Two Comparative study for viability of yogurt in traditional and lemon fruits (*Lactobacillus acidophilus* strain) isolates.

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Abstract: Recognized to confer health benefits to consumers, probiotics such as *Lactobacillus acidophilus* are commonly incorporated into fermented dairy products worldwide; among which yogurt is a popular delivery vehicle. To materialize most of the putative health benefits associated with probiotics, an adequate amount of viable cells must be delivered at the time of consumption this study focused on the viability of yogurt in traditional and effects of yogurt starter cultures on the survival of isolates probiotic *L. acidophilus* strain from lemon fruit, Differential survival behavior between *L. acidophilus* strains was further analyzed. To this end, viable cell counts of *L. acidophilus* were determined weekly during 4 °C storage in various types of yogurts made with Traditional method addition of *Streptococcus thermophilus, L. delbrueckii* ssp. *bulgaricus* alone, both species of the starter cultures, only *L. acidophilus* strain without starter culture addition Multiplication of *L. acidophilus* was not affected by the starter cultures as all strains reached high level on day 0 of the storage period. Throughout the 28-day storage period cell counts of *L. acidophilus* remained steady (~ 6×10^7 CFU/g) in yogurts made with both starter cultures with traditional method associate mix C. Delineating factors driving the differences in survival trait among probiotic strains will lead to a more efficacious delivery of health benefits in fermented dairy products through targeted technological interventions.

Introduction

Yogurt as a dairy food can be consumed in form of snack, thirst quenching beverages and as a desert. It is semi solid custard like product obtained from pasteurized or boiled milk by souring, natural or otherwise, by a harmless lactic acid or other bacterial culture. Milk is very nutritious and obligatory food for human being. But in this era of industrialization, food habit of common people is changing. They are preferable as it is healthy, delicious foods to fresh raw foods. Hence, milk is converted to various milk products, like yogurt, fermented milk, cheese, butter, yogurt, milk ice-cream etc. of which yogurt is locally available dairy product in Indian sub-continent.

Like milk, yogurt is also very nutritious as it is a good source of iodine, calcium, phosphorus, zinc, riboflavin, vitamin B5 and vitamin B12. It is also nutritionally rich in protein, molybdenum and pantothenic acid. The food rating system adopted as the government standard for food labelling that are found in the U.S food and drug administration allow yogurt to be rated as one of the world's healthiest food. Yogurt bacteria are also capable of manufacturing the entire range of B- complex vitamins in the intestine. This is very important because many modern drugs kill intestinal flora. Another advantage of yogurt is that people who are allergic to milk can generally eat it safely.

"Probiotics" are defined as "live microorganisms which when their viability in the products has been cited as an important administered in adequate amounts confer a health benefit on the prerequisite for achieving beneficial health effects (Galdeano and host" (FAO/WHO, 2001). Numerous reports have suggested probiotics Perdigón, 2004). Hence, different forms of delivery vehicles should bestudied and optimized to ensure that probiotics are viable and delivered in sufficient numbers before the expiration date (Godward et al., 2000). Based on previous studies characterizing a wide range of probiotic species and strain, a very high dose – minimum of 10^8 CFU/day, mostly in the range of 10^{10}–10^{11} CFU/day – was required for the respective health benefits (Lourens-Hattingh and Viljoen, 2001; Parvez et al., 2006; Tamime et al., 2005). For instance, *Lactobacillus acidophilus*, a common probiotic species, was administered at a minimum level of 10^9 CFU daily to prevent or treat some gastroin testinal (GI) disorders (reviewed by WGO, 2008). Kailasapathy and Chin (2000) also suggested that the minimum therapeutic dose of probiotics should be 10^8 to 10^{10} CFU/day. This amount could be translated into $\geq 10^6$ CFU/g/day of probiotics-containing yogurt given that 100 g is the daily serving portion. High dosage is required to compensate for the loss of cells during the passage through the upper and lower parts of the GI tract (Tamime et al., 2005). For probiotics delivered through a food vehicle, additional amounts of cells are likely required prior to processing to account for the loss of cells during the processing and/or storage phases.

Maintaining a high level of viable probiotic cell count in yogurts throughout the shelf life, however, is not a simple task. Many factors influence the viability of probiotics in yogurts: strain variation, acid accumulation, with starter cultures, level of dissolved oxygen and hydrogen peroxide (H_2O_2), and storage condition (Donkor et al., 2006; Gilliland and Speck, 1977; Nighswonger et al., 1996; Talwalkar and Kailasapathy, 2003). Evidently, several studies reported that some commercially available dairy products contain insufficient number of viable probiotics (as defined by b 10^6 CFU/g or mL before the expiration date), thereby diminishing the potential health benefits conferred by these products (Coeuret et al., 2004; Huys et al., 2006; Lin et al., 2006; Tharmaraj and Shah, 2003). Thus, understanding the survival of probiotics and developing methods to maintain and/or to promote their viability throughout the product shelf life continues to be an important subject of research in this field.

Many previous studies focused on devising strategies to improve the viability of particular *L. acidophilus* strains that showed suboptimal survival in yogurts (reviewed by Shah, 2000). These include reducing the oxygen content in the food by adding ascorbic acid (Dave and Shah, 1997a), and protecting the probiotics by means of encapsulation or addition of cry protectants (Capela et al., 2006). This study, on the other hand, undertook a comparative approach to probe the effects of acid and the presence of starter cultures on the survival of *L. acidophilus* in yogurts during the storage phase. This will help pinpoint the causes of suboptimal survival and provide a basis to develop more effective measures. Thus, the objectives were to determine the survival of five different *L. acidophilus* strains in yogurts made with different combinations of yogurt starter cultures, and to determine their survival independent of the starter culture fermentation by using an acidulate. The best and worst survival strains were chosen to further investigate factors attributing to the differential viabilities.

Enhancing our understanding of *L. acidophilus* survivability in yogurts may provide a foundation to improving probiotic strains and/ or starter cultures, and subsequently lead to a more effective delivery of probiotic-associated health benefits via fermented dairy products.

MATERIAL AND METHODS

Preparation of traditional yogurt sample and inoculation-:

The yogurt sample used in this study was a traditional type.it is prepared by using traditional method using amul dairy yogurt (dahi) as a starter culture.

For the preparation of traditional yogurt take the whole full fat milk fat=6, snf=9, of amul 500ml. And then added amul dairy yogurt (dahi) approx. = 1.5 to gm (one table spoon) (2.8×10^7 CFU/ml) as the starter culture, at the addition of starter culture the boiling temperature of the milk should be 40ºC to 45ºC.then stirring the milk with the help of spoon that the culture should be mix uniformly, leave the culture added milk 7 to 8 h at the room temp. After 7 to 8 h check the visibly texture of the prepared traditional yogurt and storage under the 4ºC.

(a) In another experiment isolated *L. acidophilus* strain were propagated twice in MRS broth and inoculated at 37°C for 16 h. the viable cell counts of this suspension was determine by enumeration on MRS ager plate and incubation at 37°C for 48 h in anaerobic.120µl of this concentrate (about 2.8×10^7 CFU/ml) was introduced into 500 ml milk and fallowing the same process as making of traditional yogurt (dahi).

(b) Yogurt made with probiotic culture (*Lactobacillus acidophilus* strain) without starter culture.

In this process isolated *L. Acidophilus* strain 120µl of this concentrate (about 2.8×10^7 CFU/ml) was added in whole full fat milk (500 ml) at 35ºC to37ºC milk temperature. Leave the strain added milk 15 to 16h at the room temp.

Table 1

S.NO.	Sample	Incubation Temperature (ºC)	Incubation Time (h)
1	Control A	42	4
2	Traditional B	30 to 35	7 to 8
3	Associate mix C	30 to 35	6
4	Without starter culture only strain D	37	15 to 16

Assessment of *L. acidophilus* viability in all type traditional yoghurt (dahi)

From the inoculated and non-inoculated traditional yogurt (dahi) sample, an aliquot of 10gm was taken as 1-week interval for viable count. Ten-fold serial dilution were done in 0.1% peptone water while homogenizing by overtaxing, followed by viable cell count by pour plate technique using MRS ager. Plate was inoculated anaerobic ally at 37ºCfor 48h.

Evaluation of physio- chemical characteristics of all type traditional yogurt (dahi) sample during storage. (1, 7, 14,21d) period.

Physio chemical characteristic frequently used as the best indicators for quality and stability of traditional yogurt(dahi)such as pH, Tritrable acidity texture and storage temperature were determine, the pH was measured by 410A pH meter () after calibrating with fresh pH 4.0 and 7.0 Standard buffers. The Tritable acidity was determined after mixing a yogurt sample with 10mL of hot distilled water and titrating with 0.1N NaoH using 0.5% phenolphthalein indicator to the end point of faint pink colour that persisted for at least30 seconds.

The texture profile analysis of yogurt samples was determined using texture analyzer (Texture Technologies Corp., Hamilton, MA, USA) equipped with load cell of 5 kg and a cylindrical probe (25.4 mm in diameters) supplied with texture exponent programs (Exponent, Version 6.0.6.0., Texture Technologies Corp.). Before TPA analysis, the samples were left at 25 ºC. TPA was performed by compressed twice using probe to make 10-mm penetration with the speed of 5 mm/s. Hardness, springiness, adhesiveness, cohesiveness, chewiness, were determined from TPA by using software. All measurements were carried out in triplicate for each samples.

RESULTS AND DISCUSSION

Viability of bacterial count (*L. acidophilus*) in prepared traditional yoghurt (dahi)

Table 2 Comparison of the number of viable cells counts in all type traditional yoghurt (dahi) during storage 4°C for 21 days.

Time	Control Sample (A)	Traditional (B)	Traditional +*L .acidophilus* strain (Associate mix) (C)	Without starter culture Only *L .acidophilus* strain (D)
0hrs		$30x10^6$	$86x10^8$	$50x10^6$
1d	$260x10^6$	$242x10^6$	$400x10^8$	$120x10^6$
7d	$150x10^6$	$140x10^5$	$240x10^7$	$90x10^4$
14d	$90x10^5$	$70x10^5$	$120x10^6$	$40x10^4$
21d	$27x10^5$	$22x10^5$	$90x10^6$	$15x10^3$

The count of *L. acidophilus* increase gradually during initial period of storage and faster decay was observed thereafter. The viability was found to be dependent on the associative yogurt organism C (traditional +*L .acidophilus* strain) was up to satisfactory level (10^6 cfu.g^{-1}) the pH was found to be the most crucial factor for this *L. acidophilus* culture. The associative yogurt organism has greater impact on the viability of the L *.acidophilus*. Yogurt B (Traditional) showed poor viability of *L. acidophilus* as compared to associate yogurt (C). The variation is 30 to $\log_{10}$ .27 CFU/gm .the possible reason could be strain variation and the differences in incubation time to reach the pH of 4.5 by these cultures. The control sample (A) shows good viability as compare to traditional(B) but shows poor viability compare to associate mix (C). This happen due to presence of *L .delbrueckii* spp. *Bulgaricus* in the market yogurt and this organism has been reported to produce hydrogen peroxide as observed with the studies. *L. delbrueckii ssp. bulgaricus* could drop the pH during storage, as observed in market yoghurts, which has an adverse effect on the viability of probiotic organisms (Holcomb et al, 1991). The most poor viability of micro-organism is in without starter culture (D) yogurt as compare to all type of yogurt ,A,B,C, the reason could be take long incubation period 15 to16 h and post acidification of milk and other factor may be also responsible such as pH , TA (Titratable acidity) and most important is starter culture bacteria absence. So the viability of (*Lactobacillus acidophilus*) this organism in yogurt made with associate yogurt (C)

(traditional +*L. acidophilus*) was just near the recommended level of >10^6 (kurmann and rasic, 1991) after 21d.

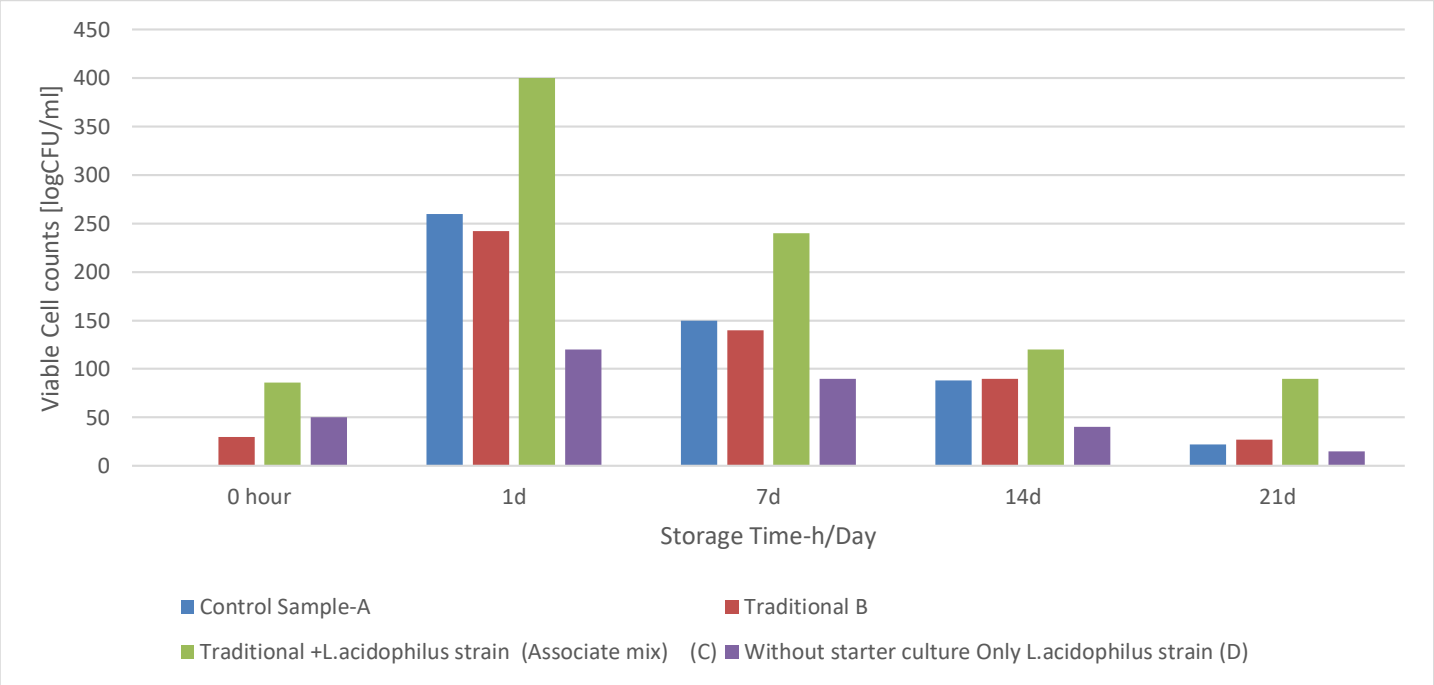

Fig 1 Viable cell count of microbes during storage period

Change in physio-chemical characteristics of all type traditional yogurt (dahi) sample during storage.

Table 3 pH of all type of yogurt sample at 4ºC storage

Time	Control (A)	Traditional (B)	Traditional +*L .acidophilus* strain (Associate mix) (C)	Without starter culture Only *L. acidophilus* strain (D)
0h		6.00	6.53	6.55
1d	4.50	4.43	4.60	4.40
7d	4.45	4.39	4.45	4.30
14d	4.36	4.26	4.31	4.21
21d	4.16	4.10	4.30	4.0

The pH, titratable acidity and texture analysis are amongst the physio-chemical characteristic commonly used as main indicators of quality and stability of traditional yogurt (Dahi). After 21days of storage at 4°C no significant change was observed in the physio-chemical parameters of the associated mix yogurt (c) (traditional +*L. acidophilus*) in Table 3 the pH, from 6.53 to 4.30, for traditional (B) the value of these parameters range from5.50 to 4.10 and for control yogurt (A) the value for these parameter range from 4.50 to 4.16 and for sample (D) the pH range from 4.60 to 4.0 The change in the pH values of yogurt during storage shown the trend of decrease in pH were identical at the recommended level of inoculation these result were in agreement with Singh et al. and ozer et al. (2005). It appears that the composition of starter culture, fermentation temperature and storage period could influence the overall level of acidity and pH of stored yogurt samples.

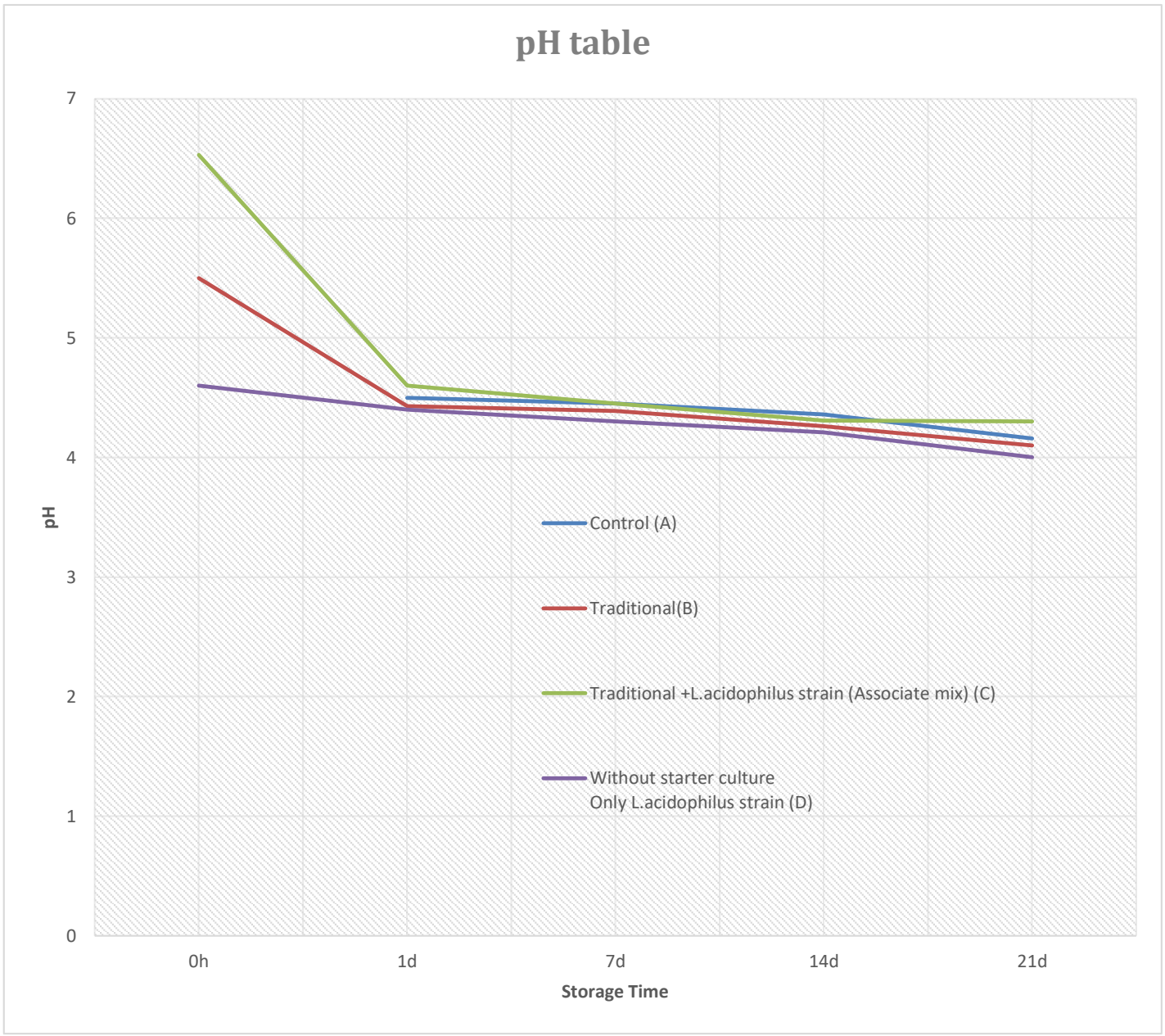

Fig 2 pH of all yogurt during storage period 4℃

Table 4 Titratable acidity of all yoghurt during storage period at 4℃

Time	Control (A)	Traditional (B)	Traditional +*L. acidophilus* strain (Associate mix) (C)	Without starter culture Only *L. acidophilus* strain (D)
0h		0.14	0.16	0.15
1d	0.78	0.76	0.80	0.67
7d	0.81	0.80	0.88	0.70
14d	0.85	082	0.92	0.73
21d	0.88	0.85	0.96	0.77

The change in TA (Titratable acidity) shown in table 4 the TA increase slightly all type of yogurt for control (A) the range from 0.78 to 0.88, for traditional the TA value increase 0.14 to 0.85, and TA value increase from 0.16 to 0.96 for Associate mix (C) .for sample (D) the range 0.15 to 0.77 increase as compare to all sample each other the sample (C) Associate mix show high TA (Titratable acidity) The change in the pH values and that of titratable acidity (TA) of yogurt during storage shown the trend of decrease in pH or increase in TA were identical at the recommended level of inoculation and the decrease in pH or increase in TA was higher in these yogurt showed the least post acidification at all the inoculum levels.

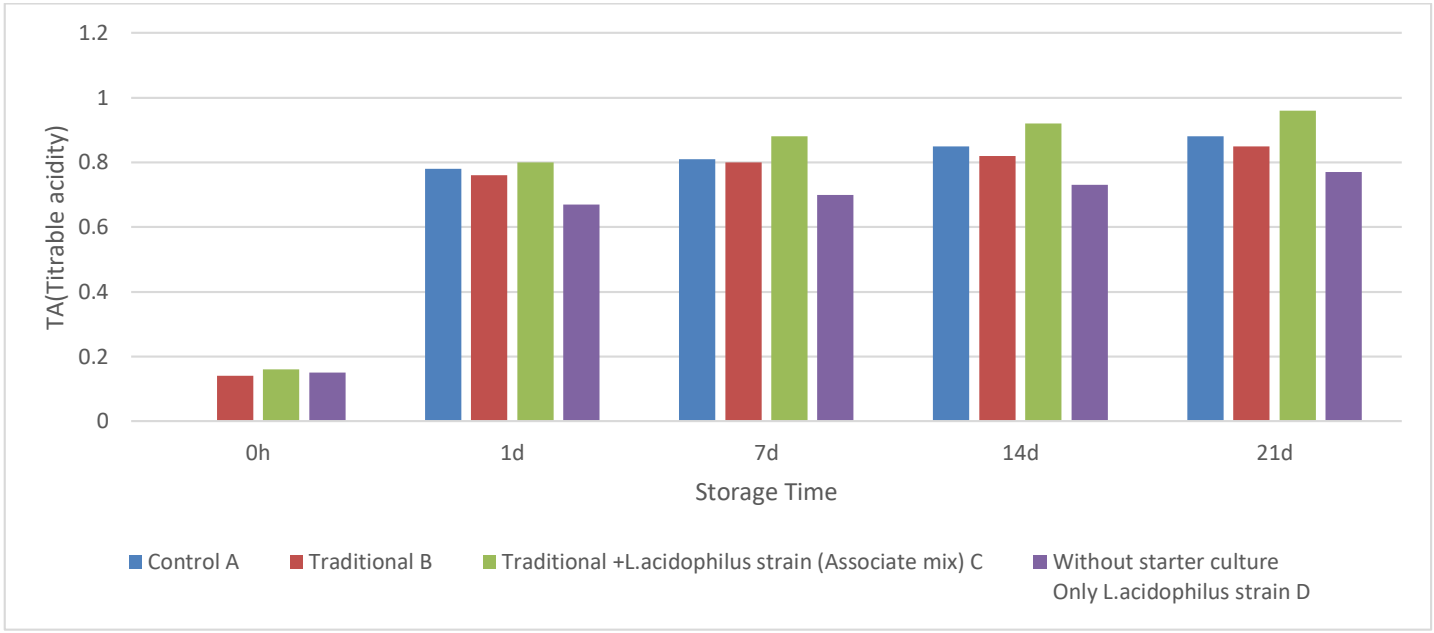

Fig 3 Titratable acidity of all type yoghurt during storage period at 4℃

Texture analyses

It is well known that various properties (texture, functionality and appearance) of foods were greatly affected by their structure. Texture properties of the fermented dairy products are depending on their structural arrangement and microstructure of the protein network (Delikanli and Ozcan, 2017). TPA parameters (Hardness, springiness, adhesiveness cohesiveness, chewiness) of the yogurt samples are given in Table 4 Hardness, or firmness, is the most important characteristic in determining of yogurt texture. It is regarded as the force required to attain a certain deformation and is considered as a measure of hardness of the yogurt (Mudgil et al., 2017). As seen Table, 4 whereas hardness values of control yogurt samples ranged from 130.36 to 198.36 g, traditional yogurt samples ranged from 59.35 to 87.00 g. And associated mix yogurt samples ranged from 82.93 to 98.28 g. As shown in the table, control yogurt samples have higher hardness values than traditional yogurt samples and associated mix yogurt samples. It is thought that the homogenization process is not applied in the yogurt produced by the traditional method. Because, large fat globules as present in unhomogenised milk may decrease hardness of fermented products by interrupting the gel network (Aguilera and Kessler, 1988). These differences are thought to be due to the incubation temperature, time and the amount of culture. That a higher hardness of yogurt samples has also been related to a longer incubation time. Specially, lower yogurt incubation time can negatively affect the textural properties of yogurt. In another study, Lee and Lucey (2003) determined that higher temperatures during the incubation could give rise to a weaker protein network and a lower gel firmness. Adhesiveness is the force necessary to remove the material that adheres to the mouth during eating (Ganesh, 2006).it can be seen from Table 4 the maximum and minimum adhesiveness values (g.s) have been determined in traditional yogurts and values of the samples ranged from -2.39 to -3.77. These values were lower than the value determined by Hashim et al. (2009). Springiness value of the sample represents the recovery ability of the sample against first deformation applied during analysis. As known, food products are subjected to different forces, resulting in deformation of the product, during transportation and storage. Hence, springiness value of the product is very important for the product quality with desired level (Yildiz et al., 2015). Springiness (%) values of the yogurt samples ranged from 48.27 to 84.03. Ocak and Kose (2010) expressed that the protein matrix is responsible for the springiness and hardness of yogurt. The cohesiveness indicates the strength of internal bonds making up the body of food and the degree to which a food can be deformed before it breaks (Chandra and Shamasundar, 2015). The maximum and minimum cohesiveness values have been determined in control yogurt samples and the values of the yogurt samples are changed 0.76 to 0.84. Cohesiveness is defined as the ratio of the positive force area during the second penetration to that of the first penetration. It may be measured as the rate at which the material is disintegrated under mechanical action. Tensile strength is a manifestation of cohesiveness. The cohesiveness indicates the ability of the product to hold together (Chandra and Shamasundar, 2015).comparing all yogurt texture quality the most poor quality of sample D it shows the Syneresis is generally defined as separation of aqueous phase from continuous phase or gel network, which is an undesirable

property in fermented milk products . The highest syneresis at the end of the storage was determined in sample D and whey off of the sample .the possible reason could be long fermentation time, inoculation dose or without starter culture addition.

Chewiness is measured in terms of the energy required to masticate a solid food and should be calculated in TPA of solid food. It is calculated as the product of hardness x springiness x cohesiveness of sample (Mehta et al., 2012) and is affected by change any of these parameters. The chewiness value of the yogurt samples is changed 31.96 to 75.66. Chewiness is the most difficult characteristic to measure distinctly. Because mastication involves compressing, shearing, piercing, tearing, grinding and cutting along with adequate lubrication by saliva at body temperatures.

Table 5 texture analysis

Storage Time	Sample of yogurt	Hardness G	Adhesiveness g.s	Springiness %	Cohesiveness	Chewiness
1d	Control A	174.57±1.8	-17.90±3.27	48.27±0.70	0.84±0.07	55.16±2.29
	Traditional B	59.35±0.24	-2.39±0.23	56.93±1.05	0.67±0.05	44.51±0.61
	Associated mix C	82.93±2.55	-3.77±0.07	69.43±1.00	0.79±0.02	60.04±3.17
	Only Strain D	44.23±0.12	-1.11±0.2	50.63±3.00	0.43±0.01	30.15±0.23
7d	Control A	198.36±0.07	-16.49±0.34	54.95±12.95	0.89±0.01	60.04±3.17
	Traditional B	61.62±0.89	-2.13±0.10	53.95±1.40	0.68±0.03	31.96±1.52
	Associated mix C	87.80±0.85	-3.77±0.07	69.55±1.20	0.73±0.05	44.76±1.20
	Only Strain D	55..24±1.01	-1.15±0.04	51.44±2.00	0.55±1.00	26.03±0.03
14d	Control A	130.36±0.10	-19.89±0.43	63.74±1.57	0.80±0.03	73.11±0.04
	Traditional B	63.69±2.73	-3.39±0.07	64.94±2.23	0.68±0.05	44.08±0.20
	Associated mix C	96.28±1.50	-4.46±1.36	65.96±2.36	0.80±0.03	49.43±3.03
	Only Strain D	53.33±.040	-2.10±1.06	52.33±1.22	0.60±2.00	30.12±1.00
21d	Control A	178.78±3.37	-25.90±1.88	68.07±2.10	0.76±0.01	75.66±0.05
	Traditional B	87.00±0.85	-3.77±0.07	69.55±1.40	0.69±0.03	36.30±0.17
	Associated mix C	98.28±1.50	-4.68±1.10	59.90±1.50	0.79±0.02	44.51±0.6
	Only Strain D	52.22±1.03	-2.00±1.05	51.22±0.12	0.61±1.00	30.11±.01

Yogurt is traditionally produced in the small dairy farms of families and small-scale dairies. The composition of raw milk, pH of milk, manufacturing process, starter culture and storage conditions, could affect the texture profiles of yogurt samples. It has been determined that the textural parameters of industrial yogurt samples were closer to each other and there is a standard among samples according to the results of yogurt samples.

Conclusion

Probiotic bacteria used were found to survive throughout storage period and are suitable to provide sufficient number of viable bacteria counts at the time of product consumption.

In this study we demonstrated the viability of *L. acidophilus* in different type yogurt made with traditional method. Four yogurt sample prepared for this study, one is control A, second made with starter culture B, the third one Associate mix C made with (starter culture +*L. acidophilus* strain), fourth made with only use of strain (*L. acidophilus*).All prepared yogurt sample characterize with physio chemical property such as, pH ,TA (Titratable acidity) Texture analysis during (0,1,7,14,21d) storage period. After investigation find that Associate mix yogurt C (Dahi) has been found to be good matrix to convey this probiotic strain into was up to satisfactory level (10^6 cfu.g^{-1}) throughout 21d stored at 4ºC. These results are promising and more studies are necessary to investigate the survival of other probiotic strains in traditional yogurt (Dahi) and the *in vivo* antimicrobial activity of the inoculated traditional yogurt (Dahi) sample.

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