Significance of storage study on α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity of fermented milk-based beverage

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Abstract: In the study, 3 potent *Lactobacillus* cultures were considered to prepare fermented milk-based beverage. Three products were prepared by using the three cultures namely *L. fermentum* (M2), *L. fermentum* (M7) and *L. paracasei* (M11) in 1:1 ratio. Based on sensory evaluation, combination of M2:M7 (1:1) product was selected during storage study (upto 9 days) determining pH, acidity (% lactic acid), α -amylase, α -glucosidase and pancreatic lipase inhibitory activities. Self-life study was also done for this selected final product. Decrease in pH and increase in acidity of product were also observed during storage. Slight reduction of *Lactobacillus* count was found (from 8.64 to 8.32 log cfu/mL) for both flavored and without flavored products (8.71 to 8.38 log cfu/mL). However, at 0 day, α -amylase, α -glucosidase and pancreatic lipase inhibitory activities of fresh products (fermented milk without flavor) were 51.49%, 38.10% and 98.71% respectively while product with added flavor also produced 41.28%, 69.55% and 96.12% inhibitions respectively. Best results were observed for lipase inhibitory activity throughout the storage periods for both the products.

Keywords: Fermented milk beverage, Lipase inhibitory activity, *Lactobacillus*, α -Amylase inhibitory activity, α -Glucosidase inhibitory activity, Self-life study

Introduction

The metabolic disorder is firmly identified with way of life and central obesity, filling in as a hazard factor for metabolic sicknesses, for example, type 2 diabetes and cardiovascular ailment (Naydenov et al. 2012). The quantity of individuals with diabetes has ascended from 108 million out of 1980 to 422 million of every 2014. The worldwide commonness of diabetes among adults more than 18 years old has ascended from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more quickly in center and low-pay nations (Emerging Risk Factors, 2010). Diabetes is a noteworthy reason for visual impairment, kidney failure, heart attacks, stroke and lower appendage removal. In 2016, an expected 1.6 million passing were legitimately brought about by diabetes. Another 2.2 million passing were inferable from high blood glucose in 2012. Practically, 50% of all deaths inferable from high blood glucose happen before the age of 70 years. WHO states that diabetes was the seventh driving reason for death in 2016. A large number of individuals around the globe live with diabetes. Cho et al. (2018) assessed that in 2017 there are 451 million (age 18-99/ years) individuals with diabetes around the world. These figures were relied upon to increment to 693 million by 2045. It was assessed that practically 50% surprisingly (49.7%) living with diabetes are undiscovered.

Diabetes mellitus (DM) is arriving at conceivably plague extents in India. DM keeps on expanding because of fast cultural and social changes, which incorporate ageing populations, expanding urbanization, dietary changes, diminished physical activity and unfortunate behavior (Tripathy et al. 2017). As per International Diabetes Federation estimates, around 415 million individuals had DM in 2015 and this number is relied upon to ascend to 642 million by 2040 (<http://www.idf.org/idf-diabetes-atlas-seventh-edition>).

There are different treatments available for preventing or managing diabetes, but many researches have been done to prove that probiotics have antidiabetic properties (Zhang et al. 2016). The term probiotic is a moderately new word signifying “for life” and it is as of now used to name microbes (friendly bacteria) related with valuable impacts for people and creatures (Metchnikoff, 1907). Fermentation is an old development and

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various civic establishments in different pieces of the world have utilized it to improve the capacity characteristics and nutritive estimation of numerous transitory sustenance, for example, milk, vegetables, meat, fish and grains. Lactic acid bacteria (LAB) is the major group of organisms that play key role in such kind of fermentation. Their utilization as dairy starter societies have turned into an industry where they are principally connected with assembling of matured dairy items, for example, cheddar, yogurt, buttermilk, sour cream, and so forth. LAB establish an enormous heterogeneous gathering of microorganisms of extensive significance for industrial applications, for example, the production of healthy sustenance and feeds. LAB share the property of changing over fermentable starches essentially to lactic acid (Ljungh and Wadstrom, 2006). Phenolic rich compounds from fruits generally shows the antioxidative and antidiabetic activity (Gajera et al. 2017). It has been proven through research that Jamun has antidiabetic properties. Jamun seed and pulp invigorated the release of insulin from the cultured Langerhans cells from both normal and diabetic rats (Baliga et al. 2013). The aim of the study is to evaluate the potentiality of α -Amylase inhibitory activity, α -Glucosidase inhibitory activity, Lipase inhibitory activity of milk-based beverage prepared with lactobacillus cultures.

Materials and Methods

Collection of LAB isolates and their maintenance

The LAB cultures used in the present study were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand (Table 1). The LAB cultures were propagated in sterilized reconstituted skim milk. The transfer was given every week during the study. Before use in the study, they were activated by propagation in MRS broth by daily transfers.

Product preparation

The pasteurized and standardized milk were purchased from Amul shop, Anand, India for making curd/dahi. Batches for preparation of the products using combination of selected cultures.

Combinations of cultures were taken as follows:

Heat treatment was given to milk at 90°C for 10 min
↓
Allowed to cool down (37°C)
↓
Transfer the milk to six sterile clean and dry beakers in equal volume (500 mL)
↓
Inoculation of selected cultures in combination to each beaker at the rate of 2% (v/v)
↓
Mix all the beakers thoroughly
↓
Incubation at 37°C for 9 h
↓
Curd formation
↓
Transfer all beakers to refrigerator at 6±2°C for 2 h
↓
Flavor [2% salt and cumin, 12% Jamun (*Syzygium cumini* L.) pulp] was added after blending curd

Combination-1: *L. fermentum* (M2) and *L. fermentum* (M7)

Combination-2: *L. fermentum* (M2) and *L. paracasei* (M11)

Combination-3: *L. fermentum* (M7) and *L. paracasei* (M11)

Milk without inoculation of cultures was taken as control.

Sensory evaluation

The products were subjected to the sensory evaluation by an expert trained panel of judges using nine-point hedonic scale (Rahman et al. 2009). The product was served at 10°C. The score for colour and appearance, flavour, body and texture and overall acceptability were recorded as nine-point hedonic scale (Rahman et al. 2009).

Evaluation of final product during storage

Product made by combination of M2 and M7 cultures were selected based on its acidity and palatability. This product was

Table 1 *Lactobacillus* cultures used in the study

Sr.No.	Culture name	Source of isolation	Selective Media	Growth conditions	Gene bank accession no.
1	<i>Lb. fermentum</i> (M2)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951094
2	<i>Lb. fermentum</i> (M7)	Fermented rice beverage	MRS Agar	37°C for 24h	MF951099
3	<i>Lb. paracasei</i> (M11)	Fermented rice beverage	MRS Agar	37°C for 24h	MG027695

also stored for 9 days and evaluated on 0th day, 3rd day, 6th day and 9th day by performing *in vitro* tests as follows.

Determination of pH

pH of control (milk), product without flavour (fermented milk) and product with flavour (beverage) were measured by using digital pH meter (Cole Parmer, India).

Determination of titratable acidity

The titratable acidity of products was estimated by the procedure described in (IS: 1479, Part I, 1960). Ten mL of sample was taken into a porcelain dish and an equal volume of distilled water was added to it. Then 1 mL of phenolphthalein indicator was added, and it was titrated against 0.1 [N] NaOH till the appearance of light pink colour, which persisted for 30 seconds in the solution. Titratable acidity was calculated by the following formula:

$$\text{Acidity (\% Lactic acid)} = \frac{9 \times V \times N}{X} \times 100$$

Where,

V = Volume (mL) of 0.1 [N] NaOH required for the titration

N = Normality of NaOH solution

X = Volume of milk (mL) taken for titration

Determination of *Lactobacillus* counts

Lactobacilli counts of bacterial cultures were determined as per the method described by IDF standards (117A:1989: doi: 10.1016/0168-1605(93)90043-G). One mL sample was taken out from the tubes and added to 9 mL phosphate buffer tubes (1:10 dilution). Similarly, required numbers of serial dilutions were prepared. One mL diluted sample from appropriate tubes was transferred to labelled petri plates (performed in triplicates), then 15-20 mL of melted and cooled (45°C) MRS agar was poured to respective petri plates. The content was mixed thoroughly by tilting and rotating the plates and allowed it to solidify and then additional layer (5-7 mL) of the same agar was poured completely over the solidified medium. Again, allow it to solidify, then incubated at 37°C for 24-48 h in inverted position. Typical colonies were calculated, and the counts were expressed as cfu/mL.

α -Amylase inhibitory activity

Reaction mixture containing 200 μ L phosphate buffer (100 mM, pH = 6.8), 10 μ L α -amylase (2 U/mL), and 100 μ L supernatant of different cultures was preincubated at 37°C for 5 min. Then, the 100 μ L 1% soluble starch (100 mM phosphate buffer, pH 6.8) was added as a substrate and incubated further at 37°C for 20 min; 1 mL of the DNSA colour reagent was then added and boiled for 10

min. The absorbance of the resulting mixture was measured at 540 nm using Systronic PC based double beam Spectrophotometer, 2206 (Telagari and Hullatti, 2015). The results are expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where,

A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

α -Glucosidase inhibitory activity

Reaction mixture containing 1.5 mL phosphate buffer (100 mM, pH = 6.8), 2.5 μ L α -glucosidase (1 U/mL), and 100 μ L supernatant of different cultures was preincubated at 37°C for 5 min. Then, 500 μ L P-NPG (5 mM) as a substrate was added and incubated further at 37°C for 10 min. The reaction was stopped by adding 100 μ L Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Systronic PC based double beam Spectrophotometer, 2206 following the method Shai et al. (2011). The results are expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where, A_s is the absorbance in the presence of test substance and A_c is the absorbance of control.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity was carried out by measuring the release of 4-methylumbelliferone (4MU) from the substrate 4-methylumbelliferyl oleate (4MUO) (Sergent et al. 2012) with some modifications. Reaction mixture containing 1.7 mL phosphate buffer (100 mM, pH = 6.8), 100 μ L supernatant of different cultures, 100 μ L 0.25 mM 4MUO, and 2 μ L pancreatic lipase (1 U/mL) were mixed and incubated at 37°C for 30 min. Then, 100 μ L 0.1 M sodium citrate was added to stop the reaction. Fluorescence from the release of 4MU was measured at 260 nm using Systronic PC based double beam Spectrophotometer, 2206 (Add reference). The percentage of pancreatic lipase inhibitory activity was calculated as follows:

$$\text{Pancreatic lipase inhibitory activity (\%)} = ((A_c - A_s) \times A_c) \times 100$$

Where, A_c is the absorbance of the control and A_s is the absorbance of the sample.

This test was performed for control (milk), product without flavour (fermented milk) and product with flavour (beverage) on different days of storage period.

Statistical Analysis

All the data were subjected to statistical analysis using one factor and two factor Completely Randomized Design (CRD) as per the requirement. The significance was tested on basis of comparison between calculated value and Table F-value. Standard error of mean value, co-efficient of variance (C.V.) and critical difference (C.D.) were determined. The values for microbial counts were log transformed before analysis.

Results and Discussion

Formulation of product

The fermented milk-based beverage appeared most appropriate for use as a vehicle for M2, M7 and M11 cultures. Therefore, three variants of the product were made using combinations of selected *Lactobacillus* cultures. To improve the palatability of the product, Jamun pulp, salt and cumin were added.

Sensory evaluation of products prepared using selected cultures

Pasteurized and standardized milk (4.5% Fat, 8.5% SNF) were used to prepare products using different combinations of selected cultures. Milks were inoculated with 2% rate of cultures and incubated for 9 h. When the curd was formed and transferred to refrigerator for 2 h and then the product was evaluated by an expert panel of judges for evaluating the flavour, body and texture, acidity, colour and appearance and overall acceptability using nine-point hedonic scale (Table 2).

The flavour score, body and texture score, acidity score, colour and appearance score and overall acceptability score of the variants of product were obtained in the range of 5.87 to 9, 7.62 to 8.62, 6 to 8.75, 7.5 to 8.25 and 6.12 to 8.5 respectively. The product prepared with M2 and M7 in combination showed distinctly higher scores. Hence these cultures (M2 and M7) were selected based on the sensory attributes and considered for further use.

Analysis of the selected product during storage

Product was prepared with M2 and M7 in combination, stored and studied for 9 days. Different testing parameters were determined during storage i.e. pH, acidity, *Lactobacillus* count, sensory quality, α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity of the product. Milk was used as control for all the experiments.

pH of the product

pH of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were checked at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage. Product was stored under refrigeration condition at $7 \pm 2^\circ\text{C}$. Changes were observed and is depicted in Fig. 1.

Based on statistical analysis, it was observed that there is no significant ($P > 0.05$) difference between flavoured and without flavoured product in terms of their pH. In fact, difference in pH of the product from 0th day to 9th day was also not significant ($P > 0.05$).

pH of stored buttermilk prepared by using five strains of *L. acidophilus* and one strain of *L. casei* were studied by

Fig. 1 Changes in pH during storage of product

Table 2 Sensory Score (9-point Hedonic scale) of three products

Variants of product	Flavour score	Body & Texture score	Acidity score	Colour & Appearance score	Overall acceptability score
M2-M7	8.81 $\pm$ 0.11	8.27 $\pm$ 0.19	8.58 $\pm$ 0.09	8.10 $\pm$ 0.11	8.44 $\pm$ 0.06
M2-M11	6.04 $\pm$ 0.13	7.75 $\pm$ 0.07	6.15 $\pm$ 0.08	7.79 $\pm$ 0.18	6.17 $\pm$ 0.05
M7-M11	7.00 $\pm$ 0.10	7.83 $\pm$ 0.11	6.60 $\pm$ 0.22	7.96 $\pm$ 0.15	7.19 $\pm$ 0.11

Each observation is mean of six replicates (n=6)

Nighswonger et al. (1996). On the 0th day, they observed pH of cultured buttermilk samples (n=3) were in the range of 4.5 to 4.6 and did not change during storage period (28 days) at 5°C. They also checked pH of stored yogurt in which initial pH of three batches of yogurt (made with culture CM-2) were 4.5 to 5 which decreased to 4.2 to 4.4 during storage period upto 28 days at 7°C. In our study, pH was dropped from 6.1 to 5.7 during storage period (upto 9 days) and similarly, pH of fermented milk did not change during storage and pH of the stored products which did not vary significantly during storage periods was also supported by Irigoyen et al. (2005).

Acidity of the product

Acidity of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes was observed and the trend is presented in Fig.2.

It was observed that there is no significant ($P>0.05$) difference between flavoured and without flavoured product in terms of their acidity. In fact, difference in acidity of the product from 0th day to 9th day was also not significant. But slight variation in acidity of the product was observed.

Mani-Lopez et al. (2014) had studied the titratable acidity of fermented products during storage of 35 days. They recorded the highest acidity values of yogurt containing *L. delbrueckii* ssp. *bulgaricus*. Lactic acid (%) ranged from 0.72 to 0.74% at initial stage and increased during storage period from 0.82 to 1.04%. Acidity in fermented milks without *L. delbrueckii* ssp. *bulgaricus* varied from 0.67 to 0.79% and from 0.74 to 0.83% at the end of the storage. Dave and Shah (1997) reported initial acidity in its probiotic yogurts and fermented milk of 0.68 and 0.77%, separately; they likewise found after 5 days at 4°C to estimations of 0.82 to 0.84%, and steadily qualities were observed (30 days) from the day 6 of storage. Another study by Donkor et al. (2007) evaluated the generation of acetic and lactic acids in milk fermented by *L. acidophilus* and *L. casei* related with yogurt bacteria during 28 days of cold storage. Korbekandi et al. (2009) announced comparative outcomes in yogurts with *L. casei*. In our study, acidity of fermented milk without flavor was ranged from 0.87 to 0.97% and acidity of fermented milk with Jamun flavor was ranged from 0.79 to 1.15%. These values are approximately similar with Mani-Lopez et al. (2014).

Analysis of product based on viable count of *Lactobacillus*

Lactobacillus count of fermented milk (product without flavour) and beverage (product with flavour) were taken at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes was observed and that trend is exhibited in Fig.3.

Fig. 2 Changes in acidity during storage

Fig. 3 Total viable count (cfu/ml) of *Lactobacillus* during storage

From the Fig. 3, *Lactobacillus* count in fermented milk and beverage was same at 3rd day and almost similar at 6th day of storage. Thus, Jamun did not show any inhibitory effect on viable counts of *Lactobacillus*. *Lactobacillus* count were higher in fresh product and then decreased gradually during its storage.

Viability of *L. acidophilus* and *L. casei* in fermented milk products (yogurt and buttermilk) were investigated by Nighswonger et al. (1996) during refrigerated storage at 5 to 7°C. They observed that total number of lactobacilli in cultured buttermilk containing different strains of *L. acidophilus* declined significantly during storage (28 days) at 5°C and increased slightly ($P>0.05$) beyond

7 days. They concluded that all strains retained viable population at 28th day of storage above 10⁶ CFU/g in the cultured buttermilk stored at 5°C. Significant ($P<0.05$) decrease of viable counts were also observed in stored yogurt at 7°C except for *L. acidophilus* L-1. They did this experiment on two different agar medium and observed different results. In our study, viable counts of *Lactobacillus* ranged from 8.71 to 8.38 log cfu/ml and 8.64 to 8.32 log cfu/ml were observed in case of fermented milk product without Jamun flavour as well as fermented milk with Jamun flavour (beverage) respectively.

α -Amylase inhibitory activity of the product

α -Amylase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes in inhibition rate was observed and the trend is presented in Table 3.

Significant difference ($P<0.05$) was observed between flavoured and without flavoured product in terms of their inhibition capacity. Results showed reduction in inhibition of α -amylase activity from 0th day to 9th day which suggest that diabetic patient needs to consume product when it is fresh for more benefits and inhibition was ranged from 51.49% to 30.48% for fermented milk (without flavoured product) and 41.28% to 3.27% for beverage (flavoured product). Vankudre et al. (2015) had performed comparative analysis of whey from cow and buffalo milk fermented with *Lactobacillus* species. They observed the highest α -amylase inhibition in product fermented by *L. delbeurkii* and noted that cow milk whey (46.59%) has shown higher α -amylase inhibitory activity than buffalo milk whey (32.29%).

α -Glucosidase inhibitory activity of the product

α -glucosidase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product. Product was stored at 7±2°C. Changes in inhibition rate was observed and trend is depicted in Table 3.

Significant difference ($P<0.05$) was observed between flavoured and without flavoured product in terms of their inhibition capacity. Reduction in inhibition of α -glucosidase activity was observed from 0th day to 6th day for fermented milk product and after that product lost the capacity to inhibit activity and therefore table shows negative activity on 9th day which suggest that diabetic patient can consume product till 6th day of the storage. On the other hand, flavoured product (beverage) showed better ability to inhibit α -glucosidase activity and difference in inhibition rate was not significant ($P>0.05$) during storage of it. In fact, beverage has capacity to inhibit α -glucosidase activity on 9th day was almost similar with capacity of without flavoured product to inhibit α -glucosidase activity on 0th day. Inhibition ranged from 38.10% to 17.89% for fermented milk (without flavoured product) and 69.55% to 34.73% for beverage (flavoured product).

Shori and Baba (2014) had checked α -glucosidase inhibitory activity of cow milk yogurt and camel milk yogurt during 21 days of storage period. They observed increased ($P<0.05$) inhibition of α -glucosidase for plain-camel milk yogurt (8.4% to 13.7%) and Allium sativum-camel milk yogurt (11.7% to 18.8%). Inhibition reduction was observed for plain-cow milk yogurt from 11.3% to 5.5%. α -Glucosidase inhibition by Allium sativum cow milk yogurt was reduced (15.2% to 12.8%) after 7 days of storage but it was still higher than inhibition by plain-cow milk yogurt during storage study. Ramchandran and Shah (2008) had also stated that fermented milk products have α -glucosidase inhibitory activities.

Pancreatic lipase inhibitory activity of the product

Pancreatic lipase inhibition ability of milk (control), fermented milk (product without flavour) and beverage (product with flavour) were measured at 0th day (very first day of product made), 3rd day, 6th day and 9th day of storage period of product at 7±2°C (Table 3).

Based on statistical analysis, there was no significant ($P>0.05$) difference observed between flavoured and without flavoured product. Fermented milk and beverage, both showed excellent ability to inhibit lipase activity till last storage day of the product about 85.93% and 81.88%, respectively. Inhibition ranged from 98.71% to 85.93% for fermented milk (without flavoured product)

Table 3 Evaluation of selected product for three enzyme inhibition activity during storage

Storage period (days)	Inhibition (%)					
	α -Amylase activity		α -Glucosidase activity		Lipase activity	
	Fermented milk	Beverage	Fermented milk	Beverage	Fermented milk	Beverage
0	51.49±0.23	41.28±0.18	38.10±0.01	69.55±0.00	98.71±0.04	96.12±0.06
3	29.71±0.22	20.50±0.31	19.97±0.02	63.20±0.01	95.63±0.01	91.99±0.00
6	26.85±0.07	07.97±0.14	17.89±0.02	59.65±0.00	87.10±0.21	84.13±0.24
9	30.48±0.05	03.27±0.12	-41.80±0.02	34.73±0.00	85.93±0.06	81.88±0.06

Each observation is mean of three replicates (n=3)

Fig. 4 Sensory evaluation of products during storage period

and 96.12% to 81.88% for beverage (flavoured product). Skim milk was fermented by using different strains of *L. helveticus*, *L. acidophilus*, *L. lactis*, *L. brevis*, *L. plantarum*, *L. delbrueskiissp bulgaricus* and *Pediococcus pentosaceus* for checking its lipase inhibitory activity at different temperatures (30°C, 33°C, 37°C and 42°C). Graph indicated that SC45 strain of *L. helveticus* has the highest ability to inhibit lipase at 42°C in compare to other strains ($49.75 \pm 2.13\%$). At 42°C, more than 30% lipase inhibition was reported for different strains such as SC77 and SC40 of *L. delbrueskiissp bulgaricus*, SC1 and SC63 of *L. acidophilus*, SC18 and SC34 of *L. lactis*, SC57 of *Pediococcus pentosaceus* and SC80 of *L. plantarum*. It was concluded that all different strains of LAB species have less or more capability to inhibit pancreatic lipase at different temperatures (Gil-Rodriguez and Beresford, 2019). α -Amylase and α -glucosidase play key role in diabetes, but obesity is the strongest risk factor for diabetes and therefore fermented milk-based product should have ability to inhibit pancreatic lipase which is responsible for fat metabolism.

Sensory evaluation of the product

The pasteurized and standardized milk was used to prepare product using M2 and M7 in combination. They were inoculated at 2% rate and incubated for 9 h. The curd was transferred to refrigerator for 2 h and then the product was judged by an expert panel of judges using nine-point hedonic scale. Sensory evaluation of final product (n=4) for different attributes was done during storage period using nine-point hedonic scale as mentioned above. It is depicted in Fig.4.

The flavour score, body and appearance score, acidity score, colour & appearance score and overall acceptability score of the products were obtained in the range of 7.22 to 9, 7 to 8.47, 7.47 to

8.22, 7.78 to 8.22 and 7.38 to 8.34 respectively. Highest score for all the sensory attributes was observed at the 3rd day of storage. Sensory scores indicated that beverage was acceptable to consume upto 9th day.

Conclusions

Product shows the highest α -amylase inhibitory activity, α -glucosidase inhibitory activity and pancreatic lipase inhibitory activity at 0th day for both flavoured (51.49%, 38.10% and 98.71% respectively) and without flavoured products (41.28%, 69.55% and 96.12% respectively). The results showed that microorganisms differed in growth, acid production and antidiabetic activities. Fermented milk without flavor (Jamun) showed slightly higher *Lactobacillus* growth in compare to flavored product (beverage). Jamun boost antidiabetic activity in α -glucosidase activity. Sensory results also indicated that beverage produced by using combination of cultures was acceptable upto 9th day. Further study is required to validate the health claim of antidiabetic activity of the fermented products.

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