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Development and characterization of indigenous value added *Greek strained dahi*

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Fermented dairy products provide healthy good bacteria in the intestinal tract and beneficial effects to the host by changing the equilibrium and metabolism of the intestinal microflora. *Dahi* is an indigenous Indian fermented milk product and is a good source of B vitamins, proteins, and calcium, which is easier for the body to digest than that, is present in fresh milk. Different types of *dahi* such as sweet *dahi*, sour *dahi* and flavored *dahi* are usually found in the markets but value added products like that of its counterpart yoghurt such as fruit yoghurt are not available. Hence, the objective of this study was to develop value added *dahi* products. In this report we have developed and characterized *Greek* strained *dahi* and probiotic *Greek strained dahi* and have also compared it with traditional *dahi*. We have assessed physicochemical, microbiological characteristics, as well as shelf life and sensory acceptance of all these products. For this study four *dahi* formulations were prepared: traditional *dahi* (type A), probiotic *dahi* (type B), *Greek dahi* (type C) and probiotic *Greek dahi* (type D). Probiotic *dahi* has significantly less pH and reducing sugar compare to the traditional *dahi*. Whereas probiotic *dahi* has significantly higher acidity, TSS, moisture, fat, protein and antioxidant activity than traditional *dahi*. Probiotic *Greek dahi* has significantly ($p<0.05$) decreased pH, moisture and reducing sugar compare to all three types of *dahi* and, significantly ($p<0.05$) increased in acidity, TSS, fat, protein and antioxidant as compared to all three *dahis*. All *dahi* samples were stored up to 24 days at (4^0C) refrigeration temperature. Microbiologically, *dahi* samples were stable and with satisfactory sanitary conditions for consumption but pH decreasing and acidity increasing vice versa were present with increasing the day of storage. Probiotic *Greek dahi* showed the greatest shelf life and acceptance by consumers. In conclusion, Probiotic *Greek Dahi* is a good food alternative, because in addition to market innovation, it has high nutritional value and good acceptance by consumers.

Key Words : *Greek dahi*, Probiotic, Self lifem, Physico-chemical

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INTRODUCTION

Dahi is an indigenous Indian fermented milk product known for its stimulating taste, palatability and curative values (Lal, 1980) otherwise called as 'curd'. It is yoghurt like product made in India and neighboring countries. About 7 per cent of the total milk produced in India is transformed into fermented milk products (Singh, 2007). According to Bureau of Indian Standards (BIS) (1980), *Dahi* is a product obtained by lactic fermentation of cow or buffalo milk or mixed milk through the action of single or mixed strains of lactic acid bacteria or by lactic acid fermentation accompanied by alcoholic fermentation by yeast. As per PFA rules (1988), *dahi* or curd is a product

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obtained from pasteurized or boiled milk fermented with a culture. The different starter culture used in the manufacture of *dahi* includes *Lactococcus. lactis, L. cremoris, Streptococcus thermophilus, Lactobacillus bulgaricus, L. plantarum* and lactose fermenting yeasts. A good quality *dahi* is of firm and uniform consistency with a sweet aroma and clean acid taste, the surface is smooth and glossy and a cut surface is trim and free from cracks and air bubbles. *Dahi* or curd is a good source of B vitamins, proteins, and calcium which are much easier for the body to digest than when they are present in fresh milk. *Dahi* contains 85-88 per cent water, 5-8 per cent fat, 3.2-3.4 per cent protein, 4.6- 5.2 per cent lactose, 0.5-1.1 per cent lactic acid, 0.70-0.75 per cent ash, 0.12-0.14 per cent calcium and 0.09-0.1 per cent phosphorus (Laxminarayana *et al.*, 1952). The Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) set broader international standards for yogurt in the *Codex Standard for Fermented Milks* (2003), yogurt may be the result of fermentation by *Streptococcus thermophilus* and *Lactobacillus delbruekii* ssp. *bulgaricus* cultures, and contains a minimum of 2.7 per cent milk protein, less than 15 per cent milk fat, and at least 0.6 per cent titratable 0.10 per cent vitamin B6 and iron 0.10 per cent (The Dairy Council, 2013). *Dahi* is very healthy, nutritious dairy product, valued for its control in the growth of bacteria that incur intestinal disease like constipation, diarrhea and dysentery (Shahani and Chandan, 1979). Different types of *dahi* such as sweet *dahi*, sour *dahi* and flavored *dahi* are usually found in the market but *Greek* or strained *dahi* is not found in the Indian market. In India system of medicine (Ayurveda), *dahi* has been strongly recommended for curing ailments like dyspepsia, dysentery and other gastrointestinal disorders and also effective in lowering the blood cholesterol (Mann and Spoerry, 1974).

"Good bacteria" present in dahi help reduce harmful bacterial organisms in the human gut and potentially encourage the response of the immune system when it's needed and can help with several digestive issues and improved lactose digestion which aids lactose intolerant individuals (Gilliland, 1985), decrease in serum cholesterol levels (Gilliland and Kim, 1984). As with regular yoghurt, *Greek* yoghurt is also made from fermented milk. *Greek* yoghurt is also called strained / drained yogurt (Tamime and Robinson, 1999; Robinson, 2003). Strained yogurt is typical of the Mediterranean region, including North Africa, Southern Greek yogurt. Perhaps this is because of the generally positive image of Greek cuisine in New York City and the importance of the Greek dairy company Fage in introducing their strained yogurt product, made in Greece, to New York markets beginning in 1998. Like many dairy products, there is a federal standard for identification of yogurt (*http://milkfacts.info/Milk%20Processing/Yogurt%20Production.htm)*, but strained or Greek yogurt is not a uniquely defined product. Strained yogurt is typical of the Mediterranean region, including North Africa, Southern Europe and West Asia. In the US, generically refer to this type of yogurt as *Greek* yoghurt is heavily strained to remove liquid whey (containing water, lactose, some protein and minerals) leaving behind a tangy, creamy product (Ramos *et al.*, 2009). *Greek* yoghurt has doubled the protein, half the carbohydrates and half the sodium of the regular variety. As a result, *Greek* yoghurt has a creamier, thicker texture and rich flavor. In addition, the removal of whey results in a healthier yoghurt with 40 per cent less sugar, 38 percent less sodium, fat content of 7 – 10 per cent , solids not fat (SNF) of 10 – 12 per cent, total solids from 14 per cent to 21 – 23 per cent and more than twice the amount of protein than traditional yoghurt (Tamime and Robinson, 1999; Robinson, 2003). *Greek* yoghurt is better than traditional yoghurt because *Greek* yoghurt provides important nutrients, such as calcium, magnesium, phosphorus, potassium and protein, which work together to promote strong, healthy bones (Boynton and Novakovic, 2014). *Dahi* is a very nourishing food and is available as source of protein, essential vitamin, minerals and also a rich source of calcium and riboflavin. The proteins in curd are more readily digested than the protein in milk. It has been estimated that regular milk is only 32 per cent digested after an hour in the digestive tract, whereas 91 per cent of curd is digested within the same period of time. The addition of various probiotic strains has not shown to affect the flavor or consumer perception of dairy products (Hekmat and Reid, 2006) but contributes to an improvement in the balance of microbiota in the human body which can result in physiological benefits (Fuller, 1992). It is, therefore, an ideal diet for those with sensitive digestive systems, particularly young children and elderly persons. The intestine friendly bacterial cultures in curd can keep colon

healthy and reduces the risk of colon cancer (Aso and Akazan, 1992; Aso *et al.*, 1995). Lactose intolerance is the inability to metabolize lactose, because of a lack of the required enzyme lactase in the digestive system. It is estimated that 75 per cent of adults worldwide show some decrease in lactase activity during adulthood. The good bacteria (live, active cultures) found in yogurt will help digest the lactose. *Dahi* has lots of benefits for health, hence the reason we made indigenous *Greek dahi*. With the aim that *Greek dahi* will provide higher amount of bacteria, very less lactose, high protein, high fat, and high micronutrient in less volume of *dahi*. Yoghurt has only two bacterial strains *(S. thermophilus* and *L. bulgaricus)* where as *Greek* probiotic *dahi* we have made mixed (more than two) bacterial culture and probiotic culture (such as *Lactococcus. lactis, L. cremoris, Streptococcus thermophilus, Lactobacillus bulgaricus, L. acidophilus, L. plantarum, L. casei*) these mixed culture will provided more beneficial effect on the host.

METHODOLOGY

Toned skim milk was collected from Amul Company and starter cultures of *dahi* and probiotic culture (*Lactobacillus acidophillus, L. casei* and *L. plantarum*) collected from NDRI (National Dairy Research Institute) Karnal and Haryana, India.

Preparation of different types of dahi :

An important aim of this study was to study the impact of the starter cultures of *dahi* and probiotic culture on the sensory parameter, biochemical parameters, shelf life and cell viability of the different types of *dahi*. The preparation of plain *dahi* has been investigated by a number of researchers in different parts of the world (Desai *et al.*, 1994; Shukla *et al*., 1987). Collected milk samples were heated to boiling temperature. After desired heating milk pan was taken out from the heater and allowed to cool down to near 42°C and inoculated with 2 per cent starter culture of dahi and for preparation of probiotic *dahi,* inoculated with 2 per cent starter cultures of *dahi* and 1 per cent probiotic culture (*Lactobacillus acidophillus, L. casei, L. plantarum*). After inoculation milk kept in an incubation temperature of 37°C until complete coagulation. After complete coagulation (6-8 hrs), coagulated *dahi* and probiotic *dahi* strained through muslin cloth for preparation of *Greek* plain *dahi* and probiotic *Greek dahi*. Due to the straining process to remove excess whey, even non-fat varieties of strained *dahi* are much thicker, richer, and creamier than the conventional, then different *dahi* samples stored in a refrigerator at about 4°C for analysis. Four types of *dahi* were prepared designated as follows:

A = Plain *dahi*
B = Probiotic *Dahi*
C = *Greek Dahi*
D = Probiotic *Greek dahi*

Analysis of dahi :

Chemical tests :

The different types of *dahi* samples were chemically analyzed (Acidity, pH, moisture, Fat, total soluble solids, crude protein were estimated according to the method of AOAC (2002). pH of *dahi* sample was done by using pH meter. Titratable acidity of samples was determined by titrating with 0.1 N NaOH and the acidity was calculated as percentage lactic acid (Vijayendra and Gupta, 2013). The fat content of the *dahi* samples was determined using either Gerber method (Kleyn *et al.*, 2001) and the fat content was expressed in percentage. The protein content of *dahi* samples was estimated using the micro Kjedahl method (AOAC, 1990) and protein content was calculated using the factor 6.38. The antioxidant activity of the *dahi* was studied by to scavenge 2, 2- diphenyl picryl hydrazyl free radical (DDPH) method. Reducing sugar of the *dahi* analyzed by DNS method.

Microbiological test:

The *Lactobacillus casei, L. acidophilus,* and *L. plantarum,* were grown in a sterile, liquid MRS medium, at a temperature of 30°C, in glass tubes (10 cm^3) under anaerobic condition. Total viable counts of *dahi* samples were determined according to the method as described in the "Standard Methods for Examination of Dairy Products" American Public Health Association (APHA, 1998). Procedures given in APHA (1998) method were followed for microbiological analysis. 11 g of *dahi* (dairy product) was suspended uniformly in 99 ml of dilution blank. Serial dilution was also prepared. After incubation in each Petri plate, the average count of colonies present on Petri plates were multiplied by dilution factor and expressed as colony forming units per gram (cfu/g). The diluted samples were spread on MRS-maltose agar, a selective medium for *L. acidophilus* (IDF, 1995). De Man Rogosa Sharpe agar with vancomycin (1 mg/L) was used

selective medium for *L. casei* (Tharmajar and Shah, 2003). Pantothenate culture agar (1 mg/L) was used selective medium for counting of *L. plantarum.*

Sensory evaluation :

Sensory evaluation of the *dahi* samples was evaluated for its acceptability, during the process and storage studies. *Dahi* samples were judged individually by an expert judge team for sensory evaluation on the basis of 9 point hedonic scale. Sensory parameters measured by expert judges.

Statistical analysis :

The number of treatments was four (4) and each treatment was replicated three (3) times. Data were analyzed by using one way analysis of variance test (CRD) as per SPSS (16.0) statistical program using computer and the results were represented as mean±SD. The results of physico-chemical, microbial, cell viability and sensory tests were analyzed by one-way analysis of variance (ANOVA).

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Biochemical and physical parameters :

pH :

Analysis of pH of *dahi* is necessary for viability of *dahi* bacterial strains and probiotic bacterial strains. According to Charalampopoulos *et al*. (2002) and Patel *et al.* (2004) *L. acidophilus, L. casei* and *L. plantarum* are most resistant at pH 4.0 and at this pH increased their viable count is increased. Since *dahi* bacteria are one of the most important components of the *dahi*, we have measured pH of the sample as described in material and methods by pH meter. The pH of traditional *dahi* is (4.23±0.06), probiotic *dahi* (4.18±0.04), *Greek dahi* (4.11±0.04) and probiotic *Greek dahi* (4.08±0.02). There was significant difference (P<0.05) between traditional *dahi* (A) and probiotic *Dahi* (B); probiotic *dahi* (B) and *Greek dahi* (C); *Greek dahi* (C) and probiotic *Greek dahi* (D). pH value of *dahi* samples ranged between 4.23±0.06 to 4.08±0.02, minimum in probiotic *Greek dahi* (D) and maximum in traditional *dahi* (A).

Acidity :

Titratable acidity of the *dahi* samples were evaluated determined by titrating with 0.1 N NaOH and the acidity was calculated as percentage lactic acid (Vijayendra and Gupta, 2013). It was observed from the results that the lowest acidity 0.35±0.018 found in traditional *dahi* (A), highest 0.54±0.03 found in *Greek dahi* (C) and probiotic *Greek dahi* has 0.51±0.02. The increase acidity in *Greek dahi* may be due to the removal of whey from *dahi*. But the value of the acidity was not too high to effect sensory perception and storage. Acidity of *dahi* sample is significant for sensory perception and storage. If acidity increases sensory perception of *dahi* will be reduced and hence the taste.

Total soluble solids :

The total soluble solids (TSS) of different *dahi* samples, plain *dahi* (23.88±2.64), probiotic *dahi* (27.46±1.6), *Greek dahi* (33.4±0.93) and probiotic *Greek dahi* (36.37±1.95) as shown in Table 1 and Fig. 1. In the present study, per cent of total soluble solids in probiotic

Table 1 : Summary of the result of Biochemical evaluation of different types *dahi* samples

Chemical parameter	Type of *Dahi*				LoS
	A	B	C	D	
pH	4.23^{a}±0.06	4.18^{ab}±0.04	4.11^{ab}±0.04	4.08^{b}±0.02	***
Acidity (%)	0.35^{b}±0.018	0.44^{ab}±0.057	0.54^{a}±0.03	0.51^{ab}±0.02	***
TSS (g/100g)	23.88^{b}±2.64	27.46^{ab}±1.6	33.4^{ab}±0.93	36.37^{a}±1.95	*
Moisture (%)	85.00^{b}±0.20	89.5^{a}±0.31	54.9^{c}±0.26	43.4^{d}±0.17	*
Fat (%)	4.4^{d}±0.50	5.23^{c}±0.23	8.1^{b}±0.16	9.73^{a}±0.12	***
Protein (%)	2.86^{c}±0.169	3.18^{bc}±0.074	4.61^{b}±0.179	5.43^{a}±0.197	***
Antioxidant activity (DPPH) (%)	43.63^{c}±0.71	47.01^{ab}±0.21	48.78^{b}±0.19	53.18^{a}±0.33	***
Reducing sugar (%)	110.27^{a}±1.27	97.29^{b}±1.73	88.39^{c}±0.56	80.33^{d}±1.12	*

* and ** indicate significance of values at P <0.001 and <0.05, NS= Non significant
LoS= Level of Significant, Standard deviation (±SD) calculated with 95 per cent confidence,
Type A= Plain *dahi*, Type B= probiotic plain *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*.

Greek dahi was higher than that of other samples. The highest total soluble solids 36.37±1.95 g/100g found in probiotic *Greek dahi* (D) and lowest 23.88±2.64 g/100g found in traditional *dahi* (A).

Moisture content :

The moisture content of product is measure for shelf life, lower the moisture content maximizes shelf life. The moisture content of traditional *dahi* (85.0±0.2), probiotic *dahi* (89.5±0.31), *Greek dahi* (54.9±0.26) and probiotic *Greek dahi* (43.4±0.17) as represented in the Table 1 and Fig.1. There was a significant difference ($P<0.05$) between traditional *dahi* (A) and probiotic *dahi* (B); and significant difference ($P<0.01$) between probiotic *dahi* (B) and *Greek dahi* (C); *Greek dahi* (C) and probiotic *Greek dahi* (D). The highest moisture content was observed in probiotic *dahi* (89.5±0.31%) and the lowest were in the probiotic *Greek dahi* (43.4±0.17).

Fat content :

The results showed significant difference ($P<0.01$) between traditional *dahi* (A) and *Greek dahi* (C); plain *dahi* (A) and probiotic *Greek dahi* (D); probiotic *dahi* (B) and *Greek dahi* (C); probiotic *dahi* (B) and probiotic *Greek dahi* (D) and significant difference ($P<0.05$) between plain *dahi* (A) and probiotic *dahi* (B); *Greek dahi* (C) and probiotic *Greek dahi* (D). The highest fat content was observed in probiotic *Greek dahi* 9.73 and the lowest was in the traditional *dahi* 4.4 (Table 1).

Protein content :

In order to evaluate the protein content of dahi we used the micro Kjedahl method (AOAC, 1990) and protein content was calculated using the factor 6.38. The present study showed that protein content in traditional *dahi* (2.86±0.16), probiotic *dahi* (3.18±0.074), *Greek dahi* (4.61±0.17) and probiotic *Greek dahi* (5.43±0.19) are given in Table 1 and Fig.1. The protein content was higher in probiotic *Greek dahi*.

Antioxidant activity :

To analyzed the Antioxidant activity of different types of dahi prepared in this study we used DPPH method. The present study showed that the range of antioxidant activity from 53.18±0.33 found in probiotic *Greek dahi* to 43.63±0.71 in traditional *dahi* (Table 1). The results showed that there were significant differences ($P<0.01$) between plain *dahi* (A) and probiotic *dahi* (B); Plain *dahi* (A) and *Greek dahi* (C); plain *dahi* (A) and probiotic *Greek dahi* (D); probiotic *dahi* (B) and probiotic *Greek dahi* (D); *Greek dahi* (C) and probiotic *Greek dahi* (D), but there was a significant difference ($P<0.05$) between probiotic *dahi* (B) and *Greek dahi* (C).

Reducing sugar :

In the order to evaluate the reducing sugar of dahi, we used DNS method. The highest Reducing Sugar 110.27±1.27 g/kg found in traditional *dahi* (A) and lowest 80.33±1.12 g/kg in probiotic *Greek dahi* (D) (Table 1).

Table 2 : Mean values (M±SD) of viable count (Log10) of the different probiotic strain per ml of *dahi*

Microbial parameters	Type of *dahi*				LoS
	Plain *dahi*(A)	Probiotic *dahi* (B)	*Greek dahi* (C)	Probiotic greek *dahi* (D)	
Total viabilty (log10 CFU/g) $x10^7$	1.190±0.06	1.617±0.11	1.453±0.11	2.529±0.09	***
Probiotic strain					
L. acidophillus (log10 CFU/g) $x10^7$	0.177±0.03	0.439±0.04	0.271±0.04	0.576±0.04	*
L. casei (log10 CFU/g) $x10^7$	0.146±0.03	0.385±0.05	0.223±0.04	0.507±0.06	*
L. plantarum (log10 CFU/g) $x10^7$	0.190±0.04	0.470±0.05	0.308±0.04	0.621±0.06	*

* and *** indicate significance of values at P <0.001 and 0.05; NS= Non-significant,
LoS= Level of Significant, Standard Deviation (±SD) calculated with 95 per cent confidence.

Table 3 : Summary of the result of sensory evaluation of different *dahi* samples

Sensory parameters	Types of *Dahi*				LoS
	Sample A	Sample B	Sample C	Sample D	
Color/Appearance	6.91^{b}±0.311	7.25^{ab}±0.322	7.37^{ab}±0.239	7.91^{a}±0.235	***
Body/Texture	6.12^{c}±0.42	6.58^{c}±0.311	7.91^{b}±0.235	8.5^{a}±0.25	***
Smell/Taste	6.66^{c}±0.186	7.41^{b}±0.276	7.75^{b}±0.204	8.04^{a}±0.335	*
Overall acceptance	6.87^{c}±0.279	7.29^{b}±0.224	8.06^{ab}±0.226	8.79^{a}±0.303	*

* and *** indicate significance of values at P *<0.001* and *0.05;* LoS= Level of Significant, Standard deviation (±SD) calculated with 95 per cent confidence
Type A= Plain *dahi*, Type B= probiotic plain *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*.

Cell viability analysis :

The average total viability per ml of *dahi* samples are presented in Table 1. There was significant (P<0.05) difference between plain *dahi* (A) and probiotic *dahi* (B); plain *dahi* (A) and *Greek dahi* (C); plain *dahi* (A) and probiotic *Greek dahi* (D); probiotic *dahi* (B) and *Greek dahi* (C), but there was no significant difference between probiotic *dahi* (B) and probiotic *Greek dahi* (D). The average total viability of different probiotic strain per ml of *dahi* samples are presented in Table 2 and Fig. 2. It was observed that the individual and total viability of different probiotic strain per ml of plain *dahi* (A), *Greek dahi* (C) and probiotic *Greek dahi* (D) were significantly (p<0.05) increased.

Cell viability during storage, the average total viability per ml of *dahi* samples are presented in Table 5. There was significant (P<0.05) difference between plain *dahi* (A) and probiotic *dahi* (B); plain *dahi* (A) and *Greek dahi* (C); plain *dahi* (A) and probiotic *Greek dahi* (D); probiotic *dahi* (B) and *Greek dahi* (C), but there significant difference between probiotic *dahi* (B) and probiotic *Greek dahi* (D). The average total viability of different probiotic strain per ml of *dahi* samples are decreasing during storage (Table 5). It was observed that the individual and total viability of different probiotic strain per ml of plain *dahi* (A), *Greek dahi* (C) and probiotic *Greek dahi* (D) were significantly (p<0.05) increased. Total cell viability levels, in the case of probiotic *greek dahi* (1.116±0.02) and lower in the traditional dahi (0.615±0.07).

Sensory analysis :

In fermented probiotic products, probiotic cultures contributes significantly towards sensory properties. Therefore it is quite common to use probiotic bacteria mixed together with other types of bacteria suited for the fermentation of the specific product. The score of color/appearance, body and texture, smell/taste and overall acceptability were complied in Table 3. It was observed that the individual and total color /appearance and body and texture significantly increased (p<0.05), whereas smell/taste and overall acceptability significantly (p<0.001) increased. The highest overall acceptability 8.79±0.30 found in probiotic *Greek dahi* (D) and lowest 6.87±0.27 in traditional *dahi* (A) (Table 3).

Shelf-life study :

The mean pH values (Table 4) of formulations ranged from 4.29 to 3.78, decreasing during the storage period, corroborating with the results of Oliveira and Damin (2002), who observed slight decrease in pH studying the viability of *dahi* bacteria and probiotic cultures in fermented *dahi* under refrigeration at 4°C during the storage period. In this study we found that 24 days shelf-life of probiotic *Greek dahi* (D) under refrigeration temperature (4^0 C) and 12 days shelf life of traditional *dahi* (A) under refrigeration temperature (4^0 C).

In the present study the mean pH values (Table 1) of *dahi* ranged from 4.23 to 4.08, decreasing during the storage period. An important characteristic of a probiotic is its survival at low pH (Brink *et al.*, 2006). In this study acidity of *Greek dahi* is 0.54±0.03 (C) and lowest 0.35±0.018 found in plain *dahi* (A). Acidity of *Greek* dahi is more, as explained by Boynton and Novakovic (2014) that liquid whey is to drained out, the resulting yogurt is thicker consistency and a more tart taste (more acidic) than unstrained yogurt. The acidity of *dahi* is increased with time of storage, this increased acidity is because of the continued fermentation process by lactic acid bacteria during the storage period as the result of post acidification of products with lactic acid production (Aportela Palacios *et al.*, 2005), in which lactose is converted into *lactic* acid (Pereira *et al.*, 2012). Beal *et al*. (1999) in a study on the combined effects of culture conditions and storage time on acidification, and viscosity of yogurt, reported that yogurt will always show a decrease in pH and increase in acidity during storage under, refrigeration. In the present study our data on greek or strained dahi which is reported first time also accepts with reported data. Total soluble solids (TSS) 36.37±1.95 g/100g were found in probiotic *Greek dahi* (D) and lowest 23.88±2.64 g/100g found in plain *dahi* (A). The total soluble solids (TSS) is required for estimation of total solids present in the *dahi* which increases the taste and sensory. Ghosh and Rajorhia (1987) observed that total solids content of *dahi* varied from 26.92 to 43.04 g/100g with an average value of 33.96 g/100g. In this study the highest moisture content was observed in probiotic *dahi* (89.5±0.31%) and the lowest were in the probiotic *Greek dahi* (43.4±0.17%). Avlesen *et al.* (1979) observed that a good flavour *dahi* contained 77.0 g/100g or less water. Since moisture content and shelf life are inversely

Table 4 : Shelf life study of different types of *dahi* under refrigeration temperature (4^0C)

Storage interval (days)	pH				Acidity (%)			
	Type A	Type B	Type C	Type D	Type A	Type B	Type C	Type D
0 day	4.23±0.06	4.18±0.04	4.11±0.04	4.08±0.02	0.35±.018	0.44±0.012	0.54±0.02	0.51±0.02
4 days	4.18±0.02	4.11±0.03	4.09±0.01	4.05±0.06	0.39±0.04	0.48±0.06	0.57±0.02	0.53±0.01
8days	4.01±0.04	4.08±0.14	4.02±0.16	4.01±0.04	0.51±0.06	0.54±0.08	0.61±0.08	0.62±0.04
12 days	3.86±0.02	4±0.04	3.96±0.08	3.98±0.08	0.67±0.04	0.61±0.15	0.63±0.23	0.64±0.21
16days	spoiled	3.84±0.02	3.91±0.06	3.96±0.24	spoiled	0.69±0.12	0.66±0.17	0.68±0.08
20days	spoiled	spoiled	3.81±0.03	3.88±0.01	spoiled	spoiled	0.71±0.06	0.69±0.02
24 days	spoiled	spoiled	spoiled	3.79±0.08	spoiled	spoiled	spoiled	0.73±0.6
28 days	spoiled	spoiled	spoiled	spoiled	spoiled	spoiled	spoiled	spoiled

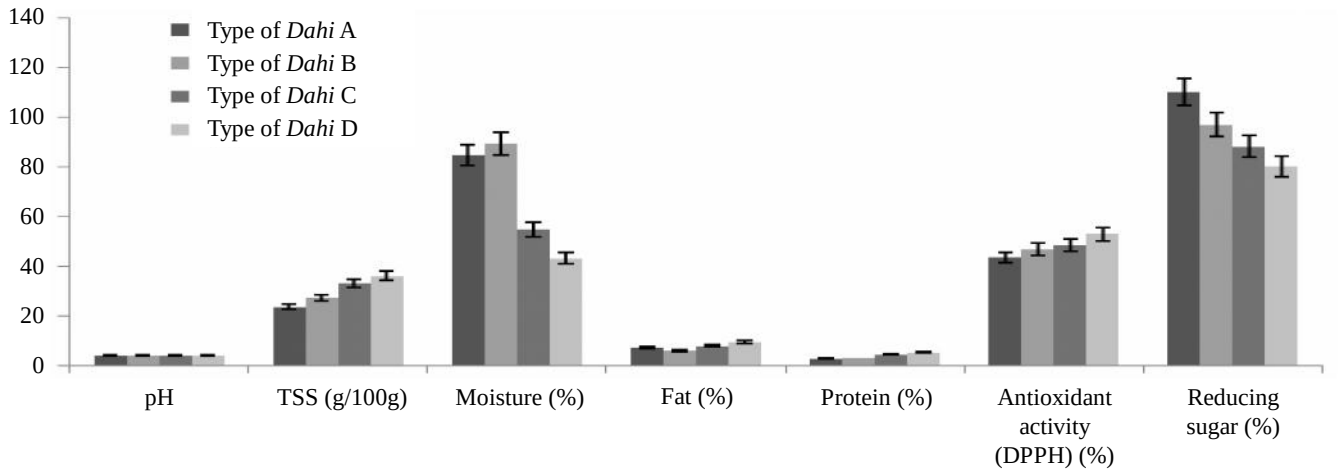
Standard deviation (±SD) calculated with 95 per cent confidence, Type A= Traditional *dahi*, Type B= probiotic *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*.

Table 5 : Cell viability count of different types of *dahi* during storage under refrigeration temperature (4^0C)

Cell viability	Storage time	Type of *dahi*			
		A	B	C	D
Total viability count	0 days	1.190±0.06	1.617±0.11	1.453±0.02	2.529±0.09
	4 days	1.132±0.02	1.328±0.05	1.298±0.04	2.287±0.06
	8days	1.081±0.04	1.192±0.05	1.159±0.06	2.184±0.12
	12 days	0.990±0.11	1.087±0.04	1.12±0.06	1.864±0.02
	16 days	0.783±0.06	0.962±0.02	1.014±0.04	1.656±0.06
	20 days	0.694±0.08	0.836±0.01	0.879±0.02	1.378±0.07
	24 days	0.647±0.02	0.797±0.11	0.824±0.04	1.291±0.05
	28 days	0.615±0.07	0.716±0.08	0.764±0.07	1.116±0.02
Lactobacillus. acidophillus	0 days	0.177±0.03	0.414±0.03	0.271±0.04	0.576±0.04
	4 days	0.152±0.03	0.395±0.06	0.231±0.04	0.541±0.04
	8days	0.146±0.05	0.378±0.05	0.218±0.03	0.528±0.06
	12 days	0.131±0.02	0.324±0.02	0.192±0.03	0.492±0.04
	16 days	0.112±0.04	0.286±0.05	0.184±0.06	0.477±0.03
	20 days	0.108±0.02	0.255±0.03	0.173±0.06	0.434±0.02
	24 days	0.0984±0.04	0.216±0.02	0.158±0.04	0.412±0.03
	28 days	0.0912±0.03	0.198±0.02	0.144±0.04	0.389±0.04
Lactobacillus. casei	0 days	0.147±0.03	0.172±0.03	0.124±0.04	0.356±0.06
	4 days	0.132±0.04	0.161±0.03	0.112±0.04	0.332±0.04
	8days	0.127±0.03	0.154±0.05	0.104±0.06	0.318±0.08
	12 days	0.118±0.04	0.148±0.03	0.0946±0.04	0.296±0.04
	16 days	0.101±0.03	0.122±0.04	0.0923±0.08	0.266±0.02
	20 days	0.092±0.08	0.111±0.03	0.0862±0.02	0.232±0.04
	24 days	0.081±0.03	0.102±0.04	0.0818±0.03	0.214±0.04
	28 days	0.068±0.04	0.821±0.02	0.0786±0.04	0.182±0.06
Lactobacillus. plantarum	0 days	0.197±0.04	0.470±0.05	0.308±0.04	0.621±0.06
	4 days	0.172±0.05	0.459±0.04	0.282±0.05	0.595±0.04
	8days	0.156±0.03	0.438±0.03	0.269±0.02	0.572±0.08
	12 days	0.134±0.04	0.421±0.04	0.244±0.03	0.544±0.06
	16 days	0.121±0.02	0.408±0.02	0.228±0.04	0.512±0.06
	20 days	0.102±0.02	0.382±0.06	0.197±0.03	0.482±0.04
	24 days	0.084±0.04	0.356±0.04	0.174±0.04	0.429±0.06
	28 days	0.073±0.02	0.326±0.03	0.142±0.03	0.375±0.02

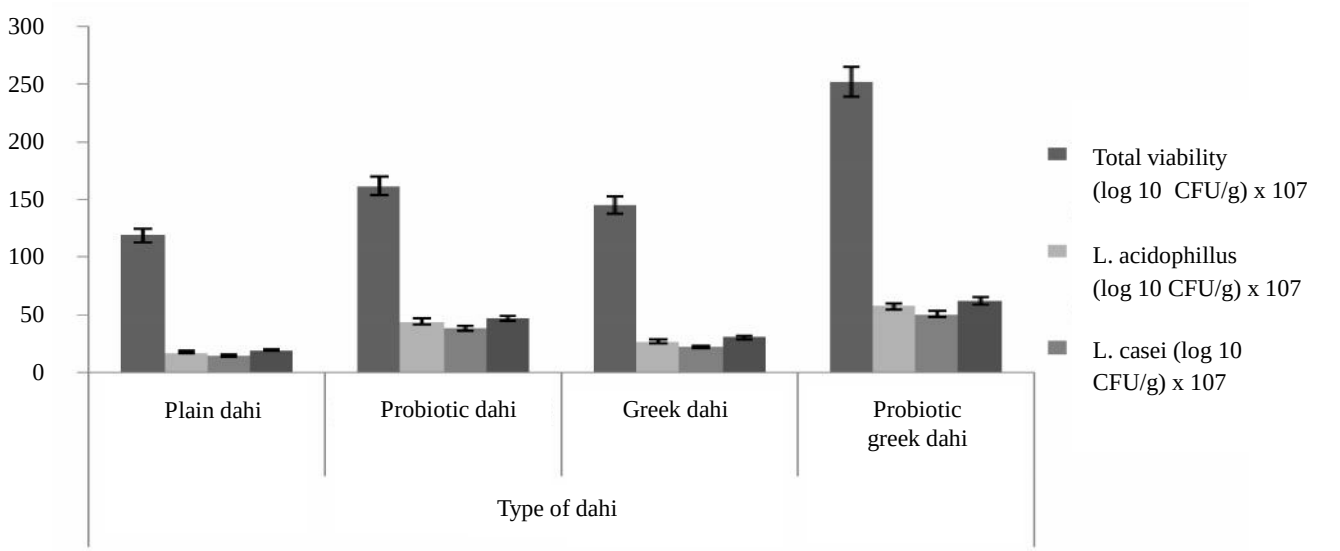
proportional, *Greek dahi* would have higher shelf-life.

The highest fat content was observed in type D (probiotic *Greek dahi*) 9.73 and the lowest was in the type A (plain *dahi*) 4.4. The Dairy Council (2013) showed that greek yoghut have higher fat percentage than plain yoghurt or unstrained yoghurt. Fat content influence flavors and taste of the product. The digestibility of fat is improved during fermentation and in the present study *Greek* dahi have higher fat content will have good digestibility compared with full fat milk. Fermented milk product is a rich source of whey proteins such as lactalbumin, lactoglobulin, lactoferrin, lactoperoxidase, immunoglobulins and variety of growth factors. The present study protein content was higher in probiotic *Greek dahi* followed by *Greek dahi* (C), probiotic plain *dahi* (B) and plain *dahi* (A). The range of antioxidant activity from 53.18±0.33 found in *Greek dahi* (C) and 43.63±0.71 in plain *dahi* (A). Strained yogurt contains a higher percentage of protein than unstrained yogurt simply because of the removal of a significant portion of



Standard deviation (±SD) calculated with 95 per cent confidence, Type A= Plain *dahi*, Type B= probiotic plain *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*

Fig. 1 : The result of physico-chemical analysis of different *dahi* samples



Standard deviation (±SD) calculated with 95 per cent confidence, Type A= Plain *dahi*, Type B= probiotic plain *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*

Fig. 2 : Result of the cell viability of the different probiotic strain in per ml of *dahi*

the whey (Boynton and Novakovic, 2014). McIntosh *et al.* (1998) revealed that proteins have demonstrated a number of biological effects ranging from anti-carcinogenic activity to different effects on the digestive function therefore our *Greek dahi* would be beneficial to health than normal dahi. DPPH (2, 2- diphenyl picryl hydrazyl free radical) radical scavenging studies showed that fermentation of *dahi* and selected probiotic starter strains increased the antioxidant activity. Lactose is the main source of reducing sugar present in the dairy products. In this study plain *dahi* (A) have the highest reducing sugar is 110.27±1.27 g/kg and lowest 80.33±1.12 g/kg in probiotic *Greek dahi* (D). Because of probiotic *Greek* dahi have lower lactose content, it can be tolerated by people with a reduced ability to digest lactose and thus promotes digestibility, and inhibits the growth of potentially harmful bacteria. This also influences the physical properties of casein improves the utilization of calcium and other minerals (Buttriss, 1997; McBean, 1999). Viability of probiotic bacteria in fermented products declines over time because of the acidity of the product, storage temperature, storage time, and depletion of nutrients (Dave and Shah, 1997) but in case bacteria remained above 10^7cfu/g for the duration of storage (24 days), thereby providing a population needed to a probiotic benefit. In a study Kurmann and Rasic (1991) explain that to obtain the desired health effects, probiotic bacteria must be able to grow in milk/ soymilk and survive in sufficient numbers. It has been suggested that probiotic organisms should be present in a food at a minimum concentration of 10^5-cfu/g (Kurmann and Rasic, 1991; Rybka and Fleet, 1997) or the daily intake should be about 10^8 cfu/g (Gomes and Malcata, 1999). The viability of the *Lactobacillus* species was investigated during storage at storage 4°C on the viable count of all strain from 0 to 28 days. In this study probiotic *Greek* dahi have maximum of 24 days shelf life with total viability count of 2.529±0.09 where as traditional dahi have maximum of 12 days shelf life with total count 1.190±0.06. Low temperature storage of dahi enables use of these lactic acid bacteria (LAB) species as probiotics, these results agree with that of Ashrad *et al.* (2009). Soares *et al.* (2011) observed that yoghurt cultures such as *Lactobacillus. bulgaricus* and *S. thermophilus* remain active even at refrigeration temperature and are able to produce small quantities of lactic acid through lactose fermentation, resulting in noticeable decrease in pH. Similarly in present study study also we have used dahi culture which is mixed, cultures and contains *Lactobacillus acidophilus, L. casei* and *L. plantarum* in addition other bacteria. Sensory evaluation revealed that the overall acceptability of all products was good but probiotic greek dahi stands first with 8.79±0.30 of overall acceptability.

In conclusion, different types of *dahi* prepared in the present study has significant differences with respect to its pH, titratable acidity, TSS, moisture, fat, protein, antioxidant activity, reducing sugar and total viability count. All types of *dahi* were produced acceptable physico-chemical, probiotic strain viability and sensory qualities. These new types of probiotic Greek dahi have highest protein, TSS, Fat, reducing sugar, antioxidant activity and sensory characteristics and 24 days shelf life under refrigeration temperature (4^0 C). It could be concluded that the delicious probiotic *Greek dahi* with pleasant aroma can be prepared by using probiotic cultures *viz*., *lactobacillus acidophilus, lactobacillus casei* and *lactobacillus plantarum* in combination containing viable lactobacilli counts more than 10^7 c.f.u/ g. According to Gorbach (1990), it is known that certain *Lactobacilli* species adhere to the gut mucosal surface and in a certain way inhibit the attachment of gram-negative bacteria. Hence our newly prepared probiotic *Greek dahi* will have more nutritional value, and can be used by lactose sensitive patients, and also can be used by diabetes.

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