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Evaluation of Various Physico-Chemical Properties of *Hibiscus sabdariffa* and *L. casei* Incorporated Probiotic Yoghurt

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Abstract: The present investigation was carried out to examine the effect of *Hibiscus sabdariffa* Calyx extract on the physico-chemical properties, sensory attributes, texture and microbial analysis of *L. casei* incorporated in probiotic yoghurt after manufacture and during storage. Incorporation of *Hibiscus sabdariffa* Calyx extract into the probiotic yoghurt resulted into decrease in coagulation time by 25 min. The pH ranged from 4.39 to 4.59, TA 0.81 to 1.14%, moisture 3.05 to 3.37 g%, syneresis 18.85 to 24.90 mL/50 g of sample, % inhibition 12.32 to 59.43, TS 21.27 to 24.90 g% and β-galactosidase activity 1.041 to 3.277. The protein content ranged between 4.11 and 4.14 g% while the fat content ranged between 3.43 and 3.49 g%. No major changes in sensory evaluation were observed on the day of manufacture and during storage for 7 days. Sabdariffa added yoghurt showed a higher score in almost all sensory attributes. Microbial analysis showed a total plate count ranging from 1.8×10^4 to 1.85×10^7 cfu mL^{-1}. Yeast and mold counts were negligible in the Sabdariffa yoghurts. Thus the study concludes that incorporation of *Hibiscus sabdariffa* extract in yoghurt improved the total antioxidant property, organoleptic qualities and decreased the exudation of whey proteins (Syneresis). Thus, *Hibiscus sabdariffa* Calyces has beneficial influence on the quality of *L. casei* incorporated probiotic yoghurt.

Key words: Antioxidant activity of yoghurt, *Hibiscuss sabdariffa*, *L. casei*, texture analysis, β-galactosidase assay

INTRODUCTION

The significant growth in the consumption of yogurt has been reported in many countries during the past decade (Tamime, 2002). The increase in yogurt consumption is probably due to its high organoleptic quality and potential health-enhancing effects. The nutritional benefit of yogurt is due to milk constituents and exogenous living lactic acid bacteria. There have been many attempts to make yogurt-like products from a variety of food resources.

Probiotics are defined as living microbial feed supplements added to the diet and provide beneficial effects on the host by improving their intestinal microflora balance (Fuller, 1989). Probiotic bacteria beneficially affect human health by improving the gut microbiota balance and the defenses against pathogens. Additional health benefits attributed to probiotics are the stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities (Heenan *et al*., 2004). Two other important criteria to determine the efficacy and the success of the product containing probiotics are the acceptance of the product by the consumers and the survival of probiotic microorganisms during its production (Heenan *et al*., 2004). In general, the food industry has applied the recommended level of 10^6 cfu/g of probiotic bacteria at the time of consumption for *Lactobacillus acidophilus*, bifidobacteria and other probiotic bacteria (Boylston *et al*., 2004).

Yoghurts are traditionally made with cultures composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. However, in the last decade, yoghurts have increasingly been enriched with various species of Bifidobacterium, cultures of *Lactobacillus acidophilus* and more recently, *Lactobacillus casei*. This trend is a response by industry and consumers to data on potential probiotic properties of these species.

L. casei is the latest of the probiotic adjunct to be added to yoghurts or in probiotic cultures (McCann *et al*., 1996). In addition to its health potential, *L. casei* is of interest since it seems to be relatively stable during storage (Nighswonger *et al*., 1996), compared to the other yogurt cultures and particularly bifidobacteria (Shah *et al*., 1995).

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Hibiscus sabdariffa L. (family Malvaceae), commonly known as roselle, red sorrel, or karkadè, is widely grown in Africa, South East Asia and some tropical countries of America (Farombi-Olatunde, 2003). The fleshy flowers provide a soft drink consumed as a cold or hot beverage (Babalola *et al*., 2001; Ali-Bradeldin *et al*., 2005). The daily consumption of this beverage, called flor de jamaica in Mexico and sobo in Nigeria is high because of the sensation of freshness conveyed.

Pharmacological actions have been identified in *H. sabdariffa* L. flowers, petals and seeds. The healthy effects are numerous: cardioprotective action (Chang-Che *et al*., 2003) reduction of urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium, phosphate, antihypertensive action; effectiveness against low-density lipoprotein oxidation and hyperlipidemia (Chang-Che *et al*., 2003).

In the present study, an effort has been made to develop *H. sabdariffa* and *L. casei* incorporated yoghurt product and evaluation of its various physico-chemical properties.

MATERIALS AND METHODS

Selection and maintenance of culture: *Lactobacillus acidophilus* NCDC 013, *Streptococcus thermophilus* NCDC 075, *Lactobacillus bulgaricus* NCDC 25, *Lactobacillus casei* NCDC 297 were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal. The method of culture control/maintenance is very important in yoghurt manufacture and for this reason stock (mother) cultures are best maintained individually, rather than mixed. Mother starter was prepared as follows: Fresh or reconstituted skim milk (Sagar Brand) was autoclaved at 15 psi for 15 min, cooled to 41°C and inoculated with 1% inoculum. Cultures of *L. acidophilus*, *L. bulgaricus*, *L. casei* and *S. thermophilus* were incubated at 37°C. Normal coagulation occurred in 12-18 h, at this time the cultures were cooled to 5°C. Transfers were made once in two days. Activation of cultures was done by three successive transfers in 10% sterile skim milk (10 g skim milk powder dissolved in 100 mL distilled water and sterilized at 15 psi for 15 min). The culture was transferred (4% of total volume of milk) and incubated at 37°C for 8 h or till setting.

Preparation of aqueous extract of *Hibiscus sabdariffa* Calyx: The present research study was carried out at Food Biotechnology Laboratory, Sardar Patel University during November 2007 to May 2008. *Hibiscus sabdariffa* calyces were obtained from Dabhou, Anand District. Powdered form of *Hibiscus sabdariffa* calyces (2 g) were extracted under reflux with sterile water (50 mL) at 100°C for 4 h. The resulting extract was filtered using cheesecloth and the sub-filtrate was sterilized using a 45 μm Millipore filter to remove microbial contaminants. The resulting filtered extract was reconstituted with sterile water to 40 mg mL^{-1} final concentration and kept in air-tight container at 4°C prior to use as a flavouring agent.

Control yoghurt preparation: Standardized Amul milk (4.5% fat, 8.5% SNF) was taken in a stainless steel vessel. Milk was first boiled at 45-50°C. Three percent Skim milk powder was added to standardized milk to increase the solids- not- fat content and 7% sugar was added. The contents were mixes properly. Heat treatment was given at 90°C for 5 min in a waterbath. The temperature was monitored constantly by a thermometer followed by cooling to 42-44°C. Activated culture of *L. acidophilus*, *L. bulgaricus*, *L. casei* and *S. thermophilus* were added at a concentration of 4%. The content was mixed properly. The vessel was covered with aluminum foil and incubated in an incubator at 37°C till acidity reached to 0.9%. It was then placed under refrigeration to cool to 5-7°C.

Experimental yoghurt I and yoghurt II: In experimental yoghurt I and II the aqueous extract of *Hibiscus sabdariffa* calyces was added in a quantity of 1, 100 mL and 2, 100 mL of yoghurt, respectively after inoculation. The remaining process was same as for the control yoghurt.

Physico-chemical analysis: The pH values of yoghurt samples were determined with a digital pH meter. Moisture content, titrable acidity and total solids were measured according to the method of AOAC (1990). Titrable acidity was expressed in terms of mL/100 g. Fat content was measured by Gerber method using gerber butyrometer tubes (British Standard Institute, 1988). Protein estimation was carried out by Kjeldahl method (AOAC, 1990).

Syneresis determination: Syneresis was determined by dispensing 125 g of yogurt into a cheesecloth line funnel placed on top of a graduated cylinder. The amount of whey, milk serum in mL was measured after manufacture and on days 3, 5 and 7 of storage at refrigeration temperature (8±1°C). The amount of whey drained off (expressed as mL per 125 g of sample) was calculated as the syneresis index.

β-galactosidase assay: The activity of (3-galactosidase in each of the yogurt products after manufacture and during storage at 8±1°C was carried out according to Hughes and

Hoover (1995). To 1.0 mL of yogurt sample, 4.0 mL of 0.05 M sodium phosphate buffer (pH 7.0) and chloroform (0.2 mL) were added. The mixture was then centrifuged at 29,000 g for 15 min. After the removal of supernatant, the cell debris was mixed 1% Triton X -100 (1 mL) in sodium phosphate buffer (2 mL) and incubated at 37°C for 15 min. This was followed by the addition of 15 mM 0-nitrophenyl-p-galactoside substrate (1.0 mL) and further incubation at 37°C for 10 min. The reaction was stopped by the addition of 1.0 mL of 0.1 M Na_2CO_3 followed by centrifugation at 29,000 g for 15 min to remove cell debris. The absorbance of the recovered supernatant was then measured at 405 nm using a UV-visible spectrophotometer to determine the concentration of 0-nitrophenol liberated by extrapolation from a standard o-nitrophenol curve (50-3000 μmol). One unit of β-galactosidase activity is defined as the amount of enzyme required to liberate 1 μmol of o-nitrophenol per min at 37°C.

Textural analysis: Texture analyzer TA.XT express (Stable Micro Systems, UK.) was used to measure and analyze fundamental, empirical and imitative tests related to texture analysis, material science as well as rheology of solid, semi-solid, viscous liquid, powder and granulate materials. Parameters analyzed were stickiness, firmness, consistency, index of viscosity and distance.

Microbiological analysis: Microbiological analysis of control yoghurt and experimental yoghurt I and II was carried out at storage temperature of yoghurt samples at 4°C for a period of 7 days. Sampling was carried out on 0th and 7th day. At each sampling day, 10 g samples were collected aseptically and blended with 90 mL of 0.1% sterile peptone water and submitted to serial dilutions. Yeast and mould count was carried out according to Bacteriological Analytical Manual (1998). Enumeration of yeast and moulds were carried out on Potato Dextrose Agar (PDA). After incubation time, the colonies were counted and the results were expressed as colony forming units per gram of product (cfu g^{-1}). Lactobacillus MRS Agar, pH 6.5±0.2 was used for total lactobacilli count. 10^{-1}, 10^{-2} ….10^{-6} dilutions were prepared from the samples. From this 0.1 mL of aliquote was taken from each dilution (control and experimental yoghurts) on the solidified MRS agar plates and spread the sample on the respective plates. These plates were incubated at 37°C for 48 h.

Radical scavenging antioxidant activity of yoghurt samples by DPPH method: The antioxidant activity of yoghurt samples was determined as the ability of each extract to scavenge. 1,1 diphenyl - 2 - picrylhydrazyl (DPPH) radicals, by using a previously described method by Brand-Williams *et al.* (1995). One g of yoghurt was taken in a tube and it was homogenized by vortexing. It was then filtered using muslin cloth. For the assay, 50 μL filtrate was taken in a test tube and the volume was made up to 1 mL with methanol. Three milliliters of the freshly prepared solution of DPPH (200 μM) in methanol was added to the sample tube and mixed vigorously for 15 sec. The sample tube was then kept at 37°C for 20 min. The absorbance of the sample was measured at 517 nm by using UV spectrophotometer (Hitachi 220S). For control, in 1 mL methanol, 3 mL DPPH reagent was added and the colour was read immediately at 517 nm. Methanol was used as blank. The DPPH radical scavenging effect was expressed as % inhibition.

Sensory evaluation: For the sensory evaluation of control and experimental yoghurt, the panel consisted of six judges. All of them were research students of the Foods Nutrition/Food biotechnology division. Organoleptic studies included the appearance, consistency, texture, flavor and overall acceptability. Sensory evaluation was carried out on the day of manufacturing. The scoring of samples was done by using the modified composite scoring test having overall acceptability score of 25.

Data analysis: The results obtained are analyzed using SPSS for windows 10.0. Differences between variables were tested for significance by using one-way analysis of variance (Duncan).

RESULTS AND DISCUSSION

pH and titratable acidity: The mean values of pH of control and experimental yoghurts after manufacture and during the 7 days storage period at 4°C temperature are presented in Table 1. Comparing the pH values of non-sabdariffa (control) and sabdariffa yoghurts (yoghurts I and II) on the day of manufacture indicated that the highest pH value (4.59) was observed in yoghurt prepared by adding 1% of sabdariffa extract that showed a significant difference with the pH value of control yoghurt (4.47). The yoghurt prepared by addition of 2% sabdariffa showed a pH value of 4.57 which was significantly higher than the control yoghurt and non-significantly lower than experimental yoghurt I. On the 3rd day of storage similar trend was observed. The pH value (4.57) of yoghurt I was significantly higher compared to yoghurt II (4.47) and the control yoghurt (4.23). On the 7th day of storage, pH value (4.47) of control yoghurt was significantly higher compared to yoghurt I and II (4.39). Iwalokun and Shittu (2007) observed pH range of 4.41-4.53 in their four

Table 1: Different parameters of the control and experimental yoghurts after manufacture and during 7 day of storage period at refrigeration temperature (4°C)

		Storage days			
Parameters	Samples	0	3	5	7
pH	C	4.47±0.033^{bc}	4.23±0.033^{aa}	4.53±0.033^{bd}	4.47±0.014^{bcd}
	I	4.59±0.006^{be}	4.57±0.014^{be}	4.55±0.028^{be}	4.39±0.003^{ab}
	II	4.57±0.014^{ce}	4.47±0.014^{bcd}	4.59 ±0.005^{ce}	4.39±0.003^{ab}
Titrable acidity	C	0.81±0.008^{aa}	1.13±0008^{bd}	1.17±0.005^{ce}	1.19±0.005^{ce}
	I	0.81±0.005^{aa}	1.03±0.005^{bb}	1.07±0.015^{cc}	1.07±0.008^{cc}
	II	0.83±0.002^{aa}	1.04±0.003^{bb}	1.08±0.002^{cc}	1.14±0.002^{dd}
Moisture	C	5.16±0.10^{ce}	3.03±0.031^{aa}	3.22±0.011^{bbc}	3.22±0.017^{bbc}
	I	3.37±0.011^{cd}	3.05±0.028^{aa}	3.20±0.008^{bbc}	3.24±0.026^{bc}
	II	3.21±0.014^{bbc}	3.11±0.005^{aa}	3.26±0.005^{cc}	3.21±0.005^{bbc}
Syneresis	C	22.50±0.29^{be}	19.23±0.15^{ab}	23.73±0.15^{eg}	27.93±0.033^{dl}
	I	20.75±0.14^{bc}	18.95±0.028^{aa}	22.93±0.033^{df}	21.90±0.057^{cd}
	II	18.85±0.086^{aa}	24.90±0.057^{ch}	22.80±0.12^{be}	22.90±0.057^{bf}
Inhibition (%)	C	11.50±0.011^{aa}	25.23±0.017^{de}	23.92±0.046^{cd}	20.11±0.15^{bc}
	I	12.32±0.040^{ab}	39.04±0.014^{di}	32.29±0.29^{ch}	30.17±0.36^{bg}
	II	28.12±0.0141^{af}	59.43±0.020^{dl}	48.55±0.17^{ck}	42.69±0.13^{bj}
Total solids	C	25.47±0.017^{dg}	24.95±0.028^{ef}	21.45±0.028^{bb}	21.21±0.008^{aa}
	I	24.85±0.086^{df}	24.46±0.023^{ce}	23.03±0.020^{bc}	21.40±0.028^{ab}
	II	24.90±0.057^{df}	24.47±0.014^{ce}	23.72±0.014^{bd}	21.27±0.011^{aa}

Values are Mean±SD of three observations. Values bearing different superscript within the column are significantly different ($p<0.05$), C: Control yoghurt, I: Experimental yoghurt I, II: Experimental yoghurt II

yoghurt brands after manufacture were within the ranges reported for yoghurts in previous studies (Haddadin, 2005; Muhammad *et al*., 2005; Salvador and Fizman, 2004). Thus the decrease in pH values in all the three products was not sufficient to impair the survival of probiotic micro-organisms present in the yoghurt products. Thus, it can be observed that addition of hibiscus sabdariffa did not affect the pH of the products.

The mean values of titratable acidity of control and experimental yoghurts after manufacture and during the 7 days storage period at 4°C temperature are presented in Table 1. The titratable acidity of control yoghurt, yoghurt I and yoghurt II ranged from 0.81 to 1.19%, 0.81 to 1.07% and 0.83 to 1.14% of lactic acid, respectively on the day of manufacture to 7 days of storage. Comparing the titratable acidity of non-sabdariffa (control) and sabdariffa yoghurts (yoghurts I and II) on the day of manufacture indicated that the highest titratable acidity (0.83%) was observed in yoghurt prepared by adding 2% of sabdariffa extract that showed a non-significant difference with the control yoghurt and yoghurt I (0.81%). These results are partially supported by Inoue *et al.* (1998) who reported no appreciable change in acidity during storage of yoghurt.

Iwalokun and Shittu (2007) observed TA values of 0.9-1.2% lactic acid on days 0 and 3, while > 1.2-1.39% values recorded from day 5 are lower than 1.9 reported for labneh (Haddadin, 2005) but comparable to 1.31-1.51% reported for skim milk yoghurt by Muhanimed *et al.* (2005). Titratable acidity as high as 6.8% lactic acid has also been reported for skimmed yoghurt after 91 days of storage at 10°C (Salvador and Fizman, 2004). The present study observed TA values of 0.81-1.19% lactic acid which is near to the acceptable range set by the International dairy guidelines.

Moisture: The mean values of moisture content of control and experimental yoghurts after manufacture and during the 7 days storage period at 4°C temperature are presented in Table 1. Comparing the moisture content of non sabdariffa (Control) and sabdariffa yoghurts (yoghurts I and II) on the day of manufacture indicated that the highest moisture value (5.16) was observed in control yoghurt. On the 7th day of storage were observed that is the moisture content of yoghurt I was 3.24 g % which was significantly higher compared to the control (3.22) and yoghurt II (3.21).

Syneresis: α-lactalbumin and β-lactoglobulin from yoghurts is a well-established rheological factor responsible for decrease nutritional quality and organoleptic failure of yoghurt products (Salvador and Fiszman, 2004; Trachoo and Mistry, 1998). Low fat yoghurts are known for poor textural characteristics owing to their low total solids contents, which make them susceptible to syneresis unless they are heavily stabilized (Trachoo and Mistry, 1998). The mean values of syneresis (released serum) of control and experimental yoghurt after manufactured and during the 7 days storage period at 4°C temperature are presented in Table 1. The syneresis of control sanıple ranged from 19.23 to 27.93 mL/50 g of sample on the day of manufacture to 7 days of storage. On the day of manufacture the syneresis value of control sanıple was 22.50 mL which significantly decreased to 19.23 mL on the 3rd of storage. The syneresis values

significantly increased on 5th day of storage to 23.73 mL while a slight increased was observed (27.93) on the 7th day of storage compared to 5th day. The syneresis of yoghurt I sample ranged from 18.95 to 22.93 mL/50 g of sample on the day of manufacture to 7 days of storage. On the day of manufacture the syneresis value of yoghurt I sample was 20.75 mL which significantly decreased to 18.95 mL on the 3rd day of storage. The syneresis values significantly increased on the 5th day of storage to 22.93 mL while a slight decrease was observed (21.90 mL) on the 7th day of storage.

The syneresis of yoghurt II sample ranged from 18.85 to 24.90 mL/50 g of sample on the day of manufacture to 7 days of storage. On the day of manufacture the syneresis value of yoghurt II was 18.85 mL which significantly increased to 24.90 mL on the 3rd day of storage. The syneresis values significantly decreased on 5th day of storage to 22.80 mL, while a slight decrease was observed (22.90 mL) on the 7th day of storage. Iwalokun and Shittu (2007) reported a high degree of syneresis in control yoghurts compared to sabdariffa yoghurts after manufacture and during 7 day study period. Similar trend was observed in the present study except on the 3rd and 5th day of storage. The above findings provide a strong indication for the presence of stabilizing agents in *Hibiscus sabdariffa*. The stabilization factors may be contributed by the dietary fibers endowment in *Hibiscus* sp. (Punna *et al*., 2004). Modler *et al*. (1983) found that with increase in protein in yoghurt the syneresis was decreased.

Antioxidants activity by DPPH method: The mean values of % inhibition by control and experimental yoghurts after manufacture and during 7 days storage period at 4°C temperature are presented in Table 1. Comparing the % inhibition by non-sabdariffa (control) and sabdariffa yoghurts (yoghurts I and II) on the day of manufacture indicated that the highest inhibition (59.43%) was observed in yoghurt prepared by adding 2% sabdariffa extract that showed a significant difference with the % inhibition values of control yoghurt (11.50%). The yoghurt prepared by addition of 1% sabdariffa showed% inhibition of 12.32 which was significantly higher than the control yoghurt and significantly lower than yoghurt II. On the 7th day of storage similar trend was observed i.e., inhibition (42.69) by yoghurt II was significantly higher compared to the control (20.11%) and yoghurt I (30.17%). Chang-Che *et al*. (2003) showed that adding an aqueous extract of *H. sabdariffa* flowers at 0.5 and 1.0% of the diet of New Zealand white rabbits inhibited serum lipids and had an antiatherosclerotic activity. They also studied the effect of two types of *H. sabdariffa* phenolic compounds, protocatechuic acid as an LDL oxidation inhibitor and *Hibiscus* anthocyanins as antioxidants.

Total solids: The mean values of total solids content of control and experimental yoghurts after manufacture and during the 7 days storage period at 4°C temperature are presented in Table 1. The total solids content of control yoghurt, yoghurt I and yoghurt II ranged from 21.21 to 25.47 g %, 21.40 to 24.85 g % and 21.27 to 24.90 g %, respectively on the day of manufacture to 7 days of storage. The total solids content of yoghurt I ranged from 21.40 to 24.85 g % on the day of manufacture to 7 days of storage. On the day of manufacture the total solids content of yoghurt I was 24.85 g % which significantly decreased to 24.46 g % on the 3rd day of storage. The total solids content significantly decreased on 5th day of storage to 23.03 g %. While a slight decrease was observed (21.40 g %) on the 7th day of storage. No significant trend was observed in the total solids of control and experimental yoghurts during storage.

β-galactosidase assay: β-galactosidase activity of control and experimental yoghurts after manufacture and during the 3rd day storage period at 4°C temperature are presented in Table 2. β-galactosidase activity of control and experimental yoghurts ranged from 1.13 to 3.52 U mL^{-1} on the day of manufacture (0 day) and on 3rd of storage. The degradation of lactose depends upon the β-galactosidase activity produced by starter cultures.

Fat and protein content: Mean values of protein and fat content of control and experimental yoghurts after manufacture are presented in Table 3. The fat content of control and experimental yoghurt I and II was found to be 4.4, 3.49 and 3.43 g%, respectively. The control yoghurt had significantly higher fat content compared to yoghurt I and II. Protein content of control and experimental

Table 2: β-galactosidase activity of the control and experimental yoghurts after manufacture and during the 3-day of storage period at refrigeration temperature (4°C)

	β-galactosidase activity (U mL^{-1})	
Yoghurt samples	0 day	3 days
C	1.130	3.521
I	1.041	3.028
II	1.066	3.277

Values are Mean of three observations, C: Control yoghurt, I: Experimental yoghurt I, II: Experimental yoghurt II

Table 3: Protein and fat content of control and experimental yoghurts during manufacture

Yoghurt samples	Protein (g %)	Fat (g %)
C	4.11±0.001[a]	4.40±0.01[c]
I	4.14±0.015[a]	3.49±0.01[b]
II	4.11±0.06[a]	3.43±0.015[a]

Values are Mean±SD of three observations. Values bearing different superscript within the column are significantly different ($p<0.05$), C: Control yoghurt, I: Experimental yoghurt I, II: Experimental yoghurt II

Table 4: Textural analysis of control and experimental yoghurts on the day of manufacture

Textural parameters	C	I	II
Stickiness	4.96±0.1008[b]	5.10±0.1269[a]	5.28±0.3495[a]
Consistency	308.99±8.7747[a]	328.90±3.6383[b]	308.1427±8.5815[a]
Firmness	20.39±0.3264[ab]	20.96±0.1326[b]	19.71±0.4022[a]
Index of Viscosity	55.78±5.0598[a]	58.65±2.6618[c]	54.19±2.7997[a]
Distance	19.94±0.043	19.51±0.4667	19.87±0.060

Values are Mean±SD of three observations. Values bearing different superscript within the column are significantly different. ($p<0.05$)

Table 5: Sensory profile of control and experimental yoghurts (0 day)

Samples	Flavour (10)	Body texture (5)	Appearance (5)	Product acidity (3)	Container (2)	Total score (25)
Control yoghurt	7.83±0.31	3.92±0.20	3.75±0.25	1.92±0.083	1.25±0.17	18.67±0.73
Yoghurt I	8.58±0.27	3.42±0.27	3.75±0.25	2.00±0.29	1.17±0.17	18.92±1.05
Yoghurt II	8.58±0.44	4.00±0.26	4.33±0.21	2.17±0.33	1.50±0.34	20.58±1.33

Values are Mean±SD of a composite scoring by a Panel of 6 Judges×3 replications. Values in parentheses in the title of the column indicate maximum scores

yoghurt was found to be 4.11, 4.14 and 4.11 g %, respectively. No significant difference was observed in the values of all three yoghurts.

Textural analysis: From the prepared yoghurt products rheological studies are performed as a quality control method and as a technique to study the structure of the product (Tunick, 2000). Textural analysis of control and experimental yoghurts on the day of manufacture are presented in Table 4. The mean for stickiness ranged from 4.96 to 5.28. The highest stickiness was observed in experimental yoghurt II while the lowest was observed in control yoghurt. The mean for consistency ranged from 308.14 to 328.90. The highest consistency was observed in yoghurt I while the lowest was observed in yoghurt II. The mean for firmness ranged from 19.71 to 20.96. The highest firmness was observed in yoghurt I while the lowest was observed in yoghurt II. The mean for index of viscosity ranged from 54.19 to 58.65. The highest index of viscosity was observed in yoghurt I while lowest was observed in yoghurt II. The mean for distance ranged from 19.51 to 19.94. The highest distance was observed in control yoghurt while lowest was observed in yoghurt II.

Sensory evaluation: Yoghurt should firm, free from any whey separation and creamy layer. Generally the appearance of yoghurt should convey smooth, homogeneous, moderately firm gel or custard like body and texture and a uniform off white colour. The mean sensory scores of control yoghurt on the day of manufacture and during storage are presented in Table 5. Yoghurt I and II had the same scores for flavour (8.58), while the control yoghurt had a lower score (7.83) as compared to experimental yoghurts. No significant difference was observed between these values.

The scores of body texture revealed the yoghurt prepared by adding 2% extract of *Hibiscus sabdariffa* calyce had the highest scores (4) compared to the control yoghurt (3.92) and exp. yoghurt I (3.42) in which 1% extract was added. No significant difference was observed between these values. The scores of appearance revealed the yoghurt prepared by adding 2% extract of *Hibiscus sabdariffa* calyce had the highest scores (4.33) compared to the control yoghurt (3.75) and exp. yoghurt I (3.75) in which 1% extract was added. No significant difference was observed between these values. The scores of product acidity revealed the yoghurt prepared by adding 2% extract of *Hibiscus sabdariffa* calyce and the highest scores (2.17) compared to the control yoghurt (1.92) and exp. yoghurt I (2.00) in which 1% extract was added. No significant difference was observed between these values. The scores of container revealed the yoghurt prepared by adding 2% extract of *Hibiscus sabdariffa* calyce had the highest scores (1.50) compared to the control yoghurt (1.25) and exp. yoghurt I (1.17) in which 1% extract was added. No significant difference was observed between these values. The total score revealed the yoghurt prepared by adding 2% extract of *Hibiscus sabdariffa* calyce had the highest scores (20.58) compared to the control yoghurt (18.67) and exp. yoghurt I (18.92) in which 1% extract was added. No significant difference was observed between these values.

Salvador and Fiszman (2004) reported that firmness in yogurt significantly increased with storage and the firmness values for whole yogurt were lower than for nonfat yogurt.

Iwalokun and Shittu (2007) reported that *Hibiscus* calyces incorporated yoghurt showed reduction in scores for aroma, taste and overall acceptability when compared to their non-sabdariffa counterparts. These results are not in accordance to the results obtained in the present study where sabdariffa added yoghurt showed a higher score in almost all sensory attributes.

Microbial analysis: Microbial analysis of the control and experimental yoghurts after manufacture and during storage for 7 days at refrigeration temperature (4°C) are presented in Table 6. Microbial analysis was done to check its acceptability by analyzing for Lactobacillus count and yeast and mold count. Microbial assessment of the yoghurt products revealed the absent of yeast and

Table 6: Lactobacilli count and yeast and mould count in the three yoghurts after manufacture and storage of 7 day

		No. of storage days	
	Samples	0	7
Lactobacilli count	C	5.19×10^7	4.8×10^6
	I	5.37×10^7	4.1×10^6
	II	5.27×10^7	3.8×10^6
Yeast and mould count	C	--	1.1×10^2
	I	--	1.3×10^2
	II	--	1.1×10^2

Results are mean of three replications. Results are expressed in cfu mL^{-1}. C = Control yoghurt, I = yoghurt I, II- yoghurt II

mold in control and experimental yoghurt after the manufactured. A general decline in lactic acid bacteria count in all yoghurt samples was found. At the day of inoculation, the lactic acid bacterial count was 5.19×10^7, 5.37×10^7 and 5.27×10^7 cfu mL^{-1} in control yoghurt, yoghurt I and yoghurt II, respectively. As inoculation time increased the bacterial count was decrease and after a week of inoculation the count was 4.8×10^6, 4.1×10^6 and 3.8×10^6 cfu mL^{-1} control, yoghurt I and yoghurt II, respectively.

Thus the study concludes that incorporation of *Hibiscus sabdariffa* extract into yoghurt prepared with *L. casei* improved the total antioxidant property, organoleptic qualities and decreased the exudation of whey proteins (Syneresis). Thus, *Hibiscus sabdariffa* Calyces has beneficial influence on the quality of yoghurt. The present study demonstrated the successful incorporation of *Hibiscus sabdariffa* and *L. casei* for the preparation of probiotic yoghurt and its storage stability. Further research can be carried out using different antioxidant rich plant extracts and different strains of probiotic organisms.

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