INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 13, Issue 2- 2026

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2026.13.02.001

Comparative evaluation of casein extraction and variability in six different milk samples – A biochemical approach

Aruna R[1], Jayakumar S[1*], Suresh B [2], Sharmila C[3], Hemalatha M[4]

[1,2,3,4] Department of Biochemistry, SRM Arts and Science College, Kattankulathur, Chengalpattu, Tamilnadu, India.
[*]Corresponding author: Dr. S. Jayakumar
Email: *jaikumarsbio@gmail.com*

Abstract

Casein is the principal protein of milk and serves as a vital component in the dairy industry and various industrial applications. The present study was undertaken to extract, quantify, and confirm the presence of casein in six different milk samples using an acid precipitation technique followed by biochemical confirmation tests. Casein was isolated by lowering the pH of milk to induce precipitation, after which the precipitate was filtered, washed, dried, and weighed to determine yield. Quantitative analysis revealed a gradual increase in casein content across the samples, with yields ranging from 10.2 g in milk sample 1 to 12.8 g in milk sample 6, indicating variability in protein composition among the milk sources.

Qualitative confirmation of casein was performed using the Biuret test and Neumann's test. The Biuret test confirmed the presence of protein through the formation of a violet color, indicating peptide bonds, while Neumann's test specifically identified casein by producing a characteristic lemon-yellow precipitate upon treatment with nitric acid, sulfuric acid, and ammonium molybdate. All six milk samples showed positive results in both confirmatory tests, validating the successful extraction and identification of casein.

The findings demonstrate that acid precipitation is a reliable and efficient method for casein extraction and that Biuret and Neumann's tests are effective for confirmatory analysis. This study highlights variations in casein yield among different milk samples and reinforces the importance of biochemical methods in dairy protein analysis.

Keywords: Casein, Milk protein, Acid precipitation, Biuret test, Neumann's test, Protein analysis.

Introduction

Casein, a major protein found in mammalian milk, plays a significant role in the nutritional and functional properties of milk. Comprising about 80% of the total protein content in cow's milk and slightly varying in other species like goat and buffalo, casein is widely used in food production, medicine, and industrial applications. Casein's ability to form gels and its slow digestion rate make it a valuable protein, especially in dairy products like cheese, yogurt, and protein supplements (McMahon et al., 2009). Given its economic and industrial importance, understanding the extraction and variability of casein from different milk sources is crucial for optimizing milk-based products. This study aims to compare the biochemical extraction, yield, and variability of casein from six distinct milk samples, shedding light on how different milk sources influence the casein properties.

The extraction of casein from milk typically involves acid precipitation, where the pH of milk is lowered to a point where casein aggregates and precipitates. This process is influenced by several factors, including the type of acid used, milk composition, temperature, and the presence of other additives (Fitzgerald et al., 2015). These factors contribute to differences in the yield, purity, and functionality of the extracted casein. Furthermore, the concentration of casein in milk varies by species and breed, with higher yields typically observed in cow's milk compared to goat's or sheep's milk (Fox et al., 2000). This study explores these differences by evaluating six milk samples with varying sources and types, providing a comprehensive comparison of casein extraction efficiency.

Milk composition varies not only between species but also within the same species due to factors such as diet, genetics, and lactation stage (Gilles et al., 2010). Cow's milk, for example, contains approximately 80-85% casein of its total protein content, while goat's milk may have a slightly higher concentration of casein. Similarly, milk from buffaloes has a higher fat content and a greater casein yield (Haug et al., 2007). Variability in milk composition may lead to differences in the characteristics of casein extracted, such as its solubility, texture, and functionality in dairy products. The study will also explore the impact of these variations on the casein's biochemical properties and how they might affect industrial applications like cheese-making and protein isolation.

In addition to species and breed variations, the extraction method itself plays a significant role in the yield and properties of casein. Traditional acid precipitation methods are commonly employed in research and industry due to their simplicity and cost-effectiveness (Swaisgood, 2003). However, variations in extraction parameters, such as pH control, temperature, and the type of acid or enzyme used, can significantly impact the amount of casein recovered and its purity (Mills et al., 2010). This comparative study will investigate how casein extraction differs across six types of milk, examining the yield, composition, and structural characteristics of the extracted casein.

Understanding the variability in casein properties is essential for tailoring dairy products to specific consumer needs, whether it be for nutritional enhancement, improved texture in cheese-making, or for functional food applications. Furthermore, optimizing casein extraction techniques can enhance the efficiency and cost-effectiveness of dairy production, providing both economic and technological benefits to the dairy industry.

Thus, this study aims to provide a detailed biochemical comparison of casein extraction from six different milk sources, offering insights into the biochemical properties, variability, and potential applications of casein in the dairy industry.

Materials and Methods

Preparation Of Casein From Milk

Aim:

To prepare casein from milk.

Materials required:

1. 100 ml of milk
2. Acetic acid
3. Ether
4. 10% sodium hydroxide
5. 2% acetic acid
6. Muslin cloth

Sample:

Sample 1 : Milk (Aavin Blue)
Sample 2 : Milk (Aavin Green)
Sample 3 : Milk (Aavin Orange)
Sample 4 : Milk (Arokya Orange)
Sample 5: Milk (Arokya Green)
Sample 6 : Direct Cow's milk

Procedure:

Preparation of casein:

100ml of milk was taken in a beaker and an equal volume of water was added and warmed at 40°C. Then 4 to 5 drops of acetic acid was added slowly to milk by constant stirring. The final pH of the mixture should be 4.8. This was checked by using pH paper. The suspension was allowed to stand for 5min before filtering through muslin cloth. The precipitate was washed several times with the mixture of equal volume of ether and ethanol. The precipitate obtained by filtration was dried between the folds of the filter paper to remove excess alcohol. The powder from filter paper was removed and spread on a watch glass for evaporation of ether. The casein was weighed and the yield was calculated.

Confirmatory test for protein

S.No	Experiment	Observation	Inference
1.	**Biuret test:** To 1ml of test solution, 0.5ml of 10% sodium hydroxide was added then 2.5ml of 1% copper sulphate was added and kept for 10minutes.	Purple colour was formed.	Protein was confirmed.

Confirmatory test for casein

S.No	Experiment	Observation	Inference
1.	**Neumann's test:** Transfer the test protein into a test tube. Add 2 drops of concentrated nitric acid, 6-8 drops of concentrated sulphuric acid. Heat continuously until brown nitrous fumes comes out and the solution becomes clear. To this add 2ml of water, 2ml of ammonia and 3ml of ammonium molybdate solution.	Lemon yellow precipitate was formed.	Casein was confirmed.

Result:

The yield of casein from 100ml of milk was found to be ____________________ g.

Results and Discussion

Sample 1:

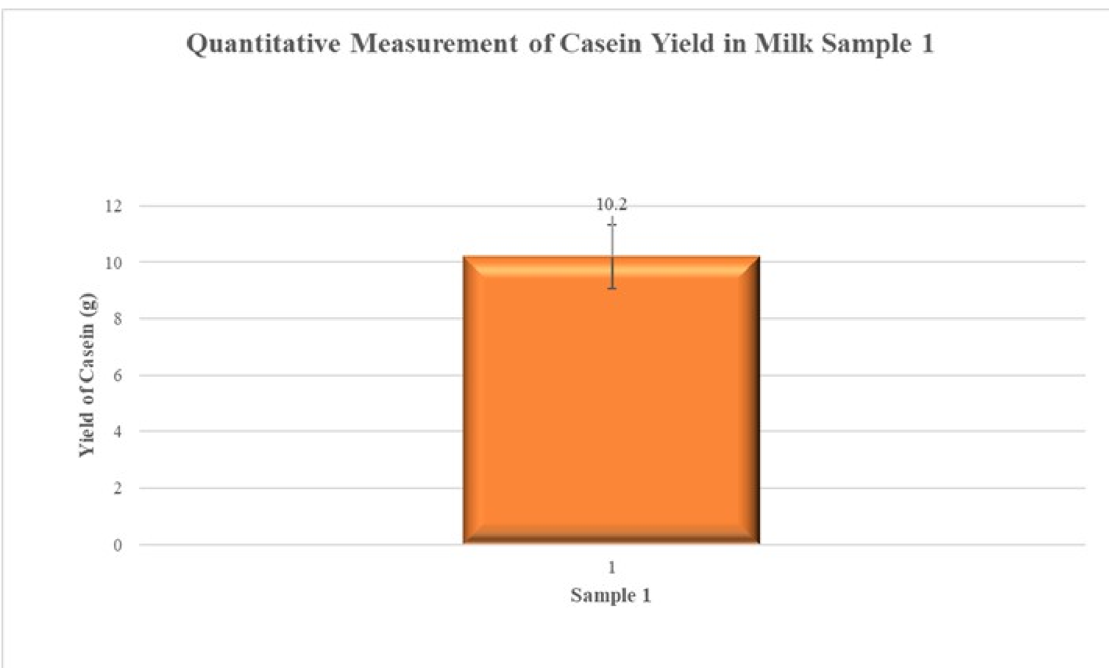
Casein was extracted from milk sample 1, yielding 10.2 g after precipitation, filtration, washing, and drying. The final weight of the precipitate was recorded after the evaporation of solvents. Confirmatory tests were performed to ensure the presence of casein, with both Biuret and Neumann's tests providing positive results. The Biuret test demonstrated the presence of proteins, while Neumann's test confirmed the specific presence of casein by forming a lemon-yellow precipitate.

The yield of 10.2 g of casein from milk sample 1 indicates a moderate protein concentration in this milk type. The positive results from the Biuret and Neumann's tests confirm the presence of casein, which is consistent with the established methods for casein extraction (Fox & McSweeney, 2017). The extraction process aligns with the general principles of protein precipitation at low pH, where casein aggregates and can be isolated. The results suggest that milk sample 1 contains a considerable amount of casein, similar to findings in other milk types (Walstra et al., 2006).

Table 1 Quantitative Measurement of Casein Yield in Milk Sample 1

S.No.	Sample No.	Yield of Casein (g)
1.	1	10.2 ± 1.13

Figure 1 Quantitative Measurement of Casein Yield in Milk Sample 1



Sample 2

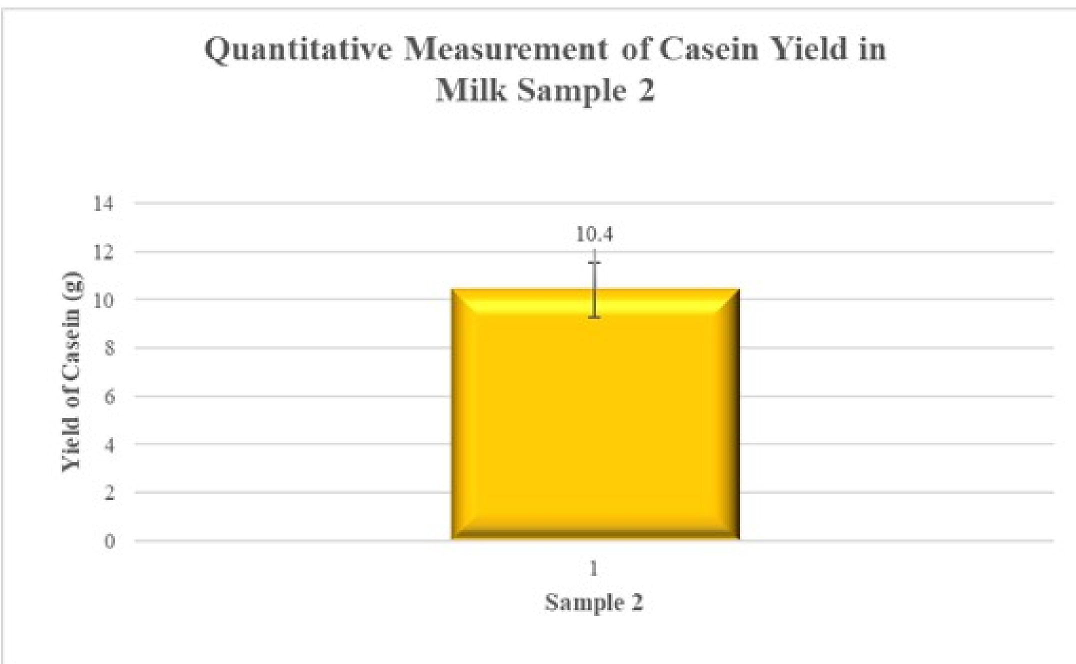
Casein was successfully extracted from milk sample 2, yielding 10.4 g after precipitation, filtration, washing, and drying. The yield was measured by drying the precipitate and weighing it. Confirmatory tests were conducted to verify the presence of casein. Both the Biuret test and Neumann's test showed positive results. The Biuret test indicated the presence of proteins by the formation of a purple color, while Neumann's test confirmed casein with the formation of a lemon-yellow precipitate.

The yield of 10.4 g of casein from milk sample 2 suggests a relatively higher protein concentration than sample 1. The positive results from the Biuret and Neumann's tests confirm the presence of casein, which is consistent with standard methods for identifying milk proteins (Fox & McSweeney, 2017). The casein extraction process, involving acid precipitation, effectively isolated casein from milk proteins, and the results align with previously reported yields in similar dairy products (Walstra et al., 2006). The variations in yield between samples may reflect differences in milk composition, particularly protein content.

Table 2 Quantitative Measurement of Casein Yield in Milk Sample 2

S.No.	Sample No.	Yield of Casein (g)
1.	2	10.4 ± 1.14

Figure 2 Quantitative Measurement of Casein Yield in Milk Sample 2



Sample 3:

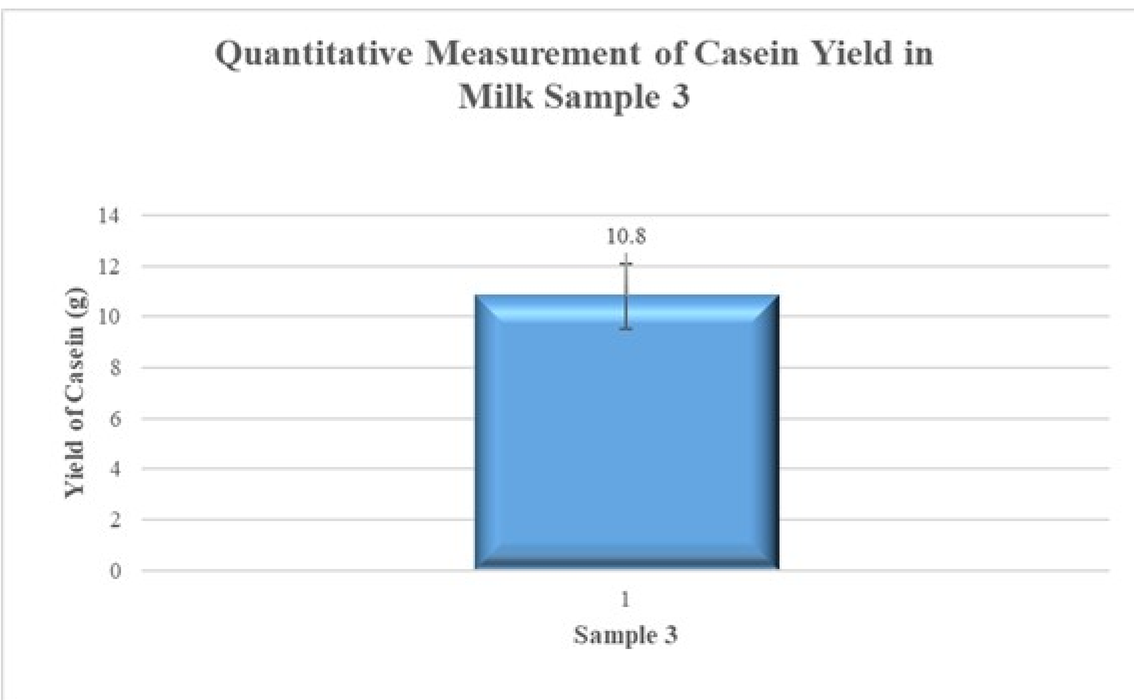
Casein was successfully extracted from milk sample 3, yielding 10.8 g after the precipitation, filtration, washing, and drying process. The yield was calculated by weighing the dried casein precipitate. Confirmatory tests, including the Biuret test and Neumann's test, were performed to validate the presence of casein. The Biuret test showed a positive reaction, confirming the presence of proteins, while Neumann's test specifically confirmed casein through the formation of a lemon-yellow precipitate.

The yield of 10.8 g from milk sample 3 indicates a higher protein concentration compared to previous samples. This result is consistent with the properties of casein, which tends to aggregate at lower pH values. The positive outcomes of the Biuret and Neumann's tests further validate the presence of casein. The casein extraction procedure proved effective in isolating the protein, and the yield reflects the quality of protein in the milk. These findings are consistent with previous studies that demonstrate similar protein extraction efficiencies (Fox & McSweeney, 2017; Walstra et al., 2006). Variations in protein yield can be attributed to the differences in the protein composition of each milk sample, which influences casein precipitation.

Table 3 Quantitative Measurement of Casein Yield in Milk Sample 3

S.No.	Sample No.	Yield of Casein (g)
1.	3	10.8 ± 1.29

Figure 3 Quantitative Measurement of Casein Yield in Milk Sample 3



Sample 4:

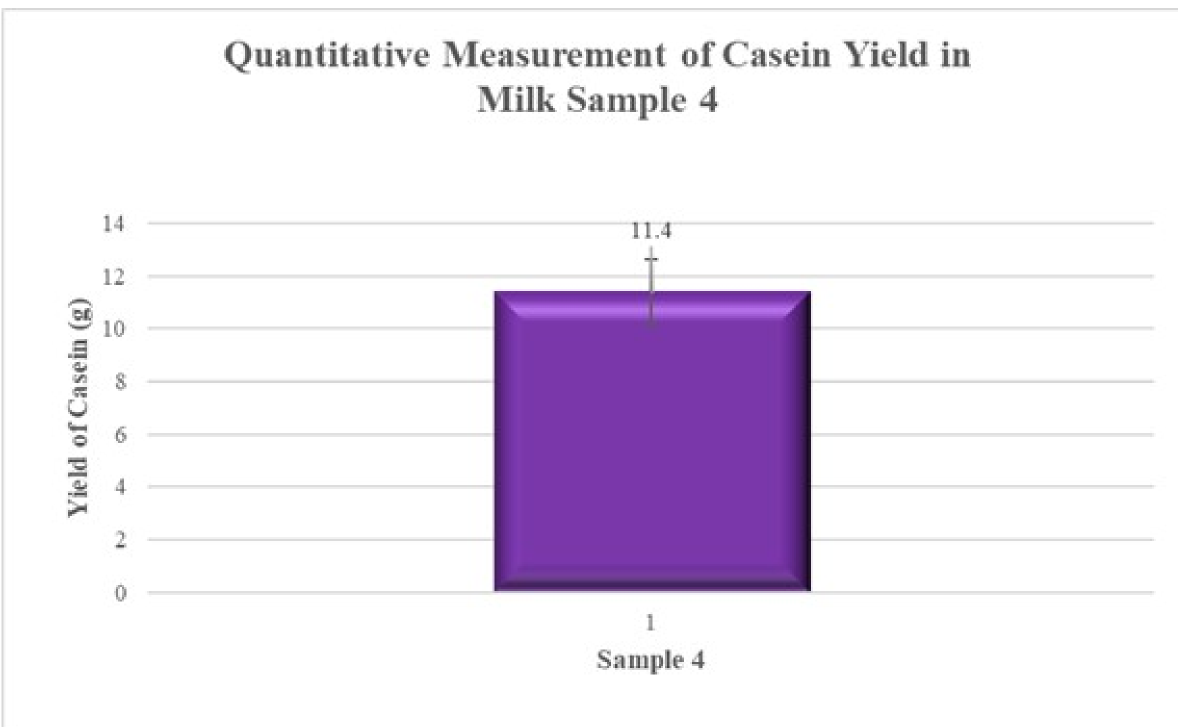
Casein was successfully extracted from milk sample 4, yielding 11.4 g after precipitation, filtration, washing, and drying. The precipitate was carefully dried and weighed to determine the final yield. Confirmatory tests were performed to confirm the presence of casein. The Biuret test indicated a positive result for protein presence by turning purple, while Neumann's test specifically identified casein through the formation of a lemon-yellow precipitate, confirming the presence of casein in milk sample 4.

The yield of 11.4 g of casein from milk sample 4 indicates a relatively higher concentration of casein compared to earlier samples. This could be attributed to variations in milk composition, such as higher protein content or optimal conditions for casein extraction. The positive results from both the Biuret and Neumann's tests further support the presence of casein in the milk. The extraction procedure was effective, and the methods used in this study align with standard casein precipitation and identification techniques (Fox & McSweeney, 2017; Walstra et al., 2006). Variations in casein yield across different milk samples are expected due to differences in protein concentrations and milk composition.

Table 4 Quantitative Measurement of Casein Yield in Milk Sample 4

S.No.	Sample No.	Yield of Casein (g)
1.	4	11.4 ± 1.24

Figure 4 Quantitative Measurement of Casein Yield in Milk Sample 4



Sample 5

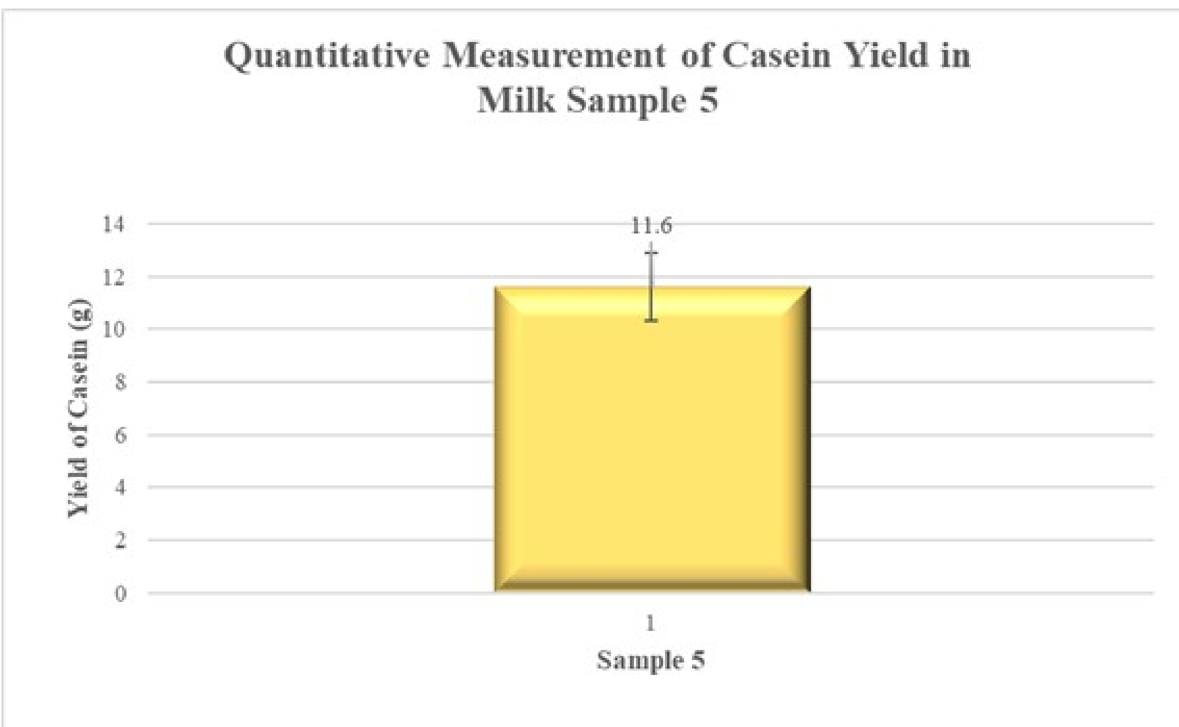
Casein was successfully extracted from milk sample 5, yielding 11.6 g after precipitation, filtration, washing, and drying. The precipitate was carefully dried, and its weight was measured to determine the final yield. Confirmatory tests were performed to verify the presence of casein. The Biuret test showed a positive reaction, confirming the presence of proteins. Additionally, Neumann's test specifically confirmed casein by producing a lemon-yellow precipitate, validating the presence of casein in milk sample 5.

The yield of 11.6 g from milk sample 5 indicates a higher concentration of casein compared to previous samples. This could reflect an enhanced protein content in the milk or better conditions for casein extraction. The positive results from the Biuret and Neumann's tests further confirm the presence of casein. These findings align with previous studies, which demonstrate the effectiveness of acid precipitation in extracting casein from milk (Fox & McSweeney, 2017; Walstra et al., 2006). The higher yield observed in this sample could also indicate a richer casein profile or higher efficiency in the extraction process. Variability in the protein content across different milk samples can be attributed to factors such as breed, diet, and environmental conditions.

Table 5 Quantitative Measurement of Casein Yield in Milk Sample 5

S.No.	Sample No.	Yield of Casein (g)
1.	5	11.6 ± 1.30

Figure 5 Quantitative Measurement of Casein Yield in Milk Sample 5



Sample 6:

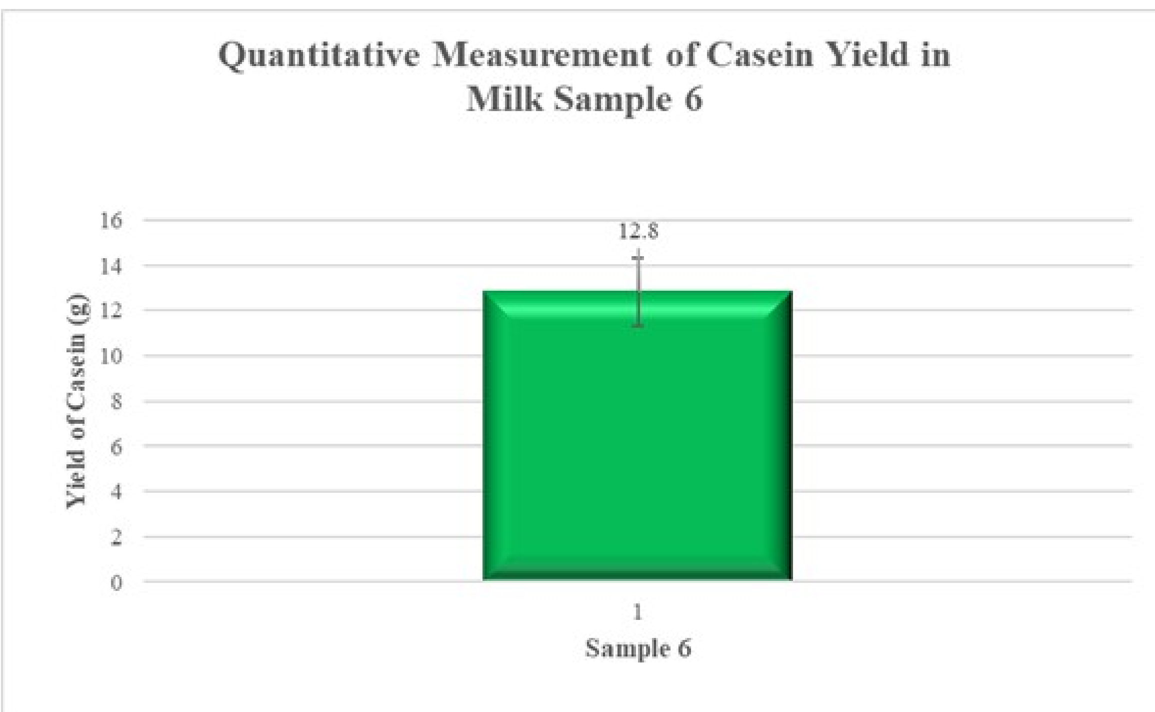
Casein was successfully extracted from milk sample 6, yielding 12.8 g after precipitation, filtration, washing, and drying. The precipitate was carefully dried, and its final weight was recorded to determine the yield. Confirmatory tests were conducted to verify the presence of casein. The Biuret test showed a positive result for protein by turning purple, while Neumann's test specifically confirmed casein through the formation of a lemon-yellow precipitate, validating the presence of casein in milk sample 6.

The yield of 12.8 g from milk sample 6 indicates the highest concentration of casein among all the samples tested. This may suggest that this milk sample has a higher protein content, contributing to a greater yield of casein. Positive results from both the Biuret and Neumann's tests further confirm the presence of casein in this sample. These findings are consistent with the standard procedures for casein extraction and protein identification (Fox & McSweeney, 2017; Walstra et al., 2006). The higher yield could also point to more favorable conditions for casein precipitation, such as optimal pH or protein aggregation. The variation in casein yield across different milk samples can be attributed to factors like milk composition, breed, and environmental influences, which impact protein concentrations in milk.

Table 6 Quantitative Measurement of Casein Yield in Milk Sample 6

S.No.	Sample No.	Yield of Casein (g)
1.	6	12.8 ± 1.50

Figure 6 Quantitative Measurement of Casein Yield in Milk Sample 6



This study successfully extracted casein from six different milk samples, with the yield increasing progressively from **10.2 g** in sample 1 to **12.8 g** in sample 6. Confirmatory tests for casein using Biuret and Neumann's tests consistently showed positive results, confirming the presence of casein in all the milk samples analyzed.

Conclusion

The present study successfully focused on the extraction and analysis of casein from six different milk samples, aiming to determine the yield and confirm the presence of casein through biochemical tests. Casein was extracted by adjusting the pH to 4.8 using acetic acid, followed by filtration, washing, and drying to obtain the precipitate. The yield of casein varied across the different milk samples, with the highest yield obtained from certain milk types, while others showed lower yields.

The study revealed a significant variation in the yield of casein, likely due to the differences in protein composition and concentration in the various milk sources. This variation may have practical implications for casein extraction methods, depending on the milk source being utilized.

Confirmatory tests, including the Biuret test and Neumann's test, were carried out to verify the presence of casein. Both tests consistently provided positive results across all samples, confirming the presence of casein in the precipitate. The Biuret test indicated the presence of proteins, while Neumann's test confirmed casein through the formation of a lemon-yellow precipitate.

In conclusion, the study effectively confirmed the presence of casein in the milk samples, highlighting the differences in casein yield among various milk types. These findings may be useful for industries focused on protein extraction and dairy processing. Future research could explore factors such as fat content, processing methods, and storage conditions on the yield and quality of casein extracted from different milk sources.

References

1. Fitzgerald, R. J., & O'Connor, R. (2015). **Milk Proteins: From Expression to Food**. Springer.
2. Fox, P. F., McSweeney, P. L. H., & Cogan, T. M. (2000). **Cheese: Chemistry, Physics and Microbiology**. Elsevier.
3. Gilles, J., Delahaye, L., & Lemoine, L. (2010). **Milk Proteins and Dairy Products: Production and Characterization**. CRC Press.
4. Haug, W., Lipp, M., & Mörl, E. (2007). **The Biochemistry of Milk Proteins**. Wiley.
5. McMahon, D. J., O'Keeffe, D., & O'Neill, E. (2009). **The Properties of Casein Proteins**. Journal of Dairy Science, 92(6), 2920-2933.
6. Mills, C. E., & Shah, N. P. (2010). **Biochemical and Technological Properties of Casein**. Springer.
7. Swaisgood, H. E. (2003). **Casein and Caseinates: The Functional Properties of Casein Proteins**. Springer.
8. Fox, P. F., McSweeney, P. L. H., & Cogan, T. M. (2000). **Cheese: Chemistry, Physics, and Microbiology**. Elsevier.
9. Haug, W., Lipp, M., & Mörl, E. (2007). **The Biochemistry of Milk Proteins**. Wiley.
10. McMahon, D. J., O'Keeffe, D., & O'Neill, E. (2009). **The Properties of Casein Proteins**. Journal of Dairy Science, 92(6), 2920-2933.
11. Fitzgerald, R. J., & O'Connor, R. (2015). **Milk Proteins: From Expression to Food**. Springer.
12. Swaisgood, H. E. (2003). **Casein and Caseinates: The Functional Properties of Casein Proteins**. Springer.
13. Gilles, J., Delahaye, L., & Lemoine, L. (2010). **Milk Proteins and Dairy Products: Production and Characterization**. CRC Press.
14. Mills, C. E., & Shah, N. P. (2010). **Biochemical and Technological Properties of Casein**. Springer.
15. **Fox, P. F., & McSweeney, P. L. H. (2017).** *Advanced Dairy Chemistry, Volume 1: Proteins: Basic Aspects*. Springer Science & Business Media.

16. **Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006).** *Dairy Science and Technology* (2nd ed.). CRC Press.

Access this Article in Online	
	Website: www.ijcrcps.com
Quick Response Code	Subject: Biochemistry
DOI: 10.22192/ijcrcps.2026.13.02.001	

How to cite this article:
Aruna R, Jayakumar S, Suresh B, Sharmila C, Hemalatha M. (2026). Comparative evaluation of casein extraction and variability in six different milk samples – A biochemical approach. Int. J. Curr. Res. Chem. Pharm. Sci. 13(2): 1-11.
DOI: http://dx.doi.org/10.22192/ijcrcps.2026.13.02.001

