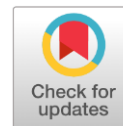


Nutritional Evaluation of Protein Content in Commercial UHT and Raw Bovine Milk Samples Collected from Lahore

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ABSTRACT

Background: Milk protein content is considered as important parameter of nutrition, authenticity and safety of milk. The protein levels of processed and raw milk are not necessarily the same due to processing methods, storage conditions, environmental influences and adulteration practices. It is therefore essential to monitor milk quality regularly, to ensure the nutritional adequacy and safeguarding of public health.

Objectives: To estimate and compare protein content of commercially processed UHT milk and raw bovine “gawala” milk samples from various areas in Lahore, Pakistan.

Methods: This analytical cross-sectional study included milk samples collected from 15 urban and peri-urban locations of Lahore. There were two types of UHT milk samples, all commercially available and “gawala” milk samples from local vendors as raw milk. The protein concentration was spectrophotometrically measured by the photometric colorimetric Biuret method using a reagent kit from HUMAN Germany (Ref-10570). The absorbance was measured at 546 nm and protein was quantified by standard methods after incubation.

Results: Commercial UHT milk demonstrated protein concentrations ranging from 2.2 to 3.2 g/100 ml. Milkpak and Olpers maintained relatively stable protein levels near 2.7 g/100 ml, while Haleeb variants ranged from 2.2–3.0 g/100 ml. Among different processed milk brands, the protein content was highest in Nurpur 3.2g/100ml. Raw “gawala” milk exhibited comparatively higher protein concentrations (3.5–4.2 g/100 ml) but demonstrated greater variability across sources.

Conclusions: Commercial milk brands were relatively uniform in protein content and raw milk was higher in protein potential but was not uniform. It is essential to have continuous monitoring of the nutritional status and to monitor the quality of the milk for enhancing milk safety and to eliminate the possible risks of adulteration.

Keywords: Bovine milk, Protein estimation, UHT milk, Raw milk, Biuret method, Nutritional quality



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INTRODUCTION

Milk is one of the most critical foods in human diet and a rich source of various nutrients needed for growth, development and maintenance of health throughout life [1]. It is loaded with proteins, fats, carbohydrates, minerals, vitamins and several bioactive compounds, which makes it very nutritional. Milk is consumed by a very large population and is an important food source; the

safety, quality and authenticity of milk has become a major concern in public health [2].

With the increasing concern about milk adulteration and milk quality differences around the world, the need for constant monitoring of important physico-chemical parameters especially protein concentration [3] is highlighted. One of the important parameters to assess the nutritional value and genuineness of milk is protein content which is frequently adulterated in milk. During the

dilution with water and addition of other ingredients such as urea, preservative and stabiliser, the milk composition may be altered and the safety of the consumer may be jeopardised [4]. Therefore, the analysis of milk protein content is also important for nutrition assessment, quality control and regulation checks [5].

Milk is a complex biological fluid, the major components of bovine milk are water, proteins, fats, lactose, minerals, vitamins, enzymes and immunologically active compounds. The content of bovine milk protein usually ranges at 3.0-3.5% of the total milk, which can be divided into two classes: caseins and whey proteins [6]. Caseins constitute almost 80% of the total protein in milk and whey proteins about 20% and are significant sources of amino acids, calcium and phosphorus, while the importance of whey proteins in immunity and tissue repair (immunoglobulins, lactoferrin, beta-lactoglobulin etc.) is well established [7].

Several factors including breed of animal, management, environment, stage of lactation, parity, season and health affect milk composition and its quality [8]. The concentration of milk proteins was found to be significantly affected by lactation period, age of cattle, and nutritional status of dairy animal as shown in previous studies [9]. Furthermore, the analysis of milk proteins has been improved, and it has been discovered that processing (pasteurization, homogenization, ultra-high temperature processing) has an impact on the structure of the milk proteins, digestibility and the release of bioactive peptides [10].

Commercial milk products which are typically processed are standardized to maintain uniformity, shelf stability and microbiological safety [11]. But in Pakistan, the milk usually given as “gawala” is crude, not standardized and has a lot of variations in its composition. The composition of this milk can be a mixture of buffalo and cow milk and can be affected by season, transportation conditions and adulteration (dilution with water) [12]. Despite the possible higher protein levels of raw milk, standardization and microbiological safety are serious issues [13].

Lahore is an urban city having dense population and different dairy value chains [14] and high demand of milk products and milk from the consumers. The differences in production, transport and retail distribution practices may result in the nutritional quality of the commercially available milk being different from that of the raw milk. Despite the high consumption of milk in Lahore, limited information is available in the region to compare protein content of various milk brands and raw milk sources [15].

Therefore, the present study was conducted to estimate and compare the protein contents of commercially processed UHT milk and raw “gawala” bovine milk samples collected from different regions of Lahore. The results of this study could be used as baseline information on the nutritional quality of milk products, as a quality

control tool and for public health surveillance of adulterated or nutritionally compromised milk products [12].

MATERIALS AND METHODS

This analytical cross-sectional study was conducted from January 2025 to July 2025 to evaluate and compare the protein concentrations of commercially processed and raw bovine milk samples obtained from different regions of Lahore, Pakistan. The study was approved by the Ethical Review Committee of Rashid Latif Khan University (RLKU), Lahore, under the approval number: RLKU/ERC/2025/007. All the study procedures were performed as per the institutional ethical and laboratory guidelines.

To ensure the representative geographical distribution, milk samples from 15 different places were taken out of Lahore (urban, suburban, peri-urban). The samples collected were ultra-high temperature (UHT) milk brands in the market in the departmental stores and raw “gawala” milk from the local milk vendors. Commercial milk samples contained several popular commercial brands with different commercial formulations and package labels. The milk samples were collected in a sterile airtight container in hygienic conditions and sent to the laboratory as soon as possible under proper storage conditions to prevent biochemical changes before analysis. Milk samples that had expired, were contaminated, damaged, or had been improperly stored were not included in the study.

Protein estimation was accomplished by using a photometric colorimetric method for quantitative determination of total protein concentration in milk samples using the Biuret method. A commercially available reagent kit (HUMAN Diagnostics, Germany; Ref-10570) with assay linearity extending up to 12 g/dl was utilized according to the manufacturer's instructions. Twenty microlitres of each of the milk samples were thoroughly mixed with 1000 µl of prepared Biuret reagent for laboratory analysis. The reaction mixtures were left to sit at 25°C for 10 minutes for proper color formation. The absorbance values were then taken in a calibrated spectrophotometer at 546 nm. Protein concentration was calculated using the standard conversion formula: $C \text{ (g/dl)} = 19 \times \Delta A$, where ΔA represented the absorbance difference of the analyzed sample.

All data collected were coded and analyzed with the help of SPSS version 26.0. Protein concentrations are reported as mean $\pm$ standard deviation (SD) or as percentage as appropriate. The variation in protein content and nutritional quality of the samples of various commercial brands of UHT milk and raw “gawala” milk was determined by comparative analysis. The data was summarized in tables and figures.

RESULTS

Protein estimation analysis showed that there were measurable differences between various concentrates of protein in the different commercially processed ultra high temperature (UHT) milk brands and raw bovine milk samples collected from different areas of Lahore. The protein concentrations were more similar for the different commercial milk brands and reasonably in the acceptable range for nutritional applications which indicates more or less similar processing and manufacturing process. However, there were some differences detected among each brand and milk type, suggesting that the milk composition and/or milk production characteristics, processing technologies, and milk type may matter.

Protein content was found to be different in the processed milk brands in the commercial label evaluation. MilkPak (Nestlé) full cream UHT milk demonstrated a protein concentration of 2.7 g/100 ml, equivalent to approximately 6.75 g protein per 250 ml serving. Olpers UHT milk showed a protein value of 2.7–3.2 g/100 ml. Haleeb variants exhibited comparatively wider variation, ranging from 2.2 to 3.0 g/100 ml, while Haleeb Asli demonstrated the lowest declared protein concentration at 2.2 g/100 ml. Milk of the brand 'Nurpur' had the highest labelled protein concentration (3.2 g/100 ml) corresponding to approximately 8 g protein in a 250 ml glass. Raw “gawala” milk demonstrated estimated protein concentrations ranging from 3.5–4.2 g/100 ml, indicating comparatively greater protein potential but substantial variability due to lack of standardization (Table 1).

Laboratory analysis showed that there were variations in the mean protein concentrations of milk types collected from Lahore. The highest mean protein content (4.45%)

was observed in raw “gawala” milk as compared to other brands of processed milk. Among commercial brands, MilkPak demonstrated the highest mean protein concentration (4.08%), followed by Olpers (3.95%), Haleeb (3.87%), and Nurpur (3.86%). The protein profiles of commercial milk brands showed similar characteristics to each other, which may be attributed to standardisation in the milk production process and quality control by the industry. The differences observed indicate that raw milk could have a relatively higher protein content, but there is also a lot of variation between raw milk sources (Table 2).

Proportional distribution of mean protein concentration for each milk category collected from Lahore was further depicted graphically as shown in Figure 1. Raw “gawala” milk contributed the largest proportion (22.02%) of overall protein distribution, followed by MilkPak (20.19%), Olpers (19.54%), Haleeb (19.15%), and Nurpur (19.10%). There is no significant difference in the proportions of the various brands commercially processed, which indicates uniformity in manufacture and standardization. However, raw milk had a higher relative presence of proteins, suggesting laboratory findings of higher protein levels in raw milk. The pie chart illustrates the relative contribution of the various types of milk analyzed; the largest contribution comes from raw milk, while the distributions of commercial UHT brands are relatively similar. Commercial UHT milk overall showed fairly constant protein levels, indicating standardization of the manufacturing process and of the nutritional stability. Protein content, however, of raw “gawala” milk was significantly higher with higher variation and the effect of source characteristics and non-standardized processing was reflected.

Table 1: Protein Contents Based on Nutritional Labels

Brand	Variant	Protein Content	Findings
Milkpak (Nestlé)	Full Cream UHT Milk	2.7 g/100 ml	Standardized UHT processing; ~6.75 g protein per 250 ml serving
Olpers (Engro)	Full Cream UHT Milk	2.7–3.2 g/100 ml	Consistent protein concentration
Haleeb	Full Cream/Premium UHT Milk	2.2–3.0 g/100 ml	Variable concentrations across subvariants
Haleeb Asli	Full Cream UHT Milk	2.2 g/100 ml	Lowest declared protein concentration
Nurpur (Fauji)	Full Cream/Original UHT Milk	3.2 g/100 ml	Highest labeled protein concentration among commercial brands
Gawala Milk (Raw)	Unprocessed and unstandardized	3.5–4.2 g/100 ml	Greater variability and higher protein potential

Table 2: Mean Values of Protein Contents in Milk Samples

Milk Sample	Mean Protein Content (%)	Remarks
MilkPak	4.08	Highest among commercial UHT brands
Olpers	3.95	Within acceptable nutritional range
Nurpur	3.86	Comparable with other processed brands
Haleeb	3.87	Slightly lower than MilkPak
Gawala (Raw Milk)	4.45	Highest overall protein concentration

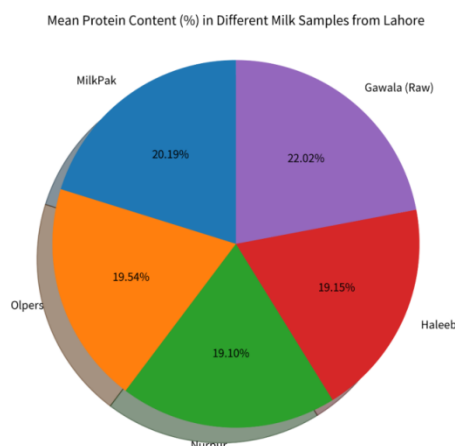


Figure 1: Graph Showing Mean Protein Percentage Distribution in Different Milk Samples from Lahore

DISCUSSION

The present analytical study evaluated protein concentrations in commercially processed ultra-high temperature (UHT) milk brands and raw bovine milk samples collected from different regions of Lahore, Pakistan [1]. Results showed that there were significant differences in crude protein among the milk types, and raw “gawala” milk had the highest mean crude protein (4.45%), whereas the protein content of milk marketed in the commercial market had been relatively standardized between 3.86% and 4.08%. MilkPak had the highest mean protein concentration, while Haleeb and Nurple had comparatively similar values among the processed milk products [2]. The results reveal the effects of milk processing, milk standardization, and source variations on the protein composition of bovine milk [3].

To date, one of the most significant dairy nutritional quality and commercial value indicators is milk protein concentration [4]. Protein components, especially casein and whey proteins, play a significant role in the availability of amino acids, the development of tissues, the regulation of metabolism, and the maintenance of physiological processes. Thus for carrying out nutritional surveillance, quality assurance and to detect practices which could influence the composition of milk and consumer safety, it is necessary to accurately quantify the protein [5].

There are several analytical methods for the estimation of milk protein which include the Kjeldahl method, Bradford assay, Lowry assay, spectrophotometric and chromatography [6]. While these traditional methods are still applied owing to their high repeatability and low cost, advanced techniques like high throughput methods, high-performance liquid chromatography, near infrared spectroscopy and metabolomic techniques provide high throughput and analytical accuracy. Protein was measured by Photo Colorimetric Biuret method which is still applicable in the present investigation for the routine

biochemical analysis and a reliable method for estimation of total protein concentration [7].

This increased protein content of raw “gawala” milk may be due to the less industrial treatment and differences in the source composition. Local raw milk is sometimes blended with cow milk and buffalo milk that contain different proteins [8]. In addition, environmental factors, breed of animals, nutrient content of the feed, stage of lactation, parity, health status and seasonality have a major impact on the protein synthesis in the cow and the nutritional composition of milk. Also, the different dairy production systems and regional farming practices might be a factor to account for the variability seen [9].

The commercial UHT brands had more uniform protein concentrations, which resulted from the standardisation in the industrial production of UHT products and quality control [10]. Processing techniques such as ultra-high temperature and homogenisation improve the microbiological safety and stability of the product, but may have an impact on protein structure, digestibility and bioactive peptide content due to the thermal exposure and processing conditions. The protein concentration of the samples in the present study, although subjected to changes, remained acceptable and the extent of variation was relatively less in the commercially processed samples than in the raw milk samples [11].

An important result emerging from this study is the difference in protein concentration observed between commercial milk products and raw milk samples [12]. The results highlight the need to enhance dairy quality control systems with regular quality monitoring and evaluation of laboratories. Uniformity in nutrition through the milk chain is quite significant in respect of quality of food and consumers protection especially in cities where the urban population is dense like Lahore [13].

The present study has provided baseline information on some of the data pertaining to variation of proteins in bovine milk and milk products marketed in Lahore. However, there are a few restrictions [14,15]. That

investigation has been targeted only towards the total protein content and not on the protein fractions, amino acid profile, fat contents, specific adulterants, and any possible microbial contaminations. The present study did not include the effects of season and breed. Future research with larger sample sizes and more comprehensive nutritional characterization may be helpful in the understanding of the nutritional variability in bovine milk quality [16,17].

Overall, these findings indicate that there is a need for regular nutritional monitoring and for the development of standardized quality assurance systems which would help to enhance the uniformity of dairy products, food safety and consumer confidence in the products available in the market and raw milk [18-20].

CONCLUSION

The present study indicated that there was a significant difference in protein concentration between commercially processed ultra-high temperature (UHT) milk and raw bovine milk from various regions of Lahore. Protein concentration in the commercial milk brands was generally relatively constant, suggesting uniformity in the processing and quality control procedures of the industry. Processed milk products showed the highest declared protein concentration for Nurpur, while MilkPak and Olpers had fairly stable and nutritionally consistent protein content. Moderate variability was observed between the Haleeb variants based on the formulations. Raw “gawala” milk exhibited relatively higher protein levels than the commercially processed milk products, although there was significant variation among raw milk sources, likely due to variations in production practices, source, and lack of standardization. While raw milk may have a higher protein potential, consistency and uniformity in quality are factors to be taken into account. Commercial processing of milk does offer high quality and uniformity of product, and raw milk has considerable variability, but its protein content is relatively high. It is important to continue with the nutritional monitoring of the continuous supply, the continuation of Quality assurance measures, and frequent laboratory analysis of milk composition to ensure food safety monitoring, nutritional adequacy, and consumer confidence in dairy foods supplied by local supply systems.

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Authors' Contributions: Y.J. conceived and designed the study and prepared the manuscript. A.N. and S.F. contributed to sample collection, laboratory analysis, and data compilation. U.A. contributed to data interpretation, manuscript revision, and supervision. All authors reviewed and approved the final manuscript.

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Data Availability: The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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