

# Comparative evaluation of sensory properties and viscosity in fruit, cereal and millet based probiotic *Lassi*

H A Sojitra<sup>1</sup>, A M Patel<sup>2</sup>(✉), Smitha Balakrishnan<sup>3</sup> and J M Mallik<sup>1</sup>

Received: 25 July 2025 / Accepted: 11 October 2025 / Published online: 23 February 2026

© Indian Dairy Association (India) 2026

**Abstract:** A probiotic *lassi* was developed by incorporating *dahi* and Fruit, Cereal and Millet blend (FCM). To determine the optimal formulation levels of *dahi* and FCM blend, response surface methodology (RSM) was employed. The optimization process was based on sensory characteristics such as colour and appearance, flavour, mouthfeel/consistency, sweetness and overall acceptability—as well as viscosity of the product. RSM optimization yielded ideal proportions of 71.07% *dahi* and 9.07% FCM blend. The formulation prepared using these optimized values was compared to a control probiotic *lassi*. Both samples exhibited statistically significant difference in composition in most aspects. Additionally, the experimental probiotic *lassi* had notably higher titratable acidity (% LA), viscosity, tyrosine values, degree of hydrolysis, ACE inhibitory activity, DPPH activity and ABTS activity. Sensory evaluations revealed that the FCM based probiotic *lassi* outperformed the control in overall acceptability. Experimental product also showed significantly higher mineral enrichment than control. From a microbiological perspective, both variants remained free from coliforms and yeast and moulds, indicating good microbial quality, while fulfilling the criteria for probiotic count.

**Keywords:** Cereal and Millet based probiotic *lassi*, *dahi*, Fruit, Probiotic

<sup>1</sup>Department of Dairy Technology, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

<sup>2</sup>Department of Dairy Processing and Operations, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

<sup>3</sup>Department of Dairy Chemistry, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

AM Patel(✉)

Department of Dairy Processing and Operations, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

Email: [amitmpatel@kamdhenuuni.edu.in](mailto:amitmpatel@kamdhenuuni.edu.in)

Mobile #: +91 93761 32364

## Introduction

*Lassi* is a traditional fermented dairy beverage characterized by its viscous consistency, creamy-white appearance, and distinct sweetish aroma with mild to pronounced acidic notes. Its flavour profile varies regionally, with common additions including salt, sugar, or other flavoring agents to suit local preferences. According to FSSAI (2023), *lassi* is classified as a composite product under fermented milk, prepared by mixing fermented milk with potable water, with or without the inclusion of whey, other milk-derived ingredients, approved non-dairy components, and flavours. Fermented milk-based beverages must contain at least 40 per cent (m/m) fermented milk. Additionally, microorganisms beyond the standard starter cultures may also be incorporated.

Fermented dairy products are considered the most effective medium for delivering live probiotics (Andrade et al. 2012). According to the FAO/WHO (2002), probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Mangoes are rich in essential nutrients and phytochemicals. They provide a significant amount of prebiotic dietary fiber, vitamin C, various polyphenolic compounds, and provitamin A carotenoids (Ajila and Rao, 2008). Oats are notable for their abundant soluble dietary fiber, particularly  $\beta$ -glucan, and also contain significant levels of phenolic compounds and niacin (Ramzan, 2020). Oat-based products are known to lower cholesterol levels in both individuals with normal cholesterol and those with high cholesterol. They lead to a notable decrease in Low Density Lipoprotein (LDL) levels (Truswell, 2002). LDL is often referred to as bad cholesterol because it can travel through the bloodstream and adhere to artery walls, potentially causing blockages that impede blood flow. Millets are widely recognized for their abundance of bioactive compounds and their promising functional properties. Among different millet varieties, *kodo* millet has been reported to possess the highest antioxidant potential (Deshpande et al. 2015).

The current study focused on developing the product containing combination of traditional ingredients like (*Kodo* millet & Oats) and added probiotics to meets the growing demand for functional foods.

The primary objective is to determine the optimal levels of *dahi* and FCM blend using response surface methodology (RSM) to enhance sensory attributes while also improving the viscosity and nutritional content of the probiotic *lassi*. *Lassi*, a traditional fermented dairy drink, serves as an excellent vehicle for the delivery of probiotics. Integrating the nutritional richness of fruits, cereals, and millets into *lassi* can enhance its functional value and consumer acceptance. By utilizing *dahi* and FCM blend, this study aims to formulate a value-added *lassi* incorporating mango, *kodo* millet, and oats, along with probiotic cultures, to enhance both nutritional quality and digestive health.

## Materials and Methods

The FCM based probiotic *lassi* was formulated using toned milk (3.0% fat & 8.5% SNF) for *dahi* from Vidya dairy & FCM blend from Fruit formulation Pvt. Ltd. Non-dairy ingredients included a starter culture (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *Bulgaricus* and *Bifidobacterium bifidum* (probiotic) from DSM Firmenich, Netherland; crystalline sugar from local market and high methoxy pectin from LOBA chemie. The probiotic *lassi* was packed in polyethylene terephthalate (200 mL) with of 650-micron thickness purchased from an authorized dealer.

### Preparation of *dahi*

For the preparation of *dahi*, toned milk was heated to 90! for 15 minutes. Milk was cooled to 42±2! followed by inoculation with Direct-to-Vat Starter (DVS) culture (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Bifidobacterium bifidum*) and incubation at that temperature for 3-4 h (or until acidity of 0.7% lactic acid was obtained). *Dahi* was then immediately cooled to refrigeration temperature to arrest the development of acidity. After cooling for 1-hour *dahi* was ready to use for preparation of *lassi*.

### Preparation of FCM Base

Calculated amount of water (by difference of total composition) was taken and heated to 60°C. Then, all the dry ingredients (sugar and pectin) were added. Then FCM blend was added to it and the mixture was heated to 85°C. This preparation was then cooled to 20!.

### Preparation of FCM based probiotic *lassi*

Both, *dahi* and FCM base was blended with the help of hand blender to form *lassi*. Prepared *lassi* was packed in polyethylene terephthalate (PET) bottles (capacity: 200mL) in such a way to maintain minimum air in the head space and stored at refrigeration temperature of 7±2°C.

### Analysis of FCM based probiotic *lassi*

Toned milk was analyzed for total solids, fat, protein, ash and titratable acidity. Titratable acidity was determined by method IS: SP-18, Part XI, 1981. Fat content and total solids was analyzed using FSSAI (2022). Protein was determined by the Block Digestion/Steam Distillation method (FSSAI, 2022). Ash content was analyzed using IS 1479, Part-II-1961 method. *Dahi* was analyzed for titratable acidity, total solids, fat and protein using the method given in FSSAI (2022) and ash using method IS 1479, Part-II-1961. FCM based probiotic *lassi* was analyzed for total solids, fat and ash as per method for *dahi* and protein was estimated as per method for milk. Carbohydrate was calculated by difference. The titratable acidity was measured as per method of *dahi*, water activity was measured with a water activity meter and viscosity using brookfield viscometer. Quantification of selected minerals, Calcium and magnesium was estimated as per (BS EN 16943: 2017); sodium and potassium were estimated as per (ISO 8070: 1987 E) and phosphorus using Vanado-molybdate method (Hanson, 1950). ACE-inhibitory activity was estimated according to the method described by Cushman and Cheung (1971) based on the reaction of hydrolysis of N-Hippuryl-His-Leu (HHL) into hippuric acid (HA) and His-Leu (HL) catalysed by the ACE. Total phenolics content was estimated as per Singleton and Rossi (1965). The degree of hydrolysis was measured according to Kumar et al. (2016). Protein breakdown, specifically in terms of tyrosine content, was evaluated using the procedure given by Hull (1947). The antioxidant activity of FCM based probiotic *lassi* was estimated by ABTS radical scavenging activity as per Re et al. (1999) and DPPH radical scavenging activity as per McCune and Johns (2002). *Lactobacillus delbrueckii* ssp *bulgaricus* count and *streptococcus thermophilus* count was estimated by APHA, 2004. *Bifidobacterium bifidum* (probiotic) count was estimated by ISO 29981/IDF 220 (2010). Coliform and yeast and mould count was measured as per IS:5401, Part I, 2012 and IS:5403, 1999 respectively.

### Sensory evaluation of FCM based probiotic *lassi*

The sensory evaluation of FCM based probiotic *lassi* was done by panel of 7 judges using a 9-point hedonic scale. The panellist included scientists, technical officers/assistants and students of the institute. Each panellist was asked to taste the samples and evaluate the sensory parameters on a 9-point hedonic scale. They were asked a series of questions pertaining to colour and appearance, flavour, mouthfeel/consistency, sweetness and overall acceptability of each sample. Panellists were requested to give the scores and comments on a sensory evaluation score card. Saline water was provided to rinse the palate before and after tasting the sample. Sensory responses were evaluated based on a 9-point hedonic scale (Meilgaard et al. 1999). Mean score was calculated from the responses of panellists for each set of samples.

## Statistical analysis

Design Expert software is to be used for all statistical work including the selection of the number of trials, range of parameters to be studied, number of replications and final analysis of the data generated. Two elements, to prepare probiotic *lassi*, the rate of *dahi* and FCM blend was optimized using the Face Centred Composite Rotatable Design of Response Surface Methodology (RSM). The experiment was conducted using various combination of treatment with some range of the parameters under study for manufacture of an acceptable quality of FCM based probiotic *lassi*. On the basis of preliminary trials, the range of variables was obtained. RSM with faced centred rotatable design (FCRD) for two variables at five levels and six replicates at central point was adopted to optimize the quality of FCM based probiotic *lassi* with respect to selected sensory responses. The result of the 13 trials (Table 1) formed the base for an optimized level *dahi* and FCM blend which were suggested by software. The optimized level of ingredients was then replicated seven times and actual values of sensory analysis was compared with predicated value. The p-value is a statistical measure that shows the probability of obtaining the observed results if the null hypothesis is true. It helps to determine whether the differences seen in the data are statistically meaningful or not. The p-value is calculated from the test statistic using the sample data and its distribution. A value of  $p < 0.05$  is generally considered statistically significant. In contrast, a higher p-value suggests that the observed differences may be due to random variation. Statistically significant data are shown as ( $p < 0.05$ ).

## Results and Discussion

The optimization process for *dahi* and FCM blend was carried out by evaluating sensory parameters such as colour and appearance, flavour, mouthfeel/consistency, sweetness and overall acceptability, along with physical characteristic like

viscosity. Regression analysis resulted in quadratic models for each parameter, with coefficients of determination ( $R^2$ ) ranging from 0.80 to 0.91 (Table 2), indicating good model fit. The model F-values were statistically significant, and the non-significant lack of fit validated the suitability of these models for predicting responses across the tested range. Furthermore, the models showed Adequate Precision Values (APVs) between 7.92 and 13.30, all well above the acceptable limit of 4, affirming that the models had a strong signal-to-noise ratio and were robust for optimizing the formulation of the FCM based probiotic *lassi*.

### Effect on colour and appearance scores

The colour and appearance of *lassi*, including its hue, uniformity, and texture, significantly influence consumer perception and initial acceptability. These visual attributes serve as primary indicators of quality about freshness and taste. In FCM based probiotic *lassi*, the colour and appearance scored ranged from 7.83 to 8.67 on a 9-point hedonic scale (Table 1). The formulation with 70% *dahi* and 10% FCM blend received the highest panelist score, while the one with 70% *dahi* and 5% FCM blend scored the lowest. A high  $R^2$  value of 0.82 and an APV of 8.00 (above the threshold of 4.00) indicate a strong model fit and adequate signal, supported by a significant model F-value of 6.33. The colour and appearance of the product were positively influenced by both *dahi* and FCM blend ( $p < 0.05$ ) at linear level. However, the interactive effect and quadratic effects of FCM blend negatively impacted the response. Mital (2023) demonstrated that pomegranate-beetroot preparation had significant positive effect on the colour & appearance score ( $p \leq 0.05$ ) at linear term of *lassi*. Hussain (2013) noted that milk fat and aloe vera juice greatly influenced the colour and appearance score of the APL (Aloe vera supplemented probiotic *lassi*).

### Effect on flavour scores

Table 1: Experimental design matrix, sensory attributes & viscosity of FCM based probiotic *lassi*

Run No.	<i>Dahi</i>	FCM	Sensory scores				Overall Acceptability	Viscosity @ 20°C (cP)
			Colour & Appearance	Flavour	Mouthfeel/ Consistency	Sweetness		
1	68.00	10.00	8.65	8.29	8.49	8.28	8.33	342
2	70.00	10.00	8.67	8.55	8.67	8.43	8.58	527
3	70.00	7.50	8.21	8.24	8.25	8.39	8.29	445
4	70.00	7.50	8.54	8.25	8.62	8.29	8.31	446
5	70.00	7.50	8.31	8.27	8.37	8.26	8.26	424
6	68.00	7.50	8.50	8.11	8.33	8.13	8.42	308
7	68.00	5.00	7.93	7.86	7.79	7.79	7.82	271
8	70.00	7.50	8.41	8.29	8.58	8.33	8.29	460
9	70.00	7.50	8.10	7.85	8.33	8.27	8.24	410
10	72.00	10.00	8.64	8.68	8.61	8.39	8.68	560
11	72.00	7.50	8.50	8.38	8.39	8.38	8.46	540
12	70.00	5.00	7.83	7.94	7.83	8.01	7.85	517
13	72.00	5.00	8.30	8.10	8.30	8.50	8.40	384

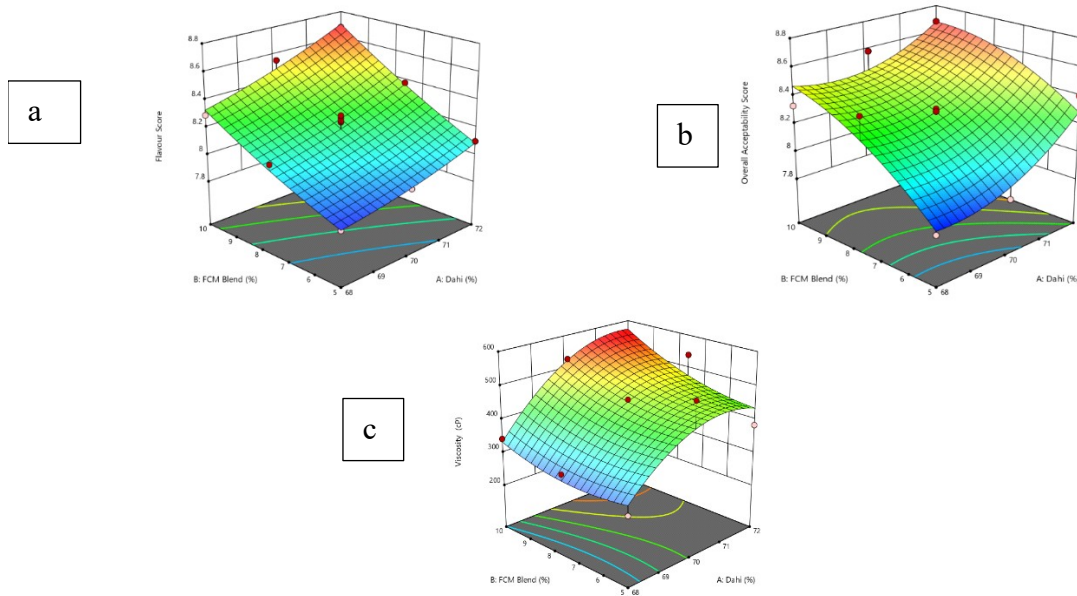


Fig. 1 Response surface plots as influenced by different variables of FCM based probiotic *lassi*. a: Flavour, b: Overall acceptability, c: Viscosity (cP)

**Table 2:** Partial coefficients of regression equations of suggested models for sensory attributes and viscosity of FCM based probiotic *lassi*

Terms	Colour & appearance	Sensory scores (9-point hedonics scale)			Overall Acceptability	Viscosity (cP)
		Flavour	Mouthfeel/ Consistency	Sweetness		
Intercept	8.32	8.19	8.41	8.30	8.29	453.52
A: <i>Dahi</i>	0.06	0.15*	0.12	0.18*	0.16*	93.83*
B: FCM blend	0.32*	0.27*	0.31*	0.13*	0.25*	42.83
AB	- 0.10	0.04	- 0.10	- 0.15*	- 0.06	26.25
A <sup>2</sup>	0.16	0.03	- 0.01	- 0.02	0.13	- 70.81*
B <sup>2</sup>	- 0.09	0.03	- 0.12	- 0.05	- 0.10	27.19
R <sup>2</sup>	0.82	0.80	0.81	0.91	0.85	0.82
Model F-Value	6.33	5.60	5.99	13.61	8.18	6.54
APV	8.00	8.56	7.92	13.30	10.02	8.42
Suggested Model	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic

\*:  $p < 0.05$ ; APV= Adequate Precision Value,  $R^2$  = Coefficient of determination

Flavour represents a critical sensory attribute that determines product acceptability through its complex interplay of taste, aroma, and mouthfeel. The flavour score of FCM based probiotic *lassi* were observed in the range of 7.85 to 8.68 on a 9-point hedonic scale. The formulation with 72% *dahi* and 10% FCM blend scored highest, while the one with 70% *dahi* and 7.5% FCM blend scored lowest among panelists. The coefficient of determination ( $R^2$ ) of 0.80 indicates a strong model fit, while an APV of 8.00 (above the desired threshold of 4.00) confirms the model's adequacy for design navigation. The model F-value of

5.60 further supports its statistical significance. *Dahi* and FCM blend at linear level contributed positively, enhancing the flavour profile ( $p < 0.05$ ). The interactive effect and quadratic effect had positive but negligible impacts. Hussain (2013) noted milk fat level showed an enhancing effect on flavour score, whereas Aloe vera juice showed a negative effect. Interaction effect of milk fat and MSNF (milk solids-not-fat) positively affected the flavour. Whereas, interaction effect of MSNF with Aloe vera juice negatively affected the flavour. Mital (2023) demonstrated that both *dahi* and pomegranate-beetroot preparation had significant

effect on the flavour score at linear term ( $p < 0.05$ ), while at quadratic level also *dahi* has significant effect on flavour scores ( $p < 0.05$ ) of *lassi*.

**Effect on mouthfeel/consistency scores**

Mouthfeel encompasses the tactile sensations experienced during consumption, determined by a product’s viscosity, texture, and structural properties. The mouthfeel/consistency score of *lassi* samples were observed in the range of 7.79 to 8.67 on a 9-point hedonic scale. The formulation with 70% *dahi* and 10% FCM blend received the highest panelist score, while the one with 70% *dahi* and 5% FCM blend received the lowest. An  $R^2$  value of 0.81 indicates a strong model fit, while an APV of 7.92 (above the threshold of 4.00) confirms its adequacy for design navigation. The model F-value of 5.99 further supports its statistical significance. Mouthfeel/consistency were significantly enhanced by FCM blend, highlighting its role in improving the thickness and creaminess of the product ( $p < 0.05$ ). While *dahi* had a minimal positive effect. The interactive effect and quadratic effect of *dahi* and FCM level had minimal negative effect. Hussain (2013) demonstrated that Milk fat level showed an enhancing effect on consistency, whereas Aloe vera juice showed a negative

effect. Interaction effect of milk fat and MSNF positively affected the consistency. Whereas, interaction effect of MSNF with Aloe vera juice negatively affected the consistency. Mital (2023) noted that both *dahi* and pomegranate-beetroot preparation had significant positive effect on *lassi* on mouthfeel/consistency score at linear term ( $p < 0.05$ ) and at quadratic level also both has significant positive effect.

**Effect on sweetness scores**

In fermented dairy products like *lassi*, balanced sweetness is essential for masking acidity while enhancing overall palatability. The sweetness score of *lassi* samples were observed in the range of 7.79 to 8.50 on a 9-point hedonic scale. The formulation with 72% *dahi* and 5% FCM blend received the highest panelist score, while the one with 68% *dahi* and 5% FCM blend received the lowest. An  $R^2$  value of 0.91 indicates a strong model fit, while an APV of 13.30 (well above the threshold of 4.00) confirms the model’s suitability for design navigation. The model F-value of 13.61 further supports its statistical reliability. *Dahi* and FCM blend at linear level contributed positively, enhancing the sweetness score ( $p < 0.05$ ). While the interaction term contributed negatively to the sweetness score ( $p < 0.05$ ). The quadratic terms

**Table 3:** Criteria/responses chosen for optimization of FCM based probiotic *lassi*

Sr No.	Parameter	Units	Goal	Lower Limit	Upper Limit	Level of Importance
1.	A: <i>Dahi</i>	%	In range	68	72	3
2.	B: FCM blend	%	In range	5	10	3
3.	Colour & appearance	Out of 9	In range	7.83	8.67	3
4.	Flavour	Out of 9	In range	7.85	8.68	5
5.	Mouthfeel/Consistency	Out of 9	In range	7.79	8.67	3
6.	Sweetness	Out of 9	In range	7.79	8.50	3
7.	Overall acceptability	Out of 9	In range	7.82	8.68	4
8.	Viscosity	cP	In range	271	560	3

**Table 4:** Comparison of predicted v/s actual values of responses used for optimization of FCM based probiotic *lassi*

Response	P Value	Predicted Value *	Actual Value @	Cal. t-Value#	Level of Significance
Colour & Appearance	0.34	8.53	8.61 ± 0.20	1.04	NS
Flavour	0.07	8.47	8.57 ± 0.12	2.20	NS
Mouthfeel/Consistency	0.31	8.58	8.68 ± 0.24	1.10	NS
Sweetness	0.60	8.40	8.46 ± 0.30	0.56	NS
Overall Acceptability	0.24	8.51	8.61 ± 0.20	1.31	NS
Viscosity	0.17	530.27	533.00 ± 4.69	1.54	NS

\* Predicted values of Design Expert 13.0.1.0 package

@ Actual values are average of seven trials for optimized product

# t-values at 5 per cent level of significance

NS = non-significant

Tabulated t-value = 2.447 (cal. t-value less than tabulated value)

have minimal negative effect. Mital (2023) demonstrated that both *dahi* and pomegranate-beetroot preparation had significant effect on the sweetness score of *lassi* at linear level ( $p < 0.05$ ).

### Effect on overall acceptability scores

Overall acceptability represents the composite sensory evaluation of a product, integrating key attributes like flavour, mouthfeel/consistency, colour and appearance, and sweetness into a holistic quality assessment. The overall acceptability score of *lassi* samples were observed in the range of 7.82 to 8.68 on a 9-point hedonic scale. The formulation with 72% *dahi* and 10% FCM blend received the highest score from the panelists, while the one with 68% *dahi* and 5% FCM blend scored the lowest. An  $R^2$  value of 0.85 indicates a strong model fit, while an APV of 10.02 (above the threshold of 4.00) supports its suitability for design navigation. The model F-value of 8.18 further confirms its statistical significance. Overall acceptability was positively influenced by *dahi* and FCM blend indicating their contribution towards increased overall acceptability scores ( $p < 0.05$ ). While interaction term showed a minimal negative effect. At quadratic level *dahi* showed minimal positive effect and FCM blend showed

minimal negative effect. Hussain (2013) noted that milk fat level showed an enhancing effect on overall acceptability score, whereas Aloe vera juice showed a negative effect. Interaction effect of milk fat and MSNF positively affected the flavour. Whereas, interaction effect of MSNF with Aloe vera juice negatively affected the overall acceptability. Mital (2023) demonstrated that both *dahi* and pomegranate-beetroot preparation had significant effect on the overall acceptability score of *lassi* at linear level ( $p < 0.05$ ) while, pomegranate-beetroot preparation had significant negative effect ( $p < 0.1$ ).

### Effect on viscosity

Viscosity quantifies a fluid's resistance to flow, measured in centipoise (cP) or mPa·s. The viscosity score of *lassi* samples were observed in the range of 560 cP to 271 cP. The formulation with 72% *dahi* and 10% FCM blend achieved the highest panelist score, whereas the one with 68% *dahi* and 5% FCM blend received the lowest. An  $R^2$  value of 0.82 indicates a strong model fit, while an APV of 8.42 (above the threshold of 4.00) supports its adequacy for design navigation. The model F-value of 6.54 further confirms its statistical significance. Viscosity was significantly enhanced

**Table 5:** Comparison of FCM based probiotic *lassi* with control *lassi* (n=5)

Parameter	Control <i>lassi</i>	FCM based probiotic <i>lassi</i>	CD (0.05)
Chemical composition			
Total solids, %	18.63 ± 0.02	23.05 ± 0.04	0.05
Fat, %	2.33 ± 0.01	2.45 ± 0.03	0.02
Protein, %	2.42 ± 0.01	2.96 ± 0.05	0.02
Ash, %	0.57 ± 0.01	0.63 ± 0.01	0.01
Carbohydrates, %	13.30 ± 0.03	17.00 ± 0.04	0.05
Physico-chemical properties			
Acidity (% LA)	0.61 ± 0.005	0.64 ± 0.005	0.01
Water activity	0.971 ± 0.002	0.969 ± 0.001	NS
Viscosity (cP)	331.00 ± 1.00	538.00 ± 2.55	2.82
Sensory attributes			
Colour & appearance	8.42 ± 0.12	8.83 ± 0.16	0.20
Flavour	8.36 ± 0.10	8.83 ± 0.09	0.13
Mouthfeel/ Consistency	8.25 ± 0.14	8.76 ± 0.07	0.14
Sweetness	8.37 ± 0.19	8.63 ± 0.13	0.23
Overall acceptability	8.38 ± 0.25	8.75 ± 0.08	0.27
Biofunctional properties			
Total phenolic content (µg GAE/ g)	23.48 ± 0.55	40.34 ± 0.61	0.95
Tyrosine content (µg tyrosine/5ml filtrate)	13.56 ± 0.28	18.93 ± 0.11	0.49
Degree of hydrolysis (%)	1.59 ± 0.005	1.80 ± 0.021	0.04
ACE inhibitory (%)	6.21 ± 0.31	12.35 ± 0.35	0.48
ABTS (µM TE/ mg protein)	83.45 ± 2.88	215.54 ± 3.08	6.76
DPPH (% inhibition)	5.74 ± 0.10	15.60 ± 0.15	0.29
Microbial analysis			
<i>L. bulgaricus</i> (log <sub>10</sub> cfu/mL)	8.96 ± 0.04	8.97 ± 0.07	NS
<i>S. thermophilus</i> (log <sub>10</sub> cfu/mL)	9.09 ± 0.01	9.11 ± 0.03	NS
<i>B. bifidum</i> (log <sub>10</sub> cfu/mL)	8.03 ± 0.012	8.15 ± 0.004	0.013
Coliform	Absent/mL		
Yeast and mold	Absent/mL		

**Table 6:** Comparison of mineral content in FCM based probiotic *lassi* and control *lassi* (n=5)

Parameter	Control <i>lassi</i>	FCM based probiotic <i>lassi</i>	CD (0.05)
Mineral Quantification			
Calcium	92.34 ± 5.21	101.81 ± 2.94	6.17
Magnesium	7.35 ± 0.64	11.14 ± 0.11	0.69
Sodium	21.48 ± 0.96	23.53 ± 0.87	1.33
Potassium	68.65 ± 5.09	77.02 ± 2.95	6.07
Phosphorus	87.6 ± 0.97	99.31 ± 3.60	3.85

by *dahi* at linear level, highlighting its role in improving the thickness and creaminess of the product ( $p < 0.05$ ). While FCM blend at linear level had a minimal positive effect. The interaction term and quadratic terms of FCM blend level indicated that minimal positive effect towards viscosity score. While at quadratic level of *dahi* gave significant negative effect ( $p < 0.05$ ). Hussain (2013) demonstrated that Milk fat and MSNF levels had beneficial role in increasing viscosity, whereas Aloe vera juice had a negative effect. Interaction effect of milk fat and MSNF positively affected the viscosity. Whereas, interaction effect of MSNF with Aloe vera juice negatively affected the viscosity. Mital (2023) noted that *dahi* and pomegranate-beetroot preparation had significant positive effect ( $p < 0.05$ ) on viscosity at linear term while, at quadratic level *dahi* had a significant negative effect on viscosity ( $p < 0.05$ ).

#### Optimization of product formulation for FCM based probiotic *lassi*

The optimization of the process for the manufacture of FCM based probiotic *lassi* was carried out with the objective of determining the best proportion of *dahi* and FCM blend that would lead to the most acceptable product in terms of sensory attributes and viscosity. The goals that were set for obtaining the best combination are illustrated in Table 3. The goal was to optimize various parameters including *dahi* and FCM blend percentages while maximizing sensory attributes. In the optimization process, *dahi* and FCM blend were maintained within their specified ranges (68-72% and 5-10% respectively). The sensory attributes and viscosity were also kept in range. The flavour and overall acceptability were given higher importance. The RSM suggested optimal levels of 71.07 per cent *dahi* and 9.07 per cent FCM blend, achieving a desirability of 1.00. FCM based probiotic *lassi* was prepared by adding *dahi* and FCM blend as suggested by RSM. The predicted values for colour and appearance, flavour, mouthfeel/consistency, sweetness, overall acceptability and viscosity were 8.53, 8.47, 8.58, 8.40, 8.51 and 530.27 cP respectively. The observed values for these parameters were not significantly different from the predicted values (Table 4), confirming that the selected levels of *dahi* and FCM blend are optimal for achieving desirable sensory and viscosity in the FCM based probiotic *lassi*.

#### Analysis of FCM based probiotic *lassi*

The FCM based probiotic *lassi* ( $T_2$ ) was analysed and compared with the control *lassi* ( $T_1$ ) for its proximate composition, physico-chemical properties sensory attributes, biofunctional properties and mineral quantification, with results statistically analysed using a completely randomized design (CRD) as shown in Table 5 and Table 6.  $T_2$  exhibited slightly higher fat content than  $T_1$ . Total solids were significantly higher in  $T_2$  compared to  $T_1$ . Protein content also increased in  $T_2$  over  $T_1$ , highlighting nutritional enhancement. Ash content was marginally higher in  $T_2$  than  $T_1$ , while total carbohydrates were notably higher in  $T_2$  compared to  $T_1$ . Sensory evaluation indicated that the FCM-based probiotic *lassi* ( $T_2$ ) was significantly preferred over the control ( $T_1$ ) across various attributes.  $T_2$  scored higher for colour and appearance, mouthfeel/consistency, sweetness and flavour compared to  $T_1$ . Overall acceptability was also significantly higher in  $T_2$ . All differences were statistically significant ( $p < 0.05$ ). Titratable acidity and viscosity were significantly higher in  $T_2$  than in  $T_1$  whereas water activity ( $a_w$ ) showed no significant difference between  $T_1$  and  $T_2$ . The total phenolic content and angiotensin-converting enzyme (ACE) inhibitory activity was significantly higher in the FCM-based probiotic *lassi* ( $T_2$ ), compared to the control *lassi* ( $T_1$ ). FCM based probiotic *lassi* showed significantly higher degree of hydrolysis, tyrosine content, DPPH activity and ABTS activity compared to control *lassi*. Mineral quantification also showed that  $T_2$  exhibited significantly higher levels of selected minerals compared to  $T_1$ . *Lactobacillus delbruekii ssp bulgaricus* count and *streptococcus thermophilus* count was comparable in both. *B. bifidum* (probiotic) count was significantly higher in  $T_2$  than  $T_1$  may be attributed to the presence of high nutritional ingredients like oats and *kodo* millet in the FCM blend. Microbiologically, both the experimental and control samples were free from coliform count and yeast and mould count.

#### Conclusions

A fruit, cereal and millet (FCM)-based probiotic *lassi* was optimized using Response Surface Methodology (RSM). *Dahi* significantly improved flavour, sweetness, overall acceptability and viscosity, while the FCM blend enhanced colour & appearance, flavour, sweetness, mouthfeel, and overall acceptability. Interaction effects showed a negative influence on sweetness but minor effects on other attributes. At the quadratic level, *dahi* reduced viscosity, whereas the FCM blend had minor

negative effects on sensory traits. The optimized formulation (71.07% *dahi* and 9.07% FCM blend) showed predicted values closely aligned with experimental results, confirming the model's reliability. Thus, RSM successfully facilitated the development of a probiotic *lassi* with superior sensory quality and nutritional value.

### Acknowledgement

The authors of this article duly acknowledge the support provided by Mr. Pravin Singh, Key Account Manager, DSM Firmenich, Mumbai for providing starter culture samples and Mr. Nikhil Kapoor, Chief Executive Officer, Fruit Formulations Pvt. Ltd., Pune for providing Fruit, Cereal and Millet blend.

### References

- Ajila CM, & Rao UP (2008) Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. *Food Chem Toxicol* 46:303-309.
- Andrade E, Santos Pires, AC dos Soares M, Jan G, de Carvalho AF (2012). Probiotics in Dairy Fermented Products. In: E. Rigobelo (Eds.), Probiotics, In Tech, pp. 129-148. <http://dx.doi.org/10.5772/51939>
- APHA (2004). In Wehr HM & Frank JF (Eds.), Standard methods for the examination of dairy products, American Public Health Association, Washington, DC, pp. 327-404.
- BS EN 16943:2017 Foodstuffs - Determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulphur and zinc by ICP-OES.
- Cushman DW, Cheung HS (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol* 20(7):1637-1648. [https://doi.org/10.1016/0006-2952\(71\)90292-9](https://doi.org/10.1016/0006-2952(71)90292-9).
- Deshpande SS, Mohapatra D, Tripathi MK, Sadvatha, RH (2015) *Kodo* millet-nutritional value and utilization in Indian foods. *JGPS* 2:16-23.
- FAO / WHO (2002) Report of a Joint FAO/WHO Working Group 'Guidelines for the Evaluation of Probiotics in Food' London, Ont. Canada. <https://openknowledge.fao.org/server/api/core/bitstreams/382476b3-4d54-4175-803f-2f26f3526256/content>
- FSSAI (2022) Manual of methods of analysis of foods: Dairy and Dairy Products. Food Safety and Standard Authority of India, New Delhi
- FSSAI (2023) Food Product Standards. Retrieved March 10, 2025, from [https://www.fssai.gov.in/upload/uploadfiles/files/2\\_%20Chapter%202\\_1%20\(Dairy%20products%20and%20analogues\).pdf](https://www.fssai.gov.in/upload/uploadfiles/files/2_%20Chapter%202_1%20(Dairy%20products%20and%20analogues).pdf)
- Hanson WC (1950) The photometric determination of phosphorus in fertilizers using the phosphovanado molybdate complex. *J Sci Food Agric* 1:172-173
- Hull M (1947) Studies on Milk Proteins. II. Colorimetric determination of the partial hydrolysis of the proteins in milk. *J Dairy Sci* 30:881-884. [https://doi.org/10.3168/jds.s0022-0302\(47\)92412-0](https://doi.org/10.3168/jds.s0022-0302(47)92412-0)
- Hussain SA (2013) Development of a technology for the manufacture of Aloe vera supplemented probiotic *lassi*. Doctoral Thesis, NDRI, Karnal.
- IS: 1479 (Part II) (1961) Method of test for dairy industry. Chemical analysis of milk. Bureau of Indian Standards, New Delhi
- IS: 5401 (Part I). (2012) Microbiology of Food and Animal Feeding Stuff - Horizontal Method for the Detection and Enumeration of Coliforms, Part 1: Colony Count Technique (Second Revision). Bureau of Indian Standards, New Delhi
- IS: 5403 (1999) Yeast and Mold Count of Foodstuffs and Animal feeds (first revision). Bureau of Indian Standards, New Delhi, 209
- IS: SP-18 (XI). (1981) *Handbook of food analysis*. Milk and milk products. Bureau of Indian standard, New Delhi
- ISO 29981/IDF 220: 2010: Milk products, Enumeration of presumptive bifidobacteria, Colony count technique at 37 °C, International Organization for Standardization.
- ISO 8070:1987 (E) Dried milk - Determination of sodium and potassium Contents-Flame emission spectrometric method.
- Kumar D, Chatli, MK, Singh R, Mehta N, Kumar P (2016) Enzymatic hydrolysis of camel milk casein and its antioxidant properties. *DST* 96:391-404. <https://doi.org/10.1007/s13594-015-0275-9>
- McCune LM, Johns T (2002) Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J Ethnopharmacol* 82:197-205. [https://doi.org/10.1016/S0378-8741\(02\)00180-0](https://doi.org/10.1016/S0378-8741(02)00180-0).
- Meilgaard MC, Carr BT, Civille GV (1999) Sensory evaluation techniques. CRC press. <https://doi.org/10.1201/9781003040729>
- Mital SA (2023) Development of technology for the manufacture of pomegranate-beetroot *lassi*. M. Tech. Thesis, Kamdhenu University, Gandhinagar
- Ramzan S (2020) Oat: A novel therapeutic ingredient for food applications. *J Microbiol Biotechnol Food Sci* 9:756-760
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231-1237.
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16:144-158.
- Truswell AS (2002) Cereal grains and coronary heart disease. *Eur J Clin Nutr* 56:1-14