Evaluation of potential of selected prebiotics on probiotic *L. acidophilus* and *L. casei* strains

H. C. Devaraja^{1*}, H. M. Jayaprakasha² and K. Jayaraj Rao³

¹ Dairy Technology section, ICAR-National Dairy Research Institute, Adugodi, Bengaluru - 560 030, Karnataka, India; ²Dairy Science College, Department of Dairy Technology, Hebbal-560 024 KVAFSU, Bidar, India; ³Dairy Technology section, ICAR-National Dairy Research Institute, Adugodi, Bengaluru - 560 030, Karnataka, India

Abstract

Attempts were made to understand the fermentation kinetics of fructo-oligosaccharides (FOS), inulin and isomalto-oligosaccharides (IMO) supplemented skim milk samples and its effect on direct microscopic clump (DMC) count of *L. acidophilus* and *L. casei* strains in skim milk medium. It was found that supplementation of FOS at 2% level significantly ($p<0.05$) increased the rate of acidification by 21.43% and 17.74% in *L. acidophilus* and *L. casei* inoculated sample compared to control, respectively. Skim milk supplemented with 3% FOS and inoculated with 4% *L. casei* culture recorded highest DMC count ($9.28 \log_{10}$ cfu/g) followed by IMO ($9.20 \log_{10}$ cfu/g) and Inulin ($9.16 \log_{10}$ cfu/g) supplemented sample inoculated with *L. casei*.

Key words: Fructo-oligosaccharides, Inulin, Isomalto-oligosaccharides, *Lactobacillus acidophilus*, *Lactobacillus casei*

Highlights

- Addition of prebiotics viz. FOS, inulin and IMO increased the rate of acidification and DMC count.
- *L. acidophilus* performed better compared to *L. casei* in terms of acid production.
- Addition of FOS at the rate of 3 per cent recorded the highest mean DMC count of $8.84 \log_{10}$ cells/g.
- *L. casei* added at the rate of 5% recorded significantly ($p<0.05$) higher DMC count.

INTRODUCTION

Oligosaccharides are short chain carbohydrates, having two to ten monosaccharides units linked together with glycosidic bonds. Some of the oligosaccharides, known as functional oligosaccharides, are resistant to digestion by human gut enzymes and selectively fermented by specific genera/species of resident gut microbes resulting in targeted increase in specific bacterial population that confers health benefits to the host (Roberfroid *et al.*, 2010). The common known functional oligosaccharides are fructo-oligosaccharides, galacto-oligosaccharides, malto-oligosaccharides, isomalto-oligosaccharides etc. (Ibrahim, 2018).

Inulin, a mixture of oligo- and polysaccharides, is composed of fructose moieties joined by β (2 $\rightarrow$ 1) linkages in linear chains. The above oligosaccharides and inulin exhibit excellent prebiotic potential, which promotes the growth of probiotic bacterial strains (Oliveira *et al.*, 2013; Canbulat and Ozcan, 2015). The application of oligosaccharides as a prebiotic ingredient in dairy products and other foods such as breakfast cereal and soft drinks is increasing exponentially.

In recent times, probiotic food and beverages segment is attracting more popularity in urban population due to growing health and diet

*Corresponding Author, E Mail: dev.rajds@gmail.com

consciousness among the consumer group. Probiotic food is defined as a processed product which contains viable probiotic microorganisms in a suitable matrix and in sufficient concentration (Parvez *et al.*, 2006). Probiotic bacteria have been recommended for the treatment of atopic dermatitis, chronic liver disease, allergic disease, necrotizing enterocolitis, pseudo-membranous colitis and food allergy (Salminen *et al.*, 2005). Dairy foods are one of the most versatile food segments, appealing to many palates and suitable for all age groups, and also offers versatile opportunities as a probiotic food carrier (Shah, 2007). Among the probiotics, *L. acidophilus* and *L. casei* had been studied extensively for their growth kinetics in milk medium and their contribution to gut health in humans.

MATERIALS AND METHODS

Oligosaccharides such as FOS (FOSSENCE™) and IMO (VitaFibre™) as well as inulin (Frutafit® HD) were procured from Tata Chemicals Ltd, India; Sensus, Netherlands and Bioneutra Inc., North America, respectively. Freeze-dried DVS probiotic strains of *Lactobacillus acidophilus* (La-5) and *Lactobacillus casei* (*L. casei* 431®) were obtained from Chr. Hansen Lab. (Hoersholm, Denmark) as free samples. Milk was collected from the dairy farm of ICAR-National Dairy Research Institute, Bengaluru and was standardised to <0.5% fat and 8.7% SNF for use as growth medium.

Supplementation of skim milk with selected prebiotics: The milk standardised to <0.5% fat and 8.7% SNF was thermally processed to 90°C for 10 min. The selected prebiotics were added at the rate of 2% to the processed skim milk. The probiotic culture was added at the rate of 4% and incubated at 40±2°C. The samples were analysed for pH and acidity at every two hours interval. The pH of the samples was measured using a pH meter (Labindia, India) and the titratable acidity in terms of % lactic acid was determined using the method described by AOAC (1995). The change in pH during

fermentation was monitored at every 2 h intervals upto 6 h and final acidification rate (V_p) was calculated as the time variation of pH using formula (dpH/dt) and expressed in 10^{-3} pH units/min.

Enumeration of direct microscopic clump count (DMCC):

The DMC count was enumerated using the method described by Harrigan (1998). The well mixed samples were diluted to 1:10 using sterile physiological saline as diluent. Sample of 0.01 mL (10 µL) was transferred using a micropipette onto a marked slide of 1cm² and smear was prepared by spreading evenly on the marked area. The smear was fixed using ethanol for 2 min and stained using borax methylene blue for 5 min and then rinsed with tap water. After air drying, the smear was observed under the oil immersion objective and organisms were counted in each of the field. The average number of organisms per field was calculated and multiplied by microscopic factor, dilution factor and 100. The DMC count was expressed as log₁₀ cells per gram of the sample.

RESULTS

Effect of selected prebiotics on pH of milk: As depicted in Fig. 1A, skim milk supplemented with selected prebiotics recorded significantly lower pH compared to control, upon incubation for 6 h with probiotic *L. acidophilus* and *L. casei* strains, inoculated at 4% level. The skim milk sample supplemented with 2% FOS, recorded very low pH of 4.35 compared to inulin (4.55) and IMO (4.56) supplemented samples. It is clear from Table 1 that the sample supplemented with FOS and inoculated with *L. acidophilus* recorded significantly ($p<0.05$) higher acidification rate of 6.139×10^{-3} pH units min⁻¹ and 21.43% more compared to control (5.056×10^{-3} pH units min⁻¹), followed by inulin (5.667×10^{-3} pH units min⁻¹) and IMO (5.639×10^{-3} pH units min⁻¹). The β co-efficient of regression equations presented in Table 2 indicates that the interaction of FOS with *L. acidophilus* reduced the pH of milk at a faster rate compared to all other combinations.

Effect of selected prebiotics on acidity of milk:

The skim milk samples inoculated with *L. acidophilus* and added with selected prebiotics recorded significant ($p < 0.05$) rise in acidity from 0.18 to 0.77% LA over 6 h of incubation (Fig. 1B). The control skim milk sample recorded the lowest acidity (0.51% LA) while the milk added with FOS recorded significantly ($p < 0.05$) higher (0.77% LA) rise in acidity after 6 h of incubation at $40 \pm 2^\circ\text{C}$ followed by IMO (0.69% LA) and inulin (0.68% LA). The skim milk samples without addition of prebiotics recorded lowest acidity

of 0.57 and 0.51% LA when inoculated with *L. acidophilus* and *L. casei*, respectively. The acidity of milk, inoculated with *L. casei* was more influenced by FOS and thus the milk sample with FOS and *L. casei* combination recorded the highest acidity of 0.65% LA as compared to *L. casei* with inulin (0.60% LA) and IMO (0.63% LA) combinations. However, the magnitude of β co-efficient of regression equations clearly depicts that the interaction of FOS with *L. casei* increased the acidity of skim milk at a faster rate compared to all other combinations (Table 2).

FOS: Fructo-oligosaccharides, IMO: Isomalto-oligosaccharides, LA: *Lactobacillus acidophilus*, LC: *Lactobacillus casei*

Fig. 1. Effect of selected prebiotics on pH (A) and acidity (B) of skim milk inoculated with *L. acidophilus* and *L. casei*

Table 1. Mathematical model adjusted to the experimental data of pH and acidity with model parameters and coefficient of determination (*Adj R*²)

Parameter	Probiotic culture	Prebiotics	Model equation	Adj R ²	F value
pH	<i>L. acidophilus</i>	Control	$y = -0.295x + 6.752$	0.98	845.19**
		FOS	$y = -0.370x + 6.82$	0.98	1306.72**
		Inulin	$y = -0.35x + 6.823$	0.91	213.16**
		IMO	$y = -0.333x + 6.821$	0.96	445.41**
Acidity	<i>L. casei</i>	Control	$y = -0.317x + 6.792$	0.97	758.57**
		FOS	$y = -0.351x + 6.76$	0.98	996.35**
		Inulin	$y = -0.325x + 6.771$	0.98	886.40**
		IMO	$y = -0.334x + 6.767$	0.96	474.76**
	<i>L. acidophilus</i>	Control	$y = 0.058x + 0.119$	0.94	309.24**
		FOS	$y = 0.014x^2 - 0.014x + 0.19$	0.92	230.02**
		Inulin	$y = 0.087x + 0.069$	0.97	774.84**
		IMO	$y = 0.0065x^2 + 0.0382x + 0.1281$	0.96	505.88**
	<i>L. casei</i>	Control	$y = 0.053x + 0.138$	0.97	658.71**
		FOS	$y = 0.078x + 0.092$	0.99	1574.66**
		Inulin	$y = 0.074x + 0.082$	0.97	566.30**
		IMO	$y = 0.073x + 0.092$	0.98	928.74**

FOS: Fructo-oligosaccharides, IMO: Isomalto-oligosaccharides

Table 2. Effect of selected prebiotics on acidification rate

Substrate	<i>L. acidophilus</i> (4%)		<i>L. casei</i> (4%)	
	Acidification rate (V _p) (10 ⁻³ pH units/min)	% Increase	Acidification rate (V _p) (10 ⁻³ pH units/min)	% Increase
Control	5.056±0.01 ^a	-	5.167±0.02 ^a	-
2% FOS	6.139±0.01 ^b	21.43 ^a	6.083±0.02 ^b	17.74 ^a
2% Inulin	5.667±0.02 ^c	12.09 ^b	5.444±0.02 ^c	5.38 ^b
2% IMO	5.639±0.02 ^c	11.54 ^c	5.722±0.01 ^d	10.75 ^c

* n=3; Mean±SE; Values with different superscript letters within a column are significantly (p<0.05) different; FOS: Fructo-oligosaccharides, IMO: Isomalto-oligosaccharides

Effect of selected prebiotics on direct microscopic clump count (log₁₀ cells/g) of *L. acidophilus* and *L. casei*: The skim milk sample standardised to <0.5% fat and 8.7% SNF was filled into 10 mL test tubes and selected prebiotic was added at 1%, 2% and 3% levels and sterilised at 120°C/15 min. After cooling to 40°C, *L. acidophilus* and *L. casei* at 3%, 4% and 5% levels were added in different

combinations with prebiotics and incubated at 40±2°C for 8 h. The direct microscopic clump count was enumerated by pour plate method.

Effect of fructo-oligosaccharides (FOS): Table 3 illustrates that, the rate of addition of FOS and probiotic strains, individually and in combination significantly (p<0.05) influenced the DMC count of the fermented skim milk. The

Table 3. Effect of fructo-oligosaccharides on direct microscopic clumpcount (log₁₀ cells/g) of *L. acidophilus* and *L. casei*

% Culture addition		Direct microscopic clump count (log ₁₀ cfu/g)			Statistical mean	Statistical mean
		Fructo-oligosaccharides				
		1%	2%	3%		
<i>L. acidophilus</i>	3	8.00±0.01 ^{aA}	8.20±0.04 ^{aA}	8.35±0.03 ^{aA}	8.21±0.15 ^a	8.33±0.15 ^a
	4	8.25±0.05 ^{abA}	8.34±0.05 ^{aA}	8.47±0.01 ^{aA}	8.35±0.10 ^b	
	5	8.41±0.02 ^{bA}	8.48±0.03 ^{aA}	8.51±0.05 ^{aA}	8.47±0.05 ^c	
<i>L. casei</i>	3	8.79±0.03 ^{cA}	9.16±0.03 ^{bB}	9.20±0.04 ^{bB}	9.05±0.20 ^d	9.10±0.20 ^b
	4	8.85±0.05 ^{cA}	9.26±0.03 ^{bB}	9.28±0.02 ^{bB}	9.13±0.21 ^e	
	5	8.88±0.04 ^{cA}	9.26±0.02 ^{bB}	9.29±0.03 ^{bB}	9.14±0.19 ^e	
Statistical mean		8.53±0.34 ^A	8.78±0.46 ^B	8.84±0.42 ^B		

* n=3; Mean±SE; Values with different superscript with small letters within a column are significantly (p<0.05) different; Values with different superscript with capital letters within a row are significantly (p<0.05) different

skim milk sample added with 1% FOS recorded significantly (p<0.05) lower (8.53 log₁₀ cfu/mL) DMC count compared to that of the samples added with 2% (8.78 log₁₀ cfu/mL) and 3% (8.84 log₁₀ cfu/mL) of FOS. However, there was non-significant difference between DMC count of samples added with FOS at 2 and 3% levels. The mean DMC count of the samples inoculated with *L. acidophilus* differed significantly (p< 0.05) at all the three levels of addition i.e. 3%, 4% and 5%, which recorded 8.21 log₁₀ cfu/mL, 8.35 log₁₀ cfu/mL and 8.47 log₁₀ cfu/mL, respectively.

The skim milk sample inoculated with *L. casei* at 3% level exhibited significantly (p<0.05) lower mean DMC count of 9.05 log₁₀ cfu/mL compared to the samples added with 4% (9.13 log₁₀ cfu/mL) and 5% (9.14 log₁₀ cfu/mL) of *L. casei* culture. However, there was no significant difference between DMC count of samples added with *L. casei* at 4 and 5% levels. It is also clear from the Table 3 that *L. casei* inoculated samples recorded significantly (p<0.05) higher overall mean DMC count (9.10 log₁₀ cfu/g) compared to that of *L. acidophilus* (8.33 log₁₀ cfu/g).

Effect of inulin: Increasing the level of inulin significantly (p<0.05) increased the DMC count of the fermented skim milk. Inulin added at the rate of 1, 2 and 3% recorded the mean DMC

count of 8.28 log₁₀ cells/g, 8.55 log₁₀ cells/g and 8.67 log₁₀ cells/g, respectively (Table 4). Similarly, the DMC count of *L. acidophilus* and *L. casei*, added individually at different levels exhibited significant (p<0.05) differences within and between the cultures. The DMC count of the sample inoculated with *L. acidophilus* differed significantly (p<0.05) at all the three levels of addition i.e. 3, 4 and 5%, which recorded the mean DMC count of 7.81 log₁₀ cells/g, 8.05 log₁₀ cells/g and 8.30 log₁₀ cells/g, respectively.

The skim milk sample inoculated with *L. casei* at 3% level exhibited significantly (p<0.05%) lower mean DMC count of 8.89 log₁₀ cells/g as compared to the samples added with 4% (8.97 log₁₀ cells/g) and 5% (9.00 log₁₀ cells/g) of the said culture. It is also clear from Table 4 that, the skim milk sample inoculated with *L. casei* recorded nearly one log higher mean DMC count (8.95 log₁₀ cells/g) as compared to that of *L. acidophilus* (8.04 log₁₀ cells/g).

Effect of isomalto-oligosaccharides (IMO):

The IMO added at the rate of 1, 2 and 3% recorded significantly (p<0.05) different mean DMC count of 8.35 log₁₀ cells/g, 8.56 log₁₀ cells/g and 8.70 log₁₀ cells/g, respectively.

L. acidophilus inoculated samples recorded 7.85 log₁₀ cells/g, 8.07 log₁₀ cells/g and 8.28 log₁₀ cells/g (Table 5) when added at the rate

Table 4. Effect of inulin on direct microscopic clump count ($\log_{10}$ cells/g) of *L. acidophilus* and *L. casei*

% Culture addition	Direct microscopic clump count (log ₁₀ cells/g)					Statistical mean	Statistical mean
	Inulin						
		1 %	2 %	3 %			
<i>L. acidophilus</i>	3	7.70±0.02 ^{aA}	7.83±0.03 ^{aA}	7.86±0.05 ^{aA}	7.81±0.15 ^a	8.04±0.26 ^a	
	4	7.84±0.04 ^{abA}	7.98±0.04 ^{abAB}	8.35±0.04 ^{bB}	8.05±0.23 ^b		
	5	8.11±0.03 ^{bA}	8.35±0.02 ^{bAB}	8.44±0.05 ^{bB}	8.30±0.15 ^c		
<i>L. casei</i>	3	8.61±0.04 ^{cA}	8.97±0.03 ^{cB}	9.10±0.03 ^{cB}	8.89±0.22 ^d	8.95±0.21 ^b	
	4	8.69±0.06 ^{cA}	9.09±0.04 ^{cB}	9.14±0.05 ^{cB}	8.97±0.21 ^c		
	5	8.73±0.05 ^{cA}	9.11±0.05 ^{cB}	9.16±0.04 ^{cB}	9.00±0.20 ^c		
Statistical mean		8.28±0.42 ^A	8.55±0.54 ^B	8.67±0.50 ^C			

* n=3; Mean±SD; Values with different superscripts (abcde) within a column are significantly (p<0.05) different; Values with different superscripts (ABC) within a row are significantly (p<0.05) different

Table 5. Effect of Isomalto-oligosaccharides on direct microscopic clump count ($\log_{10}$ cells/g) of *L. acidophilus* and *L. casei*

% Culture addition	Direct microscopic clump count				Statistical mean (within culture)	Statistical mean (between culture)
	(log ₁₀ cells/g)					
	Isomalto-oligosaccharides					
	1 %	2 %	3 %			
<i>L. acidophilus</i>	3	7.73±0.03 ^{aA}	7.88±0.05 ^{aA}	7.97±0.04 ^{aA}	7.85±0.10 ^a	8.07±0.22 ^a
	4	7.89±0.05 ^{abA}	8.01±0.03 ^{abAB}	8.31±0.03 ^{abB}	8.07±0.19 ^b	
	5	8.11±0.04 ^{bA}	8.32±0.05 ^{aA}	8.39±0.04 ^{aA}	8.28±0.12 ^c	
<i>L.casei</i>	3	8.69±0.03 ^{cA}	9.01±0.04 ^{bAB}	9.16±0.05 ^{bB}	8.95±0.21 ^d	9.01±0.18 ^b
	4	8.80±0.02 ^{cA}	9.10±0.04 ^{bAB}	9.18±0.02 ^{bB}	9.03±0.17 ^{dc}	
	5	8.86±0.04 ^{cA}	9.11±0.05 ^{bA}	9.20±0.05 ^{bA}	9.06±0.15 ^e	
Statistical mean		8.35±0.46 ^A	8.56±0.53 ^B	8.70±0.51 ^C		

* n=3; Mean±SD; Values with different superscripts (abcde) within a column are significantly (p<0.05) different; Values with different superscripts (ABC) within a row are significantly (p<0.05) different

of 3, 4 and 5%, respectively which was statistically significant (p<0.05) at all levels. In case of *L. casei*, the sample added with 3% culture recorded significantly (p<0.05) lower DMC count (8.95 $\log_{10}$ cells/g) as compared to the samples inoculated with 4% (9.03 $\log_{10}$ cells/g) and 5% (9.06 $\log_{10}$ cells/g) of the *L. casei* culture.

The result indicates that supplementation of milk with prebiotics significantly (p<0.05) increased the acidification rate and reduced the fermentation time. Among the selected prebiotics, FOS significantly (p<0.05) improved

the activity of probiotic *L. acidophilus* and *L. casei* strains, followed by inulin and IMO. However, non-significant difference between inulin and IMO was noticed. The milk supplemented with 4% FOS and inoculated with *L. casei* recorded highest DMC count compared to all the other combinations studied.

DISCUSSION

Addition of prebiotics namely FOS, inulin and IMO at the rate of 2% resulted in significant (p<0.05) decrease in pH of cultured milk at a faster rate as compared to control samples. Skim

milk sample added with FOS and inoculated with *L. acidophilus* at 4% level recorded very low pH of 4.35 by the end of incubation time of 6 h, followed by inulin (pH 4.55) and IMO (pH 4.56).

The added prebiotics significantly improved the culture activity and thereby increased the rate of acidification. Among the probiotic cultures used, *L. acidophilus* performed better as compared to *L. casei*. Amongst prebiotics, FOS influenced the acidification rate more than inulin and IMO. The high acidification rate of *L. acidophilus* compared to *L. casei* could be attributed to their ability to produce high lactic acid (Yoon *et al.*, 2005). The better performance of *L. acidophilus* over *L. casei* and influence of prebiotics on acidification rate is consistent with the observations of Oliveira *et al.* (2013), who reported that FOS and inulin significantly increase the acidification rate and decrease the fermentation time as compared to the samples without prebiotics in low fat milk. Similar results were also observed by Rodrigues *et al.* (2011) when *L. casei*-01, *L. acidophilus* La-5 and *B. lactis* B94 strains were added with FOS : inulin (50:50). The lower degree of polymerization (5–8) in case of FOS as compared to inulin (~25) renders FOS to be hydrolysed quicker by microbes and grow faster in presence of FOS (Angus *et al.*, 2005). Olson and Aryana (2012) also observed that inulin added at 1% and 3% levels to skim milk and inoculated with *L. acidophilus* exhibited faster development of pH during incubation as compared to control samples.

Similar trend was observed in the present study for development of acidity in skim milk samples added with selected prebiotics and probiotics. The performance of *L. acidophilus* and FOS combination was found to be significantly better in development of acidity as compared to all other combination of prebiotics and probiotics tried. This could be attributed to the high acid tolerance and faster lactic acid production ability of *L. acidophilus* as compared to *L. casei* (Yoon *et al.*, 2005). Nonsignificant difference was noticed between

the effect of inulin and IMO on acid production by both the probiotics.

Effect of prebiotics on direct microscopic clump count (DMCC) of *L. acidophilus* and *L. casei*: FOS and probiotic cultures, individually and in combination significantly ($p < 0.05$) influenced the DMC count of the fermented milk. Increasing the rate of addition of FOS and probiotic culture increased the DMC count of the fermented milk. The DMC count of *L. casei* was significantly ($p < 0.05$) higher as compared to *L. acidophilus*. The results are in agreement with the observations of Oliveira *et al.* (2013) who observed significant influence of 4% FOS in low fat milk on the growth of *L. acidophilus* (7.66 log cfu/mL) as compared to control sample (7.35 log cfu/mL). Moreno *et al.* (2006) reported higher viable cells of *L. casei* (9.01 log cfu/mL) in fermented milk as compared to *L. acidophilus* (8.36 log cfu/mL) indicating better growth, multiplication and survivability of *L. casei* in fermented milks as compared to *L. acidophilus*.

Increasing the level of inulin addition significantly ($p < 0.05$) increased the DMC count of fermented milk. Similarly, increased rate of addition of culture also showed significant ($p < 0.05$) increase in the DMC count of *L. acidophilus* and *L. casei*. Oliveira *et al.* (2011) noted that the inulin supplementation in skim milk considerably increased the growth and sustainability of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium lactis* in non-fat fermented milk. Inulin promoted the growth and antibacterial activity of *L. paracasei* TD3 (Mahboubi and Kazempour, 2016). Inulin added at the rate of 1% and 3% to skim milk and inoculated with *L. acidophilus* exhibited increased growth and faster development of pH during incubation as compared to control samples (Olson and Aryana, 2012). Bedani *et al.* (2013) reported that addition of inulin at 3% level to soy milk increased the growth and survivability of *L. acidophilus*. Similarly, it has been reported by Akalin and Erişir (2008) that the viable

counts of *L. acidophilus* La-5 and *B. animalis* Bb-12 significantly ($P<0.05$) increased in the probiotic ice cream mix added with oligofructose. Inulin added at the rate of 4% to ice cream mix also improved the viability of *L. acidophilus* La-5 and *B. animalis* Bb-12.

Addition of IMO to the milk increased the DMC count of *L. acidophilus* and *L. casei*. Among the prebiotics, FOS stands out to be the best prebiotic, which significantly ($p<0.05$) increased the total viable probiotic count as compared to inulin and IMO. Oliveira *et al.* (2013) demonstrated that the low-fat milk supplemented with 4% FOS exhibited highest acidification rate 23.17 (10-3 pH units/min), decreased fermentation time (7.44 h) and high viable cell count as compared to 4% inulin supplemented sample. Considering the significantly ($p<0.05$) higher DMC count of *L. casei* as compared to *L. acidophilus* in the skim milk, inoculation with *L. casei* at 4% level was recommended for further optimization of product parameters.

The results clearly indicated that, the addition of prebiotics viz. FOS, inulin and IMO significantly ($p<0.05$) improved the culture activity and thereby, increased the rate of acidification as compared to control. Among the probiotic cultures used, *L. acidophilus*

performed better than *L. casei* in terms of acid production. Addition of prebiotics namely FOS, inulin and IMO (at the rate of 2% in each case), resulted in a significant decrease in pH at a faster rate as compared to control samples. The performance of *L. acidophilus* and FOS combination was found to be significantly ($p<0.05$) better for development of acidity as compared to all other combinations of prebiotics and probiotics tried. Addition of FOS at the rate of 3% recorded the highest mean DMC count of 8.84 $\log_{10}$ cells/g and the *L. casei* added at the rate of 5% recorded significantly ($p<0.05$) higher DMC count (9.14 $\log_{10}$ cells/g) as compared to all the other combinations tried within and between the strains.

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Author's contribution: JHM: Conceived the idea and guided the technical program; DHC: Carried out the experiments and wrote the manuscript; JRK: Supervised the work and edited the manuscript.

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