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RESEARCH ARTICLE

Dairy Technology

Physicochemical and sensory attributes of inulin incorporated set yoghurt prepared using cow and buffalo milk

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Abstract: The demand for probiotic food products has been increased over the last few decades. The current study aimed to evaluate the microbiological and physicochemical properties of two set yoghurt types prepared using cow milk (CM) and buffalo milk (BM) with three different concentrations of inulin (1%, 2% and 3% w/v). Two types of starter cultures BB12 (*Bifidobacterium bifidum* – BB) and YC-X11 (*Streptococcus thermophilus* – ST and *Lactobacillus bulgaricus* subspp. *Bulgaricus* – LB) were used as probiotic sources. Probiotic viable cell counts, syneresis, total acidity, pH, organoleptic properties, and viscosity were evaluated during 21 days of storage at 4 °C. Both inulins incorporated CMY and BMY showed significantly high ($p < 0.05$) ST and LB counts at the end of the enumeration period (~8.0 log cfu/mL) while control samples of each group showed significantly lower (~7.0 log cfu/mL) probiotic counts. 3% CMY and BMY reported the highest probiotic survivability. Significantly ($p < 0.05$) high viscosity was observed in 2% inulin incorporated yoghurts which were highly acceptable and showed better stability during storage. This study concludes that the addition of inulin improves the overall probiotic survivability of the tested microorganisms while ensuring consumer acceptance without drastically altering the physicochemical properties of yoghurt.

Keywords: *Bifidobacterium,* buffalo milk, inulin, *Lactobacillus,* probiotic, yoghurt.

INTRODUCTION

The consumer approach towards eating healthy foods has evolved during the last decade, enhancing the potential to develop various functional foods that provide more benefits than simply nutritional value. Among the functional foods, much attention has been focused on probiotic products. Probiotics are health-promoting viable microorganisms such as *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Bifidobacterium longum* that exhibit a beneficial effect on human health, by improving the intestinal microbial balance (Martin-Diana *et al.,* 2003). In addition, probiotics improve lactose utilization by producing lactase enzyme which helps to digest lactose, preventing intestinal infections by producing organic acids and other antibacterial agents, reducing cancer risk, improving immunity, lowering cholesterol and blood pressure, and protecting against diarrhoea, constipation and osteoporosis (Ranadheera *et al.,* 2010). Several studies have demonstrated the modes of actions of probiotics as antioxidants, antimicrobial compounds, and agents that improve bowel functions (Beena *et al.,* 2012).

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In order to ensure the survival of probiotic strains, most probiotic-containing foods also include prebiotic sources. A prebiotic is defined as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving the health of the host' (Gibson *et al.,* 2004). Ingestion of prebiotics significantly enhances the proliferation of colon microflora by acting as a substrate for probiotics, while improving the colonization rate of bacterial strains such as *Lactobacillus* and *Bifidobacterium* which have high probiotic activity. The prebiotics can balance the composition of microflora in the human gut (Ranadheera *et al.,* 2010). In many foods, non-digestible oligosaccharides and fructooligosaccharides are used as prebiotics. The most utilized prebiotic in yoghurt manufacturing is inulin. It is a non-digestible and fermentable fructan (Ehsani *et al*., 2016) that stimulates the growth and activity of probiotic bacteria. Inulin consumption significantly increases faeces loading and improves faecal excretion in humans. Thus, inulin is effective against colon disorders such as constipation.

In the food industry, inulin is often used as a low-calorie sweetener and a non-digestible fibre that forms gels which increase viscosity and enhance organoleptic properties (Meyer *et al*., 2011). Considering these beneficial health effects imparted by both prebiotic compounds and probiotic microbial strains, current food manufacturers tend to produce symbiotic foods. Yoghurt is such a food that is widely consumed and produced by incorporating prebiotics with the probiotics *Lactobacillus acidophilus, Lactobacillus casei,* and *Bifidobacterium* to enhance their therapeutic value. The viability of the probiotic bacterial count should be as high as 10^6-10^7 CFU/mL at consumption to achieve the aforementioned benefits (de Souza Oliveira *et al.,* 2011). Hence, it is essential to ensure sufficient and viable probiotic bacteria throughout the shelf life of the yoghurt. Recent studies have shown that most probiotic foods have a low population of probiotic bacteria at the time of consumption. The main dairy source of yoghurt is fresh cow milk. When compared to cow milk, buffalo milk has a higher content of fat, protein, lactose and total solids (Abesinghe *et al.,* 2020). These properties of buffalo milk give a rich flavour and taste and make it very suitable for the manufacture of a wide variety of milk products (Han *et al.,* 2012b). Moreover, recent studies on the shelf life of buffalo milk yoghurt have indicated that it could be a good vehicle for probiotics (Han *et al.,* 2012a). As a result, the potential of utilizing buffalo milk to produce a symbiotic yoghurt was examined. The present study aims to explore and compare the physicochemical and probiotic activity of cow and buffalo milk set yoghurt to identify the best probiotic carrier with a suitable level of prebiotic inulin addition.

MATERIALS AND METHODS

Preparation of set type yoghurt

Cow milk set yoghurt (CMY) and buffalo milk set yoghurt (BMY) were prepared according to the following methodology. Milk was pre-heated at 65 °C and inulin powder (Orafti® HPX, High-Performance Inulin Powder, Mannheim, Germany) was added in three concentrations of 1%, 2% and 3% (w/v). Then, skim milk powder (SMP) was added at concentrations of 3% (w/v) for the control, 2% (w/v) SMP for 1% inulin treated sample and 1% (w/v) SMP for 2% and 3% inulin treated samples respectively. The mixtures were homogenized for 15-20 min at 5 Mpa. The milk was then pasteurized at 90-95 °C for 5-10 min followed by cooling down to 36 °C. A starter culture containing *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) (Chr. Hansen YC-X11, Hoersholm, Denmark) and *Bifidobacterium bifidum* (BB) (Chr. Hansen, Hoersholm, Denmark) were added in 0.002% (w/v) concentration. The yoghurts were incubated in cups at 42 °C for 4 h. Finally, the samples were stored at 4 °C pending analyses.

Preparation of microbiological isolates

The pour plate method was used to evaluate the growth and viability of yoghurt bacteria, and bifidobacterial (Shori & Baba, 2012) MRS media (M641, HIMEDIA, Mumbai, India) was used to isolate *Lactobacillus.* Commercial M17 agar base (HIMEDIA, Mumbai, India) was used for isolation of the *S. thermophilus* strain, and commercial Bifidobacterium agar (HIMEDIA, Mumbai, India) was used to isolate *Bifidobacterium* strains of the samples. A dilution series was prepared by using 0.1% (w/w) peptone (CDH, India) solution for all microbiological analyses. All culture media were incubated at 35-37 °C for 48-72 h in the laboratory incubator (IN- 601/GEMMYCO) in anaerobic conditions. Colony counts were obtained at 1, 7, 14, and 21 ds by hand enumeration through a colony counter (Witeg Colony Counter Galaxy 330, Germany). For each microbial strain, three nearby dilutions were prepared and for each dilution, duplicate plates were prepared and enumerated. The microbial counts were expressed as log colony forming units per millilitre (log CFU/mL).

Physicochemical analyses

pH measurement

Changes in the pH during the storage period were measured using a multi-parameter pH meter (35 Series, Eutech Instruments, Singapore). The pH probe was immersed in 25 mL of homogenized sample and the readings were taken in relation to room temperature (27±2 ºC) in triplicate. Readings were collected on the 1st, 7th, 14th, and 21st ds after manufacturing.

Titratable acidity

A slurry of each sample was made by mixing 10 g of yoghurt with 5 mL of distilled water. Samples were titrated against 0.1 N NaOH solution, in the presence of 1% phenolphthalein until a persistent pink colour was observed. Acidity is expressed as a percentage of lactic acid (Jayarathna *et al.,* 2020). Analysis was carried out in triplicate.

Syneresis

Twenty grams of each sample was placed on a Whatman No.1 filter paper and the weight of the filtrate was measured for 2 h with 30 min time intervals in triplicate. Syneresis is expressed as the percentage of the initial sample (Amal *et al.,* 2016).

Texture

Texture analyses were completed using a programmable digital viscometer (Brookfield DVE Digital Viscometer, Massachusetts, USA) at 4 °C, using spindle numbers 6 and 7 at speed of 10 rpm. Readings were recorded after 30 s and expressed in millipascal-second (mPa.s) on the 7th day after manufacturing.

Sensory evaluation

Sensory evaluation of inulin incorporated cow milk and buffalo milk yoghurt was carried out with 30 untrained panelists (aged 22-55 years, women 60%, men 40%) according to a randomized design in order to find out the degree of likeability of the probiotic yoghurt with the most preferable level of inulin. The study was carried out at least 2 h after a main meal and smokers, betel chewers, or alcohol consumers were not recruited as panelists. Each panelist evaluated the final yoghurt product according to a five-point hedonic scale (1-5, from strongly disliked to strongly liked, respectively) using a simplified and structured sensory evaluation ballot at 25 °C.

Statistical analysis

Physicochemical data of the yoghurt were analysed by using one-way ANOVA of Minitab 18 software (Minitab® 17.1.0, UK) with a 95% confidence interval (significance at the $p < 0.05$) while the Friedman test was used to analyze sensory data. Microbiological data were analysed via one way ANOVA of the SAS program (Version 9.0, SAS Inc, North Carolina, USA).

RESULTS AND DISCUSSION

Changes in the microbial count during the storage

In consideration of variations of viable ST counts in CMY during the end of storage, there was a significant difference ($p < 0.05$) of viability between 2% and 3% inulin incorporated yoghurt samples in contrast to the control and 1% inulin incorporated samples (Table 1). The highest ST count throughout the storage period in both CMY and BMY was recorded from 3% inulin incorporated samples (9.11 ± 0.07 log CFU/mL to 8.72 ± 0.14 log CFU/mL). There was no significant difference in initial viable counts among the samples, although there was a significant reduction of the viable counts in both 2 % and 3 % CMY on the 21st day of storage. The viable counts in CMY followed a gradual decline towards the end of the storage period, yet they were remaining in the range of critical microbial count which is $>10^6$ CFU/mL.

It is evident that ST has dominantly grown in both cow milk and buffalo milk set yoghurts. Oliveira *et al.* (2002), reported that ST predominated in all products, showing higher counts than 9 log CFU/mL in fermented milk prepared with mixed culture.

The yoghurt samples prepared with buffalo milk showed a significant difference ($p < 0.05$) in microbial viability of ST among inulin incorporated samples and the control sample at the end of storage. The highest viable count on the 21st day of storage was reported from the 3% inulin incorporated BMY sample (8.23 ± 0.12 log CFU/mL). Considering the BMY, they showed no significant difference ($p > 0.05$) compared to their initial viable count throughout the storage up to the 14th day, yet they showed a significant reduction ($p < 0.05$) when comparing the initial stage and the final day of storage. However, the viable ST counts were still above the critical count. This prominent and better viability of ST is likely due to its enhanced proteolytic activity combined with higher lactose utilization, compared to the other two probiotic strains (Özer & Kirmaci, 2010).

Table 1: Viable counts of *Streptococcus thermophilus* (ST) in cow and buffalo milk set yoghurt stored at 4 °C during 21 days of storage (log CFU/mL)

Treatment	Period of storage in days (Mean ± SD)			
Cow milk yoghurt	1	7	14	21
Control	8.34 ± 0.14^{Ba}	8.27 ± 0.17^{Ca}	8.13 ± 0.15^{Ba}	7.93 ± 0.12^{Ca}
1% Inulin	8.46 ± 0.1^{Ba}	8.49 ± 0.06^{BCa}	8.41 ± 0.12^{Ba}	8.27 ± 0.17^{Ba}
2% Inulin	8.82 ± 0.14^{ABa}	8.75 ± 0.13^{ABab}	8.65 ± 0.09^{Bab}	8.49 ± 0.06^{ABb}
3% Inulin	9.11 ± 0.07^{Aa}	9.08 ± 0.08^{Aa}	9.04 ± 0.08^{Aab}	8.78 ± 0.12^{Ab}
Buffalo milk yoghurt				
Control	8.13 ± 0.15^{Ba}	8.08 ± 0.09^{Ca}	7.78 ± 0.33^{Bab}	7.54 ± 0.16^{Bb}
1% Inulin	8.38 ± 0.09^{ABa}	8.31 ± 0.10^{BCa}	8.13 ± 0.15^{ABab}	7.93 ± 0.12^{Ab}
2% Inulin	8.63 ± 0.12^{ABa}	8.58 ± 0.11^{ABa}	8.43 ± 0.15^{Aab}	8.13 ± 0.15^{Ab}
3% Inulin	8.72 ± 0.14^{Aa}	8.65 ± 0.10^{Aa}	8.55 ± 0.14^{Aab}	8.23 ± 0.12^{Ab}

Means in the same row without a common simple letter superscript significantly ($p < 0.05$) differ for each treatment; means in the same column without a common capital letter superscript significantly ($p < 0.05$) differ among the treatments per microbial analysis.

Table 2: Viable counts of *Bifidobacterium bifidum* (BB) in cow and buffalo milk set yoghurt stored at 4 °C during 21 days of storage (log CFU/mL)

Treatment	Period of storage in days (Mean ± SD)			
Cow milk yoghurt	1	7	14	21
Control	8.31 ± 0.10^{Ba}	8.23 ± 0.12^{Ca}	8.13 ± 0.15^{Ba}	7.69 ± 0.21^{Bb}
1% Inulin	8.63 ± 0.12^{ABa}	8.49 ± 0.06^{Ca}	8.49 ± 0.14^{ABa}	8.31 ± 0.10^{Aa}
2% Inulin	8.75 ± 0.13^{Aa}	8.67 ± 0.07^{ABab}	8.60 ± 0.08^{Aab}	8.32 ± 0.1^{Ab}
3% Inulin	8.91 ± 0.15^{Aa}	8.86 ± 0.13^{Aa}	8.78 ± 0.12^{Aa}	8.65 ± 0.04^{Aa}
Buffalo milk yoghurt				
Control	8.00 ± 0.21^{Ba}	8.67 ± 0.10^{Bab}	8.46 ± 0.10^{Bb}	7.39 ± 0.21^{Bab}
1% Inulin	8.35 ± 0.04^{Aa}	8.31 ± 0.10^{Aa}	8.28 ± 0.05^{Aa}	8.17 ± 0.21^{Aa}
2% Inulin	8.58 ± 0.05^{Aa}	8.46 ± 0.10^{Aa}	8.41 ± 0.12^{Aa}	8.35 ± 0.04^{Aa}
3% Inulin	8.67 ± 0.10^{Aa}	8.46 ± 0.10^{Aa}	8.39 ± 0.21^{Aa}	8.27 ± 0.17^{Aa}

Means in the same row without a common simple letter superscript significantly ($p < 0.05$) differ for each treatment; means in the same column without a common capital letter superscript significantl y ($p < 0.05$) differ among the treatments per microbial analysis.

According to Table 2, it is evident that the addition of inulin, the duration of storage, and their collective interaction have had significant ($p < 0.05$) effects upon the BB viability in both CMY and BMY samples. Especially, considering the 1st and 21st days of CMY samples, the control yoghurt sample has reported the lowest BB viability (8.31 ± 0.10 log CFU/mL and 7.69 ± 0.21 log CFU/mL, respectively) in contrast to other samples, although the viable BB count has not remarkably varied among the inulin incorporated samples. A recent study by Kamel *et al.* (2021) concludes that increasing inulin concentrations helped to amplify the viability of BB significantly ($p < 0.05$) during storage, as well as acting as an antifungal agent of probiotic yoghurt, suggesting that inulin can be used as a suitable and a successful nutrient for BB.

Table 2 shows that significantly lower ($p < 0.05$) viable BB counts were observed with time in each BMY sample, compared to their initial counts, though the viable counts were reported above the critical microbial count up to the 21st day. Similarly, Han *et al.* (2012a) reported that *Bifidobacterium* spp. remained viable throughout 10 weeks of storage when incorporated in low-fat symbiotic yoghurt. The highest viable counts observed in 3% inulin incorporated yoghurt sample on 1st and 21st days of storage (8.67 ± 0.1 log CFU/mL to 8.27 ± 0.17 log CFU/mL) suggesting that BM yoghurt could provide a suitable environment for the growth of probiotic microbes and maintain therapeutic levels until the end of the storage period.

Table 3: Viable counts of *Lactobacillus bulgaricus* (LB) in cow and buffalo milk set yoghurt stored at 4 °C during 21 days of storage (log CFU/mL)

Treatment	Period of storage in days (Mean ± SD)			
Cow milk yoghurt	1	7	14	21
Control	8.28 ± 0.05^{BCa}	8.08 ± 0.09^{Ca}	7.93 ± 0.12^{Bab}	7.69 ± 0.21^{Bb}
1% Inulin	8.54 ± 0.06^{ACa}	8.43 ± 0.15^{BCa}	8.39 ± 0.21^{Aa}	8.27 ± 0.17^{Aa}
2% Inulin	8.79 ± 0.10^{ABa}	8.82 ± 0.07^{ABa}	8.52 ± 0.09^{Aab}	8.39 ± 0.21^{Ab}
3% Inulin	8.96 ± 0.16^{Aa}	8.86 ± 0.08^{Aab}	8.74 ± 0.11^{Aab}	8.59 ± 0.13^{Ab}
Buffalo milk yoghurt				
Control	8.08 ± 0.09^{Ca}	7.93 ± 0.12^{Cab}	7.80 ± 0.70^{Bb}	7.54 ± 0.39^{Bc}
1% Inulin	8.45 ± 0.07^{Ba}	8.38 ± 0.09^{Ba}	8.27 ± 0.17^{Aa}	8.04 ± 0.27^{ABa}
2% Inulin	8.64 ± 0.02^{ABa}	8.44 ± 0.07^{ABab}	8.31 ± 0.10^{ABbc}	8.13 ± 0.15^{Ac}
3% Inulin	8.73 ± 0.09^{Aa}	8.59 ± 0.13^{Aab}	8.50 ± 0.16^{Aab}	8.31 ± 0.10^{Ab}

Mean values in the same row without a common simple letter superscript significantly ($p < 0.05$) differ for each treatment; means in the same column without a common capital letter superscript significantly ($p < 0.05$) differ among the treatments per microbial analysis.

According to Table 3, there is a significant difference ($p < 0.05$) in LB viability between inulin incorporated samples and control samples made with cow milk. The viable counts of LB ranged from 8.28 ± 0.05 log CFU/mL to 7.69 ± 0.21 log CFU/mL on the 1st and 21st day in storage, while the highest viable LB counts were observed in 3% inulin incorporated yoghurt sample, on the 21st day of storage (8.59 ± 0.13 log CFU/mL). However, the viability of LB counts significantly declined with time compared to their initial values yet remained in the critical microbial count even on the final day of storage. A similar study (Saccaro *et al.,* 2009) revealed that the viability of LB was reduced by 1.51 log CFU/mL when grown in co-culture with ST and in association with probiotic microorganisms, which explains the receding nature of LB viability in these mixed culture CMY samples.

The viability of LB in BMY samples also shows a significant difference between inulin incorporated and control samples, where the viable counts of LB in the control sample gave values of 8.08 ± 0.09 log CFU/mL and 7.54 ± 0.39 log CFU/mL on 1st and 21st day of storage, respectively. However, a significantly different ($p < 0.05$) value for the highest viability of LB was reported from the 3% inulin incorporated sample on the 21st day, which was 8.31 ± 0.1 log CFU/mL. Significantly low viable counts were observed in each treatment compared to their initial counts and the rate of reduction of viable counts was higher in the control sample though the counts remained in the range of critical microbial count up to the 21st day ($>10^6$ CFU/mL).

The results of the study showed the increase of probiotic bacterial counts with increasing inulin concentrations for both milk types. Inulin is a fructan that can be effectively used as a nutrient for microbial growth (Srisuvor *et al.,* 2013). Inulin enhances probiotic proliferation by acting as dietary fibre (Ramirez-Santiago *et al.,* 2010). In addition, lactic acid bacteria (LAB) are capable of producing exopolysaccharides (EPS) during their growth, and these EPS are essential for LAB-host interaction mechanisms such as bacterial tolerance to gut conditions and their immune-modulatory activities, which helps them to survive in the host's gastrointestinal tract (Caggianello *et al.,,* 2016), The increase in inulin levels may lead to high concentrations of EPS amounts with a positive effect on microbial growth.

Lower viability was observed for LB bacteria in both yoghurt types, which may be due to the post acidification (Sendra *et al.,* 2008) leading to lower pH and low availability of bacteria in the commercial starter culture (Chr. Hansen YC-X11, Hoersholm, Denmark). The results of the present study showed that the viability of microbes decreased with time, yet the decrease of LAB viability was less with high concentrations of inulin. These results agree with Ehsani *et al.* (2016), who also observed that counts of probiotic strains declined over four weeks of storage. Donkor *et al.* (2007) suggested that ultimate pH and accumulation of organic acids had a notable impact on the survival of LB and BB in yoghurt. Therefore, considering the results obtained in this study, reduction of energy source with time would be another reason for the declining survival rate of microbes with time, apart from previously mentioned factors.

Changes in pH, titratable acidity, syneresis and texture

Variations of pH in different yoghurts

According to the pH variation data of CMY, there were no significant differences ($p > 0.05$) among the samples throughout the storage period and a decrease of pH during storage was observed in all the treatments with time (Figure 1a). At the end of the experiment, the control sample recorded the lowest pH (4.13 ± 0.01) while the 1% inulin incorporated sample recorded the highest pH value (4.24 ± 0.01).

When considering the pH variation of BMY, there were significant differences ($p < 0.05$) in pH among the treatments throughout the storage period (Figure 1b). The pH varied from 4.86 ± 0.01 to 4.96 ± 0.00 from the 1st day to the 21st. On the 14th day, 1% CMY recorded a significantly higher ($p < 0.05$) pH value compared to other samples (4.97 ± 0.01). However, the pH at the end of the storage period of yoghurts manufactured with inulin addition showed no significant difference ($p > 0.05$) among samples.

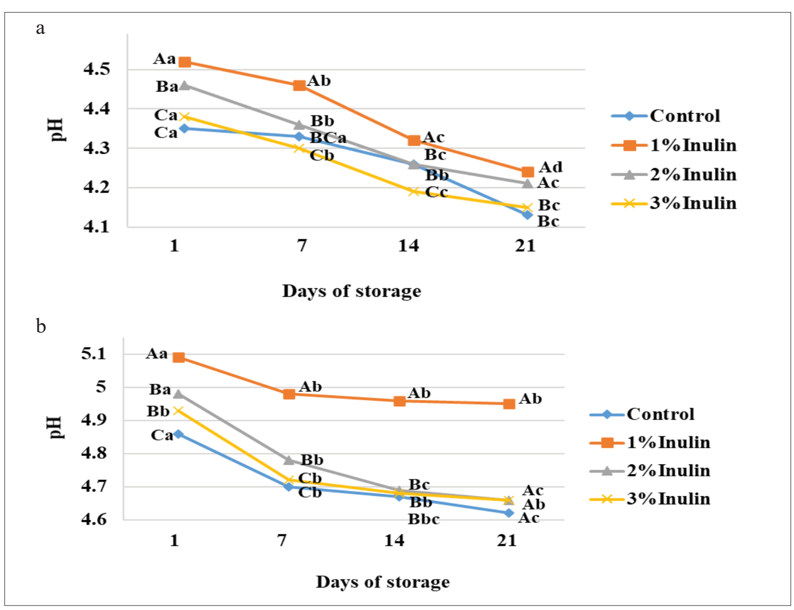

Figure 1: a) Variation in pH of CMY with different inulin concentrations stored at 4 °C during 21 days of storage b) Variation in pH of BMY with different inulin concentrations stored at 4 °C during 21 days of storage.

[ABC] Means with different uppercase letters are significantly different between each treatment.
[abc] Means with different lowercase letters are significantly different between days of storage.

In yoghurt processing, lactic acidification is the result of lactose fermentation by the associative growth of the two thermophilic, homofermentative, lactic acid bacteria, ST and LB. Increasing H^+ concentration in the yoghurt due to acid formation leads to low pH. Similar results were reported by Tiwari *et al.* (2015). The accumulation of organic acids contributes to the continuous reduction of pH throughout the storage. Results of the present study also showed that pH decreased with increasing inulin levels. Continued acidification during the storage can be linked to a progressive transformation of lactose into lactic acid. However, in yoghurts containing high soluble fibre components, other mechanisms could also promote a further increase in acidity level (Ramirez-Santiago *et al.,* 2010). According to Sendra *et al.* (2008), fibre addition itself contributes to a decrease in pH but the increased metabolic activity of the probiotics would also contribute to drastic pH reduction. Increasing inulin levels lead to high bacterial growth, consequently increasing acid production, which tends to lower the pH over time.

Changes in titratable acidity of yoghurts

The titratable acidity (TA) of CMY showed significant differences ($p < 0.05$) between control and inulin added yoghurt samples while there were no significant differences in acidity among treatments throughout 21 days of storage period (Figure 2a). Throughout the storage, the control sample reported the highest titratable acidity levels whereas, the 1% sample reported the lowest titratable acidity values.

According to data of BMY, there was no significant difference in TA between the control sample and inulin added yoghurt samples throughout the storage period. A significant ($p < 0.05$) increase of TA during storage was observed in all the treatments. The TA values were ranged from 0.94 - 0.95% and 0.96 - 0.97% during the storage period of CMY and BMY, respectively. The 1% inulin incorporated BMY sample showed a lower TA throughout the storage period while the 3% inulin incorporated BMY sample showed the highest TA. However, there

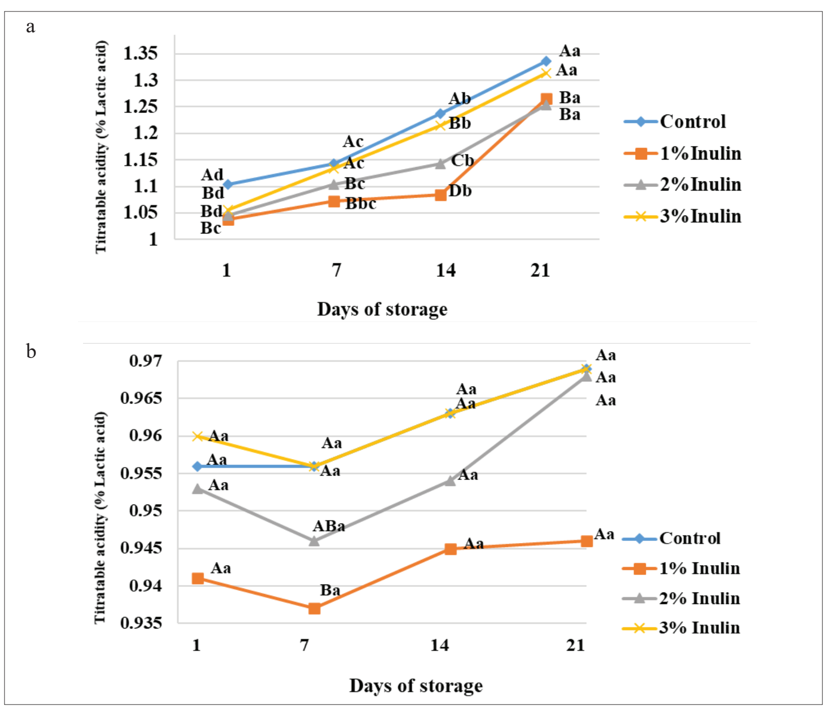

Figure 2: a) Variation in titratable acidity of CMY with different inulin concentration levels stored at 4 °C during 21 days of storage. b) Variation in titratable acidity of BMY with different inulin concentration levels stored at 4 °C during 21 days of storage.

ABC Means with different uppercase letters are significantly different between each treatment.
abc Means with different lowercase letters are significantly different between days of storage.

was an overall increase in titratable acidity while the pH dropped marginally throughout the storage period. These phenomena indicate continuously ongoing enzymic and metabolic activities of mixed starter cultures in low-temperature storage. These results agree with the findings of Canbulat and Ozcan (2015) who also reported a similar type of pH drop and proportional increase in titratable acidity with inulin incorporated set yoghurts. These results confirmed that there is no drastic change in both pH and titratable acidity with the incorporation of inulin into CM and BM yoghurts.

Variations of syneresis in yoghurt during storage

The results of CMY showed a significant ($p < 0.05$) difference in syneresis among treatments throughout the storage period (Figure 3a). On the 1st day, the highest syneresis was observed in the 3% CMY sample (32.51 ± 0.50%) and the lowest value in the 2% CMY (30.37 ± 0.38%). On the 21st day, the lowest syneresis was observed with the yoghurt produced with the 2% inulin CMY (14.3 ± 0.28%), while the highest value was observed in the 3% inulin CMY (16.3 ± 0.0%).

With BMY, the control sample showed significantly ($p < 0.05$) lowest syneresis (23.81 ± 0.08%) while the 3% inulin BMY sample gave the highest syneresis value (26.12 ± 0.10%) on the 1st day of storage. At the end of the storage period, the highest syneresis of 14.3 ± 0.28% was observed in the 3% inulin inclusion, while the lowest syneresis value of 9.27 ± 0.28% was observed in the 2% inulin incorporated BMY sample. A significant ($p < 0.05$) decrease of syneresis during the storage time was observed in all the BMY yoghurts throughout the storage period.

Inulin, when incorporated into the protein matrices, forms micro-crystals that interact with each other to form small aggregates. These aggregates are capable of encapsulating water, decreasing the whey removal (syneresis), while creating a smooth and creamy mouthfeel (Kip *et al.,* 2006). It was observed that the 2% inulin incorporated yoghurt samples displayed a similar pattern of behaviour to that of the control sample when compared with the other two concentrations. The 1% and 3% inulin incorporated CMY and BMY samples had higher syneresis levels than the 2% inulin yoghurts.

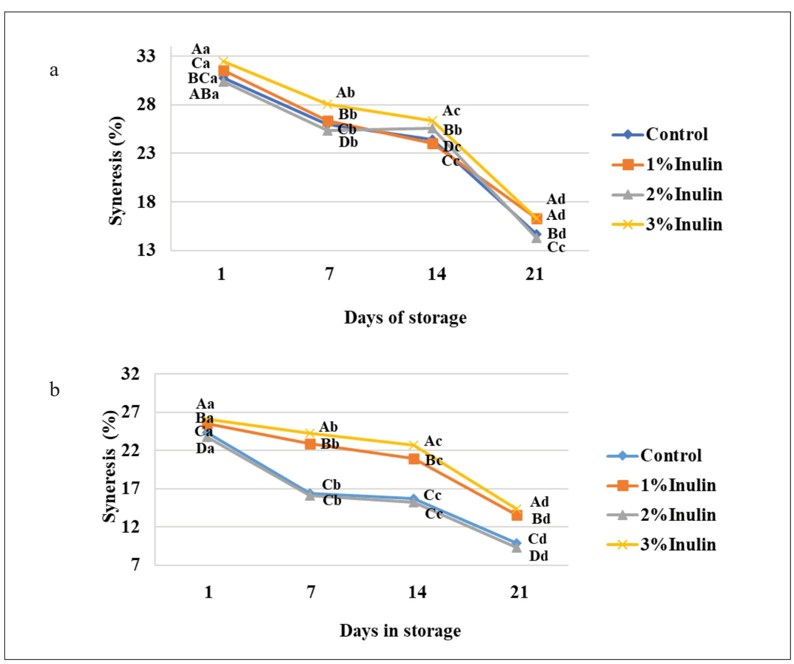

Figure 3: a) Variation in syneresis (%) of CMY with different inulin concentration levels stored at 4 °C during 21 days of storage. b) Variation in syneresis (%) of BMY with different inulin concentration levels stored at 4 °C during 21 days of storage.

ABC Means with different uppercase letters are significantly different between each treatment.
abc Means with different lowercase letters are significantly different between days of storage.

Perhaps, the 1% inulin concentration was insufficient to from a strong tridimensional network of of insoluble submicron crystalline inulin particles that entraps large amounts of water, hence the rate of syneresis was higher. In contrast, it has been observed that gel stiffness becomes lower when the inulin concentration is increased. Therefore, the highest syneresis levels observed by 3% inulin incorporated CMY and BMY samples can be explained in terms of the weak network resulting from decreased amounts of casein-casein bonds. It was observed that 2% inulin incorporation level has the ability to retain the strength and rigidity of the yogurt gel matrix without affecting its structure or the rate of whey removal in a drastic manner. Amatayakul *et al.* (2006) observed a reduction in whey separation when using exopolysaccharide (EPS) producing LAB starter cultures. YCX-11 the commercial starter culture used during this study can produce EPS which can facilitate the water binding capacity and improve the yoghurt microstructure (Han *et al.,* 2016). The resulting increase in inulin level could enhance the EPS production reducing the syneresis process compared to the control sample.

Table 4: Changes in the viscosity of CMY and BMY with refrigerated storage (4 °C) for 21 days.

Treatment	Viscosity ($x10^3$ mPa.s) (Mean ± SD)
Cow milk yoghurt	
Control	49.850 ± 0.07^{C}
1% inulin	46.75 ± 0.07^{D}
2% inulin	55.85 ± 0.07^{A}
3% inulin	54.05 ± 0.07^{B}
Buffalo milk yoghurt	
Control	70.85 ± 0.07^{B}
1% inulin	62.85 ± 0.07^{C}
2% inulin	86.45 ± 0.07^{A}
3% inulin	86.40 ± 0.06^{A}

Means in the same column without a common capital letter superscript significantly ($p<0.05$) differ for each treatment.

Viscosity changes in yoghurt

Overall, there seems to be an increased viscosity in BMY yoghurts when compared to CMY (Table 4). This phenomenon can be linked to the differences in the two types of milk. The average amount of total solids in buffalo milk is 16.3%, whereas in cow milk it is 12.8%. In addition, buffalo milk has high amounts of protein, ash, fat and lactose content (Abesinghe *et al.,* 2020).

Viscosity is directly related to the curd stability of yoghurt. According to Srisuvor *et al.* (2013), the curd stability of the yoghurt is one of the important physical properties in yoghurt matrices. Parameters such as total solids and protein content, the heating process, homogenization, the acidity of yoghurt, and the activity of starter culture bacteria generally influence curd stability. The structure and the chemical composition of the added ingredients mainly affect the strength of the casein aggregate. The generated branching structure enhances the protein and carbohydrate interaction giving the gel better curd stability. According to the results, 2% inulin incorporated yoghurt samples had a much higher viscosity when compared to 1% inulin incorporated yoghurts, though there was only a slightly lower viscosity at the 3% incorporation level compared to the 2% incorporation level. Viscosity reduction may be due to the incompatibility of gel structure formed with the interactions of inulin. Increasing inulin levels developed the casein aggregates only up to a certain limit, if inulin addition exceeded the critical value, layer separation could happen, and leading to lower viscosity. Similar observations were made by Helal *et al.* (2018), where the addition of 1% and 2% inulin increased the viscosity of a low-fat yoghurt by 14.7 % and 17.9% respectively, yet the addition of 3% inulin only showed an 8% increase in the viscosity of the yoghurt samples.

Sensory evaluation

According to Figure 4a), 2% CMY gave the highest preference for the odour, colour, texture, taste and overall acceptability while the control sample showed the lowest acceptability. Figure 4b) shows that panelist perception for odour and taste was higher in 3% IBM while colour, texture and overall acceptability were higher in 2% IBM. The control yoghurt sample had the lowest acceptability. However, the sensory results of the present study directly implied inulin addition improved the organoleptic properties of set yoghurt without affecting negatively the taste, odour and colour of the yoghurt. The addition of inulin influences the perception of 'creaminess.' Therefore, among these samples, with increasing inulin concentrations, the perception of 'creaminess' has increased as well. Increasing inulin levels lead to high acceptability via taste and odour while excess addition of inulin lowers the acceptability due to texture degradation. The acceptability may increase due to inulin addition. Inulin enhances the taste (due to fructose), odour, and colour of the yoghurt, though it adversely affects improving the texture by adding additional total soluble solids to yoghurt. Staffolo *et al.* (2004) stated that yoghurt supplemented with inulin maintained its colour stability during storage, and Seydim *et al.* (2005) stated that yoghurt made with inulin addition had more perceptive flavour and a smooth texture.

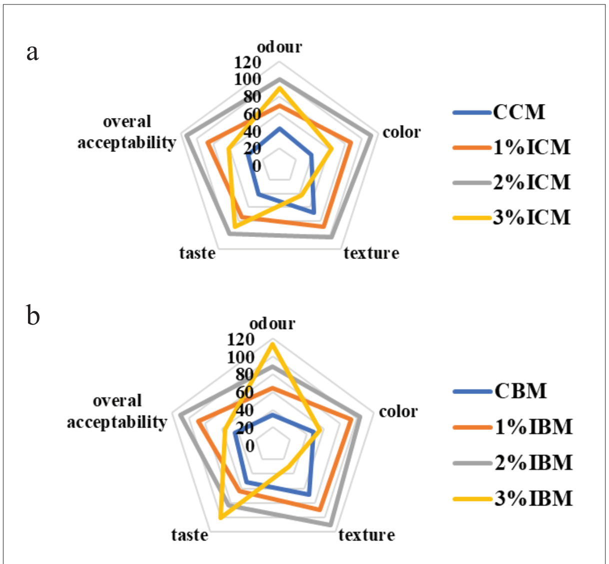

Figure 4: a) Variation of sensory properties in CMY with varying levels of inulin incorporation. CCM, 1% ICM, 2% ICM and 3% ICM samples contain varying levels of inulin, w/w = 0% (Control), 1%, 2%, and 3%, respectively. b) Variation of sensory properties in BMY with varying levels of inulin incorporation. CBM, 1% IBM, 2% IBM and 3% IBM samples contain varying levels of inulin, w/w = 0% (Control), 1%, 2%, and 3%, respectively.

CONCLUSION

The finding of the present study concludes that incorporation of inulin enhances the survival of *S. thermophiles*, *L. bulgaricus,* and *B. bifidum* in set type cow milk and buffalo milk yoghurts during refrigerated storage for 21 days. Cow milk was shown to be a better probiotic carrier than buffalo milk during the storage. It confirms that the addition of 2% inulin into cow milk and buffalo milk yoghurts results in low syneresis without altering the textural properties of the yoghurt matrices. The sensory evaluation results showed that additional inulin concentrations help to increase the 'creaminess' in yoghurts, hence indicating the 2% inulin incorporation as the optimal condition for yoghurt with both cow milk and buffalo milk.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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