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Research Paper

Effective Utilization of Whey to Produce Protein Based Bioproduct

Muhammad Monis Imdad

Dairyland Private Limited, Gharao, Thatta, Pakistan.

ABSTRACT

Whey protein contains globular proteins like α-Lactalbumin and β-lactoglobulin that play an important role in the innate immune system. In this study, fresh bovine milk whey was utilized to develop a 'whey protein' drink with a protein content of 3.8%. Biochemical testing of the 'whey protein' drink was performed to identify its total protein content, types of protein, and protein stability for four months. Fresh bovine milk whey was collected from cheese curd followed by standardization, formulation, and homogenization. The product was then moved toward plate heat exchangers for pasteurization and ultra-high treatment. Later, the product was filled in 6-layered packaging. Protein quantification and SDS-PAGE were performed to characterize the protein present and its stability concerning the shelf life. The size exclusion HPLC was performed to analyze the proteins in whey drink. The amount of protein decreased gradually throughout the shelf life. Bands of α-Lactalbumin and β-Lactoglobulin could be seen in all samples, however, the band of bovine serum albumin became lighter at the end of the shelf-life period. In HPLC chromatograms, two peaks were observed in fresh, 30-day, and 60-day-old samples. Whereas in 90-day and 120-day-old samples, the peaks were merged indicating a conformational change in the whey proteins and probable protein-complex formation.

KEYWORDS: Lactoglobulin, Lactalbumin, SDS-PAGE, Whey protein.

INTRODUCTION

Protein malnutrition, also known as protein-energy malnutrition (PEM), is a significant health problem worldwide. According to the World Health Organization (WHO), an estimated 149 million children under the age of five were stunted in 2020 due to malnutrition, with protein deficiency being one of the leading causes. The Global Nutrition Report 2020 found that around one in nine people worldwide (9.9%) were undernourished, with the highest prevalence in sub-Saharan Africa (21.3%) and South Asia (13.7%). In 2019, the prevalence of global acute malnutrition (GAM) among children under the age of five was 7.9%, with the highest prevalence in sub-Saharan Africa (13.8%). The Lancet in 2019 estimated that the burden of malnutrition in all its forms, including protein malnutrition, resulted in 11 million deaths and 255 million disability-adjusted life-years lost in 2017 [1].

The whey protein is a term that describes the group of milk proteins that are soluble at pH 4.6 and 20°C. It is a byproduct of the cheese-making process and constitutes about 20% of the total protein in milk [2]. It is a complex protein that consists of a mixture of globular proteins, including beta-lactoglobulin, alpha-lactalbumin, serum albumin, and immunoglobulins [3]. Whey protein contains bioactive peptides that have been shown to have numerous health benefits, such as reducing blood pressure, improving immune function, and enhancing antioxidant activity [4]. Whey protein is particularly rich in essential amino acids, particularly leucine, which are crucial for stimulating muscle protein synthesis and promoting muscle hypertrophy [3]. One of the most well-established benefits of whey protein is its

ability to promote muscle growth and repair. Fresh whey has been shown to enhance immune function [5]. Fresh whey has been used to develop yogurt, smoothies, and protein bars that are fortified with immune-enhancing compounds [6]. Fresh whey has been incorporated into infant formulas to improve the immune system of babies [7]. Whey protein may also have benefits for weight management. Some studies suggest that whey protein can increase satiety, leading to reduced calorie intake and weight loss [8]. One study found that consuming whey protein before a meal led to significantly lower calorie intake compared to consuming a placebo. Whey protein may also help to preserve lean body mass during weight loss, preventing muscle loss and promoting fat loss [9]. Consuming fresh whey-based drink for four weeks led to a significant increase in the levels of immunoglobulins and cytokines in healthy adults, suggesting an improvement in immune function [10]. It has been reported that consuming a fresh whey-based supplement for 12 weeks improved the immune response in elderly individuals, potentially reducing the risk of infections [11].

Whey protein is widely used in the food industry due to its high nutritional value and functional properties. It is commonly utilized as an ingredient in various food products, including protein bars, sports drinks, and infant formula to enhance the texture and flavor of dairy products such as yogurt and ice cream [12]. Whey protein is used as a nutritional supplement for livestock to improve the protein content and nutritional value of their feed [13], which can be especially beneficial for young animals or animals that are stressed or recovering from illness. Whey protein is also used in non-food applications, particularly in the pharmaceutical industry. It is a source of bioactive peptides, which are small protein fragments with antioxidant and antimicrobial properties that have been linked to several health benefits [14]. These peptides were extracted from whey protein and utilized as functional ingredients in pharmaceutical products, such as wound healing products [15]. Whey protein is easily digestible and rapidly absorbed by the body, making it a desirable ingredient for sports nutrition products and dietary supplements [3]. Whey protein is a versatile ingredient that has numerous applications in various industries, making it a valuable commodity in the market [16].

The non-alcoholic beverage industry is a significant segment of the food and beverage market worldwide, experiencing rapid growth due to changing lifestyles, increasing disposable incomes, and growing health consciousness. By 2025, the global non-alcoholic beverage market is expected to reach a value of USD 1.6 trillion, with a CAGR of 4.6% from 2020 to 2025. The US and Europe represent the largest markets for soft drinks and bottled water, respectively, while the Asia-Pacific region is the fastest-growing market due to the rise in the middle-class population and increased disposable incomes. Apart from soft drinks and bottled water, the beverage market also includes energy drinks, sports drinks, RTD tea and coffee, and functional beverages, which have gained popularity due to their perceived health benefits. In the coming years, manufacturers are expected to focus on developing innovative and functional beverages to meet the growing consumer demand for unique health benefits [17].

The beverage industry in Pakistan is a growing sector, with a diverse range of products ranging from carbonated soft drinks, juices, tea, coffee, and energy drinks. The carbonated soft drink segment

dominates the Pakistan beverage industry, accounting for approximately 55% of total sales. The leading companies in this segment are PepsiCo and Coca-Cola, which control more than 95% of the market share. However, the juice and bottled water segments are also growing rapidly, with local companies such as Nestle and Shezan International gaining market share. The leading company in the health drink sector in Pakistan is Nestle, which offers a range of products such as Nesvita Pro-Bone milk, Nesfruta, and Nestle Juices. Another major player is Engro Foods, which offers a range of health drinks under the brand name Olpers, including Olpers Pro-Cal, Olwell, and O'Live. The government has also implemented policies to ensure the safety and quality of products, including the Pakistan Standards and Quality Control Authority (PSQCA) and the Punjab Food Authority (PFA).

The dairy industry in Pakistan is an important sector of the economy, providing employment to millions of people and contributing significantly to the country's GDP. According to a report by the Pakistan Dairy Development Company, the dairy industry in Pakistan is worth approximately PKR 1 trillion and the cheese industry in Pakistan is worth approximately PKR 5 billion and has an annual growth rate of 10-12%. In Pakistan's cheese industry, whey is typically considered as a waste and is often disposed of or used for low-value applications such as animal feed or fertilizer [18]. However, there is growing interest in finding new ways to utilize whey in the Pakistani cheese industry to reduce waste and increase profitability. Utilizing fresh whey in food product development provides an opportunity to create new functional foods with good nutritional properties.

This study was designed to develop and characterize value-added whey protein drinks in terms of nutrition and taste by utilizing fresh whey a byproduct in cheese processing by dairy industries in Pakistan.

MATERIALS AND METHODS

Whey samples have been collected directly from the curd trolley outlet after the production of mozzarella cheese at the temperature of 25°C. Samples were stored for aging at 4°C for 24 hours to separate a thin film of fat at the top. The top layer of fat was removed by filtration and samples were ready for biochemical characterization.

Fresh whey has been standardized at 4.2% protein, 0.8% fat, and 5% carbohydrates using concentrated whey protein, fresh cow milk cream, and sugar, respectively.

A total of 1175 liters of Mozzarella whey was taken in a batch silo tank mixer and heated. BS3 was connected to the mixing station. Dry ingredients such as sugar and acids were added to the mixing station when the temperature had reached 65°C. The high-shear mixer/agitator was kept off all the time to avoid foaming. Once all the ingredients were dosed, the slurry was circulated for 20-30 minutes to ensure proper mixing at a temperature of 65°C using a high shear mixer at 1800 RPM. The level was increased to 1250 liters by adding water to the mixer. The circulation was then stopped, and the entire batch was transferred to the Batch Silo tank.

The temperature of the batch decreased to below 5 °C and it was hydrated in Batch Silo for 01 hours at less than 10°C. It was ensured that the temperature was less than 10°C within 2 hours. After the hydration time was completed, the pH of the batch was checked using the pH meter. Citric acid was added to set the pH at 4.3 – 4.38. The batch was then agitated for 15 minutes after

adding the flavor. The batch was transferred to the UHT section through a centrifugal pump and homogenized at a pressure of 200-250 Bar. The temperature at the homogenization inlet was maintained at 65 ± 3°C.

Once the batch was homogenized, it was subjected to UHT treatment at 120°C for 30 seconds at a flow rate of 2500 liters/hour. The batch was then transferred into ATS, and the agitator was kept on. The product was packed in 250 ml Tetra packaging as 250 ml PET bottles. The product was then transferred to a warehouse for storage at ambient temperature and then proceeded for biochemical characterization.

Protein estimation of whey protein drink samples was carried out by Lowry's method. SDS page has been done for the analysis of types of protein present in whey protein drink samples and their stability in a four-month shelf-life period. To assess the fat content of milk and dairy products, including fresh whey, the Gerber technique is often used in the dairy business. The Gerber machine method may be used to ascertain the fat content of fresh whey. The pH meter and moisture analyzer were employed to record the pH and moisture content of fresh whey samples, respectively. The presence of soluble solids in fresh whey was also determined using a Refractometer:

Following that size exclusion-high-performance liquid chromatography was also carried out to determine and quantify the aggregates and fragments of protein or protein-derived content in the whey samples under isocratic conditions while the flow rate was maintained at 1 ml/min.

RESULTS AND DISCUSSION

During this food technology study, we have prepared a whey protein drink for commercial utility. Fresh whey (91%) was used in the formulation of whey drink to maximize the total protein content in the final product. To resolve the taste pungency, artificial fruit flavors were added. An appropriate blend of stabilizers was used to minimize the sedimentation and separation of whey drink components. Moreover, homogenization at 250 Bar played a significant role in product stabilization. The product was stable in terms of taste and physical attributes. There was no change in color, nor any separation and sedimentation observed throughout the shelf life. The details of whey drink samples taken for protein analysis are shown in Table 1.

Table 1: Whey protein drink samples.

No.	Sample code	Manufacturing date	Aging days
1	WP-4M	10-Sep-2022	122 days
2	WP-3M	22-Oct-2022	90 days
3	WP-2M	19-Nov-2022	60 days
4	WP-1M	17-Dec-2022	33 days
5	WP-0M	21-Jan-2023	Fresh (1 day)

Different chemical tests were performed using samples mentioned in table 1. These tests were carried out by Gerber milk testing equipment, pH meter, Moisture analyzer, and Refractometer. Test results are given in table 2. Five whey drink samples in triplicates were analyzed (fresh drink, 1-, 2-, 3-, and 4-months old whey drinks).

Protein estimation was carried out by the Lowry method. Bovine serum albumin was used as the standard protein.

Table 2: Composition of whey drink.

No.	Component/ parameter	WP-0M	WP-1M	WP-2M	WP-3M	WP-4M
1	Protein (%)	3.82	3.08	2.14	1.69	1.62
3	Fat (%)	5.60	5.60	5.50	5.60	5.50
4	pH	4.37	4.35	4.33	4.33	4.29
5	Moisture (%)	85	85	85	85	85
6	Soluble solids (°brix)	8	8	8	8	8

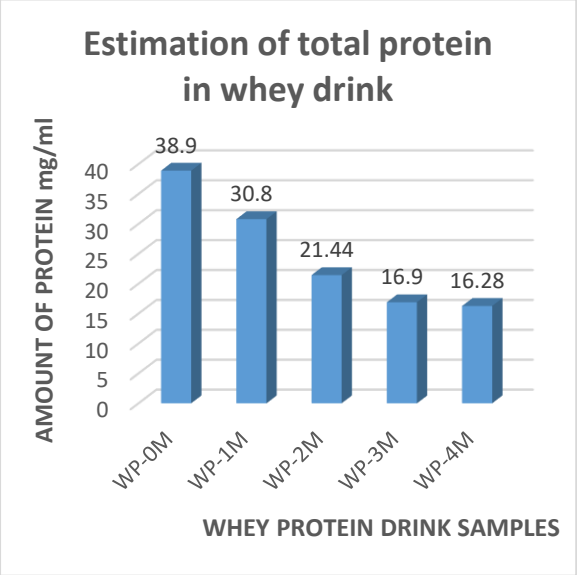

Figure 1: Estimation of total proteins whey drink samples.

The fresh whey drink sample (WP-0M) contained 38.9 mg/ml protein. The amount of protein in 1-month-old (WP-1M) sample was 30.8 mg/ml which indicated 20.8% protein loss. Likewise, in 2-month-old (WP-2M) sample, 44.9% of protein decreased. The amount of protein decreased in (WP-3M) and (WP-4M) samples was 56.5% and 58.1% respectively as shown in figure 1.

SDS PAGE analysis of whey drink samples was done to find out the types of protein present and to notice if there is any change in the proteins that occurs due to aging. Five samples were taken in triplicates, given the names of their months of manufacture.

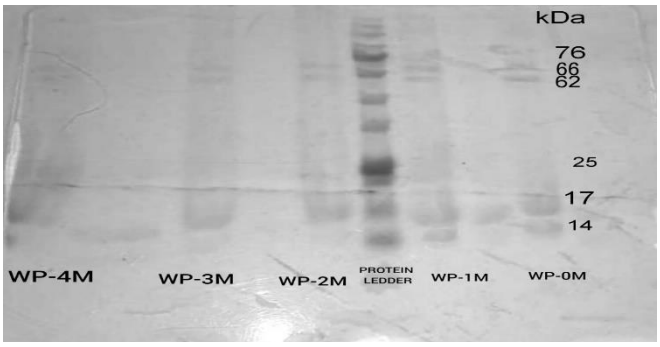

Figure 2: SDS-PAGE of proteins extracted from whey drinks samples.

The SDS PAGE of proteins extracted from whey drink samples is shown in figures 2 and 3. Four bands of different molecular masses have been observed. The band near the bottom with the molecular mass of 14 kDa was α-lactalbumin and the band above it was β-lactoglobulin. The protein ladder was used to identify the molecular masses of bands.

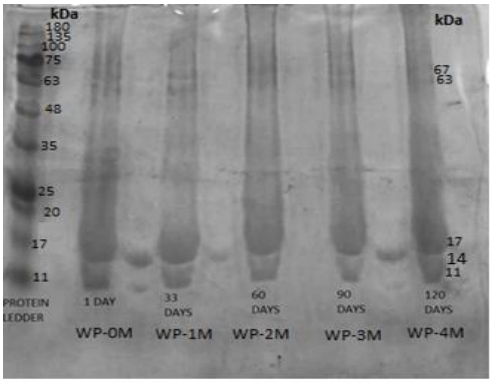

Figure 3: Electrophoresis Gel 2 of whey drinks samples.

The bands became lighter as the shelf life progressed. The fresh whey drink samples showed the most clearly observed bands and the lighter ones were of the 4-months-old samples (WP-4M).

Proteins present in 5 whey drink samples were analyzed by size exclusion high pressure liquid chromatography (SEC-HPLC). The chromatograms of SEC-HPLC are given in Figures 7-11.

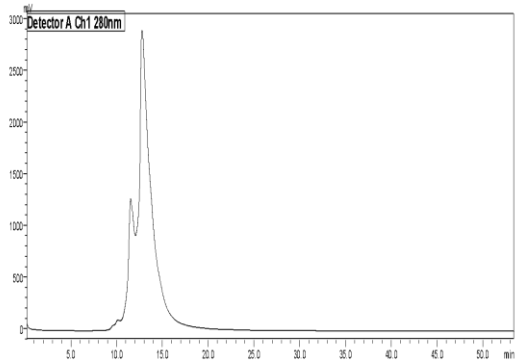

Figure 4: GFC by HPLC of fresh of whey drink (WP-0M).

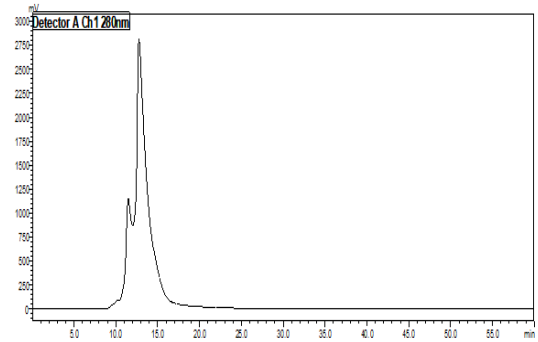

Figure 5: GFC by HPLC of One-month-old whey drink (WP-1M).

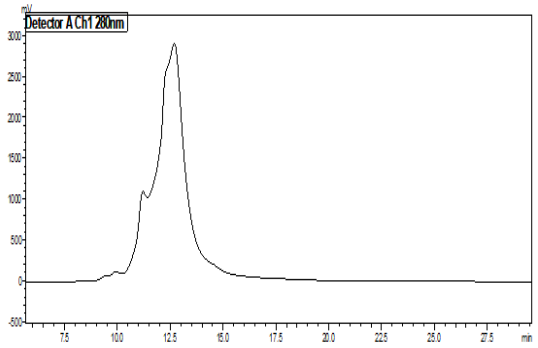

Figure 6: GFC by HPLC two-month-old whey drink (WP-2M).

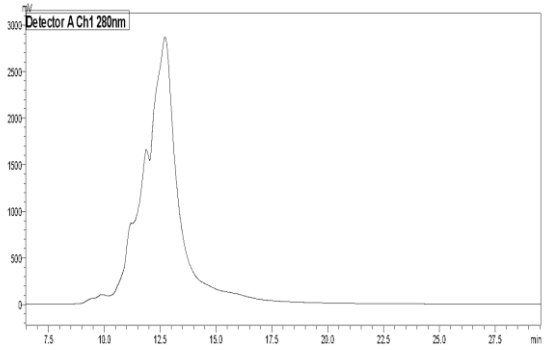

Figure 7: GFC by HPLC of three months old whey drink (WP-3M).

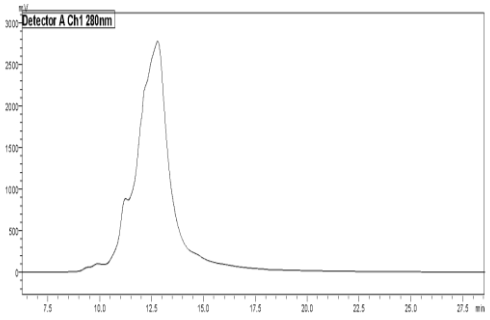

Figure 8: GFC by HPLC of four-months old whey drink (WP-4M).

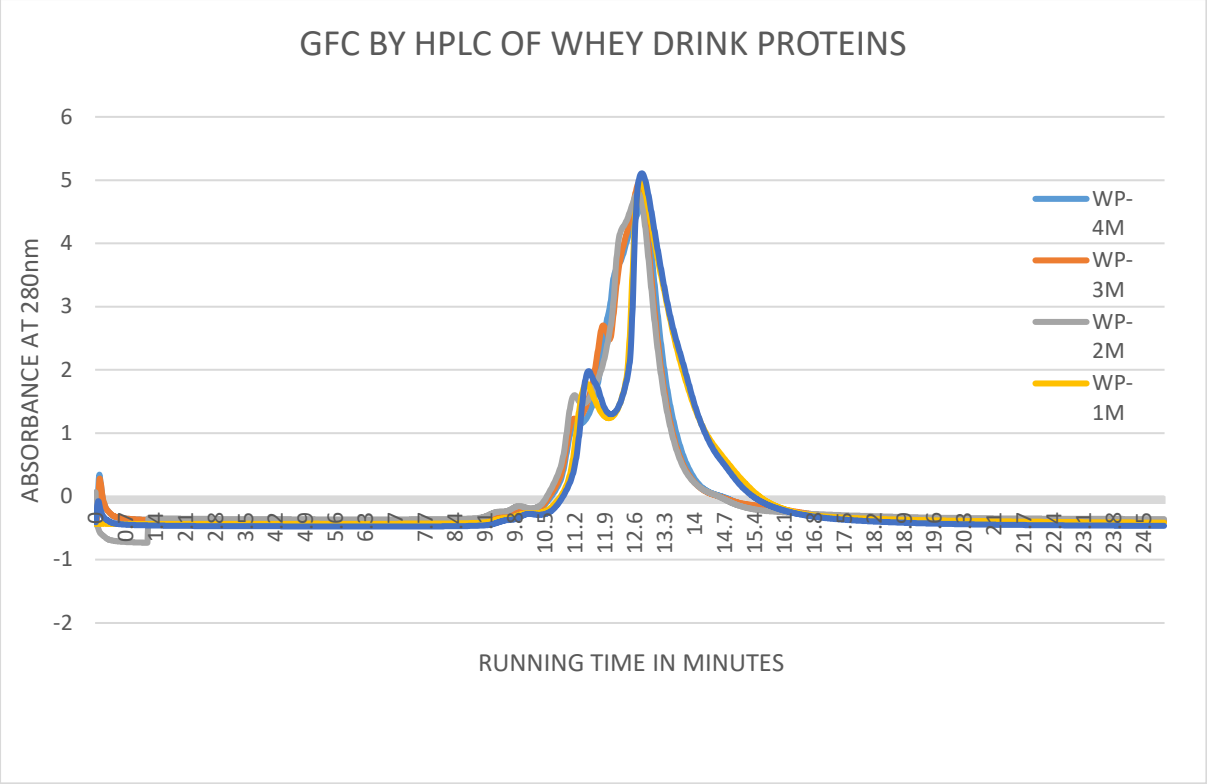

Figure 9: Superimposed HP-Gel Filtration Chromatograms of whey drink samples WP-0M, WP-1M, WP-2M, WP-3M, and WP-4M.

The fresh whey sample (code: WP-0M) showed two peaks. The first peak was eluted at 11.4 minutes and the second peak was eluted at 13 minutes as shown in figure 9. A similar trend was observed for the 1-month-old sample (WP-1M) indicating no change in the protein. The sample of two-month-old (WP-2M) showed two peaks like previous samples but there is a slight change in the elution time. First Peak was observed at 11.2 minutes and the second peak was eluted at 12 minutes indicating the start of conformational changes in proteins. In the 3 and 4-month-old samples (WP-3M and WP-4M), two separate peaks merged.

CONCLUSION

In this study, a whey protein drink was developed by utilizing the cheese by-product, “the fresh whey”. Freshly collected whey from the cheese curd trolley was chemically analyzed and standardized according to manufacturing order. Homogenization and pasteurization were done at the tetra pack plant followed by the 6-layer tetra packaging. Biochemical analysis was performed to determine the protein content and protein stability throughout the shelf life. The pH was in the range of 4.3-4.4 with a fat content of 5.6%, protein 3.8%, moisture 85%, and soluble solids 8%. Five samples were taken with a difference of approximately 30 days aging time. The amount of protein in the one-day-old whey drink sample was 3.89% gradually decreased to the level of 1.62% in the 122 days-old-sample. Gel electrophoresis results showed stable and sustained bands of α-Lactalbumin and β-Lactoglobulin. Bovine serum albumin was also detected in each of the samples with the lighter bands as the aging days proceeded. The Size exclusion-high-performance liquid chromatography (SEC-HPLC) indicated the conformational changes in the proteins after 60-days of the

shelf-life period and the two fine peaks observed in 1-day, 33-days and 60-days old samples were merged, showing a single peak in 90- and 122-days old samples. The presence of α-Lactalbumin and β-Lactoglobulin indicated the immune booster abilities and the whey protein amount ranges 1.6-3.9% makes this whey drink a healthy beverage in countering the protein malnutrition.

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