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ECONOMIC ANALYSIS OF A BIOPROCESS FOR PRODUCING DAIRY FORMULA HAVING LOWER ALLERGENIC ACTIVITY

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CONTENTS

1 INTRODUCTION AND OBJECTIVES

Cow's milk protein allergy (CMPA) is one of the most common food allergies in early childhood, affecting approximately 2–3% of infants (Cleveland Clinic, n.d.; Malik & Kaul, 2024). Milk allergies pose a significant public health concern because the primary allergens in cow's milk-including casein, β-lactoglobulin, and α-lactalbumin-can trigger a range of symptoms from mild gastrointestinal discomfort to severe anaphylaxis (Mayo Clinic, n.d.; Bu et al., 2013). In CMPA, the immune system erroneously recognizes certain milk proteins as harmful, leading to an immune-mediated reaction. This reaction manifests through the production of IgE antibodies in IgE-mediated responses or via non-IgE pathways, which in some cases produce a mixed clinical profile (Vandenplas et al., 2023; Cuomo et al., 2017).

Dairy foods have long been recognized not only for their nutritional benefits-providing high-quality proteins, essential amino acids, and favorable digestibility profiles (Mathai et al., 2017)-but also for their potential role in ameliorating a wide range of diseases (Shiby & Mishra, 2013; Achaglinkame et al.,2023). However, the consumption of dairy products presents a dual challenge. On one hand, they contribute about 18–20% of total protein intake in adults and are a rich source of amino acids, such as ketogenic (KAAs), glucogenic (GAAs), essential (EAAs), and branched-chain amino acids (BCAAs) (Garau et al., 2021). On the other hand, some individuals, particularly infants and young children, experience milk allergies and lactose intolerance, making the nutritional benefits inaccessible without risking health (Lawson et al., 2024).

The allergenic potential of dairy proteins is not solely dependent on their primary structures. Instead, the heterogeneity of the human IgE response plays a crucial role, as even small protein fragments can possess antigenic epitopes (Wal.,2002). Furthermore, dairy proteins are often present in processed foods beyond traditional dairy, which increases the likelihood of unintended allergenic exposure (E. G.J. et al., 1991). As cow and buffalo milk are significant sources of high-quality proteins (30–39 $g \cdot L^{-1}$ and 22–47 $g \cdot L^{-1}$, respectively) (Garau et al., 2021), eliminating them from the diet may lead to nutritional deficiencies, especially in vulnerable populations such as infants and children (Lawson et al., 2024).

To reduce the risk of allergic reactions while preserving the nutritional and functional properties of dairy proteins, various bioprocessing techniques have been explored. Traditional approaches—such as heat treatment, high-pressure homogenization, enzymatic hydrolysis, and

fermentation—have been employed to modify the structure of dairy proteins, thereby reducing their allergenic potential (Bu et al., 2013; van Lieshout et al., 2020). However, these methods come with challenges; for example, while physical treatments may diminish antigenicity, the correlation between process parameters and the reduction in allergenic activity is often nonlinear. Such treatments can also alter protein folding, leading to changes in lactosylation, oxidation, and cross-linking, which may affect nutritional quality (Buňka et al., 2009; Danielsen et al., 2020).

Enzymatic hydrolysis has emerged as a promising technique due to its recognized safety by the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) (Abd El-Salam & El-Shibiny., 2017). Although enzyme-mediated hydrolysis can sometimes generate antigenic peptides with immunomodulatory activity (Murtaza et al., 2022), these processes are generally preferred over acid or alkali hydrolysis, which can damage essential amino acids (Buňka et al., 2009; Danielsen et al., 2020). Hypoallergenic formulas, such as extensively hydrolyzed formulas (eHF) and amino acid-based formulas (AAF), rely on these biochemical modifications to reduce antigenicity and are widely used in managing CMPA (DRACMA Guidelines, 2012; Fiocchi et al., 2009).

Given the dual challenge of reducing allergenic potential while maintaining nutritional integrity, this study aims to develop a dairy formula with reduced allergenic activity using a combined enzymatic and microbial hydrolysis approach. By modifying milk proteins without resorting to complex isolation and purification processes, the proposed method seeks to balance allergen reduction, nutritional value, and cost-effectiveness (Milk Genomics, n.d.). In addition to assessing the allergenic potential, the research will evaluate the bioactive properties of the derived peptides, which could offer additional health benefits (Bu et al., 2013). Furthermore, an economic analysis will be conducted to compare production costs and feasibility with conventional dairy processing methods, drawing insights from previous economic evaluations of dairy bioprocessing techniques (Agile BioFoundry, n.d.; Colacicco et al., 2024).

GOAL OF THE STUDY

Milk and dairy foods have been considered as a well-balanced diet regardless of sociodemographic (geographic continent, urban or rural region and the composition of the population), cultural, lifestyle and environmental factors. However; the role of dairy foods in the amelioration of wide ranges of diseases and their potentiality to ensure sustainable wellness, dairy foods sensitive community is often experienced with lactose intolerance and protein-mediated allergenic activity. Dairy food allergy may be categorized as IgE-mediated, non-IgE

mediated and mixed (combining IgE with non-IgE) based on pathophysiological condition. Therefore, the reduction of allergenic activity of dairy proteins might be beneficial from a nutritional viewpoint. It was found that hypoallergenic dairy protein formula could be produced by sequential tryptic and microbial hydrolysis of dairy proteins in a lab-scale experiment (50 mL) (Nath et al., 2021); however, economic analysis of this approach was not explored. The objective of this investigation is to perform economic analysis of the proposed process scheme. Results were compared with other approaches dedicated to producing dairy protein formulas having lower allergenic activity. Information from articles published in peer-reviewed scientific journals about microwave heating, high hydrostatic pressure, enzymatic and microbial hydrolysis alone and with combination were taken into consideration for this aid. The SuperPro Designer software (v. 12, Intelligen, Inc., USA) and concepts from chemical engineering were considered to perform economic analysis of mentioned process schemes.

Specific Objectives of the Study

The specific objectives of the study are:

- To optimize enzymatic and microbial hydrolysis parameters (e.g., enzyme concentration, pH, temperature, and fermentation time) for effectively reducing the allergenic activity of milk proteins.
- To evaluate the impact of hydrolysis on allergenic proteins by assessing the structural and immunological changes in β-lactoglobulin, α-lactalbumin, and caseins.
- To analyze the nutritional and sensory properties of the hypoallergenic dairy formula to ensure it maintains desirable taste, texture, and nutrient composition.
- To investigate the bioactive properties of peptides generated from enzymatic and microbial hydrolysis, focusing on their antioxidant, antihypertensive, and immunomodulatory effects.
- To assess the economic feasibility of the bioprocess by conducting cost estimation, profitability analysis, and market potential evaluation for large-scale production.
- To evaluate the scalability of the proposed bioprocess by determining its technical feasibility from laboratory to industrial-scale dairy production.
- To compare the newly developed hypoallergenic dairy formula with existing products in terms of allergenicity reduction, cost-effectiveness, and consumer acceptability.

2 LITERATURE REVIEW

2.1 Characteristics of Cow's Milk Proteins

Cow’s milk is a complex and nutritionally rich fluid that contains a variety of bioactive compounds, with proteins being among the most important constituents. Milk proteins account for approximately 3.2–3.5% of its total composition, playing a crucial role in human nutrition and food processing. These proteins are broadly categorized into caseins, which make up around 80% of total milk proteins, and whey proteins, which comprise the remaining 20%. Each protein fraction exhibits distinct structural, functional, and allergenic properties, which influence their digestibility, nutritional benefits, and potential to induce allergic reactions (Wal., 1998; Fox & McSweeney., 2015).

2.2 Casein Proteins

Caseins exist in milk as colloidal particles known as micelles, which provide stability to the milk matrix and contribute to its white appearance. The four major caseins—αs1-casein, αs2-casein, β-casein, and κ-casein—differ in their amino acid composition, structural characteristics, and functional properties. These proteins are known for their high phosphorus content, ability to bind calcium, and role in the formation of dairy products such as cheese and yogurt (Miciński et al.,2013). Additionally, caseins are amphiphilic proteins, meaning they possess both hydrophilic and hydrophobic regions, which allows them to form stable colloidal suspensions in milk (Villa et al.,2017). Their structural flexibility enables them to act as excellent emulsifiers in food applications, further enhancing their functional importance. Moreover, casein micelles serve as a natural delivery system for calcium and phosphate, facilitating their bioavailability in the human diet (Fox.,2001).

2.2.1 αs1-Casein

This is the most abundant casein protein, comprising approximately 30–40% of total milk protein. It is highly phosphorylated, which enhances its ability to bind calcium and contribute to micelle stability. Due to its strong immunogenic properties, αs1-casein is considered a major allergen in cow’s milk allergy (CMA), with studies indicating that it can elicit strong IgE-mediated immune responses in sensitive individuals (Wal.,1998; Villa et al.,2017).

2.2.2 αs2-Casein

αs2-Casein**,** although present in lower quantities (8–10% of total milk protein), plays an essential role in micelle structure and calcium binding. Its allergenicity is lower than that of αs1-casein, but it is still implicated in allergic reactions, particularly in individuals with severe CMA (Järvinen & Chatchatee.,2009). Some studies suggest that variations in αs2-casein expression among different cow breeds may influence milk digestibility and allergenicity (Monaci & Trégoat., 2006).

2.2.3 β-Casein

β-Casein accounts for 25–35% of milk proteins and exists in multiple genetic variants, the most well-known being A1 and A2 β-casein. Research has linked A1 β-casein to the production of β-casomorphin-7 (BCM-7) during digestion, which has been suggested to cause gastrointestinal inflammation and discomfort in some individuals (Lohner et al.,2022). In contrast, A2 β-casein is associated with improved digestibility and is considered a preferable alternative for individuals with milk intolerance (Fox & Brodkorb,2008). Additionally, hydrolysis of β-casein produces bioactive peptides with potential antihypertensive and immunomodulatory effects (Korhonen & Pihlanto.,2006).

2.2.4 κ-Casein

κ-Casein constitutes approximately 8–12% of the total casein fraction and plays a vital role in stabilizing casein micelles through steric repulsion and electrostatic interactions, particularly in the presence of calcium (Fox, 2001). During cheese manufacturing, κ-casein is enzymatically cleaved by chymosin at the Phe105-Met106 bond, producing para-κ-casein and glycomacropeptide (GMP), which is essential for curd formation and influences the functional characteristics of cheese (Fox.,2001; FrieslandCampina Institute, n.d.). GMP is a bioactive peptide with documented prebiotic activity and potential immune-modulating properties, which may contribute to improved gut health and influence immune responses in sensitive individuals (Nowak-Wegrzyn & Fiocchi.,2009; FrieslandCampina Institute, n.d.).

2.2.5 Whey Proteins

Whey proteins are globular proteins that remain in the liquid fraction of milk after casein precipitation. They are highly digestible, rich in essential amino acids, and possess various functional and bioactive properties, including immunomodulatory, antimicrobial, and antioxidant activities (Villa et al.,2017; Fiocchi et al.,2016). The primary whey proteins include

β-lactoglobulin, α-lactalbumin, bovine serum albumin, and Lactoferrin. These proteins play a crucial role in the nutritional and functional properties of dairy products, influencing their solubility, gelation, and emulsification capabilities (Miciński et al.,2013). Additionally, whey proteins are widely utilized in the development of hypoallergenic dairy formulas due to their ability to be hydrolyzed into bioactive peptides with reduced allergenicity (Wal.,1998; Huang et al.,2022).

2.2.6 β-Lactoglobulin

β-Lactoglobulin (β-LG) is a predominant whey protein found in the milk of most mammalian species, except camelids, rodents, and humans. It constitutes about 56% of the total whey protein content in cow's milk and is absent in human milk, making it a significant allergen for infants. β-LG is a small protein with a molecular weight of approximately 18.3 kDa and consists of 162 amino acids (Lajnaf et al.,2022). Its tertiary structure includes two anti-parallel β-sheets forming a binding pocket, connected by an α-helical strand, allowing interactions with various molecules. β-LG often exists as dimers and can adopt other quaternary structures, enabling interactions with different protein molecules (Bu et al.,2013). With one unpaired cysteine and two disulfide linkages, β-LG exhibits resistance to acid hydrolysis and enzyme activity, allowing it to traverse the gastrointestinal tract without significant breakdown. This resistance can lead to immunological responses when undigested β-LG encounters immune cells in the gut, potentially triggering allergic reactions in sensitive individuals (Wal., 1998). Epitopes for both IgE and IgG have been identified within the β-LG structure, reacting with blood samples from individuals with cow's milk allergy. Food allergies to β-LG may impact up to 80% of the population (Miciński et al.,2013).

2.2.7 α-Lactalbumin (α-LA)

α-LA has a lower allergenicity compared to β-LG due to its similar chemical composition in bovine and human milk. It constitutes approximately 22% of the total whey protein content in cow's milk. α-LA is a monomeric protein with a globular structure, participating in lactose formation and binding metals such as cobalt, magnesium, and zinc. α-LA is well soluble in water (Lajnaf et al.,2022). It has 123 amino acids, a molecular weight of approximately 14.2 kDa, and three genetic variations. The protein contains four disulfide bridges, contributing to its stability (Wal.,2002). α-LA has high thermal stability and refolding ability, with a highly structured secondary configuration resulting in a compact, spherical tertiary structure. Despite a relatively low denaturation temperature of roughly 64°C, its structural arrangement provides

greater resistance to heat-induced protein aggregation (Hochwallner et al., 2014; Villa et al., 2017).

2.2.8 Bovine Serum Albumin (BSA)

BSA comprises 582 amino acids and has a stable tertiary structure with a molecular weight of 66.3 kDa. It is found in cow's milk in amounts of about 20 mg per 100 mL. BSA's principal biological role is to transport, metabolize, and distribute chemicals such as fatty acids, ions, and hormones, and protect against free radicals (Hochwallner et al., 2014). BSA is divided into three domains, each with nine loops connected by 17 disulfide connections, many of which are well-protected within the protein's core, making them less accessible. These disulfide connections are critical for sustaining the protein's innate antigenic properties, owing to its extraordinary stability in maintaining its three-dimensional structure even under conditions that normally break proteins (Restani et al., 2004). When exposed to temperatures ranging from 70°C to 80°C, BSA loses its function. Among all the proteins found in cow's milk, BSA is expected to retain its potential to elicit an immunological response even after heat treatment (Miciski et al., 2013). BSA may be a minor allergen in cow's milk, and individuals allergic to beef may also be at risk of being allergic to cow's milk due to the presence of BSA (Fuc et al., 2019).

2.2.9 Lactoferrin

Lactoferrin is an iron-binding glycoprotein belonging to the transferrin protein family. It can bind and chelate iron, depriving bacteria of this essential nutrient, making LF a natural antimicrobial protein found in milk. However, LF has been linked to allergic reactions. Research indicates that LF can be detected by IgE antibodies in people allergic to cow's milk, with the proportion of allergic patients having IgE antibodies to lactoferrin ranging from 5% to 66%. Despite these findings, the clinical significance of lactoferrin in milk allergy remains unclear. While lactoferrin-specific IgE antibodies have been found in the sera of cow's milk allergic patients, the extent of its allergenic activity and impact on the severity of clinical symptoms has not been fully studied. Further research is needed to determine the precise role and clinical importance of lactoferrin in milk allergies (Linhart et al., 2019)

2.3 Regulatory and Safety Considerations

The development of hypoallergenic dairy products must adhere to strict food safety

regulations to ensure they meet international standards. Regulatory bodies such as the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) have established guidelines for allergen labelling and permissible levels of residual allergenic proteins in dairy formulas (EFSA, 2014). Ensuring compliance with these standards is critical for the commercial viability of hypoallergenic dairy products. Analytical methods such as ELISA, mass spectrometry, and immunoblotting are commonly used to assess allergenicity levels and confirm the safety of processed dairy products (Fiocchi et al., 2016; Huang et al., 2023).

2.4 Epitopes in Cow Milk Protein

Cow's milk allergy (CMA) is one of the most prevalent food allergies, particularly affecting infants and young children. The complexity of this allergy lies in the diverse immune responses triggered by the proteins in cow's milk, which vary significantly between individuals. Central to this immune reaction are epitopes—specific regions of milk proteins that are recognized by the immune system, especially IgE antibodies. These epitopes, present in proteins like caseins, β-lactoglobulin (β-LG), and α-lactalbumin (α-LA), play a pivotal role in determining the allergenic potential of cow's milk, making the study of these protein regions crucial for understanding and managing CMA effectively (Wal.,1998; Villa et al., 2017).

Epitopes can be classified into two main types: linear (sequential) and conformational (discontinuous). Linear epitopes are continuous sequences of amino acids that remain intact even when the protein's structure is altered, such as through heat or enzymatic treatment. These epitopes are more resistant to digestion and processing, meaning they often retain their ability to trigger allergic reactions even after the milk has been subjected to various treatments (Monaci & Trégoat., 2006). For instance, β-LG, a major allergenic protein in cow's milk, contains linear epitopes that persist through cooking and other processing methods, contributing to more persistent allergic reactions (Korhonen & Pihlanto., 2006).

In contrast, conformational epitopes depend on the three-dimensional structure of the protein. These epitopes are typically disrupted by heat, fermentation, or enzymatic hydrolysis, which makes them more susceptible to being neutralized during processing. For example, α-LA's allergenic potential is reduced when it undergoes such treatments (Fiocchi et al., 2016). This distinction between linear and conformational epitopes is vital in developing processing

methods that reduce the allergenic potential of milk proteins.

Further complicating the allergy landscape is the genetic diversity found in bovine milk proteins. Variations in the genetic makeup of these proteins can alter their epitope structures, which in turn affects how these proteins are recognized by the immune system (Abd El-Salam & ElShibiny., 2019). As a result, allergic responses to milk proteins can vary widely from one individual to another, necessitating personalized approaches to diagnosis and treatment. For example, αs1-casein contains several highly immunogenic linear epitopes that are commonly recognized by IgE antibodies in allergic individuals, and understanding these regions is crucial for customizing allergy management (Järvinen & Chatchatee., 2009).

Moreover, allergic reactions can also occur through skin contact with milk proteins, highlighting the need for comprehensive strategies that address both ingestion and external exposure (El-Agamy.,2007).

Recent advances in processing techniques offer promising solutions to reduce milk protein allergenicity. Enzymatic hydrolysis and microbial fermentation are key methods that can target specific epitopes, effectively reducing their ability to trigger immune responses. For example, proteolytic enzymes like trypsin and pepsin can break down the allergenic epitopes in β-LG and caseins, while fermentation with lactic acid bacteria can further degrade these proteins and even generate bioactive peptides with additional health benefits (Bu et al.,2009; Korhonen & Pihlanto.,2006; Pessato et al.,2020). These bioprocessing approaches are paving the way for hypoallergenic dairy products, offering potential relief for individuals with CMA.

Understanding the epitopes in cow's milk proteins is crucial for developing strategies to reduce their allergenicity and improve the safety of milk products for allergic individuals. By identifying and targeting the specific epitopes responsible for allergic reactions, researchers can develop more effective strategies for managing and mitigating this allergy, ultimately improving the quality of life for those affected

2.5 Influence of Technological Processing on Cow's Milk Protein

Technological processing methods have a significant impact on the structural, functional, and nutritional properties of cow’s milk proteins. These changes influence their digestibility, bioavailability, and allergenic potential. From the moment milk is collected on the farm to when it reaches retail shelves, it undergoes several key steps to ensure microbial safety, optimize

protein functionality, and extend shelf life.

Immediately after milking, the milk is rapidly cooled to about 4°C to preserve its freshness and inhibit microbial growth. It is then transported in temperature-controlled stainless-steel tanks to maintain its integrity. Upon arrival at processing facilities, centrifugation is used to separate milk fat from skimmed milk. This is followed by standardization, where precise amounts of fat are reintroduced to achieve the desired composition (Verhoeckx et al., 2015; Borad et al., 2017). Heat treatment is a critical step in processing that enhances milk safety by eliminating pathogenic microorganisms while also altering protein structure and functionality. Pasteurization, which involves heating milk to 70–80°C for 15–20 seconds, effectively inactivates most harmful bacteria while preserving the nutritional and sensory attributes. Sterilization (110–120°C for 10–20 minutes) ensures broader microbial destruction, while ultra-high-temperature (UHT) processing (135–145°C for 0.5–4 seconds) provides extended shelf stability with minimal Maillard reaction effects (Borad et al., 2017). However, these thermal treatments can cause protein denaturation and aggregation, leading to changes in solubility, emulsification, and foaming properties (Augustin & Udabage, 2007). Denaturation may disrupt conformational epitopes, reducing immunoglobulin E (IgE) binding and potentially lowering allergenicity, but sequential epitopes often remain intact. Moreover, high-temperature treatments may promote the formation of neoepitopes, increasing allergenic potential (Nowak-Wegrzyn & Fiocchi, 2009).

Beyond thermal treatments, homogenization plays a crucial role in modifying milk protein interactions. By mechanically reducing fat globule size, homogenization improves product stability and texture without significantly altering the primary structure of proteins (FrieslandCampina Institute, n.d.). Fermentation, another widely used processing method, further enhances milk protein functionality by promoting partial hydrolysis, resulting in bioactive peptides with improved digestibility and reduced allergenicity (Kopf-Bolanz, 2017; Lajnaf et al., 2022). Recent research highlights the potential of enzymatic hydrolysis in mitigating allergenic properties, as observed in studies using extended shelf-life (ESL) and UHT milk processed into yogurt through papain and microbial hydrolysis (Pessato et al., 2020). Advancements in dairy processing techniques continue to refine methods for preserving or enhancing milk protein functionality while minimizing negative structural modifications. For instance, novel enzymatic and fermentation approaches aim to optimize protein digestibility while ensuring microbial safety. Additionally, research into alternative processing methods, such as high-pressure processing (HPP) and pulsed electric field (PEF) technologies, suggests

promising avenues for reducing allergenicity while maintaining nutritional quality (Li et al., 2025). These findings emphasize the intricate interplay between technological interventions and milk protein quality, highlighting the need for continued innovation to maximize consumer health benefits and dairy product performance.

2.6 Effect of Heat Treatment

Heat treatment is a widely used processing method in the dairy industry to ensure the microbial safety of milk and to improve its shelf life. In addition to its safety role, heat treatment induces various structural and functional modifications in milk proteins, which affect their nutritional quality, digestibility, and allergenic potential. These changes depend significantly on the processing temperature, duration, and specific method used (Borad et al., 2017).

Casein proteins, which constitute most milk proteins, exhibit relative stability under moderate heat conditions. In contrast, whey proteins such as β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) begin to denature at temperatures above 65°C. Pasteurization, typically carried out at 70–80°C for 15–20 seconds, causes partial unfolding of these proteins. This denaturation can disrupt conformational IgE-binding epitopes, potentially reducing the allergenicity of whey proteins (Nowak-Wegrzyn & Fiocchi, 2009; Fox, 2001).

More intensive processes such as ultra-high temperature (UHT) treatment (135–145°C for a few seconds) and sterilization (110–120°C for up to 20 minutes), cause extensive protein unfolding and aggregation. These changes can decrease protein solubility and bioavailability by promoting interactions between denatured whey proteins and caseins, often through disulfide bonding and hydrophobic interactions. The free thiol group in β-LG plays a central role in these heat-induced interactions (Fox, 2001; FrieslandCampina Institute, n.d.).

Additionally, severe heat treatment facilitates non-enzymatic browning reactions such as the Maillard reaction, which involves reducing sugars and amino acids, particularly lysine. This process can result in the loss of essential amino acids and negatively impact the sensory and functional properties of milk, including color, flavor, and emulsifying ability (Borad et al., 2017; Augustin & Udabage, 2007).

The effect of thermal processing on milk allergenicity is variable. While moderate heating can reduce IgE-binding capacity and sensitizing potential of certain proteins, excessive heating may create neoepitopes that elicit immune responses in sensitive individuals. Moreover, the presence of other food matrix components such as fats and carbohydrates can influence the exposure or

masking of allergenic sites (Nowak-Wegrzyn & Fiocchi, 2009; Bu et al., 2013). These effects are summarized in Table 1, which highlights how different heat treatments influence the allergenicity of major milk proteins.

Although heat treatment can reduce the nutritional value of milk by degrading heat-sensitive vitamins such as B12 and thiamin, it is still considered a vital step in dairy processing. Innovations such as combining mild heat treatments with enzymatic hydrolysis have been explored to enhance digestibility and reduce allergenicity while preserving nutritional and sensory quality (Fox, 2001; Borad et al., 2017).

1. Table: Effects of Heat treatment on milk allergens

Matrix	**Allergen**	**Findings**	**Reference**
Purified protein, skim milk and sweet whey	β-LG	Temperatures from 80 to 90°C, the allergenicity of β-LG rose. However, it decreased when exposed to temperatures over 100°C	(Kleber et al., 2007)
Whey protein isolates	α-LA, β- LG	Denaturation starts above 65°C, altering the native structure. Pasteurization improves digestibility and reduces allergenicity by disrupting conformational epitopes.	Borad et al., 2017; Nowak-Wegrzyn & Fiocchi, 2009
Whole milk and β-LG	β-LG	Denaturation and aggregation processes occurred between 90°C	(Asghar TaheriKafrani et

solution		and 95°C, the IgE-binding ability of β-LG dropped slightly but significantly.	al., 2009)
UHT-treated milk	β-LG, α-LA	Formation of Maillard reaction products leads to decreased lysine bioavailability and off-flavors; may increase or decrease allergenicity depending on context.	Nowak-Wegrzyn et al., 2009
Fresh skim milk	β-LG, α- L	β-LG and α-LA showed a decrease in allergenicity at 90 to 100°C after 20 min	(Bloom et al., 2014)

2.7 Effects of Homogenization

Homogenization is a fundamental mechanical process in the dairy industry, widely applied to enhance the physical stability and sensory quality of milk. This process involves subjecting milk to high pressures—typically between 20 and 100 MPa—which breaks down fat globules into smaller, uniformly distributed particles, thereby preventing cream separation and creating a stable emulsion (Gonçalves, 2022).

While primarily used for improving texture and appearance, homogenization may also influence the allergenicity of milk proteins. The reduction in fat globule size increases the total surface area available for protein adsorption, potentially altering the presentation of allergenic epitopes (Miciński et al., 2013). In raw milk, many allergenic proteins, particularly those associated with casein micelles, are partially shielded. Homogenization may increase the exposure of these proteins by modifying the milk fat globule membrane and promoting interactions between caseins and whey proteins, thereby enhancing the antigenic potential of the milk matrix.

Evidence regarding the immunological consequences of homogenization is mixed. Poulsen et al. (1987), in a study using a mouse model, observed anaphylactic reactions following the ingestion of homogenized and pasteurized milk, whereas raw milk did not elicit similar responses. This finding has led to concerns about the possible enhancement of allergenicity due to homogenization. Conversely, Pelto et al. (2000), in a randomized, double-blind, cross-over study in humans, found no significant difference in allergic outcomes between homogenized and non-homogenized milk, suggesting that homogenization alone may not be a critical factor in determining allergenic responses.

Due to such conflicting findings, the precise role of homogenization in modifying milk protein allergenicity remains uncertain. It is likely that homogenization interacts with other processing factors, such as pasteurization, storage conditions, and the composition of the food matrix, to determine its overall impact. Therefore, further research is required to clarify the immunological implications of homogenized milk, especially in sensitive populations (Gonçalves, 2022; Miciński et al., 2013; Pelto et al., 2000; Poulsen et al., 1987).

2.8 Effects of Fermentation

Fermentation is a widely applied process in the food industry that can significantly influence the allergenic properties of milk proteins. Fermented dairy products, such as yoghurt, contribute to human health by enzymatically hydrolyzing major dietary proteins, leading to the generation of bioactive peptides. Lactic acid bacteria (LAB), which are commonly involved in fermentation, facilitate the breakdown of milk proteins into smaller peptides, free amino acids, and exopolysaccharides (Wang et al., 2021). This proteolytic activity has been associated with a reduction in the allergenicity of milk proteins.

LAB utilizes intricate proteolytic systems—comprising proteinases, peptidases, and specific transport mechanisms—that support their growth in milk and dairy matrices. These enzymatic systems are capable of cleaving immunologically relevant epitopes on milk proteins, thereby diminishing their allergenic potential. Furthermore, fermentation by-products such as lactic acid can modify the secondary and tertiary structures of proteins, potentially altering epitope configurations and reducing antigenicity (Mu et al., 2021).

In contrast to thermal processing, LAB fermentation is more effective at degrading casein. Certain strains, such as Enterococcus faecium, which are prevalent in fermented milk and

cheese, produce enzymes including metalloproteases and cell envelope proteinases that are instrumental in the hydrolysis of casein (Wang et al., 2021). Additionally, Mu et al. (2021) reported that *Lactobacillus fermentum* IF3956 could efficiently hydrolyze milk proteins, with a particular affinity for α-casein and β-lactoglobulin. However, the extent of allergenicity reduction during fermentation depends on several variables, including the bacterial species employed, fermentation conditions (e.g., time, temperature, pH), and the specific milk protein involved (Abd El-Salam & El-Shibiny, 2019).

Beyond the degradation of allergens, the consumption of probiotics and fermented foods may alleviate allergic symptoms and reduce the incidence of atopic conditions through immune modulation. LAB-containing fermented products have been shown to enhance systemic expression and secretion of both Type I and Type II interferons, contributing to improved immune regulation (Bu et al., 2010).

Table 2 summarizes the impact of various bacterial strains on the antigenicity and allergenicity of bovine milk proteins.

2. Table: Fermentation effect on the milk allergenicity

Bacterial Strain	**Milk Protein**	**Findings**	**Reference**
Lactobacillus fermentum IF3956	α-casein, β-lactoglobulin	Efficient hydrolysis of milk proteins; significantly reduced antigenicity due to enzymatic cleavage of allergenic epitopes	Mu et al., 2021
Enterococcus faecium	Casein	Produces metalloproteases and cell envelope proteinases; aids in the degradation of casein, lowering allergenic	Wang et al., 2021

		potential decreased by 32% due to the degradation of the three primary epitopes of β-LG.	
Lb. bulgaricus, Lb. helveticus, S. thermophilus (single; mixed 1:1)	α-LA, β-LG	Combination of Lb. helveticus, S. thermophilus resulted in the most significant reduction in immunoreactivity, with 87% for α-LA and 95% for β-LG	(Bu et al., 2010)
Various LAB strains	Casein, β-lactoglobulin, α-lactalbumin	Allergenicity reduction depends on strain specificity, fermentation conditions, and protein type	Abd El-Salam & El-Shibiny, 2019
Lb.casei	α-LA, β-LG, Caseins	Fermentation with or without simulated digestion: decreased allergenicity (higher with digestion)	(Wróblewska et al., 2016)

2.9 Effect of Enzymatic Hydrolysis

Enzymatic hydrolysis is a widely accepted and effective strategy for reducing the allergenic potential of milk proteins. This process involves the application of proteolytic enzymes to cleave specific peptide or disulfide bonds within the protein structure. As a result, the conformational and linear epitopes—responsible for eliciting immune responses—can be disrupted or masked, leading to decreased allergenicity (Bu et al., 2013; Nowak-Wegrzyn &

Fiocchi, 2009).

The hydrolysis process breaks milk proteins down into smaller peptides and free amino acids. Since allergenic epitopes are typically buried within the core of intact protein structures, enzymatic breakdown increases the likelihood that these regions are altered or rendered inaccessible, thus reducing their immunogenicity (Bu et al., 2013). For example, hydrolysis with enzymes like Alcalase has been shown to significantly reduce the antigenicity of β-lactoglobulin (β-LG) and α-lactalbumin (α-LA), two major milk allergens (Liang et al., 2020). Moreover, using combinations of enzymes, such as Alcalase and papain in a two-step process, has proven more effective in reducing the immunoreactivity of whey proteins compared to using a single enzyme alone (Wroblewska et al., 2004).

Despite its effectiveness, extensive hydrolysis can also lead to undesirable sensory characteristics, including bitterness, altered taste, and reduced emulsifying capacity. These challenges, along with high production costs and high osmolality, often limit the application of extensively hydrolyzed milk proteins in standard food products and infant formula (Abd El-Salam & El-Shibiny, 2019; FrieslandCampina Institute, n.d.). Consequently, recent research has focused on optimizing hydrolysis conditions—such as enzyme selection, reaction duration, and hydrolysis extent—to achieve a balance between allergenicity reduction and acceptable product quality (Bu et al., 2013).

It is important to note that even after hydrolysis, some residual epitopes may persist. The degree of allergenicity reduction depends on several factors, including the enzyme type, hydrolysis model, and processing parameters (Nowak-Wegrzyn & Fiocchi, 2009). Therefore, while enzymatic hydrolysis represents a promising approach for producing hypoallergenic milk protein ingredients, further refinement is necessary to enhance its effectiveness without compromising sensory and functional properties.

Table 3 summarizes the impact of enzymatic hydrolysis on the antigenicity of milk proteins.

3. Table: Effect of enzymatic hydrolysis on milk allergenicity

Enzyme	**Milk Allergen**	**Findings**	**Reference**
Alcalase,		The antigenicity of milk proteins was	(Wroblewska et

Papain	β-LG, α-LA	decreased, and the two-step hydrolysis process proved to be more effective in reducing antigenicity. However, it was not entirely successful in eliminating allergenic epitopes	al., 2004)
Protease of Bacillus licheniformis	Whey protein concentrate	A hydrolysate with an average peptide chain length of around 4 amino acids was obtained. The antigenic whey protein in the product was reduced 99.97%,	(Guadix et al., 2006)
Trypsin, chymotrypsin	Whey protein isolate	Enzymatic hydrolysis combined with glycation significantly reduced the IgE binding capacity of whey protein isolate	(Xu et al., 2020)
Papain	β-LG	The hydrolysates demonstrated a complete elimination of allergenicity	(LópezExpósito et al., 2012)
Alcalase, Protamex	Casein and β- L	Efficiently reduced the antigenicity of milk allergens	(Liang et al., 2020)
Alcalase, Protamex, and Flavourzyme	Caseins, β-LG, and α-LA	The IgE-binding and IgG-binding ability in hydrolyzed were significantly reduced.	(Liang et al., 2021)

2.10 Effect of Combined treatment

Combining multiple processing methods is often more effective at reducing milk allergenicity than relying on a single approach, as each technique presents its own strengths and limitations. For example, heat treatment can denature milk proteins, potentially lowering allergenicity. However, it may also lead to the formation of new allergenic compounds. Enzymatic hydrolysis breaks proteins into smaller peptides, which are often less allergenic, but this process can introduce bitter flavors or expose new allergenic epitopes. Similarly, lactic acid fermentation alters protein structures and can reduce allergenicity, though it may also result in the formation of neo-allergens.

By integrating different processing strategies, it is possible to overcome individual limitations and achieve a more comprehensive reduction in allergenicity (Villa et al., 2017). For instance, pre-treating milk proteins with heat has been shown to enhance enzymatic hydrolysis by unfolding protein structures such as α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), thereby exposing more enzymatic cleavage sites and facilitating more effective degradation (Bu et al., 2013).

Mecherfi et al. (2011) investigated the combination of microwaving and enzymatic hydrolysis on β-LG and other bovine whey proteins. Their results indicated that microwave pre-treatment significantly improved protein breakdown compared to conventional heating, leading to a marked reduction in allergenic potential. Additionally, microwave-assisted hydrolysis was found to improve both the solubility and digestibility of milk proteins (Izquierdo et al., 2008).

More recently, Ye et al. (2023) explored the synergistic effects of enzymatic hydrolysis and LAB fermentation. Their study demonstrated that using an alkaline protease in combination with Lactobacillus helveticus effectively eliminated allergenic proteins while preserving desirable functional and sensory properties in the final product. This combined strategy presents a promising solution for producing hypoallergenic dairy products without compromising quality.

Table 4 summarizes the different combined processing techniques and their respective impacts on milk allergenicity.

4. Table: Effect of combined method on milk allergenicity

Treatment	Matrix	Allergen	Findings	Reference
Heat treatment and enzymatic hydrolysis	Purified whey	α-LA, β-LG	Enzymatic hydrolysis with heat treatment significantly improved the breakdown α-LA and β-LG by tryptic and peptic enzymes, leading to a reduction in the allergenicity of milk.	(BertrandHarb et al., 2002; Peyron et al., 2006)
Heat and enzymatic hydrolysis	Purified proteins	α-Casein, β-L	Heat treatment decreased β-LG by altering its structure and making it more vulnerable to enzymatic digestion, affecting B cell epitopes, while it had no impact on the allergenicity of α- casein	(Morisawa et al., 2009)

Heat and enzymatic hydrolysis	Whey protein concentrate	Whey protein	Heated WPC had lower antigenicity compared to native WPC when treated with various enzymes, and the most significant reduction in antigenicity occurred when using a high E/S ratio of pepsin followed by trypsin.	(Kim et al., 2007)
microwaves combined with enzymatic hydrolysis	Whey protein isolate	β-LG	Microwave treatment at 200 W not only improved the breakdown of β-lactoglobulin by pepsin within just 3 minutes but also substantially reduced its immunoreactivity	(El Mecherfi et al., 2011)

Enzymatic hydrolysis and LAB fermentation	Cow's milk	Whey and casein proteins	Allergenicity reduction of cow milk treated by alkaline protease combined with Lactobacillus Plantarum and Lactobacillus helveticus based on epitopes	(Ye et al., 2023)

2.11 Economic Considerations in Hypoallergenic Dairy Production

Developing dairy products with reduced allergenicity requires careful consideration of both technical feasibility and economic viability. One of the primary cost factors in enzymatic and microbial processing for allergen reduction is the expense of enzymes and microbial cultures, which can significantly impact production costs (Agile BioFoundry, n.d.). Additionally, the scalability of these bioprocessing methods remains a challenge, as maintaining consistency and efficiency in large-scale production requires optimization of processing conditions and raw material utilization (Colacicco et al.,2024).

The industrial feasibility of allergen-reduction techniques also depends on their integration into existing dairy processing systems. Traditional heat treatments and homogenization are well-established and cost-effective, whereas advanced enzymatic and microbial approaches may require additional investments in specialized equipment and process control technologies (National Renewable Energy Laboratory, n.d.). Conducting economic assessments can help determine whether these innovations offer a sustainable return on investment while maintaining consumer affordability.

Market demand for allergen-reduced dairy is growing, driven by increased awareness of milk allergies and intolerances. However, the commercial success of such products depends on consumer perception, labelling regulations, and competitive pricing compared to conventional dairy alternatives (Milk Genomics, n.d.). Regulatory compliance is another crucial factor, as allergen-reduction processes must meet stringent food safety and labelling requirements across

different regions (FrieslandCampina Institute, n.d.).

By evaluating the balance between cost, scalability, market potential, and regulatory considerations, this study aims to provide insights into the feasibility of producing allergen-reduced dairy products at an industrial scale. Addressing these economic challenges is essential for ensuring that such products are both accessible and commercially viable.

2.12 Gaps in Existing Research and Need for Further Studies

Despite advancements in allergen reduction techniques, several gaps remain in current research that need to be addressed to enhance the development of hypoallergenic dairy products.

One major limitation is that existing enzymatic and microbial processing methods often result in partial hydrolysis, leaving residual allergenic peptides intact. While hydrolysis can disrupt conformational epitopes, sequential epitopes may persist, maintaining allergenic potential (Nowak-Wegrzyn & Fiocchi, 2009). Further studies are required to optimize enzyme selection, reaction conditions, and hydrolysis efficiency to achieve a more complete reduction in allergenicity without compromising the functional properties of milk proteins.

Additionally, research on the combined use of enzymatic and microbial hydrolysis remains limited. Most studies focus on either enzymatic or microbial treatments separately, overlooking the potential synergies that could enhance protein modification and allergen reduction. Future investigations should explore multi-step bioprocessing approaches that integrate these techniques, assessing their impact on allergenicity, bioactive peptide formation, and overall protein digestibility.

Another significant gap is the lack of comprehensive economical evaluations of large-scale hypoallergenic dairy production. While studies have assessed the effectiveness of allergen reduction techniques at the laboratory level, their industrial feasibility remains uncertain. There is a need for cost-benefit analyses, market studies, and pilot-scale production trials to determine the scalability and economic sustainability of these processes (Agile BioFoundry, n.d.; National Renewable Energy Laboratory, n.d.).

Furthermore, research should focus on developing hypoallergenic dairy formulations that maintain both nutritional balance and sensory quality. Many allergen-reduced dairy products suffer from altered texture, taste, or nutritional losses due to extensive processing. Future studies should prioritize consumer acceptability testing and formulation improvements to ensure that hypoallergenic dairy remains a viable alternative for both allergic individuals and

the general population.

Addressing these research gaps will be crucial in advancing the development of cost-effective, safe, and high-quality hypoallergenic dairy products. A multidisciplinary approach, integrating food biotechnology, process engineering, and economic analysis, is essential for bridging the gap between laboratory research and large-scale production.

3 MATERIALS AND METHODS

3.1 Description of simulation

In this investigation, the economic analysis of one of our previously published bioprocesses for producing dairy protein formula with lower allergenic activity of proteins (Nath et al., 2021) are compared to six other bioprocess schemes dedicated for the development of dairy protein formula with lower allergenic activity. Economic analysis of enzymatic hydrolysis (Liang et al., 2022), microbial hydrolysis (Song et al., 2023), microwave heating (Grar et al.,2009), high pressure (Jiang et al.,2023) treatments alone and their combination (Izquierdo et al.,2008 ; Peñas,et al.,2006) mentioned in Table 5 have been performed by SuperPro Designer software (v. 12, Intelligen, Inc., USA) for the comparison purpose. The detailed descriptions of process schemes are mentioned subsequently.

5. Table: Process schemes (I-VI) for producing dairy protein formulas with lower allergenic activity.

Process scheme		**Feedstock**	**Reference**
(I)	Sequential tryptic and microbial hydrolysis	Skimmed milk protein concentrate	(Nath et al., 2021)
(II)	Hydrolysis by alcalase	Skimmed milk protein	(Liang et al.,2022)
(III)	Hydrolysis by microbes	Skimmed milk protein	(Song et al.,2023)
(IV)	Microwave heating	Whey protein obtained by acid precipitation of milk protein	(Grar et al.,2009)
(V)	High pressure treatment	Whey protein concentrate	(Jiang et al.,2023)
(VI)	Hydrolysis by alcalase and microwave heating	Whey protein concentrate	(Izquierdo et al.,2008)

(VII)	Hydrolysis by pepsin and high-pressure treatment	Whey protein obtained by acid precipitation of milk protein	(Peñas et al.,2006)

Dairy protein formula with lower allergenic activity by sequential tryptic and microbial hydrolysis of dairy proteins was produced from liquid milk protein concentrate (LMPC) (Nath et al., 2021). Information about whole milk protein and whey protein concentrate (WPC) as feedstock for producing lower allergenic dairy protein formulas were taken into consideration for the comparison purpose due to the lack of information in peer-reviewed journals. The composition of cow milk and WPC used in this investigation to perform economic analysis by the SuperPro Designer software are mentioned in Table 6

6. Table: Compositions of cow milk and whey protein concentrate.

Cow milk		Whey protein concentrate	
Constituent	**Concentration (% wt)**	Constituent	Concentration (% wt)
Anions	0.4	Water	3.62
Cations	0.3	Protein	80.6
Casein	2.7	Lactose	4.45
Fats	3.9	Ash	4.16
Lactose	4,9	Fat	7.17
Whey protein	0.6		
Water	87.2		

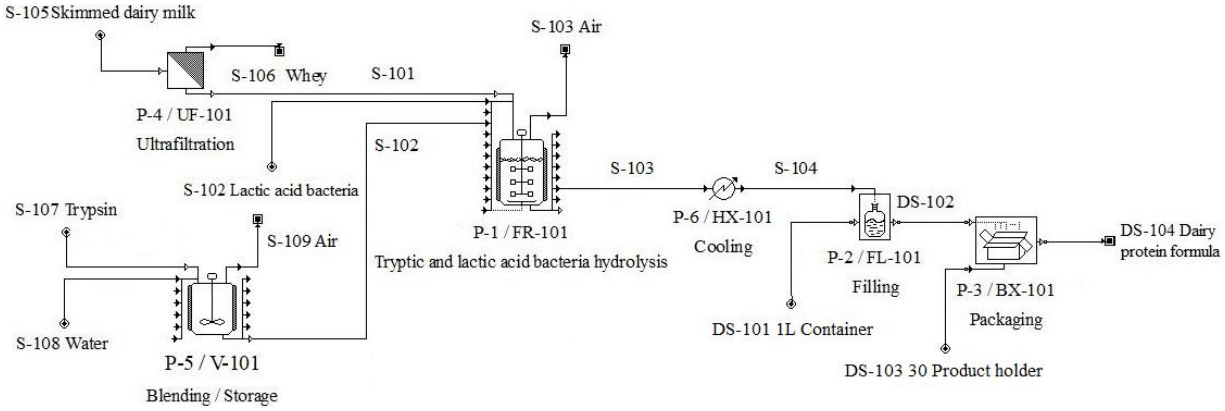
3.2 Descriptions of process schemes

Information from laboratory-scale experiments and literatures about process schemes for processing dairy protein formulas having lower allergenic activity were used to develop models by the SuperPro Designer software. These are mentioned in subsequent sections. It was considered 16 Ton and 8 Ton of milk as a feedstock to execute model (I) and model (III). In the first case, 16 Ton of milk was concentrated to 8 Ton by the membrane filtration prior to enzymatic and microbial hydrolysis. To maintain the similar volume, 8 Ton milk was considered for microbial hydrolysis in the latter case. In other process schemes, 2 Ton milk or

WPI was considered for execution. The reason behind this assumption is microbial-based dairy foods are considered as high-valued commodity food products and their higher market share; whereas, enzyme-based dairy protein formula is limited up to the diet chart of athletes.

3.2.1 Process scheme (I)

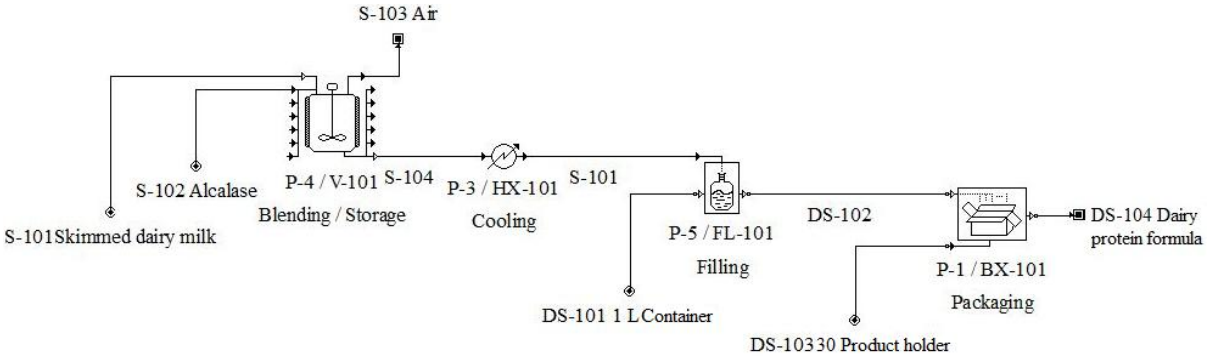
In process scheme (I), a dairy protein formula with lower allergenic activity was developed by sequential tryptic and microbial hydrolysis of dairy proteins. The detailed laboratory-based process scheme was described by Nath et al. (2021). Briefly, LMPC was developed from skimmed milk by a membrane filtration technology (membrane filtration area 59 m2), operated in batch-mode. The filtration process was performed with average permeate flow flux 33 L·m-2·h-1 until volumetric concentration ratio (VCR) 2 was achieved. The protein concentrated from the retentate side of the membrane was considered for the tryptic hydrolysis in a temperature and pH-controlled well-equipped bioreactor. In the tryptic hydrolysis reaction, concentration of protein and trypsin were 66 g·L-1 and 0.016 g·L-1, respectively. Temperature 40^{o}C for 10 min reaction time was considered for the tryptic hydrolysis of LMPC and subsequently, temperature 70^{o}C for 20 min incubation time was considered for the deactivation of trypsin. When the temperature of the tryptic-hydrolyzed LMPC was reduced from 70^{o}C to 45^{o}C, it was further hydrolyzed by lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophiles*) for 6 hours. For the SuperPro Designer model, 16 Ton skimmed milk/batch was considered as feedstock and the protein concentrates from the retentate side of membrane (8 Ton LMPC/batch) was considered for the development of dairy protein formula with lower allergenic activity. It may be felt that milk proteins were unfolded and partially hydrolyzed by trypsin which resulted in lower molecular weight of peptides with antigenic activity. These peptides were consumed by microbes and produced amino acids. The probable biochemical mechanisms are already mentioned in the previous article (Nath et al., 2021). In the next step, the temperature of protein hydrolysate was reduced from 45^{o}C to 10^{o}C by a heat-exchanger. the product was transferred to the filling machine and subsequently, packaging. The process scheme for developing a dairy protein formula having lower allergenic activity by sequential tryptic and microbial hydrolysis of milk proteins is presented in Fig. 1.



1. Figure: Process scheme (I): Sequential tryptic and microbial hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity.

3.2.2 Process scheme (II)

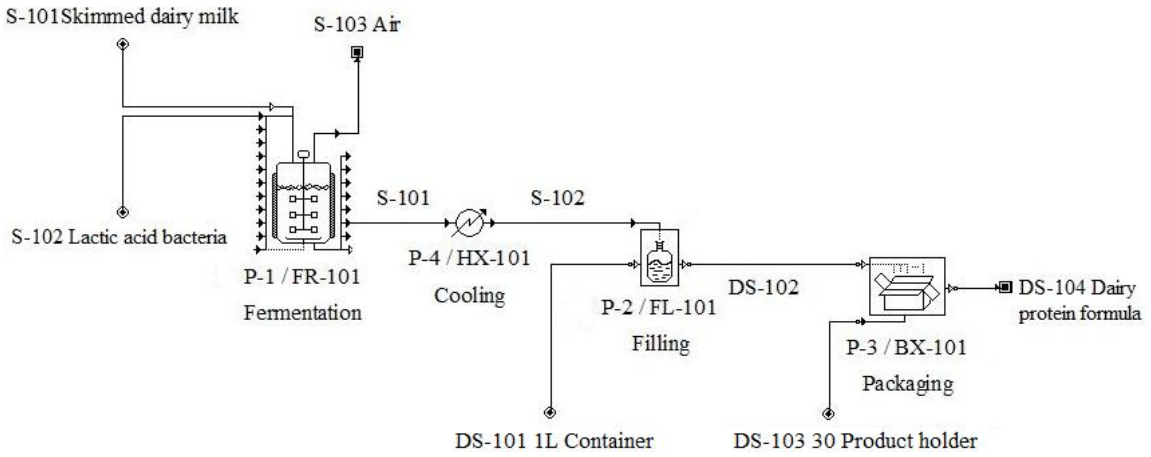
In this process scheme, skimmed milk protein was hydrolyzed by alcalase in a temperature and pH-controlled tank reactor operated in batch-mode. During the enzymatic reaction, temperature 55^{o}C was maintained for 2-hours and subsequently, temperature 90^{o}C for 10 min was considered for the deactivation of alcalase (Liang et al.,2022). Investigators implemented different food-grade enzymes, such as alcalase, neutrase, flavourzyme, protamex, papain, and pepsin on the antigenicity of casein, β-lactoglobulin, and α-lactalbumin in cow milk. Alcalase exhibited pronounced hydrolytic activity and could efficiently reduce the antigenicity of casein and whey proteins among all. The enzymatic hydrolysis was performed considering the concentrations of protein and alcalase, 33 g·L-1 and 10 mL, respectively. In the SuperPro Designer model, the amount of skimmed milk (feedstock) was considered 2 tons, and after the enzymatic bioprocess dairy protein formula of 2 tons in a batch was produced. In the next step, the enzymatically hydrolyzed milk protein formula was cooled down to 55 °C by a heat exchanger. Subsequently, the enzyme-hydrolyzed milk protein was considered for the filling and packaging. The process scheme for developing a protein formula with lower allergenic activity by the enzymatic hydrolysis of skimmed milk proteins is presented in Fig. 2.



2. Figure: Process scheme (II): Enzymatic hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity.

3.2.3 Process scheme (III)

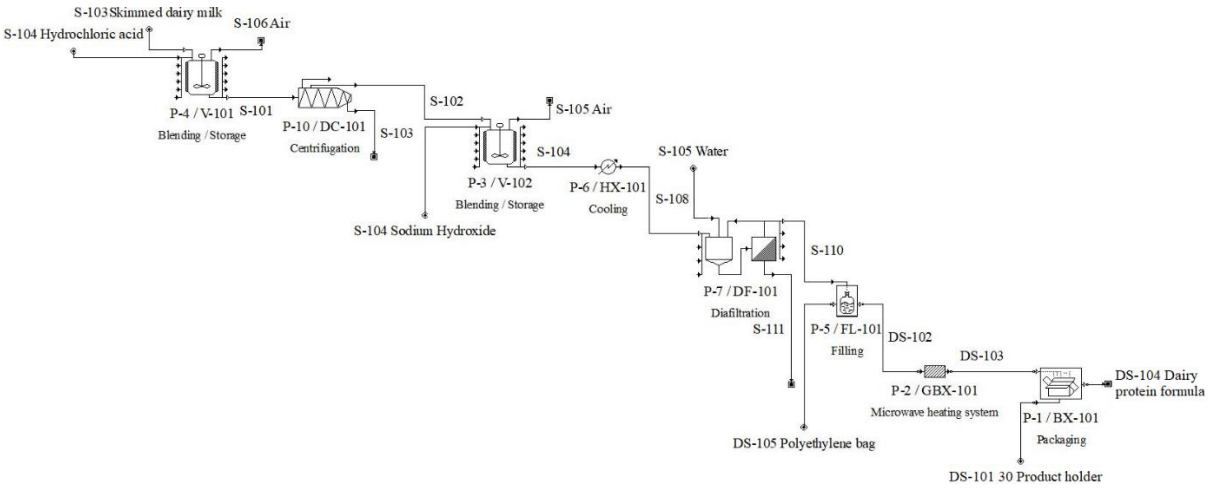
The process scheme for producing a dairy protein formula with lower allergenic activity by the microbial hydrolysis of skimmed milk protein was considered according to Song et al. (2023). Skimmed milk protein was hydrolyzed by lactic acid bacteria (2.5% (w/v)), namely *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* according to the manufacturer's instructions (Chr Hansen Co., Ltd., Hirschholm, Denmark). The microbial hydrolysis milk protein was performed in a temperature and pH-controlled well-equipped bioreactor. The microbial hydrolysis of dairy proteins was performed at a temperature 45^{o}C for 6 hours. The proteolytic system of lactic acid bacteria hydrolyses milk proteins to peptides (2–20 amino acid residues, 4 mg of peptides/ 100 g of microbial hydrolyzed product) and to some extent amino acids (5.23 mg/ 100 g of microbial hydrolyzed product). In the SuperPro Designer model, 8 Ton/batch skimmed milk was used as feedstock and after the microbial bioprocess, 8 Ton dairy protein formula/batch was produced. After the microbial hydrolysis of dairy proteins, the temperature of protein hydrolysate was reduced from 45^{o}C to 10^{o}C by a heat-exchanger. The product was transferred to the filling machine and subsequently, packaging. The schematic diagram of the proposed process for producing a protein formula with lower allergenic activity by the microbial hydrolysis of skimmed milk proteins is presented in Fig. 3.



3. Figure: Process scheme (III): Microbial hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity.

3.2.4 Process scheme (IV)

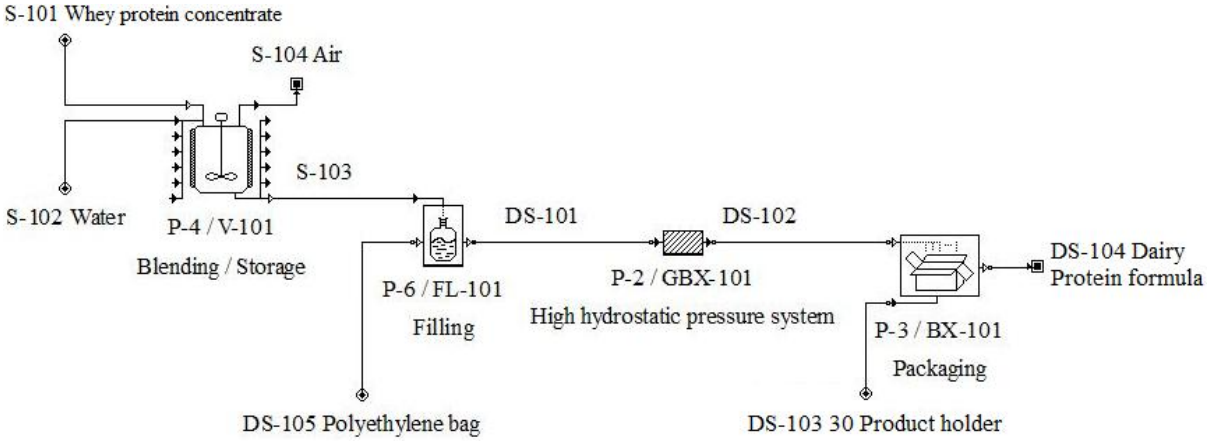
In process scheme (IV), a dairy protein formula with lower allergenic activity was developed by microwave heating. The detailed laboratory-based process scheme was described by Grar et al. (2009). Briefly, casein proteins in skimmed milk were precipitated by 0.1 N HCl, pH 4.6 at temperature 70^{o}C for 30 minutes. Mixture was centrifuged at 3,500 rpm for 15 minutes to collect whey as supernatant and the pH of whey was neutralized (pH 6.8) by 1 N sodium hydroxide. The temperature of pH-neutralized whey was reduced to 25^{o}C by a heat exchanger prior to removing lactose and salts by a membrane-based process. Whey protein concentrate was filled into 1 L of heat-resistant polyethylene bottle which was further considered for the microwave heating. The microwave heat treatment was carried out by 2450 MHz frequency with 700 W power for 10 minutes, final temperatures reached 102°C. In the SuperPro Designer model, 2 Ton/batch skimmed milk was used as feedstock and after the mentioned bioprocess, 2 Ton dairy protein formula/batch was produced. Bottles were considered for packaging after microwave heat treatment. The process scheme for microwave heat treatment for producing a dairy protein formula with lower allergenic activity is presented in Fig. 4.



4. Figure: Process scheme (IV): Microwave heat treatment for producing a dairy protein formula with lower allergenic activity.

3.2.5 Process scheme (V)

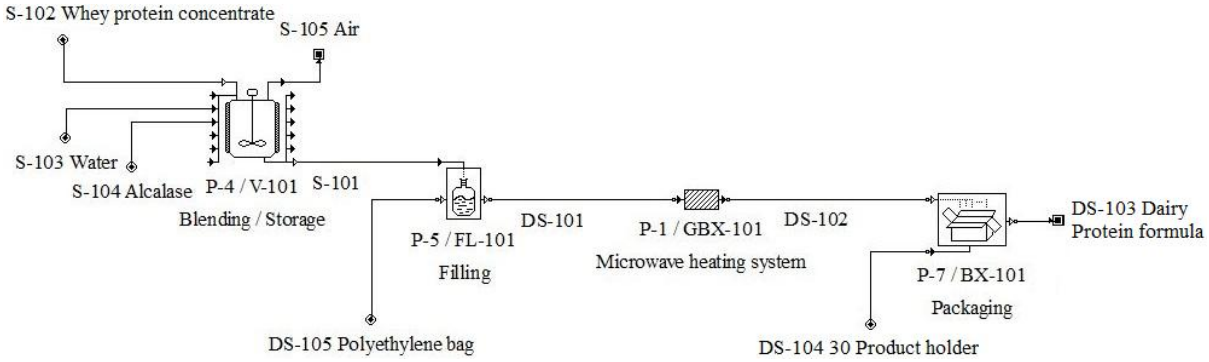
In this process scheme, WPC was dissolved in distilled water to make the concentration 50 g·L-1. WPC solution was filled into 1 L pressure-resistant polyethylene bottle followed by high pressure treatment where constant pressure 500 MPa for 10 minutes was considered (Jiang et al.,2023). In the SuperPro Designer model, 2 Ton of WPC solution/batch was considered for high pressure treatment and 2 Ton dairy protein formula having lower allergenic activity was produced. For the commercialization purpose, finally, bottles were considered for the packaging. The process scheme for developing a dairy protein formula with lower allergenic activity by high pressure treatment is presented in Fig. 5.



5. Figure: Process scheme (V): High pressure treatment for producing a dairy protein formula with lower allergenic activity.

3.2.6 Process scheme (VI)

Similar to before, WPC was dissolved in water to make the final concentration 50 g·L-1 and the WPC solution was hydrolyzed by alcalase. The enzymatic hydrolysis was performed considering 100 mL enzyme (2 mg·mL−1) and 100 mL of substrate in the final volume of solution 300 mL. In the biochemical reaction, temperature 50°C for 5 minutes was maintained and subsequently, the reaction mixture was heated to temperature 80°C for 5 minutes to deactivate the enzyme. Enzyme hydrolyzed WPC was filled into a heat-resistant polyethylene bottle (1 L sample holding capacity) similar to before and considered for microwave heat treatment. The microwave heat treatment of the enzyme hydrolyzed WPC was carried out by 2450 MHz frequency with 532 W power for 5 minutes (Izquierdo et al.,2008). In the SuperPro Designer model, 2 Ton WPC was considered as a feedstock and finally 2 Ton protein formula with lower allergenic activity (~99%) in a batch was produced. In this process, high temperature could unfold the whey protein structure resulting in exposure of conformational epitopes. The proteolytic enzyme alcalase could hydrolyze both linear and conformational epitopes resulting in a protein formula with lower allergenic activity. In the last step of the process scheme, bottles were packed for the marketing. The process scheme for developing a dairy protein formula having lower allergenic activity by enzymatic hydrolysis and microwave heat treatment is presented in Fig. 6.

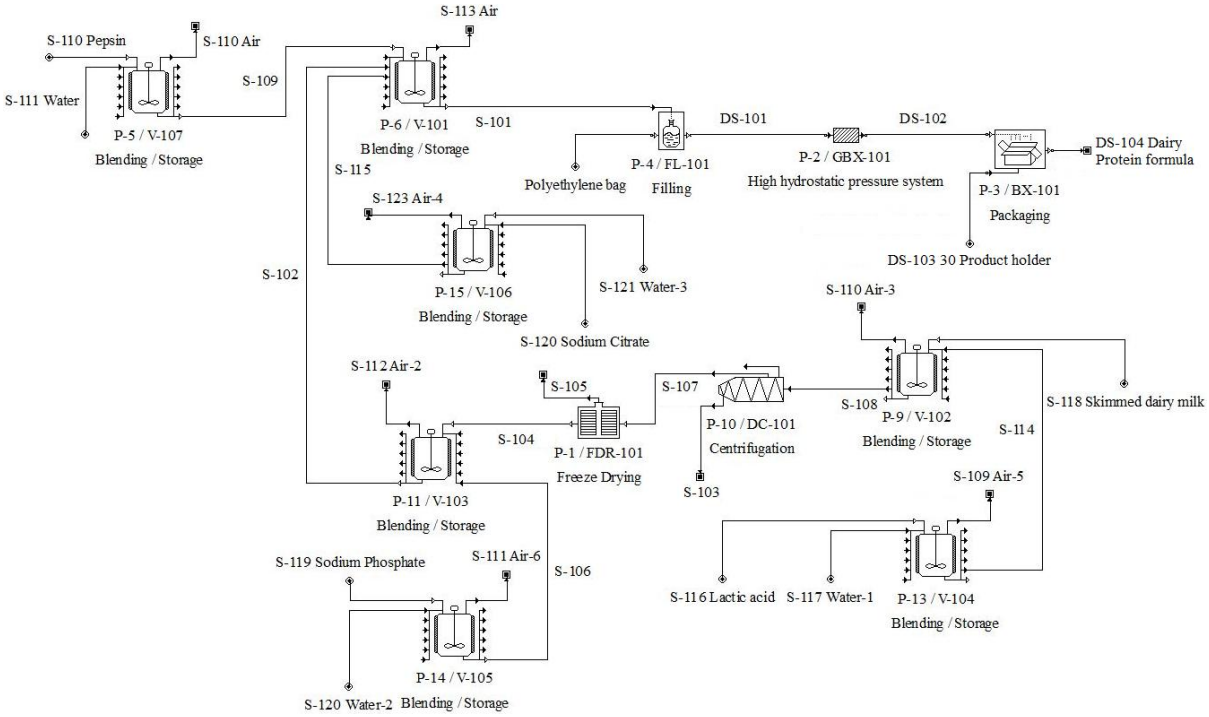


6. Figure: Process scheme (VI): Enzymatic hydrolysis and microwave heat treatment for producing a dairy protein formula with lower allergenic activity.

3.2.7 Process scheme (VII)

The process scheme for producing a dairy protein formula with lower allergenic activity by enzymatic hydrolysis and high-pressure treatment of skimmed milk protein was considered according to Peñas et al. (2006). In the first step of the process scheme, proteins in skimmed bovine milk were precipitated by 4 M lactic acid, and subsequently, the protein fraction was

separated by centrifugation. Lyophilized protein fraction having moisture content of 5% was obtained by freeze-drying. The lyophilized protein was suspended in 50 mM sodium phosphate buffer, pH 8.0, to get a concentration 50 $g \cdot L^{-1}$. Before the pepsin hydrolysis, the pH of the protein solution was maintained at 4.0 with 50 mM sodium citrate buffer. A total volume of 1.250 L was used for the proteolysis reaction considering 1.1 L of buffer (50 mM sodium citrate at pH 4 for pepsin) and 0.025 mL of enzyme (5 $mg \cdot mL^{-1}$) and 0.125 mL of substrate. Pepsin hydrolysis of milk proteins was performed at a temperature of 37 ^{o}C for 30 min followed by the deactivation of pepsin at a temperature 90 ^{o}C for 5 minutes. Whey protein hydrolysate was filled into a pressure-resistant polyethylene bottle (1 L sample holding capacity) and considered for high pressure treatment with 300 MPa pressure for 15 minutes. In the SuperPro Designer model, 2 tons of skimmed milk was considered as a feedstock, and finally 4 Ton protein formula with lower allergenic activity in a batch was produced. At the end, bottles were considered for the packaging. The process scheme for developing a dairy protein formula with lower allergenic activity by enzymatic hydrolysis and high pressure treatment is presented in Fig. 7.



7. Figure: Process scheme (VII): Enzymatic hydrolysis and high-pressure treatment for producing a dairy protein formula with lower allergenic activity.

3.3 Economic parameters

In the present study, different economic parameters, such as direct fixed-capital cost (DFC), annual operating cost (AOC), revenue, gross profit (GP), net profit (NP), gross margin (GM), return on investment (ROI), payback time (PBT), internal rate of return (IRR) and net present value (NPV) have been considered to evaluate the economic outcomes of seven process schemes. The SuperPro Designer software (v. 12, Intelligen, Inc., USA) was used for this purpose. All prices were considered in US$ for economic analysis of all process schemes. Furthermore, the construction and start-up periods were not considered in this investigation. All calculations were performed considering the production from day 1.

3.3.1 Direct fixed-capital cost (DFC)

The direct fixed-capital cost (DFC) is contributed by direct, indirect and miscellaneous costs that are associated with the capital investment of a plant. Hence, it is related to total plant cost (TPC) and contractor's fee and contingency (CFC). Total plant cost (TPC) depends on total plant direct cost (TPDC) and total plant indirect cost (TPIC) (Brigham & Houston, 2018a). Therefore,

TPC (US$) = TPDC + TPIC (Shiby et al.,2013)

DFC (US$) = TPC + CFC (Achaglinkame et al.,2023)

It is necessary to mention that TPDC depends on equipment purchase cost, installation of equipment and apparatus for different unit operations, accessories of equipment, joints and fittings, process piping, instrumentation and controls, electrical items, insulation of electric items, buildings, yard improvement and auxiliary facilities. TPIC depends on engineering and construction. CFC is contributed by contractor's fee and contingency. Purchasing costs of individual equipment were collected from the Alibaba online shopping website (Alibaba.com, 2025) because in most of the industries utmost equipment are purchased from renounced international companies. The updated cost of equipment may provide a more realistic economic view of different process schemes because it shares a significant contribution to the TPDC. Costs of equipment available on the website were considered without international and local taxes (corporation tax), shipping and delivery. Currency exchange rate was considered according to 2025, if required (Magyar Nemzeti Bank, 2025). Since the costs of equipment were provided up-to-date, cost adjustment with the Chemical Engineering Plant Cost Index was

not considered. The default prices in the SuperPro Designer software for the installation of equipment and apparatus for different unit operations, accessories of equipment, joints and fittings, process piping, instrumentation and controls, electrical items, insulation of electric items, buildings, yard improvement, auxiliary facilities, engineering, construction, contractor's fee and contingency were considered for the economic analysis of different process schemes because these are mostly provided by local suppliers. Hence; their costs depend on the geographic region. In this investigation, the land cost was not taken into consideration because it depends on the personal communication between the property management companies and the investor of the project. The costs of equipment and apparatus present in different process schemes (Fig. 1-Fig. 7) are mentioned in subsequent tables (Table 7-Table 13).

7. Table: Costs of equipment for the process scheme (I).

Item code	**Equipment**	**Number of units**	**Cost (US$)/ Item**
UF-101	Cross-flow ultrafiltration membrane with module (Membrane Area = 59.00 m^2)	1	100,000
FR-101	Fermenter (Vessel Volume = 8.78 m^3)	5	100,000
FL-101	Filler (Discrete Throughput = 7740.81 entities/h)	2	100,000
V-101	Blending tank (Vessel Volume = 44.83 L)	1	65,000
HX-101	Heat exchanger (Heat Exchange Area = 11.45 m^2)	1	10,000
CIP-101	Clean in place (Volume = 1.77 m^3)	1	23,000
Total			798,000

8. Table: Costs of equipment for the process scheme (II).

Item code	Equipment	Number of units	Cost (US$)/ Item
V-101	Blending tank (Vessel Volume = 2,28 m^3)	1	65,000
FL-101	Filler (Discrete Throughput = 2001.02 entities/h)	1	100,000
HX-101	Heat exchanger (Heat Exchange Area = 2.13 m^2)	1	10,000
CIP-101	Clean in place (Volume = 0.31 m^3)	1	23,000
Total			198,000

9. Table: Costs of equipment for the process scheme (III).

Item code	Equipment	Number of units	Cost (US$)/ Item
FR-101	Fermenter (Vessel Volume = 8.96 m^3)	5	100,000
FL-101	Filler (Discrete Throughput = 7955.21 entities/h)	2	100,000
HX-101	Heat exchanger (Heat Exchange Area = 12.86 m^2)	1	10,000
CIP-101	Clean in place (Volume = 0.49 m^3)	1	23,000
Total			633,000

10. Table: Equipment costs for the process scheme (IV).

Item code	Equipment	Number of units	Cost (US$)/ Item
V-101, V-102	Blending tank (Vessel Volume = 3132.18 L)	2	65,000
HX-101	Heat exchanger (Heat Exchange Area = 3.05 m^2)	1	10,000
GBX-101	Microwave heating system (Throughput = 11855.90 entities/h)	1	100,000
DF-101	Cross-flow nanofiltration membrane with module (Membrane Area = 19.41 m^2)		120,000

DC-101	Decanter centrifuge (Throughput = 11275,86 L/h)	1	60,000
FL-101	Filler (Discrete Throughput = 1975.98 entities/h)	1	100,000
CIP-101, CIP-102	Clean in place (Volume = 0.35 m^3 for CIP-101 and Volume = 0.42 m^3 for CIP-102)	2	23,000
Total			566,000

11. Table: Equipment costs for the process scheme (V).

Item code	**Equipment**	**Number of units**	**Cost (US$)/ Item**
V-101	Blending tank (Vessel Volume = 2.33 m^3)	1	65,000
FL-101	Filler (Discrete Throughput = 2095.55 entities/h)	1	100,000
GBX-101	High pressure system (Throughput = 12573.30 entities/h)	1	100,000
CIP-107	Clean in place (Volume = 0.31 m^3)	1	23,000
Total			288,000

12. Table: Equipment costs for the process scheme (VI).

Item code	**Equipment**	**Number of units**	**Cost (US$)/ Item**
V-101	Blending tank (Vessel Volume = 2.35 m^3)	1	65,000
FL-101	Filler (Discrete Throughput = 2096.56 entities/h)	1	100,000
GBX-101	Microwave heating system (Throughput = 25158.66 entities/h)	1	100,000
CIP-101	Clean in place (Volume = 0.31 m^3)	1	23,000
Total			288,000

13. Table: Costs of equipment for the process scheme (VII).

Item code	Equipment	Number of units	Cost (US$)/ Item
V-101, V-102, V-103, V-104, V-105, V-106, V-107	Blending tank (Vessel Volume = 3.62 m^3 for Vessel 1, Vessel Volume = 2.24 m^3 for Vessel 2, Vessel Volume = 2.38 m^3 for Vessel 3, Vessel Volume = 0.01 m^3 for Vessel 4, Vessel Volume = 2.23 m^3 for Vessel 5, Vessel Volume = 0.11 m^3 for Vessel 6, Vessel Volume = 1.12 m^3 for Vessel 7)	7	65,000
BGBX-101	High pressure system (Throughput = 13040.22 entities/h)	1	100,000
FDR-101	Freeze dryer (Sublimation Capacity = 1794.86 kg)	1	150,000
FL-101,	Filler (Discrete Throughput = 3260.06 entities/h)	1	100,000
DC-101	Decanter centrifuge (Throughput = 8049.92 L/h)	1	60,000
CIP-101, CIP-102, CIP-103, CIP-104, CIP-105, CIP-106, CIP-107, CIP-108	Clean in place (Volume = 0.06 m^3 for CIP-101, Volume = 0.31 m^3 for CIP-102, Volume = 0.32 m^3 for CIP-103, Volume = 0.11 m^3 for CIP-104, Volume = 0.31 m^3 for CIP-105, Volume = 0.36 m^3 for CIP-106, Volume = 0.03 m^3 for CIP-107, Volume = 0.24 m^3 for CIP-108)	8	23,000
Total			1,049,000

3.3.2 Annual operating cost (AOC)

The annual operating cost (AOC) expressed by US$ year-1 is composed of different factors. Among them, costs of feedstock (milk and WPC), chemicals, enzymes, microbial culture, water and packaging items are significant (Brigham & Houston, 2018b). Information about them was

collected from the Alibaba online shopping website (Alibaba.com, 2025) with 2025 currency exchange rate (Magyar Nemzeti Bank, 2025); however, costs for international and local taxes, shipping and delivery were not considered, similar to before (section 2.2.1). The costs of feedstock, chemicals, enzyme, microbial culture, water and packaging items present in different process schemes (Fig. 1-Fig. 7) are mentioned in Table 14.

14. Table: Costs of feedstock, chemicals, enzyme, microbial culture, water and packaging items for the process schemes (I-VII).

Feedstock ($·kg^{-1})		**Biochemicals and microbial culture ($·kg^{-1})**		**Inorganic chemicals ($·kg^{-1})**		**Packaging items ($/entity)**	
Skimmed dairy milk	0.5	Trypsin	2,200	Sodium hydroxide	0.1	200 mL container	0.1
WPC	10	Pepsin	1,000	Phosphoric acid	0.1	Product holder (Box)	0.1
		Alcalase	200	Sodium phosphate	0.2	Polyethylene bag	0.05
		Lactic acid bacteria	10	Sodium citrate	0.1		
		Lactic acid	1.2	Hydrochloric acid	0.1		
				Water	0.1		
				De-ionized water	0.1		

Costs for facility-dependent, consumables, waste treatment/disposal, and utilities of electric

power and steam also influence the AOC. Their default values in the SuperPro Designer software were considered for the economic analysis. Furthermore, AOC is labor-dependent. In this investigation, it was considered that one worker of each equipment and one quality control (QC) analyst are necessary for each process scheme. The labor costs for individual worker and QC analyst were US$ 23/ hour and US$ 46/ hour, respectively. The default prices in the SuperPro Designer software for electricity, chilled water (5-10 ^{o}C) and regular water (25-30 ^{o}C) were considered for the economic analysis of different process schemes because it depends on the geographic region.

3.3.3 Revenue

The revenue is mainly generated from sales of products (reduced allergen-free dairy protein formulas). In this investigation, some assumptions for producer prices of dairy protein formulas with lower allergenic activity were taken into consideration because allergen free dairy foods are limited in the marketplace. The assumptions for dairy protein formulas with lower allergenic activity were considered to get positive economic indicators. The producer prices of dairy protein formulas in different process schemes are mentioned in Table 15.

15. Table: Producer prices of dairy protein formulas from different process schemes (I-VII).

Producer price	**Cost (US$)**						
	Process scheme (I)	**Process scheme (II)**	**Process scheme (III)**	**Process scheme (IV)**	**Process scheme (V)**	**Process scheme (VI)**	**Process scheme (VII)**
1 L in a bottle	2.0	1.43	0.93	2.8	1.4	1.53	5.4
Total 30 bottles in a box	60	43	28	84	42	46	162

For each operating year during the expected lifetime of a project, the revenues are calculated by multiplying the calculated annual revenues with the fraction (f_Q, of total capacity that corresponds to the operating capacity for that year) and with the months (t, operation for that

year) (Fabozzi & Peterson, 2003a). Revenues can be expressed according to Eq. 3.

Revenues (US$.year^{-1}) =$f_Q$ × t × Annual revenues (Malik et al.,2024)

Annual revenue of a process is calculated as the sum of the revenues of all streams. Therefore, it is associated with unit production cost revenue and cost basis annual rate.

Unit production cost revenues ($/entity) defined as a per-unit-of-reference revenue term that is calculated by dividing the annual revenues by the unit reference rate, mentioned in Eq. 4 (Brigham & Houston, 2018c).

Unit production cost revenues ($/entity) =Revenues (savings)/

Revenues (saving reference rates) (Vandenplas et al.,2023)

Cost basis annual rate can be defined as the flow basis (total flow or component flow) used to convert the annual operating cost to unit production cost. Cost basis is the original value or purchase price of an asset or investment for tax purposes. It is used to convert other annual cost items from an annual basis to a per-unit-reference basis (Brigham & Houston, 2018d).

3.3.4 Gross profit (GP)

The gross profit (GP) was evaluated by the difference of revenues and AOC, mentioned in Eq. 5 (Fabozzi & Peterson, 2003b).

GP (US$ year-1) = Revenues – AOC (Cuomo et al.,2017)

3.3.5 Net profit (NP)

The net profit (NP) was evaluated by GP, taxes and depreciation. In this investigation, a 9% corporate tax rate was considered. This (relatively low) corporate tax rate has been applied in Hungary since 2017 (Csaba, 2025). The value of the depreciation of the fixed capital investment was calculated using the straight line method on 10 years' lifetime with negligible salvage values. The NP can be calculated according to Eq. 6 (Fabozzi & Peterson, 2003c).

NP (US$ year-1) = GP – 0.09×GP + Depreciation (Wal, 2002)

3.3.6 Gross margin (GM)

The gross margin (GM) is a measure of profit which represents a percentage of the annual revenues left over after subtracting the cost of item (Fabozzi & Peterson, 2003d). The gross

margin (GM) was calculated according to Eq. 7.

GM = (Revenue - Cost of item)/Revenue × 100 (E. et al., 1991)

Return on investment (ROI)

The return on investment (ROI) is a type of profitability indicator which evaluates the viability of an investment or to compare the profitability of a number of different investments. It is calculated by dividing the annual net profit by the total capital investment charged to a project (Brigham & Houston, 2018e). The ROI for different process schemes was calculated according to Eq. 8.

ROI (%) = (Gain from investment - Cost of investment)×100/ Cost of investment (Garau et al.,2021)

3.3.7 Payback time (PBT)

The payback time (PBT) is a measure of the time (usually measured in years) needed for the total capital investment to be exactly balanced by the cumulative NP. It is calculated by dividing the total capital investment charged to this project by the annual NP (Brigham & Houston, 2018f). The PBT was calculated according to Eq. 9.

PBT (year) = Total capital investment/ Annual net profit (Mathai.,2017)

3.3.8 Internal rate of return (IRR)

The internal rate of return (IRR), which is also known as discounted cash rate of return (DCRR) is the annual rate of growth that an investment is expected to generate. It is calculated based on cash flows before and after income taxes. The cash flow before income taxes is calculated as the net cash flow and the income taxes, and the cash flow after income taxes corresponds to the net cash flow (Brigham & Houston, 2018g). In this investigation, IRR (in %) was calculated for 10 years.

3.3.9 Net present value (NPV)

The net present value (NPV) represents the total value of future net cash flows (spread over the lifetime of a project), at the beginning of the project. If the NPV is zero, the investment does not generate any additional return compared to the alternative investment. A positive value of NPV indicates an additional return compared to the alternative investment. The PBT was calculated according to Eq. 10.

$$NPV(US\$) = PV - Co = \sum_{t=0}^{T} \frac{C_t}{(1+r)^t} - Co$$ (Achaglinkame et al., 2023)

where, PV is the present value, Ct is the net cash inflow during the period t, Co is the total initial investment cost, IRR is the discount rate and t is the time (10-year consideration) (Brigham & Houston, 2018f).

4 RESULTS AND DISCUSSION

4.1 Comparison of product quality for process schemes (I-VII)

It is necessary to mention that concentration of proteins and residual allergenic activity of proteins influence the economic indicators because it influences consumer's acceptance and market viability. According to the SuperPro Designer model, concentration of protein in the final dairy protein formula was evaluated, mentioned in Table 12. Concentration of protein in the dairy protein formula produced by the process scheme (I) was higher than other cases. It may be due to the application of membrane separation process for the dewatering of skimmed milk prior to tryptic and microbial hydrolysis of dairy proteins (Nath et al., 2021). Development of a protein formula with lower allergenic activity from whole milk protein is beneficial in nutritional viewpoints because casein contains a higher proportion of different types of EAAs, such as His, Met, Phe and Val, than whey proteins. Furthermore, several non-EAAs, such as Arg, Glu, Pro, Ser and Tyr in casein are abundant. Whey protein contains a higher proportion of BCAAs, such as Leu, Ile, and Val, compared to casein (Sindayikengera & Xia, 2006). Caseins agglomerate in the acidic environment of the stomach. Therefore, it leads to late gastric emptying, and a steady and prolonged release of amino acids into the bloodstream. On the other hand, whey proteins are highly soluble in the food matrix which leads to their faster absorption than casein in the gastrointestinal tract. Therefore, whey proteins lead to a dramatic short-lived rise in plasma amino acids (Boirie et al.,1997; Paul ,2009). Attempts were made to produce dairy protein formula with lower allergenic activity by process schemes (IV) (Grar et al.,2009), (V) (Jiang et al.,2023), (VI) (Izquierdo et al., 2008) and (VII) (Peñas et al.,2006). Concentrations of protein in dairy protein formulas produced by process schemes (IV) and (VII) are significantly lower than their feedstocks because in those cases whey proteins were isolated by acid precipitation and centrifugation ((Grar et al., 2009; Peñas et al.,2006). In the process scheme (II), temperature 90^{o}C for 10 min was considered for the deactivation of enzyme alcalase. It may be realized that at 90^{o}C, some whey proteins could be denatured and precipitate,

but casein could be unaffected. Investigators did not mention this issue in their manuscript (Liang et al., 2022). Microwave heating can unfold the protein structure and degrade epitopes in protein structure; whereas, some investigators mentioned that microwave heating can lead to the exposure of hidden epitopes which increase the allergenic activity of proteins (Grar et al., 2009). High pressure treatment significantly induces structural changes in whey protein, including the unfolding of protein structure, the formation of macromolecular aggregates linked by disulfide bond, increasing in β-sheet content in protein structure and decreasing the content of tight helical structures. Furthermore, high pressure treatment promotes the exposure of internal hydrophobic groups which leads to increased surface hydrophobicity and altered allergenic activity of protein molecules (Jiang et al.,2023). It is quite difficult to comprehend the effects of complex processes (enzymatic hydrolysis with microwave heating, enzymatic hydrolysis with high pressure treatment) on the modulation of protein structure and their antigenic activity because biochemical mechanisms are moderately multifaceted.

Allergenic activity of dairy protein formula along with assay method are mentioned in Table 16. It is noted that there was a residual allergenic activity in all dairy protein formulas except the dairy protein formula from process scheme (I); however, this claim may raise an argument. Immunoblot was used to understand the residual allergenic activity of the dairy protein formula produced by the process scheme (I); whereas, enzyme-linked immunosorbent assay (ELISA) was used to understand the residual allergenic activity of the dairy protein formula in other process schemes. In the process scheme (I), allergenic activity of dairy proteins was measured by polyclonal primary antibodies from rabbit (Rb), such as anti-casein, anti-α-lactalbumin and anti-β-lactoglobulin, and cow's milk positive pooled human serum together with peroxidase-labelled goat anti-Rb IgG secondary antibody in the immunoblot. The reduction of allergenic activity of individual dairy proteins by the mentioned technology (process scheme (I)) was published in our previous publication. It was found that the allergenic activity of casein was reduced by more than 99%. Allergens β-lactoglobulin and α-lactalbumin were retained; however, they were reduced compared to the control by the sequential tryptic and microbial hydrolysis of LMPC according to Rb polyclonal antibody in the immunoblot. Furthermore, it was found that allergenic activities of casein, β-lactoglobulin, and α-lactalbumin were reduced more than 99% according to cow's milk positive pooled human serum in the immunoblot (Nath et al., 2021). Similarly, Rb polyclonal primary IgG antibodies (anti-casein, anti-α-lactalbumin and anti-β-lactoglobulin) with secondary alkaline phosphatase-conjugated goat anti-Rb IgG antibodies in the immunoblot were used by Liang et al, 2021 (Liang et al., 2022) in the process

scheme (II). Furthermore, investigators used ELISA to understand the reduction of allergenic activity of mentioned proteins in a quantitative way (the IgG reactivity inhibition (%)). The IgE-binding inhibition rate of proteins (α-lactalbumin, β- lactoglobulin, α-casein and β-casein) in ELISA was carried out by Song et al, (2023) in the process scheme (III). In the process scheme (IV), Gar et al, (2009) measured antigenic activity of β-lactoglobulin in WPC by ELISA considering goat anti-rabbit IgG peroxidase conjugate as secondary antibody. ELISA for β-lactoglobulin in WPC was performed considering anti-β-lactoglobulin as a primary antibody and Horseradish Peroxidase-conjugated antibody as a secondary antibody in the process scheme (V) by Jiang et al, (2023). ELISA was conducted using human pool serum, considering anti-α-lactalbumin and anti-β-lactoglobulin as primary antibody and goat anti-human peroxidase-labeled IgE as secondary antibody in the process scheme (VI) by Izquierdo et al, (2008). Similar ELISA was performed by Peñas et al, (2006) in the process scheme (VII).

Furthermore, sensory properties (texture and flavor) and digestibility of the final dairy protein formula could play a pivotal role on the economic indicators because it influences consumers' acceptance and market viability. Enzymatic hydrolysate of dairy protein formulas generally has a bitter taste (Nath et al.,2022). In another investigation, it has been reported that pretreatment of milk protein concentrates either by microwave irradiation or ultrasound with enzymatic hydrolysis in a sequential way produces bitterness of the hydrolysates (Uluko et al.,2013). However, enzymatic hydrolysis of dairy proteins could produce bitterness, it may improve digestibility (Medeiros et al.,2014). Thermal and non-thermal technologies, such as microwave heating and high-pressure treatment, respectively, induce the change of protein structure (denaturation, conformational changes, and unfolding), thereby increasing their susceptibility to digestion (Bhat et al.,2021). Contradictorily, results were also published. The digestibility of dairy proteins could be reduced due to the glycation of milk proteins by heat treatment of dairy foods. Other chemical modifications, including dephosphorylation and cross-linking of dairy proteins by heat treatment on digestibility are less studied, but may also result in decreased amino acid bioavailability (van Lieshout et al.,2020). In the present investigation, these factors were not considered because of the limited information in the articles mentioned.

16. Table: Comparison of the protein concentrations, amount and hypoallergenic status for all process schemes (I-VII).

Process scheme/ Reference	Initial protein concentration and amount	Final protein concentration and amount (Estimated)	Allergenic activity	Analytical Methods
(I)/ (Nath et al., 2021)	33 g/L in 16 tons of milk/batch	66 g/L in 8 tons of dairy protein formula/batch	Allergens casein was reduced more than 99%; however, β-lactoglobulin and α-lactalbumin retained according to Rb polyclonal antibody in the immunoblot. Allergens casein, β-lactoglobulin and α-lactalbumin were reduced more than 99% according to cow's milk positive pooled human serum in the immunoblot.	Immunoblots for Rb-IgE, and IgE and IgG for cow's milk positive pooled human serum
(II)/(Liang et al., 2022)	33 g/L in 2 tons of milk/batch	33 g/L in 2 tons of dairy protein formula/batch	Allergen (casein, α-lactalbumin and β-lactoglobulin) retained according to immunoblot. Reduction 90.25%, 91.21%	Immunoblot and ELISA for IgG

			and 10% reduction for casein, β-lactoglobulin and α-lactalbumin, respectively according to ELISA.	
(III)/ (Song et al.,2023)	33 g/L in 8 tons of milk/batch	33 g/L in 8 tons of dairy protein formula/batch	Retained 48.8%, 30.25%, 52.52% and 40.8% antigenicity of α-lactalbumin, β-lactoglobulin, α-casein and β-casein, respectively	ELISA for IgE
(IV)/ (Grar et al.,2009)	33 g/L in 2 tons of milk/batch	6 g/L in 2 tons of dairy protein formula/batch	Retained β- lactoglobulin antigenicity 41.16%	ELISA for IgE
(V)/ (Jiang et al.,2023)	38 g/L in 2 tons of WPC/batch	38 g/L in 2 tons of dairy protein formula/batch	Retained antigenicity in WPC 76.19%	ELISA for IgE
(VI)/ (Izquierdo et al.,2008)	38 g/L in 2 tons of WPC/batch	38 g/L in 2 tons of dairy protein formula/batch	Retained α-lactalbumin and β-lactoglobulin antigenicity 2%	ELISA for IgE
(VII)/ (Peñas et al.,2006)	33 g/L in 2 tons of milk/batch	4 g/L in 4 tons of dairy protein formula/batch	Retained antigenicity of whey proteins 4%	ELISA for IgE

4.2 Costs for investment

The direct fixed-capital cost (DFC) for process schemes (I-VII) is presented in Table 17. It may be realized that the development of a complex process could be a major factor for the higher value of DFC because it depends on the (i) expenses of equipment and accessories, (ii) equipment installation, (iii) process piping, (iv) instrumentation and control, (v) electrical system and insulation, (vi) buildings, (vii) yard improvements, (viii) auxiliary facilities, (ix) engineering, (x) construction expenses, (xi) contractor's fee and (xii) contingency.

It is noted that DFCs for process schemes (VII) is quite higher than other process schemes. The higher value of DFC for process scheme (VII) is mainly due to the construction of the complex process scheme. Therefore, expenditure of (i) equipment for different unit operations, (ii) accessories of equipment, joints and fittings, (iii) equipment installation, (iv) process piping, (v) instrumentation and control of the process, (vi) electrical systems and insulation, (vii) building, (viii) engineering and (ix) contingency mainly attribute the higher value of DFC of the process scheme (I). Without any contradiction, it can be realized that higher values of DFC for the process schemes (I) than the process scheme (III) due to the application of (i) membrane separation process to produce LMPC from milk, (ii) the construction of whole process (accessories of equipment, joints and fittings, equipment installation, process piping, instrumentation and controls, electrical system and insulation, and engineering), (ii) enzyme for the hydrolysis of LMPC and (iii) contingency. Interestingly, the DFC for the process scheme (III) is greater than the process schemes (IV); however, it is less complex. The higher value of DFC for the process scheme (III) is mainly attributed to the application of temperature-controlled well-equipped fermenter, engineering and construction expenses. The DFC for process scheme (IV) is higher than process schemes (II), (V) and (VI). This may be due to the complicated process scheme involving wide ranges of unit operations, such as acid precipitation of skimmed milk proteins, decanting whey by centrifugation, diafiltration to concentrate whey protein and microwave heating of concentrated whey protein. DFC values for process schemes (V) and (VI) are higher than the process scheme (II) for the similar reason.

17. Table: Direct fixed-capital cost (DFC) for process schemes (I-VII).

Items	**Process scheme**	**Process scheme**	**Process scheme**	**Process scheme**	**Process scheme**	**Process scheme**	**Process scheme**

	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
Costs of equipment (US$)	798,000	198,000	633,000	566,000	288,000	288,000	1,049,000
Accessories of equipment, joints and fittings (US$)	194,000	44,000	153,000	130,000	66,000	66,000	216,000
Equipment installation (US$)	371,000	96,000	281,000	299,000	153,000	153,000	397,000
Process piping (US$)	339,000	77,000	267,000	228,000	116,000	116,000	378,000
Instrumentation and controls (US$)	388,000	88,000	305,000	260,000	133,000	133,000	433,000
Insulation (US$)	29,000	7,000	23,000	20,000	10,000	10,000	32,000
Electrical system (US$)	97,000	22,000	76,000	65,000	33,000	33,000	108,000
Buildings (US$)	436,000	98,000	343,000	293,000	149,000	149,000	487,000
Yard improvements (US$)	145,000	33,000	114,000	98,000	50,000	50,000	162,000
Auxiliary facilities (US$)	388,000	88,000	305,000	260,000	133,000	133,000	433,000
Engineering (US$)	790,000	182,000	619,000	543,000	277,000	277,000	878,000
Construction expenses (US$)	1,106,000	254,000	867,000	760,000	387,000	387,000	1,229,000
Contractor's fee (US$)	253,000	58,000	198,000	174,000	89,000	89,000	281,000
Contingency (US$)	506,000	116,000	396,000	347,000	177,000	177,000	562,000
Total cost (US$)	5,840,000	1,360,000	4,580,000	4,043,000	2,061,000	2,061,000	6,645,000

4.3 Annual operating cost

Annual operating cost is one of the important issue to continue a process with time progress in a process industry. It may be realized that the AOC could be increased if a process scheme is complex; however, it is dependent on costs for raw materials, labor-dependent, facility-dependent, laboratory analysis for quality control (QC) and quality assurance (QA), consumables, waste treatment, and utilities. The annual operation costs for all process schemes are presented in Table 18. It is noted that AOC for process schemes (I) and (III) are significantly higher among all process schemes. AOC for process schemes are comparable for process schemes (IV) and (VII); likewise, process schemes (II) and (VI). Furthermore, it is noted that AOC for process scheme (V) is relatively higher than process schemes (II) and (VI) but lower than process schemes (IV) and (VII). The variation of AOC among different process schemes is discussed in detail in subsequent sections.

Raw materials

The costs of raw materials on an annual basis for process schemes (I) and (III) are higher than other process schemes (Table 18). This could be mainly due to the higher amount of feedstock (16 Ton and 8 Ton skimmed milks in the process scheme (I) and the process scheme (III), respectively). The maximum number of batch·year-1 also influences the annual expenditure of raw materials and total AOC. For instance, the maximum number of batch·year-1 is higher for the process scheme (III) than all other process schemes (Table 19) which provides the higher expenditure of raw materials for the process scheme (I). Additionally, the costs of enzyme, water, and chemicals for cleaning of systems and waste treatment (whey from skimmed milk) could also have a great contribution on the higher costs of raw materials per year for process schemes. Solid organic waste and liquid waste water were produced due to the precipitation of protein and diafiltration membrane permeate, respectively in the process scheme (IV). The cost of chemicals for these waste treatment is responsible for the higher value of raw material for the process scheme (IV) than process schemes (II), (V), (VI) and (VII). Similar analogy could be implying for the process scheme (I) where 8 Ton of whey were produced from 16 Ton of milk. The process scheme (VII) is composed of a wide range of equipment and unit operations, where annual expenditures due to chemicals for cleaning and waste treatment after each batch operation could be significant; however, the maximum number of batch·year-1 is lower for the process scheme (VII) than all other process schemes (Table 19). The annual expenditure of raw materials for the process scheme (V) is higher than process schemes (VI) and (II); however, the initial amount of feedstock was similar for process schemes (V), (VI) and (II). This might be

due to the higher maximum number of batches·year-1 for process scheme (V) (Table 19). Furthermore, annual expenditure due to chemicals for cleaning and waste treatment for the process scheme (V) is higher than process schemes (VI) and (II). The expenditure of raw materials per year for process schemes depends on the characteristics of feedstock also. For instance, WPC was used in process schemes (V) and (VI) having price US$ 10·kg-1 which is higher than skimmed milk (US$ 0.5·kg^{-1}) used in process scheme (II).

4.4 Labor-dependent

Annual labor-dependent costs for process schemes (III) is quite higher than other process schemes (Table 18), which is attributed by the maximum number of batch·$year^{-1}$ (Table 19) and consequently, involvement of laborers. Process schemes (I) and (III) are not complex compared to process scheme (VII) and (IV); however, membrane filtration to produce LMPC in process scheme (I) and microbial fermentation in process schemes (I) and (III) are main cause of the higher batch time. Annual labor-dependent-dependent expenditures for process schemes (VII) and (IV) are greater than process schemes (II), (V) and (VI) (Table 18) which might be due to the complex process scheme where more labors are required. Annual labor-dependent expenditures for process schemes (II), (V) and (VI) are almost similar (Table 18). This might be due to almost similar numbers of steps involved in the mentioned process schemes.

4.5 Facility-dependent

Annual facility-dependent costs associated with equipment maintenance were varied among process schemes. The process scheme (VII) is quite complex, developed by wide ranges of equipment and unit operations which is associated with high facility-dependent cost similar to process scheme (I) (Table 18). Process schemes (I), (II), (III), (V) and (VI) are not complex; however, facility-dependent costs for process schemes (I) and (III) are quite high (Table 18). A well-equipped temperature-controlled fermenter was used in process schemes (I) and (III). Its sensors need proper maintenance after each operation. A membrane filtration process was used in process schemes (I) and (IV) where maintenance of membrane is an important issue to reduce the concentration polarization on the membrane surface and subsequently membrane fouling which might be a reason for the higher facility-dependent cost of mentioned process schemes

(Table 18).

4.6 Laboratory analysis for quality control (QC) and quality assurance (QA)

Annual expenditure for laboratory QC and QA of products for the process scheme (III) is higher than other process schemes (Table 18) because of the higher number of batches per year which generates a higher number of samples. Furthermore, annual expenditure for laboratory QC and QA of products for the process scheme (I) is higher than process schemes (II), (IV), (V), (VI) and (VII) (Table 18); however, maximum numbers of batch·year-1 are almost similar for process schemes (II), (IV), (V) and (VI) (Table 19). In the process scheme (I), investigators used western-blot technique to understand the reduction of allergenic activity of dairy protein formula; whereas, other investigators used ELISA. Without any contradiction, costs for reagents, antibodies and chemicals, gel-electrophoresis system, trans-blot semi-dry protein transfer cell and gel-doc costs for western-blot technique are quite higher than ELISA kit. Furthermore, annual expenditures for quality analysis of dairy protein formulas from process schemes (I) and (III) are quite higher than others (Table 18) which is attributed by the microbiological investigation. Annual expenditure for the quality analysis of dairy protein formulas produced by process schemes (VII) is relatively higher than process schemes (II), (IV), (V) and (VI) (Table 18). It might be due to the complex process schemes where quality analysis is required in different points of the process scheme.

4.7 Consumables

Annual expenditures of consumables for process schemes (I) and (IV) are caused mainly by membrane used to produce protein concentrate from milk and removal of lactose and salt from whey, respectively. Expenditure of consumables for the process scheme (I) is higher than the process scheme (III) because membrane with larger surface area was used in the process scheme (I). Furthermore, items for microbiological purposes (Erlenmeyer flask, petri plate, safety cabinet) contribute some amount of annual expenditure; however, it is negligible (Table 18).

4.8 Waste treatment

Annual expenditures for waste treatment for process schemes (I) and (IV) are significantly higher than all other process schemes (Table 18). A huge amount of liquid whey (8 Ton whey from 16 Ton of skimmed milk) was produced due to production of LMPC by the membrane

filtration process in the process scheme (I). Similarly, solid waste and liquid waste were produced by centrifugation to produce whey and whey protein concentrate by diafiltration membrane permeate, respectively in the process scheme (IV). In all other process schemes annual waste treatment cost is almost comparable (Table 18) which is mostly caused by cleaning of equipment, pipes and so on.

4.9 Utilities

Expenditures of utility·year^{-1} for a process is mainly due to the consumption of heat transfer agent (warm water/ steam, chilled water) and electricity. Annual expenditures of utilities for process schemes (III) and (I) are significantly higher than all other process schemes (Table 18). These results could be attributed to several factors. In the process scheme (III), a temperature 45^{o}C for 6 hours was maintained during the hydrolysis of milk proteins by lactic acid bacteria in the fermenter. It is necessary to mention that a heat exchanger was used to reduce the temperature (90 °C to 10 °C within 1 hour) after the microbial hydrolysis of milk proteins in the fermenter prior to filling, which consumes water and steam. However, this process is simple; the higher number of batches·year^{-1} (Table 19) is the cause of higher expenditure of utility·year^{-1}. In the process scheme (I), around 8 tons of LMPC was produced from 16 tons of skimmed milk within 4 hours. A temperature 25^{o}C was maintained in the retentate tank by water circulation within the jacket of the mentioned tank. Subsequently, LMPC was hydrolyzed within a temperature-controlled fermenter. The enzymatic hydrolysis was performed at temperature 40^{o}C for 10 min followed by enzyme deactivation at temperature 70^{o}C for 30 min. When the temperature of enzyme-hydrolyzed LMPC was reduced to 45^{o}C (within time 30 min), it was further hydrolyzed by lactic acid bacteria at temperature 45^{o}C for 6 hours. Subsequently. the temperature of protein hydrolysate was reduced from 45^{o}C to 10^{o}C by a heat-exchanger prior to filling. In all cases, temperatures in the filtration process and biochemical reactions were maintained within the fermenter by water circulation within the jacket of the fermenter. The annual expenditure of utilities for the process scheme (VII) is quite higher than process schemes (II), (IV), (V) and (VI) (Table 18) which is caused by the consumption of greater amounts of water and electricity due to the complex process scheme. The consumption of higher amounts of water during diafiltration of whey protein is the reason for the annual expenditure of utility for the process scheme (IV).

18. Table: Annual operating cost for process schemes (I-VII).

Items	**Process scheme (I)**	**Process scheme (II)**	**Process scheme (III)**	**Process scheme (IV)**	**Process scheme (V)**	**Process scheme (VI)**	**Process scheme (VII)**
Raw materials (US$·year^{-1})	15,490,000	2,867,000	17,606,000	4,234,000	3,382,000	2,720,000	3,975,000
Labor-dependent (US$·year^{-1})	1,454,000	422,000	2,619,000	473,000	414,000	418,000	935,000
Facility-dependent (US$·year^{-1})	1,098,000	254,000	861,000	755,000	385,000	385,000	1,235,000
Laboratory analysis for QC and QA (US$·year^{-1})	421,000	163,000	560,000	171,000	162,000	163,000	240,000
Consumables (US$·year^{-1})	187,000	0	1,000	48,000	0	0	0
Waste treatment (US$·year^{-1})	1,226,000	53,000	105,000	1,056,000	60,000	49,000	64,000
Utilities (US$·year^{-1})	298,000	35,000	479,000	33,000	1,000	14,000	84,000
Total cost (US$·year^{-1})	20,174,000	3,794,000	22,231,000	6,771,000	4,404,000	3,749,000	6,533,000

19. Table: Batch time for process schemes (I-VII).

Items	Process scheme (I)	Process scheme (II)	Process scheme (III)	Process scheme (IV)	Process scheme (V)	Process scheme (VI)	Process scheme (VII)
Batch time (h)	16.54	6.5	12.17	14.49	4.93	6.1	26.22
Maximum number of batch·year^{-1}	1,482	1,484	3,250	1,356	1,662	1,335	441

4.10 Production cost

To understand the production cost of a product is a key feature of any process scheme because it determines the market price of the product. Furthermore, if a product produced from a unique or innovative technology of a process scheme had a similar or even lower calculated price than a product from an already existing technology or process scheme can be referred as a viable candidate. The production costs of different dairy protein formulas were calculated by the SuperPro Designer software and those are mentioned in Table 20.

It is noted that the production costs of a dairy protein formula per container estimated for the process scheme (III) is lower than all other process schemes. Simplest process scheme and operational strategy could be the reason of it. The production cost of a dairy protein formula per container estimated for the process scheme (I) is higher than process schemes (II), (III), (V) and (VI). Higher production cost of dairy protein formula could be justified by several reasons, such as (i) the higher value of DFC, (ii) the cost of higher amount of feedstock for process scheme (I) than other process schemes (16 Ton of milk for process scheme (I)), (iii) the application of membrane filtration process to produce LMPC prior to tryptic hydrolysis in case of process scheme (I), (iv) the application of trypsin for the hydrolysis of concentrated milk proteins prior to their microbial hydrolysis, (v) higher facility-dependent cost, (vi) higher expenditure for laboratory analysis for QC and QA, (vii) higher expenditure for consumables and (viii) cost for waste treatment. The production cost of dairy protein formulas per container for process scheme (VII) is quite higher than process schemes (II), (IV), (V) and (VI). The higher production cost of dairy protein formulas in these cases could be mainly caused by the (i) construction of complex process schemes, (ii) costs of equipment or unit operations and (iii) involvement of a higher number of operators and laborers. Due to the similar reason, the

production cost of dairy protein formulas per container for process scheme (IV) is higher than process schemes (I), (II), (III), (V) and (VI).

20. Table: Production costs of dairy protein formulas from different process schemes (I-VI).

Production cost	**Cost (US$)**						
	Process scheme (I)	**Process scheme (II)**	**Process scheme (III)**	**Process scheme (IV)**	**Process scheme (V)**	**Process scheme (VI)**	**Process scheme (VII)**
1 L in a bottle	1.78	1.28	0.87	2.55	1.28	1.35	4.59
Total 30 bottles in a box	53.29	38.32	26.06	76.57	38.32	40.6	137.7

4.11 Profitability indicators

Based on DFC, annual operation cost and revenue, mentioned above, profitability indicators were evaluated for the process schemes (I-VI). Results are summarized in Table 22. Profitability indicators, such as GP, NP, GM, revenue, NPV, IRR, ROI and PBT for process schemes were considered with the aid of comparison purpose.

GP and NP are quite higher for the process scheme (I) compared to other process schemes. The considerable producer price of dairy protein formula with lower allergenic activity for the process scheme (I) is quite higher than other process schemes; however, the DFC value for the process scheme (I) is quite higher. It may be because the mentioned dairy protein formula had not only lower allergenic activity, additionally it had antioxidant capacity, anti-angiotensin activity and antibacterial activity. Furthermore, the protein formula from the process scheme (I) has quite a higher concentration of protein because LMPC was used (Nath et al., 2021). NP and GP for the process scheme (III) are also appreciable. The quite higher values of GP and NP for the process scheme (III) is due to the higher number of batch·year^{-1}. GP and NP for the process scheme (VII) is substantially higher than process schemes (II), (IV), (V) and (VI) because the considerable producer price was quite higher. GMs of different process schemes were varied based on revenue and cost of items. Cost-based annual rate and unit production revenues for all process schemes are mentioned in Table 21).

Table 21: Cost basis annual rate and unit production revenues for process schemes (I-VII).

Column1	Process scheme (I)	Process scheme (II)	Process scheme (III)	Process scheme (IV)	Process scheme (V)	Process scheme (VI)	Process scheme (VII)
Cost basis annual rate (MP Entities·year $^{-1}$)	378,572	98,984	853,196	88,421	114,933	92,364	47,444
Unit production revenues ($·entity $^{-1}$)	60	43	28	84	42	46	162

It is noted that higher revenue could be obtained by process schemes (I) and (III) than others. The higher revenue for the process scheme (I) could be the reason for the application of trypsin and microbes in a sequential way to produce concentrated dairy protein formula with lower allergenic activity. Therefore, it could be considered a relatively high-valued commodity dairy product. In the process scheme (III), lactic acid bacteria were used to produce dairy protein formula with lower allergenic activity which could be considered as regular fermented dairy foods. Furthermore, revenues for process schemes (IV) and (VII) are quite similar and higher than process schemes (II), (V) and (VI). The considerable producer prices of dairy protein formulas (Table 15) for process schemes (IV) and (VII) are comparatively quite higher than other process schemes due to the complex process schemes which are the cause of higher revenue. Almost similar revenue was found for process schemes (II), (V) and (VI) due to almost identical producer prices of dairy protein formulas (Table 15). It is because process schemes are simpler and developed by almost identical numbers of unit operations. Higher NPV value could be obtained by the process scheme (I) because the dairy protein formula had the higher concentration of protein with less allergen and could provide additional health benefits. Furthermore, process schemes (III) and (VII) could provide higher NPV value compared to

process schemes (II), (IV), (V) and (VI). Positive IRR values were found for all process schemes. It is noted that IRR values are quite similar for process schemes (I) and (II) and higher than other process schemes. Similarly, IRR values are quite similar for process schemes (III) and (VI), and (V) and (VII); however, changes are not significant. The IRR value was lower for the process scheme (IV) among all process schemes. ROI values of all process schemes represent the viability of an investment. However, total capital investment was higher for the process scheme (I), it could provide a higher ROI value because the NP of the process scheme (I) was higher among all process schemes. Due to the similar reason, the ROI value of the process scheme (II) is higher than process schemes (III), (IV), (V), (VI) and (VII). The ROI value of the process scheme (IV) is lower among all process schemes because the total capital investment was very high compared to NP. It is noted that PBT for the process scheme (I) is quite lower than all other process schemes. It is expected that dairy protein formula from the process scheme (I) could be attractive for consumers due to higher concentration of protein, lower antigenic activity and its unique biofunctional activity. Therefore, its demand will be very high, which could provide higher economic benefits and may cause a lower PBT. PBT for the process scheme (III) is higher than the process scheme (I) because the producer price was significantly lower. PBT for process schemes (IV) and (VII) are quite higher than process schemes (II), (V) and (VI). It is due to a complex process scheme which generates lower IRR and ROI.

22. Table: Profitability indicators for process schemes (I-VII).

Profitability indicators	**Process scheme (I)**	**Process scheme (II)**	**Process scheme (III)**	**Process scheme (IV)**	**Process scheme (V)**	**Process scheme (VI)**	**Process scheme (VII)**
Gross profit (GP, US$·year^{-1})	2,487,000	463,000	1,659,000	657,000	423,000	500,000	1,153,000
Net profit (NP, US$·year^{-1})	2,884,000	550,000	1,945,000	982,000	580,000	650,000	1,681,000
Gross margin (GM, %)	9.60	10.87	6.94	8.84	8.76	11.76	15
Revenues (US$·year^{-1})	25,908,075	4,256,312	23,889,000	7,427,364	4,827,186	4,248,744	7,685,928
Net present value (NPV, US$, at 7.0% interest)	11,572,000	2,095,000	7,799,000	2,343,000	1,596,000	2,118,000	4,405,000
Internal rate of return (IRR, % after taxes)	22.86	21.61	18.43	13.98	15.53	18.19	15.17
Return on investment (ROI, %)	32.66	31.73	29.02	20.59	23.1	26.51	22.62
Payback time (PBT, year)	3.06	3.15	3.45	4.86	4.33	3.77	4.42

5 CONCLUSION AND PROPOSAL

Milk and dairy foods have been considered as a well-balanced diet regardless of sociodemographic (geographic continent, urban or rural region and the composition of the population), cultural, lifestyle and environmental factors. Dairy foods are members of 'Functional foods' which is a rich source of proteins, carbohydrates, minerals and fat. Despite this fact, dairy foods are not considered as suitable dietary intake for dairy foods sensitive consumers, frequently experienced with protein allergy and lactose intolerance. However, lactose-free dairy formulas are already commercialized; information about dairy foods with lower allergenic activity is restricted in scholarly databases. However, it was published that tryptic and microbial treatment of milk proteins in sequential ways could reduce the allergenic activity of milk proteins in a substantial way; the economic analysis of this scheme was not explored. Therefore, efforts were placed on economic analysis of the mentioned process scheme compared with other technologies dedicated for the reduction of allergenic activity of milk proteins. It may be realized that a protein formula with higher concentration of protein and lower allergenic activity could be quite attractable to consumers which is the cause of higher profitability indicator for the process scheme (I). The dairy protein formula with lower allergenic activity produced from the process scheme (I) had higher concentration of proteins where essential, non-essential, ketogenic and glucogenic amino acids are abundant. Therefore, it will gain attention from consumers interested in protein supplement and sustainable diet.

The results of allergenic activity are not directly comparable because Nath et al. used immunoblot; whereas other investigators type of analytical method, i.e. ELISA where associated protocols could be varied based on manufacturers. Furthermore, the organoleptic properties of the final product were not considered, which is a considerable crucial factor for the development of food products because it is associated with consumer acceptance, the commercialization and market value of the food products. The legal framework from the dairy food stakeholders' point of view might be a considerable challenging issue because the applications of any other proteolytic enzymes other than rennet and lactase for producing dairy foods are still not considered by food safety and regulation authorities, regardless of their nutritional value.

Dairy foods have immense potential as a sustainable protein source; nonetheless, there is a limited concern about allergenic activity of dairy foods. More than a decade, consumers have

been more interested in value-driven dairy foods than conventional and volume-based dairy foods. It may be expected that the proposed process scheme (I) will reduce the limitations of dairy food consumption. The dairy industry is consistently seeking a recipe and technology to develop and supply dairy foods which offer more amounts of protein and health benefits compared to traditional dairy foods. Therefore, it is anticipated that the proposed process scheme (sequential tryptic and microbial hydrolysis) will bring an economic boom besides the commercialization of regular dairy foods and a new arena in the functional food sector might be explored.

6 SUMMARY

Milk and dairy products are widely recognized as nutritionally complete foods, rich in proteins, fats, carbohydrates, and essential micronutrients, and are consumed globally across diverse sociocultural and demographic settings. Despite their nutritional value, dairy consumption poses challenges for individuals with lactose intolerance or milk protein allergies, limiting their access to these functional foods. While lactose-free dairy products are available on the market, research and commercial development of dairy formulations with reduced allergenicity remain limited.

This study explored a sequential enzymatic treatment process—combining tryptic digestion and microbial hydrolysis—as a strategy to reduce the allergenic potential of milk proteins. The findings suggest that this process effectively diminishes allergenicity while preserving or enhancing the protein content and amino acid profile of the final product. The resulting formula demonstrated high concentrations of essential, non-essential, ketogenic, and glucogenic amino acids, making it a promising candidate for use in protein supplements and sustainable dietary interventions.

An economic assessment of the proposed process (Scheme I) revealed favorable profitability indicators, suggesting its commercial viability when compared to other allergen-reduction technologies. However, variability in analytical methods used to evaluate allergenicity across studies (e.g., immunoblot vs. ELISA) and the absence of sensory evaluation in this work highlight the need for standardized protocols and consumer-focused product development.

Furthermore, regulatory challenges remain a significant barrier, as current food safety frameworks limit the application of proteolytic enzymes beyond those traditionally used in dairy processing. Nevertheless, as consumer demand shifts toward high-value, health-oriented dairy products, the integration of such innovative processes offers significant potential for expanding the functional food market.

In conclusion, the sequential enzymatic hydrolysis approach presents a promising avenue for producing hypoallergenic, high-protein dairy products. This strategy not only addresses the dietary needs of sensitive populations but also aligns with the dairy industry’s goal of delivering health-focused, functional products with strong market potential.

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LIST OF TABLES AND FIGURES

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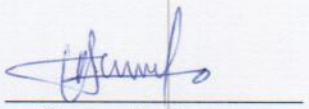

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