Significance of storage study on \(\alpha\)-amylase inhibitory activity, \(\alpha\)-glucosidase inhibitory activity and pancreatic lipase inhibitory activity of fermented milk-based beverage

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Abstract: In the study, 3 potent \textit{Lactobacillus} cultures were considered to prepare fermented milk-based beverage. Three products were prepared by using the three cultures namely \textit{L. fermentum} (M2), \textit{L. fermentum} (M7) and \textit{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), \(\alpha\)-amylase, \(\alpha\)-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 \textit{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, \(\alpha\)-amylase, \(\alpha\)-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, \textit{Lactobacillus}, \(\alpha\)-Amylase inhibitory activity, \(\alpha\)-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 inerfable from high blood glucose in 2012. Practically, 50% of all deaths inerfable 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
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:

Table 1 Lactobacillus cultures used in the study

| Sr.No. | Culture name          | Source of isolation      | Selective Media | Growth conditions | Gene bank accession no. |
|--------|-----------------------|--------------------------|-----------------|-------------------|-------------------------|
| 1      | Lb. fermentum (M2)    | Fermented rice beverage  | MRS Agar        | 37°C for 24h      | MF951094                |
| 2      | Lb. fermentum (M7)    | Fermented rice beverage  | MRS Agar        | 37°C for 24h      | MF951099                |
| 3      | Lb. paracasei (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 Parmar, India).

**Determination of titratable acidity**

The titratable acidity of productswas 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:

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

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 (117 A: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 μL phosphate buffer (100 mM, pH = 6.8), 10 μL α-amylase (2 U/mL), and 100 μL supernatant of different cultures was preincubated at 37°C for 5 min. Then, 100 μL 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,

\[
\text{Inhibitory activity} \ (%) = (1 - \frac{A_s}{A_c}) \times 100
\]

Where,

As is the absorbance in the presence of test substance and Ac 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 μL α-glucosidase (1 U/mL), and 100 μL supernatant of different cultures was preincubated at 37°C for 5 min. Then, 500 μL 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 μL Na₂CO₃ (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,

\[
\text{Inhibitory activity} \ (%) = (1 - \frac{A_s}{A_c}) \times 100
\]

Where, As is the absorbance in the presence of test substance and Ac 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 μL supernatant of different cultures, 100 μL 0.25 mM 4MUO, and 2 μL pancreatic lipase (1U/mL) were mixed and incubated at 37°C for 30 min. Then, 100 μL 0.1 M sodium citrate was added to stop the reaction. Fluorescence from the release of 4MU was measured at 260nm using Systronic PC based double beam Spectrophotometer, 2206 (Add reference). The percentage of pancreatic lipase inhibitory activity was calculated as follows:

\[
\text{Pancreatic lipase inhibitory activity} \ (%) = \left(\frac{(A_c - A_s)}{A_c}\right) \times 100
\]
Where, Ac is the absorbance of the control and As 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, coefficient 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±2°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

**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±0.11     | 8.27±0.19            | 8.58±0.09     | 8.10±0.11                | 8.44±0.06                 |
| M2-M11              | 6.04±0.13     | 7.75±0.07            | 6.15±0.08     | 7.79±0.18                | 6.17±0.05                 |
| M7-M11              | 7.00±0.10     | 7.83±0.11            | 6.60±0.22     | 7.96±0.15                | 7.19±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 cultureCM-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.

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 10^6 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 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) | α-Amylase activity | β-Glucosidase activity | Lipase activity |
|-----------------------|--------------------|------------------------|-----------------|
|                       | Fermented milk     | Beverage               | Fermented milk  |
|                       | Inhibition (%)     |                        | Inhibition (%)  |
|                       |                    |                        | Fermented milk  |
|                       |                    |                        | Inhibition (%)  |
| 0                     | 51.49±0.23         | 41.28±0.18             | 38.10±0.01      |
| 3                     | 29.71±0.22         | 20.50±0.31             | 19.97±0.02      |
| 6                     | 26.85±0.07         | 07.97±0.14             | 17.89±0.02      |
| 9                     | 30.48±0.05         | 03.27±0.12             | -41.80±0.02     |

Each observation is mean of three replicates (n=3)
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. delbrueksii*sp 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 ± 2.13%). At 42°C, more than 30% lipase inhibition was reported for different strains such as SC77 and SC40 of *L. delbrueksii* sp 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-Rodríguez 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 up to 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 antiadiabetic activities. Fermented milk without flavor (Jamun) showed slightly higher *Lactobacillus* growth in compare to flavored product (beverage). Jamun boost antiadiabetic activity in α-glucosidase activity. Sensory results also indicated that beverage produced by using combination of cultures was acceptable up to 9th day. Further study is required to validate the health claim of antiadiabetic activity of the fermented products.

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**References**

Baliga MS, Fernandes S, Thilakchand KR, D’souza P, Rao S (2013) Scientific validation of the antidiabetic effects of Syzygiumjambolanum DC (black plum), a traditional medicinal plant of India. J Altern Complem Med 19: 191-197

Cho N, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B (2018) IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138: 271-281

Dave RI and Shah NP (1997) Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Int Dairy J: 31-41

Donkor ON, Nlimini SL, Stolic P, Vasiljevic T, Shah NP (2007) Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int Dairy J 17: 657-665

Emerging Risk Factors Collaboration (2010) Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. The Lancet 375: 2215-2222

Gajera HP, Gevariya SN, Hirpara DG, Patel SV, Golakiya BA (2017) Antidiabetic and antioxidant functionality associated with phenolic constituents from fruit parts of indigenous black jamun (*Syzygium cumini* L.) landraces. J Food Sci Technol 54: 3180-3191

Gil-Rodríguez AM and Beresford TP (2019) Lipase inhibitory activity of skim milk fermented with different strains of lactic acid bacteria. J Funct Foods 60: 103413. https://doi.org/10.1016/j.jff.2019.06.015

Indian Standards (1960) IS: 1479. Methods of testing for dairy industry. Indian Standards Institution, New Delhi

International Diabetes Federation (2016) IDF Diabetic Atlas 7th Edition. http://www.idf.org/idf-diabetes-atlas-seventh-edition
Irigoyen A, Arana I, Castiella M, Torre P, Ibanez FC (2005) Microbiological, physicochemical, and sensory characteristics of kefir during storage. Food Chem 90: 613-620
Korbekandi H, Jahadi M, Maracy M, Abedi D, Jalali M (2009) Production and evaluation of a probiotic yogurt using Lactobacillus casei ssp. casei. Int J Dairy Technol 62: 75-79.
Ljungh A and Wadstrom T (2006) Lactic acid bacteria as probiotics. Curr Issues Intestinal Microbiol 7: 73-90
Mani-López E, Palou E, López-Malo A (2014) Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. J Dairy Sci 97: 2578-2590
Metchnikoff E (1907) Lactic acid as inhibiting intestinal putrefaction. In: The prolongation of life: Optimistic studies. W. Heinemann, London, pp 161-183
Naydenov C, Anastasov A, Avramova M, Mindov I, Tacheva T, Tolekova A, Vlaykova T (2012) Probiotics and diabetes mellitus. TJS 10:300-306
Nighswonger BD, Brashears MM, Gilliland SE (1996) Viability of Lactobacillus acidophilus and Lactobacillus casei in fermented milk products during refrigerated storage. J Dairy Sci 79: 212-219
Nikbakht E, Khalesi S, Singh I, Williams LT, West NP, Colson N (2016) Effect of probiotics and synbiotics on blood glucose: A systematic review and meta-analysis of controlled trials. European J Nutr 57: 95-106
Rahman IE, Dirar HA, Osman MA (2009) Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures. African J Food Sci 3: 398-405
Ramechandran I. and Shah NP (2008) Proteolytic Profiles and Angiotensin I Converting Enzyme and α Glucosidase Inhibitory Activities of Selected Lactic Acid Bacteria. J Food Sci 73: 75-81
Sergent T, Vanderstraeten J, Winand J, Beguin P and Schneider YJ (2012) Phenolic compounds and plant extracts as potential natural anti-obesity substances. Food Chem 135: 68-73
Shai L, Magano SR, Lebolo SL, Mogale AM (2011) Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. J Med Plants Res 5: 2863-2867
Shori AB and Baba AS (2014) Comparative antioxidant activity, proteolysis and in vitro α-amylase and α-glucosidase inhibition of Allium sativum-yogurts made from cow and camel milk. J Saudi Chem Soc 18: 456-463
Telagari M and Hullatti K (2015) In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. Indian J Pharmacol 47: 425
Tripathy JP, Thakur JS, Jeet G, Chawla S, Jain S, Pal A, Prasad R, Saran R (2017) Prevalence and risk factors of diabetes in a large community-based study in North India: Results from a STEPS survey in Punjab, India. Diabetol Metab Syndrome 9: 8. https://doi.org/10.1186/s13098-017-0207-3
Vankudre M, Balpande A, Athale M (2015) Comparative analysis of α-amylase inhibition and antioxidant activity of whey from cow and buffalo milk fermented with Lactobacillus species. Bio Sci Biotech Res Comm 8: 25-28
Zhang Q, Wu Y, Fei X (2016) Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. Medicina 52: 28-34