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ORIGINAL ARTICLE

Preliminary analysis of milk samples: evaluation of its constituents, phenolic content, and nutritional value to humans

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ABSTRACT
Indicators of raw cow milk (lactose, fat, whey protein, total protein content, and pH profiles) were evaluated in this study, which took place from July to September of 2024 in several locations in the Jammu district, Jammu and Kashmir, India. 50 mL of the fresh morning raw cow milk sample was aseptically taken from different residential areas using a sterile sampling vial. In general, cows' milk production is determined by calculating the amount of milk during control milking, sampling the milk from each cow, and evaluating its fat, lactose, pH, and protein content for each milking; these indications are the same for the day. The following fifteen days were spent repeating this technique. Standard protocols were adhered to during milk sampling, transit, and handling. Furthermore, the rate of somatic cells varies significantly between cow's milk collected from respective places. In summary, cow's milk is an excellent protein resource because it contains all of the essential amino acids as well as lactose and fats. In other words, saturated fat, which can block muscle mass from being used as an energy source, is another abundant energy source found in whole milk.
Key words: *milk; cow; protein; fat; somatic cells*

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INTRODUCTION

A healthy diet must include milk, also referred to as "white liquid food," which is a valuable commodity rich in vitamins, minerals (particularly calcium), lipids, and high-quality protein. The six billion people who drink milk globally span a wide age range, from newborns to adults over 70. Such a high rate of consumption guarantees the nutrition and security of a diet. It has grown in popularity as a result of its well-known nutritional benefits and low price (**1, 2**). From 2019 to 2023, India's total domestic milk usage was around 207 million metric tons, according to statista.com. Human growth and development are significantly influenced by milk. Milk has been a helpful food to prevent illnesses in addition to promoting growth. Because most of the supplemental milk used for both adults and newborns is produced by cows, the term "gau mata (kindness, materialism, purity, wealth, prestige, and power in Hindu mythology) refers to them (**3, 4**). Cow urine, or gomutra, is a liquid by-product of metabolism in cows. In the literature, Gomutra Plus Capsule, which contains a concentrated form of gomutra powder blended with the goodness of Triphala (Amla, Beheda, and Harda), is an innovative product of Pitambari Products in India (**5**).

Casein and whey proteins, which have multiple benefits of antibacterial, antioxidant, and immunomodulatory activities, among others, are the primary sources of active compounds found in milk. The majority of physiologically active proteins require proteolysis in order to fully express their functions. Apart from the digestive system, coagulants or lactic acid bacteria—which are frequently used in the food industry—can also start proteolysis (**6, 7**). Proteolysis is a naturally occurring process in milk. One of the bioactive molecules, casein, corresponds to the family of milk proteins that incorporate phosphorus (phosphoprotein) and sugar (glycoprotein) groups; it is made up of around 20 protein components. The phosphoproteins found in casein control how the body uses calcium and phosphate. After menopause, approaches showed a spike in bone mineral formation in experimental animals. Furthermore, casein proteins prevent dental decay by raising the calcium phosphate level in dental plaque. Similarly, another bioactive component, i.e., lactoferrin, was initially extracted from milk from cows in 1939 and then from human milk around 1960. It possesses iron-binding capabilities similar to those of transferrin proteins. One polypeptide chain, made up of two spherical lobes joined by a hinge region, makes up the monomeric

glycoprotein lactoferrin (**8**). A protein called lactoferrin can be found in a variety of bodily fluids, including milk, colostrum, tears, saliva, nasal secretions, and vaginal secretions. Additionally, neutrophils create a significant amount of it. The antiviral, antifungal, antiparasitic, anticancer, bacteriostatic, and antioxidant qualities of lactoferrin are shown. In addition, α-Lactalbumin (under the category of whey protein; globular protein; hydrophilic albumin), β-Lactoglobulin (whey protein; globular protein; five cysteine residues where four are involved in the formation of disulphide bridges stabilizing the quaternary structure), and Proline-Rich Peptide (PRP; 32 peptides having 500–3000 kDa) are also present (**9, 10**). In this study, we collected raw milk samples from different residential places in the Jammu region and compared or analysed the casein, whey protein, and fat samples with fresh, collected raw milk samples from cow.

MATERIAL AND METHODS

Collection of samples

Use sterile bottles or containers, and clean all collecting instruments thoroughly. To avoid an infection, apply disinfectant to our hands and wear protective gloves. Milk should be collected straight from the udder or milking gear of animals right after milking. The majority of tests require 100–200 mL. Refrigerate raw milk samples between 18 and 25 degrees Celsius while collecting them, and transport them using ice packs or a refrigerator to avoid spoilage.

Determination and composition of milk components

Take a milk sample (0.5 ml) and dilute it with double the quantity of distilled water. The dilution of milk samples may help to reduce the concentration of components in the milk, making it easier to isolate and analyze them. Place the diluted milk samples in a centrifuge. The centrifuge spins the samples at 3500 rpm for 10 minutes, causing the different components in the milk to separate based on their densities. Typically, heavier components, such as fats and proteins, will form a pellet at the bottom, while the lighter components, like water and dissolved substances, will remain in the supernatant. Carefully remove the supernatant and collect the pellet. After isolation, weigh the different components separately. This will give us their quantities and proportions in the milk sample.

The chemical composition (i.e., fat, protein, lactose) was determined with an automatic milk analyzer which is a Fourier transform infrared spectroscope based on the infrared absorption principle, operating in the mid-infrared range (FT-MIR spectroscope) (**11**). The results of chemical composition analysis are given in percentage or mg/ml.

Estimation of phenolic content from milk samples

Milk samples' total phenolic content was quantified spectrophotometrically employing the Folin-Ciocalteu technique by Singleton and Rossi, with gallic acid as the standard. The working solution was made by diluting the Folin-Ciocalteu reagent (1:10) with clean water. After mixing the sample or standard (1 mL) with the Folin-Ciocalteu working solution (5 mL) and letting it sit for three minutes, 4 mL of sodium carbonate (75 g/L) was added to the mixture. The samples were centrifuged at 12,000 g for two minutes following a two-hour dark incubation period at room temperature. The absorbance values of the samples were measured at 760 nm against distilled water using a spectrophotometer. The gallic acid equivalents (GAE) per litre of milk samples were used to express the results (**12, 13**).

Estimation of vitamin in milk samples using Vitamin D3 oral solution (60,000 IU, DA Farma, Chandigarh)

Milk samples were purchased from the local market of the Jammu region, India. All sample preparation was carried out in subdued light and in amber glass vials. The vitamin D standard (vitamin D3, 1000 IU) was dissolved in absolute ethanol to form stock solutions (500 IU). Aliquots of the vitamin D stock solutions were mixed and diluted with ethanol to obtain a series of standard calibration solutions ranging from 15.6 to 500 IU. These calibration solutions underwent the derivatization step as described below.

A standard curve of vitamin D3 was prepared by adding 1 ml of vitamin D3 standard solution to approximately 1 ml of ethanol along with or without milk samples, which were collected from respective places in the Jammu region. The area of the vitamin D3 peak was adjusted by deducting the area in the control (ethanol and milk, with or without added vitamin D3) and plotted against a known concentration of vitamin D3 (i.e., the amount actually added). Finally, optical density was measured at 570 nm using a UV-visible spectrophotometer.

RESULTS

Estimation of milk components

We observed seasonal variations in milk protein concentration. The protein, lipid, and lactose levels were determined in August and September. Milk fat concentrations increased during the milking season. Changes

in the dairy feed composition may cause seasonal fluctuations of protein and fat concentrations in raw milk (**Table 1**).

Table 1. Variance in milk quality and composition

Milk samples	Fat (%)in 100 ml milk	Total Protein (%) in 100 ml milk	Lactose (%) in 100 ml milk	SCC (10^5cells/ml)	pH	Calcium (mg/100 ml)	Whey protein (mg/100 ml)
JMU1	2.68	3.28	3.22	< 2	6.82	570.32	4.68
JMU2	3.12	2.43	2.98	< 2	6.58	594.46	4.38
JMU3	2.98	2.77	4.36	< 2	6.74	512.84	3.97
JMU4	3.18	2.83	3.92	< 2	6.82	598.24	4.29
JMU5	2.77	2.91	4.04	< 2	6.62	539.18	5.08
JMU6	2.56	2.58	3.38	< 2	6.66	555.16	5.56
JMU7	2.84	2.80	4.76	< 2	6.57	504.72	5.88
JMU8	3.07	3.36	3.92	< 2	6.81	515.74	6.12
JMU9	3.17	3.11	2.78	< 2	6.75	501.62	4.97
JMU10	3.18	2.92	3.76	< 2	6.63	508.62	5.16
JMU11	2.79	2.88	3.66	< 2	6.54	518.82	5.38
JMU12	2.96	3.04	4.38	< 2	6.38	592.44	5.55
JMU13	3.19	3.35	3.90	< 2	6.52	552.18	5.76
JMU14	3.18	3.13	4.16	< 2	6.47	556.72	5.46
JMU15	2.82	2.98	4.28	< 2	6.66	578.14	5.69
Cow milk (healthy)	3.19	3.31	4.84	< 2	6.61	692.70	5.84

Phenolic content

Our results showed that raw cow milk was collected from respective places, which exerted a significant amount of phenolic content in vitro, with full cream milk used as a control for the study. This study revealed that raw cow milk has high phenolic content but less in comparison with full cream milk, which has a significant amount of phenolic content, as shown in **Table 2**.

Table 2. Estimation of phenolic content in milk samples

S.No.	Milk samples	Phenolic content (GAE per litre of milk samples)
1	JMU1	439.12 ± 5.69
2	JMU2	454.16 ± 6.23
3	JMU3	441.18 ± 7.04
4	JMU4	481.58 ± 5.17
5	JMU5	442.14 ± 6.58
6	JMU6	471.22 ± 9.64
7	JMU7	462.48 ± 3.66
8	JMU8	426.66 ± 8.18
9	JMU9	451.14 ± 5.02
10	JMU10	466.28 ± 4.19
11	JMU11	487.14 ± 4.37
12	JMU12	434.18 ± 5.95
13	JMU13	477.76 ± 6.81
14	JMU14	457.21 ± 7.92
15	JMU15	409.58 ± 7.36
16	Cow milk (healthy)	478.06 ± 9.88
17	Buffalo milk (healthy)	592.19 ± 10.22

Estimation of vitamin D3

Milk samples were collected from different places in the Jammu region, and their vitamin D3 level was estimated. For these studies, vitamin D standards (vitamin D3, 1000 IU) were used to form stock solutions (500 IU) in ethanol and determine their content in milk samples. The studies revealed that milk has a vitamin D level in the range of 29 to 48 IU.

DISCUSSION

Inflammation-related events in the mammary gland lead to the formation of somatic cells, which change the composition of milk in cow samples to more closely resemble blood. This is brought on by a rise in the blood-mammary barrier's permeability, which allows more ions, proteins, and inflammatory cells to enter the milk. Lower milk yields primarily correspond to higher numbers of somatic cells within milk; decreased pH, protein, lactose, and fat content are also seen. The drop in milk supply associated with an increase in milk somatic cell counts is mostly due to physical injury to the milk-producing epithelial cells, resulting in a significant reduction in the mammary gland's synthetic and secretory capability **(8-10**). The quantity of total protein, casein, lactose, ash, and mineral element (Ca) in the milk samples was also measured using a variety of chemical assays, and the results are shown in **Tables 1 and 2**. The samples' chemical properties varied widely, with each sample outperforming the others in a particular area. Total protein and calcium levels ranged from (2.43 ± 0.78%) to (3.36 ± 0.44%) and (501.62 ± 8.56%) to (598.24 ± 11.37%), respectively.

The sweet substance found abundantly in milk has been identified as lactose. Despite being a sugar, lactose is found instinctively in milk and does not undergo processing to make it sweeter. Every newborn needs milk to survive. They may readily absorb this milk's ingredients and use them for growth. However, human digestion evolved throughout time, and production of the enzyme lactase ceased once children began to consume meals other than milk (14, 15). Several people in European, African, and Asian nations developed the capacity to break down milk after they had grown up, between 5,000 and 10,000 years ago. This resulted from an adaptation of the gene that is accountable for manufacturing the enzyme lactase. Due to this genetic alteration, milk suddenly became a source of vital nutrients, giving evolution a boost. Lactose intolerance could arise from an inability to properly digest lactose (14, 15). Lactose was found in milk samples taken from several locations in the Jammu area for this investigation.

In the literature, it has been claimed that the observed antioxidant activity is due to phenolic chemicals found naturally in milk. Although some may be byproducts of amino acid catabolism, feed is assumed to be the primary source of the phenolic chemicals found naturally in cow's milk. Changes, either metabolic or intestinal, result in distinct secondary dairy phenolic compounds that are distinct from the natural ones found in feeds. These secondary dairy molecules are seldom found in typical sources of phenolic compounds in the human diet, such as fruits and vegetables, yet they may have substantial biological activity in *vivo*, such as antioxidant and anti-inflammatory properties (15).

Milk consumption has fallen in recent years, so has dietary intake of vitamin D from fresh milk, although the usage of cheese has elevated dramatically (by about 100%) since 1980. The large rise in human population and alterations in dietary patterns have rendered it practicable to produce additional fortified food items that can supply the required amount of vitamin D in the diet for humans (**16**). Milk does not meet the daily needs of vitamin D, but cheese constitutes the proper form of food for the necessary daily consumption of this nutrient; in the United States, the fortification amount of vitamin D in cheese is rigorously monitored by the US Food and Drug Administration. By appropriately regulating the body's calcium and phosphorus concentrations, fat-soluble vitamin D is crucial for bone metabolism. Numerous studies have demonstrated the relationship between vitamin D ingestion and improvements in a number of disorders, including glycemic index in people with type 2 diabetes. In this investigation, we discovered that the vitamin D3 level in the raw milk samples was significantly greater in buffalo milk than in raw cow raw milk because buffalo milk has a significantly higher fat content. These investigations demonstrated the low vitamin D3 concentration of milk.

CONCLUSION

The current study aimed to determine the physicochemical properties and nutritional quality of several milk samples that were sold in Jammu, India, through preliminary studies. According to the findings, every characteristic of the analyzed milk samples fell below the acceptable nutritional levels, with the exception of mineral components. These findings may assist relevant governmental parties in checking the quality of milk products on the Jammu, India, market. If more research is done to look at additional organic and inorganic ingredients in the milk sold in the markets, that would be really interesting.

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