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REVIEW ARTICLE

# A review of various protein separation techniques and valorization of cheese whey and buttermilk

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**Abstract**
The dairy industry generates large quantities of nutrient-rich by-products, particularly cheese whey, and buttermilk, which are valuable sources of bioactive proteins and phospholipids. Whey proteins exhibit notable therapeutic potential, offering various health benefits such as antihypertensive effects, immune modulation, and opioid antagonism. Similarly, buttermilk-derived phospholipids demonstrate bioactive properties, including cholesterol reduction, anti-inflammatory activity, and neuroprotective effects. This review critically assesses recent advances in protein separation techniques, comparing conventional thermal methods, known to potentially compromise protein functionality, with emerging non-thermal approaches such as high-pressure processing and membrane filtration, which enable efficient recovery while preserving bioactivity. Additionally, advanced methodologies such as multi-level processing and enzymatic hydrolysis further expand the protein separation techniques. Key technical challenges, including high mineral content in whey and the low solids concentration in buttermilk, are discussed alongside potential innovative solutions. Whey's elevated mineral levels can reduce protein isolation efficiency and diminish the emulsifying properties of whey proteins, potentially restricting their functional applications. Meanwhile, the low total solids content in buttermilk poses challenges in achieving high protein concentration yields, requiring advanced separation techniques. Further, this review examines contemporary trends in cheese whey and buttermilk valorization, highlighting their opportunities for enhanced food applications. It also explores cross-sectoral utilization in biofuels, biodegradable materials, pharmaceuticals, and agriculture, supporting economic and sustainability goals in the dairy sector.

**Keywords** Buttermilk · Cheese whey · Dairy by-product · Dairy industry · Protein separation · Valorization

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## Introduction

Dairy production is a cornerstone of the global agricultural economy, supplying essential nutrients to billions of people while driving multi-billions of dollars in industry across the globe. As of 2023, worldwide milk production has surpassed 936 billion kilograms, significantly influencing global nutrition and economic stability. Furthermore, the global dairy market is continuously increasing and is estimated to exceed $1 trillion by 2028, reflecting the sector's robust growth and ongoing economic importance [1].

Dairy industry provides various products—ranging from fresh milk to cheese, yogurt, butter, and beyond—providing essential nutrition and becoming a staple in nearly every household worldwide. The Macronutrient and micronutrient compositions of milk and dairy products are shown in Table 1. Cheese, one product of dairy processing, has been around for nearly 7000 years. It began in ancient civilizations such as Egypt and was well-established in Classical Rome. Initially, cheese was produced on farms, but as cheese demand grew in the mid-nineteenth century, it transitioned to industrial production [2].

Cheese manufacturers produce cheese by fermenting pasteurized and standardized milk. Lactic acid bacteria starters in a cheese vat. After fermentation, rennet and other coagulants are added. This induces curd formation and separate it from its liquid whey. Then, the curd-whey mixture is separated, and cheese whey is generated as by-product. The cheese curds go through pressing, salting, molding, and ripening until they are ready to sell as commercial cheese [5, 6].

From the growing history of cheese, there are now more than 1000 types of cheese that are classified based on the milk used, its consistency, fat content, fermentation type, and surface appearance [7, 8]. Today's cheese production symbolizes historical work and modern-day technology, playing an integral role in shaping the global food landscape.

Butter represents a significant global dairy commodity, accounting for a substantial share of total milk fat utilization. During the 2022 period, India dominated butter production with 6.5 million tonnes, followed by the European Union with 2.2 million tonnes [9]. As a premium dairy fat, butter offers significant nutritional advantages and bioactive properties, complemented by its characteristic sensory attributes. Its standardized composition mandates a fat concentration between 80 and 90%, positioning it as one of the richest natural sources of milk fat [8].

Further, butter is another product of milk processing, which has been used as an important ingredient in cooking and baking. Cream separation from milk on an industrial scale began in the 1890s, and combined with refrigeration, it facilitated the large-scale production of sweet cream butter. By the 1950s, continuous churning processes had replaced the earlier used batch method, enlarging the production scale today [10], butter is classified into two main types: sweet butter and cultured butter, depending on its milk or cream origin [11, 12].

The manufacture of butter starts with separating skim milk and cream from raw milk, which then goes through pasteurization. Pasteurized cream goes through ageing (optional, to produce cultured cream) or churning. The churning process separates butterfat from the liquid. This produces butter as the main product and buttermilk as a by-product. The raw butter then undergoes final processing. This includes salting before being sold as finished butter [11, 13]. Improved production methods have made butter a globally recognized product. It comes in diverse varieties and is widely used in cooking. Today, butter remains an irreplaceable ingredient in many cultures.

Therefore, this review study emphasizes the sustainable utilization of dairy by-products and their potential applications in the food and non-food industries. However, to the best of our knowledge, no comprehensive review study has systematically discussed protein recovery techniques from dairy by-products, specifically buttermilk and cheese whey. Furthermore, the challenges associated with protein separation from these by-products and their subsequent industrial applications remain poorly characterized.

**Table 1** Macronutrient and micronutrient compositions of milk and dairy products

| Compositions | Milk and dairy products | | | |
|---|---|---|---|---|
| | Milk | Cream | Cheddar cheese | Yoghurt |
| Macronutrient (%) [3] | | | | |
| Protein | 3.5 | – | – | – |
| Fat | 3.9 | – | – | – |
| Lactose | 4.9 | – | – | – |
| Micronutrient (mg/100 g) [4] | | | | |
| Ca | 119 | 80 | 721 | 121 |
| P | 93 | 104 | 512 | 95 |
| Mg | 13 | 6 | 28 | 12 |
| K | 151 | 119 | 98 | 155 |
| Vitamin $B_2$ (Riboflavin) | 0.162 | 0.197 | 0.375 | 0.142 |
| Vitamin $B_5$ (Pantothenic acid) | 0.314 | 0.271 | 0.413 | 0.389 |
| Vitamin $B_{12}$ (Cobalamine) | 0.357 | 0.424 | 0.827 | 0.372 |

## Dairy waste and its by-products

Dairy processing operations are characterized by high water consumption and the production of nutrient-rich wastewater containing significant organic content. Liquid waste from this sector presents more critical environmental concerns than solid or gaseous waste streams from other industries

[14]. However, this growing production also generates substantial by-products, primarily cheese whey, and buttermilk. These two by-products are produced in large quantities during cheese and butter production and are often underutilized despite their rich nutritional and functional potential. The annual dairy waste residue, including solid waste and effluents, amounts to approximately 4 to 11 million tonnes globally. In Europe alone, nearly 29 million tonnes of dairy products are wasted each year. Apart from dairy by-products, a substantial amount of dairy products also go out of the consumption chain for various reasons, including expiration of shelf-life, packaging defects, and other microbiological and chemical defects [15]. Underscoring the dairy industry need for more sustainable waste management practices.

## Cheese whey

Cheese whey, a by-product of cheese and casein production, is produced in large volumes worldwide, with an estimated 190 million tons generated annually [16]. However, only about 50% is processed and utilized for low-value purposes such as animal feed and fertilizer, while the rest is often discarded or left untreated [17]. In the European Union, over 55 million tons of cheese whey were produced in 2022 [18]. The processed whey is primarily transformed into various food and feed products, with about half of it used directly in liquid form, 30% converted into powdered cheese whey, 15% used as lactose and its by-products, and the remainder concentrated into cheese whey-protein concentrate [19]. Despite its widespread production, a significant part of the potential of cheese whey is still waiting to be explored, especially in creating high-value functional food ingredients.

In the past, cheese whey waste was discharged via land application, including direct discharge into water bodies without pretreatment, stored in tanks, or released into sewage systems. To reduce the environmental impact of whey disposal and enhance by-product utilization, most whey components can now be extracted and transformed into high-value added products. Modern whey processing follows three main approaches: first, conversion of whey into value-added products; second, biological or chemical conversion into electricity, biogas, or biochemicals; and third, the separation of individual components to recover valuable products [20]. Figure 1 presents a schematic overview of both conventional and innovative food-grade applications currently being explored for whey utilization.

Although alternative methods like its usage as a nutrient-rich fertilizer or animal feed are now available, these practices still pose significant environmental challenges. Due to its high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), untreated cheese whey can severely

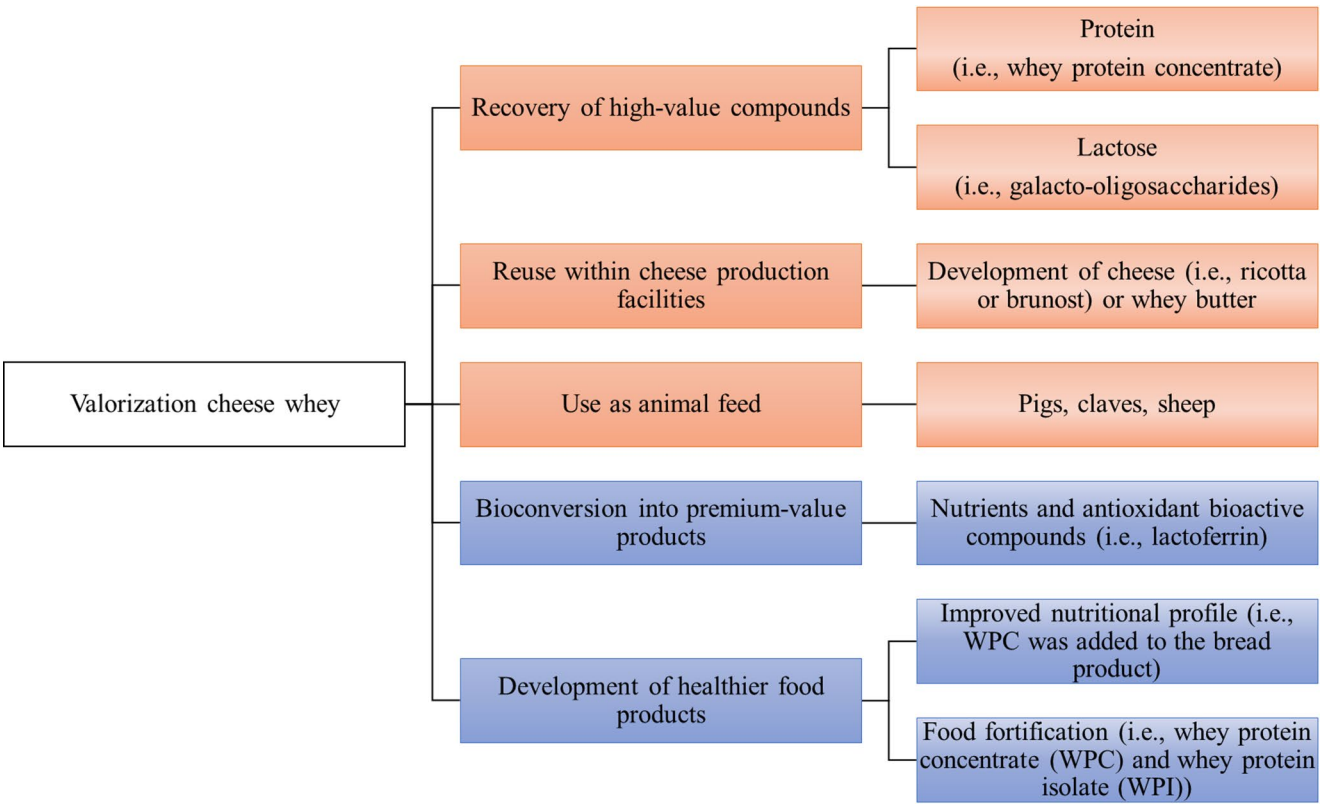


**Fig. 1** Conventional and innovative cheese whey applications [21]

pollute water systems, leading to eutrophication and harming aquatic ecosystems [22, 23].

A study by Peters [24], highlighted the significant economic potential of cheese whey when processed into high-value products. The study suggests that processing whey can be more cost-effective than traditional disposal, as its intrinsic economic value can double its market price. In addition to cost savings, processing whey enables the production of high-value products like whey protein concentrate (WPC) and whey protein isolate (WPI), which are currently in high demand in multiple industries. Expanding into these markets offers manufacturers a competitive edge and an opportunity to diversify their revenue streams. The environmental impact and economic potential discussed highlight the critical importance of cheese whey processing to minimize waste, reduce environmental harm, and unlock significant value across the food, nutrition, and industrial sectors.

## Cheese whey components

Cheese whey is a rich source of bioactive components, particularly milk serum proteins, constituting approximately 20% of its composition. Two major protein fractions, α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), account for nearly 70% of the total protein content in whey. These proteins are valuable sources of amino acids essential for various nutritional applications, particularly in sports and dietary nutrition [25]. α-La contains high levels of tryptophan, an amino acid known for its positive effects on serotonin release, enhancing psychological well-being, especially for patients under stress. Purified α-La is also used in infant food products, due to its reduced allergenic potential compared to β-Lg, a major allergen absent in human milk [25]. Due to its heat-sensitive nature, whey protein undergoes denaturation during heat treatment especially above 70 °C. To mitigate this issue, whey proteins can be structurally modified through various techniques [26]. In addition to these benefits, whey proteins have been shown to possess remarkable therapeutic properties. They can inhibit tumor growth more effectively than other food proteins and provide a range of health benefits, such as lowering blood pressure, stimulating the immune system, and acting as opioid antagonists [27]. Other functional properties include antibacterial and antifungal activities, improved mineral transport, and weight management benefits. Whey protein peptides have also demonstrated the ability to inhibit oxidative processes, blood platelet aggregation, and even HIV proteinase, making them a promising functional ingredient in therapeutic and nutritional applications [27]. The proximate analysis of cheese whey, according to Kaminarides et al., [28], is shown in Table 2. Meanwhile cheese whey predominantly contains β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, caseinomacropeptide and several other protein types. β-lactoglobulin is the main protein in cheese whey with a molecular weight of 18.3 kDa. Whey proteins are broadly defined as the fraction of milk proteins that remain soluble under acidic conditions (pH 4.6) at 20 °C [29, 30].

**Table 2** Proximate components of cheese Whey [28]

| Composition | Content (%) |
|---|---|
| Protein | 14.21 ± 1.34 |
| Fat | 11.79 ± 1.20 |
| Ash | 0.65 ± 0.10 |
| Lactose | 3.68 ± 0.31 |

## Buttermilk

Buttermilk is recognized as a functional food primarily because of its water-soluble constituents, particularly polar lipids and milk fat globule membrane (MFGM) components. This byproduct of butter manufacturing consists of milk's aqueous phase, which retains water-soluble elements, including casein, lactose, whey proteins, and bioactive MFGM-derived compounds [31]. As the principal by-product of butter manufacturing, buttermilk exhibits a composition comparable to skim milk but with significantly higher phospholipid concentrations. Industrial butter production demonstrates an approximate 5:4 input-output ratio, generating 166 kg of buttermilk per 200 kg of butter [32]. Buttermilk is the liquid that results when cream is churned during butter production, causing fat globules to clump together and their membranes to break. This process releases membrane material and most water-soluble components into the buttermilk, making it a valuable by-product [33]. In the European Union, over 29% of the milk process is used for butter production, with 1.6 million tonnes of butter produced in 2023 alone. Since butter production yields approximately 50%, buttermilk production is estimated to be roughly the same volume [12]. Within the dairy industry, buttermilk comes in various types, including those obtained from sour cream, sweet cream, and whey [31]. Depending on its acidity, buttermilk is classified into two main types: sweet buttermilk (from sweet cream) and cultured buttermilk (from fermenting cultured cream or sweet buttermilk) [12].

In Switzerland, buttermilk is a significant side-stream, contributing to more than half of the total food loss in the dairy processing industry. Of the buttermilk produced, 63% is used as livestock feed, while 37% is consumed as human food [34]. Some butter manufacturers return buttermilk to the churning process to maximize resource usage and minimize waste generation. This process also supports the production of fermented butter and ghee, implementing

a circular economy in producing a food staple in many cultures.

The discharge of dairy by-products, such as buttermilk, into aquatic systems contributes to water pollution. Given its high organic load, with BOD and COD values around 1000–1500 mg/L, direct disposal of buttermilk into water bodies may impose environmental risks [35]. This may translate into alteration of soil properties, leading to crop yield reduction, and potentially polluting groundwater. High organic content may also lead to soil acidification [36]. The high BOD and COD contents may also lead to eutrophication in waterbodies, making them susceptible to excessive algal growth, depletion of oxygen levels, and harm to aquatic life [37].

## Buttermilk components

Buttermilk has been gaining recognition as a valuable source of functional ingredients and highly nutritious. Commercial buttermilk is an excellent source of phospholipids, which are derived from the milk fat globule membrane (MFGM). The potential phospholipid yield from MFGM in buttermilk is approximately 0.25 mg/mg protein [33]. Moreover, butter contains fat-soluble vitamins, including A, D, and E [38]. Buttermilk comprises polar lipids and proteins that play a crucial role in stabilizing fat droplets in milk and cream, ensuring their integrity. The polar lipids in MFGM, including phospholipids and sphingolipids, are found to be five times more concentrated in buttermilk than in whole milk. Phospholipids have been shown to have cholesterol-lowering and anti-inflammatory effects, and positive impacts on the nervous system. These components also exhibit antioxidant and antimicrobial properties, making them particularly beneficial for improving the immune defence system of new-borns [12]. Buttermilk has a reduced amount of lactose, making it a digestive aid for lactose-intolerant people [31]. The nutritional and functional benefits of buttermilk highlight its potential as a functional food ingredient, with applications in health and wellness. The proximate analysis of fresh buttermilk, as conducted by Astaire et al., [39], is shown in Table 3.

As butter churning happens at a high shear rate, the milk fat globules are broken down, and parts of it are recovered in the buttermilk [40]. Close to 20% of buttermilk proteins originate from the MFGM, as these proteins are solubilized during churning [41]. The process of MFGM forming starts during lactation, when the milk fat droplets are created and released from the mammary cells. This substance is a complex structure with three parts: an inner layer of phospholipids and protein, a middle layer of proteins, and an outer layer of phospholipids with a sugary coating [42].

**Table 3** Proximate analysis of fresh buttermilk [39]

| Composition | Content (%) |
|---|---|
| Fat | 10.15 ± 0.95 |
| Protein | 31.60 ± 3.33 |
| Ash | 6.52 ± 0.47 |
| Lactose (by-difference) | 51.73 |

Various analytical techniques can be used to quantify MFGM components, including proteomic analyses [43], immunoassays [44], mass spectrometry [45], and microfiltration membrane [46]. The composition of the constituents may also alter depending on the treatment used during processing. For example, according to Conway et al., [41], polar lipids are a small part in cream, making up only about 0.9% of the total fat. But in buttermilk, they are more concentrated, accounting for over 4.5% of the fat. In contrast, polar lipids account for only 0.2% of fat in butter. This explains how the processing method directly impacts the fat or protein content in the product.

According to Lopez [47], proteins constitute around 25–60% of the MFGM mass, with variations depending on the method used for isolation. This study also claims that the main protein constituent of MFGM is butyrophilin (BTN), which accounts for 40% of the protein components in MFGM. Another study, however, quantified the MFGM protein of buttermilk and resulted with Periodic Acid-Schiff (PAS 6/7) as the main protein constituent of MFGM, accounting for almost 50% of the MFGM protein content [48]. Such discrepancies can be attributed to differences in sample preparation methods or analytical techniques used during quantification. This discrepancy results mainly from the washing step of butter-making, which can release proteins from the MFGM [49]. Furthermore, the detailed protein composition of MFGM remains elusive due to the absence of standardized analytical techniques [48, 50]. Holzmüller & Kulozik [48], reported from multiple studies that the protein content of MFGM structures ranges widely between 25% and 70% of its dry matter, and this large variation poses challenges for further processing of these proteins into consistent, high-value applications. However, it can be concluded that the main protein components of MFGM are butyrophilin (BTN), Periodic Acid-Schiff (PAS 6/7), and Xanthine Oxidase/Dehydrogenase (XO/XDH) [47, 48, 50, 51].

These findings of the composition of buttermilk, especially MFGM proteins, have led to a wide range of applications, due to its numerous health benefits. MFGM is known to have support in cholesterol-lowering, anti-inflammation, chemotherapeutic, and neuroprotective effects, due to the action of the polar lipid in its structure. Other than that, it is also known that MFGM proteins have crucial bioactive components [41, 52]. The high amount of minerals and vitamins, especially vitamin B12, riboflavin, and potassium in MFGM makes it an important substance for amino acid and

fatty acid synthesis in the body. Vitamin B12 is known to have the ability to decrease stress, and anaemia, also aid nerve cell growth [31].

## Protein separation techniques

To fully utilize the protein and its health benefits from the materials, the correct separation technique must be used. Current conventional techniques used, mostly involving heat, have disadvantages, particularly regarding the stability of the protein [53]. Thermal processing effectively inactivates microbes and denatures proteins, ensuring product safety and extended shelf life, but may degrade nutritional and sensory qualities [54]. In contrast, non-thermal processing maintains functionality and nutritional value while achieving microbial inactivation [55, 56]. However, industrial implementation of non-thermal processing has a barrier due to high equipment costs [57].

The selection between these approaches ultimately depends on the specific goals of the processing, prioritizing either scalability, cost-effectiveness and established safety (particularly for small-to-medium-sized dairy operations) or preserving nutritional and sensory qualities (non-thermal). Moreover, certain non-thermal processing methods demonstrate significant nutritional benefits. High-pressure processing effectively preserves vitamin content, enhances calcium bioavailability, and promotes the formation of bioactive peptides [58]. Similarly, membrane filtration efficiently isolates dairy proteins capable of producing functional peptides through hydrolysis while simultaneously inhibiting undesirable enzymatic activity [56].

Protein separation employs three key approaches: recovery (extracting proteins from dairy by-products), purification (isolating specific proteins with high purity levels), and fractionation (separating protein mixtures into functional fractions) [59–61]. Therefore, current research is focused on coming up with novel technologies that can separate the needed proteins, without reducing their active compounds and bioactivity. The advantages and disadvantages of protein separation techniques are shown in Table 4.

**Table 4** Protein separation techniques

| No | Protein Separation Techniques | Methods | Advantages | Disadvantages | References |
|---|---|---|---|---|---|
| 1 | Thermal Processing Techniques | Heat Precipitation | Simplicity | Reduced Selectivity | [62] |
| | | | Does not require chemical precipitants | Irreversible denaturation | |
| | | Heat Coagulation | Simplicity | Requires time and energy-intensive | [63, 64] |
| | | | | Loss of protein solubility | |
| 2 | Non-Thermal Processing Techniques | High Pressure Processing | Food size/shape Independence | Pressure resistance for bacterial spores and food enzymes | [65] |
| | | | Shortening processing time | Inactivation requirement | |
| | | Membrane Technology | Readily for commercial production | Biofouling | [64, 66] |
| | | | Bioactivity Retention | | |

## Thermal processing techniques

### Heat precipitation

Heat precipitation is a widely used processing technique for pre-treating biomolecules, where heat induces protein coagulation and precipitation at specific temperatures. This process is driven by entropy shifts and the interactions between water and non-polar protein groups at increased temperatures [67]. The method is valued for its simplicity, efficiency, and ability to exploit the unique thermal properties of proteins, making it effective for selective isolation with high recovery rates [68].

However, this technique is unsuitable for whey protein as it induces protein denaturation. β-Lactoglobulin is a primary whey protein. Its denaturation impairs both structure and function, limiting its applications in food and pharmaceutical industries. Moreover, excessive heating can lead to nutrient degradation and undesirable changes in texture and flavor, reducing overall product quality [53]. Raut et al., [69], evaluated a whey protein powder (WPP) recovery method combining heat treatment with polyelectrolyte precipitation. The results demonstrated 60% protein recovery through thermal treatment at 90 °C, while carboxymethyl cellulose (CMC) addition enhanced recovery by 10–12%.

### Heat coagulation

Heat coagulation is another widely used protein separation technique that uses the heat sensitivity of proteins. During this process, whey is exposed to high temperatures for a specific duration, leading to protein denaturation, which depends on time, temperature, and pH conditions. Research indicates that whey proteins undergo optimal denaturation at 95 °C for 120 min, whereas buttermilk proteins exhibit effective molecular interactions at 65–80 °C for 25 min [70, 71]. Heat-coagulated whey proteins retain functional

properties including viscosity, elasticity, adhesion, foaming capacity, and gelation. However, their insolubility and gritty texture compared to undenatured whey proteins restrict practical applications [72, 73].

Despite the limitations, heat-coagulated whey proteins show potential for high-protein foods. However, research and industry currently prioritize lactose recovery over protein optimization [74]. Additionally, like other heat-based processing technologies, the application of this technique may lead to nutrient degradation, loss of bioactive compounds, and undesirable sensory changes [53]. Therefore, future innovations in processing technologies that could isolate needed compounds without decreasing their nutritional value are in high demand. Giroux et al., [75], investigated a heat-denaturation process for whey protein (WP) recovery in artisanal cheese production. Sheep whey subjected to 80 °C for 30 min exhibited significantly higher ($P \leq 0.05$) denaturation rates compared to cow and goat whey. Recovery efficiencies reached 65.18% and 65.90% using sweet whey and acid whey, respectively.

## Non-thermal processing techniques

### High pressure processing

Pressure-based non-thermal processing commonly known as High Pressure Processing (HPP), is a method used in dairy processing typically to isolate protein and ensure microbial safety without heat exposure. HPP is a physical method based on the principle of uniform pressure transmission via a hydrostatic fluid medium, ensuring consistent treatment throughout the food matrix [76]. By applying pressures between 100 and 500 MPa, this method effectively pressurizes both solid and liquid food products and achieves pasteurization without the need for high temperatures [77].

HPP reduces environmental impact through lower energy and water consumption, decreased $CO_2$ emissions, and diminished pollution generation [78]. Unlike thermal processing techniques, this technology is increasingly used in food processing. It enhances safety and shelf life while preserving nutrition and sensory attributes [79]. Consumer evaluations indicated that HPP products were primarily valued for their perceived naturalness, improved taste, and nutritional quality, aligning with food quality and convenience expectations. However, insufficient technological knowledge and consumer skepticism appeared as major barriers to acceptance [80, 81].

The process applies uniform pressure, causing a controlled temperature rise of 2–3 °C per 100 MPa due to adiabatic heating. HPP inhibits microbial growth through cell disruption and enzyme deactivation while maintaining organoleptic and rheological properties. This technique follows three fundamental principles: Le Chatelier, isostatic principle, and microscopic ordering principle [82]. Studies by Graikini et al., [83], and Parrón et al., [84], have shown that treating whey and buttermilk below 500 MPa for 15 min preserves its natural antirotaviral activity, highlighting their potential for functional food applications, particularly in regions with high rotavirus prevalence. Despite its benefits, the application of HPP on an industrial scale faces some drawbacks, especially due to its high cost, which ranges from USD 0.10 to 0.20 per liter, significantly higher than using conventional heat treatment (USD 0.02–0.04 per liter). However, as demand for pressurized (or cold-pressed) dairy products increases, production costs are expected to decrease [82].

### Membrane technology

Membrane technology plays a vital role in the dairy industry, serving multiple functions, including spore removal, purification of bioactive compounds, fractionation of milk proteins, and by-product valorization. Due to its superior efficiency and compatibility with continuous processing, cross-flow membrane filtration has largely supplanted conventional dead-end filtration in industrial applications [85].

Modern membrane technologies including ultrafiltration, microfiltration, and reverse osmosis are increasingly employed in cheese production in the dairy industry. These processes effectively separate whey components while retaining valuable whey proteins and other beneficial constituents in the cheese product [86]. Milk and whey proteins demonstrate multifunctional characteristics, including emulsifying capacity, foaming ability, and gel formation. These properties are essential for effective protein recovery from dairy by-products. Membrane filtration technologies, particularly ultrafiltration and microfiltration, significantly optimize these functional attributes, thereby increasing the utility of dairy proteins in diverse food applications [87–89].

According to Blais et al., [90], there are four broad categories of membrane filtration commonly used in the dairy industry. Firstly, microfiltration (MF) is a filtration technique that uses membranes with a pore size of 0.1–10 µm to filter out fat globules, somatic cells, bacteria, spores, and large protein clusters from dairy products, or to separate large macromolecules. Transmembrane pressure (TMP) refers to the pressure difference between the two sides of the membrane used during filtration. Generally, an increase in TMP enhances permeate flow, but too high of TMP may also lead to increased fouling and overall filtration inefficiency [91]. A microfiltration process has a TMP ranging from 0.03 to 0.2 MPa.

Second, ultrafiltration (UF) is a technique that operates using a membrane small enough to separate the molecules

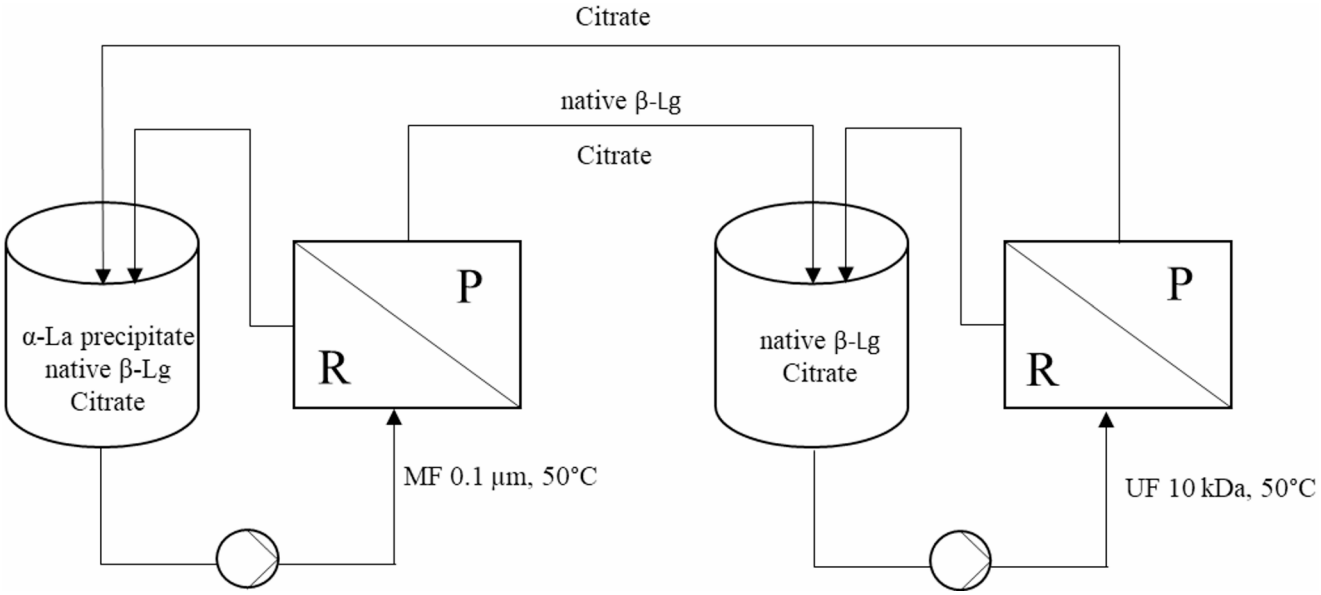


**Fig. 2** α-La and β-Lg MF/UF separation scheme [25]

based on their molecular weight, commonly ranging from 1 to 800 kDa. This technique is coupled with a bigger TMP range of 0.1-1 MPa, as smaller pores have higher resistance to permeate flux. UF is commonly used in the production of protein concentrates or isolates from milk or whey (e.g. whey protein concentrates). The membranes with lower molecular cut-off are used to remove salts, lactose, non-protein nitrogen, and other soluble solutes. Other than that, permeates from UF processes are usually used in standardization processes or lactose production.

Third, nanofiltration (NF) employs membranes with a pore size up to 150–700 Da, paired with a TMP of 1–3 MPa. In this technique, mineral salts can be removed depending on the charge of the minerals. A study by Daufin et al., [92], showed that NF can concentrate skim milk up to 22% of dry matter, while removing 25–50% of its monovalent ions (e.g. sodium). This removal efficiency can be increased up to 90% by coupling it with a process called diafiltration.

Finally, reverse osmosis (RO) is another membrane filtration method frequently used to concentrate milk or whey up to 27% of its dry matter, before coupling it with evaporation. This technique helps minimize overall energy consumption; however, it is limited by factors such as osmotic pressure and viscosity of the retentate. These limitations can result in a decrease in crossflow velocity and cause fouling when accumulated over time.

## Multi-level processing techniques

Each processing technique possesses distinct advantages and limitations. Therefore, the integration of multiple technologies is implemented to maximize the benefits of each individual process. By leveraging the specific characteristics of each process, multi-level processing techniques may enhance both concentration and fractionation efficiency in dairy products and their derivatives, offering better performance compared to single-stage processing [90].

A study conducted by Astaire et al., [39], introduced a two-step approach to produce buttermilk-derived ingredients with enhanced concentrations of milk fat globule membrane (MFGM) through microfiltration and supercritical fluid extraction (SFE). The process involved microfiltration with a 0.8 µm, then followed by SFE using supercritical $CO_2$ to selectively remove non-polar lipid materials. These combined techniques provide environmentally friendly alternatives to traditional lipid extraction using toxic solvents. Additionally, lipid analysis confirmed a significant increase in polar lipid concentration in the final product. Supercritical $CO_2$ technology is widely favored for developing environmentally sustainable processes that align with natural product requirements. Concurrently, consumer preference is shifting toward supercritical $CO_2$ processed products owing to their perceived safety and additive-free natural characteristics [93, 94].

Research conducted by Toro-Sierra et al., [25], evaluated the fractionation method for α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) on a pilot scale (Fig. 2). This study implemented both heat treatment and membrane-based separation of the whey protein isolate. Heat treatment was used to selectively precipitate α-La by raising the temperature of the solution up to 50 °C at a 1 °C/min rate for 2 h. The process was followed by microfiltration to separate the aggregated α-La from the native β-Lg using a microfiltration module, coupled with an ultrafiltration membrane

operated in diafiltration mode, as visualized in Fig. 2. This approach achieved purities of 91.3% for α-La and 97.2% for β-Lg, demonstrating its potential for pilot-scale implementation and possible industrial applications in producing native α-La and/or β-Lg fractions.

Touhami et al., [95], also combined HHP (high hydrostatic pressure) and UF to improve the recovery and purification rate of α-La from skimmed milk. This resulted in an increase in α-La/β-Lg ratio in the UF permeate. However, it also revealed that the implementation of heat treatment decreased filtration performance, which consequently decreased the recovery and purity of α-La. The author suggested the use of a centrifugal or decanter centrifuge as a pre-treatment in future experiments to improve UF performance.

A financial analysis by Macedo et al., [96], indicates that installing an industrial membrane unit in a small cheese dairy is a profitable investment with a positive return. Sensitivity analysis ran by the author highlighted while some factors could impact returns, e.g. stagnant sales or increased labor costs, the project remains financially viable. Additionally, implementing multi-level processing technology enables the production of value-added dairy products, such as whey protein concentrates, which can open new market opportunities. Therefore, a more detailed market study is recommended to be done to understand potential customers and industries better.

### Enzyme hydrolysis

Enzyme hydrolysis is an established method for modifying lactose and proteins in whey and buttermilk, improving their nutritional quality and functional properties. Specifically, exhibit enhanced biological value, reduced allergenicity, and superior functionality, making them ideal for functional food applications [97, 98]. A variety of enzyme preparations (EPs), including Alcalase, Flavourzyme, Neutrase, Promod 439 L, and Flavorpro 766MDP, are employed for targeted protein hydrolysis [97–99].

Enzyme selection depends on their specificity for peptide bonds, which governs the degree of hydrolysis and the resulting peptide profile. A key advantage of proteolysis is the generation of bioaccessible short-chain peptides with potential physiological benefits [99]. Despite its advantages, enzymatic hydrolysis faces several practical limitations. Key challenges include the elevated costs of commercial enzyme preparations, the energy-intensive thermal inactivation required to terminate reactions, and operational difficulties in implementing continuous processing systems. These factors currently restrict broader industrial adoption of this technology [100, 101].

Controlled hydrolysis enhances milk protein functionality by increasing solubility, heat stability, emulsification, foaming capacity, and surface hydrophobicity traits, which are essential for ingredient applications in dairy and other food systems [102]. Process efficiency and product quality depend on hydrolysis parameters (pH, temperature, duration, enzyme-to-substrate ratio) [102], which must be optimized to produce cost-effective, high-value results.

## Challenges in protein separation from dairy By-Products

### Challenges in cheese Whey protein separation

In cheese whey, a high salt content is added during cheese production, which, unfortunately, affects the efficiency of the whey protein concentration process. High salt concentrations in the whey can lead to selective coagulation, where certain proteins precipitate, while others remain soluble. For example, β-lactoglobulin (β-Lg) may remain in solution, while casein-macropeptide precipitates at high NaCl concentrations. This selective behavior complicates the concentration process, as valuable proteins may be lost during processing [103]. Additionally, the presence of salt can increase the viscosity of whey protein solutions, which reduces mass transfer during concentration techniques like freeze-concentration. This reduces efficiency when isolating proteins from whey [104]. However, it is also important to note that increased salt concentrations can enhance the solubility of whey proteins at specific pH levels. An optimum salt concentration must be identified and maintained, as excessive salt can reduce the emulsifying capacity of whey proteins, potentially limiting their functional applications [105]. To overcome this challenge, a methoxy-polyethylene glycol-block-sulfonated polyacrylonitrile (mPEG-b-SPAN/PAN) ultrafiltration membrane can be employed, offering superior selectivity and antifouling properties for efficient protein-salt separation in whey [106].

Another problem with cheese whey is that it contains high levels of lactose, which can influence protein behavior during processing and affect the overall protein concentration in whey products. High lactose content can lead to increased protein aggregation, especially when the whey is heat-treated, forming larger and denser protein aggregates. This aggregation can affect the overall functionality of the proteins [107]. Additionally, higher lactose concentrations correlate with a more porous aggregate structure, which may impact the protein's functional properties, such as texture and stability. Lactose also plays a significant role in the gelation process of whey proteins. Increased lactose levels can reduce gel hardness, indicating a negative effect on

gelation performance. For example, in goat whey protein, increasing protein levels typically leads to firmer gels, but excessive lactose content may interfere with the bonding of proteins, resulting in a weaker gel [108]. When attempting to isolate pure whey protein from cheese whey, the gelation caused by high lactose content can complicate the process. To address this, techniques such as ultrafiltration are commonly employed to effectively separate lactose from whey, ensuring protein concentrate can be isolated without interference from lactose.

### Challenges in buttermilk protein separation

Concentrating protein from buttermilk presents several significant challenges, primarily due to its low total solids content, high moisture and fat content, and poor heat stability. As a by-product of butter production, buttermilk has a low concentration of total solids, which makes achieving a high yield of protein concentration difficult [109]. Low protein content in buttermilk limits the effectiveness of traditional concentration methods, such as ultrafiltration, and necessitates the use of more advanced techniques like reverse osmosis (RO) or nanofiltration (NF) to enhance protein recovery and concentration [110]. Furthermore, buttermilk's high moisture content, a result of the butter-making process, further complicates protein concentration. The churning cream to make butter retains significant amounts of water, diluting protein concentration and reducing the overall yield. In food applications, higher moisture levels are typically undesirable as they affect protein content, shorten shelf life, and reduce the overall usability of the product [109].

In addition to the challenges posed by moisture content, buttermilk's high fat content also plays a crucial role in the difficulty of protein concentration. The fat, a natural by-product of butter production, interacts with proteins, potentially altering protein solubility and reducing the effectiveness of separation processes [33, 111]. This interaction can complicate the recovery of milk fat globule membrane (MFGM) proteins, which are valuable for their functional properties but add another layer of complexity to the concentration process. Moreover, the proteins in buttermilk exhibit poor heat stability, making them prone to denaturation during processing, which can further hinder concentration efforts [112]. Despite these challenges, innovative methods such as nanofiltration and reverse osmosis show promise in improving both the concentration and stability of proteins from buttermilk. However, the inherent properties of buttermilk continue to present significant hurdles, underscoring the need for ongoing research and development. One promising approach involves supercritical $CO_2$ extraction of phospholipids from buttermilk, which enables the separation of MFGM proteins from milk fat components.

## Food industry applications

In response to the growing demand for nutritious, functional, and sustainable food products, the food industry has turned to utilizing their by-products, such as cheese whey and buttermilk, into new products. The utilization of these products provides not only a cost-effective solution but also enhances the nutritional value and functionality of many popular food products. For example, the addition of whey protein concentrate (WPC) in comminuted products like sausages, luncheon meats, or surimi. The addition of WPC facilitates fat reduction while maintaining desired textural and organoleptic properties. A common challenge with creating low-fat processed meat products is increased water content, which changes the toughness, juiciness, and texture of the products. Besides that, adding extra water to these meat products can lead to more liquid being lost during cooking or storing, reducing the product's final weight. Incorporating WPC into these formulations helps improve the products' functionality and structure. In the case of sausage production, higher concentrations of WPC contributed to creating an improved matrix uniformity and reduced granulation. This results in the improvement of textural qualities, stability, and sensory acceptance, overall highlighting the potential of WPC to create healthier and low-fat meat product options [113, 114].

Dairy by-products have high potential as protein-concentrated raw materials, as they are abundant in quantity, could cause environmental problems (when not discarded properly), yet are highly nutritious. Whey has one of the best protein efficiency ratio, biological value, and net protein utilization among all protein sources [115]. Therefore, concentrating these proteins allows for their use in various applications, maximizing their nutritional benefits, especially in the food industry.

In the current years, whey-based functional beverages fortified with probiotics are growing rapidly in technology and market. Probiotic-fortified beverages are may alleviate gastrointestinal disorders linked with the digestive system, as it promotes the digestive system as they promote the activity of composition and activity of intestinal microbiota [116]. Another emerging trend in the food industry is the development of ready-to-serve whey-based beverages. These drinks, enriched with carbohydrates and elevated salt levels, have potential applications in sports drink formulations to support post-exercise recovery in athletes. Given whey's high protein efficiency ratio, the drink will be important in supporting muscle recovery, supports lean muscle synthesis, and overcoming adenine depletion [115]. Whey also has a wide range of applications in the food industry. Its emulsifying ability confers excellent emulsifying properties for processing cheese, salad dressings, and adding creaminess to drinks. The gel-forming ability makes whey a good

agent for gelation and water binding in meat and fish products like ham and surimi [117].

Similarly, concentrated protein from buttermilk offers significant applications in the food industry. Hameed et al., [116], highlighted that incorporating buttermilk in milkshake production enhances foam formation and reduces surface tension, contributing to improved texture and stability of the beverage. Additionally, buttermilk is rich in phospholipids, enhancing the beverage's nutritional profile. When concentrated, buttermilk-derived proteins can function as natural emulsifiers, improving the texture and stability of various food products. However, current research indicates that the production of buttermilk-based beverages remains the most viable innovation from both technological and economic perspectives.

## Other industry applications

Waste reduction and by-product valorization constitute critical components of sustainable dairy processing systems. The industry produces multiple by-products, which possess significant potential for conversion into value-added products [118]. While cheese whey and buttermilk are traditionally utilized in the food industry, their potential extends to offering valuable opportunities for other sectors. These dairy by-products have been explored in bioethanol and biofilm production for biodegradable materials. They also hold promise for the cosmeceutical industry for their bioactive compounds, contributing to developing anti-aging formulations. Furthermore, the nutrient-rich composition of cheese, whey, and buttermilk makes them valuable for livestock feed and organic fertilizers. Implementing circular economy principles enables dairy processors to achieve dual benefits: minimizing environmental impact through waste mitigation while creating supplementary economic value through resource recovery [118].

### Bioethanol production

Cheese whey contains a huge amount of lactose, which can be used as a fermentation substrate to produce bioethanol. The process begins with the pretreatment of the lactose in cheese whey, converting it into simpler sugars such as glucose and galactose. Pretreatment methods include enzymatic hydrolysis, microbial hydrolysis, and membrane filtration [18, 119]. Following pretreatment, yeast strains such as *Saccharomyces cerevisiae* are employed to ferment the sugars into ethanol. To further enhance fermentation efficiency, co-culturing with lactose-utilizing microorganisms is often applied, as *S. cerevisiae* is unable to directly ferment lactose unless the whey is pretreated [119, 120]. Optimal processing conditions must be achieved to enhance the ethanol yield and reduce chemical oxygen demand in the whey [121]. After fermentation, ethanol is typically recovered through distillation. This process is crucial to obtain bioethanol with a high purity level, so it can be used as biofuel [122]. Some studies reported that the fermentation using S. cerevisiae resulted in a notable ethanol concentration of 9.2 g/L, corresponding to an ethanol yield of 0.23 g/L, 42% of the theoretical yield. Extrapolating these findings, it is theoretically feasible to produce up to 5 million tons of bioethanol annually using cheese whey as a feedstock [120, 122].

### Biofilm and biodegradable polymer

Another potential way of valorization of cheese whey is the production of biofilm or bioplastic. Polyhydroxyalkanoate (PHA) is a biodegradable polymer with properties comparable to petroleum-derived plastics, and it is a highly researched polymer that can replace petroleum-derived plastics. This polymer can be synthesized using cheese whey as a substrate, which involves microorganisms like *Ralstonia eutropha* and *P. hydrogenovora* [19]. Since carbon source is a significant cost in bioplastic production, cheese whey, an effluent from the dairy industry, has become a potential cost-effective and environmentally friendly carbon source for PHA production [123].

Biofilm production with cheese whey protein was done in the study of Uribarrena et al., [124], where cheese whey was ultrafiltered to obtain the whey protein. After that, the protein undergoes a process of dissolution, pH adjustment, glycerol addition, and heating. After heating, the solution goes through freeze-drying to remove the water content. Finally, films were obtained by applying thermal compression to the powdered solution. Extracted whey protein was of 91.6% purity, and SEM imagery revealed the presence of lactose in the extracted protein. The biodegradability test shows that the biofilm is rapidly compostable in any industrial composting facility. Further challenges in implementing this innovation are scaling up the manufacturing process since it needs a lot of optimization and contributes the most to environmental problems.

### Cosmeceutical applications

Cheese whey is also a potential substrate in the cosmeceutical industry, primarily due to its rich array of bioactive compounds, such as proteins, peptides, and lactose derivatives [125]. These compounds are important not only for skin health but also for functional roles in cosmetic formulations. For instance, bioactive peptides such as lactoferrin and lactoperoxidase possess antimicrobial and antiviral

properties that help reduce skin infections. Immunoglobulins in cheese whey also help enhance skin immunity and serve as protection against environmental stressors [126]. Furthermore, whey-derived compounds like lactobionic acid exhibit antioxidant properties, which help protect the skin from oxidative stress and free radicals [125, 126]. The prebiotic and moisturizing effects attributed to lactose and oligosaccharides present in whey help enhance skin barrier function and hydration, thereby improving skin texture and elasticity [127]. However, challenges arise in the realization of cheese whey's full potential due to logistic and financial challenges. These obstacles are particularly evident during the collection and processing stages, hindering the efficient utilization of this valuable by-product [125].

## Pharmaceutical applications

As for buttermilk, recent studies have revealed the potential of buttermilk in the pharmaceutical industry, particularly for the encapsulation of sensitive ingredients like omega-3-rich oils. When used as a wall material, buttermilk offers excellent protection against environmental stressors like heat, light, and oxygen, which can degrade active compounds. Specifically, buttermilk has shown high encapsulation efficiency in microencapsulating algal oil, which is rich in omega-3 oils and prone to oxidation [128, 129].

Techniques like spray drying further enhance the stability and effectiveness of the encapsulation process, improving the incorporation of these oils into food and pharmaceutical products [130]. The fat globule membrane in buttermilk plays a critical role in stabilizing omega-3 oils, reducing oxidation and prolonging shelf life, making it a valuable material for functional food applications [131]. This ability to protect and deliver sensitive ingredients underscores buttermilk's potential as a sustainable and efficient tool in food and pharmaceutical applications.

Several studies have investigated the potential anticancer effects of individual whey proteins. For instance, lactoferrin may sequester iron in tissues, thereby reducing oxidant-induced carcinogenesis [20, 132]. Additionally, in vitro studies demonstrate that bovine serum albumin (BSA) suppresses the proliferation of the human breast cancer cell line MCF-7 when co-cultured with tumor cells [132].

## Animal feed

Both cheese whey and buttermilk have demonstrated significant potential as cost-effective and nutritionally valuable resources for animal feed. The utilization of these by-products offers promising benefits for livestock and poultry production. Buttermilk has shown remarkable results in enhancing growth performance and overall efficiency in livestock. A study by Karim & Mahmood [133], indicates that feeding lambs with concentrate pellets supplemented with buttermilk significantly improved growth performance compared to the feed without buttermilk. Additionally, lambs on buttermilk-enriched diets exhibited reduced growth periods, lower fat thickness, and decreased total costs, resulting in overall higher profitability. Similarly, the use of buttermilk-coated diets in crossbred chickens produced similar results to commercial diets in terms of live weight, feed intake, and organ development, despite the need for slightly larger feed quantities to achieve a kilogram of weight gain. However, these diets were the most cost-effective, reducing production costs, and boosting feed conversion efficiency, compared to commercial diets. This reduction in feed costs could play a crucial role in narrowing the gap between chicken meat demand and supply [134].

Cheese whey also has emerged as another valuable by-product for animal feed, due to its rich nutritional composition. Research by Caltzontzin-Rabell et al., [135], highlights its utility in producing high-protein feed through black soldier fly larvae (BSFL) cultivation. When grown on cheese whey, BSFL efficiently converts the whey into nutrient-dense larval biomass. The larvae, enriched with fats and proteins, provide a sustainable and high-quality feed alternative for livestock, helping to reduce reliance on convention feed sources and lowering feed costs. Additionally, the study noted that the accumulation of fats in the larvae can be processed into biofuels, offering a renewable energy source and contributing to the diversification of energy resources. These findings underscore the potential of cheese whey and buttermilk to revolutionize animal feed practices. Repurposing these by-products will help the dairy industry reduce waste while supporting more sustainable and economically viable livestock systems.

## Fertilizer production

Another potential use of cheese whey, and buttermilk is utilizing their nutritional and organic compounds as a fertilizer. A study by Abdelraof et al., [136], found that an addition of 1% of cheese whey could substitute for chemical fertilizer used in growing maize. As level of cheese whey increases, the growth traits (e.g. shoot heights and leaf numbers) increase as well. It is known that applying cheese whey at a 1% concentration can meet the NPK (nitrogen, phosphorus, and potassium) needs of maize crops. Therefore, this finding recommends the use of cheese whey as an organic fertilizer to substitute for costly and environmentally dangerous chemical fertilizer. Another study by Grosu et al., [137] reinforces the idea that cheese whey is a potential substitute for chemical fertilizer. Wheat, soy, and broccoli have shown positive effects when grown with the addition of whey in all

stages of growth. Whey addition during cultivation also has a positive impact on the overall biosynthesis and accumulation of bioactive compounds of the three plants, making it a promising and more sustainable substitute for fertilizer. As for buttermilk, the study of its utilization as a plant fertilizer goes way back as old as 1926. The study by Coville [138], shows that buttermilk appears to have a positive impact on plant growth, shown by physical appearance, height increase, and new shoot growth, which suggests that buttermilk may contain essential nutrients, such as nitrogenous compounds and other growth-promoting factors.

## Conclusions

The dairy processing industry is a critical global sector, producing essential nutritional commodities such as milk, cheese, yogurt, and butter to meet increasing nutritional foods. However, this large-scale production generates substantial by-products, notably cheese whey and buttermilk, which possess significant untapped potential. Cheese whey is a rich source of bioactive compounds containing approximately 20% milk serum proteins by composition, with α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) constituting ~ 70% of its total protein fraction. Meanwhile, buttermilk, a derivative of butter manufacture, has emerged as a functionally valuable ingredient due to its high phospholipid content, primarily sourced from the milk fat globule membrane (MFGM). Conventional protein separation techniques predominantly employ thermal treatments, including heat precipitation and coagulation, which may induce protein denaturation and impair functionality. In contrast, non-thermal processing methods such as high-pressure processing (HPP) and membrane filtration offer promising alternatives for protein recovery while preserving structural integrity and ensuring microbial inactivation. Nevertheless, challenges persist in the efficient separation of proteins from dairy by-products. Cheese whey's elevated mineral content, can reduce whey protein concentration efficiency. Similarly, buttermilk's low total solids content complicates the concentration of its protein fraction, necessitating advanced separation strategies to optimize yield. Selecting appropriate separation methodologies is imperative to maximize the utilization of these bioactive components. Further, the food industry has increasingly valorized whey and buttermilk by integrating them into novel food formulations, enhancing economic feasibility and nutritional functionality. Beyond conventional applications, these by-products hold substantial promise for diversified industrial uses, including bioethanol production, biodegradable polymer fabrication, cosmeceutical and pharmaceutical formulations, as well as animal feed and fertilizer production applications.

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## Declarations

**Conflict of interest** There is no conflict of interest/competing interest.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Research involving humans and animals partipants** Not applicable.

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