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Development of foxtail millet (*Setaria italica*) milk; a novel beverage

A thesis submitted in partial fulfilment of the requirement for the degree of Master of Food
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ABSTRACT

Growing environmental and health concerns related to animal-based food production has led to environmental and health concerns recently, which has resulted in plant-based foods increase in popularity. Plant-based milk alternatives, made from sources like legumes and seeds, are becoming more popular. In this study, foxtail millet is explored as a gluten-free nutritious and sustainable plant-based milk alternative.

Phase I of the study focused on investigating the extraction of foxtail millet extracts (FME) by analysing three key parameters: colour, pH, and total soluble solids. Dry milling with a higher millet-to-water ratio (millet percentage) produced FME with colour similar to cow's milk. The pH of dry-milled FME ranged from 6.38 to 6.67, slightly higher with wet milling. The total soluble solids (°Brix) were found higher processed with dry milling, extracted with higher percentage of amylase rate and millet percentage. Focus group sensory evaluation was conducted and determined parameters including dry milling, 0.20% amylase, and 10% and 12% millet grain were decided for further investigation.

In Phase II, FMEs underwent emulsification with varying millet grain rates, added oil, and lecithin. Physicochemical properties including whiteness, pH, total soluble solids, particle size, viscosity, and gravitational separation rate were measured to screen 12 formulations. Higher millet grain percentage increased whiteness and soluble solids, while more added oil raised whiteness and viscosity. Particle size decreased with homogenisation but was not significantly affected by other factors. Sensory evaluation favoured formulations with 12% millet grain and 0.8% oil. The optimal formulation for further study was identified as having 12% millet grain, 0.8% oil, and 5% lecithin.

Phase III investigated the physicochemical properties of the selected foxtail millet milk formulation and its shelf-life during storage at 4°C for 4 weeks. Analysis of foxtail millet milk suggested improved microbial stability and while maintaining a similar moisture content to cow skim milk but with lower ash content. It contained 0.5% protein, 1.3% unsaturated fats, 7.6% carbohydrates, and 0.4% dietary fibre which is absent in cow's milk. It offers a healthier profile than saturated fat-rich or high-sodium alternatives.

In conclusion, the foxtail millet milk developed in this study demonstrated good appearance, sensory acceptance, microbial stability, and served as a good source for carbohydrates.

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LIST OF ABBREVIATIONS

CIE	International Commission on Illumination
SOP	Standard operation procedure
AHSM	After high shear mixing
AHT	High-temperature α -amylase
ANOVA	Analysis of variance
AOAC	Association of official analytical collaboration
APH	After primary homogenisation
BHSM	Before high shear mixing
CVD	Cardiovascular disease
EFME	Emulsified foxtail millet extract
FAO	Food and Agriculture Organisation
FME	Foxtail millet extract
GRAS	Generally recognised as safe
HLB	Hydrophilic-lipophilic balance
HP	Horsepower
HPVH	High-pressure valve homogeniser
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
LDL	Low-density lipoprotein
MA	Massachusetts
NZD	New Zealand dollar
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PSD	Particle size distribution
RO	Reverse osmosis
SD	Standard deviation
TSS	Total soluble solids
UHT	Ultra-high temperature
UK	The United Kingdom
USA	The United States of America
WI	Whiteness index

CHAPTER 1 INTRODUCTION

Over the last century, advances in agricultural and food technology have been significant in order to meet the demands of a growing global population. As a result, the production of animal-based food dramatically increased by 58% between 1998 and 2018, and animal products have become a staple in the daily diets of many individuals (Curtain & Grafenauer, 2019). However, the high consumption of animal products has led to growing environmental concerns, as the meat and dairy industries are known for being responsible for significant greenhouse gas emissions that contribute to climate change (Ettinger et al., 2022). Additionally, the high intake of animal products and processed foods has led to an increase in diet-related diseases such as obesity, diabetes, and cardiovascular disease (McClements & Grossmann, 2021).

On the other hand, plant-based foods, such as cereals, legumes, seeds, and nuts, are rich in dietary fibre, minerals, vitamin, and antioxidants, which are considered beneficial for human health (Aydar et al., 2020a). As a result, there has been a rising demand for plant-based food options in recent years, with more people transitioning to vegetarian or vegan lifestyles due to health concerns and growing awareness of the importance of sustainable food production. This growing demand for plant-based food options shows the increasing awareness of the importance of sustainable food production and the negative impact of animal-based food on both human health and the environment. This has led to an increase in plant-based food ingredients being incorporated into daily meals (Aydar et al., 2020a). In recent years, plant-based food including plant-based milk, plant-based meat, plant-based yoghurt, and plant-based cheese are increasing in more countries and supermarkets (McClements & Grossmann, 2021).

The milk alternatives are made from a variety of sources, including legumes, oil seeds, cereals, and pseudocereals, and have a similar appearance to bovine milk (Mäkinen et al., 2015). They are now a crucial component of many people's diets, both on their own and as ingredients in other plant-based meals. According to the Passport report, the dairy industry in New Zealand generated 3,3 billion NZD in 2020. Also, there has been a noticeable trend towards increased sales of milk alternatives, which rose from 8.3 million NZD in 2015 to 13.9 million NZD in 2020, indicating a promising market potential for plant-based dairy alternatives (International, 2020).

While bovine milk has been consumed worldwide as an important source of protein, carbohydrates, and fat, there are some concerns about including it in one's diet (Vanga & Raghavan, 2018). One major concern is the presence of food pathogens in bovine milk, such as *Salmonella spp.* and *Escherichia coli*, which have caused food pathogenic diseases globally. Additionally, a significant number of people are allergic to bovine milk protein and suffer from lactose intolerance. Around 80% of Africans and an even greater percentage of East Asians are lactose intolerant (Vanga & Raghavan, 2018). Around 86% of northerners from China suffer from the same condition (Yongfa et al., 1984). Plant-based milk can be a good milk alternative to address these health issues. Common use milk alternatives include soy milk, coconut milk, oat milk, almond milk, and rice milk (McClements & Grossmann, 2021). The growing interest in plant-based milk alternatives may encourage the dairy industry to expand the range of plant materials used in these products, as they play a crucial role in determining the taste, texture, and nutritional value of the final product (McClements & Grossmann, 2021). As the demand for alternatives continue, there is a growing need to develop new products that provide a range of sensory experiences and improved nutritional profiles.

In this study, foxtail millet was investigated as a potential plant-based material for producing a plant-based milk alternative. Foxtail millet belongs to the *Poaceae* family with other crops such as maize, wheat, rice, and barley. Foxtail millet is also called Italian millet, German millet, or Hungarian millet. Foxtail millet are cereal plants that commonly found in most of African and Asian countries, and they are known for their high resistance to pest, diseases, and drought which explains their popularity in tropical regions (He et al., 2015). Foxtail millet is one of the oldest crops in the world with high production yield. It has been used to feed people for a long time particularly in China, India, Japan, and some African countries. Foxtail millet is gluten-free and non-allergic. It is considered as a good healthy food ingredient as foxtail millet contains higher amounts of protein and less carbohydrates than rice, maize, and sorghum (He et al., 2015). Foxtail millet contains 2.5 times more dietary fibre than rice (He et al., 2015). Foxtail millet has been consumed in the forms of porridge, bread, noodles, including fermented foxtail millet food (Sachdev et al., 2021). This study explores the potential of foxtail millet as a plant-based milk alternative, adding to the growing interest in plant-based diets and sustainable food sources. The following sections will discuss the methodology and results of

the study, providing insights into the feasibility of using foxtail millet for producing a milk alternative.

AIM AND OBJECTIVES

1.2.1 Aim

The aim of this research was to develop a plant-based foxtail millet milk.

1.2.2 Objectives

1. To extract foxtail millet grain into water using suitable extraction method by investigating the physiochemical and sensory properties of the foxtail millet water mixtures.
2. To determine the effects of adding canola oil as fat content and soy lecithin as emulsifier in foxtail millet milk emulsion.
3. To measure the particle size distributions of foxtail millet milk emulsions at different homogenisation pressures.
4. To select the optimal foxtail millet milk formulation based on the physiochemical characteristics and sensory acceptance of a plant-based milk beverage.
5. To determine the physical, nutritional properties, and sensory properties of the selected foxtail millet milk formulation.
6. To determine the shelf-life stability of foxtail millet milk when stored at refrigeration temperature (4°C) by investigating its physiochemical properties and conducting microbial tests.

CHAPTER 2 LITERATURE REVIEW

2.1 Millet and foxtail millet

2.1.1 Millet

Millet belongs to the *Poaceae* family and has been recognized as a significant food crop for a long time (Sharma & Niranjana, 2018). This cereal is cultivated in about 26 countries, predominantly in Central Africa and Asia producing about 95% of global millet yield (Li et al., 2021; Saleh et al., 2013; Sharma & Niranjana, 2018). Figure 2.1 shows the distribution and production of millet in the top ten producing countries in 2019. According to Food and Agriculture Organisation (FAO) (2019), India, Niger, and China are the leading countries in millet production. The cultivation of millet has also expanded to North, and South America, and Australia. Millet is gaining popularity as a gluten-free alternative grain (Caballero et al., 2015).

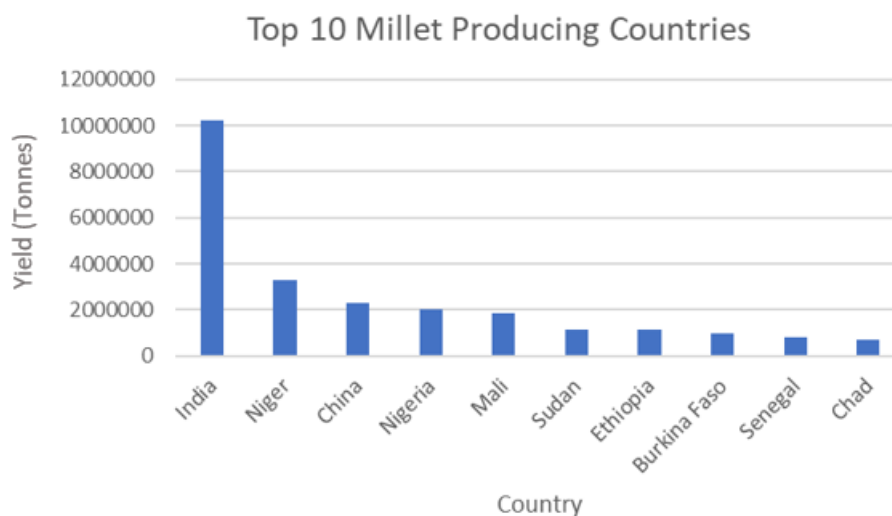


Figure 2.1 The top ten millt producing countries and their production in 2019 (FAO, 2019).

Millet plays a crucial role, particularly in developing countries, as it serves as an important food for both humans and livestock. It has high resistance to drought, pests and plant diseases which makes it a resilient crop. Compared to other major cereals such as oat and rice, millet has a relatively shorter growing period, leading to increased production. In fact, millet production ranks as the sixth highest among cereal crops (Saleh et al., 2013).



Figure 2.2 The appearance of primary varieties of millets. (a) White fonio; (b) Pearl millet; (c) Proso millet; (d) Finger millet; (e) Teff; (f) Foxtail millet (Taylor, 2019).

Millet is generally small grained, with the size and colour of millet depending on the variety (Figure 2.2). Common millet varieties include pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), finger millet (*Eleusine coracana L. Gaertn*), and many more (Caballero et al., 2016). There are some variations in nutritional content among the varieties (Table 2.1).

Table 2.1 The nutritional properties of main varieties of millet (Sharma & Niranjana, 2018).

Millet Type	Crude Protein	Carbohydrates	Fat	Crude Fibre	Mineral	Energy
	(g) in 100 g of grain					(kcal)
Pearl	11.6	67.5	5	1.2	2.3	363
Finger	7.3	72	1.3	3.6	2.7	336
Proso	12.5	70.4	1.1	2.2	1.9	364
Foxtail	12.3	60.9	8	8	3.3	351

2.1.2 Foxtail millet

2.1.2.1 Origin, distribution, and current use

Foxtail millet is also known as Italian millet, German millet, Chinese millet, and Siberian millet. The earliest archeological remains of foxtail millet were found in northern China approximately 7400 years ago (Sharma & Niranjana, 2018). Processing millet has resulted in the development of food products such as beverages, porridges, bread, and noodles (Figure 2.1) (Saleh et al., 2013).



Figure 2.3 Traditional food made from millets (Caballero et al., 2016); (a) Pouring injera batter (teff) (b) Cooking uji (finger millet) a. (c) Pearl millet and sorghum couscous agglomeration (d) Foxtail millet and yak milk tea (e) Finger millet porridge (f) Foxtail millet gruel (g) White fonio rice and peanut sauce.

Millet is known for its hardiness in dry conditions, and foxtail millet is even more well-adapted to dry and cool environments than other millets. Therefore, it has adapted to the tropical, subtropical, and temperate regions. Foxtail millet can tolerate sandy and loamy soils, and it requires one third less water than that required for growing corn. The cereal can also tolerate soil pH ranging from 5.5-7 (Sharma & Niranjana, 2018; Sheahan, 2014). Foxtail millet is tolerant to high salinity and the grain is flexible with altitude; it can grow in elevations as high as 1500 m and as low as sea-level. Millet is free of pest, however, the summer annual weeds and *Pyricularia* leaf spot may threaten the survival of foxtail millet (Sheahan, 2014).

2.1.3 Structure of foxtail millet

Foxtail millet grains are covered with a pericarp in the form of an utricule (Figure 2.4). Consequently, the harvesting process for foxtail millet involves dehulling to remove the attached pericarp (Caballero et al., 2015). The husk and bran of foxtail millet account for approximately 13.5% and 1.5-2% of the grain weight, respectively. Foxtail millet is typically planted in spring, and its growth cycle ranges from five to eight weeks with seed maturation occurring within 8-15 weeks (Sharma & Niranjana, 2018). A mature foxtail millet plant can reach a height of 120-200 cm (Roshan Kumar Singh, 2017).

Figure 2.4 *Structure of foxtail millet grain (Sharma & Niranjana, 2018).*

2.1.4 Nutritional composition and health impact of foxtail millet

Foxtail millet is an important crop in Asian countries. There are more than 3000 foxtail millet seed accessions kept by Chinese Crop Germplasm Resources Information System representing 26 different colours, mainly shown as yellow, white, golden, black, and grey (He et al., 2015). The nutritional content of foxtail millet cultivars with identical colours can be varied (Li et al., 2021).

Protein content of foxtail millet varies between different cultivars which ranges from 10.6-15.2% (Taira, 1968), surpassing the protein content of rice, maize, sorghum, and other millets (FAO, 1995; He et al., 2015). The protein of foxtail millet mainly consists of albumin, globulin, prolamin, and glutelin (He et al., 2015). Foxtail millet contains a range of non-essential amino acids, with high glutamic acid. According to Yang et al. (2008), different foxtail millet cultivars exhibit high variability in non-essential amino acids, while the content of essential amino acids are stable within a narrow range. Regarding essential amino acids millet is higher in valine, leucine, isoleucine, phenylalanine, and threonine. The content of tryptophan, lysine, cysteine, and methionine is relatively low (Li et al., 2021). However, when comparing with other cereal crops, the amount of methionine and tryptophan in foxtail millet is higher (He et al., 2015). Raw foxtail millet has protein digestibility ranging from 75.5% to 79.3%, which is higher than that of raw finger millet (67.4%-74.7%), pearl millet (70.4%-72.7%), and proso millet (68.4%-72.9%). Protein digestibility of foxtail millet can be further enhanced from 90.4% to 93.8% after cooking (Pawar & Machewad, 2006; Ravindran, 1992; Annor et al., 2017). This makes foxtail millet a superior plant-based protein source for human consumption.

Foxtail millet contains varying levels of fatty acids, typically ranging from 12 to 62 g/kg (Zhang et al., 2015). Linoleic acid makes up approximately 67% of the fatty acid composition in foxtail millet, followed by oleic acid (16.11%), palmitic acid (7.42%), and stearic acid (6.84%) (Zhang et al., 2015).

Table 2.2 *The proximate and nutritional composition of foxtail millet (Amadou et al., 2014; Li et al., 2021).*

Parameter	Content
Moisture (%)	12.27 ± 0.55
Protein (%)	12.02 ± 0.68
pH (%)	5.93 ± 0.43
Ash (%)	0.89 ± 0.09
Carbohydrates (%)	83.77 ± 0.22
Crude fat (%)	4.03 ± 0.15
Soluble sugar (%)	2.54 ± 0.1
Total starch (%)	15.78 ± 0.17
Rapidly digestible starch (%)	1.43 ± 0.47
Slowly digestible starch (%)	6.83 ± 0.74
Resistant starch (%)	7.61 ± 1.62
Zinc (mg/kg)	29.33 ± 1.15
Magnesium (g/kg)	1.01 ± 0.03
Iron (mg/kg)	53.10 ± 1.28
Potassium (g/kg)	3.23 ± 0.05
Sodium (mg/kg)	38.20 ± 0.70
Calcium (mg/kg)	153.33 ± 0.5

carbohydrates in foxtail millet mainly consist of starch, reducing sugars, and cellulose. Starch is the predominant form of carbohydrates in foxtail millet, and the (millet) starch has higher agglutination stability, swelling point, and gelatinisation temperature than maize (Laxmi et al., 2015). Besides normal starch, foxtail millet contains resistant starch, a type of carbohydrate that resists digestion in the human digestive system (Bangoura et al., 2012). This property makes foxtail millet beneficial on human health, particularly for individuals with diabetes. The chemical components, morphology, structures, and physicochemical properties of foxtail millet starch can vary among different cultivars and growing conditions (Sharma & Niranjana, 2018).

Table 2.2 shows that foxtail millet contains minerals such as calcium, magnesium, phosphorus, iron, and zinc which are considered higher than in rice (Verma et al., 2015). The nutrient density of foxtail millet makes it a healthy diet option, especially considering its gluten-free properties.

2.2 Gelatinisation and rheological properties

The main component of foxtail millet is starch, which plays a vital role in its gelatinisation behavior and digestibility (Sharma & Niranjana, 2018). Gelatinisation is primarily influenced by the starch content in the food. Starch is insoluble in cold water, but when mixed with hot water, the hydrogen bonds break allowing water molecules to bind with amylose and amylopectin molecules within the starch. The hydration process leads to the swelling of starch granules, thereby trapping more water molecules and leading to an increase in viscosity (Tako et al., 2014). Amylose has a linear structure, whereas amylopectin has a branched structure. The ratio of amylose to amylopectin determines the structure of the starch granules, which affects the functionality and application of the starches. Having a higher amylose content means more rigid starch granules, whereas having a higher amylopectin content results in more branched but less rigid starch granules. Consequently, the size, shape, molecular structure of the granules, swelling ability, and gelatinisation temperature of starch vary depending on the source of the starch.

In foxtail millet, the starch granules are mostly polygonal with some spherical shapes (Yin et al., 2019). The starch granules extracted from foxtail millet are larger than the starch granules extracted from other millets. The average size in foxtail millet is around 7.6 μm (Annor et al., 2014). Foxtail millet starch granules gelatinisation starts at around 66.7°C, and the gelatinisation peaks are in the range of 67.9–72.2 °C. Foxtail millet exhibits a narrow gelatinisation temperature range, with only 1.7°C between onset and conclusion, ranging from 72.1 to 73.8°C., the difference it may be contributed by the variations of amylase content in the millets (Li et al., 2019).

Viscosity of millet can be readily impacted by change in temperature and shear. The change in viscosity in 5% foxtail millet flour slurry is shown in Figure 2.5. The viscosity increases with initial heating and reaches peak viscosity (A) at around 95°C. However, maintaining this constant temperature results in the rapid drop of viscosity (B), upon cooling, the viscosity rises again (C), this is due to retrogradation of the starch. This phenomenon can be explained by the amylose molecules that get aligned in parallel forming aggregates with low solubility which results in higher thickness and viscosity (Leelavathi et al., 1987). The peak viscosity of foxtail

millet during heating is 0.166 centipoise (cP), whereas, for hot paste viscosity (viscosity after temperature holding) is 0.073cP, and cold paste viscosity is 0.150 cP (Shinoj et al., 2006).

Figure 2.5 *The viscosity of flour slurries of 5% foxtail millet with temperature change (Sharma & Niranjana, 2018).*

Note: (A): peak first viscosity during heating; (B): constant viscosity; (C): viscosity rise during cooling.

2.3 Plant-Based Milk Emulsion

2.3.1 Definitions and principles of food emulsions

An emulsion involves the dispersion of two immiscible liquids, commonly water and oil, where one phase is dispersed as small spherical droplets within the other phase. Emulsion technology is widely used in different kinds of food products. The droplet sizes in food emulsions commonly range from 0.1 to 100 μm (Serdaroğlu et al., 2015). Emulsions can be primarily classified as oil-in-water (O/W) and water-in-oil (W/O) (Dalglish, 2001).

When oil droplets are dispersed within an aqueous phase, an oil-in-water emulsion is formed. The oil acts as the dispersed phase and water as the continuous phase. Common examples of oil-in-water food emulsions are milk, cream, salad dressings, beverage and soups (McClements et al., 2019). Whereas, water-in-oil emulsions involve water droplets dispersed within an oil phase, with water acting as the dispersed phase and oil as the continuous phase. Butter and margarine are examples of such emulsions (McClements, 2016). Additionally, multiple

emulsions can also be produced, like oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W) (Dalgleish, 2001).

Emulsion technology is also used in the delivery systems for functional food ingredients such as vitamins, colours, flavours, and preservatives, mainly participating in encapsulation, protection, and release during their use (McClements, 2016). This technology enhances the handling ability, stability, and efficacy of these ingredients. Food emulsions consist of a wide range of components, including oils, water, emulsifiers, gelling agents, colourants, salts and chelating agents (Dalgleish, 2001). Each of these components exhibit distinct physicochemical, sensory, and nutritional properties, making the selection of specific ingredients vital to achieve the desired properties of the emulsion (McClements et al., 2007).

2.3.2 Formation of food emulsions

The natural composition of common processed foods, such as homogenised milk, cream, and certain dairy products can form emulsions (Friberg et al., 2004). These foods naturally provide sufficient amounts of fats and proteins, which are key components for the formation of emulsions. However, in some other foods, these key components may be lacking, thus additional ingredients need to be incorporated to create a stable emulsion (Friberg et al., 2004). For example, in salad dressings, protein, and polysaccharides are typically added to produce a stable emulsion.

When the ingredients suitable for formation of an emulsion are present, homogenisation is introduced to mix the immiscible liquids into an emulsion (Dalgleish, 2001). This process is crucial for enhancing the stability of the system by reducing the droplet sizes in the emulsion, thus achieving desired physicochemical properties (Friberg et al., 2004). During homogenisation, several processes should be considered as they contribute to the formation and quality of the emulsion (Figure 2.6) (McClements, 2016).

Before homogenisation, it is important to mix the functional ingredients in the most soluble phase. Lipophilic ingredients such as colours, antioxidants, and oil-soluble emulsifiers are

mixed and dissolved in oil, while hydrophilic ingredients like sugars, salts, proteins, water-soluble emulsifiers and polysaccharides are dissolved in water (McClements, 2016). This process can improve dispersion and dissolution of the functional ingredients during homogenisation.

Figure 2.6 *Processes of pre-homogenisation, homogenisation and post-homogenisation during emulsion production (McClements, 2016).*

The selection of a suitable homogenisation method is vital. High-shear mixers, colloid mills, ultrasonic homogenisers, and high-pressure valve homogenisers are commonly used in the food industry (Figure 2.7) (McClements et al., 2019). The homogenisation process can be categorized into primary homogenisation and secondary homogenisation (Figure 2.8), which are usually carried out sequentially (McClements, 2016). The former involves homogenising two immiscible liquids into an emulsion, while the latter focuses on reducing the size of droplets in an existing emulsion. In food production, primary homogenisation is typically

carried out using high-shear mixers, while secondary homogenisation is accomplished using high-pressure valve homogenisers (McClements, 2016).

Figure 2.7 *Common mechanical devices used for homogenising plant-based milk (McClements et al., 2019).*

Depending on the specific requirements, primary and secondary homogenisation can be used individually or in combination in the food emulsions. Selection of ingredients during homogenisation contributes to the overall quality and stability of the emulsion in the final food product (McClements, 2016).

Figure 2.8 *Creation of oil-in-water emulsion by conducting primary and secondary homogenisation (McClements, 2016).*

2.3.3 Physical mechanism of the emulsion formation

The formation of the droplet in an emulsion during production is dependent on the interfacial and disruptive forces. The interfacial force holds the droplet together against breaking of the droplet (Dickinson, 2012). Thus, the disruption of the droplets needs external force to break the droplets apart, and this force is defined as disruptive force. To break a droplet, the disruptive force that is applied to the droplet must be significantly larger than the interfacial force within the droplet (Friberg et al., 2004).

Within a droplet, the pressure difference between inside and outside is defined as the Laplace pressure ΔP_L (McClements, 2016) (Equation 1).

$$\Delta P_L = \frac{4\gamma}{d} \text{-----}[1]$$

Where

γ is the interfacial tension between oil phase and water phase

d is the diameter of the droplet

Equation 1 shows that increase of the interfacial tension and decrease of the droplet size leads to the increase of the Laplace pressure. Therefore, the pressure generated by the disruptive

force must achieve higher level to meet the requirement to break the droplet. Moreover, the time of the disruptive force applied to the droplet is an important parameter for the disruption process (Dalglish, 2001).

The disruption can be also characterised by the Weber number (We), which is derived from disruptive force/ interfacial force. When the Weber number reaches a certain level, the droplet will be disrupted (McClements, 2016). Considering the shear stress that affect the droplet, the Weber number can also be explained with Equation [2].

$$We = \frac{\text{Disruptive force}}{\text{Interfacial force}} = \frac{Gd\eta_c}{2\gamma} \text{-----}[2]$$

Where

G is the shear rate

η_c is the viscosity of the continuous phase

d is a diameter of the droplet

As shown by the Weber number in Equation 2, the higher viscosity of the continuous phase and the higher shear rate applied to the droplet can result in the higher value of the Weber number which indicates the droplet can be broken easier.

2.3.4 Role of emulsifiers in plant-based milk production

In the production of plant-based milk, emulsifiers play a vital role in achieving a stable emulsion system (McClements, 2016). The presence of liquid and oil phases may exist in certain food materials, while a stable food emulsion system cannot be formed through homogenisation alone. In such cases, emulsifiers are used as surface-active substances. Emulsifiers possess both hydrophilic and lipophilic structures, allowing them to adsorb at the interface between the water and oil phases (Branen, 2002). By binding the water and oil droplets together, emulsifiers can prevent droplet aggregation, and ensuring the formation of a stable emulsion (Branen, 2002).

The emulsification ability of each emulsifier can be determined by two parameters: the minimum emulsifier concentration required to create small oil droplets and the minimum size of the formed oil droplets. Higher surface loads necessitate a higher concentration of emulsifiers (McClements et al., 2019).

The emulsifier serves two primary functions in emulsion production. Firstly, it reduces the interfacial tension and decreases the required free energy to disrupt the droplets. Secondly, the emulsifier forms a protective coat around the droplets, preventing aggregation (Hartel & Hasenhuettl, 2019).

Emulsifiers can be classified based on different principles, with the most common classification being based on ionic properties and hydrophilic/lipophilic characteristics. According to Bancroft's rule (Figure 2.10), an emulsifier is more soluble in a particular phase, which then becomes the continuous phase of the emulsion. If the emulsifier is more water-soluble, the emulsion will be an oil-in-water emulsion. Conversely, if the emulsifier is more soluble in oil, the emulsion will be a water-in-oil emulsion (McClements, 2016).

The Hydrophilic-Lipophilic Balance (HLB) is another important indicator of the hydrophilic/lipophilic properties of emulsifiers, widely used for classification of emulsifiers (Narsimhan et al., 2019). The HLB value is determined by the emulsifier's chemical properties and can be calculated using Davis Equation [3] (McClements, 2016).

$$HLB = 7 + \sum (\text{hydrophilic group numbers}) - \sum (\text{lipophilic group numbers}) \text{ -----}[3]$$

HLB = Hydrophilic-Lipophilic Balance

As shown in Equation 3, the more hydrophilic structure an emulsifier possesses, the higher its HLB value. Emulsifiers with high HLB values exhibit more hydrophilic characteristics than lipophilic characteristics, resulting in a greater possibility of forming an oil-in-water emulsion. Conversely, if an emulsifier has more lipophilic structures than hydrophilic structures, it will have a lower HLB value which is normally used in production of water-in-oil emulsions. In

general, emulsifiers with high HLB numbers (10-18) are considered hydrophilic, while those with low HLB numbers (3-6) are considered lipophilic (Narsimhan et al., 2019).

Emulsifiers also contribute to the stability by generating electrostatic or steric repulsion between emulsifier-coated oil droplets. When selecting an emulsifier for a food product, it is important to consider the performance of the emulsifier in different environmental conditions in food (McClements et al., 2019). These environmental conditions may include variations in ionic strength, pH, temperature, and interactions of ingredients (McClements & Grossmann, 2022). For instance, using plant proteins as emulsifiers to coat oil droplets may lead to aggregation when the pH approaches the isoelectric point of the protein or under high salt and high-temperature conditions. Aggregation occurs when proteins lose surface charge as they approach the isoelectric point, and they tend to unfold when heated above the denaturation point (McClements, 2016). In comparison, polysaccharide-coated fat droplets are less susceptible to pH changes and they provide strong steric repulsion instead of electrostatic repulsion generated by proteins (McClements, 2004). However, polysaccharide-coated oil droplets, being relatively larger, are more prone to creaming (McClements & Grossmann, 2022).

In selecting food emulsifiers, other factors should also be considered, such as the legality in the country, effectiveness, processing methodology, and the cost (Branen, 2002).

Figure 2.9 *Water soluble surfactant produce oil-in water emulsion (McClements, 2016).*

2.3.5 Common emulsifiers used in plant-based milk

Lecithin

Lecithin is a plant-based emulsifier (E322) which is widely applied in the food industry. It can be extracted from various plant and animal sources, including soybeans, sunflowers, eggs, and milk. Lecithin is naturally present in the cell and organelle walls of plants, animals, and microorganisms as a barrier material (McClements, 2016).

Commercial soybean lecithin consists of three primary phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). The standard form of commercial lecithin is liquid, other forms such as powder and granules are also available (Branen, 2002). Natural liquid lecithin typically has a hydrophilic-lipophilic balance (HLB) number ranging from 2 to 8, which is desired for producing water-in-oil emulsions (McClements, 2016).

However, certain modifications can be applied to lecithin to increase its hydrophilic properties. Using chemical and enzymatic treatments, such as using hydrogen peroxide and lactic acid or acetic acid, can induce hydroxylation of double bonds in lecithin. This process can result in increased hydrophilic characteristics (Whitehurst, 2004). The degree of hydrolysis of lecithin impacts its emulsification capacity. As shown in Figure 2.10, high levels of hydrolysis can produce smaller droplet sizes during emulsification (Whitehurst, 2004). Hydrolysed lecithin is commonly used to produce oil-in-water emulsions.

Lecithin powder or granules undergo further modification and de-oiling. De-oiling is typically carried out using acetone, which dissolves most of the natural lipids. De-oiled lecithin generally exhibits an HLB number ranging from 7 to 10. These types of lecithin demonstrate improved emulsifying properties and dispersibility in oil-in-water emulsions. Additionally, de-oiled lecithin shows a more natural taste compared to liquid lecithin (Branen, 2002).

Soy lecithin, as derived from natural sources has a GRAS (Generally Recognised As Safe) status. Besides its emulsifying properties, lecithin also contributes to the stabilisation of some

food products. Furthermore, lecithin can be utilised as an antioxidant in certain food products (Branen, 2002).

Figure 2.10 *The mean diameter of droplet sizes of emulsion decreases with higher degree of hydrolysed lecithin (Whitehurst, 2004).*

Plant protein

The addition of plant proteins to plant-based beverages offers several advantages, including improved nutritional value, enhanced sensory attributes, and functional benefits (Qamar et al., 2020). Most globular proteins or proteins with a random coil structure can effectively function as emulsifiers (Zhang et al., 2023). Plant proteins possess surface activity, enabling them to adsorb onto droplet surfaces and modify interfacial properties, particularly in the creation of oil-in-water emulsions. The mechanism underlying plant protein emulsification is illustrated in Figure 2.12 (Zhang et al., 2023). In the initial stage, proteins migrate and attach to the droplet surface. Subsequently, plant proteins undergo partial unfolding and reorientation, exposing previously concealed hydrophobic regions within the core of the protein (Kim et al., 2020). These hydrophobic regions adsorb onto the surface of the oil droplets, while the hydrophilic regions of the proteins bind to the water phase. Finally, the protein particles positioned between the water and oil phases form a viscoelastic film that stabilises the emulsion through partial wettability (Kim et al., 2020).

Figure 2.11 *Application of protein as an emulsifier in oil-in-water emulsion (Zhang et al., 2023).*

Plant-based proteins exhibit significantly lower emulsifying ability compared to animal proteins (Sagis & Yang, 2022). This disparity can be attributed to the larger size of plant-based proteins, which render them slower and more challenging to adsorb onto the surfaces of droplets. Moreover, the rigid structure of plant proteins contributes to the formation of a weaker interfacial film. However, some undesired extraction processes of plant-based proteins may lead to protein aggregation, consequently reducing their emulsifying ability and product quality (Sagis & Yang, 2022).

The surface-active properties of plant proteins in food emulsions are influenced by several factors, including the source, protein structure, protein concentration, pH, temperature, ion concentration, and the presence of polysaccharides in the food emulsion (Qamar et al., 2020; Zhang et al., 2023). Plant proteins can be categorised into three main groups based on their origin: nuts (almond, walnut), legumes (soybeans, peas, chickpeas) and cereals (oats, rice, sorghum) (Zhang et al., 2023). Proteins derived from different sources exhibit varying emulsifying abilities. However, the diverse conformation of plant proteins limits their application in food emulsions (Zhang et al., 2023).

2.4 Plant-based milk

2.4.1 Production of plant-based milk

As an alternative to dairy milk, the plant-based milk analogue is expected to have more similarities with dairy milk in terms of appearance, texture, mouthfeel and flavour attributes. Plant-based milk is an oil-in-water emulsion like dairy milk. The fat globules in bovine milk play a significant role in providing those sensory features.

Thus, to make the texture of plant-based milk closer to that of bovine milk, production of plant-based milk is commonly conducted by two methods based on the fat content. Firstly, by disrupting the plant tissues and using the natural oil bodies from the plant to make an oil-water emulsion. Secondly, by creating an emulsion with fat globules sourced from plant material, plant-based protein, emulsifier, water, and other ingredients (McClements & Grossmann, 2022).

2.4.2 Method 1: Disruption of plant tissue method

2.4.2.1 Soaking, Grinding, and Separation

General processes of disrupting the plant tissue to produce plant-based milk are shown in Figure 2.13 (McClements & Grossmann, 2022). The plant tissue firstly undergoes soaking to soften which allows it to be easily broken down in the equipment. Soaking also removes enzyme inhibitors, thus improving the nutrient digestibility and bioavailability of the plant-based product (McHugh, 2018). During grinding, oil bodies, starch granules and cell wall fragments are released from the plant. This is followed by separation which removes the unwanted plant materials. This step can be achieved by using gravitational separation, centrifugation and filtration (Reyes-Jurado et al., 2023).

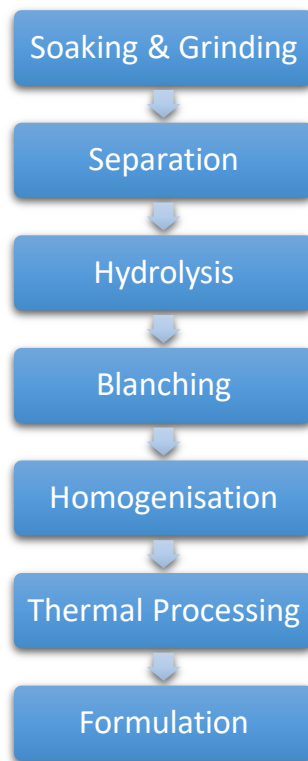


Figure 2.12 *Disruption of plant tissue method to produce plant-based milk (McClements & Grossmann, 2022a).*

2.4.2.2 Hydrolysis

After separation, the plant material can be subjected to chemical or enzymatic actions (McClements & Grossmann, 2022a). Hydrolysis of starch or plant fibre is commonly used in producing plant-based food to improve extraction yields and the overall quality. This process can be achieved by using chemical or enzymatic methods. For instance, mineral acid can be applied to the starch slurry below gelatinisation temperature to degrade the starch chemically. The degree of acid hydrolysis of starch is determined by the acid concentration, reaction time, and reaction temperature (Deswal et al., 2014). For some plant materials, the application of the cell wall degrading enzymes such as polygalacturonases, pectate lyases, or pectin methyl esterase elevate the extraction of other substrates mainly including protein and fat (Dhankhar & Kundu, 2021). The application of cell wall degrading enzymes after homogenisation can reduce the droplet particle size in food emulsion, thereby improving the emulsion stability (Dhankhar & Kundu, 2021).

High starch content in some plant material may impact on the palate of the plant product negatively (Dhankhar & Kundu, 2021). Amylase enzyme is commonly used for starch hydrolysis to degrade and remove the starches in plant-based milk. Enzymatic liquefaction is used to decrease viscosity in plant-based product by partially hydrolysing the starch. It can also increase yield by improving the filtration with less byproducts and improve the overall physicochemical properties of the product (Aiyer, 2005; Babolanmogadam et al., 2022; Deswal et al., 2014). The enzymatic hydrolysis method not only has higher yield than acid hydrolysis, it also requires milder condition thus the product will be less affected (Aiyer, 2005).

2.4.2.3 Blanching

Blanching is a thermal pretreatment that is commonly applied on some plant materials to reduce inactivating enzymes and the microbial load (Safarzadeh Markhali, 2021; Severini et al., 2016). As a result, blanching can help to elongate the shelf-life of the plant-based product. Also, it can decrease the beany flavour of soy milk by inactivating lipoxygenase (Aydar et al., 2020a). According to Archana et al. (1998), blanching pearl millet in hot water can significantly reduce the content of polyphenol and phytic acids by 28% and 38%, respectively (Sehga & Kawatra, 1998). Polyphenol compounds can serve as antioxidants which can be used to prevent oxidation. However, they also commonly considered showing some antinutritive effect, as polyphenols can binding with certain nutrients, making them unavailable for human body to digest or absorb (Pawase et al., 2021). The presence of polyphenols in some beverages can be unpleasant due to their bitterness (Soares et al., 2013). Blanching can effectively reduce the antinutritional factors, rancidity and bitterness of pearl millet, (Pawase et al., 2021).

2.4.2.4 Homogenisation

During the production of plant-based milk, various particles such as proteins, starch, fibre, and other cellular materials dispersed within the matrix, tend to settle. Homogenisation can be used to reduce the size of these particles to avoid settling caused by gravity (Dhankhar & Kundu, 2021). Additionally, emulsifiersthat are able to adhere to oil and water interface, can be added to improve the stability of the plant-based milk alternatives (Dhankhar & Kundu, 2021).

During the homogenisation process, parameters including homogenisation pressure and number of passes are important for producing the desired final product. With a higher homogenisation pressure, properties such as stability, clarity and whiteness index can be increased – this is critical in producing a good plant-based milk (Aydar et al., 2020b). Homogenisation pressure ranging from 20-60 MPa is commonly used for producing plant-based milk under non-ultra-high pressure. The homogenisation effect can also be improved by increasing homogenisation pass time which has been shown in previous studies (Dhankhar & Kundu, 2021).

2.4.2.5 Thermal Processing

In order to ensure compliance to food safety, like cow milk, plant-based milk undergoes thermal pasteurisation treatment to eliminate microorganisms and food pathogens and prevent spoilage in food. Alongside pasteurisation, other thermal processing methods such as sterilisation and ultra-high temperature (UHT) can be used to improve the storage of plant-based milk. These treatments can work in some cases by inactivating enzymes and microorganisms that are considered problematic for food preservation (Sala et al., 1995).

The common thermal treatment of plant-based milk includes pasteurisation at 65°C for 30 minutes (Nakamura et al., 2016; Yazici et al., 1997), sterilisation at 121°C for 15-30 minutes (Maghsoudlou et al., 2016; Tiravibulsin et al., 2021) and ultra-high temperature (UHT) treatment at 135-140°C for 2-20 seconds (Kwok et al., 2002).

2.4.2.6 Formulation

Plant-based milk can be customised by incorporating various ingredients to modify its properties. These modifications often involve the use of food additives, which are commonly used to improve factors such as colour, flavour, nutritional content, stability and also extend shelf life (Reyes-Jurado et al., 2023).

When compared to conventional bovine milk, plant-based milk alternatives are typically lower in protein content (Tachie et al., 2023). Thus, plant-based milk can be blended with high-protein plant materials such as peas and soybeans to increase the overall protein levels (Romulo,

2022). Additionally, commercial plant-based milk products are frequently fortified with essential nutrients like Vitamin D and Vitamin B12 to enhance the vitamin content (Romulo, 2022).

2.4.3 Method 2: Emulsification of plant material with added oil

Emulsification method can be used to produce plant-based milk from plant materials that are low in fat content and surface-active substance (McClements & Grossmann, 2022a). The emulsification of plant-based milk is produced with a mixture that contains plant material, added plant-based oil, emulsifier and water. The mixture normally goes through coarse emulsification using a blender first, then homogenised to produce fine emulsion with smaller particles. This process can enhance the stability of the plant milk emulsion as shown in Figure 2.14 (McClements & Grossmann, 2022a).

Figure 2.13 *Generalised process of the emulsification of plant-based milk (McClements & Grossmann, 2022a).*

2.4.3.1 Key ingredients of producing plant-based milk emulsion

Oil

Oil is the key ingredient of the emulsification method. It is used for creating fat globules in the plant-based milk emulsion system. Oils that are extracted from coconut, corn, sunflower, canola, soybean, flaxseed, olive and palmare commonly used in producing food emulsions (McClements, 2016). These oils mainly compose of triacylglycerol with different positions, chain lengthand types of fatty acid.

The selection of oil is important due to their differences in oil composition which can contribute to the various physiochemical, nutritional and sensory properties of the produced food emulsion (McClements & Grossmann, 2022a). For instance, coconut oil contains higher saturated fat than flaxseed oil. Similar to milk fat, saturated fat in coconut oil has low crystalline point which can create the mouthfeel of plant-based milk closer to dairy milk. However, due to its high saturated fat content, coconut oil is more prone to oxidation than flaxseed oil. Health impact of the oil also need to be considered. For instance, the consumption of saturated fatty acids can increase the risks of cardiovascular diseases (McClements et al., 2019).

Canola oil

Canola oil is derived from rapeseed (*Brassica napus*, *Brassica rapa L.*and *Brassica juncea L.*) and is sometimes labeled as rapeseed oil in food products (Grossmann et al., 2021). Natural rapeseed oil has limited use in food due to its high content of erucic fatty acid ($\approx 45\%$), which has been associated with cardiac issues and heart muscle lesions (Decloedt et al., 2017; O'Brien, 2009). However, genetically modified rapeseed cultivars with low erucic acid content ($<1\%$) have been use and deemed to be generally safe with the GRAS status (O'Brien, 2009). Canola refers specifically to cultivars with $<2\%$ erucic acid (Gunstone, 2004).

Canola oil has low saturated fatand containsomega-6 and omega-3 fatty acids, which are essential for human health (O'Brien, 2009). As a member of the *Brassica* family, canola seeds produce high levels of brassicasterol (≈ 48.8 mg/100 g canola oil), which can reduce serum

cholesterol levels and promote cardiovascular health (Decloedt et al., 2017; Xu et al., 2020). The mild odor of canola oil also enhances its application in food products (Shahidi, 1990).

Water

Water is another major ingredient of an emulsion and plays a vital role in impacting the bulk physicochemical and organoleptic characteristics of the food emulsion. The pH of mineral content of potable water may vary with location and time (Navarini & Rivetti, 2010). Water may also have possibility that contain organic matter that influence the emulsification by interacting with the emulsifier or other ingredients in the food system. Therefore, water may need to be treated before using to produce a stable plant-based milk emulsion (McClements & Grossmann, 2022a).

2.5 Sensory Evaluation

Plant-based milk serves as a viable alternative to bovine milk. Bovine milk is typically perceived as a low viscosity liquid with a mild flavour and an opaque white colour (Reyes-Jurado et al., 2023). With plant-based milk alternatives, consumers often seek for similar characteristics. However, some consumers may not accept some unique attributes of plant-based milks. For instance, milk alternatives derived from legumes, their distinct beany and earthy aroma are not accepted by some consumers (Reyes-Jurado et al., 2023). Therefore, it is vital to use sensory evaluation techniques to assess the sensory attributes of plant-based milk and gain a deeper understanding of consumer preferences. By using sensory analysis, manufacturers can modify their plant-based milk products accordingly to better meet consumer expectations and enhance overall consumer satisfaction (Grossmann et al., 2021).

Various approaches are commonly used to determine the sensory attributes of plant-based milk alternatives. These methods encompass both qualitative and quantitative techniques (Grossmann et al., 2021).

Descriptive analysis tests involve trained panelists who carefully evaluate and describe the sensory characteristics of plant-based milks. This method requires a detailed understanding of attributes such as taste, texture, aroma and appearance (Grossmann et al., 2021).

Discrimination tests are used to determine perceptible differences between different samples of plant-based milks. The tests help to identify variations in sensory properties and can be conducted through methods such as triangle tests or duo-trio tests (Kemp et al., 2009).

Consumer tests play a vital role in understanding the preferences and acceptance of plant-based milk products. Qualitative methods such as focus group discussions enable in-depth exploration of consumer perceptions, attitudes and preferences (Kemp et al., 2009). The participants in focus group are asked to respond a series of questions as part of the in-depth interview to provide a better understanding the decision behind the attitude and behavior and help the evaluators come a mutual understanding after discussion (Massey, 2011). Meanwhile, quantitative methods like hedonic response and overall acceptance tests provide numerical data to indicate consumers' liking (Kemp et al., 2009).

By using sensory analysis methods, researchers and manufacturers can gain valuable insights into the sensory attributes of plant-based milk alternatives. This information can be used to improve product formulation, optimise sensory characteristics and meet consumer preferences in the rapidly growing market of plant-based milks (Grossmann et al., 2021)

2.6 Conclusion

Plant-based milk has become a popular dairy alternative accepted by many consumers worldwide. This review covered the important processing and ingredient considerations for producing plant-based milks. Highlighted the advantages of using foxtail millet, including its high yield, nutritional value and health benefits, suggesting its potential as a novel ingredient to produce foxtail millet milk as a milk alternative.

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental design

The experimental design consisted of three phases. The first phase investigated the milling method and the percentage of millet in water. The effect of enzyme treatment (amylases) on foxtail millet-water mixture was also investigated in phase one.

In the second phase, canola oil and granule lecithin were added as emulsifier to the coarse-filtered millet milk at different ratios. In this phase, the samples were analysed for their stability by determining the particle size, separation rate and sensory acceptance by focus group. The optimal formulation was selected for investigated in the subsequent phase.

In the third phase, the nutritional value of the selected millet formulation was analysed and the stability was evaluated during storage for 28 days at 4°C.

3.2 Materials

Foxtail millet (Ceres Organics Ltd, Auckland, New Zealand), reverse osmosis (RO) water, α -amylases (total 24540 Unit) (FoodPro® AHT, Danisco), food dehydrator (Ezidri, ULTRA FD1000, New Zealand), pH meter (3540 pH & conductivity meter, Jenway, UK), colorimeter (CR-300, Konica Minolta, Osaka, Japan), blender (Waring BB155), digital hotplate with stirrer (152, Stuart, New Zealand), two-stage high-pressure homogeniser (APV 2000, Copenhagen, Denmark), water bath (TC120, Grant, UK), plate Count Agar (Thermo Scientific™ Oxoid™) and Petri dishes (Thermo Scientific™ Oxoid™), high shear mixer (Silverson, L504, Chesham, England) and particle size analyser (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK).

3.3 Phase I: Preparation and extraction of foxtail millet milk before stabilisation

Foxtail millet water mixtures were prepared and extracted following previous studies with minor modifications as shown in Figure 3.1 (McClements & Grossmann, 2022a; Reyes-Jurado et al., 2023). The investigation focused on the percentage of foxtail millet grain, milling method and the rate enzyme addition. Measurements of Brix, pH, whiteness index and a focus group discussion were conducted to define the most suitable preparation and extraction methods. These parameters were used to evaluate the effectiveness of different variables of the process.

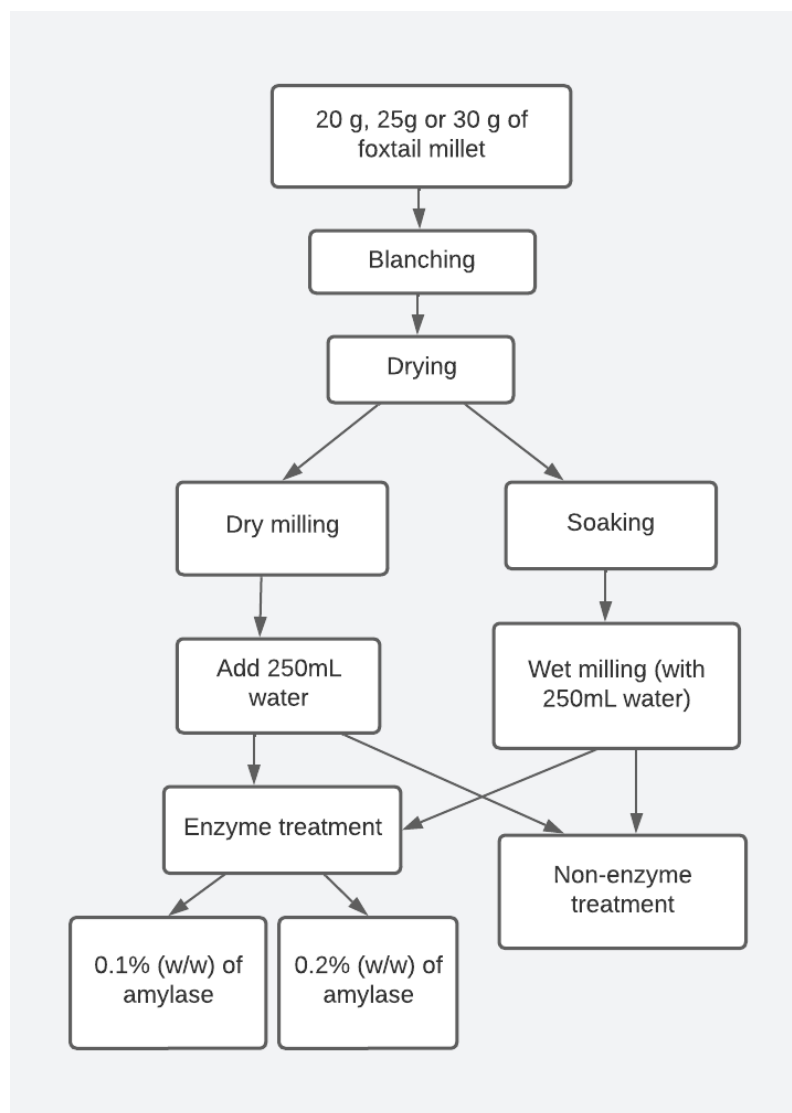


Figure 3.1 The preparation and extraction process of foxtail millet water mixture.

3.3.1 Preparation

Blanching and Drying

Foxtail millet samples (20 g, 25 g and 30 g) were transferred into boiling water (98°C) for 30 seconds for blanching (Archana et al., 1998). After draining with a sieve (Living & Co, New Zealand), the blanched foxtail millet grains were transferred into a food dehydrator (Ezidri, ULTRA FD1000, New Zealand) for drying at 55°C for 1 h (Rani et al., 2018).

3.3.2 Extraction

3.3.2.1 Milling

Dry milling: Blanched and dried foxtail millet was transferred into a grinder (Living & Co, The Warehouse, New Zealand). The lid was closed and the millet grains were grinded for 30 seconds. The ground foxtail millet powder was transferred into separate glass jars. Each glass jar was filled with 250 mL of RO water and mixed with the powder to ensure full hydration. This resulted in millet grain-water mixture containing 8%, 10% and 12% millet powders.

Wet milling: Blanched and dried foxtail millet was completely immersed in Reverse Osmosis (RO) water overnight (8-12 hours), according to (Nair UK et al., 2020). After soaking, foxtail millet was rinsed with RO water and drained using a sieve at room temperature (20°C). The soaked millet was transferred into a blender (Waring BB155) and blended at high speed for 2 min (3/4 HP) with 250 mL of RO water to mix thoroughly. Once blended, the mixtures were transferred into glass jars to create an extract of wet-milled foxtail millet. Three concentrations (8%, 10% and 12%) were prepared.

3.3.2.2 Enzyme treatment

The three foxtail millet mixtures in glass jars, prepared with varying percentage of foxtail millet grain in water. The enzyme used in this study was FoodPro AHT (High-temperature α -amylase). The samples were heated up to 95°C on a hot water bath, following the recommendations of the supplier. The millet-water mixtures were treated with amylases at two different additional rates, 0.10% (w/w) and 0.20% (w/w), for a duration of 60 minutes (Di Stefano et al., 2017). The

amylase addition rates studied were selected based on the pre-experiment trial and the unit load of α -amylases used in starch hydrolysis by Yang and colleagues (2021) (Yang et al., 2022). The specific enzyme weights used in the experiment are provided in the Table 3.1.

Table 3.1 *The amylase addition rate at 0.1% (w/w) and 0.2% (w/w) for foxtail millet mixture preparation.*

Experiment code	Milling method	% of Foxtail millet grains in water (grams used)	Enzyme addition rate % (w/w)	Enzyme addition weight (g)
D8-1	Dry	8% (20 g)	0.1	0.020
D8-2	Dry	8% (20 g)	0.2	0.040
D10-1	Dry	10% (25 g)	0.1	0.025
D10-2	Dry	10% (25 g)	0.2	0.050
D12-1	Dry	12% (30 g)	0.1	0.030
D12-2	Dry	12% (30 g)	0.2	0.060
W8-1	Wet	8% (20 g)	0.1	0.020
W8-2	Wet	8% (20 g)	0.2	0.040
W10-1	Wet	10% (25 g)	0.1	0.025
W10-2	Wet	10% (25 g)	0.2	0.050
W12-1	Wet	12% (30 g)	0.1	0.030
W12-2	Wet	12% (30 g)	0.2	0.060

Note: The enzyme addition rate was based on the weight of millet and the weight of amylase.

3.3.2 Characterisation of the millet-water extraction

3.3.2.1 Determination of pH

The pH value of the foxtail millet water mixture was determined according to the guidelines of by Tyl and Sadler (2017). A pH meter (3540 pH & conductivity meter, Jenway, UK) was used to measure the pH of the millet-water mixture. The pH electrode was calibrated with standard buffers (pH 4 and pH 7 standard solutions) at room temperature (25°C). Subsequently, the pH

electrode was immersed in a beaker containing the foxtail millet water mixture sample. The pH of each sample was recorded once the reading had stabilised.

3.3.2.2 Determination of total soluble solids

The total soluble solids of foxtail millet water mixture samples were determined by using a refractometer (Atago, pr-32 alpha, UK) based on Mauer & Bradley, 2017. The refractometer was calibrated with RO water prior to use. Subsequently, 2-3 drops of the well-mixed foxtail millet water mixtures at 20 °C were transferred/pipetted on to the prism of the refractometer. The °Brix (g of sucrose/100 g of sample) value was recorded once stabilised. The refractometer was rinsed with RO water and dried with clean lens tissue between measuring each sample.

3.3.2.3 Determination of the whiteness index

The colour of the foxtail millet-water mixture samples was measured with a colorimeter (CR-300, Konica Minolta, Osaka, Japan) and the parameters were determined using CIE* $L^*a^*b^*$ colour system with the illuminant D65. Where the L^* represented the lightness (black to white, 0 to 100), $+a^*$ represented the redness, $-a^*$ represented the greenness, $+b^*$ represented the yellowness and $-b^*$ represented blueness (McClements & Grossmann, 2022a), as illustrated in Figure 3.2.

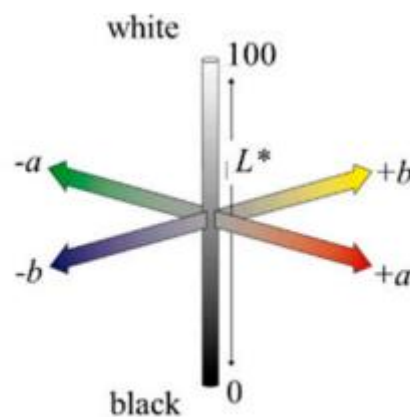


Figure 3.2 The CIE* $L^*a^*b^*$ colour system (Torres-González et al., 2023).

Colour measurement of the millet-water samples was conducted according to (Aydar et al., 2021). The colorimeter was calibrated with a white calibration plate standardize the equipment before colour measurements. Subsequently, the samples were measured by placing on the light-projection tube with standard black background. The L*a*b* of each sample were recorded.

Whiteness index (WI) indicates the overall whiteness of food products, which is widely applied as an important index for appearance in the development of plant-based (Jeske et al., 2017; Kuru & Tontul, 2020; McClements et al., 2019). The whiteness index of each sample was calculated according to the Equation [4] (Jeske et al., 2017):

$$\text{Whiteness Index} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \text{ -----[4]}$$

3.3.2.4 Sensory evaluation by the focus group

Before conducting sensory evaluation, the enzyme-treated millet mixtures were mixed with a high shear mixer (Silverson, L504) at 8000 rpm for 4 min (Naylor, 2021), followed by filtration using 160 mesh filter bags (Jinchennilong, Guangzhou, China). Filtered millet milk samples were pasteurised in a water bath at 65°C for 30 min to eliminate microbes in the samples before the focus group sensory evaluation (da Silva et al., 2023; Mäkinen et al., 2016; Özer & Yaman, 2014).

The primary objective of the sensory evaluation was to identify the most acceptable formulation for the initial phase of the project, which involved determining the optimal method for extracting and preparing foxtail millet to produce a plant-based milk. A focus group method with the participation of eight individuals representing both genders was employed. The researcher acted as the host, providing an introductory overview of the study, background information on foxtail millet and the specific objective of the sensory evaluation.

The focus group sensory evaluation was held in a closed ambient room with moderate temperature (22°C) and white light. The session lasted for approximately 60 min. A visual representation of the sensory evaluation setup was presented to the participants at the start.

Each participant was then served with samples of enzyme-treated millet mixtures individually, allowing them to assess and discuss sensory attributes, with particular emphasis on aroma, flavour and texture. Participants were provided with papers, water and plain crackers to clean their palate between samples. The samples were randomly labelled and participants were not provided with any information regarding the specific details of each formulation.

Following the presentation of each fresh millet mixture sample, the host posed questions to evoke the opinions and comments on the taste and flavour. Comments and audio were recorded during the session. The discussions were guided by the questions shown in Appendix. In the next phase of the study, the selected formulations will be further investigated. These formulations were chosen based on the outcomes of the focus group discussions, as well as the analysis of their physicochemical properties.

3.4 Phase II: Stabilisation of the foxtail millet milk and selection of formulation

In phase two of the study, the dry-milled (10% and 12% grain-to-water percentage) samples with 0.2% enzyme treatment were selected for further investigation. The selected formulation was subjected to stabilisation, which involved coarse homogenisation, filtration and the addition of fat and emulsifier, followed by high-pressure homogenisation, as shown in Figure 3.3.

During phase two, experiments were primarily focused on evaluating the impact of varying levels of oil and emulsifier on the stability and sensory properties of foxtail millet milk. Additionally, the homogenisation pressure was examined to determine the optimal final formulation.

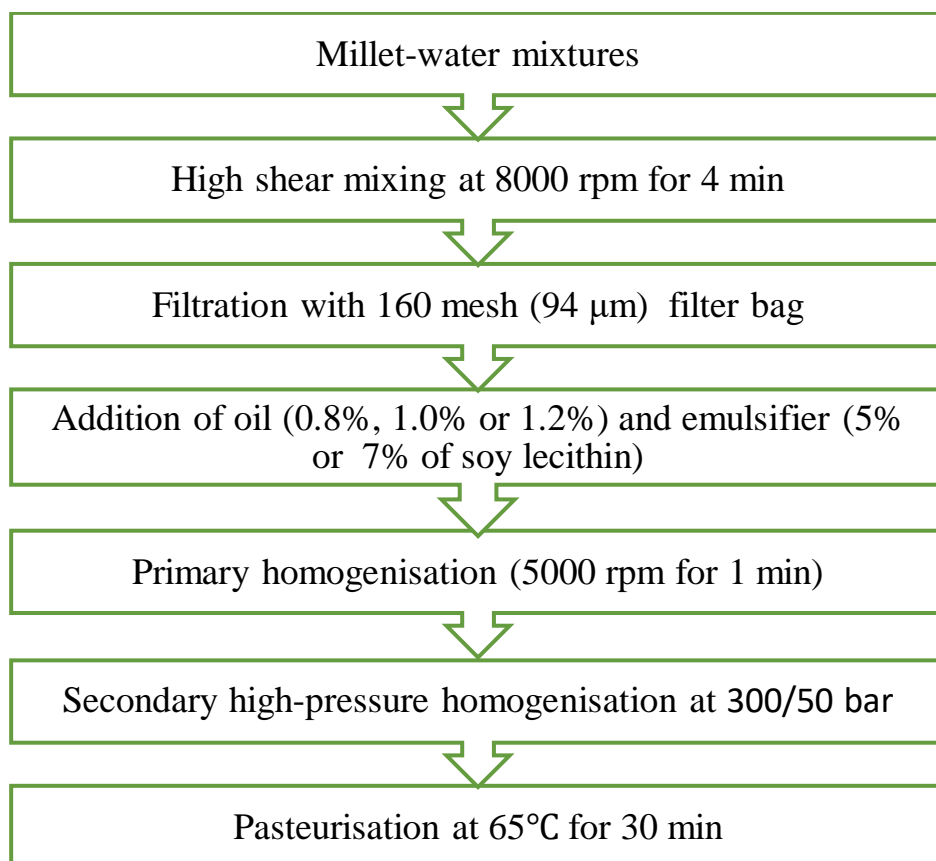


Figure 3.3 *The stabilisation of foxtail millet milk.*

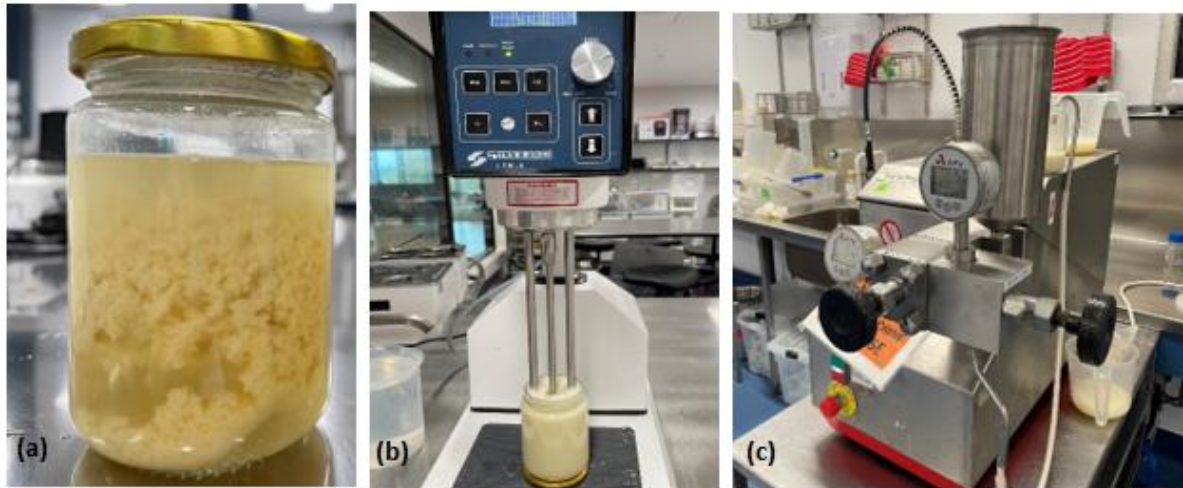


Figure 3.4 *Processing foxtail millet milk, (a) Millet-water mixture, (b) High shear mixing millet milk, (c) High-pressure homogenisation of millet milk.*

3.4.1 Production of foxtail millet mixture stabilisation

3.4.1.1 High shear mixing millet mixtures

The purpose of this step was to further reduce the size of the particles after enzyme treatment and minimise losses during filtration process. The enzyme-treated millet samples were mixed using a high shear mixer (Silverson L5M-A). The height was adjusted using the control panel, then the rotor stator head was gently immersed into the samples. The speed controller was then gradually rotated to reach 8000 rpm, making sure no sample spilled. Once the desired speed was achieved, a timer was set for 4 min.

3.4.1.2 Filtration

The millet slurry was filtered to separate the insoluble large-sized particles from the slurry. This was achieved using a 160-mesh filter bag, resulting in a filtered particle size of approximately 100 μm as reported by Wang et al (2022) (Wang et al., 2022).

To maintain the standards of sanitation, the filtering bags were securely attached to a clean and sanitised jug. The millet slurry was then carefully poured into the bag and any solid particles larger than 100 µm were effectively captured inside the bag. The filtration process was monitored to avoid over-flow. The filtrate was transferred into clean glass bottles, labelled and stored in refrigerator at 4°C.

3.4.1.3 Addition of oil and emulsifier

Due to low fat content (4%) in foxtail millet, the oil and emulsifier were added to stabilise the foxtail millet-water mixture as reported by (McClements & Grossmann, 2022c). Canola oil, a common oil found in commercial plant-based milk, was chosen for its widespread use and acceptance. As a natural and healthy emulsifier, soy lecithin was selected due to its ability to partially replace sodium caseinate in milk for emulsification properties (Chung et al., 2019; McClements, 2020) and successfully applied in several previous studies (Chung et al., 2019; Lee et al., 2019; Wang et al., 2022). The specific amounts of oil and lecithin added to the foxtail millet slurry are shown in Table 3.2.

Table 3.2 Amounts of oil and lecithin added to the foxtail millet mixture test samples.

Sample ID	Oil (%)	Lecithin/Oil (%)	Lecithin (%)	Sample ID	Oil (%)	Lecithin/Oil (%)	Lecithin (%)
A5	0.8	5	0.04	A5*	0.8	5	0.04
B5	1.0	5	0.05	B5*	1.0	5	0.05
C5	1.2	5	0.06	C5*	1.2	5	0.06
A7	0.8	7	0.56	A7*	0.8	7	0.56
B7	1.0	7	0.07	B7*	1.0	7	0.07
C7	1.2	7	0.084	C7*	1.2	7	0.084

Note: Samples IDs contain 5 represent the samples with 5% lecithin addition rate, while samples IDs contain 7 represent samples with lecithin addition rate at 7%. A, B, C represented samples with 0.80%, 1.00% and 1.20%, respectively. Sample contains coded with ‘*’ representing 12% millet grain in water rate. This process was duplicated and replicated.

The fat content of plant-based milk available in the New Zealand market varies between 10 g-30 g/L, while cereal milk ranges from 12 g-18 g/L (Smith et al., 2022). In this study, to achieve

similar fat content as commercial milk alternatives, canola oil was added at rates of 0.8% , 1% and 1.2% (w/w) on top of 4% fat naturally present in foxtail millet (Sharma & Niranjana, 2018).

The addition of lecithin was based on previous study conducted by Zhu et al (2021) (Zhu et al., 2021), where 5% (w/w) and 7% (w/w) lecithin markedly improved the physical stability of infant formula emulsions. Thus, 5% and 7% (w/w) addition levels of soy lecithin were chosen for investigation in this study.

Oil and lecithin were quantified according to Table 3.3. To prepare the oil and lecithin for homogenisation, the canola oil was pre-heated in a 25 mL beaker on a hotplate (152, Stuart, New Zealand) to optimize the temperature for dissolving the soy lecithin. Once the oil temperature had reached 55°C, different rates of lecithin were added to the oil separately according to the specified amounts (Zhu et al., 2021). The mixture was maintained at 55°C with stirring for at least one hour using magnetic stir-bar. Complete dissolution of lecithin for accuracy was ensured. The oil-lecithin mixture was kept heated at 55°C until just before homogenisation (Zhu et al., 2021).

3.4.1.4 Primary homogenisation

Primary homogenisation was achieved using a high shear mixer to blend the added oil and lecithin with the foxtail millet mixtures to produce a coarse emulsion with lecithin-coated fat droplets (McClements & Grossmann, 2022). Each sample of foxtail millet mixture with added oil and lecithin was carefully transferred into a clean 500 mL beaker. The high shear mixing was carried out in the same manner as described in 3.4.1.1. The coarse emulsion was formed at mixing speed 5000 rpm for 1 min (Zhou et al., 2023). The effect of the primary homogenisation was also investigated by measuring the particle size of the emulsified foxtail millet extract.

3.4.1.5 Secondary high-pressure homogenisation

The two-stage high-pressure homogeniser was thoroughly rinsed with RO water at least three times. This was accomplished by operating the equipment without setting pressure on the two valves. After rinsing the equipment, the coarse millet emulsion was carefully guided into the hopper of the homogeniser and the homogeniser was started by pressing the start/stop button. The first 20 mL of liquid from homogeniser was discarded to prevent any dilution caused by residual water during rinsing.

The coarse emulsion of millet milk was processed using a two-stage high-pressure homogeniser (APV 2000, Copenhagen, Denmark) with a pressure setting of 30/5 MPa (300/50 bar). The selection of the homogenisation pressure was determined through pre-trials.

A clean glass bottle was then positioned below the homogenisation outlet to collect the homogenised emulsion. The high-pressure homogenisation of the foxtail millet milk coarse emulsion was treated in three cycles to ensure the full homogenisation. After the third cycle of homogenisation, the millet milk emulsion was collected and sealed in appropriately labelled glass jars, with each jar containing a separate batch of the homogenised product.

3.4.1.6 Pasteurisation

The glass jars containing the high-pressure homogenised foxtail millet milk samples were carefully placed into a water bath for pasteurisation. The water bath was filled with potable water, which was slightly above the liquid level inside the sample jars but below the lid level to avoid water contamination during the process. The temperature of the water bath was set to 65°C.

The samples were heated in the hot water bath for 30 min once the sample temperature had reached 65°C. To maintain a consistent temperature and prevent water and heat loss, the water bath was covered with a lid during pasteurisation process.

Figure 3.5 *Primary homogenisation using high-shear mixer and high-pressure homogenisation using HPVH (High pressure valve homogeniser) (McClements & Grossmann, 2022a).*

3.4.2 Determination of properties of foxtail millet milk stabilised by canola oil and lecithin

3.4.2.1 Determination of pH, total soluble solids and whiteness index

Total soluble solids, pH and whiteness index of stabilised foxtail millet milk samples were determined using the methods described in 3.3.2.1, 3.3.2.2 and 3.3.2.3, respectively.

3.4.2.2 Determination of separation rate

Milk alternatives are expected to exhibit similar stability to bovine milk during the storage period. However, during storage, instability may occur due to the impact of gravity, resulting in a phenomenon such as creaming and sedimentation. These processes lead to the accumulation of under/over-sized particles at the top or bottom of the milk alternative, which is undesirable for consumers (McClements & Grossmann, 2022c). The determination of the separation rate is based on the previous study conducted by (Rincon et al., 2020). Ten mL of millet milk sample from each formulation were transferred into a 15 mL falcon tube. The samples were stored at refrigeration temperature (4°C) and their separation rate was evaluated

every 24 hours for 3 days. After each 24-hour period, the volumes of the creaming or sedimentation phase were marked. The separation rate was calculated using Equation [5] (Rincon et al., 2020).

$$\text{Separation rate} = \frac{\text{Volume of creaming or sedimentation (mL)}}{10\text{mL}} \times 100\% \text{ -----[5]}$$

3.4.2.3 Determination of particle size distribution (PSD)

Particle size distribution (PSD) analysis provides valuable insights into the concentration of different-sized particles in an emulsion, while the mean particle diameter is a key parameter for the stability of the foxtail millet milk emulsion. In this study, the volume-weighted (d_{43}) and surface-weighted (d_{32}) mean particle diameters, commonly used to determine properties of plant-based milk, were also measured (Grossmann et al., 2021).

The PSD and mean particle diameters of millet milk samples were determined using a static laser light diffraction unit (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK). The equipment is equipped with optical unit, a wet dispersion unit, a wet cell and Mastersizer application software installed on a computer. The measurement SOP was set up for non-spherical particles with a refractive index of 1.48. Obscuration level at 10% was used for measurement.

Prior to each measurement, the equipment was thoroughly rinsed with RO water three times to ensure that the light energy was under 100 units at detector 1 and less than 20 units at detector 20. The millet milk samples were then added to the dispersion unit, where RO water was used as the dispersion medium and the unit was operated at 2000 rpm. The equipment was operated to obtain particle size information and each sample was measured five times. Between measurement of each sample, the Mastersizer was rinsed three times. The obtained PSD data for each millet milk sample were recorded for further analysis.

Selection of secondary homogenisation pressure by determining the particle size of the emulsified foxtail millet extract (EFME)

To determine the appropriate secondary homogenisation pressure to achieve the desired particle size of emulsified foxtail millet extract (EFME), a preliminary trial was conducted. Foxtail millet extracts, added with 1.0% canola oil and 5% soy lecithin were subjected to emulsification using a two-stage homogeniser at varying homogenisation pressures. The pressures used were 200 bar, 300 bar, 450 bar or 600 bar. The EFMEs were then subjected to particle size distribution analysis, following the procedure described in the previous section.

Effect of primary homogenisation and coarse emulsification on the particle size distribution of foxtail millet extract during production

An experiment was carried out to investigate the impact of primary homogenisation and coarse emulsification on particle size change during production, the sample investigated was foxtail millet extract with 12% millet grain ratio, 1.0% oil addition and 5% lecithin addition rate. The particle size distribution of same foxtail millet extract sample was measured at three different production points which including after enzyme treatment, after first high-shear mix and after coarse emulsification.

3.4.2.4 Determination of apparent viscosity

In this study, the apparent viscosity of the millet milk samples was considered a crucial parameter for evaluating the rheological properties of the milk analogues, as well as gaining insights into their oral perception. The viscosity was measured using a viscometer (DV-II+, Brookfield Engineering Laboratories, Stoughton, MA, USA) following previous studies (Beaulieu et al., 2020; Gama et al., 2019; Mattison et al., 2020) with minor modifications.

Before conducting the measurements, the viscometer was properly positioned for calibration. To obtain accurate viscosity values, the spindle LV1 and shear speed at 50 rpm were selected based on pretrials, ensuring that the torque value exceeded the threshold of 10%. The shear rate was around 10/s based on the setting, it was calculated based on the data retrieved from the Brookfield viscometer guidance (Laboratories., 2017). Each millet milk sample (about 200 mL) at room temperature (20°C) was placed in a 250-mL beaker (Schott, Duran, Germany). The spindle was carefully inserted into the sample and the viscometer was set to begin the

measurements. The viscosity readings for each millet milk sample were recorded after at least three minutes of running time to ensure stable measurements.

3.4.2.5 Determination of sensory properties of test samples by conducting a focus group sensory evaluation

The focus group sensory evaluation was carried out based on the set up and procedures described in 3.3.2.5. Twelve high pressure homogenised millet milk samples were evaluated by the panellists.

3.5.2 Consumer sensory evaluation of selected foxtail millet milk formulation

A total of 100 panellists were recruited randomly through posters and email communications. The sensory evaluation was conducted in the Innovation Complex building. Six portable booths were set up to provide individual tasting space for the panellist. Ample natural light was available during the tasting. Participants were introduced to the project's purpose and completed an Ethics Form approved by the Massey University Human Ethics Committee (Approval Number: 4000026371). The composition of the millet milk was disclosed to each participant to prevent any potential allergy incidents.

A 9-point hedonic scale was used to evaluate various sensory aspects of the selected millet milk formulation which composed colour, aroma, flavour, mouthfeel and overall liking. An online sensory evaluation system known as RedJade was used for this purpose. Questionnaire setup was completed online, with the design details provided in the Appendix C. A QR code was generated by the application, which panellists scanned using their smartphones to access the sensory questionnaire.

During the evaluation, panellists were served with pasteurised 20 mL samples of foxtail millet milk at room temperature (20°C). The samples were presented in transparent 25 mL plastic cups covered with lids. Additionally, panellists were invited to express the features of the millet milk they appreciated or disliked, based on their overall responses.

3.5 Phase III: Physicochemical properties and shelf life stability of foxtail millet milk

3.5.1 Analysis of proximate and mineral compositions of foxtail millet milk

The proximate analysis of the selected foxtail millet milk formulations was conducted by accredited nutrition laboratory of Massey University Palmerston North (<https://www.massey.ac.nz/about/massey-subsidiaries-and-commercial-ventures/nutrition-laboratory/>). The proximate analysis composed analysis of content of moisture, ash, crude protein, fat, carbohydrates and total dietary fibre, while analysis of mineral compositions are consisted of calcium, potassium, sodium and phosphorus.

The moisture content was determined according to the AOAC 925.10, 930.15 and ash content was determined following the AOAC 942.05 using furnace at 550°C. Crude protein content of the millet milk was analysed by the Dumas method (AOAC 968.06), using 5.83 as the nitrogen-protein conversion factor. The fat content was analysed by the Mojonnier method based on AOAC 922.06. Total dietary fibre content was determined using the enzymatic kits of the Megazyme, according to the AOAC 991.43. The carbohydrate content was determined by difference.

For the sensory evaluation, panellists were presented with 20 mL samples of pasteurised foxtail millet milk at room temperature (20°C). The samples were served in transparent 25 mL plastic cups covered with lids. Panellists were encouraged to assess both the positive and negative properties of the millet milk based on their overall sensory experience.

3.5.2 Determination of physicochemical and microbiological properties of foxtail millet milk during storage at 4°C

3.5.2.1 Determination of pH, Brix, whiteness index, particle size, viscosity and separation rate

In phase III, physicochemical properties of the selected foxtail millet formulation were determined during the refrigerated storage. These parameters including pH, °Brix, whiteness index, particle size distribution, viscosity and separation rate which were determined at

refrigerated temperature at 4°C, the process of determination can be referred to the Sections 3.3-3.4.

3.5.2.2 Aerobic plate total counts

The microbial stability of foxtail millet milk was determined by conducting aerobic mesophilic counts over a 4-week period. Briefly, dilutions of millet milk samples (10^0 , 10^{-1} and 10^{-2}) were examined. One mL of each diluted sample was carefully pipetted into 9 mL of sterile peptone water. This dilution was mixed using a vortex mixer (VM-10, WiseMix®, Germany) and plated in molten plate count agar (Oxoid™ Plate Count Agar, Thermo Fisher Scientific). The solidified plates were incubated at 30°C for 72h.

3.6 Statistical analysis

Most experiments were repeated twice and analysis/ measurements were conducted at least in duplication. The processes involved in foxtail millet milk production were duplicated. Measurements of the physicochemical properties, including total soluble solids, whiteness index, and pH, were also duplicated. The particle size distribution measurement was replicated five times and duplicated. The data were analysed by Minitab 21. (Minitab Inc., State College, PA, USA) software using descriptive statistics, One-Way Analysis of Variance (ANOVA) and fit regression model to determine significant differences of the means ($p < 0.05$). All data are shown as mean \pm standard deviation (SD). The ANOVA was used to data collected from various preparation, extraction and emulsification methods and determine its impact on the physicochemical properties of the foxtail millet extract. Significant differences of the means between groups (95% C.I.) were compared using Tukey's test.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Phase I Preparation and extraction of foxtail millet milk

4.1.1 Foxtail millet extraction: effects on colour

Colour is vital for the appearance of food as it influences the consumer perception of the product before consumption (Hutchings, 2011). Plant-based milk is an alternative to the traditional dairy milk especially bovine milk. Therefore, it should exhibit a colour similar to that of cow's milk. However, consumer expectations of plant-based milk can vary based on factors such as the raw materials used or the flavour profile of the milk substitute (McClements, 2020). Typically, plant-based milk beverages have a creamy colour. Therefore, the whiteness and lightness of the milk alternatives are often the most important attributes for evaluating their appearances (Tobolková & Durec, 2023).

The colour of extracted foxtail millet was investigated in Phase I. Figure 4.1 shows that milling method and percentage of millet in water impacted on the whiteness of foxtail millet water extract ($p < 0.05$), while the rate of enzyme added had little to no effect.

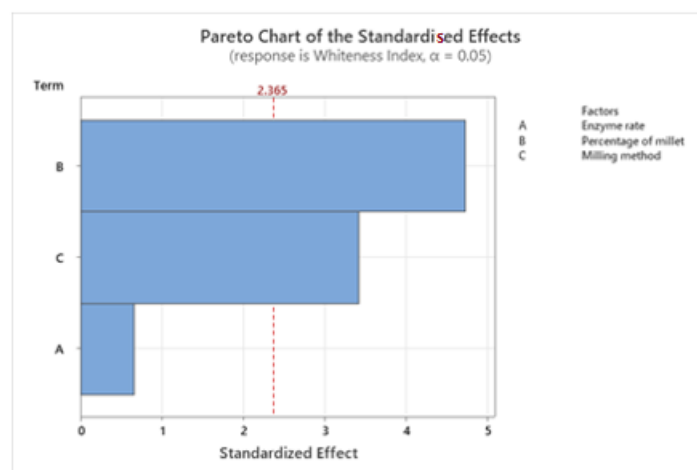


Figure 4.1 Pareto chart of standardized effects for whiteness index of foxtail millet extract. The factors that surpassed the red broken line were considered statistically significant ($p < 0.05$).

The percentage of millet in water was the most significant factor, with the higher percentage of millet in water showing a higher whiteness and lightness, irrespective of the milling method, whether it was dry or wet (Table 4.1).

Table 4.1 *The average values for L*, a*, b* and whiteness index of foxtail millet water mixture extracted with different milling methods and amount (%) of millet in water.*

Milling method	% millet in water	L*	a*	b*	Whiteness Index
Dry	8.00	39.67 ± 1.84 ^a	-0.01 ± 0.30 ^a	-1.57 ± 2.27 ^a	39.62 ± 0.62 ^a
Dry	10.00	40.21 ± 1.84 ^a	-0.41 ± 0.28 ^a	-1.91 ± 0.76 ^{ab}	40.17 ± 1.13 ^a
Dry	12.00	46.87 ± 4.19 ^b	-1.24 ± 0.51 ^b	0.17 ± 1.17 ^b	46.84 ± 1.19 ^b
Wet	8.00	37.67 ± 0.86 ^c	-0.20 ± 0.38 ^c	-2.46 ± 0.89 ^a	37.60 ± 0.56 ^c
Wet	10.00	38.25 ± 0.02 ^c	-0.18 ± 0.32 ^c	-1.28 ± 1.03 ^{ab}	38.19 ± 0.23 ^c
Wet	12.00	41.79 ± 0.90 ^d	0.03 ± 0.28 ^c	-1.80 ± 0.03 ^b	41.77 ± 0.34 ^d

Note: L* = lightness (black to white, 0 to 100), a* = redness, -a* = greenness, b* = yellowness and -b* = blueness. Within the same column, data labelled with different letters is statistically different.

The foxtail millet mixture had a higher lightness as the millet concentration used was increased, particularly when the ratio surpassed 10% (Figure 4.2). This observation agrees with Klevenst and Oppenheimer (1964) who reported a link between higher concentration particles and shorter wavelengths, which yield a pronounced light scattering effect.

With respect to 12% millet ratio, the density of millet mixture was considerably higher than in the 8% and 10% millet in water. Therefore, the higher concentration of particles within the medium the more particles interact with light waves leading to more extensive light scattering effects which lead to higher levels of both lightness and the whiteness index (Chantrapornchai et al., 1999).

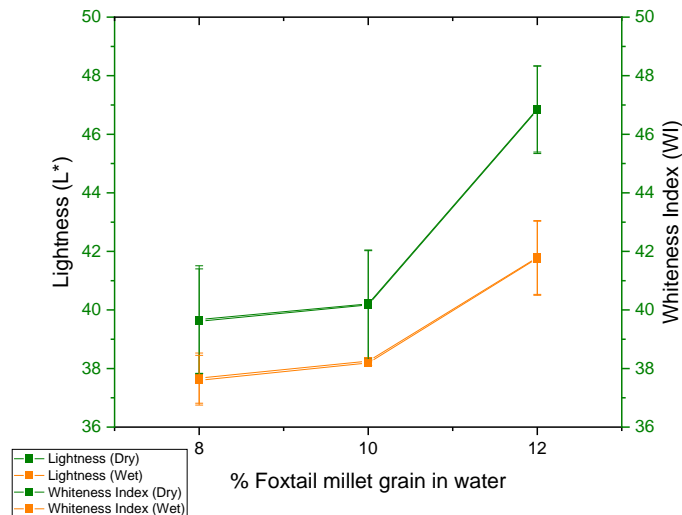


Figure 4.2 Lightness (L^*) and whiteness index (WI) of dry-milled and wet-milled foxtail millet extract with variable millet in water.

Note: Lightness (Dry) = Lightness (L^*) of dry-milled foxtail millet water extract; Lightness (Wet) = Lightness (L^*) of wet-milled foxtail millet water extract; Whiteness Index (Dry) = Whiteness Index (WI) of dry-milled foxtail millet water extract; Whiteness Index (Wet) = Whiteness Index (WI) of wet-milled foxtail millet water extract.

The lightness observed in the initial phase of foxtail millet extraction appears to fall within a similar range around 45-60 as reported by Bembem and Agrahar-Murugkar (2020). However, the average yellowness and blueness (b^* value) of the extracted foxtail millet was approximately -1.48 which was considerably lower than previous studies including foxtail millet in China (18.5-20.7) (Shen et al., 2015) and domestically cultivated foxtail millet varieties in Korea (20 - 23) (Jeon et al., 2011). This indicates that the low yellowness colour of foxtail millet was extracted.

This phenomenon can be attributed to the cooking method that is used for the foxtail millet. Carotenoids, the compounds responsible for the yellowness in foxtail millet grain have been reported to be susceptible to degradation under thermal and pressure treatments (Shen et al., 2015). The loss of yellowness (b^* value) is associated with the degradation of carotenoids during cooking. Moreover, blanching has been shown to have a significant impact on the reduction in b^* value (yellowness) of millet skim milk beverages (Pan et al., 2019). Thus, the low b^* value of the millet extraction in this study can be attributed to the combined effects of blanching and the thermal conditions used during the extraction process.

There are some differences ($p < 0.05$) between the lightness and whiteness indices of dry-milled and wet-milled foxtail millet-water extract, which may be attributed to various factors such as particle size and distribution caused by the milling methods used (Li et al., 2020). The particles of foxtail millet obtained by wet milling might not have been ground as finely as those produced through dry milling, resulting in larger particle sizes. Therefore, the particle concentration of the wet-milled-extract may have been lower. A larger particle size results in a smaller surface area, resulting in reduced scattering of light (MacDougall, 2010). This probably contributed to the lower lightness observed in the wet-milled extract comparing with the dry-milled extracts (Figure 4.2). Conversely, when particles are reduced to small sizes, they exhibit a larger surface area, which scatter more light as the number of particles has increased. Therefore, extracts of smaller particle sizes tend to appear lighter in colour (MacDougall, 2010).

The results obtained in study may differ from findings in previous studies where wet-milling produced finer particles compared to dry-milling (Lee et al., 2015; McHugh, 2018). However, the results of the present study showed that dry milling was more effective in reducing particle size than wet milling.

4.1.2 Foxtail millet water extraction: effects on pH

When testing the impact of milling method, millet ratio and enzyme rate on the pH level, it was observed that the pH levels of the foxtail millet milled extracts were primarily influenced by the milling method (Figure 4.3).

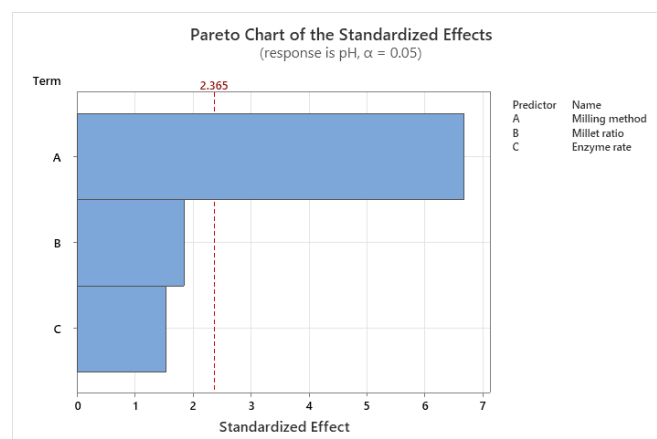


Figure 4.3 Pareto chart of standardised effects for pH of FME (foxtail millet extract).

Note: The factors that surpassed the red broken line were considered significant (p-value). Overall, the wet-milled foxtail millet extract exhibited a significantly higher pH

compared to the dry-milled millet extract ($p < 0.05$). The mean pH of the wet-milled foxtail millet water extract was 6.60 ± 0.06 , whereas the average pH of the dry-milled extract was 6.42 ± 0.05 (Figure 4.4). The differences in pH may be attributed to the soaking carried out prior to wet milling.

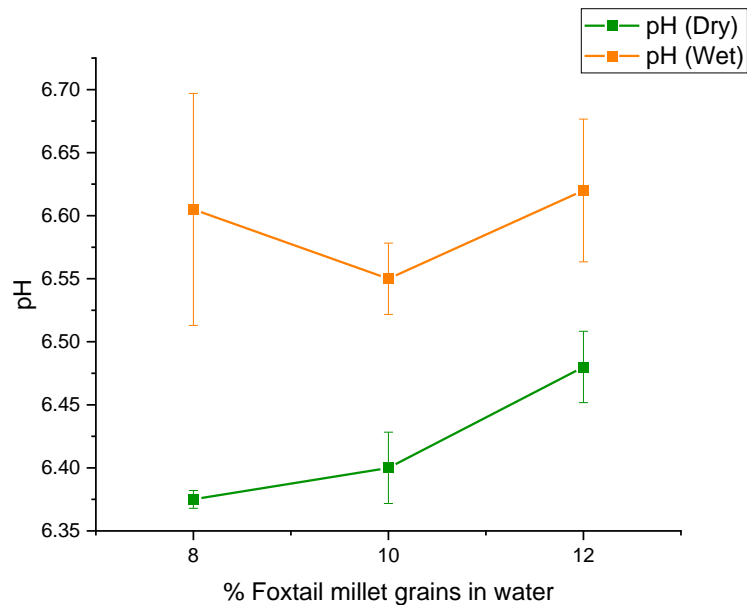


Figure 4.4 pH of dry-milled and wet-milled foxtail millet extracts with variable percentages of millet in water.

Note: Green line indicates wet-milled; orange line indicates dry-milled.

The effect of soaking on pH agreed with findings from earlier studies (Gupta et al., 2015; Lokeswari et al., 2021; Shigihalli et al., 2018). Phytic acid impacts on pH and is commonly present in plant materials, particularly seeds and grains. It serves as a storage form of phosphate in plants and its concentration is relatively high in cereal bran. Phytic acid is often considered an anti-nutrient component due to its strong chelating properties with iron and zinc ions (Kumar & Anand, 2021).

Pawar and Machewad (2006) reported significant reductions in phytic acid content, as well as total iron and total zinc levels, in soaked and dehulled foxtail millet compared to non-soaked dehulled millet (Pawar & Machewad, 2006). During the soaking process, phytic acid and certain mineral ions are released into the soaking water and subsequently removed through the

draining process. The observed higher pH in wet-milled foxtail millet extract compared to dry-milled extract may be a result of the loss of the phytic acid and mineral ions.

4.1.3 Foxtail millet water extraction: effects on total soluble solids (TSS)

Total soluble solids (TSS), also known as °Brix, of extracted foxtail millet water extracts are presented in Figure 4.5. Millet in water, milling methods and amylase level impacted on the TSS of the millet extract ($p < 0.05$).

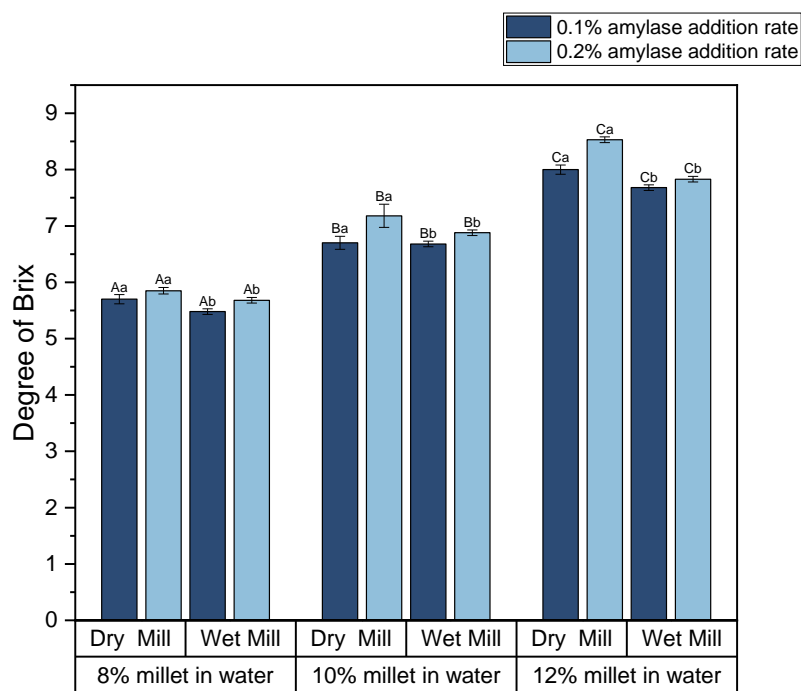


Figure 4.5 Total soluble solids (°Brix) at different percentage of millet in water, milling method and amylase addition rate.

Note: The data labelled with A, B and C indicates the significant difference at different millet percentage; data labelled with a, b, c indicates the significant difference using different milling methods. The data bar in dark blue is significantly different from the data with light blue due to the different amylase addition rates.

Dry-milled foxtail millet extracts (FME) exhibited higher °Brix values compared to wet-milled extracts. The average °Brix value for dry-milled extract was 6.99 ± 1.14 , whereas the average wet-milled extract was 6.70 ± 0.98 . The difference can be attributed to the pH variation resulting from the soaking process during wet milling, which removed some soluble solids, such as

phytic acid, polyphenols and minerals from the millet grains as discussed in Section 4.12. Additionally, it may also be attributed to the degree of the starch damage during both dry and wet milling. The damaged starch granules have great surface areas which are more susceptible to enzymatic hydrolysis compared to intact starch granules. This potential indicates that dry-milling shows greater milling ability than wet-milling (Lumdubwong & Seib, 2000).

An increasing percentage of millet grains and amylase addition rates led to an increase in the total soluble solids in the extracted foxtail millet water extract. In Figure 4.5, the millet extract treated with 0.20% (w/w) amylase exhibited a higher average °Brix compared to the extract treated with only 0.10% (w/w) amylase, with averages of 6.99 ± 1.12 and 6.71 ± 1.01 , respectively.

In terms of plant-based milk production, amylase is primarily used to hydrolyse starch in plant materials. In this study, the foxtail millet-water mixture was soaked for 30 min in hot water bath at 95°C, resulting in full gelatinisation of the foxtail millet starch content (gelatinisation begins at 72.1°C) (Li et al., 2019). When the starch completely loses its ordered structure and becomes fully disordered, the α -amylase can break it down more easily. (Tester et al., 2006). The α -amylase applied in foxtail millet water mixture catalysed the hydrolysis of internal α -1,4-glycosidic linkages in millet starch (Mobini-Dehkordi & Javan, 2012). The end-products of starch hydrolysis by α -amylase are shorter oligosaccharides, typically a mixture of maltose, maltotriose and oligosaccharides containing 6-8 glucose units (Souza, 2010). The amylase-driven hydrolysis converted the water-insoluble starch into water-soluble sugars, resulting in an increase in the total soluble solids in the foxtail millet extract.

Comparing with the 0.10% (w/w) amylase addition, more starch content in the FME was hydrolysed with 0.20% (w/w) amylase addition which resulted in higher TSS. In contrast, the addition of 0.10% (w/w) amylase resulted in incomplete starch hydrolysis, leaving some gelatinised starch, which contributed to a higher viscosity extract. The amylase addition rate was calculated based on the weight of foxtail millet added, therefore, according to the amount of units in amylase applied in this study, in every one litre of the foxtail millet water mixture at 10% grain to water ratio, around 2454 units and 4908 units of amylases were used for 0.10%

(w/w) and 0.20% (w/w) addition rates, respectively. The use of 0.20% (w/w) addition rate was similar to the study done by Yang et al. (2022), which used 5186 units of amylase and aminoglycosidases to a mixture of cereal materials at 10% seeds in water (Yang et al., 2022).

A higher TSS results in more sugars being released during starch hydrolysis, this is important as it impacts the sensory properties of foxtail millet extracts. The end products of starch hydrolysis are maltose and maltotriose which account for 30-50% of the sweetness of sucrose (Tiefenbacher, 2017). A higher concentration of sugar molecules theoretically results in a sweeter taste that can be perceived by sensory panels.

To further enhance foxtail millet milk production, transitioning to a low-temperature amylase could also be considered. This would minimise protein denaturation and reduce energy consumption. A selection of other hydrolysing enzymes such as glucosidase, β -amylase and xylanase may also improve the hydrolysis of the starch and other other plant components as shown in some previous studies (Chantrapornchai et al., 1999; Silva et al., 2020).

4.1.4 Sensory evaluation by focus group

The focus group sensory evaluation was conducted by seven panellists and the summary is presented in Table 4.2.

Table 4.2 Comments of the foxtail millet extracts prepared using different milling methods, percentages of millet in water and amylase addition rate.

Sample ID	Summary of comments		
	Aroma	Flavour	Texture
W8-1	Light aroma	Taste bland, rice flavour	Slight thick texture
W8-2	Cereal aroma	Slight astringent, taste and aroma are not strong enough	Light texture, watery taste
W10-1	Medium intensity of cereal aroma	Cereal, rice milk taste	Too thick in texture
W10-2	Good rice aroma, quite plain	Cereal taste	Light but slight gritty texture
W12-1	Good cereal aroma	Taste grainy	Too thick in texture, porridge-texture
W12-2	More intensity in taste and cereal aroma	Slight sweetness	Medium texture, with grainy
D8-1	Light cereal aroma	Slight astringent	Slight thick texture
D8-2	Light rice aroma	Bland and light taste	Light texture, too watery
D10-1	Medium intensity of aroma,	No bitterness, slight rice/cereal taste	Thick texture, high viscosity
D10-2	Medium intensity of cereal aroma	Cereal, rice milk taste	Light in texture
D12-1	High intensity of aroma	Cereal porridge like taste	Too thick in texture, porridge-like texture
D12-2	Good cereal aroma	Slight sweetness, similar to soy milk, more intense taste	Medium intensity texture

Note: W8-1: 8% wet-milled extract with 0.1% enzyme treatment; W8-2: 8% wet-milled extract with 0.2% enzyme treatment; W10-1: 10% wet-milled extract with 0.1% enzyme treatment; W10-2: 10% wet-milled extract with 0.2% enzyme treatment; W12-1: 12% wet-milled extract with 0.1% enzyme treatment; W12-2: 12% wet-milled extract with 0.2% enzyme treatment; D8-1: 8% dry-milled extract with 0.1% enzyme treatment; D8-2: 8% dry-milled extract with 0.2% enzyme treatment; D10-1: 10% dry-milled extract with 0.1% enzyme treatment; D10-2: 10% dry-milled extract with 0.2% enzyme treatment; D12-1: 12% dry-milled extract with 0.1% enzyme treatment; D12-2: 12% dry-milled extract with 0.2% enzyme treatment.

The focus group discussions revealed that the FMEs were consistently described as having a rice-like cereal aroma, with a taste profile resembling a blend of rice milk and soy milk. However, the texture and intensity of both taste and aroma exhibited variations depending on different extraction and preparation methods.

Based on the observations recorded in Table 4.2, FME processed with 8% millet-grain to water ratio was described as having lower intensity in both taste and aroma compared to those prepared with 10% and 12% ratios. This may be attributed to the lower concentration of millet grain used, resulting in a decreased concentration of flavour and aroma compounds. Panellists also noted that FME treated with a 0.10% amylase addition rate exhibited an excessively thick texture, showing a porridge-like consistency, which was not expected for a milk alternative product, as indicated by most panellists. While the FME produced with a 12% ratio and hydrolysed with 0.20% amylase had a pleasant, slight sweetness by resulting from the end-product of starch hydrolysis.

The high viscosity was particularly evident in FME with 10% and 12% millet-grain in water. The application of a 0.10% amylase addition rate in FME did not fully hydrolyse the starch content in foxtail millet, leaving some starch residues that were gelatinised during high-temperature treatment, contributing to the viscosity of the FME, as also noted in other studies (Mahajan et al., 2021).

In the sensory evaluation, panellists observed the wet-milled FME having a grainier texture compared to dry-milled FME. This was interesting as wet milling is more commonly used for producing plant-based milk (McHugh, 2018), however, dry-milled FME samples were preferred by the panellists. The graininess reported by panellists may result from differences in millet particle size, with wet milling using a juice blender possibly producing larger millet grain particles. Particles that are generally considered responsible for the grainy and gritty sensations are usually found to be greater than 100µm in size (Godoi et al., 2021; McClements & Grossmann, 2022c). These observations aligned with the results and discussions on colour differences in wet-milled and dry-milled FME, as discussed in Section 4.1.1.

Dry-milled FME using coffee grinder may produce better grinding results than wet-milled FME using a juice blender. It is important to note that further study of improving the milling process of foxtail millet milk can be conducted by using more suitable milling equipment like colloidal mill and hammer mill (McClements, 2004). These improvements have the potential to enhance sensory properties through more effective milling.

4.1.5 Summary and conclusion of Phase I

In Phase I of this study, examinations of three key extraction and preparation parameters were conducted to evaluate their impact on the physicochemical attributes of foxtail millet extracts. The quantified variables included colour attributes (lightness and whiteness index), pH and total soluble solids ($^{\circ}$ Brix). The purpose of this investigation was to identify an optimal formulation for subsequent phases of the study. Additionally, sensory evaluation through focus group discussions was used as a screening tool.

The dry milling process for FME, with a higher percentage of millet in water produced FME samples with higher levels of lightness and whiteness indices, thereby closely resembling bovine milk in terms of colour. The pH of FME ranged from 6.38 to 6.67, while the wet-milled FME showed slightly higher average pH around 6.6 ± 0.06 .

In contrast, the $^{\circ}$ Brix was impacted by all three factors including milling method, amylase addition rate and percentage of millet under consideration. Higher level of starch hydrolysis was observed with dry mill indicating better milling ability of dry milling method. The FMEs processed with dry milling, a higher percentage of millet in water and the addition of 0.20% amylase had a higher $^{\circ}$ Brix, indicative of greater starch hydrolysis and a potential increase in sweetness in the FME. This observation was noted during focus group discussions, where panellists expressed a preference for samples with higher millet and enzyme concentrations due to their more pronounced taste, aroma and sweetness.

In conclusion, the following parameters were selected for further investigation in the subsequent phase: dry milling, a 0.20% amylase addition rate and millet grain-to-water ratios

of 10% and 12%. These selections were based on their notable impact on the physicochemical properties of FME and the sensory results of the focus group panellists.

4.2 Phase II: Stability of the selected foxtail millet extract (FME)

The foxtail millet extract (FME) formulations were selected through screening experiments conducted in phase I. In phase II, the FMEs were homogenised after addition of oil, using lecithin to stabilise the fat phase in the beverage. Various physicochemical properties were determined (pH, total soluble solids, whiteness index, particle size distribution and separation rate) were determined for selecting an optimum formulation. A focus group sensory evaluation was also conducted in this phase to select an optimal formulation of stabilised foxtail millet extract. Alongside this, a consumer sensory evaluation was conducted to evaluate the sensory acceptance of the optimised formulation.

4.2.1 The colour of the emulsified foxtail millet extract

The colour of the FMEs underwent changes during the emulsification process (Table 4.3). The whiteness index of the emulsified foxtail millet extractions (EFME) ranged from 53.39 to 61.06, with an average value of 58.26 ± 2.11 . This average whiteness index was higher than the non-emulsified FMEs, which averaged 40.74 ± 3.34 . Both L* and b* values of the EFMEs increased compared to the FMEs, while the a* value decreased. These changes in chroma indicated that foxtail millet extracts, after undergoing emulsification, exhibited higher lightness and more pronounced whiteness, greenness and yellowness in their colour profiles.

Table 4.3 The average chroma values of L*, a*, b* and whiteness index of the stabilised foxtail millet milk with different addition levels of oil, lecithin and millet grain in water.

Sample #	Whiteness Index	Lecithin (%)	L*	a*	b*
A5	53.39 ± 0.38	0.04	53.43 ± 0.40	-1.50 ± 0.16	1.05 ± 0.66
B5	57.85 ± 0.56	0.05	57.95 ± 0.57	-1.78 ± 0.09	2.32 ± 0.17
C5	59.12 ± 1.08	0.06	59.19 ± 1.10	-1.72 ± 0.19	1.69 ± 0.57
A7	56.47 ± 0.50	0.56	56.51 ± 0.50	-1.64 ± 0.18	0.75 ± 0.60
B7	57.30 ± 0.59	0.07	57.33 ± 0.60	-1.41 ± 0.10	0.96 ± 0.25
C7	60.61 ± 1.00	0.084	60.80 ± 1.06	-1.91 ± 0.16	3.26 ± 0.7
A5*	57.72 ± 0.16	0.04	57.88 ± 0.16	-2.02 ± 0.05	3.08 ± 0.15
B5*	58.09 ± 0.44	0.05	58.22 ± 0.41	-1.78 ± 0.20	2.64 ± 0.43
C5*	60.64 ± 0.53	0.06	60.97 ± 0.62	-2.19 ± 0.15	4.56 ± 0.99
A7*	57.79 ± 0.15	0.56	57.89 ± 0.12	-1.90 ± 0.18	2.19 ± 0.51
B7*	59.13 ± 1.20	0.07	59.29 ± 1.21	-1.99 ± 0.14	2.98 ± 0.13
C7*	61.06 ± 0.22	0.084	61.33 ± 0.24	-2.16 ± 0.06	4.02 ± 0.23

Note: Samples coded with '5' represent samples with 5% lecithin addition rate based on added oil weight, samples coded with '7' represent samples with lecithin addition rate at 7% based on added oil weight. A, B, C represented samples with 0.80%, 1.00% and 1.20% oil addition, respectively. Sample contains a symbol *

representing 12% millet grain in water. Detailed preparation of foxtail millet extract can be found in Table 3.3. L* stands for the lightness (black to white, 0 to 100); +a* represented the redness, -a* represented the greenness, +b* shows yellowness and -b* shows blueness. All the data within same column presented in the Table 4.3 are statistically different.

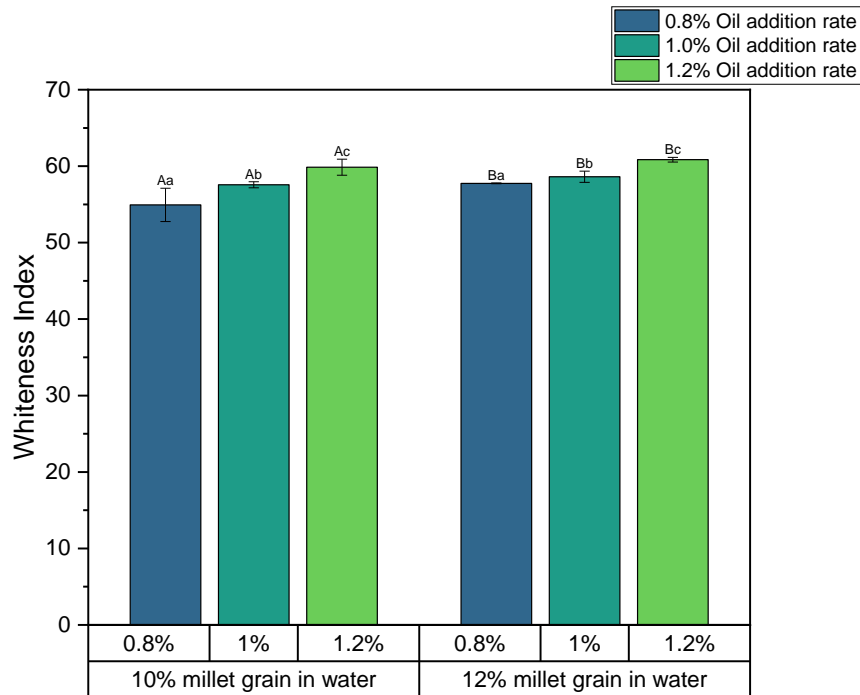


Figure 4.6 The whiteness Index of emulsified foxtail millet extract with different oil addition level at 10% and 12% millet grain in water.

Note: The data labelled with A or B indicates the significant difference at different millet percentage; data labelled with a, b or c indicates the significant difference at different levels of oil addition level.

Data analysis revealed that the colour of EFME was impacted largely impacted by two factors: the percentage of millet in water and the rate of oil addition. Firstly, a higher whiteness index was observed for EFME with a 12% millet grain when compared to a 10% millet grain extract. The average whiteness index for the 12% millet EFME was 59.07 ± 1.47 , while the 10% millet EFME was 57.45 ± 2.46 . The variance in the whiteness index can be attributed to higher concentrated particles enhancing the light scattering in the emulsion which results in the increase of the lightness and whiteness index (Chantrapornchai et al., 1999).

Secondly, the level of oil also showed an impact on the whiteness index of EFME. Figure 4.6 shows that an increase in the amount of oil addition in foxtail millet extract resulted in a higher

whiteness index during emulsification. The average whiteness index of EFMEs with 10% millet increased to 54.93, 57.57 and 59.86 with oil addition of 0.8%, 1.0% and 1.2%, respectively. Similarly, EFMEs exhibited increasing whiteness index values of 57.75, 58.61 and 60.85 for the same respective oil addition levels with 12% millet. The results on the whiteness index of the EFMEs agreed with previous studies which reported that the increased oil content in emulsions lead to higher whiteness index due to an increase in droplet concentrations (Chanamai & McClements, 2001; McClements, 2002a, 2002b). McClements also reported that the lightness (L^*) of emulsions rose with increased droplet concentration within the range of 0 to 20 wt% (McClements, 2002a). During homogenisation, the added oil is transformed into fat globules that are stabilised by emulsifiers, resulting in a greater number of colloidal particles within the emulsion system and more scattered light as result (McClements et al., 2019). The whiteness index is calculated based on the L^* , a^* and b^* values, with the primary factor being the lightness value, as outlined in the equation presented in Section 3.3.2.3. An increase in whiteness index is directly associated with an increase in the lightness value (McClements et al., 2019).

Besides lightness changes with the levels of oil addition, the chroma values a^* and b^* also showed a significant change ($p < 0.05$) with the level of oil addition as shown in Figure 4.7. With increasing fat content in the foxtail millet milk, the a^* value decreased, while the b^* increased, this agreed with previous studies (McClements et al., 2019). The milk emulsion also tends to show more greenness and yellowness and less redness and blueness.

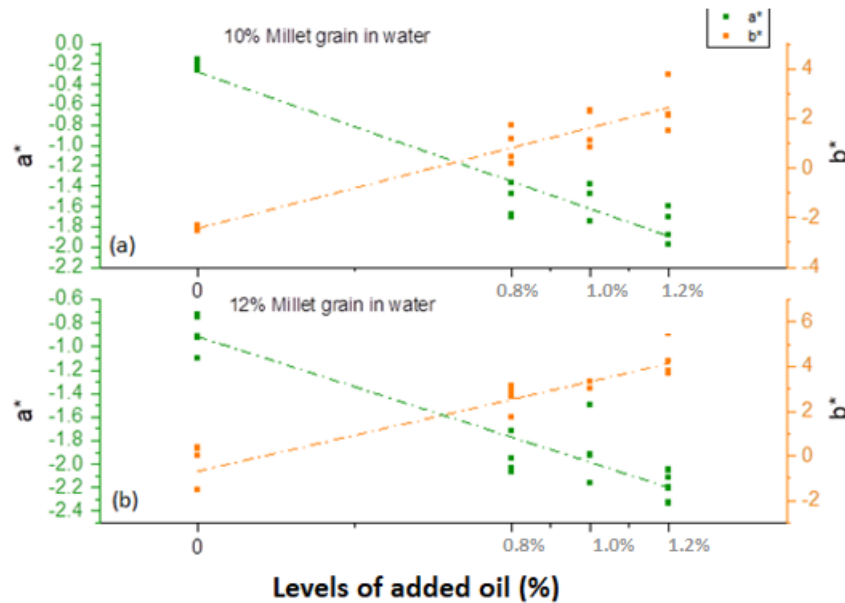


Figure 4.7 The average chroma a^* and b^* value of EFMEs with 10% and 12% millet grain in water at 0.8%, 1.0% and 1.2% oil addition level.

Note: Graph (a) represents the change of chroma values in EFME with 10% millet grain in water, graph (b) represents the change of chroma values in EFME with 12% millet grain in water. The green broken line represents the change of a^* value and orange broken line represents the change of b^* value.

The average whiteness index of EFME (58.26) was lower than the whiteness index of bovine milk which was reported around 81.9. Furthermore, several other plant-based milk varieties, including almond milk (72.6), cashew milk (65.6), hemp milk (68.5), rice milk (66.5) and soy milk (70.3) also reported higher whiteness indices than the average whiteness of EFME (Jeske et al., 2017; Reyes-Jurado et al., 2023). However, other milks such as hazelnut milk, macadamia milk and oat milk have whiteness index values of 56.3, 51.7 and 60.2, respectively, which are lower than EFME (Jeske et al., 2017).

It is essential to note that the colour of plant-based milk is variable. For instance, the whiteness indices of almond milk ranges from 52 to 73, while whiteness index of soy milk falls within the range of 69 to 75 (McClements, 2020). Following the principles of colour and droplet concentration, adjustments to the fat content can be used to modify and optimise the colour of plant-based milk (McClements et al., 2019). The addition of plant-based proteins or fragments of plant tissue can be used to enhance the creamy colour of plant-based milk (McClements et al., 2019).

4.2.2 The pH of the emulsified foxtail millet extract

The pH of the EFME in this study ranged from 6.55 to 6.65, with an overall average of 6.60 ± 0.04 , as shown in Table 4.3. Notably, these pH values remained relatively stable during the refrigeration storage for at least one week and were not significantly impacted by the stabilisation processes used.

Comparing our findings to recent research by Reyes-Jurado et al. (2023), the pH of EFMEs agree with the pH levels observed in various types of milk, including both bovine milk and plant-based alternatives. Among these, the pH of EFMEs were more similar to hazelnut milk, bovine milk, soy milk and cashew milk. In contrast, the pH values of chickpea milk, rice milk and almond milk were slightly higher than those of EFMEs (Reyes-Jurado et al., 2023).

It is essential to acknowledge that the pH of plant-based milk can be influenced by several factors, including water quality and the composition of the added ingredients (JEMAA et al., 2021). The pH of plant-based milk may slightly varied if produced with commercially sourced water which may contain different types and amount of minerals (Reyes-Jurado et al., 2023). This kind of water may consist of purified water, spring water, bottled water, or from other sources.

Table 4.4 pH and °Brix (total soluble solids) of emulsified foxtail millet extract.

Sample ID	Lecithin (%)	pH	°Brix	Whiteness Index
A5	0.040	6.63 ± 0.01^a	7.75 ± 0.07^a	53.39 ± 0.38
B5	0.050	6.58 ± 0.04^a	7.40 ± 0.00^a	57.85 ± 0.56
C5	0.060	6.59 ± 0.06^a	6.85 ± 0.07^a	59.12 ± 1.08
A7	0.560	6.67 ± 0.02^a	8.05 ± 0.07^a	56.47 ± 0.50
B7	0.070	6.66 ± 0.01^a	7.60 ± 0.00^a	57.30 ± 0.59
C7	0.084	6.56 ± 0.04^a	7.50 ± 0.14^a	60.61 ± 1.00
A5*	0.040	6.55 ± 0.07^a	9.15 ± 0.21^b	57.72 ± 0.16
B5*	0.050	6.56 ± 0.01^a	9.35 ± 0.35^b	58.09 ± 0.44
C5*	0.060	6.60 ± 0.02^a	9.60 ± 0.00^b	60.64 ± 0.53
A7*	0.560	6.59 ± 0.06^a	9.60 ± 0.00^b	57.79 ± 0.15
B7*	0.070	6.61 ± 0.11^a	9.55 ± 0.07^b	59.13 ± 1.20
C7*	0.084	6.62 ± 0.05^a	9.15 ± 0.07^b	61.06 ± 0.22

Note: Samples coded with '5' represent samples with 5% lecithin addition rate based on added oil weight, samples coded with '7' represent samples with lecithin addition rate at 7% based on added oil weight. A, B, C

represented samples with 0.80%, 1.00% and 1.20% oil addition, respectively. Sample contains a symbol * representing 12% millet grain in water. Detailed preparation of foxtail millet extract can be found in Table 3.3. L* stands for the lightness (black to white, 0 to 100); +a* represented the redness, -a* represented the greenness, +b* shows yellowness and -b* shows blueness. All the data within same column presented in the Table 4.3 are statistically different.

4.2.3 Total soluble solids (TSS) in stabilised foxtail millet milk

As shown in Table 4.4 and Figure 4.8, the TSS of the EFMEs fell within the range of 6.85 to 9.60, with a mean value of 8.46 ± 0.30 . The TSS was significantly affected by the percentage of millet in water ($p < 0.05$). The EFME samples with a 10% millet had a lower °Brix value compared to the samples with 12% millet grain. The average TSS value for samples with 10% millet grains was 7.52 ± 0.16 , while the mean °Brix for EFME samples with 12% millet grain was 9.40 ± 0.09 . The high TSS can be attributed to the higher concentration of soluble substances resulting from the increased millet concentration, as discussed in Section 4.13.

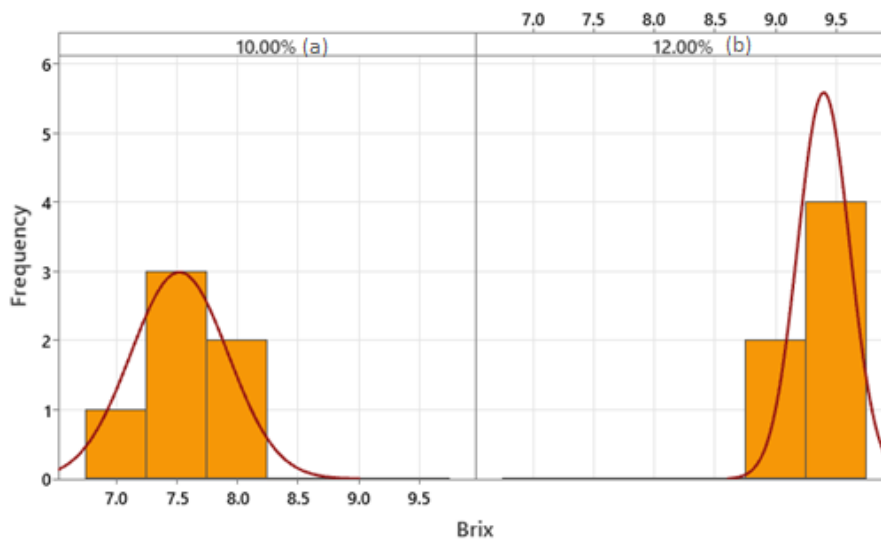


Figure 4.8 Distribution of frequency of °Brix of the emulsified foxtail millet extract processed with (a) 10% and (b) 12% millet grain in water ratio.

It is worth noting that neither the addition of oil nor lecithin had an impact on TSS ($p > 0.05$). In the case of oil addition, this may be due to the limited solubility of canola oil. While for the lecithin, which is used as an emulsifier to produce oil-in-water emulsion, it functions as the surface-active substance that adsorbs on the oil-water interface. Thus, the lecithin will not be presented in a water-soluble form in the foxtail millet milk emulsion (McClements, 2004).

The °Brix of EFMEs were lower than those of cow's milk, which have been reported at 12.4 (Shakerardekani et al., 2013). The difference shows that cow's milk is richer in soluble components, notably lactose, which contributes to its sweetness. In addition to sugar content, cow's milk contains various minerals, including calcium, phosphorus, potassium and magnesium, which are present in a soluble form and contribute to its TSS (Foroutan et al., 2019). Cow's milk also has a higher content of soluble substances such as proteins, vitamins and organic acids compared to plant-based milk alternatives (Foroutan et al., 2019).

When compared to other plant-based milk alternatives such as chickpea milk (4.0°Brix), almond milk (7.0°Brix) and pistachio milk (3.40°Brix), EFMEs produced a higher average TSS (Rincon et al., 2020, Maghsoudlou et al., 2016, Shakerardekani et al., 2013). Meanwhile, starch-rich plant-based milk, such as oat milk, tends to have a higher TSS level, reaching up to 15.6°Brix (Olson, 2021). The variations in TSS data among different beverages underscore the influence of composition, production parameters (e.g., material concentration, enzyme treatment and the addition of soluble ingredients) and ingredient characteristics in plant-based milk alternatives.

4.2.4 Particle size distribution of foxtail millet extract and emulsified foxtail millet milk

4.2.4.1 Particle size of foxtail millet extract following high shear mix and primary homogenisation

Three processing points are measured for the volume-weighted ($d_{4,3}$) and surface-weighted ($d_{3,2}$) mean particle diameters of FME Samples: before high shear mixing (BHSM), after high shear mixing (AHSM) and after primary homogenisation (APH) – which was processed with the addition of oil and emulsifier (Figure 4.9). A significant reduction in both $d_{3,2}$ and $d_{4,3}$ was observed between BHSM and AHSM. Specifically, the mean $d_{3,2}$ and $d_{4,3}$ of the foxtail millet extract decreased from 120.4 and 549.7 μm to 20.1 μm and 46.04 μm , respectively.

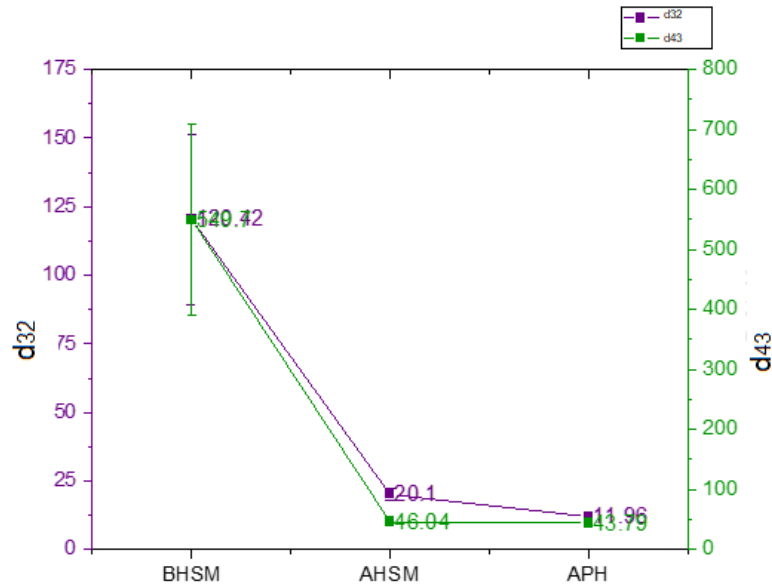


Figure 4.9 The average particle size of the foxtail millet extract at three processing points, BHSM (before high shear mixing), AHSM (after high shear mixing) and APH (after primary homogenisation).

Note: d_{43} represents the volume-weighted mean particle diameter; and d_{32} represents the surface-weighted mean particle diameter.)

This reduction in particle size after high shear mixing is attributed to the longitudinal, rotational and radial velocity gradients within the fluids generated from the rotation of the mixing heads (McClements, 2004). Importantly, the high shear mixing procedure effectively breaks down the particle size, leading to a decrease in the loss of FME solids during filtration.

As shown in Figure 4.9, the mean $d_{3,2}$ and $d_{4,3}$ of FME exhibited a decrease after primary homogenisation, $d_{3,2}$ reduced from 20.1 μm to 12.0 μm and $d_{4,3}$ reduced from 46.0 μm to 43.8 μm , respectively. However, the difference was not statistically significant between AHSM and APH ($p > 0.05$). Notably, prior to primary homogenisation, oil and emulsifier were introduced into the FME during high shear mixing. The implementation of primary homogenisation resulted in the creation of an emulsion – oil in water in the FME. The mean particle size at APH showed that alongside the canola oil and soy lecithin being mixed in the water phase of the FME, but they were also broken down into smaller particles through primary homogenisation.

According to previous studies, high shear mixing can reduce particle size ranging from 1 to 10 μ m (McClements, 2004). However, in this study, such reduction may not have been realized, possibly due to limitations imposed by the type of particles, shearing speed, shearing time and shearing temperature applied in this study (De Hert & Rodgers, 2017). Nevertheless, the changes in particle sizes of the FME in this study still shows that the high shear mixing can be effective for reducing particle size in foxtail millet milk production.

4.2.4.2 Determination of particle size of the EFME under different homogenisation pressure

The volume-weighted ($d_{4,3}$) and surface-weighted ($d_{3,2}$) particle diameters of the trial EFME samples after homogenisation at different pressures is shown in Figure 4.10. The average values of $d_{4,3}$ and $d_{3,2}$ of the samples homogenised at 200 bars showed a larger diameter than at other pressures. Both $d_{4,3}$ and $d_{3,2}$ showed a declining trend with increase in homogenisation pressure. This observation agreed with several studies which reported that the increase of the homogenisation pressure reduces the particle size of emulsions (McClements, 2004; Qian & McClements, 2011; Schulz & Daniels, 2000).

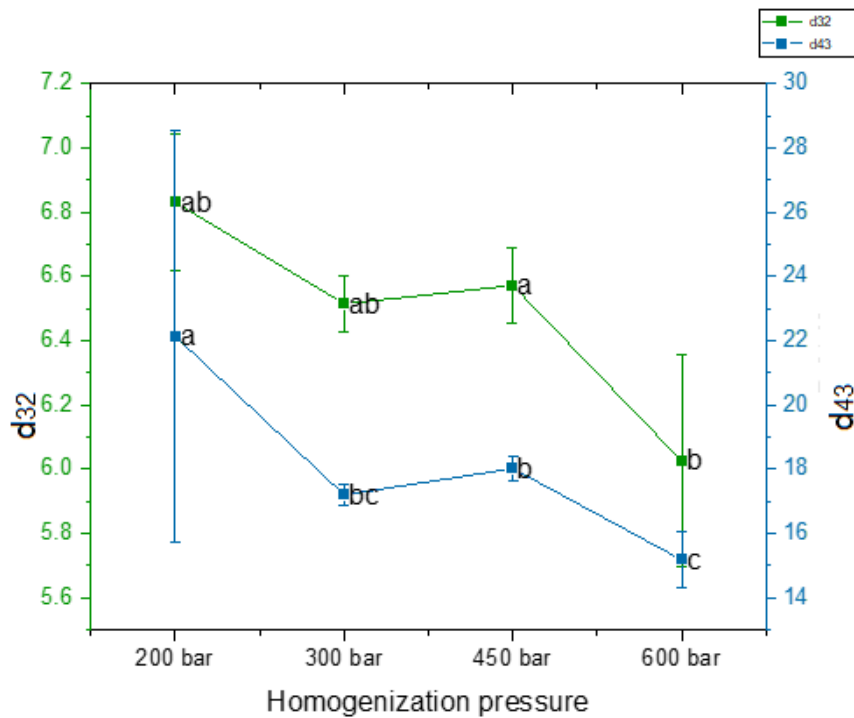


Figure 4.10 The volume-weighted ($d_{4,3}$) and surface-weighted ($d_{3,2}$) mean particle diameters of the trial emulsified foxtail millet extract samples processed with homogenisation pressure at 200 bar, 300 bar, 450 bar and 600 bar, respectively.

Note: The data that labelled with the same letters are not significantly different, whereas the data labelled with different letters are significant different; $d_{4,3}$ represents the volume-weighted mean particle diameters; and $d_{3,2}$ represents the surface-weighted mean particle diameters.

As shown in Figure 4.10, The $d_{3,2}$ and $d_{4,3}$ of the samples homogenised with 600 bars were showing a significant difference between both samples homogenised with 200 bars and 450 bars. However, samples that underwent 300 bars homogenisation pressure not significantly different from the particle size processed under 450 bar or 600 bar pressure. Therefore, 300 bars homogenisation pressure was selected as for further investigation.

4.2.4.3 Particle size distributions of the emulsified foxtail millet extract with different levels of added oil and soy lecithin

The results of the mean particle sizes $d_{3,2}$ and $d_{4,3}$ of the EFME with various oil and soy lecithin were illustrated in Table 4.5 and Figure 4.11. The average value of the $d_{3,2}$ of the EFMEs was $6.35 \pm 0.50 \mu\text{m}$ and ranged from $5.72 \mu\text{m}$ to $6.18 \mu\text{m}$, whereas the $d_{4,3}$ of the EFMEs ranged from $12.25 \mu\text{m}$ to $15.25 \mu\text{m}$, with a mean of $13.77 \pm 0.91 \mu\text{m}$.

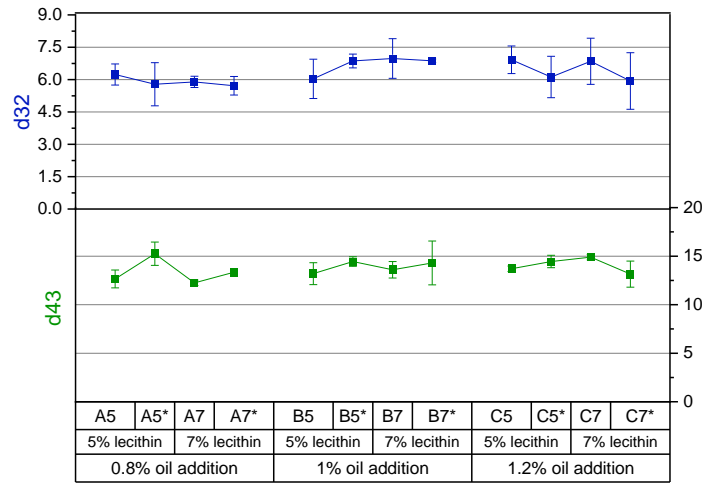


Figure 4.11 The volume-weighted ($d_{4,3}$) and surface-weighted ($d_{3,2}$) mean particle diameters of emulsified foxtail millet extracts (EFMEs) with 10% and 12% millet grain in water, at levels of 0.8%, 1.0% and 1.2% oil added, 5% (w/w) and 7% (w/w) lecithin was added to samples.

Note: Samples titled with 5 represent samples with 5% lecithin addition rate based on added oil weight, samples coded with 7 represent samples with lecithin addition rate at 7% based on added oil weight. A, B, C represented samples with 0.80%, 1.00% and 1.20%, respectively. Sample contains coded with '*' representing 12% millet grain in water rate.

Table 4.5 The mean particle size $d_{3,2}$ and $d_{4,3}$ of the foxtail millet extract emulsified with different levels of oil and soy lecithin addition.

Sample	Millet (%)	Added oil	Lecithin (%)	$d_{4,3}$	$d_{3,2}$
A5	10%	0.8%	0.04	12.65 ± 0.92	6.24 ± 0.49
B5	10%	0.8%	0.05	13.20 ± 1.13	5.80 ± 1.00
C5	10%	1.0%	0.06	13.70 ± 0.28	5.90 ± 0.26
A7	10%	1.0%	0.56	12.25 ± 0.28	5.715 ± 0.43
B7	10%	1.2%	0.07	13.60 ± 0.85	6.035 ± 0.91
C7	10%	1.2%	0.084	14.90 ± 0.28	6.865 ± 0.32
A5*	12%	0.8%	0.04	15.25 ± 1.20	6.98 ± 0.92
B5*	12%	0.8%	0.05	14.45 ± 0.49	6.87 ± 0.08
C5*	12%	1.0%	0.06	14.45 ± 0.64	6.92 ± 0.64
A7*	12%	1.0%	0.56	13.35 ± 0.07	6.12 ± 0.96
B7*	12%	1.2%	0.07	14.30 ± 2.26	6.85 ± 1.07
C7*	12%	1.2%	0.084	13.15 ± 1.34	5.94 ± 1.31

Note: $d_{4,3}$ represents the volume-weighted mean particle diameters; and $d_{3,2}$ represents the surface-weighted mean particle diameters. The data presented in Table 4.4 was not statistically significant.

According to Jeske et al. (2017), variations exist in both $d_{3,2}$ and $d_{4,3}$ values among commercial milk alternatives. Specifically, the $d_{3,2}$ values range from 0.36 μm to 2.36 μm , while the $d_{4,3}$ values range from 0.60 μm to 81.47 μm , with the majority falling below 10 μm (Jeske et al., 2017). In comparison, the foxtail millet milk in this study has demonstrated higher values for both $d_{3,2}$ and $d_{4,3}$ than most commercial sources, although not the highest in the observed range.

In this study, variations in the percentage of millet grain, canola oil addition level and lecithin addition rate did not yield statistically significant changes ($p > 0.05$) in the mean particle size of the FME. This observation disagreed with some relevant studies, which reported an increase in emulsifier addition rate lead to reduced particle size following homogenisation (McClements & Grossmann, 2022c; Qian & McClements, 2011). According to McClements and Grossman (2022), when the concentration of emulsifier has exceeded the optimal level, the increasing concentration of emulsifier will not show any significant reduction in particle size. However, in the study a low concentration of soy lecithin was used, which may impact the final result.

As reported by the Pan et al (2004), when lecithin was used as emulsifier in an oil-in-water emulsion, the concentration of lecithin at 0, 0.1% and 0.5% did not impact the $d_{4,3}$ of the particle size in the emulsion. The rate of lecithin used in this study was based on the oil addition, however, this amount of lecithin addition rate up to 0.084% in this study may not reach the critical concentration which is required to have an impact on the particle size of the emulsion (Pan et al., 2004).

Both $d_{3,2}$ and $d_{4,3}$ were used to report the mean particle diameter, the $d_{3,2}$ is representing the size of the majority of particles in the EFMEs, while the $d_{4,3}$ is more sensitive to the larger particles and aggregates presented in the emulsion (Grossmann et al., 2021). Based on the data retrieved from this study, the $d_{4,3}$ values of EFMS were higher than $d_{3,2}$ values, which may indicate the presence of large particles or aggregates in EFME.

Additionally, as shown in the PSD curves (Figure 4.12), which were not a monomodal distributions, as a minority of particles were between 1.0 μm - 10 μm in diameter, while the majority of the particles were above 10 μm in diameter. This indicates a bimodal PSD in the EFME, which can be undesirable as it may cause instability of the milk emulsion. Several production operations are considered to influence the PSD of plant-based milk, including emulsifier concentration, homogeniser type, homogenisation pressure and the number of passes. It is worth noting that ultra-high-pressure homogenisation under 200-600 MPa have been widely used in the production of milk alternatives due to their ability to form smaller and more uniformed particles (Swati Sethi et al., 2016).

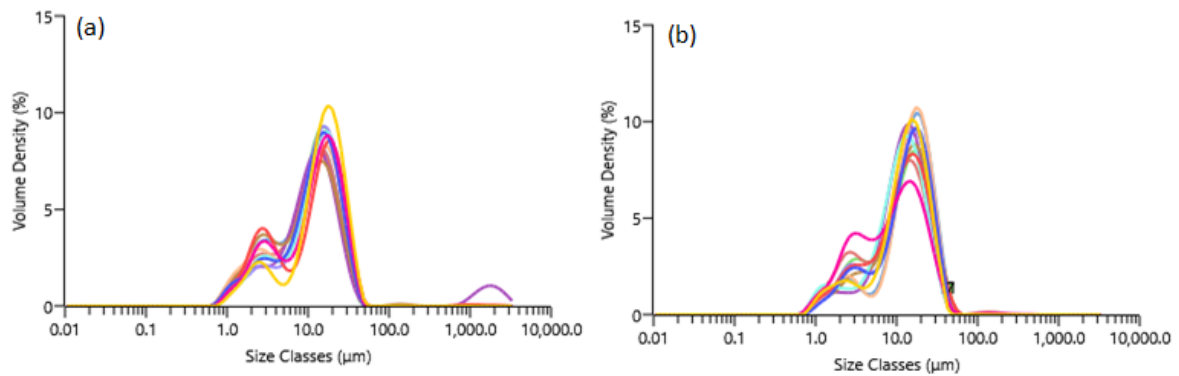


Figure 4.12 The particle size distribution in emulsified foxtail millet extract (EFME), (a) with 10% millet grain and (b) with 12% millet grain

These observations suggest that there is room for further improvement in reducing the particle size of foxtail millet milk. This potential enhancement in particle size reduction could lead to the development of a product that has more similar characteristics to commercial milk alternatives.

4.2.4.4 The effect of particle size on the colour of EFME

The particle size of the EFME distribution showed impact on the colour of the emulsion. The levels of millet grain and added oil addition were two factors that studies found to have an impact the colour of the EFME (Chantrapornchai et al., 1999; McClements & Grossmann, 2022c).

At the same level of millet grain and oil addition, the particle size and whiteness index of EFME were detailed in Table 4.4. The data illustrates that samples with a smaller mean particle diameter of EFMEs are associated with higher whiteness index values. This finding aligns with the observations of McClements (2002), who reported that the lightness (L^*) of the emulsion increases with the particle radius until it reaches 100nm, beyond 100nm, the lightness of the emulsion decreases. In this study, the mean particle diameter of the EFME samples exceeded 100 nm, resulting in a higher whiteness index obtained from emulsions with smaller particle sizes.

Given the understanding of the effect of particle size on the colour of the emulsion, reducing the particle diameter in the emulsified foxtail millet extract may contribute to achieving a more desired colour, one which is closer to that of bovine milk, characterised by higher lightness and whiteness index.

4.2.5 The separation rate of the emulsified foxtail millet milk

By using this certain observation method as described in Chapter 3, no visible phase separation was detected in the period of 72 h. Through visual observation, this would indicate the EFMEs werestable emulsions. However, the results of particle size distribution were $>10 \mu\text{m}$, which would indicate the possible phase separation caused by sedimentation (McClements & Grossmann, 2022c).

4.2.6 Apparent viscosity of the emulsified foxtail millet extract

The mean apparent viscosity of the EFME was 15.86 ± 2.37 mPa.s. at 10/s shear rate. The result indicates that viscosity of EFMEs was significantly affected by the addition of oil ($p < 0.05$). A higher viscosity was obtained with addition of oil at a higher concentration (Figure 4.13). The viscosity of EFMEs ranged from 13.69 ± 0.52 to 18.49 ± 1.93 with the varying additions of oil at 0.8%, 1.0% and 1.2%.

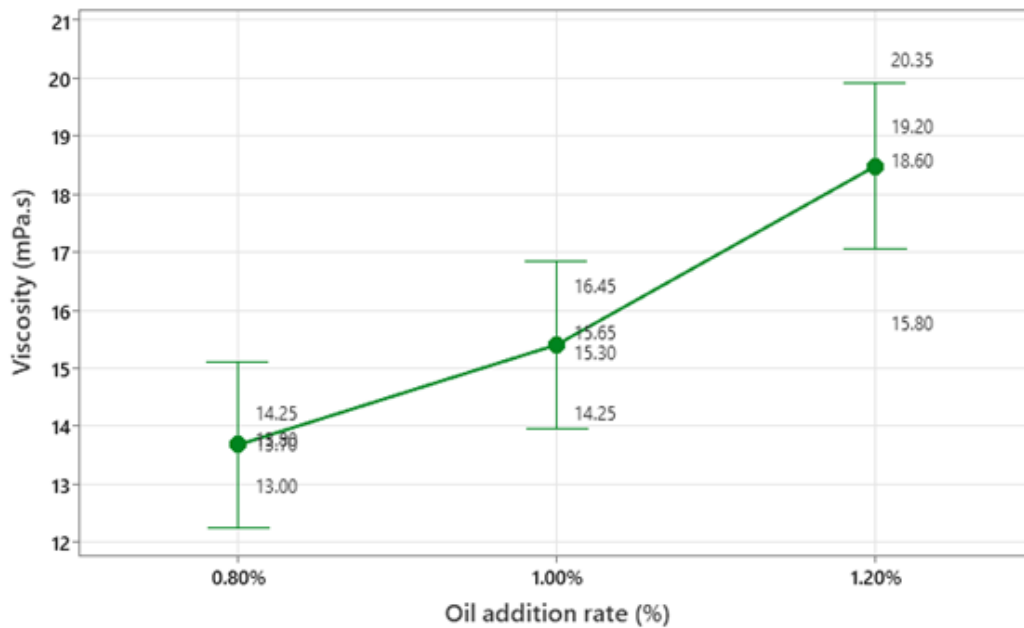


Figure 4.13 Viscosity of emulsified foxtail millet extracts after addition of 0.80%, 1.00% and 1.20% oil addition level measurements ($n = 8$) at 20°C and 10/s.

Higher viscosities due to higher oil addition rate levels were observed in the EFME. It is likely attributed to an increase in droplet concentration within the emulsion. As the quantity of added oil increased, a higher volume of fat droplets was produced through the homogenisation process, this subsequently increased the droplet concentration within the foxtail millet milk. More presence of fat droplets in the emulsion system contributed to increased flow resistance, leading to a higher viscosity of the emulsion. This phenomenon agrees with findings from other studies (McClements & Grossmann, 2022c; Phipps, 1969; Simuang et al., 2004).

The increase in apparent viscosity can also be explained by Einstein's equation [3], which is applicable to dilute emulsion systems containing less than 5% volume fraction and non-

interacting spherical particles. This equation shows the relationship between apparent viscosity and particle concentration in such systems (McClements & Grossmann, 2022c).

$$\eta = \eta_1 (1 + 2.5\phi) \text{-----}[3](\text{McClements \& Grossmann, 2022c})$$

Where η_1 is the viscosity of the dispersed medium and the ϕ is the volume fraction of the particles. This equation shows that when the volume fraction is less than 5%, the increase in the volume fraction increases the viscosity of the emulsion linearly.

The apparent viscosity at shear rate of 10/s of bovine milk and other commercial plant-based milk alternatives were reported in previous studies (Jeske et al., 2017; McClements & Grossmann, 2022c). There were variations between different milks ranging from 2.2 to 48 mPa.s. In this study, the average apparent viscosity of the foxtail millet milk (15.9 mPa.s) was less than some milk alternatives such as coconut milk (48 mPa.s), hemp milk (25 mPa.s) and hazelnut milk (25 mPa.s). Foxtail millet milk shared similar viscosity with quinoa milk (13 mPa.s), but a higher viscosity than that of soy milk (2.6 mPa.s), oat milk (6.8 mPa.s), cashew milk (5.6 mPa.s) and bovine milk (3.2 mPa.s).

It is worth to mentioning that that there also some variations within the same type of milk alternatives, for instance, the apparent viscosity of commercial almond milk ranges from 3.9 to 26.3 mPa.s. The viscosity of the milk alternatives is mainly dependent on the concentration of the fat droplet, oil bodies, cell wall fragments and thickening agent. Among the same type of milk, the rheological properties of milk alternatives can vary due to the different ingredients from suppliers or batches (McClements & Grossmann, 2022b).

Furthermore, bovine milk is considered a Newtonian (ideal) fluid which is a fluid that has low apparent viscosity and remains stable with changes in shear rate. However, plant-based milk alternatives show relatively high viscosity with shear thinning properties, which mean there is a decrease in viscosity when the shear rate increases (McClements & Grossmann, 2022b).

The differences in viscosity of various milk types can also impact sensory properties associated with the texture. Based on the apparent viscosity obtained from previous study, the foxtail

millet milk might present a thicker texture and mouthfeel than lower viscosity milks like bovine milk, soy milk and oat milk. However, in contrast to higher viscosity milks such as coconut milk, hazelnut milk and hemp milk, foxtail millet milk is likely to offer a lighter mouthfeel.

Apart from oil addition, the viscosity of the plant-based milk alternatives can also be affected by temperature change. The low viscosity of food emulsion can be induced by high temperature. This phenomenon is due to the high level of molecular movement and flexibility caused by increasing temperature (McClements & Grossmann, 2022d).

4.2.7 Sensory evaluation of the emulsified foxtail millet milk

The colour, aroma, taste, texture (mouthfeel) and appearance of the EFME samples were assessed during the focus group discussion. The summary of the comments of these samples are shown in the Table 4.6.

Table 4.6 *The summary of sensory properties of the emulsified foxtail millet extract samples obtained from focus group discussion.*

Sample ID	Summary of comments			
	Appearance	Aroma	Flavour	Texture
A5	Light creamy colour, no observed phase separation	Cereal and creamy aroma	Light cereal-like taste	Medium consistency
B5	Light creamy colour, no observed phase separation	Light in aroma	Bland cereal-like taste	Medium low consistency texture
C5	Creamy colour; light in aroma, no observed phase separation	Cereal and creamy aroma	Bland cereal-like taste	Thick consistency
A7	Light creamy colour, no observed phase separation	Light in aroma.	Light cereal-like taste	Light consistency
B7	Light creamy colour, no observed phase separation.	Medium intensity of aroma	Bland cereal-like taste	Thick consistency
C7	Creamy colour; light in aroma, no observed phase separation	Light in aroma; texture	Bland cereal-like taste	Thick consistency
A5*	Light creamy colour, no observed phase separation.	Medium intensity of cereal aroma	Medium intensity balanced taste and texture; taste like oat milk; slight sweetness	Medium low consistency texture
B5*	Light creamy colour, no observed phase separation.	Medium intensity of cereal aroma	Oat-like taste; slight sweetness	Slight mouth coating texture
C5*	Creamy colour; light in aroma, no observed phase separation	Medium intensity of cereal aroma	Oat-like taste; slight sweetness; a slight grainy mouthfeel	Thick consistency, slight mouth coating texture
A7*	Light creamy colour, no observed phase separation.	Medium intensity of cereal aroma	Cereal-like taste; slight sweetness	Medium low thickness; no observed phase separation
B7*	Light creamy colour, no observed phase separation.	Medium low intensity of cereal aroma	Cereal-like taste; slight sweetness	Medium consistency.
C7*	Creamy colour; light in aroma, no observed phase separation	Medium low intensity of cereal aroma	Oat-like taste; a slight sweetness	Thick consistency, unpleasant mouth coating texture

Note: Samples titled with 5 represent samples with 5% lecithin addition rate based on oil weight, samples titled with 7 represent samples with lecithin addition rate at 7% based on oil weight. A, B, C represented samples with 0.80%, 1.00% and 1.20%, respectively. Sample contains a symbol * representing 12% millet grain in water rate. Detailed preparation of foxtail millet extract refers in Table 3.3.

The results from the sensory evaluation of the EFME samples revealed distinct characteristics which include a creamy-like colour, an oat-like taste and no apparent phase separation. Notably, samples with a 12% millet grain content exhibited a more pronounced aroma, taste and a lighter mouthfeel compared to those with 10% millet grain content. As a result, the focus group panellists expressed a stronger preference for the samples with a higher percentage of millet grain. This preference may be attributed to the greater concentration of foxtail millet in the emulsion, resulting in higher levels of aroma compounds and hydrolysed free sugars derived from starch, which would have contributed to a subtle sweetness in the samples (Arora et al., 2023)

Interestingly, the majority of panellists leaned towards slightly less white-coloured samples in the context of colour evaluation, as these samples conveyed more distinct plant-based characteristics. Thus, samples with lower oil additions were favoured, given that the whiteness index increased with higher rates of oil addition, as discussed in Section 4.2.1.

Furthermore, the results show that the level of oil addition in EFMEs impacted the perceived texture. Samples with higher level of oil added exhibited increased thickness in texture, some samples even displayed a mouth-coating sensation which agrees with findings reported by (McCarthy et al., 2017). However, the mouth-coating texture of some EFMEs treated with higher level of oil added (1.0%;1.2%) was consequently seen to be overwhelming and unpleasant. This resulted in samples with the 0.8% oil added in EFMEs emerging as the preferred choice for further work during the focus group discussion.

Thus, formulations A5* and A7*, both processed with 12% millet grain, 0.8% canola oil added and different levels of lecithin percentages (5% and 7% based on weight of oil added,

respectively), were considered the most desirable among the 12 samples in terms of sensory properties.

4.2.8 Summary of stabilisation of foxtail millet extract and selection of formulation

In Phase II of this study, samples of foxtail millet extract were subjected to emulsification, various levels of millet grain rate, added oil and added lecithin. Several physicochemical properties of the emulsified foxtail millet extracts (EFMEs) were determined including the whiteness index, pH, total soluble solids, particle size distribution, separation rate, apparent viscosity and sensory attributes. The properties were used to evaluate and refine 12 formulations in the selection of an optimal formulation for in-depth testing in the subsequent phase.

The percentage of millet grain used exhibited a significant influence on the whiteness index and total soluble solids of the EFMEs ($p < 0.05$). Formulations with a higher millet grain rate demonstrated elevated values for both whiteness index and total soluble solids. Conversely, the levels of added oil impacted the whiteness index and apparent viscosity of the EFMEs ($p < 0.05$). EFMEs Increased oil addition level correlated with higher whiteness index and viscosity ($p < 0.05$; Figure 2.7 and Figure 4.19).

The PSD analysis results of the EFMEs demonstrated the effects of primary and secondary homogenisation in reducing particle size. However, EFMEs samples processed with varying levels of millet grain, oil and lecithin did not exhibit significant differences in particle size distribution ($p > 0.05$). Moreover, no phase separation was observed in the EFMEs for 72 h.

The sensory evaluation was conducted through focus group discussions. The results indicated a preference for two samples prepared with 12% millet grain rate and 0.8% oil addition (A5* and A7*). The addition of lecithin did not show a significant influence on the physicochemical properties of the EFMEs. Therefore, the sample prepared with less added lecithin which made from 12% millet grain and 0.8% oil addition and a 5% lecithin addition (A5*) were identified as the optimal formulation for further investigation.

4.3 Consumer evaluation of the selected formulation

The sensory acceptance results of the selected foxtail millet milk formulation (A5*), evaluated using the 9-point hedonic system, is shown in Figure 4.14. Sensory attributes evaluated were colour, aroma, taste/flavour, texture/mouthfeel and overall acceptance.

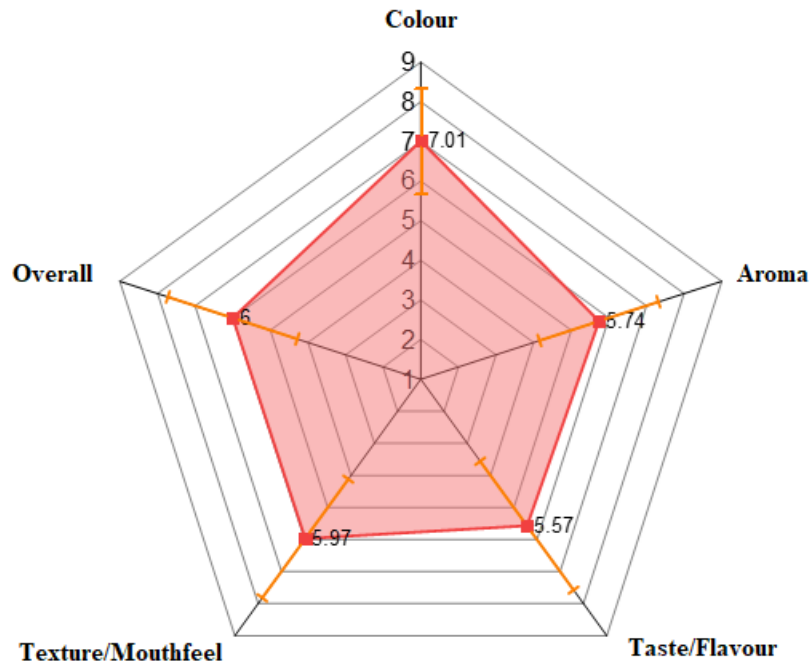


Figure 4.14 The sensory evaluation on colour, aroma, taste/flavour, texture/mouthfeel and overall opinion of the selected foxtail millet milk formulation using 9-point hedonic system ($n=100$, the data were mean scores).

Note: The scores of the 9-point hedonic system presented: dislike extremely (1), dislike very much (2), dislike moderately (3), dislike like slightly (4), neither like nor dislike (5), like slightly (6), like moderately (7), like very much (8), like extremely (9).

The colour of the foxtail millet milk formulation had the highest score among all attributes evaluated. It scored on average 7.01 ± 1.33 with over 70% acceptance evaluated by 100 panellists. The average high mean score indicates the colour of the foxtail millet milk was highly accepted by the panellists.

Meanwhile, the aroma and taste/flavour only scored 5.7 ± 1.6 and 5.6 ± 2.00 , respectively. These attributes were characterised by a neutral response, neither strongly liked nor disliked. The high standard deviation indicated that a wide range of the acceptances regarding these two aspects. The relatively low acceptance of these attributes could be attributed to the absence of additional flavour components, such as sugar, coffee and chocolate. Nevertheless, this suggests

there is potential for improving the aroma and flavor profile of foxtail millet milk by incorporating additional flavor ingredients.. (Rincon et al., 2020). The average scores of texture/mouthfeels were at 6.0 ± 1.9 and overall acceptance and at 6.00 ± 1.7 which indicated as 'slightly liked' on the 9-point hedonic scale. There were 65% of scores ≥ 6 , 12% of scores equal to 5 and 23% scores < 5 .

Based on a previous study (Rincon et al., 2020), the product is considered accepted when it receives an overall acceptance score of ≥ 7.0 on a 1 to 9 point hedonic point scale. The average overall score of 6 suggests a positive potential towards the foxtail millet milk formulation studied in this thesis, there is potential for increasing consumer acceptance through improvements, especially by incorporating flavour ingredients.

4.3 Phase III Physicochemical properties and shelf life of foxtail millet milk

4.3.1 Proximate and mineral composition

4.3.1.1 Moisture content

The selected foxtail millet milk contained 90.1% moisture content, slightly higher than bovine milk which comprises of full-fat milk (88.13%), 1% fat milk (89.21%), 2% fat milk (89.9%) and skim milk (90.84%) (Chalupa-Krebzdak et al., 2018). Compared to other plant-based milks, the moisture content of foxtail millet milk falls between rice milk (89.23%) and almond milk (97.05%). The variability in moisture content among plant-based milks can be attributed to the differences in their composition (Chalupa-Krebzdak et al., 2018). The high moisture content in foxtail millet milk may be caused by the lower total solids (9.9%) in the emulsion (Bradley, 2010). Consequently, the moisture content is an important indicator of the concentrations of solids in foxtail millet milk which impacts on the physicochemical properties, sensory attributes and shelf-life (Roos, 1997).

4.3.1.2 Ash content

The ash content in food represents the inorganic matter remaining after ignition or complete oxidation, which gives insights into the mineral composition of the food (Nielsen & Marshall, 2010). In this study, the foxtail millet milk formulation contained 0.1% ash. The ash content in foxtail millet milk was relatively low compared to bovine milk and other plant-based milks alternatives (Reyes-Jurado et al.; 2021). Bovine milk contains 6-8 times more ash content than foxtail millet milk, whereas other plant-based milks including soymilk, rice milk, almond milk, cashew milk and hazelnut milk contain 4 to 30 times more ash than the foxtail millet milk produced in this study. The low ash content might indicate loss of inorganic matter like minerals in the production process of foxtail millet milk possibly during blanching and filtering (Ayo-Omogie et al., 2021). The relatively low ash content in foxtail millet milk compared to other plant-based milk may also result from the lack of fortification of salt and other mineral contents commonly used in plant-based milk production (Drewnowski, 2021).

4.3.1.3 Crude protein content

Foxtail millet milk contained 0.5% (w/w) crude protein content. Based on the protein content (approx. 12%) of foxtail millet grain used for millet milk production, the protein content in this millet milk was expected to be higher than the determined value which is around 1.45% (w/w), it may suggest a potential loss of protein during production (Amadou et al., 2013). The loss of protein could be attributed to protein denaturation during high-temperature treatment and filtration (Swati Sethi et al., 2016).

The protein content of foxtail millet milk in this study was lower than most of milk types reported (Chalupa-Krebzdak et al., 2018). Bovine milk has the highest protein levels ranging from 3.15 to 3.37 g per 100 mL followed by soy milk with 2.47 to 3.16 g per 100 mL (Chalupa-Krebzdak et al., 2018; Pointke et al., 2022; Reyes-Jurado et al., 2023). Previous studies reported protein content of oat milk ranging from 1.35g per100mL to 1.9g per100 mL (Drewnowski (2021), Reyes-Jurado et al (2023). This indicates that there are large variations between different milk alternatives from different sources.

Additionally, there are some variations between plant-based milk made from the same plant material. According to previous studies the protein content in hemp milk and coconut milk were reported higher than foxtail millet milk which are 1.99 g per100 mL and 2.00 gper100 mL, respectively (Reyes-Jurado et al., 2021; Chalupa-Krebzdak et al., 2018). However, the protein content of the these two plant-based milks were also found to be relatively low, around 0.08 g per100 g of protein content which is lower than the protein content in foxtail millet milk (Drewnowski, 2021; Jeske et al., 2017). This may be contributed to variations may be attributed to production processes including extraction, heat treatment, enzyme treatmentand fortification (McClements, 2002).

Other alternatives such as almond milk and rice milk were reported to contain less protein content than the foxtail millet milk in this study. Protein content in almond milk ranges from 0.42 to 0.59 g per 100 mL(Chalupa-Krebzdak et al., 2018; Pointke et al., 2022)and rice milk was reported to range from 0.28g per 100 mL to 0.5 g per100 mL (Chalupa-Krebzdak et al. (2018) and Drewnowski (2021).

Despite the relatively low protein content in foxtail millet milk is low compared to bovine milk, it still maintains higher or similar level of protein content compared with plant-based milks. Further improvements to the protein content of foxtail millet milk can be undertaken by optimising the production process and fortification of alternative protein sources.

4.3.1.4 Fat content

Foxtail millet milk contained 1.3% (w/w) fat which was composed of natural fat and addition of canola oil. In comparison to bovine milk, foxtail millet milk contains less fat than whole fat and 2% fat bovine milk, which have approximately 3.3% and 2.0% fat, respectively. Conversely, foxtail millet milk contains higher fat than low fat bovine milk and skimmed bovine milk (Chalupa-Krebzdak et al., 2018). Among other plant-based milks, fat content varies based on the plant material and production methods. Generally, soy milk, coconut milk and oat milk contain higher amounts of fat than foxtail millet milk, while almond milk and hemp milk have a similar level of fat with foxtail millet milk and rice milk has the lowest fat (Chalupa-Krebzdak et al., 2018; Paul et al., 2020; Romulo, 2022).

The fat content of processed foxtail millet milk comes from the millet grain and canola oil addition. Both sources of fat are composed of unsaturated fatty acids. Whereas bovine milk and coconut milk contain mainly saturated fatty acids, the unsaturated fatty acids in foxtail millet milk may be healthier, due to the saturated fatty acids being associated with increasing low-density lipoprotein (LDL) cholesterol in the human body, potentially elevating the risk of cardiovascular disease (CVD) (Briggs et al., 2017; Pehowich et al., 2000).

4.3.1.5 Carbohydrates and total dietary fibre

Carbohydrates make up 7.6% of foxtail millet milk and serve as the primary source of energy. The predominant carbohydrate in foxtail millet grain is starch, hydrolysed by α -amylase and converted into free sugars. With an approximately 63% natural carbohydrate in grain, foxtail millet milk exhibits relatively higher carbohydrate levels compared to bovine milk and other common plant-based milks such as soy milk, almond milk and hazelnut milk (Paul et al., 2020;

Scholz-Ahrens et al., 2020). However, the carbohydrate content in foxtail millet milk aligns closely with those reported in oat milk, rice milk and coconut milk.

The total dietary fibre accounts for 0.4% (w/w) of the total carbohydrates in foxtail millet milk. Dietary fibre is known for its health benefits, including the reduction of risks associated with diseases such as diabetes, stroke, coronary heart disease and gastrointestinal disorders (Anderson et al., 2009). The presence of dietary fibre makes foxtail millet milk a preferable option for those seeking to increase their dietary fibre intake, as dietary fibre is absent in bovine milk.

According to Fructuoso et al. (2021), dietary fibre content in foxtail millet milk surpasses some almond-based, coconut-based, hemp-based and rice-based milk alternatives. However, the dietary fibre content in foxtail millet milk falls below the levels found in soy milk and oat milk. The average dietary fibre content in almond milk, oat milk and soy milk is approximately 0.27% (w/w), 1.24% (w/w) and 3.15% (w/w), respectively (Pointke et al. 2022). The presence of dietary fibre further enhances the nutritional profile of foxtail millet milk, making it a good choice for individuals prioritising dietary fibre in their diet (Pointke et al., 2022).

4.3.1.6 Mineral content

Calcium

Calcium, an essential mineral for the human body, plays a vital role in bone health, normal muscle function, blood flow and the nervous system (Zhang et al., 2022). The calcium level in foxtail millet milk (9.1 mg/kg) is considered low compared to bovine milk and some other fortified plant-based milks, given the natural low calcium content in foxtail millet and the absence of mineral fortification (Fructuoso et al., 2021). However, like most unfortified plant-based milk, the calcium content only ranges from 0-12mg/100g which aligns the calcium content in foxtail millet milk (Chlupa-Krebzdak et al, 2018). To mimic the calcium content in the bovine milk, the fortification of calcium is widely used in plant-based milk production. Common forms of calcium addition in plant-based milk includes calcium carbonate and

tricalcium phosphate, as observed in studies on almond milk, cashew milk, coconut milk and oat milk (Chalupa-Krebzdak et al., 2018; Fructuoso et al., 2021).

Potassium

The measured potassium content in foxtail millet milk was 165 mg/kg. Potassium is crucial for regulating fluid balance and participating in nerve signals and muscle contraction (Kowey, 2002). The recommended daily intake of potassium is approximately 3510 mg, taking this into account, foxtail millet milk would be considered to have low levels (Drewnowski et al., 2021). The proposed level of potassium for plant-based milk is 258 mg/100 g. However, the addition of potassium sources is generally optional and less common than calcium addition in plant-based milk. Addition of potassium may impact the sensory property of the milk negatively and is less friendly to consumers who have chronic kidney diseases. Potassium citrate can be found as an additive to regulate acidity in some plant-based milks such as almond milk and coconut milk (Fructuoso et al., 2021).

Sodium

Foxtail millet milk had a low sodium content at 12 mg/kg. Sodium is typically added in the form of salt to enhance flavour and increase milk stability in plant-based milk production. However, excessive salt intake has been linked to some health risks, particularly cardiovascular diseases (Graudal et al., 2014). The sodium standard for plant-based milk is proposed to be less than 120 mg per 100 g (Drewnowski et al., 2021). Thus, the sodium level in foxtail millet milk was considerably below the threshold which can be categorised as a low-sodium drink. Foxtail millet milk contains less sodium than most plant-based milks reported, including almond milk (56 mg/100 g), cashew nut milk (52 mg/100 g), coconut milk (52 mg/100 g), oat milk (48 mg/100 g) and sapucaia nut milk (315.8 mg/100 g) (Fructuoso et al., 2021, Drewnowski et al., 2021).

Phosphorus

The phosphorus content in foxtail millet milk was approximately 210 mg/kg. Phosphorus is essential for the metabolism and maintenance of the body, but excessive intake has been

associated with chronic kidney disease and heart disease (Calvo & Uribarri, 2013; Chen et al., 2018). The recommended daily allowance of phosphorus is 700 mg. Therefore, normal consumption of foxtail millet milk does not lead to excessive phosphorus intake. Phosphorus can be added in the plant-based milk in the form of tricalcium phosphate, calcium phosphate and magnesium phosphate to fortify the mineral content in plant-based milk (Chlupa-Krebzdak et al., 2018).

4.3.2 Aerobic Plate Total Count

There was no visible microbial growth of plated samples of foxtail millet milk stored for four weeks at 4°C. The total count was zero. The result suggested an acceptable level of microbial stability of the foxtail millet milk during refrigerated storage.

4.3.3 Physicochemical characteristics and stability of foxtail millet milk during storage (4°C)

4.3.3.1 Particle size of foxtail millet milk during storage (4°C)

An increasing trend of mean particle size in foxtail millet milk was observed during storage (Figure 4.15). The surface-weighted mean particle diameter ($d_{3,2}$) of foxtail millet milk increased from 6.95 to 12.24 μm , while the volume-weighted mean particle diameter ($d_{4,3}$) increased from 14.55 to 24.86 μm . The results also suggest that the particle sizes of foxtail millet milk were significantly increased in the first three weeks of storage and then stabilised during the third and fourth weeks.

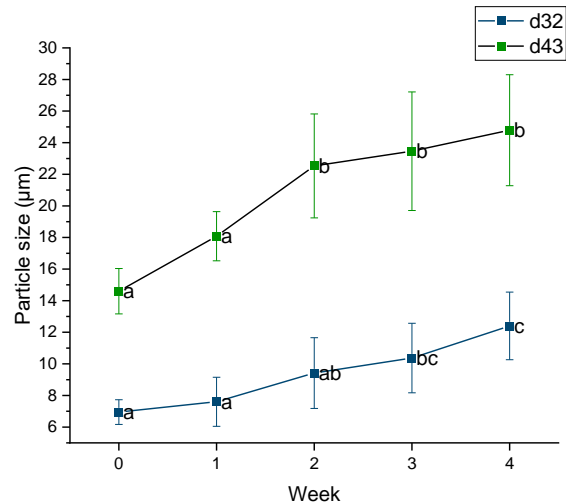


Figure 4.15 The mean particle size $d_{3,2}$ and $d_{4,3}$ of the foxtail millet milk during 4 weeks of refrigeration storage.

Note: $d_{4,3}$ represents the volume-weighted mean particle diameter; and $d_{3,2}$ represents the surface-weighted mean particle diameter

The observed increase in particle size could negatively impact the mouthfeel of the foxtail millet milk, potentially leading to a gritty texture (S. Sethi et al., 2016). Increasing particle size trend was also observed in flaxseed milk during storage (Meng et al., 2023). This increasing trend of particle sizes at 10-100 μm in plant-based milk might be attributed to the aggregation of the oil droplet and protein (Meng et al., 2023; Mu et al., 2022). The larger particles may result from the coalescence of smaller droplets caused by attractive forces between particles (McClements, 2002). In commercial plant-based milk production, the stability of particle size can be enhanced by adding additives such as polysaccharides, proteins and charged surfactants, which increase steric and electrostatic repulsion to counteract attractive forces and prevent aggregation (McClements, 2002). For instance, plant-based milk that contains proteins with neutral pH, polysaccharides including carageenans and gellan gum may be used to improve the stability (S. Sethi et al., 2016).

4.3.3.2 pH and °Brix of foxtail millet milk during storage (4 °C)

The pH of foxtail millet milk ranged from 6.56 to 6.64. The pH remained stable at approximately 6.63 and 6.64 during the first three weeks of storage. Beyond 21 days of storage, a minor decrease in pH occurred, though not statistically significant ($p > 0.05$). A similar trend

was observed with °Brix of foxtail millet milk. During the initial three weeks of storage, °Brix was 9.3 and from week 3 to week 4, it decreased slightly to 9.2 ($p > 0.05$). The decreasing of pH and total soluble solids (°Brix) in foxtail millet milk decreased is similar to results reported for coconut milk and orange juice (Tarek et al., 2020; Zulueta et al., 2013).

4.3.3.3 Whiteness index and apparent viscosity of the foxtail millet milk during storage (4°C)

The whiteness index and apparent viscosity of foxtail millet milk exhibited a decreasing trend during the 4-week storage period (4°C). The average whiteness index of foxtail millet milk decreased from 57.34 to 53.70 after 4 weeks ($p < 0.05$). Similarly, a significant decrease in the apparent viscosity of foxtail millet milk was observed between week 0 (14.30) and week 4 (13.60) ($p < 0.05$), although a significant drop in viscosity was not observed between consecutive weeks ($p > 0.05$).

The decrease in the whiteness index of foxtail millet milk may be attributed to changes in the particle size distribution of the milk system during storage (McClements, 2002). The increase in both surface-weighted mean particle diameter (d_{32}) and volume-weighted mean particle diameter (d_{43}) suggested potential oil droplet or protein aggregations occurring during storage. The aggregation of colloidal particles in foxtail millet milk can lead to a lower concentration of particles in the milk system, resulting in reduced light scattering by particles, which contribute to the decrease in lightness and whiteness indices (Chanamai & McClements, 2001; McClements, 2002a, 2002b).

Moreover, as discussed in Section 4.2.4.5, the colour of the emulsion is also associated with colloidal particle size. When the particle size exceeds 100 nm, the lightness of the emulsion decreases with increasing particle size (McClements, 2002). The whiteness index of foxtail millet milk after 4 weeks of storage was similar to almond milk (Jeske et al., 2017).

The decrease in apparent viscosity may also be linked to changes in colloidal particle conditions in foxtail millet milk. The low concentration of particles resulting from particle

aggregation in the emulsion is associated with a reduction in viscosity (McClements, 2002). As oil droplets form a network structure that impedes the flow of the liquid, a higher concentration of oil droplets in the foxtail millet milk emulsion leads to higher viscosity (Ushikubo & Cunha, 2014). Conversely, the reduction in particle concentrations in foxtail millet milk shows lower viscosity.

Furthermore, an increase in droplet size in the emulsion indicates a larger mean distance of separation between particles, leading to a looser packing of particles and reduced resistance to flow which can contribute to the decrease in viscosity (Pal, 1996). The increase in particle size also indicates less surface area between the interface of the oil and water phase, lowering interfacial tension and viscosity (Pu et al., 2016; Schroën et al., 2020).

4.3.3.4 Separation of the foxtail millet milk during storage (4°C)

Separation in foxtail millet milk is shown in Figure 4.16. At week 0, no visible phase separation was observed. However, by week 1, there was apparent sedimentation observed due to a transparent top layer. In the falcon tube containing 10 ml of foxtail millet milk, the liquid phase below 9.7 ml appeared denser, indicating that the upper phase of the millet milk at week 1 constituted around 3%. Similarly, phase separation was observed in the subsequent weeks (weeks 2, 3 and 4), with approximately 5% upper phase of the volume becoming clearer during storage.

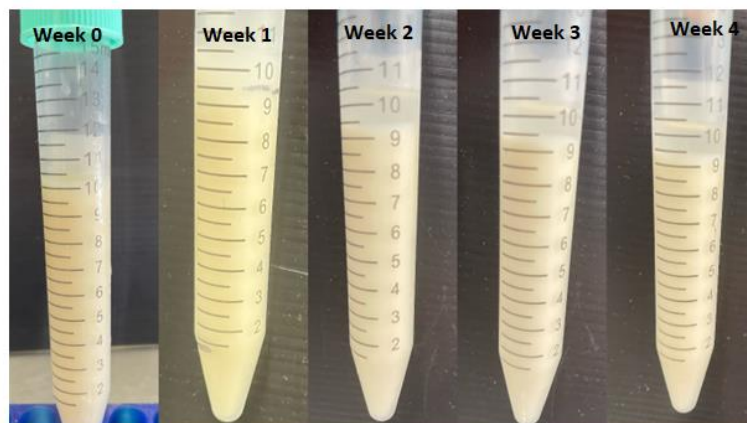


Figure 4.16 The gravitational separation of the foxtail millet milk during 4 weeks of storage (4°C).

This outcome indicated that the gravitational stability of foxtail millet milk decreased during the first two weeks and stabilises after two weeks, with a top layer of around 5%. The outcomes were similar to previous findings on coconut milk (Ricon et al.,2020). The presence of phase separation may indicate instability in foxtail millet milk, potentially leading to a decrease in overall quality.

The phase separation in foxtail millet milk was likely linked to the enlarged particle sizes in the milk emulsion. During the storage period, the coalescence of droplets led to a phase separation, according to the Stokes' law, an increase in particle diameter results in higher sedimentation velocity. Homogenisation of foxtail millet extract during stabilisation plays an important role in determining particle size. To enhance the stability of foxtail millet milk, different homogenisers, pressure levels and treatments can be used to reduce the particles in the milk. A study by Mu et al. (2022) showed that the application of high-intensity ultrasound in soy milk significantly decreased particle size and increased stability during storage.

In addition to particle size reduction, increased viscosity can improve the physical stability of foxtail millet milk. Therefore, common thickening agents used in plant-based milk, such as starch, locust bean gum, xanthan gum and other polysaccharide hydrocolloids, could be considered to enhance gravitational stability (Hadidi et al., 2023; Karimidastjerd & Kilic-Akyilmaz, 2021; Syed et al., 2020).

4.3.4 Summary of physicochemical properties and shelf life of foxtail millet milk

The moisture content of foxtail millet milk was 90.1%, similar to skimmed bovine milk. A low ash content indicated fewer minerals in the foxtail millet milk than other plant-based milks. Due to the foxtail millet milk not being fortified, the mineral content is lower than most of the other commercial plant-based milk. However, foxtail millet milk has good level of fat and carbohydrates, while being low in protein. Nevertheless, foxtail millet milk offers a healthier profile compared to alternatives rich in saturated fats, sodium and phosphorus.

Microbial test results indicated that foxtail millet milk was stable for 4 weeks storage (4°C). Particle size increased during storage for four weeks thereby affecting whiteness and viscosity. The pH and °Brix remained stable, whereas, gravitational separation occurred, indicating potential instability. Improvement in homogenisation or addition of thickening agents may contribute to better stability.

In conclusion, foxtail millet milk as a milk alternative has shown good potential. However, improvements in protein retention, mineral content and physical stability during storage may be desirable to make the product more competitive as a bovine milk alternative.

CHAPTER 5 OVERALL CONCLUSION AND RECOMMENDATIONS

Foxtail millet was investigated as the main ingredient for developing a novel plant-based milk in this study. The foxtail millet milk was extracted in water with 12% millet grain (dry-milled) and 0.2% of α -amylase addition. followed by pressure homogenisation and formulation with 0.8% (v/w) canola oil and 5% (w/w) lecithin. The selected foxtail millet milk formulation was accepted by 65% of consumer sensory panellists using hedonic rating scale. The foxtail millet milk contains a good profile of carbohydrates and fat and it has also shown microbial stability during the 4 weeks of storage at 4 °C. Therefore, foxtail millet milk could be a potential alternative for bovine milk, which can be commercialised due to its novelty.

This study has also shown some limitations on optimising properties including particle size and gravitational stability of the foxtail millet milk. Further investigations on improving these properties are recommended. Some of these recommendations are as follows:

- Investigate particle size and gravitational stability of foxtail millet milk for optimization. Such as using ultra high-pressure homogenisation (200-600 MPa, 30-85 °C) and other technique like ultrasound homogeniser to reduce particle size and improve stability.
- Use different enzymes, emulsifiers and other stabilisers like guar gum, xanthan gum to enhance the storage stability.
- Use different levels of oil and emulsifier addition rate.
- Quantify the viscosity of the milk at different levels of shear rate. Use more rheological measurements to determine the texture of the foxtail millet milk.
- Improve quantification of physical stability using equipment like Lumisizer™ and Zetasizer for more precise results
- Improve the sensory profile of the foxtail millet milk by using different flavour additives.

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APPENDICES

A. Raw data

Table A.1 °Brix, pH and colour of foxtail millet extract during extraction in Phase I.

Mill	Millet ratio	Enzyme rate	Brix1	Brix2	pH1	pH2	L1	a1	b1	WI1	L1	a2	b2	WI2
Dry	8.00%	0.10%	5.80	5.7	6.38	6.38	40.91	0.18	-3.28	40.82	40.2	0.18	-3.28	40.11
Dry	8.00%	0.10%	5.60	5.7	6.38	6.38	41.37	0.07	-3.39	41.27	41.41	0.4	-2.75	41.34
Dry	8.00%	0.20%	5.90	5.9	6.37	6.37	39.42	-0.36	-1.71	39.39	39.07	-0.3	1.79	39.04
Dry	8.00%	0.20%	5.80	5.8	6.36	6.36	35.94	0.06	-1.74	35.92	39.07	-0.3	1.79	39.04
Dry	10.00%	0.10%	6.80	6.8	6.41	6.41	37.4	0.04	-2.09	37.37	38.41	-0.31	-2.15	38.37
Dry	10.00%	0.10%	6.60	6.6	6.43	6.43	46.32	-1.2	-0.26	46.31	43.9	-0.98	-0.98	43.88
Dry	10.00%	0.20%	7.40	7.3	6.43	6.43	37.98	-0.24	-2.33	37.94	38.83	-0.16	-2.47	38.78
Dry	10.00%	0.20%	7.00	7	6.35	6.35	39.02	-0.26	-2.51	38.97	39.79	-0.2	-2.47	39.74
Dry	12.00%	0.10%	8.00	8	6.44	6.44	47.97	-1.4	0.09	47.95	48.6	-1.49	0.51	48.58
Dry	12.00%	0.10%	7.90	8.1	6.48	6.48	51.27	-1.76	1.7	51.21	51.46	-1.76	1.66	51.4
Dry	12.00%	0.20%	8.50	8.5	6.52	6.52	44.17	-0.73	-1.5	44.15	44.17	-0.75	-1.51	44.14
Dry	12.00%	0.20%	8.60	8.5	6.48	6.48	43.28	-0.92	0.02	43.27	44	-1.1	0.35	43.99
Wet	8.00%	0.10%	5.50	5.5	6.53	6.53	37.17	0.19	-3	37.10	37.08	0.01	-3.11	37
Wet	8.00%	0.10%	5.40	5.5	6.55	6.55	37.02	0.07	-3.13	36.94	37	0.01	-3.11	36.94
Wet	8.00%	0.20%	5.60	5.6	6.98	6.98	37.17	-0.56	-1.47	37.15	41.58	-0.67	-1.37	41.56
Wet	8.00%	0.20%	5.60	5.5	6.96	6.96	37.28	-0.67	-1.39	37.26	37.08	0.01	-3.11	37
Wet	10.00%	0.10%	6.70	6.7	6.5	6.5	39.15	-0.15	-2.05	39.12	36.88	0.3	-1.88	36.85
Wet	10.00%	0.10%	6.60	6.7	6.55	6.55	38.16	0.19	-2.05	38.13	38.75	-0.15	-2.05	38.72
Wet	10.00%	0.20%	6.80	6.8	6.55	6.55	38.23	-0.16	1.41	38.21	38.28	-0.6	-1.08	38.27
Wet	10.00%	0.20%	6.70	6.8	6.58	6.58	38.36	-0.43	-1.27	38.35	38.21	-0.43	-1.27	38.2
Wet	12.00%	0.10%	7.70	7.7	6.58	6.58	42.85	-0.14	-1.83	42.82	42.89	-0.04	-1.8	42.86
Wet	12.00%	0.10%	7.60	7.7	6.57	6.57	42.52	-0.2	-1.81	42.49	42.48	-0.18	-1.83	42.45
Wet	12.00%	0.20%	7.90	7.8	6.64	6.64	40.98	0.17	-1.76	40.95	40.98	0.17	-1.76	40.95
Wet	12.00%	0.20%	7.80	7.8	6.68	6.68	40.82	0.22	-1.79	40.79	40.82	0.22	-1.79	40.79

Table A.2- Viscosity, pH, °Brix and colour of the emulsified foxtail millet extract in Phase II.

Sample	Millet %	Oil addition rate	Lecithin Rate	Viscosity	pH	brix	L	a	b	WI	L-2	a-2	b-2	WI-2
A5	10%	0.80%	5.00%	13.8	6.5	9.3	57.72	-2.03	2.87	57.57411	57.92	-2.07	3.06	57.75814
B5	10%	1.00%	5.00%	15.1	6.55	9.6	58.24	-1.93	2.86	58.09771	58.07	-1.92	2.89	57.92669
C5	10%	1.20%	5.00%	18.3	6.61	9.6	61.88	-2.33	5.46	61.42054	60.84	-2.31	5.37	60.40608
A7	10%	0.80%	7.00%	14.5	6.55	9.6	57.95	-1.72	1.73	57.87929	58	-1.76	1.78	57.92547
B7	10%	1.00%	7.00%	16	6.53	9.6	59.35	-1.92	3.04	59.19129	58.1	-1.84	2.79	57.96692
C7	10%	1.20%	7.00%	19.8	6.58	9.2	61.54	-2.11	4.22	61.25168	61.53	-2.1	4.21	61.24339
A5-2	10%	0.80%	5.00%	13.6	6.6	9	58.09	-1.95	3.16	57.92583	57.8	-2.01	3.21	57.63039
B5-2	10%	1.00%	5.00%	15.5	6.57	9.1	58.77	-1.5	2	58.69428	57.79	-1.77	2.79	57.66088
C5-2	10%	1.20%	5.00%	18.9	6.58	9.6	60.51	-2.05	3.68	60.28596	60.66	-2.08	3.73	60.42886
A7-2	10%	0.80%	7.00%	14	6.63	9.6	57.88	-2.07	2.65	57.74598	57.73	-2.03	2.61	57.60087
B7-2	10%	1.00%	7.00%	15.3	6.68	9.5	60.94	-2.16	3.01	60.76469	58.78	-2.02	3.09	58.61502
C7-2	10%	1.20%	7.00%	20.9	6.65	9.1	61.1	-2.2	3.78	60.85491	61.15	-2.22	3.87	60.89466
A5*	12%	0.80%	5.00%	13.6	6.64	7.8	53.01	-1.37	0.47	52.98768	53.16	-1.37	0.49	53.13741
B5*	12%	1.00%	5.00%	14.3	6.55	7.4	57.81	-1.74	2.29	57.71208	57.47	-1.72	2.14	57.38147
C5*	12%	1.20%	5.00%	15.9	6.54	6.8	58.62	-1.6	1.51	58.56156	58.24	-1.51	0.96	58.20168
A7*	12%	0.80%	7.00%	13.6	6.65	8	56.26	-1.48	0.19	56.23456	56.36	-1.51	0.27	56.33305
B7*	12%	1.00%	7.00%	16.2	6.67	7.6	57.46	-1.48	1.13	57.41927	57.69	-1.49	1.19	57.64705
C7*	12%	1.20%	7.00%	19.2	6.58	7.4	59.43	-1.7	2.12	59.33909	61.98	-2.07	3.53	61.76041
A5*-2	12%	0.80%	5.00%	14	6.62	7.7	53.73	-1.7	1.71	53.66721	53.81	-1.56	1.54	53.75801
B5*-2	12%	1.00%	5.00%	14.2	6.6	7.4	57.73	-1.74	2.31	57.63118	58.78	-1.91	2.54	58.65767
C5*-2	12%	1.20%	5.00%	15.7	6.63	6.9	59.16	-1.88	2.15	59.06026	60.75	-1.88	2.15	60.64623
A7*-2	12%	0.80%	7.00%	12.4	6.68	8.1	57.26	-1.68	1.15	57.21154	56.17	-1.87	1.37	56.10874
B7*-2	12%	1.00%	7.00%	16.7	6.65	7.6	57.72	-1.38	0.85	57.68895	56.45	-1.28	0.65	56.42635
C7*-2	12%	1.20%	7.00%	19.2	6.53	7.6	61.09	-1.97	3.78	60.85722	60.68	-1.9	3.59	60.47076

Note: Column marked with '-2' represents the duplications.

Table A.3- Particle size of d_{32} and d_{43} of the emulsified foxtail millet extract in Phase II.

Sample Name	Millet %	d32	d43	Sample Name	Millet %	d32	d43
A5-1	10%	6.17	12.9	A5 -1	12%	8.18	18.1
A5-1	10%	5.86	12	A5 -1	12%	7.66	16.2
A5-1	10%	5.8	11.8	A5 -1	12%	7.52	15.7
A5-1	10%	5.8	11.7	A5 -1	12%	7.45	15.4
A5-1	10%	5.82	11.8	A5 -1	12%	7.4	15.2
A5-2	10%	6.88	14.3	A5-2	12%	6.66	14.4
A5-2	10%	6.56	13.2	A5-2	12%	6.05	12.6
A5-2	10%	6.51	13	A5-2	12%	6.61	240
A5-2	10%	6.49	12.9	A5-2	12%	5.72	12.7
A5-2	10%	6.49	12.9	A5-2	12%	6.75	299
A7-1	10%	5.47	12.2	A7-1	12%	6.08	17.3
A7-1	10%	5.34	11.9	A7-1	12%	5.5	12.5
A7-1	10%	5.36	12	A7-1	12%	5.3	11.8
A7-1	10%	5.43	12.2	A7-1	12%	5.25	12.8
A7-1	10%	5.46	12.3	A7-1	12%	5.15	12.5
A7-2	10%	6.08	160	A7-1	12%	6.86	13.3
A7-2	10%	5.54	11.6	A7-1	12%	6.78	13.2
A7-2	10%	5.68	12.1	A7-1	12%	6.77	13.2
B5-1	10%	5.36	12.5	A7-1	12%	6.79	13.2
B5-1	10%	5.14	11.8	A7-1	12%	6.82	13.3
B5-1	10%	5.09	13.7	B5-1	12%	7.82	17.5
B5-1	10%	4.97	12.5	B5-1	12%	7.02	15.2
B5-1	10%	4.87	11.3	B5-1	12%	6.67	14.3
B5-2	10%	6.63	14.5	B5-1	12%	6.45	13.7
B5-2	10%	6.43	13.8	B5-1	12%	6.3	13.3
B5-2	10%	6.43	13.8	B5-2	12%	7.31	15.5
B5-2	10%	6.44	13.8	B5-2	12%	6.98	14.3
B5-2	10%	6.51	14	B5-2	12%	6.83	13.7
B7-1	10%	5.3	12.8	B5-2	12%	6.8	13.6
B7-1	10%	5.33	12.8	B5-2	12%	6.78	13.4
B7-1	10%	5.38	13	B7-1	12%	8.26	18
B7-1	10%	5.44	13.2	B7-1	12%	7.66	16.1
B7-1	10%	5.5	13.4	B7-1	12%	7.49	15.5
B7-2	10%	6.87	14.8	B7-1	12%	7.39	15.1
B7-2	10%	6.63	14.1	B7-1	12%	7.32	14.9
B7-2	10%	6.62	14.1	B7-2	12%	6.22	13
B7-2	10%	6.63	14	B7-2	12%	6.02	12.5
B7-2	10%	6.67	14.1	B7-2	12%	6.04	12.5
C5-1	10%	5.99	15.5	B7-2	12%	6.07	12.6
C5-1	10%	5.69	14	B7-2	12%	6.11	12.7
C5-1	10%	5.6	13.6	C5-1	12%	7.1	15.6
C5-1	10%	5.77	62.6	C5-1	12%	6.62	14.2
C5-1	10%	5.54	13.3	C5-1	12%	6.39	13.6
C5-2	10%	6.16	13.7	C5-1	12%	6.22	13.4
C5-2	10%	5.99	13	C5-1	12%	6.12	13.2
C5-2	10%	6.02	13.1	C5-2	12%	7.78	16.3
C5-2	10%	6.07	13.2	C5-2	12%	7.39	15
C5-2	10%	6.14	13.3	C5-2	12%	7.28	14.6
C7-1	10%	6.34	14.7	C5-2	12%	7.23	14.4
C7-1	10%	6.1	13.7	C5-2	12%	7.2	14.2
C7-1	10%	6.08	13.7	C7-1	12%	5.51	14
C7-1	10%	6.08	13.6	C7-1	12%	5.1	11.6
C7-1	10%	6.09	13.6	C7-1	12%	4.98	14.3
C7-2	10%	8.1	17.8	C7-1	12%	4.79	10.6
C7-2	10%	7.62	16	C7-1	12%	4.73	10.4
C7-2	10%	7.5	15.6	C7-2	12%	7.2	15
C7-2	10%	7.4	15.2	C7-2	12%	6.84	14.1
C7-2	10%	7.36	15.1	C7-2	12%	6.77	13.8

Table A.4- Particle size of foxtail millet extract at different homogenisation pressure (bar).

Bar	d32	d43
200	7.14	19.6
200	6.93	29.4
200	6.42	17.4
450	7.03	18.6
450	6.56	18.2
450	6.46	17.7
450	6.42	17.8
450	6.39	17.7
300	6.56	18.4
300	6.54	17.2
300	6.43	16.9
300	6.4	16.8
300	6.61	16.7
600	6.3	15.3
600	6.16	15.2
600	5.41	14.7
600	6.25	15.5

Table A.5- Particle size of foxtail millet extract at different processing stage.

Process	d32	d43
BHSM	76.3	296
BHSM	98.1	483
BHSM	122	576
BHSM	107	661
BHSM	93.8	601
BHSM	145	614
BHSM	129	482
BHSM	189	883
BHSM	120	427
BHSM	124	474
AHSM	18.5	47
AHSM	17.8	46.1
AHSM	17.7	48
AHSM	17.1	45.4
AHSM	24.6	49.3
AHSM	22.1	45.4
AHSM	21.3	44.5
AHSM	20.9	44.5
AHSM	20.4	43.3
APH	11.9	41.9
APH	12.1	43
APH	12.4	43.3
APH	12.7	42.9
APH	12.8	42.1
APH	11.3	43.9
APH	11.3	43.8
APH	11.5	44.9
APH	11.6	44.5

Table A.6- Particle size of foxtail millet milk during 4 weeks of storage at 4°C.

Week	d32	d43
0	7.66	16.2
0	7.52	15.7
0	7.45	15.4
0	7.4	15.2
0	6.66	14.4
0	6.05	12.6
0	5.72	12.7
1	11.7	16.5
1	7.55	16.7
1	6.96	15.4
1	6.67	15.6
1	6.99	18.2
1	6.96	17.6
1	7.42	15.2
1	7.15	14
1	7.05	13.5
2	9.31	22.5
2	8.06	19
2	7.58	17.8
2	7.33	17.3
2	14.6	16.2
2	11	26.6
2	9.61	23
2	8.89	20.9
2	8.43	19.5
3	13.9	27
3	10.3	27.3
3	9.02	23.5
3	8.34	21.5
3	7.85	19.9
3	14.3	27.7
3	11.3	21.9
3	10.1	19.6
3	9.51	18.4
3	9.11	17.8
4	10.1	21.7
4	10.6	23
4	11.3	24.9
4	12.9	28.9
4	16.1	25.8
4	16	29.2
4	13.1	23.4
4	11.9	21.1
4	11.2	19.8
4	10.8	19.1

B. Statistical outputs

B.1 Phase I: One-way ANOVA analysis of colour results

One-way ANOVA: Whiteness Index versus millet ratio

Analysis of variance

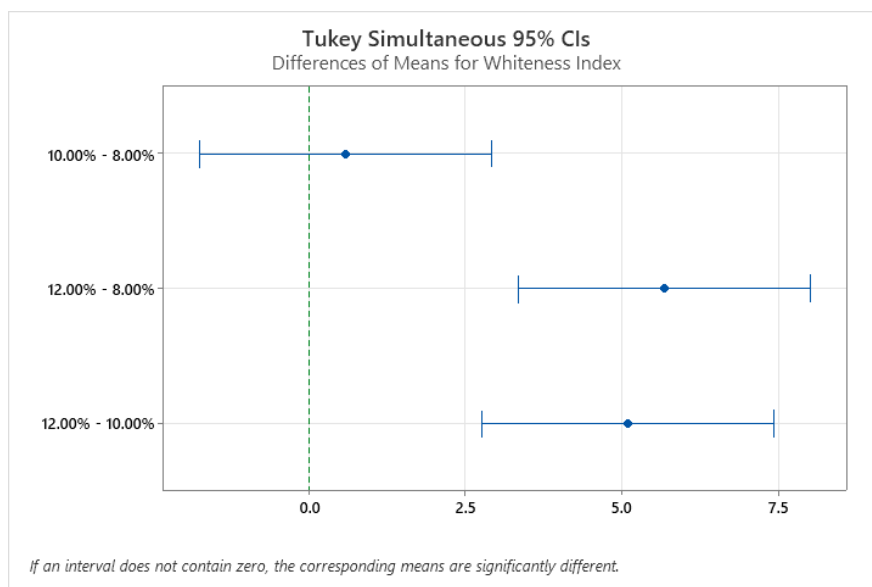
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Millet ratio	2	80.09	40.047	30.78	0.000
Error	21	27.32	1.301		
Total	23	107.41			

Tukey Pairwise Comparisons

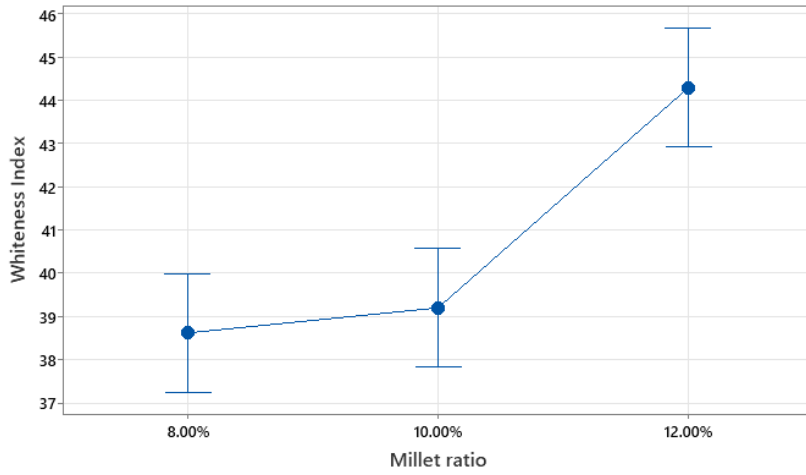
Grouping Information Using the Tukey Method and 95% Confidence

Millet ratio	N	Mean	Grouping
12.00%	16	44.300	A
10.00%	16	39.199	B
8.00%	16	38.618	B

Means that do not share a letter are significantly different.

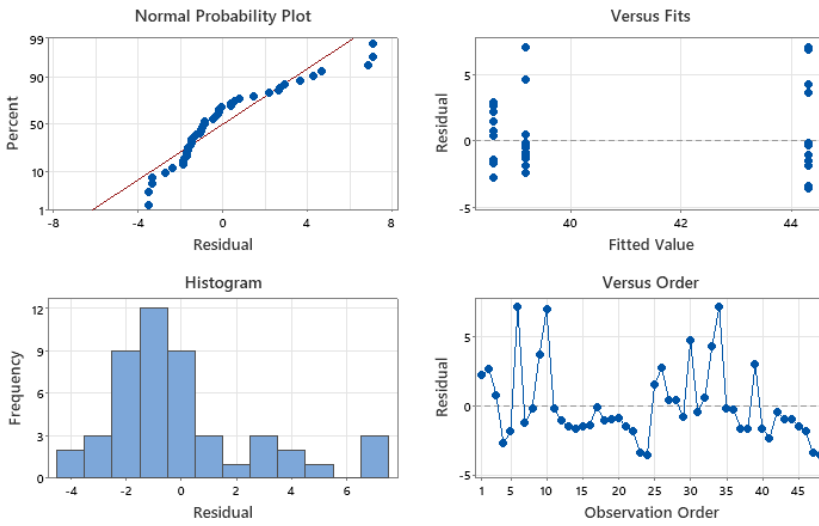


Interval Plot of Whiteness Index vs Millet ratio
95% CI for the Mean



The pooled standard deviation is used to calculate the intervals.

Residual Plots for Whiteness Index



One-way ANOVA: a* versus millet ratio

Analysis of Variance

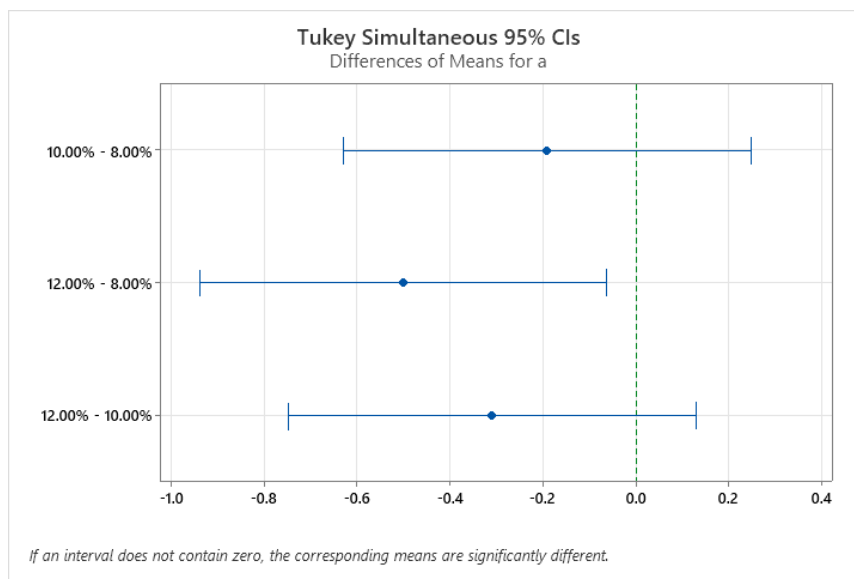
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Millet ratio	2	2.042	1.0211	3.91	0.027
Error	45	11.737	0.2608		
Total	47	13.779			

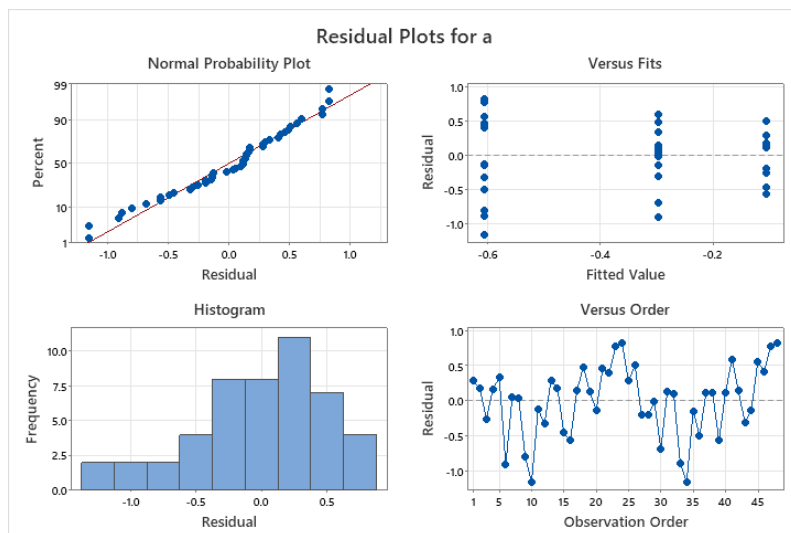
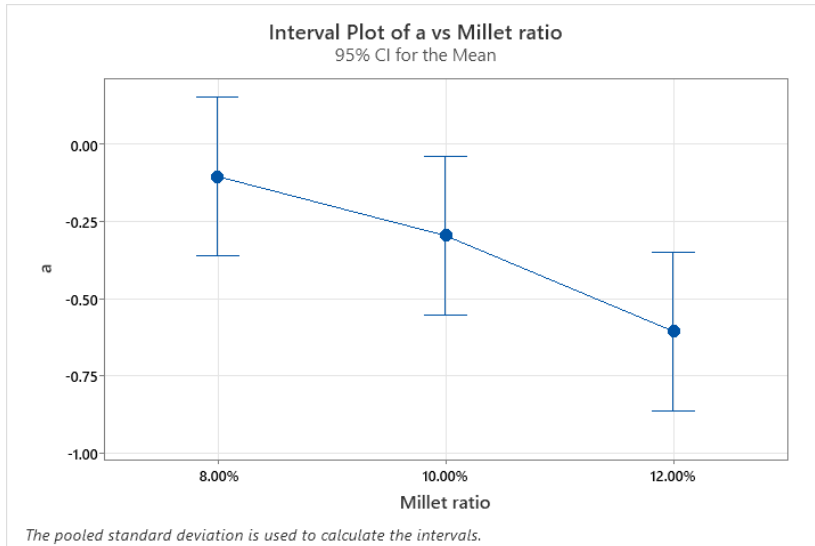
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Millet ratio	N	Mean	Grouping
8.00%	16	-0.1050	A
10.00%	16	-0.2963	A B
12.00%	16	-0.606	B

Means that do not share a letter are significantly different.





One-way ANOVA: b* versus Millet ratio

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Millet ratio	2	11.87	5.935	3.21	0.050
Error	45	83.24	1.850		
Total	47	95.11			

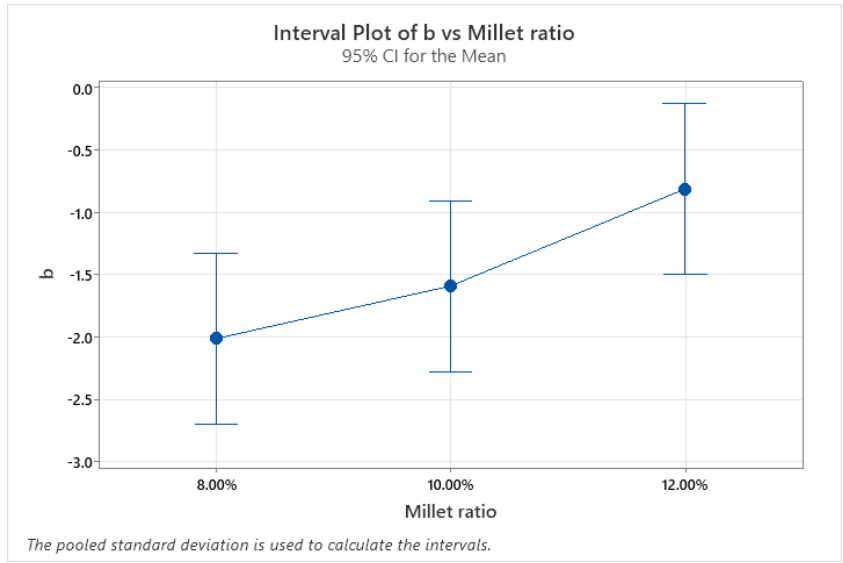
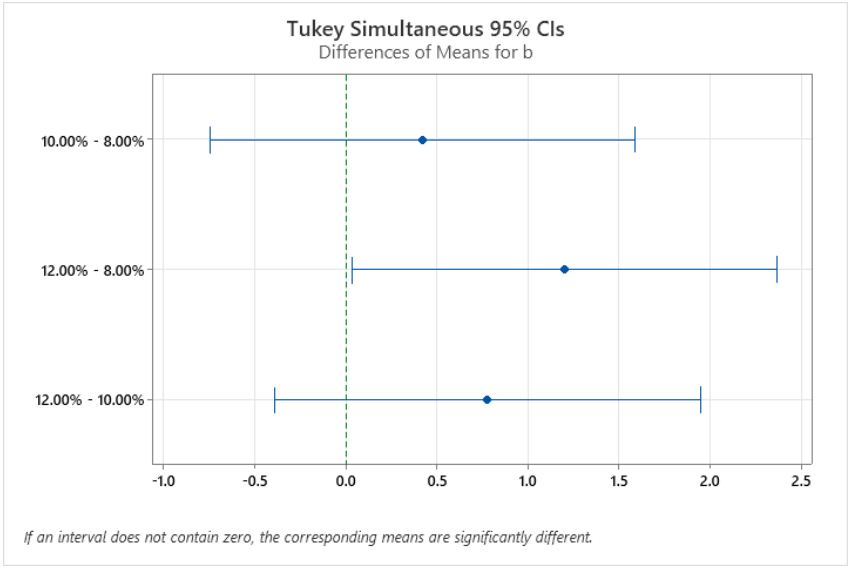
Tukey Pairwise Comparisons

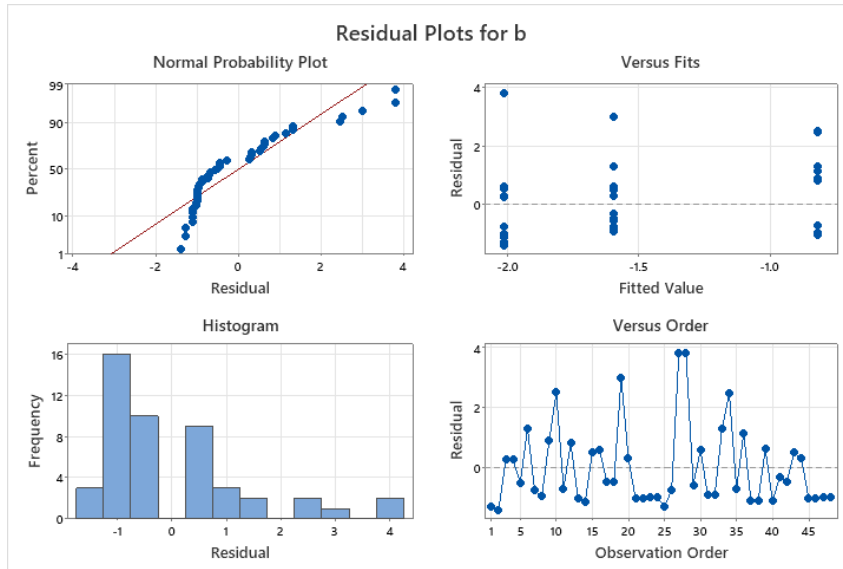
Grouping Information Using the Tukey Method and 95% Confidence

Millet ratio	N	Mean	Grouping
12.00%	16	-0.816	A

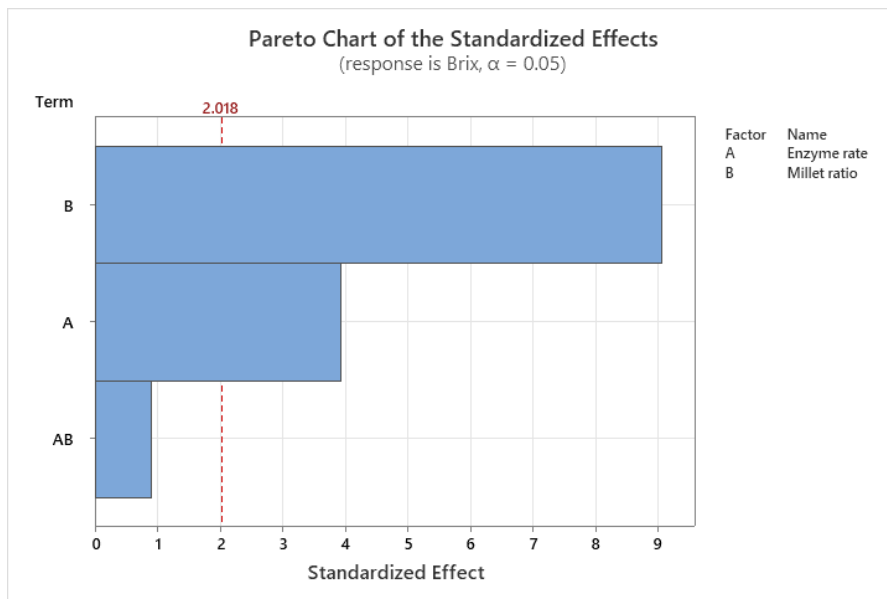
10.00%	16	-1.594	A	B
8.00%	16	-2.016		B

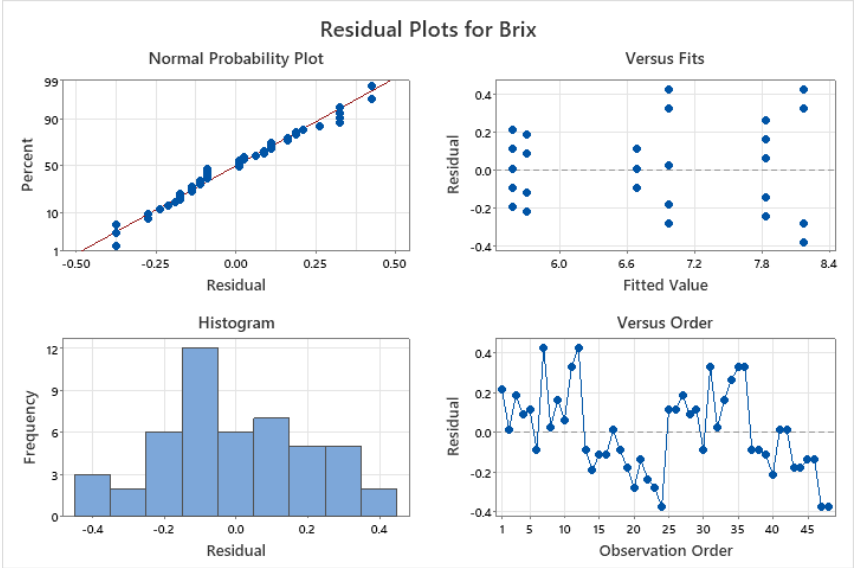
Means that do not share a letter are significantly different.





B.2 Phase I: General Factorial Regression: Brix versus Enzyme rate, Millet ratio





B.3 Phase I: One-way ANOVA: pH versus Mill

Analysis of Variance

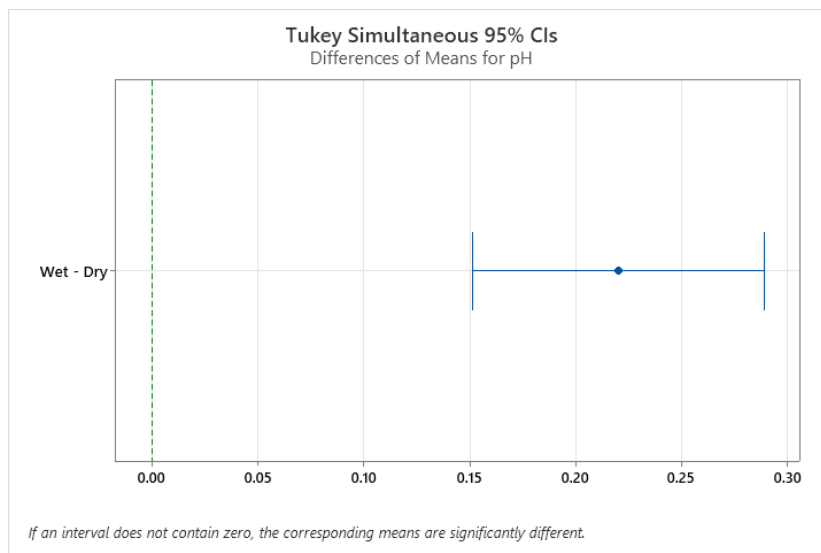
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Mill	1	0.5808	0.58080	41.77	0.000
Error	46	0.6396	0.01390		
Total	47	1.2204			

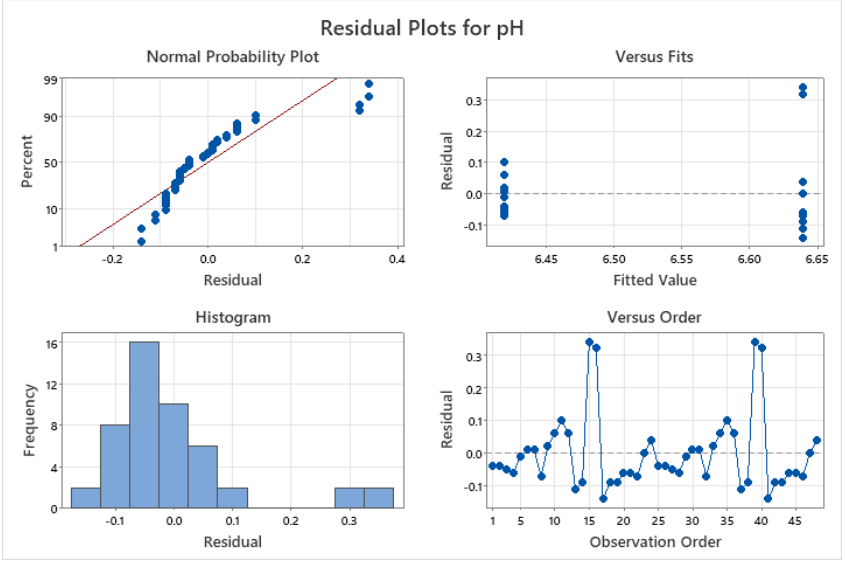
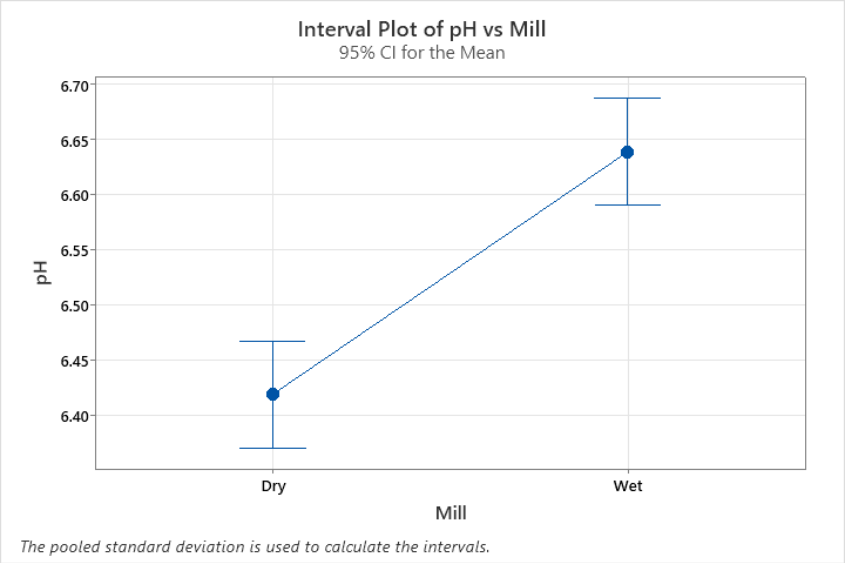
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Mill	N	Mean	Grouping
Wet	24	6.6392	A
Dry	24	6.4192	B

Means that do not share a letter are significantly different.





B.4 Phase II: One-way ANOVA analysis of colour results

One-way ANOVA: Whiteness Index (WI) versus Millet rate

Analysis of Variance

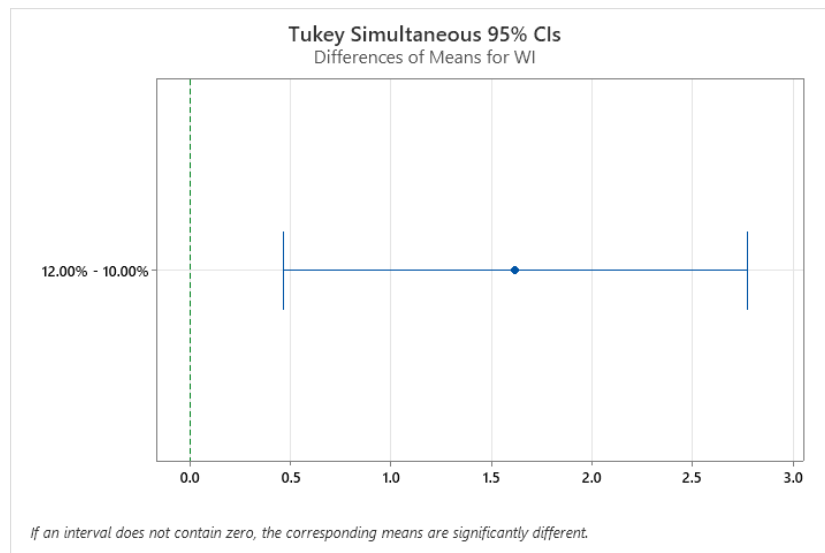
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Millet rate	1	31.43	31.435	8.01	0.007
Error	46	180.54	3.925		
Total	47	211.97			

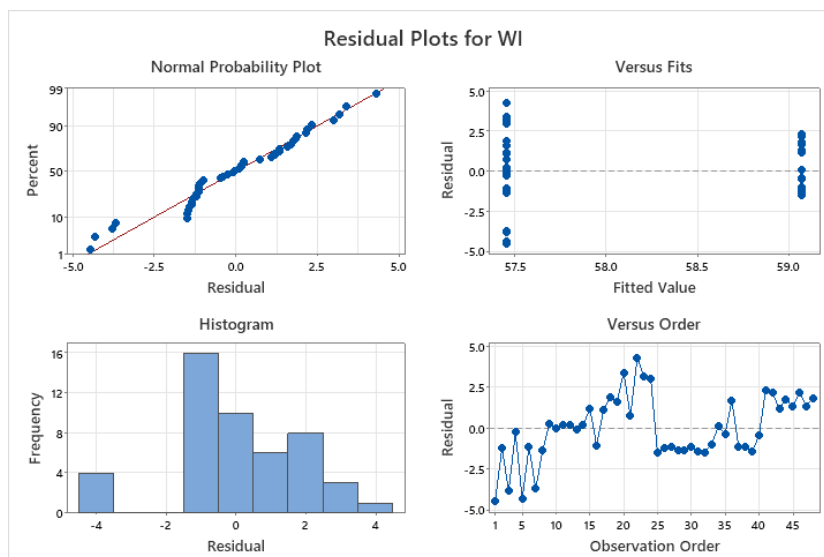
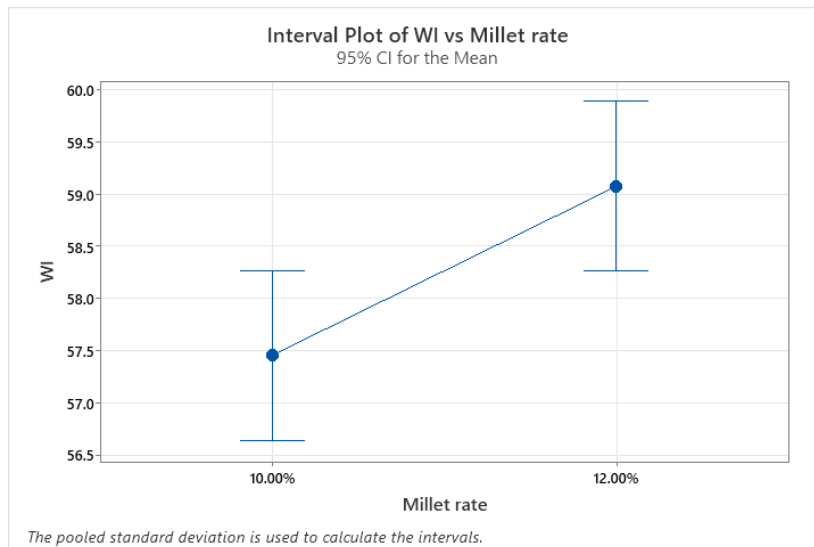
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Millet rate	N	Mean	Grouping
12.00%	24	59.073	A
10.00%	24	57.454	B

Means that do not share a letter are significantly different.





One-way ANOVA: Whiteness Index versus Oil addition level

Analysis of Variance

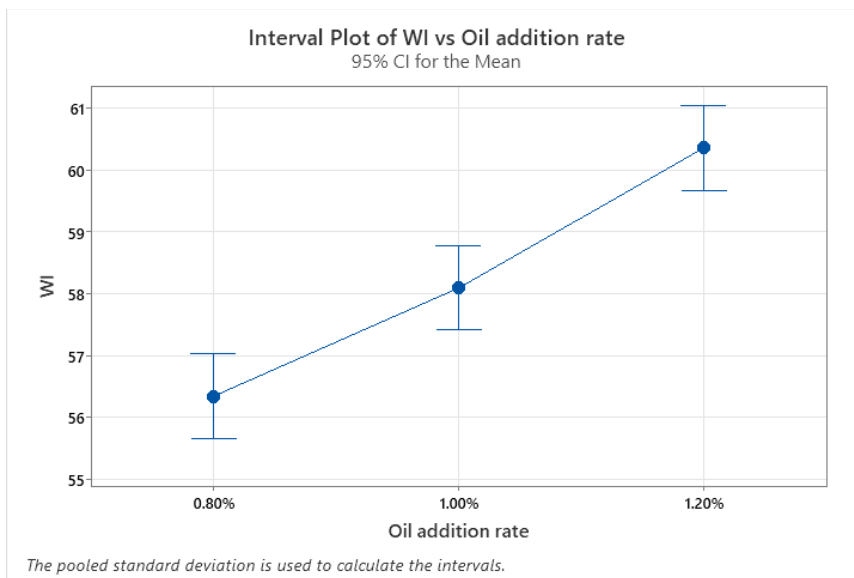
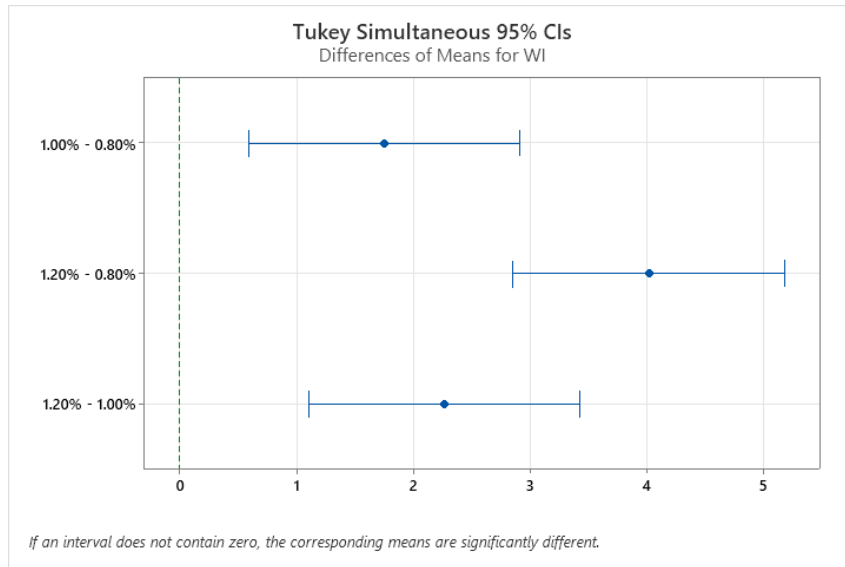
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Oil addition level	2	129.52	64.761	35.35	0.000
Error	45	82.45	1.832		
Total	47	211.97			

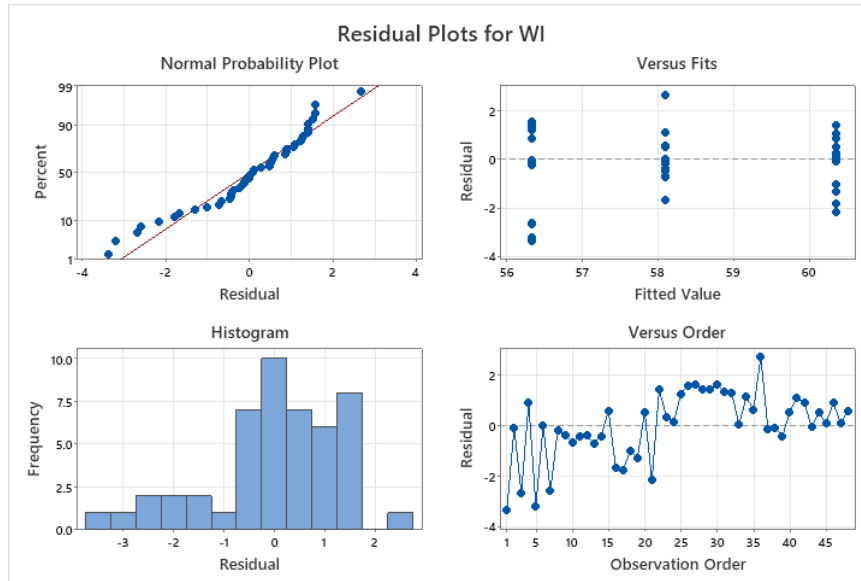
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Oil addition level	N	Mean	Grouping
1.20%	16	60.355	A
1.00%	16	58.093	B
0.80%	16	56.342	C

Means that do not share a letter are significantly different.





One-way ANOVA: a* versus Oil addition level

Analysis of Variance

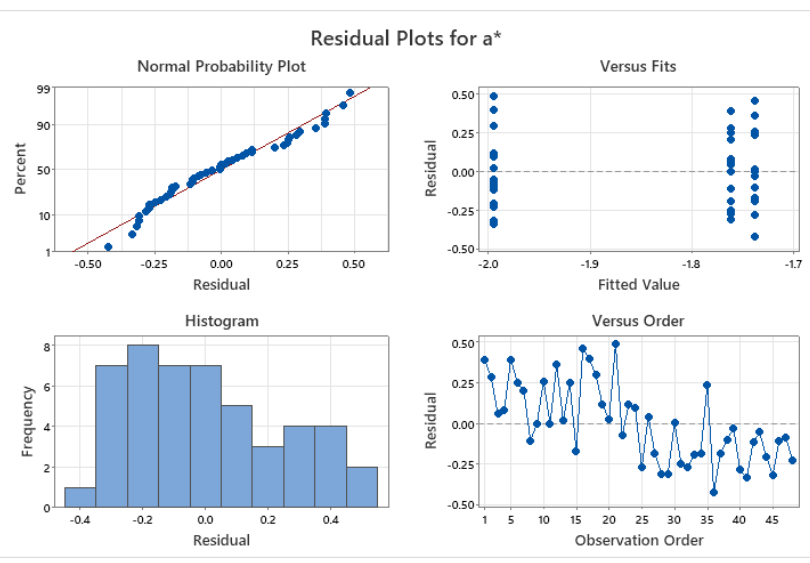
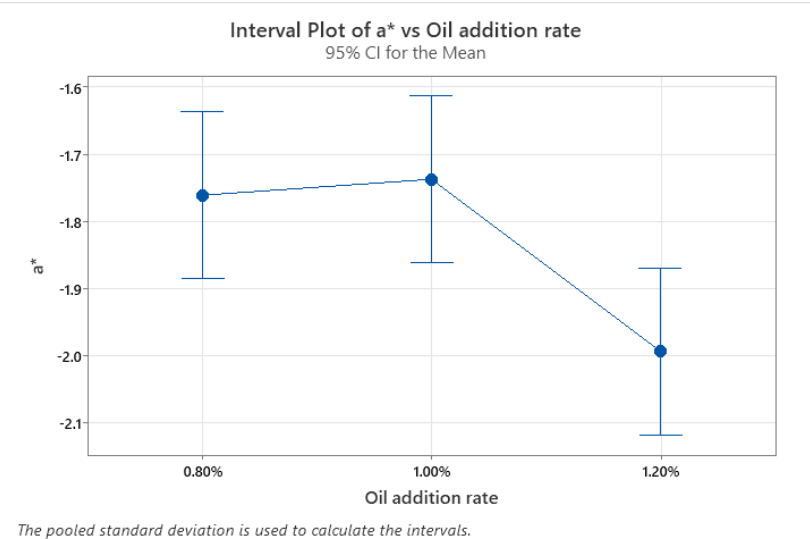
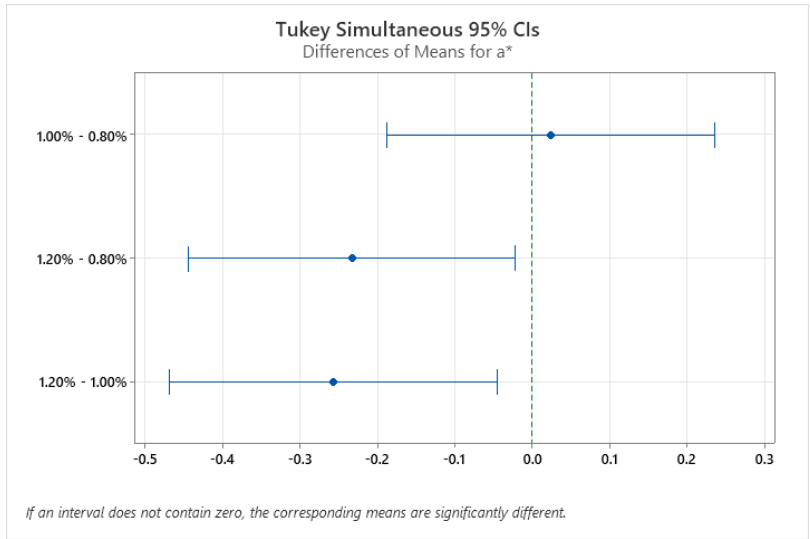
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Oil addition level	2	0.6448	0.32239	5.32	0.008
Error	45	2.7287	0.06064		
Total	47	3.3734			

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Oil addition level	N	Mean	Grouping
1.00%	16	-1.7375	A
0.80%	16	-1.7612	A
1.20%	16	-1.9944	B

Means that do not share a letter are significantly different.



One-way ANOVA: b* versus Oil addition level

Analysis of Variance

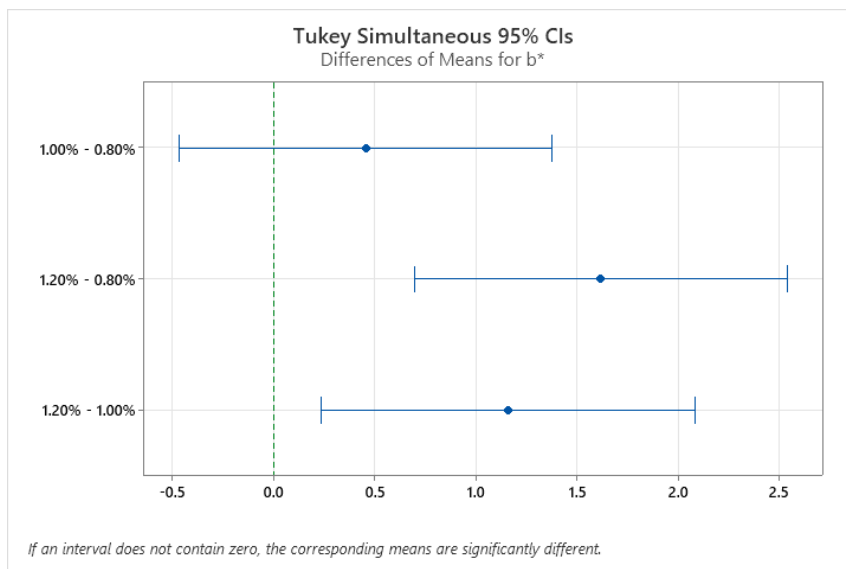
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Oil addition level	2	22.20	11.098	9.63	0.000
Error	45	51.85	1.152		
Total	47	74.04			

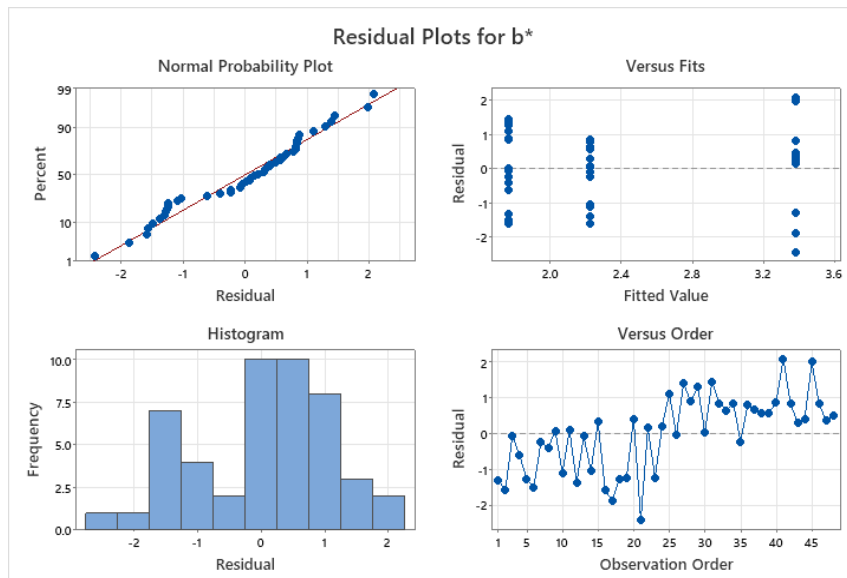
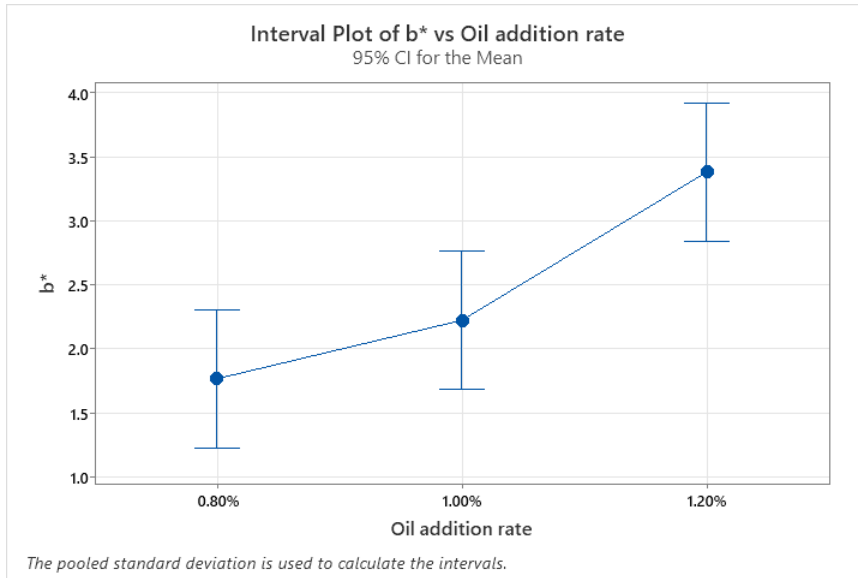
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Oil addition level	N	Mean	Grouping
1.20%	16	3.382	A
1.00%	16	2.223	B
0.80%	16	1.766	B

Means that do not share a letter are significantly different.





B.5 Phase II: One-way ANOVA: d_{43} versus processing stage

Analysis of Variance

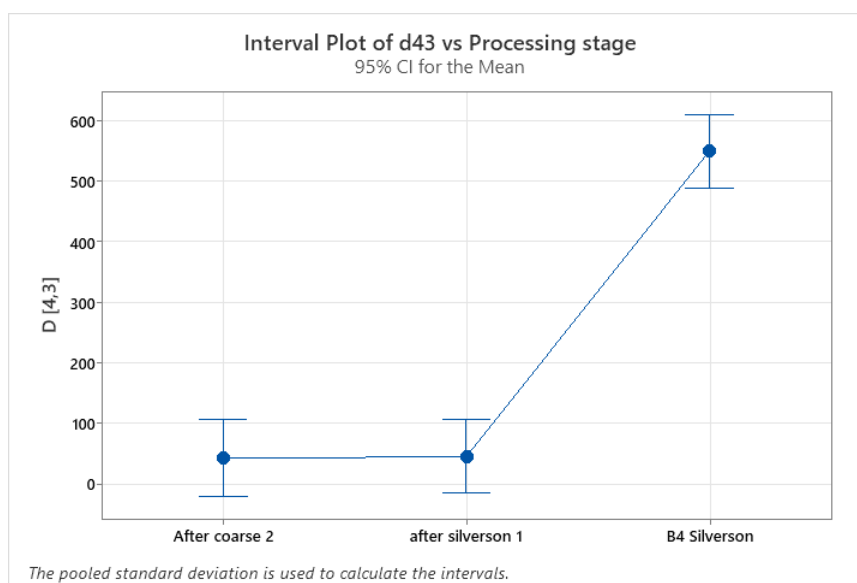
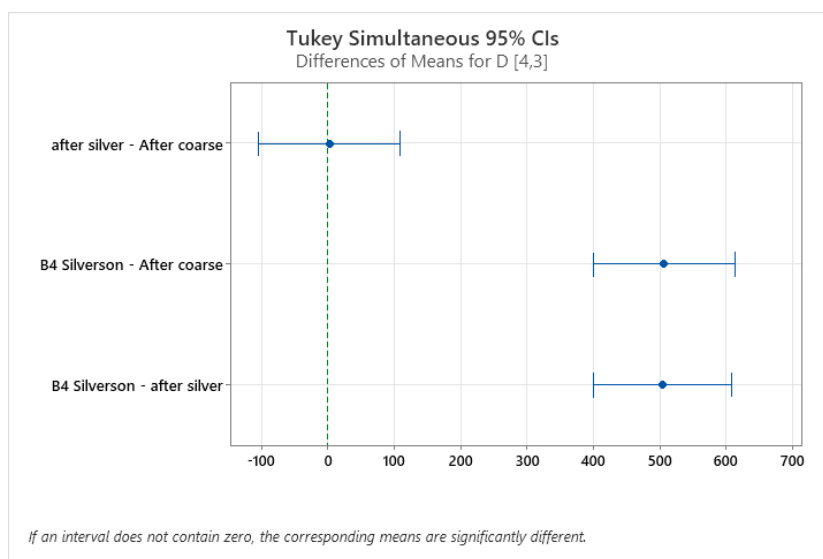
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample Name	2	1670400	835200	96.45	0.000
Error	26	225154	8660		
Total	28	1895553			

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample Name	N	Mean	Grouping
B4 Silverson	10	549.7	A
after silverson 1	10	46.040	B
After coarse 2	9	43.367	B

Means that do not share a letter are significantly different.



B.6 Phase II: One-way ANOVA: d₄₃ versus Homogenisation pressure

Analysis of Variance

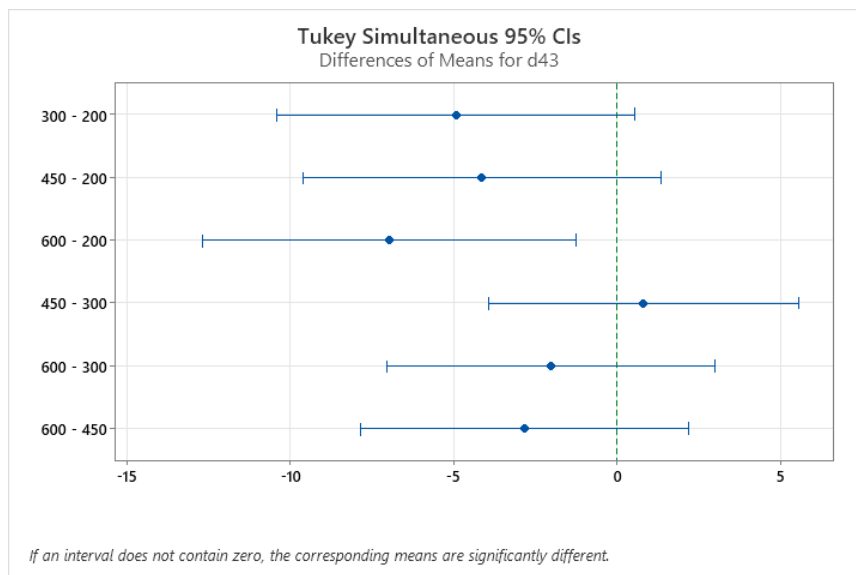
Source	Adj SS	Adj MS	F-Value	P-Value
Bar	85.88	28.627	4.40	0.024
Error	84.53	6.503		
Total	170.42			

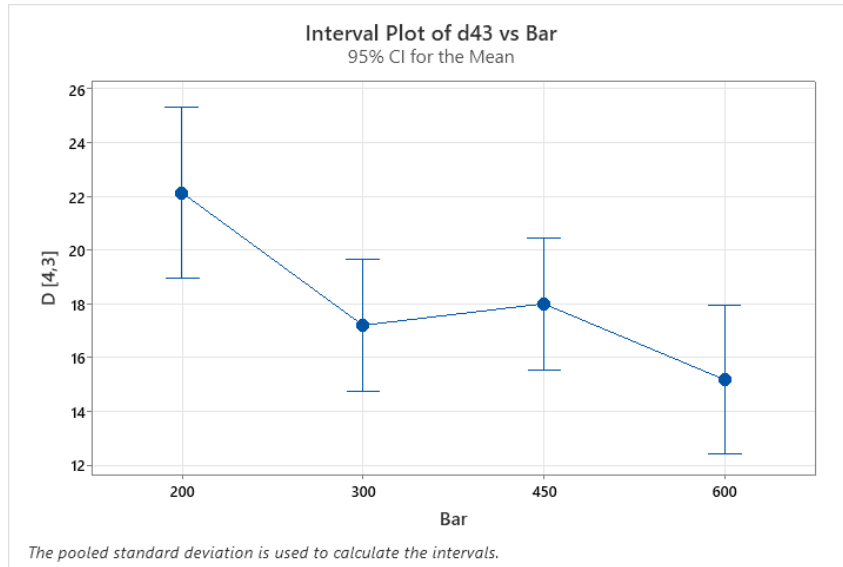
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Bar	N	Mean	Grouping	
200	10	22.13	A	
450	10	18.000	A	B
300	10	17.200	A	B
600	10	15.175	B	

Means that do not share a letter are significantly different.





B.6 Phase II: One-way ANOVA: d₃₂ versus Homogenisation pressure

Analysis of Variance

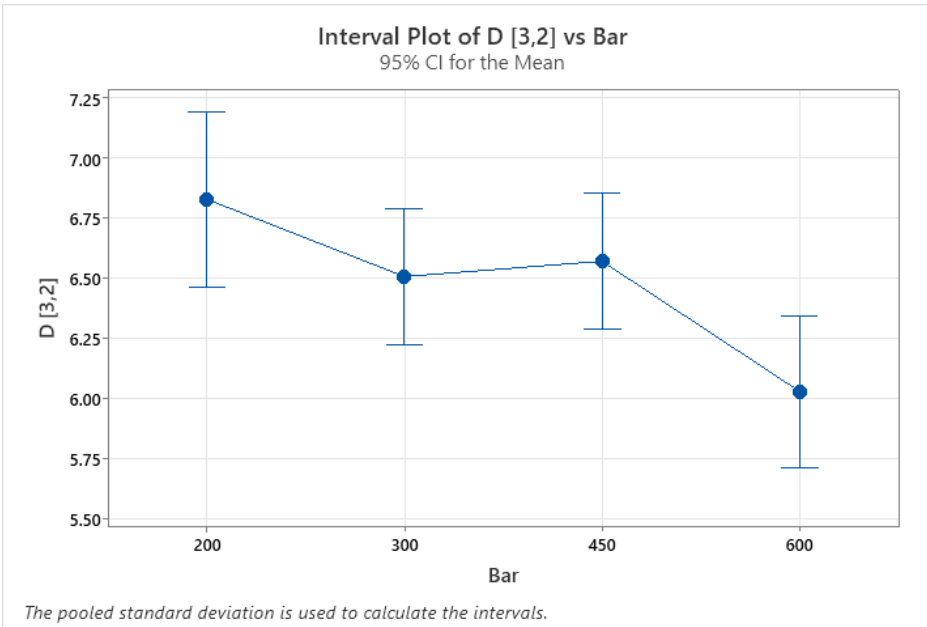
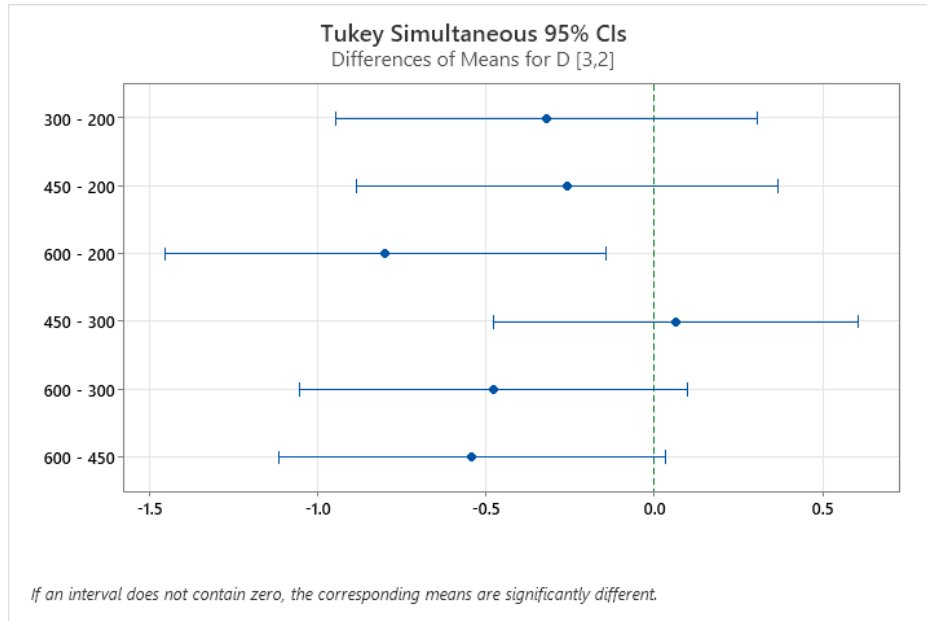
Source	Adj SS	Adj MS	F-Value	P-Value
Bar	1.222	0.40747	4.78	0.019
Error	1.107	0.08518		
Total	2.330			

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Bar	N	Mean	Grouping	
200	10	6.830	A	
450	10	6.572	A	B
300	10	6.5080	A	B
600	10	6.030		B

Means that do not share a letter are significantly different.



B.6 Phase III: One-way ANOVA: d₄₃ versus week during storage

Analysis of Variance

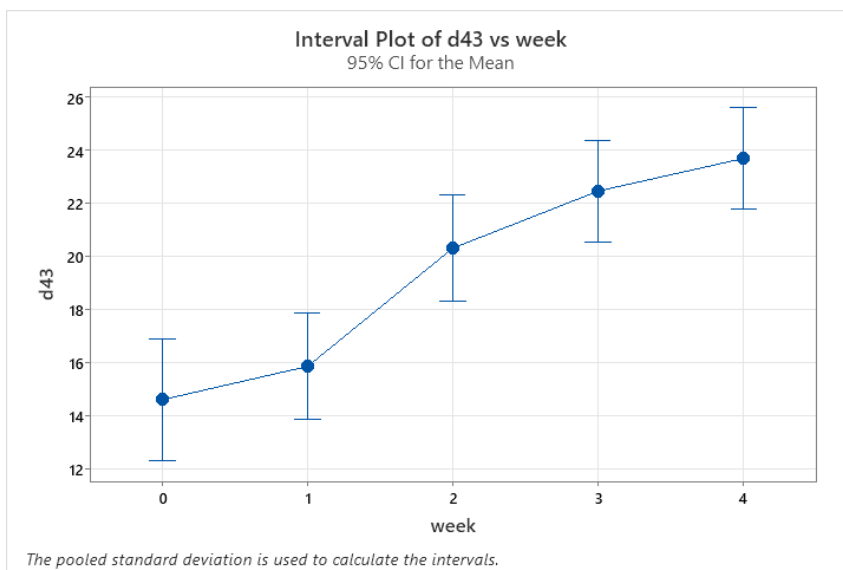
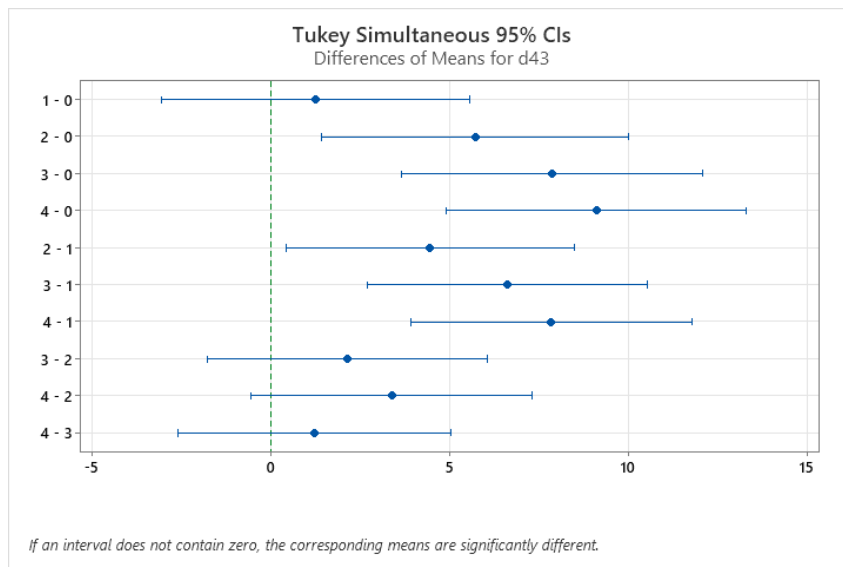
Source	DF	Adj SS	Adj MS	F-Value	P-Value
week	4	553.7	138.416	15.55	0.000
Error	40	355.9	8.899		
Total	44	909.6			

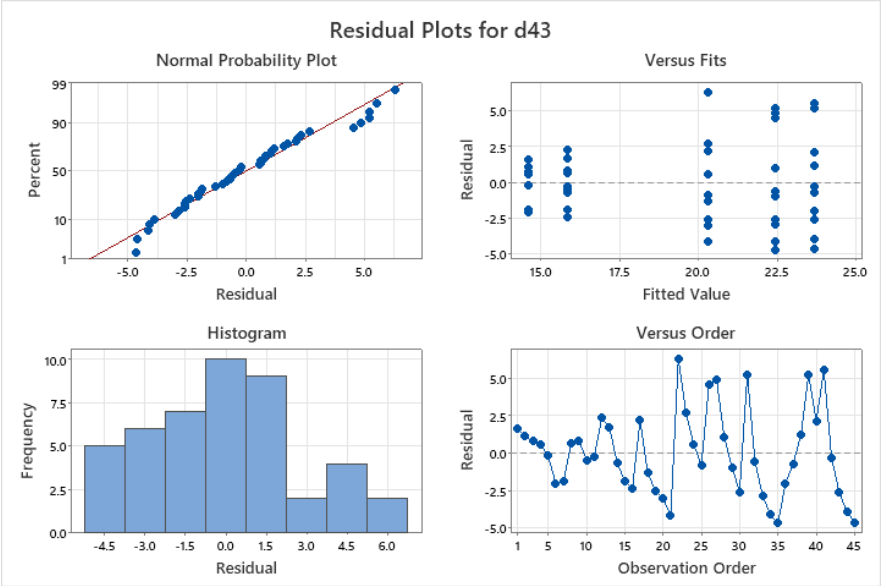
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Week	N	Mean	Grouping
4	10	23.69	A
3	10	22.46	A
2	10	20.31	A
1	10	15.856	B
0	10	14.600	B

Means that do not share a letter are significantly different.





C. Sensory evaluation set up and questionnaire



Could you let us know how frequently do you normally consume plant-based milk?

Daily

Weekly

Occasionally

Never

Please select the phrase that best describes how much you like or dislike the **Colour** of this product.

Dislike Extremely (1)	Very Dislike (2)	Dislike Moderately (3)	Dislike slightly (4)	Neutral (5)	Like Slightly (6)	Like Moderately (7)	Like Very Much (8)	Like Externely (9)
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Please select the phrase that best describes how much you like or dislike the **Aroma** of this product.

Dislike Extremely (1)	Very Dislike (2)	Dislike Moderately (3)	Dislike slightly (4)	Neutral (5)	Like Slightly (6)	Like Moderately (7)	Like Very Much (8)	Like Externely (9)
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Please select the phrase that best describes how much you like or dislike the **Flavor** of this product.

Dislike Extremely (1)	Very Dislike (2)	Dislike Moderately (3)	Dislike slightly (4)	Neutra l (5)	Like Slightly (6)	Like Moderately (7)	Like Very Much (8)	Like Externely (9)
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Please select the phrase that best describes how much you like or dislike the **Mouthfeel** of this product.

Dislike Extremely (1)	Very Dislike (2)	Dislike Moderately (3)	Dislike slightly (4)	Neutral (5)	Like Slightly (6)	Like Moderately (7)	Like Very Much (8)	Like Externely (9)
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Please select the phrase that best describes your **Overall Opinion** of this product.

Dislike Extremely (1)	Very Dislike (2)	Dislike Moderately (3)	Dislike slightly (4)	Neutral (5)	Like Slightly (6)	Like Moderately (7)	Like Very Much (8)	Like Externely (9)
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