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ORIGINAL ARTICLE

Preparation, characterization and in vitro antioxidative potential of synbiotic fermented dairy products

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Abstract The present study, evaluates the antioxidative potential of two synbiotic dairy products viz. synbiotic lassi with honey and whey based synbiotic drink with inulin and orange juice, along with their physicochemical and microbiological activity during storage period. Antioxidative potential of raw ingredients and probiotic cultures used to prepare synbiotic products was also evaluated. Synbiotic lassi with honey was prepared using *Streptococcus thermophilus* MTCC 5460 (MD2) and *Lactobacillus helveticus* MTCC 5463 (V3) as probiotics and honey as prebiotic. For preparation of whey based synbiotic drink, *Lactobacillus helveticus* MTCC 5463 and inulin were used as probiotic and prebiotic, respectively and orange juice was also incorporated. Titratable acidity and pH of both synbiotic products followed a similar pattern of increase or decrease during storage. Furthermore, no major changes were observed in viability of probiotic cultures under storage conditions adapted. The hydroxyl radical scavenging activity of synbiotic lassi with honey was found to significantly decrease from 107.76 to 79.41 % at the end of storage whereas, the activity of whey based synbiotic drink was 100.32 % which declined sharply to 79.21 % on 7th day but further increased to 102.59 % on 14th day. The DPPH (α, α-Diphenyl-β-Picrylhydrazyl) radical scavenging activity of freshly prepared synbiotic lassi with honey was 28.43 % which decreased to 23.03 % on 7th day while for whey based synbiotic drink decreased from 26.85 % (0 day) to 17.12 % (7th day) and continued to decline. Moreover, probiotic strains used for synbiotic preparation also demonstrated good antioxidative activity.

Keywords Antioxidants · Lactic acid bacteria · Probiotics · Synbiotics

Introduction

Over the years many microbial species have been used as probiotics. The term "probiotic" is used to describe live lactic acid bacteria (LAB) in fermented foods and was probably first defined by Kollath (Kollath 1953). Since then the definition has been modified number of times. EU Expert Group on Functional Food in Europe (FUFOSE) defined Probiotics as "viable preparation in food or dietary supplements to improve the health of humans and animals" (FUFOSE 1999). FAO/WHO (2002) defined probiotics as, "Live microorganisms which when administered in adequate amounts confer health benefit to the host." These definitions have set the foundations for assessment of health promoting probiotic properties of microbes. Probiotic organisms mainly comprise lactic acid producing bacteria (Lactobacilli, Streptococci, Enterococci and Lactococci), *Bifidobacteria* and also some *Bacillus* sp. as well as some yeast like *Saccharomyces* spp. (Ziemer and Gibson 1998; Ashwell 2002; Fooks and Gibson 2002; Saarela et al. 2002). Various therapeutic and nutritional benefits have been attributed to probiotics including alleviation of lactose intolerance, immune modulation, reduction of serum cholesterol and allergic reactions, anti carcinogenic effects, modulation of metabolic activities of colonic microbes and maintenance of mucosal integrity (Holzapfel and Schillinger 2002; Kullisaar et al. 2002). To derive maximum benefits from

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probiotics, number of strategies has been applied; one of which is combination of prebiotics with probiotics making synbiotics. Prebiotics are defined as non-digestible nutritional ingredients which positively affect the host, by selectively stimulating the growth and the action of one or more beneficial intestinal bacteria (Gibson and Roberfroid 1995; Nagpal et al. 2007). Studies have also revealed that consumption of synbiotic products has greater beneficial effect on the human health than probiotic and prebiotic alone. Synbiotics refer to a form of synergism where combining of probiotics and prebiotics is done in single food, which improve the survival of probiotic bacteria during the storage conditions as well as during passage through intestinal tract, hence selective growth of indigenous gut bacteria is promoted (Mishra et al. 2001; Roberfroid 2000; Schrezenmeir and de Vrese 2001). In addition, the synbiotic product may permit an efficient implantation of probiotic microbes in colon, because prebiotic has stimulating effect on the growth and other activities of the probiotic bacteria. Several synbiotic fermented milk products have been reported, in which the strains of *L. acidophilus*, *L. casei* and *Bifidobacterium* ssp. have been used as probiotic and fructo-oligosaccharides, galacto-oligosaccharides, lactulose, inulin-derived products etc. used as prebiotics (Klaenhammer and Kullen 1999; Ziemer and Gibson 1998). Among various attributes of synbiotics, antioxidative property is being investigated recently. Antioxidants in food are of interest because of their ability to protect components of the food itself against oxidative damage (Halliwell et al. 2000). Besides the probiotic properties, there are some reports of the antioxidative potentials of lactic acid bacteria, including starter cultures (Ahire et al. 2013; Kullisaar et al. 2002; Achuthan et al. 2012; Lin and Chang 2000). But very few reports are available on antioxidative potential of probiotics and synbiotic products. The present study was conducted with an aim to asses the antioxidative functionality of two synbiotic products i.e. lassi with honey and synbiotic whey with inulin and orange juice. The products were developed in our lab and contained probiotic strain *L. helveticus* MTCC 5463 (Prajapati et al. 2011). Honey and inulin were incorporated as their prebiotic potential has been well established. Inulin is legally classified as a food or food ingredient in all countries where it is used. It is well accepted for food use without limitations (Coussement 1999).

Materials and methods

Chemicals All the chemicals used in this study were of analytical grade and procured from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. All microbiological media were procured from Hi-Media (Hi-Media, Mumbai, India).

Microorganisms and culture conditions The cultures used in the present study, viz., *Streptococcus thermophilus* MTCC 5460 (MD 2), *Lactobacillus helveticus* MTCC 5463 (V3), were obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat. All the cultures were maintained by propagating in sterilized skim milk and stored at 5 ± 2 °C. Prior to use, cultures were activated in suitable medium by incubating at 37 °C for 18 h.

Preparation of synbiotic fermented dairy products and their storage

Synbiotic lassi with honey Fresh, double toned milk and honey collected from local market were used for preparation of synbiotic Lassi with honey. The double toned milk was preheated to 45 °C, and at this stage ingredients such as 5 % sugar and 5 % honey were added at optimum level. This was followed by heating to 90 °C for 5 min and cooling to 40 ± 2 °C. Probiotic cultures *L. helveticus* MTCC 5463 and *S. thermophilus* MTCC 5460 were inoculated into above said mixture at 1 % *v*/*v* each. The preparation was mixed homogenously and incubated at 37 ± 2 °C until 0.6–0.7 % titratable acidity was achieved, followed by cooling to 5 ± 2 °C. Synbiotic Lassi was prepared by breaking the curd using mechanical stirrer and filled into sterile polypropylene plastic cups sealed with caps and stored at <5 °C for 28 days (cups were treated with 32 % hydrogen peroxide and dried in oven. Swab tests were conducted to check sterility). Samples were drawn at interval of 7 days up to 28 days for analysis.

Synbiotic whey drink with inulin and orange juice Raw materials such as sugar, double toned milk, inulin, UHT sterilised and aseptically packed commercial brand orange juice were procured from local market and used for preparation of synbiotic dairy product preparation. Filtered double toned milk was heated at 95 °C for 5 min and cooled at 70 °C, followed by whey separation using addition of 1.5 % citric acid solution and allowed to settle for 10 min. After complete settlement, whey was separated by removal of coagulated mass. Whey was boiled at atmospheric pressure and cooled to 37 °C. At this stage, ingredients such as sugar (10 % and, inulin (3 %) were added followed by heating to 95 °C for 5 min and cooling to 37 °C. Probiotic culture, *L. helveticus* MTCC 5463 (2% *v*/*v*) was aseptically inoculated and mixture was kept for incubation at 37 °C till titratable acidity reached to 0.7 % of lactic acid. Orange juice was added into whey mixture at 10 % *v*/*v* and filled into sterile glass bottles (200 mL/bottle) with crown capping. Synbiotic whey drink thus obtained was stored at 4 ± 1 °C for 28 days. Samples were drawn at 7 day intervals up to 28 days for analysis.

Chemical analysis of synbiotic dairy products Titratable acidity (TA) of both synbiotic products were estimated by titration with 0.1 N NaOH solution and expressed as percent lactic acid (AOAC 1984). The pH of synbiotic product was determined by using digital electronic pH meter (Cyberscan 2100, Eutech Instruments, Singapore). pH and TA were measured on a weekly basis during storage condition up to 28 days.

Viability of probiotic cultures during storage conditions The survival of *S. thermophilus* MTCC 5460 *and L. helveticus* MTCC 5463 in both synbiotic fermented dairy samples stored at 4 °C and were determined by using M17 and MRS agar plate respectively using standard laboratory procedures at 1 week interval. A 100 µl of sample was dissolved and diluted serially in phosphate buffered saline (PBS) pH 7.3. Growth of probiotic cultures was expressed as colony forming units per milliliter ($\log_{10}$ CFU/ml) by spreading appropriate dilution of suitable agar medium at 37 °C for 72 h and 48 h respectively.

Studies on antioxidative potential of synbiotic dairy products, probiotic cultures and raw materials

The antioxidative potentials of synbiotic products and intra cellular cell free extract and intact cells of probiotic cultures used in development of synbiotic product were determined using different in vitro methods. In addition, antioxidative activity of raw materials such as milk, honey, inulin, orange juice and whey (samples prepared using suitable diluents) was determined by hydroxyl radical scavenging activity and ABTS assays. To assess activity of products, 10 ml of selected product was taken into sterile tubes and mixed thoroughly. The synbiotic product was then centrifuged at 4000 × g for 15 min. The supernatant was used as sample for determination of antioxidative activity.

Preparation of intracellular cell free extracts (ICFE) It was prepared as per the method of Lin and Yen (1999) with minor modifications. Probiotic bacterial cells, *L. helveticus* MTCC 5463, *S. thermophilus* MTCC 5460 were harvested by centrifugation at 4000 × g for 15 min after 18 h of incubation in MRS and M17 broth at 37 °C respectively. Cell pellets were then quickly washed twice with deionized water and resuspended in same and allowed for ultrasonic disruption. Sonication was performed at 130 W with 30 % amplitude for 5 min in 50 s on/10 s off cycle by keeping it in ice bath using sonicator (VCX 130, Sonics Vibra cell, USA). Cell debris was removed by centrifugation at 6000 × g for 15 min. Resulting supernatant was used as ICFE for antioxidative studies.

Hydroxyl radical scavenging activity The hydroxyl radical scavenging activity was measured using the method of de Avellar et al. (2004) with some modification. One ml of 0.75 mM/l 10- Phenanthroline, 1.5 ml of 0.15 M Sodium Phosphate Buffer (pH 7.4), 1 ml of 0.75 mM FeSO4 and 1 ml of H_2O_2 (0.01 % *V/V*) and 0.5 ml of sample were added in a test tube and mixed homogenously. The mixture was incubated at 37 °C for 30 min and A_{536} was measured using following equation:

The % hydroxyl radical scavenging activity

$$= \frac{A\,test - Ablank}{A' - Ablank} \times 100$$

Where A'was the absorbance of the deionised water instead of H_2O_2. Deionised water was used as blank/control.

DPPH radical scavenging activity Free radical scavenging activity was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Son and Lewis 2002) with minor modifications. 20 µl sample of honey based synbiotic lassi/20 µl sample of whey based drink sample was reacted with 2 ml of freshly prepared DPPH (2 mM/l in methanol) and reaction was allowed for 30 min in dark and A_{517} was recorded. DPPH scavenging activity was calculated using following equation:

$$\%\,\text{DPPH scavenging activity} = \frac{A\text{blank} - Atest}{A\text{blank}} \times 100$$

$$\text{Deionized Water} = \text{As a blank/control.}$$

2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging ability Free radical scavenging activity (RSA) was determined by the ABTS method (Pellegrini et al. 1999; Hernández-Ledesma et al. 2005). 20 µl sample of honey based synbiotic lassi/20 µl sample of whey based drink was taken and 2 ml of diluted ABTS was added to it. The mix was incubated at room temperature for 30 min and A_{734} was recorded. The % ABTS $\text{activity} = \frac{A\text{blank} - A\text{test}}{A\text{blank}} \times 100$ Deionised Water = as a blank/control.

Statistical analysis

At least 3 observations were taken for each experiment. The data obtained in the study was subjected to statistical analysis for drawing meaningful information. Optimization study data were analyzed by Completely Randomized Design as per the methods (Steel and Torrie 1980). Storage study data were examined using Factorial CRD. The level of significance is presented at $P \leq 0.05$. Correlation coefficients were derived for different parameters.

Results and discussion

Titratable acidity and pH Changes in titratable acidity and pH of synbiotic lassi with honey and whey based synbiotic drink during refrigerated storage conditions were measured and presented in Table 1. TA of synbiotic lassi with honey increased during storage. From 0 to 14th day the TA increased from 0.70 to 0.83 % while, from 14 to 28th day TA increased from 0,83 to 1.15 %. Titratable acidity of whey based synbiotic drink increased up to 14 days storage but, decreased for next week i.e. 21st day and again increased for 28th day of storage. For both synbiotic products pH was found to be decreased as the storage period increased. The rate of increase in acidity for synbiotic lassi with honey was higher than whey based synbiotic drink. The findings indicate that prebiotic sources (honey and inulin) did not have effect on the post-acidification of both lassi and whey based products. Usually, a low pH and high TA value indicate enhanced acid production due to high bacterial activity. Our findings are in agreement with Gandhi (1989), who reported increase in titratable acidity of patented acido whey- lactic fermented non carbonated beverage. Other studies on honey based fermented dairy products confirm the present trend of decline in pH. Chick et al. (2001) also reported decrease in pH of fermented milk products prepared separately by fermentation of 12 % non fat dry milk by *S. thermophilus* and *L. acidophilus*. In another study, yoghurt fortified with different concentrations of honey, a non significant decrease in pH was observed during 6 week storage of product at 4 °C (Varga 2006).

Viability of probiotic cultures in synbiotic dairy products during storage Survival of probiotic cultures during storage of synbiotic dairy products is presented in Table 2. Survival of probiotic bacteria *L. helveticus* and *S. thermophilus* in synbiotic lassi with honey steadily decreased up to 8.36 and 8.55 $\log_{10}$ CFU/ml respectively during refrigerated storage conditions up to 28 days and data suggested that statistical analysis of treatment was significant ($P<0.05$) and insignificant ($P>0.05$) respectively. On the other hand, CFU of *L. helveticus* in whey based synbiotic drink was found to be 8.53 during 7, 14 and 21 days of storage which decreased to 8.39 on 28 days of storage and decline in count was statistically insignificant ($P>0.05$). The survival of probiotic bacteria in synbiotic product is important parameter in the context of positive health effects on host. Our results are in agreement with earlier studies such as Hosny et al. (2009) who studied the yogurt fortified with different concentrations of honey and reported drop in viable lactobacilli count during storage for 5 days. However, addition of prebiotic like FOS could either serve as additional nutrients or modify the unfavorable environmental conditions, resulting in enhanced viability of probiotic (Desai et al. 2004; Madhu and Prapulla 2012; Makras et al. 2005).

Studies of antioxidant activity of synbiotic dairy products Oxidative metabolism can produce extremely reactive free radicals which are molecules having unpaired electron and are capable of carrying out a rapid change reaction which can destabilize other molecules and generate free radicals. Free radicals are known to play a crucial role in development of chronic diseases like cancer, cardiovascular diseases, Alzheimer's disease and Rheumatoid arthritis and oxidative damage of DNA which is responsible for ageing (Seifried et al. 2007; Valko et al. 2007; Verma and Banerjee 2010). Accordingly, the antioxidative potential of synbiotic dairy products, probiotic cultures and raw material used for synbiotic products preparation was determined using different in vitro antioxidant assays.

Hydroxyl radical scavenging activity of the freshly prepared synbiotic lassi with honey (Fig. 1) was found to be 107.76 % while, during refrigerated storage conditions, the activity gradually declined as the storage period increases such as 105.53, 100.92, 96.20 and 79.41 % on 7, 14, 21 and 28th day respectively. The decline during early stages i.e. up to 14th day was insignificant. In addition, interesting results were obtained for scavenging activity of whey based synbiotic product (Fig. 2). The activity of fresh product was 100.32 % which declined sharply to 79.21 % on 7th day. The activity

Table 1 Changes in titratable acidity (TA; percentage lactic acid) and pH of synbiotic dairy products during storage conditions

Storage (days)	TA (%)		pH	
	Synbiotic Lassi with honey	Whey based synbiotic drink	Synbiotic Lassi with honey	Whey based synbiotic drink
1	0.70 ± 0.02	0.80 ± 0.01	4.59 ± 0.17	3.82 ± 0.009
7	0.77 ± 0.05	0.82 ± 0.005	4.43 ± 0.02	3.78 ± 0.005
14	0.83 ± 0.01	0.91 ± 0.02	4.43 ± 0.01	3.76 ± 0.005
21	1.09 ± 0.01	0.75 ± 0.01	4.25 ± 0.09	3.75 ± 0.06
28	1.15 ± 0.09	0.96 ± 0.005	4.13 ± 0.02	3.67 ± 0.07
SEM[a]	0.026	0.007	0.046	0.022

[a] Pooled standard error of mean

Table 2 Viability of probiotic cultures during storage conditions of synbiotic dairy products

Viability of probiotic cultures during storage of synbiotic dairy products (log_{10}CFU/mL)			
Storage (days)	Synbiotic Lassi with honey *L. helveticus* MTCC 5463 (V3)	*S. thermophilus* MTCC 5460 (MD 2)	Whey based synbiotic drink *L. helveticus* MTCC 5463 (V3)
1	8.64 ± 0.34	9.20 ± 0.61	8.72 ± 0.24
7	8.47 ± 0.04	9.04 ± 0.61	8.53 ± 0.17
14	8.33 ± 0.12	8.83 ± 0.35	8.53 ± 0.47
21	8.21 ± 0.09	8.56 ± 0.63	8.53 ± 0.01
28	8.36 ± 0.04	8.55 ± 0.45	8.39 ± 0.02
SEM[a]	0.086	0.126	0.273

[a] Pooled standard error of mean

further increased to 102.59 % on 14th day and remained at 100.68 % on 21st day. The activity again declined on 28th day of storage. The statistical analysis found the treatments to be significant. Free hydroxyl radicals are extremely reactive by either removing hydrogen from or adding hydroxyl radicals to biological entities and are also involved in several pathological conditions as well as known for lipids peroxidation and DNA damages (Halliwell and Chirico 1993). Hydroxyl radicals are formed through Fenton reaction by using iron (Ahire et al. 2013).

DPPH radical scavenging activity of both synbiotic products (Fig. 1) presents contrasting results. For synbiotic lassi with honey, the DPPH scavenging activity of fresh product was found to be 28.43 % and the activity declined slightly to 23.03 % on 7th day but, increased to 23.20, 25.94 and 35.93 % on 14th, 21st and 28th day of refrigerated storage conditions respectively. DPPH activity of whey based synbiotic product (Fig. 2) was in contrast to honey based product. The activity of fresh product was 26.85 %, but the activity declined to 17.12 % on 7th day and continued to decline significantly and reduced to 10.74 % on 28th day. Variations in DPPH radical scavenging activity of both products may be attributed to differences in their composition even though literature suggested no explanation.

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, and its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically. The ABTS activity of freshly prepared honey based (Fig. 1) and whey based synbiotic products (Fig. 2) was found to be 56.23 and 55.11 % respectively. Thereafter further storage showed different results in activity. For honey based product, the activity increased significantly to 73.74 and 74.75 % on day 7 and 14 respectively. The activity stabilised on further storage as it was recorded as 71.71 and 71.63 % on day 21 and 28 respectively.

The initial ABTS activity of whey based synbiotic drink was similar to honey based product but the value declined to 44.42 and 38.95 % on 7th and 14th day of storage respectively. Thereafter the activity increased to 40.88 % on day 21

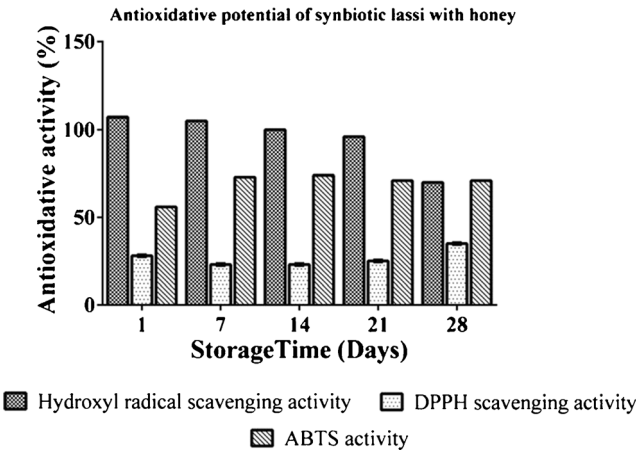

Fig. 1 Effect of storage time (28 days) on antioxidative potentials of synbiotic lassi with honey. A pooled standard error of the mean was calculated for each antioxidative assay such as, DPPH: 4.012; Hydroxyl radical scavenging activity: 1.145; ABTS activity: 1.233. The significant difference in different samples was observed ($P < 0.05$)

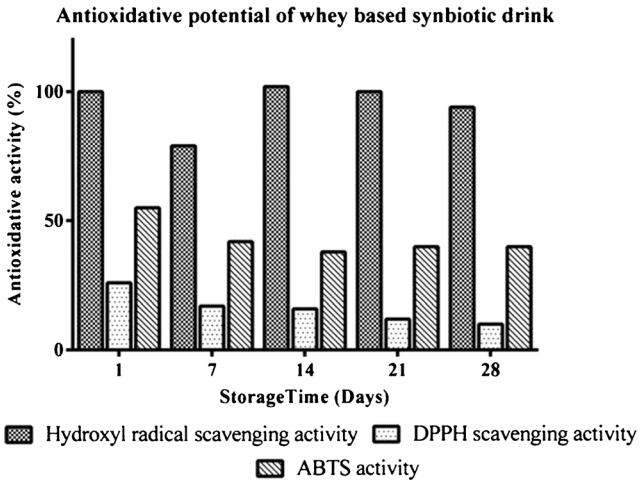

Fig. 2 Effect of storage time (28 days) on antioxidative potentials of Whey based synbiotic drink. A pooled standard error of the mean was calculated for each antioxidative assay such as, DPPH: 2.322; Hydroxyl radical scavenging activity: 2.320; ABTS activity: 0.977. The significant difference in different samples was observed ($P < 0.05$)

Table 3 Antioxidative potential of raw material used for preparation of synbiotic dairy products

Antioxidative potential (%)	Raw material					
	Honey	Milk	Whey	Orange juice	Inulin	SEM[a]
Hydroxyl radical scavenging activity	134.07	97.28	92.67	78.23	93.90	2.322
ABTS activity	31.14	38.87	09.17	84.61	08.89	0.977

[a] Pooled standard error of mean

which declined to 40.65 % on 28th day. In both the products, it was observed that there was variation in ABTS activity up to 21st day of storage and the values stabilised after 3 weeks of storage. Statistical analysis of above described antioxidative assays were found to be significant ($P<0.05$).

It was observed that out of both the products studied, the synbiotic lassi containing honey had better antioxidative potential as revealed by DPPH and ABTS methods. Studies conducted elsewhere support the present result as (Schramm et al. 2003; Al-Waili 2003) reported that consumption of honey increased antioxidant levels and decreased serum levels and they also increased Vitamin C concentration by 47 %, β-carotene by 3 %, uric acid by 12 % and glutathione reductase by 7 %. Hutt et al. (2009) reported that consumption of a synbiotic product reduced the systemic oxidative stress due to colonization of *Helicobacter pylori*. Kaushal and Kansal (2012) reported that probiotic dahi containing *L. acidophilus* and *B. bifidum* reduces age-inflicted oxidative stress as studied by expression of biomarkers of ageing in mice. Moreover, results of Madhu et al. (2012) revealed that synbiotic yogurt samples containing *L. plantarum* and *L. fermentum*, the DPPH radical inhibition was 85 and 82 %, respectively, at day 1 and the values increased as the storage time increased. In this study, fructo-oligosaccharide was used as prebiotic for preparation of synbiotic yogurt. The results observed and subsequent discussions strongly support the presumption that fermented dairy products contain antioxidative components. But the role of individual components in development of synbiotic products is still to be comprehensively studied as scarce literature is available in this area. The methods used for such studies are usually applied for plant based foods hence there is need to standardize methods for dairy based foods.

Studies on antioxidant potentials of raw materials used for synbiotic product preparation Food components are considered best sources of antioxidants and in present study too it was shown that ingredients used for development of synbiotic products posses antioxidative activity. The hydroxyl radical scavenging activity of honey, milk, whey, orange juice and inulin is showed in (Table 3). Honey had highest activity at 134.07 %, while orange juice had lowest activity at 78.23 % among all the ingredients. The ABTS activity of orange juice was observed to be highest (84.61 %) followed by honey (31.14 %) and inulin had lowest activity (8.89 %). The results thus lead to inference that food ingredients posses antioxidative activity but the nature of antioxidants may differ. Mohamed et al. (2010) studied the antioxidative activity of honey by DPPH assay and reported 41.3 ± 0.78 % activity. Similarly Al et al. (2009) reported antioxidative activity of Romanian acacia honey in range of 35.80–45.27 %. These reports confirm the results of present study. Antioxidative potential of raw material was found to be significant.

Antioxidative activities of ICFE and intact cells of probiotic cultures The hydroxyl radical scavenging activity of ICFE and intact cell of probiotic cultures is given in Table 4. Intact cells of *L. helveticus* MTCC 5463 (V3) showed 97.39 % hydroxyl radical scavenging activity in comparison to 48.79 % for intra cellular cell-free extract (ICFE). In addition,

Table 4 Antioxidative potential of intracellular cell free-extract (ICFE) and intact cells of probiotic cultures used for synbiotic dairy products

Antioxidative potential (%)	ICFE			Intact cells		
	V3	MD2	V3 + MD2	V3	MD2	V3 + MD2
Hydroxyl radical scavenging activity	48.79	146.67	164.46	97.39	95.64	102.27
DPPH radicalbscavenging activity	27.18	03.83	6.48	0.76	02.37	1.70
ABTS activity	08.73	07.72	02.57	0.47	3.01	5.46
SEM[a]		0.677			0.466	

[a] Pooled standard error of mean, V3: *L. helveticus* MTCC 5463, MD2: *S. thermophilius* MTCC 5460, V3+ MD2, *L. helveticus* MTCC 5463 + *S. thermophilius* MTCC 5460

activity of ICFE of *S. thermophilus* (MD2) was (146.47 %) significantly higher ($P<0.05$) than that of intact cell 95.64 %. Similar results were also observed when both cells were used in combination [*L. helveticus* MTCC 5463 (V3) + *S. thermophilus* MTCC 5460 (MD2)]. The activity of combination of intact cells V3 + MD2 was 102.27 % while activity of cell free extract was 164.46 %. The DPPH radical scavenging activity of ICFE (Table 4) was found to be more than that of intact cell (Table 4) for both cultures in separate or when cultures were mixed. Activity of *L. helveticus* intact cells was 0.76 % while for ICFE was significantly higher as 27.18 %. The scavenging activity of *S. thermophilus* cells was observed as 2.37 % while its extract showed 3.83 %. When both cultures were combined (V3 + MD2), the cellular fractions showed 6.48 % activity in comparison to 1.70 % for intact cells. The ABTS activity of intact cells and ICFE of probiotic cultures is reported in Table 4. The intact cells of V3, MD2 and V3 + MD2 showed 0.47, 3.01 and 5.46 % ABTS scavenging activity respectively while the activity of their respective extracts was 8.73, 7.72 and 2.47 % respectively. In this case, the activity of cell free extracts of both the cultures was higher than their intact cells. Antioxidant activity of microbial cultures used for the development of probiotic and synbiotic products is a subject of in vitro and in vivo studies in recent times. Cultures having such activity along with other probiotic attributes are considered as better organisms for development of product. Sourabh et al. (2011) reported antioxidative activity of four potential probiotic yeasts isolates by DPPH method and found that maximum activity was 22.07 % for isolate Sc 16 and minimum activity was 11.43 for isolate Sc 01 for whole cells. It was reported that activity slightly increased when cell extracts were used as sample. In present study, the activity of cell extracts was more than the intact cells. Kullisaar et al. (2002) comprehensively studied *L. fermentum* E-3 and E-18 strains and showed both strains posses strong antioxidative activities. Wang et al. (2009) studied the in vitro free radical-scavenging activity of *L. fermentum* using DPPH, superoxide and hydroxyl radical scavenging methods. The activity was comparable to present results. Ahire et al. (2013) studied antioxidative activity of intact cells and ICFE of *L. helveticus* CD6. The DPPH activity of intact cells was found to be 24.7 ± 10.9 %, more than the cell extracts (6.8 ± 2.0 %). This study was in contrast to our results where the DPPH activity of cell extracts was more than intact cells. They also reported hydroxyl radical scavenging activity of cell extract as 20.8 ± 0.9 %, which was equivalent to ascorbate. In this study, the activity by this method for intact cells and their extracts was found to be much higher. Correlation coefficient.

The data obtained after experiments for both the products for titratable acidity, pH, *Lactobacillus helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5460 count, hydroxyl radical scavenging activity, DPPH and ABTS activity were analyzed statistically for correlation. Coefficient of correlation (r) was studied among different parameters for both products. Table 5 represents correlation among different parameters for synbiotic lassi with honey. The titratable acidity was significantly ($P<0.05$) and negatively correlated with pH (r = −0.858*), *L. helveticus* MTCC 5463 count (r = −0.496*), *S. thermophilus* MTCC 5460 count (r = −0.458*) and hydroxyl radical scavenging activity ($P<0.01$) while there was positive but significant correlation with DPPH activity.

DPPH activity had significant ($P<0.01$) and positive correlation with *Lactobacillus helveticus* MTCC 5463 count (r = 0.622, $P<0.01$), which indicate that microbial activity can significantly contribute to antioxidative activity of synbiotic fermented product. ABTS activity was significantly positively correlated (r = 0.553, $P<0.05$) with pH of product.

Table 5 Correlation coefficient of synbiotic lassi based with honey to its acidity, pH, probiotic cultures, and antioxidative potential

	Acidity	pH	*L. helveticus* MTCC 5463	*S. thermophilus* MTCC 5460	Hydroxyl radical scavenging activity	DPPH activity	ABTS activity
Acidity	1						
pH	−0.858*	1					
L. helveticus MTCC 5463	−0.496*	0.281	1				
S. thermophilus MTCC 5460	−0.458*	0.28	0.649**	1			
Hydroxyl radical scavenging activity	−0.699**	0.774**	0.277	0.67	1		
DPPH activity	0.503*	−0.433	0.009	−0.307	−0.522*	1	
ABTS activity	0.429	−0.522*	−0.474*	−0.273	−0.308	−0.213	1

**$P<0.01$ (very significant), *$P<0.05$ (significant)

Conclusion

In present study two synbiotic products were studied and in both products the probiotic count after storage for 28 days at 4 ± 1 °C was maintained at more than 8 $\log_{10}$ CFU/ml. Both products were found to posses antioxidative activity in terms of hydroxyl radical scavenging activity, radical scavenging activity and anti-radical scavenging activity. The storage of products could have adverse effect on these activities as for whey based drink, the activity reduced for all parameters studied. It can also be concluded that composition of synbiotic products also affect the antioxidative activity indicated by contrasting results of both products for DPPH and ABTS activity during storage. Use of such potential synbiotic food products may be encouraged, and antioxidant properties might be an additional promising parameter to confer health benefits to host.

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