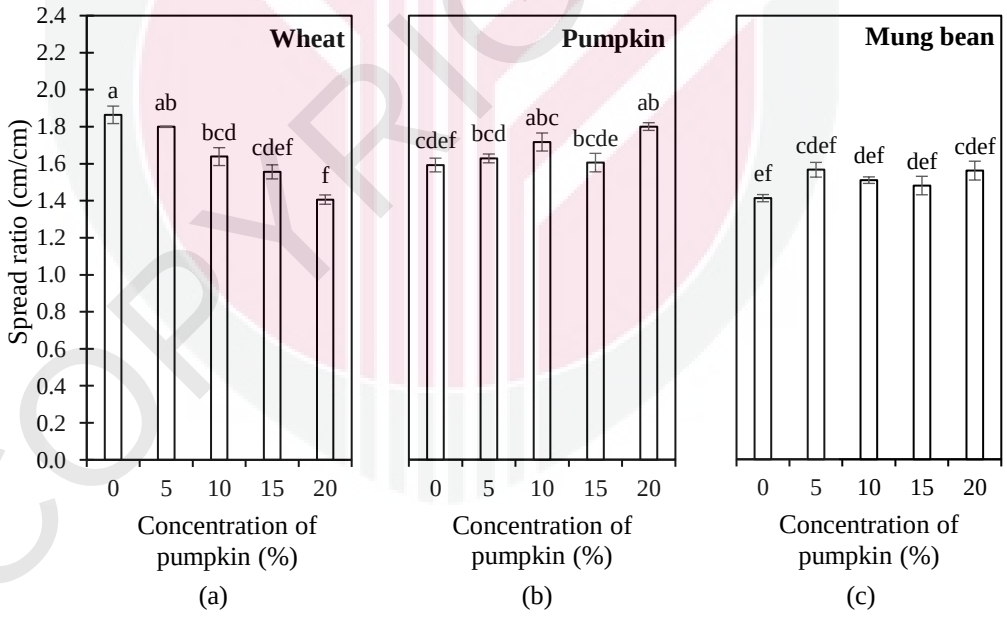

Figure 4.7: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on spread ratio of SSBs with 0 to 20% of pumpkin fortification

4.3.3 Colour

Among the physical characteristics of steamed bread, colour appears to be a special one. It is normally vital as the first impression (S. Ma et al., 2014) yet the standard is flexible enough for newly developed products. Food ingredients come with their own natural colour, therefore the incorporation of non-cereals is indirectly bringing fresh new image to steamed bread. Instead of the expected white colour (Zhu, 2014), steamed bread can also appear in orangish colour after the fortification of pumpkin. The colour changes in SSBs with different types of sourdough starters and different degrees of pumpkin fortification were determined in this study (Figure 4.8). The colour of each SSB was represented by numerical values, in terms of L*, a* and b*.

From Figure 4.9, L* values of SSBs were significantly affected ($p \leqslant 0.05$) by the different types of sourdough starters and degrees of pumpkin fortification. The use of wheat-pumpkin sourdough starter decreased the L* value (lightness) of control SSB from 56.97 to 49.35, whereas the value was raised to 61.89 with the presence of wheat-mung bean sourdough starter. Overall, L* decreased with the increase in degrees of pumpkin fortification. The colour of SSB was darker and deviated further from the natural white colour of wheat flour as more pumpkin was incorporated. This agrees with the study of El-Demery (2011) that showed L* values ranged between 26.57 and 27.88 for toast breads with 5 to 20% pumpkin fortification. L* values were higher at 38.72-61.89 in this study without baking at high temperature, thus omitting the darkening by Maillard reaction (Sahin, Zannini, et al., 2019).

Figure 4.8: The colour changes in SSBs using (a) wheat (control) starter, (b) wheat-pumpkin and wheat-mung bean composite starters at increasing pumpkin fortification of 0, 5, 10, 15 or 20% (from left to right)

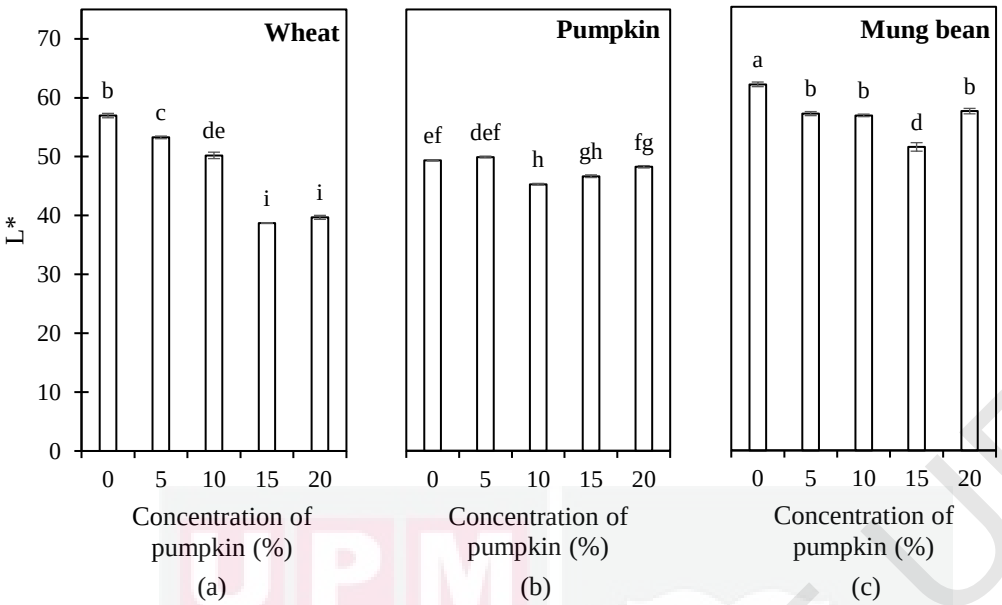

Figure 4.9: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on L* values of SSBs with 0 to 20% pumpkin fortification

Figure 4.10 shows that the a* values measured for SSBs without pumpkin fortification were minimal between 0.36 and 0.99 disregard of the types of sourdough starters used. A significant increase was observed with increasing pumpkin fortification up to 15% in steamed buns, especially for the buns made using wheat sourdough starter where values raised from 0.99 at 0% to 45.98 at 15% fortification from Figure 4.10(a). This highlighted the effect of pumpkin fortification in contributing to a* values. This effect seemed to be more pronounced in SSB than in baked sandwich breads which only showed a* values ranged between 10.61 and 10.92 with a 10-20% pumpkin fortification (Pongjanta et al., 2006). The appearance of SSB was believed to be more attractive than the typical white steamed bread since a* values or redness increased (El-Demery, 2011).

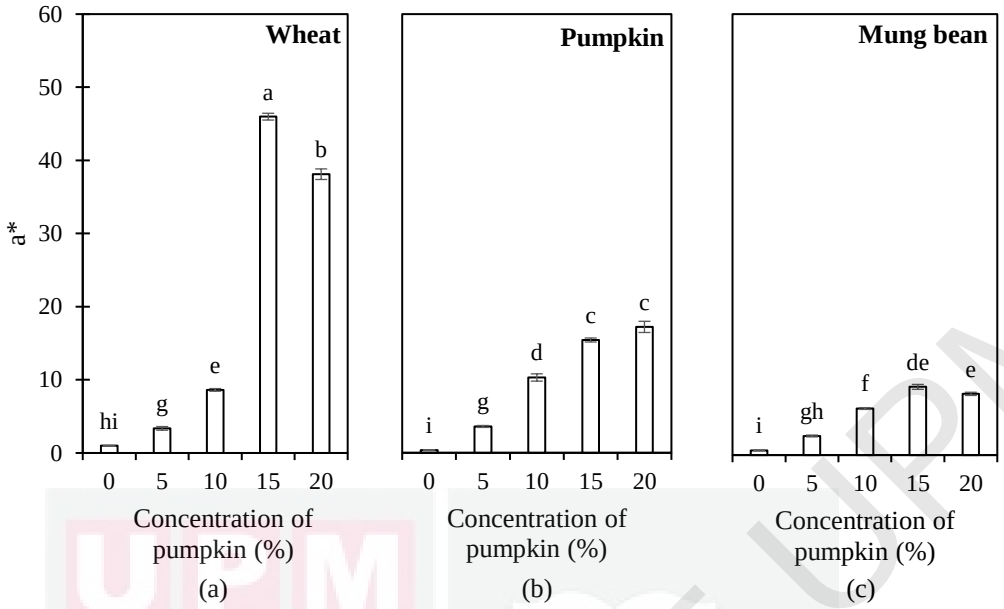

Figure 4.10: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on a* values of SSBs with 0 to 20% pumpkin fortification

By comparing between Figures 4.11(a) and 4.11(b), the value of b* increased from 16.59 to 22.95 when using wheat-pumpkin composite starter, while between Figures 4.11(a) and 4.11(c), insignificantly decreased to 13.65 with wheat-mung bean sourdough starter. It could be interpreted that SSB was more yellow in colour with pumpkin starter at higher b*, while SSB with mung bean starter was slightly more bluish as compared to control SSB. In terms of pumpkin fortification, b* values were generally raised in a concentration-dependent manner with each sourdough starter (Figure 4.11). Pumpkin fortification resulted in orange colour with greater yellow intensity in SSB. The SSB existed with higher level of yellowness (positive b* values), for example from 33.89 to 77.14 as compared to redness with positive a* values of 3.36-45.98 at 5-20% pumpkin fortification with wheat sourdough starter (Table A.4 in Appendix A). Such outcome was originated from the high beta-carotene content in pumpkin (Pongjanta et al., 2006). Apart from improving the bread acceptability with its colour (Różyło et al., 2014), beta-carotene also enhanced the nutritional value as an

antioxidant (Tsao & Lo, 2006; Ware, 2019). Similar to a* values, the b* values were lower in baked bread, *i.e.*, ranged from 10.37 to 11.25 at similar pumpkin concentrations (El-Demery, 2011).

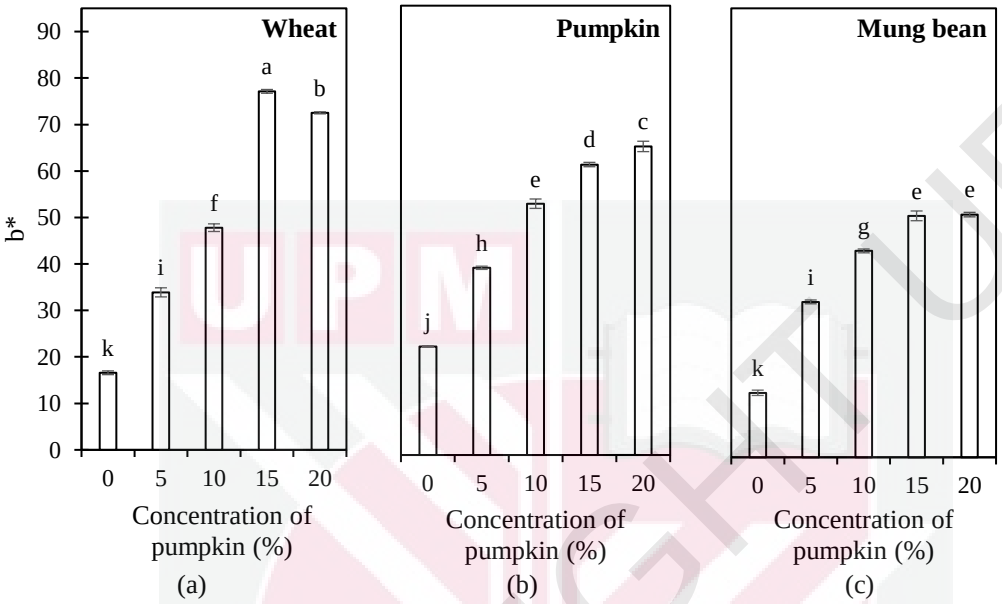

Figure 4.11: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on b* values of SSBs with 0 to 20% pumpkin fortification

Total colour difference of SSBs was determined by calculating ΔE values using Equation 3.7 with reference to control bun without any pumpkin fortification, *i.e.* 0%. Figures 4.12(b) and 4.12(c) show significant colour difference of 9.95 and 5.76 respectively in SSBs with wheat-pumpkin or wheat-mung bean composite sourdough starters from the control SSB. The inherent colour of sourdough starters was one of the possible factors. The 15% pumpkin fortification has raised ΔE values the most by 77.61 with wheat starter from Figure 4.12(a), followed by 48.21 and 35.97 respectively in SSBs made from wheat-pumpkin and wheat-mung bean starters. The white wheat sourdough starter allowed greater impact of pumpkin fortification on SSB colour

although the concentration of coloured non-cereals was greater with composite starters.

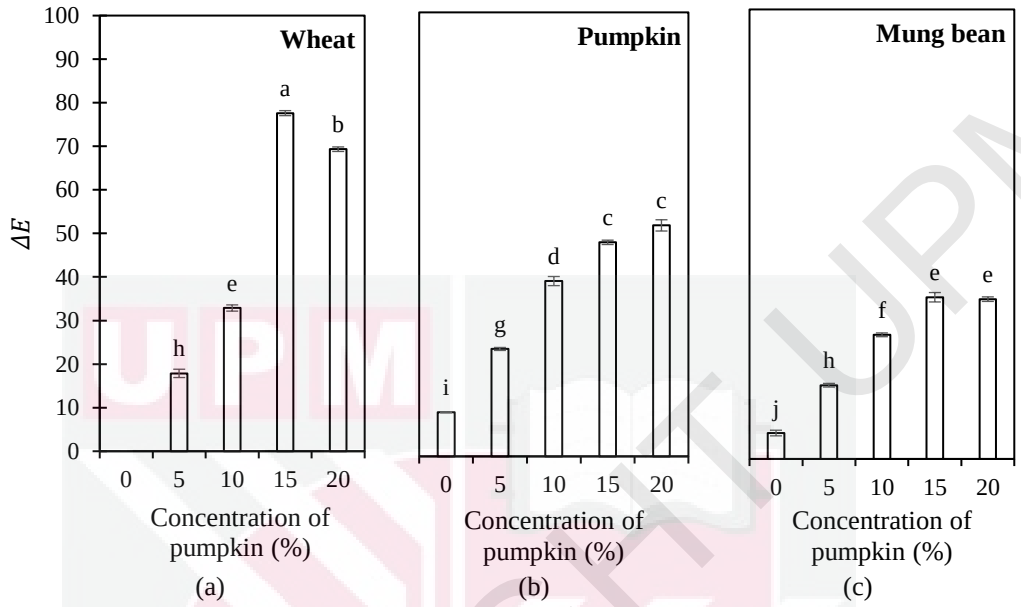

Figure 4.12: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on *ΔE* values of SSBs with 0 to 20% pumpkin fortification

4.3.4 Texture

Hardness as the maximum force on the first bite (Bourne, 2014), is usually in negative correlation with bread quality (Li et al., 2021; C. Liu et al., 2015). The usage of wheat-pumpkin and wheat-mung bean composite sourdough starters significantly ($p \leqslant 0.05$) reduced the hardness values of SSBs displayed in Figure 4.13, from 7353 gram-force (gf) to 2991 and 2605 gf respectively. This may be related to their enhanced leavening abilities, hence retaining more gas cells and decreasing hardness within the buns. Figure 4.14 is a cross-sectional diagram that shows the gas cells in a steamed sourdough bun. Table A.5 in Appendix A and Figure 4.13(a) show that the lowest hardness value of 6279 gf was achieved at 10% pumpkin fortification among SSBs

with wheat sourdough starter. The softness was possibly attributed by the surface activity of pumpkin pectin which could stabilise the gas cells in dough (Ptitchkina et al., 1998; Sadeghi et al., 2019). Conversely, the excess pumpkin fortification of 15% and 20% led to unappealing, hard SSBs. This is in agreement with other studies that utilised different ingredients, such as foxtail millet (Li et al., 2021), quinoa (S. Wang et al., 2015) and wheat germ (S. Ma et al., 2014). From Figure 4.13(c), the SSBs with wheat-mung bean sourdough starter were the exceptions with lesser impact of pumpkin fortification, reflected by the narrower range of hardness values between 3045 and 3848 gf.

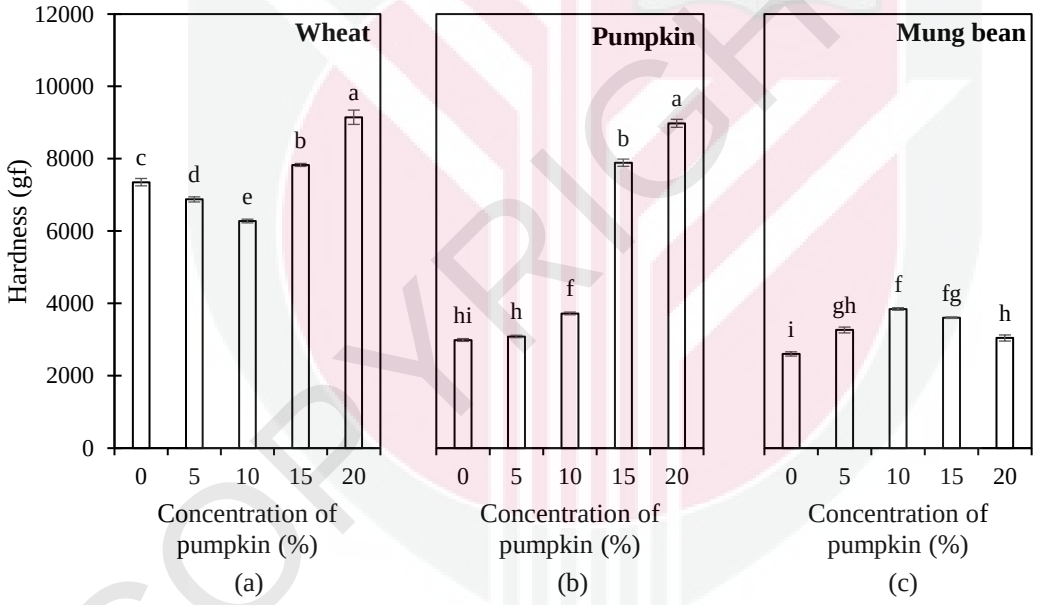

Figure 4.13: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on hardness of SSBs with 0 to 20% pumpkin fortification

Figure 4.14: The gas cells in the cross-section of a steamed sourdough bun

From Figure 4.15, the respective springiness rise from 0.93 to 0.99 and significantly ($p \leqslant 0.05$) to 1.02 was contributed by involving pumpkin and mung bean composite starters as compared to control SSB with wheat starter. At 20% pumpkin fortification, the highest value of 1.03 was achieved among buns containing wheat sourdough starter. Unlike hardness, springiness is one of the positive qualities in SSB that satisfies expectation of consumers. Springiness, sometimes known as elasticity, demonstrated the capability of SSB to return to its initial shape after the removal of external force (Li et al., 2021). The dominant factor in affecting springiness is probably the gas-holding capability of gluten network in dough, owing to the concentration of gluten, especially the glutenin (Li et al., 2021; X.-Y. Wang et al., 2016). Hence, greater springiness was accomplished by composite sourdough starters with their improved leavening performances and sufficient pumpkin fortification, consistent with the previous study on bread using wheat-legume sourdough (Rizzello et al., 2014). Nevertheless, the springiness values dropped significantly in excessive pumpkin fortification at 20% with composite sourdough starters, possibly attributed to the weakened supporting network since gluten was diluted by the incorporated pumpkin (Yang, 2016).

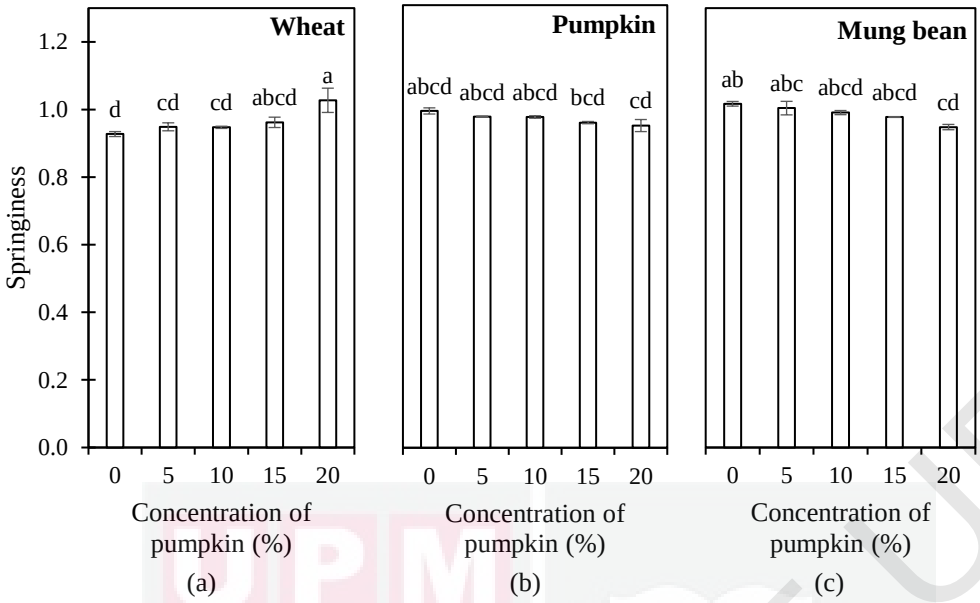

Figure 4.15: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on springiness of SSBs with 0 to 20% pumpkin fortification

Cohesiveness portrays the extent to resist the second deformation in relation to first deformation in steamed bread (Li et al., 2021). Similar to springiness, Figure 4.16 shows that the cohesiveness of control SSB at 0.77 was significantly ($p \leqslant 0.05$) increased to 0.86 and 0.91 respectively by the usage of composite sourdough starters with pumpkin and mung bean, whereas by increasing pumpkin fortification to 10%, the value was raised to 0.87 (Table A.5). For SSBs using composite sourdough starters, the negative influence of pumpkin fortification was also observed in cohesiveness and other textural parameters. Pumpkin fortification may disturb the gluten network by competing hydration, hence decreasing the elasticity and eventually cohesiveness in steamed bread (Laohaprasit & Sricharoenpong, 2018).

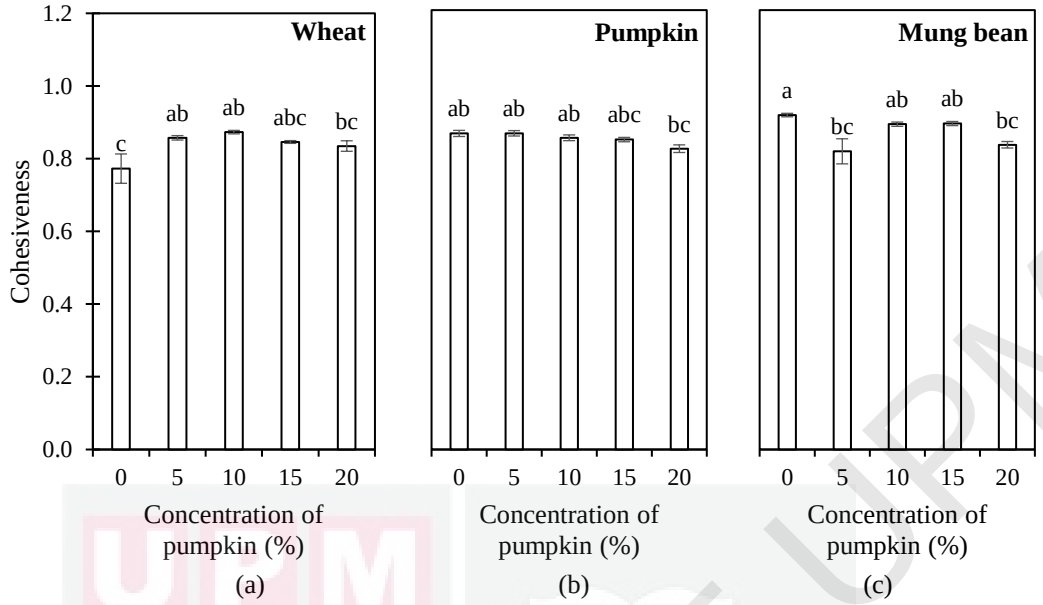

Figure 4.16: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on cohesiveness of SSBs with 0 to 20% pumpkin fortification

Chewiness is obtained as the product of hardness, springiness and cohesiveness. Figure 4.17 shows that chewiness of SSBs made using pumpkin and mung bean composite sourdough starters reduced significantly ($p \leqslant 0.05$) to 2553 and 2418 gf when compared to the one using wheat starter at 5262 gf. Less force was required to chew SSBs from composite starters likely related to its bigger volume (Różyło et al., 2014), improved leavening and presence of more air pockets inside. In general, fortification of pumpkin flour resulted rise in chewiness in all SSBs except for the wheat-mung bean. The significant increase especially at 20% pumpkin fortification was possibly associated with the weakened gluten network (Li et al., 2021) and lower specific volume at 0.95 and 1.63 cm^3/g, as compared to 1.05 and 1.88 cm^3/g respectively in steamed breads with wheat and wheat-pumpkin starters without fortification (Figure 4.5). However, SSBs containing mung bean starter were excluded, probably prior to the stronger leavening capability that achieved 3.12 cm^3/g at the third activation stage from Figure 4.3.

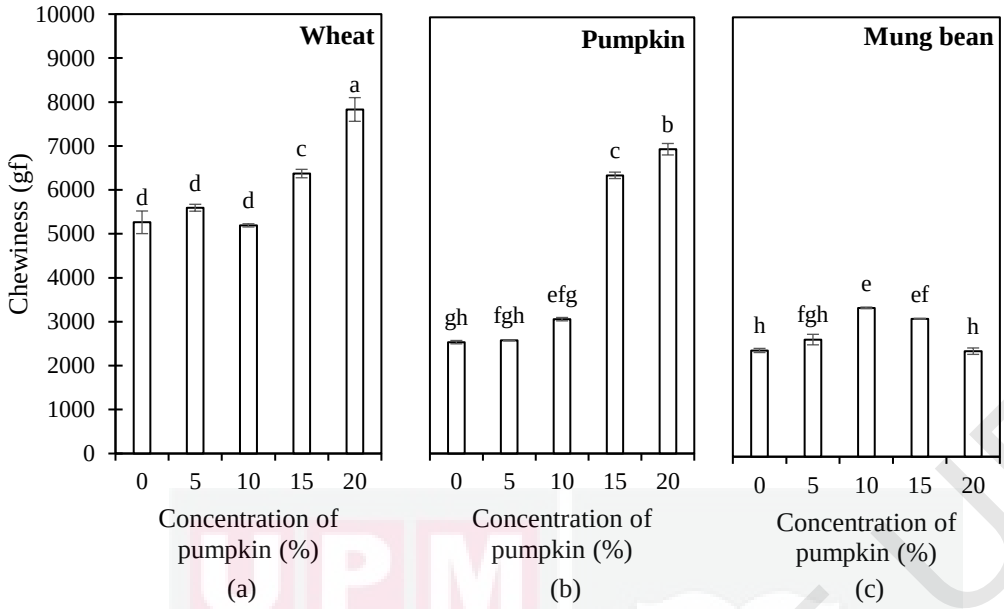

Figure 4.17: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on chewiness of SSBs with 0 to 20% pumpkin fortification

The resilience of SSB describing elasticity as the ability to recover after first bite is presented in Figure 4.18. A significant increase from 0.32 for control SSB to 0.39 and 0.50 respectively was found when the starter was replaced by wheat-pumpkin and wheat-mung bean. This could be related to the stronger gas trapping capability and greater viscoelasticity of composite sourdough starters (S. Ma et al., 2014). From Table A.5, the resilience of 0.39 was the highest at 10% pumpkin fortification among SSBs with wheat starter. With composite starters, resilience generally decreased with increasing pumpkin fortification. There was possibly a moisture competition between pumpkin flour and gluten in wheat flour (Li et al., 2021). The pumpkin fortification in SSBs with pumpkin starter may blend in the dough structure better with the initial presence of pumpkin in starter, hence showing insignificance on resilience values.

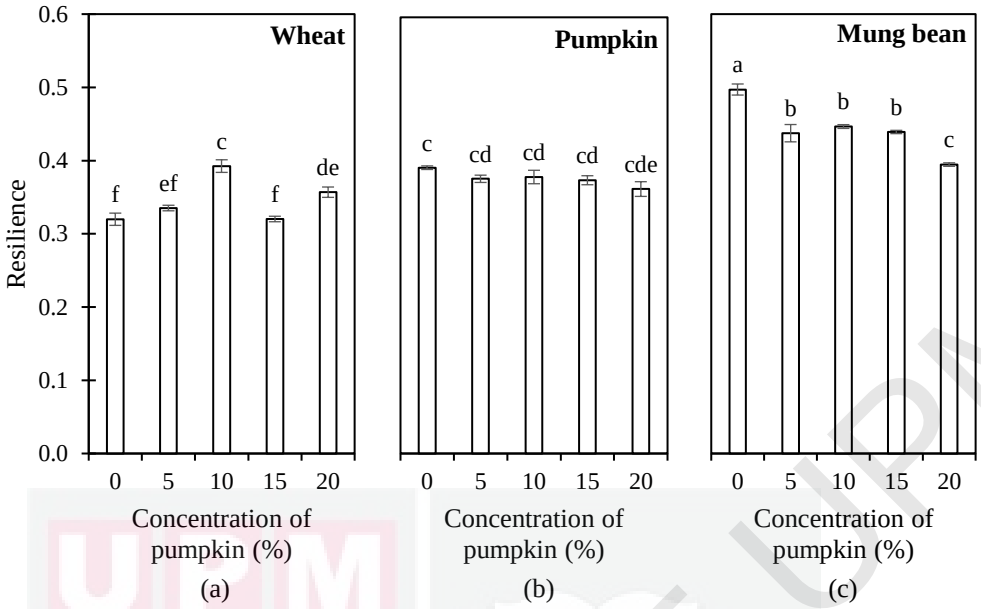

Figure 4.18: The effects of using (a) wheat, (b) wheat-pumpkin and (c) wheat-mung bean composite sourdough starters on resilience of SSBs with 0 to 20% pumpkin fortification

In general, textural properties of SSBs involving pumpkin and mung bean starters has improved excellently at 5-10% pumpkin fortification. By gathering the values from Table A.5 (Appendix A), Figure 4.19 shows the comparison of textural parameters *i.e.* hardness, springiness, cohesiveness, chewiness and resilience for SSBs containing composite starters at 5% and 10% pumpkin fortification with control SSB containing wheat starter without pumpkin fortification. For easy comparation, values of hardness and chewiness were presented at their ten thousandth (10^{-4}) numbers. Low hardness and chewiness were preferrable for SSB while for springiness, cohesiveness and resilience, higher values were more satisfying.

From Figure 4.19, the hardness and chewiness of SSBs were all lower at 5% pumpkin fortification, the cohesiveness was higher at 10% pumpkin fortification with mung bean starter, whereas all values of springiness and resilience were insignificantly different for both levels of pumpkin fortification. The specific volume and spread ratio

of SSBs were also outstanding at 5% pumpkin fortification from Figures 4.5 and 4.6. Therefore, both SSBs with composite sourdough starters at the optimum level of 5% pumpkin fortification were selected together with control SSB to progress with proximate and microbiological analyses.

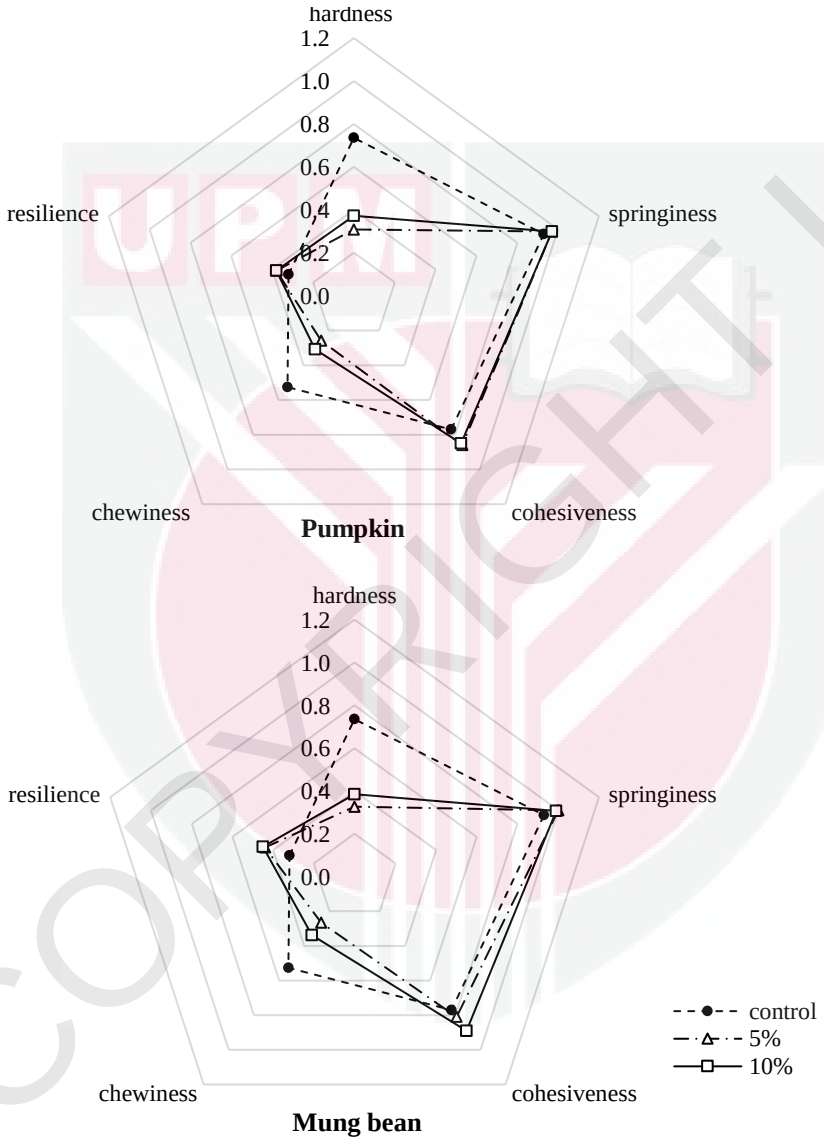

Figure 4.19: The effects of 5% (–▵–) and 10% (—□—) pumpkin fortification on textural parameters of SSBs with wheat-pumpkin and wheat-mung bean composite sourdough starters as compared to control SSB (--●--)

4.3.5 Sensory Qualities

Sensory qualities should not be compromised while using atypical ingredients in food products. The qualities of SSB samples with different degrees of pumpkin fortification and different types of sourdough starters were evaluated by 15 untrained sensorial panellists through 9-point hedonic tests. The samples were served after being reheated and cooled. After the sensorial test, the sensorial scores of all SSB samples given by panellists in terms of texture, colour, taste, aroma and overall liking are summarised in Figure 4.20 and Table A.6 (Appendix A).

In terms of texture, both wheat-pumpkin and wheat-mung bean sourdough starters have markedly boosted the score of control SSB from 5.5 to 7.0 and 7.5 respectively. Similarly, wheat-legume sourdough starter improved the texture score in terms of elasticity in baked wheat bread (Rizzello et al., 2014). When using wheat starter, 5 and 10% pumpkin fortification gave the highest texture scores of 7.1 and 6.8 respectively (Table A.6). Texture could be determined by distribution and amount of gas cells in SSB (Sadeghi et al., 2019). The stability of gas cells thus was improved by the little yet adequate amount of pumpkin particularly the contained pectin (Ptitchkina et al., 1998). The further increase of pumpkin fortification up to 20% negatively affected the texture score of all SSBs, as confirmed by the study of Pongjanta et al. (2006) using sandwich and sweet breads fortified with pumpkin powder. The scores for texture of SSBs were generally in relation to the measured hardness and specific volume. For example, 5% pumpkin fortification, which contributed the higher texture scores of 6.8-7.1, gave relatively lower hardness of 3088-6876 gf (Table A.5) and bigger specific volume of 1.06-2.18 in SSB with each starter (Table A.4).

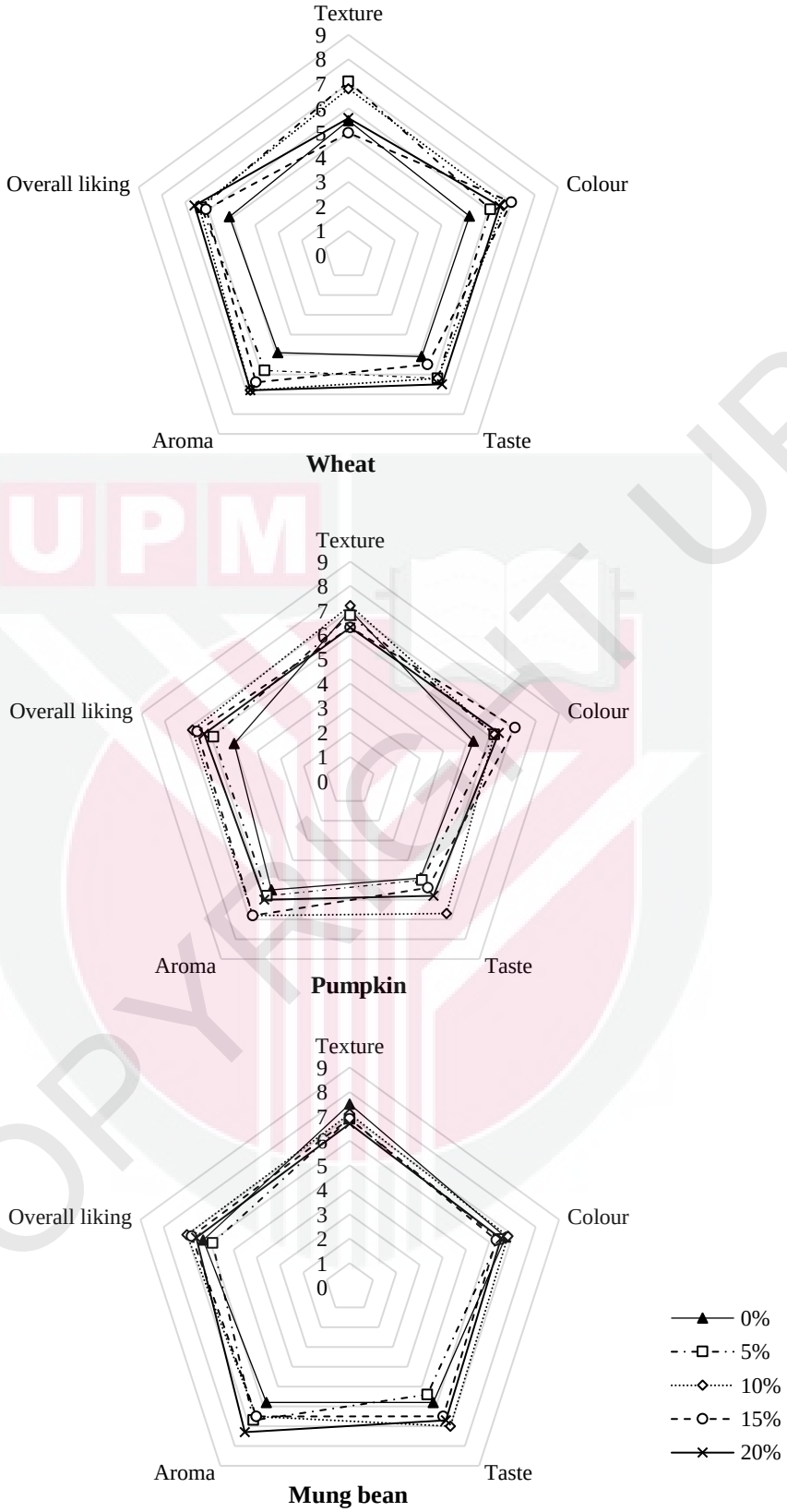

Figure 4.20: The effects of 0% (—▲—), 5% (- -□- -), 10% (····◇····), 15% (- -○- -) and 20% (—×—) pumpkin fortification on sensorial scores of SSBs with wheat, wheat-pumpkin and wheat-mung bean composite sourdough starters

The colour score of 5.2 for control SSB was similar at 5.3 by using wheat-pumpkin sourdough starter but was raised to 6.7 by using the starter with mung bean. Pumpkin fortification at 5-20% also increased all colour scores to above average, *i.e.* within 6.1 and 7.1. From human eyes, colour differences caused by different types of sourdough starters and degrees of pumpkin fortification may not be distinguishable. Overall, the degree of pumpkin fortification was positively correlated to the colour score although the impact was less obvious with wheat-mung bean starter. The liking scores were possibly improved by beta-carotene in pumpkin (Pongjanta et al., 2006) that manipulates the a* and b* values. The carotene pigments are helpful to provide food products, such as pumpkin chips, pumpkin juice, dairy and bakery products with their notable yet natural orangish-yellow colour (Hussain et al., 2022).

Similar to texture and colour scores, the positive effect of wheat-mung bean composite sourdough starter on taste score was observed by slightly raising it from 5.1 to 5.8. Legumes, which mung bean belongs to, could enrich the overall taste of SSB by causing saltiness taste without salt addition (Rizzello et al., 2014). From Table A.6, the highest taste score at 7.0 was contributed by the wheat-mung bean sourdough starter with 10% pumpkin fortification. The highly subjective taste gave rise to fluctuating scores but most panellists preferred 10 and 20% pumpkin fortification in SSBs. The increasing pumpkin fortification played more pronounced role than composite starters, probably by adding some natural sweetness (Kamarubahrin et al., 2018) to mask the slightly sour taste of SSB.

Table A.6 shows that the panellists rated the aroma of SSB from 4.9 to 7.3, indicating levels ranging from “neither like nor dislike” to “like moderately”. Among the SSBs, the highest average aroma score at 6.6 was recorded when wheat-mung bean

sourdough starter was used, while wheat-pumpkin and wheat sourdough starters achieved lower average aroma score of 6.2 and 6.1. Use of sourdough in steamed bread provides a pleasant, buttery aroma which is supplied through lactic acid fermentation of sourdough microbiota (Petrova & Petrov, 2020). As seen from Figure 4.20, the scores for aroma rised generally with increasing degrees of pumpkin fortification in SSB. The natural, pleasing aroma of pumpkin (Hussain et al., 2022) is likely to enhance the aroma scores with its increasing proportion in SSB.

The overall liking scores of SSB were improved by incorporating composite sourdough starters and by fortifying with pumpkin flour (Figure 4.20). All the buns achieved positive scores of at least 5 out of 9 from Table A.6. The overall liking score of control SSB was 5.1, 5.0 for pumpkin starter and higher at 6.3 for mung bean starter. For wheat starter alone in SSB, the effect of pumpkin fortification was not significant although at 20%, overall liking score of 6.6 was the highest. For composite starters containing pumpkin and mung bean, 10% fortification was rated the best by panellists with scores of 6.8 and 7.0 respectively. Sadeghi et al. (2019) also deduced that overall acceptability was improved with 10% pumpkin puree addition in bread rolls.

4.3.6 Comparison on Proximate Compositions

Table 4.2 shows the proximate compositions of SSBs made using wheat-pumpkin and wheat-mung bean sourdough starters respectively at 5% pumpkin fortification along with control (wheat at 0% pumpkin fortification) and a commercial steamed bun that was manufactured by QL Figo Foods Sdn. Bhd. (Johor, Malaysia). The proximate composition in SSB was comparable and improved by using starters containing pumpkin and mung bean, especially referring to the contents of total carbohydrates

and dietary fibre. The proximate composition for each SSB was comparable with up to 3.8% differences, as compared to a commercial steamed bun containing baker's yeast and baking powder. A sourdough bread of good quality is expected to possess fewer carbohydrate and greater protein, ash and fibre contents (Catană et al., 2022).

Table 4.2: Proximate composition of SSBs using wheat, wheat-pumpkin and wheat-mung bean sourdough starters

Proximate composition	Steamed buns with different sourdough starters			Commercial steamed bun
	Wheat	Wheat-pumpkin	Wheat-mung bean	
Energy (kJ/100g)	1090 ± 218	1030 ± 206	1070 ± 214	1151
(kcal/100g)	259 ± 52	246 ± 49	254 ± 51	274
Total carbohydrates (%)	54.2 ± 10.8	50.8 ± 10.2	52.5 ± 10.5	54.6
Total dietary fibre (%)	2.6 ± 0.2	3.0 ± 0.2	3.3 ± 0.2	3.3
Ash (%)	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	-
Protein (%)	8.0 ± 0.4	8.0 ± 0.4	8.4 ± 0.5	7.4
Moisture (%)	36.5 ± 1.9	39.7 ± 2.0	37.6 ± 1.9	-
Fat (%)	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	2.9

The total carbohydrate content reduced from 54.2 to 50.8 and 52.5%, respectively with pumpkin and mung bean starters. Similar trend was observed for energy values too, from 259 to 246 and 254 kcal/100g since carbohydrates are main sources of energy. The results were consistent with the findings of El-Demery (2011) and González-Montemayor et al. (2021), showing lower carbohydrate content by 1.8% with 5% pumpkin and by 5.4% with 20% legumes (green bean, pea and mesquite) flours fortification respectively. This is possibly caused by the partial replacement of wheat flour by non-cereals with lower carbohydrate content. The values may also be attributed to the variation in contents of other components including fat, moisture, protein and ash.

The total dietary fibre content of steamed buns was raised from 2.6 to 3.0 and 3.3% respectively with pumpkin and mung bean sourdough starters, while the ash content also increased from 0.1 to 0.3% for both composite starters. This was in line with the

studies of Bayramov et al. (2022) and Rizzello et al. (2014) that recorded an increase by 0.09% and 0.2% in fibre content of baked breads with 10% pumpkin puree and wheat-legume composite starter respectively. Both pumpkin and mung beans that contain more fibre and ash, contributed to the improvement on nutritional aspect of SSB, including the calorie level (Bayramov et al., 2022), mineral content (Noor Aziah et al., 2012) and starch hydrolysis index (Rizzello et al., 2014).

SSB with composite starters had relatively higher moisture and protein contents. SSBs with pumpkin and mung bean starters had greater moisture content of 39.7 and 37.6% respectively, as compared to wheat at 36.5%. SSB with mung bean starter had higher protein content by 0.4%. Wheat protein, particularly gluten, builds interconnected network to support the volume and enhance the texture in bread through moisture absorption (Boz & Karaoglu, 2013). The plain flour used in the present study formed weaker gluten network since it has poorer moisture absorption capacity with its low contents of protein (Boz & Karaoglu, 2013) at 11% and of fibre (Bae et al., 2014) at 3.5%. Therefore, components such as mung bean proteins and pumpkin fibre, were believed to bind with water and form a non-gluten network, thus supporting dough matrix and improving physical qualities of SSB (Monteiro et al., 2021; Sadeghi et al., 2019).

4.3.7 Summary

The addition of non-cereals improved the qualities of SSB, especially involving wheat-pumpkin and wheat-mung bean composite starters at 5-10% pumpkin fortification. The fortification at levels higher than that brought adverse impacts instead. In terms of specific volume, wheat-pumpkin and wheat-mung bean sourdough starters

enhanced the values from 1.05 to 1.88 and 2.22 cm^3/g, while in terms of spread ratio, from 1.86 to 1.59 and 1.41 respectively. With the aid of each composite starter, values of specific volume were peaked at 10% and 5% pumpkin fortification respectively. For the colour of SSB, L* decreased with both a* and b* values increased with increasing levels of pumpkin fortification, in relation to the natural orange colour. At 5% pumpkin fortification, the most optimum texture could be indicated by the lowest hardness at 3088 and 3266 gf respectively among SSBs containing pumpkin and mung bean starters. The sensory panellists preferred 10% pumpkin fortified steamed buns and gave overall liking scores of 6.8 and 7.0 respectively for the pumpkin and mung bean starters. The sensorial attributes of texture, colour, taste and aroma all scored satisfactory ranging between 6.2 and 7.2 with this pumpkin fortification level. In short, such outcomes are encouraging for usage of composite sourdough starters and pumpkin fortification in SSB with positive feedback received from panellists. Ash and total dietary fibre contents in steamed buns with composite starters at 5% pumpkin fortification were raised while carbohydrate content and energy values dropped.

4.4 Microorganisms in Sourdoughs

Microorganisms in sourdoughs were investigated via bacterial and fungal screening using NGS. The focus was to compare the differences of microorganisms in starters and breads with controls. The activated composite sourdough starters with 15% non-cereal flours, *i.e.* pumpkin (SWP) or mung bean flours (SWM), steamed bun products from them respectively with pumpkin-fortified flour at 5%, labelled BWP and BWM along with the wheat starter (SWW) and the control steamed bun (BWW) containing

SWW were the major samples chosen. Other fermented food samples that were outsourced externally for comparison purposes included wheat sourdough starter (SB1), rye sourdough starter (SR2), baked yeasted bread (BW15), baked sourdough bread (BW25), kefir (KFR) and yogurt (YGT). Among these products of lactic acid fermentation, kefir and yogurt provide different forms of fermentation substrates from milk, rather than from wheat for sourdough. DNA extraction was conducted for all the 12 samples. Every DNA was then sent as template for both 16S and ITS rRNA amplicon sequencing respectively for bacterial and fungal identification.

During bioinformatic analysis, 720000 raw reads in total were generated for 16S and ITS. The reads went through filtering, denoising, merging of forward and reverse reads and chimera removal processes. A total of 118677 and 136324 trimmed reads were then obtained for 16S and ITS respectively. Then, the amplicon sequence variants (ASVs) determined were used to identify and assign the taxonomy identity for microbiota in the tested samples. The outcome shows a total of 11 phyla, 16 classes, 38 orders, 59 families and 74 genera of bacteria within all the samples. The fungal communities comprised 6 phyla, 17 classes, 35 orders, 55 families and 69 genera in total.

4.4.1 Microbiota Diversity in Sourdough Starters and Buns

Figures 4.21 and 4.22 show alpha rarefaction curves for bacterial and fungal amplicons respectively in sourdough samples. The flattened curves at their maximum values for all samples indicate that the sequence depth chosen was sufficient to fully represent the microbial complexity of samples so that none of the microorganisms was missed out (Coda, Kianjam, et al., 2017; Ercolini et al., 2013; Minervini et al., 2016). The

sequencing numbers to reach plateau were less than 2500, generally lower for sourdough starters than buns in bacterial communities while in fungal communities (Figure 4.22), the sequencing numbers were higher at about 10000 for sourdough starters, except for the one with mung bean. This indicated that as compared to buns, bacterial diversities in starters were more simple (Ercolini et al., 2013) but the fungal profiles were more diverse in wheat and wheat-pumpkin sourdough starters.

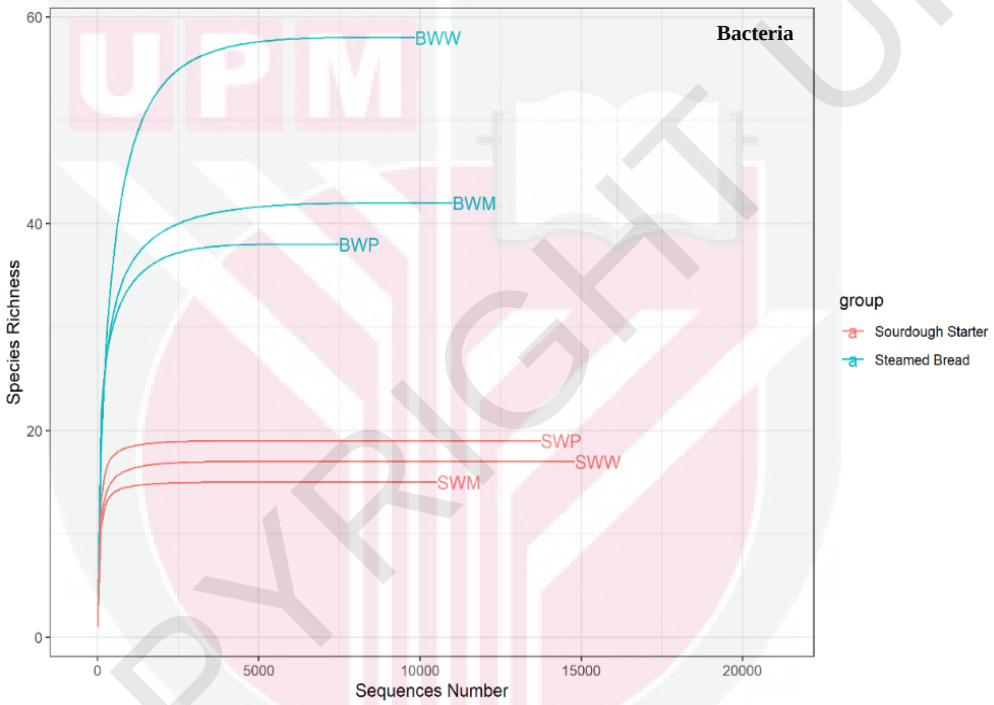

Figure 4.21: Alpha rarefraction curves showing bacterial species richness for main samples of sourdough starters (SWW: wheat; SWP: wheat-pumpkin; SWM: wheat-mung bean) and buns (BWW: wheat starter; BWP: wheat-pumpkin starter; BWM: wheat-mung bean starter)

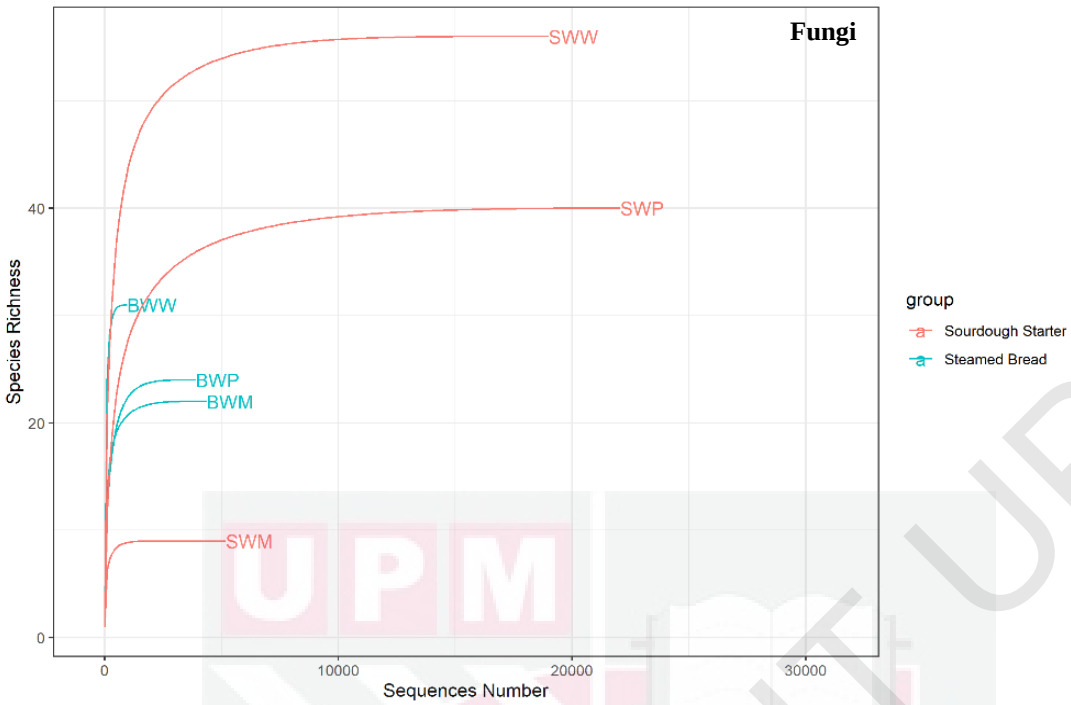

Figure 4.22: Alpha rarefraction curves showing fungal species richness for main samples of sourdough starters (SWW, SWP, SWM) and buns (BWW, BWP, BWM)

The microbiota is analysed by determining the alpha diversities within each sourdough starter or product (Urien et al., 2019). The alpha diversities, presented in the form of Chao1 richness and Shannon diversity indices, within each sourdough microbiota was compared in Table 4.3. Chao1 indices refer to number of species and Shannon indices refer to both number and evenness of species (Safari et al., 2020). The greater the indices, the more diverse the microbiological profiles. In general, the values of Chao1 index reduced for both bacterial and fungal communities when involving non-cereals except for SWP's bacterial communities in agreement with the findings of Minervini et al. (2016). For example, after including pumpkin and mung bean starters, the Chao1 value reduced from 58 to 38 and 42 respectively in the bacterial community of buns. For Shannon indices, the greatest values of 2.6 and 2.8 in the fungal community were achieved by wheat starter and wheat bun respectively, while in the bacterial community, the greatest indices of 2.2 and 2.9 were achieved by involving pumpkin

starter. In both indices, the lowest diversity was obtained by starters with mung bean, possibly due to the absence of mung bean hull, that is the main source of microbial contamination (Coda, Kianjam, et al., 2017). The more diverse fungal communities in wheat and wheat-pumpkin sourdough starters supported the previous results shown by alpha rarefaction curves.

Table 4.3: The Chao1 and Shannon indices for bacterial and fungal communities in sourdough starters (SWW, SWP, SWM) and buns (BWW, BWP, BWM)

Sample			Chao1		Shannon	
Group		**Pumpkin fortification**	**Bacterial**	**Fungal**	**Bacterial**	**Fungal**
Starter						
SWW	Wheat	-	17	56	2.14	2.60
SWP	Wheat-pumpkin	-	19	40	2.24	1.13
SWM	Wheat-mung bean	-	15	9	2.06	0.97
Steamed bun						
BWW	Using SWW starter	0%	58	31	2.40	2.84
BWP	Using SWP starter	5%	38	24	2.93	1.33
BWM	Using SWM starter	5%	42	22	2.86	1.58

Figure 4.23 shows the collective indices for both Chao1 and Shannon for bacterial and fungal communities. In general, bacteria have higher diversity than fungi except for starters in Chao1 index. Unlike for Chao1, the Shannon index of fungi is lower than bacteria, probably due to the uneven, biased fungal community structure (Safari et al., 2020) especially in wheat-pumpkin starter. This also accords with the outcomes of Ercolini et al. (2013) and De Vuyst et al. (2009). Apart from that, the diversity of bun group was generally greater than sourdough starter group. The introduction of innate microbes by adding water and flour to form main doughs led to the enhancement of microbial richness and diversity (Minervini et al., 2016; X. Wang et al., 2020). The low diversity in sourdough starters also potentially indicated the microbiota was stable and mature since it was only inhabited by highly adapted microbes (Coda, Kianjam, et al., 2017; Ercolini et al., 2013; Minervini et al., 2016; X. Wang et al., 2020).

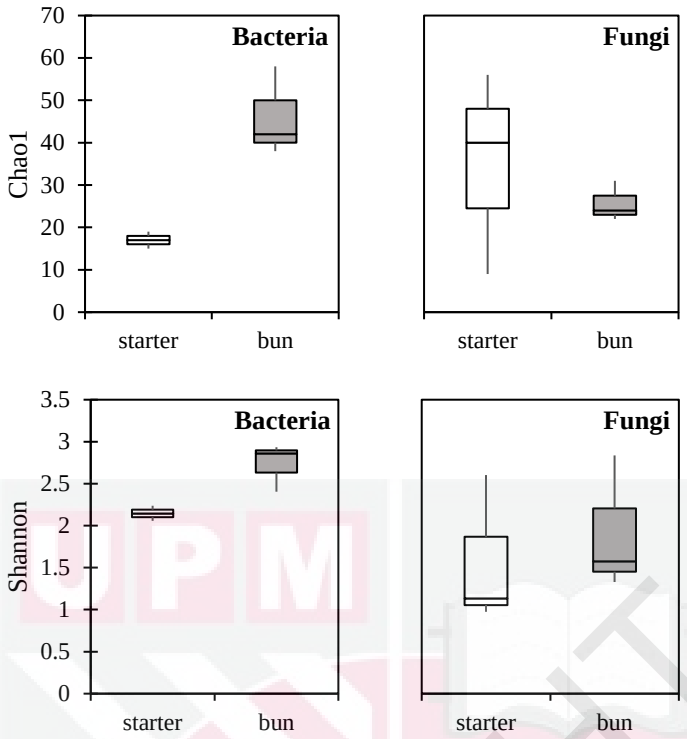

Figure 4.23: The Chao1 and Shannon indices for bacterial (left) and fungal (right) communities in sourdough starters and buns

The beta diversity for comparing bacterial and fungal communities in starters and buns using PCoA plot provides clear differentiation of the microbial diversities among the samples (Urien et al., 2019). Figure 4.24(a) shows that sourdough starters formed a smaller cluster, indicating higher degree of similarity in bacterial diversity as compared to fungal diversity in Figure 4.24(b). The fungal community in starter with mung bean significantly deviated from the other two starters as the alpha diversity was the lowest from Table 4.3 using dehulled mung bean flour. The buns from composite starters, BWP and BWM are distanced more closely compared to BWW for both the bacteria and fungi communities. For fungi, Figure 4.24(b) shows cluster of BWM and BWP suggesting similarities in fungi communities and its retention through the bun making process from dough formation, proofing and steaming into buns.

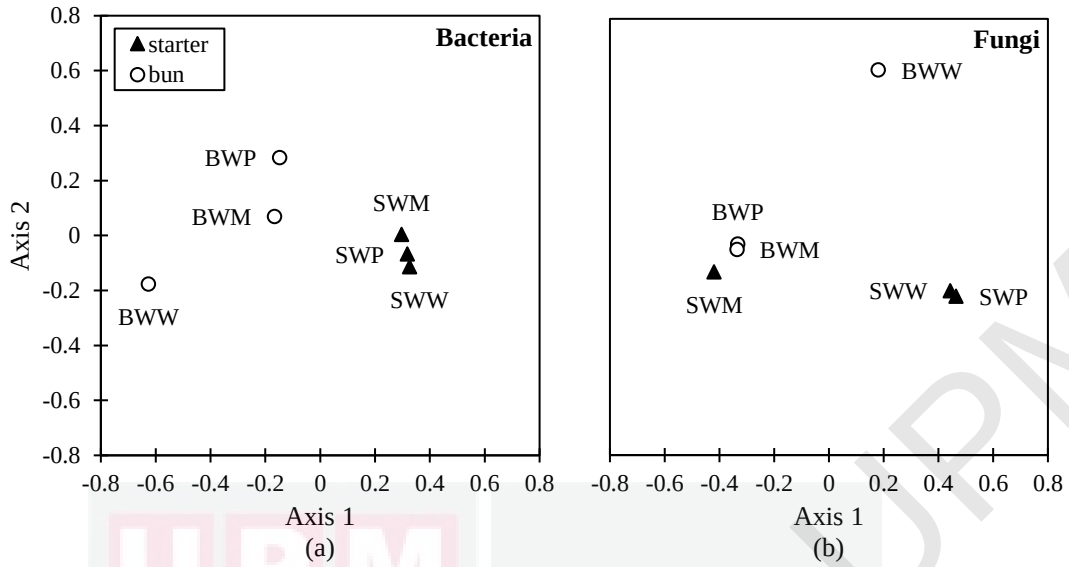

Figure 4.24: Principal Coordinate Analysis (PCoA) plot for (a) 16S (bacteria) and (b) ITS (fungi) amplicon sequences in sourdough starters and buns based on Bray-Curtis dissimilarity

4.4.2 Sourdough Microbiota Comparison with Other Fermented Foods

The alpha rarefaction curves for bacterial and fungal amplicons in all samples are respectively reported in Figures 4.25 and 4.26. The species richness values increased with number of sequences and eventually reached plateau. All species were likely to be included as there was no change in species richness with any further increase in number of sequences. Similar to curves in Figures 4.21 and 4.22 in Section 4.4.1, the sequence depth was proven to satisfactorily cover the microbial complexity of all samples (Coda, Kianjam, et al., 2017; Ercolini et al., 2013; Minervini et al., 2016). In bacterial community, the necessary sequence numbers were about the same among sourdough starters, yet species richness of outsourced wheat (SB1) and rye (SR2) sourdough starters were slightly lower than sourdough starters developed in this study (Figure 4.25). The bacterial diversities in baked breads (BW15 and BW25) were generally lower among the steamed buns. Meanwhile the least bacterial diversity was

detected in yogurt (YGT) with less than 1000 sequence number to flatten the curve, whereas for kefir (KFR), the value was comparable with sourdough starters. Figure 4.26 shows a lower species richness of outsourced samples including SB1, SR2, KFR and YGT, in the fungal community but a higher species richness for outsourced baked bread, BW25 which was actually sourdough based and made through overnight retarded fermentation process.

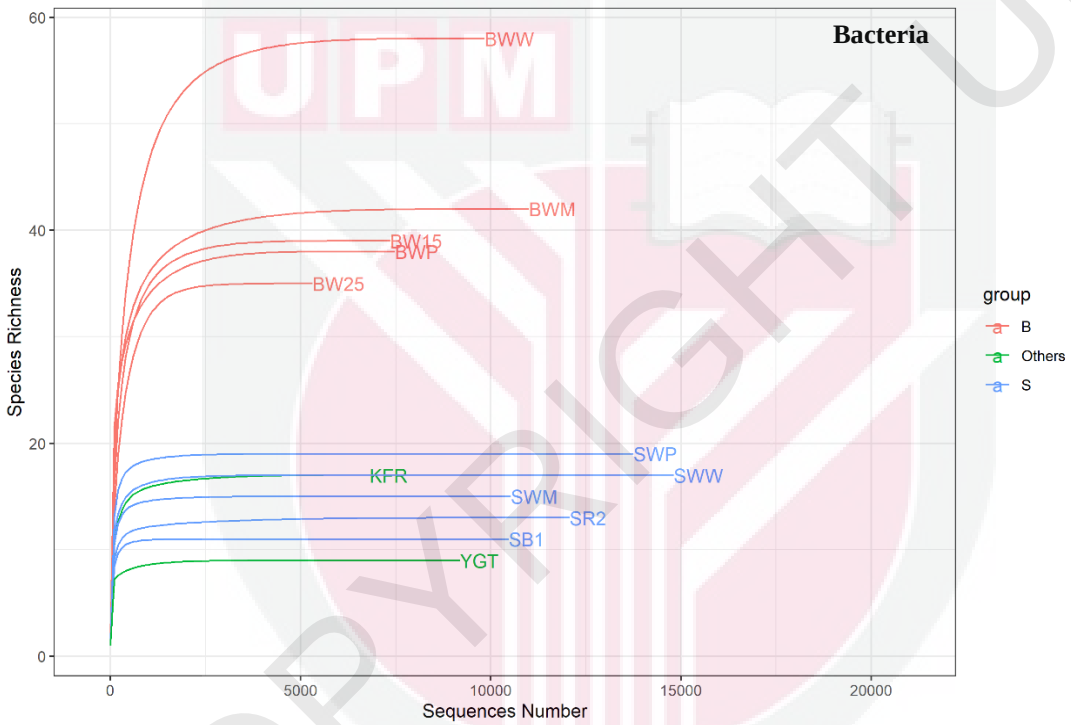

Figure 4.25: Alpha rarefraction curves showing bacterial species richness for sourdough starters (SWW: wheat; SWP: wheat-pumpkin; SWM: wheat-mung bean; SB1: outsourced wheat starter; SR2: outsourced rye starter), breads either steamed (BWW: wheat starter; BWP: wheat-pumpkin starter; BWM: wheat-mung bean starter) or baked (BW15: yeast; BW25: starter) and other fermented foods (KFR: kefir; YGT: yogurt)

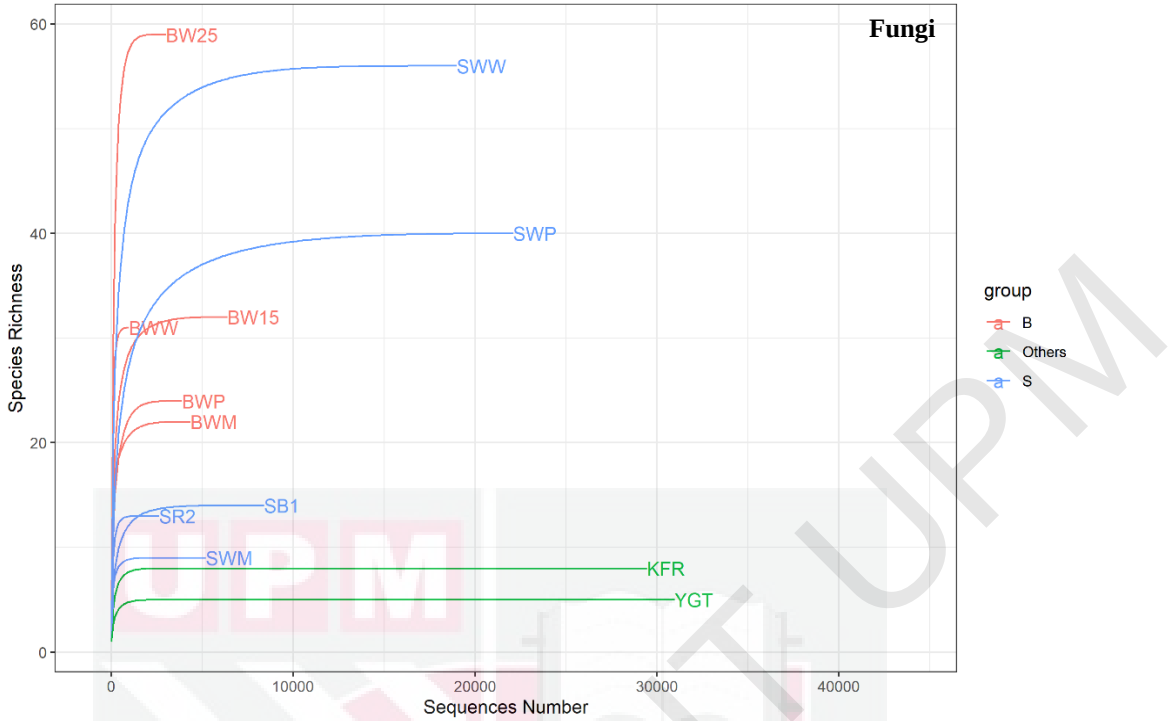

Figure 4.26: Alpha rarefraction curves showing fungal species richness for sourdough starters (SWW, SWP, SWM, SB1, SR2), breads either steamed (BWW, BWP, BWM) or baked (BW15, BW25) and other fermented foods (KFR, YGT)

Table 4.4 lists the alpha diversities in terms of Chao1 richness and Shannon diversity indices for samples categorised into sourdough starter, bread and other fermented foods. For Chao1 indices, SB1 and SR2 from external source showed lower diversities in both bacterial and fungal communities possibly due to different ingredients used in relation to the house microbiota (Minervini et al., 2015). Shannon index for SR2 was high at 1.4 despite its low Chao1 index implying a more balanced fungal distribution (Safari et al., 2020). For breads, bacterial diversities of outsourced breads were also low, as BW15 was made using baker's yeast while BW25 made using SB1 which had a lower bacterial diversity from its start. The fungal diversity was the highest in BW25, possibly due to the long and retarded refrigerated overnight dough fermentation process (Table B.1) that allowed further growth of fungal diversity. The least Chao1 indices was found in yogurt (YGT) at 9 for bacterial community and 5 for fungal

community. The microbial diversity may be limited by the initial inoculation of selected microbial strains instead of spontaneous fermentation initiated from innate, diverse microbiota.

Table 4.4: The Chao1 and Shannon indices for bacterial and fungal communities in sourdough starters (SWW, SWP, SWM, SB1, SR2), breads (BWW, BWP, BWM, BW15, BW25) and other fermented foods (KFR, YGT)

Sample			Chao1		Shannon	
Group		**Pumpkin fortification**	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**
Starter						
SWW	Wheat	-	17	56	2.14	2.60
SWP	Wheat-pumpkin	-	19	40	2.24	1.13
SWM	Wheat-mung bean	-	15	9	2.06	0.97
SB1	Wheat	-	11	14	1.76	0.59
SR2	Rye	-	13	13	1.83	1.42
Bread						
BWW	Steamed, SWW starter	0%	58	31	2.40	2.84
BWP	Steamed, SWP starter	5%	38	24	2.93	1.33
BWM	Steamed, SWM starter	5%	42	22	2.86	1.58
BW15	Baked, baker's yeast	0%	39	32	2.26	1.86
BW25	Baked, SB1 starter	0%	35	59	2.14	3.13
Others						
KFR	Kefir	-	17	8	1.93	0.360
YGT	Yogurt	-	9	5	1.83	0.160

Figure 4.27 shows the collective Chao1 and Shannon indices for the bacterial and fungal communities to compare sourdough samples in current study with outsourced samples. The trend of less diversity in fungal community compared to the bacterial is still visible as observed in earlier Section 4.4.1. The diversity of bread group was also generally greater than sourdough starter group. Referring to Chao1 indices in Figure 4.23, the size of the box plot for bread group in Figure 4.27 decreased when BW15 and BW25 were included indicating higher resemblance of bacterial species richness with the buns prepared. For Shannon indices, similarity of fungal species richness and evenness of outsourced starters improved from the decrease of box plot size in Figure 4.27 compared to Figure 4.23. The microbial diversities for yogurt and kefir were

simpler than sourdough products with high similarities likely due to the association with their common fermentation substrate, *i.e.*, milk. The similarity between them may be supported by the claim that kefir is also the 'yogurt of the 21st century' (Simova et al., 2002).

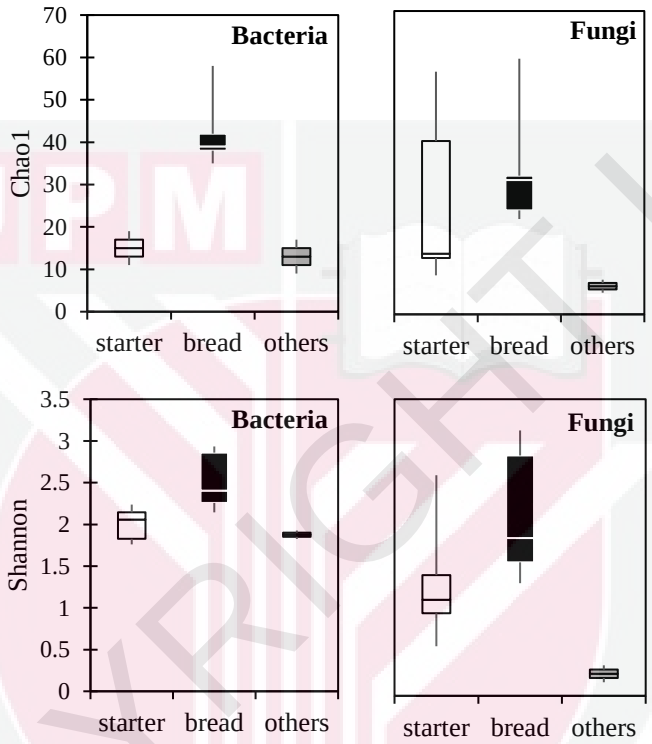

Figure 4.27: The Chao1 and Shannon indices for bacterial (left) and fungal (right) communities in sourdough starters, breads and other fermented foods

Figure 4.28(a) shows that an obvious variation in bacterial communities was observed between the outsourced starters (SB1 and SR2) with the ones developed in this study (SWW, SWP, SWM). SR2 possessed a separated fungal profile by its own in Figure 4.28(b) due to its different origin from rye. For breads, the outsourced baked breads (BW15 and BW25) have clustered with BWW in bacterial communities due to the source similar ingredient, solely wheat-based starter instead of composite starters. In terms of fungal communities, Figure 4.28(b) shows that BW15 and BW25 were clearly differentiated due to the use of different leavening agents, where BW15 was baked

using yeast while BW25 was using starter SB1. The composite sourdough breads, BWM and BWP has high similarities for both bacterial and fungi communities. Specifically for fungi, the usage of composite sourdough starters succeeded in creating fungal communities that were comparable to the one using baker's yeast in leavening breads (BW15). The close distance between BWW (steamed) and BW25 (baked) also implied that the different breadmaking process of baking and steaming did not affect bacterial and fungal communities in sourdough breads.

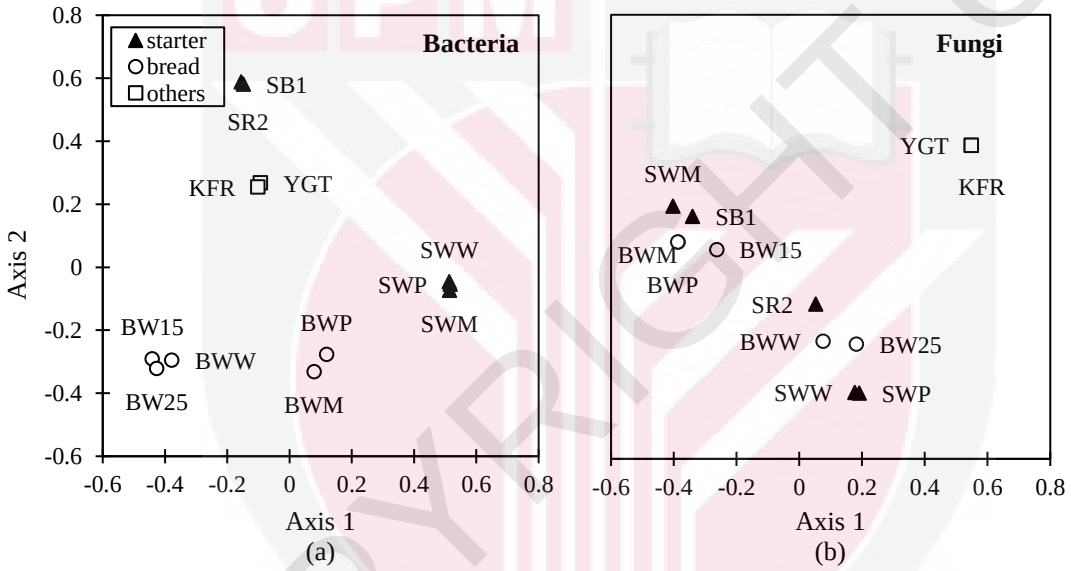

Figure 4.28: Principal Coordinate Analysis (PCoA) plot for (a) 16S (bacteria) and (b) ITS (fungi) amplicon sequences in sourdough starters, breads and other fermented foods based on Bray-Curtis dissimilarity

4.4.3 Sourdough Starters Microbiota at Class and Genus Levels

Zooming into specific microbiota analysis, Figures 4.29 and 4.30 are presented to show the identified top 10 classes and genera respectively. Figure 4.29 concludes that the bacterial profiles were about the same for all five starters, *i.e.*, the abundance of Bacilli significantly overtaking the Alphaproteobacteria and Oxyphotobacteria while

containing traces of Gammaproteobacteria. The majority, 92.9% of the bacteria in wheat starter was under class of Bacilli, while Alphaproteobacteria and Oxyphotobacteria class constituted 5.8% and 1.3% respectively (Table A.8). Figure 4.30 shows that the main genera found in all starters are the *Lactobacillus* of 92.8-97.4% and those unidentified was up to 5.2%. The microbial diversities of all starters were low at genus level as there were only 1-3 genera detected in each starter excluding the unidentified. This suggests that all starters samples were matured and suitable to be used in sourdough products (Calvert et al., 2021).

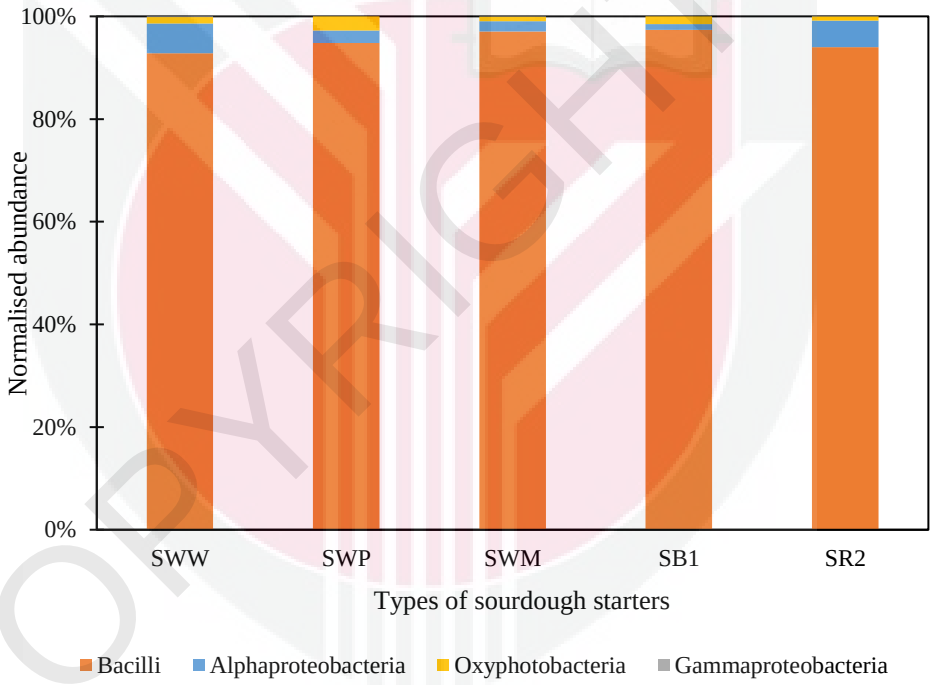

Figure 4.29: Major bacterial diversity and abundance at class level for sourdough starters (SWW, SWP, SWM, SB1, SR2)

For detailed analysis at class level in Figure 4.29, the normalised abundance of the predominant Bacilli class was the highest in SWM and the outsourced SB1 at 97.1% and 97.4% respectively, followed by SWP (94.8%), outsourced SR2 (94.0%) and lastly SWW (92.9%). Being one of the most essential bacteria for sourdough

fermentation, LAB which are under the Bacilli class give rise to markedly high abundances of Bacilli (De Angelis & Gobbetti, 2011). At genus level (Figure 4.30), the bacteria in sourdough starters were mainly *Lactobacillus* under class of Bacilli. It was recorded that *Lactobacillus* contributed to 92.8%, 94.6% and 96.7% of the bacterial communities in wheat (SWW), wheat-pumpkin (SWP) and wheat-mung bean (SWM) sourdough starters respectively. The genera under Bacilli class isolated in sourdough starters were *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Bacillus* and *Lactococcus*. Similar to starters developed in the present study, the outsourced SB1 and SR2 were also both mainly inhabited by *Lactobacillus*, as the only genus under Bacilli class, at 97.4% and 94.0% each (Figure 4.30). The significance in majority of LAB suggested the maturity in leavening, providing acidic flavour and signature aroma, and inhibiting growth of undesired microbes.

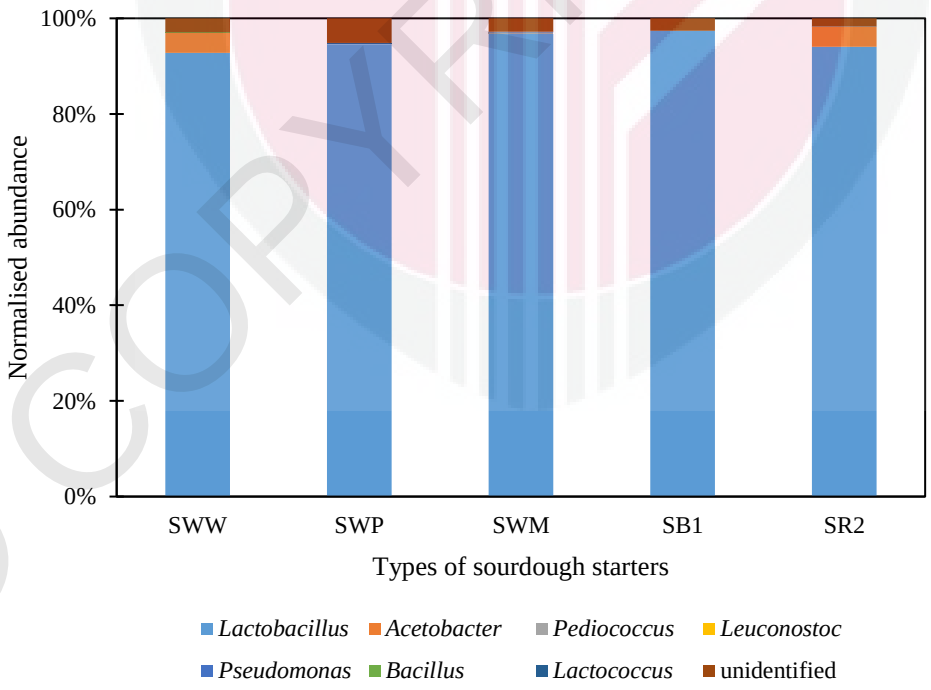

Figure 4.30: Major bacterial diversity and abundance at genus level for sourdough starters (SWW, SWP, SWM, SB1, SR2)

Zooming into the analysis at species level from data presented in Table A.16 (Appendix A), the major *Lactobacillus* species found in the main starter samples was *Lb. fermentum*. The starter with mung bean contained the highest proportion of *Lb. fermentum* at 94.5%, followed by wheat at 91.0% and SWP at 90.3%. This bacterium adapts well under acidic condition with its stable presence, even during extended fermentation of mature sourdough starter (Van der Meulen et al., 2007). Besides sourdough starter, *Lb. fermentum* has been detected in other fermented cereal products, such as *ting*, *boza* and *maegu* (Nyanzi & Jooste, 2012). *Lb. fermentum* produces carbon dioxide during sugar metabolism, hence contributes well to leavening of sourdough starters.

The coexistence of *Lb. fermentum* and *Lb. brevis* was also observed in the present study. *Lb. brevis* comprised of 0.6%, 2.8% and 1.1% in wheat, wheat-pumpkin and wheat-mung bean sourdough starters accordingly (Table A.16). It is an obligately heterofermentative LAB found in wheat sourdough (Menezes et al., 2020; Oshiro et al., 2020; Taccari et al., 2016; Van Kerrebroeck et al., 2016), einkorn sourdough (Çakır et al., 2020), barley sourdough (Harth et al., 2016), milk and cheese (De Angelis & Gobbetti, 2011; Teixeira, 2014). Among the samples, the population of *Lb. brevis* was the biggest when pumpkin, a type of vegetable, was involved. This may be in association with its common presence in fermented vegetables (Teixeira, 2014). *Lb. brevis* has been competent in tolerating harsh conditions with low pH (Oshiro et al., 2020) and presence of salt, *i.e.*, 6% sodium chloride and 100 ppm potassium sorbate (Çakır et al., 2020). The bacteria also release organic acids by themselves, further reducing the pH conditions that are not conducive for most pathogenic fungi and bacteria. Therefore, some manufacturers have isolated *Lb. brevis* for freeze-drying so that it could aid in shelf-life extension and convenient breadmaking (Teixeira, 2014).

Referring to Table A.16, *Lb. pontis* was solely detected in outsourced starters, at 96.5% for wheat and 92.9% for rye. This bacterium is acid-tolerant, as the minimum pH for its survival is low, *i.e.*, around 3.5 (Corsetti, 2013). It inhabits frequently in type II sourdough starters, indicating its great competitiveness despite experiencing infrequent backslopping, high temperatures and high acidity (Gänzle, 2014). The sole presence of *Lb. pontis* in outsourced starters may be associated with the different backslopping cycles and fermentation temperatures from the starters developed in the present study. The bacterium was classified as obligately heterofermentative like the mentioned *Lb. fermentum* and *Lb. brevis*, thus aiding pH drop and leavening in sourdough starters and products (Hansen, 2006). Thiele et al. (2002) also claimed that ornithine, an amino acid that may improve roasty flavour in bread crust, was exclusively found in dough containing *Lb. pontis*.

There was presence of *Acetobacter* at 4.2% in wheat (SWW) and rye (SR2) starters (Table A.8). *Acetobacter* is collectively known as acetic acid bacteria (AAB) with other genera in the family of *Acetobacteraceae*, *e.g. Gluconobacter* (De Vuyst et al., 2017). The aerobic AAB grow optimally at temperature ranged between 25 and 30 ºC and release acetic acid and gluconic acid as metabolic by-products (Hommel, 2014). The spontaneous fermentation of sourdough involving *Acetobacter* was infrequent and insignificant (Lhomme et al., 2015; Minervini et al., 2012; Scheirlinck et al., 2008) as compared to LAB. *Acetobacter* is more often detected in wine, vinegar, and fermented cocoa instead (Scheirlinck et al., 2008), since this bacterium adapts better in liquid medium theoretically (Di Cagno et al., 2014). From the studies of Lynch et al. (2019) and Siepmann et al. (2017), *Acetobacter* contributes to faster acidification of starter, altered volatile compounds for signature flavour and production of levan (homoexopolysaccharide) that potentially helps in leavening and textural properties.

Hence, the lowest pH of wheat starter was probably influenced by the presence of *Acetobacter* that released more acetic acid.

For fungal class analysis, Figure 4.31 shows that Saccharomycetes and Dothideomycetes were the fungal classes that inhabited all starters including SB1 and SR2, where the former class was the major in most starters except SWW. The fungal profile of wheat starter with more even distribution was significantly different from composite starters as Table A.9 (Appendix A) demonstrated the major fungal class as Leotiomycetes (58.5%), followed by Dothideomycetes and Sordariomycetes classes constituting 27.3% and 6.6% respectively. Leotiomycetes was determined in wheat tissues, therefore potentially entered the wheat starter through wheat flour (Xu et al., 2021). The fungi from class of Sordariomycetes was scarcely detected at 0.3% in both the outsourced wheat and rye starters. From Figure 4.32, the major genus of SB1 was *Saccharomyces*, similar to SWP and SWM, but SR2 was mainly harboured by *Kazachstania*. *Blumeria*, *Alternaria*, *Didymella*, *Mycosphaerella* and *Gibberella* were the minor classes that constituted the fungal profiles of outsourced samples at less than 5% respectively.

Unlike in bacterial community, the wheat starter did not share the common major fungal class of Saccharomycetes with composite starters (Figure 4.31). Saccharomycetes possessed the most substantial proportion of 81.7% and 72.9% respectively in wheat-pumpkin and wheat-mung bean sourdough starters. The rye starter also comparably consisted of 80.6% Saccharomycetes class (Table A.9 in Appendix A). The normalised abundance of Saccharomycetes in wheat starter was less than 0.1%. The prevalence of Saccharomycetes class was the most significant in SB1 at 95.7% and the *Saccharomyces* was harbouring at 72.9-95.9% in SB1 and composite

starters as the major genus under this class. *Kazachstania* (67.7%), that belongs to the same class, was more dominant in SR2 than *Saccharomyces* (13.5%). These are natural yeasts which function as leavening and flavouring agents of sourdough products (T. Liu et al., 2018). This class is also where most sourdough yeasts belong to, for example, *S. cereviasiae*, *K. exigua*, *K. humilis* and *Candida holmii* (De Vuyst et al., 2014; Hammes & Gänzle, 1998).

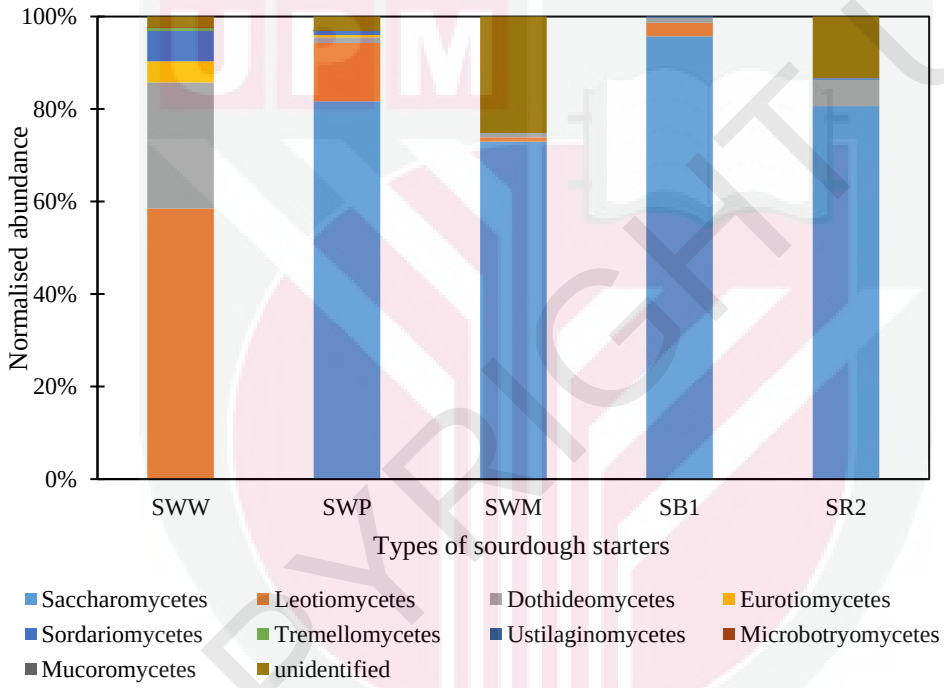

Figure 4.31: Major fungal diversity and abundance at class level for sourdough starters (SWW, SWP, SWM, SB1, SR2)

The fungi under *Saccharomyces* genus were identified only up to genus. In general, *S. cerevisiae* is most frequently detected among the sourdough yeasts (Hansen, 2006; Lhomme et al., 2015; Urien et al., 2019). It is also the typical baker's yeast and brewer's yeast that plays vital roles in the fermentation industry. This is possibly in relation to its stronger gas-releasing ability among sourdough yeasts (Gobbetti, 1998). Hence, without the aid of *Saccharomyces* spp. in releasing carbon dioxide, wheat

starter demonstrated weaker leavening performances. This may be related to the greater amount of free water in wheat starter, which was generally less favourable for yeast growth (Calvert et al., 2021). The absence of *Saccharomyces* spp. in wheat starter could be the result of the comparatively lower pH that was suppressed due to presence of AAB (Calvert et al., 2021). Besides *S. cerevisiae*, *S. cariocanus* (Comasio et al., 2021), *S. bayanus* (Ercolini et al., 2013) and *S. exiguus* (Gobbetti, 1998) are other isolated sourdough yeasts under genus of *Saccharomyces*.

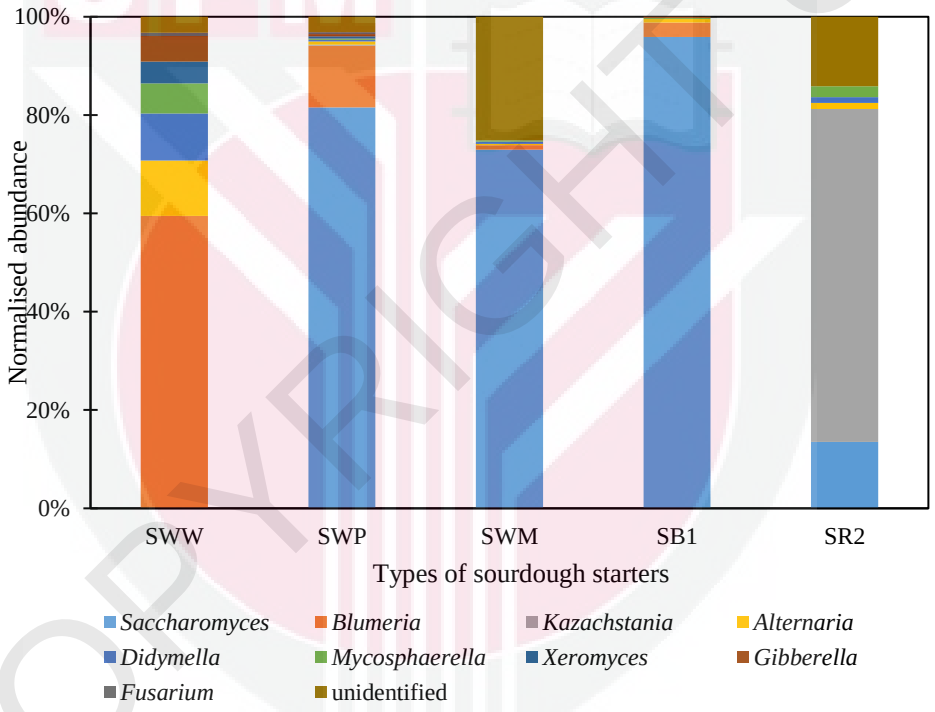

Figure 4.32: Major fungal diversity and abundance at genus level for sourdough starters (SWW, SWP, SWM, SB1, SR2)

The fungal community in wheat starter displayed pronouncedly different profiles from the other starters. The fungal diversity in SWW as shown in Figure 4.32, were greater and more even, confirming the high Chao1 index in Section 4.4.1. Referring to Table A.9, the *Blumeria* genus under Leotiomycetes class outcompeted the others including *Saccharomyces* at 59.5% in SWW. The fungus, *Blumeria graminis* f. sp. *tritici*, may

lead to powdery mildew, which is a biotrophic wheat disease that absorbs plant nutrients without causing cell death (Dutilloy et al., 2022). *Alternaria*, another common fungus in bakery products (Calasso et al., 2023), was also detected in wheat starter (11.3%). Nevertheless, these fungal genera are not relevant in sourdough fermentation. They were potentially inherited from the wheat flour during dough formation.

From Table A.16, the predominant fungal species in rye starter was *Kazachstania humilis* (*Candida humilis*) at relative abundance of 67.2%. It has been widely encountered as important yeast in sourdough starters (De Vuyst et al., 2014; Di Cagno et al., 2014; Ercolini et al., 2013; T. Liu et al., 2018; X. Wang et al., 2020), often in Italian and French bakery sourdough (De Vuyst et al., 2016). The exceptional coexistence of *K. humilis* and *Lb. sanfranciscensis* is often found in San Francisco sourdough, possibly in relation to their trophic associations, *i.e.*, nutritional mutualism whereby the LAB metabolises maltose but the yeast does not (De Vuyst et al., 2014; X. Wang et al., 2020). In accordance with the results of outsourced rye starter, X. Wang et al. (2020) deduced that the abundance of *K. humilis* surpassed *S. cerevisiae* in sourdough, even in the form of main dough. *K.* humilis, which is highly acid-tolerant (De Vuyst et al., 2014), adapted better in SR2 despite the greater amount of acetic acid released by AAB.

4.4.4 Sourdough Buns Microbiota at Class and Genus Levels

The comparison of microbial diversities and composition in sourdough steam breads, BWW, BWP and BWM along with two bread samples obtained externally, *i.e.* baked bread using baker's yeast (BW15) and baked bread using SB1 (BW25) are shown in

Figures 4.33 and 4.34 showing major bacterial communities at class and genus level respectively. The wheat bun was dominated by Gammaproteobacteria at 74.2%, which is also major class in outsourced breads, whereas the steamed buns containing composite starters were mainly Bacilli class as summarised in Table A.10 (Appendix A). Within the class of Gammaproteobacteria (from Figure 4.33), *Pseudomonas* (from Figure 4.34) one of the common innate bacteria in wheat flour (De Angelis et al., 2018; Ercolini et al., 2013) was the genus that showed its presence in all bread samples.

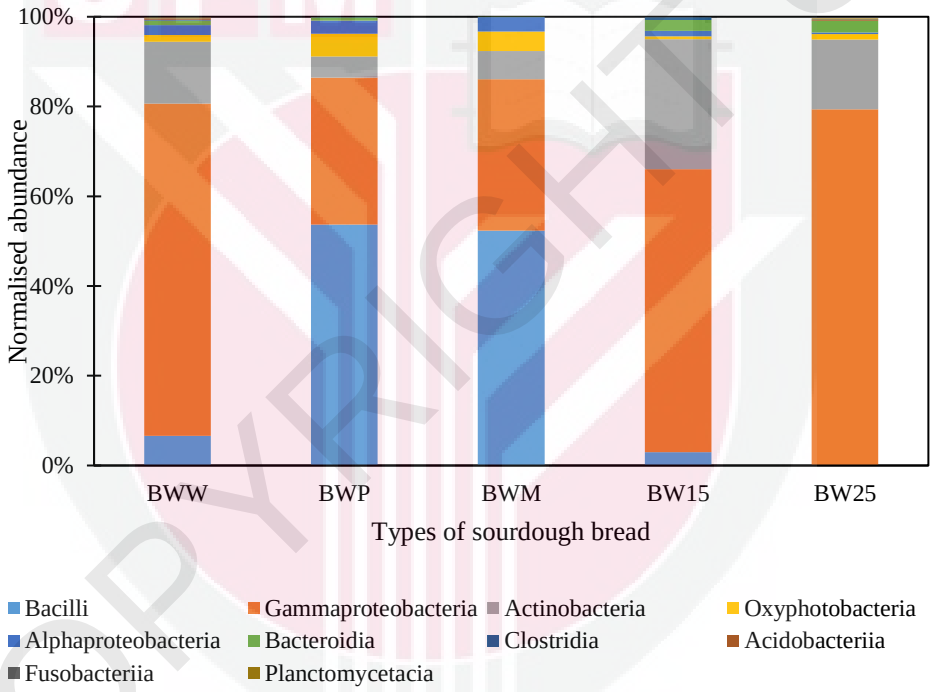

Figure 4.33: Major bacterial diversity and abundance at class level for steamed breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

From Table A.10 (Appendix A), Bacilli class dominated BWP and BWM, with normalised abundances of 53.7% and 52.3%. Bacilli was not the main class in BWW, BW15 and BW25 standing only at 6.7%, 3.0% and 0.2%, respectively. The Gammaproteobacteria was the dominant class at 74.2% for BWW, at 63.1% for BW15 and at 79.5% for BW25. These bacteria, especially those from *Pseudomonas* genus,

are naturally found in wheat flour (De Angelis et al., 2018; Ercolini et al., 2013), thus potentially entered the microbiota of bread samples during formation of dough. Despite the different processing environment, methods and temperature, the bacterial diversity of wheat bun was found to be similar to the outsourced bread samples, possibly because of their common usage of wheat flour as main ingredients.

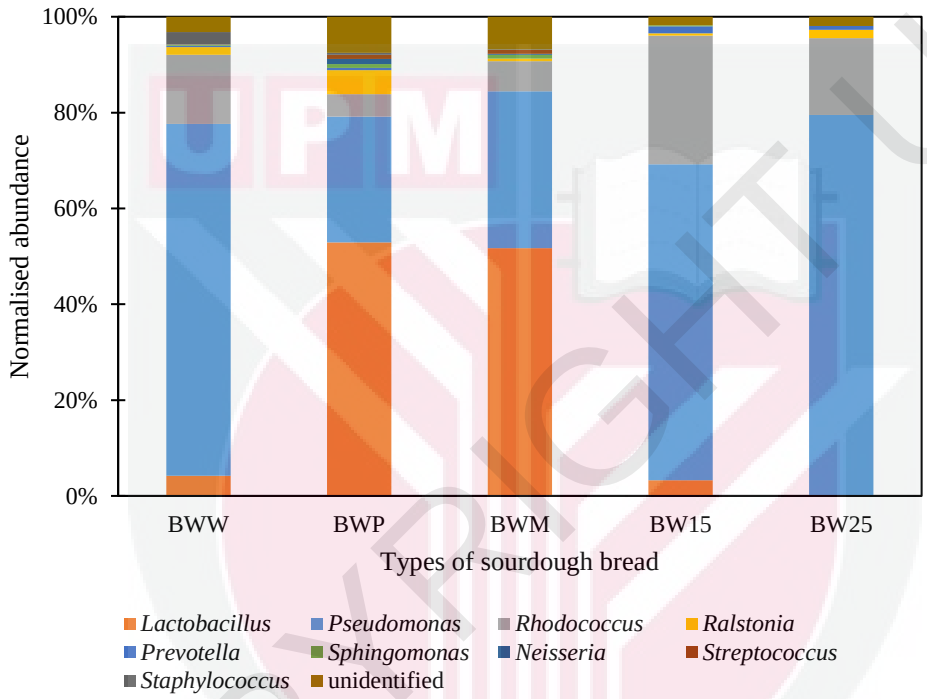

Figure 4.34: Major bacterial diversity and abundance at genus level for steamed breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

Lb. fermentum by itself took up 3.1%, 50.7% and 48.3% of respective bacterial communities in BWW, BWP and BWM, as shown in Table A.16. The higher abundances of this obligately heterofermentative LAB in BWP and BWM reflected their more excellent leavening performances that aids in improving properties of steamed buns, especially the volume and texture explained in Sections 4.3.1 and 4.3.4. Harth et al. (2016) realised the predominance of *Lb. plantarum*, *Lb. fermentum* and *Lb. brevis* in microbial community of uncommon barley sourdough that contributed to

flavourful barley bread. The genus under Bacilli class in outsourced bread samples was solely *Lactobacillus*, however the diversity was higher with other genera, including *Pediococcus*, *Streptococcus*, *Staphylococcus* and *Gemella*, in the bun samples at low abundances.

The differences among fungal communities in bread samples were greater than the bacterial communities, as shown in Figures 4.35 and 4.36. From Figure 4.35, Saccharomycetes, the important class of common yeasts in bread samples was occupying 20.7 to 72.5% in the fungal microbiota. Leotiomycetes and Dothideomycetes were the classes of highest normalised abundances in wheat bun (36.0%) and yeasted, baked bread (54.5%) respectively. At genus level, BWP and BWM were dominated by the *Saccharomyces* genus, while the greatest normalised abundances were respectively *Blumeria* at 52.8% in BWW, *Aureobasidium* at 56.4% in BW15 and *Debaryomyces* at 26.1% in BW25 (Figure 4.36). Among them, only *Aureobasidium pullulans* was detected in the study of Urien et al. (2019). The non-yeast fungi including *Blumeria* and *Aureobasidium*, might be occasionally introduced by minor contamination when handling the samples or by the natural microbiota of ingredients. They are not involved for the flavour and texture formation in sourdough.

The class of Saccharomycetes dominated the fungal communities of respective steamed bun with wheat-pumpkin starter at 72.5% and wheat-mung bean starter at 62.5% as summarised in Table A.11 (Appendix A). This class was also significant at 20.7% in steamed wheat bun, 35.0% in baked, yeasted bread and 29.8% in baked sourdough bread. The baker's yeast used for leavening BW15 was *S. cerevisiae* that falls under this class. As for BW25, the normalised abundance of *Debaryomyces* genus (26.1%) from the same class surpassed *Saccharomyces* (0.5%) from Table A.11. Table

A.16 shows the unique and atypical presence of *D. hansenii* in sourdough (BW25) which may be encouraged by the addition of salt in breadmaking as the yeast was usually isolated from ocean and salted food products such as cheese and fermented meat (Wrent et al., 2014). The yeasts from this class contributed to leavening of outsourced bread samples, possibly aided by other subdominant yeasts, *e.g.*, *Candida parapsilosis*, *Candida glaebosa*, *Cyberlindnera jadinii*, *Kluyveromyces* spp. and *Meyerozyma* spp.

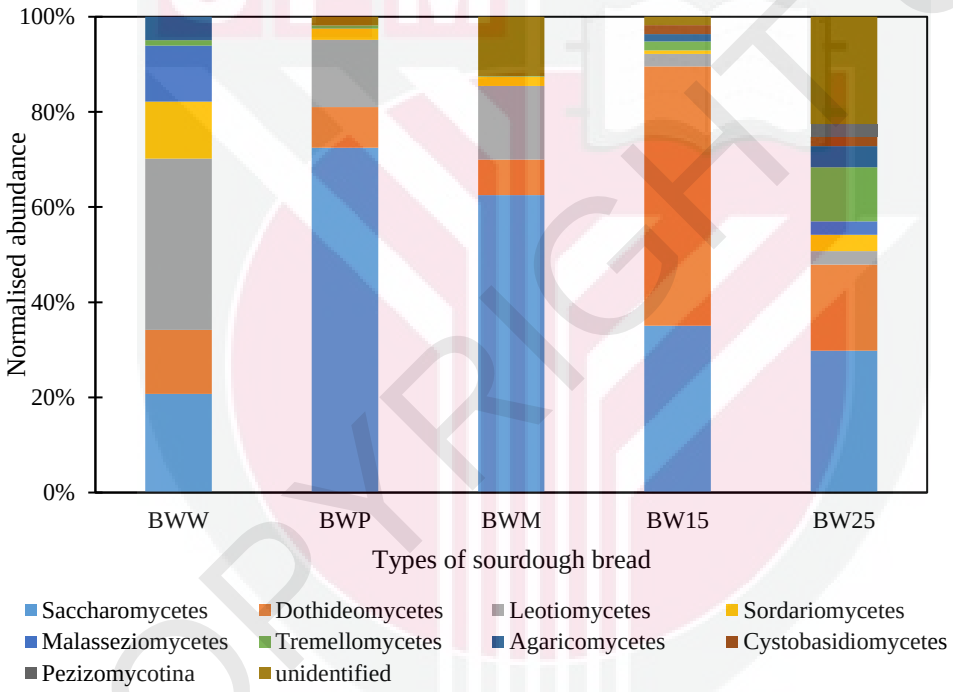

Figure 4.35: Major fungal diversity and abundance at class level for steamed breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

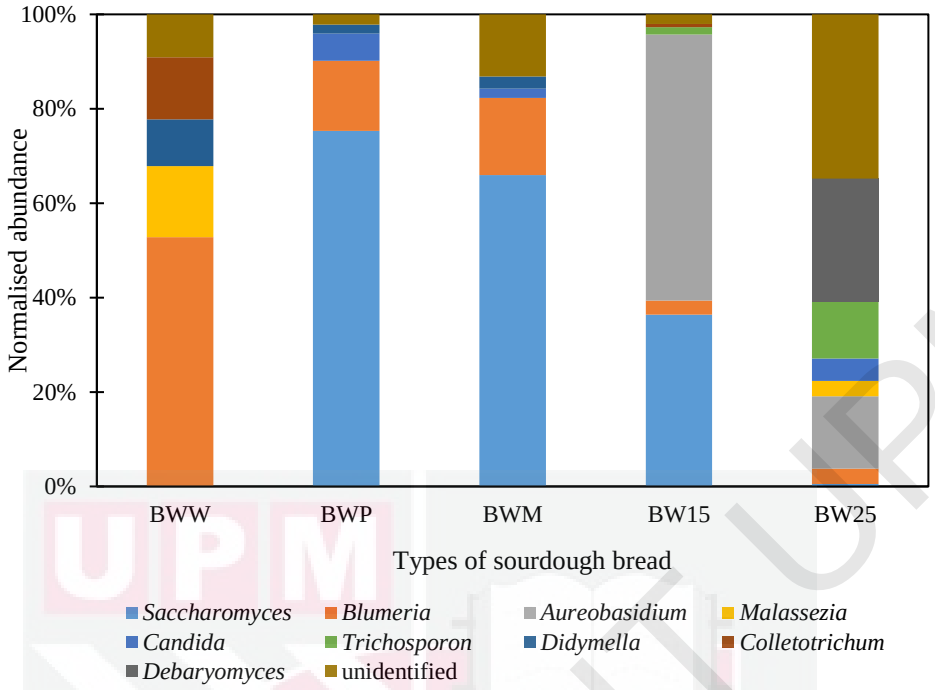

Figure 4.36: Major fungal diversity and abundance at genus level for steamed breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

4.4.5 Progression of Microbiota from Sourdough Starter to Bun

From Figure 4.37 and Table A.12 (Appendix A), the major bacteria in starters, under class of Bacilli was also found in the buns with more significant amount passed on to the buns for the composite starters than the wheat. Similarly, in Figure 4.38, *Lactobacillus* as the main genus was also passed on to the buns more significantly for the composite sourdoughs. The buns were also found to have increased in *Pseudomonas* of the Gammaproteobacteria and *Rhodococcus* of the Actinobacteria. The general increase of bacterial diversity and richness in buns were possibly due to the dough formation process during bun making where additional flour was added.

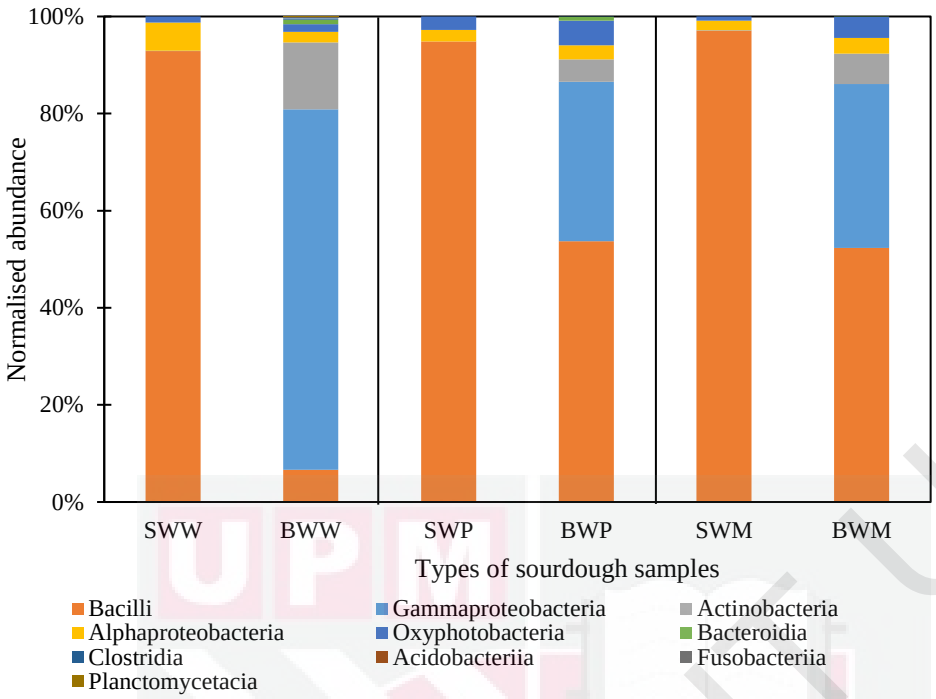

Figure 4.37: Changes in major bacterial diversity and abundance at class level from sourdough starter samples (SWW, SWP, SWM) to steamed buns samples (BWW, BWP, BWM)

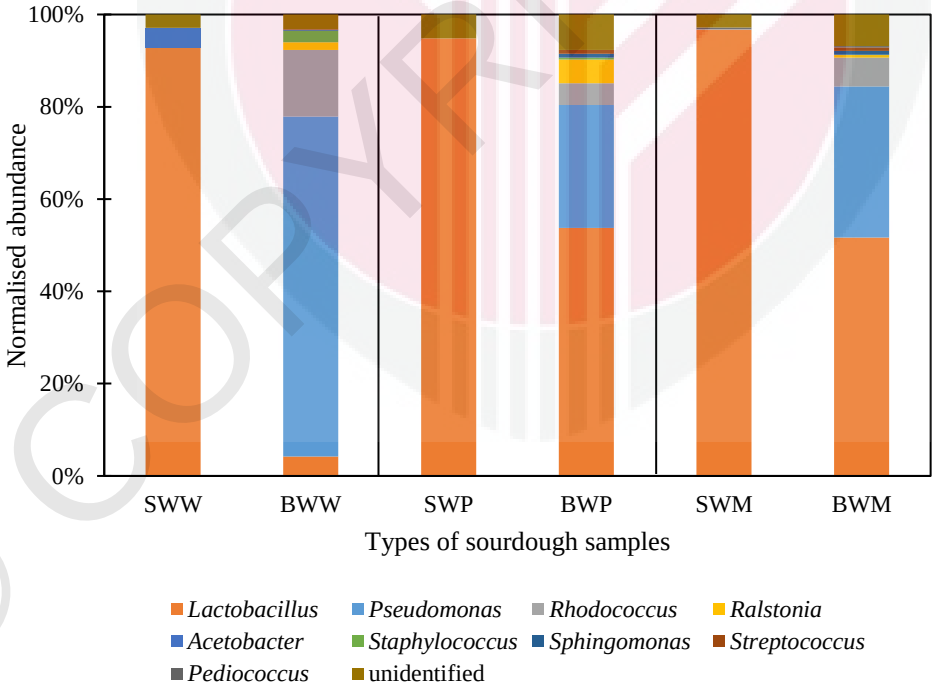

Figure 4.38: Changes in major bacterial diversity and abundance at genus level from sourdough starter samples (SWW, SWP, SWM) to steamed buns samples (BWW, BWP, BWM)

For sourdoughs from wheat, although the Bacilli and *Lactobacillus* were the main class and genus of bacteria in wheat starter, the wheat buns however contained Gammaproteobacteria and *Pseudomonas* as the most prevalent class and genus, respectively (Table A.12). *Pseudomonas* was possibly introduced in steamed buns as innate bacterium in wheat flour (De Angelis et al., 2018; Ercolini et al., 2013) and is usually inhibited with other foreign bacteria (X. Wang et al., 2020) after fermentation. This is potentially in association with the slow, incomplete dough proofing process by less active LAB in wheat starter, as shown by its weaker leavening performances too in Section 4.2. Another possible factor could be the high fermentation temperature at around 30 °C, which is still favourable for growth of *Pseudomonas* (Menezes et al., 2020). The fermentation condition in wheat bun also caused the disappearance of *Acetobacter* that favoured the liquid medium at starter stage (Di Cagno et al., 2014).

Traces of other beneficial LAB from *Streptococcus* and *Pediococcus* genera which share the same class were detected in buns (Table A.12) although their abundances were lower than the *Lactobacillus*. Table A.16 shows that *Lb. fermentum* existed predominantly in the starter samples at 90.3-94.5% but the abundance decreased to 3.1-50.7% in steamed buns, while for *Lb. brevis* had a better retention in the composite sourdough buns at 1.1% and 0.7% for the BWP and BWM, respectively.

For the fungal community, Saccharomycetes dominated the composite sourdough starters (Figure 4.39 and Table A.13) and its predominance was maintained in the buns, BWP and BWM. At genus level, Figure 4.40 shows that *Saccharomyces* from the class of Saccharomycetes was also dominating in the composite sourdough communities passing on 82.8% to 75.0% for the pumpkin and 72.9% to 63.8% for the mung bean. In wheat starter, the major fungal class was Leotiomycetes (58.5%), followed by

Dothideomycetes and Sordariomycetes classes constituting 27.3% and 6.6% respectively. A more diverse fungal community was observed in wheat bun (Figure 4.40 and Table A.13) as shown by the greater number of colours in the column. Apart from the major *Blumeria* genus in both wheat starter and bun, Table A.13 lists the other subdominant fungi that also inhabited the wheat bun, including *Didymella* (7.6%), *Malassezia* (11.6%), *Colletotrichum* (10.2%), *Dekkera* (8.0%), *Brettanomyces* (7.4%) and *Alternaria* (3.5%). In general, although the abundances in both bacterial and yeast communities were diluted from starter to buns, they were diversified during the dough formation.

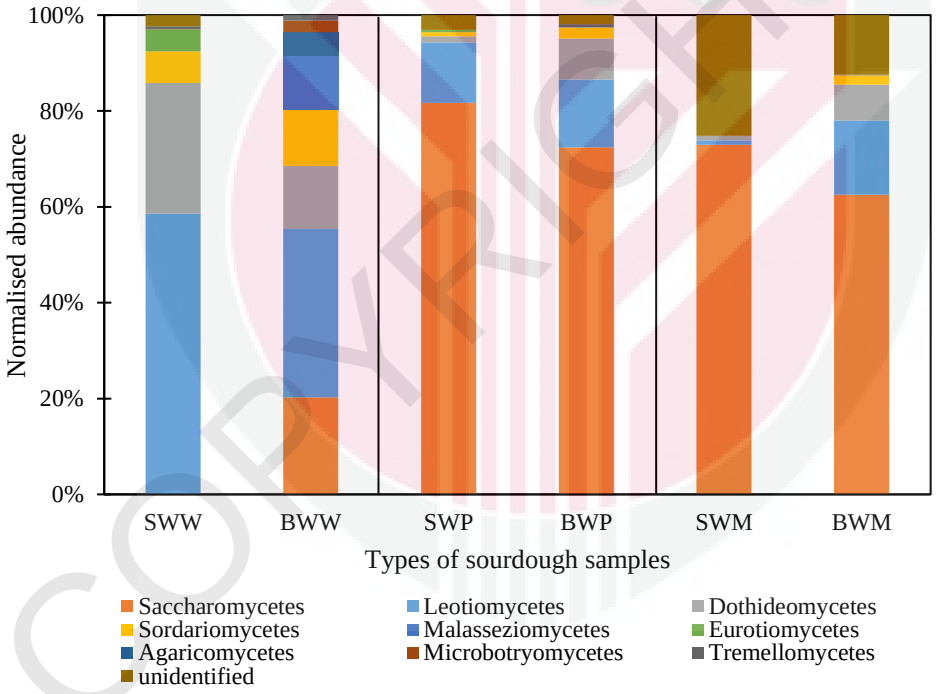

Figure 4.39: Changes in major fungal diversity and abundance at class level from sourdough starter samples (SWW, SWP, SWM) to steamed buns samples (BWW, BWP, BWM)

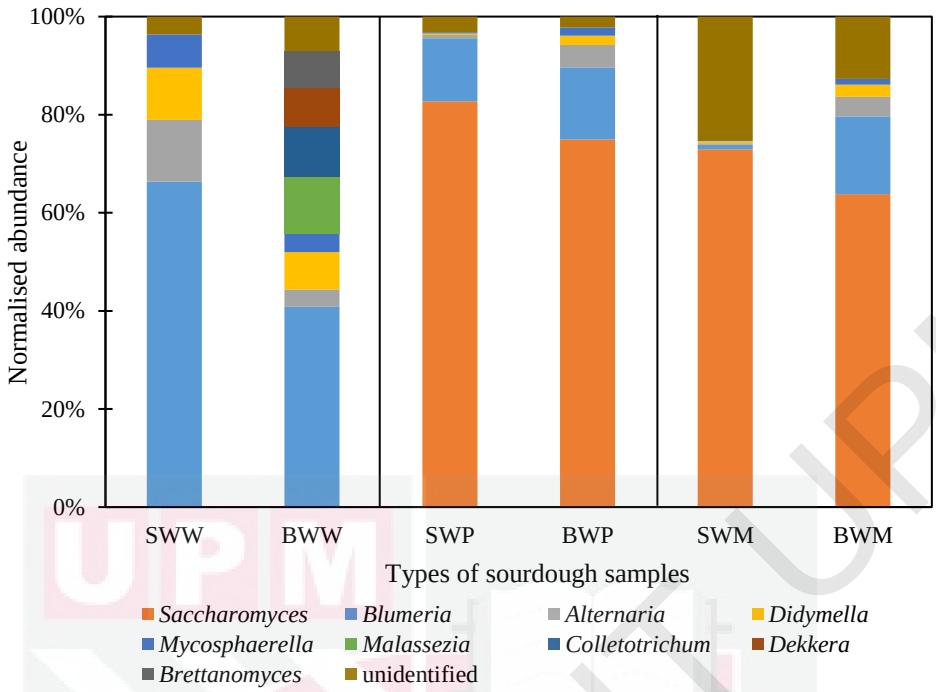

Figure 4.40: Changes in major fungal diversity and abundance at genus level from sourdough starter samples (SWW, SWP, SWM) to steamed buns samples (BWW, BWP, BWM)

4.4.6 Fermented Foods Microbiota at Class and Genus Levels

The bacterial profiles of two other fermented foods, kefir (KFR) and yogurt (YGT) were examined along with sourdough products of starters and breads in this study. At class level, Figure 4.41 shows that Bacilli occupied the biggest proportion of bacterial communities in most samples, including kefir and yogurt. Wheat bun and baked breads were the exceptions dominated by Gammaproteobacteria class that was mainly *Pseudomonas* genus. The subdominant classes only appeared in selective groups of samples, for example, Actinobacteria in breads and kefir while Alphaproteobacteria and Oxyphotobacteria in starters and breads. At genus level, Figure 4.42 shows the prevalence of genera under Bacilli, *i.e. Lactococcus* in kefir and *Lactobacillus* in sourdough starters, steamed buns with composite starters and yogurt.

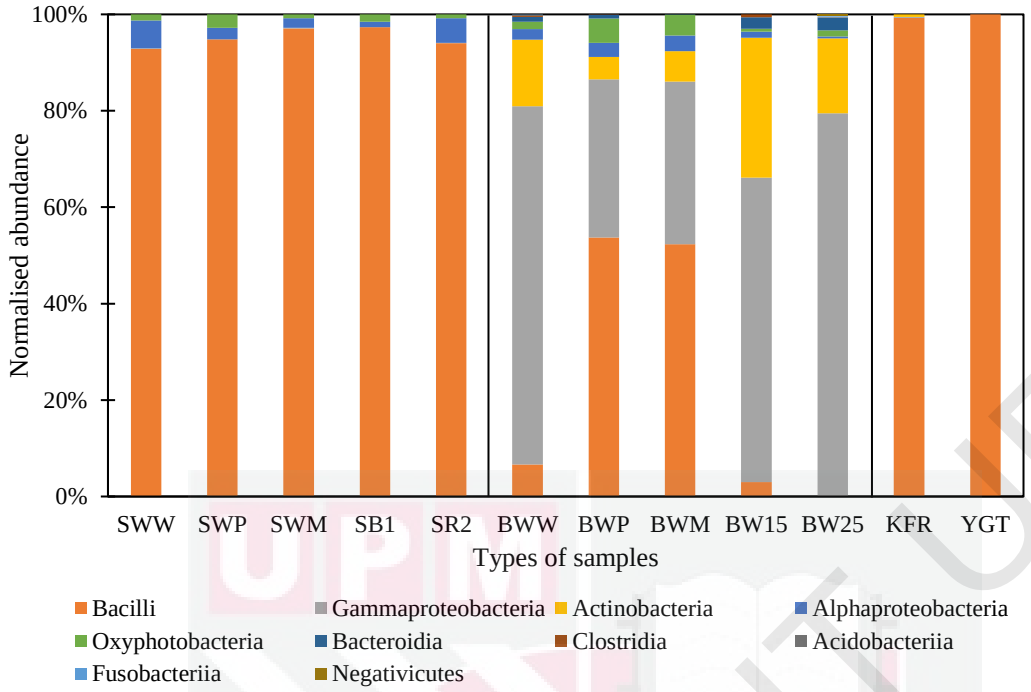

Figure 4.41: Comparing bacterial diversity and abundance at class level in other fermented foods of kefir (KFR) and yogurt (YGT) with sourdough starters (SWW, SWP, SWM, SB1, SR2), steamed sourdough breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

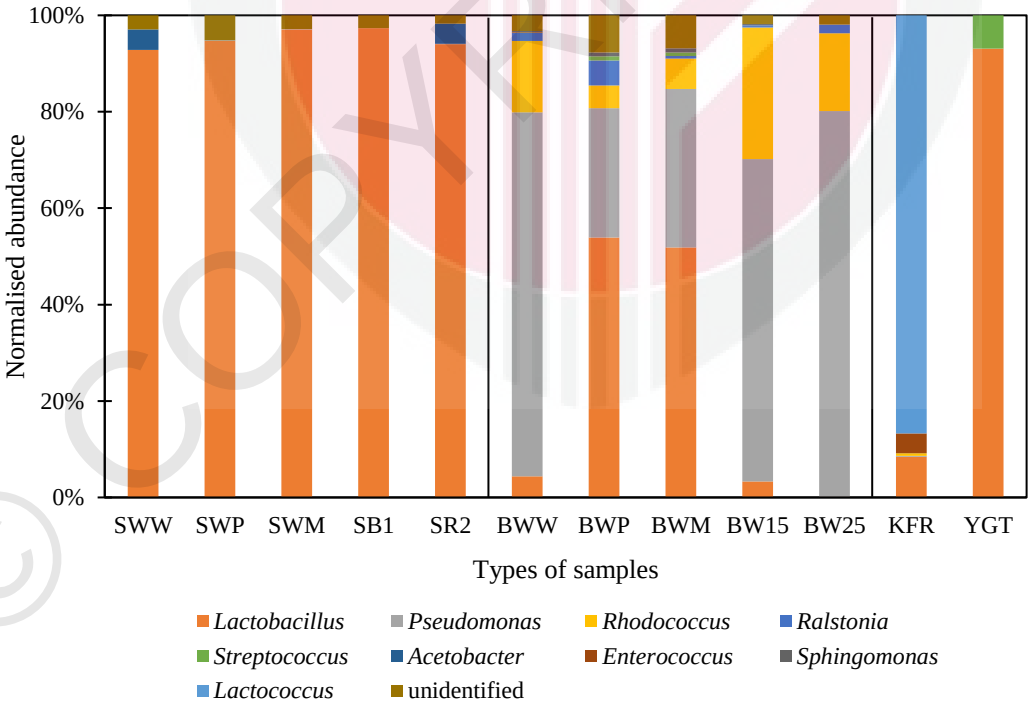

Figure 4.42: Comparing bacterial diversity and abundance at genus level in other fermented foods of kefir (KFR) and yogurt (YGT) with sourdough starters (SWW, SWP, SWM, SB1, SR2), steamed sourdough breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

Bacilli class harboured the bacterial community of yogurt completely (Figure 4.41). The situation was also similar in kefir with less than 1% other bacteria. These fermented milk products have low bacterial diversities as they were inoculated by carefully chosen and cultured LAB or kefir grains for optimised fermentation. The bacterial diversities were relatively higher in the spontaneously fermented sourdough starter samples with up to four classes, and the highest in bread samples that contained up to nine classes. As for Gammaproteobacteria class, the bacteria were assumed to be absent (< 1%) in sourdough starters, yogurt and kefir, but they dominated the communities in steamed wheat bun and the baked wheat breads (Table A.14 in Appendix A). The absence in kefir and yogurt further supported the discussions in Sections 4.4.4 and 4.4.5 that the Gammaproteobacteria class bacteria, especially *Pseudomonas* spp., were likely sourced from wheat flour (De Angelis et al., 2018; Ercolini et al., 2013).

Lactobacillus from class of Bacilli, was the major genus that comprised more than 90% in sourdough starters, more than 50% in steamed buns with composite sourdough starters and 93.1% in yogurt (Figure 4.42). The normalised abundance of *Lactobacillus* was only 8.4% in kefir as the bacterial community was dominated by another genus under the same class, *i.e.*, *Lactococcus*. Although lactic acid fermentation was conducted by LAB in sourdough starters, kefir and yogurt, the inhabited LAB were not necessarily the same. Each of them has their own typical LAB, *e.g.*, *Lb. fermentum*, *Lb. brevis* and *Lb. pontis* in sourdough starters (Minervini et al., 2016; Thiele et al., 2002; Van der Meulen et al., 2007), *Lc. lactis* and *Lb. kefiri* in kefir (Dobson et al., 2011; Magalhães et al., 2011) and *Lb. delbrueckii* subsp. *bulgaricus* in yogurt (Helal et al., 2018).

Table A.14 shows that 86.7% of detected bacteria in kefir was from the dominating genus of *Lactococcus*. Among them, *Lc. lactis* was identified. This bacterium has been common in kefir (Cais-Sokolińska et al., 2016; Dobson et al., 2011; Magalhães et al., 2011; Simova et al., 2002) and occasionally found in firm sourdough (Di Cagno et al., 2014; Ercolini et al., 2013). Dobson et al (2011) mentioned the potential antimicrobial characteristic of *Lc. lactis* DPC3147 through the release of lacticin 3147 in kefir. The LAB is homofermentative (Simova et al., 2002), hence it metabolises glucose and releases mainly lactic acid through different pathway as other heterofermentative LAB (Chavan & Chavan, 2011). In other words, *Lc. lactis* is mainly responsible on the sour taste of kefir, whereas the carbonated mouthfeel may be contributed by yeast or other heterofermentative LAB, such as *Lb. kefiri*, *Leuconostoc mesenteroides* and *Leuconostoc lactis* from Table A.16.

The *Lactobacillus* genus made up 93.1% of bacterial community in yogurt, whereas under the genus, *Lb. delbrueckii* subsp. *bulgaricus* was detected at 35.4% of the overall bacterial community (Table A.16). This probiotic bacterium is frequently utilised for Italian cheese, yogurt and other fermented milk production (De Angelis & Gobbetti, 2011). Balanced bacterial communities have been always maintained by *Lb. delbrueckii* subsp. *bulgaricus* and the yogurt starter (*Streptococcus salivarius* subsp. *thermophilus*) in yogurt (De Angelis & Gobbetti, 2011; Helal et al., 2018). When yogurt was added in sourdough fermentation, these thermophilic bacteria were replaced by *Pediococcus pentosaceus* in the final bacterial community (Gordún et al., 2014). Gordún et al. (2014) suggested that this may be associated with the lower fermentation temperature for sourdough which was not favourable by the bacteria. This also explained the exclusive presence of *Lb. delbrueckii* subsp. *bulgaricus* in yogurt from Table A.16 but not in the sourdough starters developed for this study.

Figures 4.43 and 4.44 provide the taxonomic profiles of fungal classes and genera respectively in sourdough starters, steamed sourdough buns, and other fermented foods. Saccharomycetes was found in all samples and dominated kefir and yogurt, as illustrated in Figure 4.43. The class is also the majority in most sourdough samples, except wheat starter (SWW) and wheat bun (BWW). Figure 4.44 shows *Saccharomyces* as dominating genus for most sourdough samples, *Kazachstania* for rye starter, *Debaryomyces* for baked sourdough bread and *Kluyveromyces* for kefir and yogurt. The mentioned genera collectively belong to Saccharomycetes class. *Blumeria* (Leotiomycetes class) was the major genus in SWW and BWW while *Aureobasidium* (Dothideomycetes class) dominated baked yeasted bread (BW15).

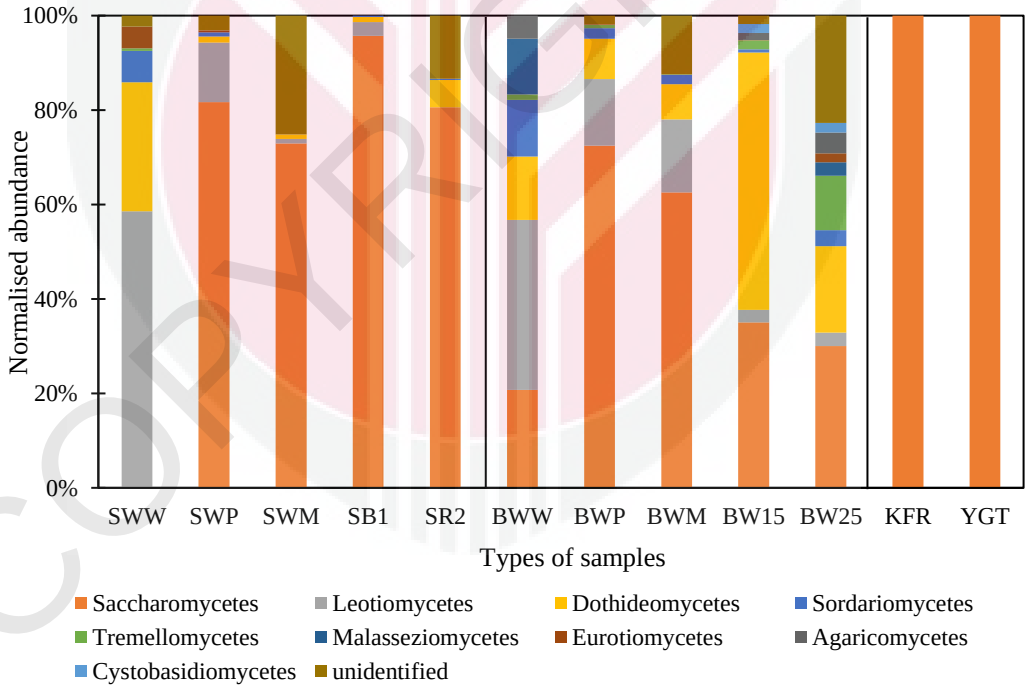

Figure 4.43: Comparing fungal diversity and abundance at class level in other fermented foods of kefir (KFR) and yogurt (YGT) with sourdough starters (SWW, SWP, SWM, SB1, SR2), steamed sourdough breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

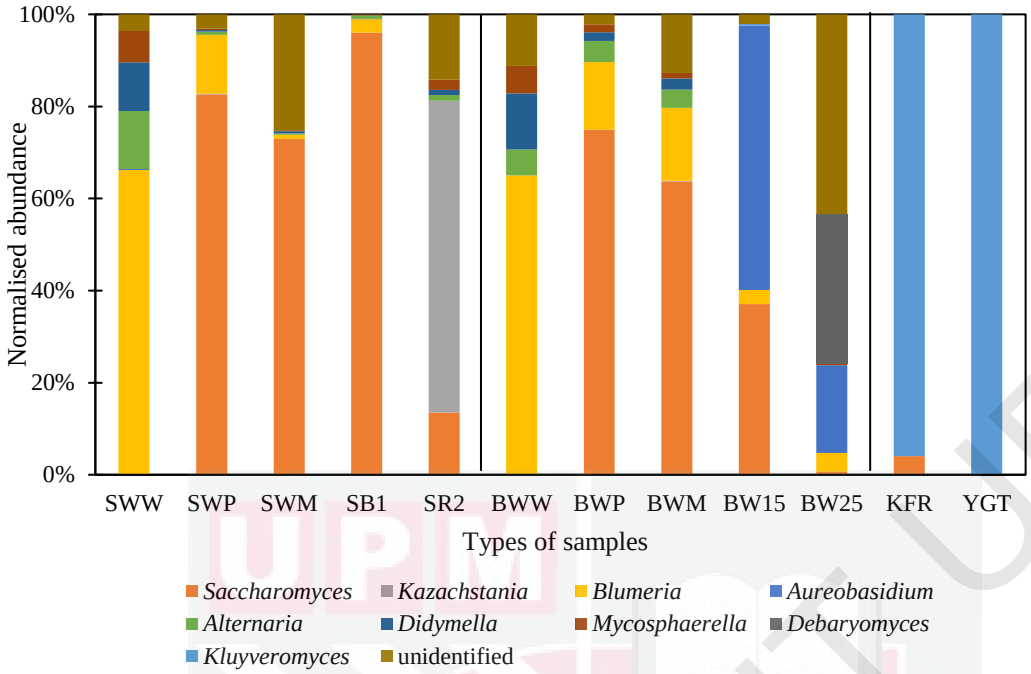

Figure 4.44: Comparing fungal diversity and abundance at genus level in other fermented foods of kefir (KFR) and yogurt (YGT) with sourdough starters (SWW, SWP, SWM, SB1, SR2), steamed sourdough breads (BWW, BWP, BWM) and baked breads (BW15, BW25)

The normalised incidence of Saccharomycetes as major class was the highest at 100% in yogurt and kefir, followed by 72.9–95.7% in sourdough starters, 62.5–72.4% in steamed buns and 30.0% in baked sourdough bread except SWW, BWW and BW15. By referring to Table A.15 in Appendix A, the most well-known yeast genus, *i.e.*, *Saccharomyces*, made up of 63.7–96.1% in outsourced wheat sourdough starter (SB1), composite sourdough starters and steamed buns using composite starters. Under the same class, *Kazachstania* genus comprised of the highest normalised abundance at 67.7% in SR2 while for BW25, *Debaryomyces* was the major genus that occupied 32.5%. As for kefir and yogurt, *Kluyveromyces* genus, which is also belonged to class of Saccharomycetes, was the majority with the normalised abundances of 96.0% and 100% respectively.

Kluyveromyces marxianus is a common yeast found in fermented dairy products, such as kefir, cheese and koumiss (Homayouni-Rad et al., 2020) among the *Kluyveromyces* genus. It has great potential in being acknowledged as probiotics since it could be grown on industrial scale while surviving in production and human digestion processes to bring health benefits, as proposed by Homayouni-Rad et al. (2020). *Kl. marxianus* was tested on its leavening ability and the outcome was comparable with *S. cerevisiae* in lean dough (Caballero et al., 1995). The inoculation of this yeast and *Lb. delbrueckii* subsp. *bulgaricus* also provided improvement on sensory characteristics of sourdough bread (Plessas et al., 2008). Thus, it is encouraging to involve kefir or yogurt in sourdough fermentation for more satisfying food products.

4.4.7 Summary

Among sourdough, the microbial diversities of wheat starter and wheat bun were generally reduced by involving non-cereals, reported in terms of Chao1 richness and Shannon diversity indices. The fungal diversities were usually lesser than the bacterial. With higher similarities among bacterial diversities, the diversities of sourdough starter group were lesser than bread group. By comparing with outsourced sourdough starters and baked breads, the microbial diversities of those prepared in the present study were mostly higher. The simplest microbial profiles were observed in the outsourced kefir and yogurt, followed by sourdough starters and lastly the breads, which showed the most diversified microbial profiles.

Overall, *Lactobacillus* and *Saccharomyces* were primary sourdough LAB and yeasts that dominated the microbiota and provided unique properties of sourdough starters and products. In composite starters, *Lb. brevis* and *Lb. fermentum* coexisted

harmoniously with the main yeast of *Saccharomyces* spp., while *Lb. pontis* harboured only in outsourced starters with *Saccharomyces* spp. and *Kazachstania humilis*. *Lactobacillus* genus under Bacilli class was the most prevailing in bacterial communities of all sourdough starters at more than 90%. The predominance remained at lower abundances (51.8-53.9%) in breads with composite starters. Meanwhile, for fungal communities, *Saccharomyces* genus from the class of Saccharomycetes dominated at 72.9–82.6% in composite starters and 63.7–75.0% in steamed buns containing them. In wheat starter, LAB were possibly less active and yeast was almost absent, thus resulting in weak leavening performances and relatively poor properties in wheat bun. As for kefir and yogurt, they shared the same major bacterial class with sourdough samples but the dominating genera were *Lactococcus* and *Lactobacillus* respectively. The fungal communities of kefir and yogurt were mostly *Kluyveromyces* genus that was also from Saccharomycetes class.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The effects of adding pumpkin and mung bean in wheat sourdough starters were significant in the major properties studied in the present study. From the aspect of chemical properties, the greater the proportion of non-cereals, the higher the final pH and TTA values, thus the more comparable the values to sourdough starters from other literatures. The final pH of wheat sourdough starter was raised from 3.31 to 3.38-3.50 and 3.35-3.50 respectively with the addition of pumpkin and mung bean flours, meanwhile for TTA, from 14.0 ml to 15.1-18.4 ml and 17.7-20.1 ml. As fermentation progressed, pH decreased while TTA increased in sourdough starters irrespective of pumpkin and mung bean presence. A substantial change of chemical properties, including the greatest pH drop by 32.5% with 5% mung bean and the greatest TTA increase by 17.1 ml with 15% mung bean, was observed in the first three days. The pH values converged since day 4 whereas for TTA, smaller fluctuations appeared only after another two peaks on day 5 and day 11. The three peaks of TTA values indicated the adaptation of microbes within the acidic condition, followed by convergence that confirmed sourdough maturity.

The addition of pumpkin and mung bean markedly aided leavening improvement in sourdough starters. In terms of specific volume, the maximum value during stage 1 was boosted to 240.5 and 196.7 cm^3/100g in starters containing pumpkin and mung bean respectively, as compared to wheat starter at 153.9 cm^3/100g. The activation raised the specific volume of wheat starter up to 175.3 cm^3/100g and subsequently 205.3 cm^3/100g in following stages. The leavening performances, which were expressed by volume expansion ratio, were accurately described using Gompertz

model with most R^2 > 0.97. The activated sourdough starters acquired more outstanding leavening capabilities with pumpkin or mung bean addition, as demonstrated by the raised maximum volume expansion ratio from 0.40 to 1.72 and 2.03 cm^3/cm^3, maximum volume expansion rate from 0.04 to 0.51 and 0.57 h^{-1}, reduced leavening lag time from 6.39 to 2.13 and 2.35 h and overall fermentation time from 18 to 7 and 8 h respectively.

Generally, non-cereal incorporation, especially involving wheat-pumpkin and wheat-mung bean composite starters at 5-10% pumpkin fortification, ameliorated the qualities of sourdough breads prepared in the form of steamed sourdough buns. Conversely, steamed buns fortified with too much pumpkin, *i.e.* 15-20% showed unfavourable properties. The specific volume of wheat-pumpkin and wheat-mung bean sourdough starters was improved from 1.05 to 1.88 and 2.22 cm^3/g, while for spread ratio, from 1.86 to 1.59 and 1.41 respectively. With the aid of pumpkin and mung bean starters, specific volume were peaked at 10% and 5% pumpkin fortification respectively. The greater the degree of pumpkin fortification, the lower the L*, the higher the a* and b*, hence implying the darker and more orangish colour. For textural properties, 5% pumpkin fortification was the most satisfactory with the lowest hardness at 3088-3266 gf among buns containing composite starters. 10% pumpkin fortification meanwhile was favoured by the sensory panellists since the highest overall liking scores of 6.8 and 7.0 were given for buns with starters containing pumpkin and mung bean. The decent scores of 6.2-7.2 were also given for steamed buns with 10% pumpkin fortification, in terms of texture, colour, taste and aroma. Energy values and carbohydrate content were lower whereas ash and total dietary fibre contents were higher when composite sourdough starters were utilised with 5% pumpkin fortification in steamed sourdough buns.

Overall, the involvement of pumpkin and mung bean flours reduced microbial diversities of sourdough starters and steamed sourdough buns in terms of Chao1 richness and Shannon diversity indices. The fungal diversities were relatively simpler than bacteria. The microbiological profiles were the simplest in the outsourced kefir and yogurt, followed by sourdough starters and lastly breads that contained the most diversified microbial communities. The diversities were shown more directly in bacterial and fungal abundances. *Lactobacillus* and *Saccharomyces* are typical, dominating sourdough LAB and yeasts which provide distinctive features of sourdough starters and products. *Lb. brevis* and *Lb. fermentum* coexisted harmoniously with the main yeast of *Saccharomyces* spp. in sourdough starters.

The incorporation of pumpkin and mung bean flours is believed to have increased possibilties of LAB and yeast retention in steamed buns and yeast survival in sourdough starters. *Lactobacillus* (Bacilli) was the most prevailing genus in bacterial communities of all sourdough starters at more than 90%. In buns, the predominance remained at lower occurrences of 51.8-53.9% with starters containing pumpkin and mung bean. Yeasts from *Saccharomyces* genus of Saccharomycetes class dominated the fungal communities at 72.9–82.6% in composite starters and 63.7–75.0% in steamed buns containing them. Yeast was almost absent in wheat sourdough starter and LAB were possibly less active, hence relatively poorer leavening performances and associating properties were demonstrated in wheat buns.

Unified and interdependent relationship was discovered among the studied properties of sourdough starters and steamed sourdough buns. The pH and TTA were associated with the organic acids released by sourdough microbiota while the microbiological profiles were also influenced by acidity. The LAB activities and yeast presence were

potential factors that affect the amount of carbon dioxide released, thus affecting leavening performances of sourdough starters. The acidity and leavening whereas brought key effects on physical properties and sensory qualities of steamed buns. In short, the sourdough starters involved should be well maintained and activated to guarantee qualities of sourdough products such as steamed buns as example.

With the positive outcomes of present research that were partially published as stated in the list of publication, it is anticipated that the food industry, especially for sourdough products, to be flourished by introducing more beneficial non-cereal ingredients in sourdough, such as yogurt and kefir which also contained *Lactobacillus* and *Saccharomycetes*. The investigation on health or product quality improving effects of specific or coexisting sourdough LAB, has a great potential to be further developed. The management of sourdough fermentation can also be easier and less time-consuming by optimising sourdough qualities through activation system and mathematical modelling. By doing so, the attention received on sourdough by bakers, researchers and manufacturers will be enhanced. A wider range of sourdough products can be developed, thus making full use of sourdough starters including those discarded through backslopping process.

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APPENDICES

Appendix A: Tables of Results

Table A.1: Evolution of pH in wheat-pumpkin and wheat-mung bean composite sourdough starters during the 14-day development

Samples	Fermentation time (day)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
00W (control)	5.30 ± 0.18	4.57 ± 0.00	3.87 ± 0.25	3.67 ± 0.04	3.55 ± 0.04	3.53 ± 0.05	3.53 ± 0.08	3.51 ± 0.06	3.43 ± 0.05	3.43 ± 0.01	3.42 ± 0.02	3.41 ± 0.03	3.42 ± 0.00	3.37 ± 0.01	3.31 ± 0.00
05P	5.38 ± 0.08	4.17 ± 0.23	3.80 ± 0.20	3.77 ± 0.10	3.59 ± 0.01	3.52 ± 0.05	3.43 ± 0.03	3.55 ± 0.00	3.44 ± 0.00	3.52 ± 0.02	3.48 ± 0.02	3.42 ± 0.06	3.42 ± 0.02	3.40 ± 0.00	3.40 ± 0.01
10P	5.32 ± 0.05	4.45 ± 0.16	4.00 ± 0.47	3.67 ± 0.01	3.53 ± 0.02	3.54 ± 0.00	3.45 ± 0.03	3.54 ± 0.03	3.44 ± 0.05	3.52 ± 0.14	3.52 ± 0.00	3.48 ± 0.04	3.49 ± 0.00	3.48 ± 0.04	3.38 ± 0.00
15P	5.38 ± 0.10	4.13 ± 0.11	4.09 ± 0.03	3.65 ± 0.13	3.64 ± 0.09	3.54 ± 0.12	3.60 ± 0.14	3.54 ± 0.04	3.55 ± 0.09	3.61 ± 0.15	3.53 ± 0.08	3.49 ± 0.05	3.49 ± 0.05	3.48 ± 0.04	3.41 ± 0.03
20P	5.34 ± 0.08	3.66 ± 0.04	3.66 ± 0.05	3.68 ± 0.04	3.64 ± 0.02	3.50 ± 0.01	3.64 ± 0.02	3.53 ± 0.08	3.54 ± 0.06	3.58 ± 0.10	3.65 ± 0.05	3.57 ± 0.02	3.58 ± 0.00	3.54 ± 0.09	3.50 ± 0.02
05M	5.37 ± 0.34	4.41 ± 0.05	3.88 ± 0.06	3.63 ± 0.01	3.57 ± 0.04	3.54 ± 0.02	3.47 ± 0.03	3.53 ± 0.00	3.51 ± 0.08	3.54 ± 0.03	3.52 ± 0.02	3.53 ± 0.02	3.50 ± 0.07	3.43 ± 0.00	3.35 ± 0.02
10M	5.20 ± 0.10	4.84 ± 0.03	3.81 ± 0.08	3.76 ± 0.13	3.60 ± 0.05	3.58 ± 0.01	3.47 ± 0.02	3.56 ± 0.01	3.57 ± 0.11	3.56 ± 0.00	3.55 ± 0.00	3.53 ± 0.01	3.53 ± 0.04	3.47 ± 0.00	3.40 ± 0.02
15M	5.15 ± 0.10	4.91 ± 0.08	3.91 ± 0.13	4.00 ± 0.30	3.62 ± 0.06	3.60 ± 0.00	3.56 ± 0.01	3.58 ± 0.02	3.54 ± 0.04	3.68 ± 0.04	3.68 ± 0.07	3.59 ± 0.00	3.62 ± 0.02	3.56 ± 0.01	3.49 ± 0.02
20M	5.34 ± 0.16	5.00 ± 0.06	3.93 ± 0.04	3.77 ± 0.07	3.66 ± 0.03	3.67 ± 0.05	3.60 ± 0.11	3.56 ± 0.04	3.66 ± 0.02	3.66 ± 0.04	3.63 ± 0.09	3.60 ± 0.02	3.62 ± 0.04	3.57 ± 0.01	3.50 ± 0.01

Table A.2: Evolution of TTA in wheat-pumpkin and wheat-mung bean composite sourdough starters during the 14-day development

Sample	TTA (ml of 0.1 N NaOH)														
	Fermentation time (day)														
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
00W (control)	3.2 ± 1.4	8.8 ± 0.0	13.2 ± 1.3	14.8 ± 1.1	14.8 ± 0.8	16.2 ± 1.0	13.9 ± 0.6	15.2 ± 0.0	15.7 ± 0.6	12.7 ± 2.1	14.5 ± 2.1	19.3 ± 2.1	12.7 ± 0.6	12.4 ± 0.0	14.0 ± 1.3
05P	4.5 ± 0.6	7.4 ± 2.0	15.0 ± 1.4	13.5 ± 0.7	16.7 ± 0.9	17.9 ± 1.2	15.7 ± 1.8	17.3 ± 1.4	15.4 ± 2.1	14.3 ± 0.4	17.4 ± 1.7	20.5 ± 0.7	17.1 ± 1.3	13.9 ± 1.3	16.8 ± 1.1
10P	5.8 ± 0.0	5.8 ± 1.1	15.0 ± 1.4	15.0 ± 1.1	15.5 ± 2.1	16.9 ± 1.2	17.8 ± 0.6	15.4 ± 2.0	15.3 ± 1.3	15.0 ± 1.3	17.1 ± 1.2	16.8 ± 0.6	16.7 ± 2.0	14.5 ± 1.3	18.4 ± 0.4
15P	4.6 ± 0.5	9.1 ± 0.0	14.8 ± 2.0	19.3 ± 0.8	18.8 ± 2.0	16.9 ± 2.1	19.5 ± 2.1	18.1 ± 1.8	15.3 ± 1.3	16.2 ± 1.4	16.6 ± 0.8	19.9 ± 1.3	14.4 ± 1.4	14.7 ± 0.7	15.1 ± 1.2
20P	4.0 ± 0.0	10.7 ± 0.6	19.1 ± 1.1	19.6 ± 0.6	19.3 ± 1.4	20.6 ± 0.6	20.8 ± 0.6	23.2 ± 1.2	17.0 ± 1.3	16.7 ± 0.6	21.0 ± 1.6	20.2 ± 1.3	21.5 ± 2.1	16.1 ± 1.1	18.1 ± 1.6
05M	4.7 ± 2.0	15.0 ± 1.3	16.9 ± 1.6	19.1 ± 0.9	19.3 ± 1.5	17.5 ± 0.7	17.4 ± 0.8	16.3 ± 0.9	16.7 ± 0.6	16.2 ± 1.4	19.0 ± 1.1	22.2 ± 1.1	16.7 ± 2.1	14.7 ± 0.8	18.5 ± 1.9
10M	5.3 ± 1.1	13.0 ± 2.1	16.7 ± 1.0	21.1 ± 1.3	18.9 ± 0.8	20.1 ± 0.0	21.1 ± 1.1	18.9 ± 1.8	15.5 ± 0.7	15.7 ± 0.4	19.1 ± 0.9	23.3 ± 1.3	18.3 ± 1.6	14.9 ± 0.7	17.7 ± 0.6
15M	5.2 ± 1.2	15.8 ± 1.3	21.6 ± 2.1	22.3 ± 0.0	22.1 ± 0.0	21.4 ± 0.6	21.7 ± 0.5	20.7 ± 0.8	19.6 ± 2.1	17.2 ± 0.0	17.7 ± 0.0	20.1 ± 1.1	20.6 ± 1.9	16.7 ± 0.8	18.2 ± 1.3
20M	5.1 ± 1.2	14.6 ± 0.6	22.7 ± 0.6	21.4 ± 1.4	22.3 ± 0.0	24.7 ± 0.6	26.7 ± 0.4	26.5 ± 0.4	24.7 ± 0.6	18.3 ± 0.0	19.9 ± 0.0	21.4 ± 2.0	19.8 ± 1.5	18.0 ± 1.4	20.1 ± 0.0

Table A.3: Specific volume at three activation stages for sourdough starters using wheat (00W) as control and composite flours containing pumpkin (15WP) and mung bean (15WM)

Time (hour)	Specific volume (cm^3/100g)								
	Activation stage 1			Activation stage 2			Activation stage 3		
	00W	15WP	15WM	00W	15WP	15WM	00W	15WP	15WM
0	111.2 ± 0.0	117.8 ± 0.0	119.7 ± 0.0	106.9 ± 6.0	112.9 ± 6.9	119.7 ± 0.0	115.5 ± 6.0	112.9 ± 6.9	106.9 ± 6.0
1	115.5 ± 6.0	117.8 ± 0.0	119.7 ± 0.0	106.9 ± 6.0	127.6 ± 0.0	119.7 ± 0.0	124.0 ± 6.0	122.7 ± 6.9	106.9 ± 6.0
2	115.5 ± 6.0	122.7 ± 6.9	119.7 ± 0.0	106.9 ± 6.0	127.6 ± 0.0	119.7 ± 0.0	128.3 ± 0.0	132.5 ± 6.9	124.0 ± 6.0
3	115.5 ± 6.0	122.7 ± 6.9	119.7 ± 0.0	111.2 ± 0.0	127.6 ± 0.0	124.0 ± 6.0	132.6 ± 6.0	157.1 ± 0.0	162.5 ± 0.0
4	119.7 ± 12.1	122.7 ± 6.9	124.0 ± 6.0	115.5 ± 6.0	137.4 ± 13.9	145.4 ± 0.0	149.7 ± 6.0	216.0 ± 13.9	201.0 ± 18.1
5	119.7 ± 12.1	132.5 ± 6.9	124.0 ± 6.0	128.3 ± 0.0	166.9 ± 13.9	166.8 ± 6.0	179.6 ± 12.1	270.0 ± 20.8	243.7 ± 18.1
6	119.7 ± 12.1	132.5 ± 6.9	124.0 ± 6.0	136.8 ± 0.0	186.5 ± 13.9	205.3 ± 0.0	192.4 ± 6.0	289.6 ± 6.9	299.3 ± 0.0
7	124.0 ± 6.0	132.5 ± 6.9	128.3 ± 0.0	136.8 ± 0.0	211.1 ± 6.9	235.2 ± 6.0	205.3 ± 0.0	289.6 ± 6.9	312.2 ± 6.0
8	124.0 ± 6.0	132.5 ± 6.9	128.3 ± 0.0	153.9 ± 0.0	235.6 ± 13.9	273.7 ± 0.0	205.3 ± 0.0		312.2 ± 6.0
9	124.0 ± 6.0	137.4 ± 13.9	128.3 ± 0.0	158.2 ± 6.0	250.3 ± 20.8	290.8 ± 12.1			
10	132.6 ± 6.0	137.4 ± 13.9	136.8 ± 0.0	166.8 ± 6.0	250.3 ± 20.8				
11	132.6 ± 6.0	152.2 ± 6.9	136.8 ± 0.0	175.3 ± 6.0					
12	141.1 ± 6.0	152.2 ± 6.9	153.9 ± 12.1						
13	145.4 ± 0.0	157.1 ± 13.9	158.2 ± 6.0						
14	145.4 ± 0.0	166.9 ± 0.0	166.8 ± 6.0						
15	149.7 ± 6.0	171.8 ± 6.9	179.6 ± 0.0						
16	153.9 ± 0.0	176.7 ± 13.9	183.9 ± 6.0						
17	153.9 ± 0.0	196.3 ± 13.9	196.7 ± 0.0						
18	153.9 ± 0.0	211.1 ± 20.8							
19		240.5 ± 6.9							
20		240.5 ± 6.9							

Table A.4: Specific volume, spread ratio and colour parameters of SSBs containing wheat, wheat-pumpkin and wheat-mung bean sourdough starters at 0-20% pumpkin fortification

Type of starter	Concentration of pumpkin (%)	Specific volume (cm^3/g)	Spread ratio (cm/cm)	Colour			
				*L**	*a**	*b**	*ΔE*
Control	0	1.05 ± 0.07^{f}	1.86 ± 0.08^{a}	56.97 ± 0.75^{b}	0.99 ± 0.10^{hi}	16.59 ± 0.80^{k}	0.00
	5	1.06 ± 0.03^{f}	1.80 ± 0.01^{ab}	53.27 ± 0.54^{c}	3.36 ± 0.61^{g}	33.89 ± 2.39^{i}	17.87 ± 2.34^{h}
	10	1.09 ± 0.04^{f}	1.64 ± 0.08^{bcd}	50.21 ± 1.34^{de}	8.63 ± 0.44^{e}	47.81 ± 2.01^{f}	32.88 ± 1.76^{e}
	15	1.05 ± 0.08^{f}	1.56 ± 0.07^{cdef}	38.72 ± 0.13^{i}	45.98 ± 0.93^{a}	77.14 ± 0.81^{a}	77.61 ± 1.15^{a}
	20	0.95 ± 0.04^{f}	1.41 ± 0.04^{f}	39.68 ± 0.83^{i}	38.12 ± 1.77^{b}	72.51 ± 0.53^{b}	69.33 ± 1.28^{b}
Pumpkin	0	1.88 ± 0.03^{cd}	1.59 ± 0.06^{cdef}	49.35 ± 0.23^{ef}	0.36 ± 0.09^{i}	22.95 ± 0.31^{j}	9.95 ± 0.27^{i}
	5	2.05 ± 0.08^{abc}	1.63 ± 0.04^{bcd}	49.93 ± 0.36^{def}	3.60 ± 0.32^{g}	39.59 ± 0.83^{h}	24.20 ± 0.82^{g}
	10	2.12 ± 0.09^{ab}	1.72 ± 0.08^{abc}	45.30 ± 0.33^{h}	10.30 ± 1.22^{d}	53.13 ± 2.43^{e}	39.49 ± 2.52^{d}
	15	1.63 ± 0.09^{e}	1.61 ± 0.09^{bcde}	46.66 ± 0.45^{gh}	15.43 ± 0.55^{c}	61.41 ± 0.92^{d}	48.21 ± 0.98^{c}
	20	1.63 ± 0.08^{e}	1.80 ± 0.04^{ab}	48.26 ± 0.39^{fg}	17.22 ± 1.53^{c}	65.24 ± 2.20^{c}	52.03 ± 2.53^{c}
Mung bean	0	2.22 ± 0.06^{a}	1.41 ± 0.03^{ef}	61.89 ± 0.78^{a}	0.64 ± 0.10^{i}	13.65 ± 1.13^{k}	5.76 ± 1.25^{j}
	5	2.18 ± 0.10^{a}	1.57 ± 0.07^{cdef}	56.94 ± 0.83^{b}	2.60 ± 0.30^{gh}	32.89 ± 0.99^{i}	16.40 ± 0.99^{h}
	10	1.88 ± 0.09^{cd}	1.51 ± 0.03^{def}	56.64 ± 0.52^{b}	6.34 ± 0.29^{f}	43.68 ± 1.00^{g}	27.61 ± 1.03^{f}
	15	1.92 ± 0.09^{bcd}	1.48 ± 0.09^{def}	51.31 ± 1.61^{d}	9.28 ± 0.74^{de}	51.10 ± 2.30^{e}	35.97 ± 2.35^{e}
	20	1.76 ± 0.09^{de}	1.56 ± 0.09^{cdef}	57.36 ± 0.92^{b}	8.33 ± 0.43^{e}	51.36 ± 0.94^{e}	35.55 ± 1.01^{e}

Table A.5: Textural parameters of SSBs containing wheat, wheat-pumpkin and wheat-mung bean sourdough starters at 0-20% pumpkin fortification

Type of starter	Concentration of pumpkin (%)	Texture				
		Hardness (gf)	Springiness	Cohesiveness	Chewiness (gf)	Resilience
Control	0	7353 ± 250^{c}	0.93 ± 0.02^{d}	0.77 ± 0.10^{c}	5262 ± 630^{d}	0.32 ± 0.02^{f}
	5	6876 ± 173^{d}	0.95 ± 0.03^{cd}	0.86 ± 0.01^{ab}	5593 ± 192^{d}	0.34 ± 0.01^{ef}
	10	6279 ± 126^{e}	0.95 ± 0.01^{cd}	0.87 ± 0.01^{ab}	5192 ± 89^{d}	0.39 ± 0.02^{c}
	15	7830 ± 101^{b}	0.96 ± 0.04^{abcd}	0.85 ± 0.01^{abc}	6372 ± 235^{c}	0.32 ± 0.01^{f}
	20	9148 ± 485^{a}	1.03 ± 0.09^{a}	0.84 ± 0.04^{bc}	7830 ± 661^{a}	0.36 ± 0.02^{de}
Pumpkin	0	2991 ± 95^{hi}	0.99 ± 0.02^{abcd}	0.86 ± 0.02^{ab}	2553 ± 93^{gh}	0.39 ± 0.01^{c}
	5	3088 ± 80^{h}	0.97 ± 0.00^{abcd}	0.86 ± 0.02^{ab}	2592 ± 21^{fgh}	0.38 ± 0.01^{cd}
	10	3724 ± 89^{f}	0.97 ± 0.01^{abcd}	0.85 ± 0.02^{ab}	3080 ± 94^{efg}	0.38 ± 0.02^{cd}
	15	7891 ± 244^{b}	0.96 ± 0.01^{bcd}	0.85 ± 0.01^{abc}	6377 ± 180^{c}	0.38 ± 0.02^{cd}
	20	8980 ± 270^{a}	0.95 ± 0.04^{cd}	0.82 ± 0.03^{bc}	6977 ± 322^{b}	0.36 ± 0.02^{cde}
Mung bean	0	2605 ± 143^{i}	1.02 ± 0.02^{ab}	0.91 ± 0.01^{a}	2418 ± 116^{h}	0.50 ± 0.02^{a}
	5	3266 ± 198^{gh}	1.00 ± 0.05^{abc}	0.81 ± 0.08^{bc}	2666 ± 293^{fgh}	0.44 ± 0.03^{b}
	10	3848 ± 85^{f}	0.99 ± 0.01^{abcd}	0.89 ± 0.01^{ab}	3387 ± 44^{e}	0.45 ± 0.01^{b}
	15	3607 ± 50^{fg}	0.98 ± 0.00^{abcd}	0.89 ± 0.01^{ab}	3139 ± 24^{ef}	0.44 ± 0.01^{b}
	20	3045 ± 207^{h}	0.95 ± 0.02^{cd}	0.83 ± 0.02^{bc}	2402 ± 179^{h}	0.40 ± 0.01^{c}

Table A.6: Sensory qualities of SSBs containing control and composite starters containing pumpkin and mung bean at 0-20% pumpkin fortification

Type of starter	Concentration of pumpkin (%)	Sensory qualities				
		Texture	Colour	Taste	Aroma	Overall liking
Control	0	5.5 ± 1.2^{cd}	5.2 ± 0.8^{c}	5.1 ± 0.5^{cd}	4.9 ± 0.8^{d}	5.1 ± 0.4^{bc}
	5	7.1 ± 0.8^{a}	6.1 ± 0.9^{abc}	6.2 ± 0.8^{abcd}	5.8 ± 1.3^{bcd}	6.3 ± 1.0^{a}
	10	6.8 ± 0.9^{ab}	6.7 ± 1.0^{a}	6.2 ± 0.7^{abcd}	6.8 ± 0.7^{ab}	6.4 ± 0.6^{a}
	15	5.0 ± 0.9^{d}	7.0 ± 0.9^{a}	5.5 ± 1.1^{bcd}	6.4 ± 0.7^{abc}	6.1 ± 0.7^{ab}
	20	5.6 ± 0.9^{bcd}	6.5 ± 1.0^{a}	6.5 ± 1.3^{abc}	6.8 ± 0.8^{ab}	6.6 ± 1.0^{a}
Pumpkin	0	7.0 ± 1.1^{a}	5.3 ± 0.8^{bc}	4.9 ± 1.1^{d}	5.5 ± 0.9^{cd}	5.0 ± 0.5^{c}
	5	6.8 ± 1.3^{ab}	6.2 ± 1.0^{abc}	5.0 ± 1.5^{d}	5.8 ± 0.7^{bcd}	5.9 ± 1.0^{abc}
	10	7.2 ± 0.7^{a}	6.2 ± 1.1^{abc}	6.7 ± 1.6^{ab}	6.8 ± 0.4^{ab}	6.8 ± 1.2^{a}
	15	6.3 ± 0.9^{abc}	7.1 ± 0.7^{a}	5.4 ± 1.5^{bcd}	6.8 ± 1.3^{ab}	6.6 ± 0.8^{a}
	20	6.3 ± 1.1^{abc}	6.4 ± 0.5^{ab}	5.8 ± 1.2^{abcd}	6.0 ± 0.7^{bc}	6.3 ± 1.0^{a}
Mung bean	0	7.5 ± 0.7^{a}	6.7 ± 0.8^{a}	5.8 ± 1.0^{abcd}	5.8 ± 0.7^{bcd}	6.3 ± 0.8^{a}
	5	6.9 ± 0.9^{a}	6.4 ± 0.9^{ab}	5.4 ± 1.2^{bcd}	6.7 ± 0.7^{ab}	5.9 ± 1.0^{abc}
	10	7.1 ± 1.1^{a}	6.8 ± 0.8^{a}	7.0 ± 0.9^{a}	6.5 ± 1.5^{abc}	7.0 ± 0.9^{a}
	15	6.9 ± 1.3^{a}	6.3 ± 0.6^{abc}	6.5 ± 0.6^{ab}	6.5 ± 0.7^{abc}	6.8 ± 1.0^{a}
	20	6.7 ± 1.0^{abc}	6.5 ± 1.1^{a}	6.7 ± 1.3^{ab}	7.3 ± 0.5^{a}	6.6 ± 0.9^{a}

Table A.7: Room temperature and humidity during 14-day sourdough starter development

Day	Temperature (°C)	Humidity (%)
0	30.1 ± 0.2	85.8 ± 5.5
1	28.9 ± 0.3	87.2 ± 6.7
2	29.2 ± 0.2	87.6 ± 5.9
3	29.7 ± 0.2	88.3 ± 6.5
4	29.5 ± 0.4	83.9 ± 6.7
5	29.1 ± 0.4	84.6 ± 6.2
6	29.4 ± 0.4	84.2 ± 6.8
7	29.7 ± 0.2	87.4 ± 5.9
8	29.6 ± 0.2	79.5 ± 7.8
9	30.1 ± 0.2	78.8 ± 8.3
10	30.5 ± 0.3	83.4 ± 8.6
11	30.0 ± 0.3	85.3 ± 5.8
12	29.9 ± 0.2	82.2 ± 7.2
13	29.7 ± 0.4	87.2 ± 6.2
14	29.4 ± 0.1	89.4 ± 3.2

Table A.8: Top ten bacterial classes and genera in sourdough starters

Bacterial community	Normalised abundance (%)				
	SWW	SWP	SWM	SB1	SR2
Class					
Bacilli	92.9	94.8	97.1	97.4	94.0
Alphaproteobacteria	5.8	2.4	2.0	1.1	5.1
Oxyphotobacteria	1.3	2.8	0.8	1.5	0.8
Gammaproteobacteria	0.1	0.0	0.1	0.0	<0.1
Genus					
Lactobacillus	92.8	94.6	96.7	97.4	94.0
Acetobacter	4.2	0.0	0.0	0.0	4.2
Pediococcus	0.0	0.0	0.4	0.0	0.0
Leuconostoc	0.0	0.2	0.0	0.0	0.0
Pseudomonas	0.0	0.0	0.1	0.0	<0.1
Bacillus	0.1	0.0	0.0	0.0	0.0
Lactococcus	0.0	0.1	0.0	0.0	0.0
unidentified	2.9	5.2	2.8	2.6	1.8

Table A.9: Top ten fungal classes and genera in sourdough starters

Fungal community	Normalised abundance (%)				
	SWW	SWP	SWM	SB1	SR2
Class					
Saccharomycetes	<0.1	81.7	72.9	95.7	80.6
Leotiomycetes	58.5	12.6	0.9	2.9	0.0
Dothideomycetes	27.3	1.3	1.0	1.1	5.7
Eurotiomycetes	4.6	0.4	0.0	0.0	0.0
Sordariomycetes	6.6	0.9	0.0	0.3	0.3
Tremellomycetes	0.6	0.0	0.0	0.0	0.0
Ustilaginomycetes	0.1	0.0	0.0	0.0	0.0
Microbotryomycetes	0.1	0.0	0.0	0.0	0.0
Mucoromycetes	0.0	<0.1	0.0	0.0	0.0
unidentified	2.3	3.1	25.2	0.0	13.4
Genus					
Saccharomyces	0.0	81.5	72.9	95.9	13.5
Blumeria	59.5	12.6	0.9	2.9	0.0
Kazachstania	0.0	0.1	0.0	0.0	67.7
Alternaria	11.3	0.7	0.3	0.7	1.2
Didymella	9.5	0.3	0.4	0.0	1.2
Mycosphaerella	6.1	0.3	0.2	0.2	2.2
Xeromyces	4.4	0.4	0.0	0.0	0.0
Gibberella	5.2	0.5	0.0	0.3	0.0
Fusarium	0.7	0.4	0.0	0.0	0.0
unidentified	3.2	3.1	25.2	0.0	14.2

Table A.10: Top ten bacterial classes and genera in breads

Bacterial community	Normalised abundance (%)				
	BWW	BWP	BWM	BW15	BW25
Class					
Bacilli	6.7	53.7	52.3	3.0	0.2
Gammaproteobacteria	74.2	32.8	33.7	63.1	79.5
Actinobacteria	13.8	4.7	6.3	29.0	15.6
Oxyphotobacteria	1.5	5.1	4.3	0.6	1.3
Alphaproteobacteria	2.2	2.9	3.3	1.3	0.4
Bacteroidia	1.0	0.7	0.1	2.4	2.6
Clostridia	0.3	0.1	0.0	0.6	0.0
Acidobacteriia	0.2	0.0	0.0	0.0	0.1
Fusobacteriia	0.1	0.0	0.0	0.0	0.2
Planctomycetacia	0.1	0.0	0.0	0.0	0.2
Genus					
Pseudomonas	73.4	26.3	32.8	65.9	79.3
Lactobacillus	4.2	52.9	51.7	3.3	0.2
Rhodococcus	14.4	4.6	6.3	26.9	16.0
Ralstonia	1.6	5.1	0.5	0.5	1.7
Prevotella	0.3	0.4	0.0	1.4	0.8
Sphingomonas	0.2	0.8	0.8	0.2	0.0
Neisseria	<0.1	1.1	0.2	0.0	0.0
Streptococcus	0.1	0.8	0.8	0.0	0.0
Staphylococcus	2.5	0.4	0.1	0.0	0.0
unidentified	3.2	7.6	6.8	1.8	1.9

Table A.11: Top ten fungal classes and genera in breads

Fungal community	Normalised abundance (%)				
	BWW	BWP	BWM	BW15	BW25
Class					
Saccharomycetes	20.7	72.5	62.5	35.0	29.8
Dothideomycetes	13.5	8.5	7.5	54.5	18.1
Leotiomycetes	36.0	14.1	15.5	2.7	2.9
Sordariomycetes	12.0	2.3	2.0	0.7	3.4
Malasseziomycetes	11.8	0.0	0.0	0.0	2.8
Tremellomycetes	1.2	0.7	0.1	1.9	11.4
Agaricomycetes	4.9	0.0	0.0	1.5	4.4
Cystobasidiomycetes	0.0	0.0	0.0	1.9	2.0
Pezizomycotina	0.0	0.0	0.0	0.0	2.7
unidentified	0.0	1.9	12.4	1.7	22.6
Genus					
Saccharomyces	0.0	75.3	66.0	36.4	0.5
Blumeria	52.8	14.8	16.4	3.0	3.3
Aureobasidium	0.0	0.0	0.0	56.4	15.3
Malassezia	15.0	0.0	0.0	0.0	3.2
Candida	0.0	5.8	2.0	0.0	4.7
Trichosporon	0.0	0.0	0.0	1.6	12.0
Didymella	9.9	1.9	2.6	0.0	0.0
Colletotrichum	13.2	0.0	0.0	0.7	0.0
Debaryomyces	0.0	0.0	0.0	0.0	26.1
unidentified	9.1	2.2	13.1	2.0	34.8

Table A.12: Top ten bacterial classes and genera in main sourdough starters and SSBs

Bacterial community	Normalised abundance (%)					
	Wheat starter		Pumpkin starter		Mung bean starter	
	SWW	BWW	SWP	BWP	SWM	BWM
Class						
Bacilli	92.9	6.7	94.8	53.7	97.1	52.3
Gammaproteobacteria	0.1	74.2	0.0	32.8	0.1	33.7
Actinobacteria	0.0	13.8	0.0	4.7	0.0	6.3
Alphaproteobacteria	5.8	2.2	2.4	2.9	2.0	3.3
Oxyphotobacteria	1.3	1.5	2.8	5.1	0.8	4.3
Bacteroidia	0.0	1.0	0.0	0.7	0.0	0.1
Clostridia	0.0	0.3	0.0	0.1	0.0	0.0
Acidobacteriia	0.0	0.2	0.0	0.0	0.0	0.0
Fusobacteriia	0.0	0.1	0.0	0.0	0.0	0.0
Planctomycetacia	0.0	0.1	0.0	0.0	0.0	0.0
Genus						
Lactobacillus	92.8	4.2	94.8	53.7	96.7	51.6
Pseudomonas	0.0	73.6	0.0	26.7	0.1	32.8
Rhodococcus	0.0	14.4	0.0	4.7	0.0	6.3
Ralstonia	0.0	1.6	0.0	5.1	0.0	0.5
Acetobacter	4.2	0.0	0.0	0.0	0.0	0.0
Staphylococcus	0.0	2.6	0.0	0.4	0.0	0.1
Sphingomonas	0.0	0.2	0.0	0.8	0.0	0.8
Streptococcus	0.0	0.1	0.0	0.8	0.0	0.8
Pediococcus	0.0	0.0	0.0	0.0	0.4	0.3
unidentified	2.9	3.2	5.2	7.7	2.8	6.8

Table A.13: Top ten fungal classes and genera in main sourdough starters and SSBs

Fungal community	Normalised abundance (%)					
	Wheat starter		Pumpkin starter		Mung bean starter	
	SWW	BWW	SWP	BWP	SWM	BWM
Class						
Saccharomycetes	<0.1	20.2	81.7	72.4	72.9	62.5
Leotiomycetes	58.5	35.1	12.6	14.1	0.9	15.5
Dothideomycetes	27.3	13.1	1.3	8.5	1.0	7.5
Sordariomycetes	6.6	11.7	0.9	2.3	0.0	2.0
Malasseziomycetes	0.0	11.5	0.0	0.0	0.0	0.0
Eurotiomycetes	4.6	0.0	0.4	0.1	0.0	0.0
Agaricomycetes	0.0	4.8	0.0	0.0	0.0	0.0
Microbotryomycetes	0.1	2.4	0.0	0.0	0.0	0.0
Tremellomycetes	0.6	1.1	0.0	0.7	0.0	0.1
unidentified	2.3	0.0	3.1	1.9	25.2	12.4
Genus						
Saccharomyces	0.0	0.0	82.8	75.0	72.9	63.8
Blumeria	66.4	40.8	12.8	14.7	0.9	15.8
Alternaria	12.6	3.5	0.7	4.6	0.3	4.0
Didymella	10.6	7.6	0.3	1.9	0.4	2.5
Mycosphaerella	6.8	3.8	0.3	1.7	0.2	1.2
Malassezia	0.0	11.6	0.0	0.0	0.0	0.0
Colletotrichum	0.0	10.2	0.0	0.0	0.0	0.0
Dekkera	0.0	8.0	0.0	0.0	0.0	0.0
Brettanomyces	0.0	7.4	0.0	0.0	0.0	0.0
unidentified	3.6	7.0	3.2	2.2	25.2	12.7

Table A.14: Top ten bacterial classes and genera in sourdough starters, SSBs, baked breads plus other fermented foods

Bacterial community	Normalised abundance (%)											
	Sourdough starter					Bread					Others	
	SWW	SWP	SWM	SB1	SR2	BWW	BWP	BWM	BW15	BW25	KFR	YGT
Class												
Bacilli	92.9	94.8	97.1	97.4	94.0	6.7	53.7	52.3	3.0	0.2	99.3	100.0
Gammaproteobacteria	0.1	0.0	0.1	0.0	<0.1	74.3	32.8	33.7	63.1	79.3	0.2	0.0
Actinobacteria	0.0	0.0	0.0	0.0	0.0	13.8	4.7	6.3	29.0	15.6	0.5	0.0
Alphaproteobacteria	5.8	2.4	2.0	1.1	5.1	2.2	2.9	3.3	1.3	0.4	0.0	0.0
Oxyphotobacteria	1.3	2.8	0.8	1.5	0.8	1.5	5.1	4.3	0.6	1.3	0.0	0.0
Bacteroidia	0.0	0.0	0.0	0.0	0.0	1.0	0.7	0.1	2.4	2.6	0.0	0.0
Clostridia	0.0	0.0	0.0	0.0	0.0	0.3	0.1	0.0	0.6	0.0	0.0	0.0
Acidobacteriia	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0.0
Fusobacteriia	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0
Negativicutes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
Genus												
Lactobacillus	92.8	94.7	97.1	97.4	94.0	4.4	53.9	51.8	3.3	0.2	8.4	93.1
Pseudomonas	0.0	0.0	0.1	0.0	<0.1	75.5	26.8	32.9	66.9	80.0	0.3	0.0
Rhodococcus	0.0	0.0	0.0	0.0	0.0	14.8	4.7	6.3	27.3	16.2	0.5	0.0
Ralstonia	0.0	0.0	0.0	0.0	0.0	1.7	5.2	0.5	0.5	1.8	0.0	0.0
Streptococcus	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.8	0.0	0.0	0.0	6.9
Acetobacter	4.2	0.0	0.0	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enterococcus	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	4.1	0.0
Sphingomonas	0.0	0.0	0.0	0.0	0.0	0.2	0.8	0.8	0.2	0.0	0.0	0.0
Lactococcus	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	86.7	0.0
unidentified	2.9	5.2	2.8	2.6	1.8	3.3	7.7	6.8	1.8	2.0	0.0	0.0

Table A.15: Top ten fungal classes and genera in sourdough starters, SSBs, baked breads plus other fermented foods

Fungal community	Normalised abundance (%)											
	Sourdough starter					Bread					Others	
	SWW	SWP	SWM	SB1	SR2	BWW	BWP	BWM	BW15	BW25	KFR	YGT
Class												
Saccharomycetes	<0.1	81.7	72.9	95.7	80.6	20.7	72.4	62.5	35.0	30.0	100.0	100.0
Leotiomycetes	58.6	12.6	0.9	2.9	0.0	36.0	14.1	15.5	2.7	2.9	0.0	0.0
Dothideomycetes	27.3	1.3	1.0	1.1	5.7	13.5	8.5	7.5	54.5	18.3	0.0	0.0
Sordariomycetes	6.6	0.9	0.0	0.2	0.3	12.0	2.3	2.0	0.7	3.4	0.0	0.0
Tremellomycetes	0.6	0.0	0.0	0.0	0.0	1.2	0.7	0.1	1.9	11.5	0.0	0.0
Malasseziomycetes	0.0	0.0	0.0	0.0	0.0	11.8	0.0	0.0	0.0	2.8	0.0	0.0
Eurotiomycetes	4.6	0.4	0.0	0.0	0.0	0.0	0.1	0.0	0.1	1.9	0.0	0.0
Agaricomycetes	0.0	0.0	0.0	0.0	0.0	4.9	0.0	0.0	1.5	4.4	0.0	0.0
Cystobasidiomycetes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	2.0	0.0	0.0
unidentified	2.3	3.1	25.2	0.0	13.4	0.0	1.9	12.4	1.7	22.7	0.0	0.0
Genus												
Saccharomyces	0.0	82.6	72.9	96.1	13.5	0.0	75.0	63.7	37.1	0.6	4.0	0.0
Kazachstania	0.0	0.2	0.0	0.0	67.7	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Blumeria	66.2	12.8	0.9	2.9	0.0	65.1	14.7	15.8	3.0	4.1	0.0	0.0
Aureobasidium	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	57.4	19.1	0.0	0.0
Alternaria	12.5	0.7	0.3	0.7	1.2	5.6	4.6	4.0	0.0	0.0	0.0	0.0
Didymella	10.6	0.3	0.4	0.0	1.2	12.2	1.9	2.5	0.0	0.0	0.0	0.0
Mycosphaerella	6.8	0.3	0.2	0.2	2.2	6.0	1.7	1.2	0.0	0.3	0.0	0.0
Debaryomyces	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.5	0.0	0.0
Kluyveromyces	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	96.0	100.0
unidentified	3.6	3.1	25.2	0.0	14.2	11.2	2.2	12.7	2.1	43.4	0.0	0.0

Table A.16: Lactic acid bacteria (LAB) and yeast species detected in sourdough starters, SSBs, baked breads plus other fermented foods

Species	Relative abundance (%)											
	Sourdough starter					Bread					Others	
	SWW	SWP	SWM	SB1	SR2	BWW	BWP	BWM	BW15	BW25	KFR	YGT
LAB												
Lb. brevis	0.6	2.8	1.1	0.0	0.0	0.0	1.1	0.7	0.0	0.0	0.0	0.0
Lb. fermentum	91.0	90.3	94.5	0.0	0.0	3.1	50.7	48.3	0.0	0.0	0.0	0.0
Lb. pontis	0.0	0.0	0.0	96.5	92.9	0.6	0.0	1.9	2.7	0.2	0.0	0.0
Lb. delbrueckii subsp. *bulgaricus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.4
Lb. kefiri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0
Lc. lactis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<0.1	0.0
L. lactis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0
L. mesenteroides	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0
P. acidilactici	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Strep. thermophilus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.9
Yeast												
C. glaebosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
C. parapsilosis	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0	1.4	5.6	0.0	0.0
Cyberlindnera jadinii	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.2	0.0	0.0	0.0
Debaryomyces hansenii	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.7	0.0	0.0
Dekkera anomala	0.0	0.0	0.0	0.0	0.0	6.8	0.0	0.0	0.0	0.0	0.0	0.0
K. exigua	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
K. humilis	0.0	0.1	0.0	0.0	67.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lachancea kluyveri	0.0	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T. delbrueckii	0.0	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wi. anomalus	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix B: Method References

Table B.1: The ingredients and breadmaking steps for the outsourced, baked breads using dry yeast (BW15) and sourdough starter (BW25)

Baked bread	BW15	BW25
Ingredients	bread flour sugar salt dry yeast milk egg butter	bread flour water sourdough starter salt
Basic steps	mixing (all except butter) ↓ kneading (15 min) ↓ adding butter ↓ kneading ↓ dividing ↓ proofing ↓ baking (150 °C, 40-45 min) ↓ cooling	mixing (flour + water) ↓ resting (1 hr) ↓ adding starter ↓ resting (10 min) ↓ adding salt ↓ resting (30 min) ↓ 4 cycles of kneading + resting (30 min) ↓ resting (0.5 - 1 hr) ↓ 3 cycles of kneading + resting (0.5 -1 hr) ↓ cold retard fermentation (overnight or 8 – 12 hr) ↓ baking with lid (250 °C, 20min) ↓ baking without lid (200 °C, 20min) ↓ cooling

Table B.2: The method references of proximate analysis for each parameter used by ALS Technichem Sdn. Bhd.

Parameter	Method references	References
Total carbohydrates	Method of Analysis for Nutrition Labeling (1993), pg 106	(Sullivan & Carpenter, 1993)
Energy	Method of Analysis for Nutrition Labeling (1993), pg 5 & 106	
Protein	AOAC Official Method 972.43	
Total dietary fibre	AOAC Official Method 985.29	
Moisture	In-house Method QWI-OF/17-38 Moisture Analyzer	
Ash	Method of Analysis for Nutrition Labeling (1993), Chapter 10	
Fat	Method of Analysis for Nutrition Labeling (1993), Chapter 18 & Pearson's (1991), pg 24	(Kirk & Sawyer, 1991; Sullivan & Carpenter, 1993)

Table B.3: The descriptions for sensory attributes of steamed sourdough buns

Attributes	Descriptions
Texture	A quarter of the sample is pressed with the finger. If the bread regains its original form, the texture is regarded as satisfactory. Score: 1 = no springback; 9 = fast and full springback
Colour	The scale goes from dull white to bright orange Score: 1 = dull white; 9 = bright orange
Taste	The scale goes from sharp sour taste to sweet taste with slight tart Score: 1 = sharp sour taste; 9 = sweet taste with slight tart
Aroma	Combination of intensities of smell and taste Score: 1 = low intensity; 9 = high intensity
Overall liking	The scale goes from very unpleasant to very satisfying Score: 1 = very unpleasant; 9 = very satisfying

Appendix C: Experimental Images

(a) (b) (c)

Figure C.1: Flours made of (a) wheat, (b) pumpkin and (c) mung bean used for sourdough fermentation

Appendix D: **Zwitering Re-parameterisation of Gompertz Equation**

$$w(t) = a\exp[-\exp(b-ct)] \qquad (2.4)$$

Let $r = -\exp(b-ct)$,

$$\frac{dr}{dt} = -(-c)\exp(b-ct) = c\exp(b-ct)$$

$$w(r) = a\exp(r), \frac{dw}{dr} = a\exp(r)$$

$$\therefore \frac{dw}{dt} = \frac{dw}{dr}\cdot\frac{dr}{dt}$$

$$= a\exp(r)\cdot c\exp(b-ct)$$

$$= ac\exp[-\exp(b-ct)]\cdot\exp(b-ct)$$

Let $u = ac\exp[-\exp(b-ct)]$, $v = \exp(b-ct)$

$$\frac{du}{dt} = ac\exp[-\exp(b-ct)]\cdot c\exp(b-ct)$$

$$= ac^2\exp[-\exp(b-ct)]\cdot\exp(b-ct)$$

$$\frac{dv}{dt} = -c\exp(b-ct)$$

$$\frac{d^2w}{dt^2} = u\frac{dv}{dt} + v\frac{du}{dt}$$

$$\frac{d^2w}{dt^2} = ac\cdot\exp[-\exp(b-ct)]\cdot -c\exp(b-ct) +$$

$$\exp(b-ct)\cdot ac^2\exp[-\exp(b-ct)]\cdot\exp(b-ct)$$

$$= -ac^2\exp[-\exp(b-ct)]\cdot\exp(b-ct) +$$

$$ac^2\exp(b-ct)\cdot\exp[-\exp(b-ct)]\cdot\exp(b-ct)$$

$$= ac^2\exp[-\exp(b-ct)]\cdot\exp(b-ct)\cdot[-1+\exp(b-ct)]$$

At inflection point, $\frac{d^2w}{dt^2} = 0$

$\therefore ac^2 \exp[-\exp(b-ct)] \cdot \exp(b-ct) \cdot [-1+\exp(b-ct)] = 0$

To solve for t at inflection point, t_i

$ac^2 \exp[-\exp(b-ct_i)] = 0$ or $\exp(b-ct_i) = 0$ or $[-1+\exp(b-ct_i)] = 0$

Since any exponent will not equal to zero, only $[-1+\exp(b-ct_i)] = 0$ is valid.

$[-1+\exp(b-ct_i)] = 0$

$\exp(b-ct_i) = 1$

$b - ct_i = 0, \therefore t_i = \frac{b}{c}$

$$w(t_i) = a\exp\{-\exp[b-c\left(\frac{b}{c}\right)]\}$$
$$= a\exp[-\exp(b-b)]$$
$$= a\exp[-\exp(0)]$$
$$= a\exp(-1)$$
$$= \frac{a}{e}$$

Substitute $t = t_i = \frac{b}{c}$,

$$\frac{dw}{dt} = ac\exp\{-\exp[b-c\left(\frac{b}{c}\right)]\} \cdot \exp[b-c\left(\frac{b}{c}\right)]$$
$$= ac\exp[-\exp(b-b)] \cdot \exp(b-b)$$
$$= ac\exp[-\exp(0)] \cdot \exp(0)$$
$$= ac\exp(-1) \cdot 1$$
$$= \frac{ac}{e}$$

$\frac{dw}{dt}$ refers to gradient at inflection point $(\frac{b}{c}, \frac{a}{e})$, $\therefore \frac{ac}{e}$ was represented by μ.

Let the equation of tangent at inflection point to be in the form of $y = mx + c$,

m refers to the gradient, thus $m = \mu$,

whereas for c, the interception, the unknown is represented by C to avoid confusion.

$y = \mu t + C$

At inflection point, $(t, y) = (\frac{b}{c}, \frac{a}{e})$ and $\mu = \frac{ac}{e}$,

$$\frac{a}{e} = \frac{ac}{e}(\frac{b}{c}) + C$$

$$\frac{a}{e} = \frac{ab}{e} + C$$

$$C = \frac{a}{e} - \frac{ab}{e}$$

$$= \frac{a}{e}(1 - b)$$

$$\therefore y = \frac{ac}{e}t + \frac{a}{e}(1 - b)$$

The t-axis intercept is re-parameterised as lag time at $(t, y) = (t_{lag}, 0)$

$$0 = \frac{ac}{e}t_{lag} + \frac{a}{e}(1 - b)$$

$$-\frac{a}{e}(1 - b) = \frac{ac}{e}t_{lag}$$

$$t_{lag} = -\frac{a}{e}(1 - b)(\frac{e}{ac})$$

$$= -\frac{(1 - b)}{c}$$

$$= \frac{b - 1}{c}$$

To find the asymptote,

$$\lim_{t\to\infty}[w(t)] = \lim_{t\to\infty}\{a \exp[-\exp(b - ct)]\}$$

Since $\lim_{x\to a}[c \cdot f(x)] = c \cdot \lim_{x\to a}[f(x)]$,

$$\lim_{t\to\infty}[w(t)] = a \cdot \lim_{t\to\infty}\{\exp[-\exp(b - ct)]\} = a$$

Therefore, asymptote is a, that is re-parameterised as A.

$w(t) = a\exp[-\exp(b-ct)]$ is re-parameterised as $L(t)$ with

$a = A$,

$$\mu = \frac{ac}{e}$$

$$= \frac{Ac}{e}$$

$\therefore c = \frac{\mu e}{A}$,

$$t_{lag} = \frac{b-1}{c}$$

$$(t_{lag})(c) = b - 1$$

$$b = (t_{lag})(c) + 1$$

$$= (t_{lag})\left(\frac{\mu e}{A}\right) + 1$$

$$\therefore L(t) = A\exp\{-\exp\left[t_{lag}\left(\frac{\mu e}{A}\right) + 1 - \left(\frac{\mu e}{A}\right)t\right]\}$$

$$= A\exp\{-\exp\left[\frac{\mu e}{A}(t_{lag} - t) + 1\right]\}$$

LIST OF PUBLICATIONS

Lau, S. W., Chong, A. Q., Chin, N. L., Talib, R. A., & Basha, R. K. (2021).Sourdough microbiome comparison and benefits. Microorganisms, 9(7), 1355. https://doi.org/10.3390/microorganisms9071355

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Lau, S. W., Chin, N. L., Talib, R. A., & Basha, R. K. (2024). Modelling the three-stage activation process of composite sourdough starters developed using pumpkin and mung bean flours. Journal of Agriculture and Food Research, 15, 100982. https://doi.org/10.1016/j.jafr.2024.100982