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Determinants of refrigerated cow milk curd pH based on starter cultures and incubation temperatures

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Abstract
Accurate regulation of cow milk curd pH is mandatory for processing and maintenance of shelf life; Dysregulation of cow milk curd pH has been associated with easy spoilage of curd. Curd manufacture in India needs improvements in the existing methods of production and marketing so as to ensure a uniformly high standard in the quality of curd.
Research was undergone to identify the starter cultures and incubation temperatures, which can maintain desirable pH and in turn increase the shelf life without compromising with the standard curd quality. Significant (p<0.01) difference between the control and treatment samples was observed. The cow curd samples prepared with starter culture combination of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *Lactis biovar diacetylactis* showed desirable pH during storage than other curd samples incubated at 37 °C and 40 °C. On storage at 5.00±0.5 °C, the pH of the *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis biovar diacetylactis* combined cultures curd sample incubated at 37 °C was higher compared to *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *Lactis biovar diacetylactis* combined cultures incubated at 40 °C. Compared to other lactic starter cultures The cow curd samples prepared with starter culture combination of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *Lactis biovar diacetylactis* showed desirable pH during storage than other curd samples incubated at 37 °C and 40 °C.

Keywords: Cow milk curd-pH, refrigeration-starter cultures-incubation temperatures, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*

Introduction
The critical role of pH in milk and milk products has been recognized from the earliest days of fermentation. The pH differences generate remarkable changes in taste, keeping quality and serve as an interconvertible source of metabolic energy. The central role of organellar pH regulation in cellular homeostasis and survival is therefore evident and unquestionable. In this light, it is remarkable that our knowledge of the determinants of cow milk curd pH is rather primitive. The pH remains understudied as well as underappreciated perhaps because of the multiplicity of variables involved in pH regulation and the technical challenges it presents.
Preservation of dairy foods with the help of specific group of bacteria known as lactic acid bacteria has been the most common practice. There are a variety of control measures and processes to prevent microbial contamination, some of which are even capable of extending shelf life. Research in the area of extending shelf life of dairy foods is very aggressive. Thus it is immensely important to meet the current market trend of dairy products. With this view the main objective of this Research work is to find starter culture combinations and incubation temperatures prominent for cow milk curd production with a better shelf life.

Materials and Methods
Cow milk curd and buffalo milk curd were prepared and incubated at 37 °C and 40 °C separately using 5 per cent fat and 9 per cent SNF combination in cow milk using the starter culture combination viz. Dahi culture, *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Lactococcus lactis*

subsp. *lactis* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis subsp. lactis biovar diacetylactis*, *Leuconostoc lactis*, *Ln. mesenteroides* subsp. *cremoris*, *Leuconostoc lactis* + *Ln. mesenteroides* subsp. *cremoris*, *Lactobacillus plantarum*, *Brevibacillus brevis*, *Lactobacillus paraplantarum*, *Streptococcus salivarius* subsp. *thermophilus*.

pH

The pH was measured by Electronic digital type Hanna pH meter No. H 8416 according to method No. 981.12 of AOAC (2016) [11]

Results and Discussion

The pH of cow milk curd samples prepared by using different starter cultures and incubated at 37 °C and 40 °C and stored at 5 °C is presented in Tables 1 and 2 respectively. The initial pH of cow milk curd incubated at 37 °C and 40 °C on day 0 of storage ranged from 4.52 to 4.59 and 4.51 to 4.57, respectively. There was a decrease in the pH during the entire storage period. The pH of cow milk curd incubated at 37 °C and 40 °C on day 6 day ranged from 4.11 to 4.37 and 3.92 to 4.08, respectively. The pH was high in cow milk curd samples prepared using *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures on day 0 as well on day 6 of storage at refrigeration temperature. On storage at 5.00±0.5 °C, the pH of the cow milk curd sample prepared using the starter cultures *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures incubated at 37 °C was higher compared to *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures incubated at 40 °C.

The data pertaining to the pH of the cow milk curd samples prepared by inoculating different starter cultures at incubation temperature of 37º and 40 °C at refrigeration is presented in Table 1 and 2 respectively. Significant (p<0.01) difference between the control and treatment samples was observed. The *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* sample showed desirable pH during storage than other curd samples incubated at 37 °C and 40 °C of incubation.

Gandhi, *et al* 2022 [5]; Jaros and Rohm (2003) [7] analyzed that enhanced acidification to pH values below 4.0 may lead to body and texture defects such as gel shrinkage and syneresis. They recorded that the average pH value of laboratory made curd samples was 4.55 and average pH value curd samples from different local markets varied between 4.37, 4.17 and 4.10. In this study, few cow curd samples were rejected for further analysis. This is justified by Beena, 2010 [1]; Kroger, 1976 [9] who had stated that a weak coagulum is obtained above the pH of 4.5. For a lower pH, 4.5 the gel is generally firmer, presumably because the protein-protein bonds are stronger (Ray 2025]; Nishinari 2021; Walstra *et al*, 1999) [12, 10, 5].

The results were in accordance with Coetzer 2012[3] and Beyene *et al.* (1998) [2] who had reported that a total of 25 single strains of *Lactococcus lactis* cultures capable of reducing pH of milk to below 4.6 within 24hrs at 30 °C incubation at 1 per cent inoculation rate.

L. plantarum strains and *L. paraplantarum strains* grow poorly in milk without supplementation (Siezen *et al*., 2010; Zalan *et al.*, 2010; Corsetti *et al.*, 2011) [13, 15, 3], which suggest that *L. plantarum* could not efficiently use some milk ingredients such as casein. Kiran *et al*. (2012) [8] recorded that *Brevibacillus brevis* MMB 12 does not have the ability to ferment lactose and observed that the culture did not develop acidity.

Vannini and Siroli (2025) [14]; Holland and Liu (2011) [6] stated that *Leuconostocs* are mesophilic grows poorly in milk. *Leuconostocs* usually grow associatively with lactococci in milk, although strains of *Ln. lactis* can grow independently and produce acid in milk.

It is well known that curd cannot be kept for long at ordinary conditions. Therefore different methods are applied to restrict the growth of undesirable microorganisms. One of the valuable properties of starter culture is their ability to inhibit growth of undesirable microorganisms. The main preservative action of lactic starter culture is a result of acid production. Factors such as reduced water content, acidity and pH of the product ensure that milk solids can be stored for a prolonged time.

Table 1: pH of cow milk curd with various starter cultures incubated at 37 °C and stored at refrigeration temperature

Storage period in Days	Starter cultures							
	C1C	C1Ll	C1Lc	C1Ld	C1Ll+Ld	C1Lc+Ld	C1Ll+Lc+Ld	C1St
0	4.58^{dA}±0.10	4.56^{dA}±0.10	4.59^{dA}±0.10	4.57^{dA}±0.10	4.52^{eA}±0.10	4.56^{eA}±0.10	4.58^{dA}±0.10	4.53^{eA}±0.10
2	4.32^{bA}±0.10	4.31^{A}±0.10	4.31^{bA}±0.10	4.30^{bA}±0.10	4.21^{cA}±0.10	4.38^{bA}±0.10	4.43^{aA}±0.10	4.32^{bA}±0.10
4	4.32^{bcA}±0.10	4.31^{cA}±0.10	4.30^{bcA}±0.10	4.29^{bA}±0.10	4.21^{dA}±0.10	4.38^{bA}±0.10	4.41^{aA}±0.10	4.31^{cA}±0.10
6	4.29^{bA}±0.10	4.12^{cB}±0.10	4.28^{bA}±0.10	4.11^{cB}±0.10	3.95^{dB}±0.10	4.13^{cB}±0.10	4.37^{aA}±0.12	4.17^{bA}±0.10
8	4.27^{aA}±0.12	4.02^{bB}±0.10	4.26^{aA}±0.12	3.94^{cB}±0.10	3.93^{cB}±0.10	3.92^{cB}±0.10	4.21^{cAB}±0.10	3.89^{cB}±0.10
10	3.98^{aB}±0.10	3.87^{bC}±0.10	3.98^{aB}±0.00	3.78^{cC}±0.10	3.89^{aB}±0.10	3.88^{aB}±0.10	4.10^{aB}±0.10	3.76^{cB}±0.12

Mean ± Standard error values from six trials.

Mean values bearing different lowercase superscripts in a same row differ significantly (p<0.01)

Mean values bearing different uppercase superscripts in same column differed significantly (p<0.01)

Table 2: pH of cow milk curd with various starter cultures incubated at 40 °C and stored at refrigeration temperature

Storage period in Days	Starter cultures							
	C2C	C2Ll	C2Lc	C2Ld	C2Ll+Ld	C2Lc+Ld	C2Ll+Lc+Ld	C2St
0	4.57^{eA}±0.10	4.55^{eA}±0.10	4.52^{fA}±0.10	4.55^{eA}±0.10	4.51^{fA}±0.10	4.54^{eA}±0.10	4.58^{eA}±0.10	4.51^{fA}±0.10
2	4.28^{bA}±0.10	4.37^{aA}±0.10	4.30^{bA}±0.10	4.30^{bA}±0.10	4.30^{bA}±0.12	4.29^{bA}±0.10	4.41^{aA}±0.10	4.20^{cA}±0.10
4	4.11^{cA}±0.10	4.16^{cA}±0.10	4.26^{bA}±0.10	4.09^{cA}±0.10	4.01^{dA}±0.10	3.94^{eB}±0.10	4.29^{aA}±0.10	4.29^{aA}±0.10
6	4.08^{bA}±0.03	3.98^{cB}±0.10	4.09^{bA}±0.10	3.96^{cB}±0.10	3.94^{cB}±0.10	3.92^{dB}±0.10	4.17^{aA}±0.10	4.15^{aA}±0.10
8	4.01^{aB}±0.10	3.92^{bB}±0.10	4.06^{aA}±0.10	3.87^{cB}±0.10	3.89^{cB}±0.10	3.92^{bB}±0.10	3.99^{cB}±0.12	3.87^{cB}±0.10
10	3.77^{aB}±0.10	3.97^{aB}±0.10	3.96^{aB}±0.10	3.87^{aB}±0.10	3.87^{aB}±0.10	3.86^{aB}±0.10	3.97^{aB}±0.10	3.74^{aB}±0.10

Mean ± Standard error values from six trials.
Mean values bearing different lower case superscripts in a same row differ significantly (p<0.01)
Mean values bearing different upper case superscripts in a same column differed significantly (p<0.01)

Conclusion

The pH was high in cow milk curd samples prepared using *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures on day 0 as well on day 6 of storage at refrigeration temperature. On storage at 5.00±0.5 °C, the pH of the *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures curd sample incubated at 37 °C was higher compared *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures incubated at 40 °C. Thus based on this research, compared to other starter cultures cow milk curd samples prepared using *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures incubated at 37 °C can be kept under refrigeration temperature for about 6 days without any defects.

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