PHYSICO-CHEMICAL CHARACTERIZATION OF RAW MILK: EXPERIMENTAL DETERMINATION OF ACIDITY AND CASEIN CONTENT

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RESEARCH ARTICLE

Abstract

Milk represents a complex biological fluid whose composition and physicochemical characteristics are essential indicators of its freshness and quality. Raw cow's milk samples collected from local markets in Oradea, Romania, were analyzed to evaluate key quality parameters, including acidity, ethanol stability, and casein content. Six samples from different local producers underwent organoleptic examination, titratable acidity determination, and protein quantification. The sensory evaluation showed uniform color, texture, odor, and taste, consistent with fresh milk characteristics. Acidity values ranged from 16.0 to 23.0 ºT, where samples with higher acidity formed aggregates in the ethanol test, indicating reduced freshness. Casein concentrations varied between 31.0 and 52.6 g/L (3.1–5.3%), with higher values associated with better protein stability and lower acidity levels. These findings highlight the strong relationship between acidity, casein concentration, and overall milk quality, emphasizing their combined relevance as indicators of freshness and suitability for consumption or further processing.

Keywords: casein quantification, milk acidity, physicochemical parameters, freshness indicators
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INTRODUCTION

Milk is one of the most complete and valuable natural foods, providing essential nutrients such as proteins, fats, carbohydrates, vitamins, and minerals that support human growth and health. It plays a central role in the diet of both children and adults and serves as an important raw material for the dairy industry. Globally, milk production exceeds 900 million tons per year, emphasizing its nutritional and economic significance (Acquavia et al., 2025; Górska-Warsewicz et al., 2019; Sarode et al., 2016).

Milk represents a complex colloidal system in which fat globules, casein micelles, and whey proteins are dispersed in an aqueous phase containing lactose and mineral salts. Its physicochemical characteristics are influenced by both intrinsic compositional factors and extrinsic factors such as temperature variations and post-milking handling. Among milk proteins, casein constitutes about 80% of the total protein content and plays a crucial role in determining milk stability, processing behavior, and nutritional value (Corredig et al., 2019; Zheng et al., 2023).

The acidity of milk is a key parameter for evaluating its freshness and quality. Natural acidity arises from normal milk constituents such as casein, phosphates, and citrates, while microbial activity during storage leads to the conversion of lactose into lactic acid, resulting in increased titratable acidity and lower pH values. Monitoring these parameters provides insight into both the chemical balance and hygienic status of milk (Aydogdu et al., 2023; Linehan et al., 2024).

This study focuses on assessing the physicochemical profile of raw cow's milk collected from local markets, aiming to highlight variations in composition and quality among samples obtained from different local producers.

MATERIAL AND METHOD

SAMPLES

Six cow milk samples were collected from distinct local producers at a fresh food market in Oradea, Romania. The samples were maintained in sealed containers at ambient temperature until analysis to preserve their original physicochemical properties.

REAGENTS AND SOLUTIONS

Analytical grade reagents were used throughout the study. Sodium hydroxide (NaOH) 0.1 N (Chimreactiv, Romania); hydrochloric acid (HCl) 0.1 N (Chimreactiv,

Romania); and 0.5 N, sulfuric acid (H_2SO_4) 0.1 N (Chimreactiv, Romania); acetic acid 1 N (Chimreactiv, Romania); ethanol p.a 59–61 % v/v (Chimreactiv, Romania); phenolphthalein 1 % alcoholic solution (Silver Chemicals) ; and methyl red 0.2 % alcoholic solution (Remed Prodimpex); distilled water.

METHODS

Organoleptic Evaluation: Milk samples were evaluated for color, opacity, consistency, appearance, odor, and taste. Observations were made under natural light, and consistency and appearance were assessed visually, while odor and taste were determined by sensory evaluation.

Free Acidity (pH): The pH of milk was measured using pH indicator paper, with results compared against a standard color scale to determine free acidity. Normal cow milk has a slightly acidic pH ranging from 6.4 to 6.7.

Titratable Acidity: Milk titratable acidity was determined by titration with NaOH in the presence of phenolphthalein (Miere et al.,2012). Results were expressed in degrees Thörner (ºT) using the formula:

$$\text{Acidity (ºThörner)} = N \cdot F \cdot 10$$

N - volume of 0.1 N NaOH used for titration (mL);
10 - factor to refer the result to 100 mL of milk;
F- factor of the solution NaOH 0,1 N.

Casein Content: Casein was precipitated by adding acetic acid to milk in the presence of methyl red. The precipitate was filtered, washed to neutrality, and dissolved in NaOH. Excess NaOH was titrated with H_2SO_4 using phenolphthalein as an indicator (Husnaeni et al.,2019; Kumaresan et al., 2017). Casein content (g/L milk) was calculated using:

$$\text{Casein (g/L)} = 11 \cdot (V_1 - V_2)$$

V_1 - volume of NaOH 0,1 N used to dissolve the casein;
V_2 - volume of H_2SO_4 0,1 N used to neutralize the excess NaOH 0,1 N.

RESULTS AND DISCUSSIONS

The organoleptic evaluation of the cow milk samples revealed slight variations in color, ranging from white to pale yellow, with a homogeneous and fluid consistency in all cases. No flakes or sediment were observed, and all samples presented the characteristic odor of fresh raw milk. Heating the samples to 35–40°C did not reveal any abnormal odors, and the taste was consistent with fresh milk, slightly sweet and pleasant.

The main physicochemical parameters of the milk samples, including acidity, ethanol test results, and casein content, are summarized in Table 1.

Table 1

Physicochemical parameters and casein composition of cow milk

Sample	Acidity (°Thörner)	Ethanol test	Casein content (g/L)	Casein percentage (%)
1	23.0	Numerous large coagulates formed, indicating high acidity and low freshness	34.87 ± 1.2	3.49 ± 0.12
2	17.0	Small precipitate deposited on the tube walls, indicating moderate acidity and medium freshness	42.02 ± 1.4	4.20 ± 0.14
3	20.5	Precipitate observed on the tube walls	45.10 ± 1.0	4.51 ± 0.10
4	16.0	No coagulation observed, indicating low acidity and very fresh milk	52.58 ± 1.6	5.26 ± 0.16
5	16.5	Minor precipitate deposited on the tube walls, acidity within normal limits	46.75 ± 1.3	4.68 ± 0.13
6	20.0	Precipitate observed on the tube walls	31.02 ± 1.5	3.10 ± 0.15

The acidity of the samples, expressed in Thörner degrees (ºT), varied between 16.0 and 23.0 ºT. According to the literature, normal milk acidity ranges between 16–18 ºT or, depending on the source, 15–20 ºT. Some samples fell within this range, indicating freshness, while others exhibited elevated acidity, suggesting a decline in quality. This was further confirmed by the ethanol test: samples with higher acidity formed numerous aggregates, whereas those with moderate acidity showed minor precipitate formation, and samples with low acidity remained clear, consistent with very fresh milk. This reaction is known to occur when milk proteins (particularly casein) destabilize under increased ionic and acidic stress, suggesting a correlation between ethanol stability and freshness.

Casein content varied considerably among the analyzed samples, ranging from 31.0 to 52.6 g/L, corresponding to 3.1–5.3% of the

milk. These concentrations are consistent with previously reported data, where average casein levels in bovine milk were found to be around 29.5 g/L (Davoodi et al., 2016) and between 4.47–4.77% in cow milk samples (Pamarthy et al., 2016). A clear relationship was observed between milk freshness and protein content: the freshest samples with low acidity exhibited the highest casein concentrations, whereas samples with elevated acidity contained lower casein levels, reflecting potential protein degradation.

CONCLUSIONS

The analyzed milk samples exhibited moderate variability in their physicochemical properties, reflecting differences in freshness and overall quality. Samples with elevated acidity showed signs of reduced protein stability, whereas those with acidity values within the normal range maintained characteristics consistent with fresh milk. Casein content proved to be a critical determinant of milk quality, with higher concentrations associated with improved protein stability and indicators of freshness.

These observations underscore the interrelated roles of acidity and casein concentration in defining the quality of raw cow's milk, providing a reliable basis for assessing its suitability for consumption and further processing.

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