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RESEARCH PAPER

Development and Characterization of *Greek Probiotic Dahi* Fortified with Pomegranate Pulp

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Abstract

Dahi is an indigenous Indian fermented milk product and is a good source of B vitamins, proteins, and calcium. Different types of *Dahi* such as sweet and flavored *Dahis* are found in the markets but value added products like that of its counterpart yoghurt such as fruit yoghurt are not available. Hence, Probiotic *Greek* (*strained) Dahi* fortified with pomegranate pulp has been developed and compared with traditional *Dahi*. Its physico-chemical, microbiological characteristics, shelf-life and sensory acceptance of all these products was made. Initially four *dahi* formulations were prepared: traditional *Dahi*, probiotic *Dahi*, *Greek Dahi* and probiotic *Greek Dahi* and analysed. Probiotic *Greek Dahi* had significantly decreased pH, moisture and reducing sugar and increased acidity, TSS, fat, protein and antioxidant compared to all the three *dahis*. The probiotic greek *dahi* was selected and was fortified with varied amount of pomegranate pulp. Probiotic Greek *Dahi* with 20% the pomegranate pulp had significantly decreased pH, but titratable acidity, fat, total solid, reducing sugar, carbohydrate and ash percentage were significantly increased compared to the control probiotic *Greek Dahi*. All the *Dahi* samples up to 24 days of storage showed acceptable pH and acidity values. Microbiologically, *Dahi* samples were stable with satisfactory quality for consumption. Probiotic *Greek Dahi* with 20% pomegranate pulp showed the highest quality acceptance by consumers. *Greek Probiotic Dahi* fortified with pomegranate pulp is a nutritions food with high acceptance by consumers.

Keywords: Greek *dahi*, probiotic, pomegranate pulp, antioxidant activity, curd

Dahi is an indigenous Indian fermented milk product known for its stimulating taste, palatability and curative values (Madan Lal *et al.,* 1980) also called as 'curd'. It is yoghurt like product made in India and neighboring countries. About 7% 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 obtained from pasteurized or boiled milk fermented with a culture. The different starter culture used in the manufacture of *dahi* includes *Lactococcus. lactis, Lactobaccilus. cremoris, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus. plantarum* and lactose fermenting yeasts. A good quality *dahi* is of firm and uniform consistency with a sweet aroma and clean acid taste. It should be with smooth and glossy surface, and a cut surface is trim and free from cracks and air bubbles. *Dahi* is a very nourishing food and is a source of protein, essential vitamin, minerals calcium and riboflavin. The proteins in curd are more readily digested than the protein in milk as

regular milk is only 32% digested after an hour in the digestive tract, whereas 91% of curd is digested of present in fresh milk.

The word 'probiotic' is coined by Kollath (1953) and is derived from the Greek language, which means for life. According to Lilly and Stillwell (1965), probiotics are substances produced by microorganisms that promote the growth of other microorganisms. However, the widely adopted definition states probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Probiotic bacteria have been used in dairy products like yoghurt and other fermented dairy foods to modulate the immune system, block pro-inflammatory molecule to increase mucin production (Tien *et al.,* 2006) and to reduce the serum cholesterol content (Vijayendra and Gupta, 2012). Fruits and vegetables have been suggested as ideal media for probiotic growth because they inherently contain essential nutrients (Luckow and Delahunty, 2004; Sheehan *et al.,* 2007). The addition of various probiotic strains has not been 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 confer physiological benefits (Fuller, 1992). It is, therefore, an ideal diet for those with sensitive digestive systems, particularly young children and elderly persons and lactose intolerant person. Lactose intolerance is the inability to metabolize lactose, because of a lack of the required enzyme lactase in the digestive system. Probiotic dairy product like yoghurt, and curd alleviate lactose intolerance. The intestine friendly bacterial cultures in curd keep colon healthy and also reduce the risk of colon cancer (Aso and Akazan, 1992; Aso *et al.* 1995).

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. In order to prolong the shelf-life of food, synthetic antioxidants such as (butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used in industrial processing. But those are restricted by legislative rules, related to their potential health risks and toxicity (Imaida *et al.* 1983; Kahl and Kappus, 1993). Therefore, naturally occurring antioxidants mainly plant sources as food additives medicinal materials as alternative for synthetic antioxidants are being searched (Proestos *et al.* 2005; D'Abrosca *et al.* 2007; Annegowda *et al.* 2013). Natural antioxidants such as phenolic compounds, flavonoids and other phytochemicals can function as free radical scavengers, reducing agents, chelators of prooxidant metals, or as quenchers of singlet oxygen and thus delay the oxidation process in food products (Rice-Evans *et al.* 1997; Kahkonen *et al.* 1999; Miraliakbari and Shahidi, 2008; Sun *et al.* 2009; Amarowicz *et al.* 2010). Pomegranate is a natural source of antioxidant and has higher levels of antioxidants than many other fruits, attributed to diverse phenolic compounds in the pulp. It is believed that pomegranate has cancer fighting properties and a glass full of pomegranate juice is said to contain more antioxidants than 10 cups of green tea (Anon, 2005).

Today, as a consequence of the consumer's desire for a healthy and fresh diet that is also low in calories, a wide range of fruit yoghurt can be found in food stores. The aim of this study was to develop indigenous probiotic that *Greek* style *dahi* fortified with pomegranate pulp that could provide higher amount of probiotic bacteria, very less lactose, high protein, and high micronutrients as well as antioxidants, flavonoids and total polyphenols in less volume of *dahi*.

Materials and Methods

Materials

Toned skim milk was collected from Amul Company and starter cultures of *dahi* and probiotic culture (*Lactobacillus acidophilus* NCDC195, *Lactobacillus. case* NDRI 184, and *Lactobacillus. plantarum* NDRI RTS) collected from NDRI (National Dairy Research Institute) Karnal, and Haryana, India. Fresh red pomegranate fruits were procured from the local market of Varanasi.

Preparation of pomegranate pulp

Pomegranate fruits were washed with clean water

and the skin was separated with the help of knife, aseptically. Arils of pomegranate fruits were manually separated from the peels and piths, and their pulp was extracted using an electric pulper (Sunbeam, model IE-AD, Milan, Italy). After extracting, the pulp was passed through a clean muslin cloth (hot water washed). These were kept in plastic containers and stored at freezing temperature (-0°C), until preparation of *dahi*.

Preparation of different types of greek style probiotic dahi fortified with pomegranate pulp

Toned milk was pasteurized and heated at boiling temperature. During boiling, it was stirred continuously with a stirrer to avoid formation of cream layer. After desired heating, milk was allowed to cool. When the temperature was about 40°C, milk was divided into five equal portions and different types of *dahi* were prepared from each portion. Pomegranate pulp was added before incubation with respective starter culture as suggested by Guven and Karaca (2002). The pasteurized pomegranate pulp was incorporated into milk at 5%, 10%, 15% and 20% level in different cups except the control. Milk was inoculated with desirable proportion of starter culture of *dahi* (1%) and probiotic culture (1%). The samples were incubated at 37°C until the complete curd formation/coagulation of *dahi* (8-12 hrs) took place. After complete coagulation, probiotic *dahi* was strained through a muslin cloth for preparation of *Greek dahi.* The *dahi* samples were stored at about 4°C.

To achieve this aim first four *dahi* formulations were prepared: traditional *dahi* (type A), probiotic *Dahi* (type B), *Greek Dahi* (type C) and probiotic *Greek Dahi* (type D).

Physicochemical analysis of products

The fortified probiotic greek *dahi* samples were chemically analyzed for various physico-chemical and other charactersestics. Total solids, carbohydrate and ash content of greek *dahi* samples were estimated according to the method of AOAC (2002). The reducing sugars were measured by DNS method. pH of *Greek 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 % lactic acid (Vijayendra and Gupta, 2013). The fat content of the samples was determined using Gerber method (Kleyn *et al.* 2001). The protein content of the greek *dahi* samples was estimated using the micro Kjedahl method (AOAC, 1990) and crude protein content was calculated using the factor 6.38.

In order to determine total polyphenols, total flavonoids, and antioxidant activity, the sample was extracted by the use of 80 % methanol acidified with HCl (0.5%). Polyphenols were determined by the Folin-Ciocalteu method (Singleton *et al.,* 1999). Briefly, 0.125 ml of Folin-Ciocalteu reagent and 0.25 ml of 25% sodium carbonate were added to the extract previously diluted with deionised water. After 60 min, the absorbance at 765 nm was measured. Polyphenol content was found from a standard curve plotted for (+) - catechin.

Total flavonoid content was measured by aluminium chloride assay (Zhishen *et al.* 1999; Ardestani and Yazdanparast, 2007). Briefly, after appropriate dilution of the extract with deionised water, $NaNO_2$, $AlCl_3$ and NaOH were added; the samples then thoroughly vortex mixed and placed in darkness for 15 min. Afterwards, the absorbance at 510 nm was measured. Total flavonoid content was calculated from the standard curve plotted for (+)-catechin. Antioxidant activity was determined by means of three spectrophotometric methods: as scavenging activity against DPPH (1.1-diphenyl-2-picrylhydrazyl) free radical (Pekkarinen *et al.,* 1999); applying ABTS (2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) cation radical (Re *et al.,* 1999); and by the ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996).

Antioxidant activity was determined by means of three spectro-photometric methods: as scavenging activity against DPPH (1.1-diphenyl-2-picrylhydrazyl) free radical (Dorman *et al.,* 2004). 2 ml of methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1 ml of extract were placed in cuvettes. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then,

the absorbance was measured at 517 nm against methanol as a blank in spectro-photometer. The DPPH free radical concentration was calculated using the following equation:

DPPH scavenging effect (%) = (A0−A1/A1) × 100

Where, A0 was the absorbance of the negative control or blank and A1 was the absorbance of reaction mixture or standards.

Microbiological analysis

Prepared *dahi* samples were examined for total viable count, total coliform count, total yeast and mold count. Total viable count and yeast and mold count were determined according to the "Standard Methods for Examination of Dairy Products" by American Public Health Association (APHA, 1998). Total coliform (MPN/g) was counted by MPN (Most probable number) method.

For cell viability of probiotic bacteria of *dahi* samples were examined by a selective media. MRS-maltose agar used for *L. acidophilus* (IDF, 1995). De Man Rogosa Sharpe agar with vancomycin (1 mg/L) was used as 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 analysis

The Fortified probiotic greek *dahi* samples were judged individually by an expert judge team for sensory evaluation on the basis of 9 point hedonic scale.

Statistical analysis

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, microbiological and sensory tests were analyzed by one-way analysis of variance (ANOVA).

Results and Discussion

Physico-chemical analysis

The mean pH values (Table 1) of *dahi* ranged from 4.23 to 4.08, decreasing further during the storage period. An important characteristic of a probiotic is its survival at low pH (Brink *et al.* 2006). It was observed from the results that the lowest acidity 0.35±0.018 was found in traditional *dahi* (A), highest 0.54±0.03 found in *Greek dahi* (C) and probiotic *Greek dahi* had 0.51±0.02. Acidity of *Greek dahi* is more, as explained by Boynton, R.D. and Novakovic, A.M., (2014) that liquid whey is to drained out, the resulting yogurt is thicker consistency and has a more tart taste (more acidic) than unstrained yogurt. The increase in 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 the sensory perception and storage behaviour. The acidity of *dahi* is increased with time of storage, this increased acidity, thus, is because of the continued fermentation process by lactic acid bacteria during the storage period as 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).

As in Table 1, total soluble solids (TSS) percent in probiotic *Greek dahi* was higher than that of other samples. 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. The moisture content of product is measured for shelf-life, lower the moisture content maximum 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 shown in the Table 1. Since moisture content and shelf-life are inversely proportional, *Greek dahi* should have higher shelf-life.

The highest fat content was observed in probiotic *Greek dahi* 9.73 while the lowest was in the traditional *dahi* 4.4 (Table 1). The Dairy Council, (2013) showed that greek yoghut had higher fat percentage than plain yoghurt or unstrained yoghurt. Fat content influence flavors and taste of the product. Further, the digestibility of fat is improved during fermentation

Table 1: Summary of the result of Biochemical, sensory and microbial evaluation of different types of *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}±0.01	0.44^{ab}±0.05	0.54^{a}±0.03	0.51^{ab}±0.02	***
TSS (g/100g)	23.88^{b}±2.6	27.46^{ab}±1.6	33.4^{ab}±0.93	36.37^{a}±1.95	*
Moisture (%)	85.00^{b}±0.2	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}±0.16	3.18^{bc}±0.07	4.61^{b}±0.17	5.43^{a}±0.197	***
Reducing Sugar (%)	110.2^{a}±1.2	97.29^{b}±1.7	88.39^{c}±0.56	80.33^{d}±1.12	*
Sensory Parameter					
Color/Appearance	6.91^{b}±0.31	7.25^{ab}±0.32	7.37^{ab}±0.23	7.91^{a}±0.235	***
Body/Texture	6.12^{c}±0.42	6.58^{c}±0.31	7.91^{b}±0.235	8.5^{a}±0.25	***
Smell/Taste	6.66^{c}±0.18	7.41^{b}±0.27	7.75^{b}±0.204	8.04^{a}±0.335	*
Overall acceptance	6.87^{c}±0.27	7.29^{b}±0.22	8.06^{ab}±0.22	8.79^{a}±0.303	*
Microbial Parameter					
Total viabilty (log10 CFU/g) $\times 10^{7}$	1.190±0.06	1.617±0.11	1.453±0.11	2.529±0.09	***
Probiotic Strain					
L. acidophillus (log10 CFU/g) $\times 10^{7}$	0.177±0.03	0.439±0.04	0.271±0.04	0.576±0.04	*
L. casei (log10 CFU/g) $\times 10^{7}$	0.146±0.03	0.385±0.05	0.223±0.04	0.507±0.06	*
L. plantarum (log10 CFU/g) $\times 10^{7}$	0.190±0.04	0.470±0.05	0.308±0.04	0.621±0.06	*

*= $p<0.001$; ***= $p<0.05$, NS= Non significant LoS= Level of Significant, Standard deviation (±SD) calculated with 95% confidence, Type A= Plain *dahi*, Type B= probiotic plain *dahi*, Type C=*Greek dahi*, Type D= Probiotic *Greek dahi*.

and in the present study *Greek dahi* have higher fat content will have good digestibility compared with full fat milk. Table 1 shown that the protein content was higher in probiotic *Greek dahi*. McIntosh *et al.* (1998) have shown that proteins have 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*. The highest reducing sugars 110.27±1.27 g/Kg were found in traditional *dahi* (A) and lowest 80.33±1.12 g/Kg in probiotic *Greek dahi* (D) (Table 1). Because the probiotic *Greek dahi* have low 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 and improves the utilization of calcium and other minerals. (But riss, 1997; McBean, 1999). The average total viability of different probiotic strain per ml of *dahi* samples are presented in Table 1. It was observed that total viable count of the bacteria were significantly more in sample B and D. which may be due to addition of probiotic strains. We have also measured the individual viability of probiotic strain per ml. *Greek dahi* (D) had highest viable count than sample A, B and C. All types of *dahi* produced had acceptable sensory qualities.

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.

These results shown that probiotic greek *dahi* has many desirable characteristics. Therefore we have to chosen probiotic greek *dahi* to make probiotic greek *dahi* fortified with pomegranate pulp. Statistical analysis showed also that the differences of pH, titratable acidity, fat percent, total solid,

protein percent, reducing sugar, carbohydrate and ash percent *of probiotic greek dahis* fortified with pomegranate pulp were significant ($p<0.05$).

In this study, four Greek probiotic *dahi* were prepared: no addition of pomegranate pulp (T1), 5% (T2), 10% (T3), 15% (T4) and 20% pomegranate pulp (T 5). All the preparations were analyzed physico-chemically.

Titratable acidity, fat, total solid, reducing sugar, carbohydrate and ash percentage were increasing, with addition of increasing concentration of pomegranate pulp which was lowest in control probiotic greek *dahi* (T1) and highest in 20% pomegranate pulp probiotic greek *dahi* (T5).

Titratable acidity ranged from 0.51 to 1.26. Khalid Khan *et al.* (2008) have reported that incorporation of fruit pulp increases the acidity of yoghurt, whereas the pH was significantly decreased ($P<0.05$) among the *probiotic greek dahi* samples. The pH was decreasing; with increasing concentration of pomegranate pulp which was the highest in control *dahi* (4.08) and lowest in 20% pomegranate pulp *dahi* (3.81). The results of the present study experiment agreed with the findings of Mustafa (1997) who recorded that pH of *dahi* decreased after addition of fruit juice.

Generally fruit contains low level of fat. So, the addition of pomegranate pulp might have little increase in the fat percentage. Fat percent ranged 5.12 to 5.42. Ghosh and Rajorhia (1987) found that fat percent of plain misty *dahi* varied from 4.3 to 8.8% with an average of 3.78 percent. The results of the study agrees with the findings of Desai *et al.* (1994) and Mustafa (1997) who found that the titratable acidity, total solids, carbohydrate and ash content of fruit was significantly increased by the addition of fruit juice/ pulp. Total solid was recorded from 36.37 to 41.32 which indicated that total solid content increased with increasing concentration of pomegranate pulp. Ghosh and Rajorhia (1987) observed that total solids content of *dahi* varied from 269.2 to 430.4 g/kg with an average value of 339.6 g/kg. Ghosh and Rajorhia (1987) observed that solids content of plain market *dahi* varied from 26.92 to 43.04% with an average value of 34.64 percent.

The protein content was not significant ($p<0.05$) among the different treatments because fruit juice contains lower proteins than milk. These results agrees with the work of Mustafa *et al.* (1997) who found that plain *dahi* contain higher amount of protein than fruit *dahi*. The highest value of reducing sugar was recorded in case of greek style probiotic *dahi* (4.53) with 20 % pomegranate pulp and minimum in control greek *dahi* (2.46). This indicates that reducing sugar is increased with the addition of pomegranate pulp in the *dahi*.

The carbohydrates content was the highest in probiotic greek *dahi* (12.86) with 20 % pomegranate pulp and minimum in control probiotic greek *dahi* (11.21) (Table 2). Maximum ash content was seen in probiotic greek *dahi* (0.68) with 20% pomegranate pulp and minimum in control probiotic greek *dahi* (0.48). Generally fruit contains high level of carbohydrates and ash. Therefore, the variation might have come from chemical composition of pomegranate pulp.

Total phenolic and flavonoids content

The results of total phenolic content (TPCs) and flavonoids content of investigated fortified probiotic greek *dahi* are summarized in Table 3 and Fig. 1. TPCs of *dahi* samples ranged from 218 to 376 (mg/100g). The addition of pomegranate pulp (Pp) in the range of 0 to 20% to probiotic greek *dahi* led to an increase in TPCs of the probiotic greek *dahi*. The probiotic greek *dahi* containing 20% pomegranate pulp showed highest concentrations of TPC of all the samples measured. Phenolic compounds play important role in delaying the development of chronic diseases such as cardiovascular diseases, cancer, inflammatory bowel syndrome and Alzheimer's diseases (Chun *et al.* 2005). The antioxidant activity of phenolic compounds is mainly attributed to their redox actions, neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Flavonoids of *dahi* samples ranged from 112 to 204 (mg/100g). Flavonoids were increased with increase of pomegranate concentration. The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and

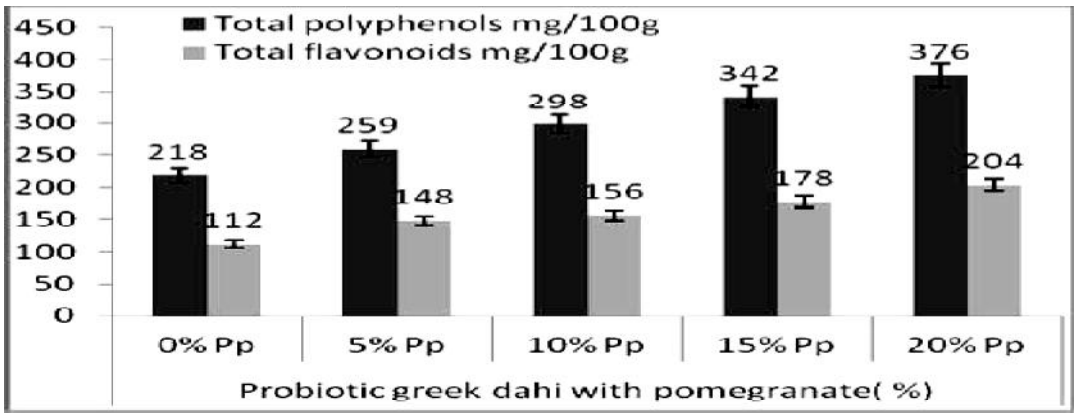

Fig. 1: Changes in total phenols and total flavonoids of greek style probiotic *dahi* with different proportion of pomegranate pulp (pp = pulp)

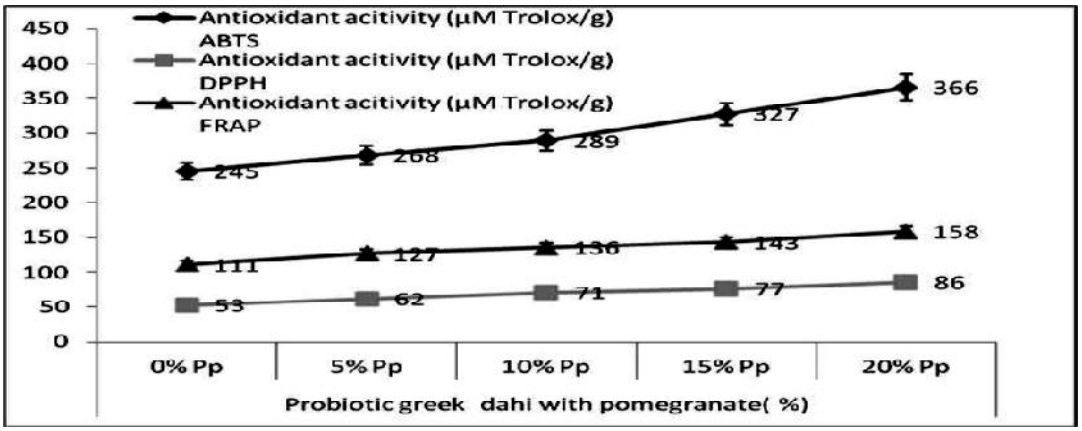

Fig. 2: Changes in Antioxidant activity by ABTS, DPPH and FRAP method of greek style probiotic *dahi* with different proportion of pomegranate pulp (pp = pulp)

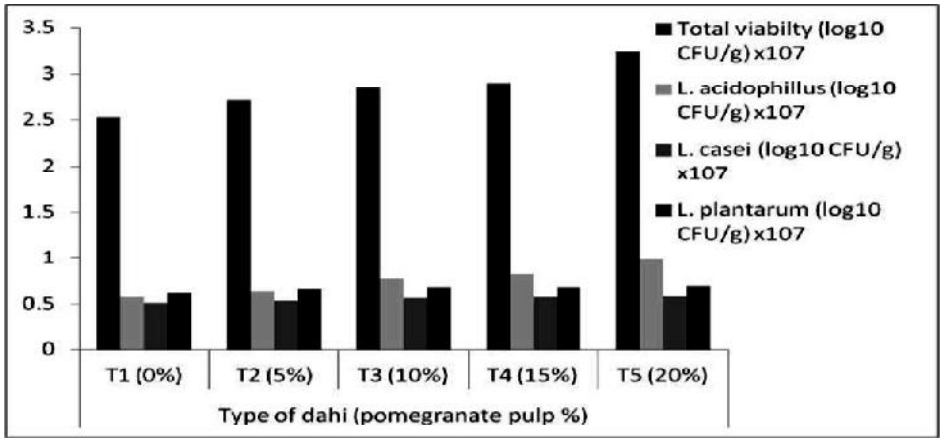

Fig. 3: Effect of pomegrante pulp concentration on total viability count and probiotic strain counts of greek style probiotic *dahi* with different proportion of pomegranate pulp (pp = pulp)

Table 2: Physico-chemical characteristics of greek style probiotic *dahi* fortified with different level of pomegranate pulp

Physico-chemical Parameters	Probiotic greek dahi with (%) Pomegranate pulp				
	0%	5%	10%	15%	20%
Acidity (%)	0.51±0.02	0.86±0.04	0.92±0.03	1.11±0.02	1.26±0.01
pH	4.08 ±0.02	4.0 ±0.03	3.97 ±0.01	3.89 ±0.03	3.81 ±0.02
Fat (%)	5.12±0.12	5.18±0.08	5.26±0.06	5.35±0.03	5.42±0.01
Total solid (%)	36.37±0.19	37.18±0.19	37.49±0.09	40.95±0.06	41.32±0.10
Protein (%)	5.43±0.19	5.46±0.26	5.51±0.24	5.57±0.21	5.6±0.21
Reducing sugar (%)	2.46±0.12	3.32±0.38	3.75±0.21	4.26±0.09	4.53±0.24
CHO (%)	11.21±0.41	11.69±0.41	11.89±0.36	12.13±0.28	12.86±0.11
Ash (%)	0.48±0.032	0.51±0.02	0.59±0.04	0.64±0.02	0.68±0.02
	Descriptive Statistics				
	N statistics	**Minimum Statistics**	**Maximum Statistics**	**Mean Std. Error**	**Std. Deviation**
Acidity (%)	4	0.51	1.26	0.04761	0.09522
pH	4	3.81	4.08	0.13769	0.27538
Fat (%)	4	5.12	5.42	0.19259	0.38517
Total solid (%)	4	36.37	41.32	1.15220	2.30441
Protein (%)	4	5.43	5.6	0.14442	0.28883
Reducing sugar (%)	4	2.46	4.53	0.0352	0.0743
CHO (%)	4	11.21	12.86	0.99733	1.99467
Ash (%)	4	0.48	0.68	0.02858	0.05715

Standard deviation (±SD) calculated with 95% confidence.

Table 3: Total phenols, flavonoids and antioxidant activity of probiotic greek *dahi* with pomegranate concentration

Sample of probiotic greek dahi (% PP)	Total polyphenols mg/100g	Total flavonoids mg/100g	Antioxidant acitivity		
			ABTS	DPPH	FRAP
			µM Trlox/g	µM Trolox/g	µM Trolox/g
0%	218±17	112±2	245±8	53±4	111±2
5 %	259±16	148±1	268±2	62±4	127±5
10 %	298±12	156±6	289±4	71±2	136±7
15 %	342±13	178±4	327±7	77±7	143±4
20%	376±16	204±3	366±8	86±3	158±4

Standard deviation (±SD) calculated with 95% confidence.

metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation. Fruits and vegetables are rich source of flavonoids (Ioannou and Ghoul, 2012), thus might be responsible for increased content of flavonoids in the pomegranate fortified *dahi*.

Total antioxidant activity

High antioxidant potential of the pomegranate pulp-fortified *dahi* in terms of DPPH radical scavenging activity may be due to the existence of numerous hydroxyls in pomegranate pulp molecule, which could serve as electron donator and transfer of electron to DPPH free radical (Krings and Berger, 2001). As shown in Table 3 and Fig.3, ABTS, FRAP and the percentage of free radical DPPH• inhibition were increased with the increasing concentration of pomegranate pulp (Pp). A significant difference in the amount of DPPH scavenging activity, ABTS and FRAP were found among the probiotic greek *dahi* samples T2 to T5. ABTS was varied from 245 µM Trlox/g in control probiotic greek *dahi* to 366 µM Trlox/g in samples T5 containing 20% Pp. FRAP was varied from 111 µM Trlox/g in control probiotic greek *dahi* to 158 µM Trlox/g in samples T5 containing 20% Pp. DPPH scavenging activity was varied from 53 µM Trlox/g in control probiotic greek *dahi* to 86 µM Trlox/g in samples T5 containing 20% Pp. Pomegranate is known to be a rich source of antioxidant therefore antioxidant acivity of probiotic greek *dahi* increases due to increase percentage of pomegranate pulp.

Sensory evaluation of Greek Probiotic Dahi fortified with pomegranate pulp

Control probiotic greek *dahi* (0% Pp) was compared with probiotic greek *dahis* incorporating different concentrations (5%, 10%, 15% and 20%) of pomegranate pulp. Results of the sensory parameters were presented in Table 4. Based on the scores given by the panelists, there was significantly decrease ($p<0.05$) among the colour of *dahi* (Table 4). The best sample with the highest score of body and texture, smell/ flavour and overall acceptability was for the greek probiotic *dahi* supplemented with 20 % pulp (Pp) (Table 4). However, the lowest scores for body and texture, smell/ flavour and overall acceptability respectively were for samples containing control *dahi* (0% Pp). Statistical analysis showed that there was significant difference ($p<0.05$) among the body and texture, color and overall acceptability score of different probiotic *greek dahi*. The highest score of body and texture (8.16) was probiotic greek *dahi* with 20% Pp and the lowest score (6.19) was seen in case of control probiotic greek *dahi*. The result of this experiment supports the findings of Desai *et al.* (1994) who observed that addition of fruit juice improved the body and texture of yoghurt. There was significant difference ($p<0.05$) for smell and taste scores of among different types of probiotic greek *dahi*. The results agrees with the work of Desai *et al.* (1994) who found that smell and taste of mango and pineapple yoghurt were higher than that of control yoghurt. Mustafa (1997) and Keating and White (1991) also found that the addition of fruit juice improved physical properties of *dahi*.

Microbiological evaluation of Greek Probiotic Dahi fortified with pomegranate pulp

Microbiological characteristics are indicators of safety, quality and shelf-life of prepared probiotic greek *dahi*. Total viable count, viability count of probiotic strain, total coliform count and yeast and mold count of the fortified probiotic greek *dahi* was determined, results obtained are shown in Table 5.

Total viable Count and viability count of probiotic strain

Result of total viable Count and viability count of probiotic strain are shown in Table 5. Average highest total viable count was seen in probiotic greek *dahi* with 20% percent pomegranate pulp. The total viable count per ml of 0%, 5%, 10%, 15% and 20% pomegranate pulp *probiotic greek dahis* were 2.52±0.09, 2.71±0.11, 2.85±0.07, 2.9±0.04 and 3.24±0.04 (log cfu/ml $x10^7$) respectively (Table 5). It was revealed that there were significant ($p<0.01$) differences amongst *dahi* samples. The variations in total viable count in probiotic greek *dahi* samples might be due to the

Table 4: Sensory evaluation of greek style probiotic *dahi* fortified with different levels of pomegranate pulp

Sensory parameters	Pomegranate pulp (%)				
	0%	5%	10%	15%	20%
Color/Appearance	7.83±0.06	7.78±0.02	7.53±0.03	7.28±0.08	7.15±0.05
Body/ Texture	6.91±0.38	7.12±0.25	7.25±0.43	7.33±0.76	8.16±0.14
Smell/ Taste	7.08±0.14	7.15±0.66	7.25±0.66	7.27±0.52	7.55±0.37
Overall acceptance	6.58±0.38	7.25±0.14	7.50±0.38	7.58±0.5	8.25±0.20

Standard deviation (±SD) calculated with 95% confidence

Table 5: Mean values (M±SD) of total viable count (Log10) and viability of probiotic strain count (Log10) in per ml of *dahi* sample

Microbial Parameters	Type of *dahi* (pomegranate pulp %)				
	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	T5 (20%)
Total viabilty (log10 CFU/g) $\times 10^7$	2.52±0.09	2.71±0.11	2.85±0.02	2.9±0.07	3.24±0.04
Probiotic strain					
L. acidophillus (log10 CFU/g) $\times 10^7$	0.576±0.04	0.639±0.04	0.771±0.04	0.82±0.04	0.976±0.04
L. casei (log10 CFU/g) $\times 10^7$	0.507±0.06	0.534±0.05	0.564±0.04	0.57±0.06	0.588±0.06
L. plantarum (log10 CFU/g) $\times 10^7$	0.621±0.06	0.660±0.05	0.678±0.04	0.68±0.06	0.691±0.06
Coliform count (log cfu/ml)	0.0	0.0	0.02± 0.08	0.02± 0.09	0.03 ± 0.02
Yeast and mould count (log cfu/ml)	0.0	0.0	0.03 ± 0.02	0.09± 0.05	0.12± 0.03

Standard Deviation (±SD) calculated with 95% confidence

addition of pomegranate pulp which has increased acidity may be due to increased growth of acidophilic bacteria. As the percentage of pomegranate pulp is increasing, the total viable count also increase. Then to confirm, the total count was measured and *Lactobacillus acidophilus, L. casei, and L. plantarum* were taken. As table 5 and Fig. 3 shown *L. casei, and L. plantarum* are grown constantly but growth of *Lactobacillus acidophilus* were observed to be the highest amongst all the samples.

Coliform count and Yeast and mould count

Results corresponding to the coliforms and yeast and mold count of different *dahi* samples are shown in Table 5. The average Coliform count per ml. of control probiotic greek *dahi*, 5%, 10%, 15% and 20% pomegranate pulp probiotic greek *dahis* were 0.0, 0.0, 0.02±0.08, 0.02±0.09 and 0.3±0.02, respectively. The average yeast and mould count per ml. of control probiotic greek *dahi*, 5%, 10%, 15% and 20% pomegranate pulp probiotic greek *dahis* were 0.0, 0.0, 0.03±0.02, 0.09±0.05 and 0.12±0.03 respectively. Presence of yeast and mould count was the indication of contamination. No yeast was grown in our experiment which shows greater quality of Bogra *dahi* samples. Dastum (1956); Henricks and Deconick (1965) reported that yoghurt did not grow any yeast.

Shelf-life study of dahi during storage under refrigeration temperature (4°C)

The mean pH values (Table 6) of formulations ranged from 4.08 to 3.53, decreasing during the storage period while acidity ranged from 0.51 to 1.62, increasing during the storage period. Oliveira and Damin (2002), who observed fermented milk product pH slightly decreases and increase acidy

Table 6: Shelf-life study on the basis of pH and acidity of greek style probiotic *dahi* with different concentration of pomegranate pulp under refrigeration temperature (4°C)

Storage interval (days)	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	T5 (20%)
			pH		
0 day	4.08±0.06	4.0±0.04	3.97±0.04	3.89±0.02	3.81±0.11
4 days	4.06±0.02	3.91±0.03	3.92±0.01	4.05±0.06	3.75±0.06
8days	4.01±0.04	3.88±0.14	3.88±0.16	4.01±0.04	3.71±0.04
12 days	3.86±0.02	3.75±0.04	3.81±0.08	3.98±0.08	3.68±0.08
16days	spoiled	3.71±0.02	3.76±0.06	3.96±0.24	3.63±0.24
20days	—	3.65±0.04	3.72±0.03	3.88±0.01	3.58±0.01
24 days	—	3.6±0.04	3.64±0.04	3.79±0.08	3.53±0.08
28 days	—	Spoiled	Spoiled	Spoiled	Spoiled
			Titrable Acidity		
0 day	0.51±.018	0.86±0.11	0.92±0.02	1.11±0.02	1.26±0.5
4 days	0.69±0.04	0.88±0.06	0.96±0.02	1.13±0.01	1.33±0.01
8days	0.78±0.06	0.94±0.08	0.99±0.08	1.19±0.04	1.40±0.04
12 days	0.87±0.04	0.98±0.15	1.13±0.23	1.24±0.21	1.46±0.21
16days	spoiled	1.09±0.12	1.16±0.17	1.28±0.13	1.51±0.28
20days	—	1.18±0.21	1.27±0.41	1.36±0.08	1.54±0.18
24days	-	1.29±0.16	1.36±0.3	1.48±0.04	1.62±0.31
28days	—	Spoiled	Spoiled	Spoiled	Spoiled

Standard deviation (±SD) calculated with 95% confidence.

during storage study. In this study, it was found that the shelf-life of fortified probiotic greek *dahi* was 24 days and control probiotic greek *dahi* was 12 days. Addition of pomegranate pulp increased the shelf-life may be because of antioxidant activity of pomegranate. Thus, control *dahi* (0% Pp) has shelf-life of only 12 days where as the pomegranate pulp containing samples T2-T5 had 24 days shelf life.

Conclusion

In conclusion, different types of probiotic greek *dahi* fortified with pomegranate pulp 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 and also emphasizing that pomegranate fruit can be a good source of nutrients and antioxidant. All types of probiotic greek *dahi* were produced acceptable physico-chemical, probiotic strain viability and sensory qualities.

These new types of probiotic Greek *dahi* fortified with 20% pomegranate pulp have highest protein, TSS, Fat, reducing sugar, antioxidant activity and sensory characteristics and 24 days shelf life under refrigeration temperature (4°C). It could be concluded that the delicious probiotic *Greek dahi* with 20% percent pomegranate pulp, with pleasant aroma can be prepared by using probiotic cultures *viz.*, *lactobacillus acidophilus, lactobacillus casei* and *lactobacillus plantarum* in combination, containing viable lactobacilli counts of more than 10^7 c.f.u/gm. According to Gorbach (1990), it is known that certain *Lactobacilli* species adhere to the gut mucosal surface and in a certain way inhibit the at achment of gram-negative bacteria.

Hence the newly prepared probiotic *Greek dahi* with 20% pomegranate pulp have more nutritional value, and can be used by lactose sensitive patients.

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