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Standardization and Evaluation of Synbiotic Yoghurt

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A B S T R A C T

The present investigation was carried out to standardize and evaluate plain, probiotic, prebiotic and synbiotic yoghurts using selected yoghurt commercial starter culture, probiotic cultures (L. *acidophilus*, L. *casei*, L. *plantarum*, and L. *rhamnosus*), and prebiotic fibre sources *viz*., oat flour, inulin and banana flour. Based on the acceptance of the product from the sensory point of view, the overall acceptability score for plain and probiotic yoghurt prepared with 2 % inoculation was higher than 1 % inoculation. Prebiotic yoghurt prepared with 0.5% oat flour, 1.0% inulin and 1.5% banana flour obtained higher scores when compared to the other prebiotic samples. Synbiotic yoghurt developed with 0.5% oat flour scored highest values among the other oat flour added samples. Synbiotic yoghurt developed with 1.0% inulin scored maximum values than other inulin added samples. Synbiotic yoghurt developed with 1.5% banana flour scored more values than the other banana flour added samples. The pH of the yoghurt samples was ranged from 3.20 to 3.41, total solid content 8.54 to 8.79 %, protein content 3.51 to 3.78 % and fat content 3.01 to 3.07 % in all the yoghurt samples. The viability of probiotics and total bacterial population was found to be highest in the treatment of T_{10} (Yoghurt commercial starter culture- 1 % + *L. rhamnosus*- 1 %+ 0.5 % oat flour). Growth of probiotic bacteria have been shown to be enhanced with prebiotics. It was found that till the end of storage there was no yeast and mold growth in the dilution factors 10^{-1} and 10^{-2}. Hence it may be inferred that the prepared yoghurts can be deemed fit for storage up to 21 days at 4°C.

Introduction

The most popular milk-based products in the world are fermented milk products due to their high nutritional and therapeutic values (Aneja *et al.*, 2002). They play an important role in the human daily diet. Some of the popular fermented milk products are yoghurt, shrikhand, dahi, lassi, kumiss, kefir and acidophilus milk. Yoghurt is one of the most popular products worldwide which have great consumer acceptability due to its health benefits other than its basic nutrition, which is produced by lactic acid fermentation of milk, generally by mixed cultures of lactic acid bacteria (Chandan, 2006). Yoghurt becomes a functional food upon incorporating probiotics-live microorganisms which when adequately administered confer health benefits. Prebiotics are fermentable fibres that nourish beneficial gastrointestinal microflora enhancing the functionality of probiotics (Stephanie *et al.,* 2011). Production of functional foods containing prebiotic ingredients is an area that has dominant featuring in the food industry in recent years, and a very promising market, not only for economic reasons but by scientific evidence of its benefits (Burgain *et al*., 2011). Aiming to satisfy this new market, some prebiotics, have been incorporated into a wide variety of foods and beverages that are part of a natural diet as dairy products, breads, cereals, dietary supplements, and others. Through this growth consumers can appreciate tasty meals while promoting beneficial effects to their own health (Coman *et al*., 2012).

Consumers often take in moderate levels of prebiotics naturally from many fruits and vegetables including leeks, artichokes, tomatoes, chicory, onion, garlic, banana, and asparagus, but the levels of prebiotics in these food sources are generally too low to exhibit any significant effect on the composition of intestinal microflora (Manning and Gibson, 2004). Thus, prebiotics are commercially extracted and concentrated from fruits and vegetables through the hydrolysis of polysaccharides from dietary fibers or starch, or through enzymatic generation. Prebiotics are mixtures of indigestible oligosaccharides, except for inulin which is a mixture of fructooligo and polysaccharides (Gibson and Fuller, 2000).

Addition of inulin to food products like low fat yoghurt not only makes them rich in dietary fiber, but also improves their physicochemical and sensory properties by imparting fat like textures. Other effects of inulin include providing a sense of fullness, lowering serum cholesterol, and incidence of colon cancer (Jenkins *et al.,* 1999). Unripe banana have attracted much attention recently due to its prebiotic potential activity (Niba and Rose, 2003) and which has shown beneficial effects in disease preventions including modulation of Glycemic index (GI), diabetes, cholesterol lowering capability, and weight management (Thakorlal *et al.,* 2010). Nutritionally, oats are an excellent source of soluble fiber in the form of beta-glucan, alpha tocopherols, B vitamins, minerals, proteins, and plant fats (Rashid *et al.,* 2007).

Combination of both probiotics and prebiotics is known as Synbiotics. The combination of both probiotic and prebiotic has the ability to heal and regulate the intestinal flora, particularly after the destruction of microorganisms following antibiotic, chemotherapy, or radiation therapies. Incorporation of prebiotics into yoghurt containing probiotics would probably lead to healthier yoghurt. The beneficial nutritional properties of oats, banana, and inulin have attracted attention from researchers and have resulted in the food industry wishing to use those as a food ingredient more extensively than today and therefore more studies are needed in this area. The aim of this study was to develop synbiotic yoghurt, by combining the health benefits of probiotic culture with the prebiotic through fermentation.

Materials and Methods

Selection and preparation of starter culture

All the selected lyophilized starter cultures *viz.,*Yoghurt Commercial Starter Culture (NCDC 260)**,** *Lactobacillus acidophilus* (NCDC 14), *Lactobacillus casei* (NCDC 298), *Lactobacillus plantarum* (NCDC 25), *Lactobacillus rhamnosus* (NCDC 19) obtained from NCDC, NDRI, Karnal were revived in sterile 12 per cent reconstituted skim milk by incubating at 37±2 ºC for 24-48 h. The cultures were then activated in MRS broth and incubated for 12-16 h at 37±2 ºC which exhibited good growth. All the activated cultures were stored in 30 % glycerol stock at -20 ºC. The activated cultures were inoculated in 10 ml of sterile 12 % reconstituted skim milk and incubated for 12-16 h at 40±1ºC which was used as starter for yoghurt preparation.

Preparation of plain yoghurt

Toned milk (3.0 % fat, 8.5 % SNF) was heated to 90°C for 3-5 min for pasteurization, and then cooled to 40°C. It was then inoculated with 1 % / 2 % yoghurt commercial starter culture (NCDC 260). Yoghurt samples were elaborated of quantities of 50 ml for each sample and the experiments were carried out. The inoculated milk was incubated to 40°C until a pH of 4.5 was attained (approximately 6-8 h). When the pH end point was achieved, the yoghurt samples were cooled at 4°C, stored at the same temperature throughout the period of post-acidification (21 days).

Preparation of probiotic yoghurt

Toned milk (3.0 % fat, 8.5 % SNF) was heated to 90°C for 3-5 min for pasteurization, and then cooled to 40°C. It was then inoculated with 1 % starter culture in 1:1 ratio (0.5 % of a yoghurt commercial starter culture (NCDC 260) and 0.5 % of probiotic cultures including *Lactobacillus acidophilus* (NCDC 14)/ *Lactobacillus casei* (NCDC 298)/ *Lactobacillus plantarum* (NCDC 25)/ *Lactobacillus rhamnosus* (NCDC 19)) and 2 % starter culture in 1:1 ratio (1 % of a yoghurt commercial starter culture (NCDC 260) and 1 % of probiotic cultures including *Lactobacillus acidophilus* (NCDC 14)/ *Lactobacillus casei* (NCDC 298)/ *Lactobacillus plantarum* (NCDC 25)/ *Lactobacillus rhamnosus* (NCDC 19)). Yoghurt samples were elaborated of quantities of 50 ml for each sample and the experiments were carried out. The inoculated milk was incubated to 40°C until a pH of 4.5 was attained in approximately 6-8 h. After clean curdling and attainment of required pH, the yoghurt samples were cooled at 4°C, stored at the same temperature throughout the period of post-acidification (21 days).

Processing of oat flour

The oat grains (moisture content of 10 %) were ground into fine oat flour using a mixer grinder and the oat flour was stored in airtight containers (PET) and was used as a substrate in different concentrations 0.5 %, 1.0 %, and 1.5 %. The oat flour was blended with 50 ml of distilled water to make into slurry. The slurry was then used in the preparation of synbiotic yoghurt.

Processing of banana powder

The raw bananas were peeled and cut into small pieces. These pieces were water blanched with 0.2 % KMS at 92°C for 3 min and dehydrated for 10-12 h at 45-50°C to a moisture content of 8.5 %. Then the slices were ground and powdered by the use of mixer grinder and the banana powder was stored in airtight containers (PET) and was used as a substrate in different concentrations 0.5 %, 1.0 %, and 1.5 %. The banana flour was blended with 50 ml of distilled water to make into slurry. The slurry was then used in the preparation of synbiotic yoghurt.

Preparation of prebiotic yoghurt

Toned milk (3.0 % fat, 8.5 % SNF) was heated to 90°C for 3-5 min for pasteurization, at the end point of heating process the prebiotic fibre (oat flour/ inulin/ banana flour) was added, and then cooled to 40°C. It was then inoculated with 2 % of a yoghurt commercial starter culture (NCDC 260).

Yoghurt samples were elaborated of quantities of 50 ml for each sample and the experiments were carried out. The inoculated milk was incubated to 40°C until a pH of 4.5 was attained in approximately 6-8 h. When the pH end point was achieved, the yoghurt samples were cooled at 4°C, stored at the same temperature throughout the period of post-acidification (21 days).

Preparation of synbiotic yoghurt

Toned milk (3.0 % fat, 8.5 % SNF) was heated to 90°C, at which stage the oat flour/ banana flour/ inulin were added. The oat flour, banana flour, and inulin (Plate 6) were added each at 0.5 %, 1.0 %, and 1.5 %. Then the milk samples were cooled down to 40°C for inoculation.

The samples were inoculated with yoghurt culture (1 %) and probiotic culture (1 %). The inoculated samples were mixed thoroughly and dispensed in 50 ml polystyrene cups with lids then incubated at 40 °C until the pH dropped to 4.5. The fermentation was stopped by transferring the cups immediately to the refrigerator maintained at 4°C.

Analysis of physico - chemical characteristics and nutritional components of yoghurt

The physico- chemical and nutritional components *viz.,* colour, pH, acidity, syneresis, total solid content, protein, fat, and crude fibre of the prepared yoghurt samples were analysed

Colour value

Colour is an important attribute in food. It is the first characteristic perceived by the consumers and thus often influences the consumer's preference. Colour measurements (L* a* b* values) of the developed products were determined using the Hunter Laboratory chromometer (Model # Lovibond RT 100) with the Lovibond RT Colour software (Version 3.0). The pH of the sample was estimated by the method described by Jayaraman (1985). Acidity was estimated by the method given by (Saini *et al.*, 2001).

The extent of syneresis of yoghurt after 12 h of cold storage was analyzed using a slight modification of the centrifugation method of Amatayakul *et al.,* (2006) and Purwandari *et al.,* (2007). The total solids of the samples were determined by the method (Richardson, 1985). Protein was analyzed by the amount of nitrogen available in the sample by micro kjeldhal method (Ma and Zuazaga, 1942). The fat content of the sample was estimated by the method described by Cohen (1917). The crude fibre content was determined by the method described by Maynard (1976).

Storage studies of yoghurt samples

The plain, prebiotic and synbiotic yoghurt were prepared and stored in polystyrene cups under refrigerated condition (4°C). The prepared yoghurt samples conforming to the different treatments were studied for storage stability during a storage period of 21 days under refrigeration temperature (4°C). The chemical, microbial and sensory analysis of stored samples were analysed at regular intervals of once in 7 days during the period of storage of 21 days.

Organoleptic Evaluation

Organoleptic evaluation of the yoghurt samples was done by 20 semi trained judges at regular intervals of 7 days during 21 days of storage study. The nine points hedonic rating scale was used to grade plain, probiotic, pebiotic and synbiotic yoghurt samples with the scores ranging from like extremely to dislike extremely (Amerine *et al.*, 1965).

Microbial studies

Viability of probiotic bacteria in yoghurt

Viability of probiotic bacteria was determined during the storage by serial decimal dilutions in sterilized phosphate buffer at 7 days intervals. One gram of yoghurt sample was weighed and serially diluted up to 10^{12} dilutions. The probiotic bacterial population was enumerated using the pour plate technique (using MRS media) after incubation of plates at 40°C for 48 h.

Microscopic examination of probiotic bacterial cultures

The selected actively grown starter cultures such as yoghurt commercial starter culture (NCDC 260), *L.acidophilus* (NCDC 14), *L. casei* (NCDC 298), *L. plantarum* (NCDC 25) and *L. rhamnosus* (NCDC 19) were mounted on glass slide (1.27 cm x 1.27 cm) covered with cover slip. The purity and morphological examination of the starter cultures were studied through the Binocular Microscope.

Microbial quality of yoghurt samples

The microbial load of the stored samples was enumerated by serial dilution and plating technique using phosphate buffer for dilution as described by Istavankiss (1984) and assessed on 0, 7, 14 and 21 days of storage. MRS agar medium was used for probiotic count, nutrient agar medium for total plate count, potato dextrose agar medium for yeast and mold count and violet red bile agar medium for coliforms and *E. coli* count.

Statistical analysis

The data obtained were subjected to statistical analysis to find out the impact of treatments, storage periods and their interaction on the quality of plain, probiotic, prebiotic and synbiotic yoghurt. Factorial Completely Randomized Design (FCRD) was applied for the statistical analysis using Statistical Analysis System (SAS) software (Rangaswamy, 1995).

Results and Discussion

Standardization of plain, probiotic, prebiotic and synbiotic yoghurt

Organoleptic characteristics of plain, probiotic, prebiotic and synbiotic yoghurt

Table 1 revealed that the overall acceptability score for plain and probiotic yoghurt prepared with 2 % inoculation (T_1b, T_2b, T_3b, T_4b and T_5b) was higher than 1 % inoculation. Prebiotic yoghurt prepared with 0.5% oat flour, 1.0% inulin and 1.5% banana flour (T_6a, T_{11}b and T_{16}c) obtained higher scores when compared to the other prebiotic samples. Synbiotic yoghurt developed with 0.5% oat flour (T_7a, T_8a, T_9a and T_{10}a) scored highest values among the other oat flour added samples. Synbiotic yoghurt developed with 1.0% inulin (T_{12}b, T_{13}b, T_{14}b and T_{15}b) scored maximum values than other inulin added samples. Synbiotic yoghurt developed with 1.5% banana flour (T_{17}c, T_{18}c, T_{19}c and T_{20}c) scored more values than the other banana flour added samples.

Similarly Shireesha *et al.,* (2014) also developed synbiotic yoghurt with both probiotic and prebiotic incorporation. The probiotics used were *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as live starter cultures and the prebiotic was Fructo-oligosaccharide. In addition to this sweet potato was also added for the stabilization of yoghurt. The product formulations were done in three combinations with different proportions of ingredients. The proportions prepared as T_1 (Plain yoghurt), T_2 (Plain Yoghurt + Fructo-oligosaccharide), T_3 (Live

Active Culture 3 %). The results from sensory analysis showed that the product T3 yoghurt sample was the best suited with correct proportion of ingredients and 30 % sweet potato was suited for good quality product development.

Colour measurements of plain, probiotic, prebiotic and synbiotic yoghurt

From the table 2, it was observed that the colour of yoghurt samples were between the range of 176.64 (L*), 50.00 (a*), -72.45 (b*) and 371.65 (L*), 67.02 (a*), -92.04 (b*) with the minimum and maximum levels recorded. The L* and a* values were reduced when prebiotic sources such as oat flour, inulin and banana flour added into the yoghurt formulation. The results indicated that the plain and probiotic yoghurts were whiter in colour when compared to the prebiotic and synbiotic yoghurts.

Chemical characteristics of plain, probiotic, prebiotic and synbiotic yoghurts

The pH of the yoghurt samples was ranged from 3.20 to 3.41 (Table 3). Similarly Mahrous *et al.,* (2014) also developed synbiotic yoghurt with the addition of probiotic and prebiotic combination and observed pH value which is ranged from 4.65 to 4.50. The titratable acidity ranged from 1.03 to 1.18 % in all the prepared yoghurts. Similar results were reported by Gueimonde *et al.,* (2004) who developed 14 commercial fermented milks and determined acidity values that varied from 0.79 to 1.16 %.

The syneresis content was in the range of 4.13 to 4.84 %. The total solid content of all the yoghurt samples was in the range of 8.54 to 8.79 % with the minimum and maximum levels recorded. Amatayakul *et al.,* (2006) also reported that syneresis values >5 % and 8.0-8.5 % total solids in yoghurts and fermented with exopolysaccharide producing starter cultures.

Nutritional components of plain, probiotic, prebiotic and synbiotic yoghurt

Table 4. shows the nutritional components of yoghurt samples. The protein content of all the yoghurt samples was in the range of 3.51 to 3.78 %. Noelia *et al.,* (2014) also formulated synbiotic non-fat yoghurt by combining prebiotics and probiotic microorganisms and found that the protein content was ranged between 3.90 and 4.04 %. The data obtained revealed that the fat content of all the yoghurt samples ranged from 3.01 to 3.07 %. Similar results were reported by Sucre and Ruiz (2013) who developed caramel flavored yoghurt and observed that the fat content was ranged from 1.7 to 3.2 per cent.

The crude fibre content was in the range of 0.05 % to 0.15 % in all the yoghurt samples. It was observed that the synbiotic yoghurt prepared from oat flour (YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 0.5 % oat flour) obtained the higher crude fibre content when compared to other yoghurt samples. Shireesha *et al.,* (2014) also prepared synbiotic yoghurt with both probiotic and prebiotic fibre sources and found that the fibre content were ranged from 0.6 to 0.11 per cent.

Storage qualities of plain, probiotic, prebiotic and synbiotic yoghurt

Changes in colour measurements of plain, probiotic, prebiotic and synbiotic yoghurt

The L* values ranged from 437.24 to 668.03, a* values ranged from 82.57 to 159.85 and the b* values ranged from -42.87 to -68.01. Colour differences were observed because of the combination of different strains and the addition of various prebiotic sources. From the results of statistical analysis, it was concluded that significant difference in colour was found within the treatments, between treatments and storage period.

Changes in chemical characteristics of plain, probiotic, prebiotic and synbiotic yoghurt during storage

pH

The tendency of pH of yoghurt to decrease during storage. The pH of the yoghurt samples was found to decrease to a range of 3.15 to 3.32 after 7 days of storage, 3.08 to 3.23 after 14 days of storage and from 3.01 to 3.19 after 21 days of storage. Statistical analysis recorded significant difference in terms of pH among the different treatments and also in terms of storage period. Declining pH can be attributed to the residual post acidification activity of microorganisms. These results were in conformity with the finding of Akalin (2007) who reported that the pH values of yogurt containing prebiotics was found to be lower than yoghurt without supplementation during refrigerated storage for up to 28 days. Boeni and Pourahmad (2012) prepared yoghurt containing 1 % inulin and found that during cold storage, pH of the sample decreased. These findings are in line with the results of present study, corroborating the residual acidification during storage.

Acidity

The acidity of yoghurt samples were increased to1.11 to 1.23 % after 7 days, 1.17 to 1.28 % after 14 days, and 1.21 to 1.31 % at the end of the storage after 21 days (Table 6). Similar to pH changes, the observed acidity changes could be attributed to the residual activity of the lactic acid bacteria. Statistical analysis revealed significant difference in terms of acidity among the different treatments and also during the storage period. These results agree with the findings of Hassan and Amjad (2010) who prepared yoghurt with two different types of starter cultures; *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* at 3, 4 and 5 % concentrations. The results showed that the protein, acidity (0.87-1.60 percent) and total solid were slightly increased while pH and moisture values gradually decreased during the storage period of 12 days.

Total solids

There was a corresponding increase in total solid content in the entire yoghurt samples during storage. The total solid content was 8.73 to 8.98 % at the end of storage period of 21 days. Similar results were reported by Shilpi and Kumar (2013) who prepared mango soy fortified probiotic yoghurt by using blends of toned milk, soymilk and mango pulp which had total solids 8.7%.

Changes in nutritional components of plain, probiotic, prebiotic and synbiotic yoghurt during storage

Protein

While analyzing the changes in the protein content of the yoghurt samples conforming to the different treatments during storage revealed a slight increase in protein content in all the yoghurt samples. The increase in protein content of all the yoghurt samples during storage ranged from 3.59 to 3.93 % after 7 days, from 3.69 to 4.18 % after 14 days and from 3.75 to 4.23 % after 21 days of storage. Among all the yoghurt samples, the maximum range were recorded in the synbiotic yoghurt prepared from oat flour (YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 0.5 % oat flour) (4.23 %) followed by synbiotic yoghurt prepared from using banana flour (YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 1.5 % banana flour) (4.19 %) when compared to other yoghurt samples. Statistical analysis recorded significant difference in terms of protein among the different treatments and also in terms of storage period. Similar results were reported by Bibiana *et al.,* (2014) prepared yoghurts with the starter cultures of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* and found that the protein content of the sample ranged from 3.02 % to 6.14 % in yoghurt and were significantly different ($p<0.05$).

Fat

Fat content was observed to be reduced in all the yoghurt samples. The loss of fat content during storage ranged from 2.98 to 3.05 % after 7 days, 2.93 to 3.03 % after 14 days and from 2.92 to 2.99 % at the end of storage period of 21 days. Statistical analysis revealed significant difference in terms of fat content among the different treatments and also during the storage period. Schneeman and Onyeneke (2013) also studied that the fat content in yoghurt samples ranged from 3.12 to 3.25 g/100g.

Crude fibre

Based on the results, the crude fibre content was decreased during storage of yoghurt samples at the end of storage period (21 days). The crude fibre content during storage ranged from 0.04 to 0.13 % after 7 days, 0.03 to 0.12 % after 14 days and from 0.02 to 0.10 % at the end of storage period of 21 days.From the results, it was concluded that the synbiotic yoghurt prepared from using oat flour (YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 0.5 % oat flour) had the higher crude fibre content during storage followed by other synbiotic combinations. From the statistical analysis, it was concluded that significant difference in crude fibre was found within the treatments, between the treatments and storage period. Schneeman (2002) also reported that the crude fibre of the yoghurt samples ranged from 0.21 % to 0.51 % and it differed significantly ($p<0.05$) among the treatments and storage period.

Changes in organoleptic characteristics of plain, probiotic, prebiotic and synbiotic yoghurt during storage

The overall acceptability of plain, probiotic, prebiotic and synbiotic yoghurt samples during storage is discussed here. The synbiotic yoghurt prepared from oat flour (T_{10}) had the highly acceptable score of 8.2. The prebiotic yoghurt made with inulin (T_{11}) has minimum acceptability score value of 6.9 during storage. Noh *et al.,* (2013) also reported an overall acceptability of 6.7 which is based on the sensory attributes of colour, texture, and flavour on the 21st day of storage. The shelf-life of the of the product could be maintained steadily when it was stored at refrigerated temperature (4°C) and the product could be stored satisfactorily for a period of 25 days.

Viability of probiotic bacteria and Total bacterial population in plain, probiotic, prebiotic and synbiotic yoghurt during storage

Initially the viability of the probiotics was found to be high in the treatment T_{10} (Yoghurt commercial starter culture- 1 %+ *L. rhamnosus*- 1 %+ 0.5 % oat flour) 51×10^{12} cfu ml^{-1} and the lowest probiotic count was seen in T_1 (Yoghurt commercial starter culture (NCDC 260) – 2 %) 16×10^{12} cfu ml^{-1}. During storage also there was good viability of probiotic count because there existed of suitable pH (3.8 to 4) for the growth of probiotics. The probiotic count ranged from

9×10^{12} cfu ml^{-1} to 29×10^{12} after 21 days of storage period. These results agree with the findings of Tungrugsasut *et al.,* (2012) who reported that the initial count of probiotics in probiotic yoghurt made with 3 per cent probiotic was 126x10^{8}cfu ml^{-1} and that of 4 per cent probiotic was 129x10^{8}cfu ml^{-1}. At 30 days of storage, the counts of probiotic in 3 per cent probiotic and 4 per cent probiotic were 61x10^{8}cfu ml^{-1}and 64x10^{8}cfu ml^{-1} respectively.

Initially the viability of total bacterial population was found to be highest in the treatment of T_{10} (Yoghurt commercial starter culture- 1 % + *L. rhamnosus*- 1 %+ 0.5 % oat flour) 69×10^{8} cfu ml^{-1} and the lowest total bacterial population was seen in T_1 (Yoghurt commercial starter culture (NCDC 260) – 2 %) 25×10^{8} cfu ml^{-1}. During storage the viability of total bacterial population was slightly decreased and ranged from 17×10^{8} to 39×10^{8} cfu ml^{-1} after 21 days of storage period. Similar results were reported by Sadek *et al.,* (2004) also reported that all the yoghurt samples had more than 99 % of viable bacteria during storage. Variable results have been found when incorporating of prebiotic in plain and probiotic yoghurt. Growth of probiotic bacteria such as *L. acidophilus L. plantarum* and *L rhamnosus* have been shown to be enhanced with prebiotics.

Table.1 Organoleptic characteristics of plain yoghurt

Plain yoghurt	**T_1**		-	-	-	-	-	-	-	-	-	-
T_1 - Plain yoghurt - Yoghurt commercial starter culture (NCDC 260) – 1 % / 2 % **a - 1 % inoculation** **b -2 % inoculation**	**a**	**b**	-	-	-	-	-	-	-	-	-	-
Mean scores	8.0	8.8	-	-	-	-	-	-	-	-	-	-
Probiotic yoghurt	**T_2**		**T_3**		**T_4**		**T_5**		-	-	-	-
T_2 -Probiotic yoghurt - YCSC (NCDC 260) – 0.5 %/ 1 % + *L. acidophilus* (NCDC 14) – 0.5 % / 1 %. **T_3 -Probiotic yoghurt - YCSC (NCDC 260) – 0.5 % / 1 % + *L. casei* (NCDC 298) –0.5 % / 1 %** **T_4 -Probiotic yoghurt - YCSC (NCDC 260) – 0.5 % / 1 % + *L. plantarum* (NCDC 25) – 0.5 % / 1 %** **T_5 -Probiotic yoghurt - YCSC (NCDC 260) – 0.5 % / 1 % + *L. rhamnosus* (NCDC 19) – 0.5 % / 1 %** **a - 1 % inoculation** **b -2 % inoculation**	a	b	a	b	a	b	a	b	-	-	-	-
Mean scores	7.6	8.6	7.8	8.6	7.4	8.4	7.8	8.8	-	-	-	-

Prebiotic yoghurt	**T_6**			**T_{11}**			**T_{16}**			**-**	**-**	**-**
T6 -Prebiotic yoghurt - YCSC (NCDC 260) - 2 % + 0.5 % oat flour (a)/ 1 % oat flour (b)/1.5 % oat flour (c) T11 -Prebiotic yoghurt- YCSC (NCDC 260) - 2 % + 0.5 % inulin (a)/ 1 % inulin (b)/ 1.5 % inulin (c) T16 -Prebiotic yoghurt- YCSC (NCDC 260) - 2 % + 0.5 % banana flour (a)/ 1 % banana flour (b)/ 1.5 % banana flour (c)	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**-**	**-**	**-**
Mean scores	8.2	7.4	7.8	7.4	8.0	7.2	7.6	7.4	8.2	-	-	-
Synbiotic yoghurt- oat flour	**T_7**			**T_8**			**T_9**			**T_{10}**		
T7 -Synbiotic yoghurt - YCSC (NCDC 260) - 1 % + L. acidophilus (NCDC 14) - 1 % + 0.5 % oat flour (a)/ 1 % oat flour (b)/ 1.5 % oat flour (c) T8 -Synbiotic yoghurt - YCSC (NCDC 260) - 1 % + L. casei (NCDC 298) - 1 % + 0.5 % oat flour (a)/ 1 % oat flour (b)/ 1.5 % oat flour (c) T9 -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + L. plantarum (NCDC 25) - 1 % + 0.5 % oat flour (a)/ 1 % oat flour (b)/ 1.5 % oat flour (c) T10 -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + L. rhamnosus (NCDC 19) - 1 % + 0.5 % oat flour (a)/ 1 % oat flour (b)/ 1.5 % oat flour (c)	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
Mean scores	8.8	7.8	7.6	8.4	7.8	7.4	8.6	7.6	7.4	8.8	8.0	7.8
Synbiotic yoghurt- inulin	**T_{12}**			**T_{13}**			**T_{14}**			**T_{15}**		
T_{12} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. acidophilus* (NCDC 14) - 1 % + 0.5 % inulin (a)/ 1 % inulin (b)/ 1.5 % inulin (c) T_{13} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 %+ *L. casei* (NCDC 298) - 1 % + 0.5 %	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**

inulin (a)/ 1 % inulin (b)/ 1.5% inulin (c) **T_{14} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. plantarum* (NCDC 25) - 1 % + 0.5 % inulin (a)/ 1 % inulin (b)/ 1.5 % inulin (c)** **T_{15} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 0.5 % inulin (a)/ 1 % inulin (b)/ 1.5 % inulin (c)**												
Mean scores	7.6	8.2	7.4	7.6	8.2	7.2	7.4	8.0	7.6	7.8	8.2	7.6
Synbiotic yoghurt- banana flour	**T_{17}**			**T_{18}**			**T_{19}**			**T_{20}**		
T_{17} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *Lactobacillus acidophilus* (NCDC 14) - 1 % + 0.5 % banana flour (a)/ 1 % banana flour (b)/ 1.5 % banana flour (c) **T_{18} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. casei* (NCDC 298) - 1 % + 0.5 % banana flour (a)/ 1 % banana flour (b)/ 1.5 % banana flour (c)** **T_{19} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. plantarum* (NCDC 25) - 1 % + 0.5 % banana flour (a)/ 1 % banana flour (b)/ 1.5 % banana flour (c)** **T_{20} -Synbiotic yoghurt- YCSC (NCDC 260) - 1 % + *L. rhamnosus* (NCDC 19) - 1 % + 0.5 % banana flour (a)/ 1 % banana flour (b)/ 1.5 % banana flour (c)**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
Mean scores	7.8	8.2	8.6	7.4	7.8	8.4	7.8	7.6	8.6	7.8	8.0	8.8

Table.2 Colour measurements of plain, probiotic, prebiotic and synbiotic yoghurts

Treatments	Colour value			Treatments	Colour value		
	L*	a*	b*		L*	a*	b*
T_1	363.94	66.46	-90.53	T_{11}	203.41	52.60	-88.57
T_2	365.81	65.68	-89.75	T_{12}	205.59	51.49	-85.56
T_3	371.65	67.02	-92.04	T_{13}	203.89	52.92	-88.46
T_4	369.58	65.57	-90.27	T_{14}	208.47	52.66	-87.33
T_5	367.42	64.31	-89.81	T_{15}	202.57	50.00	-86.52
T_6	235.59	51.97	-81.00	T_{16}	187.19	58.22	-79.72
T_7	232.73	51.51	-79.25	T_{17}	185.69	57.34	-85.44
T_8	233.21	50.81	-78.06	T_{18}	190.10	55.97	-79.73
T_9	241.48	54.61	-87.72	T_{19}	184.17	59.77	-87.28
T_{10}	230.20	50.10	-72.45	T_{20}	176.64	56.70	-81.49

Table.3 Chemical characteristics of plain, probiotic, prebiotic and symbiotic yoghurt

Treatments	pH	Acidity (%)	Syneresis (%)	Total solids (%)	pH	Acidity (%)	Syneresis (%)	Total solids (%)	Total solids (%)
T_1	3.36	1.03	4.13	8.59	T_{11}	3.33	1.07	4.57	8.54
T_2	3.21	1.18	4.28	8.71	T_{12}	3.35	1.15	4.54	8.65
T_3	3.29	1.12	4.46	8.69	T_{13}	3.27	1.13	4.50	8.59
T_4	3.20	1.17	4.57	8.73	T_{14}	3.28	1.12	4.47	8.63
T_5	3.25	1.15	4.35	8.77	T_{15}	3.36	1.09	4.65	8.71
T_6	3.41	1.08	4.84	8.61	T_{16}	3.39	1.05	4.59	8.60
T_7	3.29	1.13	4.41	8.73	T_{17}	3.23	1.14	4.73	8.72
T_8	3.38	1.07	4.63	8.68	T_{18}	3.37	1.09	4.61	8.67
T_9	3.37	1.09	4.59	8.75	T_{19}	3.32	1.11	4.58	8.76
T_{10}	3.39	1.05	4.68	8.79	T_{20}	3.35	1.07	4.54	8.78

Table.4 Nutritional components of plain, probiotic, prebiotic and symbiotic yoghurt

Treatments	Nutritional components			Treatments	Nutritional components		
	Protein (%)	Fat (%)	Crude fibre (%)		Protein (%)	Fat (%)	Crude fibre (%)
T_1	3.51	3.01	0.05	T_{11}	3.53	3.05	0.09
T_2	3.63	3.06	0.07	T_{12}	3.69	3.02	0.11
T_3	3.55	3.05	0.06	T_{13}	3.59	3.05	0.08
T_4	3.64	3.04	0.05	T_{14}	3.68	3.03	0.09
T_5	3.74	3.00	0.06	T_{15}	3.76	3.02	0.12
T_6	3.65	3.06	0.13	T_{16}	3.63	3.04	0.08
T_7	3.73	3.05	0.14	T_{17}	3.71	3.05	0.10
T_8	3.67	3.07	0.13	T_{18}	3.62	3.06	0.09
T_9	3.61	3.06	0.12	T_{19}	3.59	3.05	0.08
T_{10}	3.78	3.03	0.15	T_{20}	3.75	3.04	0.09

Microbiological quality of plain, probiotic, prebiotic and synbiotic yoghurt during storage

Based on the observation it was found that till the end of storage there was no yeast and mold growth in the dilution factors 10^{-1} and 10^{-2}. Hence it may be inferred that the yoghurt can be deemed fit for storage upto 21 days at 4°C.

In conclusion, plain yoghurt prepared with 2 % inoculation using commercial yogurt starter culture was most preferred when compared to the 1 % level. The probiotic yoghurt prepared with 1 % yoghurt commercial starter culture and 1 % probiotic culture of *L. rhamnosus* was most acceptable than other probiotic cultures *viz., L. acidophilus, L. casei,* and *L. plantarum.* The prebiotic yoghurt made with 1 % yoghurt commercial starter culture and 0.5 % oat flour was found to be most acceptable when compared to the other yoghurts. The synbiotic yoghurt made with 1 % yoghurt commercial starter culture, 1 % probiotic culture (*L. rhamnosus*) and 0.5 % oat flour was most acceptable rather than other probiotic and prebiotic combinations.

The pH, acidity, syneresis, protein, fat and crude fibre content of the synbiotic yoghurt was high among all the other yoghurt samples. Storage studies revealed that the plain, probiotic, prebiotic and synbiotic yoghurt samples had good storage stability during the period of study (21 days) at refrigerated temperature (4°C). The viability of probiotics was good in synbiotic yoghurt made with both the probiotic (*L. rhamnosus*) and prebiotic fibre source (0.5 % oat flour) up to 21 days of storage. The synbiotic yoghurt could thus serve as functional food and good carrier for probiotic to gut and improve gut health with regard to a possible role to reduce the risk of diseases.

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