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From gutter to value: Valorization of acid whey from fresh cheese using membrane filtration

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ABSTRACT

Acid whey, often regarded as a byproduct of fresh cheese production, particularly Jben cheese in Morocco, holds untapped potential as a valuable resource. Through advanced membrane filtration technologies, such as ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), acid whey can be transformed into high-value components, including proteins, lactose, and minerals. These fractions can be utilized in various applications, ranging from enriched dairy products and nutritional supplements to biomedical uses. The methodology focuses on optimizing filtration parameters, including diafiltration and pH adjustment, to improve protein retention, stabilize the fractions, and minimize precipitation. Our results show that the application of these techniques enables effective valorization of acid whey, generating functional fractions. The integration of diafiltration improves protein retention, while pH adjustment with citric acid stabilizes the product by reducing precipitation and improving fraction homogeneity. Statistical analysis using ANOVA revealed significant differences ($p < 0.05$), confirming the positive impact of these strategies on the physicochemical properties of the obtained fractions.

Keywords: acid whey, fresh cheese, membrane filtration technologies, new products, circular economy.

INTRODUCTION

The cheese industry generates a significant amount of by-products, including whey, which represents approximately 85 to 90% of the total volume of milk used in cheese production (Pires et al., 2021). While whey can be a valuable source of nutrients, its treatment and management present a major challenge, particularly due to its high organic load, which can lead to environmental pollution if the by-product is discharged without proper treatment (Panghal et al., 2018). Whey can be classified into two main types based on the coagulation method used (Moatsou and Moschopoulou, 2021). Sweet whey is derived from enzymatic coagulation using rennet, and it is typically produced in the making of cheeses such as Gouda, Cheddar, or Parmesan (Benitez et Ortero, 2012). Acid whey is produced through lactic coagulation and is primarily associated with the production of fresh cheeses (Papademas and Bintsis, 2017), such as Jben in Morocco, a traditional, soft, and fresh cheese. This study focuses particularly on acid whey, mainly derived from the production of fresh cheeses, a sector that holds a significant share of the Moroccan cheese market. This whey is rich in soluble proteins, lactose, and minerals (Hejtmánková et al., 2012), compounds of interest that can be valorized in various agro-food and biotechnological applications. However, advanced studies on the valorization of acid whey remain limited, and this by-product is often considered an industrial waste. In this context, membrane filtration appears as an innovative and sustainable solution for the valorization of acid whey (Le et al., 2014; Reig et al., 2021). Unlike chemical treatments, membrane filtration allows for the selective separation of the components of whey (proteins, lactose, and minerals) while preserving their functional and nutritional quality. This technology is divided into several processes, including microfiltration, ultrafiltration, Nanofiltration, and reverse osmosis (Rektor et al., 2004). Microfiltration eliminates larger particles and certain bacteria, often acting as a preliminary step to protect the subsequent filters (Argenta and Scheer, 2020). Ultrafiltration primarily separates proteins while allowing lactose and minerals to pass through. It is therefore ideal for concentrating whey proteins (Reig et al., 2021). Nanofiltration partially retains minerals and lactose, creating fractions that can be used for various applications (Koca, 2018). Reverse osmosis enables advanced purification of whey by eliminating almost all solutes and producing a concentrated fraction (Sathya et al. 2023). Various studies have investigated microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) for the valorization of whey and for the extraction of protein fractions and lactose. (Rektor and Vatai, 2004; Minhalma et al., 2007; De Souza et al., 2010; Yorgun, et al., 2008; Cuartas-Uribe et al., 2009). These membrane filtration technologies offer a more ecological and sustainable approach to whey treatment, providing new perspectives for its valorization in food, nutraceutical, and biotechnological products (Rosseto et al., 2024; Das et al., 2022). The main objective of this study is to examine the effectiveness of membrane filtration techniques for valorizing acid whey from fresh Jben cheese. The study focuses on the separation and concentration of proteins, lactose, and minerals, optimizing the use of diafiltration and pH adjustment to improve the quality and stability of the obtained fractions. The aim is to propose practical and economically viable solutions for the dairy sector in Morocco.

MATERIAL AND METHODS

Raw material

The acid whey used in this study was obtained from an industrial fresh cheese production process. This by-product was collected immediately after the coagulation and draining of the curd. Once recovered, it was filtered through a 100 μm stainless steel mesh to remove solid particles, such as curd residues and protein aggregates. To prevent microbial growth and biochemical changes, the whey was stored at 4 °C in airtight polyethylene containers. Prior to use in experimental testing, it was homogenized using a magnetic stirrer at a speed of 300 rpm for 30 minutes to ensure an even distribution of the components within the whey.The experiments were conducted at a pilot scale using 100 L of this whey.

Initial physicochemical analysis

The raw acid whey was analyzed through several physicochemical tests to determine its properties and valorization potential, providing a detailed profile before membrane filtration. A summary of the analyses performed and the methods used for each test is provided in Table 1.

Membrane filtration system and operating conditions

In this study, three membrane filtration processes were implemented to fractionate and concentrate acid whey: ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Each technique enables the separation of components based on their molecular size, with

Table 1. Summary of analytical methods and physicochemical analysis for acid whey

Analysis	Method
pH	Measurement with a calibrated pH meter (HANNA HI 2211) at room temperature
Titratable acidity (°D)	Measurement with the HI 84529 automatic mini-titrator using a 0.1 N NaOH solution, with results expressed in Dornic degrees (°D)
Total solids	Measurement by the gravimetric method, involving oven drying at 105 °C until a constant weight was achieved
Soluble proteins	Kjeldahl method to measure total nitrogen, followed by protein calculation using a conversion factor
Lactose	Polarimetric method
Fats	Gerber method to determine the fat content
Ash	Incineration in a muffle furnace at 550 °C until constant weight
Electrical conductivity	Measurement with conductivity meter (WTW cond 3110), with the results expressed in (mS/cm)
Turbidity	Measurement with turbidimeter, with results expressed in nephelometric turbidity units (NTU)

transmembrane pressure (TMP) acting as the driving force and molecular weight cut-off (MWCO) as a key parameter. Microfiltration (MF) utilizes membranes with an MWCO greater than 100.000 Da under a TMP ranging from 0.1 to 2 bar, while UF operates with an MWCO between 1.000 and 100.000 Da and a TMP of 2 to 10 bar. NF, on the other hand, functions with an MWCO between 100 and 1.000 Da and a TMP varying from 5 to 40 bar, whereas RO employs membranes with an MWCO between 1 and 100 Da under a TMP of 30 to 100 bar (Hinkova et al., 2012). In this work, a multifunctional ceramic membrane filtration unit was used, allowing the application of different membrane techniques (MF, UF, NF, and RO) by replacing membrane elements with membranes of specific porosity and MWCO characteristics. All experiments were conducted using ceramic membranes, selected due to their numerous advantages over polymeric membranes. Their high mechanical strength and enhanced thermal and chemical resistance (withstanding pH levels from 1 to 13 and temperatures above 80 °C) make them a preferred choice. Additionally, their low susceptibility to fouling facilitates cleaning and extends their lifespan, thereby improving efficiency in industrial settings (Mostafavi et al., 2019). The efficiency of ceramic membranes in whey valorization has been widely studied (Carter et al., 2021) demonstrated their significantly higher resistance to fouling compared to polymeric membranes (Sathya et al., 2023) highlighted their effectiveness in retaining bioactive peptides (Shekin, 2021) showed that reverse osmosis using ceramic membranes enables optimal lactose recovery and efficient purification of residual water. Finally, (CUNHA et al., 2022) investigated filtration flux stability in industrial environments and emphasized the low tendency of these membranes to foul.Several manufacturers offer ceramic membranes specifically designed for whey filtration processes, including TAMI Industries (France), Pall Corporation (USA), Atech Innovations GmbH (Germany), Membralox (Parker Hannifin, France), and Metawater (Japan). These companies develop specialized solutions for dairy applications, ensuring optimal separation of acid whey fractions. Thus, the use of ceramic membranes represents a high-performance and sustainable approach for recovering valuable compounds, maximizing both the quality and yield of the obtained products.

Experimental setup for the treatment of 100 L of whey

The clarified whey is initially stored in a 200 L tank before being transferred to the filtration circuit. To ensure optimal homogenization of the circulating liquid, continuous agitation is maintained throughout the process. The system has been designed to process 100 L batches of whey per cycle, with an optimized configuration to achieve efficient component separation:

- Feed tank (200 L): stores the whey and ensures a continuous supply to the filtration circuit.
- Recirculation pump: maintains a tangential flow through the membranes to reduce fouling and enhance separation performance.
- Filtration units: ceramic membrane modules integrating different filtration techniques depending on the treatment requirements (ultrafiltration, nanofiltration, or reverse osmosis).
- Monitoring and control system:
  - manometers positioned at the module's inlet and outlet monitor the transmembrane pressure (TMP).
  - flow meters track the permeate and retentate fluxes.
  - pH and temperature sensors adjust the operating conditions according to process requirements.
- Collection tanks:
  - a dedicated tank for the permeate.
  - another tank for collecting the retentate.
  - in diafiltration mode, water is gradually added to the retentate, and multiple cycles are performed to maximize the extraction of valuable components.

The schematic diagram of the membrane system can be observed in Figure 1.The operational parameters were defined according to the membrane manufacturer's recommendations and were maintained throughout the trials, which were conducted in three repetitions. The operating conditions applied at each filtration stage are presented in Table 2. Two different approaches were evaluated separately to assess the effects of specific operational parameters on the efficiency of the filtration process:

- Approach 1: this approach involved applying diafiltration (DF) to the UF retentate, both with and without diafiltration. DF involves diluting the retentate with water, followed by reconcentration. This step is essential for enhancing the purification of components rejected by the

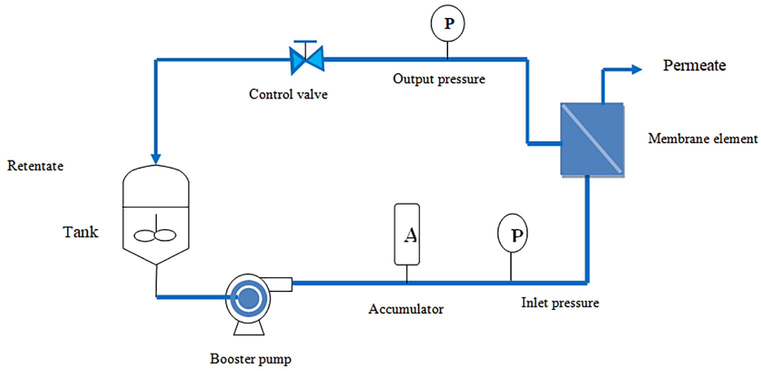

Figure 1. Shematic diagram of the membrane system

Table 2. Operational parameters of different filtration techniques

Membrane techniques	Type of membrane	Operating parameters of membranes (MWCO)	Operating temperature (°C)	TMP (bar)	FCV
Ultrafiltration (NF)	Ceramic α-Al_2O_3, TiO_2	6-8 KDa	20	3	3.5
Nanofiltration (NF)	Ceramic α-Al_2O_3, TiO_2	400 Da	20	15	3.9
Osmose inverse (OI)	Ceramic α-Al_2O_3, TiO_2	<100 Da	20	45	10

membrane by selectively removing molecules such as lactose and salts while maintaining a constant volume of retentate. The main goal of DF is to increase the purity of the components while preserving the proteins. The amount of water added is determined based on the quantity of whey processed, with two tested incorporation coefficients (0.100 and 0.145), in accordance with supplier recommendations, to identify the optimal coefficient. This method also aims to evaluate the impact of diafiltration on the quality of both the retentate and the permeate. Although there is limited research and studies on this topic, this investigation is crucial for understanding the influence of DF on the separation of components and the quality of the final products.

- Approach 2: retentate NF – with and without pH adjustment of the permeate before concentration. The objective of this second approach was to evaluate the impact of pH adjustment on the efficiency of the filtration process, with a particular focus on the quality of the retentate and permeate obtained.

Fractions obtained

At each filtration stage, two primary fractions were collected: the retentate (also know concentrate), which is held back by the membrane, and the permeate (filtrate), which flows through the membrane (Le et al., 2014; Subhir et al., 2022).The products of interest may be found in either the retentate, the permeate, or both. Each fraction (permeate and retentate) was characterized in terms of composition, molecular weight distribution. A series of physicochemical analyses were performed on these fractions to determine their purity, concentration, and the efficiency of separation at each filtration step.

Statistical analysis

A statistical analysis was performed using one-way ANOVA (analysis of variance) to identify significant differences between the different filtration approaches and operational conditions.

RESULTS ANDDISCUSSION

Initial characteristics of whey

Table 3 presents the physicochemical characteristics of the whey analyzed in this study, highlighting its potential as a valuable source of nutrients for the food industry. This by-product contains a significant amount of essential components

Table 3. Summary of the average analyses of initial whey

pH	°D	Dry matter (g/kg)	Proteins content (g/kg)	Fat content (g/kg)	Lactose (g/L)	Ash (g/L)	Electrical conductivity (µS/cm)	Turbidity (NTU)
4.45±0.05	55±1	60.55±0.5	4.12±0.15	0	44.3±0.2	7.52±0.03	5200±20	784±10

such as proteins, lactose, and minerals, which can be utilized in various applications. However, its relatively high turbidity may limit certain industrial uses, requiring a clarification step to improve its transparency and facilitate its integration into food formulations. Compared to the results reported by (Nova et al., 2022) the turbidity level measured in this study is lower, which could be attributed to differences in production conditions, including the type of milk used, the cheese-making process, and whey separation parameters. Similar variations have also been observed in other studies, notably those by (Hejtmánková et al., 2012), who emphasized the impact of the coagulation process on whey composition. Additionally, the ash content recorded in our study is lower than that reported in comparable research, which could be due to variations in raw material composition or technological adjustments applied during processing. These findings confirm that whey composition can fluctuate depending on production parameters, as demonstrated by (Reig et al., 2021), who explored the influence of filtration techniques on the concentration of protein and mineral fractions. Therefore, incorporating pretreatment steps such as clarification and standardization could enhance its industrial usability and promote its integration into food formulations.

Analysis of filtration-derived fractions

The fractions obtained after each filtration step, namely the permeate and retentate, were subjected to physicochemical analyses similar to those performed on raw whey to assess the modifications induced by the different separation techniques (Figure 2). The study of fractions derived from ultrafiltration (FVC 3.5), nanofiltration (FVC 3.9), and reverse osmosis (FVC 10) highlights notable differences in composition. These membrane filtration processes enable the selective separation of acid whey constituents, leading to significant variations in the concentrations of proteins, lactose, and other soluble compounds. Ultrafiltration (UF), for instance, primarily retains macromolecules such as proteins while allowing smaller molecules to pass through, thereby enriching the retentate in proteins and the permeate in lactose and minerals. This selective separation has been demonstrated in the work of (Pouliot 2008) who highlighted the potential of UF for

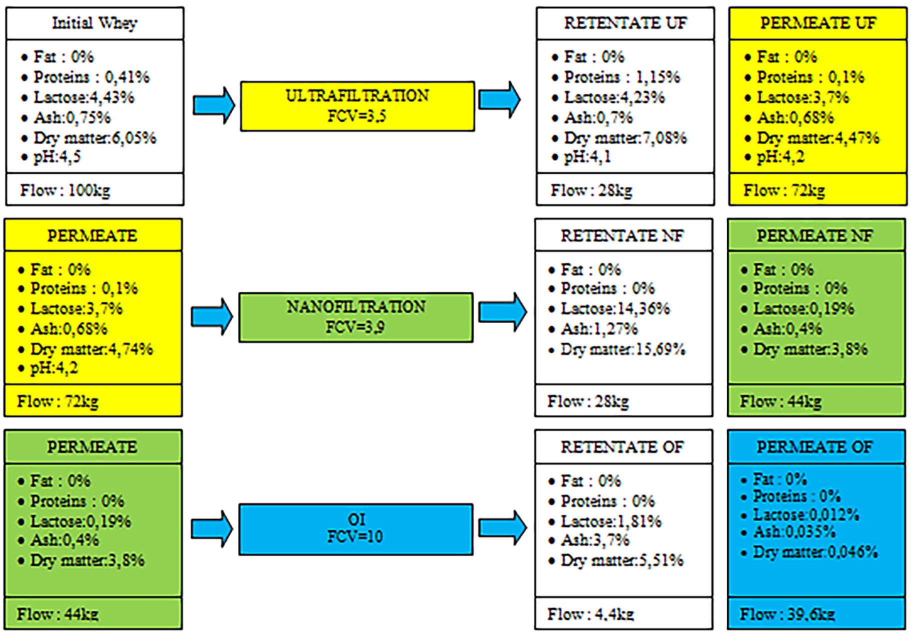

Figure 2. Composition and properties of filtration fractions

concentrating whey proteins and enhancing their valorization in the dairy industry. Nanofiltration (NF), on the other hand, removes a portion of salts and lactose while retaining a significant fraction of proteins, making the retentate particularly suitable for applications requiring a reduction in mineral content.

This phenomenon was described by (Gésan-Guiziou et al., 2002), who emphasized the impact of NF membranes on improving the quality of whey protein fractions. Finally, reverse osmosis (RO) is a technique that retains nearly all dissolved solids, thereby significantly concentrating whey constituents and producing a permeate composed mainly of purified water, which can be used for industrial applications, particularly as process water, as suggested by the work of (Madaeni and Mansourpanah, 2004 ; Marx et al., 2018) (Chamberland et al., 2020).

These findings confirm that each filtration technique plays a key role in the separation and concentration of acid whey components, offering various opportunities for industrial exploitation. The efficiency of these processes has been highlighted in several recent studies, including those by (Reig, Vecino, et Cortina 2021), which demonstrated that optimizing filtration conditions could significantly influence the composition of the obtained fractions and their potential applications. Furthermore, the protein-rich fraction obtained from ultrafiltration can be used for the formulation of protein-enriched dairy products or nutritional supplements designed for athletes and individuals suffering from malnutrition (Rocha-Mendoza et al., 2021; Krissansen, 2007). Meanwhile, the nanofiltration retentate, with an intermediate composition, could serve as a base for formulations requiring a balance between proteins and lactose (Altuntas and Hapoglu, 2019; Reig et al., 2021), while the water recovered after reverse osmosis could be reused in industrial processes, contributing to better water resource management in dairy processing plant (Brião et al., 2024).

Comparison of filtration performance with and without diafiltration

In this study, a comparative analysis of filtration performance with and without diafiltration was conducted to evaluate the impact of this additional step on the separation of acid whey components. Without the application of diafiltration, the results showed an effective separation of the main whey constituents, particularly proteins, lactose, and mineral salts, in accordance with previous observations made by (Almécija et al., 2007; Nova et al., 2022) (Figure 2). However, to further refine this separation and optimize the concentration of target fractions, diafiltration was applied using two different coefficients: 0.100 and 0.145. This approach allowed for a comparison of the effects of two diafiltration intensities on protein enrichment and purification of the fractions.The results obtained with diafiltration showed a significant improvement in protein retention, with an increase in protein concentration in the retentate, rising from 1.15% to 1.25% using a diafiltration coefficient of 0.145. This increase indicates a higher efficiency in protein concentration, confirming the findings of (Baldasso et al., 2022), who emphasized the importance of diafiltration in the selective concentration of proteins by removing small molecules such as lactose and mineral salts. The more effective separation of proteins allows for the production of a protein-rich retentate, which is ideal for industrial applications such as the development of high-protein dairy products or nutritional supplements.

These findings align with those of (Mestawet et al., 2024), who demonstrated the potential of diafiltration for concentrating and enriching whey proteins for food and dietary formulations. The impact of diafiltration on the composition of acid whey was further evidenced by the reduction in lactose and ash concentration. These changes are directly related to the selective removal of small molecules during the diafiltration process, there by promoting a more advanced purification of the retentate. This phenomenon aligns with the conclusions of (Reig et al., 2021), who demonstrated that diafiltration improves the quality of whey protein fractions by reducing mineral content and increasing protein purity, which is particularly important for the formulation of high-value-added products.Another significant aspect of this study was the increase in the retentate pH, from 4.5 to 5.2, when diafiltration was applied. This pH variation can be attributed to several factors. Firstly, the addition of water during diafiltration dilutes the acids present in whey, thereby reducing their impact on the overall acidity of the solution. Additionally, the removal of small acidic molecules, such as lactic acid, also contributes to the pH increase, which has been observed in the work of (Altuntas et Hapoglu, 2019).

This pH elevation is further reinforced by the higher protein concentration, as proteins have a buffering effect that helps stabilize the pH at a higher level. This pH improvement is beneficial as it helps create products with a milder and more pleasant organoleptic profile. Similar findings were reported by (Hejtmánková et al., 2012), who observed comparable pH modifications in whey filtration systems.Finally, sensory analyses confirmed that diafiltration had a positive impact on the acidity and organoleptic profile of the fractions. The reduction in acidity and the improved taste of diafiltered fractions make them an attractive choice for low-acidity dairy products or formulations intended for consumers sensitive to acidity, such as athletes or individuals with specific nutritional needs. This phenomenon was also highlighted by (Lameloise, 2021), who studied the impact of diafiltration on optimizing the taste profile of whey fractions, demonstrating the improved sensory attributes of products obtained through this technology.

The results obtained in this study confirm the relevance of diafiltration as a key technique in the valorization of acid whey. Not only does it optimize protein separation, but it also opens promising perspectives for the reuse of process water in the dairy industry, which could contribute to better water resource management in processing plants. The visual illustration presented in Figure 3, comparing the initial whey, the permeate, and the retentate after diafiltration, highlights the increased concentration of macromolecules, particularly proteins, in the retentate, demonstrating the effectiveness of diafiltration in this process. Additionally, Table 4 provides a comparative analysis of filtration results with and without diafiltration, offering valuable data on variations in fraction composition and the impact of each approach on the physicochemical characteristics of whey.These observations reinforce the significance of membrane technologies in a sustainable whey valorization approach, aligning with circular economy strategies and waste reduction initiatives in the food industry.

Figure 3. Initial whey, permeate, and retentate after diafiltration

Effect of pH adjustment on the stability of UF permeate in nanofiltration

In this study, we examined the impact of pH adjustment of ultrafiltration (UF) permeate before concentration by nanofiltration (NF). The main objective was to understand how this pH modification affects the stability of the final product and the physicochemical properties of the resulting retentate. This process aims to improve the quality of the permeate by reducing the instability of the solution, particularly by limiting the precipitation of proteins and minerals, a phenomenon that can harm the purity of the collected fractions. Previous studies, including those by (Chandrapala et al., 2016; Brião et al., 2024), have shown that precise

Table 4. Comparative table of results with and without diafiltration

Criteria	Initial whey	Without DA (FVC 3.5)		DA (0.10) (FVC 3.5)		DA (0.145) (FVC 3.5)	
		Retentate	Permeate	Retentate	Permeate	Retentate	Permeate
Fat%	0	0	0	0	0	0	0
Proteins%	0.41	1.15	0.1	1.18	0.07	1.25	0.01
Lactose%	4.43	4.23	3.7	4.1	3.83	3.8	4.13
Ash%	0.75	0.7	0.68	0.65	0.62	0.63	0.64
Dry matter%	6.05	7.08	4.47	6.08	4.52	5.72	4.78
pH	4.5	4.1	4.2	4.8	4.3	5.2	4.4
Sensory Analysis	Slight acceptable acidity	Acidic taste, less balanced	-	Milder and more balanced taste	-	More pleasant taste, low acidity	-

pH control plays a critical role in managing turbidity and optimizing filtration results by keeping proteins in solution and preventing their precipitation.

The visual results of this study revealed a significant difference between the samples before and after pH adjustment ($p < 0.05$). Prior to the adjustment, the permeate exhibited marked turbidity and clear signs of precipitation, indicating chemical and physical instability of the solution. However, after pH adjustment with citric acid, the permeate became clearer, more stable, and free from visible precipitation, as shown in Figure 4. This visual change suggests a significant improvement in the overall quality of the permeate, thus reducing the risk of quality loss during the subsequent nanofiltration concentration step (Table 5). These observations confirm the importance of pH adjustment, which, as shown by (Reig et al., 2021) helps improve component separation and reduce contamination by undesirable elements.

The physicochemical analyses conducted in parallel corroborated these visual observations, showing that pH adjustment significantly reduced the concentration of unwanted components such as mineral salts and weak acids, while increasing the protein concentration in the retentate. This phenomenon is crucial for industrial applications, particularly in the production of protein-enriched dairy products, where a pure protein fraction is sought. These results align with the research by (Steinhauer et al., 2015), who also highlighted that pH adjustment can optimize the permeate composition and improve the efficiency of protein concentration.

The results obtained were also confirmed by statistical analysis using one-way ANOVA ($p < 0.05$), which showed significant differences between the treatments, particularly the addition of diafiltration and pH adjustment. These results showed an increase in protein concentration in the retentate, accompanied by a reduction in mineral salts and lactose. This demonstrates the effectiveness of this combination of techniques in improving the quality and purity of the obtained fractions (Bhattacharjee et al., 2006) had already observed that pH adjustment, when combined with membrane filtration, plays a key role in stabilizing the solution and optimizing filtration results.Thus, this study confirms that

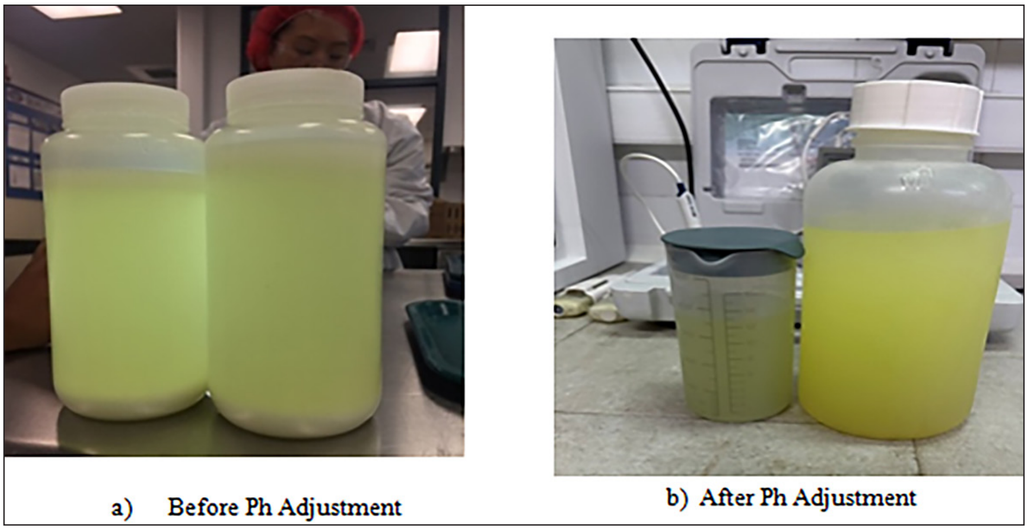

Figure 4. Visual illustration of the product's evolution before and after pH adjustment

Table 5. Comparison of parameters before and after pH adjustment

Parameters	Before pH adjustment	After pH adjustment
pH	4.2	4.8
Visual observation	Decantation and precipitation	Homogeneous
Turbidity (NTU)	95	20
Proteins%	0.1	0.11
Lactose%	3.7	3.7
Ash%	0.68	0.68

pH adjustment before nanofiltration is an effective strategy for improving protein separation and concentration while reducing impurities. These results are part of an approach to valorize whey, which has the potential to become a valuable resource for the dairy industry by offering high-quality protein fractions.

CONCLUSIONS

This study highlights the significant potential of acid whey valorization from fresh cheese through membrane filtration, as it is a rich source of essential fractions such as proteins, lactose, and other bioactive compounds. Rather than being considered waste, this by-product can be transformed into high-value ingredients using membrane filtration techniques. Our results demonstrated that the application of ultrafiltration, nanofiltration, and reverse osmosis can convert acid whey into a valuable resource, generating high-added-value fractions. For instance, these fractions can be incorporated into formulations for dairy products, dietary supplements, or even health-related applications. The integration of diafiltration and pH adjustment further optimizes this valorization. Specifically, diafiltration enhances protein retention, while pH adjustment with citric acid stabilizes the product by reducing precipitation and improving the homogeneity of the fractions. Statistical analysis using ANOVA revealed significant differences ($p < 0.05$) between the applied treatments, confirming the positive impact of these techniques on the physicochemical properties of whey fractions. In a context where the agri-food industry faces increasing environmental and economic challenges, whey valorization represents a sustainable and economically viable alternative. In Morocco, where the dairy sector plays a key role in the national economy, integrating membrane filtration technologies could not only reduce the environmental footprint of dairy production but also open new market opportunities through the development of functional and innovative products.

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