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Comparative Study on the Impact of Lactic Acid Formation Over Time on Calcium Levels in Curd Made from Cow's and Buffalo's Milk

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Abstract: This study explores the process of lactic acid production in milk through natural fermentation, primarily driven by *Lactobacillus* species, which convert lactose into lactic acid. This acidification lowers the milk's pH, leading to the coagulation of proteins like casein and resulting in the distinct texture and flavor of fermented dairy products such as curd and yogurt. The research emphasizes that optimal temperature conditions (30°C to 40°C) are crucial for proper curd formation. It also highlights the differences between cow and buffalo milk, noting that cow milk acidifies more slowly even at optimal temperatures, making it more effective in retaining calcium. In contrast, buffalo milk ferments more rapidly, requiring careful temperature control to prevent excessive calcium loss. Additionally, the study stresses the importance of public awareness regarding nutrient preservation. Educational initiatives through schools, health programs, and media can encourage better food preparation and storage practices. Promoting the use of fresh, local ingredients and understanding food labels can help individuals, especially vulnerable groups, make informed dietary choices. Overall, the findings underscore the need to preserve nutrients during food processing and raise awareness so that essential nutrients can be retained or supplemented as needed for individual health and well-being.A comparison of lactic acid production at different temperatures shows that the inoculum becomes more active and multiplies more rapidly at temperatures optimal for *Lactobacillus*, leading to faster acid development. Conversely, at lower temperatures, the growth of the bacteria slows down, resulting in a slower rate of acid formation. Interestingly, buffalo milk tends to exhibit a quicker rise in acidity than cow milk, suggesting that fermentation occurs at a faster pace. However, this rapid acid production in buffalo milk can lead to increased calcium loss, as the rising acidity dissolves calcium phosphate, which is then released into the whey and removed during curd formation

Keywords: *Milk, Lactic Acid, Curd, Temperature, Calcium.*

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I. INTRODUCTION

Lactobacillus is a genus of beneficial bacteria commonly found in fermented dairy products like curd. In curd production, Lactobacillus delbrueckii subsp. bulgaricus plays a crucial role by converting lactose, the natural sugar in milk, into lactic acid through the process of fermentation. This acidification causes the milk proteins, mainly casein, to coagulate, leading to the thick texture and tangy flavor characteristic of curd. Additionally, Lactobacillus acts as a probiotic, contributing to gut health by promoting the growth of good bacteria and aiding in digestion. Its presence in curd not only enhances the taste and texture but also provides nutritional and health benefits, making it a valuable component of a balanced diet (Tamime & Robinson, Yoghurt: Science and Technology, 2007).

Cow's milk is a naturally rich source of essential nutrients that support overall health and development. It provides high-quality protein, containing all the essential amino acids needed by the body for growth, repair, and maintenance. One of its most important nutrients is calcium, which plays a key role in building and maintaining strong bones and teeth. In addition to calcium, cow's milk offers other vital minerals such as potassium, phosphorus, magnesium, and zinc, each contributing to muscle function, bone health, and cellular activity. It is also a good source of vitamins, including vitamin B12, which supports nerve health and blood formation, and riboflavin (vitamin B2), which

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helps in energy production. Fortified milk often contains added vitamin D to aid in calcium absorption, and it naturally contains vitamin A for immune support and vision. Depending on the fat content (whole, low-fat, or skim), milk can also provide energy and fat-soluble vitamins. These qualities make cow's milk a valuable part of a balanced diet (U.S. Department of Agriculture, 2020; National Institutes of Health, Office of Dietary Supplements).

Buffalo milk is nutritionally dense and offers several key health benefits. Compared to cow's milk, it contains a higher amount of fat, which results in a creamier texture and provides more energy per serving. This increased fat content also boosts levels of fat-soluble vitamins, particularly vitamin A and vitamin E. It is also a rich source of high-quality protein, supplying all the essential amino acids needed for body growth and tissue repair.

When it comes to minerals, buffalo milk surpasses cow's milk in calcium and phosphorus, both crucial for maintaining strong bones and teeth. It also includes important minerals like potassium, magnesium, and zinc, which support cardiovascular health, bone strength, and immune function. Additionally, buffalo milk is high in vitamin B12 and riboflavin (B2), which play essential roles in energy metabolism and nervous system function. Because of its richness and thickness, buffalo milk is commonly used in making products such as ghee, paneer, and yogurt (Ahmad et al., Buffalo Milk: Chemistry and Technology, 2013).

Commercial milk is milk that has been processed, packaged, and sold for everyday consumer use. Before reaching the market, it usually goes through pasteurization, a heating process designed to eliminate harmful microorganisms while preserving its nutritional content. Most commercial milk is also homogenized, a method that evenly distributes fat throughout the liquid, giving it a smooth and uniform consistency. To improve its nutritional value, commercial milk is often fortified with extra nutrients like vitamin D and calcium.

There are various forms of commercial milk available, such as whole, low-fat, skim, and even flavored or plant-based versions, allowing consumers to choose according to their dietary needs. Although the fat levels may differ, commercial milk still provides essential nutrients like protein, calcium, vitamin B12, and vitamin B2 (riboflavin). Strict food safety and quality standards are followed during production to ensure the milk is both safe and consistent for public consumption (FDA, 2020).

II. REVIEW OF LITRATURE

Lactic acid in milk is produced through the natural process of fermentation, where specific bacteria, primarily Lactobacillus species, convert the milk sugar lactose into lactic acid. This transformation typically occurs when milk is left to ferment or during the production of dairy products like curd, yogurt, and cheese. The formation of lactic acid lowers the pH of milk, causing it to become sour and leading to the coagulation of proteins, particularly casein. This is what gives fermented dairy products their distinct texture and tangy taste.

Lactic acid not only enhances flavor and texture but also acts as a natural preservative by inhibiting the growth of harmful bacteria. Additionally, it aids in digestion and contributes to the probiotic qualities of fermented milk products. This makes lactic acid an important compound in both the nutritional value and shelf-life of dairy foods (Fox et al., Fundamentals of Cheese Science, 2017).

Lactobacillus plays a significant role in improving the nutritional quality of fermented foods by altering their nutrient composition during fermentation. One of its primary functions is breaking down lactose into lactic acid, which not only gives fermented products like curd their sour taste but also makes them easier to digest, particularly for individuals with lactose intolerance. Additionally, the fermentation process can enhance the bioavailability of minerals such as calcium and magnesium, making them more readily absorbed by the body. Some strains of Lactobacillus are also known to synthesize certain B vitamins, including riboflavin and folate, thereby increasing their concentration in the final product. Moreover, Lactobacillus helps reduce anti-nutritional factors like phytates, which can interfere with mineral absorption. By producing beneficial compounds and supporting gut health through probiotics, Lactobacillus contributes to both the digestibility and nutritional value of fermented foods (Marco et al., Annual Review of Food Science and Technology, 2017).

Curd prepared from cow's milk and buffalo milk shows noticeable differences because of the varying nutritional content in each type of milk. Curd made using cow's milk tends to be lighter and softer in consistency, with a milder flavor. Since cow's milk has lower fat and solid content, the curd is easier to digest, making it a suitable choice for children, the elderly, and individuals with sensitive digestion.

In contrast, curd made from buffalo milk is thicker, creamier, and richer, owing to its higher levels of fat, protein, and minerals like calcium. It has a denser texture and a slightly stronger taste. This type of curd is commonly used in preparing other dairy products like paneer and thick yogurt because of its richness and firmness.

Overall, cow milk curd is lighter and more digestible, while buffalo milk curd offers a creamier and more nutrient-dense option (Ahmad et al., Buffalo Milk: Chemistry and Technology, 2013).

The process of curd formation requires specific temperature and time conditions to ensure proper fermentation. Generally, curd sets best at a temperature range of 30°C to 40°C. Within this range, the Lactobacillus bacteria become active and convert lactose (milk sugar) into lactic acid, which helps the milk coagulate.

At around 37°C (close to human body temperature), curd usually sets within 6 to 8 hours. However, the time can vary depending on the ambient temperature, type of milk

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used, and quantity of starter culture (previous curd). In warmer climates or during summer, curd may set faster — in 4 to 6 hours, while in colder conditions, it may take up to 10–12 hours or more. For better results in cooler weather, the milk can be kept in a warm place or wrapped in a cloth to retain heat during fermentation.

In summary, curd typically sets at 30–40°C over a period of 6–8 hours, with variations based on environmental and preparation factors (Tamime & Robinson, Yoghurt: Science and Technology, 2007).

An increase in temperature beyond the optimal range (30°C to 40°C) can negatively affect curd formation. When the temperature rises above 45°C, the Lactobacillus bacteria responsible for fermentation may become inactive or die, as they are sensitive to excessive heat. This disrupts the conversion of lactose into lactic acid, preventing the milk from coagulating properly.

As a result, the curd may fail to set, or it may develop a grainy, watery texture and an unpleasant sour taste. Extremely high temperatures can also encourage the growth of undesirable bacteria, leading to spoilage and reduced quality. Therefore, maintaining a stable, moderate temperature during curd setting is crucial for achieving proper consistency, taste, and safety (Tamime & Robinson, Yoghurt: Science and Technology, 2007).

The acid value and calcium content are important indicators of the quality and nutritional value of milk and curd. During fermentation, lactic acid bacteria such as Lactobacillus convert lactose into lactic acid, increasing the acidity. While fresh milk typically has a low acidity of about 0.13–0.17%, well-set curd reaches an acid value of approximately 0.6–0.8%, which contributes to its sour flavor and protein coagulation. Calcium, a key mineral in milk, is present at around 120 mg per 100 ml in cow's milk and about 180 mg per 100 ml in buffalo milk due to its higher solid content. Most of this calcium remains in curd, and fermentation may enhance its absorption in the body, making curd a rich and bioavailable source of calcium (Tamime & Robinson, Yoghurt: Science and Technology, 2007; USDA FoodData Central).

A rise in acidity during the curd-making process can noticeably influence its taste and texture. As lactic acid builds up, the curd develops a stronger sourness, which may be acceptable to some extent but can become unpleasant if the acid level is too high. This increase in acidity can also lead to changes in the consistency, often resulting in curd that is too thick, grainy, or watery, rather than smooth and creamy. Over-acidification may be a sign of prolonged fermentation, which can negatively affect both the flavor and nutritional quality of the curd. To produce curd with the best taste and texture, it is essential to keep acidity within an ideal range (Tamime & Robinson, Yoghurt: Science and Technology, 2007).

III. METHODOLOGY

Cow milk and buffalo milk were taken as sample 1 and sample 2 respectively.Both the samples were boiled and cooled up 45 to 50 degree centigrade and 30 to 40 degree centigrade considering the temperature conditions to be 1 and 2 then the inoculums or the curd sample of 10 ml were added in 100 ml of milk.Then the samples were left undisturbed for 6 hours for the settling of milk into curd.After 6 hours the texture was observed in the sample 1 and 2 under two different temperatures. Standard procedure for finding the acid values in the samples . The acid content in a curd sample can be measured using a titration method, which determines the percentage of lactic acid produced during fermentation. In this method, a known amount of curd is diluted with distilled water and titrated against a standard solution of sodium hydroxide (NaOH) using phenolphthalein as an indicator. The sodium hydroxide neutralizes the lactic acid present in the curd, and the end point is identified by the appearance of a faint pink color that remains stable for about 30 seconds. The volume of NaOH used is then applied in a standard formula to calculate the percentage of lactic acid. This method is widely used because of its accuracy and simplicity in assessing the acidity level in fermented dairy products like curd. Accurate measurement of acidity is essential for quality control, as it affects the taste, texture, and safety of the product (Tamime & Robinson, *Yoghurt: Science and Technology*, Procedure repeated for the samples with the time variation in order to find the acidity created in the curd of cows milk and buffalo milk.acidity levels of sample 1 and sample 2 were compared to analyse the stability of the curd.

Increase in acidity leads to the alteration in the levels of calcium.

Table 1 Calcium Value of Cow's Milk and Baffalo's Milk

Type of Milk	Calcium in Raw Milk (mg/100 ml)	Calcium in Curd (approx., mg/100 g)
Cow's Milk	120–125 mg	80–100 mg
Buffalo's Milk	180–190 mg	100–120 mg

Table 2 Lactic Acid Content (Titratable Acidity in % Lactic Acid) (Optimal Temperature)

Time After Inoculation	Cow Milk Curd (%)	Buffalo 0Milk Curd (%)
6 hours	0.45%	0.50%
8 hours	0.60%	0.70%
10 hours	0.72%	0.85%

Table 3 Lactic Acid Content Higher Temperature (Titratable Acidity in % Lactic Acid)

Time After Inoculation	Cow Milk Curd (%)	Buffalo 0Milk Curd (%)
6 hours	0.40%	0.43%
8 hours	0.55%	0.68%
10 hours	0.68%	0.78%

IV. DISCUSSION

Increased acidity during curd formation can lead to a decrease in its calcium content. As the milk becomes more acidic—whether through the action of starter cultures or the direct addition of acid—the pH drops, triggering a chemical shift that affects the calcium bound within the casein micelles. Under these acidic conditions, calcium phosphate begins to dissolve and separate from the casein structures, becoming more soluble in the liquid portion of milk, known as whey. When the whey is drained during curd production, the dissolved calcium is carried away with it. This results in less calcium being retained in the final curd.

The reduction in calcium not only affects its nutritional value but also influences the texture and firmness of the curd, potentially altering its functional and sensory properties. In summary, higher acidity promotes calcium loss during whey separation, thereby reducing the overall calcium content in the final curd product (Fox et al., Fundamentals of Cheese Science, 2017). Value for acidity is compared for milk of cow and milk of buffalo is compared. Acidity was taken for 6 hours after 8 hours and 10 hours were taken.where gradual increase of acidity shown with the increase in time.

A comparison of lactic acid production at different temperatures shows that the inoculum becomes more active and multiplies more rapidly at temperatures optimal for Lactobacillus, leading to faster acid development. Conversely, at lower temperatures, the growth of the bacteria slows down, resulting in a slower rate of acid formation. Interestingly, buffalo milk tends to exhibit a quicker rise in acidity than cow milk, suggesting that fermentation occurs at a faster pace. However, this rapid acid production in buffalo milk can lead to increased calcium loss, as the rising acidity dissolves calcium phosphate, which is then released into the whey and removed during curd formation.

V. RESULT

A comparison of lactic acid levels under varying temperature conditions reveals that the inoculum grows more actively and rapidly at temperatures ideal for Lactobacillus, leading to quicker acid production. In contrast, lower temperatures slow bacterial activity, resulting in reduced acidity. Notably, buffalo milk shows a faster increase in acidity compared to cow milk, indicating more rapid fermentation. However, this accelerated acid formation in buffalo milk may cause greater calcium loss, as the acid breaks down calcium phosphate, allowing it to escape with the whey.

To prevent this, adding the inoculum at lower temperatures can help moderate the acid development, especially in buffalo milk, thereby preserving more calcium in the final curd. While cow milk is also affected by temperature, it naturally acidifies more slowly, helping retain calcium over a longer period. Using the optimal temperature range (around 37°C to 42°C) is ideal when quick curd setting is needed, but for better nutrient preservation, initiating fermentation at a lower temperature is more beneficial.

In cow milk, even when fermented at optimal temperatures, the acidity does not increase as rapidly as in buffalo milk, which makes it more suitable for calcium retention. Overall, the study shows that buffalo milk requires careful temperature management to avoid excessive acid production, while cow milk's slower acidification rate helps maintain its calcium content more effectively under similar condion.

VI. CONCLUSION

Preserving nutrients in food is essential for maintaining its health benefits, ensuring that the body receives the vitamins, minerals, and other bioactive compounds needed for proper growth, energy, immunity, and overall well-being. Nutrients such as proteins, vitamins (like A, B-complex, C, and D), minerals (such as calcium, iron, and zinc), and essential fats can be sensitive to heat, light, air, and improper storage or processing methods. Overcooking, exposure to high temperatures, or prolonged storage can degrade these nutrients, reducing the food's nutritional value.

Raising awareness about preventing nutrient loss in food is essential for improving dietary quality and public health. Many common food handling practices—such as excessive boiling, overcooking, or improper storage—can significantly reduce the levels of essential nutrients like vitamins and minerals. Educating individuals on better cooking methods, such as steaming, stir-frying, or minimal peeling, can help retain more of the original nutrient content. For example, cooking with less water or using pressure cookers can preserve water-soluble vitamins like vitamin C and B-complex.

Awareness campaigns conducted through schools, community health programs, and media can help people make informed decisions about food preparation and storage. Encouraging the use of fresh, local produce and explaining the importance of reading nutritional labels are also important strategies. Such knowledge enables individuals and families to adopt healthier food practices, contributing to improved nutrition, particularly among vulnerable groups like children, pregnant women, and the elderly. Enhancing public understanding of nutrient preservation aligns with global health efforts to combat malnutrition and promote food security (FAO/WHO, Guidelines on Nutrition and Food Processing, 2019).

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This study provides the ways by which the nutrient can be preserved and also create awrness on the loss of nutrient .So that required nutrients can be supplemented as required by the individual

RECOMMENDATIONS

Further research can be carried out to explore the types and effectiveness of preservative materials used in commercial curd to control acidity, especially over short storage periods such as two days. Unlike homemade curd, which tends to become increasingly sour due to ongoing fermentation, commercial curd remains stable in taste and texture for a longer time. This is often attributed to the use of food-grade preservatives and controlled processing techniques. Studying the preservatives used—such as potassium sorbate, sodium benzoate, or natamycin—can help understand how they slow down acid production by inhibiting the activity of lactic acid bacteria or delaying post-fermentation acidification.

Additionally, analyzing the mechanisms by which commercial curd resists rapid acidification could offer insights into improved shelf-life without compromising quality. Factors such as packaging conditions, pasteurization before fermentation, and starter culture control may also play roles in maintaining low acid development.

Another key area of investigation involves examining how these preservatives might alter the nutrient profile of curd. While preservatives are added in minimal quantities, their interaction with nutrients—especially proteins, vitamins, and minerals—needs to be studied carefully. For instance, certain preservatives might interfere with calcium availability, B-vitamin stability, or probiotic viability, which can impact the overall nutritional value of the product.

➢ *Research in this Area could Focus on:*

- Identifying commonly used preservatives in commercial curd
- Evaluating their impact on acid formation, microbial activity, and shelf life
- Measuring any nutrient degradation or alteration caused by preservative interactions
- Developing safer and more natural alternatives (like plant-based antimicrobials) that can offer similar benefits without reducing curd quality

Such studies would contribute to safer, more nutritious commercial curd with improved shelf life, and help balance consumer health, product stability, and regulatory compliance in the dairy industry (Tamime & Robinson, Yoghurt: Science and Technology, 2007; Farkas, Preservation and Shelf-Life Extension, 2004).

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