

INNOVATIVE APPROACHES TO OPTIMIZING PROTEIN YIELDS
FROM food waste (whey)

مناهج مبتكرة لتحسين إنتاج البروتين من نفايات الطعام (مصل اللبن)



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المخلص

تركز هذه الدراسة على استخلاص وتركيز بروتينات مصّل اللبن، والتي تعدّ مكونات أساسية في مصّل اللبن ونواتج ثانوية لإنتاج الجبن. يعدّ كل من بروتين مصّل اللبن المعزول (WPI) وبروتين مصّل اللبن المركز (WPC) من المنتجات الغذائية المهمة المستخدمة في مجموعة متنوعة من السلع الاستهلاكية، بما في ذلك المخبوزات والحلويات والأيس كريم وأغذية الأطفال. تم إجراء البحث في عام 2024 في مختبرات الكيمياء بكلية الزراعة في جامعة جرش. بهدف تحديد الطريقة الأكثر فاعلية لاستخلاص بروتينات مصّل اللبن باستخدام أربع تقنيات مختلفة: الترسيب الحراري، والترشيح الفائق، والرغوة الفقاعية، وسليولوز الكربوكسي ميثيل الحمضي المتعادل (CMC-NA). أظهرت النتائج أن محتوى البروتين زاد بشكل ملحوظ مع الترشيح الفائق مقارنة بالطرق الأخرى. بينما ظل محتوى الدهون ثابتاً عبر جميع تقنيات الاستخلاص، انخفضت نسبة اللاكتوز مع جميع الطرق الأربعة. حيث سجلت أكبر نسبة انخفاض في طريقة الترسيب الحراري. أشارت أخطاء المتوسطات القياسية إلى وجود تباين معتدل، حيث أظهر كل من البروتين واللاكتوز أكبر التقلبات بعد عملية الترشيح. تسلط الدراسة الضوء على التفاعلات المعقدة بين المكونات الكبرى للحليب (البروتين، والدهون، واللاكتوز) والتقنيات المحددة لاستخلاصه، بالإضافة إلى ظروف المعالجة التي تؤثر على النتائج. ختاماً، لا يزال تطوير تقنيات فعالة لاستخلاص بروتينات مصّل اللبن يمثل تحدياً، ولكنه ضروري لتعزيز صناعات الأغذية والتعبئة والتغليف. ومن خلال تحسين هذه الأساليب لاستعادة مصّل اللبن، الذي يُعتبر تقليدياً منتجاً ثانوياً، تهدف هذه الدراسة إلى تحويله إلى مورد قيم لاستخدامات متعددة. الكلمات المفتاحية: طرق الجمع، تقنيات التركيز، معالجة الألبان، عمليات الفصل، عزل البروتين، استرداد بروتين مصّل اللبن.

ABSTRACT

ABSTRACT

This study focuses on the extraction and concentration of whey proteins, which are essential components of whey and byproducts of cheese production. Whey protein isolate (WPI) and whey protein concentrate (WPC) are important food products used in a variety of consumer goods, including baked goods, candy, ice cream, and baby food. The research was conducted in 2024 in the chemistry laboratories of the Faculty of Agriculture at Jerash University. It aimed to determine the most effective method for extracting whey proteins using four different techniques: thermal precipitation, ultrafiltration, bubble foaming, and neutral acid carboxymethyl cellulose (NA-CMC). The results showed that protein content increased significantly with ultrafiltration compared to the other methods, while fat content remained consistent across all extraction techniques. Lactose content decreased with all four methods, with the greatest reduction observed in heat precipitation. The standard errors of the means indicated moderate variability, with protein and lactose showing the largest fluctuations after filtration. The study highlights the complex interactions between the macro components of milk (protein, fat, and lactose) and the specific extraction methods and processing conditions that influence the results. In conclusion, developing efficient extraction techniques for whey proteins remains challenging but is crucial for advancing the food and packaging industries. By optimizing these methods to recover whey, traditionally considered a byproduct, this study aimed to transform it into a valuable resource for various applications.

Keywords: Collection Methods, Concentration Techniques, Dairy Processing, Separation Processes, Protein Isolation, Whey Protein Recovery.

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Innovative Strategies for Maximizing Whey Protein Recovery from Dairy Byproducts

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Abstract

This study focuses on the extraction and concentration of whey proteins, essential components of whey, and byproducts of cheese production. Whey protein isolate (WPI) and whey protein concentrate (WPC) are important food products used in various consumer goods, including baked goods, candy, ice cream, and baby food. The research was conducted in Jan-2022 to Mar 2023 in the chemistry laboratories of the Faculty of Agriculture at Jerash University. It aimed to determine the most effective method for extracting whey proteins using four different techniques: thermal precipitation, ultrafiltration, bubble foaming, and neutral acid carboxymethyl cellulose (NA-CMC). SAS (GLM) was used to analyze the effects of dairy plants, extraction methods, sample stages, and their interactions on cow milk fat, protein, and lactose, with significance tested by Duncan's Multiple Range test. The results showed that protein content increased significantly with ultrafiltration compared to the other methods, while fat content remained consistent across all extraction techniques. Lactose content decreased with all four methods, with the greatest reduction observed in heat precipitation. The standard errors of the

means indicated moderate variability, with protein and lactose showing the largest fluctuations after filtration. The study highlights the complex interactions between the macro components of cow milk (protein, fat, and lactose) and the specific extraction methods and processing conditions that influence the results.

In conclusion, developing efficient extraction techniques for whey proteins remains challenging but is crucial for advancing the food and packaging industries. By optimizing these methods to recover whey, traditionally considered a byproduct, this study aimed to transform it into a valuable resource for various applications.

Keywords: Collection Methods, Concentration Techniques, Dairy Processing, Separation Processes.

1. Introduction

Whey, a valuable by-product of cheese manufacturing, has emerged as a star ingredient in processed foods such as candy, baked goods, ice cream, and baby food, thanks to its transformation into whey protein isolate (WPI) and whey protein concentrate (WPC) (1).

Whey Protein Isolate (WPI) contains at least 90% protein, making it a highly concentrated source of protein, while Whey Protein Concentrate (WPC) has protein levels ranging from 30% to 70%. Both types of whey protein are commonly used in food products and are driving innovations in edible film technology (2, 3). Whey protein-based films are known for their clarity, odorless nature, and flexibility, and they are setting new standards in the development of smart and active packaging solutions. These films offer potential benefits in enhancing food preservation and sustainability (4).

The production of WPI involves sophisticated ion-exchange chromatographic techniques, leveraging whey proteins' unique charge properties at varying pH levels for precise protein separation and concentration (5). While these methods yield high-protein, low-lactose products suitable for diverse applications, they face challenges, including the high cost of ultrafiltration, extensive washing requirements, and risks of microbial contamination in reactors (6).

In Jordan, ensuring food security and promoting sustainable agriculture requires a comprehensive approach. Financial support, including agricultural loans [7] and aid programs [8], plays a crucial role in enhancing productivity and supporting small-scale farmers. The adoption of advanced, crop-specific technologies [9] enhances both efficiency and sustainability in farming practices. Furthermore, empowering rural youth [10] through targeted initiatives fosters a more inclusive and resilient agricultural sector. While Jordan maintains moderate food security [11], challenges such as significant post-harvest losses, particularly in potato production, remain a pressing issue. These losses, coupled with growing demand, highlight the urgent need for innovative storage and preservation solutions [12]. This study is an example of the applications of whey proteins [13], which explored the impact of whey protein coatings on potato quality, revealing minimal effects on dry matter and firmness but a slight reduction in total soluble solids. Importantly, refrigeration was found to be the most effective preservation method, especially for storage periods exceeding 32 days, emphasizing its potential to reduce post-harvest losses and improve food security.

The significance of this topic lies in its dual impact: reducing food industry waste and enhancing food security through sustainable innovation. By converting surplus whey into high-value proteins and smart packaging materials, we address both environmental and nutritional challenges. This aligns with global goals for circular economies and resource efficiency, especially in regions like Jordan where agricultural resources are limited and food demand is rising.

This research aimed to identify the most efficient whey protein extraction method by evaluating four techniques in dairy factories. It analyzed the process before and after extraction and explored innovative methods such as heat precipitation, ultrafiltration, and foaming bubbles to improve yield and protein composition.

Methodology

This research was conducted in Jan 2022 to Feb 2023 in the Chemistry Laboratories of the Faculty of Agriculture at Jerash University, Jordan, using advanced equipment to ensure accurate and reliable results.

All milking procedures followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) and complied with IACUC-approved animal welfare protocols and Directive 2010/63/EU, ensuring minimal harm and distress to animals with continuous monitoring for ethical compliance.

2.1 Whey Protein Collection and Extraction

Whey protein was collected from dairy factories in Jerash and Irbid cities, as well as from the Jerash University Dairy Factory, following strict safety protocols. The acid whey was stored in 2-liter plastic bags, transported in ice boxes, and refrigerated at 4°C for 12 hours before use, ensuring freshness for one business day. Various extraction methods, including thermal precipitation, ultrafiltration, bubble foaming, and neutral acid carboxymethyl cellulose (NA-CMC), were tested based on a comprehensive literature review. The most effective method, which provided the highest protein yield and optimal separation efficiency, was selected for further use.

2.2 Whey Protein Extraction Methods

2.2.1 Heat Precipitation Method

The heat precipitation process for isolating whey protein begins by acidifying the whey to pH 3, which initiates the formation of small protein clumps (Figure 1). The solution is then heated to 90°C, promoting further protein precipitation. Subsequently, pH was raised to 4.5 to encourage the formation of larger protein aggregates, which are then separated through centrifugation. Finally, the isolated protein is neutralized to pH 6.0, resulting in the final whey protein isolate (14). This method, which relies on precise pH adjustments, produces high-quality protein concentrates and isolates.

2.2.2 Ultrafiltration Method

Whey protein is isolated using ultrafiltration (Figure 2), where fresh whey, initially at pH 5.2 and stored at 8°C for up to seven days, is forced through a 30-kDa membrane. The whey is first heated to 60°C for 15 minutes with sonication (ultrasonic heating) and agitation to assist in protein separation. It is then clarified by centrifugation. The clarified whey is filtered using an Amicon cell and tangential flow filtration (TFF) to improve separation efficiency and increase yield. Further purification is achieved with a 10 kDa membrane and ultracentrifugation. Lactose content is measured spectrophotometrically, while protein concentration is determined via the Bradford assay, which utilizes the reaction between protein and the Quance phenol dye (15).

<p>Acidification of whey to pH=3.</p> <p>Protein + H⁺ → Protein Clumps</p> <p>Heating to 90 °C to enhance precipitation</p> <p>Protein Clumps $\xrightarrow{90^{\circ}C}$ Precipitated Protein</p> <p>Raising pH to 4.5 to form larger aggregates:</p> <p>Precipitated Protein + OH⁻ → Larger Protein Aggregates</p> <p>Separation of proteins via centrifugation (a physical process).</p> <p>Neutralization of the isolated protein to pH 6.0:</p> <p>Larger protein Aggregates + OH⁻ → Neutralized Whey Protein</p>	<p>Initial heating and sonication:</p> <p><i>Protein (soluble)</i> $\xrightarrow{60^{\circ}C, \text{sonication}}$ <i>Protein</i> (Partially separated)</p> <p>Filtration through a 30-kDa membrane:</p> <p>Protein > 30 kDa (Retentate)</p> <p>Protein < 30 kDa (Permeate)</p> <p>Further purification with a 10-kDa membrane:</p> <p>Protein > 10 kDa (Retentate)</p> <p>Protein < 10 kDa (Permeate)</p> <p>Bradford assay reaction (protein quantification):</p> <p>Protein + Coomassie Brilliant Blue → protein-Dye Complex</p>
Figure 1: Step-by-step process of isolating whey protein by thermal precipitation.	Figure 2: Step-by-step procedure for size-exclusion of proteins using ultrasound and ultrafiltration.

2.2.3 Foaming Bubble Method

Bubble foaming is a technique for isolating proteins using a specialized column designed by a medical equipment manufacturer (Figure 3). This method relies on the interaction between proteins and bubbles in an aqueous medium. Proteins are adsorbed to the surface of bubbles or separated based on their affinity for the bubble interface. While the exact chemical reaction is not specified, the technique's effectiveness in protein separation has been demonstrated in studies, emphasizing its ability to maintain stable protein concentrations, which makes it a reliable and efficient method. The process is fundamentally based on the protein-bubble interactions within the medium (16).

2.2.4 Neutral Acid Carboxymethyl Cellulose Method

Neutral Acid Carboxymethyl Cellulose (NA-CMC) protein isolation method (Figure 4) isolates protein by adding 0.1% carboxymethyl cellulose (CMC) to one liter of acid whey and adjusting the pH to 3.2 using hydrochloric acid (HCl). The mixture is stirred for one hour to enhance protein interaction with CMC, then left undisturbed for protein precipitation. NA-CMC is made by replacing hydroxyl groups (-OH) in cellulose with carboxymethyl groups (-CH₂-COO⁻), improving solubility and hydrophilicity. Cellulose consists of β(1→4) linked glucose units. NA-CMC is prepared by treating cellulose with sodium hydroxide (NaOH), then adding sodium chloroacetate (ClCH₂COONa) to replace the hydroxyl groups and adjusting the pH to neutral (17, 18).

<p>Proteins interact with bubbles based on their hydrophobicity.</p> <p>Hydrophobic proteins adsorb onto bubble surfaces, forming a protein-bubble complex:</p> <p><i>Protein + Bubble</i> → <i>Protein-Bubble Complex</i></p> <p>They migrate to the bubble interface:</p> <p><i>Protein (Hydrophobic)</i> → <i>Bubble surface Adsorption</i></p> <p>While hydrophilic, remain in the aqueous phase:</p> <p><i>Protein (hydrophobic)</i> → <i>Aqueous Phase</i></p> <p>This enables selective protein separation while preserving structural integrity.</p>	<p>Acidification of whey:</p> <p>$HCl \rightarrow H^{+} + Cl^{-}$</p> <p><i>Whey Protein + H⁺</i> → <i>Acidified Whey Protein</i></p> <p>Preparation of NA-CMC (Carboxymethylation of Cellulose):</p> <p>$Cellulose + NaOH \rightarrow Alkali\ cellulose + H_2O$</p> <p>$Alkali\ Cellulose + ClCH_2COONa \rightarrow$ Carboxymethyl Cellulose (CMC) + NaCl</p> <p>Protein interaction with CMC and precipitation:</p> <p><i>Acidified Whey Protein + CMC</i> → <i>Protein-CMC Complex</i></p> <p><i>Protein-CMC Complex</i> $\xrightarrow{Undisturbed}$ <i>Precipitated Protein</i></p>
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Figure 3: Step-by-step procedure for protein separation by adsorption onto the bubble surface.

Figure 4: Step-by-step precipitation of whey protein using carboxymethyl cellulose (CMC).

2.3 Statistical analysis

Program SAS was used for statistical evaluation (19). General Linear Model (GLM) was used to determine the effect of the Dairy Factories, Extraction Methods, Sample Analysis Stages and the interactions between them on Fat %, Protein % and lactose % of cow milk according to the following linear model:

$$Y_{ijkl} = \mu + DF_i + EM_j + SA_k + (DF \times EM)_{ij} + (DF \times SA)_{ik} + (EM \times SA)_{jk} + I\sigma^2 e$$

Where, μ = overall mean. DF_i = effect of *i*th Dairy factories coded as *i*=1 (Jerash city), *i*=2 (Irbid city) and *i*=3 (Jerash university). EM_j = effect of *j*th Extraction Methods coded as *j*=1 (Heat precipitation), *j*=2 (Ultrafiltration), *j*=3 (Foaming Bubble), and *j*=4 (Neutral Acid Carboxymethyl Cellulose). SA_k = effect of *k*th Sample analysis stages coded as *k*=1 (Before filtration), and *k*=2 (After filtration). $(DF \times EM)_{ij}$ = effect of *ij*th interaction between Dairy factories and Extraction Methods. $(DF \times SA)_{ik}$ = effect of *ik*th interaction between Dairy factories and Sample analysis stages. $(EM \times SA)_{jk}$ = effect of *jk*th interaction between Extraction Methods and Sample analysis stages. e = random error term associated with Y_{ijkl} observations with zero mean and variance $I\sigma^2 e$. To determine significant differences between means of the Classifications effects, Duncan's multiple range test was used (20).

RESULTS

Table 1 outlines the key trends in protein isolation, emphasizing methods that yield the highest protein concentrations. Specifically, protein content remains consistent across dairy factories but varies significantly depending on the extraction technique. Notably, ultrafiltration proves to be the most effective method, achieving the highest protein concentrations, with levels increasing considerably after filtration. In contrast, fat content remains stable across different factories, extraction methods, and stages of analysis, showing no significant variation. Furthermore, lactose levels are generally stable, but a notable reduction occurs following filtration.

Table 1: Means and standard errors of protein, fat, and lactose percentages in whey protein across dairy factories, extraction methods, and sample analysis stages.

Factors	Classifications	Traits		
		Whey Protein%	Fat%	Lactose%
Dairy Factories	Jerash City	1.832±0.261a	0.258±0.009a	3.954±0.050a
	Irbid City	1.818±0.259a	0.265±0.010a	3.954±0.047a
	Jerash University	1.815±0.252a	0.266±0.009a	3.950±0.050a
Extraction Methods	Heat precipitation	1.685±0.250b	0.268±0.011a	3.922±0.059a
	Ultrafiltration	2.306±0.402a	0.252±0.012a	3.972±0.042a
	Foaming Bubble	1.659±0.243b	0.265±0.011a	3.950±0.065a
	NA-CMC	1.637±0.241b	0.269±0.010a	3.967±0.059a
Sample Analysis Stages	Before filtration	1.657±0.001b	0.267±0.008a	4.067±0.029a
	After filtration	2.987±0.099a	0.261±0.007a	3.839±0.040b
Overall Means		1.282±0.278	0.25833±0.009	0.247±0.050

NA-CMC = Neutral Acid Carboxymethyl Cellulose. Values sharing the same letter do not show significant differences ($p>0.05$).

Table 2 presents key trends in protein isolation. While fat content remains largely stable across various factories and extraction techniques, with minimal variation, protein content shows more noticeable fluctuations. Ultrafiltration is identified as the most effective method for obtaining the highest protein concentrations. In comparison, lactose percentages exhibit only slight variations and remain relatively stable across methods and factories. The standard errors further emphasize the variability in protein content, suggesting that extraction methods, particularly ultrafiltration, have a significant impact on protein levels. In contrast, fat and lactose percentages demonstrate greater consistency across different conditions.

Table 2: Means and standard errors of protein, fat, and lactose percentages in whey protein across of factors dairy factories and extraction methods.

Factors		Traits		
Dairy Factories	Extraction Methods	Whey Protein %	Fat%	Lactose%
Jerash City	Heat precipitation	1.638±1.076a	0.267±0.052a	3.833±0.344a
	Ultrafiltration	2.362±1.872a	0.250±0.051a	3.950±0.187a
	Foaming Bubble	1.700±1.144a	0.248±0.044a	4.000±0.283a
	NA-CMC	1.628±1.066a	0.268±0.037a	4.033±0.137a
Irbid City	Heat precipitation	1.695±1.145a	0.268±0.050a	3.983±0.194a
	Ultrafiltration	2.312±1.827a	0.255±0.054a	4.001±0.179a
	Foaming Bubble	1.638±1.076a	0.268±0.044a	3.833±0.344a
	NA-CMC	1.628±1.100a	0.272±0.053a	4.000±0.167a
Jerash University	Heat precipitation	1.722±1.169a	0.270±0.045a	3.950±0.197a
	Ultrafiltration	2.245±1.743a	0.252±0.050a	3.967±0.197a
	Foaming Bubble	1.638±1.076a	0.278±0.051a	4.017±0.194a
	NA-CMC	1.655±1.094a	0.267±0.049a	3.867±0.383a

NA-CMC = Neutral Acid Carboxymethyl Cellulose. Values with same letters are not significantly different ($p>0.05$).

Table 3 shows that filtration increases protein concentration, confirming its role in protein isolation. Fat content remains unchanged, while lactose content decreases slightly. Protein concentration varies the most after filtration, emphasizing its impact. These results highlight the importance of filtration in optimizing protein isolation.

Table 3: Means and standard errors for protein, fat, and lactose percentages in whey protein across different dairy factories and sample analysis stages.

Factors		Traits		
Dairy Factories	Sample Analysis Stages	Whey Protein%	Fat%	Lactose%
Jerash City	Before filtration	0.657±0.005a	0.267±0.049a	4.067±0.178a
	After filtration	3.008±0.650a	0.250±0.039a	3.842±0.261a
Irbid City	Before filtration	0.657±0.007a	0.267±0.050a	4.067±0.177a

Factors		Traits		
Dairy Factories	Sample Analysis Stages	Whey Protein%	Fat%	Lactose%
	After filtration	2.980±0.651a	0.265±0.048a	3.842±0.223a
Jerash University	Before filtration	0.657±0.006a	0.267±0.047a	4.067±0.179a
	After filtration	2.973±0.530a	0.267±0.046a	3.833±0.253a

Values with same letters are not significantly different ($p>0.05$).

Table 4 presents the impact of various protein isolation methods on protein, fat, and lactose percentages before and after filtration. Protein concentration increases with all methods, with Ultrafiltration yielding the highest levels. Fat content stays constant, while lactose decreases, with Heat Precipitation showing the greatest reduction. Standard errors reveal moderate variability, particularly in protein and lactose changes after filtration. Ultrafiltration is the most effective method for maximizing protein concentration.

Table 4: Means and standard errors for whey protein, fat, and lactose percentages in whey protein across extraction methods and sample analysis stages.

Factors		Traits		
Extraction Methods	Sample Analysis Stages	Whey Protein%	Fat%	Lactose%
Heat precipitation	Before filtration	0.657±0.006b	0.267±0.050a	4.067±0.180a
	After filtration	2.713±0.144a	0.270±0.044a	3.778±0.228a
Ultrafiltration	Before filtration	0.657±0.005b	0.267±0.051a	4.067±0.181a
	After filtration	3.956±0.240a	0.238±0.045a	3.878±0.120a
Foaming Bubble	Before filtration	0.657±0.007b	0.267±0.053a	4.067±0.182a
	After filtration	2.661±0.081a	0.263±0.043a	3.833±0.316a
NA-CMC	Before filtration	0.657±0.004b	0.267±0.052a	4.067±0.183a
	After filtration	2.618±0.225a	0.271±0.040a	3.867±0.278a

NA-CMC = Neutral Acid Carboxymethyl Cellulose. Values sharing the same letter are not significantly different ($p>0.05$).

Table 5 examined the correlations between protein, lactose, and fat content across various dairy factories, extraction methods, and sample analysis stages. In dairy factories, Jerash City showed a significant negative correlation between protein and lactose (-0.43*), with weak correlations for fat. Irbid City and Jerash University also demonstrated negative correlations between protein and lactose, with Jerash University having the strongest correlation (-0.45*). Among extraction methods, heat precipitation had the strongest negative correlation between protein and lactose (-0.58*), while ultrafiltration also showed a significant negative correlation (-0.56*). Foaming bubble and NA-CMC had weaker or non-significant correlations. Sample analysis stages revealed that before filtration, protein and lactose had a highly significant negative correlation (-0.97**), with fat showing a significant negative correlation with protein (-0.50**) and a moderate positive correlation with lactose (0.28). After filtration, protein and lactose showed a weak positive correlation (0.12), while fat had negative correlations with both protein (-0.31) and lactose (-0.26).

Table 5: Pearson correlation coefficients between protein, fat, and lactose traits in whey protein across dairy factories, extraction methods, and sample analysis stages.

Factors	Classifications	Traits	Lactose %	Protein %
Dairy Factories	Jerash City	Protein %	-0.43*	
		Fat %	-0.02	-0.24
	Irbid City	Protein %	-0.41*	
		Fat %	0.02	-0.09
	Jerash University	Protein %	-0.45*	
		Fat %	0.06	-0.08
Extraction Methods	Heat Precipitation	Protein %	-0.58*	
		Fat %	0.01	0.02
	Ultrafiltration	Protein %	-0.56*	
		Fat %	0.16	-0.27
	Foaming Bubble	Protein %	-0.41	
		Fat %	-0.02	-0.05
	NA-CMC	Protein %	-0.39	
		Fat %	-0.04	-0.01
Sample Analysis Stages	Before Filtration	Protein %	-0.97**	
		Fat %	0.28	-0.50**
	After Filtration	Protein %	0.12	
		Fat %	-0.26	-0.31

NA-CMC = Neutral Acid Carboxymethyl Cellulose. Values marked with an asterisk indicate the significance of the association, with more asterisks indicating a stronger association.

DISCUSSION

Table (1) highlights the impact of dairy processing on protein isolation and concentration. Protein content varies depending on the extraction method, with ultrafiltration yielding the highest levels. Subsequent filtration further concentrates protein significantly, demonstrating the effectiveness of these processes in protein enrichment. In contrast, fat percentages remain consistent across factories, extraction methods, and sample analysis stages, showing no significant differences. Lactose levels exhibit minor variations across factories and extraction methods but decrease substantially after filtration. These findings emphasize that while fat content remains stable, extraction and filtration play a crucial role in isolating and concentrating protein while reducing lactose levels.

Protein isolation and concentration in cow milk vary depending on the extraction method. Ultrafiltration effectively increases protein content in cow milk concentrates, with lower temperatures (15 °C) enhancing membrane performance and emulsifying properties (21). Cold microfiltration further improves protein recovery, though it may raise lactose levels in the permeate (22). Membrane technologies, including capillary gel electrophoresis, provide precise protein analysis and serve as an alternative to heat treatment, which, while ensuring cow milk safety, may degrade nutritional quality (23, 24). Filtration also helps reduce lactose content, with effectiveness depending on lactase purity and the chosen method (25).

The literature reports protein concentrations varying across different extraction techniques. Thermal precipitation yields protein concentrations between 0.5% and 1% (26), while ultracentrifugation produces values ranging from 0.6% to 1.6% (27) and 0.5% to 1.5% (28). The bubble foam method consistently achieves protein concentrations between 0.56% and 1.5% (29).

Additionally, using high-quality raw cow milk improves process efficiency without altering fat percentages (30).

Protein isolation and concentration in whey protein vary significantly based on the extraction method. Table (2) highlights that ultrafiltration consistently yields the highest protein content, with substantial variability observed across different extraction techniques. The standard errors in protein content further emphasize the strong influence of the extraction method, particularly ultrafiltration, on protein levels. In contrast, fat and lactose content remain relatively stable, showing minimal variation regardless of the extraction method or factory.

To enhance protein extraction, Hadidi et al. (31) investigate the use of ultrasound, ultrafiltration, and alkaline techniques. These methods effectively modify protein content, although, as Van Lieshout et al. (32) note, they have little impact on fat and lactose levels in dairy products. Despite advancements in extraction methods, whey protein's potential for sustainable food processing is still limited by gaps in understanding minor low-molecular-weight compounds (33).

Table (3) illustrates the impact of filtration on dairy composition, showing a significant increase in protein content after filtration, confirming its effectiveness in protein isolation and concentration. Protein levels exhibit the highest variability, influenced by factors such as cow milk source, initial protein composition, and filtration parameters. In contrast, fat content remains stable throughout the process, while lactose levels decrease slightly.

Gerardo et al. (34) emphasize the importance of optimizing filtration and centrifugation for efficient fat extraction. The combination of polymeric microfiltration membranes with ultrafiltration effectively removes whey proteins from cheese cow milk while preserving casein, leading to high-quality Cheddar cheese and valuable whey by-products (35). Additionally, precise measurement of lactose as anhydrous lactose is essential for maintaining dairy product quality, improving cow milk production, and supporting the development of new lactose-based applications (36).

Table (4) highlights the effectiveness of different extraction methods in protein isolation and concentration. Protein content increased across all methods, with ultrafiltration yielding the highest concentration. In contrast, lactose content consistently decreased, with heat precipitation causing the most significant reduction. Fat content remained stable regardless of the extraction method. Standard error analysis showed moderate variability across all components, with protein and lactose levels exhibiting the highest fluctuations, likely due to differences in cow milk composition, extraction parameters, and the sensitivity of these components to specific extraction techniques.

Ultrafiltration effectively increases protein content, while heat precipitation primarily reduces lactose, maintaining fat stability (37). However, reconstituted cow Milk Protein Concentrate powders have lower thermal stability than skim cow milk due to manufacturing differences. Heat treatments like pasteurization and sterilization improve stability and eliminate bacteria but can also cause browning, protein denaturation, and other chemical changes (38). Additionally, filtration methods such as microfiltration and ultrafiltration, along with factors like flux rate and temperature, play a crucial role in determining protein concentration (24).

Table (5) shows an inverse relationship between protein and fat in dairy products, with ultrafiltration and heat precipitation increasing protein while reducing fat, especially before filtration (-0.97** correlation). Filtration methods significantly alter protein content, while lactose weakly correlates with both protein and fat.

This protein-fat inverse relationship challenges the focus on low-fat dairy (39). Coşkun et al. (40) note that the whey protein-to-casein ratio affects cow milk's structure and viscosity. Transmission

infrared spectroscopy accurately measures fat and protein but is less effective for lactose (41). Understanding how processing techniques influence these relationships is key to optimizing protein levels in dairy.

CONCLUSIONS

This study focuses on whey protein isolation and concentration, highlighting that whey protein content is highly sensitive to both extraction and filtration methods, with ultrafiltration having the most significant impact. In contrast, fat content in whey protein remains relatively stable across different extraction methods. Lactose content shows moderate reductions post-processing, which varies depending on the specific extraction technique used. The interactions between cow milk components (fat, protein, and lactose) are complex and heavily influenced by the processing methods and conditions applied.

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استراتيجيات مبتكرة لتعظيم استعادة بروتين مصل اللبن من منتجات الألبان الثانوية

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الملخص:

تركز هذه الدراسة على استخلاص وتركيز بروتينات مصل اللبن، وهي مكونات أساسية في مصل اللبن وناجحة عن تصنيع الجبن. يُعد كل من بروتين مصل اللبن المعزول (WPI) وبروتين مصل اللبن المركز (WPC) من المنتجات الغذائية المهمة المستخدمة في مجموعة متنوعة من السلع الاستهلاكية، بما في ذلك المخبوزات، والحلويات، والآيس كريم، وأغذية الأطفال. أجريت هذه الدراسة في شهر آب 2024 داخل مختبرات الكيمياء بكلية الزراعة في جامعة جرش، وهدفت إلى تحديد الطريقة الأكثر فعالية لاستخلاص بروتينات مصل اللبن باستخدام أربع تقنيات مختلفة: الترسيب الحراري، والترشيح الفائق، وتكوين الفقاعات، وسليولوز الكربوكسي ميثيل المحايد الحمضي (NA-CMC). تم استخدام SAS (GLM) لتحليل تأثيرات مصانع الألبان، وطرق الاستخلاص، ومراحل العينة، وتفاعلاتها على الدسم، والبروتين، واللاكتوز في حليب البقر، مع اختبار الدلالة من خلال اختبار دنكان متعدد المدى. أظهرت النتائج أن محتوى البروتين قد ازداد بشكل ملحوظ باستخدام تقنية الترشيح الفائق مقارنة بالطرق الأخرى، بينما بقي محتوى الدهون ثابتاً عبر جميع طرق الاستخلاص. كما انخفض محتوى اللاكتوز باستخدام الطرق الأربع، وكان أكبر انخفاض في الترسيب الحراري. أشارت الأخطاء المعيارية للمتوسطات إلى وجود تفاوت معتدل، حيث أظهرت البروتينات واللاكتوز أعلى تقلبات بعد الترشيح. تسلطت الدراسة الضوء على التفاعلات المعقدة بين المكونات الكبرى في الحليب البقر (البروتين، والدهون، واللاكتوز) وبين طرق الاستخلاص وظروف المعالجة التي تؤثر على النتائج. الاستنتاج، لا يزال تطوير تقنيات فعالة لاستخلاص بروتينات مصل اللبن يمثل تحدياً، ولكنه ضروري لتعزيز صناعات الأغذية والتعبئة والتغليف. ومن خلال تحسين هذه الأساليب لاستعادة مصل اللبن، الذي يُعتبر تقليدياً منتجاً ثانوياً، تهدف هذه الدراسة إلى تحويله إلى مورد قيم لاستخدامات متعددة.

الكلمات المفتاحية: طرائق الجمع، تقنيات التركيز، معالجة الألبان، عمليات الفصل، عزل البروتين، استرداد بروتين مصل الحليب البقر.