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Strategies for Cheese Whey Processing and Valorization

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Sucess consists of going from failure to failure
without loss of enthusiasm.

Winston Churchill

Quisiera comenzar expresando mi más sincero agradecimiento a mi tutora, Carme Sans, por su invaluable orientación y apoyo a lo largo de este viaje académico. Su profesionalidad y paciencia han sido fundamentales para el desarrollo de mi TFG. Estoy muy agradecida por su inspiración y consejos.

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SUMMARY

The cheese industry generates significant quantities of cheese whey as a byproduct, posing substantial environmental challenges due to its rich organic matter content. Recent efforts have focused on developing environmentally sustainable methods for cheese whey utilization, aiming to transform this abundant byproduct into a valuable resource. The focus of sustainable cheese whey management predominantly lies in biotechnological and food-related applications, leading to the creation of various value-added products. These include whey powders, whey proteins, hydrolysates, peptides, amino acids, functional foods and beverages, lactic acid, and other biochemicals, along with bioplastics, biogas, and other noteworthy bioproducts. This TFG presents a comprehensive review of the sustainable use of cheese whey and its components, highlighting the direct uses, the novel refining techniques, and integrated processes. The focus of these strategies is to efficiently transform cheese whey and its key components, including lactose and whey proteins, into products with significant value derived from whey.

Keywords: cheese whey, valorization strategies, environmental impacts, life-cycle-assessment, land application, whey cheeses, value-added products, biological treatment, physicochemical treatment.

RESUM

La indústria del formatge genera quantitats significatives de xerigot com a subproducte, plantejant reptes ambientals substancials a causa del seu ric contingut en matèria orgànica. Els esforços recents s'han centrat en el desenvolupament de mètodes ambientalment sostenibles per a l'ús del xerigot, amb l'objectiu de transformar aquest subproducte abundant en un recurs valuós. L'enfocament de la gestió sostenible del xerigot es basa principalment en aplicacions biotecnològiques i alimentàries, que porten a la creació de diversos productes de valor afegit. Aquests inclouen pols de xerigot, proteïnes del sèrum, hidrolitzats, pèptids, aminoàcids, aliments i begudes funcionals, àcid làctic i altres bioquímics, així com bioplàstics, biogàs i altres bioproductes notables. Aquest TFG presenta una revisió exhaustiva de l'ús sostenible del xerigot i els seus components, destacant els usos directes, les tècniques de refinament noves i els processos integrats. L'objectiu d'aquestes estratègies és transformar eficientment el xerigot i els seus components clau, incloent la lactosa i les proteïnes del sèrum, en productes amb un valor afegit significatiu.

Paraules clau: xerigot, estratègies de valorització, impactes ambientals, avaluació del cicle de vida, aplicació terrestre, recuits, productes de valor afegit, tractament biològic, tractament fisicoquímic

SUSTAINABLE DEVELOPMENT GOALS

Cheese whey (CW), a by-product of the dairy industry, poses significant environmental challenges due to its high organic load. However, its potential as a valuable resource for various applications suggests a unique opportunity for sustainable innovation. This TFG examines the utilization and valorization of CW, emphasizing in this section its importance within the framework of the 5 Ps (People, Planet, Prosperity, Peace and Partnership) and its alignment with the UN's (United Nations) SDGs (Sustainable Development Goals) (Table 1).

Table 1. Cheese Whey Valorization: Integrating 5 Ps, SDGs, Goals, and Indicators.

Ps	SDGs	Goals	Indicators
People: The reuse of CW has the potential to improve nutrition, offering products enriched with proteins and other essential nutrients, enhancing human health and well-being.	SDG 2 (Zero Hunger).	To improve nutritional value and food security through sustainable food production systems.	The increase in the availability of food products enriched with value-added components derived from CW.
Planet: Valorization of CW contributes to environmental sustainability by proposing methods to reduce water pollution and promote waste recycling.	SDG 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy) and SDG 12 (Responsible Consumption and Production).	SDG 6: To reduce pollution, eliminate dumping and minimize the release of hazardous chemicals and materials. SDG 7: Enhance environmental sustainability by reducing waste and greenhouse gas emissions through the conversion of CW into bioenergy. SDG 12: To achieve the sustainable management and efficient use of natural resources.	SDG 6: Reduction in the volume and toxicity of CW effluent released into water bodies. SDG 7: Quantifiable reduction in waste and greenhouse gas emissions attributed to the bioenergy production from CW, alongside an increase in the percentage of CW-derived bioenergy in the overall renewable energy mix. SDG 12: Increase in the percentage of CW that is recycled and reused in the production of new products.
Prosperity: Valorizing CW can open new economic opportunities, creating added-value products and fostering industry innovation.	SDG 7 (Affordable and Clean Energy), SDG 8 (Decent Work and Economic Growth) and SDG 9 (Industry, Innovation, and Infrastructure).	SDG 7: Foster economic growth and innovation in the renewable energy sector by utilizing cheese whey for bioenergy production. SDG 8: Promote development-oriented policies that support productive activities, decent job creation, and entrepreneurship. SDG 9: Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation.	SDG 7: Measurable increase in economic value and job opportunities in the renewable energy sector, specifically from bioenergy production using CW. SDG 8: Growth in employment rates in industries related to the valorization of CW. SDG 9: The number of innovative technologies developed and implemented for CW valorization.

Table 1. (*Continued*)

Ps	ODS	Goals	Indicators
Peace and Partnerships: Indirectly, this work supports peace by promoting sustainable and ethical practices in the dairy industry and highlights the importance of partnerships between researchers, industry, and public policy.	SDG 16 (Peace, Justice, and Strong Institutions) and SDG 17 (Partnerships for the Goals).	SDG 16: Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable, and inclusive institutions at all levels. SDG 17: Strengthen the means of implementation and revitalize the global partnership for sustainable development.	SDG 16: Instances of conflict over environmental and resource management issues related to CW disposal and valorization. SDG 17: The number and quality of international and cross-sectoral partnerships developed to advance CW valorization techniques.

CW: Cheese Whey; 5 Ps: People, Planet, Prosperity, Peace and Partnership; SDGs: Sustainable Development Goals.

1. INTRODUCTION

The dairy industry operates on a global scale, and according to the Food and Agriculture Organization of the United Nations, the world's cheese production primarily utilizes four types of milk: buffalo, cow, goat, and sheep [1]. Cheese making, while based on fundamental principles that are universally applied, exhibits a variety of techniques at different stages, leading to the creation of numerous distinct varieties, often within the same production facility. Cheese production involves the coagulation of the milk protein casein, effectively capturing milk solids and fat within a curd matrix. This process starts by mixing milk with a starter culture of bacteria and adding an appropriated milk coagulant, normally an enzyme preparation that catalyses the coagulation of casein. Subsequently, the formed curd is cut into cubes, and the mixture is gently stirred to maximize the protein content in the curd. Notably, the yield of cheese from this process is about 10% when cow milk is used, with the remaining 90% constituting a liquid by-product known as cheese whey (CW). The CW is then separated and drained from the curd, which is subsequently salted, pressed, and either consumed as fresh cheese or matured and packaged as ripened cheese [2].

The principal by-product generated from the milk transformation processes is CW, and in 2021, the global production of this by-product was estimated to be around 200 million tons [3,4], with the European Union alone producing approximately 40 million tons [1,5]. This by-product presents both a challenge and an opportunity for sustainable management and valorization in the dairy industry.

Specifically, as defined by Decision 97/80/EC, whey is defined as “a by-product obtained during the manufacture of cheese or casein [6]. In its liquid form, CW retains several natural components (lactose, protein, fat, and minerals) which persist after the removal of casein and most fat from milk. Typically, CW is categorized into two primary types: acid CW and sweet CW. Acid CW results from the direct use of organic acids or through the addition of lactic cultures, whereas sweet CW is predominantly produced by the coagulation of proteins using animal, vegetable, or microbial enzymes, such as chymosin.

Following legislative measures enacted worldwide, with a few exceptions in some developing countries, the disposal of untreated CW into natural water bodies is now illegal. This regulation emerged in response to growing awareness during the latter half of the 20th century, where community groups, environmental organizations, and dairy processors collectively acknowledged the ecological harm caused by releasing untreated CW. This practice, depending on the type of whey disposed, leads to eutrophication in aquatic systems and can increase soil acidity when released directly into the ground. CW is characterized by its substantial Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), which are indicators of the potential environmental impact it can have if improperly managed. If released into aquatic systems, CW can significantly deplete dissolved oxygen levels, posing a serious threat to marine ecosystems, and consequently, to broader environmental health and human well-being [7].

Historically, CW has found re-utilization within cheese manufacturing facilities, serving as a key ingredient in the creation of fresh dairy products like whey cheeses including ricotta and requesón, as well as whey butter. Additionally, it has long been a staple in the diet of farm animals, providing nourishment to pigs, calves, goats, and sheep, as documented in various studies [5,8].

Recent efforts to enhance sustainability in the agri-food sector, in alignment with the SDGs and the principles of the circular economy, have sparked a growing interest in valorizing the resources derived from various food production processes [9,10].

In light of these environmental concerns, numerous research initiatives have focused on utilizing CW as a substrate to develop a range of products for various sectors including food, pharmaceuticals, healthcare, cosmetics, and bioenergy. The valorization of CW predominantly yields lactose and whey proteins, which are then employed as ingredients in the production of various products like infant formula, bread, confectioneries, meats, and beverages. Furthermore, when used as a carbon source in microbial processes, CW facilitates the generation of a broad spectrum of secondary metabolites. These include enzymes, bacteriocins, organic acids, flavours, vitamins, additives, amino acids, oils, polysaccharides, and proteins, among others. Notably, its use extends to the production of bioplastics, biofuels, or as feedstock for such processes.

Researched data indicates that currently, only half of the CW produced is processed and converted into various food products [1,3], a figure that is expected to rise due to ongoing research and stricter regulations on the disposal of liquid waste by casein and cheese manufacturers [11,5].

Concurrently, technologies for treating CW are being developed, and there are three primary strategies for managing them. The first involves the deployment of valorization technologies designed to reclaim valuable constituents from CW, such as proteins and lactose. Techniques like membrane processing, which include microfiltration, ultrafiltration, and reverse osmosis, are employed within the cheese industry to separate and preserve whey proteins and other beneficial components, which are then employed as ingredients in the production of various products like infant formula, bread, confectioneries, meats, and beverages [12,13]. The second strategy is grounded in biological treatments where the hydrolysis of lactose and proteins results in the creation of lactose monosaccharides (glucose and galactose), as well as peptides and amino acids. Utilizing various microorganisms in controlled fermentation processes is an approach that is gaining traction for the synthesis of a range of high-value products, including lactic acid, butyric acid, butanol, acetic acid, glycerol, acetone, ethanol, hydrogen, and single cell proteins. Also, other biological treatments use extends to the production of biofuels or bioplastics. The third strategy encompasses physicochemical treatments aimed at reduce contaminant levels, specifically organic matter, turbidity, and suspended solids, and at recovering valuable substances from CW, particularly proteins and lactose. Techniques such as coagulation-flocculation, thermal and isoelectric precipitation, thermocalcic, acid or alkaline precipitation, and electrochemical oxidation are leveraged to reduce contaminant loads [13,14]. Each of these approaches contributes to the main goal of converting CW from a waste product into a valuable resource for very different sectors such as food industry, pharmaceuticals, healthcare, cosmetics, and bioenergy.

Leveraging CW strategically not only boosts the income of cheese producers but also significantly reduces the environmental impact stemming from its disposal. Such practices are in harmony with the wider goal of fostering sustainable and environmentally sound methods within the dairy sector. With ongoing research, the scope of cheese whey's applications is broadening, presenting optimistic opportunities for economic gain and environmental stewardship.

2. OBJECTIVES

The principal objective of this study is to conduct a literature review on the environmental impacts of cheese whey as waste and its economic valorization through advanced processing techniques. This includes a special focus on cheese whey treatment methods, such as anaerobic digestion and membrane-based separation processes, aimed at transforming these effluents into commercially viable and environmentally sustainable products.

The specific objectives are:

– To study the composition, components and characteristics of main by-products generated during cheese making, including sweet and acid cheese whey and second cheese whey.

– To conduct a detailed review of existing literature from 2010 to 2023 on the various methods and technologies for cheese whey economic valorization.

– To assess and quantify the environmental impact of cheese whey waste in the dairy industry, focusing on energy demand and carbon footprint, and life cycle assessment.

– To investigate and delineate effective valorization strategies for cheese whey effluents, focusing on their direct applications and the main treatments applied, including biological and physicochemical processes. This objective aims to explore the transformation of cheese whey into value-added products, thereby enhancing its economic viability and reducing environmental impact.

– To critically analyze the current trends and challenges in the management and valorization of cheese whey, assessing advancements in technology, market dynamics, environmental impact, and regulatory frameworks. This objective seeks to identify key opportunities and obstacles that shape the future potential of cheese whey utilization in various industries.

3. METHODOLOGY

3.1. Research strategies

A systematic literature search has been carried out using Scopus as the database to collect all relevant information on the study objectives. Initially, the keyword "Cheese Whey" was used, selecting the option to find it only in the title, in order to observe all available specific information on CW and, consequently, to compile that which might be useful in determining the main points of the work.

The publication years were limited between 2010 and the present (2023), as there was an interest in classifying and organizing the latest information available on CW. The bibliographic search found a total of 1004 citations (last updated on 29 November 2023). As can be seen in Figure 1 (data obtained from Scopus), the publication of studies on this subject has shown a significant increase in recent years, specifically from 2012 and 2018 onwards (>70-80 studies/year). The graph thus shows that it is a current topic that is expanding (see Figure 1).

These citations primarily consist of Articles (908), Conference Papers (46), Reviews (29) and Book Chapters (15), among others. Notably, these studies have been published in prestigious, high-impact journals (Q1 and Q2), including the Journal of Dairy Science (38), International Dairy Journal (36), International Journal of Hydrogen Energy (31), Foods (29) and Lwt (27), among others. In terms of the countries to which the research groups behind these publications are affiliated, Brazil (142 documents), Italy (128), Spain (94), Greece (77), and Portugal (73) are the top contributors in that order. The research groups that have generated the publications belong to the following universities (Figure 1): Universidade de São Paulo, University of Patras, Consiglio Nazionale delle Ricerche, Universidade Federal de São Carlos, Universidade do Minho, Universidade Estadual de Campinas, Università degli Studi di Padova, among others. Spain holds the third position among the countries. The universities and research institutions with the highest productivity in this field within Spain include the University of Extremadura (11 publications), the

University of León (8), the University of A Coruña (8), the University of Santiago de Compostela (8) and the Spanish National Research Council (CSIC, 7).

Furthermore, these publications span across various fields of knowledge, with the majority falling within Agricultural and Biological Sciences (520), followed by Chemical Engineering (248), Biochemistry, Genetics, Molecular Biology (247), and others. It's noteworthy that Chemical Engineering occupies the second position (accounts for approximately 11.7% of the total publications) among the areas of knowledge represented in these publications. This underscores the importance of the 'cheese whey' topic within the realm of Chemical Engineering. The substantial presence of research articles and contributions from experts in this field highlights the growing significance and interest in addressing challenges related to CW processing and utilization within the Chemical Engineering community. This trend suggests a heightened focus on sustainable solutions and innovative technologies for maximizing the value of this dairy by-product, contributing to both environmental sustainability and economic efficiency.

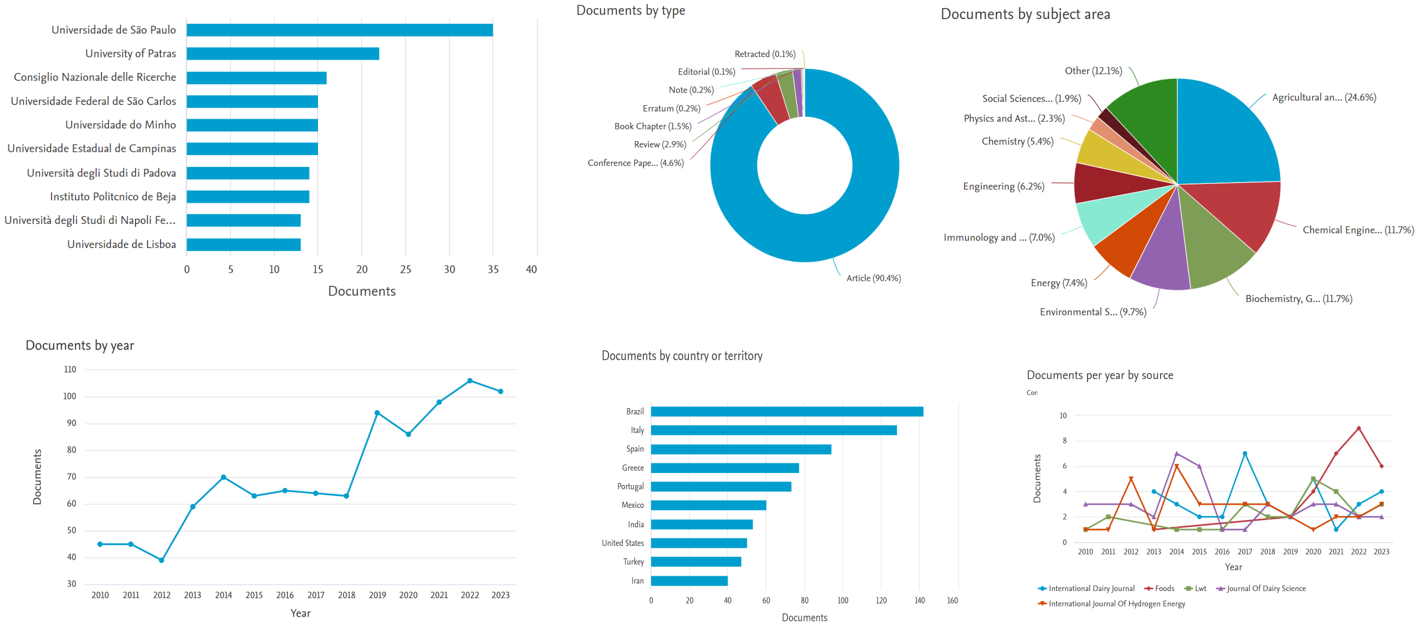

Figure 1. Publications of documents and their characteristics about the topic Cheese Whey in the year range of 2010 – 2023. Font: Scopus.

3.2. Results and Selection of articles

Figure 2 shows the search, screening, and inclusion criteria applied in the bibliographic analysis. The initial search using "Cheese Whey" yielded 1663 results (last updated on 29 November 2023) in Scopus (year range 1921-2023), searching within just the article title. Then some filters were added, raising the year range from 2010 to 2023 articles to get 1004 articles. From these documents, mainly articles, the ones that were useful according to the title and abstract and presented relevant information to meet the specific objectives were selected. Several lists were created within Scopus to organize the information found by subsections. Then, the keywords “Cheese Whey Valorization”, “Cheese Whey & Process”, “Cheese Whey & Technologies” “Cheese Whey & Value Added”, “Cheese Whey & Sustainable”, “Cheese Whey & Strategies” were also used, thus increasing the number of selected articles, and conducting a more in-depth and detailed search of each specific section. Finally, articles from other sources were added to complement specific information, resulting in a total of 123 studies. Therefore, only those research papers that present information on CW and the specific objectives of the work were included. The language of the bibliography was entirely in English.

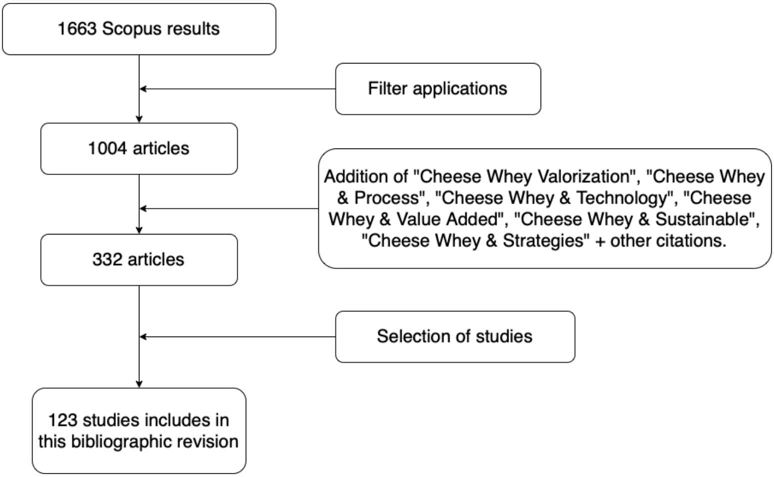

Figure 2. Flow diagram of the search and article selection process.

4. CHEESE WHEY

4.1. Cheese by-products

In the cheese-making industry, approximately 10% of the initial milk is transformed into cheese, leaving the remaining 90% as cheese whey (CW). It is estimated that the production of 1-2 kg of cheese results in the generation of 8-9 kg of whey. Thus, from every 100 kg of milk used in cheese manufacturing, around 9.3 ± 0.7 kg of cheese and nearly 90.7 kg of whey are produced [15].

Primarily, the most significant by-product in cheese making is CW, which is distinguishable by its yellow-green tint, attributed to riboflavin (B2 vitamin), and it comprises approximately 65 g of total solids per liter. Occupying 85-95% of the milk's volume, CW preserves nearly 55% of the nutrients in milk and about 20% of its total protein content [16]. The specific composition of CW is influenced by the milk's source, the variety of cheese being produced (whether using rennet or acid for coagulation), and other factors impacting milk's composition, such as the breed of the cow, seasonal variations, diet, and the phase of lactation [15,16].

Cheese whey is categorized into two main types: sweet and acid whey, with the processing method determining the type. Sweet CW typically has a pH between 6-7 [17,18]. In cheese and CW production, the initial step involves adding rennet (animal coagulant from the stomachs of lactating mammals rich in chymosin) to the milk. Rennet facilitates the coagulation of casein in the milk, resulting in curd formation, which is then separated from the remaining liquid (CW). Normally this type of CW is associated with the production of fresh cheese without lactic starter addition. Acid CW, characterized by a pH range of about 4.5-5.0 [17,18], is a by-product of acid-coagulated cheeses like Cottage cheese or a by-product of ripened cheeses where cheese milk is inoculated with different lactic starters. In both types of cheeses, the lactic acid bacteria in the starter hydrolyze lactose into lactic acid, producing the acidification of CW. While acid CW typically has a lower lactose content, its mineral content usually surpasses that of sweet CW because starter acidification produces a demineralization of the milk casein, transferring minerals, mainly calcium and phosphorus, to the CW [18,19].

Second cheese whey (SCW) is the by-product from the deproteinization process of CW. In some Mediterranean countries including Spain, CW is employed in the production of whey cheeses like Requeijão, Requesón, Ricotta, among others (see 6.1 Section). Its production involves heating the whey to temperatures of about 85-90 °C for 20-30 min, aiming to denature the proteins in the whey. The liquid remaining after whey protein separation represents more than 90% of the original whey and is called SCW. On the other hand, one of the most important and conventional solutions for the treatment of CW include the production of dehydrated whey products such as whey powder (WP), whey protein concentrates (WPC), and whey protein isolates (WPI) (see 6.2.2.2 Section). To produce these whey protein products, separation technologies utilizing ultrafiltration membranes are employed. These technologies concentrate the solids present in CW, resulting in a concentrated fraction primarily rich in protein. Simultaneously, a by-product known as permeate, which can also be referred to as SCW, is generated. Lactose (4.8–5.0%), salts (1.0–1.13%), and proteins (0.15–0.22%) generally compose SCW, depending on its CW origin [20].

4.2. Cheese whey components

As previously indicated, CW is classified into acidic CW ($pH < 5$) and sweet CW ($6 < pH < 7$), based on the casein coagulation process. Acidic CW generally has fewer proteins and a distinct flavor and salt content that limits its use in food applications [21]. The primary differences between these two types of CW lie in their mineral content, acidity, and the composition of their whey protein fractions (see Table 2).

In terms of nutritional content, CW is rich in lactose (45-50 g/L), soluble proteins (6-8 g/L), fats (4-5 g/L), and a range of mineral salts (making up 8-10% of its dry content), primarily consisting of NaCl, KCl, and various calcium salts [11]. Additionally, it comprises notable amounts of lactic and citric acids, non-protein nitrogen elements (such as urea and uric acid), and a proportion of water-soluble vitamins found in milk, specifically 55-75% of vitamin B6 and pantothenic acid to 80-90% of thiamine, nicotinic acid, folic acid, and ascorbic acid [22].

Table 2. Types of whey and their composition [21].

Type	Sweet whey	Acid Whey
Water (%)	93.0 – 94.0	93.0 – 95.0
Protein (g/L)	6 - 8	6 - 7
Lactose (g/L)	46 - 52	44 – 46
Minerals (g/L)	5.0	7.5
Lactic Acid (g/L)	2.6	4
pH (units)	6 - 7	4.5 – 5

Protein composition in CW includes primary proteins (β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, and proteose peptones) and secondary proteins (encompassing lactoferrin, lysosome, glycomacropeptide, phospholipoproteins, and lactoperoxidase) (see Table 3) [23]. These proteins have high nutritional value, comparable to egg protein, and contain all 20 amino acids, including the nine essential ones. The presence of sulfur-rich amino acids, specifically methionine and cysteine, contribute to its health benefits [24].

Table 3. Whey protein composition and characteristics [25].

Protein	Whey content (%)	Molecular mass (kDa)	Isoelectric point (pH)
β-lactoglobulin	30.0 – 55.0	18.3	5.2
α-lactalbumin	20.0 – 25.0	14.2	4.5 – 4.8
Bovine Serum Albumin (BSA)	5.0 – 10.0	66.5	4.7 – 4.9
Glycomacropeptide (GMP)	10.0 – 15.0	7.0	4.0
Immunoglobulins (Ig)	10.0	150.0 – 1000.0	5.0 – 8.3
Lactoferrin (Lf)	1.0 – 2.0	78.0	8.0
Lactoperoxidase (Lp)	0.5	80.0 – 89.0	9.5

Regarding vitamins, CW includes a proportion of the water-soluble vitamins found in milk. These range from 55-75% of vitamin B6 and pantothenic acid to 80-90% of thiamine, nicotinic acid, folic acid, and ascorbic acid [22].

This composition, along with its mineral and amino acid profile, highlights CW's potential to be exploited and valorized in numerous applications that are listed below, including the production of bio-value added food products with potential benefits health effects [26].

5. ENVIRONMENTAL IMPACT

5.1. Energy demand

In recent years, specifically over the last half-decade, cheese consumption has increased by 5%, with Cheddar cheese emerging as the preferred variety [27]. In the context of energy consumption, cheese manufacturing ranks as one of the highest sectors. Several life cycle assessment (LCA) studies have established that the production of milk is the most significant environmental concern in cheese manufacturing [28,29]. The LCA approach is crucial in calculating the carbon footprint associated with greenhouse gas (GHG) emissions for these units, expressed in terms of global warming potential (GWP) that later on will be discussed.

Regarding CW, it possesses high levels of both BOD and COD. This makes improperly disposed whey a significant environmental hazard, as outlined in Table 4. However, it is important to note that the reported values for BOD and COD in whey can vary widely.

Table 4. Biochemical and chemical oxygen demand of cheese and second whey [30,31,32].

By-product	Biochemical oxygen demand (BOD) (g/L)	Chemical oxygen demand (COD) (g/L)
Cheese Whey	30 – 50	60 – 80
Sweet Cheese Whey	40 – 102	27 – 60
Acid Cheese Whey	52 – 62	35 – 51
Second Cheese Whey	27	58 – 79

For every kilogram of milk fat, lactose, and protein, the COD values are respectively 3 kg, 1.13 kg, and 1 kg [33]. As previously mentioned, CW is composed mainly of water (93.0–94.0%), with lactose (4.5–6.0%), proteins (0.6–1.1%), minerals (0.8–1.0%) and lactic acid (0.05–0.9%). Lactose, constituting 70–75% of the total solids in CW, is the primary contributor to about 90% of its COD and BOD values. Comparatively, acid CW has a lower lactose concentration (44–46 g/L) than sweet CW (46–52 g/L), which might explain the generally lower COD and BOD values observed [19].

Although CW can be transformed into secondary products like ricotta, this process generates SCW biowaste as mentioned earlier. EU and national regulations now ban traditional disposal methods of such waste due to its environmental harm. Moreover, using waste CW as animal feed is increasingly discouraged at a high quantity due to its high lactose content potentially harming animal health. This especially occurs in areas far from processing plants, because of transport costs [34].

As stated in section 4.1, when CW is deproteinized generates the SCW. Similar to CW, the SCW is also a highly polluting effluent with a negative impact on aquatics environments. SCW has ~60% of the total solids of the original CW, maintaining a significant organic matter content (COD up to 80 g/L), and once more, lactose content (~50 g/L) is the main component responsible for the high COD (>70 g/L).

Numerous analyses of GHG emission data support the development of LCAs, with the GWP outputs from LCAs forming the basis of carbon footprint reports for products [35]. Dairy products are identified as using a lot of energy in terms of GWP, leading to a greater need for more energy-efficient heating processes [36].

Cheese whey powder manufacturing exhibits a higher GWP compared to other dairy products like milk powder. The energy needed to produce and store cheese is about 0.91 kWh per kilogram of cheese [37]. Typical energy consumption and GWP for various dairy products are summarized in Table 5 for reference.

Table 5. Total global warming potential of dairy products [38,39,40,41].

Dairy Product	Global Warming Potential (kg CO_2 eq./kg)
Whey powder	13.2
Whole milk	1.6
Butter	10 - 39
Milk powder	12.4
Dried whey (animal feed)	12.1
Cheddar cheese	14.0
Yoghurt	1.4 – 3.3

5.2. Life cycle assessment

Life cycle assessment is an organized tool, defined by ISO 14040: 2006, generally used to assess the environmental impact throughout product life cycle, from the beginning to recycle or disposal [38]. This process evaluates and reports the potential impacts on nutrient pollution and ozone harming [42]. It also determines the GWP [43,44].

While cheese production requires electricity, the by-product, CW, can be transformed into electrical power, potentially reducing the overall ecological footprint. Acid CW can be used as a feedstock for generating biogas, which in turn can produce electricity, serving as a sustainable energy alternative. Both acid CW and sweet CW can replace fossil fuels, offering the needed energy for most dairy operations through the anaerobic digestion of cheese whey. It is estimated that 78-85% of the carbon footprint in the dairy sector occurs before the product leaves the farm, primarily due to methane from livestock digestion and nitrous oxide from fertilizer use. These gases account for 70-90% of total GHG emissions at the farm level. In contrast, post-farm activities like packaging, transportation, and fossil fuel use contribute about 8, 4, and 3% to total emissions, respectively, in a typical dairy processor's supply chain [37]. There is ongoing research on LCA in livestock operations, with recent reevaluations indicating a need to reconsider the climate impact of these systems [45].

A significant advancement in LCA and carbon footprint analysis in dairy production is looking at how GHG emissions can be reduced based on efficiency, using all raw materials, and considering the amount of product made. It is pointed out that food items are often produced for multiple purposes. For instance, cattle farming produces both dairy and meat as primary products, with several other supply chains linked to the raw material. Such models have been explored using LCA techniques, proposing innovative approaches to reevaluate milk and meat systems, potentially greatly reducing their environmental impact [46].

The environmental impact of the dairy industry is difficult to define because there are so many producers and not enough data on how whey is used or disposed of. Some studies suggest that using CW wisely could reduce the environmental impact of some cheeses by up to 15% [47, 48]. However, smaller producers might not be able to use these methods due to cost and lack of resources, though they can sell the whey for other uses.

5.3. Carbon footprint assessment

Businesses around the world need tools to evaluate and lessen their role in global warming, especially now that they are required to look at GHG emissions from their supply chains and the energy they use directly. Many companies figure out their total carbon footprint (CF) by adding up emissions from energy use and transportation. However, this method does not account for differences in total production among companies, varying grazing systems, or yearly changes [37].

Arla Foods developed a way to calculate the CF from the farm to the customer for different dairy products [37]. Since the biggest part of a dairy product's CF comes from the farm, it is important to decide how to assign the emissions from raw milk to various products. Emissions linked to raw milk are divided based on fat and protein content. Arla Foods' data shows different levels of carbon dioxide equivalents for various dairy products. A key issue is how they handle the by-product CW. Their current method does not address the negative effects of producing CW, as dairy producers are not paid for the lactose in milk. Therefore, products with whey seem to have a lower impact. The model needs to be improved to accurately reflect the impact of all dairy components. This method also overlooks the environmental harm from producing CW and milk powder after leaving the farm [37,41,49].

A more universal analysis uses a matrix to measure product resource use, taking into account the advantages of not generating waste and using or valuing by-products like CW. This is known as the input-output LCA approach. It calculates average operational data for each dairy product, including the solid content of milk and dairy products. This method ensures dairy products are assigned impacts from farm activities. However, it relies on similar technology levels in different manufacturing facilities [50]. The International Dairy Federation recommends this approach, assigning specific values to different inputs, with raw milk divided based on milk solids. Thus, higher milk solids mean a higher CF, but it does not differentiate between types of milk solids [37]. This might miss the greater environmental impact of different milk components with higher COD values [33].

6. VALORIZATION STRATEGIES FOR CHEESE WHEY

The concept of CW valorization revolves around the extraction and transformation of its components into commercially and environmentally beneficial products. This approach aligns with the principles of circular economy and sustainability, aiming to minimize waste while maximizing resource efficiency.

6.1. Direct uses

The disposal of CW is distinct from its utilization. Disposing of CW often involves spreading it on fields, allowing it to oxidize in lagoons or sewage systems, diverting it into caves, discharging it into oceans, or other forms of discarding. This practice is becoming less common in developed nations but continues in some regions with lenient regulations on food industry waste. However, CW can serve beneficial purposes, such as irrigating farmland, where it delivers water and essential nutrients to the soil, enhancing plant growth [51,52].

Traditionally, CW has been applied to land due to its potential as a fertilizer, rich in salts, organic matter, and nutrients like phosphorus, nitrogen, calcium, sodium, potassium, magnesium, chloride, etc. For instance, 3.7 thousand liters of CW contain minerals equivalent to 5.9 kg of potassium sulphate, 36 kg of ammonium sulphate, 12 kg of superphosphate, and 6.8 kg of calcium carbonate. This approach is suitable for neutral to slightly alkaline soils. In contrast, acid soils may experience compaction and reduced microorganism activity, hindering nutrient biodegradation. Spraying CW is preferable as it prevents stagnant pools that can inhibit soil microorganisms' efficiency. The application limit for land fertilization is approximately 110-220 tones/ha to avoid environmental risks like pollution from runoff and odor issues. CW enhances soil fertility, improving nutrient content and soil structure for better water infiltration. However, transporting CW, which is 92-93% water, can be costly, and its application is limited by seasonal conditions [53].

Animal feed

One of the ways to utilize the CW is its direct use in the preparation of animal feed on farms is an effective method. This involves substituting fresh liquid CW for water in feed formulations, leveraging existing supplies to enhance nutrition. Ruminants (cow, goat and sheep), for instance, can consume up to 30% of their dry matter intake as liquid CW with positive outcomes. In contrast, swine exhibit diarrhea if their dry matter intake exceeds 20% liquid CW [5,8,40]. Practical experiences from various farms indicate that while most dairy cattle adapt to consuming fresh-tasting whey, particularly if introduced at a young age, a minority may reject it. These animals should be segregated and provided with water to prevent dehydration. Initially, adopting a free-feeding approach, where animals have the choice between whey and water, is advisable. Introducing CW into the diet of farm animals can lead to significant feed cost savings, estimated at about one-third of the total feed expense. This system is particularly recommended for cheese producers generating less than 125,000 kg of whey daily. To minimize transportation costs and maximize efficiency, the ideal operational radius from the cheese plant is suggested to be ~40 km.

Whey cheese

As it was commented CW is the part of milk which remains after the removal of the coagulum formed during cheese manufacture, and some cheese industries use it directly to produce the so-called whey cheeses. Whey cheeses belong to a special category of cheeses that are produced by heating the whey at high temperatures (88-92 °C) in the presence or not of acidifying agents to form a coagulum following the denaturation of whey proteins. The first small particles of denaturated whey proteins appear at temperatures ~80 °C The rate of heating is important to be such as to attain the selected temperature in 40-45 min, and another critical step is the rate of stirring. The curd obtained is left for 15-20 min on the surface at the selected temperature to lose part of its moisture and then it is scooped and molded for drainage; whey cheese is mainly consumed fresh [19,54]. In the cases in which only the whey is used for the production of whey cheese, the particles of denatured proteins floating on the surface are small and the yield is low. Usually, a certain amount of milk and/ or cream is added during heating (the quantity of milk is 30-50 mL/L) however, for higher quality cheeses, milk percentage could be 10% or higher, and cream may also be added [55].

Whey cheeses are globally produced by traditional methods either as artisanal or standardized industrial processes as a sustainable method of utilizing the CW according to the principles of circular economy [19]. There are many whey cheeses that were originally produced from sheep or goat whey as by-products. Nowadays, since their consumption has increased, dairies have started to produce whey cheese from cows' whey or add cows' milk. Most of them are heat- and/ or acid-coagulated cheeses, and their technology varies in respect of the raw material (type of whey) used, the quantity and type of the milk and/or cream added, and the cheesemaking recipe followed, and then whey cheeses are, nowadays, produced globally, under various names: Ricotta, Ricotta salata or Ricottone, and Ricotta fresca in Italy; Anthotyros, Myzithra, Manouri, Xynomyzithra, and Urda in Greece; Urda in Serbia and Romania as well as in other countries such as Israel; Lor in Turkey; Anari in Cyprus; Skuta in Croatia and Serbia; Gjetost and Brunost in Norway; Mesost and Messmor in Sweden; Mysuostur in Iceland; Myseost in Denmark; Requeijao in Portugal; and Requesón in Spain and Mexico. Among the different whey cheeses, Ricotta di Bufala Campana and Ricotta Romana in Italy, Brocciu corse in France, Manouri and Xinomyzithra Kritis in Greece, and Requeijao da Beira Baixa and Requeijao Serra da Estrela in Portugal, all having proved connection with regional history and culture, have been designated as protected designation of origin (PDO). Some important whey cheeses and their composition and processing characteristics are shown in Table 6.

Beverages direct from CW

As a recent innovation, beverages based on CW have undergone a significant transformation, advancing from being merely discarded by-products to becoming popular beverages. Their rise in popularity is due to their rich nutritional content and their versatility in meeting the evolving tastes and demands of consumers. These drinks present a wide range of choices, including protein shakes, fruit smoothies, sports drinks, and carbonated beverages, therefore offering a broad and appealing range for the consumer market [56,57,58]. A primary factor in the increasing appeal of CW-based beverages is their outstanding nutritional composition. CW is rich in essential amino acids, crucial for muscle development, restoration, and general health. Furthermore, it is a source of vitamins, minerals, and antioxidants, all contributing to overall health and wellness. The direct integration of CW into beverage formulas allow producers to provide convenient and enjoyable means for consumers to access these vital nutrients.

Table 6. Composition (%) and specific processing conditions of whey cheeses [54].

Cheese	Country	Milk source	Processing conditions	pH	Fat (%)	Protein (%)	Salt (%)	Moisture (%)
Anari (fresh)	Cyprus	S,G	Fresh Anari has a short shelf life, that is 2-3 days once packaging is opened.	-	21.7	11	–	65.4
Anthotyros	Greece	S, G	Sheep or goat cream and/ or milk is added.	6.4	16.6	6.9	–	68.4
Bracka skuta	Croatia	S	The whey is heated until proteins are coagulated and then is further boiled at 95-97 °C.	6.5	27.9	10.9	–	58.7
Lor	Turkey	S, G, C	The raw cheese is strained in a hemp or other straining cloth and press is applied.	-	5.3-15.3	9.7-13.5	0.9	64.3-72.4
Manouri PDO	Greece	S, G	Salt is added at 1% and milk and/ or cream up to a proportion of 25%.	5.9	36.7	10.9	0.8	48.1
Mesost	Sweeden	G, C	The whey is boiled to evaporate water.	–	17.0	–	–	20.0
Requeijao	Portugal	S, G	90% S whey and 10% G milk; at 95 °C for at least 15 min under stirring; this cheese is usually eaten fresh.	–	29.5	8.5	–	59.0
Requesón	Spain, Mexico	S, C, G	10% milk is added to the whey.	6.1	4.1-13.0	5.1-7.0	–	–
Ricotta di Bufala Campana PDO	Italy	C	Sweet whey is used, mixed with 5-10% milk, salt is added at 0.1% and citric acid at 0.11 kg/Land heating is carried out up to 80-85 °C	6.2-6.7	–	–	–	–

B: buffalo, C: cow, G: goat, S: sheep, –: not available.

Moreover, the popularity of CW-based drinks is driven by how they fit with current consumer trends, natural and healthy products. Today's consumers are more aware than ever of their dietary choices, preferring products crafted with natural and wholesome ingredients. Additionally, employing CW in beverage manufacturing promotes sustainability within the food sector. By transforming CW into nourishing drinks, the industry not only reduces food waste but also optimizes the use of existing resources.

6.2. Cheese whey treatment and valorization

Various treatment methodologies have been developed and optimized to harness the potential of CW. These treatments can be broadly categorized into physical, chemical, and biological methods, each with unique mechanisms and value-added products. The choice of treatment largely depends on the desired final product, economic viability, and environmental considerations.

Further exploration of the subject will reveal specific treatments available for CW valorization that will be explored, detailing their processes, applications, and the innovative products they yield. These treatments not only contribute to environmental sustainability but also add economic value to the dairy industry, demonstrating the potential of waste-to-wealth strategies in modern industrial practices. Figure 3 shows various applications and technologies associated with CW.

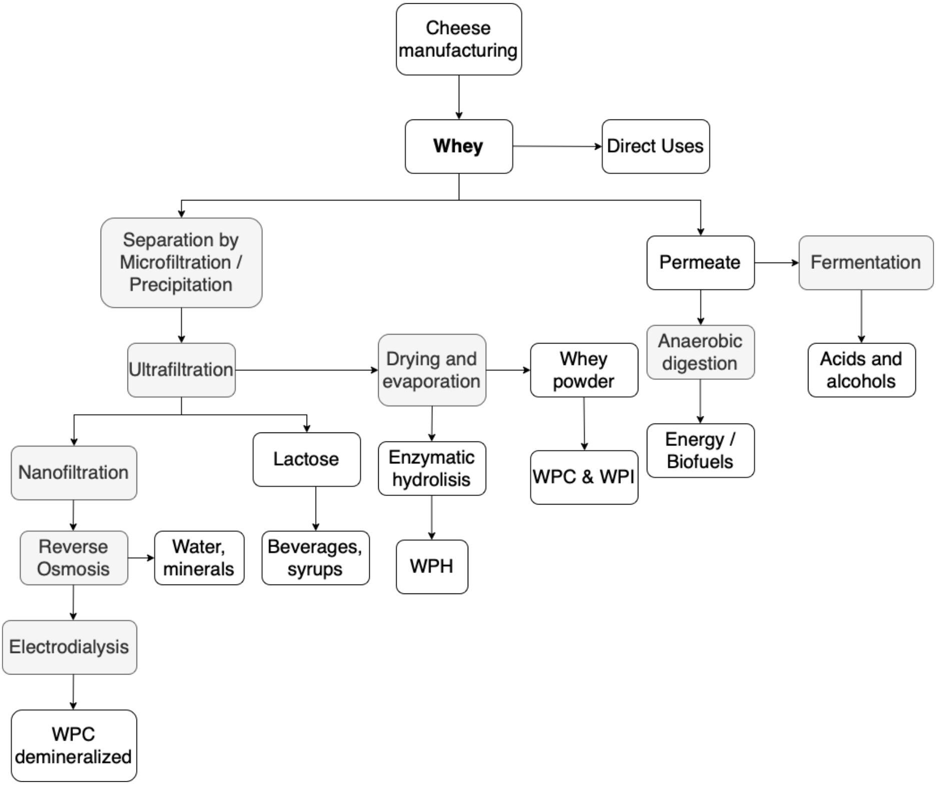

Figure 3. Various strategies and techniques utilized for converting whey into valuable products. Font: own elaboration.

6.2.1. Biological treatments

Biological processing of CW offers numerous benefits compared to direct approaches. These methods operate under normal environmental conditions and employ microorganisms to generate products with added value while also reducing the COD in CW. This makes them practical, cost-effective, and eco-friendly. Lowering the expenses associated with CW conversion renders these techniques a promising option for whey valorization, enabling its transformation into products for rising markets.

Given the variety of methods and technologies available for discussion, this TFG will focus on examining the two most significant ones: the anaerobic digestion and selected types of fermentation.

6.2.1.1. Anaerobic digestion

Anaerobic digestion (AD) can be a triple action process for CW treatment: it reduces pollution, generates energy, and recovers nutrients. The effectiveness of AD in treating CW is influenced by several factors: the composition of the CW, including organic content and tendencies towards low alkalinity and rapid acidification; the type of inoculum used, particularly its ability to buffer; and the design of the reactor, especially the use of effluent recirculation [59]. Anaerobic digestion of CW, conducted under thermophilic or mesophilic conditions, involves the hydrolysis of proteins and lactose in absence of oxygen. Proteins, including resistant ones like casein, are broken down by proteases into polypeptides, amino acids, and ammonia, while hydrocarbons like lactose are more easily converted into propionic acid, ethanol, acetate and most important, methane. However, breaking down lactose can sometimes slow down the production of methane, a key part of this process [60]. Figure 4 provides an overview on the AD process in CW, focused on obtaining methane as it is one of the primary biogas produced.

This technology is highly efficient in organic removal but faces challenges due to low alkalinity, a buildup of fatty acids, making the environment too acidic. This happens because the acids are produced faster than they can be broken down. Such imbalances can cause operational issues [60,61]. A two-phase configuration, separating acidification and methanogenic phases, has been proposed for improved efficiency and stability [62,63]. This two-phased configuration will be discussed further later on. Additionally, the biodegradation of lipids presents challenges, with fats causing sludge flotation and, furthermore, high COD concentrations in AD can increase viscosity, impacting biomass granulation and leading to flotation [64].

As previously mentioned, this process is a key method for producing bioenergy (in the form of biogas). Biogas is primarily used for combined heat and power generation and is upgraded to biomethane and hydrogen, which are its two most significant applications, for integration into natural gas networks or for transportation purposes.

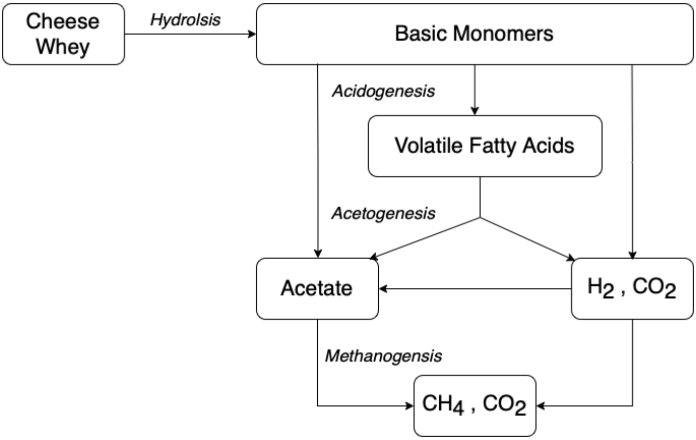

Figure 4. Phases in a CW anaerobic digestion process. Font: own elaboration.

Anaerobic digestion can be executed in two ways: as a batch process or a continuous process. In a batch process, CW is introduced into the reactor at the beginning, and then the system is sealed for the entire duration. On the other hand, in continuous digestion processes, CW is steadily or periodically added to the reactor throughout the operation. Considering these two, the majority of the mots studied and utilized processes are continuous. A variety of reactor designs are employed in the industrial anaerobic treatment. The most common types are the continuous stirred-tank reactors (CSTR), the upflow anaerobic sludge blanket (UASB) reactor and the expanded granular sludge bed (EGSB) or anaerobic fluidized bed (AFB) reactors.

Continuous stirred-tank reactor

CSTR is the basic anaerobic treatment system based in the ideal CSTR which assumes perfect mixing. This reactor maintains equal hydraulic retention time and solids retention time, usually within 15-40 days. This duration is crucial for operational efficiency and process stability. Moreover, this reactor is excellently suited for processing CW due to its high COD content [65].

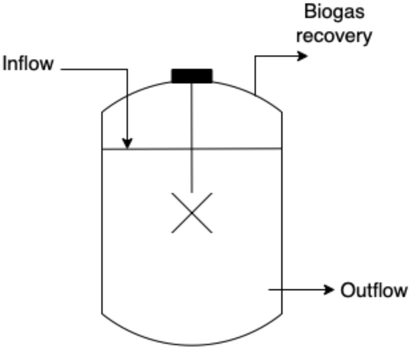

Figure 5. Continuous stirred-tank reactor (CSTR). Font: own elaboration.

Upflow anaerobic sludge blanket reactor

Effluent entering at the base of a UASB reactor moves upward through the sludge bed and exits around the periphery of a funnel-shaped outlet. This design increases the surface area for the effluent, thereby reducing upflow velocity and enhancing both the retention of solids within the reactor and the efficiency of solid separation from the outgoing wastewater. Over several weeks, granules naturally form in the reactor, predominantly comprising a dense, mixed bacterial population that facilitates the methane fermentation of substrates. UASB systems are known for their effective settleability, short retention times, absence of packing material costs, high biomass concentrations, superior solid-liquid separation, and capability to operate at very high loading rates [66].

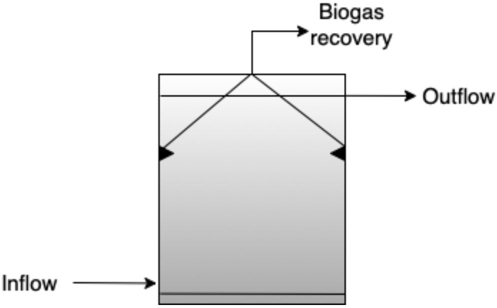

Figure 6. Upflow anaerobic sludge blanket reactor (UASB). Font: own elaboration.

Expanded granular sludge bed reactor and anaerobic fluidized bed reactor

The unique characteristic of the EGSB and AFB reactors is its design for a higher upward-flow velocity for CW wastewater moving through the sludge bed. This increased flow rate allows for a partial expansion (fluidization) of the granular sludge bed, which in turn improves the contact between the wastewater and the sludge, and also helps in separating smaller, inactive suspended particles from the sludge bed. Achieving this higher flow velocity is typically done by using taller reactors, adding an effluent recycle system, or a combination of both [67].

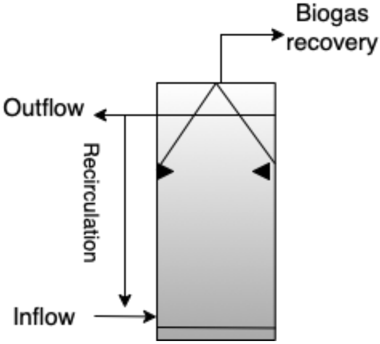

Figure 7. Expanded granular sludge bed (EGSB) or anaerobic fluidized bed (AFB) reactor. Font: own elaboration.

As already pointed out before, there are two notable AD process settings for CW treatment, the traditional single-stage anaerobic digestion and the more recent, yet increasingly significant, two-stage anaerobic digestion, particularly in terms of enhanced biogas production. Figure 8 shows both one and two-stage AD diagrams.

In a single-stage digestion system all biological processes take place in a single, enclosed reactor or tank. This approach minimizes construction expenses but offers limited control over the reactions inside the system. Acidogenesis bacteria lowers the tank's pH through acid generation. As previously mentioned, methanogenic microorganisms function within a narrowly specified pH range. Consequently, the biological activities of these varied species in a one-stage reactor may directly conflict with one another.

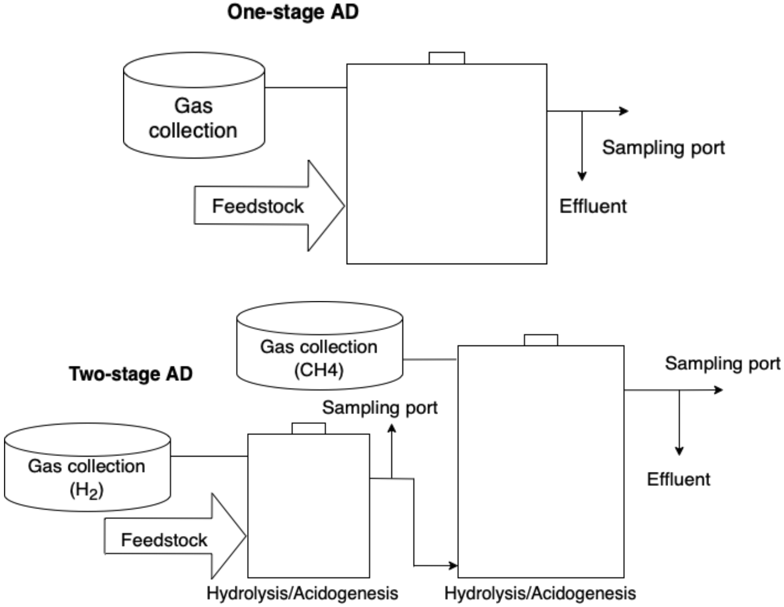

Figure 8. One-stage and two-stage anaerobic digestion diagram. Font: own elaboration.

The two-stage anaerobic digestion process, known for simultaneously generating hydrogen and methane, is gaining attention due to its several advantages. These include a higher yield of biogas, enhanced efficiency in organic matter degradation, and a reduced hydraulic retention time compared to the single-stage approach. This system involves at least two separate reactors, one for acidogenesis and another for methanogenesis. It caters to the distinct growth speeds and ideal pH levels required by acidogenic and methanogenic bacteria, each necessitating unique environmental and operational conditions [68]. Because of these benefits, dual-stage anaerobic digestion is becoming a more popular method for treating organic waste waters like CW. Furthermore, conducting the anaerobic digestion process under high temperatures (a thermophilic system) can accelerate the degradation of compounds, reduce the viscosity of the medium, and increase the solubility of gases. This leads to improved biogas production and yield, enhancing the overall digestibility of the system.

As noted in some studies [69], approximately 90% of the hydrolyzed organic material is transformed into biogas during the methanogenesis stage. Research indicates that one liter of CW can generate around 45 liters of biogas, which includes 55% methane, and the anticipated COD reduction rate is around 80%. From each liter of CW, it is possible to produce 20 L of CH_4, equating to about 700 BTU of energy [11]. Despite its energy generation potential and waste minimization benefits, AD's adoption in dairy industries is limited, primarily due to slow reaction speeds and the relative instability of the process in standard reactors [70,74]. However, as previously mentioned, it has been proven that it is a valuable source of energy. Table 7 showcases the varying levels of bioenergy (methane) production achieved by different continuous production systems, as studied in laboratory and pilot-scale experiments.

For small businesses that generate CW, the valorization of this byproduct may not be economically viable due to the limited quantities produced, which do not yield significant benefits. However, for larger companies that generate substantial amounts of CW, its valorization can be a profitable project. Despite the considerable initial investment required, these larger entities have the potential to realize meaningful profits from this process and so, mitigating the initial costs over time.

Table 7. Continuous production of methane in one-stage or two-stage reactors AD processes.

Process	Substrate	Reactor	Conditions	Methane production	COD removal (%)	Ref
One-stage	50% CW 50% cattle manure	CSTR	T = 35 ºC pH = 7.5	187 L CH_4/kg COD	82	[71]
	CW	UASB	T = 35 ºC	424 L CH_4/kg COD	95-97	[66]
	85% CW 15% dairy manure	CSTR	T = 35 ºC pH = 7	392 L CH_4/kg VS	-	[65]
Two-stage	Diluted CW	CSTR	T = 37 ºC pH = 8	300 L biogas/kg COD	99	[72]
	CW	CSTR	T = 35 ºC pH = 7.7	134 L CH_4/kg COD	95	[73]

COD: Chemical oxygen demand; VS: Volatile solids.

As an example of it Naskeo Environment [75], is a company dealing with the installation of AD systems. One of its projects is the installation of valorization of the soluble industrial CW of the Pays de Maroilles (North of France) using EGSB reactor. The average thermal power during the process is estimated 45 kW corresponding to approximately 175 m^3/day biogas production and the average effluent purifying output is approximately 99%.

6.2.1.2. Fermentation

Fermentation processes play a crucial role in the valorization of whey. It can be applied either to produce individual compounds or to formulate new foods and beverages. In the first case, a considerable amount of research has been directed to obtain biofuels able to replace those derived from petrol [76,77] like ethanol, butanol, hydrogen and lipids. In addition, the possibility of replacing petrol-derived plastics with biodegradable polymers synthesized during bacterial fermentation of CW has been sought. Further, the ability of different organisms to produce metabolites commonly used in the food and pharmaceutical industries like lactic acid, lactobionic acid, polysaccharides, etc. using CW as growth substrate has been studied. On the other hand, new low-cost functional whey-based foods and beverages leveraging the high nutritional quality of whey have been formulated, highlighting the health-promoting effects of fermented whey-derived products.

Other notable products to be discussed in this section are referred to as Aromas, Flavors, and Antioxidants (AFA), which are compounds with high market demand, widely utilized in food, cosmetic, and pharmaceutical sectors. Their primary function is to enhance the sensory qualities of products. Recognized as Generally Regarded As Safe (GRAS), these compounds are non-toxic and suitable for use in various consumer products including those ingested or applied topically. Common types of AFA include alcohols, lactones, aldehydes, ketones, fatty acids, esters, phenols, and organosulfur compounds, with many being volatile organic compounds (VOCs) [78]. The stability of aroma and flavor in products is significantly influenced by the interactions and reactivity of VOCs and adjacent substances [79]. The industrial production of VOCs is typically chemical, leading to potentially harmful byproducts like metals and persistent contaminants [80]. Cheese whey fermentation for AFA production is an alternative, offering lower energy consumption and avoidance of toxic by-products, thanks to the high efficiency of microorganisms.

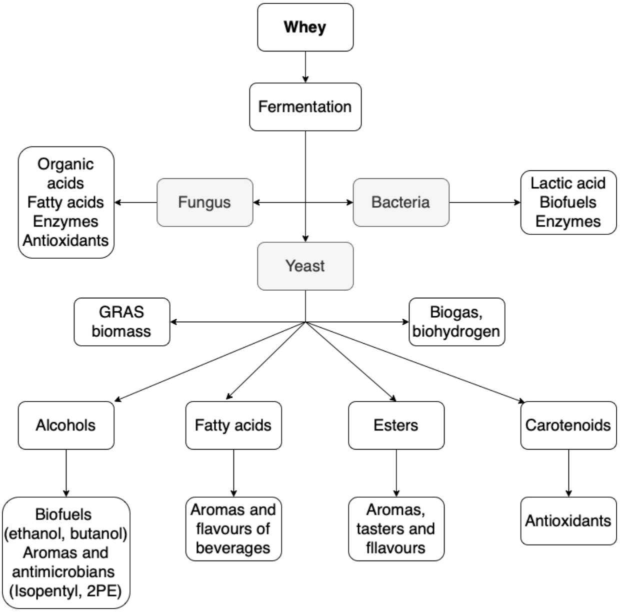

Figure 9. Products obtained by different biological treatments. Font: own elaboration.

Various fermentation techniques exist, each yielding unique end-products, fundamentally characterized by the employment of distinct microorganisms such as bacteria, fungi, and yeasts (see Figure 9).

Bacterial fermentation

Fermentation of CW using bacteria can lead to the generation of various compounds including organic acids, aldehydes, and ketones. A range of microorganisms, particularly those capable of utilizing lactose whey as a primary carbon source, are involved in this process. For instances where the microbial biomass is composed of health-beneficial microorganisms like lactobacilli and lactococci there exists potential for their use as probiotics, especially when encapsulated in stable solid formulations [81]. There are some significant compounds achievable through bacterial fermentation of CW, listed in Table 8.

Table 8. Compounds produced by whey biorefining with bacteria.

Microorganism	Substrate	Fermentation conditions	Product	Comments	Ref
Lactobacillus bulgaricus	Sweet CW	T = 37 ºC pH = 6.5 t = 72 h	Lactic acid	Use of bacteria co-culture Previous enzymatic lactose hydrolysis	[83]
Lb. helveticus	Ultrafiltrate CW	T = 42 ºC pH = 4.7 – 6.3 t = 24 h	Lactic acid	Accumulation of lactic acid. Optimum pH value of 5.9	[86]
Lactococcus lactis	CW	T = 30 ºC pH = 4.5 t = 18h	Acetoin	–	[85]
Streptococcus thermophilus	Sweet CW	T = 42 ºC pH = 4.6 t = 48 h	Acetaldehyde Acetone Acetic acid 2-propanol	Flask laboratory scale	[87]
L. lactis* subsp. *lactis	SCW	T = 30 ºC pH = 5.8 t = 24 h	Diacetyl Acetoin	Flask laboratory scale	[88]

CW: Cheese whey; SCW: Second cheese whey.

Lactic acid (LA) is a prominent value-added compound produced from whey fermentation, it serves as a food preservative and pharmaceutical precursor. Lactobacillus species are commonly used for LA production due to their selective sugar consumption ability, with a preference for glucose, followed by lactose and other sugars [82]. However, at high lactose concentrations, *Lactobacillus* sp. could inhibit substrate [83]. Also, excessive LA production can lead to acidification of the culture broth impeding bacterial growth [83,84].

Studies on CW fermentation using enterococci and lactococci strains have been conducted. It has been studied that these bacteria could produce various compounds, including furfural and ethylbenzene. However, the fermentation process has not being optimized, resulting in low yields [84]. These bacteria participate in citrate degradation during the tricarboxylic acid cycle, leading to the production of aromatic and fruity compounds.

Lactic acid bacteria also exhibit proteolytic activity, breaking down whey proteins. This activity is an adaptive response during whey fermentation and can be used to generate bioactive peptides from whey protein hydrolysate. These peptides exhibit antioxidant properties and lipid peroxidation inhibition, among other biological activities. The increase in antioxidant activity correlates with the biomass concentration and the extent of whey protein hydrolysis. These bioactive peptides can display hydrophobic characteristics, contributing to their antioxidant capacity [85].

Fungal fermentation

Fungi have advantages over bacteria due to their production of antioxidants. Table 9 shows some microorganisms for CW fermentation using fungi strains for their production. One of them is citric acid, an antioxidant that can be produced by fungi when stressed by adding substances that affect their cells, like methanol [89]. However, turning CW into citric acid using fungi has its challenges. This is because when lactose in whey is broken down, it makes galactose, a sugar that can cause issues. Galactose can interfere with how glucose is used and can affect important enzymes in the process of making citric acid [90,91].

Yeast fermentation

Yeasts are versatile in their enzymatic capabilities and can act as effective biocatalysts. For instance, certain yeast species are capable of breaking down lactose and utilizing the salts of common organic acids found in CW, like lactate and acetate [93]. These yeasts can convert biomolecules into AFA compounds even with minimal nutritional needs. Table 10 presents research on the use of yeast strains in CW fermentation for AFA compound production.

Yeasts, for example, synthesize esters through two primary enzymatic processes: esterification and alcoholysis [93]. Esterification is the combination of alcohols and carboxylic acids to form esters, whereas alcoholysis involves creating esters from alcohols and either acylglycerols or fatty acids [94,95,96].

Table 9. Compounds produced by whey biorefining with fungi.

Microorganism	Substrate	Fermentation conditions	Product	Comments	Ref
Aspergillus niger	Acid CW + sucrose	T = 30 ºC pH = 3 t = 20 days	Citric acid	Batch fermentation Improve of 750% of citric acid yield once sucrose was added	[91]
Aspergillus niger	Deproteinized sweet CW	T = 30 ºC pH = 3.5 t = 15 days	Citric acid	Batch fermentation	[89]
Blakeslea trispora	Deproteinized and hydrolized sweet CW	T = 26 ºC pH = 7 – 7.5 t = 12 days	β-Carotene γ-Carotene Lycopene	Batch fermentation	[92]
Lentinus edodes	SCW	T = 25 ºC pH = 5.5 t = 5 to 20 days	Phenols	–	[87]

CW: Cheese whey; SCW: Second cheese whey.

The literature indicates that yeast's ability to produce aromas and flavors is significantly influenced by the type and quantity of amino acids present, including branched-chain, aromatic, and sulfur-containing amino acids. These amino acids trigger the activation of genes responsible for producing fusel alcohols or acids [78]. On the other hand, ethanol is also in high demand. Ethanol production is more efficient than fusel alcohols, as it does not require additional energy input, with yeasts generating energy-rich molecules during this process. Fusel alcohol production is triggered by a lack of inorganic nitrogen, leading to the transformation of organic nitrogen into fusel alcohols [78]. Notable among fusel alcohols is 2-phenylethanol (2PE), known for its stability, rose-honey scent, flavor, and antiseptic properties [97].

Table 10. Compounds produced by whey biorefining with yeasts.

Microorganism	Substrate	Fermentation conditions	Product	Comments	Ref
Kluyveromyces marxianus	Sweet CW + $(NH_4)_2SO_4$ + L-phe	T = 30 ºC pH = 4.8 t = 96 h	2-phenylethanol	Batch fermentation Nitrogen starvation promoted	[98]
Kluyveromyces marxianus	Deproteinized acid CW	T = 30 ºC pH = 4 t = 2 h	Alcohols (isoamyl, isobutyl, 1-propanol, isopentyl) Ethyl acetate 2-phenylethanol	Continuous fermentation in air-lift bioreactor	[96]
Kluyveromyces marxianus	Sweet CW + urea	T = 32 ºC pH = 5 t = 24 h	Ethyl acetate	Batch fermentation Urea enables easy assimilation and trigger compound for EA	[95]
Rhodotorula glutinis	CW	T = 30 ºC pH = 6 t = 10 days	Carotenoids	Batch fermentation Yeast presented high affinity to lactose	[99]

CW: Cheese whey; EA: Ethyl acetate.

6.2.2. Physical treatments

Membrane separation technology stands as a groundbreaking innovation in the dairy industry. This technique involves using semi-permeable membranes to divide a liquid feed stream into two parts with different compositions [100]. The membrane allows certain substances to pass through while blocking others, and this selective process is key to the separation. The substances that go through the membrane with the liquid are called "permeates", while the liquid that does not pass through is known as "retentate" or "reject".

The two primary methods used in the dairy industry for whey protein separation are ultrafiltration (UF) and microfiltration (MF), mainly used to remove fat and proteins while nanofiltration (NF) and reverse osmosis (RO) are used for CW concentration of lactose and demineralization. However, effluents coming from micro and ultrafiltration stages still show a considerable residual COD as well as phosphate content. This is attributed to the fact that the first two still contain lactose [101,102]. Figure 10 illustrates the key features of various membrane filtration techniques, including pore size, molecular weight cut-off, and relative size of milk components, as well as the corresponding technologies such as MF, UF, NF and RO.

Membrane separation methods have unique advantages and are more effective than older methods. Firstly, they are non-thermal and eco-friendly, ensuring minimal temperature changes during the process and avoiding issues like protein damaging. These methods are also very selective, using special processes and materials that are particularly good at separating certain proteins. Advances in technology have made it possible to create custom membranes for specific industrial needs. Additionally, these systems are compact and require low maintenance, suitable for easy industrial applications and scalable by adding more modules. Importantly, the operation of these membrane modules is easy, not requiring specialized knowledge. This combination of environmental friendliness, precise selectivity, ease of scaling, and operation without high temperatures makes membrane separation a highly effective method in industries where maintaining the integrity of sensitive substances is essential [102]. Although these technologies are effective, they face economic challenges. The high-pressure requirements of membrane processes make them quite costly.

Additionally, few studies have indicated that after employing membrane separation techniques, most research focuses on applying fermentation as a post-treatment process to generate products such as ethanol, hydrogen, or lactic acid, as outlined in section 6.2.1.

As MF and UF are the most important and applied membrane separation processes to CW, they will be addressed with more emphasis in the following sections.

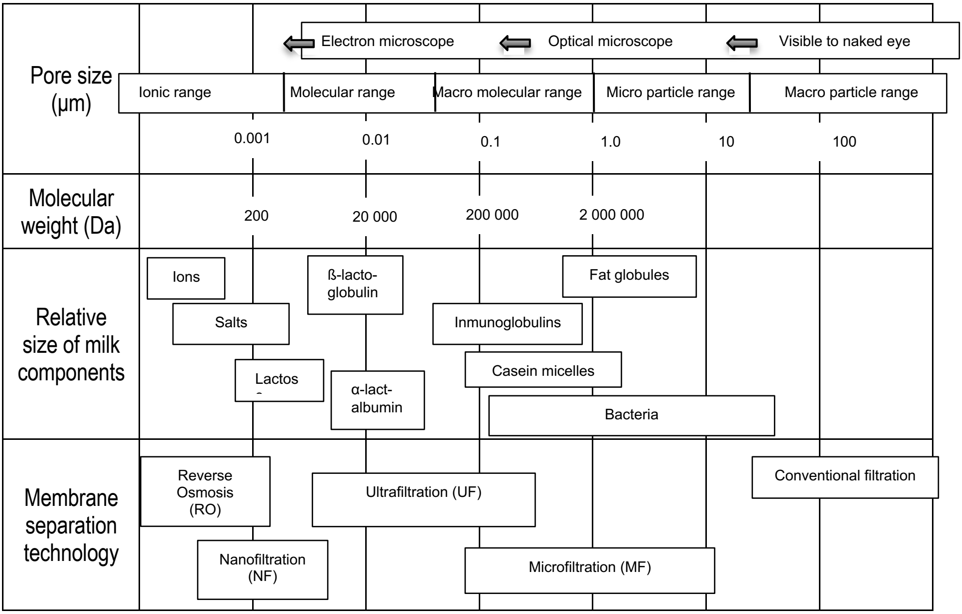

Figure 10. Membrane filtration techniques. Font: own elaboration.

6.2.2.1. *Microfiltration*

Microfiltration is a filtration method driven by differential pressure, usually under a pressure gradient between 10 and 200 kPa [103,104,105], essential for the separation of particles, organisms, and molecular entities within liquid media. MF is a critical process implemented across a multitude of industries such as pharmaceuticals, chemical production, mining, and the food sector. Functioning as an intermediary filtration technology, MF bridges the gap between UF and conventional filtration usually used a pre-treatment step to remove bacteria and fat, so that a fat-free and hygienic protein product can then be obtained by UF technique, without treating the CW with a heat treatment that would lead to denaturation of the whey protein.

As an established methodology, MF has a long history, with the earliest development of cellulose-based MF membranes traced back to more than a century ago [106]. The concept of lateral flow in MF came to prominence in 1907, recognized for its impact on the development of a filtration cake and the subsequent increase in filtration pressure. When using MF, it is important to keep the flow of filtered liquid steady, so this layer does not get too thick. MF uses special thin barriers with an average pore size between 0.02-10 µm [103,104,105]. Today's MF barriers are made with exact hole sizes, which means different parts of the same liquid can be sorted out by using a set of these barriers one after the other and use new resistant ceramic-type materials such as zirconium oxide that withstand aggressive cleaning systems.

Microfiltration, as an initial step in the processing sequence, does not yield a final product; instead, it serves as a foundational procedure in the overall treatment process.

6.2.2.2. Ultrafiltration

Ultrafiltration is a membrane separation process that targets particles approximately 0.01 to 0.1 µm in size, with pressures typically between 100 and 500 kPa. This method is particularly prevalent in the dairy industry, primarily for its capability to capture suspended solids and high molecular weight solutes when whey is propelled against a semi-permeable membrane under hydrostatic pressure [103,104]. UF stands out as the most widely used method in industry for isolating whey proteins, uniquely enabling the recovery of these proteins in their unaltered form. Utilizing this process can elevate the protein concentration in whey to as much as 85%. Numerous enhancements have been made to traditional UF techniques, aiming to boost the efficiency of whey protein recovery. The following sections will offer an overview of these advancements.

Cascaded ultrafiltration

In a cascaded ultrafiltration the output from one membrane stage (either the retained material or permeate) is channeled as the feed into the subsequent or preceding stage. Additionally, some streams might be recirculated to enhance the performance of the cascade. This setup enables further concentration of whey proteins. Patil et al. [107] explored the use of a three-stage ultrafiltration cascade for the continuous concentration of whey protein isolate. The study evaluated three different cascade configurations. The study concluded that appropriate integration of streams between different stages of the cascade can significantly improve separation efficiency compared to single-stage processes.

Bio-catalysis

Enhancing protein size during UF can help reduce problems with membrane clogging while also improving the amount of whey protein collected and the flow through the membrane. It is common for smaller proteins like peptides, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), to pass through the filter, but sometimes they get stuck and cause clogging. Research has shown that larger proteins are less troublesome for the membrane and easier to clean off than smaller proteins [108]. This has led to experiments with bio-catalysis to make proteins larger during the UF process. This approach uses special membranes that act as catalysts to modify whey proteins as they are being separated. One effective enzyme for this is transglutaminase (TG) [109]. TG creates links within and between molecules, forming larger protein polymers that are easier to catch on the filter.

A study by Wen-Qiong et al. [110] also tested a TG-based membrane for transforming whey proteins into larger structures. The study looked at how well the membrane worked after using TG on CW under different conditions. It was reported that with the TG membrane, about 15% more whey protein was recovered, lactose trapping went down by 10%, and the liquid flow through the filter increased by about 30%. These results were obtained at specific conditions.

High performance tangential flow ultrafiltration (HPTFF)

Conventional UF membranes separate proteins into permeate and retentate phases based on particle size differences whereas HPTFF not only uses size but also molecular charge characteristics for improved separation efficiency. HPTFF's unique advantage lies in its ability to separate biomolecules of identical molecular weight by their charge differences. Building upon the principles of ultrafiltration, HPTFF benefits from an established industrial framework, although it is not yet widely applied in dairy industry processes [111].

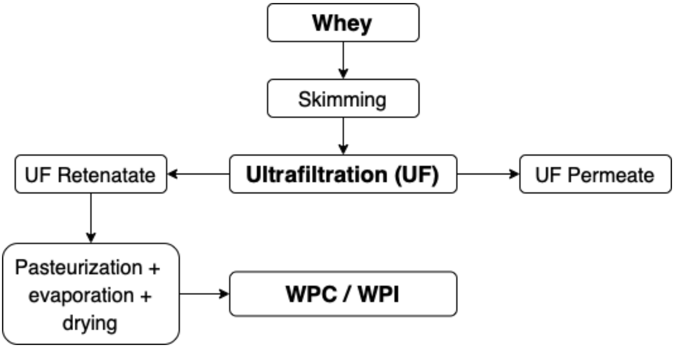

Figure 11. Representative scheme for obtaining whey protein concentrate (WPC) and whey protein isolate (WPI). Font: own elaboration.

As it has previously been discussed, UF is a crucial process in the separation of proteins from CW. Figure 11 shows the process of obtaining these proteins through UF. Proteins from CW account for approximately 20% of the total proteins found in milk, and as explored in detail in section 4.2, they hold significant importance. There are commercially available whey protein products such as whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH). WPC is a processed form of whey protein that stands out due to its minimal levels of fats and cholesterol, especially when compared to other whey varieties available commercially. This form also boasts a high concentration of bioactive compounds and includes carbohydrates, primarily lactose, with its protein percentage typically in the 65-70% range [112]. In contrast, WPI represents an advanced processing stage of whey protein. This process aims to strip away most of the fats and lactose, resulting in a reduction of bioactive compounds but an increase in protein content, often exceeding 90%. Given the high protein content of WPI, it is a more expensive option compared to WPC. Another form, WPH undergoes pre-digestion and partial hydrolysis, making it more easily metabolizable. This form is noted for its antioxidative qualities and has a protein content ranging from 70 to 80% [113]. The extensive processing required for WPH typically makes it a more expensive option than WPC and WPI.

Separation of whey proteins is merely the initial phase in rendering them commercially feasible. To enhance their market viability, these proteins must use a drying process, which is essential for reducing transportation costs and extending shelf life. While spray drying is the prevalent method, careful management of heat is crucial to prevent the denaturation of proteins. An alternative, freeze drying, conducted under vacuum conditions at lower temperatures, effectively avoids this denaturation risk. Despite its effectiveness, freeze drying is not widely used due to its high cost [114].

Once whey proteins have undergone all the necessary processing and the desired products have been obtained, they can serve a multitude of purposes. They are recognized for their versatility beyond just food and beverage applications like protein shakes and nutritional supplements or protein bars and healthy snacks, offering a wide range of uses in the food industry. Their adaptability allows them to be molded into various structures, including macro-, micro-, and nanoforms, making them ideal for encapsulating a variety of bioactive ingredients, distinct flavors, and nutritionally rich compounds. Additionally, whey proteins are frequently utilized as agents for surface activity, texture alteration, foaming, gelling, thickening, and emulsification, as noted in several studies [115,116]. The latest advancements in this area are directed towards creating innovative value-added products derived from whey protein, such as edible films, hydrogels, nanoparticles, and microencapsulated formulations [117].

Nowadays, whey protein has gained plenty of popularity among athletes, fitness fans, and those who want to increase their protein intake. Whey protein has all the important amino acids the body need and is easy to digest. It is a good choice for helping with muscle growth, recovery, and getting enough protein. With the rising trend towards being healthier, and since not everyone gets the recommended amount of protein every day, many often choose these products. These are definitely in high demand and are very profitable in the cheese whey valorization industry and while these are the most popular products, there are also several other highly sought-after items, as evidenced by the data presented in Table 11 [118].

A report by Polaris Market Research says that the global whey protein market is worth USD 9.19 billion (American) [119].

Table 11. Main industrial uses of protein whey and their functional characteristics in food manufacturing.

Food category	Uses	Functionality
Dairy Items	Reduced-fat cheese, Processed cheese, Yogurt alternatives	Fat substitutes, Emulsifying/ water binding properties, Protein enrichment/fat replacement
Beverages	Dairy-based flavored drinks, Carbonated beverages, Fruit-based beverages	Colloidal state, stability, flow resistance, creaminess, nutritional supplementation
Sport supplements	Drinks, protein enriched bars	Nutritional supplementation
Dessert products	Ice-cream, frozen juice bars, frozen dessert coatings	Whipping properties, milk powder alternatives, Emulsifying agent, body/texture, foaming.
Infant formula	Formula for infants and young children, formula for preterm and full-term babies	Nutritional balance
Dietetic foods	Therapeutic diets, nutrition for older adults	Nutritional
Convenience meals **Prepared food**	Preserved creamy soups and sauces, dehydrated cream products, salad dressings, microwavable foods, low fat convenience meals	Flavor enhancer, emulsifier, stabilizer, viscosity controller, freeze thaw stability, egg yolk replacement, water binding capacity, acid solubility
Bakery items	Pastries, loaves, cupcakes, crescent rolls, breads	Flavor, stabilization, foaming and egg substitution
Confectionery items	Whipped candy mixes, sponge desserts, meringue-based treats	Emulsifying agent, aerating properties, egg substitute, fat binding, foam stabilizer

6.2.3. Physicochemical treatments

Physicochemical methods also aim to decrease pollutants like organic matter, turbidity, and suspended solids in CW and, additionally, they help recover valuable components such as proteins and lactose. The reduction of contaminants can be achieved through coagulation and flocculation, or by employing iron electrodes in electrochemical processes, for example. To extract proteins and lactose from CW, various techniques, which will be discussed subsequently, are employed. It should be noted that these processes are commonly the initial stages in the treatment of CW, subsequently leading to further methods that yield the final products.

6.2.3.1. *Precipitation*

Precipitation utilizing coagulant/flocculant agents represents a specific approach within precipitation methodologies. This is because coagulation entails the application of a coagulant to counteract the charge on stable particles, while flocculation is a blending technique that facilitates the aggregation of particles, thereby enhancing their settlement. Consequently, these processes are efficacious strategies for the segregation of proteins and lactose from CW.

Protein removal using coagulants such as sodium polyphosphate, sodium hexametaphosphate, iron salts, and polyelectrolytes is effective, yet it is not optimal for protein recovery due to contamination from the coagulants. However, the use of natural chitosan (2-acetamido-2-deoxy-β-D-glucose), a high molecular weight linear cationic polymer derived from chitin (β (1-4)-N-acetyl-D-glucosamine) found in crustacean shells, offers a solution. This method precipitates proteins, resulting in the production of highly pure, pharmaceutical-grade lactose in the supernatant, with a purity of 99.89%. Furthermore, chitosan effectively removes various metal ions from industrial wastewater [3].

On the other hand, there are also thermal and isoelectric precipitation methods, as the precipitation of proteins from CW occurs more effectively under specific temperature and pH conditions. One example of this process is thermocalcic precipitation. This treatment process involves protein thermal precipitation through heating or autoclaving at temperatures between 90°C and 120°C, or alternatively, isoelectric precipitation achieved by adjusting the pH. Nonetheless, it is primarily utilized as an initial step before proceeding to more advanced processes, such as MF or UF, which are significantly more effective and are the ones that yield final products [3].

6.2.3.2. *Electrocoagulation*

The electrocoagulation (EC) technique presents itself as a viable alternative for the treatment of CW wastewater. EC, a form of electrolysis, effectively removes dissolved organic contaminants, turbidity, and coloration by applying an electrical current through the wastewater using specialized electrodes. This method has been shown to significantly reduce suspended colloidal particles. The efficacy of EC in processing dairy waste has particularly high removal rates of COD and oil-grease, achieving 98% and 99% effectiveness in just 7 and 1 min of electrolysis time, respectively [120].

6.2.3.3. Adsorption

Adsorption, a technique among various physicochemical CW wastewater treatment methods, is notably effective for removing organic pollutants. Activated carbon is frequently utilized as an adsorbent. However, alternative cost-effective adsorbents like rice husk ash and coal fly ash are also viable. Some studies explored using powdered activated carbon combined with other affordable adsorbents for CW treatment. When compared to other adsorbents, it was more efficient in reducing total solid content. In a similar vein, other studies employed chitosan and various inorganic coagulants, followed by powdered activated carbon adsorption, as preparatory steps before the membrane separation processes in dairy wastewater treatment [121].

In conclusion, the diverse technologies employed in the valorization of CW, encompassing biological, physical, and physicochemical processes, offer substantial benefits. These technologies not only facilitate the transformation of a by-product into a range of valuable and sustainable products, but also significantly reduce environmental impact. By optimizing the use of CW, these methods contribute to enhanced efficiency in resource utilization, reduction in waste, and promotion of circular economy principles within the dairy industry. Their implementation represents a stride forward in achieving environmental sustainability and economic viability, proving essential in the progressive evolution of the dairy sector.

7. PRESENT AND FUTURE PERSPECTIVES AND CHALLENGES

As the production of dairy products and its demand increases, CW as one of its by-products, also increases its production and it is a bigger challenge as time goes by. Despite the existing technologies and extensive research and development focused on them, obstacles such as low bioenergy production and the lack of efficient reactors for its production continue remain a barrier to the optimal valorization of CW. Additional obstacles include high investment costs associated with technologies like membranes for separating proteins and lactose from CW, or the expense of drying the final product. Consequently, disposing of waste on agricultural land as fertilizer or using it for animal feed remains one of the most common methods of waste disposal [122]. Nowadays, over 40% of CW is used for these basic purposes compared to less than 60% that is valorized into various products (see Figure 12).

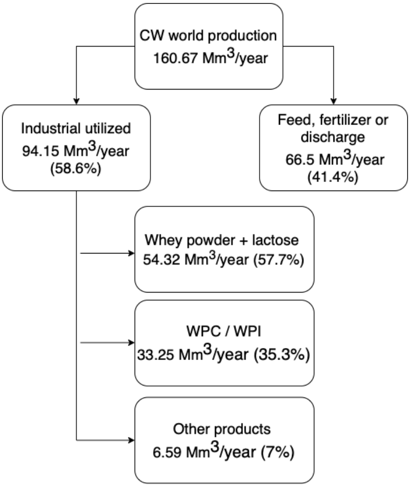

Figure 12. Global utilization of CW in 2022. Font: [123].

As shown in Figure 12 and as already studied in this TFG, the majority of CW valorization applications are in the area of powdered proteins using membrane technologies. However, it is essential to continue researching and providing opportunities for other technologies beyond the established ones.

Future research should concentrate more on optimizing strategies for biological processes like those seen in this study. Further research is required on the fermentative use of CW to produce more bioethanol, AFA, biohydrogen, and in AD to produce biogas. If the industry, researchers, and authorities agree to increase the investment in these technologies, the benefits could be much greater and help maintain a cleaner planet with green energy. Furthermore, through all these collective efforts, there exists an opportunity to enhance another critical aspect: upscaling these important processing methods from the laboratory to industrial levels.

Human health remains an important issue, and it is noteworthy that the industry develops food products enriched with protein from CW to promote better health. However, there is a world beyond CW proteins that is currently only 7% uncovered and holds a lot of potential.

8. CONCLUSIONS

In conclusion, this TFG, focused on the different technologies and valorization of CW, represents a significant step forward in understanding and harnessing the potential of this by-product in the food industry. Through an analysis of existing technologies for CW processing and the examination of key products derived from these methods, this study has illuminated the huge untapped potential that this effluent, CW, holds. Despite the advancements in technology and the development of a range of CW-based products, it is evident that there is a pressing need for further research in this area.

The technologies reviewed, starting with AD, reveal a process that is currently under-researched and underfunded, despite its potential to generate bioenergy, particularly methane. This approach could be crucial in shaping the future of energy. On another front, fermentative processes utilizing bacteria, fungi, and yeast can produce various products like lactic acid, highly sought after in the food industry. Filtration technologies, notably microfiltration as a primary step in many successful processes, and ultrafiltration, when combined with other methods, lead to the production of highly demanded protein-based products, such as WPC and WPI. These products are immensely popular among consumers because proteins derived from CW, especially β-lactoglobulin and α-lactalbumin proteins, contribute significantly to health and nutrition.

Last but not least, the sustainability aspect of whey valorization is particularly noteworthy. In an era where environmental concerns are paramount, the ability to convert a waste product into a range of useful, sustainable products, aligns perfectly with achieving the SDGs and the principles of a zero-waste, circular economy. Unfortunately, CW valorization is not always feasible, often due to the high investment costs in technology or transportation, which poses a challenge for small companies lacking the financial means to valorize a by-product with such diverse alternatives. Therefore, it is essential for larger companies to set an example and, together with researchers and relevant authorities, delve deeper into the valorization of CW. This collaborative effort is crucial to ensure that in the near future, CW can be revalorized to a greater extent.

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ACRONYMS

CW	Cheese Whey
UN	United Nations
SDGs	Sustainable Development Goals
FAOSTAT	Food and Agriculture Organization Statistics
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
SCW	Second Cheese Whey
WP	Whey Protein
WPC	Whey Protein Concentrate
WPI	Whey Protein Isolate
WPH	Whey Protein Hydrolysate
LCA	Life Cicle Assessment
GHG	Greenhouse Gas
GWP	Global Warming Potential
CF	Carbon Footprint
PDO	Protected Designation of Origin
AD	Anaerobic Digestion
CSTR	Continuous Stirred-Tank Reactor
UASB	Upflow Anaerobic Sludge Bed
EGSB	Expanded Granular Bed
AFB	Anaerobic Fluidized Bed
GRAS	Generally Regarded As Safe
AFA	Aromas, Flavors and Antioxidants
VOC	Volatile Organic Compound

LA	Lactic Acid
2PE	2 - phenylethanol
EA	Ethyl acetate
UF	Ultrafiltration
MF	Microfiltration
NF	Nanofiltration
RO	Reverse Osmosis
TG	Transglutaminase