

Factors Governing Colour and Appearance of Dahi during Fermentation

Dr. Narendra Kumar

Associate professor,

Department of A.H. & Dairying, B. R. D. P.G. College, Deoria, U. P. 274001

Abstract

The study investigated the influence of milk type, starter cultures with different inoculation levels, incubation temperatures, and incubation periods on the colour and appearance of Dahi. Cow and buffalo milk were fermented using *Lactococcus lactis subsp. lactis* and *L. lactis biovar diacetylactis* at 1%, 2%, and 3% inoculum rates under controlled incubation conditions. Sensory evaluation using a nine-point hedonic scale revealed that all major factors significantly affected the visual attributes of Dahi. Buffalo milk, especially Bhadawari, produced superior colour and appearance compared to cow milk. The best results were obtained with *S. diacetylactis* at 3% inoculum and an incubation temperature of 30°C, yielding smooth, bright, and highly acceptable curd. Although several interactions were significant, the combined four-factor interaction was non-significant. The study highlights the importance of optimizing milk composition, culture level, and incubation conditions to achieve high-quality Dahi with desirable sensory characteristics.

Keywords: Dahi, milk type, starter culture, inoculation level, incubation temperature, incubation period, colour, appearance

Introduction

Dahi, one of the most widely consumed fermented milk products in India, plays a significant role in the daily diet due to its nutritional richness, improved digestibility, and desirable sensory attributes. Among these, colour and appearance are essential quality determinants, as they strongly influence consumer acceptance and perception of freshness and purity. Previous studies have emphasized that the visual characteristics of fermented milk products are affected by several technological and compositional factors operating during processing^[12]. The type of milk used is one of the foremost contributors to the final appearance of Dahi. Buffalo milk, owing to its higher fat, protein, and total solids, often produces a thicker, creamier curd with a brighter surface, while cow milk yields a comparatively softer and lighter-coloured product. Earlier research has shown that variations in milk composition, particularly fat and casein content significantly influence curd structure and opacity in fermented dairy products^[11].

Starter cultures also profoundly influence the physical and visual qualities of Dahi. Pure cultures such as *Lactococcus lactis subsp. lactis* and *Lactococcus lactis biovar diacetylactis* differ in their acidification patterns, production of flavor compounds, and ability to form smooth and uniform curd surfaces. According to the National

Dairy Research Institute (NDRI) reports, culture activity and inoculation levels directly affect coagulation behavior, which subsequently impacts colour uniformity and surface shine ^[7]. Higher inoculation levels promote rapid acid development, reducing whey separation and enhancing visual quality ^[5].

Incubation temperature and period are equally crucial in determining the appearance of Dahi. Optimum temperatures, typically ranging from 30°C to 37°C, ensure favorable metabolic activity of starter cultures and promote desirable curd formation. Studies have demonstrated that deviations in temperature can lead to uneven coagulation, surface cracks, or whey separation, all of which negatively affect colour and appearance. Similarly, incubation duration influences the extent of fermentation; inadequate or excessive incubation affects curd smoothness and brightness due to under over acidification.

Understanding the interplay among milk type, starter culture behavior, inoculation levels, and incubation conditions is therefore essential for optimizing Dahi production. Research-based insights into these factors support the formulation of standardized production protocols aimed at enhancing sensory attributes. The present study seeks to evaluate the individual and combined effects of these parameters on the colour and appearance of Dahi, contributing to improved product quality, consumer appeal, and industry-level consistency. However, keeping the above points in view the present study is proposed to assess the effect of types of milk, starter cultures with inoculation levels, incubation temperatures and incubation periods on the colour & appearance of Dahi with the following objectives:-

1. Effect of types of milk and starter cultures with inoculation levels on colour and appearance of Dahi.
2. Effect of incubation temperatures and incubation periods on colour and appearance of Dahi.

Materials and methods

During the course of this investigation cow and buffalo milk was collected from the dairy farm of C.S. Azad University of Agriculture and Technology, Kanpur. Twenty-seven liters of raw milk of the same breed, either cow or buffalo milk were selected to represent commonly available cow and buffalo breeds in India. These included Sahiwal cow milk (A₁), Sahiwal × Jersey crossbred cow milk (A₂), Sahiwal × Friesian cross bred cow milk (A₃), Bhadawari buffalo milk (A₄) and Murrah buffalo milk (A₅) from individual milch animals for optimization of different compositional and processing parameters in order to produce the product. 100 ml capacity cups were used for Dahi making during the study. Mother culture of *S. lactis* with 1% inoculum (B1), *S. lactis* with 2% inoculum (B2), *S. lactis* with 3% inoculum (B3), *S. diacetylactis* with 1% (B4), *S. diacetylactis* 2% inoculum (B5) and *S. diacetylactis* with 3% inoculum (B6) were used for Dahi making. Three incubation temperatures 25°C (C1), 30°C (C2) and 37°C (C3) with three incubation periods 8 h (D1), 10 h (D2) and 12 h (D3) were adopted. The average composition of different types of milk was tested before preparing the final product. 100 ml capacity of plastic cups was used for Dahi making during the investigation. The cups are manufactured by the century Ultra Pack, New Delhi- 110044 and Dairy Tops Division of KFF limited, Bombay, Pune- 26 with the trade names Vulga and Yankee Doodle, respectively. These were purchased from local market of Kanpur city.

Freeze dried pure cultures namely *Lactococcus lactis subsp. lactis* (*Streptococcus lactis*) NCDC- 91 and *Lactococcus lactis biovar diacetylactis* (*Streptococcus diacetylactis*) were obtained from National Collection Centre of Dairy Cultures, Dairy Microbiology Division, National Dairy Research Institute (N.D.R.I.), Karnal- 132001, Haryana.

Analytical grade chemicals were used for chemical analysis. The vials of pure cultures were opened under aseptic conditions and 0.5 ml of sterilized skim milk was added into ampoules. The content in the ampoule was mixed thoroughly and then transferred into test tubes containing 10 ml of sterilized skin milk and incubator at 30°C for 16 hours. Thus the cultures were grown in tubes to activate them and sub-cultured 3-4 times. For Dahi making, the cultures were inoculated 1% separately in 200 ml sterilized and cooled cow milk, which was subsequently incubated at 30°C for 16 hours to prepare mother culture. The mother culture was stored in refrigerator at 5 °C $\pm$ 2 °C and it was propagated at least once every fortnight. Sterilization of milk was done at 15 PSI for 15 minutes in an autoclave and all the glassware sterilized in an oven maintained at 118°C for at least 3 hours.

Preparation of samples for analysis was done as par the method prescribed in IS: 1479 (Part-II), 1961^[3] and Hand Book of Food Analysis (Part- XI), 1981^[2].

Preparation of Dahi

27 liters of raw milk of same breed either cow or buffalo was boiled for 3-5 minutes and cool down to 40 °C. The milk was divided into three batches in sterilized containers each batch was inoculated with inoculum at the rate of 1%, 2% or 3% starter culture either *S. lactis* or *S. diacitilactis* and distributed in 100 ml capacity of plastic cups which were free from any contamination. These plastic cups were divided into three batches. Each batch was allowed to incubate at 25°C for 8, 10 and 12 hours accordingly. The same process was followed in case of 30°C and 37°C incubation temperatures. After the prescribed duration the samples were transferred into refrigerator maintained at 4°C in the further testing. Dahi samples were served for judging to panel comprising of five most experienced members from the Department of A.H. and Dairying for its score evaluation by nine point Hedonic scale ^[6]. The scores of different judges were compiled and average score was estimated for each attribute of samples as detailed below:

Evaluation Card for Numerical Scoring Test for Quality Attributes in Dahi :

Quality Grade	Description Score
Excellent	More than 8-9
Very good	More than 7-8
Good	More than 6-7
Fair	More than 5-6
Poor	Less than 5

Results and discussion

The colour and appearance of Dahi was affected significantly at the 0.1% level of significance by different types of milk. The maximum liking score of 8.26 for Dahi prepared from Bhadawari buffalo milk (A4) agrees with earlier findings showing that buffalo milk, due to its higher fat, casein, and total solids, produces a more glossy and firm curd surface ^[1-13]. The minimum score (7.69) for Dahi prepared from Sahiwal $\times$ Friesian cow milk (A3) is consistent with research indicating that cow milk forms a softer and less opaque coagulum ^[5].

The overall mean values (7.73 for cow milk and 8.22 for buffalo milk) indicate that buffalo milk Dahi was graded as excellent. This pattern has been repeatedly observed in sensory evaluations of fermented buffalo milk products due to their higher viscosity, opacity, and smooth surface structure ^[10].

Regarding the effect of starter cultures with inoculation levels, a significant difference at the 0.1% level was observed. The maximum score (8.02) for Dahi prepared with *S. diacetylactis* and 3% inoculum (B6) aligns with scientific reports that *S. diacetylactis* strains enhance diacetyl production, which improves surface shine and colour uniformity ^[7-9]. The minimum score (7.83) at 1% inoculum (B1) reflects insufficient acidification, which leads to weak curd formation and slight whey visibility, reducing appearance quality ^[8].

Table 1. Means of colour and appearance score of Dahi.

Factors		Level of means					S.E.(d)	C.D. at 5% (p)	
	A ₁	A ₂	A ₃		A ₄	A ₅			
A	7.71 ^d	7.77 ^c	7.69 ^c		8.26 ^a	8.17 ^b		0.005	0.010
Cow	7.73 ^b		Buffalo			8.22 ^a		0.003	0.006
	B ₁		B ₂	B ₃	B ₄	B ₅	B ₆		
B	7.83 ^f		7.85 ^c	7.90 ^d	7.95 ^c	7.98 ^b	8.02 ^a	0.005	0.011
S ₁	7.86 ^b		S ₂		7.98 ^a			0.003	0.006
	I ₁	I ₂		I ₃					
	7.89 ^c	7.91 ^b		7.96 ^a				0.004	0.007
	C ₁		C ₂		C ₃				
C	8.52 ^b		8.59 ^a		6.66 ^c			0.004	0.007
	D ₁		D ₂		D ₃				
D	7.90 ^c		7.93 ^b		7.94 ^a			0.004	0.007

NOTE: Figures bearing the same superscript letters within a factor do not differ significantly.

Similarly, the overall effects of selective cultures S1 and S2 (mean values 7.86 and 7.98) clearly show that both cultures produced “very good” Dahi. The slightly better performance of *S. diacetylactis* (S2) is supported by studies showing its superior flavour and curd texture development.

The effect of inoculation levels (I1, I2, I3) on colour and appearance showed increasing scores with increasing inoculum (7.89, 7.91, 7.96). Higher inoculum stimulates rapid acid development, resulting in uniform curd opacity and reduced whey separation, as noted in recent starter culture optimization studies ^[4]. The colour and appearance of Dahi were also significantly affected by incubation temperature. The maximum liking (8.59) at 30°C (C2) agrees with the widely established optimum temperature for *Lactococcus* species growth and stable gel

formation^[12]. In contrast, the minimum score (6.66) at 37°C (C3) corresponds with literature indicating that higher temperatures accelerate protein denaturation and whey expulsion, which negatively affects appearance^[11].

Table 2: Analysis of variance for colour and appearance

Source	D.F.	S.S.	M.S.S.	F	Significant
Treatment	269	730.73146	2.71649	1250.9061	***
A	4	48.03826	12.00957	5530.2717	***
B	5	3.79833	0.75967	349.8175	***
C	2	647.36614	323.68307	149052.4660	***
D	2	0.15846	0.07923	36.4845	***
A×B	20	0.13270	0.00663	3.0553	***
A×C	8	1.31198	0.16400	75.5193	***
A×D	8	0.02510	0.00314	1.4448	N.S.
B×C	10	16.66388	1.66639	767.3533	***
B×D	10	0.21493	0.02149	9.8972	***
C×D	4	12.22461	3.05615	1407.3242	***
A×B×C	40	0.14259	0.00356	1.6415	*
A×B×D	40	0.13833	0.00346	1.5925	*
A×C×D	16	0.05910	0.00369	1.7011	*
B×C×D	20	0.32363	0.01618	7.4515	***
A×B×C×D	80	0.13341	0.00167	0.7679	N.S.
Error	540	1.17267	0.00217	—	—

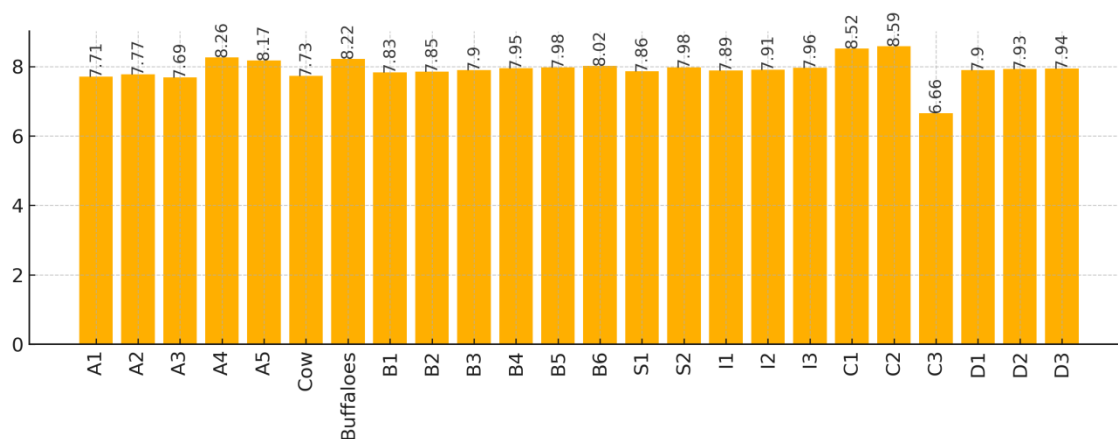
Notation: N.S. = Non-significant at 5% level of significance. * = Significant at 5% level of significance. *** = Significant at 0.1% level of significance

Incubation period significantly influenced sensory acceptability. Mean values 7.90 (D1), 7.93 (D2), and 7.94 (D3) show a gradual improvement with increasing incubation time. Longer incubation promotes more complete acidification, resulting in brighter and more uniform curd formation, as supported by controlled fermentation studies [8]. However, excessively long periods can lead to over-acidification and reduced acceptability, as also recorded in related studies^[9].

The interaction effects (A×B, A×C, C×D, B×C, B×D) were significant at the 0.1% level, indicating that milk type, culture behavior, temperature, and inoculation levels influence each other's performance, matching observations in multi-factor dairy fermentation analyses^[1]. However, the combined four-factor interaction A×B×C×D was non-significant at 5%, meaning that although individual and pair wise combinations affect appearance, the total combined effect does not produce additional complexity consistent with multifactor ANOVA patterns reported in dairy science research^[9].

The best treatment combination (A4 B6 C2 D3) recorded the highest score (8.35) and was graded excellent. This match reports that buffalo milk + *diacetylactis* culture + higher inoculum + 30°C incubation creates ideal fermentation kinetics and optimizes visual attributes ^[9-10].

The lowest score (4.90) obtained at combination (A3 B3 C3 D3) corresponds with documented issues of crossbred cow milk and elevated incubation temperature leading to coarse gel structure and whey separation, negatively affecting appearance ^[5].



It is clear from analysis of variance table- 2, that all the main effects and the interactions of first order were significant at 0. 1% level of significance except the interaction of AXD, Dahi was non-significant at 5% level of significance Interaction of the second order were significant at 5% level of significance only except the interactions of AXCXD, Dahi were significant at 0.1% level of significance. The interactions of third order were non- significant at 5% level of significance. The highly significant interaction effects indicated that the effect of one factor change the significantly with the change in the level of the other factor , non- significant interaction effects indicated that the effect of one factor changed non- significantly with the change in the level of the other factor.

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