Development and Characterisation of Plant-based (faba bean) Yoghurt

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Abstract

Increasing awareness of health, environmental, and ethical issues has recently increased the demand for plant-based alternatives to traditional dairy products. Plant-based milk might be a suitable choice for people looking for a healthier option because it often contains less fat, particularly saturated fat, and allergens. In recent years, the faba bean has gradually become popular as a plant protein due to its nutritional and health benefits to consumers.

This master's thesis investigates the possibility of producing yoghurt using faba bean milk analogue (FBM), faba bean protein isolates (FPI) and faba bean-dairy hybrid as alternatives to traditional dairy yoghurt. Also, the effects of varying ratios of dairy and faba bean proteins on the texture, rheology, microstructure, nutritional makeup, protein digestibility, and sensory characteristics of yoghurt were investigated. This study aimed to optimize the formulation of faba bean yoghurt to develop a sustainable and nutrient-rich plant-based alternative to dairy yoghurt through a series of analytical procedures.

The study's objectives include examining the interactions between faba bean milk analogue, faba bean protein isolates, and dairy milk (DM) in yoghurt production using five different formulations. These formulations include 50% dairy + 50% faba bean milk analogue (SM#2), 50% dairy + 50% faba bean protein isolates, 50% faba bean milk analogue + 50% faba bean protein isolates (SM#3), 100% faba bean milk analogue (SM#4), 50% faba bean milk analogue + 50% faba bean protein isolates (SM#5) and 100% faba bean protein isolates (SM#6). Further, assessing how these interactions affect the final product's texture, rheology, microstructure, and digestibility. The nutritional composition of various faba bean yoghurt formulations was analyzed, focusing on protein, fat, total solids, fibre, and starch content, and compared with traditional dairy yoghurt. The sensory survey was done using an internal untrained panel to evaluate the consumer preferences for different formulations based on attributes such as taste, aroma, texture, colour, and overall acceptability. Additionally, it investigated the influence of varying ratios of FBM, FPI and DM on the rate and extent of protein digestion using a static digestion (INFOGEST) model.

Results indicated that yoghurts made with FPI exhibited higher storage (G’) and loss modulus (G”) values, indicating a stronger gel structure compared to those made with DM alone (reference). The product made with DM and FPI (SM#3) showed the lowest G’ and G”. Similar behaviour was observed for apparent viscosity as well. The product made with DM and FBM (SM#2), showed comparatively similar rheological properties. All yoghurt samples' loss tangent (tan δ) values were consistently less than one, indicating a mostly solid character. Texture profile analysis results of the samples showed a significant difference in textural properties like hardness, cohesiveness, springiness, gumminess, and adhesiveness between samples ($p<0.05$). SM#2 and SM#5 showed the least deviation from the reference (100% dairy yoghurt). Faba bean yoghurt also demonstrated better water-holding capacity and lower syneresis, particularly in formulations with higher FPI (50% and 100%) content (SM#5 & SM#6). Sensory evaluations showed that consumers preferred the formulations that closely matched the texture and flavour of traditional dairy yoghurt. The hybrid formulations of DM and FBM (SM#2) showed the least deviation from the reference (100% DM). In-vitro gastrointestinal digestion was conducted following the INFOGEST method to assess the protein digestibility of the yoghurt samples. Protein hydrolysis expressed as free amino N of all yoghurt samples by pepsin during the gastric phase (0 to 120 min) was relatively lower than in the small intestinal phase. However, all samples including 100% dairy reference showed a significant increase ($p<0.05$) in the release of free amino N upon the addition of pancreatin and bile salt during the simulated small intestine phase (130-240 min) compared to the gastric phase. The free amino N release (%) was significantly lower ($p<0.05$) in 100% plant-based yoghurts (SM#4, SM#5 & SM#6) compared to dairy and plant-based hybrid samples (SM#2 & SM#3).

In conclusion, the findings suggested that faba bean yoghurt, particularly when blended with DM, can potentially meet consumer preferences and textural properties while providing a nutritious and sustainable alternative to dairy yoghurt. In addition, there is a high potential of producing 100% vegan yoghurt from faba bean proteins, but further studies on improving textural and sensory properties are needed. The study contributes to exploring the options available in the plant-based food market, addressing both environmental and nutritional needs.

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Abbreviations

LAB	Lactic Acid Bacteria
pl	Isoelectric Point
PPI	Potato Protein Isolates
DM	Dairy milk
FBM	Faba bean milk analogue
FPI	Faba bean protein isolates
TTA	Titratable Acidity
WHC	water-holding capacity
SAOR	Small amplitude oscillatory rheology
G’	Storage modulus
G”	loss modulus
LT	loss tangent
TPA	Texture Profile Analysis
CSLM	Confocal laser scanning microscopy
SSF	Simulated salivary fluid
SGF	Simulated gastric fluid
SSF	Simulated intestinal fluid

Sample (Product) Short Key

Sample Code	Product Formulation
Reference	100% DM
SM#2	50% DM + 50% FBM
SM#3	50% D M+ 50% FPI
SM#4	100% FBM
SM#5	50% FBM+ 50% FPI
SM#6	100% FPI

Chapter 1. Introduction

1.1 Background Information

Increasing awareness of health, environmental, and ethical issues has recently boosted the demand for plant-based alternatives to traditional dairy products. In many Western countries, the sale of plant-based milk is increasing while the consumption of dairy products is dropping (Islam et al., 2021). Consumers are choosing plant-based alternatives to milk and dairy products for various reasons, including increased knowledge of the negative environmental effects of dairy farming, ethical concerns, medical reasons like lactose intolerance and cow's milk allergy, and a growing belief that milk is unhealthy (McCarthy et al., 2017). Plant-based milk is a viable substitute for people who cannot drink dairy milk for medical reasons, as they are free from lactose or any of the allergens found in cow's milk. However, certain plant-based milk, such as soy or nut-based milk, contain additional allergens. People seeking lower-fat options might be suitable because they often contain less fat, particularly saturated fat. Furthermore, dietary fibre can be added to plant-based milk during its production. However, except for soy, which is equivalent to dairy milk, most currently available plant-based milk substitutes are lower in protein (Harper et al., 2022).

In recent years, the Faba bean has gradually become popular as a better alternative for plant protein. Whole faba beans have the following nutritional values: 20–35% protein, 1-2% fat, 55–65% carbohydrates, 10-15% fibre, and several vitamins and minerals like magnesium, calcium, potassium, iron, and zinc (Labba et al., 2021). Several health benefits, including antioxidant qualities and the suppression of enzyme activity during the digestion of carbohydrates, have been linked to the phytochemicals found in faba bean components (Mattila et al., 2018). Additionally, as faba beans contain L-3,4-dihydroxyphenylalanine (L-DOPA), a neurotransmitter precursor and medication used to treat Parkinson's disease, they also have medicinal value (Multari et al., 2015). As an alternative to conventional ingredients like casein, whey, soy, and wheat protein, faba beans are receiving much attention in scientific research because of their nutritional value and techno-functional qualities (Badjona et al., 2023).

It is possible to process legumes to create flours, protein isolates and concentrates with different compositions and uses. When it comes to product formulation, protein functioning is crucial because it has a direct impact on the sensory, physicochemical, and mechanical properties of the product. For example, protein components with sufficient solubility and strong emulsifying qualities are perfect for making plant-based milk substitutes. The use of fractionated protein ingredients (e.g., protein isolates) in product formulation helps in regulating product characteristics rather use of protein extracts directly from seeds. For example, faba bean protein isolates may be utilized in the development of high-protein and low-fat dairy analogues due to its good solubility and emulsifying properties (Shi & Nickerson, 2022).

The presence of certain antinutritional compounds in faba beans has limited their usage in food production (Labba et al., 2021). However, various processing methods, including soaking, germination, heating, dehulling, and roasting, reduce or eliminate antinutritional elements from pulses, improving their nutritional profiles (Cheynier, 2005).

The presence of undesired "beany" flavours and textures contributed to a reluctance to adopt plant-based milk substitutes, particularly those from legumes such as soy and faba beans. A certain amount of contributing volatile organic compounds in plant-based milk can be eliminated or reduced by fermenting the milk with various Lactic acid bacteria (LAB) (Harper et al., 2022). LAB also breaks down antinutritional components found in plants, such as tannins, saponins, phytic acid, and trypsin inhibitors, during fermentation, enhancing the nutritional value of plant-based products (Adeyemo & Onilude, 2013). Thus, fermentation and the creation of fermented products may provide an important tool for improving the nutritional value and acceptance of plant-based milk alternatives.

Although the well-documented fermentation of dairy milk by lactic acid bacteria is available, more research needs to be done on the molecular mechanisms behind fermentation in plant-based milk (Ji et al., 2021). Plant-based substitutes for dairy products like cheese and yoghurt are becoming increasingly popular, but producing them with flavours and textures that people like is a challenge (Jaeger & Giacalone, 2021).

Yoghurt, a popular dairy product, has long been accepted for its probiotic content and nutritional benefits. However, many plant-based yoghurts on the market rely heavily on soy, almond, or coconut as their base, each with its limitations (Gupta et al., 2022; Montemurro et al., 2021). Faba bean gives a novel and untapped opportunity to create a dairy-free alternative that not only mimics the creamy texture of traditional yoghurt but also offers a unique nutritional profile (Jiang et al., 2020).

This master's thesis investigates the complex relationship between flavour, texture, and nutritional value to determine the viability of faba bean either solely or combining it with dairy milk (hybrid) for formulating yoghurt.

1.2 Research Objectives

1. Examine the interactions between faba bean milk analogue, faba bean protein isolates, and dairy milk in yoghurt production, assessing how different ratios affect the final product's texture, rheology, and microstructure.
2. Analyze the proximate composition of faba bean yoghurt formulations, focusing on protein, fat, total solids, fibre, and starch content, and compare them with traditional dairy yoghurt.
3. Conduct informal sensory evaluation with an untrained consumer panel to assess consumer preferences for various faba bean yoghurt formulations, considering attributes such as taste, aroma, texture colour, and overall acceptability.
4. Investigate the influence of varying ratios of faba bean milk analogue, faba bean protein isolates, and dairy milk incorporation into yoghurt on the rate and extent of in vitro protein digestion in the gastrointestinal tract using a static digestion model.

Chapter 2. Literature Review

2.1 History and health benefits of yoghurts

Dairy products are one of the principal by-products of animal husbandry. They were crucial to human society's evolution and a significant protein source for many early human civilizations. Yoghurt was first made industrially by Isaac Carasso in 1919, while it is thought that humans created it as early as 5,000 BC on the Mesopotamian plains (Aryana & Olson, 2017).

The term "Yoghurt" refers to a milk product that has undergone fermentation using a mixed starting culture that contains *Lactobacillus delbrueckii sp. bulgaricus* and *Streptococcus thermophilus*. In the commercial manufacturing of yoghurt, milk that contains additional milk solids (not fat) and other additives is heated to 85 ^{0}C for 30 minutes, cooled to 43 ^{0}C, and then inoculated with 2% starter culture (*L bulgaricus and S thermophilus*). After incubating at 42 ^{0}C for approximately 4 hours, the inoculated yoghurt base is allowed to ferment and thicken until 0.9% acidity is obtained (Chandan et al., 2017).

Yoghurt is a nutrient-dense food with vital growth elements, including protein, vitamins, and minerals. It can enhance the overall diet quality and raise the likelihood of meeting dietary requirements such as recommended daily allowance. For those who are lactose intolerant, yoghurt is a great way to get all the advantages of milk products without having to deal with the discomforts of hypolactasia. Yoghurt functions as a food that carries probiotics and is thought to be simple to add to meals, leading to high probiotic viability. Probiotics are stated to have therapeutic benefits such as prevention of urogenital infections, easing of constipation, protection against diarrhoea, prevention of hypercholesterolemia, protection against colon/bladder cancer, and prevention of osteoporosis, etc. (Chandan et al., 2017; Weerathilake et al., 2014).

It has been observed that foods with varying physicochemical characteristics, such as viscosity and microstructure, have varying retention times in the digestive system, especially in the gastric phase. This results in variations in the rate of digestion and the amount of time that the food's constituent parts are exposed to the digestive fluids. For instance, compared to milk consumed in liquid form, milk gelled by rennet exhibited a delay in gastric digestion, which in turn resulted in a reduced quantity of protein collected at the duodenal and a lowered amino acid bioavailability (Barbe et al., 2013). Fermentation is a commonly utilized technique in the dairy industry for processing food, primarily because it can increase milk's shelf life, enhance the final products' texture and flavours, and boost their health benefits (Salampessy & Kailasapathy, 2011). Regular yoghurt consumption has been linked to several health benefits, including a suppressed acute intestinal inflammation, an 18% lower risk of type 2 diabetes, a 17% lower risk of high blood pressure, and a 21% lower risk of cardiovascular diseases, according to prior human clinical studies (Nguyen et al., 2020). Further, studies have shown that fermentation and pepsin digestion facilitate the release of bioactive peptides (Fitzgerald & Murray, 2006). The lactic acid bacteria and the gut microbiota, as well as the bioactive peptides produced during fermentation and those released during gastrointestinal digestion of the fermented product, are most likely responsible for the nutritional and health benefits of yoghurt over raw milk (Chabance et al., 1998).

2.2 Trends in plant-based yoghurt

Due to the projected rise of the world population by more than one billion people over the following 13 years, reaching 9.8 billion by 2050, the increase in global food consumption will result in a significant increase in overall food production by that time. In high-income nations, the amount of protein consumed daily has been increasing over the past 50 years. An increase of 39 to 52 g per capita was seen between 1961 and 2011, with the majority of this increase derived from meat, eggs, milk, and dairy products (Grasso et al., 2020). The Food and Agriculture Organisation projects that daily protein consumption per person will be 54 g in 2030 and 57 g in 2050, respectively (Manida, 2022). The rising living standards in developing nations and urbanization have also changed the types of food that consumers demand, and if current trends continue unchanged, this will eventually result in a rise in the production of animal food (Boland et al., 2013). However, in developed countries, there is a declining market for animal-based food due to growing consumer knowledge of the effects of food production on the environment and consumption on human health (Henchion et al., 2017). As a result, a lot of food manufacturers and researchers are concentrating on developing sustainable and healthy alternative food items to meet modern consumer needs and environmental concerns (Sethi et al., 2016).

Plant protein intake is increasing, evidenced by the yearly growth rates of 11% and 14% for dairy substitutes and meat, respectively. Among the dairy-free options, plant-based yoghurts make up a significant number that appeal to a wide range of consumers, including those with ethical concerns and dairy allergies (Grasso et al., 2020).

In the next decades, there will be a substantial need for protein due to the world's rapidly growing population. New products made from low-carbon protein sources must be invented to satisfy market demand (Gupta et al., 2022). Though conventional yoghurt is rich in protein and nutritional makeup, it may cause allergies and other health issues to consumers. Thus, lactic acid bacteria (LAB) fermenting plant raw materials has led to the development of plant-based yoghurt. The main sources of raw materials for plant-based yoghurt include nuts (e.g., almonds and walnuts), coconut, legumes (e.g., soybean, pea, and mung bean), and cereals or pseudo cereals (e.g., oat, millet, and quinoa) (Zannini et al., 2018; Zhou et al., 2019). These dairy-free yoghurts are high in protein, low in lactose and cholesterol, and appropriate for people with health concerns such as allergies to cow's milk, hyperlipidaemias, and/or lactose intolerance (Grasso et al., 2020). Further, these plant-based yoghurts are rich in unsaturated fatty acids compared to traditional dairy yoghurt (Yang et al., 2021).

The plant-based market is expected to expand at a compound annual growth rate (CAGR) of 20.62% from USD 2.56 billion in 2022 to USD 9.58 billion by 2029 (Biçer et al., 2023). While the vegan market is well-established in developed countries, it is still in its early stages in many developing countries. In developing countries, soy is the most popular vegan food source since people are unaware of alternative options like rice, oats, faba beans, and peas and need more research.

2.3 Fermentation of plant proteins

The source of bioactive peptides, which are frequently present in dairy products fermented with LAB, has been extensively explored in milk proteins (Rafiq et al., 2021). Bioactive peptides are peptides, usually ranging from three to twenty amino acids, that offer specific health advantages. These benefits may include anticancer qualities or favourable effects on the immunological, digestive, neurological, or cardiovascular systems. Bioactive peptides have also been shown to be produced by the process of fermentation of plant proteins (Rizzello et al., 2016). For instance, antihypertensive peptides were discovered to be produced during the fermentation of soy using *Lacticaseibacillus casei, Lactobacillus acidophilus, Lactobacillus Bulgaricus*, and *S. thermophilus and* by fermenting several cereal flours with Lactic acid bacteria (LAB), antioxidative peptides were generated (Harper et al., 2022). Since the lactic acid produced during fermentation causes the pH to drop, this process is crucial in facilitating the product's acidification, especially the development of a characteristic tart flavour associated with yoghurt (Harper et al., 2022).

High amounts of agalactosides, such as raffinose and stachyose, are sometimes found in many plant milk. These agalactosides are transported into the lower intestine where they are broken down by microorganisms that produce the enzyme a-galactosidase. This may produce gas, which can be uncomfortable and cause flatulence. This might discourage people from consuming plant-based foods (Harper et al., 2022). Fermentation of plant-based products can result in a decrease in a-galactosides because certain LABs include the a-galactosidase enzyme, which can break down these sugars. It is crucial to choose LAB that can use the sugars found in plant-based sources and express a-galactosidase when developing fermented plant-based dairy substitutes. This will ensure that the product is well-accepted by consumers and that LAB grows better.

A significant reduction of antinutritional compounds is another advantage of the fermenting of plant-based materials. These substances include but are not restricted to, alkaloids, lectins, pyrimidine glycosides, condensed tannins, raffinose, and protease inhibitors. It is well-recognized that these antinutritional substances can be decreased by fermentation (Montemurro et al., 2021). Therefore, Yoghurt, made from plant-based raw materials, appears to be a good way to produce food products with low antinutritional substances (McClements & Grossmann, 2024).

The current consumer trend is towards "clean label" items, specifically goods with a lower ingredient count and substances that are thought to be "more natural." One benefit of using LAB for fermentation is that it can give fermented products the desired characteristics without requiring additional ingredients to be included on the label (Perpetuini et al., 2021).

2.4 Formation mechanism of set-type plant-based yoghurt gel

The primary source of protein in milk is casein. The hydration layer, space repulsion, and negative charge that arise from the formation of a hair layer on the surface of κ-casein all contribute to the stability of casein micelles at neutral pH. When casein particles are acidified, their colloidal calcium phosphate dissolves and causes the particles to disintegrate. This ultimately results in the formation of chains and plexes, forcing the particles to re-polymerize into a certain network structure to create a gel (Yin et al., 2023).

The gel created by LAB-induced protein aggregation for proteins originating from plants is generally acid-induced. Despite the differences in composition between casein and plant proteins, their responses to acidity are similar. The process of gelation of plant-based proteins may thus be understood in terms of the production pathway of dairy yoghurt gel, which is created by casein. There are two major processes involved in building the gel network during fermentation. The first step in fermentation is the preheating process, which denatures plant proteins and causes them to agglomerate partly. Then, LAB produces lactic acid from sugar when anaerobic conditions are met. Because of a drop in net electrical charge and electrostatic repulsion between proteins, soluble protein aggregates are more likely to form at their isoelectric points (pI) when pH is lowered (Nicolai, 2019). This subsequently results in the development of a three-dimensional organized gel framework as shown in Figure 2.1

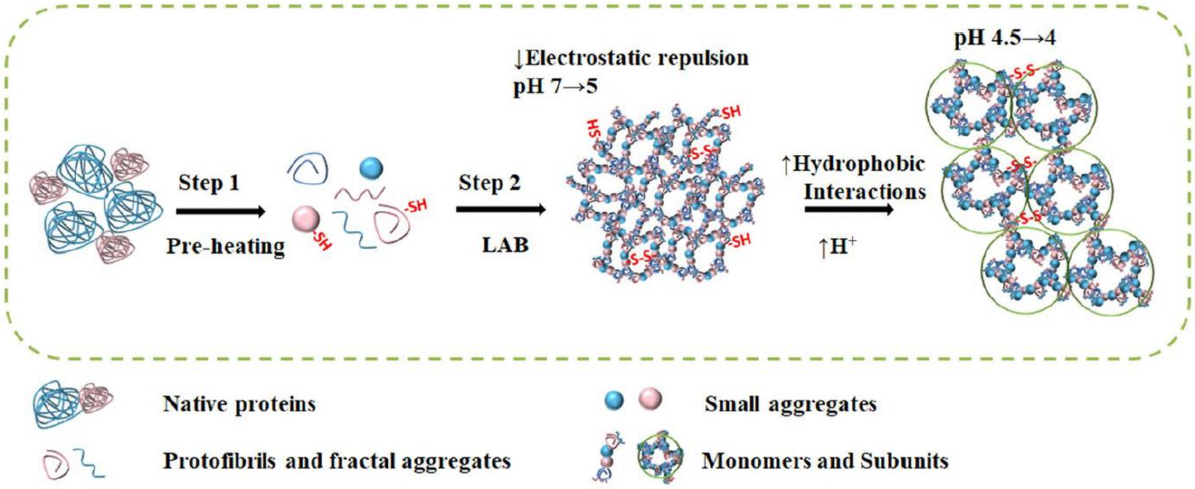


Figure 2.1 Plant-based yoghurt gel formation mechanism, source: (Yin et al., 2023), Licence number: 5766121112333

2.5 Effect of protein on plant-based yoghurt gel properties

It is important to thoroughly understand the gel properties of yoghurt to improve its quality. The gel properties of plant-based yoghurts are mainly determined by plant protein. The main gel properties of yoghurt, include mechanical properties i.e., cohesiveness, consistency, and firmness, and rheological properties like storage modulus, loss modulus, water holding capacity, syneresis, etc. (Yin et al., 2023). The texture of yoghurt is determined by its firmness, which is the force needed to distort a substance, and cohesiveness is determined by how resistant a gel is to breaking. Cohesiveness is negatively correlated with firmness. Therefore gels with greater hardness have lower cohesiveness values.

The rheological properties of yoghurt are one key element that can be modified to improve customer satisfaction. Gel network elasticity is measured by the storage modulus (G′), while viscosity is measured by the loss modulus (G″). A gel is more solid than liquid if its G′ value is much greater than its G″ value (Yin et al., 2023).

2.5.1 Effect of Plant protein components on gel formation

To effectively improve the characteristics of plant-based yoghurt gel, it is essential to understand the molecular basis of protein production. This includes an understanding of the components (e.g., cereal, legume, etc.) involved in developing gels, how secondary structures alter, and how precisely three-dimensional network structures are formed (Yin et al., 2023).

2.5.1.1 Cereal/ Pseudo cereal protein

Plant-based yoghurt alternatives are made with cereals and pseudo cereals because of their distinct flavour and nutritional content. Oat milk is highly preferred by customers due to its substantial protein content (about 15%). Oat proteins are more soluble and emulsifiable than other cereal proteins (Janssen et al., 2018), making them a potential supply of plant-based proteins and a viable ingredient for vegan yoghurt (Yin et al., 2023). Cereal proteins are often classified as albumin, globulin, gliadin, and glutenin. While salt-soluble globulins account for about 75% of the storage proteins in oats, the majority of storage proteins in several varieties of cereals are alcohol-soluble (Mel & Malalgoda, 2021). 12S globulin makes up the majority of oat protein, with 3S and 7S globulins coming in second and third, respectively (Klose & Arendt, 2012). Similar to legume globulins, the six-subunit oat globulin comprises the α-subunit, which is an acidic polypeptide, and the β-subunit, which is a basic polypeptide. They are connected through disulfide bonds (Yin et al., 2023). Since oat proteins have a high gelling capacity, plant-based yoghurt made with oat protein concentrate frequently creates a gel that cures slowly and has a diffusion network arrangement (Yang et al., 2017).

Heat treatment before acidification breaks down the hexamer structure of oat protein, and part of the monomers mix with the subunits to generate the unique forming process. These subunits and monomers interact more tightly when the pH drops, producing a large number of crosslinking sites that act as modules for forming gels. The decreased repulsion force causes them to form nuclei, which join linearly to create protofibrils and finally organize into a gel-percolating network (Yin et al., 2023).

2.5.1.2 Legume protein

The production of most acid-induced gels depends on globular proteins, which have distinct secondary and tertiary structures (Nicolai, 2019).

Albumin and globulin are the two primary types of proteins found in soybeans. Whereas 11S (legumin) is a disulfide-bonded dimer comprising α and β subunits, the globulin fraction mainly comprises 7S (vicilin) trimer subunits bound together hydrophobically (Yin et al., 2023). The 7S protein has the most impact on the properties of the gels made with soybean proteins. The isoelectric point (pI) of 7S is lower, and its gelation takes longer than 11S, however, the gel strength of soy yoghurt can be reduced if the 7S is absent. On the other hand, in the presence of sodium chloride, 7S can form a stronger gel than 11S due to the increase in its viscosity (Zhou et al., 2019).

The presence of lipoxygenase may accelerate the oxidation of sulphur-hydrate (S-H) groups during the fermentation process resulting in increased gelation time. However, the absence of lipoxygenase increases S-H groups, which helps build more crosslink sites and strengthens the 3D structure by establishing more disulfide connections (Yin et al., 2023).

As with other legumes, the majority of proteins found in faba beans are globulin-types. Based on how soluble they are in various solvents, plant proteins are divided into four major groups such as globulins, albumins, prolamins, and glutelins (Martineau-Cote et al., 2022). The ability of a protein to form gel is mostly based on contact and bonding patterns, including hydrophobic and hydrostatic interactions, as well as covalent and hydrogen bonds (Bangar & Dhull, 2022). Two main categories of faba bean globulins, legumins (11S) and vicilins (7S) are distinguished based on their sedimentation coefficients. The most prevalent globulins in faba beans are legumins, which can make up as much as 55% of the grains/legume proteins. Each subunit of their hexameric structures comprises side chains of $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ peptides bound together by a disulfide bond. Vicilins are composed of trimeric structures, with non-identical subunits. They cannot form disulfide bonds because they are cysteine-free and glycosylated. The functional characteristics of proteins are significantly influenced by the legumins to vicilins ratio, which is a crucial component in the characterization of faba bean proteins. Cultivars, processing conditions, and environmental variables are some of the elements that influence the legumin-to-vicilin ratio (Martineau-Cote et al., 2022).

2.5.2 Effects of protein type on gel formation

Protein content can impact the texture, viscoelasticity, and microstructure of plant-based yoghurt gels, leading to differences in their characteristics. Due to various proteins having varied spatial structures, buffering capacities, and gelling points, which affect hardness, viscosity, and the network of hybrid gels, their different ratios should be considered when numerous proteins are present in the emulsion system (Yin et al., 2023). When plant and dairy proteins are combined to create hybrid gels in an acidic environment, the characteristics of the gel are inversely related to the amount of plant protein (Xia et al., 2022). The pH-decreasing rate, stiffness, and water-holding capacity of acid-induced gels made with plant protein isolates is lower compared to the gels made with the same concentration of pure whey protein isolates. The plant protein's strong buffering ability causes the gelling process to proceed more slowly and to undergo weak structural rearrangements, which increase the number of pores and flocs in the gel structure. Hybrid gels have a coarser texture, a lower water holding capacity, and a storage modulus (G’) than gels made entirely of pure whey protein isolate, because of their less dense and uniform structure (Yin et al., 2023). The method of protein extraction, such as water or alkaline does not affect gelation properties, however, during heat treatment, the dispersion's pH caused the production of fine or particle gel structures at pH 7 and pH 5, respectively (Nivala et al., 2021).

2.5.3 Effects of protein concentration on gel formation

The protein concentration in plant-based yoghurt has a major impact on its gel properties. A dense gel network can only be formed by self-similar aggregation, which is characterized by a fractal dimension when the concentration of protein is above the critical gel concentration. The gel strength is directly correlated with the protein concentration, and a drop in protein concentration results in a corresponding drop in the gel strength (Nicolai, 2019). Higher protein content gel matrices exhibit increased crosslinking, giving rise to a stronger structure and higher storage modulus (G′) values (Jørgensen et al., 2019). It was found that gel made with potato protein isolates (PPI) had higher storage modulus (G′) and loss modulus (G″) compared to gel made with dairy protein. This resulted from the PPI having a high protein concentration, which raised the structural strength of the protein gel (Levy et al., 2021).

2.5.4 Intermolecular interactions of proteins involved in plant-based yoghurt gels

Long-range electrostatic and short-range attractive interactions are the main forces affecting natural globular proteins. Moreover, to keep the protein colloid solution stable, the electrostatic repulsion of oppositely charged proteins can balance the effects of electrostatic attraction. However, lowering pH may disturb the natural balance of interactions causing protein gelation and the development of new intermolecular forces (Yin et al., 2023). Solubility in various denatured solvents can be used to quantify the intermolecular interaction force present in the set-type plant-based yoghurt gel structure. Solubility in sodium chloride, β-mercaptoethanol, urea, and sodium dodecyl sulphate can express the level of ionic bonds, disulfide bonds, hydrogen bonds, and hydrophobic interaction that maintains the gel network (Klost et al., 2020). The stability of the gel network structure is primarily influenced by two forces, hydrophobic interactions and electrovalent or disulfide bonds, which can be related to variations in plant proteins. Out of these two types, hydrophobic interactions are the dominant intermolecular force (Klost et al., 2020).

Yang et al. (2021), reported that the yoghurt gels made from soybean and mung bean proteins had their hydrophobic groups exposed to the outside because these proteins unfold during fermentation. Sulfhydryl groups are also oxidized during this process, forming hydrophobic aggregates and disulfide bonds that preserve the stability of the gel network. Further, the hydrogen and ionic bonds had no significant effect on the gel's structure, and hydrophobic interactions play a significant role in the stability of the gel network of pea protein gels.

2.5.5 Effects of carbohydrates on yoghurt gel

Lactose, which affects the structure and flow behaviour of yoghurt gels, is the main ingredient in dairy yoghurt for the growth of starter cultures. Plant-based products lack lactose, thus sucrose and other sugars are needed to provide probiotics with the energy and nourishment they need to survive during fermentation. The presence of sugar can change the osmotic pressure, which in turn affects the ability of LAB to ferment (Zhao et al., 2021). Zhao et al. (2021), investigated that, the viscosity of almond yoghurt was not impacted by the sugar amount, most likely because the sugar dissolved in the almond milk and had less of an influence than the protein on the development of the gel structure. However, the storage modulus (G′) of almond yoghurt gel has increased with added sugar within its tolerance. Due to the increasing solids content following adding sugar to the mixture, the G′ value raised with the sugar concentration within the tolerances (Yin et al., 2023).

Gelatinized starch can be used as a filler to make yoghurt gels firmer when combined with protein and starch (Figure 2.2). The gelatinized starch fraction may combine with aggregated plant proteins to produce a stable three-dimensional network structure in plant-based yoghurt gels, greatly improving textural characteristics (Brückner-Gühmann et al., 2019). *Brückner-Gühmann et al. (2019)*, observed that, during fermentation, oat proteins can form aggregates at the isoelectric point due to a decrease in pH. Still, these aggregates cannot form a stable gel network structure. However, oat protein concentrates due to their gelatinized starch component can create a stable gel network. In addition, due to the higher amount of swollen starch granules, the water-holding capacity (WHC) may also increase in oat protein isolates-based gels.

2.5.6 Fats in plant-based yoghurt gels

Oil droplets stabilized in proteins have an affinity for the gel matrix and can be used as active fillers to change the characteristics of gels by raising storage modulus (G′) values and lowering loss tangent (tan δ) values (Figure 2.2). A higher fat content may have led to the development of larger fat droplets, which when filling the network structure and affecting the protein matrix increased the gel strength. This results in increased product viscosity positively correlated with particle size (Geremias-Andrade et al., 2016). However, when the amount of fat content exceeded its optimum, the fermented gels showed less apparent viscosity, this may be due to the excess amount of fat weakening the attraction between protein and fat molecules in the gel network and affecting the microstructure of the yoghurt (Yin et al., 2023).

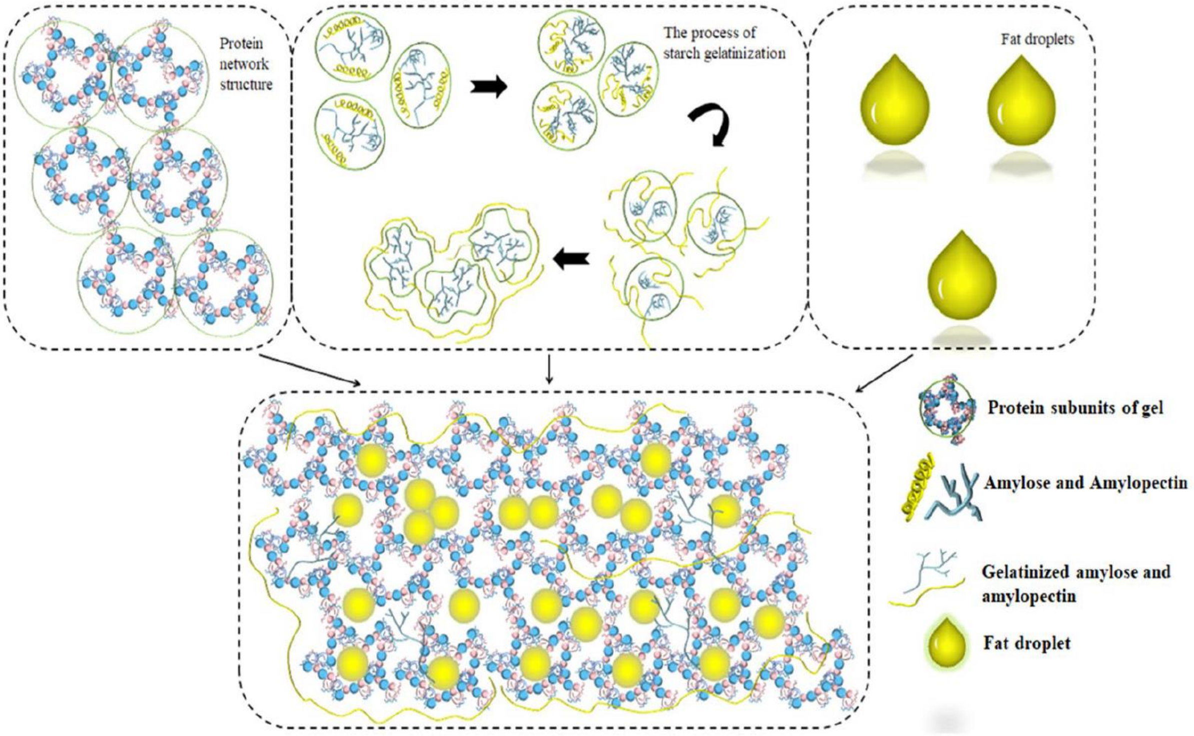


Figure 2.2: Relation between protein, fat, and carbohydrates in plant-based yoghurt gel. Source: (Yin et al., 2023), Licence number: 5766121112333

2.6 Plant-based yoghurt gel formation with pre-fermentation treatments

The majority of plant proteins have weak solubility, poor emulsification capacity, and inadequate gelation ability and they are more easily denatured by heat treatment and/or high pressure. As a result, the conversion of plant emulsions into yoghurt gels produced by fermentation is challenging. Although it is possible to create plant-based yoghurt gels with plant milk, the resulting gel texture may be poor and have low water holding capacity (WHC) and storage modulus (G') values. The functions of plant proteins, the stability of plant milk systems, and the gel qualities of plant-based yoghurt must thus be enhanced. The processing factors and/or procedures must be taken into account to increase the plant-based food industry's use of these products. These processing factors and procedures include but are not limited to pH-shifting treatments, the intervention of gelling agents, heat treatments, homogenization and enzyme treatments, etc (Jeske et al., 2018; Yin et al., 2023) .

2.6.1 pH Shifting

A protein's solubility plays a crucial role in deciding whether or not it is suitable for usage in high-moisture food materials. The inability of plant proteins to migrate to the interface and their increased susceptibility to precipitation due to their low solubility might restrict their application and impair their emulsifying action. Plant proteins often dissolve better in alkaline environments than in acidic ones. The precipitation of natural plant proteins in acidic conditions can affect the quality of plant-based yoghurt alternatives (Nicolai, 2019). The gels made from plant milk or plant protein emulsions can enhance their qualities by unfolding and changing their distinct structures. plant proteins can be treated to improve their solubility and capacity for emulsification. This can be done by changing the pH before fermentation. Compared to other technologies, the pH-shifting approach is more economical and convenient (Xia et al., 2022).

2.6.2 Heat treatments

Heat treatment can be utilized to enhance the solubility and gelling characteristics of plant proteins. High temperatures stimulate the denaturation and unfolding of native protein structures, disclosing hydrophobic groups and increasing the number of disulfide bonds and aggregation formation. These modifications may strengthen the yoghurt gel network's crosslinks, density, and formation (Grygorczyk & Corredig, 2013). Furthermore, heating treatment is necessary to sterilize plant-based milk and lower spoiling microbe levels. In addition, the type of heating technique used can have an impact on the yoghurt gel's characteristics. Pasteurization and ultra-high temperature (UHT) heating are two frequently utilized heating techniques in this industry. These techniques impact heat-labile proteins, causing them to unfold and aggregate, ultimately affecting either positively or negatively the gel's characteristics of the plant-based yoghurt (Schmidt et al., 2019). The characteristics of plant-based yoghurt gels can be affected by blanching, another heat treatment that is frequently used in soybeans. While heating can aid in the process of gelation, high levels of temperature can be detrimental to the gel's characteristics. Therefore, selecting the right heating temperature before fermentation is vital. The plant-based milk type determines the appropriate heating temperature since some plant proteins are not heat-tolerant and can result in uneven and loose emulsion systems or even heat-induced gels (Zhou et al., 2019). Plant-based gels should be firm, but not too hard since this might give the texture more of a "tofu-like" than a "yoghurt-like" consistency. The heat temperature has an impact on the hardness level as well.

2.6.3 Homogenization

The poorly functioning proteins in plant-based milk make them unstable in the absence of proper treatments. Almond protein, for example, is very hydrophobic but has a low molecular weight, making it difficult to dissolve in water. To stabilize plant proteins against sedimentation and stop fat droplets from floating to the top of the milk before fermentation, homogenization is required. Three popular techniques are employed to homogenize plant milk: high-pressure homogenization, high-shear mixing, and ultrasonication. These techniques increase the quality of food by reducing the size of scattered particles and achieving a more uniform distribution throughout the system (Koh et al., 2013). The water-holding capacity (WHC) and mechanical and rheological characteristics of plant-based yoghurt are impacted by homogenization. Proteins covered fat globules well, increasing the gels' surface area and preventing water from dripping through. After going through the mixer and colloid mill stages, plant-based milk could not be completely homogenized without homogenization. High pressure during homogenization causes microparticles to develop, giving the structure a more uniform appearance and changing the shape of the particles due to mechanical forces. Furthermore, plant proteins' ability to emulsify and gel is further improved by the reduced size and network integration of bigger globular proteins due to homogenization. However, without any pre-treatment, a single high-pressure treatment may denaturize plant proteins, and even after shearing, the plant-based emulsion may stay and separate (Yin et al., 2023).

2.6.4 Enzyme treatments

Plant proteins can change their solubility, emulsification, and dispersibility by limiting the enzymatic hydrolysis of those proteins. In addition to increasing hydrophobic areas and releasing ionizable groups, this process can also lower the molecular weight of proteins, which eventually raises their water-holding capacity (WHC). Enzymatic crosslinking and hydrolysis are two methods that can be used to perform enzymatic treatment. Plant-based yoghurt gels' characteristics are mostly dependent on the levels of hydrolysis and the ratios of hydrolyzed protein to non-hydrolyzed protein. Enzymatic hydrolysis produces small-sized peptides that can evenly extend their branching structure into the gel matrix's protein aggregates, encouraging more widespread interactions between protein molecules and enhancing the WHC (Wouters et al., 2016). However, excessive hydrolysis might lead to a softer texture and decreased hardness of the gel, as indicated by the storage modulus (G′) value, by decreasing the size of protein particles and weakening the connection between them. Protein polymers and fibres bind water in acid-induced yoghurt gels, trapping the residual water inside the gel's network. More water is retained by gels with dense and homogeneous pore structures, improving their WHC (Cikrikci et al., 2016). The protein may be modified by enzymatic hydrolysis and crosslinking, which would also enhance its solubility and gel qualities. However, the right enzymatic conditions and type of enzyme are essential for the effective production of gel during LAB-induced fermentation (Yin et al., 2023).

2.6.5 Gelling agents

Macromolecular polysaccharides and proteins, such as xanthan gum, pectin, gelatine, carboxymethyl cellulose (CMC), soy protein isolate, and polymerized whey protein, are used as thickeners to improve the texture of yoghurt. Proteins in the food system can interact with thickeners to stabilize food texture, fix the water, and inhibit yoghurt syneresis. Alternatively, immobilized solutes and solvents can form bonds with protein and polysaccharide macromolecules, changing the basic characteristics of aqueous colloids and serving as filler materials (Shi et al., 2020). The study showed that the hardness of maize yoghurt with gelatine added was considerably higher than that of yoghurt in the thickener-free control group. The gel's microstructure changed as the gelatine concentration increased, with a moderately dense gel network displaying a homogeneous, highly branching sponge-like interior structure at a high gelatine concentration. While the sample without gelatine had a very unstable structure, the sample with an intermediate concentration had fewer branches and a lower structural density. Better gel qualities are only sometimes the outcome of increasing the thickening. Highly solid and compact yoghurt could not taste very well (Supavititpatana et al., 2008). Thus, sensory testing should be done to assess the yoghurt gel's quality, as hardness cannot be the only factor in determining consumer satisfaction.

2.7 Manufacturing process of plant-based yoghurt

Though the composition differs depending on the type of yoghurt made (i.e. dairy and plant-based), the general procedure remains the same. Preparing common raw components and their calculated mixing usually comes first when manufacturing yoghurt. First, plant-based "milk" is made from one or more raw materials, such as flakes, flours, and plant-based isolates or concentrates. This procedure typically involves phases for mixing, water dispersion, and mechanical size reduction. To obtain the ideal structural qualities, the plant-based milk or dispersion can be concentrated using a filtering process to get the required dry matter content. Then the standardized mixture is homogenized, and after homogenizing the mixture is pasteurized in the next stage. Pasteurization is a heat treatment that improves the consistency of the yoghurt by denaturing the milk's protein and promoting the growth of the added culture. Once the mixture has cooled to a temperature suitable for fermentation, the mix is next inoculated with desired microorganisms incubated at 43-45 ^{0}C for 6-8 hours until the desired pH is reached (McClements & Grossmann, 2024; Weerathilake et al., 2014).

2.7.1 Blending/ Standardization

The initial step in manufacturing yoghurt is often blending. To guarantee consistent output, different dry components must be evenly distributed throughout the liquid phase. Thus, it is necessary to provide a certain shear rate and enough mixing duration. However, to avoid a high amount of oxygen dissolving and inhibiting the growth of the anaerobic culture used for fermentation, it is necessary to control the incorporation of air as much as possible during the blending process (Kilara & Chandan, 2013).

A minimum of 2.7% protein and a maximum of 15% fat are recommended for yoghurt, according to the Codex Alimentarius Commission. Yoghurt composition varies depending on the variety. As a result, yoghurt mixtures should be standardized to yield an end product that contains at least 2.7% protein, less than 15% milk fat, and a titratable acidity of at least 0.3% expressed as a percentage of lactic acid (Chandan & O'Rell, 2013; Weerathilake et al., 2014).

2.7.2 Homogenization

The homogenization process is a crucial processing step for high-fat yoghurt since it guarantees uniform dispersion of fat globules throughout the food matrix and decreases their diameter to less than 1µm. In addition, homogenization may enhance the product's digestibility to improve product consistency and stability (Kilara & Chandan, 2013). According to studies, homogenization should be used before pasteurization, since this would improve the end product's uniformity. Typically, homogenization pressures for double-stage high-pressure homogenization range from 10–20 MPa/5 MPa, for 10-17 min (Weerathilake et al., 2014), for yoghurts with higher total solids content, while relatively a lower homogenization pressure range from 6-17 MPa should be applied (Aswal et al., 2012)

2.7.3 Heat treatment/Pasteurization

The microstructure and physical characteristics of yoghurt are significantly influenced by the heat treatment of milk (dairy/plant-based), which is often regarded as a crucial phase in the production process. From a microbiological perspective, the eradication of competitive organisms creates an environment that is favourable for the growth of Lactic acid bacteria (LAB). When yoghurt is processed, it must be subjected to a high temperature that kills all of the harmful bacteria and the majority of their vegetative cells and ultimately enhances both the shelf life and food safety of the product. In addition, heat processing produces nitrogenous substances that have been broken down by proteins, releases oxygen and creates reducing conditions. All these conditions help to enhance the nutritional status of the substrate where LAB is growing. In addition, the viscosity of yoghurt is significantly impacted due to the denaturation of proteins during the heat treatment. For best results, apply a heat treatment of 90–95 ^{0}C and leave it for 5–10 minutes (Fig.2.3). Further, heat processing enhances the gastrointestinal digestibility of the products which consist of denatured proteins (Chandan & O'Rell, 2013; Weerathilake et al., 2014).

2.7.4 Starter culture inoculation and fermentation

Following the heat treatment, the yoghurt blend is then cooled to a temperature ranging from 43 to 46 ^{0}C (Fig. 2.3) before introducing yoghurt starter culture bacteria (LAB) at approximately 2% (v/v) concentration. In a conventional yoghurt culture, *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* are combined in a 1:1 ratio. In Fresh cows’ milk, 80% of proteins are represented by casein, which can keep its micelle-like structure reasonably stable. The electrical repulsion of casein particles is primarily responsible for their stability, hence the concentration of salt and the ionic balance can have a significant impact on how stable milk is. The culture breaks down lactose into lactic acid during the yoghurt fermentation process, which causes the calcium and phosphorus within the casein particles to gradually dissolve and destabilize the casein micelles. The casein finally loses its electrical repulsive force at pH 4.6–4.7, when it approaches the isoelectric point, leading to the production of a gel (Aswal et al., 2012).

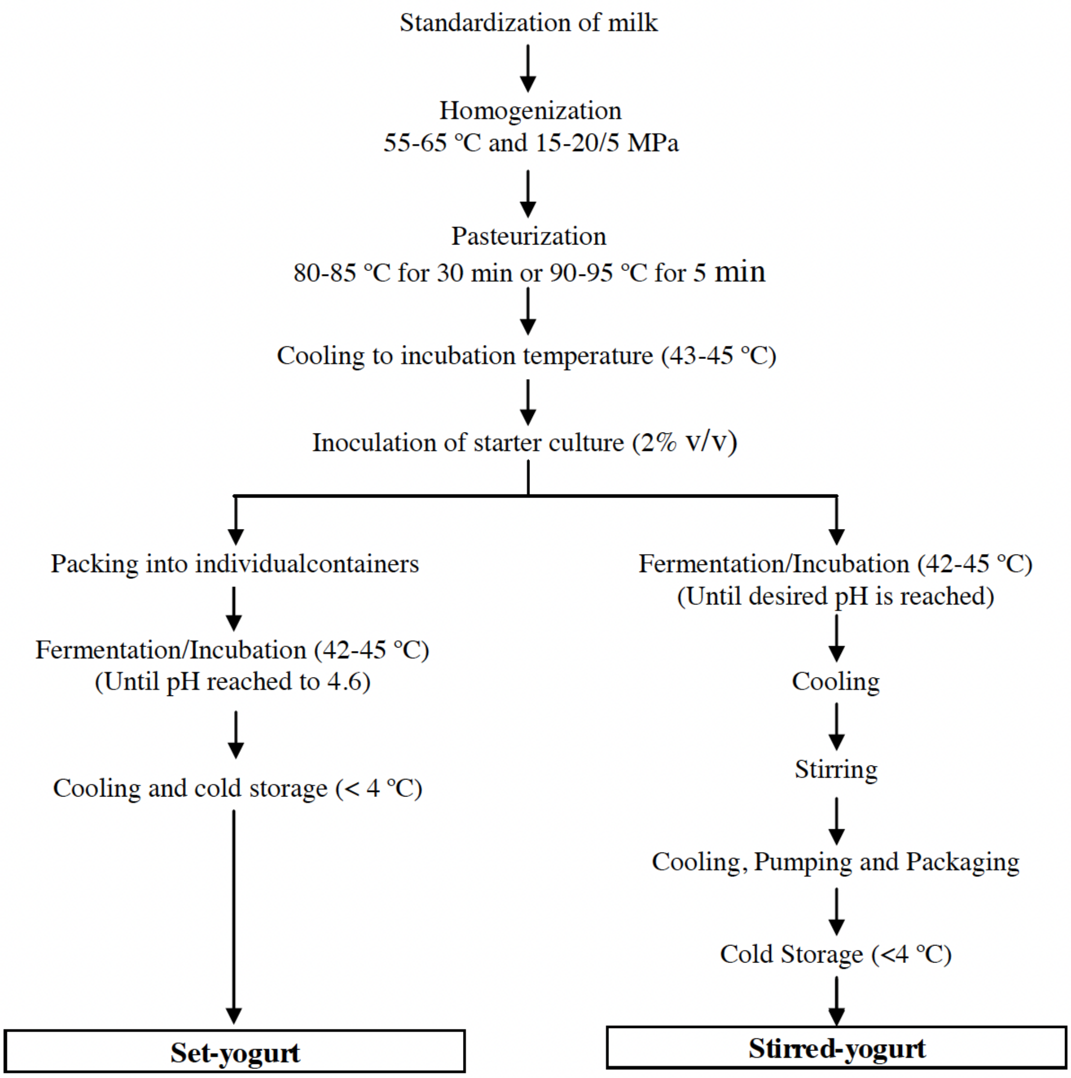


Figure 2.3: Manufacturing process steps of yoghurts (set and stirred)
Source: (Weerathilake et al., 2014)

It has been challenging to ferment plant protein into a stable gel because the gels lack the texture and fluid retention qualities of dairy yoghurt. This is because casein and plant proteins have distinct molecular structures and interactions, and certain plant-based milks have lower total protein contents than cow's milk. Compared to globular plant proteins and their aggregates, casein molecules and micelles exhibit distinct structural characteristics. Hence, compared to casein, acidification causes distinct interactions and structural arrangements in plant proteins, which results in different gel characteristics (Yin et al., 2023). Hydrocolloids like starch, guar gum, locust bean gum, xanthan gum, pectin, agar, or carrageenan are frequently added to plant-based yoghurts to improve their mechanical and structural properties.

Furthermore, it has been shown that heat treatment that partially or completely denatures plant proteins improves the ability of acid-fermented products to form structures (Boeck et al., 2021).

2.7.5 Cooling

Yoghurt should be refrigerated right away after it reaches a particular level of fermentation to preserve a certain pH of the finished product by preventing the culture from growing further. Yoghurt should usually be refrigerated once fermentation is complete when the pH hits 4.5–4.6 and a weak texture results if the yoghurt is refrigerated at pH 4.7 or above (Weerathilake et al., 2014). Furthermore, prolonged chilling promotes the growth of bacteria and may lower pH levels, which might lead to unfavourable alterations in the structure of yoghurt. As to the USDA specifications, yoghurt needs to be refrigerated and kept at a temperature not exceeding 7.2 ^{0}C following the completion of manufacture and/or packing (Lee & Lucey, 2010).

2.8 Faba bean as a useful food ingredient

Soybeans are one of the main vegetable protein sources in the world and are used in the majority of processed plant-based protein food products. Faba beans, which have around 27% protein, resemble soybeans in terms of both their morphological and functional characteristics. Compared to soybeans (18%), soybeans have a lower lipid content (1%) and less raffinose and stachyose, which cause flatulence, than cowpeas (Langton et al., 2020).

Faba bean (*Vicia faba L.*), also referred to as fava bean, is an ancient legume and annual herbaceous plant in the *Fabaceae family*. Approximately 16,000–19,000 species of faba beans are found in 750 genera under the *Fabaceae family*, which is widely dispersed throughout the world. This cool-season legume, which originated in the Middle East's ancient era, has long been used as a primary source of protein for both people and animals (Riahi & Ramaswamy, 2003). The faba bean is a high-quality protein source that is excellent in digestibility and balanced essential amino acids with some levels of anti-nutritional factors. It also has a high fibre content. Faba beans contain high-quality protein which consists of all the essential amino acids (Bangar & Dhull, 2022). A whole faba bean has 20–35% protein, 1-2% fat, 55–65% carbohydrates, 10-15% fibre, and several vitamins and minerals like magnesium, calcium, potassium, iron, and zinc. Numerous health advantages, including antioxidant qualities and the suppression of enzyme activity during the digestion of carbohydrates, have been linked to the phytochemicals found in faba bean components. Faba beans are becoming more and more popular in scientific research due to their nutritional value and techno-functional qualities. They are being used as a substitute for conventional ingredients like casein, whey, and wheat protein in a range of food products, including beverages, sausages, and meat analogues. Flavonoids, phenolic compounds, and other bioactive substances found in faba bean seeds provide several physiological advantages beyond nutrition, including antibacterial, neuroprotective, anticancer, and antioxidant properties (Nosworthy et al., 2018).

The ability to use fresh faba bean seeds in food has been restricted by several antinutritional elements. The pyrimidine glucosides convicine and vicine are the most restrictive antinutrients found in faba beans. Consuming vicine and convicine can cause haemolytic anaemia in those who lack glucose-6-phosphate dehydrogenase (Labba et al., 2021). The raffinose-series oligosaccharides have also been linked to digestive discomfort, including flatulence and gas production due to anaerobic fermentation. Tannins, phytic acid, trypsin inhibitors, and saponins are also examples of antinutritional compounds found in faba bean cultivars that vary greatly from one another. Since this restriction is easily overcome by various processing techniques, faba beans make a significant contribution to the human diet (Nosworthy et al., 2018).

2.8.1 Quality and nutrient composition of faba beans

2.8.1.1 Protein

Almost twice as much protein as cereals can be found in faba beans, which range in protein concentration from 25.3% to 27% (Table 2.1). Based on different varieties and the portion of beans in which the protein is extracted, the amount of protein may vary. Furthermore, differences in protein content are influenced by the kind of soil, irrigation techniques, growth season, and fertilization strategy (Multari et al., 2015; Yang et al., 2018). All the amino acids needed by humans are present in faba bean protein, except cystine and methionine, which make it of the highest quality. Because it has the same nutritional value as meat and fish protein, faba bean protein is sometimes referred to as the poor man's meat. The majority (60%) of faba bean protein represents globulins, while the rest is 20 % albumins, 15 % glutelins, and 5 % prolamins. Enzymes, protease inhibitors, amylase inhibitors, and lectins with molecular weights ranging from 5,000 to 80,000 Da are found in albumins, which are water-soluble protein fractions and rich with sulfur-containing amino acids and lysine. The two high-molecular-weight proteins known as legumin and vicilin (11S and 7S) comprise the globulins, the primary proteins in faba beans with molecular weights ranging from 8,000 to 600,000 Da (Bangar & Dhull, 2022; Langton et al., 2020).

2.8.1.2 Lipids

Due to their high-fat content, faba beans provide humans with a diet heavy in calories. The bean's lipid content can vary from 1.4 to 3.2% (Table 2.1). The fatty acid composition of bean lipids is composed of palmitic, stearic, myristic, pentadecanoic, arachidic, behenic, oleic, and linolenic acids. Trace quantities of hydrocarbons, steryl esters, free fatty acids, diacylglycerols, and monoacylglycerols are present in very modest proportions (1.8–2.4%), whereas the most prevalent lipid components are 47.7–50.1% triacylglycerols and 47.5–50.5% phospholipids. The three main phospholipids found in faba beans are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Phosphatidylinositol separates itself from the other two phospholipids due to its higher density of saturated fatty acid (Bangar & Dhull, 2022; Yang et al., 2018).

2.8.1.3 Carbohydrates

Faba bean seeds have a high carbohydrate content (45–49%), while starch makes up 37–43% of them (Table 2.1). The main components of starch are amylose and amylopectin, which have a substantial impact on the nutritional value and digestion of food. The starch derived from faba beans has a 40% amylose content and a 60% amylopectin level. The three most significant nutritional elements of faba beans are starch, sugar, and dietary fibre. Starch and non-starch polysaccharides are the main components of the carbohydrate fraction, however, oligosaccharides also make up a considerable fraction of this mixture (Moussou et al., 2019). The primary soluble sugars are oligosaccharides belonging to the raffinose family, namely verbascose, stachyose, and raffinose. These sugars are known to cause flatulence and restrict the amount of faba beans that may be consumed due to digestive issues (Yin et al., 2023).

The faba bean, which has soluble and insoluble fibre, is an excellent source of dietary fibre. The whole faba bean includes varying amounts of dietary fibre (15–30%), mainly hemicellulose, cellulose, and lignin. The faba bean's seed coat has a significantly higher nutritional fibre content (82.3%). As whole faba beans are a high source of dietary fibre, phenolic compounds, and minerals, consuming whole faba beans is helpful for health (Bangar & Dhull, 2022).

Table 2.1: Nutritional Composition of Faba Bean Seeds

Nutrient/Components	Concentration
Moisture	10-12%
Protein	25.3-27%
Lipids	1.4-3.2%
Carbohydrates	45-49%
Starch	37-43%
Ca	117–172 mg/100 g
P	1100–1117 mg/100 g
Mg	76–102 mg/100 g
Fe	5.44–5.48 mg/100 g
Cu	1.48–2.01 mg/100 g
Na	25–27 mg/100 g
K	1220–1285 mg/100 g
Zn	4.18–5.67 mg/100 g
Vitamin C	33 mg
Folic acid	96 µg
Tocopherol	0.08 mg
Vitamin B6	1.6 mg
Vitamin A	350 IU
Trypsin inhibitors	2.84 (TIU/g)
Chymotrypsin inhibitors	1.77 (CIU/mg)
Phytic acid	2.40%
Tannins	151 mg/100 g

Source: (Bangar & Dhull, 2022)

2.8.1.4 Minerals

Several mineral components, including potassium, calcium, sodium, and magnesium, are readily available in faba beans. It has been noted that whole and dehulled faba beans are rich in trace elements, including Cu, Zn, Mn, K, Ca, P, S, and Mg. When compared to dehulled samples, whole faba beans had a higher mineral concentration, suggesting that the seed coat has significant mineral reserves (Carmo et al., 2020). However, the bioavailability of these minerals is frequently lacking because of anti-nutritional compounds such as phytates and condensed tannins (Badjona et al., 2023). Due to the growing popularity of plant-based protein sources, especially among populations that are vulnerable to protein shortages, such as adults, children, and pregnant women, Fe and Zn are the main elements to give more concern, since research has shown that vegetarians have lower iron levels than non-vegetarians (Ali, 2019).

2.8.1.5 Amino acids

The amino acids present in faba beans include non-essential amino acids such as aspartic acid, glutamic acid, alanine, arginine, glycine, proline, and serine, and essential amino acids like isoleucine, leucine, lysine, methionine, tyrosine, phenylalanine, valine, histidine, tryptophan, while threonine, and arginine is the most abundant (6.9-12.6 g/kg) among those (Table 2.2). Faba beans are utilized as a protein supplement in a range of foods, such as bread, cookies, and oil-in-water emulsions, due to their balanced amino acid profile.

Table 2.2: Amino acid composition (%w/w) of different faba bean proteins and dairy protein (Casein*)

Amino acids	Faba bean		Protein Concentrate	Protein Isolate	Casein*	FAO/WHO Requirement	
	Whole seeds	Dehulled seeds	FBC	FBI		2-5 year old	Adults
Histidine	2.56	2.43	2.39	2.80	2.70	1.90	1.60
Isoleucine	4.1	3.97	3.73	3.80	4.90	2.80	1.30
Leucine	7.5	7.25	7.10	8.0	8.40	6.60	1.90
Lysine	6.43	6.16	6.34	7.0	7.10	5.8-	1.60
Methionine	0.89	0.79	0.60	0.10	2.60	-	-
Phenylalanine	4.25	4.07	4.13	4.90	4.50	-	-
Threonine	3.65	3.45	3.54	3.70	3.70	3.40	0.90
Valine	4.75	4.56	4.14	4.10	6.0	3.50	1.30
Alanine	3.97	3.78	3.85	4.40	2.70	-	-
Arginine	9.73	10.21	10.48	10.00	3.30	-	-
Aspartic acid	11.2	10.71	10.30	13.30	6.30	-	-
Cysteine	1.18	1.13	-	5.00	0.04	-	-
Glutamic acid	16.78	16.05	16.25	19.90	19.0	-	-
Glycine	4.38	4.06	3.81	4.90	1.60	-	-
Serine	4.98	4.74	4.87	6.30	4.60	-	-
Tyrosine	3.67	3.50	3.05	2.63	5.50	-	-
Proline	4.22	4.09	4.24	3.40	-	-	-

Source: (Badjona et al., 2023)

2.8.2 Antinutritional factors found in faba beans

There are many antinutritional components found in beans, including phytic acid, protease inhibitors, trypsin inhibitors, tannins, haemagglutinins, and oligosaccharides, including verbascose, raffinose, and stachyose. Phytic acid, which is found in faba beans in relatively high concentrations, has the potential to chelate di- and trivalent mineral ions including Ca2+, Mg2+, Zn2+, and Fe2+, therefore decreasing their availability in the digestion system. Trypsin inhibitor concentration in the hull of faba beans is double that of the cotyledon. Since these substances are fairly heat-sensitive, heat treatments can greatly lower the inhibitory action (Moussou et al., 2019).

Condensed tannins are flavonoid polymers that are mostly found in large amounts in the seed coat. They are comparatively water soluble and heat-labile. Faba beans have a similar amount of tannin to common beans, chickpeas, and peas, but nearly three times less than common beans. Phytates, which is made up of a combination of calcium and magnesium ions, is comparatively abundant in faba beans. Many seeds serve as the main source of phosphorus storage. Mineral ions including Ca2+, Mg2+, Zn2+, and Fe2+ can be chelated by phytins, reducing their availability in the digestive system (Bangar & Dhull, 2022).

Vicine and convicine are located in seed cotyledon, therefore they cannot easily be removed as tannins which are located in seed hulls through technological processes (Labba et al., 2021). In addition, vicine and convicine are heat-stable components; therefore, cooking does not eliminate them (Vilariño et al., 2009). Trypsin inhibitors can hinder the digestion of proteins, influencing their bioavailability and consequently preventing their use as a reliable source of high-quality protein. The bioavailability of the minerals needed for regular metabolic processes is hampered by the presence of phytic acid and tannins, which are known to form complexes with divalent mineral cations. This has an impact on the general health of the consumers. The availability and digestibility of proteins are negatively impacted by these antinutritional substances. Similar to this, residues of glucose and galactose found in stachyose, raffinose, and verbascose obstruct the metabolism of sugars and ferment to release gases that cause pain, discomfort, and flatulence in the stomach (Bangar & Dhull, 2022).

2.8.3 The impact of processing technologies on the activity of anti-nutritional factors

A variety of processing methods, including soaking, heating, dehulling, and roasting, are used to reduce or eliminate antinutritional elements from legumes and improve their nutritional profiles. Since heat processing denatures and unfolds protein structures and inactivates several antinutrients, it can improve the digestibility of proteins. On the other hand, some processing techniques might have an adverse effect on digestibility because they cause significant aggregation (El-Hady & Habiba, 2003). It has been shown that non-thermal food processing methods offer the least risk to the safety of the food and may be used instead of thermal methods (Badjona et al., 2023).

2.8.3.1 Soaking

Soaking is a common pre-treatment method used on faba bean seeds to reduce their antinutrient content and speed up the cooking process before they are further processed. When faba beans are soaked, their amount of condensed tannin and phytic acid can be significantly reduced compared to their raw state. This could be mainly due to the leaching of anti-nutritional compounds from the seed inside to the liquid medium (Kader, 1995). Hemagglutination activity, oxalate content, and phytic acid content were reduced after soaking faba beans in water at room temperature for four hours and *Barneveld (1999)* demonstrated that varying the soaking time with an acidic or alkaline component results in a 100% decrease in vicine and convicine levels. This is mostly because these pyrimidine glycosides hydrolyzed at an acidic or basic pH. Despite an improvement in α-amylase activity, some research indicates that soaking and germination of faba bean seeds for up to 72 hours did not significantly alter the chemical composition of the flour produced. After germination, however, notable modifications in the pasting, emulsifying, and foaming characteristics were noted. These were due to partial protein denaturation and the breakdown of proteins, which enhanced surface activity (Chaieb et al., 2011). The percentages of chymotrypsin inhibitors and trypsin inhibitors in faba beans significantly decreased after steeping. Additionally, soaking faba beans at room temperature may decrease, hemagglutination activity as well.

While antinutrients such as phytates and oligosaccharides leach during steeping, there may be a partial loss of soluble proteins. There was no relationship found between the amount of protein in faba beans and the total amount of water absorbed. Furthermore, there was a positive association between the temperature and solution strength and the rate at which the seeds absorbed water. The speed at which faba beans hydrate may be impacted by the soaking temperature. Additional advantages of the soaking process can be obtained by altering it by adding exogenous molecules as liquid media to facilitate the metabolic route, such as Ca2+, Mg2+, Na+, L-glutamic acid, L-glutamate, or other phytochemicals (Badjona et al., 2023).

2.8.3.2 Dehulling

The method of dehulling involves separating the pulses' hulls from their cotyledons. The cotyledon of seeds is a rich source of plant protein, and the hulls of the seeds may then be used to produce phytochemicals. The testa of faba bean seeds contains the majority of the seed's tannins, hence dehulling the seeds resulted in a decrease in tannin content (Kader, 1995). Since trypsin inhibitors are present in the cotyledon fraction, the trypsin inhibitory activity of dehulled beans has been shown to increase. Furthermore, increased levels of phytic acids in dehulled beans while a trace amount of phytate levels in the hull could observed. This is important because iron absorption is impeded in the presence of phytic acid, and it raises questions about the effects of hulling on the particular nutritional quality of the targeted constituents. Furthermore, it has been observed that dehulling the seeds enhances the flavour and palatability of certain legumes (Badjona et al., 2023).

2.8.3.3 Germination

Germination treatment is an efficient way to improve the techno-functional properties and has been used in a variety of applications to minimize cooking difficulties, such as shortening the cooking time, improving nutritional quality, removing or lowering levels of antinutrients, improving flavour characteristics, etc. (Coda et al., 2017). During germination, protein, and carbohydrates serve as the main energy sources. As a result, several biochemical and structural changes take place, which impact the availability of nutrients. Further, pulses have a higher amino acid content due to the hydrolysis of storage proteins during germination. Because germination increases phytase activity, several studies have demonstrated that germination is an effective way to lower the phytic acid concentration in grains and legumes. The phytate content drastically decreased with increased germination time. Therefore longer germination period is more successful in lowering the amounts of phytic acid (Kader, 1995). In addition, germination helps reduce condensed tannin and improve protein digestibility by lowering antinutrients in faba beans (Badjona et al., 2023). Due to its low processing temperature, safety, and sustainability, germination is considered one of the most effective non-thermal methods for improving the physical, chemical, nutritional, metabolic, and structural characteristics of faba beans. In addition, germinated seeds can be subjected to ultrasound or irradiation to ensure a more significant enhancement in beneficial compounds such as phenolics or reduce anti-nutrient presence (Bangar & Dhull, 2022).

2.8.3.4 Thermal processing

Depending on the duration and quantity of heat used, thermal processing can provide either a mild or severe heat treatment. Heating resulted in the whole elimination of chymotrypsin inhibitor activity, suggesting the possible use of thermal treatment in the removal of antinutritional components. *Martini et al. (2021)* discovered that the pre-soaked cooked faba bean had a 30% decrease in its total oxalate concentration. Thermal treatment can also have a significant effect on the concentration of vicine and convicine since these proteinaceous substances are prone to denaturation and degradation. A 30% and 61% decrease in vicine and convicine, respectively, was obtained by heating the faba bean to 122 ^{0}C. The microwave heating of faba bean seeds may enhance their flavour profile because it inactivates endogenous peroxidase and lipoxygenase, which give faba beans their beany flavour. In addition, was shown that the amounts of tannins and trypsin inhibitors significantly decreased while microwave heating of faba bean.

Thermal processing such as cooking and autoclaving may improve protein digestibility by removing or reducing antinutrients. In general, proteins are bound by antioxidants like polyphenols and condensed tannins to form complexes, which obstruct proteases' ability to reach peptide links. In addition to reducing these interactions, heat treatment alters the protein's structure, which may increase protease accessibility and enhance function (Badjona et al., 2023; Vidal-Valverde et al., 2002).

2.9 Food digestion mechanisms

The complex process of food digestion plays a vital role in sustaining human health and well-being. It involves extracting nutrients from the food matrix through a combination of mechanical, chemical, and enzymatic actions (Somaratne et al., 2020).

The initiation of food digestion occurs upon the entry of food into the human body. Through a series of physical and chemical processes, the food undergoes breakdown, releasing nutrients from the food matrix. These nutrients are subsequently absorbed into the body. The term "bioavailability" refers to the accessibility of nutrients for absorption in the intestines following the disintegration of food. Factors such as the mechanical properties and internal composition of food matrices impact digestion by influencing the rate of matrix breakdown and biochemical behaviour during gastrointestinal conditions (Verhoeckx et al., 2015).

The human digestive system, also known as the gastrointestinal tract (GIT), comprises the oral cavity, the oesophagus, the stomach, the small intestine (including the duodenum, jejunum, and ileum), the large intestine, and specific segments like the ascending, transverse, and descending colon, as well as the rectum and anus. The GIT performs two primary functions: mixing and peristaltic movements, facilitating food's movement to the next digestion stage. Throughout this process, the food undergoes mixing with various digestive enzymes and acids, leading to its breakdown into smaller particles. The nutrients and compounds released are then absorbed by the walls of the small intestine, entering the bloodstream and eventually reaching different parts of the body (Singh et al., 2015).

2.9.1 Different phases of food digestion

2.9.1.1 Oral phase digestion

The oral cavity serves as the initial chamber in the human gastrointestinal tract, where food undergoes exposure to various physical such as mechanical breakdown and temperature, as well as biochemical (e.g., dilution, pH regulation, the action of enzymes, presence of salts, and the influence of mucin) processes (Foegeding et al., 2011; Sarkar et al., 2009). Through mastication, the food undergoes breakdown and combines with saliva, resulting in the formation of a lubricated mass known as bolus. Mastication serves to reduce the size of food particles to approximately 0.82 - 3.04 μm, a range influenced by the nature of the food that helps easy swallowing (Lucas et al., 2002). The extent of food breakdown during the oral phase is dependent on the structural and mechanical properties of the food. Human saliva contains a crucial enzyme called α-amylase, which plays a significant role in breaking down the starch present in the food. Interactions between food particles and saliva have a significant influence on the subsequent stages of digestion. Oral breakdown includes a complex mix of physical and biochemical changes in the food characteristics (Singh et al., 2015).

2.9.1.2 Gastric phase digestion

After the oral phase, the bolus is moved from the mouth into the stomach, where it is combined with a variety of digestive fluids and enzymes, causing the food components to expand. The entry of gastric juices into the food components initiates both acid and enzymatic hydrolysis. These actions, coupled with ongoing mechanical shearing, contribute to the breakdown of the food matrix, effectively increasing the surface area for further digestion. The breakdown of proteins is not facilitated by the lower pH of the stomach; however, it does facilitate the hydrolysis of pectin, the main component of plant cell walls, hence softening (Kong & Singh, 2009). Pepsin, a crucial enzyme found in gastric juice, exhibits a wide range of activity and displays a preference for hydrophobic residues. However, the effectiveness of acid and pepsin is dependent upon the diffusion of gastric juice into the food particles within the bolus. The food's structural characteristics and material properties govern this diffusion process (Bornhorst et al., 2015).

2.9.1.3 Intestinal phase digestion

After leaving the stomach, the bolus continues its journey into the small intestine, where it undergoes further breakdown (Bornhorst et al., 2015). The small intestine comprises three segments: the duodenum, which receives digestive fluids from the liver and pancreas, along with the jejunum and ileum. Within the duodenum, sodium bicarbonate (NaHCO3) is utilized to neutralize the bolus, adjusting its pH to a neutral level to optimize enzyme activity. Various pancreatic and digestive enzymes collaborate to break down food components. Subsequently, the released nutrients are absorbed through the inner lining of the small intestine via mechanisms such as simple diffusion, facilitated diffusion, or active transport (Singh et al., 2015).

2.9.2 In Vitro food digestion method

Scientists have shown increasing interest in understanding the impact of food on human health. One approach is studying how food behaves during digestion in the gastrointestinal tract (GIT). The final state of food in the gastrointestinal tract (GIT) may be investigated using several techniques and models, such as in vivo and in vitro cultures, animal and human research, and static and dynamic in vitro models (Shani-Levi et al., 2017). In general, static in vitro digestion methods are widely popular due to their simplicity, cost-effectiveness, and lack of specialized equipment requirements. However, a significant drawback is the number of protocols available, each varying in experimental conditions such as pH levels, duration of digestion steps, and quantities of digestive enzymes and bile utilized. This variability makes it difficult to compare results across different studies (Bohn et al., 2018). To address these limitations, the COST INFOGEST protocol was introduced in 2014 (Minekus et al., 2014) and developed collaboratively by more than 200 scientists from 32 countries specializing in digestion. This protocol has gained widespread international acceptance and underwent further review and updates in 2019 (Brodkorb et al., 2019).

One of the significant limitations of static in vitro digestion models is their oversimplified nature, which fails to capture the dynamic aspects of the digestive process. Consequently, these models are primarily utilized for comparing the digestion of similar foods under standardized conditions, investigating the digestion of individual compounds, or exploring molecular-level interactions between constituents (Benede et al., 2014; Dupont et al., 2010).

There are two primary types of in vitro simulation models: *static and dynamic*. In the static simulation model, parameters such as pH, volume, and hydrodynamic mixing remain constant throughout the simulation. On the other hand, the dynamic gastric simulation model incorporates the continuous addition of gastric juices, cyclic gastric forces, and gastric emptying, aiming to create a more realistic environment resembling the human digestive tract (Kong & Singh, 2010).

2.9.3 In-Vitro digestion of protein gel products like yoghurt

Dairy products are a common source of bioactive peptides, which are essentially short chains of amino acids. A majority of these peptides are created during gastrointestinal digestion, while a small percentage may be found naturally in milk or dairy products. Caseins, which make up 70–80% of the total protein concentration in milk, are the main protein components responsible for this occurrence (Su et al., 2017). The kinetics of digestion are influenced by various factors, including the chemical composition, structure, and physicochemical properties of the food, as well as processing methods (such as heat treatments, homogenization, fermentation, etc.,) and digestion conditions. For example, heat treatment, a common step in yoghurt preparation, can alter the conformation of proteins, thereby affecting their susceptibility to enzymes (Morell et al., 2017). Processing steps involved in yoghurt production can lead to changes in digestion compared to milk. For example, heating, an essential stage in yoghurt manufacturing, unfolds the initial folded globular structure of β-lactoglobulin. This unfolding enhances the accessibility of pepsin to cleavage sites, resulting in accelerated β-lactoglobulin digestion in yoghurt compared to raw milk. Moreover, proteins in yoghurt are typically considered to be more readily digested than those in milk. This enhanced digestibility is due to partial predigestion facilitated by the proteolytic activity of lactic acid bacteria present during yoghurt fermentation (Singh et al., 2014).

A comprehensive understanding of how microstructure and physicochemical properties of food matrices relate to their behaviour during human digestion. Such an understanding could help food manufacturers develop the next generation of satiating foods with enhanced health benefits (Morell et al., 2017).

2.10 Characteristics and quality features of yoghurt

A number of tests might be conducted at various points during the yoghurt production process to ensure the product's quality. Testing for chemical composition, physical attributes, microbiological quality, and sensory aspects is often involved in yoghurt quality assessment. Minerals, fat, total solids, protein, ash, and ash are the primary criteria of yoghurt chemical testing, and other quality tests include texture, rheology, microstructure, colour, pH, etc. (Lee & Lucey, 2010).

2.10.1 Chemical composition of yoghurt

Mostly the yoghurt regulations outline the chemical composition of yoghurt to guarantee their nutritional value. This might include the final product's solid non-fat (SNF) and/ or fat percentages (Lee & Lucey, 2010). These regulations may vary slightly from country to country or region to region. In the United States and EU countries, the SNF and Fat percentages range from 8.2% to 8.65% and 2.25% to 5% respectively. However, in New Zealand and Australia, regulations are available for protein, fat, phytosterols, phytostanols, and their esters. The yoghurt made from cow's milk should contain 30g/kg of protein and no more than 1.5g of total fat/100 g (Sfakianakis & Tzia, 2014). According to studies, the ideal palatability of yoghurt is achieved when the total solids content is between 15.0 and 16.0%. Fat is another essential ingredient that may have a major effect on yoghurt's flavour. Fat is important not only for the flavour but also for the development of the yoghurt structure (Weerathilake et al., 2014).

Acidity is another significant chemical component that influences the sensory characteristics and the shelf-life of the yoghurt. The lactic acids produced by LAB during the fermentation process mainly determine the acidity of the final product. Measuring the pH is a good indicator of the acidity of the yoghurt (Jaman et al., 2022). According to the Australian and New Zealand food standards, yoghurt should have a pH of no more than 4.5 and no less than 106 cfu/g microorganisms (*Australia New Zealand Food Standards Code-Amendment 154, 2015*).

2.10.2 Physical characteristics of yoghurt

The examination of yoghurt's physical characteristics helps to improve the production process and equipment utilization and aids in improved quality. Moreover, it may be coupled with sensory experience to create yoghurt that consumers find more appealing. Several elements impact the physical characteristics of yoghurt, including not just the variety and quantity of protein in the raw material but also the dimensions and structure of the network they create.

2.10.2.1 Colour

For both fresh and processed foods, products, and their marketing, appearance is one of the most crucial sensory quality factors. Product acceptability is heavily dependent on appearance, which is the first quality criterion that customers assess. The consumer's initial perception of food’s appearance, primarily based on its colour, is used to help them decide whether or not to accept it (Nisha et al., 2010). Colour is correlated to other quality characteristics, such as sensory, nutritional, and visual or non-visual failures, and it is used indirectly to measure those quality parameters (Pathare et al., 2012). Food colour can be quantified in two ways i.e., instrumental or objective and visual or subjective. When colour is measured with an instrument, the colour coordinates are used to express colour and are measured using colourimeters or spectrophotometers.

Several colour coordinate systems can be used to characterise an object's colour. The food sector frequently employs the Hunter Lab L*, a*,b*, and the modified Commission Internationale de l’Eclairage (CIE) system known as CIELAB colour scales. The CIELAB colour space provides more uniform colour differences concerning human perception (Pathare et al., 2012).

The three coordinates (L*, a*, b*) of the CIELAB system can be read directly; L* is a psychometric indicator of luminosity, while a* and b* are colour coordinates. Positive values for reddish colours and negative values for greenish ones are assigned to the parameter a*, whereas positive values are assigned to yellowish colours and negative values to blue colours for the parameter b*. L* is an approximate measure for luminosity, where colour is regarded as being on the greyscale, which ranges from black to white, see Figure 2.4 (Granato & Masson, 2010).

The Whiteness Index (WI) is commonly measured to provide numerical values closely correlated with consumers' preferences for white colour. It mathematically combines lightness and the yellow-blue colour axis into a single parameter. WI represents the overall whiteness of food products and can indicate the degree of discolouration that may occur during processing and/or storage (Pathare et al., 2012).

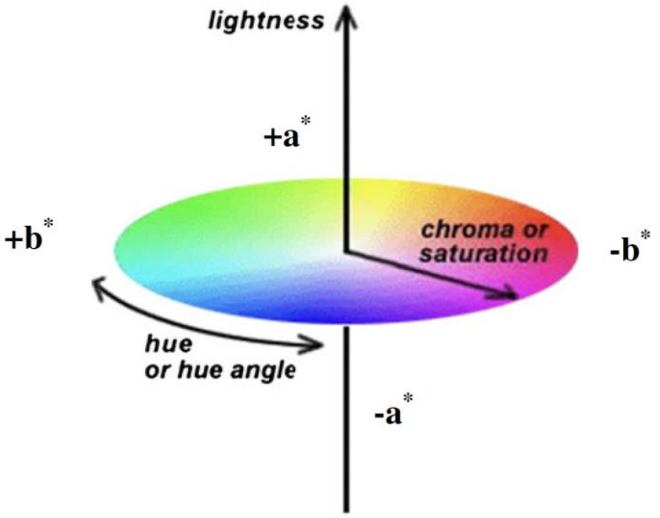


Figure 2.4 CIELAB Colour Space, Source: (Pathare et al., 2012)

2.10.2.2 Texture

As per the International Organization for Standardization (ISO), food texture includes all the rheological and structural attributes of a food product, detectable through mechanical, tactile, and sometimes visual and auditory senses. The texture of food plays a significant role in influencing customers’ overall perception of food products. It is a fundamental aspect contributing to the overall sensory experience of food, representing the structural arrangement of particles within a food item (Fox et al., 2017).

The texture of yoghurt is influenced by various factors, including the type of raw materials, the manufacturing method, the heat treatment process, the type of starter, as well as the incubation temperature and pH (Aswal et al., 2012). Texture analysis is commonly employed to assess properties such as consistency, firmness, and cohesiveness in yoghurt (Figure 2.5).

Consistency refers to the thickness or viscosity of a fluid or semi-solid substance. Typically, customers swirl the product to assess this characteristic. Firmness refers to the degree of hardness or softness of a product and indicates its ability to resist deformation. Cohesiveness refers to the product's ability to cling together, which is influenced by the intermolecular attractions of the product (Olsson et al., 2018).

For assessing the textural characteristics of different food items, texture profile analysis (TPA), is a popular double compression test. The two primary factors for characterizing texture in practice are force, or stress, and deformation, or strain. TPA measures characteristics including hardness/ firmness, cohesiveness, gumminess, adhesiveness, and chewiness (Figure 2.5). The two-bite test (named because it replicates the biting motion of the mouth), involves compressing food samples twice. Various studies have used TPA to examine the textural characteristics of different yoghurt-like products, for example, the effect of partially hydrolyzed guar gum and process variables on yoghurt texture (Mudgil et al., 2017) and the formulation of plan-based yoghurt from soybean and quinoa (Huang et al., 2022).

a) Hardness/ Firmness

Hardness is the most crucial factor in evaluating yoghurt texture. The force used to achieve a specific deformation is considered an indicator of the yoghurt's firmness. It is also described as the peak force of the product's first compression during its textural analysis (Mudgil et al., 2017).

b) Adhesiveness

Adhesiveness is defined as the amount of force needed to get the glued substance out of the mouth when chewing [The negative force area of the first compression cycle (Area 3)],. It evaluates how sticky yoghurt is and shows an inverse relationship with yoghurt's eating quality (Mudgil et al., 2017).

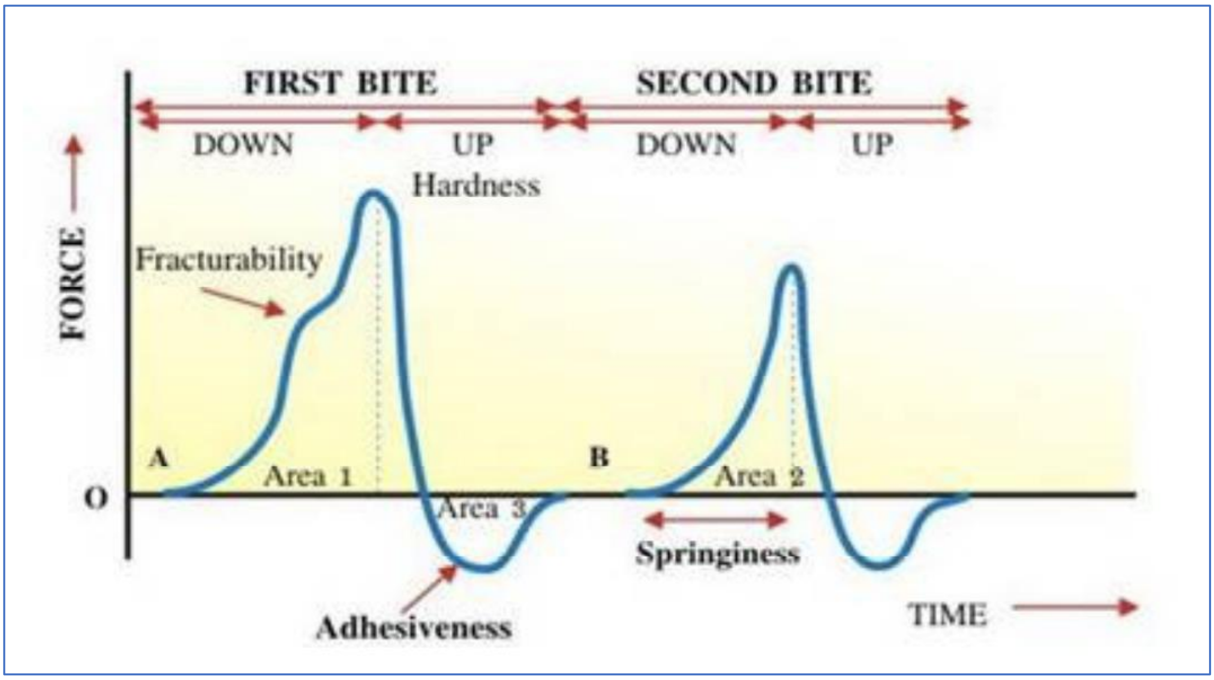


Figure 2.5 The Double-bite compression curve for texture profile analysis, Source: (Prajapati et al., 2016)

c) Cohesiveness

Cohesiveness is the ratio of the positive force area during the second compression cycle to that during the first compression cycle (Area 2/Area 1). It measures the strength of internal bonds in a material and refers to how much it may be distorted before breaking. It is a crucial characteristic for examining the texture of yoghurt and is connected to customer approval of the product (Ozcan, 2013)

d) Springiness

The rate of product return to native dimensions following the removal of the deforming force is known as springiness. It measures the degree of height recovery that occurs in the sample between the end of the first compression cycle and the beginning of the second compression cycle (Ozcan, 2013).

e) Gumminess

Gumminess is another vital parameter for yoghurt textural analysis. The consumer's acceptance of gumminess in yoghurt determines the level of acceptance. It might differ depending on the individual. Gumminess is a function of cohesion and hardness (Mudgil et al., 2017).

2.10.2.3 Rheology

Food rheology is the study of food products' flow and deformation. Since yoghurt has both viscous and elastic components, its rheological characteristics may be described using the viscous-elastic nature of milk gels. When a material is viscous-elastic, it possesses some of the solid's elastic qualities and some of the liquid's flow qualities (Lee & Lucey, 2010). Yield stress, or the point at which the shear stress starts to drop, is one of the rheological characteristics that may be found using deformation physical testing. The yoghurt gel with a lower yield value is brittle or short-skinned, whereas a greater yield value implies a stronger network of yoghurt gels (Ozcan, 2013).

Rheological characteristics of yoghurt have been studied using small amplitude oscillatory rheology (SAOR) to prevent the weak gel network from being harmed during the gel-forming process, or fermentation (Hyun et al., 2011). A minor relative deformation, such as a strain or change in dimension, is referred to as a small deformation since it does not impede the network structure's development when applied. Applying an oscillating stress or strain and observing the strain/stress responses is known as the SAOR testing (Rao & Rao, 2007). The SOAR test determines the rheological parameters such as storage modulus (G'), loss modulus (G"), and loss tangent (*tan δ*). The energy stored per deformation cycle is expressed by the storage modulus (G'), which also denotes the solid-like characteristics, and the loss modulus (G"), represents the liquid-like characteristics and shows the amount of energy dissipated as viscous dissipation every distortion cycle. The loss tangent (*tan δ*), which represents the kind of viscous-elastic characteristics present in a material, is defined as the ratio of the loss modulus to the storage modulus (G"/G'). The G" indicates the viscous or liquid nature of the gel network, whereas G' stands for elasticity. A substance exhibiting liquid-like behaviour has a high *tan δ*, value, that is, G" > G' (Ozcan, 2013).

However, in practice, a wide variety of probes, penetration methods, penetration depths, and temperatures are employed to determine the physical characteristics of yoghurt, making it nearly difficult to compare results between laboratories (Kilara & Chandan, 2013).

2.10.2.4 Microstructure

The three-dimensional arrangement and interactions between structural components greatly impact the textural qualities of a certain dairy product. Therefore, more than understanding the chemical composition and physical characteristics alone will be required to grasp the behaviour of dairy products. Also, understanding how the food microstructure is arranged is equally important (El-Bakry & Sheehan, 2014). Consequently, research on food microstructure establishes a connection between a specific product's physical and chemical characteristics, process behaviour, and organoleptic attributes.

One of the best microscopy methods for examining the microstructure of food, particularly dairy products, is confocal laser scanning microscopy (CLSM). With the help of computerized image analysis, this powerful tool can create two-dimensional micrographs and use laser scanning to visualize thin optical sections through the sample's surface without altering the internal structure. This allows for a three-dimensional analysis of the microstructure (El-Bakry & Sheehan, 2014). This is accomplished by using an argon laser to concentrate pictures up to several hundred microns in depth while seeing the specimen inside a plane both transverse to and along with the optical axis. CSLM uses lasers with specific emission wavelengths, usually between 488 and 647 nm, however, it can alternatively use ultraviolet or blue lasers at 405 nm (El-Bakry & Sheehan, 2014).

It is possible to differentiate between labelling some components, such as proteins or lipids, and get a unique insight into a food product's genuine three-dimensional structure by having the ability to observe its interior microstructure. Generic labelling is the most convenient, enabling fats and proteins to be rapidly distinguished. The dual-labeling technique involves applying a single dye combination, such as Nile Red for fat and Fast Green FCF for protein. This approach is more popular among researchers than the single-labelling technique as it is possible to see the distribution of protein and fat in mostly dairy products (Auty et al., 2001).

CSLM has been used to study fermented milk, such as yoghurts, and has the potential to characterize the aggregated protein network, fat droplet distribution, and starter microorganisms (Hassan et al., 2002). Using the dual labelling approach, CSLM has investigated the impact of high-shear milk processing on the sensory and rheological characteristics of the yoghurts (Ciron et al., 2012).

2.10.3 Protein digestibility assessment

2.10.3.1 Degree of protein hydrolysis (Ninhydrin reactive amino N)

Free amino group concentration is a measure of protein hydrolysis and can be determined using the ninhydrin test. The technique was first applied in the late 1940s to quantify amino acids; it was previously used to identify substances that contained amino groups, particularly those found in food (Sun et al., 2006).

Ruhemann's purple, a dark purple chemical, is produced when ninhydrin combines with the α-amino group of primary amino acids and is heated. The resulting chromophore remains consistent across all primary amino acids, while the colour intensity varies based on the quantity and chemical properties of the amino groups under analysis (Field & Field, 2010). The optimum pH for the entire reaction is pH 5.5. UV-visible spectroscopy measures absorbance at 570 nm, which relates to the peak absorption wavelength of Ruhemann's purple (Yemm et al., 1955).

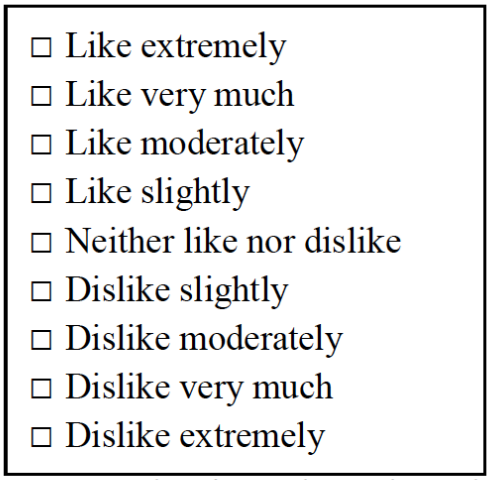
While modern analytical techniques like high-performance liquid chromatography (HPLC) are employed to identify compounds containing amino groups, the simple and practical ninhydrin method retains several benefits due to its low equipment requirements and ability to analyze large volumes of samples regularly (Sun et al., 2006). However, the O-Phthalaldehyde (OPA) method gained popularity over the ninhydrin assay in recent years for determining protein hydrolysis during digestion, it has certain disadvantages over the ninhydrin method, including a higher risk of handling hazards and false positives due to α-amino groups (Reynaud et al., 2020). On the other hand, the ninhydrin technique yields fewer levels of α-amino-group N than OPA does, and non-stoichiometric behaviour is observed in the correlation between the amount of colour formation and the variability in colour due to differences due to the nature of the product (Field & Field, 2010).

2.10.4 Evaluation of sensory characteristics

Although the yoghurt's biological, chemical, and physical characteristics may be determined, this process needs to provide a comprehensive understanding of the product's quality, since it does not account for the customer's sensory experience. Consumers may perceive various sensory experiences from chemically or physically similar or comparable things. Any fermented milk product, including yoghurt, has a texture that affects how good it tastes. The sensory perception of food products is connected to their texture. Smoothness (as opposed to lumpiness, graininess, or grittiness), thickness/viscosity, and sliminess (or ropiness) are the most often reported sensory characteristics associated with yoghurt texture. Therefore, managing the product's instrumental and sensory qualities is essential to guaranteeing its consumer acceptance (Aswal et al., 2012).

Non-descriptive hedonic tests based on product texture, appearance, and colour, as well as product odour and flavour, are often used in the sensory evaluation of fermented milk products. Though these tests focus on assessing consumer preferences, they won't represent the sensory nature of the product being tested. However, these methods are more useful during the initial product development stage (Svensson, 2012).

The emotional aspect of how consumers perceive food is measured using hedonic rating scales. The most often used hedonic rating scale is likely the 9-point degree of liking scale, sometimes known as the 9-point hedonic scale (Lawless et al., 1999). In this study, participants/customers are asked to select and mark one of nine options (from 1 = extremely like to 9 = extremely dislike) to express their hedonic view about a product sample. Nowadays, many different foam scales are used, and the verbally anchored scale (Figure 2.6) is used widely.

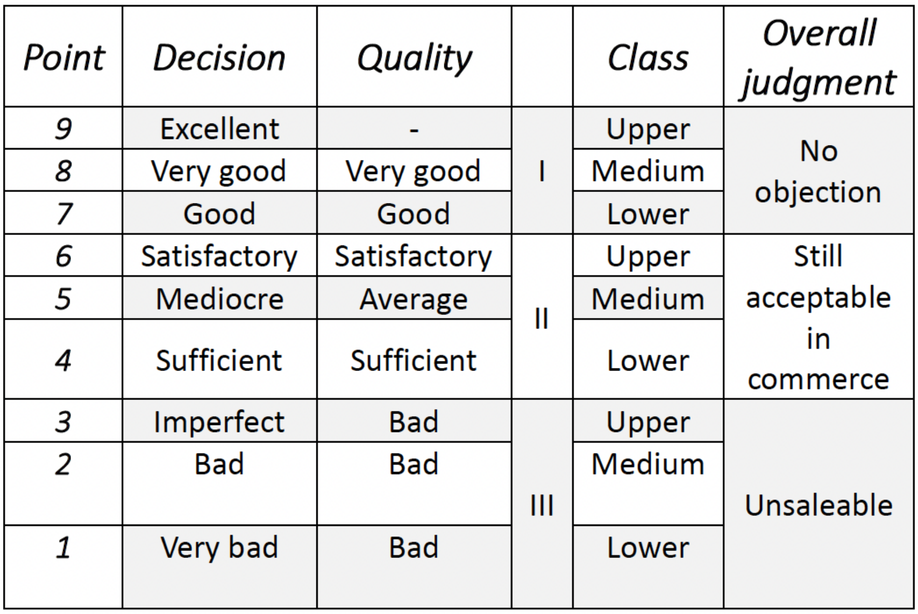


- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Figure 2.6 9-point-hedonic scale (verbally anchored)

The advantages of the 9-point hedonic scale include its ease of use, clarity, and comprehension for participants, as well as its high response stability and partial panel size flexibility (Svensson, 2012).

The Karl Ruher 9-point evaluation test is an alternative sensory assessment technique for yoghurt. The overall judgment about the product tested is decided as described in the table below (Figure 2.6) (Karagül-Yüceer & Drake, 2013).



Point	*Decision*	*Quality*		*Class*	*Overall judgment*
9	Excellent	-	I	Upper	No objection
8	Very good	Very good		Medium	
7	Good	Good		Lower	
6	Satisfactory	Satisfactory	II	Upper	Still acceptable in commerce
5	Mediocre	Average		Medium	
4	Sufficient	Sufficient		Lower	
3	Imperfect	Bad	III	Upper	Unsaleable
2	Bad	Bad		Medium	
1	Very bad	Bad		Lower	

Figure 2.7 Karl-Ruher 9-point sensory evaluation scheme

2.11 Research gaps

The research on the development of plant-based yoghurt from faba bean proteins was less evidenced, especially, in terms of its formulation with different ratios of faba bean milk, faba bean protein isolates, and dairy-faba bean hybrid products. Although faba beans have well-established nutritional and functional qualities, it is unclear how changing these ratios may affect the texture, flavour, and general quality of yoghurt. Research is required to determine the effects of varying faba bean milk analogues and protein isolate concentrations on the physico-chemical, rheological, and sensory characteristics during fermentation. Additionally, hybrid products that combine the proteins of faba beans and dairy might open up new options for protein quality and better rheological and sensory attributes, however, the best combinations are yet to be identified.

Chapter 3. Materials and Methods

3.1 Raw materials

The Faba bean (*Vicia faba L.*) seeds were locally purchased from Davis Trading Company, Palmerston North, NZ. 1 L UHT dairy milk (Meadow Fresh, NZ), 2 L Anchor "Blue" dairy milk (Fonterra, NZ), and white cane sugar were purchased from local supermarkets. Faba bean protein isolate (Unflavoured) was purchased from NZPROTEN, NZ (NZPROTEIN.CO.NZ). DELVO DSL Direct-set Lyophilised (2U DELVO YOG FVV-111) Starter Cultures, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp.* bulgaricus (DSM Food Specialties, Netherlands, www.dsmfoodspecialities.com) were supplied from "Invita" NZ Limited.

As per the label, the nutritional information of Anchor Blue Pasteurized milk and Faba bean protein Isolate used in this study is recorded in Table 3.1a and Table 3.1b respectively (see appendix A).

3.2 Preparation of plant-based and hybrid yoghurt

3.2.1 Preparation of faba bean milk analogue

The faba bean milk was prepared as shown in Figure 3.1. The purchased beans were washed and soaked in RO water overnight for 10-12 hours. Then the soaked beans were rinsed and dehulled before blending as tannins in the hulls could cross-link with protein during the blending (Jiang et al., 2020). Placed the dehulled seeds onto the mixing jar of the blender (*Sunbeam*) and RO water was added in a 1:6 (w/v) ratio and was mixed for 2-3 min at high speed until the mixture was smooth and creamy. The mixture was then manually filtered through a 150 µ filter to separate the faba bean milk from the solid contents, and the extracted milk was directly utilised as the raw material in the subsequent yoghurt-making process.

3.2.2 Preparation of faba bean protein isolate solution

The required amount of faba bean protein isolate was weighed using an electronic weighing scale (PB 3002 DeltaRange) and added to a mixing container. Then, a pre-calculated amount of RO water was gradually added while stirring continuously. Stir the mixture thoroughly to ensure even dispersion of the FPI.

For example, for making 100 ml of 4% FPI from protein isolates powder (product containing protein 85g/100g as shown in Table 3.1b), accurately weighed 4.70 g of FPI and dissolved the weighted amount in 100 ml of RO water.

The prepared faba bean protein isolate mixture was immediately utilised as the raw material in the subsequent yoghurt-making process.

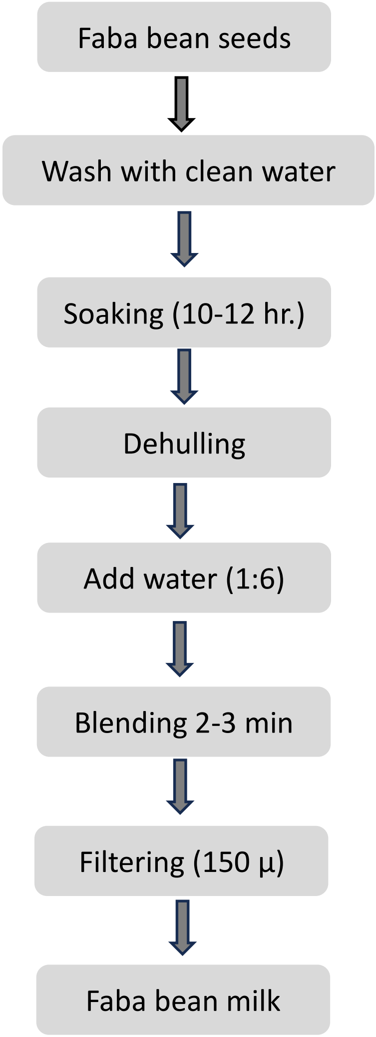


Figure 3.1 Process Flow diagram for preparation of faba bean milk analogue from faba be bean seeds

3.2.3 Preparation of culture for inoculation

The inoculation culture was prepared per the manufacturer's culture preparation instructions (*DSM Food Specialities, Netherlands)*. First, 1 litre of Duran bottle was cleaned and disinfected, and 200 ml of UHT (*Meadow Fresh, NZ*) was added to the bottle. Then the entire sachet of 2U DSM FVV-311 was poured into the Duran bottle and the rest of the UHT milk was added to obtain a final volume of 1L. Then the content was stirred well (but gently to avoid excessive foaming) until the culture was completely dispersed. Finally, the prepared culture suspension was kept at 4 ^{0}C in the refrigerator and used within 2 days from the preparation.

3.3 Preparation of yoghurt samples

A total of 6 dairy and plant-based samples have been prepared in this study as listed in Table 3.2. Each of the samples contains different ratios of the base ingredients (i.e., faba bean milk, faba bean protein isolates, and pasteurized dairy milk) of the yoghurt. All these samples were made only with the main raw material (faba bean milk (FBM), faba bean protein isolates (FPI), or dairy milk (DM)) inoculated with a starter culture. In addition, 2% (w/v) white cane sugar was added while making the samples that only had plant-based raw materials (SM#4, SM#5, and SM#6). None of the samples was homogenized during the sample preparation process. The different yoghurt samples were prepared following the basic yoghurt-making steps as shown in the flow chart (Figure 3.2).

3.3.1 Preparation of dairy-only yoghurt Sample (Reference)

The yoghurt sample contained 100% dairy and was referred to as a "reference" sample as specified in Table 3.2. The yoghurt reference sample was prepared using conventional dairy milk, as specified in Table 3.1a, by following the procedural steps outlined in the flow chart (Fig. 3.2). A stainless steel pan was used to heat 1 litter of standard dairy milk, reaching temperatures between 90-95 ^{0}C in a jacketed boiling water pan (Figure 3.3). The mixture was maintained at this temperature for 5 minutes, with continuous stirring and temperature monitoring. Following that, the milk was set in a cold-water bath and cooled until it reached a temperature range of 43-45 ^{0}C. At this point, the mixture was inoculated with the predetermined quantity (0.2% v/v) of a pre-prepared DSM starter culture and well mixed. The resulting blend was then dispensed into appropriately labelled sample containers and kept in an incubator set at 37 ^{0}C for 10-12 hours. Following incubation, the samples were stored in a chiller at 4 ^{0}C, ready for subsequent analysis.

Table 3.2 The Sample Code, product specifications, and product information of 6 different yoghurt samples used in this study (*D refers to dairy, and PB refers to plant-based*).

Sample	Sample Code	Product Specification	Product Information
1	**Reference**	100% D + 0% PB	Dairy Milk only
2	**SM#2**	50% D + 50% PB	Dairy + Faba Bean Milk
3	**SM#3**	50% D + 50% PB	Dairy + Faba Bean Protein Isolates
4	**SM#4**	0% D + 100% PB	Faba Bean Milk only
5	**SM#5**	50% PB + 50% PB	Faba Bean Milk+ Faba Bean Protein Isolates
6	**SM#6**	0% D + 100% PB	Faba bean Protein Isolates only

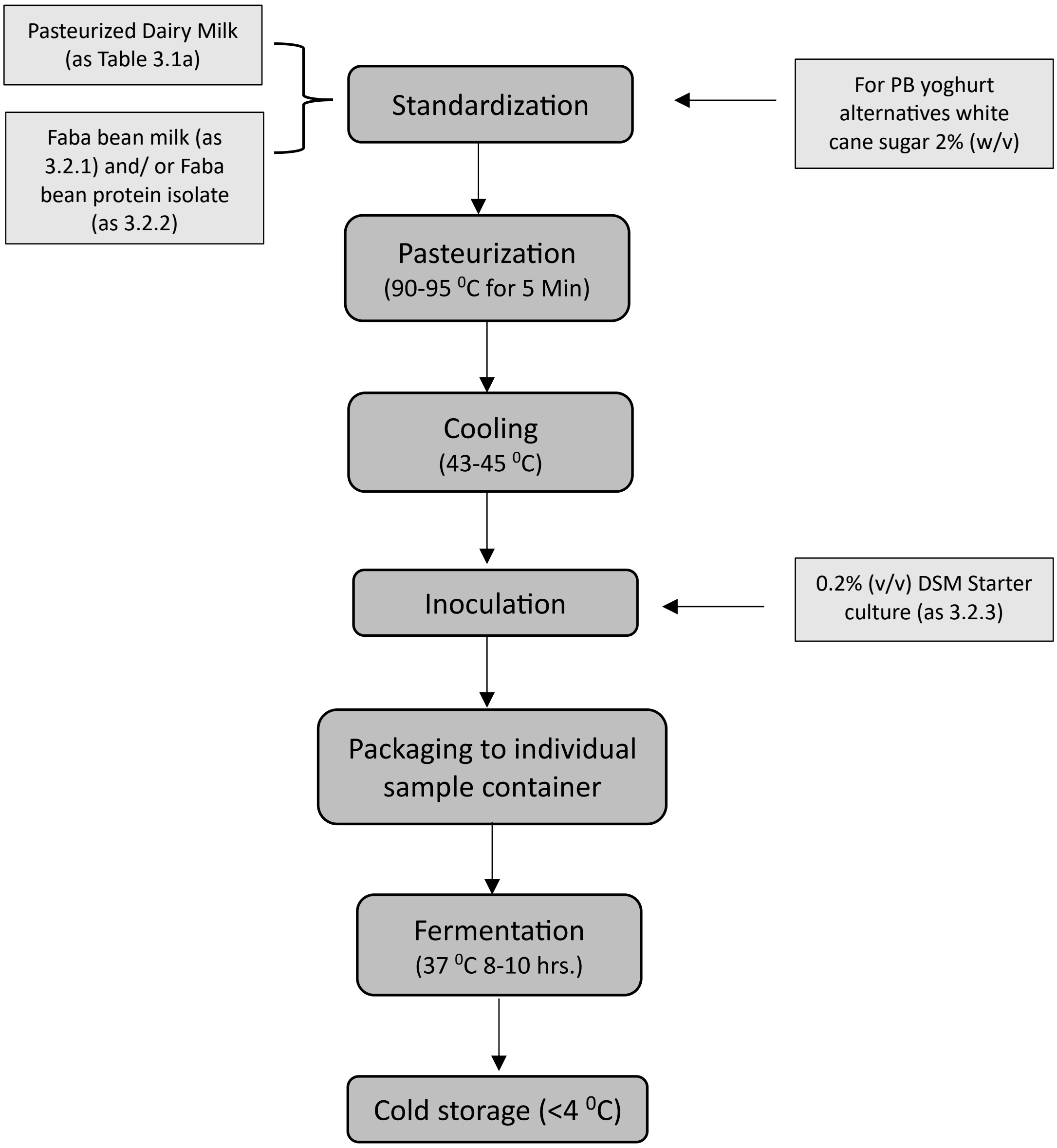


Figure 3.2 Process Flow chart for dairy and plant-based yoghurt alternatives.

3.3.2 Preparation of dairy and plant-based (hybrid) yoghurt Samples

As shown in Table 3.2, SM#2 and SM#3 were prepared as hybrid yoghurts containing 50% dairy and 50% plant-based ingredients (FBM or FPI) as the primary raw material.

3.3.2.1 Processing steps for SM# 2

1. As outlined in Table 3.1a, DM and FBM, prepared as described in 3.2.1, were accurately measured in a 1:1 ratio in a stainless steel pan. The mixture was thoroughly mixed to achieve a uniform mixture.
2. The mixture was heated to 90-95 ^{0}C by placing it in a boiling water pan (Figure 3.3). It was maintained at this temperature for 5 minutes while continuously stirring and monitoring it.
3. Following, the milk mixture was set in a cold water bath and cooled until it reached a temperature range of 43-45 ^{0}C. It was then inoculated with a pre-prepared DSM culture (0.2% v/v) and mixed thoroughly.
4. The resulting blend was then dispensed into appropriately labelled sample containers and kept in an incubator set at 37 ^{0}C for 10-12 hours.
5. Following incubation, the samples were stored in a chiller at 4 ^{0}C, ready for subsequent analysis.

Figure 3.3 Pasteurizing of milk for making yoghurt

3.3.2.2 Processing steps for SM# 3

1. As specified in Table 3.1a, DM and 4% FPI, prepared as explained in 3.2.2, were added in a 1:1 ratio to a stainless steel pan and mixed well to obtain a homogeneous mixture.

2. Steps 2 to 5, as outlined above (section 3.3.2.1), were repeated to produce SM#3, which contained 50% DM and 50% FPI.

3.3.3 Preparation of plant-based yoghurt samples

As shown in Table 3.2, SM#4, SM#5, and SM#6 were prepared as plant-based yoghurt samples that contained 100% FBM, 50% FBM, 50% FPI, and 100% FPI, respectively. Each yoghurt sample was prepared according to the steps described below.

3.3.3.1 Processing steps for SM# 4

1. A measured volume of freshly prepared FBM, as detailed in section 3.2.1, was poured into the stainless steel pan, and 2% (w/v) white cane sugar was then added and thoroughly mixed.
6. The mixture was heated to 90-95 ^{0}C by placing it in a boiling water pan. It was maintained at this temperature for 5 minutes while continuously stirring and monitoring it.
2. The milk mixture was then in a cold water bath and cooled until it reached 43-45 ^{0}C. It was then inoculated with starter culture (0.2% v/v) and mixed thoroughly.
3. The resulting blend was then dispensed into appropriately labelled sample containers and kept in an incubator set at 37 ^{0}C for 10-12 hours.
4. Following incubation, the samples were stored in a chiller at 4 ^{0}C, ready for subsequent analysis.

3.3.3.2 Processing steps for SM# 5

1. Freshly prepared FBM as described in section 3.2.1 and 4% FPI, prepared as explained in section 3.2.2 were mixed in a 1:1 ratio, and 2% (w/v) white cane sugar was then added and mixed well to obtain a homogeneous mixture.
2. Steps 2 to 5, as outlined above (section 3.3.3.1), were repeated to produce SM# 5, which contained 50% FBM and 50% FPI.

3.3.3.3 Processing steps for SM# 6

1. A measured volume of 4% FPI which was prepared as explained in section 3.2.2 was added to the stainless steel container and 2% (w/v) white cane sugar was then added and mixed well to obtain a homogeneous mixture.
2. Steps 2 to 5, as outlined above (section 3.3.3.1), were repeated to produce SM# 6, which contained 100% FPI.

3.4 Analysis of physico-chemical properties of yoghurt samples

All six yoghurt samples prepared as described above were analyzed for their Physicochemical properties.

3.4.1 Protein content

The protein content of each yoghurt sample was assessed using the Kjeldhal method, (AOAC 955.04). The nitrogen-to-protein conversion factor applied for the reference sample (100% dairy) was 6.38. In contrast, a nitrogen-to-protein conversion factor of 6.25 was used for the remaining five yoghurt samples, which contained 50% or 100% faba bean as their primary ingredient (Maubois & Lorient, 2016). Digestion with strong sulfuric acid was used in the process to convert all of the organic nitrogen to ammonium sulphate. Following an alkali distillation of ammonia to boric acid, standardized 0.1 M HCl was used to titrate the borate anions. It was determined how much crude protein each sample contained by measuring its nitrogen concentration (Conklin-Brittain et al., 1999).

The crude protein content of each sample was calculated using the following;

$$\% \text{ Nitrogen} = \frac{(A\ x\ B)\ x 14\ x\ 100}{1000\ x\ w} \qquad \ldots\ldots\ldots\ldots (1).$$

$$\% \text{ Protein} = \% \text{ N x } 6.88 \text{ (D)}, 6.25 \text{ (PB)}. \ldots\ldots\ldots\ldots (2).$$

Where A= mL HCL used, B= exact molarity of HCL, W= weight of sample (g)
D- dairy, PB-plant-based

3.4.2 Fat content

The fat content of each yoghurt sample was determined using a slightly modified version of the method as previously described by *Matela et al. (2019)* , complying with the AOAC 989.05. The yoghurt samples were mixed with ammonium hydroxide and a few drops of phenolphthalein. Afterwards, the mixture was combined with diethyl ether and petroleum ether, mixed well, and left for about an hour to separate the aqueous and organic phases. The fat content (organic phase) was then poured into a weighed flask and dried in an oven at 100 ^{0}C for 1 hour. The flask was then cooled in a desiccator and weighed to measure the mass of fat.

The crude fat content of each sample was calculated using the following;

$$\text{\% Fat content} = \frac{w2-w1}{w3} \times 100 \text{......... (3)}$$

Where, w-weight of the empty flask (g), w2-weight of flask and fat (g), and w3-weight of sample taken (g).

3.4.3 Crude fibre content

The crude fibre content of each yoghurt sample was evaluated using a slightly modified version of the method as previously described *Matela et al. (2019)* , complying with the AOAC 978.10. It was calculated as the portion that was left over after standard sulfuric acid and sodium hydroxide were used for digestion. In brief, the material was heated for thirty minutes after being hydrolyzed in a beaker with 1.25% sulfuric acid. After the mixture was vacuum-filtered, the residue was rinsed three times with hot, distilled water, heated for a further thirty minutes with 1.25% sodium hydroxide, and then filtered once more. The material that had been digested was rinsed three times with hot distilled water after being neutralized with hydrochloric acid. After the residue was placed in a crucible and dried in an oven at 100 ^{0}C for two hours, the sample was then cooled in a desiccator and weighed. The sample within the crucible was burned for 5 hours at 500 ^{0}C until all of the carbonaceous material was burned. The crucible containing the ash was then weighed after cooling in the desiccator.

The % crude fibre content of each sample was calculated using the following formula;

$$\% \text{ Crude fibre} = \frac{w1-w2}{w} \times 100 \text{...........} (4)$$

Where w1-weight of the digested sample and crucible (before ashing), w2-weight of the crucible & ash, and w-weight of the initial sample.

3.4.4 Starch content

The total starch content of yoghurt samples was analyzed in a two-step enzymatic process with amyloglucosidase and α-amylase (AOAC Method 996.11 and AACC Method 76.13) using a Megazyme total starch assay kit with slight modifications to the method previously described by *Mccleary et al. (1997).*

The yoghurt samples were accurately weighed directly into glass test tubes, (ranging from 90-100 mg). Then, 3.0 ml of thermostable $\boldsymbol{\alpha}$-amylase was added, and the contents of the tubes were mixed thoroughly on a vortex mixer to ensure complete homogenization. The tubes were immediately placed in a boiling water bath and incubated for 2 minutes. After removing them from the water bath, the contents were vigorously mixed in a vortex mixer. The tubes were then returned to the boiling water bath for 3 minutes before being thoroughly mixed.

Next, the tubes were placed in a water bath set at 50 ^{0}C and allowed to equilibrate for 5 minutes. Then, 4.0 ml of 200 mM sodium acetate buffer and 0.1 ml of amyloglucosidase solution were added to the tubes, followed by vigorous mixing. The tubes were capped and incubated for 30 minutes at 50 ^{0}C. The entire contents of each test tube were transferred to 100 ml volumetric flasks and diluted to 100 ml with Milli Q water. The contents of the flasks were thoroughly mixed, and then a portion of the suspension was centrifuged for 10 minutes at 1000 x g. Carefully, 0.1 ml portions of each supernatant were transferred to separate test tubes, using 2 tubes per supernatant. To each test tube, 3.0 ml of glucose oxidase-peroxidase-amino antipyrine buffer mixture was added, and the tubes were incubated for 20 minutes at 50 ^{0}C.

Each sample's absorbance was measured and recorded at 510 nm against a reagent blank. The average absorbance values for each sample were calculated and used in subsequent calculations using the equation (5).

$$\text{Total Starch \%} = A \times \frac{F}{W} \times 90 \text{ .... (5)}$$

Where A- absorbance of sample solutions read against reagent blank; F- factor to convert absorbance values to µg glucose; W- weight of the sample (mg).

3.4.5 Total soluble solid content

The crude fibre content of each yoghurt sample was assessed with slight modifications to the method as previously described by *Igbabul et al. (2014)*, AOAC 990.20. It was calculated as the portion that was left after all the moisture had been removed from the sample. The sample was accurately weighted to a clean and dry aluminium dish with a known weight. Then the sample was dried in an oven at 100 ^{0}C for 24 hours (until it reached the constant weight), and the final weight was measured (Matela et al., 2019). The % total solid content was calculated using the following equation.

$$\text{\% Total solids} = \frac{w2-w1}{w1-w} \times 100 \text{ .... (6)}$$

Where, w-weight of the aluminum dish, w1-weight of the dish + sample, w2-weight of the dried sample + dish

3.4.6 pH

The pH of all yoghurt samples was measured using a calibrated pH meter (ORINO STAR A211, Thermo Scientific pH meter). The pH meter was calibrated with pH 4 and pH 7 buffer solutions before measuring the sample pH. Once calibration was completed, the pH of each sample was measured in triplicates within 24 hours of sample preparation and an average value was recorded.

3.4.7 Syneresis (%)

The syneresis of the yoghurt samples was determined as previously described by *Zannini et al. (2018)* with slight modifications. The yoghurt samples as per Table 3.2, were formed in 15 ml conical plastic centrifuge tubes (Thermo Fisher Scientific, New Zealand) and stored at 4 ^{0}C. The weight % of the supernatant after centrifugation at 4000g for 15 minutes at 4 ^{0}C using a

Multifuge X Pro Series Centrifuge (Thermo Scientific, GmbH, Germany) was used to determine syneresis. The below equation (7) was used for the calculation of the degree of syneresis (Pachekrepapol et al., 2021).

$$\% \text{ Syneresis} = \frac{Weight\ of\ supernatant\ (g)}{Weight\ of\ product\ (g)} \times 100 \text{..........} \quad (7)$$

3.4.8 Total Titratable Acidity (TTA)

The total titratable acidity (TTA) of all yoghurt samples (as outlined in Table 3.2) was determined with slight modifications to the method described by *Zannini et al. (2018)*. Around 10g of each sample was placed in a 50 ml conical flask and mixed with 10 ml of distilled water. Then, 2 drops of phenolphthalein were introduced into the mixture, followed by titration using 0.1 N NaOH until the colour changed to pink and the final pH reached 8.75. The volume of NaOH used was then measured and the TTA was calculated using the equation (8) (Pachekrepapol et al., 2021).

$$\text{TTA (g/100g)} = \frac{Volume\ of\ NaOH\ (ml)\ X\ N\ X\ F}{Weight\ of\ product\ (g)} \times 100 \text{ ....} \quad (8)$$

Where N- Normality of NaOH (0.1N) and F- Factor are used to convert millilitres of the base to grams of acid (0.09 for lactic acid).

3.4.9 Colour

The six yoghurt samples used for colour measurements were prepared in 35 x 10 mm polystyrene Petri dishes (FALCON, Corning Incorporated, NY 14831, USA) following the same procedures as described in section 3.3. The colour of all yoghurt samples was measured using a colourimeter (Minolta Chromo Meter- CR-400, Minolta Co. Ltd. Japan) within 24 hours of their preparation. The equipment was calibrated using the standard colour plate (Y=86.6, x= 0.3162, and y= 0.3232). The measurements of colour were expressed as CIE L* (Lightness), a* (redness), and b* (yellowness) colour space. The colour values of the 100% dairy yoghurt sample were used as the reference values when calculating the colour difference

(ΔE) to compare how the colour of other samples deviated from the reference. The colour difference was calculated using the following equation (9) (Fernandez-Avila et al., 2017).

$$\Delta E = \sqrt{(L2 - L1)^2 + (a2 - a1)^2 + (b2 - b1)^2} \text{ ....(9)}$$

Where, L2, a2 & b2- values of the sample and L1, a1 & b1- values of reference

3.5 Analysis of structural and functional properties of yoghurt samples

All six yoghurt samples prepared as described in section 3.3 and outlined in Table 3.2 were analyzed for their structural and functional properties.

3.5.1 Rheological and Textural analysis

The rheological properties of yoghurt were studied using the small amplitude oscillatory rheology (SAOR) procedure and the textural properties of yoghurt samples (as outlined in Table 3.2) were determined with Texture Profile Analysis (TPA) as described in detail below.

3.5.1.1 Rheological properties

Dynamic oscillatory measurements of yoghurts were performed using a modular compact rheometer (Anton Paar, MCR302, Germany) with slight modifications to the previously described method *Sah et al. (2016)*. All six yoghurt samples (as outlined in Table 3.2) were analyzed for their frequency sweep, using the plate and cone geometry (40 mm diameter, 4^0 angle) with a 1 mm gap, and the test was performed at 4 ^{0}C and 10 ^{0}C temperatures. The sample was placed on the lower plate and, for structural recovery, the sample was first pre-sheared for 30 s at a shear rate of 500 s^{-1}, and then it was allowed to equilibrate for 300 s. Within the linear viscoelastic range (LVR), the frequency sweep test was conducted at 0.5% strain in the frequency range of 0.1 to 10 Hz.

The same sample was subjected to a shear rate sweep test after 300 seconds of equilibration to determine the apparent viscosity. The shear rate was increased logarithmically from 0.01 to 100 s^{-1}. The Storage modulus (G'), loss modulus (G''), loss tangent (tan δ), and apparent viscosity of each yoghurt sample were determined and recorded.

3.5.1.2 Textural properties

The Texture Profile Analysis (TPA) of yoghurts was performed with slight modifications to the previously described method *Huang et al. (2022)*. The texture analyser (TA-XT Plus, UK), with a load cell capacity of 5 kg was used for the TPA. The yoghurt samples for the TPA were formed in plastic containers (40 mm diameter and 100 mm height). A probe with a diameter of 38 mm was placed into the cylindrical cup (as described above) until it reached a depth of 30 mm. The target displacement was 15 mm, the probe testing duration was twice, and the probe velocities were 5.00 mm/s before measurement, 1.00 mm/s during measurement, and 5.00 mm/s after measurement. All tests were conducted at 25 ^{0}C, with an interval of 5s, a 38 mm cylindrical probe, and a trigger force value of 5g.

The textural qualities, including adhesiveness, hardness, gumminess, cohesiveness, and springiness, were determined and recorded using forces-time graphs (Figure 2.5), which were produced during double-compression testing of each sample.

3.5.2 Microstructure analysis

The microstructure of the six yoghurt samples, as outlined in Table 3.2, was examined using Confocal laser scanning microscopy (CLSM) with slight modifications to the method previously described by *Sah et al. (2016)*. A small amount of each sample was placed on a microscope slide in between a spacer (2 mm in thickness and 10 mm in diameter), and the yoghurt samples were stained with Fast Green FCF (for staining protein) and Nile Red (for staining fat). A glass coverslip was placed on top of the spacer in contact with the sample and kept for 5 minutes allowing dye to diffuse through the sample and remove the excess stain. The stained samples were examined with an x 63 oil immersion objective lens using an inverted confocal scanning laser microscope (Leica SP5 DM6000B, Germany). For Nile Red, the emission wavelengths were set at 488 nm/500–600 nm, and for Fast Green FCF, at 633 nm/650–710 nm.

Several images, including general observation and Airscan processing, were collected for each yoghurt sample and analyzed through Image J software. The images of representative samples of each yoghurt sample are shown in Chapter 4 under Results and Discussion.

3.5.3 Protein digestibility

3.5.3.1 In Vitro digestion experiment

The In-vitro, gastrointestinal digestion of yoghurt samples was performed based on the method described by *Nguyen et al. (2020)* using the INFOGEST method (Minekus et al., 2014), with some modifications. The in vitro digestive studies were conducted in separate double-jacketed glass reactors for each sample, one for the gastric phase and another for the intestinal phase for pre-determined time points. The human body temperature was maintained by attaching these reactors to a circulatory water bath set at 37 ± 1 ^{0}C (Figure 3.4)

Stock solutions with 1.25 X concentration of simulated salivary fluid (SSF), simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and enzyme solutions were prepared as described by *Minekus et al. (2014)*.

Oral phase

To simulate chewing and oral digestion, 8 g of sample was added to each glass reactor, and the samples were then incubated for 2 minutes at pH 7.0± 1 with 8 mL of simulated salivary fluid containing $\boldsymbol{\alpha}$-amylase (10025, Sigma Aldrich, Saint Louis, MO, USA) at the ratio 1w:1v.

8 g of yoghurt sample was added to each reactor, followed by a calculated volume of SSF and $CaCl_2$, then covered with tin foil and warmed to 37 ^{0}C. pH was adjusted to 7.0± 0.1 with 1 M NaOH and the balance volume of water was added at the ratio of 1w:1v so the total volume was 8 mL including the volume of enzyme added. Finally, the enzyme ($\boldsymbol{\alpha}$-amylase, with a concentration of 75U/mL) was added to each reactor, and after 2 minutes all the reactors were transferred to simulated gastric condition.

Gastric phase

After 2 minutes of oral stimulation, the digest in each reactor was transferred to the simulated gastric digestion as described below.

Each reactor pH was adjusted to 3.0 ± 0.1 with 0.5 M HCl, and then SGF, $CaCl_2$, and Milli Q water were added to get the final volume of 16 mL after adding of enzyme. Four glass beads

(3-5 mm) were added to each reactor to mimic food maceration in the stomach. Then the Pepsin (P7000, Sigma Aldrich, Saint Louis, MO, USA) which was dissolved in SGF was added to each reactor and the stirring speed was set at 100 rpm (3.2-3.5 gear).

The samples were conducted at each time point, (10 min, 30 min, 60 min, and 120 min) from the gastric digestion reactors. Pepstatin A (60 µl, ab141416, Abcam, Cambridge, UK) (0.5 mg/mL in methanol) was immediately added after sampling to every 5 ml of the gastric digests and mixed thoroughly for 20 seconds. The digest was then transferred to 2 mL microtubes, immersed in the ice bath, and then stored at -20 ^{0}C for further analysis.

Intestinal phase

After the above-mentioned two-hour-long gastric digestion, in vitro small intestine digestion was initiated in a second set of reactors for another 2 hours (Figure 3.4).

The small intestinal digestion process was initiated by introducing 32 millilitres of liquid, including simulated intestinal fluid (SIF), CaCl2, Milli Q water, bile extract, and pancreatin (P1750, Sigma Aldrich, Saint Louis, MO, USA) at a 1:100 protein to milk protein ratio, into the gastric digests. With continuous mixing, the digest's pH was adjusted with 1 M NaOH and kept at 7 ± 0.1.

The samples were conducted at each time point (130 min, 150 min, 180 min, and 240 min) of the small intestinal digestion. To inactivate the digestive enzymes, 2.25 ml of SIGMAFASTTM protease inhibitor cocktail solution (S8820, Sigma Aldrich, Saint Louis, MO, USA) (one tablet in 50 mL Milli-Q water) was mixed with every 5 mL of small-intestinal digest after sampling, and homogenized well by hand for 20 seconds. The digest was then transferred to 2 mL microtubes, immersed in the ice bath, and then stored at -20 ^{0}C for further analysis.

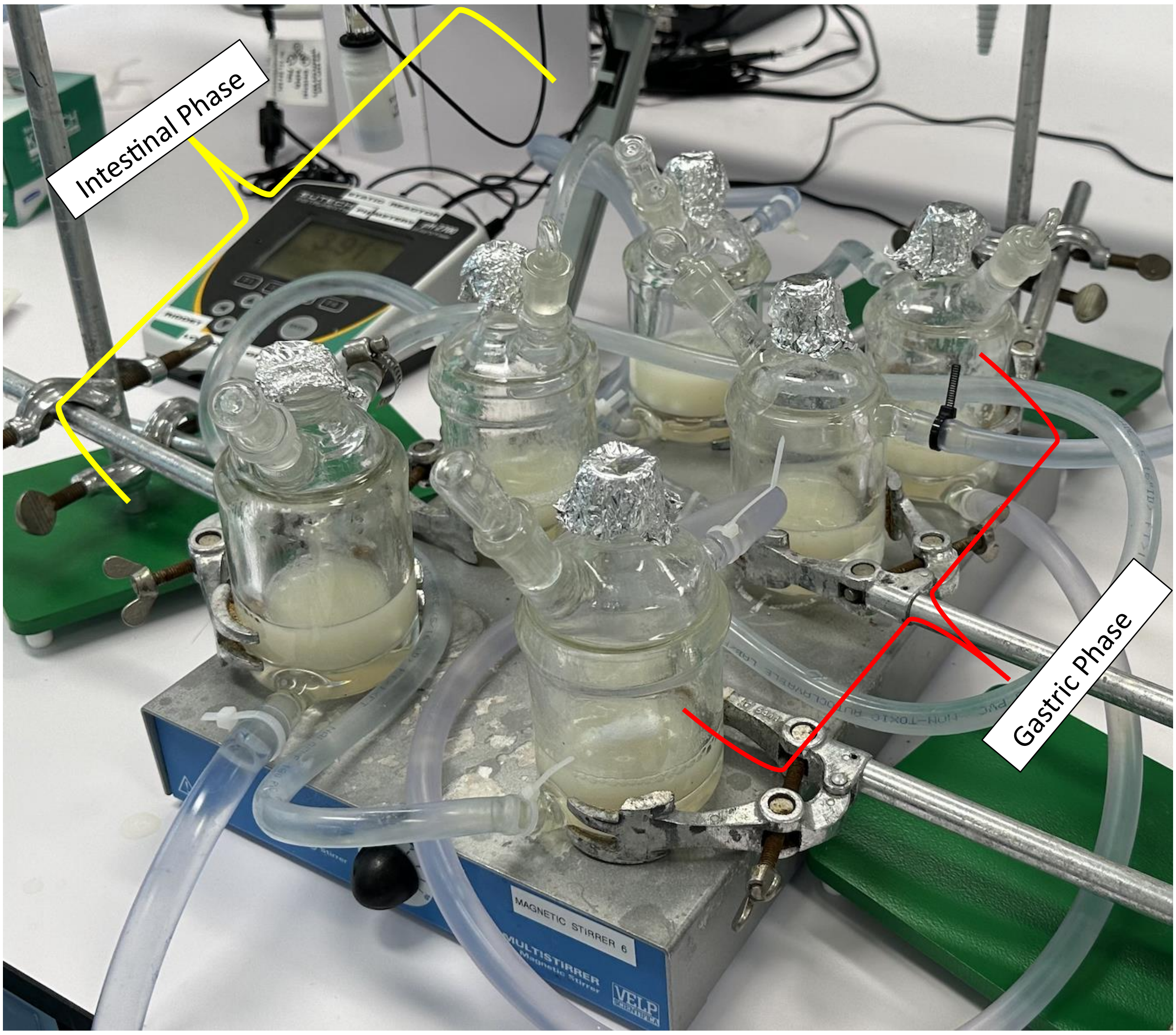


Figure 3.4 Static Gastro-Intestinal Protein Digestion Experimental Setup

3.5.3.2 Protein digestibility assessment

Ninhydrin analysis for reactive amino N

The frozen digested samples stored at -20 ^{0}C were thawed at room temperature and centrifuged for seven minutes at 14,000 rpm (Eppendorf Mini spin plus, Hamburg, Germany). The supernatant was filtered through a 0.45 µm PVDF syringe filter (Millex®, Duluth, MN, USA) (Kaur et al., 2023). Ninhydrin reagent (N7285, Sigma Aldrich Pty Ltd, USA) was used to quantify the percentage of ninhydrin reactive amino N (%) in the filtered supernatant following the method described by *Moore (1968)*.

The filtered supernatant was subjected to serial dilution with pH-adjusted Milli Q water (pH of 3.0 ± 0.1 for gastric phase samples and pH 7.0 ± 0.1 for intestinal phase samples) and made final volume to 1 ml in a Kimax tube as shown in Table 3.3. 0.5 ml of 2 % ninhydrin reagent was added to each Kimax tube followed by heating to 100 ^{0}C for 10 minutes and were cooled to room temperature. Then each Kimax tube was added with 2.5 mL of 95% ethanol.

Table 3.3 The gastric and Intestinal digesta sample preparation for Ninhydrin analysis

Time	**Gastric Phase (pH 3.0 ± 0.1)**				**Intestinal Phase (pH 7.0 ± 0.1)**			
(min)	**10 min**	**30 min**	**60 min**	**120 min**	**130 min**	**150 min**	**180 min**	**240 min**
Initial Sample volume	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl	60 µl
Milli Q Water	160 µl	160 µl	160 µl	160 µl	160 µl	160 µl	160 µl	160 µl
Diluted Sample volume	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl
Milli Q water	980 µl	980 µl	980 µl	980 µl	980 µl	980 µl	980 µl	980 µl

After obtaining the digest sample absorbance values at 570 nm, the corresponding quantities of ninhydrin-reactive amino nitrogen were calculated using the equation derived from the standard curve. A standard curve was derived from a stock solution of 50 M glycine in 0.05% glacial acetic acid (Kaur et al., 2023). Five Kimax tubes containing different concentrations of glycine were used for the standard, as shown in Table 3.4.

Table 3.4 Preparation protocol of glycine standards for STD curve

	Standard Tube				
	1	**2**	**3**	**4**	**5**
Concentration (µ mol/mL)	0.0000	0.0125	0.0250	0.0375	0.0500
50 µM glycine std (mL)	0.00	0.25	0.50	0.75	1.00
Milli-Q water (mL)	1.00	0.75	0.50	0.25	0.00
2% Ninhydrin (mL)	0.50	0.50	0.50	0.50	0.50

The standard tubes as shown in Table 3.4 were heated to 100 ^{0}C for 10 minutes and cooled to room temperature. Then, 2.5 ml of 95% ethanol was added to each Kimax tube.

The standard curve was plotted using the absorbance (Y-axis) against the glycine concentration (X-axis) after the absorbance values of the standard series were measured at 570 nm. After obtaining the absorbance values of sample digesta at 570 nm, the corresponding amount of ninhydrin-reactive amino nitrogen was calculated using the equation derived from the standard curve. Using a Vis-spectrophotometer (Helios Epsilon, Thermo Fisher Scientific Pty Ltd, U.K.), the absorbance of the samples and standards was measured. Acceptable absorbance ranges were 0.2–0.8.

3.6 Sensory evaluation

Based on composition, rheological, structural, and digestibility performances, three yoghurt samples (SM#2, SM#4, and SM#5) were subjected to sensory evaluation for comparison with the reference yoghurt sample. The sensory characteristics of the yoghurt were assessed by an untrained consumer panel consisting of 30 individuals at Massey University, Palmerston North. This panel included people from multiple ethnic backgrounds, aged 20 to 55 who were regular consumers of fermented milk products like yoghurt. The sensory evaluation was performed using the 9-point hedonic scale with slight modifications to the method described by Santiago-García et al. (2021). The sensory questionnaire used for the evaluation can be found in Appendix C. Fifty millilitres of the three experimental yoghurt samples were placed in plastic cups and stored at 4°C ± 0.5 ^{0}C. Two days after preparation, they were coded with three-digit numbers randomly and presented in a random order to panellists. The panellists were asked to rate the yoghurts' appearance, odour, texture, flavour, and overall acceptability using a 9-point hedonic scale where 9 represented "like extremely" and 1 represented "dislike extremely."

The human ethics committee of Massey University has approved this sensory test under the low-risk category with the human ethics notification number 4000028846.

3.7 Statistical data analysis

A statistical analysis was conducted on the results using Minitab (Minitab LLC, version 21.3, USA). The differences between the mean values were assessed by one-way analysis of variance (ANOVA) followed by Turkey simultaneous test with a significance level of ($p < 0.05$). All measurements were carried out in triplicate unless otherwise specifically mentioned.

Chapter 4. Results and Discussion

In this chapter, the findings of the different analytical procedures (explained in Chapter 3) applied to the yoghurt samples (as outlined in Table 3.2) are presented and discussed.

4.1 Physico-chemical properties

4.1.1 Proximate composition of yoghurt

The mean values ± SD of proximate compositions (% protein, % fat, % total solids, % crude fibre, and % starch) of six yoghurt samples are shown in Table 4.1 (appendix C) and these data are graphically represented in Figure 4.1.

The protein content of each yoghurt sample was significantly different from that of the reference (3.68 %). Out of five samples, SM#4 had a significantly lower level of protein (2.33 %) while SM#6 had a significantly higher amount of protein (4.30 %) which was higher than the reference. These differences could be mainly due to the raw material used for the production of each sample (Grasso et al., 2020). SM#4 was produced using only FBM while SM#6 was produced using FPI with an initial protein content of 85g/ 100g.

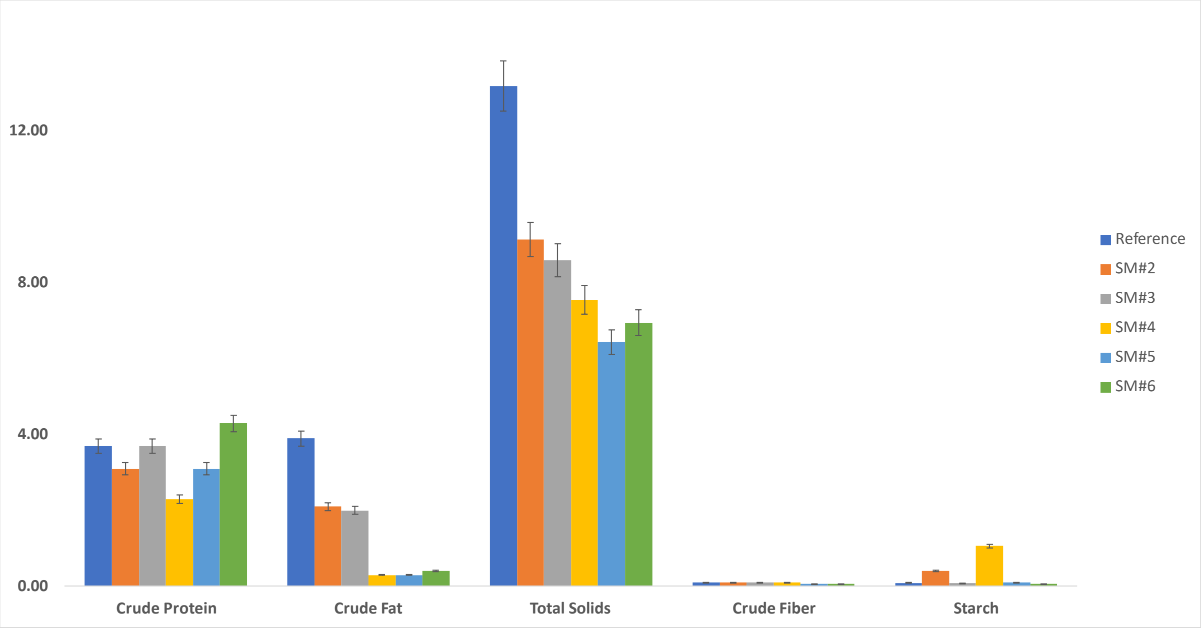


Figure 4.1 Proximities comparisons of different yoghurt samples after 24 h storage at 4^0C (**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

The % fat of each yoghurt sample significantly differed from the reference (3.88 %), which was made with 100% dairy milk. Also, a significant difference in fat content could be observed between dairy hybrid (SM#2 & SM#3) and plant-based yoghurt samples (SM#4, SM#5 & SM#6). This difference could also be due to the difference in fat content in raw materials. Dairy milk contains higher fat content compared to plant-based milk and protein isolates (Setia et al., 2019).

The % total solids content of each yoghurt sample was significantly difference from the reference yoghurt sample (13.18%). The % total solids of dairy-plant-based hybrid yoghurts were significantly higher than the plant-based yoghurt samples. This variation also could be due to the effect of the main raw material and the ratios used. The crude fibre % and starch content of all the yoghurt samples were significantly low, however, SM#4 contained a comparatively higher % of starch (1.06 %). Faba bean seed contained a higher % of starch (Millar et al., 2019).

According to the above results, the composition of the yoghurt samples differed significantly. The composition of the ingredients and the ratios used to prepare each yoghurt sample might be the reason for the variation. These differences in composition have impacted the qualitative characteristics of yoghurt, and they have been discussed in the following sections.

4.1.2 pH and Total Titratable Acidity (TTA)

As shown in Table 4.2 (appendix C), the reference sample showed significantly *($p<0.05$)* lower pH (4.17) compared to all other yoghurt samples. Figure 4.2 shows the changing pattern of pH between each yoghurt sample. The low pH resulted from the high metabolism of lactic acid bacteria (LAB) in the dairy substrate. The amount of starch in the product has a direct impact on maintaining the pH of fermented dairy products (Altemimi, 2018; Pachekrepapol et al., 2021). The hybrid yoghurt samples (SM#2 and SM#3) made as dairy and plant-based (FBM and FPI respectively) showed higher pH compared to the dairy reference sample. In addition, all plant-based yoghurt samples (SM#4, SM#5, and SM#6) also showed higher pH values compared to the 100% dairy reference sample. As reported by *Altemimi (2018)* the higher pH values of these samples could be due to the incorporation of starch (from the faba bean), which may reduce the amount of available water. Consequently, less lactic acid is created and LAB finds it more difficult to metabolize sugar.

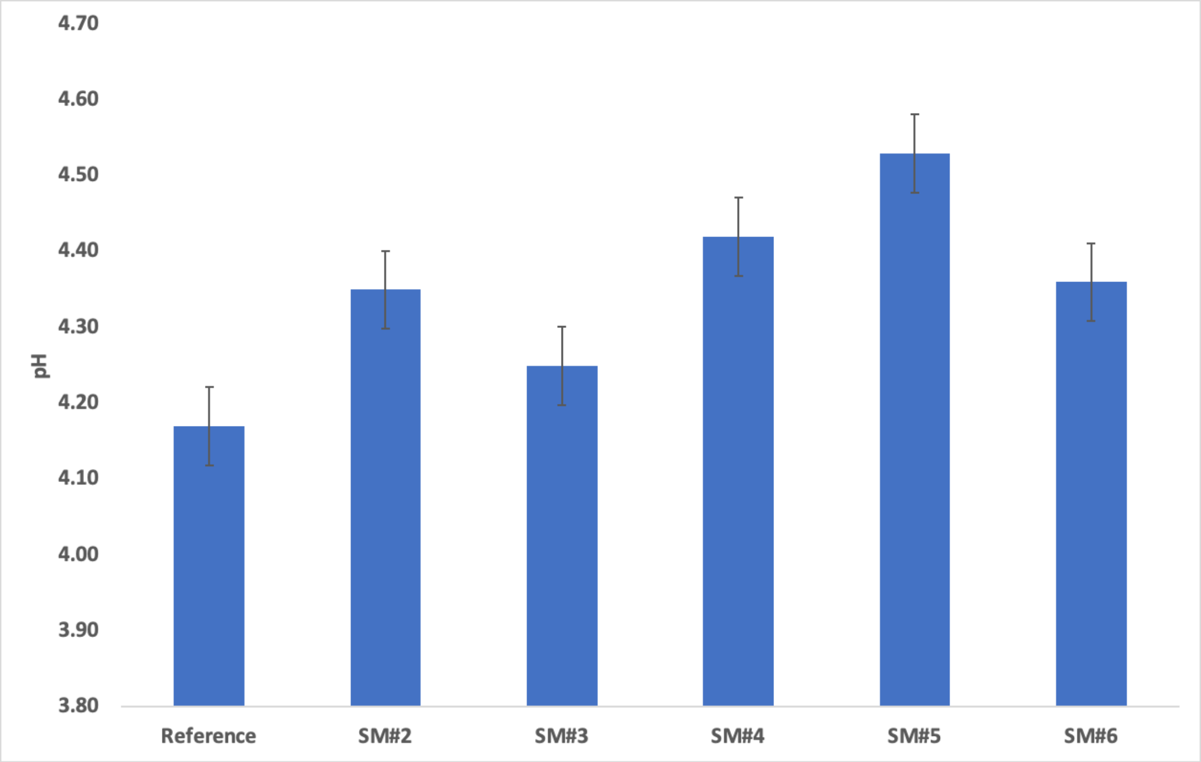


Figure 4.2 pH values of different yoghurt samples
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

As shown in Table 4.2 (appendix C), a significant difference in TTA values between the dairy-faba bean hybrid samples and samples made with 100% faba bean could observed. The reference sample showed significantly *($p<0.05$)* higher TTA (0.81) compared to all other yoghurt samples. Figure 4.3 shows TTA data of different yoghurt samples made with different ratios of dairy and faba bean (as outlined in Table 3.2) and the values agreed with the results obtained for pH. The highest TTA obtained for 100% dairy yoghurt was 0.81while the lowest value (0.24) was achieved for yoghurt made with 100 % FPI. The low TTA level indicated that faba bean would not be a suitable substrate for *S. thermophilus* and *L. delbrueckii spp.* However, the results show that faba bean supplemented with dairy milk could provide a better substrate for LAB to metabolize. *Ujiroghene et al. (2019)* reported similar results, stating that supplementing cow's milk would enhance LAB growth in quinoa.

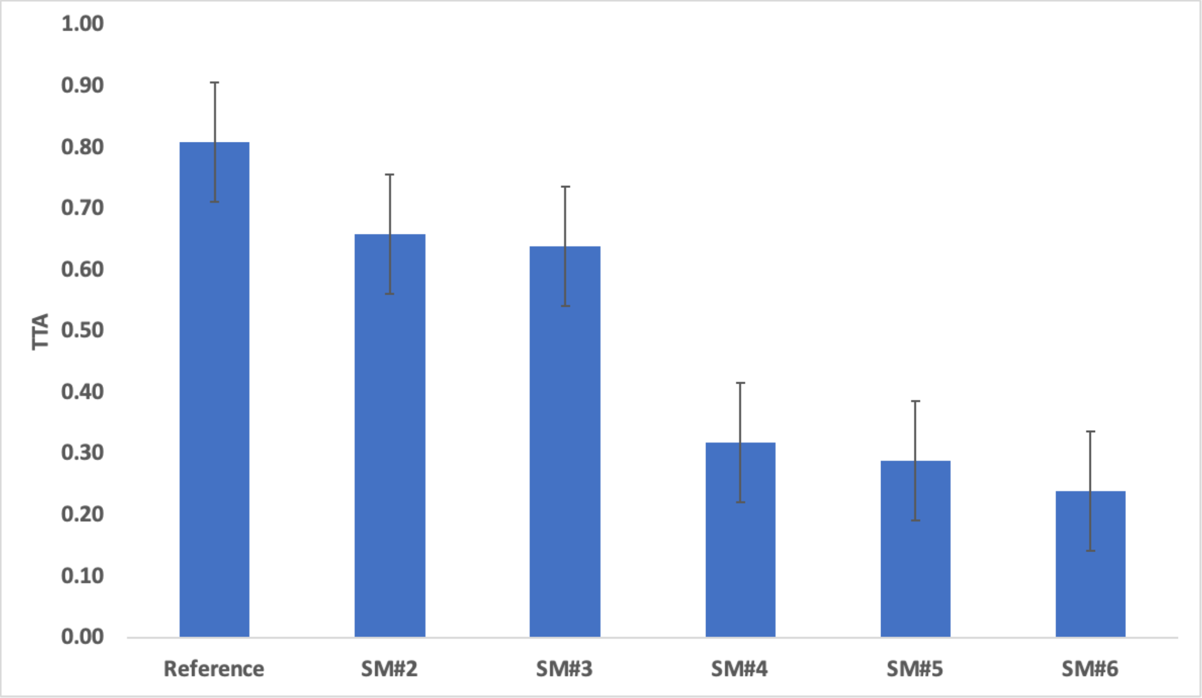


Figure 4.3 Total titratable acidity values of different yoghurt samples
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

4.1.3 Syneresis (%)

The amount of serum that is released from the gel when centrifugal force is applied is measured by a process called syneresis. It is a quality indicator that shows how much water the product can hold or its water-holding capacity (WHC) (Donmez et al., 2017). Figure 4.4 shows the changing pattern in the syneresis % while Table 4.2 (appendix C) shows the values of synaeresis of different yoghurt samples as outlined in Table 3.2. The rate of syneresis of each sample except SM#3 and SM#4 was significantly *($p<0.05$)* different. The reference yoghurt sample had nearly a 50% syneresis rate (51.8%) while SM#3 (made with dairy and FPI) showed the highest rate of syneresis (64%) and sample #6 (made with 100% FPI) had the lowest rate of syneresis (12.44%). A higher WHC might be the reason for the decreased level of syneresis in samples that had FBM and FPI, which contain reasonably higher amounts of starch and proteins. Several studies have revealed that adding starch to yoghurts reduces their amount of syneresis, e.g., low-fat yoghurt decreased syneresis when tapioca and maize starch were added (Lobato-Calleros et al., 2014). Starch, known for its thickening and stabilizing properties, has been widely employed in yoghurt production to minimize syneresis by leveraging its ability to swell and retain significant amounts of water within the yoghurt structure (Wong et al., 2020). However, the commercial plant-based yoghurts obtained a significantly lower syneresis level using starch and pectin as a mix of thickening and gelling agents (Grasso et al., 2020). Several research on the syneresis of non-dairy yoghurts have shown varying levels of syneresis, almond yoghurts achieved a syneresis between 26 and 27% when stabilizers and gelling agents such as xanthan gum, pectin, and polymerized whey protein were added (Shi et al., 2020). Yoghurt samples with 100 % FPI (SM#6) and, 50% FBM + 50% FPI (SM#5) achieved reasonably low syneresis values similar to yoghurt with almond milk added with thickening and gelling agents.

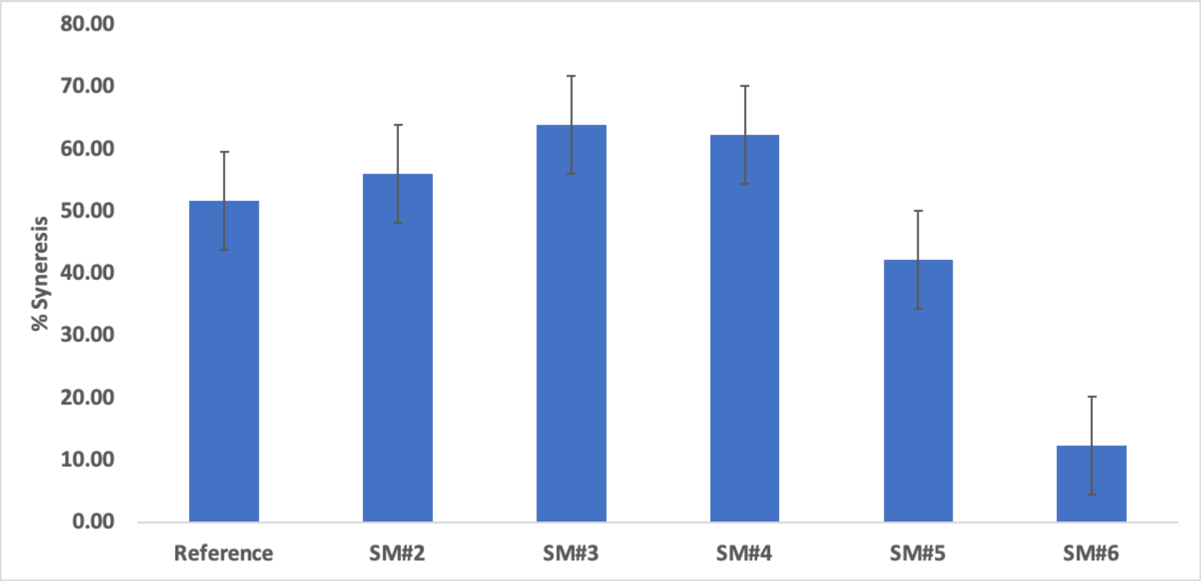


Figure 4.4 % Syneresis data of different yoghurt samples made with different dairy and faba bean ratios as outlined in Table 3.2
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

4.1.4 Colour

Food colour is an essential quality that directly affects both product attractiveness and consumer acceptance (Ramos et al., 2013). The analysed CIELAB colour space of each yoghurt sample is shown in Table 4.3. All the yoghurt samples have shown significantly lower L* values *($p<0.05$)* compared to the reference (85.85), indicating less lightness product against the reference. The yoghurt's lightness value (L*) is due to the slight scattering by the fat molecules in the product (Levy et al., 2021). Therefore, the yoghurt's lightness value directly relates to the amount of fat in each product. These results are more explainable when carefully observing the composition of each yoghurt sample as shown in Table 4.1.

The negative a* value indicates the greenish components of the yoghurt samples. The higher negative a*value indicates a higher greenish component in the sample. According to the results shown in Table 4.3, the highest negative a*value was in the reference sample (-3.22) compared to the other five yoghurt samples. Similar to value L*, value a*, is also primarily determined by fat globules in the product. The reference yoghurt sample was made with cow's milk and it had the highest fat content (3.88%) compared to other yoghurt samples, the value resulted in a* that is more related to the amount of fat in the product.

The b* value of the product indicates the yellowish component associated with the product. It could be observed that a higher b* is for products containing dairy milk, i.e., reference SM#2 and SM#3. The SM#3 has shown a very high b* value compared to the other two dairy-containing yoghurts. This was related to the product failure, i.e., SM#3 observed clear sedimentation of solid particles to the bottom (not observed in any other product) of the container and this could lead resulting a high value for b*. The other reason for showing higher b* in products containing dairy milk could be the brown pigments produced from the Maillard reaction due to the amino group and reducing sugar in the yoghurt sample (Akesowan, 2009). The SM#4 was shown as the lowest b* value (2.38) compared to all tested samples. This result was mainly related to the Maillard reaction rate in each product, as SM#4 showed the lowest amount of protein (2.33%) among the tested samples.

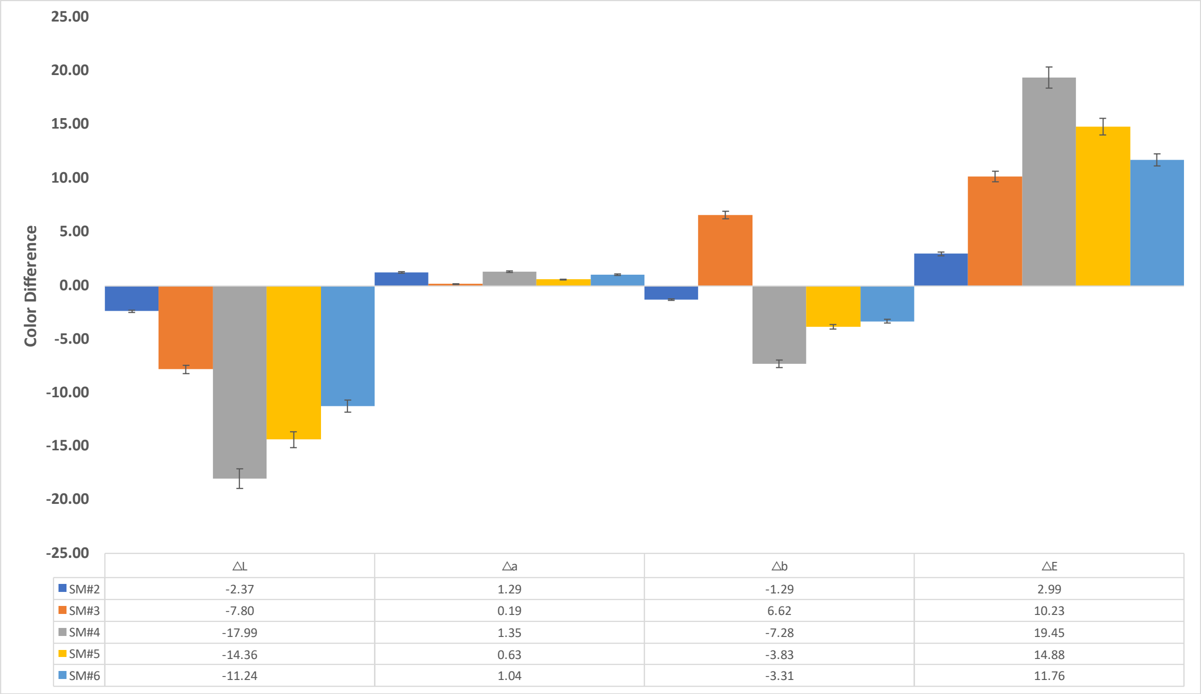
Table 4.3 CIELAB Colour coordinates (L*, a* & b*) of six yoghurt samples made as outlined in Table 3.2

Product Code	**L***	**a***	**b***
Reference	85.24 ± 0.56^{a}	-3.22 ± 0.03^{e}	9.66 ± 0.04^{b}
SM#2	82.87 ± 0.62^{b}	-1.93 ± 0.08^{a}	8.37 ± 0.13^{c}
SM#3	77.44 ± 0.24^{c}	-3.03 ± 0.03^{d}	16.28 ± 0.06^{a}
SM#4	67.25 ± 0.28^{f}	-1.87 ± 0.03^{a}	2.38 ± 0.13^{f}
SM#5	70.88 ± 0.33^{e}	-2.59 ± 0.02^{c}	5.83 ± 0.22^{e}
SM#6	74.00 ± 0.25^{d}	-2.18 ± 0.03^{b}	6.35 ± 0.29^{f}

****Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same column were significantly different (p<0.05) by the Turkey pairwise comparisons.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Total colour difference (ΔE) is a measurement that may be used to quantify colour changes. The formula (9), as described in Chapter 3, is used to get the total colour difference, which represents the degree of colour difference between any two samples (Fernandez-Avila et al., 2017). The total colour difference (ΔE) as well as the difference of individual CIELAB colour parameters (ΔL*, Δa*, and Δb*) against the reference yoghurt sample, were calculated based on formula (9) and shown in Figure 4.5. These results have shown how much individual colour parameters and the total colour have deviated from the reference sample. These data could help in understanding which product colour was closer as well as which product colour deviated more from the reference. Based on the calculated values, the total colour difference of SM#2 has shown less deviation while SM#4 has shown the highest deviation from the reference, on the other hand, the overall colour of SM#2 was closer to the reference while SM#4 was significantly difference *(<0.05)* to the reference.



	△L	△a	△b	△E
■ SM#2	-2.37	1.29	-1.29	2.99
■ SM#3	-7.80	0.19	6.62	10.23
■ SM#4	-17.99	1.35	-7.28	19.45
■ SM#5	-14.36	0.63	-3.83	14.88
■ SM#6	-11.24	1.04	-3.31	11.76

Figure 4.5 Total colour difference of each yoghurt sample (as outlined in Table 3.2, SM#2-SM#6) against the reference made with 100% dairy.
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

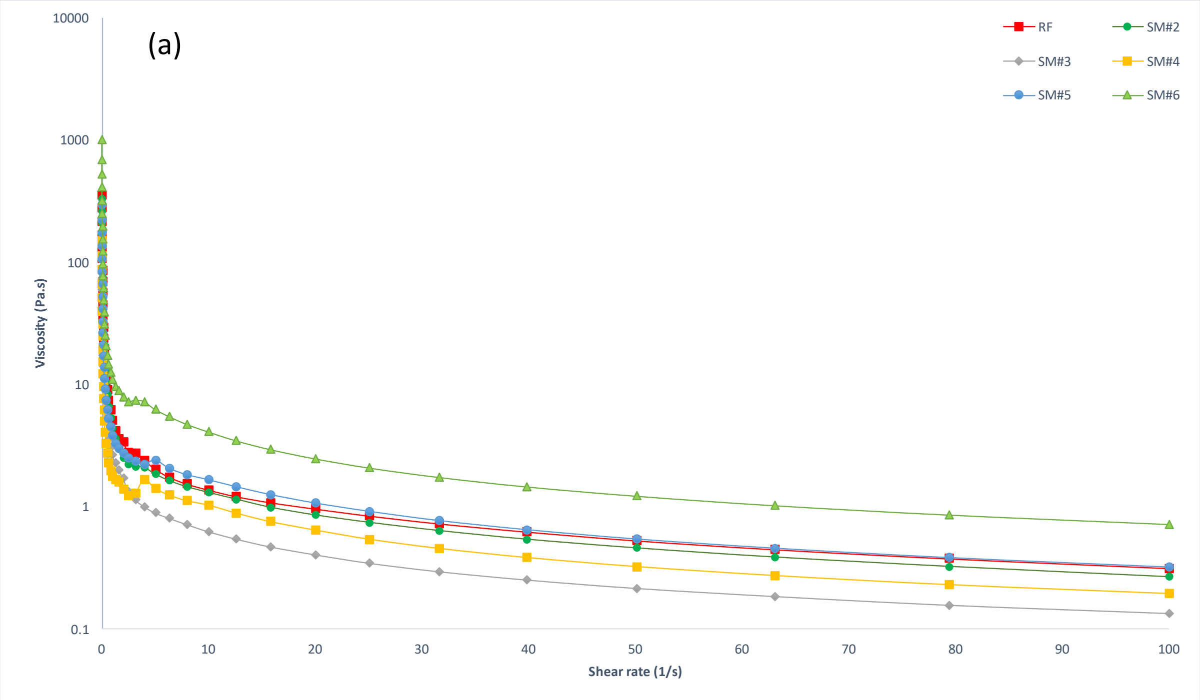
4.2 Rheological and Textural properties

4.2.1 Rheological properties

The rheological properties of all yoghurt samples were determined using the procedure described in section 3.5.1.1. Figure 4.6 illustrates how the apparent viscosity of different yoghurt samples behaved as the shear rate increased at 4 ^{0}C and 10 ^{0}C.

A non-Newtonian shear thinning behaviour was shown by the apparent viscosity decreasing with an increase in shear rate. The decrease in viscosity during the shearing demonstrated the samples' shear-thinning behaviour (Tan et al., 2018).

The viscosity of SM#6 (made with 100% FPI) was significantly higher *($p<0.05$)* than the reference sample (made with 100% DM) and the viscosity of all other yoghurt samples was lower than the reference yoghurt sample. According to these findings, the protein isolates from faba bean strengthened the gel network when the particles expanded and absorbed water in a continuous phase throughout the heating process, enhancing the connections between particles or between polysaccharides and protein. *Grasso et al. (2020)* reported an increase in the viscosity of coconut yoghurt that was added with tapioca starch.



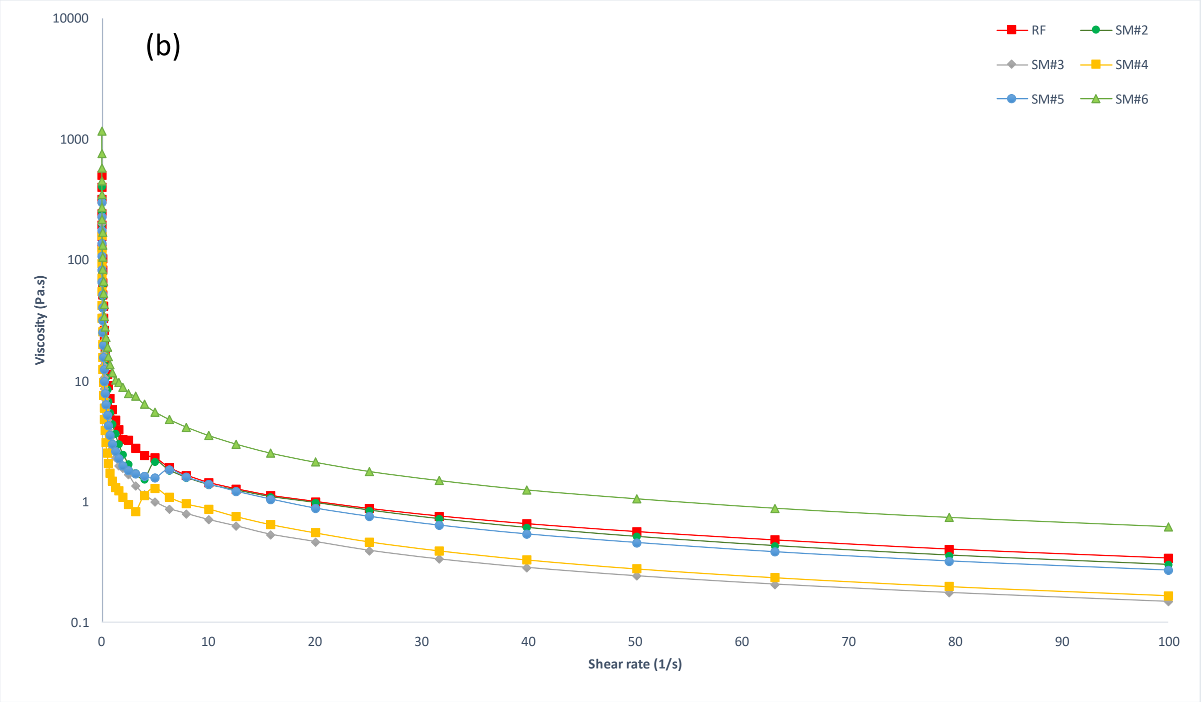


Figure 4.6 Apparent viscosity of different yoghurt samples made as outlined in Table 3.2, at 4 ^{0}C (a) and 10 ^{0}C (b)
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

According to the results obtained, there was no significant difference in the viscosity of yoghurt samples with an increase in temperature from 4 ^{0}C to 10 ^{0}C. SM#2 (50% DM and 50% FBM) and SM#5 (50% FBM and 50% FPI) had the closest viscosity to the reference yoghurt sample made with 100% DM. As previously explained, the viscosity deviation of different yoghurt samples is mainly due to their different gel structure which is more related to the product composition (Harper et al., 2022; Tan et al., 2018).

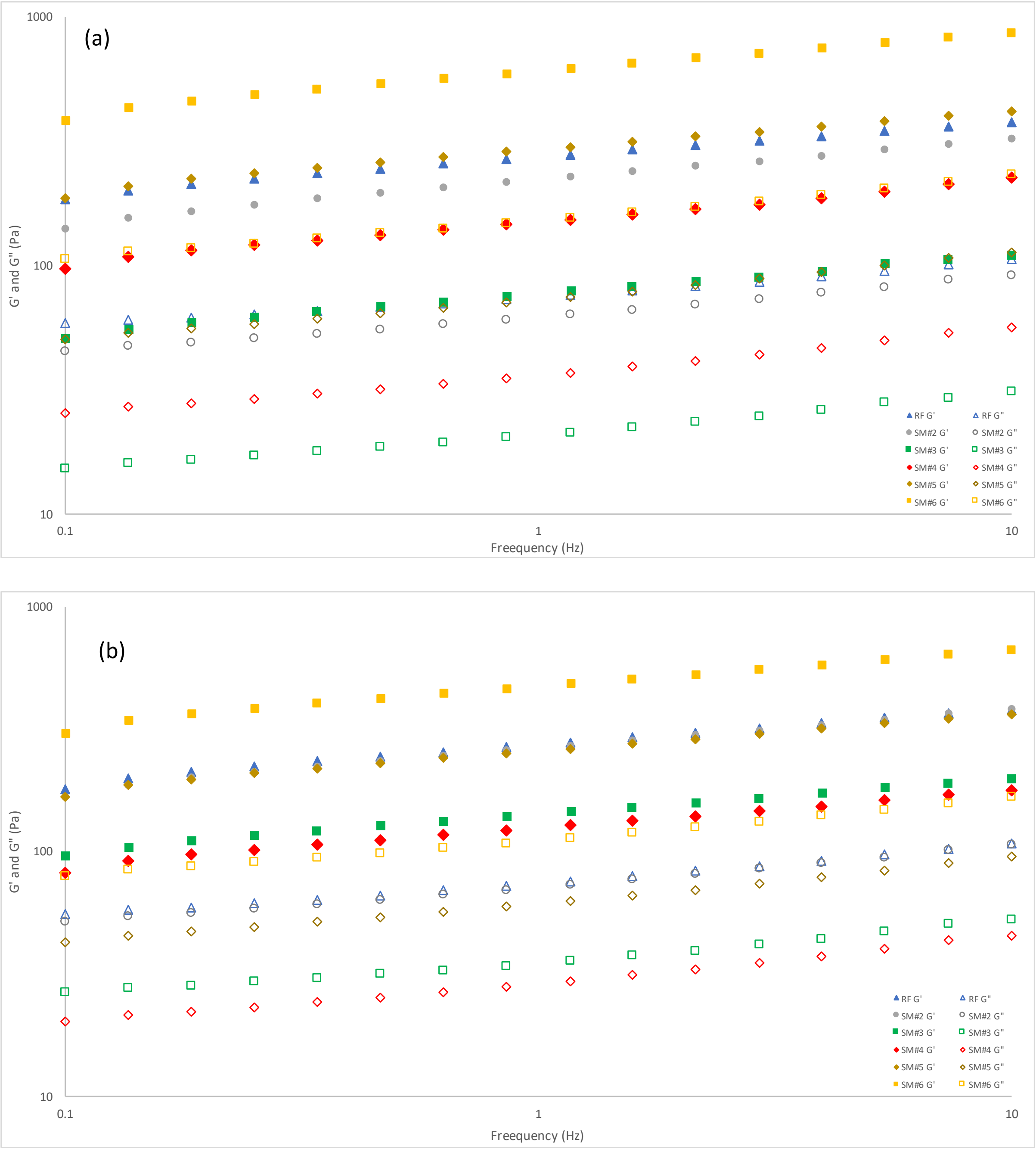


Figure 4.7 G” and G” of different yoghurt samples made as outlined in Table 3.2 at 4 ^{0}C (a) and 10 ^{0}C (b)
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Dynamic rheological testing can be used to determine yoghurt's interior structure. Figure 4.7 shows the behaviour of the storage modulus, or solid-like property (G’), and the loss modulus, or liquid-like property (G”), of all the yoghurt samples as a function of frequency at 4 ^{0}C and 10 ^{0}C. According to the results obtained all yoghurt samples showed signs of weak viscoelastic gel behaviour (G'> G”). There was no crossover between G’ and G”, indicating a strong, solid-like structure of all the samples.

SM#6 had a significantly higher *($p<0.05$)* G’ and G” compared to other tested samples. This may be due to the higher protein and starch content and their interactions compared to other yoghurt samples. Previous studies have shown the relationship between rheological characteristics and protein and starch levels (Song & Zheng, 2007). The G’ and G” of each sample behaved in similar patterns at both the temperatures tested, i.e., 4 ^{0}C and 10 ^{0}C. No significant difference existed between each sample's G’ and G’ values at 4 ^{0}C and 10 ^{0}C. These results indicate that the rheology is not affected by storage or consumption environment temperature. “According to *Lee & Lucey., (2010)*, increased protein levels resulted in improved LAB growth, resulting in higher exopolysaccharide production”. Yoghurts that included exopolysaccharides showed increased G’ due to the interactions between exopolysaccharides and the gel structure's protein. In addition, better growth of LAB has resulted in higher TTA as well. Conversely, in this study, SM#6 with higher protein content showed a significantly higher G’ but lower TTA. Therefore, the higher G’ resulted in SM#6 could be not due to the involvement of exopolysaccharides and due to some other ingredients or interactions. “According to *Pachekrepapol et al., (2021),* Increased protein and decreased starch strengthened the protein-protein complex at the acidic pH, and resulted in a higher G’ value”. However, the SM#6 (100% FPI) with higher G’ and G” had lower pH than other samples. Therefore, the effect of protein and pH on the rheological properties of faba bean yoghurt should be further investigated. SM#2 (50% DM +50%FBM) and SM#5 (50% FBM+ 50%FPI) had rheological properties closer to the reference sample (100% DM).

The loss tangent (*tan δ*), which represents the kind of viscous-elastic characteristics present in a material, is defined as the ratio of the loss modulus to the storage modulus (G”/G’). The G” indicates the viscous or liquid nature of the gel network, whereas G’ stands for elasticity. A substance exhibiting liquid-like behaviour has a high *tan δ*, value, that is, G” > G’. Tan δ values less than 1 indicate that the product's elastic character predominates over its viscous counterpart (Van Vliet et al., 1991).

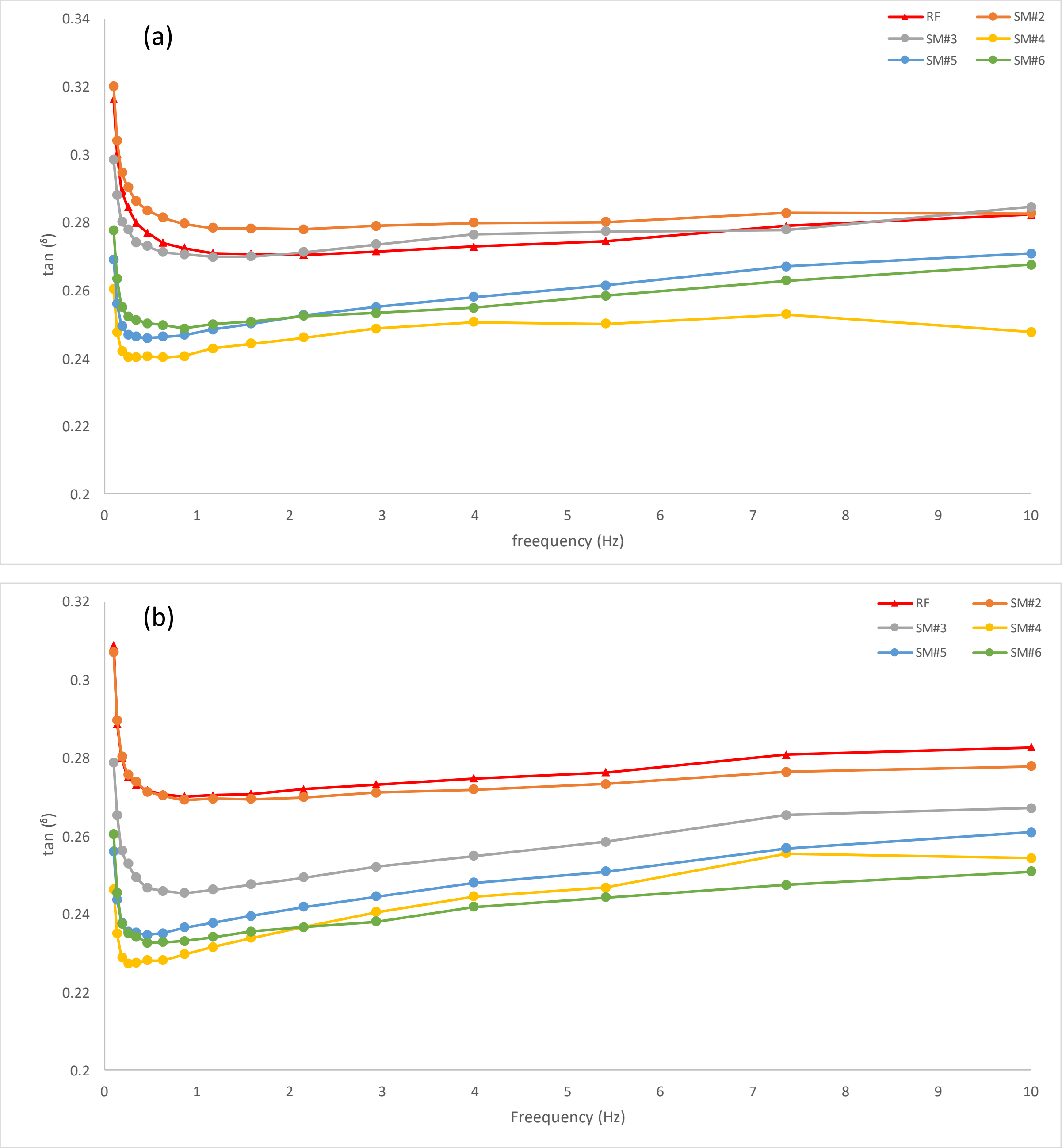


Figure 4.8 Behaviour of (*tan δ*) values against Frequency (Hz) of different yoghurt samples made as outlined in Table 3.2 at 4 ^{0}C (a) and 10 ^{0}C (b)

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

At 4 ^{0}C SM#2 (50% DM + 50% FBM showed the highest *tan δ* value (0.320) while SM#4 showed the lowest *tan δ* (0.24). The *tan δ* values of all other samples had between these maximum and minimum. These results indicate that all the yoghurt samples had solid-like properties over the liquid counterparts (Ozcan, 2013) at the storage temperature (4 ^{0}C).

The *tan δ* of the yoghurt samples also behaved similarly at 10 ^{0}C, with slightly lower values for each product than those at 4 ^{0}C. The reference sample showed the highest *tan δ* (0.308) while SM#4 (100% FBM) showed the lowest (0.022). At both temperatures, the *tan δ* showed downward trends until frequency 1 Hz and an upward trend with increasing frequency.

4.2.2 Texture analysis

Texture is a crucial sensory component that mimics how food is chewed in the mouth. The texture of formulated yoghurt samples (as outlined in Table 3.2) was determined as per the procedure described in 3.5.1.2 and the values shown in Table 4.4. Yoghurt texture is primarily determined by its hardness, also known as firmness, which reflects the force required to achieve a particular deformation to the product structure (Mudgil et al., 2017; Zannini et al., 2018).

The hardness value showed a significant difference *($p<0.05$)* between yoghurt samples but, SM#3 & SM#4 and SM#2 & SM#5 showed no significant difference between each other. The highest hardness (7.07) was observed in SM#6 (100% FPI), while SM#4 (100%. FBM) showed the lowest hardness value (1.67). According to the results, yoghurt made only with DM had a higher hardness, while mixing dairy with FBM and FPI significantly reduced the intensity of the product's hardness. This could be mainly associated with the amount of fat content of each product. Products made with 100% FPI resulted in higher hardness while incorporating FBM significantly lowered the hardness of the product. Fat regulates yoghurt's texture and perceived smoothness by creating numerous tiny, round particles when interacting with the protein structure (McCann et al., 2011). The hardness of yoghurt could be increased with the fortification of additives such as gums by forming gels with improved strengths (Raikos et al., 2020).

Adhesiveness is an indicator of how sticky yoghurt is and which has an inverse relationship with customer approval. The adhesiveness of yoghurt samples showed a similar trend for hardness, i.e., SM# 6 showed the maximum (-43.72) while SM#4 showed the minimum adhesiveness (-10.60). This could be directly associated with individual product composition.

Cohesiveness measures a material's ability to undergo deformation before rupturing (Huang et al., 2022). According to the TPA results obtained, no significant difference was observed in cohesiveness between samples. The minimum was observed in SM#6 (0.19), while the maximum was observed in SM#4 (0.27).

The yoghurt's springiness is the rate at which it returns to its original dimensions once the deforming force has been released (Mudgil et al., 2017). The results obtained for SM#5 and SM#6 significantly differed *($p<0.05$)* from other yoghurt samples. SM#6 showed the highest springiness value (8.85), while SM#2 showed the minimum (0.90).

For yoghurt textural analysis, gumminess is another crucial measure, a product of cohesiveness and hardness. A high gumminess score is correlated with a high hardness value of yoghurt. Gumminess is a quality of semisolid foods that have high cohesion and low hardness levels (Kose et al., 2018). The consumer's acceptance of yoghurt gumminess determines the acceptance level, and It might differ depending on the individual (Mudgil et al., 2017). The gumminess values showed a significant difference *($p<0.05$)* between the samples tested. The results were more correlated with the hardness values. SM#6 showed the highest (0.81) value while SM#4 showed the lowest (0.27) for the gumminess.

The TPA values show that the results obtained in this study differ from those of other studies when compared with the literature. The amount of culture, duration, and temperature of incubation, etc., are considered to be the causes of these variations. Therefore, comparing the TPA data from different studies is impossible (Kose et al., 2018).

Table 4.4 Texture profile analysis (TPA) data of different yoghurt samples

Product code	**TPA Data**				
	Hardness (g)	**Adhesiveness (g.s)**	**Cohesiveness**	**Gumminess**	**Springiness**
Reference	5.39 ± 0.90^{b}	-35.39 ± 2.34^{c}	0.20 ± 0.02^{c}	0.71 ± 0.04^{ab}	0.91 ± 0.90^{b}
SM#2	3.90 ± 0.07^{c}	-24.11 ± 2.74^{b}	0.21 ± 0.01^{bc}	0.59 ± 0.01^{b}	0.90 ± 0.11^{b}
SM#3	1.87 ± 0.03^{d}	-12.13 ± 1.19^{a}	0.25 ± 0.02^{ab}	0.69 ± 0.02^{ab}	0.93 ± 0.05^{b}
SM#4	1.67 ± 0.08^{d}	-10.60 ± 0.48^{a}	0.27 ± 0.00^{a}	0.27 ± 0.01^{c}	2.20 ± 0.06^{b}
SM#5	3.03 ± 0.29^{c}	-15.43 ± 1.30^{a}	0.21 ± 0.01^{bc}	0.33 ± 0.05^{c}	8.47 ± 0.08^{a}
SM#6	7.07 ± 0.29^{a}	-43.72 ± 1.98^{d}	0.19 ± 0.01^{c}	0.81 ± 0.07^{a}	8.85 ± 0.04^{a}

****Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same column were significantly different (p<0.05) by the Turkey pairwise comparisons.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

4.3 Microstructure analysis

The three-dimensional arrangement and interactions between structural components greatly impact the textural qualities of a certain dairy product. Therefore, more than understanding the chemical composition and bulk physical qualities alone is needed to grasp the behaviour of dairy products completely; understanding how the food microstructure is arranged is equally important (El-Bakry & Sheehan, 2014). The microstructure of all six yoghurt samples was determined according to the method described in section 3.5.2.

The yoghurt samples had a wide range of microstructures, including variations in the amount of protein aggregation and network development, significant variations in the size, shape, and integration of fat, and variations in unstained areas (that may be starch) (Figure 4.9, a-f). The variation of structures between samples could be due to changes in their composition (Table 4.1). Composition differed significantly between the yoghurt samples. Protein varied from 4.30% in SM#6 to 2.33% in SM#4. Fat varied from 3.88% in the reference sample to 0.3% in SM#4 and SM#5 and the starch was highest (1.06%) in SM#4 while 0.05% in SM#6. Further, It is anticipated that the different structures will also impact the texture and sensory experience of the product (Gupta et al., 2022).

The reference sample contained more fat than the other five yoghurt samples made with dairy and faba bean hybrid, which could be more visible in red particles within the protein network. The fat was more visible in products with more fat i.e., reference, SM#2 and SM#3 while less visible in other products. In all products, fat droplets are more integrated within the protein network.

In this study, the milk was not homogenized before fermentation. However, if the milk is homogenized before fermentation, the size of the milk fat globule may decrease and interactions with the milk protein may increase. The protein-coated fat globules contribute to developing networks at acidic pH and increase the yoghurt's hardness (Nguyen et al., 2015). Large unstained areas, possibly occupied by starch, were more visible in products that contained faba bean. All the processing conditions, including heat treatments, incubation time, temperature, etc., were the same for all the products and the type and concentration of raw materials. The type and ratios of raw materials significantly impact the differences in the microstructures of yoghurt (Gupta et al., 2022).

Previous studies have shown that gel strength is reduced when plant protein is combined with dairy protein rather than used alone. This is because plant-based and dairy-based protein sources generate separate, non-interacting networks of proteins (Schmitt et al., 2019).

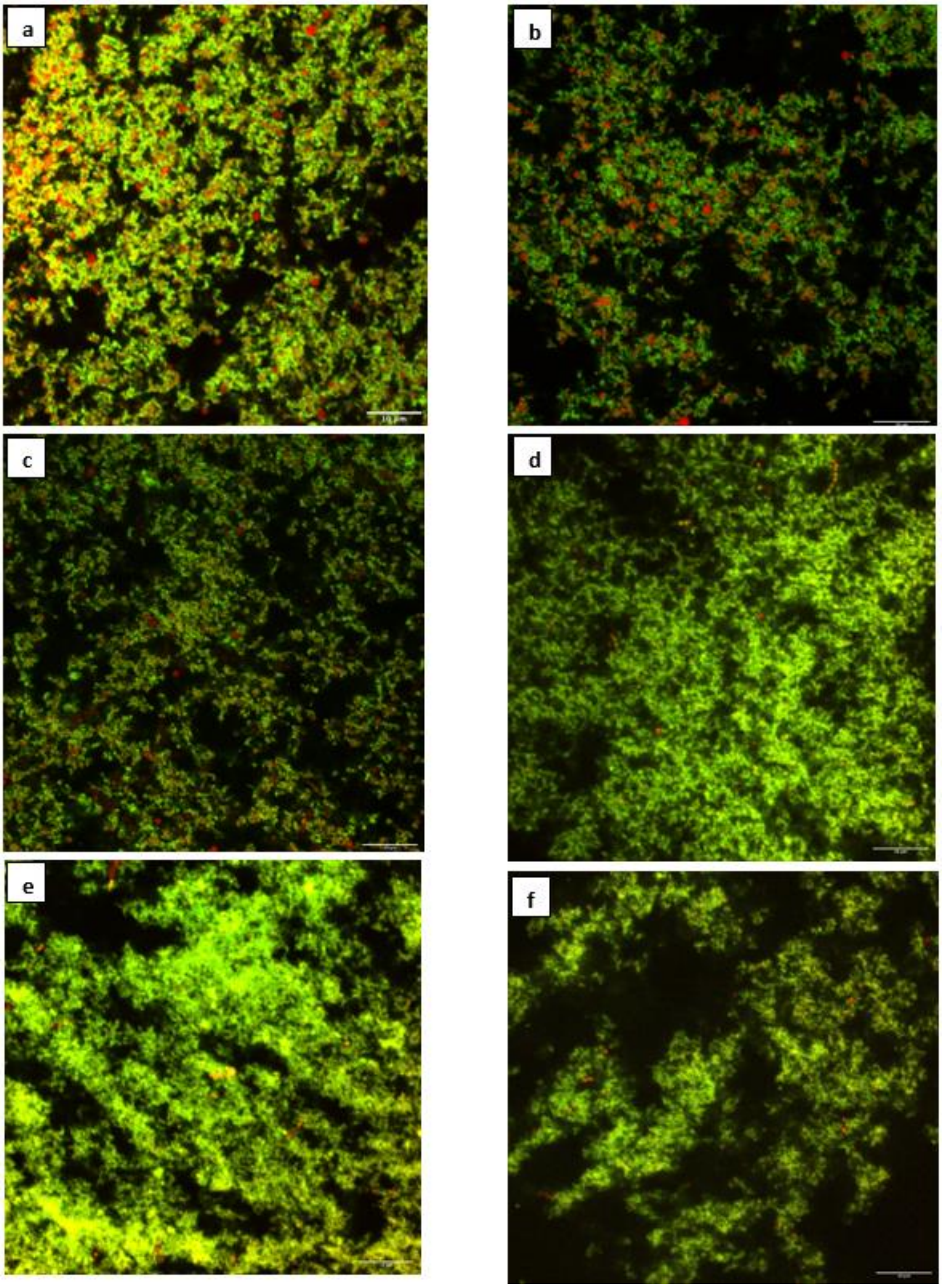


Figure 4.9 CLSM microstructure of six yoghurt samples stained with Fast green CFC (protein) and Nile red (fat), (a) Reference, (b) SM#2, (c) SM#3, (d) SM#4, (e) SM#5 and (f) SM#6. The scale bar is 10 µm.

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

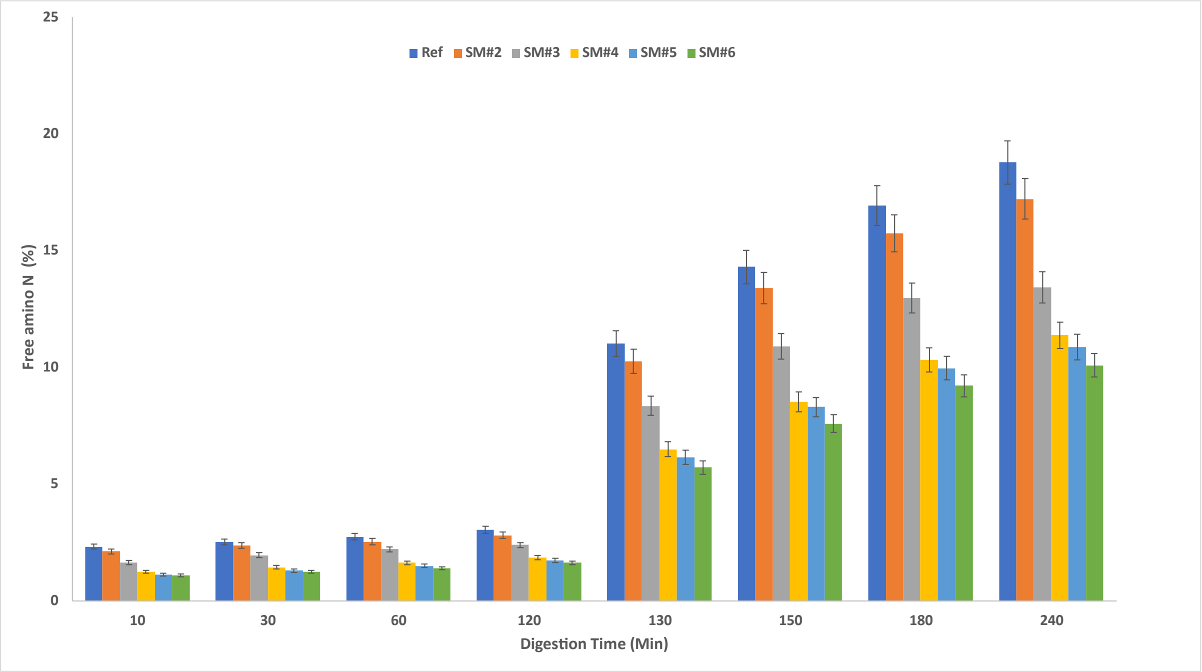
4.4 In Vitro protein digestibility assessment through ninhydrin reactive amino N (%)

The Ninhydrin test was conducted on all six yoghurt samples according to the procedure outlined in section 3.5.3.2. This test evaluated the extent of proteolytic protein breakdown in yoghurt, quantitatively measuring the free amino nitrogen released during simulated gastrointestinal digestion. As depicted in Figure 4.10, there was an increase in the release of free amino nitrogen over the digestion time (0 to 240 min) in all six yoghurt samples with similar patterns. However, a significant difference ($p<0.05$) was observed between dairy and faba bean-containing samples during both gastric and intestinal phases.

During the gastric phase (0 to 120 minutes), there was relatively less protein breakdown of all yoghurt samples by pepsin compared to the intestinal phase. However, when pancreatin and bile salt were added during the small intestinal phase (130-240 minutes), all samples showed a significant increase *($p<0.05$)* in the release of free amino N. There was a significant difference *($p<0.05$)* in the release of free amino N between the reference sample (100% DM) SM#2 (50% DM + 50% FBM) and SM#3 (50% DM + 50 % FPI) during the intestinal phase. However, there was no significant difference *($p>0.05$)* between SM#4 (100% FBM), SM#5 (50% FBM + 50 % FPI), and SM#6 (100% FPI) during the intestinal digestion phases. This suggests that adding faba bean protein decreased the release of free amino N in SM#2 and SM#3. Further, there was a significant difference between SM#2 and SM#3, suggesting that FMB showed a higher rate of free amino N release than FPI during digestion.

When small intestinal enzymes (pancreatin) were added at the beginning of the intestinal phase, a significant increase in the release of free amino N was observed compared to the gastric phase (Figure 4.10), and *Manus et al. (2021)* observed a similar trend for pea and rice protein digestion.

Legume proteins such as faba bean tend to be low digestible mainly because of factors such as anti-nutritional compounds, protein structural organization, and the formation of complexes when proteins interact with other components in the seeds. Additionally, their compact structure and stable three-dimensional shape, often due to carbohydrate components, can further contribute to reduced digestibility (Tang et al., 2009).



4.10 Percentage (%) of free amino N release during gastric (0-120 min) and small intestinal phase (130-240 min) of six yoghurt samples during static in vitro digestion

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

The presence of a β-sheet is another factor contributing to the low digestibility of legume proteins. This is similar to the β-Lactoglobulin found in cow's milk. This configuration tends to resist denaturation during digestion in the gastrointestinal tract, further reducing the overall digestibility of products (e.g., faba bean yoghurts) made from legume proteins (Carbonaro et al., 2015).

According to several digestion studies on plant proteins, the proteins containing amino acids such as proline and glutamic acid at higher concentrations were more resistant to digestion. Faba bean proteins are rich in these amino acids (Table 2.2), which could be one of the reasons behind the low digestibility of yoghurt containing faba bean protein (Savoie et al., 2005).

The research results reinforce that animal proteins are more easily digested than plant proteins (Kaur et al., 2022). Research on the digestion of products like yoghurt and cheese needs to be improved compared to pure dairy proteins like whey and casein. Consequently, there is no sufficient data to compare the digestibility of yoghurt in this study with previously reported results on similar products.

4.5 Sensory analysis

The sensory evaluation concerning flavour, aroma, texture, colour, and overall acceptability of different yoghurt tested is shown in Figure 4.12. The average sensory ratings for all tested yoghurts were between 4 and 8. As per Huang et al. (2022), this range is acceptable for plant-based yoghurts. The reference sample made with 100% dairy milk received significantly higher scores for colour, flavour, aroma, texture, and overall acceptability *($p<0.05$).* While SM#2 (formulated with 50% DM and 50% FBM) received comparatively higher scores for flavour, colour, texture, and overall acceptability *($p<0.05$).* However, all the tree samples SM#2 (50% DM + 50% FBM), SM#4 (100% FBM), and SM#5 (50% FBM + 50% FPI) received overall hedonic scores (5). According to the sensory evaluation, SM#4 and SM#5 had received similar liking for all the tested attributes. There was no difference in consumer preference for the product formulated either 100% FBM or a combination of FBM and FPI. All the tree yoghurt samples received a similar liking for aroma attribute and it was significantly lower than the reference sample *($p<0.05$)* and this indicated that the use of faba bean itself or incorporation of faba bean with dairy significantly lowered the consumer preference towards the aroma and flavour attributes. The noticeable reduction in consumer preference towards colour, texture, and overall acceptability of yoghurt incorporated with faba bean may be attributed to the beany flavour, viscosity, and water-holding capacity of faba bean. These results justify the instrumental results obtained for the colour (Table 5.2), texture (Table 5.3), and viscosity of each product.

These results pointed out that consumers preferred products that contain sensory attributes of the product made from DM. The sensory attributes of SM#2 showed the least deviation from the highest consumer preference received from dairy yoghurt, while SM#4 and SM#5 showed the highest deviation. Based on sensory evaluation SM#2 showed the best formulation and SM#4 and SM#5 showed the subsequent potential for improvement as all sensory attributes received scores within the acceptable range (above 4).

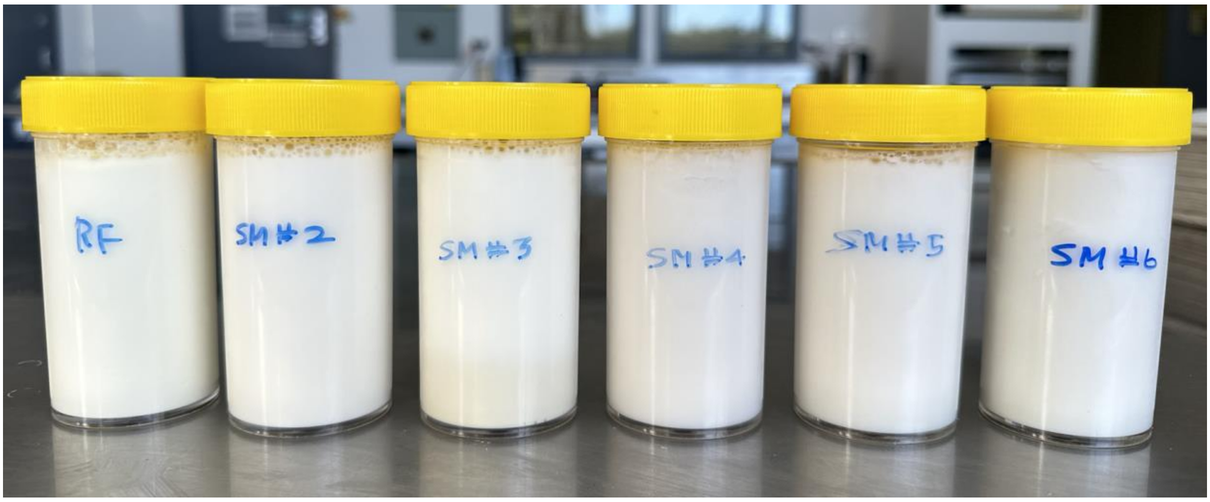


Figure 4.11 The image of yoghurt samples produced (SM#2, SM#4 and SM#5 were subjected to the sensory evaluation with the reference)

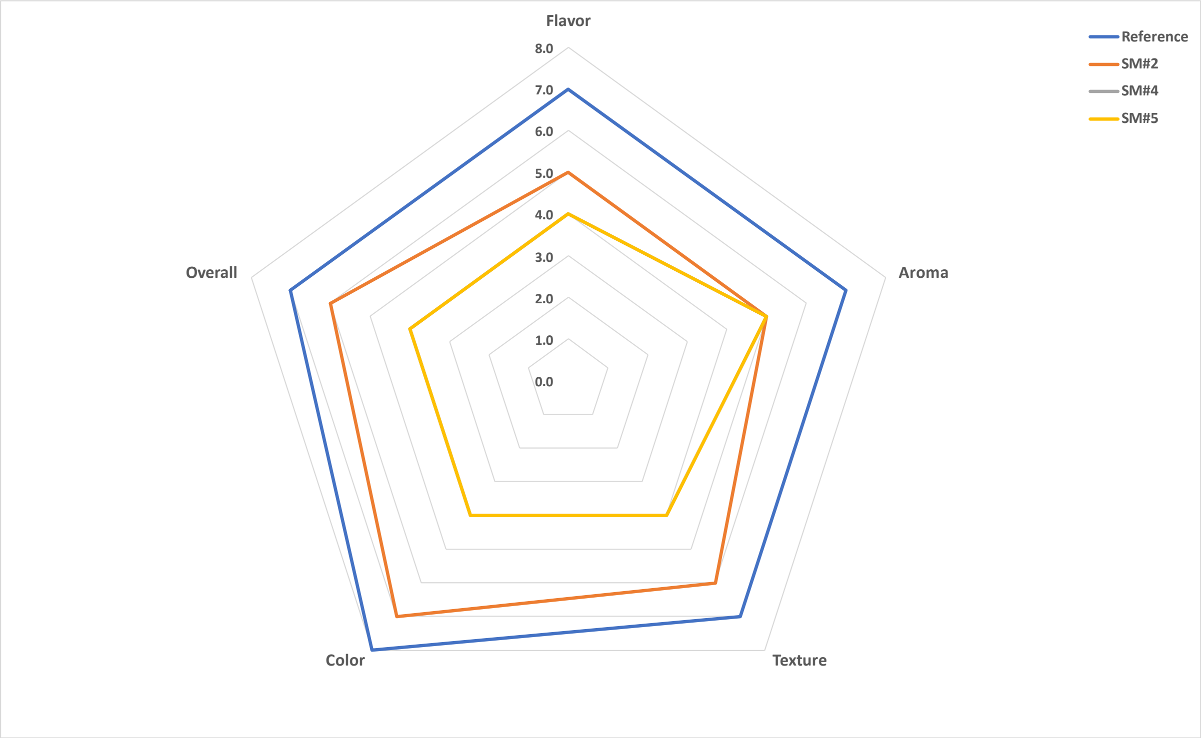


Figure 4.12 Sensory evaluation of yoghurt (formulated as outlined in Table 3.2) after 48 h storage at 4 ^{0}C
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI)

Chapter 5. Stability Evaluation of Yoghurt During Storage

5.1 Introduction

The acceptance of plain yoghurt is determined by its acidity, flavour, texture, aroma, and appearance. These are results mainly from the by-products of the fermentation process in which the LAB converts sugar into lactic acid. Acidity results from lactic acid acidification obtained at the end of incubation and post-acidification during storage. The strains used, the storage temperature, and the length of storage all have a major impact on the post-acidification of stored yoghurt. The storage and shelf life of yoghurt is impacted by modifications to its physical, chemical, or microbiological properties. Changes in these properties cause colour, aroma and textural deterioration of yoghurt, which are known as important quality criteria by consumers. Yoghurt quality is often assessed by composition and judgement, which yields a final quality score based on the presence or absence of particular defects.

Understanding how yoghurt behaves during storage is crucial to determine its shelf life. Any undesirable physical, chemical, or sensory characteristics make it unsuitable for human consumption (Salvador & Fiszman, 2004). Performing product stability evaluation would help producers better understand and accurately determine the product shelf life. The viscosity, texture, flavour, and colour of yoghurt are important quality parameters of any food product including yoghurt. Evaluating yoghurt quality often includes rheological characteristics, textural properties, acidity, and microbiological quality.

Several studies have been done to determine the changes that occurred during storage of prepared yoghurts. This study investigated sensory, biochemical, and structural changes in different faba bean yoghurt formulations (as outlined in Table 3.2) during refrigerated storage (4 ^{0}C) over 14 days.

5.2 Material and Methods

This study's yoghurt samples (as outlined in Table 3.2) were prepared with the raw material (as explained in section 3.1) and followed the methods described in sections and subsections 3.2 and 3.3. The prepared samples were stored under refrigerated conditions (4 ^{0}C) and their various physico-chemical, and rheological properties were analysed over the storage at day 1, 7 and 14 and discussed in section 5.3.

5.2.1 Colour

The colour of each sample was determined according to the producer described in 3.4.9 at the storage of yoghurt on day 1, 7, and 14. Using equation (9), the Whiteness Index (WI) (Pan et al., 2019) was determined on day 1, 7, and 14 and recorded.

$$\mathrm{WI} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \text{.............} \ (10)$$

where lightness (L), redness (a*), and yellowness (b*) of samples are represented, respectively.*

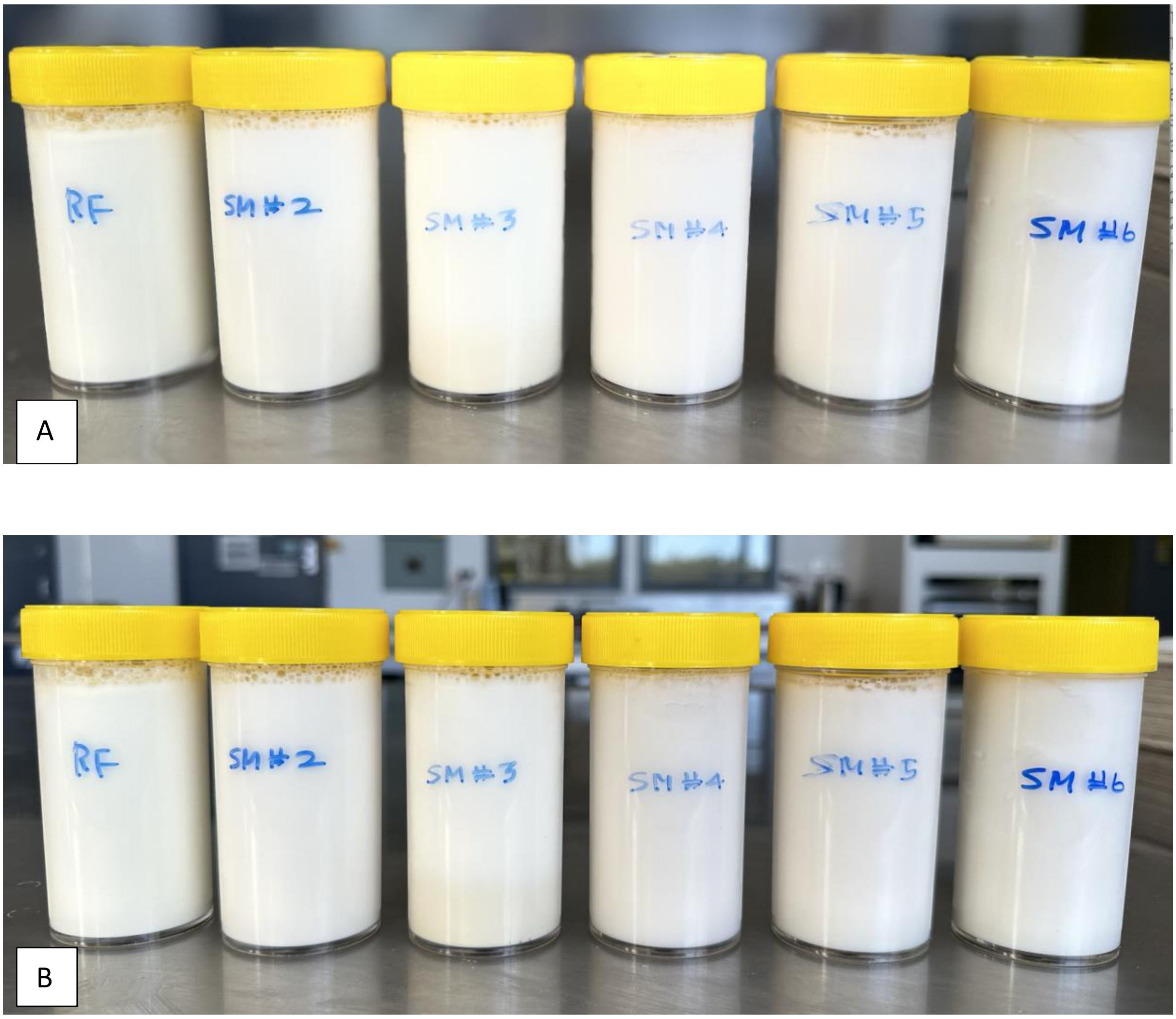


Figure 5.1 Images of yoghurt samples at (A) 7 days and (B) 14 days of storage

5.3 Results and discussion

5.3.1 pH and total titratable acidity (TTA) changes during storage

Throughout the storage period, the pH values of the yoghurt samples remained above 4.5, and the TTA stayed below 0.85, as shown in Table 5.1. The pH and TTA of the yoghurt samples exhibited minor changes during storage, indicating that weak post-acidification occurred due to the LAB present in the yoghurt. Post-acidification leading to excessive acidity and reduced shelf life is considered an undesirable process in yoghurt production such as severe acidity, syneresis gas production, etc. (Anuyahong et al., 2020; Deshwal et al., 2021). The products that contained DM (SM#2 & 3) showed low pH values compared to the products that did not contain dairy (SM# 4, SM# 5 & SM# 6). This suggests that faba bean is not a good substrate for LAB fermentation compared to dairy. As shown in Fig. 5.2, the yoghurt samples' pH had increased slightly during storage. Throughout the storage, the reference sample showed a low pH compared to other samples while SM#5 showed a higher pH compared to others. SM#4 showed the highest post-acidification of all the products during storage, indicating that LAB fermentation had continued in this product for 14 days. A reduction of pH reduction during the storage of coconut yoghurt was reported by *Pachekrepapol et al. (2021)*.

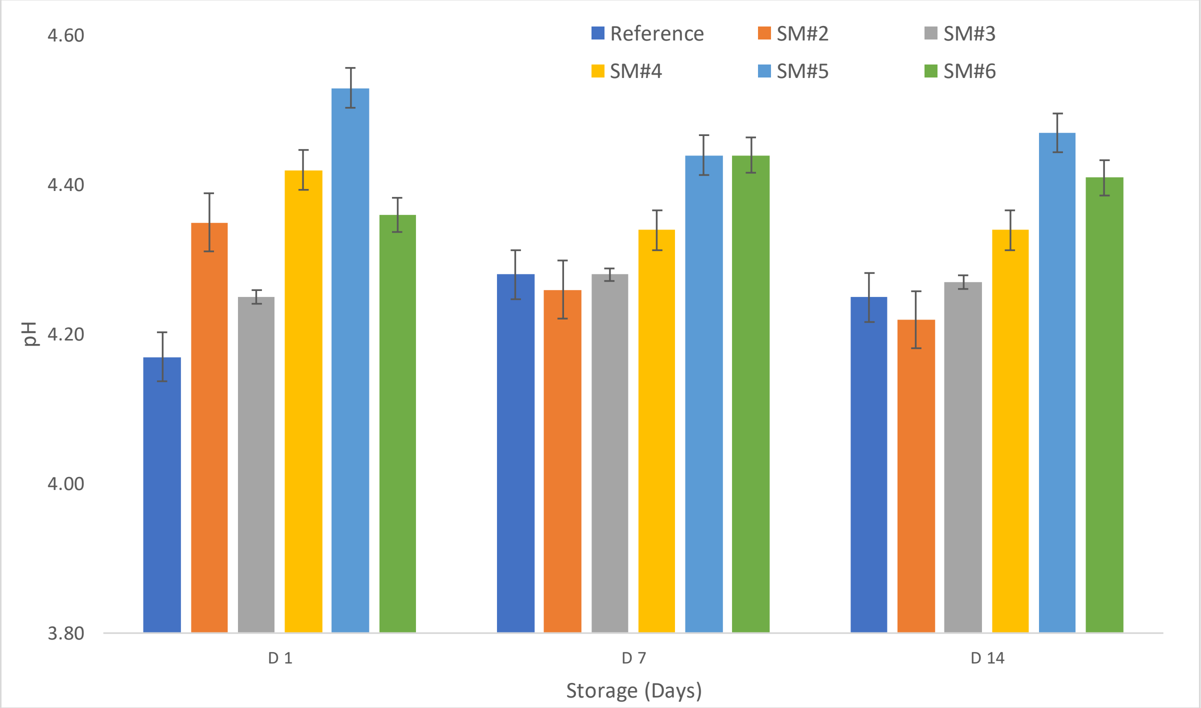


Figure 5.2 pH changes of yoghurt samples during storage at 4 ^{0}C on day 1, 7, and 14

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

As shown in Fig.5.3, the TTA of yoghurt samples exhibited similar behaviour throughout the 14 days of storage with a non-significant difference *($p<0.05$)* within the sample. The highest TTA was shown reference (100% DM) sample with a range from 0.81 (day 1) to 0.84 (day 14) and all the samples resulted in lower TTA values compared to the reference, while SM#6 showed the lowest TTA with ranging from 0.24 (day 1) to 0.25 (day 14). The products' acidity increased when the pH was decreased, and acidity decreased when the pH was increased.

According to the results obtained, SM#2 and SM#4 showed the highest post-acidification, which could be attributed to the FBM in both samples.

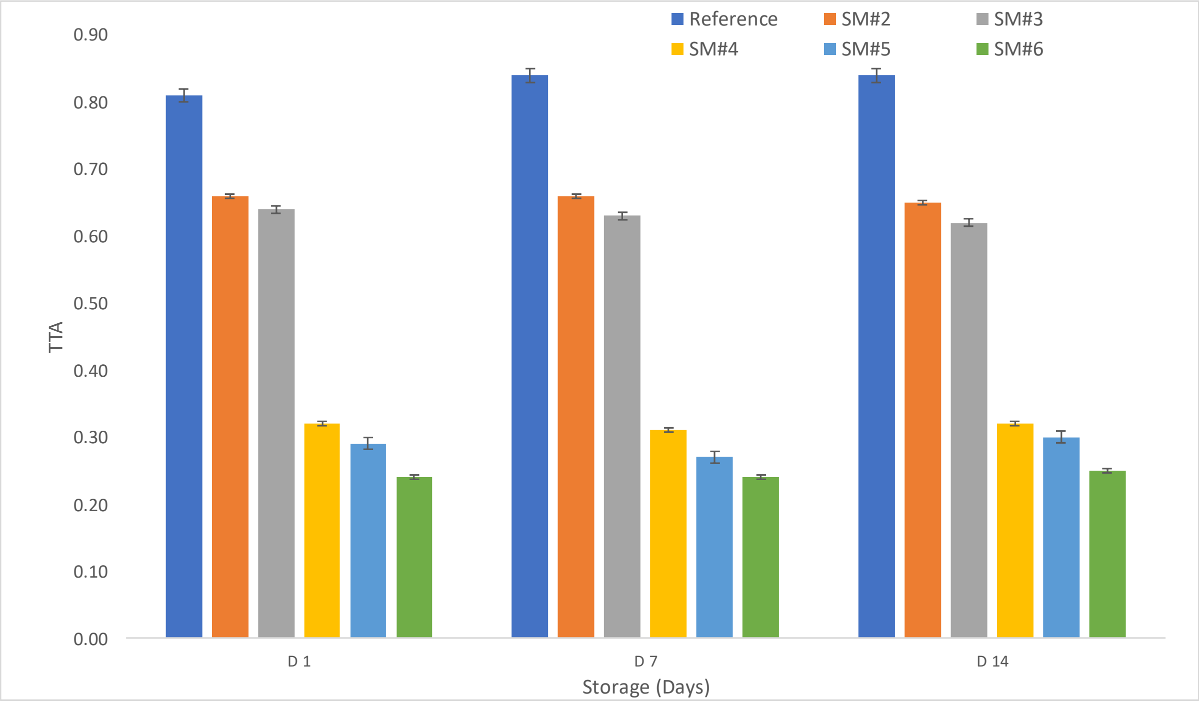


Figure 5.3 TTA changes of yoghurt samples during storage at 4 ^{0}C on day 1, 7, and 14 (**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

5.3.2 Syneresis (%) during storage

Syneresis is a measure of the serum released from the gel under centrifugal force. It serves as a quality indicator to assess the product's WHC (Donmez et al., 2017). Throughout the storage period, the syneresis values of the yoghurt samples are shown in Table 5.1. As shown in Fig. 5.4, can observe a significant difference *($p< 0.05$)* of % syneresis between yoghurt samples. SM#3 showed the highest syneresis (64%), while SM#6 showed the lowest syneresis (12.44%) on day 1 and a similar trend was observed throughout the storage. However, the syneresis %

decreased over the storage. The lower syneresis % may be attributed to increased WHC, different particles, and their interactions within the product. The % syneresis of each yoghurt sample was more related to their rheological properties, such as apparent viscosity and storage and loss modulus. The product that showed the highest % syneresis (SM#3) had the lowest apparent viscosity and loss and storage modulus, while the product that had the lowest syneresis (SM#6) showed the highest apparent viscosity and loss and storage modulus.

Commercial yoghurt's syneresis percentage was significantly reduced by combining thickening and gelling agents, such as starch and pectin. Studies on non-dairy yoghurts combined with gelling agents and stabilisers demonstrated varying syneresis percentages. For example, syneresis ranging from 26% to 27% in almond milk yoghurt was observed when stabilizers and gelling agents such as xanthan gum and pectin were used.

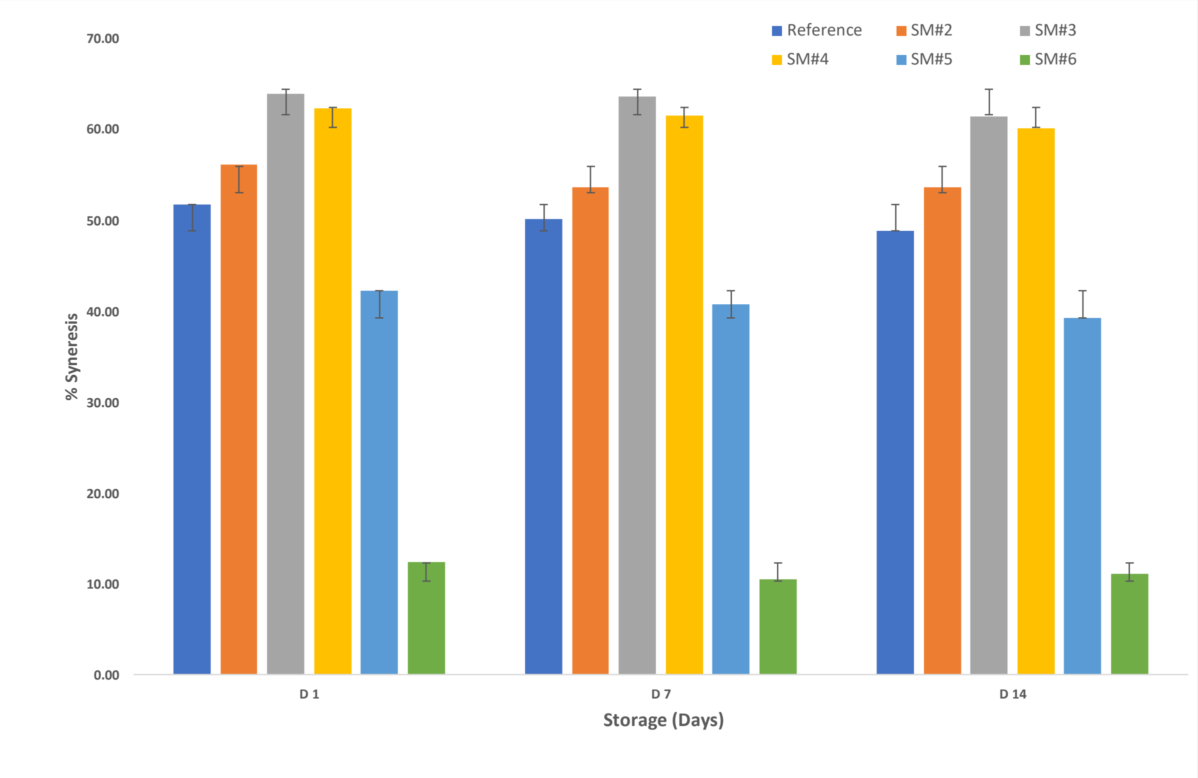


Figure 5.4 % syneresis of yoghurt samples during storage at 4 ^{0}C on day 1, 7 and 14 (**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

5.3.3 Colour changes during storage

The colour of food products, including yoghurt, plays a vital role in their visual appeal. The colour of yoghurts can be influenced by factors such as storage time, shelf-life, and colour degradation processes (Coggins et al., 2010). The colour analysis of yoghurt samples involved measuring their

CIE L*, a*, and b* values (Table 5.1). It has been noted that a difference greater than 0.5 between b* and a* values exceeded the threshold of sensory perceptibility (Anuyahong et al., 2020). In the case of yoghurt made from dairy and faba bean, the L*, b*, and a* values remained constant until the end of storage, indicating a stable state throughout the storage. Similar to the results reported by other plant-based yoghurt manufacturers (Barbosa et al., 2020), a slight reduction of L*, a*, and b* values was observed during storage. The small changes may be associated with the breakdown of phenolics and anthocyanins and the reduction of sugars in the product (Huang et al., 2022). These values were subsequently utilized to determine the overall colour change in the samples against the reference, during storage, represented as the ΔE values illustrated in Figure 5.5. According to the results, SM#2 showed less overall colour change, while SM#3 showed the highest colour change with the reference sample on day 1, and a similar trend continued during 14 days of storage. In addition, the ΔE value of each yoghurt sample was decreased gradually but slowly and evenly during storage.

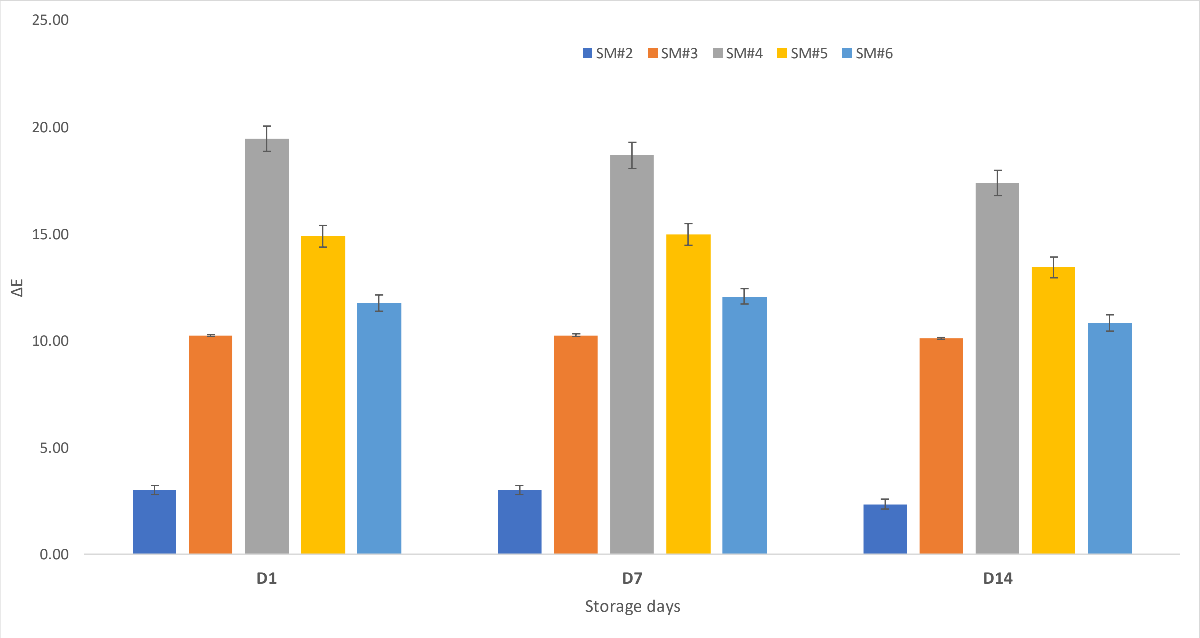


Figure 5.5 Colour change (ΔE) of different yoghurt samples compared to the reference sample stored at 4 ^{0}C on the day 1, 7 and 14
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Table 5.2 Whiteness index (WI) of different yoghurt samples during storage at 4 ^{0}C

Product code	**Whiteness Index (WI)**		
	Day 1	**Day 7**	**Day 14**
Reference	82.23 ± 0.76^{a}	82.60 ± 0.55^{a}	82.15± 0.72^{a}
SM#2	80.83 ± 0.42^{b}	81.44 ± 0.40^{a}	81.02± 0.27^{a}
SM#3	72.09 ± 0.07^{c}	72.64 ± 0.28^{b}	72.49± 0.08^{b}
SM#4	67.14 ± 0.26^{e}	67.97 ± 0.26^{d}	67.89± 0.04^{d}
SM#5	70.19 ± 0.36^{d}	70.32 ± 0.56^{c}	70.53± 0.64^{c}
SM#6	73.17 ± 0.17^{c}	72.85 ± 0.76^{b}	72.85± 0.54^{b}

****Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same column were significantly different (p<0.05) by the Turkey pairwise comparisons.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Whiteness indices (WI) are commonly measured to provide numerical values closely correlated with consumers' preferences for white colours. WI serves as a representation of the overall whiteness of food products and can indicate the degree of discolouration that may occur during storage. The resulting WI of different yoghurt samples is shown in Table 5.2 and all the values were significantly different *($p>0.05$)* from each other. On day 1 the reference sample showed the highest WI (82.23) SM#4 had the lowest WI (67.14), while SM#2 had a WI (80.83) closer to the reference sample. The WI difference on day 1 is mainly attributed to the different raw materials, ratios, and their interactions. Similar to L*, a*, and b* values, the WI of each yoghurt sample was slightly decreased during 14 days of storage. This change was non-significant *($p<0.05$)*. Even slight changes in the whiteness of samples can result from the Maillard reaction, which occurs gradually in heat-treated faba bean milk during storage. Based on these results, a yoghurt having WI closer to the reference sample can be obtained by mixing FMB with DM (50:50), rather than using FBM alone.

Table 5.1 Stability analysis of dairy-faba bean yoghurt during storage at 4 ^{0}C on day 1, 7, and 14

Items	**Storage days**	**Yoghurt Samples**					
		Reference	**SM#2**	**SM#3**	**SM#4**	**SM#5**	**SM#6**
pH	**Day1**	4.17± 0.01^{e}	4.35± 0.01^{c}	4.25± 0.01^{d}	4.42± 0.01^{b}	4.53± 0.04^{a}	4.36± 0.01^{c}
	Day7	4.28± 0.04^{ab}	4.26± 0.12^{b}	4.28± 0.03^{ab}	4.34± 0.04^{ab}	4.44± 0.04^{a}	4.44± 0.06^{a}
	Day14	4.25± 0.07^{bc}	4.22± 0.07^{c}	4.27± 0.04^{bc}	4.34± 0.07^{abc}	4.47± 0.03^{a}	4.41± 0.07^{ab}
TTA	**Day1**	0.81± 0.06^{a}	0.66± 0.00^{b}	0.64± 0.00^{b}	0.32± 0.00^{c}	0.29± 0.00^{d}	0.24± 0.00^{e}
	Day7	0.84± 0.00^{a}	0.66± 0.00^{b}	0.63± 0.01^{c}	0.31± 0.00^{d}	0.27± 0.00^{e}	0.24± 0.00^{f}
	Day14	0.84± 0.00^{a}	0.65± 0.00^{b}	0.62± 0.00^{c}	0.32± 0.00^{d}	0.30± 0.00^{e}	0.25± 0.00^{f}
Syneresis	**Day1**	51.80± 0.89^{c}	56.17± 0.20^{b}	64.00± 0.63^{a}	62.41± 0.22^{a}	42.32± 1.01^{d}	12.44± 0.39^{e}
	Day7	50.02± 0.59^{a}	53.64± 0.65^{b}	63.70± 0.43^{c}	61.60± 0.35^{d}	40.77± 0.23^{e}	11.53± 0.64^{f}
	Day14	48.90± 0.44^{b}	53.68± 0.67^{b}	61.45± 0.99^{a}	60.16± 0.63^{a}	39.31± 0.72^{c}	10.47± 0.49^{d}
L*	**Day1**	85.24± 0.56^{a}	82.87± 0.62^{b}	77.44± 0.24^{c}	67.25± 0.28^{f}	70.88± 0.33^{e}	74.00± 0.25^{d}
	Day7	85.51± 0.63^{a}	83.27± 0.48^{b}	77.95± 0.69^{c}	68.12± 0.27^{f}	70.96± 0.60^{e}	73.75± 0.73^{d}
	Day14	84.44± 0.65^{a}	82.70± 0.42^{b}	77.27± 0.24^{c}	68.05± 0.04^{f}	71.23± 0.75^{e}	73.76± 0.60^{d}
a*	**Day1**	-3.22± 0.03^{e}	-1.93± 0.08^{a}	-3.03± 0.03^{d}	-1.87± 0.03^{a}	-2.59± 0.02^{c}	-2.18± 0.03^{b}
	Day7	-3.25± 0.14^{d}	-1.69± 0.12^{a}	-2.65± 0.10^{c}	-1.89± 0.00^{a}	-2.51± 0.04^{bc}	-2.26± 0.10^{b}
	Day14	-3.00± 0.13^{d}	-1.55± 0.19^{a}	-2.37± 0.11^{c}	-1.88± 0.04^{b}	-2.53± 0.09^{c}	-2.27± 0.03^{c}
b*	**Day1**	9.66± 0.04^{b}	8.37± 0.13^{c}	16.28± 0.806^{a}	2.38± 0.13^{d}	5.83± 0.22^{e}	6.35± 0.29^{f}
	Day7	9.06± 0.14^{b}	7.85± 0.09^{c}	15.95± 0.58^{a}	2.39± 0.12^{f}	5.59± 0.42^{e}	6.55± 0.28^{d}
	Day14	8.20± 0.60^{b}	7.63± 0.38^{bc}	15.21± 0.42^{e}	2.53± 0.05^{d}	5.83± 0.48^{d}	6.57± 0.20^{cd}

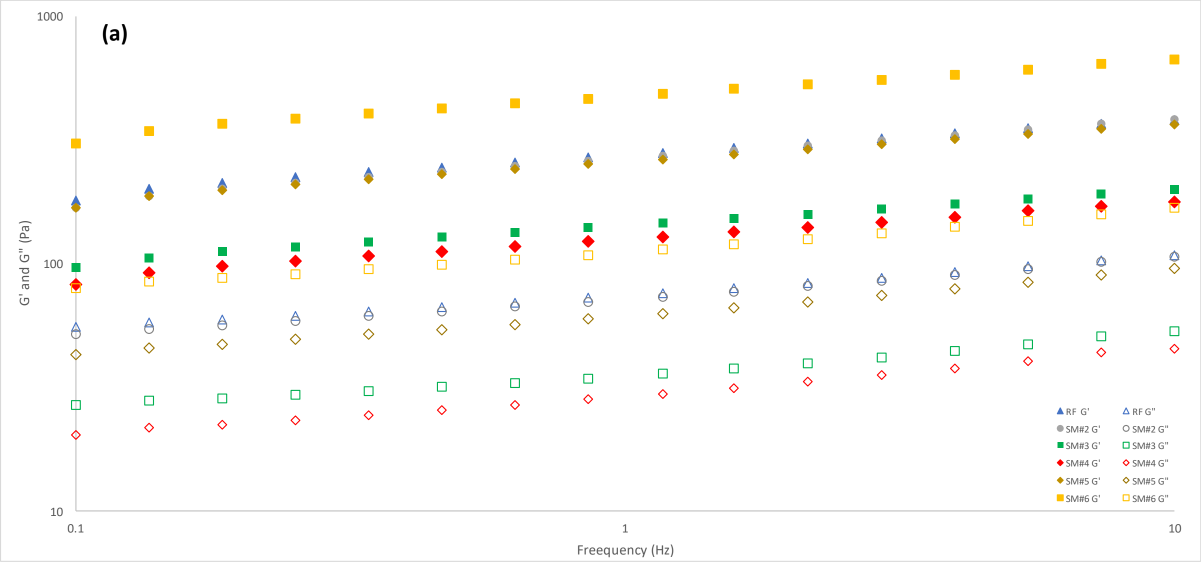
* ***Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same row for the same parameter were significantly different (p<0.05) by the Turkey pairwise comparisons. No significant differences were observed between storage times.***

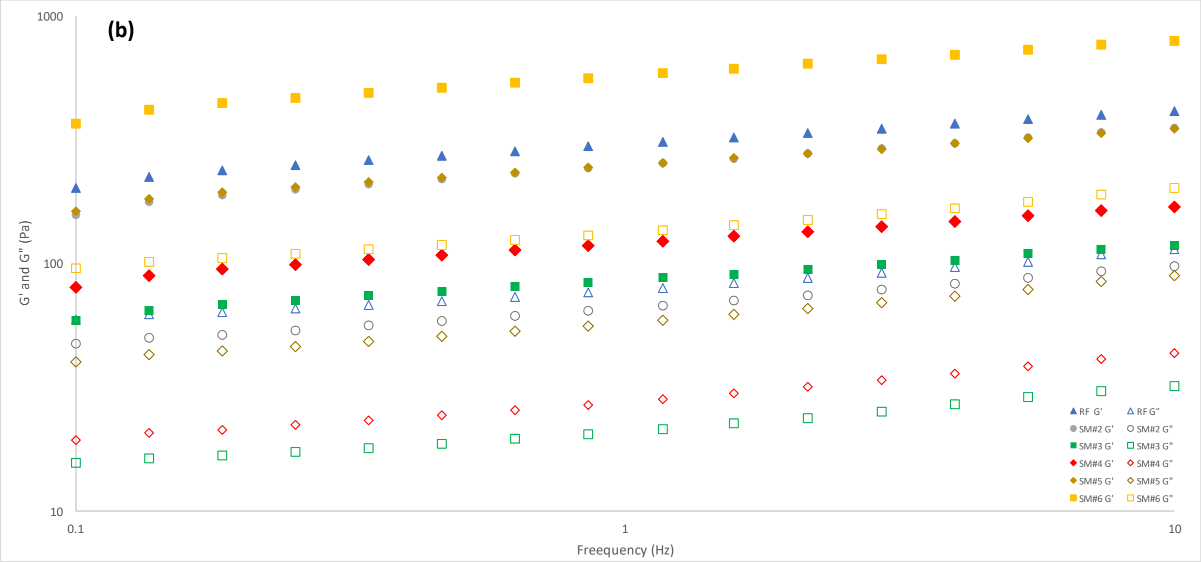
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

5.3.4 Rheological properties during storage

The dynamic rheological measurement can be used to evaluate the internal structure of the yoghurt (Pachekrepapol et al., 2021). Figure. 5.6 a, b, and c, respectively, show the storage modulus or solid-like property (G') and loss modulus or liquid-like property (G") values as a function of frequency on days 1, 7, and 14 at 10 ^{0}C. According to the results achieved, each yoghurt sample showed viscoelastic properties or formed weak gels, with solid-like properties (G') consistently higher than liquid-like properties (G") across the entire frequency range. Further, the G' and G" values were continuously increased with increasing of frequency.

Similar behaviour of G’ and G” has been shown in yoghurts prepared with coconut milk (Pachekrepapol et al., 2021). The product made with 100% FPI (SM#6) had shown higher G’ and G”, while all other samples showed lower G’ and G” compared to the reference sample (100% DM). Out of all the samples tested, SM#3, made with 50% dairy milk and 50% FPI, showed the lowest G’ and G” values. Based on the results obtained, SM#5 (50% FBM and 50% FPI) had shown the closest G’ and G” to the reference sample throughout its storage period. “According to *Pachekrepapol et al., (2021),* Increased protein and decreased starch strengthened the protein-protein complex at the acidic pH, and resulted in a higher G’ value”. A slight G’ and G’ increase in all the samples was observed during their storage period.





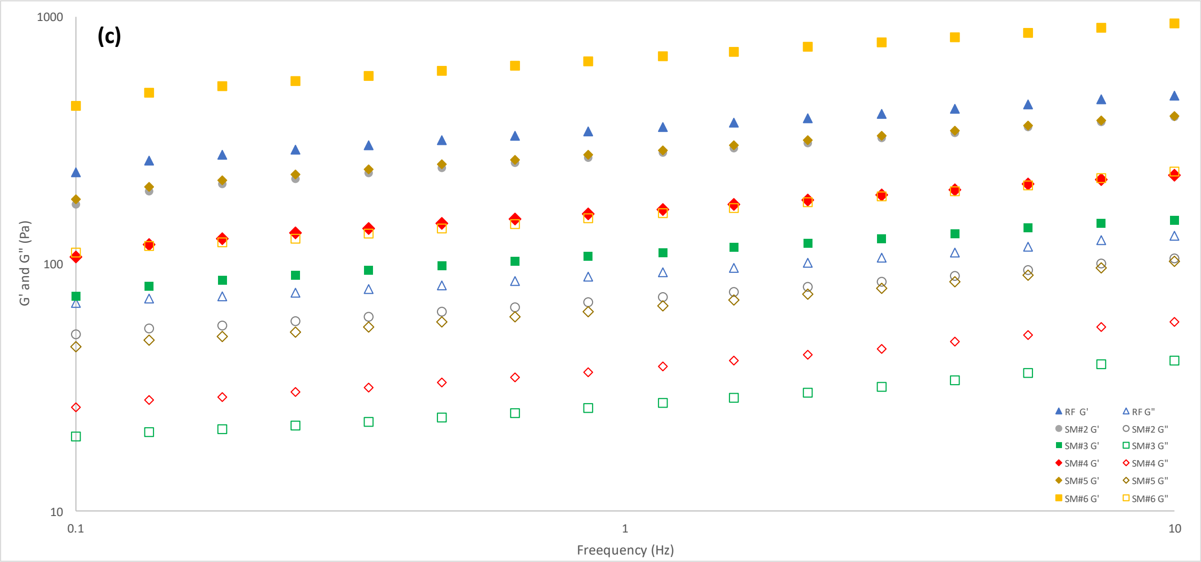
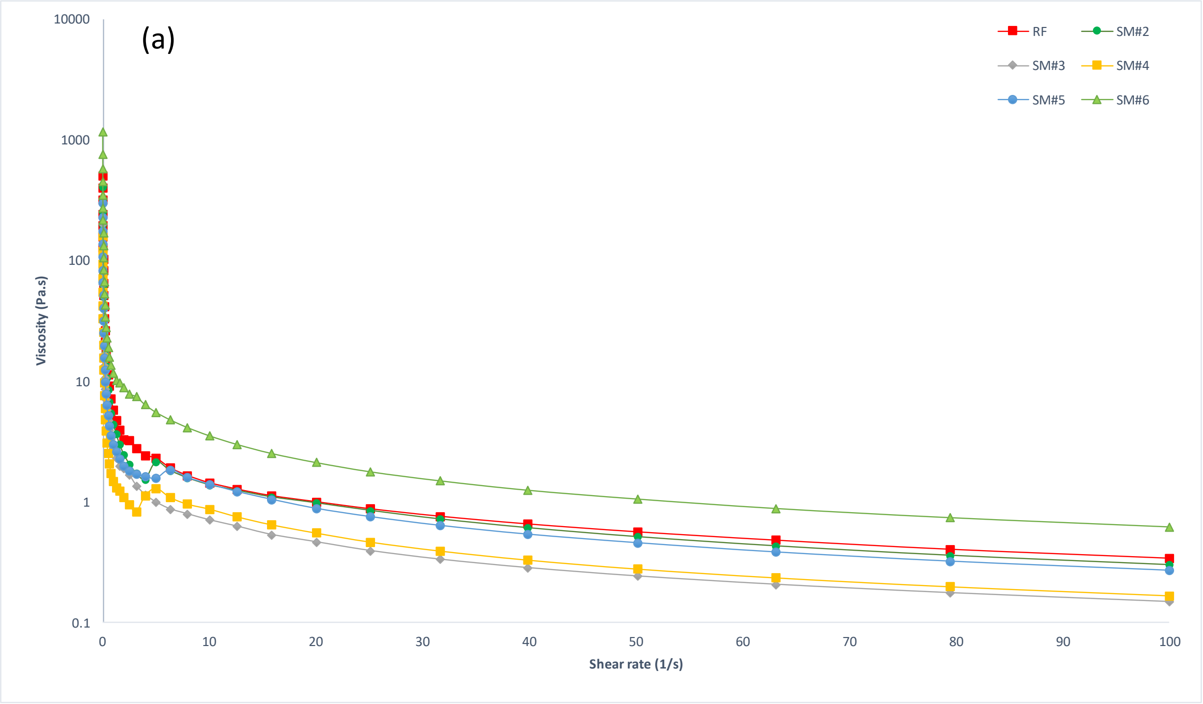


Figure 5.6 G’ and G” of yoghurt samples made from dairy and faba bean milk measured on day 1 (a), day 7 (b) and day 14 (c).
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

The apparent viscosity of all the yoghurt samples decreased with increasing shear rates as shown in Figure 5.7. a, b, and c on day 1, 7, and 14 at 10 ^{0}C respectively. The reduction in viscosity during shearing is an indication of the non-linear shear-thinning behaviour of the yoghurt samples (Pachekrepapol et al., 2021). The apparent viscosity difference of yoghurt samples depended on their raw material and ratios. The sample made with 100% FPI (SM#6) showed the highest apparent viscosity than the reference sample made with 100% DM. In contrast, all other samples’ apparent viscosity was lesser, and the lowest viscosity was observed in SM#3 (50% dairy and 50% FPI). Meanwhile, the apparent viscosity of SM#2 (50% DM + 50% FBM) and SM#5 (50% FBM + 50% FPI) showed a non-significant deviation from the reference sample. Similar behaviour was observed throughout the storage period. These findings suggested that during the heating process, when starch granules and protein particles expanded and absorbed water in the continuous phase (Pachekrepapol et al., 2021), FPI strengthened the gel network, improving protein-protein or polysaccharide-protein interactions at acidic pH conditions. However, combining FPI with DM resulted in a low apparent viscosity, which could be due to the poor interaction of the dairy protein with faba bean protein and this should be further investigated.


(a)
10000
1000
100
10
1
0.1
Viscosity (Pa.s)
0
10
20
30
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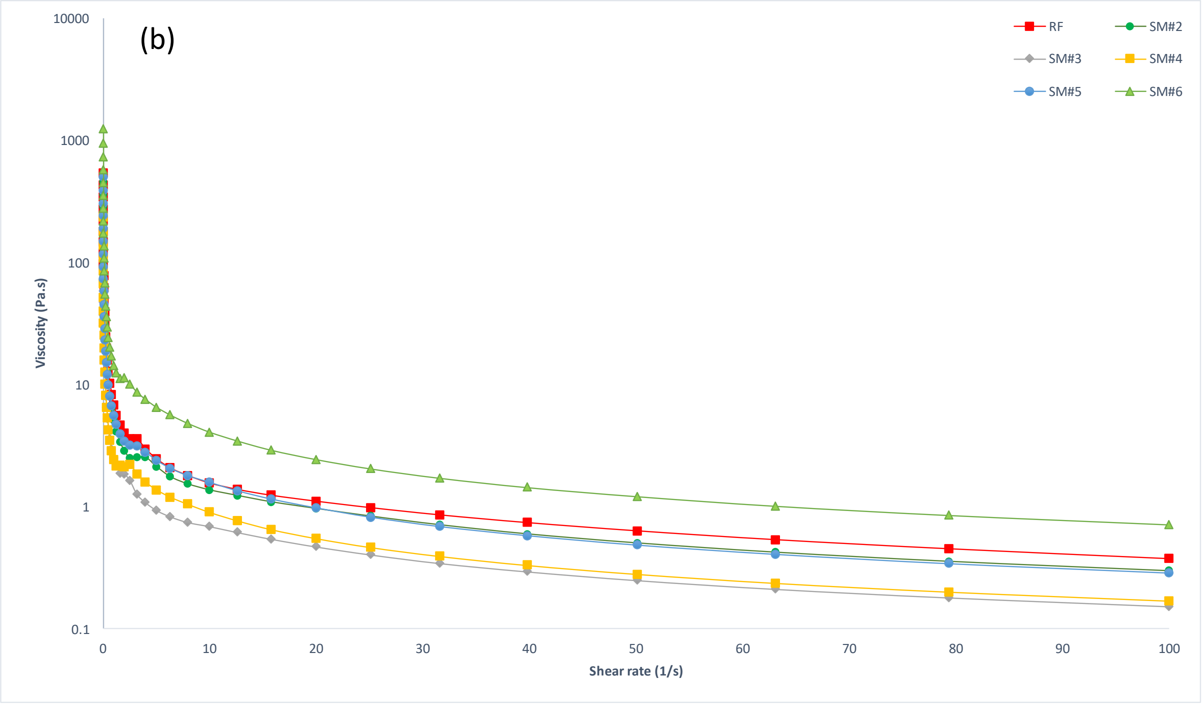
Shear rate (1/s)
RF
SM#2
SM#3
SM#4
SM#5
SM#6


(b)
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Viscosity (Pa.s)
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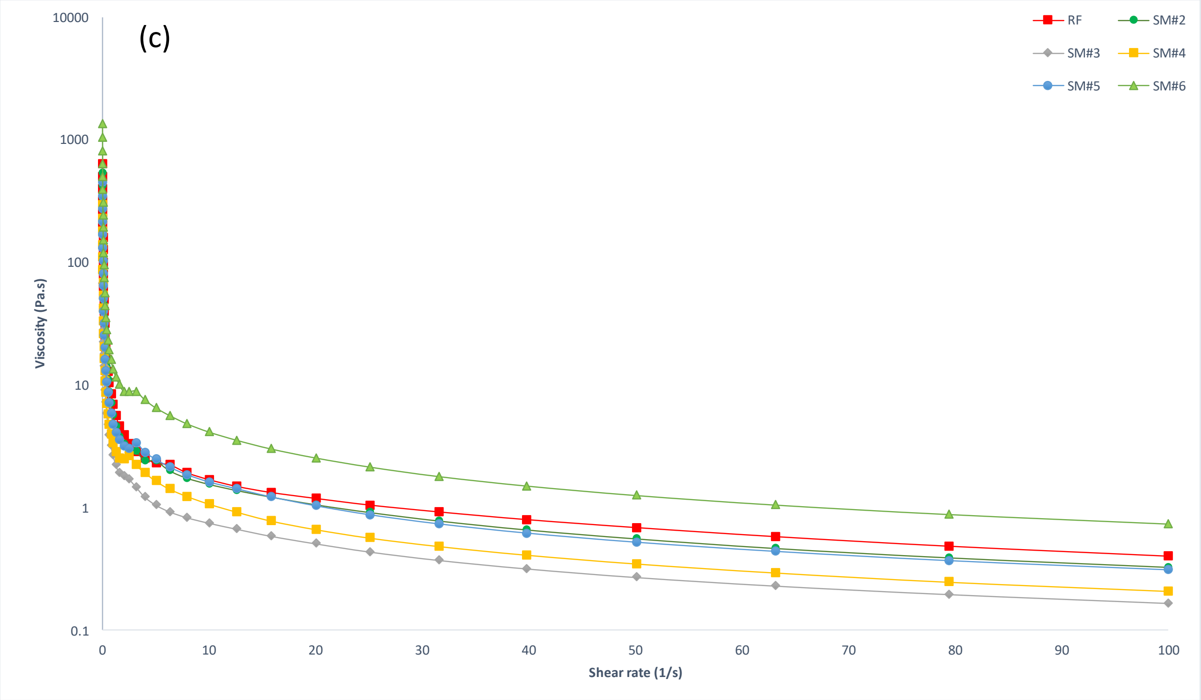

Figure 5.7 Apparent viscosity of yoghurt samples made from dairy and faba bean milk measured on day 1 (a), day 7 (b), and day 14 (c) at 10 ^{0}C
(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

5.3.5 Textural properties during storage

The texture parameters, influenced by factors such as composition and manufacturing methods, are crucial indicators for assessing the physical and sensory quality of dairy products, reflecting the structural organization of the network (Walia, 2013). As shown in Table 5.3, all the samples exhibited an increasing trend of their hardness and adhesiveness values during storage while a decreasing trend for the cohesiveness, gumminess, and springiness values compared to day 1.

Yoghurt texture is primarily determined by its hardness, also known as firmness, which reflects the force required to achieve a particular deformation to the product structure (Mudgil et al., 2017; Zannini et al., 2018). The yoghurt made with FPI showed the highest hardness (7.89) at the end of storage while the sample made with FBM showed the lowest hardness (1.67) at the end of the 14-day storage period. A similar trend was observed for the adhesiveness of the products as well; the highest resulted in SM#6 (-45.32) while the lowest value resulted in SM#4 (-10.65). Furthermore, the trend in hardness and adhesiveness corresponded with results obtained for apparent viscosity and syneresis for the products and a similar trend during yoghurt's storage analysis was reported by Karsheva et al. (2013).

Table 5.3 Textural parameters of yoghurt during storage at 4 ^{0}C on day 1, 7 and 14

Parameters	**Storage days**	**Yoghurt Samples**					
		Reference	**SM#2**	**SM#3**	**SM#4**	**SM#5**	**SM#6**
Hardness	**Day1**	5.39± 0.90^{d}	3.90± 0.07^{c}	1.87± 0.03^{d}	1.60± 0.08^{d}	2.83± 0.29^{c}	7.07± 0.29^{a}
	Day7	5.41± 0.35^{b}	4.06± 0.13^{c}	1.90± 0.05^{e}	1.63± 0.01^{e}	3.03± 0.31^{d}	7.33± 0.43^{a}
	Day14	5.68± 0.27^{b}	4.08± 0.11^{c}	1.96± 0.01^{e}	1.67± 0.05^{e}	3.15± 0.37^{d}	7.89± 0.07^{a}
Adhesiveness	**Day1**	-31.39± 2.34^{c}	-24.11± .74^{b}	-12.13± 1.19^{a}	-9.60± 0.48^{a}	-15.43± .30^{a}	-43.72± 1.98^{d}
	Day7	-35.88± 3.36^{d}	-24.63± .59^{c}	-13.53± 0.68^{ab}	-10.34± 0.22^{a}	-15.87± .99^{b}	-43.71± 1.75^{e}
	Day14	-36.37± 0.00^{d}	-27.79± .00^{c}	-13.00± 0.00^{ab}	-10.65± 0.00^{a}	-16.66±0.00^{b}	-45.32± 0.00^{e}
Cohesiveness	**Day1**	0.20± 0.02^{c}	0.22±0.0.01^{bc}	0.26± 0.02^{ab}	0.27± 0.00^{a}	0.21± 0.01^{bc}	0.20± 0.01^{c}
	Day7	0.19± 0.03^{b}	0.21± 0.00^{b}	0.25± 0.01^{a}	0.25± 0.00^{a}	0.21± 0.00^{b}	0.19± 0.01^{b}
	Day14	0.19± 0.00^{c}	0.20± 0.01^{bc}	0.24± 0.01^{ab}	0.25± 0.00^{a}	0.20± 0.01^{bc}	0.19± 0.01^{c}
Gumminess	**Day1**	0.73± 0.04^{ab}	0.59± 0.01^{b}	0.75± 0.02^{ab}	0.27± 0.01^{c}	0.34± 0.05^{c}	0.90± 0.07^{a}
	Day7	0.71± 0.08^{bc}	0.57± 0.03^{c}	0.69± 0.05^{b}	0.26± 0.01^{d}	0.33± 0.00^{d}	0.89± 0.02^{a}
	Day14	0.66± 0.02^{b}	0.57± 0.07^{b}	0.65± 0.07^{b}	0.26± 0.01^{c}	0.30± 0.03^{c}	0.81± 0.01^{a}
Springiness	**Day1**	1.01± 0.90^{b}	0.90± 0.11^{b}	1.08± 0.05^{b}	2.20± 0.06^{b}	8.47± 0.08^{a}	8.85± 0.04^{a}
	Day7	0.91± 0.28^{b}	0.84± 0.03^{b}	0.92± 0.17^{b}	1.80± 0.42^{b}	6.53±0.16^{a}	6.90± 0.94^{ab}
	Day14	0.83± 0.02^{c}	0.78± 0.09^{c}	0.78± 0.13^{c}	1.06± 0.07^{c}	5.34± 0.09^{a}	6.50±0.0.87^{b}

* ***Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same row for the same parameter were significantly differences (p<0.05) by the Turkey pairwise comparisons. No significant differences were observed between storage times.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

There was no significant difference observed for the cohesiveness of products except SM#3 and SM#4, which showed a significantly higher value compared to other samples *($p<0.05$).* Cohesiveness is defined as the ratio of the area under the positive force curve during the second compression cycle to the area under the positive force curve during the first compression cycle (Area 2/Area 1) (Figure 2.5). It measures the strength of internal bonds in a material and refers to how much it may be distorted before breaking. It is a crucial characteristic for examining the texture of yoghurt and is connected to customer approval of the product. These results pointed out that SM#3 and SM#4 have higher internal bond strength than other products. However, no significant changes in cohesiveness were observed during their storage period.

Gumminess is a function of the cohesiveness and hardness of the product. It determines the yoghurt's acceptance level and differs among individuals (Mudgil et al., 2017). Gumminess showed the highest value in SM#6 (0.90), the lowest in SM#4 (0.27), and the reference sample showed a value of 0.73 at storage on day 1. The gumminess value showed a non-significant ($p<0.05$) decreasing trend throughout the storage.

The rate of product return to native dimensions following the removal of the deforming force is known as springiness (Ozcan, 2013).

Based on these results, it can be concluded that yoghurt's hardness and adhesiveness increased during storage while gumminess, springiness, and cohesiveness decreased.

Chapter 6. Conclusions and Recommendations

One of the best ways to meet the growing demand for protein is to fully or partially replace dairy proteins with plant-based proteins. This strategy offers a great opportunity to develop new food products with a high nutritional content in an environmentally sustainable way. This study aimed to identify the possibility of developing yoghurt with full or partial replacement of dairy with plant protein. It was observed that yoghurt could be developed to either fully or partially replace DM with FBM and FPI; however, further development is needed to produce a fully successful product.

The study findings showed that yoghurt produced by partially substituting DM with FBM had a comparatively higher protein content than the product made only with FBM. However, both fully and partially substituting DM with faba bean resulted in significantly lower fat content in the yoghurt. Despite faba bean seeds containing a high protein amount, this was not fully reflected in the seed extracts, this may be due to the extraction method and other related factors. Based on the various formulations tested, it can be concluded that to create a nutritious product with high protein and low-fat content, it is beneficial to combine FBM with DM or with FPI during formulation. This approach optimizes the nutritional profile of the yoghurt.

The yoghurt produced using a combination of dairy and dairy-faba bean hybrid showed a higher syneresis rate than the product made with an FBM and protein FPI blend. The yoghurt made with DM and FPI (SM#3) had a higher syneresis percentage, whereas the product formulated with FPI (SM#6) showed the lowest syneresis. This suggests that yoghurt made with FPI has better WHC than other formulations. When compared with the reference, SM#2 (50% DM + 50% FBM) and SM#5 (50% FBM + 50% FPI) demonstrated relatively similar WHC characteristics.

The small amplitude oscillatory tests (SAOR) showed that all yoghurt samples exhibited characteristics of weak viscoelastic gel behaviour, where G' (storage modulus) was greater than G” (loss modulus). There was no crossover between G' and G”, indicating a strong, solid-like structure in the yoghurts. According to the results achieved, SM#2 (50% DM + 50% FBM) displayed slightly lower G' and G” values, while SM#5 (50% FBM + 50% FPI) exhibited slightly higher G' and G” values compared to the reference, indicating similar elastic properties across these samples. However, the dynamic rheological properties of all other samples differed significantly from the reference. This trend was also reflected in the apparent viscosity measurements among the samples, indicating variations in their rheological behaviours.

The texture profile analysis of the yoghurt samples demonstrated significant differences in textural properties such as hardness, adhesiveness, cohesiveness, springiness, and gumminess when compared to the reference. These differences can be linked to differences in composition, which directly impact the textural attributes of the yoghurts. In general, all tested samples demonstrated lower textural properties, except SM#6 (100% FPI), which displayed higher textural properties compared to the reference. However, SM#2 (50% DM + 50% FBM) and SM#5 (50% FBM + 50% FPI) showed textural properties more similar to those of the reference sample. These findings suggest that product formulations, such as those used in SM#2 and SM#5, may result in the textures closer to the desired texture as the reference. Confocal laser scanning microscopy images showed variations in microstructure among different yoghurt formulations. The dairy and faba bean hybrid yoghurts showed both protein and fat globules dispersed throughout, creating a better gel structure. On the other hand, the yoghurts made with faba bean contained a higher amount of protein and fewer fat particles. In this study, the milk was not homogenized before fermentation. Homogenization before fermentation could reduce the size of the milk fat globules and enhance their interactions with milk proteins. Protein-coated fat globules help in developing networks as the product's pH decreases during fermentation, which can increase the hardness of the yoghurt.

In vitro, gastrointestinal digestion experiments conducted on the samples indicated that the 100% faba bean yoghurt had lower protein digestibility compared to the dairy-faba bean hybrid yoghurt and the reference (100% DM). Protein hydrolysis, measured as the Free amino N (%) showed that all samples released a lower amount of free amino nitrogen during the gastric phase compared to the small intestinal phase. During the intestinal phase, a significantly lower release of free amino nitrogen was observed in the faba bean-only products (SM#4, SM#5, & SM#6) compared to the dairy-faba bean hybrid products (SM#2 & SM#3). This could be due to the lower digestibility of plant proteins compared to animal proteins (Kaur et al., 2022). The lower protein digestibility of faba bean yoghurt may be attributed to the traces of anti-nutritional factors and the formation of complexes due to the interaction of proteins with other seed components.

The sensory results indicated that consumers preferred products with sensory attributes similar to those of dairy yoghurt. The sensory attributes of SM#2 (50% DM + 50% FBM) showed the least deviation from the reference which showed the highest consumer preference. On the other hand, SM#4 (100% FBM) and SM#5 (50% FBM + 50% FPI) showed the highest deviation from consumer preferences. Based on sensory evaluation, SM#2 emerged as the best formulation, while SM#4 and SM#5 showed potential for improvement.

No significant variations were noticed in the investigated parameters (pH, TTA, Syneresis%, viscosity, and textural characteristics) during the stability analysis of yoghurt samples.

Further studies are needed to improve the textural properties of yoghurt made with faba bean. Adding hydrocolloids, such as pectin, gelatine, xanthan gum, carboxymethyl cellulose (CMC), soy protein isolate, and polymerized whey protein, can enhance yoghurt's texture and sensory properties (Shi et al., 2020).

Further, exploring various process technologies, such as homogenization and high-pressure processing, could enhance yoghurts' texture and sensory aspects. Investigating these impacts on faba bean yoghurt offers a chance to improve its overall quality.

Proteins from legumes are generally recognized for their nutty taste. Additional research suggests exploring various methods to overcome this nutty flavour, such as incorporating sweeteners or fruit purees or employing diverse processing techniques like irradiation or microwaving faba bean seeds.

The study also found that faba bean protein exhibits lower digestibility compared to dairy proteins. Low digestibility is primarily due to the presence of anti-nutritional factors in the faba bean. Various methods, such as soaking, germination, microwave treatment, irradiation, and fermentation, have been demonstrated to decrease these anti-nutritional factors and enhance the digestibility of plant proteins (Badjona et al., 2023). Therefore, investigating the impact of different processing techniques on digestibility would be an interesting area for future research, to enhance the overall quality and health benefits of faba bean yoghurt.

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Appendix

Appendix A

Table 3.1 a. Composition of Anchor Blue Milk (Fonterra, NZ)

Nutritional Information		
Nutrients	**Avg. Qty preserving 250 ml**	**Avg. Qty per 100ml**
Energy	658 kJ	263 kJ
Protein	8.3 g	3.3 g
Fat Total	8.5 g	3.4 g
Saturated	5.6 g	2.3 g
Carbohydrates	11.9 g	4.8 g
Sugars	11.9 g	4.8 g
Sodium	100 mg	40 mg
Calcium	292 mg	117 mg

Table 3.1 b. Composition of Faba Bean Protein Isolate (NZPROTEIN, NZ)

Nutritional Information	
Nutrients	**Avg. Qty per 100g**
Energy	1670 kJ
Calories	399
Protein	85 g
Gluten	0 g
Fat Total	5.4 g
Saturated	1.2 g
Carbohydrates	3.8 g
Sugars	< 1 g
Fibre	< 0.5 g
Sodium	290 mg

Appendix B

Processing of faba bean milk from faba bean seeds

Soaked Faba bean seeds

Dehulled seeds

Blending of faba bean seeds

Filtering (using 150 µ sieve)

Faba bean milk

Appendix C

Experimental Results

Table 4.1 Proximate Compositions of Different Yoghurt Samples

Product Code	% Total Solid	% Crude Protein	% Fat	% Crude Fiber	% Starch
Reference	13.18 ± 0.02^{a}	3.68 ± 0.02^{b}	3.88 ± 0.02^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
SM#2	9.13 ± 0.02^{b}	3.11 ± 0.02^{c}	2.10 ± 0.01^{b}	0.10 ± 0.00^{a}	0.39 ± 0.00^{a}
SM#3	8.60 ± 0.02^{c}	3.73 ± 0.05^{b}	2.00 ± 0.01^{c}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
SM#4	7.56 ± 0.02^{d}	2.33 ± 0.05^{d}	0.30 ± 0.00^{d}	0.10 ± 0.00^{a}	1.06 ± 0.01^{a}
SM#5	6.40 ± 0.02^{e}	3.10 ± 0.10^{c}	0.30 ± 0.00^{e}	0.09 ± 0.00^{b}	0.10 ± 0.01^{b}
SM#6	6.96 ± 0.02^{f}	4.30 ± 0.10^{a}	0.40 ± 0.00^{e}	0.09 ± 0.00^{b}	0.05 ± 0.00^{c}

****Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same column were significantly different (p<0.05) by the Turkey pairwise comparisons.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Table 4.2 pH TTA and Syneresis data of yoghurt samples

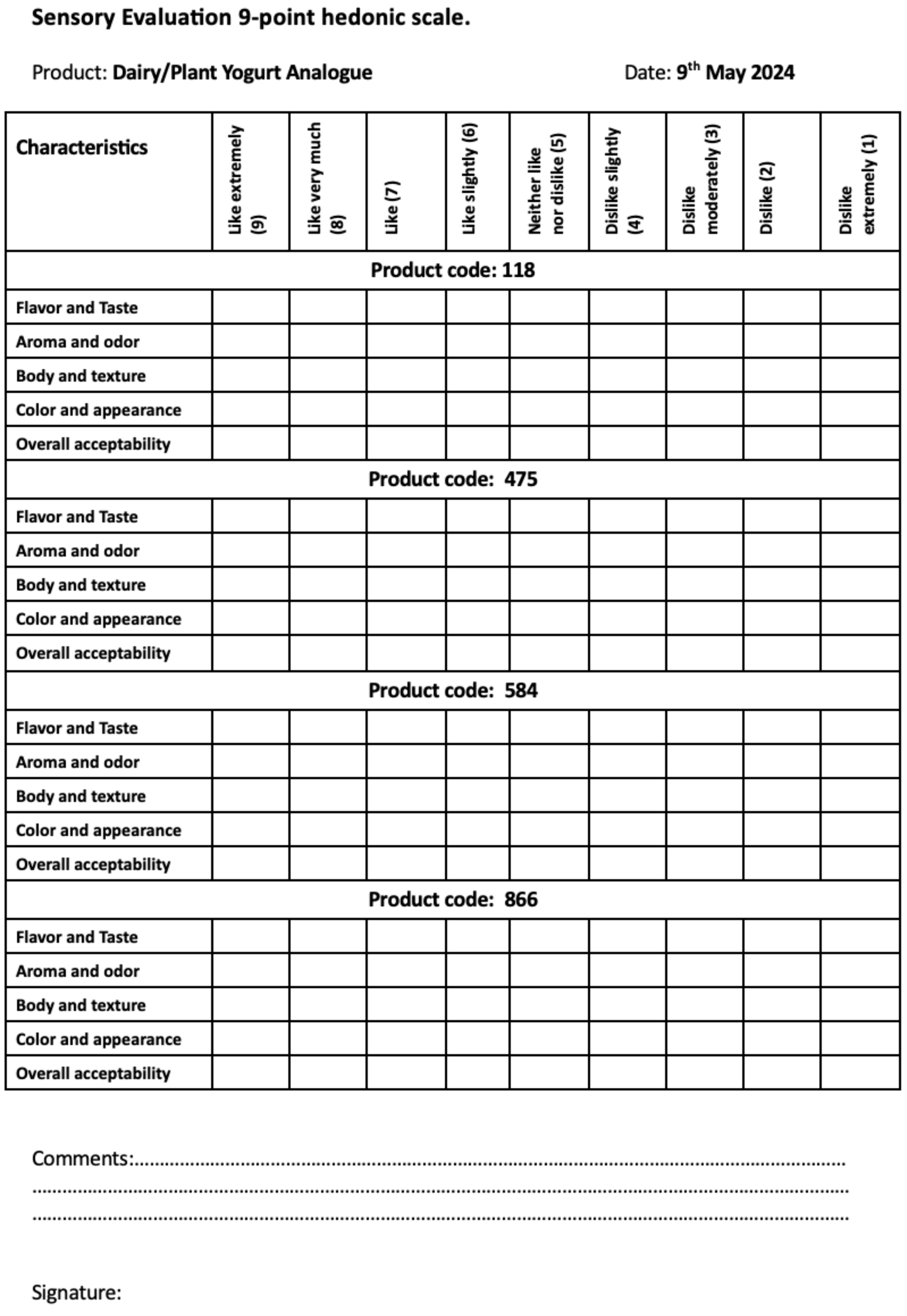
Product code	Parameters		
	pH	TTA	Syneresis
Reference	4.17 ± 0.01^{e}	0.81 ± 0.06^{a}	51.80 ± 0.89^{c}
SM#2	4.35 ± 0.01^{c}	0.66 ± 0.00^{b}	56.17 ± 0.20^{b}
SM#3	4.25 ± 0.01^{d}	0.64 ± 0.00^{b}	64.00 ± 0.63^{a}
SM#4	4.42 ± 0.01^{b}	0.32 ± 0.00^{c}	62.41 ± 0.22^{a}
SM#5	4.53 ± 0.04^{a}	0.29 ± 0.00^{d}	39.31 ± 1.01^{d}
SM#6	4.36 ± 0.01^{c}	0.24 ± 0.00^{e}	12.44 ± 0.39^{e}

****Results are expressed as mean± standard deviation (n=3), means with different superscripts in the same column were significantly different (p<0.05) by the Turkey pairwise comparisons.***

(**Ref**-100% DM, **SM#2**-50% DM+50% FBM, **SM#3**-50% DM + 50% FPI, **SM#4**-100% FBM, **SM#5**- 50% FBM + 50% FPI, **SM#6**-100% FPI)

Appendix D

Sensory Questionnaire



Sensory Evaluation 9-point hedonic scale.

Product: **Dairy/Plant Yogurt Analogue** Date: **9th May 2024**

Characteristics	**Like extremely (9)**	**Like very much (8)**	**Like (7)**	**Like slightly (6)**	**Neither like nor dislike (5)**	**Dislike slightly (4)**	**Dislike moderately (3)**	**Dislike (2)**	**Dislike extremely (1)**
Product code: 118									
Flavor and Taste									
Aroma and odor									
Body and texture									
Color and appearance									
Overall acceptability									
Product code: 475									
Flavor and Taste									
Aroma and odor									
Body and texture									
Color and appearance									
Overall acceptability									
Product code: 584									
Flavor and Taste									
Aroma and odor									
Body and texture									
Color and appearance									
Overall acceptability									
Product code: 866									
Flavor and Taste									
Aroma and odor									
Body and texture									
Color and appearance									
Overall acceptability									

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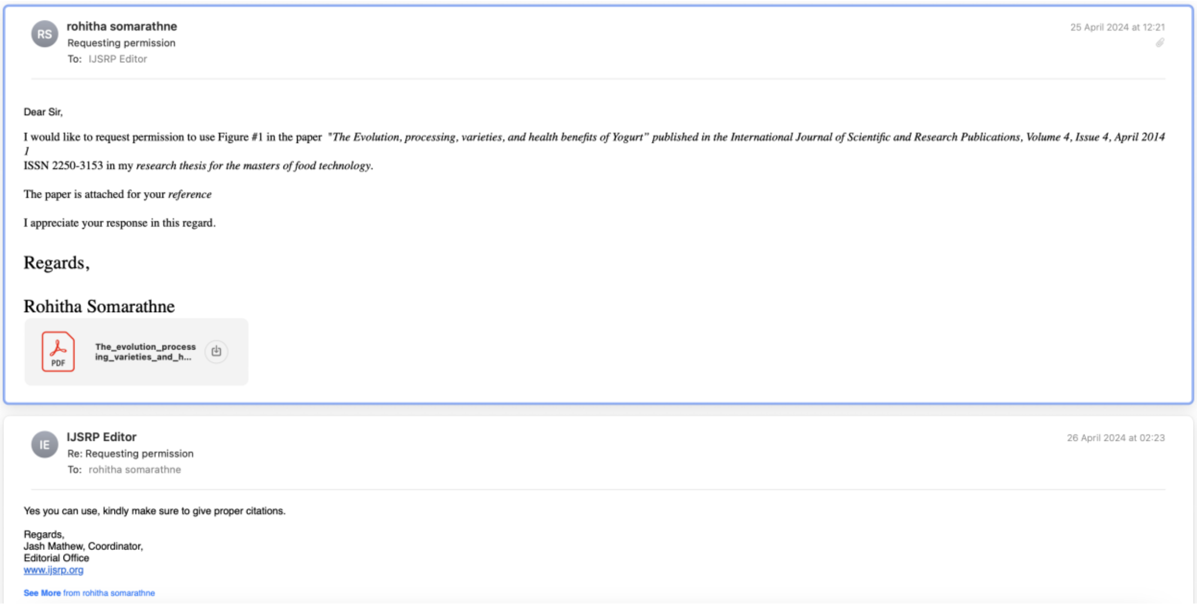

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