



Evaluation of Physicochemical Properties of Milk: A Review

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Abstract: Milk is a dilute emulsion consisting of an oil/fat dispersed phase and an aqueous colloidal continuous phase. The physical properties of milk are like those of water but are modified by the presence of various solutes in the continuous phase and by the degree of dispersion of the emulsified and colloidal components. This review explores the key aspects of milk, focusing on its composition, structure and behavior under various conditions. The physical properties of milk, such as viscosity, density, and surface tension, are discussed, shedding light on the fluid's behavior during processing and handling. Additionally, the chemical properties of milk, including pH, titratable acidity. Understanding these physiochemical properties is essential for optimizing the production, processing, and utilization of milk in the food industry and for promoting its health benefits in human nutrition. Further research in this field is guaranteed to uncover new insights and innovations that can enhance the quality and functionality of this valuable natural resource.

Keywords: Health, Milk, Natural resources, Nutrition, Physiochemical properties.

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Introduction

Milk is the most popular food for human consumption and is considered as a complete and nutritious food; not only for the new-born but for all age groups in both rural and urban people all over the world. It is a vital supply of nutrients needed for the growth, maintenance, production, and correct functioning of the bodies of mammals. However, milk and its products may be contaminated by various environmental

pollutants from agricultural, veterinary, and hygienic practices. Physicochemical analysis is an important tool to monitor the quality of milk and other dairy products. Dairy product quality starts at the farm as good dairy products can only be made from good quality raw cow milk. So, milk should have normal composition, should be not adulterated, and should be produced under hygienic conditions. Milk is an important source of animal protein, providing essential amino acids crucial for human health and cognitive development. It is a well-balanced natural food composed of proteins, fats, lactose, vitamins and minerals—offering both macronutrients and micronutrients required for maintaining good health. Physicochemical parameters of milk and milk products can be affected by adulteration, which is done either for financial gain or lack of proper hygienic conditions of processing, storing, transportation and marketing. This ultimately leads to the stage where the consumer is either cheated or often becomes a victim of diseases.

Nutritive Value of Milk

Milk is a nutrient-dense food and a powerhouse of protein that plays a critical role in human nutrition. When nutritionists refer to "complete proteins," milk immediately stands out. Unlike many plant-based sources that lack one or more essential amino acids, milk contains all nine essential amino acids that the human body cannot synthesize on its own. These amino acids are the building blocks of proteins, essential for building and repairing body tissues. A single cup of milk provides around 8 grams of high-quality protein, which includes approximately 80% casein and 20% whey proteins. While casein is digested slowly and ensures a sustained release of amino acids, whey protein is absorbed rapidly, making it ideal for muscle recovery after physical activity. This is why athletes often choose chocolate milk as a post-workout drink. Milk is also a rich source of essential minerals. A single cup provides about 300 mg of calcium, accounting for nearly 30% of an adult's daily requirement. Calcium and phosphorus, the two main minerals in milk, work together to build and maintain strong bones and teeth. The calcium-to-phosphorus ratio in milk (approximately 1.2:1) is optimal for bone mineralization, making it more effective than many calcium supplements that lack phosphorus. In addition to its role in bone health, calcium also supports nerve function, muscle contraction, and blood clotting, while phosphorus plays a key role in energy metabolism and maintaining the body's acid-base balance.

Besides calcium and phosphorus, milk also contains substantial levels of potassium, magnesium, and zinc. Potassium helps maintain healthy blood pressure and cardiovascular function, magnesium is involved in over 300 enzymatic reactions in the body, and zinc supports immune defense and wound healing. Together, these minerals make milk a well-rounded source of essential micronutrients.

Milk is beneficial across all life stages. For infants, breast milk or formula serves as a complete food. As children grow, cow milk continues to provide essential proteins and minerals necessary for development. Adults benefit from milk's role in maintaining bone density, while older adults may rely on milk's calcium and vitamin D to reduce the risk of osteoporosis. Athletes use milk for muscle recovery, and individuals watching their calorie intake can opt for low-fat or skim varieties while still gaining key nutrients.

Physicochemical Property of Milk

Milk is a complex natural fluid possessing a broad range of physicochemical properties that play a critical role in its nutritional value, quality control, and processing behavior. These properties include parameters such as pH, density, acidity, fat and protein content, viscosity, and freezing point. Composed of approximately 87% water, cow milk also contains essential nutrients like fats, proteins, lactose (milk sugar) and minerals. Its structure can be described as an oil-in-water emulsion, where fat exists in the form of

globules suspended in the aqueous phase. These globules are stabilized by membranes made of phospholipids and proteins, contributing to milk's stability and texture.

Density

Density is a fundamental physical property defined as mass per unit volume. In simpler terms, it tells us how much mass is packed into a given volume of a substance. For milk, density is an essential parameter in quality control during dairy processing. The density of milk can vary due to several factors such as fat content, temperature, and the addition of water or other substances. Density is a characteristic property of milk. Though the composition of milk is variable, the density of milk remains within a very short range. The same is true for various constituents of milk, such as milk fat. Density is an absolute value, i.e., absolute density. When density is related to a standard material like water, it is termed as specific gravity(sp.gr). Specific gravity is the ratio of the density of a substance to the density of water and is a dimensionless quantity. For milk, specific gravity typically ranges from 1.028 to 1.032 for cow milk, while buffalo milk ranges from 1.030 to 1.034. These values can be influenced by factors like fat content, temperature, and the presence of added substances. Higher fat content lowers the density, while lower fat content increases it. Skim milk, for example, has a higher density than whole milk. Temperature also affects density: as temperature increases, density decreases due to molecular expansion. Therefore, it's important to measure density at a consistent temperature, usually around 20°C. Adulteration is another factor, as the addition of water reduces the specific gravity below its normal range, serving as a key indicator of milk quality.

Viscosity

Viscosity is the measure of a fluid's resistance to flow, caused by internal friction as molecules slide past each other. The unit of viscosity is poise, but for milk, it is commonly expressed in centipoise (1/100th of a poise). The viscosity of milk at 20°C typically ranges between

1.5 to 2.0 centipoise. Milk is more viscous than water due to its fat emulsion and colloidal particles. Any change in the physical nature of fat or proteins such as during heating, cooling or homogenization can affect viscosity. Homogenization increases viscosity by breaking fat globules into smaller sizes, increasing resistance to flow. Likewise, clustering of fat globules, as seen in cream, increases viscosity. Heating and concentration processes like those used in making condensed milk also increase viscosity due to a higher proportion of total solids.

Surface Tension

Surface tension refers to the cohesive force that holds liquid molecules together at the surface, creating a skin like effect. This is caused by the inward pull on surface molecules, which lack neighboring molecules above them. In pure water, surface tension is about 72 dynes/cm at room temperature. Milk has a significantly lower surface tension, typically between 40–60 dynes/cm. This reduction is due to the presence of surfactants like proteins and fats. Proteins such as casein and whey lower surface tension by positioning themselves at the surface with their hydrophobic and hydrophilic ends. Fat globules and other milk components like lactose and minerals also play a role, though to a lesser extent.

pH of Milk

pH is the negative logarithm of the hydrogen ion concentration and indicates the true acidity or alkalinity of a substance. The pH scale ranges from 1 to 14, with 7 being neutral. Milk typically has a pH between 6.6 and 6.8, slightly acidic due to naturally occurring acids. During titration with alkali, the pH shifts due to milk's buffering capacity – its resistance to changes in hydrogen ion concentration. Buffering compounds in milk include acids, proteins, phosphate, citrate, carbonates, and dissolved CO₂. Accurate pH measurement requires calibrated pH meters and milk samples at room temperature. A deviation from the normal pH range may indicate spoilage or contamination.

Electrical Conductivity

Electrical conductivity measures a solution's ability to conduct an electric current, which occurs through ions present in the liquid. It is governed by Ohm's Law and measured as the reciprocal of specific resistance. Milk has a relatively low specific resistance, about 0.005ohm^{-1} . The major ions contributing to conductivity in milk include sodium, potassium, and chloride. During mastitis, an infection of the mammary gland, the concentration of these ions increases, which also increases conductivity. Therefore, electrical conductivity serves as a useful indicator of mastitis or abnormal milk composition.

Urea

Urea is a non-protein nitrogenous compound naturally present in cow milk. It is a by-product of protein metabolism in cows and enters milk through passive diffusion from the blood stream. Though not nutritionally essential for human consumption, the presence of urea in milk is significant as it serves as an indicator of the cow's dietary protein intake and metabolic condition. Urea in milk plays a role in maintaining the milk's osmotic balance and may affect its sensory properties when present in abnormal amounts. Milk urea is produced when dietary proteins are broken down into amino acids and excess ammonia generated in the rumen is absorbed into the bloodstream and converted to urea in the liver. While most urea is excreted through urine, a portion passes into the milk. If the cow's diet is deficient in nitrogen, the body conserves urea by recycling it into the rumen. Milk urea levels vary during early, mid and late lactation and can be influenced by the cow's water intake, general health, and type of feed. High urea content in milk generally indicates excessive protein or sufficient energy in the cow's diet, and thus serves as a valuable tool in ration balancing and herd management.

Sulphate

Sulphate is a trace mineral present in milk at concentrations of approximately 5–10mg per 100 mL. It is primarily derived from the metabolism of Sulphur containing amino acids such as methionine. Sulphate plays a critical role in supporting enzymatic activity and the synthesis of sulphur-based vitamins. Though present in small amounts, it contributes to the milk's ionic balance and buffering capacity. Sulphates can also be introduced into milk through dietary intake or as food additives. Ammonium sulphate, for example, is sometimes added legally or otherwise to increase the lactometer reading or simulate milk density. While this compound was once considered an adulterant, the Food Safety and Standards Authority of India (FSSAI) now recognizes that it can occur naturally or as a permissible additive. Apart from ammonium sulphate, milk also contains other sulphate derivatives such as oestrone sulphate, which is influenced by the reproductive cycle of the cow. Despite its low concentration, monitoring sulphate levels in milk remains important to detect adulteration and ensure consumer safety.

Phosphate

Phosphate is one of the most important inorganic constituents of milk, occurring in both soluble and colloidal forms, with concentrations ranging between 90 and 150 mg per 100 mL. It is crucial for stabilizing casein micelles the protein aggregates responsible for milk's white, opaque appearance and is also involved in pH buffering, ensuring the stability of milk during storage and processing. In cow milk, phosphorus is primarily found in the form of calcium phosphate, which is associated with casein micelles and contributes to milk's structure. The phosphorus content in milk is generally around 0.9 grams per kilogram. Phosphate is essential for both cows and humans, playing a vital role in bone formation, energy metabolism and overall growth. In dairy cows, especially those with high milk yield, a deficiency of phosphorus can lead to reduced productivity and metabolic disorders. Hormonal regulation, particularly by vitamin D, helps maintain proper phosphorus and calcium levels by promoting their absorption and mobilization. Thus, phosphate in milk not only has nutritional importance but also affects milk's biochemical and structural stability.

Calcium

Calcium is another vital mineral found in cow milk at levels ranging between 120 and 140 mg per 100mL. It exists in milk in two forms: as free ions and as colloidal calcium phosphate associated with casein micelles. Calcium is essential not just for bone and teeth development in humans but also for milk's structural integrity, texture, and thermal stability. The interaction between calcium and phosphate is fundamental to the formation and maintenance of casein micelles, which determine milk's consistency and its behavior during heating and fermentation processes. The bioavailability of calcium from cow milk is relatively high, estimated to be around 30–35%, which is significantly better than most plant-based sources. Although vegetables such as spinach contain calcium, their absorption rate is lower due to the presence of inhibitors like oxalates and phytates. For example, while cooked spinach contains 115 mg of calcium per serving, only about 5% (6 mg) is absorbed, in contrast to 32% (101 mg) from one cup of milk. Therefore, milk remains a highly effective source of dietary calcium, particularly important for children, adolescents, and pregnant women. A lack of milk or dairy products in the diet can make it difficult to meet daily calcium requirements, potentially affecting bone health and overall nutritional status.

Methodology

Physicochemical properties of Milk are important parameters in studying the compositions and nutritional aspects of milk and can be analyzed by different methods.

Measurement of Relative Density

Relative density (specific gravity) was measured using a pycnometer by the following steps:

1. Fill the pycnometer with distilled water and weight (empty reference).
2. Empty, dry thoroughly and fill the pycnometer with the milk sample.
3. Weigh the pycnometer containing milk.
4. Calculate density using:

$$\text{Density} = \frac{\text{Mass of milk} - \text{filled pycnometer} - \text{Mass of empty pycnometer}}{\text{Volume of pycnometer}}$$

Measurement of Viscosity

Viscosity was determined using an Ostwald viscometer (a U-shaped glass capillary viscometer). A known volume of milk was drawn into the viscometer above the upper bulb. The liquid was then allowed to flow under gravity through the capillary and the time taken for the meniscus to pass between two marked points was recorded. Flow time is directly proportional to viscosity; longer times indicate higher viscosity. The viscometer was calibrated using a standard fluid of known viscosity before sample measurements.

Measurement of Surface Tension

Surface tension was measured using a stalagmometer based on the principle of drop weight (or volume) as described by Tate's law. The milk sample was drawn into the capillary up to the prescribed mark, and drops were allowed to form and fall slowly. The number of drops was counted. A similar procedure was performed with a reference liquid (distilled water) to provide comparison. According to Tate's law, the drop falls when its weight(mg) equals the product of the capillary circumference and surface tension ($2\pi r \sigma$); thus, surface tension can be calculated from the drop mass and geometry.

Measurement of pH

The pH of milk was measured in the laboratory using three methods: pH paper, universal indicator solution, and an electronic pH meter. For the indicator methods, the milk sample was poured into two separate Petri dishes. pH paper was dipped into one, and a few drops of universal indicator were added to the other; after gentle mixing, the resulting color was observed and compared to the standard pH scale. For the pH meter measurement, the milk sample was placed in a beaker, the electrode was calibrated

according to manufacturer instructions, and the probe was immersed in the sample until a stable reading was obtained.

Measurement of Electrical Conductivity

Electrical conductivity of the milk samples was measured using a conductivity meter. The meter was first calibrated with a standard solution of known conductivity. The milk sample was placed in a clean beaker, and the conductivity probes were inserted into the sample. Readings were recorded once the values stabilized. Changes in conductivity can reflect ionic variations and are useful for detecting physiological conditions such as mastitis.

Detection and Quantitative Analysis of Urea

Qualitative Tests:

- **PDAB Test:** Urea in milk was detected using para-dimethyl amino benzaldehyde (PDAB) reagent, prepared by dissolving 1 g PDAB in 100 mL of 10% HCl-alcohol mixture. To 5 mL milk, 1 mL PDAB reagent was added, mixed gently and left at room temperature for 10–15 minutes. Development of yellow to dark yellow indicated the presence of urea.
- **Soybean Method:** One teaspoon of soybean powder was added to 5 mL milk and mixed; after 5–10 minutes, the sample was smelled. A pungent ammonia odor indicated urea hydrolysis.

Quantitative Analysis:

- Urea reacts with diacetyl monoxime in acidic medium in the presence of ferric ions and thiosemicarbazide to form a pink colored complex.
- **Reagents:** 2% diacetylmonoxime, 0.05% thiosemicarbazide, acid mixture (sulfuric + phosphoric acid in 9:1 ratio), 0.1% ferric chloride, distilled water, standard urea solution.
- **Procedure:** Pipette 1 mL skimmed milk sample into a test tube. Add 1 mL diacetyl monoxime, 1 mL thiosemicarbazide, 3 mL acid mixture and 0.5 mL ferric chloride. Mix and incubate in a boiling water bath for 10–15 minutes. Cool to room temperature. Measure absorbance at 540 nm. Determine urea concentration using a standard calibration curve.

Detection and Quantitative Analysis of Sulphate

Qualitative Test: Sulphate presence was tested using Barium Chloride. Ten milliliters of milk sample was mixed with 10 mL of tri chloro acetic acid (TCA) to precipitate proteins, then filtered. A few drops of 1% barium chloride solution were added to the clear filtrate. Formation of a milky white precipitate indicated the presence of sulphate (BaSO_4).

Quantitative Analysis: Sulphate concentration was determined spectrophotometrically based on turbidity from barium sulphate precipitation.

Reagents: 1% BaCl_2 solution, 0.25% gumacacia solution (stabilizer), standard sulphate solutions (for calibration), TCA or acetic acid for protein precipitation, distilled water.

Sample Prep: Mix 10 mL milk with 10 mL 10% TCA (or acetic acid); centrifuge at 3000 rpm for 10–15 minutes. Collect the supernatant.

Procedure: Take 5 mL of filtrate; add 1 mL gumacacia and 1 mL 1% BaCl_2 . Mix and let stand for 10 minutes for turbidity development. Measure absorbance at 420 nm. Construct a calibration curve using standard sulphate concentrations (e.g., 5–50 mg/L) to quantify sample sulphate.

Detection and Quantitative Analysis of Phosphate

Qualitative Test: A standard alkaline phosphatase (ALP) activity test was used, employing di sodium p-

nitro phenyl phosphate as substrate. Milk samples were incubated (e.g., at 37°C for 2 hours). Minimal or no color change indicated proper pasteurization (inactivated ALP), while development of yellow color suggested active ALP, implying inadequate heat treatment or raw milk contamination.

Quantitative Analysis: Inorganic phosphate reacts with ammonium molybdate in acidic medium to form phosphomolybdic acid, which is reduced by 1-amino-2-naphthol-4-sulphonic acid (ANSA) to yield a blue complex.

Reagents: 2.5% ammonium molybdate solution, concentrated sulfuric acid, ANSA (or ascorbic acid) reducing agent, 10% TCA for deproteinization, standard phosphate solution.

Sample Prep: Add 10 mL milk to 10 mL 10% TCA; centrifuge at 3000 rpm for 10 minutes. Collect supernatant. **Procedure:** Pipette 1 mL deproteinized sample; add 1 mL ammonium molybdate and 1 mL concentrated H₂SO₄ carefully. After 10 minutes, add 0.4 mL ANSA reagent. Allow color to develop 10–15 minutes at room temperature. Measure absorbance at 660 nm. Use a standard phosphate curve (e.g., 0.5–5 mg/dL) to determine concentration.

Detection and Quantitative Analysis of Calcium

Qualitative Test: Calcium presence was assessed using the ammonium oxalate test. Milk (5–10 mL) was acidified with dilute acetic acid or HCl to dissolve casein and release calcium, then filtered if cloudiness occurred. Ammonium oxalate solution added drop wise to the clear filtrate. Formation of a white precipitate of calcium oxalate confirmed calcium ions.

Quantitative Analysis: Calcium was quantified by complexometric titration with EDTA. Calcium forms a stable complex with EDTA in alkaline medium. Indicators such as murexide or Eriochrome Black T are used to signal the end point by a distinct color change.

Reagents: Standard EDTA solution (e.g., 0.01 M), buffer solution (pH 12, typically NH₄Cl/NH₄OH), murexide or Eriochrome Black T indicator, 10% TCA for deproteinization, distilled water, standard calcium solution for EDTA standardization.

Sample Prep: Mix 10 mL milk with 10 mL 10% TCA; filter or centrifuge to obtain clear filtration.

Procedure: Pipette 10 mL filtrate into a conical flask; add 2 mL buffer and a pinch of indicator. Titrate with 0.01 M EDTA until the color changes (e.g., pink to purple/blue). Record volume of EDTA is used to calculate calcium concentration.

Discussion

Milk possesses characteristic physicochemical properties that are specific in nature due to the unique composition of its constituents. Understanding these characteristics is crucial in milk processing and product development as they influence not only the efficiency of production but also the detection of adulteration and assessment of milk quality.

The study provides a regional snapshot of the physicochemical quality of milk. Although limited in scope, it underscores the importance of continuous monitoring and region-specific research in maintaining dairy quality standards. The variations observed across multiple parameters point to the impact of feeding practices, animal health, and environmental conditions on milk composition. Future studies should aim to expand the sample size, cover different seasons, and include more detailed biochemical analyses for a broader understanding of milk quality trends.

Conclusion

Milk possesses characteristic physicochemical properties which are specific in nature. These are due to the constituents present in milk. These physicochemical properties include specific gravity/density, pH,

buffering, viscosity, surface tension, freezing point, oxidation-reduction, specific heat, boiling point and electric conductivity. Understanding these properties can be used advantageously for processing milk & milk products. Such properties have great potential for detecting adulteration. The following recommendations are proposed for future research:

1. Seasonal and Agro-Ecological Variations: Investigate how different seasons and agro-ecological zones influence the physicochemical properties of cow milk. Such studies can help in understanding regional differences and in developing strategies to maintain milk quality throughout the year.
2. Comparative Analysis Across Species: Conduct comparative studies between cow milk and milk from other species (e.g., buffalo, goat) to identify unique physicochemical traits and nutritional benefits, which can inform consumer choices and dairy processing techniques.
3. Impact of Processing Techniques: Examine how various milk processing methods, including pasteurization and high-pressure processing, affect the physicochemical and nutritional properties of cow milk. This can aid in optimizing processing conditions to preserve milk quality.
4. Adulteration Detection and Quality Control: Develop and refine methods for detecting adulteration in cow milk, focusing on changes in physicochemical parameters. This is crucial for ensuring consumer safety and maintaining industry.
5. Advanced Analytical Techniques: Utilize advanced analytical tools, such as spectroscopy and machine learning algorithms, to predict and monitor milk quality parameters. These techniques can provide rapid and accurate assessments of milk's physicochemical properties.
6. By addressing these areas, future research can contribute to the enhancement of milk quality, safety, and processing efficiency, ultimately benefiting both producers and consumers in the dairy industry.

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