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To optimize the enzymatic process for the formulation of plant-based milk alternatives from mung beans

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ABSTRACT

The increasing demand for sustainable, lactose-free dairy alternatives has revealed limitations in existing plant-based milk products, particularly in protein content, stability, and sensory characteristics. This study addressed these challenges by optimizing enzymatic extraction and processing conditions for developing mung bean (*Vigna radiata*)–based milk. Previous studies demonstrated that enzymatic treatment and fermentation improved extraction efficiency, nutrient availability, and sensory properties; however, limited research had evaluated the interactive effects of key processing variables in mung bean systems.

The study hypothesized that soaking time, enzyme concentration, and fermentation duration significantly influenced the physicochemical properties, sensory quality, and shelf stability of mung bean milk. A quantitative factorial design was employed to evaluate soaking time (8 h and 14 h), enzyme concentration (1% and 2% w/w), and fermentation duration (2 h and 4 h). The samples were analyzed for pH, total soluble solids (TSS), water activity, color (L*, a*, b*), nutritional composition, and sensory attributes. Statistical analysis using ANOVA ($p \leq 0.05$) was conducted to determine significant effects and interactions.

The findings demonstrated that processing parameters significantly influenced product quality. Longer soaking (14 h) improved lightness and extraction characteristics, whereas shorter soaking (8 h) retained higher TSS. Increased enzyme concentration (2%) enhanced solubilization and emulsion stability, while extended fermentation (4 h) improved sensory acceptability but increased water activity. A significant interaction between enzyme concentration and fermentation duration indicated synergistic improvement in extraction efficiency. The optimized condition (14 h soaking, 2% enzyme, 4 h fermentation) produced a nutritionally balanced product with enhanced sensory quality and acceptable stability under refrigerated conditions.

These results confirmed the hypothesis and demonstrated that enzymatic-assisted processing was effective in developing high-quality mung bean milk. Future research should focus on the value-added utilization of liquid and solid by-products, fortification strategies, and process optimization to improve sustainability, functionality, and commercial scalability.

Key Words: Plant-based milk, Mung bean (*Vigna radiata*), Enzymatic extraction, Fermentation, Physicochemical properties

CHAPTER 1. INTRODUCTION

Plant-based milk, often termed as a plant-based milk alternative, is broadly defined as a water-soluble extract derived from distinct plant materials such as legumes, cereals, pseudocereals, oilseeds, or nuts, which visually and functionally resembles the color, texture, and nutritional attributes of bovine milk (Sethi et al., 2016; Makinen et al., 2016). According to the U.S. Food and Drug Administration (FDA, 2023) draft guidance permits labeling non-dairy beverages as “milk” provided the nutritional differences from dairy milk are disclosed, while the Codex Alimentarius Commission and the EU reserve the term “milk” generally for mammalian secretions except for coconut and almond, referencing compositional distinctions (FDA, 2023; European Union, 2013). National definitions differ in their strictness but converge in identifying plant-based milks as colloidal suspensions, with typical particle sizes ranging from 0.1 to 100 μm, produced by processes such as soaking, wet or dry milling, extraction, filtration, homogenization, and sometimes novel procedures like ultra-high-pressure homogenization or enzymatic hydrolysis (Makinen et al., 2016; Suryamiharja et al., 2024). These processes aim to create stable emulsions that mimic the physicochemical properties of dairy milk while meeting functional and nutritional demands (Aydar et al., 2020; Suryamiharja et al., 2024).

Comparatively, plant-based milks differ from dairy milk in several core aspects: they are naturally lactose-free and cholesterol-free, often lower in protein but higher in certain phytochemicals and fiber, and require fortification with calcium, vitamin D, or vitamin B12 to approach nutritional equivalence (Soczynska et al., 2024). Although dairy milk remains a key source of high-quality protein and micronutrients (calcium, potassium, vitamin D) in many diets, the growing variety and improving quality of plant-based milks present a dynamic challenge to the longstanding dominance of bovine milk, with differing strengths and limitations in protein quality, micronutrient content, and bioavailability (Nowson et al., 2025; Johnson et al., 2025; Suryamiharja et al., 2024).

Over the past decade, the plant-based milk market has experienced substantial growth, reflecting changing consumer preferences and increased production capacity. In 2024, the global market was valued at approximately USD 20.84 billion and is projected to grow to USD 32.35 billion by 2030, representing a compound annual growth rate (CAGR) of around 7.4% (Grand View Research, 2024). Regionally, Asia Pacific holds the largest share, accounting for over 47% of global revenues, with North America and Europe also showing significant market growth. For example, the U.S. plant-based milk market was valued at USD 3.77 billion in 2024 and is expected to reach USD 5.56 billion by 2030 (Grand View Research, 2024). Within Europe, oat milk alone has surged to capture 40% of the UK's plant-based milk volume. While the category experienced a 8.4% sales decline over two years in some markets, the overall trend remains robust, fueled by consumer interest and ongoing product innovation (Wood, 2025).

The growth of the plant-based milk sector is driven primarily by health, environmental, ethical, and dietary factors. Health-conscious consumers favor plant-based milks for their lower saturated fat and cholesterol levels, compatibility with lactose intolerance, and fortification with essential nutrients such as calcium, vitamin D, and B12 (Vanga & Raghavan, 2018; Jeske et al., 2017). Approximately 65% of the global population is lactose intolerant, which enhances the attractiveness of dairy alternatives (Misselwitz et al., 2019). Environmental sustainability also plays a vital role; plant-based milk alternatives generally have lower environmental impacts compared to dairy milk, although variations exist depending on the source and production system (Poore & Nemecek, 2018; Nowson et al., 2025). Ethical considerations regarding animal welfare and the rise of vegan, flexitarian, and vegetarian lifestyles further bolster demand (Sethi et al., 2016). Additionally, advancements in processing technologies, including ultrafiltration, high-pressure processing, and fermentation, have improved the nutritional profiles, sensory qualities, and shelf lives of plant-based milks, facilitating broader consumer acceptance (McClements, 2020).

The origins of plant-based milk date back centuries, with soy milk among the earliest recorded alternatives, dating to 14th-century China, where it was developed as a nutritious and sustainable substitute for dairy milk. Almond milk also has a long history, used in Europe during the Middle Ages, especially during Lent and other fasting periods when dairy consumption was restricted. More recently, oat milk emerged in the 1990s and gained rapid popularity in the 2010s due to its creamy texture and sustainability benefits (Sethi et al., 2016; Aydar et al., 2020). The diversity of plant sources used for milk production has expanded considerably to include legumes (soy, pea, mung bean), cereals (oat, rice), nuts (almond, cashew), seeds (flax, hemp), and emerging novel ingredients like faba bean and quinoa (Suryamiharja et al., 2024; Johnson et al., 2025).

Production methods for plant-based milk typically involve soaking the plant material, followed by grinding or milling to break down cellular structures. This slurry undergoes extraction in water, filtration to remove coarse particles, and homogenization to stabilize the emulsion. Pasteurization or UHT (ultra-high temperature) treatment extends shelf life. Fortification with vitamins and minerals such as calcium, vitamin D, and vitamin B12 is often incorporated to enhance nutritional value (Jeske et al., 2017). Innovations in processing include enzymatic hydrolysis to reduce viscosity and enhance sweetness, high-pressure homogenization to reduce particle size, fermentation to enhance flavor and confer probiotic benefits, and ultrafiltration to concentrate proteins (McClements, 2020; Aydar et al., 2020). These technologies aim to optimize sensory qualities, stability, and nutrient bioavailability, making plant-based milk more comparable to dairy milk in consumer acceptance and functional performance (Suryamiharja et al., 2024).

1.1 Research Question:

How do soaking time, mixed enzyme concentration, and fermentation duration affect the physicochemical properties, sensory quality, and nutritional value of enzymatically extracted mung bean–based plant milk?

1.2 Hypothesis

Soaking time, enzyme concentration, and fermentation duration significantly influence the physicochemical properties, nutritional composition, and sensory quality of mung bean–based plant milk. These processing variables are expected to exhibit both individual and interactive effects, resulting in variations in extraction efficiency, emulsion stability, total soluble solids, color attributes, water activity, and sensory acceptability. Optimal processing conditions are anticipated to achieve a balance between product quality, stability, and consumer acceptability.

1.3 Objectives of this research

1. To optimize the enzymatic extraction process for the development of mung bean–based plant milk using controlled variations in soaking time, enzyme concentration, and fermentation duration.
2. To evaluate the physicochemical and nutritional properties of mung bean–based plant milk, including pH, total soluble solids (TSS), water activity (aw), color parameters (L*, a*, b*), and proximate composition.

3. To assess the sensory attributes and overall consumer acceptability of the developed mung bean–based plant milk using structured sensory evaluation methods.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Types of milk

Plant-based beverages are among the brightest spots in the global food and beverage space, offering consumers lactose-free, vegetarian, and eco-friendly alternatives to traditional dairy, with various formulations developed using combinations of plant sources such as soy and almond to improve nutritional and sensory quality (Kundu et al., 2018; Velangi & Savla, 2022). With origins spanning a wide range of botanical sources, including legumes, nuts, cereals, and seeds, the beverages are highly diverse in their nutritional, sensory, and functional attributes. The source, for instance, soy, almond, oats, rice, coconut, pea, or newer sources such as mung bean and faba bean, has specific compositional strengths and technical challenges. Rationalization thereof is needed to create balanced, consumer-acceptable products. The main plant-based milk types are introduced here, with a special focus on nutritional composition, processing attributes, marketplace salience, and cross-cutting issues that compete with both quality and sustainability.

2.1.1 Mung Bean Milk:

Mung bean (*Vigna radiata*) milk is a novel legume alternative with a combination of a comparatively high protein content, a neutral flavor profile, and foam-forming properties, which can be used to produce heat-stable beverages and undergo thermal treatments (Aydar et al., 2020; Suryamiharja et al., 2024). Nutritionally, mung bean milk will offer more plant protein than most nut- or cereal-based milks. However, its amino acid profile may be limited in sulfur-containing amino acids and therefore may require formulation strategies (e.g., protein complementation or fortification) to approach the quality of bovine milk (McClements, 2020; Suryamiharja et al., 2024). At processing levels, enzymatic processing (to reduce viscosity and improve extractability) and membrane concentration (to improve protein concentration) are standard methods used to improve yield and mouthfeel (Aydar et al., 2020). Consumer acceptance remains hesitant. Initial consumers enjoy the high protein content and unflavored taste, but broader acceptance will hinge on improved labeling and more transparent communication about nutrient equivalency to cow's milk (Suryamiharja et al., 2024; Johnson et al., 2025). Functional problems include sedimentation, off-flavor resulting from lipoxygenase activity unless inactivated during processing, and protein solubility across pH ranges; these can be addressed through thermal treatment, homogenization, and stabilizer systems (McClements, 2020; Aydar et al., 2020).

2.1.2 Soy

Soy milk is the oldest industrial plant milk and is widely employed as the nutritional comparator benchmark. It contains the highest and most biologically effective plant protein of common alternatives with the highest protein content and PDCAAS (protein digestibility corrected amino acid score) closest to bovine milk of most other plant beverages (Vanga & Raghavan, 2018; Jeske et al., 2017). Soy milk contains isoflavones and other bioactive phytochemicals whose health impacts have been investigated, with beneficial effects (cardiometabolic profiles in some populations) and potentially complex effects (hormone-related results in some contexts), highlighting the need for thoughtful dietary guidance (Aydar et al., 2020). In the US, soy milk dominated the alternative milk market but has been surpassed in market share by oat and almond varieties, although it remains important commercially and nutritionally (Johnson et al., 2025). Production benefits include comparatively high protein content from whole soy and mature processing (soaking, grinding, heat treatment to denature trypsin inhibitors, and filtration). Allergenicity risk (soy is a significant allergen), off-flavor potential for beany taste unless lipoxygenases are sufficiently inactivated, and consumer dissatisfaction with genetically modified soybean origin all lead to labeling and market positioning (Mäkinen et al., 2016; Aydar et al., 2020).

2.1.3 Almond Milk

Almond milk is a profitable, high-volume retail food product due to its pleasant, slightly sweet taste and "clean" status. Almond milk is nutritionally poor in protein and relatively low in calories unless sweetened or fortified; commercially sold almond milks are usually fortified with calcium, vitamin D, and sometimes vitamin B12 to more closely resemble milk (Jeske et al., 2017; Johnson et al., 2025). Almonds are oilseed and therefore, milk contains a greater percentage of monounsaturated fats, but are inherently low in soluble fiber and protein compared to legume milks, necessitating formulation solutions (pea/soy blends, protein concentrates) to enhance the protein content if needed (McClements, 2020; Pointke et al., 2022). Environmental issues related to water use in almond cultivation are shaping consumer perceptions, despite almond milk having a relatively low greenhouse gas footprint relative to dairy (Poore & Nemecek, 2018; Ramsing et al., 2023). Functional problems in almond milk include emulsion stability (managing oil droplet size), dilution effects that lower nutrient content, and label clarity, where consumers might think there are more nutrients than actually exist (Jeske et al., 2017; Suryamiharja et al., 2024). Blending of plant-based milk sources, such as soy and almond, has been explored to improve nutritional balance and sensory acceptability, demonstrating the importance of formulation strategies in enhancing product quality (Kundu et al., 2018)

2.1.4 Oat Milk

Oat milk has been increasing rapidly in the marketplace, particularly in Europe and North America, because it is creamy and sensorially equivalent to milk in coffee and barista applications (Wood, 2025; Johnson et al., 2025). Nutritionally, oat milk contains soluble β-glucans and high carbohydrate content, which provide viscosity and valued creaminess but may increase glycaemic load compared to other milks unless reformulated (Aydar et al., 2020; Jeske et al., 2017). Oat milk will probably contain higher fiber-derived soluble polysaccharides compared to nut milk, and fortified oat milk can be as high in calcium and vitamin D (Johnson et al., 2025). US and UK production has grown exponentially in the past decade; industry reports estimate that oats' market share has increased significantly (Grand View Research, 2024; Wood, 2025). Among the challenges are maintaining enzymatic starch degradation in check (α-amylase activity contributes to sweetness and viscosity modification), preventing off-flavor formation due to lipid oxidation, and achieving sugar balance for glycaemic purposes (Jeske et al., 2017; McClements, 2020).

2.1.5 Rice Milk

Rice milk (usually made from brown or white rice) is naturally lactose-free, hypoallergenic, and of interest to multi-allergen households and, under professional advice, to infants in particular. Rice milks are generally rich in carbohydrates (starch and simple sugars after enzymatic hydrolysis) and low in protein and fat content; consequently, commercial rice milks are frequently fortified to provide calcium and micronutrients (Aydar et al., 2020; Vanga & Raghavan, 2018). The main advantages of rice milk are bland flavor and hypoallergenicity; the main drawbacks are low protein value, higher glycaemic index compared to legume or nut milks, and, in some cases, minute quantities of inorganic arsenic in rice-based products that require processing and regulatory intervention (Suryamiharja et al., 2024; Aydar et al., 2020). Functional production techniques prioritize hydrolysis of starch to enhance mouthfeel and sweetness control and filtration for stability; stabilizers and homogenization are needed for shelf life and emulsification (McClements, 2020).

2.1.6 Coconut Milk

Coconut milk (kernel) is unlike the watery, beverage-style "coconut milks" added to most processed foods and unlike canned cooking coconut milk. Beverage-style coconut milks are highly prized for their rich, creamy mouthfeel due to their high concentrations of medium-chain triglycerides, but they have very little protein and typically require supplementation when used as a dairy alternative (Aydar et al., 2020; Pointke et al., 2022). Nutritionally, coconut milks are higher in fat (mainly medium-chain fatty acids), which has led to mixed consumer perceptions: some enjoy their satiety and culinary characteristics, while others are concerned about saturated fat intake (Ramsing et al., 2023). Processing challenges include creating stable emulsions free of phase separation and reducing the perceived "coconutness" for consumers who prefer a milder flavor (McClements, 2020).

2.1.7 Pea Milk

Pea (Pisum sativum) protein milks are gaining popularity as protein-rich, allergen-friendly alternatives that bridge the gap between the protein nutritional quality of soy and the sensory quality of almond and oat (Silva et al., 2024; Suryamiharja et al., 2024). Pea protein has a relatively high lysine content and superior functional properties (gelation, solubility) following appropriate processing, rendering pea milks well-suited for high-protein applications and for mixing with cereals or nuts to create balanced amino acid formulations (McClements, 2020; Silva et al., 2024). Consumer acceptance is favorable when pea milks are positioned on protein and sustainability; however, off-flavors (earthy or "pea" taste notes) and the need for maximized texturization are formulation issues (Pointke et al., 2022; Silva et al., 2024). Pea milk is also supported by the non-GMO message in some markets and has lower water use than some tree nuts (Poore & Nemecek, 2018).

2.1.8 Emerging Sources

Another rapidly growing category is that of faba bean, quinoa, hemp, flax, lupin, and fortified blends or novel hybrid products (e.g., combinations of legumes and grains). These novel products are investigated for higher protein density, novel functional properties, or nutrient attributes (e.g., dietary fiber, omega-3 fatty acids, or novel phytochemicals) (Suryamiharja et al., 2024; Daryani et al., 2024). Faba bean and lupin offer attractive protein yields with complementary amino acid profiles but demand selective attention with the presence of anti-nutritional factors (vicine/convicine present in faba bean; alkaloids present in lupin) and the potential for novel allergen issues (Aydar et al., 2020; Daryani et al., 2024). Most novel milks from these sources enjoy very limited consumer acceptance among pioneering groups at this time; successful commercial-scale-up will require flavor masking, regulatory clearance, and assays to demonstrate safety and nutritional acceptability (Suryamiharja et al., 2024; Johnson et al., 2025). Overall, plant-based milk alternatives are increasingly positioned as functional beverages due to their bioactive components and potential health benefits, supporting their growing role in modern diets (Velangi & Savla, 2022).

Table 2.1. Proximate Nutritional Composition of Common Plant-Based Milks (per 100 mL, Typical Beverage Form). Data compiled from (Sethi et al.,2016; Aydar et al., 2020; Mäkinen et al., 2016; Jeske et al., 2017).

Plant Milk Source	Energy (kcal)	Protein (g)	Total Fat (g)	Fat (g)	CHO (g)	Sugars (g)	Fiber (g)
Mung bean milk	30–38	2.6–3.4	1.2–1.8	0.3–0.4	1.5–2.5	0.3–0.8	0.2–0.5
Soy milk	38–50	3.0–3.5	1.5–2.0	0.3–0.6	2.0–3.0	1.0–2.0	0.3–0.5
Almond milk	15–25	0.4–1.0	1.2–2.5	0.1–0.3	0.5–2.0	0.5–1.0	0.3–0.6
Oat milk	45–65	1.0–1.5	1.0–2.5	0.2–0.5	6.0–9.0	2.5–4.0	0.5–1.0
Rice milk	45–65	0.2–0.5	1.0–1.5	0.3–0.5	9.0–12	5.0–7.0	0.1–0.2
Coconut milk	25–45	0.2–0.5	1.8–3.5	1.5–2.8	1.0–2.0	0.8–1.2	0.3–0.5
Pea milk	30–45	2.5–3.5	1.0–2.0	0.2–0.4	0.5–2.0	0.3–1.0	0.3–0.6
Emerging sources (Faba, Quinoa, Lupin, Hemp)	30–55	2.0–4.0	1.0–3.0	0.3–0.8	1.0–4.0	0.5–1.5	0.4–0.8

The nutritional variability observed among plant-based milks compared to dairy highlights the need for formulation and fortification strategies to achieve nutritional adequacy (Nowson et al., 2025).

The variability in nutrient composition among plant-based milks has important implications for dietary intake, as systematic reviews indicate that reliance on these alternatives without appropriate fortification may affect growth and nutritional status in specific populations (Soczynska et al., 2024).

Table 2.2. Micronutrient Profile and Key Bioactive Components of Plant-Based Milks (per 100 mL, Fortified and Non-Fortified Forms)

Plant Milk Source	Vitamin B12(µg)	Vit D (µg)	Potassium (mg)	Iron (mg)	Calcium (mg)	Key Bioactive / Notes	Reference
Mung bean milk	0.4–0.6	1.2–1.8	120–160	1.0–1.5	100–150	Rich in phenolic acids, flavonoids, GABA, low methionine, fortified for Ca	Suryamiharja et al. (2024); Aydar et al. (2020); McClements (2020)
Soy milk	0.4–0.6	1.2–1.5	150–170	0.5–1.0	120–150	Isoflavones (genistein, daidzein), phytosterols; complete protein	Jeske et al. (2017); Vanga & Raghavan (2018); Johnson et al. (2025); Aydar et al.
Almond milk	0.3–0.5	1.0–1.2	35–70	0.2–0.4	120–140	High MUFA, vitamin E (α-tocopherol), polyphenols	Pointke et al. (2022); Jeske et al. (2017); Johnson et al. (2025)
Oat milk	0.3–0.5	1.0–1.5	100–140	0.3–0.6	120–150	Contains β-glucans (soluble fiber); lowers cholesterol	Johnson et al. (2025); Aydar et al. (2020); Jeske et al. (2017)
Rice milk	0.3–0.5	1.0–1.2	30–50	0.1–0.3	120–150	Hypoallergenic; high GI; may contain trace arsenic	Aydar et al. (2020); Vanga & Raghavan (2018); Suryamiharja et al. (2024)
Coconut milk	0.3–0.5	1.0–1.3	50–80	0.2–0.4	120–140	High MCTs (C8–C12), lauric acid; antimicrobial lipids	Pointke et al. (2022); Aydar et al. (2020); Ramsing et al. (2023)
Pea milk	0.3–0.5	1.0–1.3	100–180	1.0–1.5	120–150	High lysine, low methionine; no major allergens	Silva et al. (2024); Suryamiharja et al. (2024); Pointke et al. (2022)
Emerging sources (Faba, Quinoa, Lupin,	0.3–0.6	1.0–1.5	100–200	1.0–2.0	120–160	Contain polyphenols, omega-3s, saponins; high digestibility potential	Daryani et al. (2024); Suryamiharja et al. (2024); Aydar et al. (2020)

2.2 Technological Innovations in Production

The quality, functionality, and nutritional equivalent of plant-based milks have all been significantly impacted by developments in processing technologies. Formulation approaches, including blending different plant milk sources, have also been used to enhance physicochemical stability and sensory attributes of plant-based beverages (Kundu et al., 2018). The primary technological interventions are fermentation, emulsification, homogenization, enzymatic treatment, and new and emerging methods such as cold plasma stabilization, high-pressure processing, and ultrafiltration. These procedures complement one another to improve the nutritional value and sensory appeal of plant-based beverages while reproducing the physicochemical properties of bovine milk (Aydar et al., 2020; McClements, 2020; Suryamiharja et al., 2024). The inclusion of additives in plant-based milk systems, such as emulsifiers and stabilizers, is essential to improve texture and stability; however, their health implications and interactions with naturally occurring compounds, such as oxalates, must be carefully considered (AbuKhader et al., 2022).

2.2.1 Enzymatic Treatment

Enzyme technology is also among the most remarkable innovations for optimizing the extraction and nutritional profiles of plant-based milks. The enzymes are applied to hydrolyze macromolecules, increase solubility, decrease viscosity, and improve sweetness and flavor (Aydar et al., 2020; Mäkinen et al., 2016). In cereal-based milks such as oat and rice, starch is digested into maltose and glucose by α-amylase and glucoamylase, thereby reducing viscosity and enhancing digestibility (Jeske et al., 2017). Protease treatment improves the dispersibility of proteins and reduces the bitterness of peptides and lipoxygenase-related compounds, thereby enhancing the milk quality of legumes used as milks, such as soy, mung bean, and pea

(Suryamiharja et al., 2024). Research on mung bean milk indicates that, with the use of combined amylase-protease complexes, protein extraction yields increase up to 40%, and overall soluble solids and flavor are also elevated (Dhankhar, 2023).

Anti-nutritional components such as phytates, oligosaccharides, and oxalates are reduced through enzymatic treatments, thereby enhancing mineral bioavailability and nutritional quality (Aydar et al., 2020; AbuKhader et al., 2022). It is an energy-conserving bioprocessing process that promotes clean-label production without chemical hydrolysis. Efficacy depends on enzyme specificity, substrate concentration, and pH control during hydrolysis. Enzyme technology integrated into industrial-scale systems is instrumental in developing high-quality legume and cereal milk drinks with improved sensory and nutritional properties (McClements, 2020).

2.2.2 Fermentation Method:

Fermentation technology is also being used to enhance plant-based milk, giving it superior flavor, digestibility, and functionality. Controlled fermentation with lactic acid bacteria (LAB): *Lactobacillus plantarum, Lactobacillus rhamnosus*, and *Bifidobacterium lactis* are utilized to decrease unwanted beany flavors, lower the pH in support of microbial stability, and produce bioactive metabolites such as peptides and organic acids (Reyes-Jurado et al., 2021; Silva et al., 2024). During fermentation, phytic acids and tannins are inactivated, raising the bioavailability of calcium, zinc, and iron in soy and pea milk and improving its taste and texture (Aydar et al., 2020).

Recent research has shown that LAB strains used in the fermentation of oat and rice milks exhibit enhanced antioxidant activity and reduced glycemic indices of the products (Daryani et al., 2024). The antioxidant activity of mung bean milk co-fermented with *L.*

plantarum and *Bacillus subtilis* and GABA (γ-aminobutyric acid) is higher, and it indicates that this product can be used as a functional drink (Suryamiharja et al., 2024). Part of the flavor development involves fermentation, which also contributes to the synthesis of volatile compounds, including acetoin and diacetyl, whose characteristics resemble those of dairy (Mäkinen et al., 2016).

Processing Fermentation is one of the natural stabilizers, which form polysaccharide protein interactions, enhancing the suspension stability. Industrial producers modify starter cultures to suit plant substrates and optimize them for pH and temperature, ensuring the same flavor and shelf stability for consumers. It is an established dairy-processing method that offers probiotic potential and improved functionality of plant-based milk, helping meet the growing popularity of foods that promote gut health (McClements, 2020; Silva et al., 2024).

2.2.3 Homogenization:

Stability of emulsions, uniform particle size, and the texture of plant-based milks are essential for homogenization. It reduces the size of fat droplets (mainly 0.2 to 1.0 µm), which do not allow phase separation and contribute to mouthfeel (Mäkinen et al., 2016; Aydar et al., 2020). A common practice is high-pressure homogenization (HPH) at 100- 200MPa, which is used in soy, oat, and almond milk. It provides an even distribution of insoluble solids and decreases the rate of settlement in the store. Legume-based milk, such as mung bean and pea, can be homogenized to strengthen protein-lipid interactions, resulting in stable emulsions and reduced visible creaming (Suryamiharja et al., 2024).

Superior methods, including ultra-high-pressure homogenization (UHPH), enhance the physicochemical stability of plant-based emulsions and preserve sensitive nutrients and bioactive compounds (McClements, 2020). According to the study by Daryani et al. (2024), UHPH-processed oat milk exhibits superior thermal stability and viscosity consistency during pasteurization compared to traditional methods. Secondly, homogenization alters protein structure, unfolding globulins and exposing hydrophobic regions that support emulsifying activity (Jeske et al., 2017).

In industrial production, the particles are subjected to homogenization via enzymatic or thermal pre-treatment to achieve better colloidal stability and homogeneous dispersion. The combination of homogenization and other solutions is the secret behind the appearance and feel of dairy milk that mimics those of plant-based drinks, ensuring quality and shelf-stability (Silva et al., 2024; McClements, 2020).

2.2.4 Emulsification Techniques:

Depending on the type of plant-based milk, emulsification is the most significant element in product development, as it allows oil droplets to be suspended in the aqueous phase. The point is that plant protein complexes in emulsion form can be challenging to stabilize due to potential variation in solubility and surface activity (Aydar et al., 2020). Other natural emulsifiers include soy, pea, and mung bean proteins, which surround the fat droplets with interfacial films that prevent coalescence. In nut-based milks, e.g., almond or coconut, stabilizers for phase separation are usually added, such as lecithin, gum arabic, or polysaccharides (Pointke et al., 2022).

Ultrasound-assisted emulsification can increase droplet dispersion via cavitation, thereby improving emulsion homogeneity and stability (Suryamiharja et al., 2024). Likewise, it has been demonstrated that nano-sized droplets with high kinetic stability and optical whiteness (as in dairy milk) have been produced by micro fluidization and membrane emulsification

(McClements, 2020). An example is the examination of oat and pea products processed using micro fluidization: their droplet diameter decreases, creaming is reduced, and shelf life is 3040% higher (Daryani et al., 2024).

Not only does emulsification determine stability, but it also affects sensory features such as creaminess and opaqueness. The clean-label emulsifiers, such as oats β-glucans and legume protein isolates, have emerged in response to consumer demand for minimally processed, ingredient-free drinks. Optimizing the emulsification shear rate, surfactant concentration, and temperature is one factor that contributes to the product's texture, stability, and consumer acceptance (McClements, 2020; Silva et al., 2024).

2.2.5 Emerging Techniques

The production of plant-based milk is also being altered by new technologies that enhance safety, quality, and sustainability while reducing nutrient degradation. High-pressure processing (HPP), ultrafiltration, pulsed electric field (PEF), and cold plasma treatments are the most promising ones (McClements, 2020; Suryamiharja et al., 2024). HPP is carried out at 400-600 MPa and has been shown to effectively inactivate microorganisms that cause spoilage, without adversely affecting vitamins, flavor, or bioactive compounds. The shelf life and sensory properties of HPP milk products, oats, and almonds are higher than those of thermally pasteurized products (Daryani et al., 2024).

Ultrafiltration (UF) allows selective retention of macromolecules while concentrating proteins without isolating undesirable oligosaccharides and suspended solids. Studies with soy and pea milk demonstrate that UF can enhance the protein content of milk by 30-50 percent and increase its emulsifying capacity and nutritional enrichment (Aydar et al., 2020). Similarly, electroporation effects are produced by pulsed electric field processing and enhance extraction and improve microbial safety without altering taste, making it applicable to heat-sensitive nutrients (Dhankhar, 2023).

Another possible innovation is cold plasma technology, which produces sterilized, surface-modified plant protein by subjecting ionized gases to surface treatments to enhance emulsification and stability (Silva et al., 2024). They are non-thermal technologies applied alongside enzymatic and fermentation to support the clean-label movement by reducing the utilization of chemical preservatives. The combination of these two new technologies could help propel the next generation of high-quality, nutritionally optimized, and sensory-enhanced plant-based milk, as the industry aims for in terms of sustainability and environmental responsibility (Ramsing et al., 2023; McClements, 2020).

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

Whole dried mung beans (*Vigna radiata*) were procured from the online platform Amazon and stored under dry, ambient conditions until use. All chemicals and reagents used in the study were of analytical grade unless otherwise stated. A mixed enzyme system consisting of protease, amylase, and cellulase was obtained from Sigma-Aldrich (St. Louis, MO, USA). Potable water was used for soaking and processing mung beans. Soaked beans were ground using a Vitamix blender (Vitamix Corp., Cleveland, OH, USA). Filtration was performed using a Cuisinart mesh strainer (Walmart, Edmond, OK), followed by vacuum filtration using a Büchner funnel apparatus (Fisher Scientific, Pittsburgh, PA, USA). Filter papers with pore sizes of 100–200 µm (coarse filtration) and 0.1–10 µm (microfiltration), along with xanthan gum (Walmart, Edmond, OK), were used to obtain smooth plant-based milk.

Heat treatment and enzymatic reactions were conducted using a digital hot plate stirrer (SCI280-Pro, Scilogex, Rocky Hills, CT, USA). Homogenization was performed using a Scilogex D500 homogenizer (Marshall Scientific, Hampton, NH, USA). Pasteurization was carried out in a hot water bath (Thermo Scientific™, Waltham, MA, USA) under low-temperature, long-time (LTLT) conditions.

For physicochemical and nutritional analyses, standard laboratory equipment, including a pH meter (HI 9125, Hanna Instruments), an LS372 Colorimeter (Linshang Technology, China), a refractometer (HI 96801, Hanna Instruments), and a water activity meter (WA-160) were used. Moisture, ash, protein, fat, and carbohydrate analyses were conducted using AOAC-approved methods, including Karl Fischer titration, Combustion, and Gravimetry.

For sensory evaluation, commercially available plant-based milk products were used as control samples. All sensory materials, including coded sample cups, palate cleansers (water and crackers), and standardized sensory evaluation forms, were prepared in accordance with protocols approved by the Institutional Review Board (IRB) of the University of Central Oklahoma.

3.2 Development and optimization of milk

The process of developing mung bean-based milk used a systematic, enzyme-assisted extraction to enhance nutrient extraction, physicochemical stability, and sensory quality, and to scale up the process. Whole mung beans (*Vigna radiata*) were chosen because they contain high amounts of protein, contain low amounts of fats, and have better functional characteristics than other pulses. Optimization of the total process included consideration of key processing variables, such as soaking time, enzyme concentration, and downstream thermal and mechanical treatments, as shown in the process flowchart. To systematically evaluate the influence of processing variables, a full factorial experimental design (2^3) was employed. The independent variables included soaking time (8 h and 14 h), enzyme concentration (1% and 2%), and fermentation time (2 h and 4 h). This design enabled the assessment of both main effects and interaction effects on physicochemical, nutritional, and sensory properties of mung bean milk. All experimental runs were conducted in triplicate to ensure reproducibility and statistical reliability.

The first step involved soaking mung beans in potable water, with two soaking periods: a short (8 h) and a long (14 h). Soaking softens the seed coat, enhances hydration, triggers enzyme activity, and reduces antinutritional factors (phytic acid and enzyme inhibitors), thereby increasing extractability and nutritional value (Mudryj et al., 2014; Boye et al., 2010). After soaking, the outer hulls were manually removed to minimize fibrous material, reduce graininess, and ensure a smooth milk consistency. The dehulled beans were subsequently ground in water to make a homogeneous slurry, which was used as the substrate for enzyme treatment.

The primary optimization phase in the development of mung bean milk was enzymatic treatment. The slurry was placed in a mixed enzyme system containing proteases, amylases, and cellulases, and incubated for 2-4 h at a constant temperature and pH. Proteolytic enzymes increased the solubility of proteins, starch was hydrolyzed by amylases into soluble sugars, and polysaccharides in cell walls were dispersed, which enhanced extraction yield and stability of emulsions, as well as mouthfeel (Boye et al., 2010; Vogelsang-O'Dwyer et al., 2021). The enzyme-assisted process was chosen for its efficiency, lower energy consumption, and for producing a smoother plant milk with better nutritional content than the traditional wet-milled process.

After the enzymatic hydrolysis, the slurry was filtered in two stages to eliminate insoluble solids. The filtration (100–200 µm) was initially used to remove larger particles, followed by microfiltration (0.1–10 µm) to obtain a smooth, uniform liquid. The segregated solid residue was stored for further use as a by-product to promote sustainable processing and waste minimization. Filtration was very important for enhancing the visual appearance, overall texture, and consumer acceptability of mung bean milk (Sethi et al., 2016).

The filtered milk was then heated at 60-70 °C for 5-10 min to reduce viscosity, inactivate remaining enzymes, and prepare the system for homogenization. Homogenization was performed at 40-50ºC for 3-5 min, with the optional addition of 0.5% xanthan gum as a stabilizer to improve emulsion stability and prevent phase separation on storage. Xanthan gum is a stabilizer typically added to plant-based drinks to enhance mouthfeel and physical stability, especially in protein-rich recipes (Tangyu et al., 2019).

The milk was then pasteurized using low-temperature, long-time (LTLT) at 63ºC for 30 min to ensure microbiological safety without impairing sensory and nutritional qualities. LTLT pasteurization is most commonly used in the processing of plant-based milk, as it effectively reduces the microbial load with no significant impact on protein denaturation, solubility, or flavor degradation (Sethi et al., 2016). The mung bean milk was cooled and refrigerated after pasteurization to undergo further physicochemical, microbiological, and sensory analyses to continue the optimization study.

The optimized process demonstrated that controlled soaking, enzyme-assisted extraction, and appropriate thermal and mechanical treatments are key to producing high-quality mung bean milk with improved texture, stability, and sensory acceptability. The process flow provides a scalable platform for developing pulse-based dairy alternatives that meet current consumer demand for sustainable, nutrient-dense, and allergen-friendly drinks.

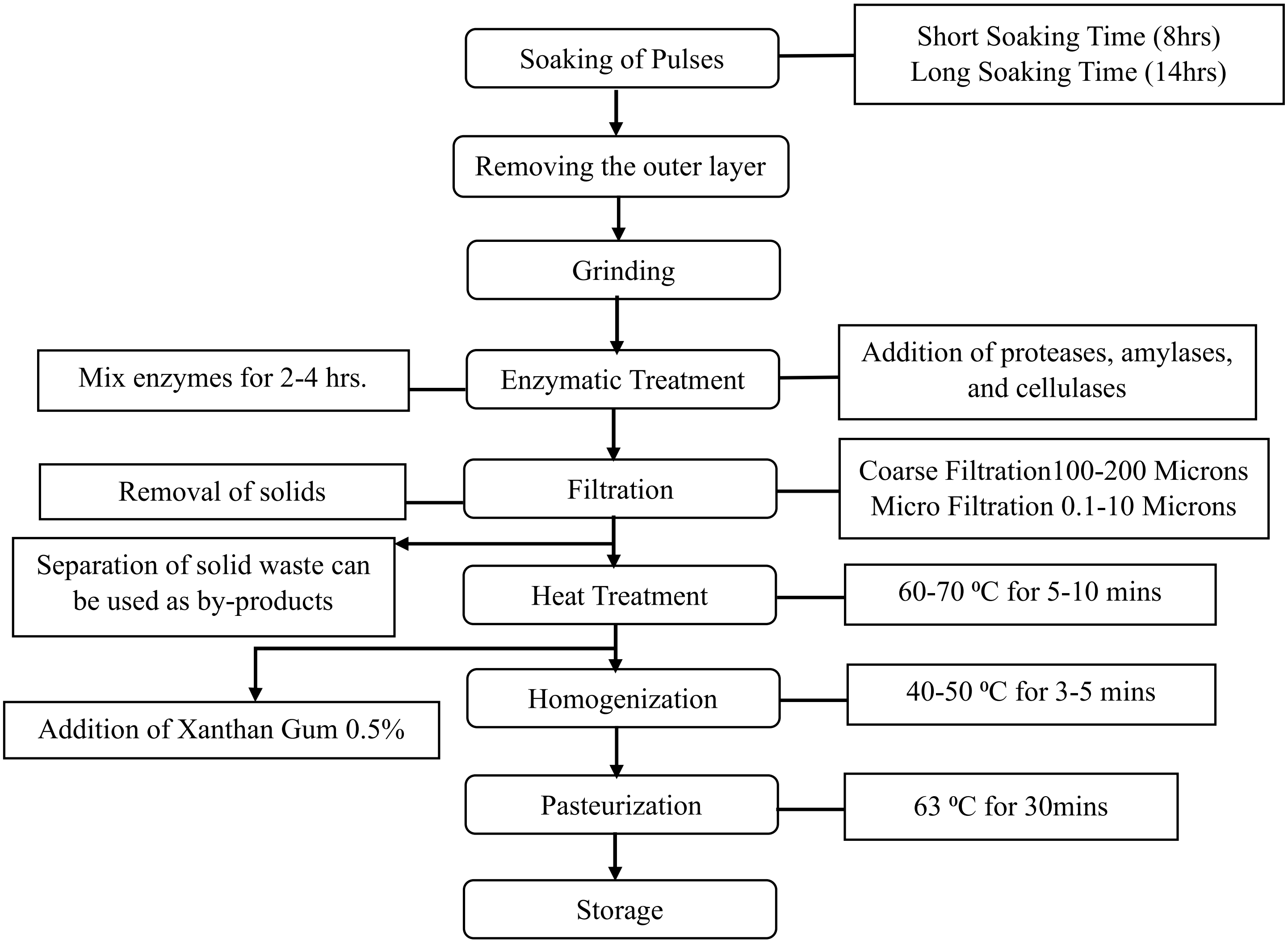

Figure 3.1. Processing flow diagram for the enzymatic production of mung bean–based plant milk

3.2.1 Experimental Design

Table 3.1: Experimental Design: Factors and Levels

Factors	**Trial 1**	**Trial 2**
A: Soaking Time	Short Soaking Time(8h)	Long Soaking Time(14 h)
B: Concentration of Enzyme Blend	1% (w/w)	2% (w/w)
C: Fermentation Time	2 hours	4 hours
A full factorial design (2^3) was employed, resulting in eight treatment combinations.		

A full factorial design (2^3) was used to investigate the effects of soaking time (A), enzyme concentration (B), and fermentation time (C) on mung bean milk properties. Each factor was evaluated at two levels, resulting in eight treatment combinations. All experiments were conducted in triplicate to ensure reproducibility. The experimental design facilitated the evaluation of main effects and interaction effects among variables.

3.3 pH Analysis

To measure changes in acidity and fermentation behavior of mung bean milk, the pH of the samples was monitored using a digital pH meter (HI 9125, Hanna Instruments) calibrated before use. To assess consistency and accuracy, measurements were performed at room temperature at specified intervals at each processing step. Before analysis, the pH meter was checked with the standard buffer solutions (pH 4.0 and 7.0). The electrode was directly plunged into the homogenized sample, and stabilized readings were taken. Measurements were conducted three times, and the average values were obtained to provide the statistical reliability and reproducibility of the results.

3.4 Total Soluble Solids (TSS)

A portable digital refractometer was used to determine total soluble solids (TSS) of the mung bean milk samples using a HI 96801 (Hanna Instruments), and the results were reported as degrees Brix (°Brix). A drop of the well-mixed sample was placed on the prism surface of the refractometer, and readings were taken after proper calibration with distilled water. The instrument is used to determine the refractive index, which is proportional to the concentration of dissolved solids such as sugars, soluble proteins, and other extractable components. Each sample was analyzed in triplicate, and the mean was calculated to ensure accuracy and statistical significance.

3.5 Colorimetry

The color values of the mung bean milk samples were measured by the colorimeter (LS372, Linshang Technology, China) according to the Hunter Lab color system. The color parameters were expressed as lightness (L*), redness/greenness (a*), and yellowness/blueness (b*). The instrument was calibrated with a white standard plate before measurements. To reduce variability, samples were placed in a standard sample holder to ensure similar surface conditions, and measurements were taken at multiple points. Measurements were performed three times, and the mean values were computed to indicate the general color traits and visual appearance of the samples.

3.6 Water Activity

A handheld water activity meter (WA-160) was used to establish water activity (aw) of the mung bean milk samples. A representative sample was placed on the sample plate so that the sensor area was well covered, and the plate was left to stabilize for at least 5 minutes until the reading stabilized. The water activity meter is used to determine the amount of free water available for microbial growth and chemical reactions, which is important for determining the stability and shelf life of the products. Each sample was repeated three times, and the average values were taken to ensure accuracy and reproducibility.

3.7 Nutrition Profile Analysis

An external accredited laboratory analysis was conducted by Eurofins Nutrition Analysis Center (Des Moines, Iowa, USA) of the nutritional composition of the developed mung bean milk samples using standardized AOAC and CFR methods. Key proximate parameters were analyzed on the samples, moisture content (AOAC 925.09) by the use of the vacuum oven method, Crude fat (AOAC 954.02) by the acid hydrolysis method, protein content (AOAC 990.03 and AOAC 992.15) using the combustion method with a nitrogen to protein ratio of 6.25, Ash content (AOAC 942.05) by the use of the gravimetric analysis method and total The difference was taken as a basis to calculate Carbohydrate content with CFR guidelines and the total calorific value was estimated through the method of Atwater calculation. All analyses were conducted under ISO/IEC 17025:2017-accredited conditions to ensure accuracy, precision, and reliability. The results were presented on an as-received basis and used to compare the nutritional profiles and functional qualities of the optimized mung bean milk samples.

3.8 Sensory Evaluation

Sensory evaluation was conducted to assess the acceptability of mung bean milk using a panel of 13 participants ($n = 13$) under controlled conditions. The study included two components: a comparison test between commercially available soy milk (Sample A) and mung bean milk (Sample B: 14 h soaking + 2% enzyme + 4 h fermentation), and a hedonic rating analysis of the mung bean milk sample. All samples were coded randomly and presented in a randomized order to minimize bias. In the comparison test, panelists evaluated overall likeability and indicated their preference between samples, while in the hedonic evaluation, attributes including appearance, aroma, texture, taste, aftertaste, and overall acceptability were assessed using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Water and plain crackers were provided as palate cleansers between samples to prevent carryover. All evaluations were conducted in accordance with Institutional Review Board (IRB) guidelines (Application no. 2026-015), and the collected data were analyzed using descriptive statistics and one-way ANOVA, with significance determined at $p < 0.05$.

3.9 Statistical Analysis

A full factorial experimental design (2^3) was used to evaluate the effects of soaking time, enzyme concentration, and fermentation duration on the properties of mung bean milk. All experiments were conducted in triplicate ($n = 3$) to ensure data reliability and reproducibility, and results were expressed as mean ± standard deviation (SD). Statistical analysis of the experimental data was performed using analysis of variance (ANOVA) to determine the significance of main effects and interaction effects among variables. The level of statistical significance was set at $p \leq 0.05$. Post hoc comparisons of treatment means were conducted using appropriate multiple-comparison tests to identify significant differences among groups. All statistical computations and graphical analyses were performed using Minitab (version 22.4.0), SPSS, and Microsoft Excel, ensuring accurate interpretation of factor interactions and treatment effects.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Effect on pH

The change in pH due to soaking time, enzyme concentration, and fermentation time was insignificant, with values falling within a narrow range, suggesting system stability. The Main Effects Plot (Figure 4.1) indicates that pH was slightly higher at 8 h of soaking than at 14 h, and this difference in pH between treatments was statistically significant ($p < 0.05$); however, the magnitude of variation was minimal ($\Delta pH \approx 0.1–0.2$), indicating limited practical relevance in terms of product stability and quality. This small rise in pH with 14h of soaking time can be explained by the leeching of acidic substances, such as phytic acid and organic acids, into the soaking medium, leading to a slight shift towards a neutral pH. A pH range of 6.2-6.8 is considered desirable in plant-based milk systems, as it enhances protein stability, inhibits coagulation, and improves sensory acceptability. Thus, both soaking conditions were acceptable in terms of pH, and a pH of about 6.2 was optimal to ensure product stability and quality.

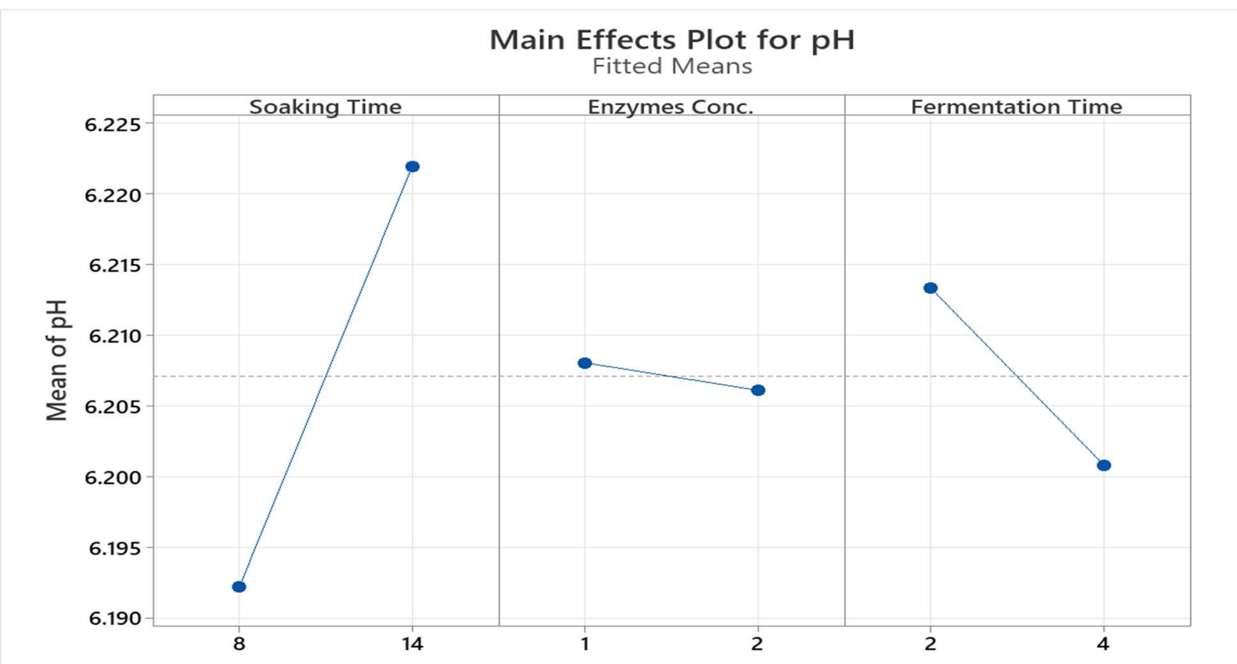

Figure 4.1. Main Effects of Plot Depicting the Individual Influence of Processing Variables on pH of Mung Bean Milk (Y-axis truncated to highlight differences. Mean of pH 6.208)

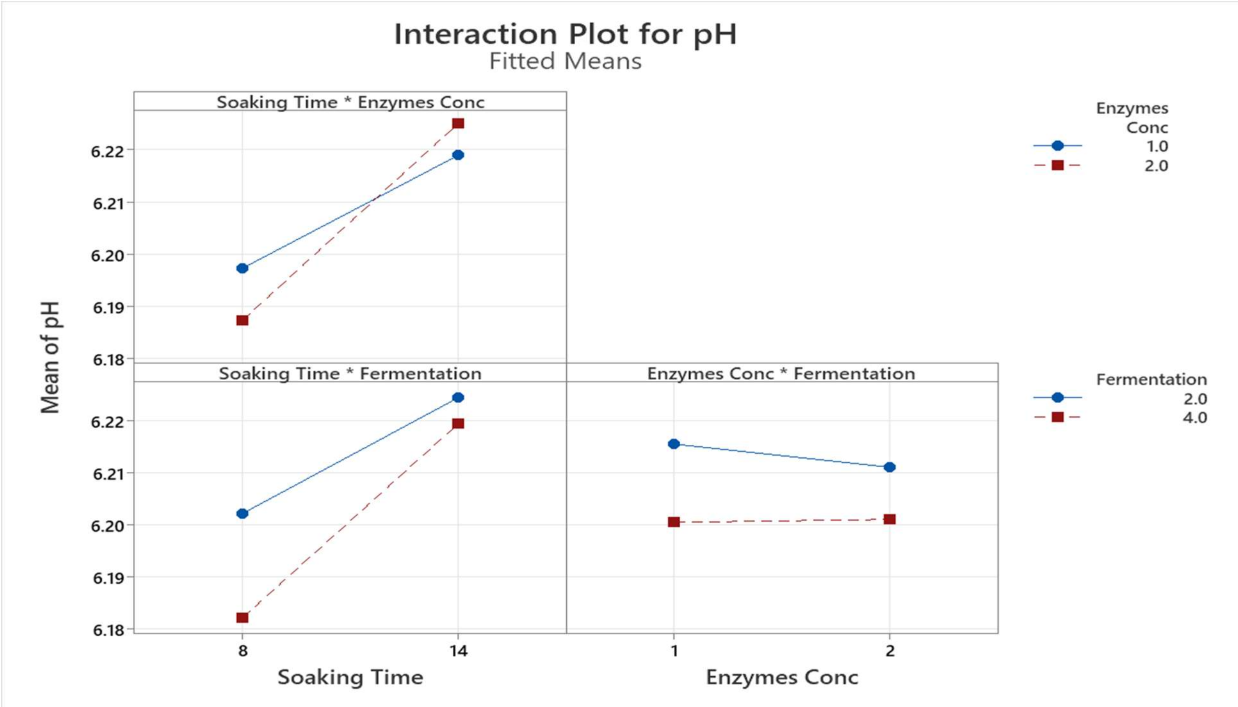

Figure 4.2. Interaction Plot Demonstrating the Combined Effect of Processing Variables on pH Stability. (Y-axis truncated to highlight differences. Mean of pH 6.208)

The pH decreased slightly (0.21) between 2 and 4 hours of fermentation, although the difference was not statistically significant (p ~0.048), suggesting little acid production during fermentation. The enzyme concentration did not significantly affect pH ($p > 0.05$), confirming that the enzymatic treatment did not significantly affect acidity. In the Interaction Plot (Figure 4.2), the lines are nearly parallel, indicating that there were no statistically significant interactions among soaking time, enzyme concentration, and fermentation time ($p > 0.05$). There were small deviations: longer soaking and higher enzyme concentration led to a slight increase in pH, possibly due to greater extraction of buffering compounds such as proteins and minerals, but none of these effects were significant. The Contour Plot (Figure 4.3) shows that pH values are evenly distributed, indicating that the process will be stable across all processing conditions. On the whole, such findings suggest that the pH is not significantly affected by interactions between processing variables and falls within the pH range conducive to product quality.

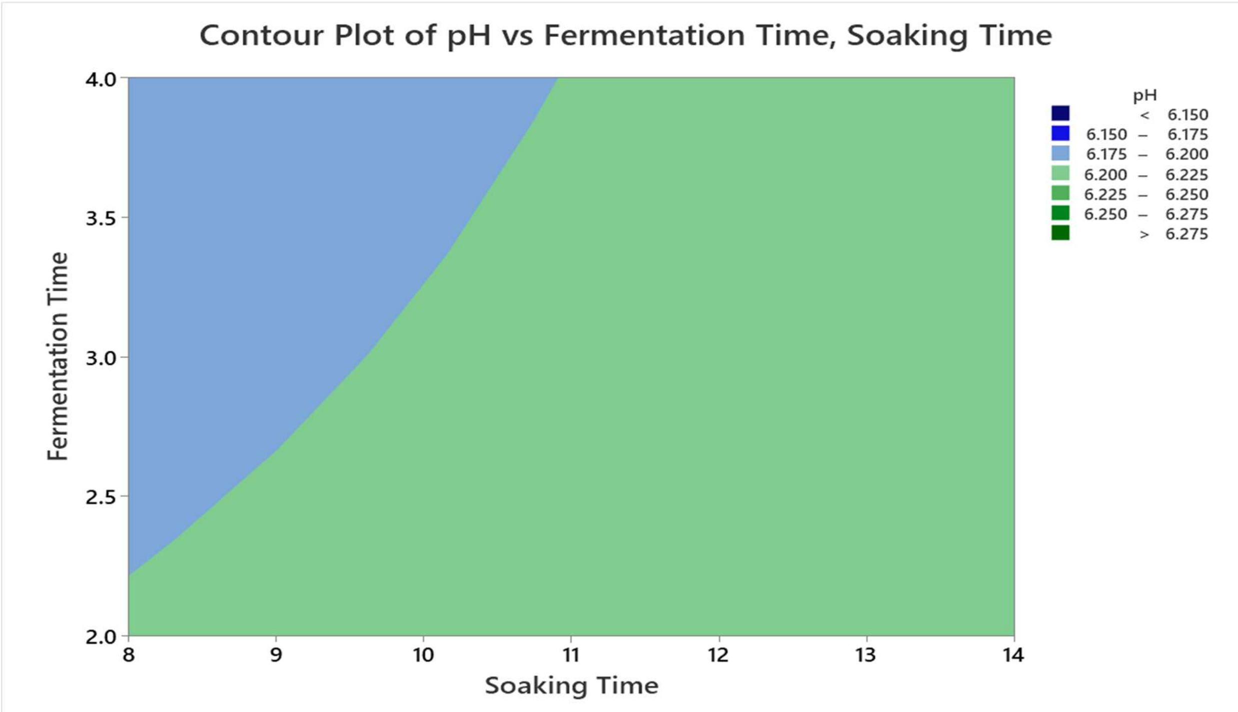

Figure 4.3. Contour Plot Illustrating the Distribution of pH Across Soaking and Fermentation Conditions. (Y-axis truncated to highlight differences. Mean of pH 6.208)

4.2 Effect on Total Soluble Solids (TSS)

The influence of soaking time, enzyme concentration, and fermentation time on total soluble solids (TSS) was assessed through statistical analysis and graphical illustration, and the results were consistent with the analysis of the individual factors. As can be seen, the Main Effects Plot (Figure 4.4) indicates that the soaking time had a negative effect of significant magnitude on TSS ($p < 0.05$), so that the values of TSS decreased (5.66 °Brix at 8 h soaking and 4.66 °Brix at 14 h soaking). This decrease indicates that soluble substances, such as sugars and oligosaccharides, have been lost during the long soaking period, likely due to diffusion into the soaking medium. Other studies have also reported the same in legume-based milk, where long soaking has led to nutrient loss and a drop in soluble solids(Jeske et al., 2017; Aydar et al., 2020). In terms of product quality, a preferable TSS for plant-based milk is 5 to 6 °Brix, as higher TSS is associated with better mouthfeel, body, and nutrient density; thus, a shorter soaking period (8 h) is more appropriate to achieve the desired TSS.

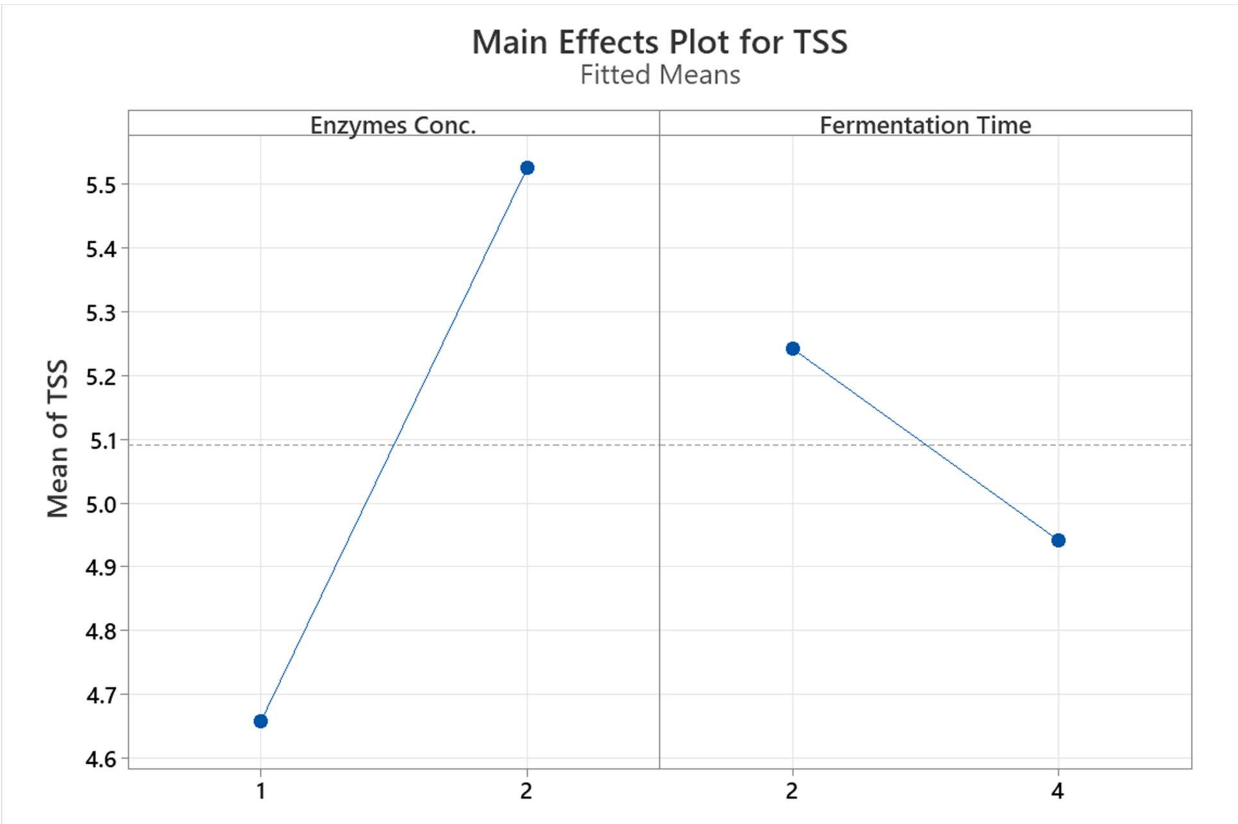

Figure 4.4. Main Effects Plot Depicting the Individual Influence of Soaking Time, Enzyme Concentration, and Fermentation Time on Total Soluble Solids (TSS) of Mung Bean Milk (Y-axis truncated to highlight differences. Mean of °Brix 5.08)

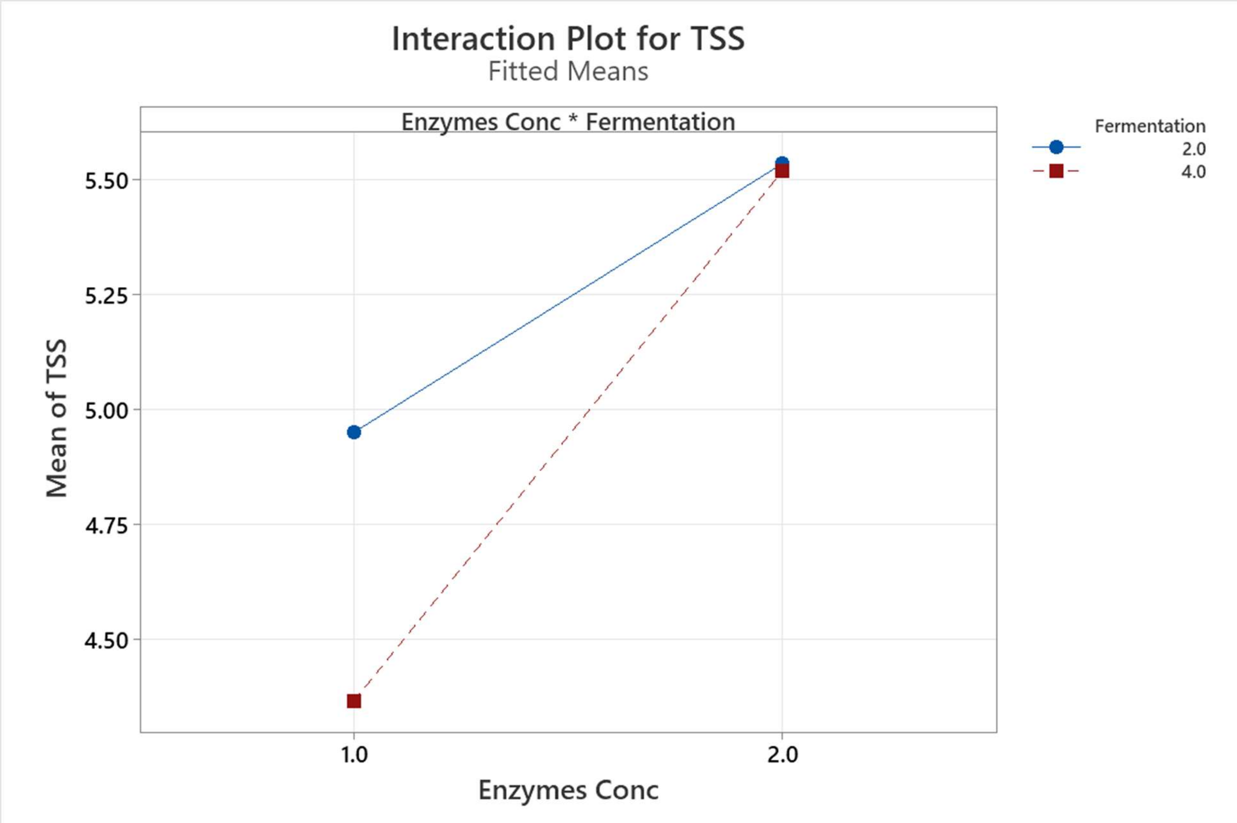

Figure 4.5. Interaction Plot Demonstrating the Synergistic and Independent Effects of Processing Variables on TSS (Y-axis truncated to highlight differences. Mean of °Brix 5.08)

Conversely, the concentration of enzyme was statistically significantly positively affected by TSS ($p < 0.05$), with TSS being 4.65 ºBrix at 1 percent enzyme concentration and 5.54 ºBrix at 2 percent enzyme concentration. This implies that increased enzyme levels promote the breakdown of cell wall structures, starch, and proteins, thereby increasing the release of soluble components. Other studies of enzymatic extraction of plant beverages have shown similar trends: higher enzyme concentration enhances extraction yield and nutrient content(Aydar et al., 2020; Dhankhar, 2023). The fermentation time did not exhibit a statistically significant change in TSS, from 5.24 ºBrix at 2 h to 4.91 ºBrix at 4 h; thus, fermentation does not significantly affect TSS.

As shown in the Interaction Plot (Figure 4.5), the interaction between enzyme concentration and fermentation time was statistically significant ($p = 0.009$), as indicated by the non-parallel lines. Increasing enzyme concentration (2%) and fermentation time (4 h) resulted in higher TSS values, suggesting that extended fermentation enhances enzymatic hydrolysis and solubilization, thereby producing a synergistic effect. Conversely, the interactions between soaking time and enzyme concentration ($p = 0.120$) and soaking time and fermentation time ($p = 0.753$) were not statistically significant, indicating that soaking time primarily exerts an independent effect on TSS.

Importantly, the three-way interaction among soaking time, enzyme concentration, and fermentation time was not statistically significant ($p = 0.088$), indicating that the combined influence of all three processing variables does not have a strong interactive effect on TSS beyond the observed main and two-way interaction effects. These results are further supported by the Contour Plot (Figure 4.6), which shows that higher TSS values correspond to higher enzyme concentrations and longer fermentation periods. Overall, these findings suggest that enzyme concentration and its interaction with fermentation time play a critical role in enhancing TSS, while soaking time independently contributes to the reduction of soluble solids.

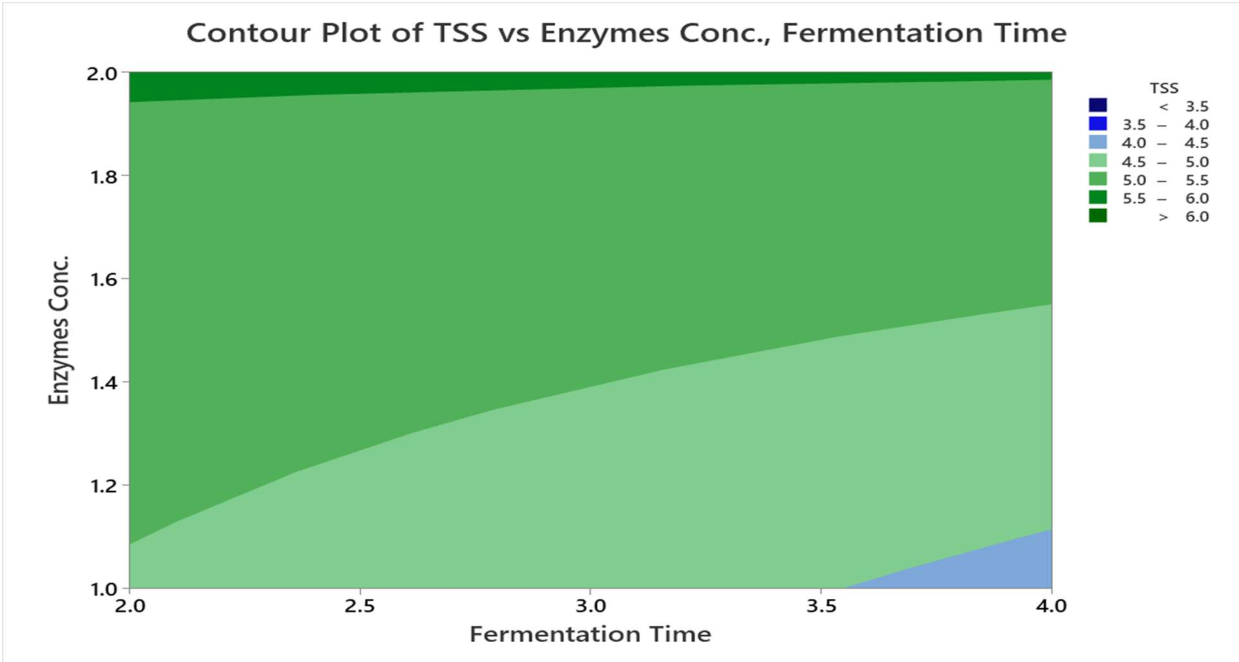

Figure 4.6. Contour Plot Illustrating the Response Surface of TSS as a Function of Enzyme Concentration and Fermentation Time(Y-axis truncated to highlight differences. Mean of °Brix 5.08)

4.3 Effect on Water Activity

The interaction between fermentation time and soaking time indicated a significant effect of soaking time, enzyme concentration, and fermentation time on water activity (a_w). Based on the Main Effects Plot (Figure 4.7), the water activity of the system rose considerably ($p < 0.05$) between 0.79 at the beginning of the fermentation process (2 h) and 0.85 at the end of the fermentation process (4 h), which demonstrates that the duration of fermentation has a strong impact on the availability of free water in the system. This growth is explained by the fact that enzymes and microbes can break down macromolecules such as proteins and polysaccharides, releasing water bound to them and thereby enhancing water mobility. In product stability terms, reduced water activity (~0.79) is preferable because it improves microbial stability and prolongs shelf life. In contrast, high water activity (~0.85) can be more desirable because it improves mouthfeel but increases microbial spoilage.

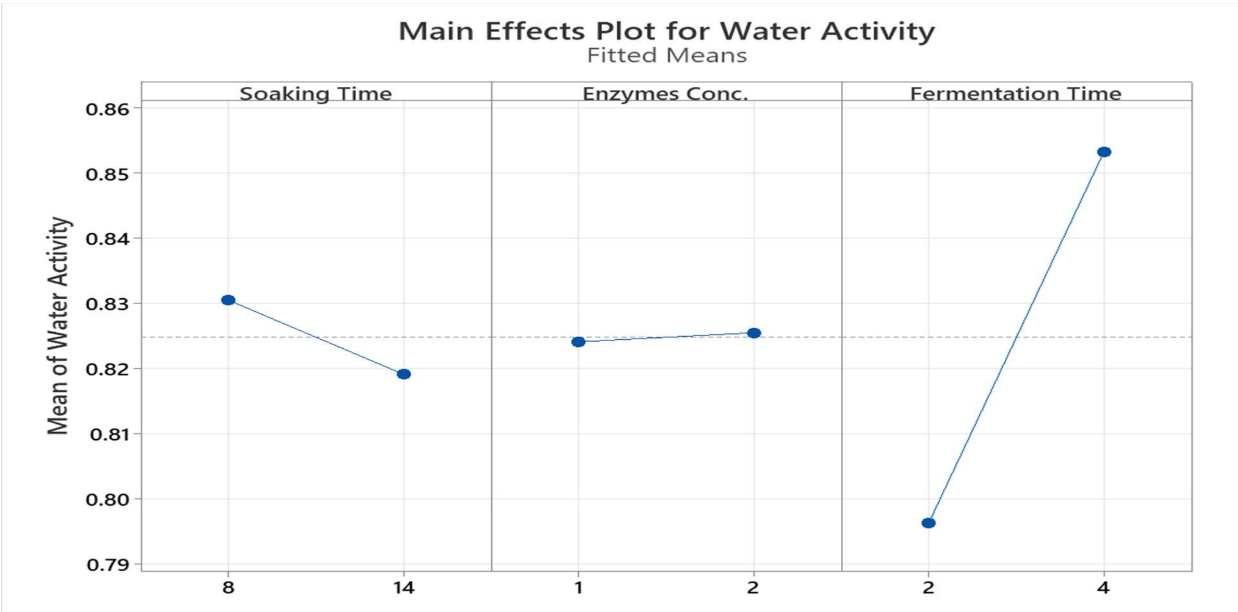

Figure 4.7. Main Effects Plot Depicting the Influence of Water Activity (a_w)

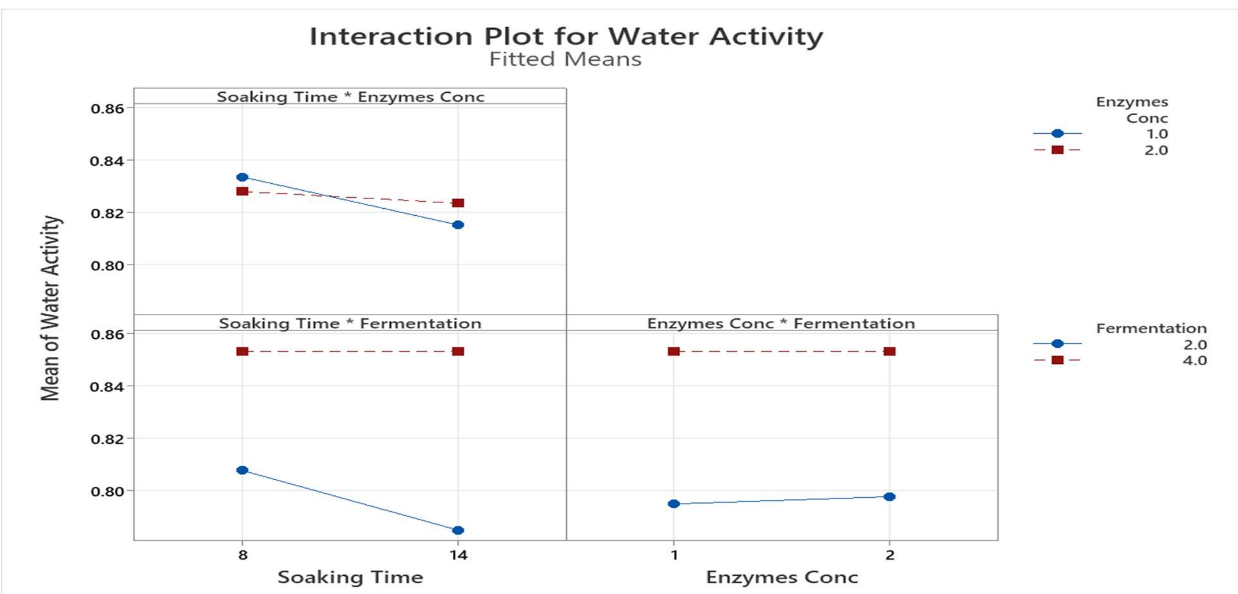

Figure4. 8. Interaction Plot Demonstrating the Combined Effect of Soaking Time and Fermentation Time on Water Activity (a_w)(Y-axis truncated to highlight differences. Mean 0.824)

The time of soaking showed a slight change in water activity, from 0.83 at 8 h to 0.81 at 14 h, but this was not significant ($p > 0.05$), so its effect was considered minimal. The enzyme concentration also had no meaningful effect on a_w ($p > 0.05$). According to the Interaction Plot (Figure 4.8), the interaction between the soaking time and the fermentation time was significant ($p < 0.05$) because the lines are not parallel. The observed effect was lower a_w at longer soaking and longer fermentation, and higher a_w at longer fermentation and longer soaking, indicating that the effect of fermentation time prevails. The relationship between the enzyme concentration and the fermentation time was not significant ($p > 0.05$). The Contour Plot (Figure 4.9) also shows distinct areas of water activity, indicating that higher values correspond to longer fermentation periods, while lower values correspond to shorter fermentation periods and longer soaking periods. The tri-way interaction was also found to be statistically significant ($p < 0.05$), indicating that all processing variables have a combined effect on water activity.

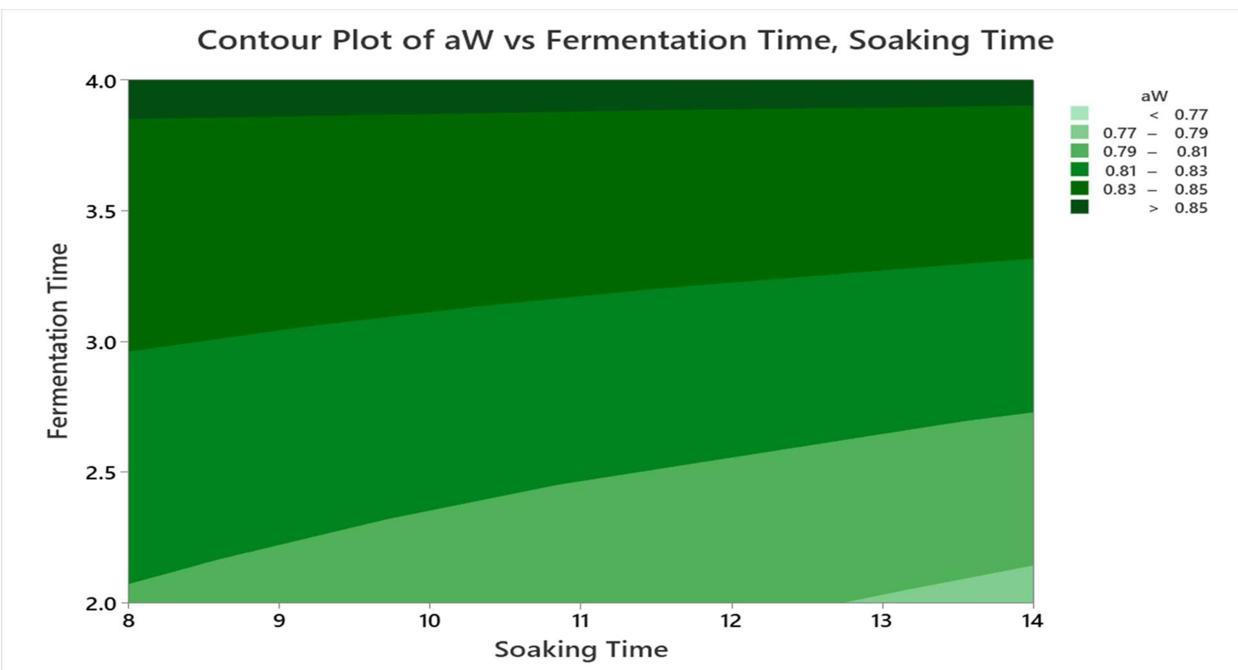

Figure 4.9. Contour Plot Illustrating the Response Surface of Water Activity as a Function of Soaking Time and Fermentation Time). (Y-axis truncated to highlight differences. Mean 0.824)

4.4 Effect on Colorimetry

The influence of soaking time, enzyme concentration, and fermentation time on the color parameters (L*, a*, and b*) was assessed using ANOVA and graphical analysis, and the results showed that the color parameters were strongly affected by the processing conditions. The results of ANOVA showed that the soaking time had a significant effect on all three-color parameters (L, a, and b) ($p < 0.05$), with the highest F-values being the highest of all factors, with the effect of soaking time considered to be the most dominant variable in color development. There were also statistically significant effects of enzyme concentration and fermentation time ($p < 0.05$), but their effects were relatively small compared to the effect of soaking time. The interaction between soaking time and fermentation time was significant ($p < 0.05$) for all color parameters, indicating that the effect of soaking time on color depends on fermentation time. The interaction between soaking time and enzyme concentration was significant ($p < 0.05$) for L and a, whereas the interaction between soaking time and b concentration was not significant ($p > 0.05$), indicating independent behavior of the factors. Moreover, three-way interactions among soaking time, enzyme concentration, and fermentation time were significant for L ($p < 0.05$) but not for a and b ($p > 0.05$), indicating that the joint effects of all factors are stronger on lightness than on chromatic properties.

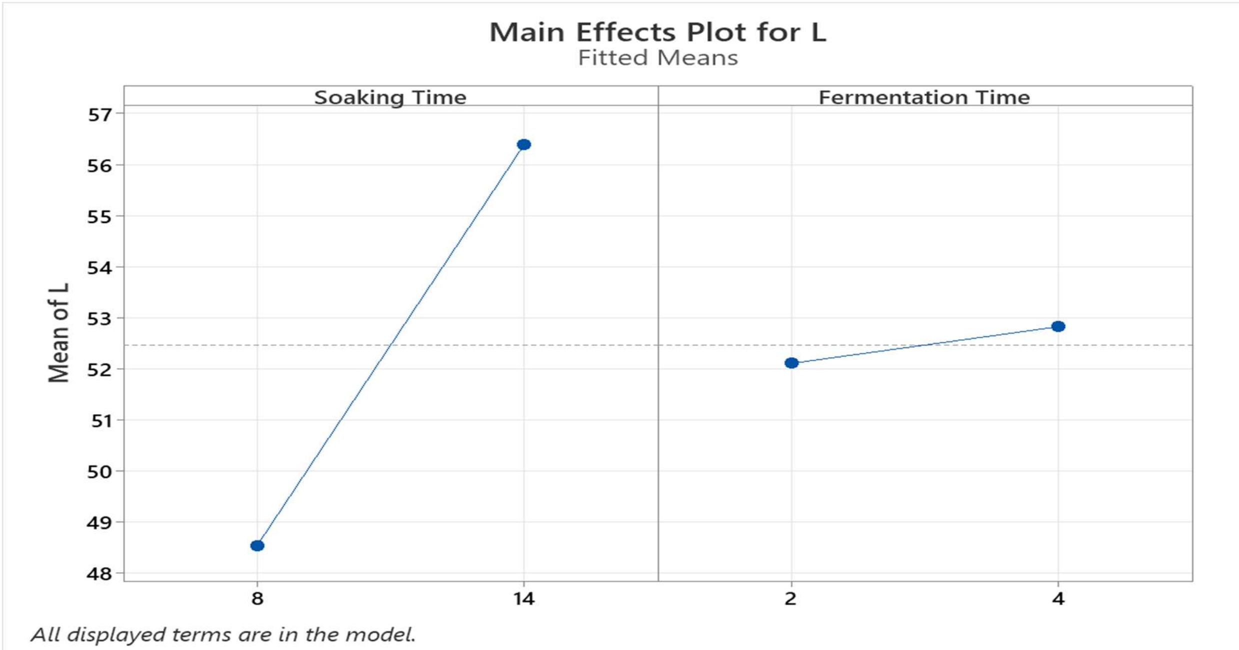

Figure 4.10. Main Effects Plot Depicting the Individual Influence of Soaking Time and Fermentation Time on Lightness (L*) of Mung Bean Milk. (Y-axis truncated to highlight differences. Mean of L* 52.4)

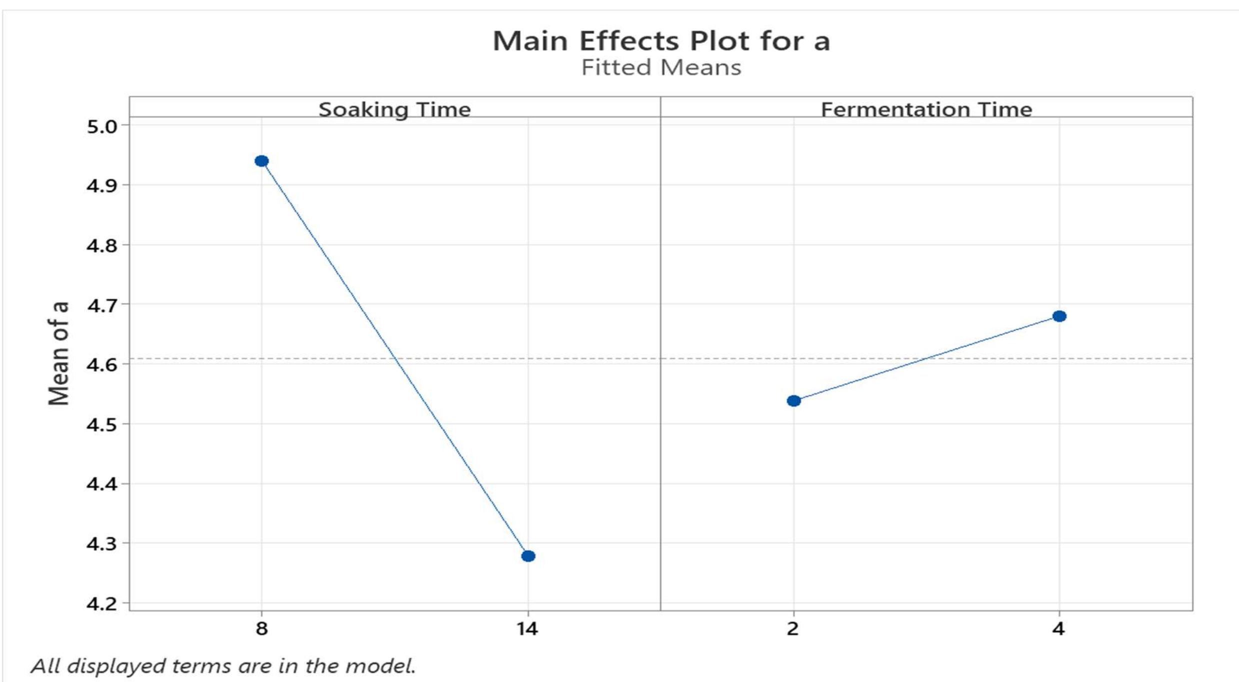

Figure 4.11. Main Effects Plot Depicting the Individual Influence of Soaking Time and Fermentation Time on Red-Green Coordinate (a*) of Mung Bean Milk. (Y-axis truncated to highlight differences. Mean of a* 4.6)

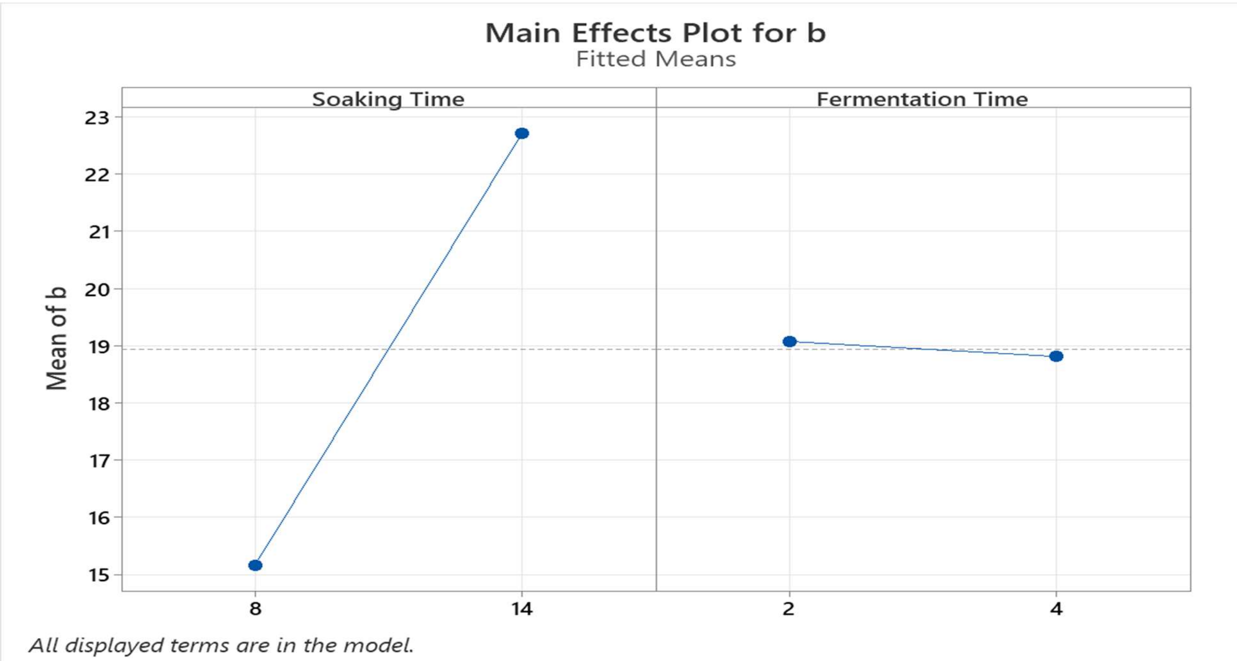

Figure 4.12. Main Effects Plot Depicting the Individual Influence of Soaking Time and Fermentation Time on the Yellow-Blue Coordinate (b*) of Mung Bean Milk (Y-axis truncated to highlight differences. Mean of b* 18.9)

It is reflected in the Main Effects Plots (Figure 4.10 -4.12) that the individual process of lightness (L) was significantly more affected by the increase of the soaking time between 8 and 14 hours ($p < 0.05$), and hence a longer soaking time led to the lighter product, possibly because of the improved hydration and lower intensity of darker pigments. The fermentation time was also slightly increased in L, indicating increased light scattering due to particle structural disintegration. Conversely, the values decreased significantly with increasing soaking time ($p < 0.05$), indicating reduced redness and a shift toward a neutral color. There was a small increase in the indication of slight alterations in the pigment composition during fermentation. The b values were significantly greater with longer soaking time ($p < 0.05$), as expected, because more natural pigments, including carotenoids, were extracted. The trend of fermentation time was slightly negative, indicating that b and a slightly degraded or transformed pigments, whereas the influence of enzyme concentration on L and a was relatively minor but significant ($p < 0.05$), and the effects of enzyme concentration on b were not significant ($p > 0.05$).

The effects of interaction are also illustrated using the contour plot (Figure 4.13) and the interaction trends in the data. The contour plot shows that lower values are more concentrated at longer soaking times, while higher values are more concentrated at shorter soaking times and longer fermentation times, confirming that soaking time is a dominant factor in reducing redness. The strong correlation between soaking time and fermentation time suggests that fermentation alters the influence of soaking, especially with shorter soaking times. Similarly, the high interactions of L* suggest that the combined effects of soaking and fermentation increase lightness at optimal conditions. Regarding b, though the soaking time has a significant impact on yellowness, the interaction effects are not as strong, meaning that b is mainly controlled by the soaking time without significant alterations in other variables, which is also a way to achieve a brighter, less reddish, and naturally appealing color sensation in the plant-based milk systems. In general, these findings indicate that soaking time is the key factor governing color properties.

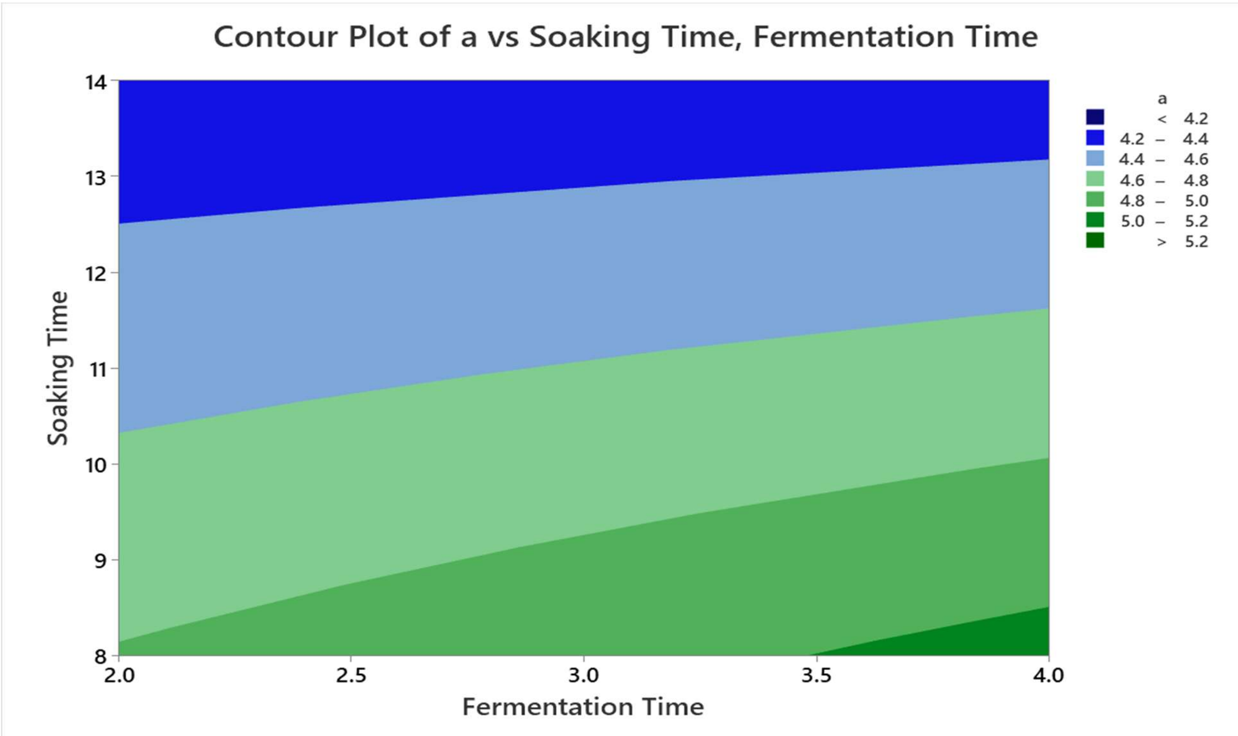

Figure 4.13. Contour Plot Illustrating the Response Surface Distribution of Red-Green Coordinate (a*) as a Function of Soaking Time and Fermentation Time in Mung Bean Milk (Y-axis truncated to highlight differences. Mean of a* 4.6)

4.5 Nutritional Profile Analysis

Standardized analytical methods were used at the Eurofins Nutrition Analysis Center to determine the nutritional content of mung bean milk prepared under long- and short-soaking time conditions (LST: 14 h, SST: 8 h) at a 2% enzyme concentration and fermented for 4 h. The analyses were performed using validated AOAC methods and CFR-based calculations, including protein by combustion (AOAC 990.03/992.15), fat by acid hydrolysis (AOAC 954.02), moisture by vacuum oven (AOAC 925.09), and ash by incineration (AOAC 942.05). The difference in carbohydrates was used to determine carbohydrate content, and the Atwater system was used to determine caloric values.

Table 4.1 presents the results of the proximate composition analysis. The Long Soaking Time (LST) sample had higher carbohydrate content (6.14%) than the Short Soaking Time (SST) sample (5.65%), indicating that the long-soaking condition produced more soluble carbohydrates. Protein content, on the other hand, was marginally higher in the SST (3.63%), relative to LST (3.50%), whereas fat content also increased marginally in SST (0.26%), as compared to LST (0.22%). The amount of moisture in LST (89.7%) was also a bit less than in SST (90.0%), indicating the presence of a relatively high total solid in the LST sample. The two samples had similar ash content (0.44% with LST and 0.46% with SST), suggesting that the mineral content was not significantly different. The caloric value also showed a similar trend, with LST having slightly higher energy (41 kcal/100 g) than SST (39 kcal/100 g). The total dietary fiber content was reported to be less than 0.2% in both samples, with negligible differences between them due to the soaking conditions.

In general, the results, presented in tabular form, show only slight changes in the macronutrient composition with soaking time, with LST exhibiting higher carbohydrate and energy levels, whereas SST retains slightly higher protein and fat levels. These differences indicate that the extraction efficiency and compositional balance differ between the two processing conditions.

Table 4.1. Nutritional Composition of Mung Bean Milk [Long Soaking Time (LST) vs Short Soaking Time (SST)]

Parameter	**Long Soaking Time (14 h)**	**Short Soaking Time (8 h)**
Carbohydrates (%)	6.14	5.65
Protein (%)	3.50	3.63
Fat (%)	0.22	0.26
Moisture (%)	89.7	90.0
Ash (%)	0.44	0.46
Calories (kcal/100 g)	41	39
Dietary Fiber (%)	<0.2	<0.2

4.6 Effect on Sensory Analysis

The sensory properties of mung bean milk were assessed using a paired comparison test (commercial vs. mung bean milk) and a hedonic rating (attribute-based assessment). The analysis was conducted by a panel of 13 participants (n = 13), and the data were analyzed using one-way ANOVA and descriptive statistics. Table 4.2 and Figures 4.14 and 4.15 present the results. To ensure comparability with the commercial product, the developed mung bean milk was standardized with the addition of sugar (3.5 g/100 mL) and vanilla flavor (5 mL/100 mL).

ANOVA was used to compare the mean scores for overall likeability between commercial soy milk and mung bean milk. The commercial sample showed a slightly higher mean score

(~3.5) compared to the mung bean milk (~3.2). However, the difference in average scores was relatively small, and the overlapping 95% confidence intervals (Figure 4.14) indicate that the sensory acceptability of the two samples was comparable. Although statistical analysis indicated a significant effect of sample type ($p < 0.05$), the magnitude of difference suggests limited practical significance. This indicates that the developed mung bean milk achieved a level of sensory acceptability similar to that of the commercial product, with only minor differences in panelist preference. Therefore, the formulated product can be considered sensorially comparable to commercially available plant-based milk.

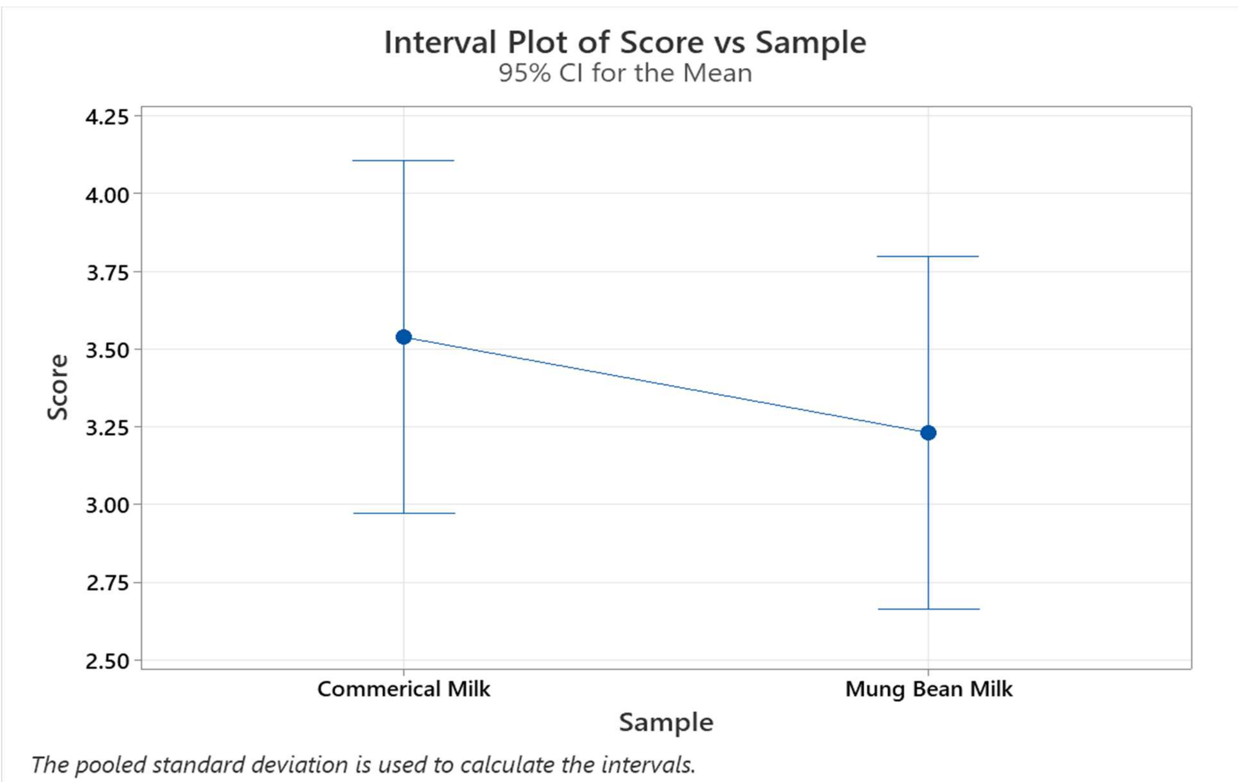

Figure 4.14. Interval Plot Showing Mean Sensory Scores and 95% Confidence Intervals for Commercial Soy Milk and Mung Bean Milk (n = 13)

In addition, the interval plot will give us an idea about the variability of the responses of the 13 panelists. Commercial soy milk has a wider confidence interval, indicating greater variation in perception, whereas the mung bean milk sample has a narrower interval, indicating less variation and more consistent ratings. This trend suggests that panelists' perceptions of mung bean milk remained consistent. The ANOVA results support this observation, indicating that the difference between the two samples is significant ($p < 0.05$).

Table 4.2. Mean Likeability Scores for Comparison Sensory Analysis (n = 13)

Sample	**Mean Score**
Commercial Soy Milk	~3.5
Mung Bean Milk	~3.2

In addition to the comparison test, a thorough hedonic analysis was conducted to evaluate the sensory qualities separately, and the findings are summarized in Table 4.3 and Figure 4.15. An average score of appearance, aroma, texture, taste, aftertaste, and overall acceptability was determined using the feedback of all 13 subjects. The findings indicate that appearance (~6.6) and texture (~6.4) scored highest, indicating that the product had acceptable visual quality and mouthfeel. The ratings for aroma (~5.9) and overall acceptability (~5.7) were moderate, whereas the rating for taste (~5.4) indicated slightly less acceptance. The lowest score was observed for aftertaste (~4.5), suggesting that the remaining flavor properties negatively affected panelist perception.

The mean score chart (Figure 4.15) clearly shows the differences in the sensory attributes and the overall perception of the 13 panelists. The appearance and texture scores were higher, indicating that enzymatic processing and fermentation had a positive impact on consistency, homogeneity, and appearance of the products. These functionalities are key to consumer acceptance in plant milk systems. Nevertheless, low scores for taste and aftertaste indicate that flavor-related characteristics are a constraint. Beany notes, bitterness, or compounds formed during fermentation can lead to lower ratings in these attributes.

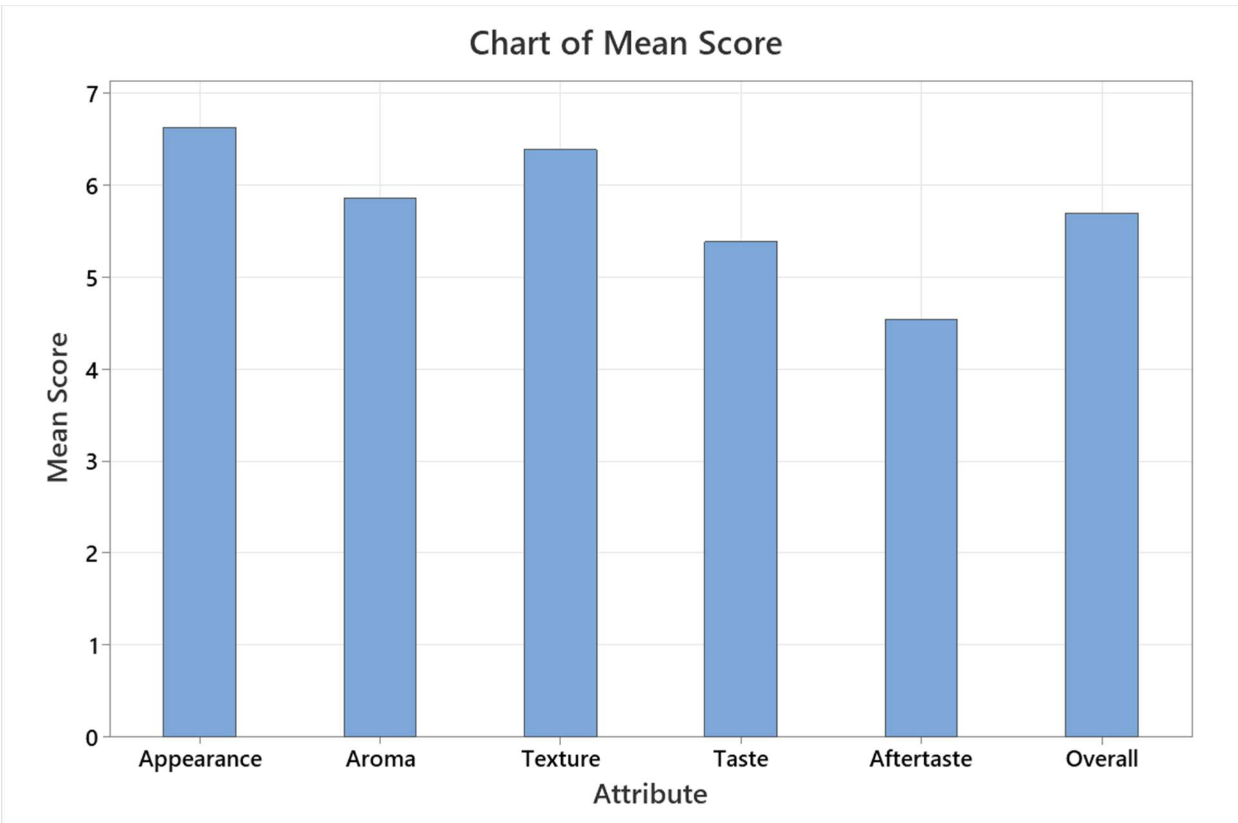

Figure 4.15. Mean Hedonic Scores of Sensory Attributes of Mung Bean Milk (n = 13)

Statistically, differences in attribute scores indicate that sensory reactions vary widely across parameters, and ANOVA confirms these differences as statistically significant ($p < 0.05$). The comparatively low aftertaste score indicates the importance of flavor persistence for overall acceptability. The overall acceptability score (~5.7) falls in the slightly to moderately liked category, but it is more influenced by physical characteristics (texture and appearance) than by flavor.

Table 4.3. Mean Hedonic Scores of Sensory Attributes (n = 13)

Attribute	**Mean Score**	**Interpretation**
Appearance	~6.6	Like moderately
Aroma	~5.9	Slightly liked
Texture	~6.4	Like moderately
Taste	~5.4	Slightly liked
Aftertaste	~4.5	Neutral to slightly disliked
Overall Acceptability	~5.7	Moderately acceptable

Overall, ANOVA and descriptive analysis indicate that sensory attributes have a significant impact on overall acceptability ($p < 0.05$), with appearance and texture positively affecting overall acceptability, while taste and aftertaste are limiting factors. The fact that mung bean milk is moderately acceptable is also corroborated by a comparison analysis, which shows that mung bean milk differs significantly from commercial soy milk in sensory perception. These results indicate that although processing parameters such as soaking, enzymatic treatment, and fermentation can improve structural and physicochemical properties, additional flavor refinement is needed to increase consumer acceptance (APENDIX A AND B).

CHAPTER 5 DISCUSSION

The current research demonstrates that optimizing enzymatic extraction parameters significantly influences the physicochemical, sensory, and nutritional characteristics of mung bean (*Vigna radiata*) milk. The application of a full factorial (2^3) design enabled systematic evaluation of soaking time, enzyme concentration, and fermentation duration, allowing identification of both main and interaction effects. These findings confirm that controlled processing conditions are critical for improving extraction efficiency, product stability, and overall quality of plant-based milk systems. This approach is consistent with previous studies that emphasize the importance of multivariate optimization for achieving desirable functional and sensory attributes in plant-based beverages (McClements, 2020). The optimized condition of 14 h soaking, 2% enzyme concentration, and 4 h fermentation resulted in a balanced product with good pH stability, improved color, enhanced extraction properties, and acceptable sensory quality.

This study shows that mung bean milk can be effectively prepared using an enzyme-assisted extraction process, with processing variables (soaking time, enzyme concentration, and fermentation time) affecting product quality differently. The results confirm that processing conditions influence the physicochemical, nutritional, and sensory qualities of mung bean plant milk. In conclusion, the optimal condition of 14 h soaking, 2% enzyme concentration, and 4 h fermentation resulted in a balanced product with good pH stability, improved color, good extraction properties, and acceptable sensory properties. This finding is significant, as plant-based milks generally exhibit low protein content, poor emulsion stability, off-flavor, separation, and reduced acceptability compared to dairy milk and commercial soy milk (Walther et al., 2022; Karoui et al., 2024). Recent comparative studies also demonstrate that plant-based beverages differ in protein quality, mineral fortification, and nutrient bioavailability, suggesting formulation and processing strategies are required to enhance their nutritional equivalence to dairy milk.

Mung bean was chosen as the raw material for this study because it has a high potential as a legume-based milk source, with protein, low flavor, starch-protein matrix, and bioactive components. Mung bean proteins are mostly globulins and albumins, including the 8S globulin, which provides emulsification, water-binding, foaming, and gelling properties (Tang & Sun, 2010; Zhu et al., 2018; Brishti et al., 2017; El-Adawy, 2000; Kudre et al., 2013). These functional characteristics are strongly influenced by the structural configuration and molecular interactions of mung bean globulins, which determine their solubility, emulsifying capacity, and gelation behavior (Tang & Sun, 2010). However, mung bean protein is deficient in sulfur-containing amino acids, particularly cysteine and methionine, indicating mung bean milk will need to be nutritionally fortified or complemented through blending or protein supplementation to be used as a dietary supplement (Zhu et al., 2018; Dahiya et al., 2015). Studies found the protein content of mung bean milk to be around 3.50-3.63%, comparable to soy milk and higher than dairy milk, and higher than other nut or cereal-based plant drinks. Zhu et al. (2018) reported that mung beans are an excellent protein source but lack sulfur-containing amino acids, which justifies blending or fortifying mung bean milk in future studies.

The nutrient analysis demonstrated that a short soaking time slightly retains more protein and fat, but a long soaking time slightly increases carbohydrate and energy content. The SST sample had 3.63% protein compared to 3.50% in LST, while LST had 6.14% carbohydrate and SST had 5.65% carbohydrate. The differences were minimal, suggesting that soaking time affected extractability but did not significantly alter the proximate composition. The higher protein in SST may result from reduced leaching of soluble nitrogenous compounds into the soaking solution. Longer soaking may allow water-soluble proteins and peptides, minerals, and low-molecular-weight carbohydrates to leach into the soaking water, thereby decreasing their concentration in the beverage (Mubarak, 2005; Pokharel et al., 2024). However, longer soaking may result in better hydration and softening of cell walls, enhancing the extraction of soluble carbohydrates derived from starch during processing and grinding. So, the nutritional difference between LST and SST is due to a combination of nutrient loss and greater extraction.

The increase in carbohydrates in the LST sample can also be attributed to the synergistic effect of soaking and amylase. Soaking can swell and loosen starch granules, making them more susceptible to enzyme action. Amylase hydrolyses starch to dextrins and smaller sugars, which may increase the recovery of total carbohydrates and contribute to body, sweetness, and mouthfeel. Similar effects have been observed in cereal- and legume-based beverages, where enzymatic breakdown improves soluble solids, decreases viscosity, and stabilizes beverages (Du et al., 2018; Cichońska et al., 2021). In this research, the increased TSS at 2% enzyme concentration supports this hypothesis, as higher enzyme concentrations likely enhanced the breakdown of starch, cell wall polysaccharides, and protein-carbohydrate complexes. Therefore, the nutritional and TSS data suggest that enzyme-assisted extraction increased the extraction of soluble nutrients into the beverage.

Protein is a critical nutritional component in the development of plant-based milk, as many commercial plant-based milks (particularly almond, rice, coconut, and oat) have much lower protein content than dairy milk. In the present study, mung bean milk contained about 3.5-3.6 g of protein per 100 g, which is nutritionally significant and similar to that of typical soy milk. This is one of the key advantages of the present product. However, protein content is not the sole factor in protein quality. Protein digestibility, amino acid composition, and antinutritional factors also affect the protein quality (Walther et al., 2022; Karoui et al., 2024; Kudre et al., 2013). Mung bean proteins are high-quality legume proteins with a high lysine content. However, their low sulfur amino acid content could limit protein quality unless combined with cereal proteins, such as those from rice, oat, and millet (Zhu et al., 2018). Thus, future product formulation may explore blends of mung bean-cereal to balance amino acids, while ensuring desirable sensory attributes.

The low-fat levels (0.22-0.26%) in both LST and SST samples indicate that the developed mung bean milk is a low-fat product. This could be a benefit for those looking for low-fat plant-based milks, but fat content also affects milk appearance, creamy texture, fat release, and satiety. The low-fat content may partly explain the lower taste and aftertaste scores compared with appearance and texture in the sensory analysis. Fat droplets in dairy milk and many commercial plant-based milks contribute to creaminess and taste. Plant-based milks with low fat content may be thinner, less rich, or less balanced in the absence of stabilizers, emulsifiers, or plant oils (McClements, 2020; Ashraf et al., 2026). So, although low-fat content is nutritionally desirable, product formulation optimization with a low percentage of healthy, unsaturated plant oils (e.g., sunflower, canola, high oleic oils) may enhance consumer acceptability without significant increases in saturated fat.

The ash values (LST 0.44% and SST 0.46%) were comparable, indicating that the mineral content was not affected by soaking time. This is a critical finding, as legumes are naturally rich in minerals such as potassium, magnesium, iron, and zinc. However, legumes contain antinutritional factors, such as phytates and tannins, which limit the bioavailability of minerals. Mung bean is rich in bioactive compounds, such as phenolic acids, flavonoids, and peptides, but it also contains antinutritional compounds, including phytic acid and trypsin inhibitors, which limit protein digestibility and mineral bioavailability (Hou et al., 2019; Pokharel et al., 2024). Soaking, germination, fermentation, and enzymatic processes reduce antinutrients and increase the nutritional value of legumes. Recent research on mung bean processing demonstrates that both traditional and thermal processing can reduce antinutrients, providing evidence for the use of soaking and enzymatic processing in this study.

Fermentation also has a nutritional value; lactic acid fermentation can increase mineral bioavailability by reducing phytate levels, increasing free amino acids, generating bioactive peptides, and improving the digestibility of legume proteins (Cichońska et al., 2021; Wu et al., 2015). In mung beans, *Lactobacillus plantarum* fermentation has been shown to enhance probiotic, antioxidant properties, and functional properties (Wu et al., 2015). Fermentation can also lead to the development of organic acids and aromas that enhance consumer acceptability but may cause sourness or off-taste when over-fermented. In the current study, the effect of fermentation time on pH was minimal, meaning low acidification, which stabilized the product and prevented sour taste. This is desirable, as a low pH can destabilize legume proteins at their isoelectric points, leading to precipitation and curdling. The pH range measured in this study suggests that the fermentation conditions were not too harsh for flavor development while maintaining protein stability.

Water activity findings showed that the availability of free water was greatly influenced by fermentation time. An increase in water activity resulting from longer fermentation may improve mouthfeel but can also lead to microbial spoilage. This is a significant consideration for product shelf-life. Plant-based milk is a high-moisture food, and water activity plays a crucial role in microbial stability. While higher water activity may lead to a smoother texture and better solubilization of solids, it also needs careful pasteurization, refrigeration, and possibly other preservation technologies. Thus, the optimized sample should be considered a cold-stored beverage rather than a shelf-stable beverage without additional processing (e.g., pasteurization), packaging (e.g., aseptic), preservation (e.g., high-pressure processing), or preservatives. Future studies should include shelf-life and microbial tests (*yeast and mold, coliforms*), as well as storage at refrigeration temperatures, to assess commercial viability.

The results for the impact on L*, a*, and b* showed that soaking time was the most significant factor. Soaking time increased lightness and decreased redness, making the beverage more appealing. This is crucial, as it is one of the first consumer uses for assessing the quality of plant-based milk. Higher lightness and uniformity with a cream color are more acceptable as they are similar to dairy milk and commercial soy milk. Soaking longer may have improved visual lightness by increasing hydration, removing seed coat pigments, reducing dark particles, and improving dispersion of suspended solids. Hydrolysis and homogenization may also improve light scattering by reducing particle size and enhancing colloidal dispersion. These results are in line with those of plant-based beverages, where the visual whiteness and opacity of beverage systems are greatly influenced by particle size, protein dispersion, and suspended solids (McClements, 2020; Daryani et al., 2024).

The sensory findings are significant as acceptability is a key issue for legume-based beverages. In the present study, the mean likability of commercial soy milk was slightly higher than that of mung bean milk. The mung bean milk had a similar level of acceptability, suggesting the formulation can be commercialized. In the sensory evaluation, appearance and texture had higher scores, aroma and overall acceptability had moderate scores, while taste and aftertaste had lower scores. This is typical of legume-based beverages, as the appearance and texture can be enhanced by filtration, homogenization, and stabilization. In contrast, taste and aftertaste can be enhanced through more complex formulation techniques.

The high appearance score indicates the process was effective in producing a visually appealing beverage. Soaking, dehulling, filtration, microfiltration, homogenization, and xanthan gum probably decreased sedimentation, making the beverage smoother and more consistent in color. The texture also rated highly, suggesting that enzyme treatment and homogenization improve texture. Cellulases and proteases may reduce particle size and improve protein-polysaccharide interactions, and xanthan gum increases the viscosity and stability of the suspension. This aligns with several plant-based milk studies showing that hydrocolloids and homogenization increase body, prevent phase separation, and increase consumer acceptance (McClements, 2020; Tangyu et al., 2019).

Aftertaste and taste were the most limiting sensory attributes. Aftertaste had the lowest score, suggesting that beany, earthy, grassy, bitter, and astringent flavors may have been present in the mung bean milk. Legume off-flavors are mainly associated with enzymes such as lipoxygenase, as well as aldehydes, ketones, alcohols, saponins, phenolics, and protein breakdown products. Mung beans are typically less off-taste than soy or pea but may still exhibit legume-like aftertaste if enzymes, pasteurization, and masking are not optimized (Roland et al., 2017; Murat et al., 2013). Enzymatic hydrolysis increases solubility but can also generate bitter peptides, depending on the degree of hydrolysis and the protease type. So, the low aftertaste score may have been due to either mung bean flavor compounds or peptides released by enzymes.

Fermentation can eliminate bean off-flavor by removing precursors and synthesizing positive volatile compounds, such as organic acids, acetoin, and diacetyl. However, fermentation should be monitored, as over-fermentation can lead to sour, bitter, or yeasty off flavors. In this study, the 4 h fermentation probably contributed to the medium aroma and acceptability, but was insufficient to remove the aftertaste. LAB strain might be key to improvement. Fermented legume-based beverages report that different *Lactobacillus* and *Bifidobacterium* strains exhibit distinct flavors, acidification, exopolysaccharide production, and bioactive compounds (Cichońska et al., 2021; Reyes-Jurado et al., 2021). Thus, future sensory improvements should include specific starter cultures such as *Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus*, and *Bifidobacterium animalis subsp*. Lactis to see how they affect the taste, aftertaste, mouthfeel, and probiotic survival.

Compared to commercial soy milk, it was justified as soy milk is one of the most nutritionally similar plant milks to dairy milk and a good benchmark for legume-based beverages. Soy milk is generally more familiar to consumers and has better commercial processing, such as improved heat treatment, deodorization, fortification, sweetening, and stabilizers. The minimal difference between commercial soy milk and mung bean milk indicates that mung bean milk is well accepted despite being a new product. However, the absolute liking scores in the comparative test were generally low, suggesting that the panelists did not like either sample very much. This may be because of panelist preference, unfamiliarity with the taste of unsweetened or low-flavored plant-based milks, or the commercial soy milk used.

The 9-point hedonic scale was scientifically valid because it is commonly used to assess consumers' liking of a product. It enables panelists to rate the intensity of preferences for appearance, aroma, taste, texture, aftertaste, and overall liking. However, the sensory panel (n=13) is small. A small panel might be useful to generate preliminary acceptability data, but it may not reflect the preferences of the average consumer. When developing products, it is generally recommended to use larger consumer panels (50-100 untrained participants) to make more reliable inferences about product acceptance. So, sensory data should be used to show preliminary acceptability, rather than consumer acceptance. Future research should involve a larger panel, segmented by demographics, purchase intention, just-about-right scaling, and descriptive sensory profiling by trained panelists.

This study demonstrates that mung bean milk is a promising, nutritious plant-based milk, particularly for its protein content and acceptable sensory qualities. Mung bean milk is a better protein source than nut and cereal milk. Compared with soy milk, mung bean milk may provide a less allergenic, milder product, but further research is needed on its allergenicity, digestibility, amino acid profile, and flavor acceptability. The nutritional findings indicate that either LST or SST processing conditions are suitable, but each has its own merits. SST was slightly better for protein and fat retention, but LST resulted in better carbohydrate extraction, color, and processing properties. Based on overall physicochemical, nutritional, and sensory results, 14 h of soaking, 2% enzyme concentration, and 4 h of fermentation are considered the best processing conditions.

Commercially, the results suggest that enzymes can improve the quality of mung bean milk. Enzymes enhance soluble solid content and texture, whereas fermentation enhances flavor and potential functional nutritional properties (An et al., 2024). However, more needs to be done in masking and aftertaste reduction, fortification, and shelf-life testing. Calcium, Vitamin D, Vitamin B12, and potentially methionine-rich protein sources would make it more nutritionally equal to dairy milk. Natural flavors such as vanilla, cocoa, cardamom, and fruit flavors may help acceptance. Blanching or light heating prior to grinding may reduce off-flavors caused by lipoxygenase.

Overall, this study demonstrates the scientific merit of the research, showing that mung bean milk can be produced as a protein-rich, low-fat, lactose-free, and potentially sustainable milk alternative. It has acceptable pH stability, appearance, and texture, adequate protein content, and sensory properties similar to those of commercial soy milk. The key challenge was the aftertaste, which could be improved by selecting a starter culture, optimizing flavor, heat treatment, and product formulation. The research contributes to the emerging field of pulse-based dairy products by demonstrating how soaking, enzyme levels, and fermentation parameters can be adjusted to enhance product attributes. Through additional research on fortification, shelf-life, and consumer testing, mung bean milk can be further developed for commercial use as a nutritious, functional plant-based milk product.

CHAPTER 6 CONCLUSION

This study has achieved its aim of optimizing the enzyme extraction procedure for mung bean (*Vigna radiata*) to improve plant milk production by systematically assessing the effects of soaking time, enzyme concentration, and fermentation time. The full factorial experimental design (2^3) enabled thorough examination of the main and interaction effects of processing variables on physicochemical properties, sensory quality, and nutritional composition. The results indicated that the enzyme is highly efficacious in controlled enzyme treatment, achieved through optimized processing conditions, thereby increasing extraction efficiency and product quality, thereby justifying the study's hypothesis.

The processing variables that had the greatest impact on total soluble solids (TSS), color attributes, and extraction efficiency were soaking time and enzyme concentration. Soluble solids were retained better when the soaking time was short (8 h), and those visual properties, like lightness, were better when soaked longer. The enzyme concentration (2%) had a significant effect on disaggregating the macromolecules, improving the extract's solubility and yield. Even though fermentation time did not significantly affect pH, it was instrumental in altering water activity and in structural and functional modifications within the system. The observed interaction between enzyme concentration and fermentation also highlighted the importance of integrated processing optimization when developing plant-based milk.

The physicochemical analysis proved that the mung bean milk developed had desirable pH stability, acceptable total soluble solids, controlled water activity, and enhanced color properties. These properties are vital in maintaining stability, shelf life, and consumer acceptability of the products. The sensory evaluation results showed that the optimized formulation achieved a satisfactory level of appearance, texture, and overall acceptability, but minor differences compared with the commercial plant-based milk were observed, suggesting the possibility of further refinement. Nutritional evaluation showed that mung bean milk has a desirable macronutrient profile, particularly its protein content, making it a strong alternative to traditional plant-based drinks made from cereals and nuts. The nutritional profile observed in this study aligns with previous research indicating that plant-based milk alternatives require careful optimization to match the protein content and micronutrient composition of dairy milk (Nowson et al., 2025).

Altogether, this study confirms that enzymatic-assisted extraction, along with optimized soaking and fermentation conditions, is an efficient and scalable method for producing high-quality mung bean milk. The research will help advance the development of sustainable, plant-based dairy substitutes by illustrating the potential of mung beans as a nutrient-rich, functional ingredient. The results provide a solid scientific basis for developing future products, streamlining processes, and commercializing pulse-based milk alternatives to meet the growing demand for sustainable, health-focused food products.

CHAPTER 7 FUTURE RESEARCH

Future studies should aim to improve the functionality, sustainability, and commercial viability of mung bean milk by refining its processing and diversifying its products. Fermented mung bean milk, which uses probiotic cultures, including *Lactobacillus plantarum* and *Bifidobacterium spp*., has a promising future as fermentation could enhance flavor, digestibility, and production of bioactive compounds, including γ-aminobutyric acid (GABA), thus making fermented drinks useful functional beverages with added health benefits (Reyes-Jurado et al., 2021). Also, fermentation conditions, such as strain selection and incubation conditions, can be optimized to further enhance sensory and nutritional quality.

The other significant field is the valorization of by-products produced during filtration. The solids left behind contain high levels of protein, dietary fiber, and bioactive compounds. They can be used in value-added products such as bakery products, plant-based protein powders, or snack formulations. This solution facilitates sustainable processing and meets the principles of a circular economy by minimizing waste and enhancing resource efficiency (McClements, 2020). The other area that needs to be researched is natural stabilizers, clean-label hydrocolloids, and advanced processing methods such as high-pressure homogenization to enhance emulsion stability and shelf life. Additionally, research on micronutrient fortification, shelf-life stability, and mung bean milk processing at scale is required to facilitate commercialization and increase product use in the expanding plant-based beverage sector.

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APPENDIX (A) : LIKEABILITY RATING AND PAIRED PREFERENCE TEST

LIKEABILITY RATING AND PAIRED PREFERENCE TEST

INSTRUCTIONS:

1. Proceed when you are ready. (Quietly so as not to distract others.)
2. Evaluate one sample at a time, working from left to right.

FOR EACH SAMPLE: Taste the sample then CIRCLE the number which best expresses your opinion of the sample.

SAMPLE CODE/NAME: _____

Like very much	Like moderately	Like slightly	Dislike	Dislike Very Much
5	4	3	2	1

SAMPLE CODE/NAME: _____

Like very much	Like moderately	Like slightly	Dislike	Dislike Very Much
5	4	3	2	1

Describe the DIFFERENCES between the two samples. (Please be specific.)

Circle the one you prefer. _____ _____

Describe the reasons why you prefer the one you chose.

APPENDIX (B): HEDONIC RATING SCALE TEST

Participant: ____________
Date: ____________
Sample #: ____________

Please record your participant number and date at the top of this form. Evaluate each sample using the 9-point hedonic scale, where 1 = Dislike Extremely and 9 = Like Extremely. Begin by observing the sample's appearance and aroma without tasting. Then take a small sip, hold it briefly in your mouth, and evaluate attributes such as texture, taste, and aftertaste. You may take additional sips if needed but are not required to consume the entire sample.

Please provide honest ratings based on your personal preference and include comments where applicable. Do not discuss your evaluation with others during the session.

Appearance:

☐ Dislike Extremely	☐ Dislike Very Much	☐ Dislike Moderately	☐ Dislike Slightly	☐ Neither Like nor Dislike	☐ Like Slightly	☐ Like Moderately	☐ Like Very Much	☐ Like extremely

Comments on Appearance: ____

Aroma:

☐ Dislike Extremely	☐ Dislike Very Much	☐ Dislike Moderately	☐ Dislike Slightly	☐ Neither Like nor Dislike	☐ Like Slightly	☐ Like Moderately	☐ Like Very Much	☐ Like extremely

Comments on Aroma: ____

Texture (Mouthfeel):

☐ Dislike Extremely	☐ Dislike Very Much	☐ Dislike Moderately	☐ Dislike Slightly	☐ Neither Like nor Dislike	☐ Like Slightly	☐ Like Moderately	☐ Like Very Much	☐ Like extremely

Comments on Texture (Mouthfeel): ____

Taste (Flavor):

☐ **Dislike Extremely**	☐ **Dislike Very Much**	☐ **Dislike Moderately**	☐ **Dislike Slightly**	☐ **Neither Like nor Dislike**	☐ **Like Slightly**	☐ **Like Moderately**	☐ **Like Very Much**	☐ **Like extremely**

Comments on Taste (Flavor): ____

Aftertaste:

☐ **Dislike Extremely**	☐ **Dislike Very Much**	☐ **Dislike Moderately**	☐ **Dislike Slightly**	☐ **Neither Like nor Dislike**	☐ **Like Slightly**	☐ **Like Moderately**	☐ **Like Very Much**	☐ **Like extremely**

Comments on Aftertaste: ____

Overall Acceptability:

☐ **Dislike Extremely**	☐ **Dislike Very Much**	☐ **Dislike Moderately**	☐ **Dislike Slightly**	☐ **Neither Like nor Dislike**	☐ **Like Slightly**	☐ **Like Moderately**	☐ **Like Very Much**	☐ **Like extremely**

Comments on Overall Acceptability: ____