

Economic analysis of a bioprocess (successive tryptic and microbial hydrolysis of milk proteins) for the producing of dairy protein formula having lower allergenic activity

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ABSTRACT

Allergenic activity of milk proteins, mainly casein, α -lactalbumin and β -globulin is one of the limiting factors for the dietary intake of dairy foods. Lab-scale experimental results about the development of dairy protein formula with lower allergenic activity by tryptic and microbial hydrolysis of dairy proteins in a

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successive way were published previously; however, the economic analysis of this bioprocess was not explored. The aim of the present investigation is to perform economic analysis of the proposed process scheme. The mentioned process schemes were developed by the SuperPro Designer software (v. 12, Intelligen, Inc., USA) and concepts from chemical engineering. According to estimated profitability indicators (gross profit, net profit, gross margin, net present value, payback time, return on investment and internal rate of return), it may be considered that successive tryptic and microbial hydrolysis of milk proteins could be a suitable process scheme to produce protein formula with lower allergenic activity in dairy industry.

KEYWORDS

dairy protein allergy, enzymatic hydrolysis, microbial hydrolysis, microwave heating, high pressure treatment, profitability indicators

1. INTRODUCTION

For a long time, the diverse health benefits of dairy products have propelled them to a leading position in the “functional food” industry. Dairy foods are recognized as a well-balanced source of nutrition across diverse socio-demographic, culture, lifestyle and environmental contexts. They deliver vital nutrients, that work synergistically to the management of deficiency syndromes, modulation of biomarkers of metabolic syndrome and support overall regular well-being (Shiby and Mishra, 2013). Global milk consumption averages 112 kg per person per year, with cows contributing the majority of the global milk supply (81%), followed by buffaloes at 15% (Achaglinkame et al., 2023). The global dairy market was valued at around \$860 billion in 2024 and is expected to exceed \$1 trillion by 2028 (Hill, 2024).

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 often experiences protein allergies and lactose intolerance. Dairy protein allergy may be categorized as IgE-mediated, non-IgE mediated and mixed (combining IgE with non-IgE) based on pathophysiological condition (Vandenplas et al., 2023). Individuals with dairy protein allergies can be categorized into different phenotypes based on their allergenic responses (Cuomo et al., 2017); however, no specific structure or function of dairy proteins has been identified as primarily responsible for their allergenic activity. As a result, the variability in human IgE responses may contribute to the allergenic potential of any dairy protein or its fragments (Wal, 2002). Lactose intolerance, on the other hand, is a clinical condition marked by symptoms resulting from improper lactose digestion. It is caused by reduced or absent activity of the lactase enzyme or its production in the intestinal lining (Catanzaro et al., 2021).

Dairy milk is an excellent source of proteins having all types of amino acids, including ketogenic amino acids (KAAs), glucogenic amino acids (GAAs), essential amino acids (EAAs) and branched-chain amino acids (BCAAs). Other advantageous aspects of dairy proteins are their exceptional digestibility quality, specifically digestible indispensable amino acid score (DIAAS) and protein digestibility-corrected amino acid score (PDCAAS) of dairy proteins, which are higher than most other protein sources (Mathai et al., 2017). These factors explain why milk and dairy products account for approximately 18–20% of adult protein intake

(Achaglinkame et al., 2023). Hence, eliminating dairy foods from the diet chart may result in nutritional deficiencies to consumers, specifically to infants and children (Lawson et al., 2024).

While lactose-free dairy products have been commercially available and steadily growing with a 7% CAGR from 2017 to 2022 and a market size of \$8.8 billion in 2022 (Li et al., 2023), there is limited focus on allergen-free dairy formulas in the industry (Nutten et al., 2020). Moderately/partially hydrolyzed dairy protein formula (Beba HA, Good Start, NAN HA, Nestlé) was first introduced in 1985 (Exl, 2001). Presently, increasing consumer awareness of lactose intolerance, food allergies and clean-label preferences are key driver factors of innovation in the dairy food sector, particularly in the development of allergen-free dairy alternatives utilizing plant-based proteins (Plamada et al., 2023) and recombinant dairy proteins produced through precision fermentation technologies (Piazenski et al., 2024).

Bu et al. discussed different unit operations and bioprocessing, generally used in dairy industries and their influence on the antigenicity of dairy proteins. The effects of heat treatment, high pressure homogenizer, and enzyme- and microbial-based bioprocess have been critically evaluated with this aid. It was mentioned that in many cases, especially physical treatment could reduce the antigenic activity; however, the correlation between reduction of antigenic activity and process parameters is not linear (Bu et al., 2013). This might be the presence of both linear and conformational epitopes in dairy proteins, where conformational epitopes could be exposed due to the unfolding of protein structure. Furthermore, both heat and pressure for the processing of dairy milk are the causes of change of protein folding lactosylation, oxidation, racemization, dephosphorylation and cross-linking of amino acids which may reduce the nutritional benefits of proteins (van Lieshout et al., 2020). Hydrolysis of dairy proteins could be performed using suitable acids or alkalis or food-grade proteolytic enzymes; however, acid and alkali treatment is not preferable due to several unfavorable reasons (Buňka et al., 2009; Danielsen et al., 2020). The hydrolysis of dairy proteins by enzyme has been declared as ‘safe’ by the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA) due to its biocompatibility (Abd El-Salam and El-Shibiny, 2017). Information about application of proteases for the modification of dairy proteins in laboratory-scale experiments was summarized. Limited hydrolysis of dairy proteins through biological routes could generate new antigenic peptides, which have immunomodulatory activity (Murtaza et al., 2022). It is important to mention that in the present decade, some dairy and biopharmaceutical companies placed attention to mitigating allergenic activity of milk proteins and to produce peptides with unique biofunctionality (Carrasco-Castilla et al., 2012); however, the information is scanty. Developing dairy protein hydrolysates is restricted to larger capacity industries; however, it may boost the economy of medium and small-scale industries generally considered the marketing of regular dairy foods (Nath et al., 2020). It may be realized that the reduction of allergenic activity of dairy proteins by biochemical route might reduce the limitation of its consumption and grab attention from dairy-food stakeholders.

Previously, we found that residual allergenic activity was present after only tryptic or microbial hydrolysis of dairy proteins. According to our findings, hypoallergenic dairy protein formula could be produced by successive tryptic and microbial hydrolysis of dairy proteins. Our experiment was performed in a lab-scale set-up (50 mL) (Nath et al., 2021); however, economic analysis of this approach was not explored. The objective of the present investigation is to perform economic analysis of the proposed process scheme. Results of the proposed scheme were compared with other process schemes dedicated for producing dairy protein formulas

having lower allergenic activity. The SuperPro Designer software (v. 12, Intelligen, Inc., USA) and concepts from chemical engineering were considered to perform economic analysis of mentioned process schemes.

2. MATERIAL AND METHODS

2.1. 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) is 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 1 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.

A dairy protein formula with reduced allergenic activity was developed from liquid milk protein concentrate (LMPC) through successive tryptic and microbial hydrolysis of dairy proteins (Nath et al., 2021). Information about whole milk protein (Liang et al., 2022; Song et al., 2023), and whey protein concentrate (WPC) (Grar et al., 2009; Jiang et al., 2023; Izquierdo et al., 2008; Peñas et al., 2006) 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 2.

2.2. 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. 16 Ton and 8 Ton

Table 1. Process schemes (I–VII) for producing dairy protein formulas with lower allergenic activity

Process scheme		Feedstock	Reference
(I)	Successive 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)

Table 2. Compositions of cow milk and whey protein concentrate

Cow milk (Reig et al., 2021)		Whey protein concentrate (Guo and Wang, 2019)	
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		

of milk as a feedstock were used 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 a 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.

2.2.1. Process scheme (I). In process scheme (I), a dairy protein formula with lower allergenic activity was developed by successive 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 °C for 10 min reaction time was considered for the tryptic hydrolysis of LMPC and subsequently, temperature 70 °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 to 45 °C, it was further hydrolyzed by lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophiles*) for 6 h. 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 mentioned article. In the next step, the temperature of protein hydrolysate was reduced from 45 to 10 °C by a heat-exchanger. The product was transferred to the filling machine and subsequently, the packaging unit. The process scheme for developing a dairy protein formula having lower allergenic activity by successive tryptic and microbial hydrolysis of milk proteins is presented in Fig. 1.

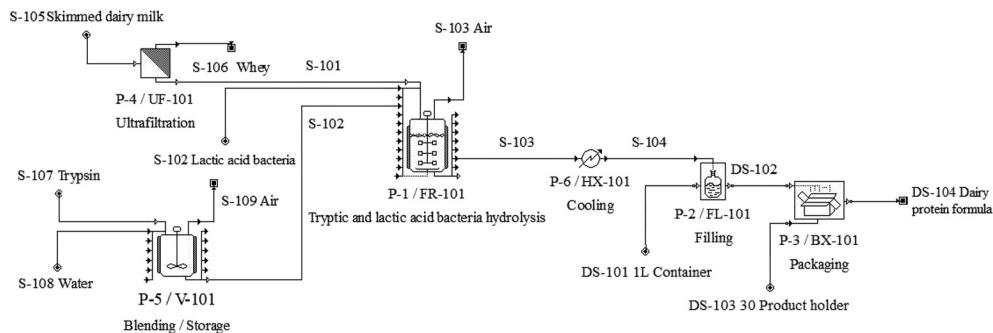


Fig. 1. Process scheme (I): successive tryptic and microbial hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity (Self-developed, Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by [Nath et al., 2021](#))

2.2.2. 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 °C was maintained for 2-h and subsequently, temperature 90 °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 $33 \text{ g}\cdot\text{L}^{-1}$ and 10 mL, respectively. In the SuperPro Designer model, the amount of skimmed milk (feedstock) was considered 2 Ton and after the enzymatic bioprocess dairy protein formula 2 Ton in a batch was produced. In the next step, the enzymatic 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](#).

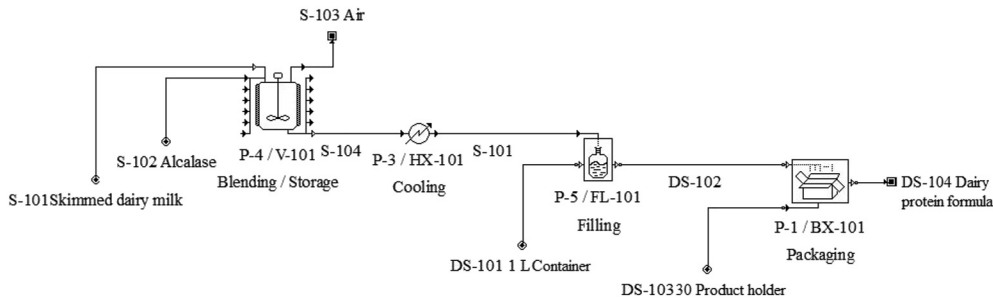


Fig. 2. Process scheme (II): enzymatic hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity (Self-developed, Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by [Liang et al., 2022](#))

2.2.3. 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 *L. 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 temperature 45 °C for 6 h. 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 to 10 °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.

2.2.4. 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 °C for 30 min. Mixture was centrifuged at 3,500 rpm for 15 min 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 °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 2,450 MHz frequency with 700 W power for 10 min, 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

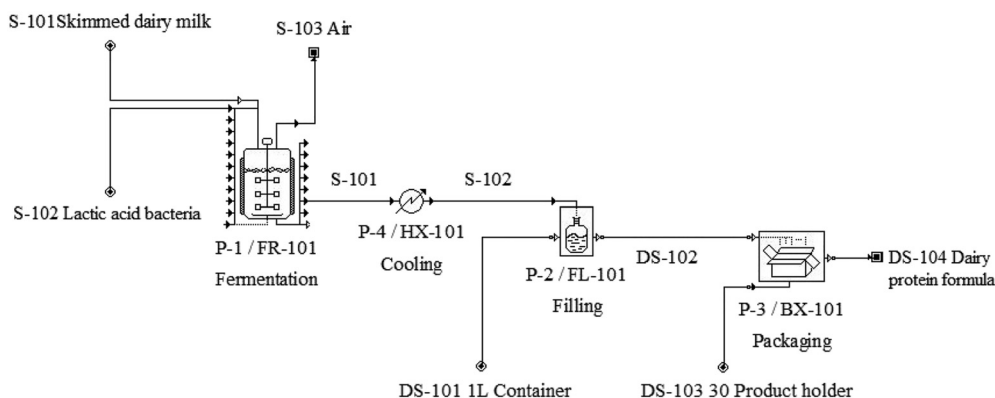


Fig. 3. Process scheme (III): microbial hydrolysis of dairy proteins for producing a dairy protein formula with lower allergenic activity (Self-developed; the Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by Song et al., 2023)

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.

2.2.5. Process scheme (V). In this process scheme, WPC was dissolved in distilled water to make the concentration $50\text{ g}\cdot\text{L}^{-1}$. WPC solution was filled into 1 L pressure-resistant polyethylene bottle followed by high pressure treatment where constant pressure 500 MPa for 10 min 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. Finally, for the commercialization purpose, bottles were delivered 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.

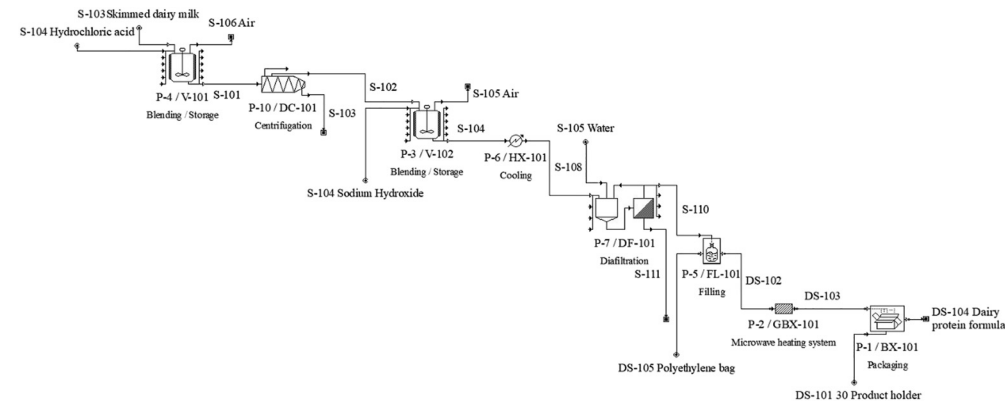


Fig. 4. Process scheme (IV): microwave heat treatment for producing a dairy protein formula with lower allergenic activity (Self-developed; the Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by Grar et al., 2009)

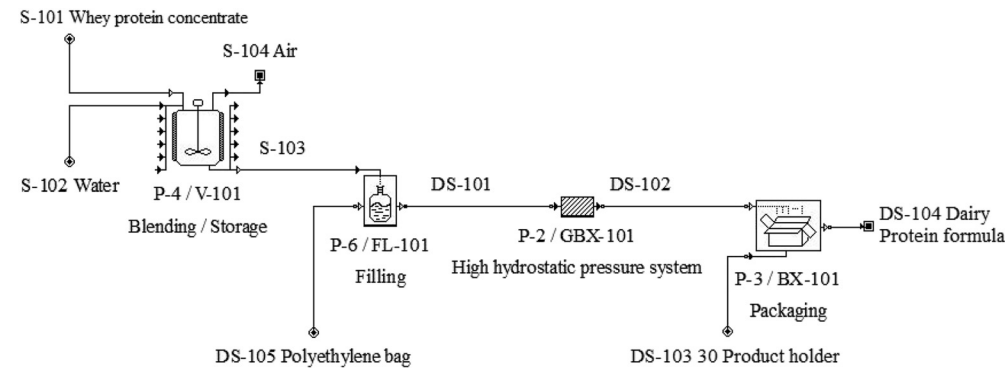


Fig. 5. Process scheme (V): high pressure treatment for producing a dairy protein formula with lower allergenic activity (Self-developed; the Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by Jiang et al., 2023)

2.2.6. Process scheme (VI). Similarly as before, WPC was dissolved in water to make the final concentration $50 \text{ g}\cdot\text{L}^{-1}$ and the WPC solution was hydrolyzed by alcalase. The enzymatic hydrolysis was performed considering 100 mL enzyme ($2 \text{ mg}\cdot\text{mL}^{-1}$) and 100 mL of substrate in the final volume of solution 300 mL. In the biochemical reaction, temperature 50°C for 5 min was maintained and subsequently the reaction mixture was heated to temperature 80°C for 5 min to deactivate the enzyme. Enzyme hydrolyzed WPC was filled into a heat-resistant polyethylene bottle (1 L sample holding capacity) similarly as before and considered for microwave heat treatment. The microwave heat treatment of the enzyme hydrolyzed WPC was carried out by 2,450 MHz frequency with 532 W power for 5 min (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 ($\sim 99\%$) 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.

2.2.7. 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, protein fraction was separated by centrifugation. Lyophilized protein fraction having moisture content 5% was obtained by freeze-drying. The lyophilized protein was suspended in 50 mM of sodium phosphate buffer, pH 8.0 to get the concentration $50 \text{ g}\cdot\text{L}^{-1}$. Prior to the pepsin hydrolysis, the pH of the protein solution was maintained at 4.0 by 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 enzyme ($5 \text{ mg}\cdot\text{mL}^{-1}$) and 0.125 mL of substrate. Pepsin hydrolysis of milk proteins was performed at temperature 37°C for 30 min followed by the deactivation of pepsin at temperature 90°C for 5 min. Whey protein hydrolysate

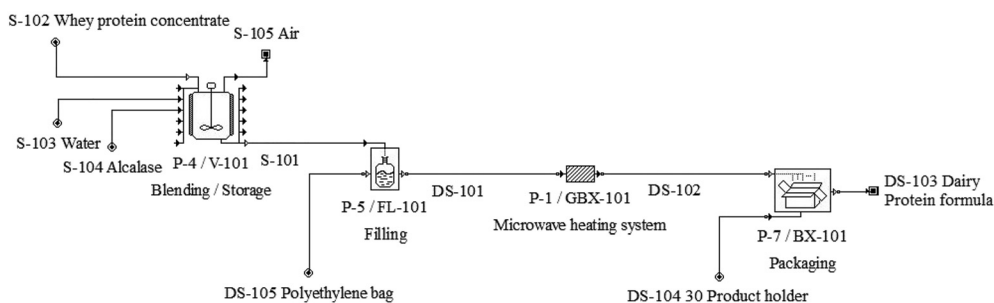


Fig. 6. Process scheme (VI): enzymatic hydrolysis and microwave heat treatment for producing a dairy protein formula with lower allergenic activity (Self-developed; the Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by Izquierdo et al., 2008)

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 min. In the SuperPro Designer model, 2 Ton 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.

2.3. 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.

2.3.1. 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 and Houston, 2018a). Therefore,

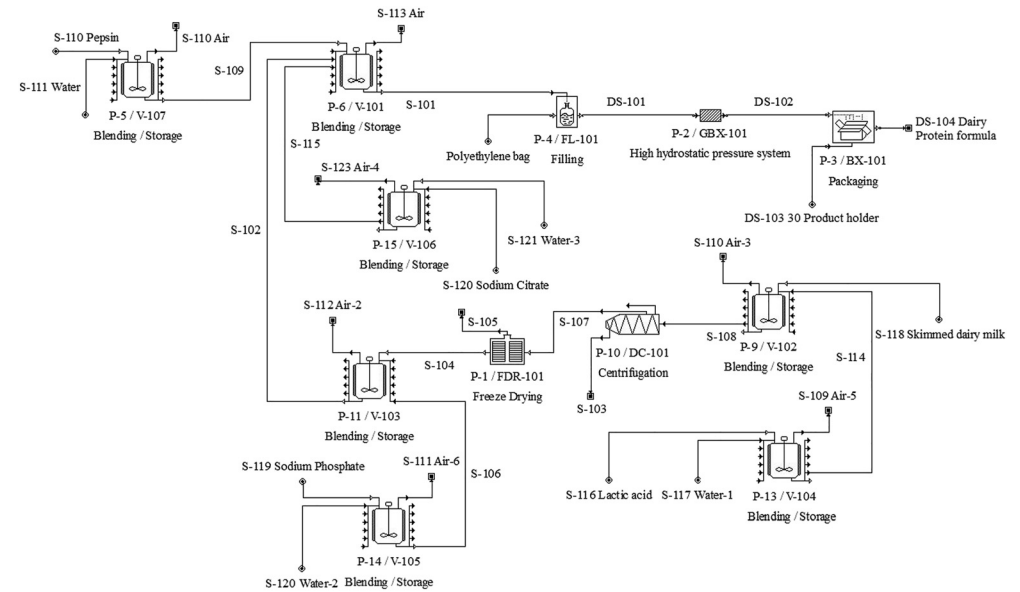


Fig. 7. Process scheme (VII): enzymatic hydrolysis and high pressure treatment for producing a dairy protein formula with lower allergenic activity (Self-developed; the Figure was developed using SuperPro Designer software (v. 12, Intelligen, Inc., USA) according to the process description by Peñas et al., 2006)

$$\text{TPC (US\$)} = \text{TPDC} + \text{TPIC} \quad (1)$$

$$\text{DFC (US\$)} = \text{TPC} + \text{CFC} \quad (2)$$

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](https://www.alibaba.com)) because in most of the industries utmost equipment are purchased from renowned 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 ([Official Daily Exchange Rates](#)). As the equipment costs were already provided in current values, no cost adjustment using the Chemical Engineering Plant Cost Index was necessary. For the economic analysis of various process schemes, the default prices in the SuperPro Designer software were used. These include costs for equipment and apparatus installation across different unit operations, equipment accessories, joints and fittings, process piping, instrumentation and controls, electrical components, insulation, buildings, yard improvements, auxiliary facilities, engineering, construction, contractor fees, and contingencies, as these values largely reflect prices from 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 (Figs 1–7) are mentioned in subsequent tables (Tables 3–9).

Table 3. 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

Table 4. 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

Table 5. 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

Table 6. 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)	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

Table 7. 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

Table 8. 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

2.3.2. 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 and Houston, 2018b). Information about them was collected from the Alibaba online shopping website (alibaba.com) with 2025 currency exchange rate (Official Daily Exchange Rates); however, costs for international and local taxes, shipping and delivery were not considered, similarly as before (section 2.2.1). The costs of feedstock, chemicals, enzyme, microbial culture, water and packaging items present in different process schemes (Figs 1–7) are mentioned in Table 10.

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/h and US\$ 46/h, respectively. The default prices in the SuperPro Designer software for electricity, chilled water (5–10 °C) and regular water (25–30 °C) were considered for the economic analysis of different process schemes because it depends on the geographic region.

2.3.3. 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 market place. The assumptions for dairy protein formulas with

Table 9. 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)	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

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 11](#).

For each operating year within the expected project lifetime, revenues are calculated by multiplying the estimated annual revenue by the fraction of total capacity utilized that year (f_Q) and the number of months of operation for that year (t) ([Fabozzi and Peterson, 2003a](#)). Revenues can be expressed according to Eq. (3).

$$\text{Revenues (US\$} \cdot \text{year}^{-1}) = f_Q \times t \times \text{Annual revenues} \quad (3)$$

The annual revenue of a process is determined by summing the revenues from all output streams. It is thus linked to both the unit production cost revenue and the annual rate used as the cost basis.

Unit production cost revenue (\$/unit) refers to the revenue generated per unit of reference, calculated by dividing the total annual revenue by the unit reference rate, as defined in Eq. (4) ([Brigham and Houston, 2018c](#)).

Table 10. 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	1 L 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		

Table 11. 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

Unit production cost revenues (\$/entity) = Revenues (savings)/ Revenues (saving reference rates)

(4)

The cost basis annual rate refers to the flow basis, either total flow or component flow, used to convert annual operating costs into unit production costs. The cost basis itself represents the original value or purchase price of an asset or investment for tax purposes. It is also used to convert other annual cost components from a yearly total to a per-unit reference basis (Brigham and Houston, 2018d).

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

$$GP \text{ (US\$ year}^{-1}\text{)} = \text{Revenues} - \text{AOC} \quad (5)$$

2.3.5. 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 depreciation of the fixed capital investment was calculated using the straight-line method over a 10-year lifespan, assuming negligible salvage value. The NP can be calculated according to Eq. (6) (Fabozzi and Peterson, 2003c).

$$NP \text{ (US\$ year}^{-1}\text{)} = GP - 0.09 \times GP + \text{Depreciation} \quad (6)$$

2.3.6. 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 items (Fabozzi and Peterson, 2003d). The gross margin (GM) was calculated according to Eq. (7).

$$GM = (\text{Revenue} - \text{Cost of item}) / \text{Revenue} \times 100 \quad (7)$$

2.3.7. Return on investment (ROI). The Return on investment (ROI) is a profitability metric used to assess the feasibility of an investment or to compare the profitability of multiple investments. It is calculated by dividing the annual net profit by the total capital invested in the project (Brigham and Houston, 2018e). The ROI for different process schemes was calculated according to Eq. (8).

$$ROI (\%) = (\text{Gain from investment} - \text{Cost of investment}) \times 100 / \text{Cost of investment} \quad (8)$$

2.3.8. Payback time (PBT). Payback time (PBT) measures the duration, typically in years, required for the total capital investment to be fully recovered by the cumulative net profit. It is calculated by dividing the total capital investment for the project by the annual net profit (Brigham and Houston, 2018f). The PBT was calculated according to Eq. (9).

$$PBT \text{ (year)} = \text{Total capital investment} / \text{Annual net profit} \quad (9)$$

2.3.9. Internal rate of return (IRR). The internal rate of return (IRR), also called the discounted cash rate of return (DCRR), represents the annual growth rate an investment is expected to achieve. It is calculated using cash flows both before and after income taxes. The cash flow before income taxes is determined by adding income taxes to the net cash flow, while the cash flow after income taxes corresponds to the net cash flow itself (Brigham and Houston, 2018g). In this investigation, IRR (in %) was calculated for 10 years.

2.3.10. Net present value (NPV). The net present value (NPV) represents the total value of future net cash flows, discounted to the start of the project, over its entire lifetime. 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).

$$NPV \text{ (US\$)} = PV - Co = \sum_{t=0}^T \frac{C_t}{(1+r)^t} - Co \quad (10)$$

Where, PV is the present value, C_t is the net cash inflow during the period t , C_o is the total initial investment cost, IRR is the discount rate and t is the time (10-year consideration) (Brigham and Houston, 2018h).

3. RESULTS AND DISCUSSION

3.1. Costs for investment

The direct fixed-capital cost (DFC) for process schemes (I–VII) is presented in Table 12. 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 for 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 assumed that higher values of DFC for the process schemes (I) than the process scheme (III) are 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.

3.2. Annual operating cost

Annual operating cost is one of the important issues 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 12. Direct fixed-capital cost (DFC) 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)
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

Table 13. 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.

3.2.1. Raw materials. The costs of raw materials on an annual basis for process schemes (I) and (III) are higher than other process schemes (Table 13). This could be mainly due to the higher

Table 13. 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

amount of feedstock (16 Ton and 8 Ton of skimmed milk 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 14), which provides 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 14). 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 14). Furthermore, annual expenditure due to chemicals for cleaning and waste treatment for the process scheme (V) is higher than process schemes (VI) and (II).

Table 14. 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

The expenditure of raw materials per year for process schemes also depends on the characteristics of feedstock. 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).

3.2.2. Labor-dependent. Annual labor-dependent costs for process schemes (III) is quite higher than other process schemes (Table 13), which is attributed by the maximum number of batch·year^{−1} (Table 14) and consequently, involvement of laborers. Process schemes (I) and (III) are not complex compared to process schemes (VII) and (IV); however, membrane filtration to produce LMPC in process scheme (I) and microbial fermentation in process schemes (I) and (III) are the 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 13), which might be due to the complex process scheme where more labor is required. Annual labor-dependent expenditures for process schemes (II), (V) and (VI) are almost similar (Table 13). This might be due to almost similar numbers of steps involved in the mentioned process schemes.

3.2.3. 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 13). 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 13). 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 13).

3.2.4. 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 13) 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 13); however, maximum numbers of batch·year^{−1} are almost similar for process schemes (II), (IV), (V) and (VI) (Table 14). 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 13), 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 13). It might be due to the complex process schemes where quality analysis is required in different points of the process scheme.

3.2.5. 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). Membrane generally suffers due to the formation of concentration polarization on its surface, which reduce the permeate flux. Therefore, alternation of membrane time to time to maintain steady operation has a significant contribution to annual expenditure. Furthermore, items for microbiological purposes (Erlenmeyer flask, petri plate, safety cabinet) contribute some amount to annual expenditure; however, it is negligible (Table 13).

3.2.6. Waste treatment. Annual expenditures for waste treatment for process schemes (I) and (IV) are significantly higher than all other process schemes (Table 13). 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 13) which is mostly caused by cleaning of equipment, pipes and so on.

3.2.7. Utilities. Expenditures of utility $\cdot\text{year}^{-1}$ for a process is mainly due to the consumptions 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 13). These results could be attributed to several factors. In the process scheme (III), temperature 45 °C for 6 h was maintained during 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 (90–10 °C within 1 h) 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 $\cdot\text{year}^{-1}$ (Table 14) is the cause of higher expenditure of utility $\cdot\text{year}^{-1}$. In the process scheme (I), around 8 Ton of LMPC was produced from 16 Ton of skimmed milk within 4 h. Temperature 25 °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 °C for 10 min followed by enzyme deactivation at temperature 70 °C for 30 min. It was further hydrolyzed by lactic acid bacteria at temperature 45 °C for 6 h when the temperature of enzyme-hydrolyzed LMPC was reduced to 45 °C (within time 30 min). Subsequently, the temperature of protein

hydrolysate was reduced from 45 to 10 °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 13), 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).

3.3. 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, it 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 15.

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).

Table 15. Production costs of dairy protein formulas from different process schemes (I–VII)

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

3.4. Profitability indicators

Based on DFC, annual operation cost and revenue, mentioned above, profitability indicators were evaluated for the process schemes (I–VII). Results are summarized in Table 16. Profitability indicators, such as GP, NP, GM, revenue, NPV, IRR, ROI and PBT for process schemes were considered for comparison.

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 a quite higher concentration of protein because LMPC was used (Nath et al., 2021). NP and GP for the process scheme (III) are also appreciable. The quiet 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 basis annual rate and unit production revenues for all process schemes are mentioned in Table A1 (Appendix). 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 successive way to produce concentrated dairy protein formula with lower allergenic activity.

Table 16. 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

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 11) for process schemes (IV) and (VII) are comparatively quite higher than other process schemes due to the complex process schemes, which is 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 11). 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. 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). 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 benefit 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.

3.5. 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. 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 from 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 and 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 °C for 10 min was considered for the deactivation of enzyme alcalase. It may be realized that at 90 °C, some whey proteins could be denatured and precipitated 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 difficult to comprehend the effects of complex processes (enzymatic hydrolysis with microwave heating and enzymatic hydrolysis with high pressure) on the modulation of protein structure and their antigenic activity because biochemical mechanisms are moderately multifaceted. In the present article, economic analysis of different process schemes was performed using skimmed milk and WPC as feedstocks. Common practices, such as milk fat standardization, homogenization, and pasteurization or high-temperature treatment prior to produce a dairy food were not included in economic analysis due to the lack of detailed information in literatures (Liang et al., 2022; Song et al., 2023; Grar et al., 2009; Jiang et al., 2023; Izquierdo et al., 2008; Peñas et al., 2006).

It should be mentioned 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 allergenic activity of casein was reduced by more than 99%. Allergens β -lactoglobulin and α -lactalbumin retained; however, they were reduced compared to control, by the successive tryptic and microbial hydrolysis of LMPC according to Rb polyclonal antibody in 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. (2022) in the process scheme (II). Furthermore, investigators used ELISA to understand the reduction of allergenic activity of the 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), Grar 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 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). The results of allergenic activity of dairy protein formulas mentioned in different articles are not directly comparable. In the process scheme (I) immunoblot was used to comprehend the allergenic activity of dairy proteins; whereas other investigators considered other types of analytical method, i.e. ELISA where associated protocols could be varied based on manufacturers.

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 consumer's 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 article, these factors were not considered because information was not available in the mentioned articles.

4. CONCLUSION

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 are a rich source of proteins, carbohydrate, 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. Dairy industry is consistently seeking a recipe and technology to develop and supply dairy foods which offer more amounts of proteins and health benefits compared to traditional dairy foods, and could bring a higher profitability. Therefore, efforts were placed on technological and economic analysis of different process schemes dedicated for the reduction of allergenic activity of milk proteins.

According to the process scheme (I), the dairy protein formula with lower allergenic activity could be produced from liquid milk protein concentrate (LMPC) through a complex and advanced bioprocess, involving nanofiltration, enzymatic hydrolysis using endo-protease and lactase, and microbial fermentation, which ensure the distinctive biofunctional characteristic of final dairy food. Tryptic hydrolysis of dairy milk proteins may produce peptides, which contributes bitterness of final dairy food formula. Efforts are necessary to investigate other plant (papain, bromelain, ficin)- or animal (chymotrypsin, pepsin)-based proteolytic enzymes in the proposed process scheme to understand their contributions on the reduction of allergenic activity of dairy proteins, biofunctional activity and sensory aspects of the mentioned dairy food formula. The legal framework from the dairy food stakeholders' point of view might be a considerable challenging issue regardless of their nutritional value. As a high-protein, allergen-free dairy food with unique health benefits produced from the process scheme (I) it needs to meet strict regulatory standards and provide scientific validation for its health and nutrition claims to gain approvals, such as "Generally Recognized as Safe" (GRAS) in USA and Novel Food status in EU. A well-structured regulatory roadmap and checklist may support smoother coordination with food safety authorities and accelerate its market entry in proper timelines.

For more than a decade, consumers have been more interested in value-driven dairy foods than conventional and volume-based dairy foods. Dairy food produced from process scheme (I) may contain higher concentration of proteins where essential, non-essential, ketogenic and glucogenic amino acids, allergen-free and provide additional health benefits (antioxidant and anti-angiotensin activities) than conventional dairy foods. Therefore, it will gain attention from consumers interested in protein supplement and sustainable diet. Furthermore, it is anticipated that the process scheme (I) could bring an economic boom, beside the commercialization of regular dairy foods, and a new arena in the functional food sector might be explored.

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REFERENCES

- Abd El-Salam, M.H. and El-Shibiny, S. (2017). Preparation, properties, and uses of enzymatic milk protein hydrolysates. *Critical Reviews in Food Science and Nutrition*, 57(6): 1119–1132, <https://doi.org/10.1080/10408398.2014.899200>

- Achaglinkame, M.A., Dari, L., and Mörlein, D. (2023). A review of dairy production and utilization in Ghana and Benin. *Discover Food*, 3(13), <https://doi.org/10.1007/s44187-023-00053-9>
- Bhat, Z.F., Morton, J.D., Bekhit, A.E.-D., Kumar, S., and Bhat, H.F. (2021). Processing technologies for improved digestibility of milk proteins. *Trends in Food Science and Technology*, 118(12): 1–16, <https://doi.org/10.1016/j.tifs.2021.09.017>
- Boirie, Y., Dangin, M., Gachon, P., Vasson, M.-P., Maubois, J.-L., and Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences of the United States of America*, 94(26): 14930–14935, <https://doi.org/10.1073/pnas.94.26.14930>
- Brigham, E.F. and Houston, J.F. (2018a). Capital structure and leverage. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 483, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018b). Cash flow estimation and risk analysis. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 425, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018c). Cash flow estimation and risk analysis. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 176, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018d). Financial statements, cash flow, and taxes. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 66, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018e). Analysis of financial statements. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 119, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018f). Analysis of financial statements. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 405, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018g). Analysis of financial statements. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 392, ISBN: 978-1-337-39525-0.
- Brigham, E.F. and Houston, J.F. (2018h). Analysis of financial statements. In: *Fundamentals of financial management*. Cengage, Boston, USA, p. 388, ISBN: 978-1-337-39525-0.
- Bu, G., Luo, Y., Chen, F., Liu, K., and Zhu, T. (2013). Milk processing as a tool to reduce cow's milk allergenicity: a mini-review. *Dairy Science and Technology*, 93(3): 211–223, <https://doi.org/10.1007/s13594-013-0113-x>
- Buňka, F., Kríž, O., Veličková, A., Buňková, L., and Kráčmar, S. (2009). Effect of acid hydrolysis time on amino acid determination in Casein and processed cheeses with different fat content. *Journal of Food Composition and Analysis*, 22(3): 224–232, <https://doi.org/10.1016/j.jfca.2008.10.023>
- Carrasco-Castilla, J., Hernández-Álvarez, A.J., Jiménez-Martínez, C., Gutiérrez-López, G.F., and Dávila-Ortiz, G. (2012). Use of proteomics and peptidomics methods in food bioactive peptide science and engineering. *Food Engineering Reviews*, 4(4): 224–243, <https://doi.org/10.1007/s12393-012-9058-8>
- Catanzaro, R., Sciuto, M., and Marotta, F. (2021). Lactose intolerance: an update on its pathogenesis, diagnosis, and treatment. *Nutrition Research*, 89: 23–34, <https://doi.org/10.1016/j.nutres.2021.02.003>
- Csaba, L. (2025). Globalization and European integration: a central European perspective. *Journal of Risk and Financial Management*, 18(2): 53, <https://doi.org/10.3390/jrfm18020053>
- Cuomo, B., Indirli, G.C., Bianchi, A., Arasi, S., Caimmi, D., Dondi, A., La Grutta, S., Panetta, V., Verga, M.C., and Calvani, M. (2017). Specific IgE and skin prick tests to diagnose allergy to fresh and baked cow's milk according to age: a systematic review. *Italian Journal of Pediatrics*, 43(1): 93, <https://doi.org/10.1186/s13052-017-0410-8>
- Danielsen, M., Nebel, C., and Dalsgaard, T.K. (2020). Simultaneous determination of L- and D-amino acids in proteins: a sensitive method using hydrolysis in deuterated acid and liquid chromatography-tandem mass spectrometry analysis. *Foods*, 9(3): 309, <https://doi.org/10.3390/foods9030309>

- Exl, B.-M. (2001). A review of recent developments in the use of moderately hydrolyzed whey formulae in infant nutrition. *Nutrition Research*, 21(1–2): 355–379, [https://doi.org/10.1016/S0271-5317\(00\)00259-1](https://doi.org/10.1016/S0271-5317(00)00259-1)
- Fabozzi, F.J. and Peterson, P.P. (2003a). Risk and expected return. In: *Financial management and analysis*. John Wiley & Sons, Inc., New Jersey, USA, p. 258, ISBN: 0-471-23484-2.
- Fabozzi, F.J. and Peterson, P.P. (2003b). Financial statements. In: *Financial management and analysis*. John Wiley & Sons, Inc., New Jersey, USA, p. 136, ISBN: 0-471-23484-2.
- Fabozzi, F.J. and Peterson, P.P. (2003c). Financial ratio analysis. In: *Financial management and analysis*. John Wiley & Sons, Inc., New Jersey, USA, p. 722, ISBN: 0-471-23484-2.
- Fabozzi, F.J. and Peterson, P.P. (2003d). Risk and expected return. In: *Financial management and analysis*. John Wiley & Sons, Inc., New Jersey, USA, p. 758, ISBN: 0-471-23484-2.
- Grar, H., Kaddouri, H., Gourine, H., Negaoui, H., Kheroua, O., and Saïdi, D. (2009). Microwave irradiation under different PH conditions induced a decrease in β -lactoglobulin antigenicity. *European Food Research and Technology*, 229(5): 779–783, <https://doi.org/10.1007/s00217-009-1114-0>
- Guo, M. and Wang, G. (2019). History of whey production and whey protein manufacturing. In: Guo, M. (Ed.), *Whey protein production, chemistry, functionality, and applications*. John Wiley & Sons, Inc., New Jersey, USA, pp. 1–12, ISBN: 9781119256052.
- Hill, J. (2024). Science, technology, and innovation in the dairy sector. *International Journal of Food Science and Technology*, 59(9): 6717–6723, <https://doi.org/10.1111/ijfs.17385>
- Izquierdo, F.J., Peñas, E., Baeza, M.L., and Gomez, R. (2008). Effects of combined microwave and enzymatic treatments on the hydrolysis and immunoreactivity of dairy whey proteins. *International Dairy Journal*, 18(9): 918–922, <https://doi.org/10.1016/j.idairyj.2008.01.005>
- Jiang, H., Zhang, Z., Wang, Y., Gao, J., Yuan, Q., and Mao, X. (2023). Effects of high hydrostatic pressure treatment on the antigenicity, structural and digestive properties of whey protein. *LWT*, 178: 114628, <https://doi.org/10.1016/j.lwt.2023.114628>
- Lawson, Y., Mpasi, P., Young, M., Comerford, K., and Mitchell, E. (2024). A review of dairy food intake for improving health among black infants, toddlers, and young children in the US. *Journal of the National Medical Association*, 116(2): 228–240, <https://doi.org/10.1016/j.jnma.2024.01.014>
- Li, A., Zheng, J., Han, X., Yang, S., Cheng, S., Zhao, J., Zhou, W., and Lu, Y. (2023). Advances in low-lactose/lactose-free dairy products and their production. *Foods*, 12(13): 2553, <https://doi.org/10.3390/foods12132553>
- Liang, X., Wang, Z., Yang, H., Luo, X., Sun, J., Yang, M., Shi, X., Yue, X., and Zheng, Y. (2022). Evaluation of allergenicity of cow milk treated with enzymatic hydrolysis through a mouse model of allergy. *Journal of Dairy Science*, 105(2): 1039–1050, <https://doi.org/10.3168/jds.2021-20686>
- Mathai, J.K., Liu, Y., and Stein, H.H. (2017). Values for digestible indispensable Amino Acid Scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). *British Journal of Nutrition*, 117(4): 490–499, <https://doi.org/10.1017/S0007114517000125>
- Medeiros, V., Rainha, N., Paiva, L., Lima, E., and Baptista, J. (2014). Bovine milk formula based on partial hydrolysis of caseins by bromelain enzyme: better digestibility and angiotensin-converting enzyme-inhibitory properties. *International Journal of Food Properties*, 17(4), <https://doi.org/10.1080/10942912.2012.675607>
- Murtaza, M.A., Irfan, S., Hafiz, I., Ranjha, M.M.A.N., Rahaman, A., Murtaza, M.S., Ibrahim, S.A., and Siddiqui, S.A. (2022). Conventional and novel technologies in the production of dairy bioactive peptides. *Frontiers in Nutrition*, 9: 780151, <https://doi.org/10.3389/fnut.2022.780151>

- Nath, A., Csighy, A., Eren, B.A., Tjandra Nugraha, D., Pásztorné-Huszár, K., Tóth, A., Takács, K., Szerdahelyi, E., Kiskó, G., Kovács, Z., Koris, A., and Vatai, Gy. (2021). Bioactive peptides from liquid milk protein concentrate by sequential tryptic and microbial hydrolysis. *Processes*, 9(10): 1688, <https://doi.org/10.3390/pr9101688>
- Nath, A., Eren, B.A., Csighy, A., Pastorne-Huszar, K., Kisko, G., Abranko, L., Toth, A., Szerdahelyi, E., Kovacs, Z., Koris, A., and Vatai, Gy. (2020). Production of liquid milk protein concentrate with antioxidant capacity, angiotensin converting enzyme inhibitory activity, antibacterial activity, and hypoallergenic property by membrane filtration and enzymatic modification of proteins. *Processes*, 8(7): 871, <https://doi.org/10.3390/pr8070871>
- Nath, A., Eren, B.A., Zinia Zaukuu, J.-L., Koris, A., Pásztorné-Huszár, K., Szerdahelyi, E., and Kovacs, Z. (2022). Detecting the bitterness of milk-protein-derived peptides using an electronic tongue. *Chemosensors*, 10(6): 215, <https://doi.org/10.1080/07315724.2009.10718113>
- Nutten, S., Schuh, S., Dutter, T., Heine, R.G., and Kuslys, M. (2020). Design, quality, safety and efficacy of extensively hydrolyzed formula for management of cow's milk protein allergy: what are the challenges? *Advances in Food and Nutrition Research*, 93: 147–204, <https://doi.org/10.1016/bs.afnr.2020.04.004>
- Official Daily Exchange Rates. The Magyar Nemzeti Bank, <https://www.mnb.hu> (Accessed 5 January 2025).
- Paul, G.L. (2009). The rationale for consuming protein blends in sports nutrition. *Journal of the American college of Nutrition*, 28(Suppl. S4): 464S–472S. <https://doi.org/10.1080/07315724.2009.10718113>
- Peñas, E., Préstamo, G., Luisa Baeza, M., Martínez-Molero, M.I., and Gomez, R. (2006). Effects of combined high pressure and enzymatic treatments on the hydrolysis and immunoreactivity of dairy whey proteins. *International Dairy Journal*, 16(8): 831–839, <https://doi.org/10.1016/j.idairyj.2005.08.009>
- Piazenski, I.N., Candelário, J.P.M., Soccol, V.T., Vandenberghe, L.P. de S., Pereira, G.V. de M., and Soccol, C.R. (2024). From lab to table: the path of recombinant milk proteins in transforming dairy production. *Trends in Food Science and Technology*, 149(12): 104562, <https://doi.org/10.1016/j.tifs.2024.104562>
- Plamada, D., Teleky, B.-E., Nemes, S.A., Mitrea, L., Szabo, K., Călinoiu, L.-F., Pascuta, M.S., Varvara, R.-A., Ciont, C., Martău, G.A., Simon, E., Barta, G., Dulf, F.V., Vodnar, D.C., and Nătescu, M. (2023). Plant-based dairy alternatives-A future direction to the Milky Way. *Foods*, 12(9): 1883, <https://doi.org/10.3390/foods12091883>
- Reig, M., Vecino, X., and Cortina, J.L. (2021). Use of membrane technologies in dairy industry: an overview. *Foods*, 10(11): 2768, <https://doi.org/10.3390/foods10112768>
- Shiby, V.K. and Mishra, H.N. (2013). Fermented milks and milk products as functional foods - a review. *Critical Reviews in Food Science and Nutrition*, 53(5): 482–496, <https://doi.org/10.1080/10408398.2010.547398>
- Sindayikengera, S. and Xia, W. (2006). Nutritional evaluation of caseins and whey proteins and their hydrolysates from Protamex. *Journal of Zhejiang University Science B*, 7(2): 90–98, <https://doi.org/10.1631/jzus.2006.B0090>
- Song, Y., Li, S., Zhang, R., Tuo, Y., Li, X., Mu, G., and Jiang, S. (2023). Physicochemical properties, antigenicity and allergenicity of yoghurt fermented by *Lactiplantibacillus Plantarum* AHQ-14 combined with starter. *International Journal of Food Science and Technology*, 58(5): 2527–2539, <https://doi.org/10.1111/ijfs.16396>
- Uluko, H., Zhang, S., Liu, L., Chen, J., Sun, Y., Su, Y., Li, H., Cui, W., and Lv, J. (2013). Effects of microwave and ultrasound pretreatments on enzymolysis of milk protein concentrate with different enzymes. *International Journal of Food Science and Technology*, 48(11): 2250–2257, <https://doi.org/10.1111/ijfs.12211>

- van Lieshout, G.A.A., Lambers, T.T., Bragt, M.C.E., and Hettinga, K.A. (2020). How processing may affect milk protein digestion and overall physiological outcomes: a systematic review. *Critical Reviews in Food Science and Nutrition*, 60(14): 2422–2445, <https://doi.org/10.1080/10408398.2019.1646703>
- Vandenplas, Y., Meyer, R., Nowak-Węgrzyn, A., Salvatore, S., Venter, C., and Vieira, M.C. (2023). The remaining challenge to diagnose and manage cow's milk allergy: an opinion paper to daily clinical practice. *Nutrients*, 15(22): 4762, <https://doi.org/10.3390/nu15224762>
- Wal, J.-M. (2002). Cow's milk proteins/allergens. *The Annals of Allergy, Asthma & Immunology*, 89(6): 3–10, [https://doi.org/10.1016/S1081-1206\(10\)62115-1](https://doi.org/10.1016/S1081-1206(10)62115-1). www.Alibaba.com (Accessed January 5, 2025).

Appendix

Table A1. Cost basis annual rate and unit production revenues for process schemes (I–VII)

	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)	60.00	43.00	28.00	84.00	42.00	46.00	162.00

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