Evaluate the physical, chemical and sensory parameters of probiotic yoghurt during storage

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Abstract

Dairy foods like yoghurt are the main types of food matrices supplemented with probiotic bacteria and they have a beneficial health effects among consumers. In this study, to evaluate the physico-chemical parameters and sensory attributers of probiotic added yoghurts using different concentrations (0%, 0.1%, 0.2%, 0.3% and 0.4%) of probiotic (Bifidobacterium spp). The samples were analyzed for physico-chemical, microbiological, and sensorial properties at a week interval during storage of four weeks. The results showed that, the quality parameters such as total solids, ash, fat, reducing sugar, total sugar, pH and titratable acidity did not show any significant difference (P>0.05) for all types of yoghurt at during the first day of storage. Syneresis was high in 0.4% probiotic added yoghurt (40.73±2.05%) and was lower in without probiotic yoghurt (37.70±1.32%). During the storage period, total solids, total sugar, reducing sugar, pH, and titratable acidity were (p < 0.05) varied among the different types of yoghurt sample. At the end of storage 0.4% probiotic added yoghurt showed higher average value of total solids (21.87±1.40%) and titratable acidity (0.67±0.04%), while yoghurt without probiotic showed higher values of reducing sugar (2.17±0.02%), total sugar (10.72±0.21%) and pH (4.38±0.01). At the end of storage, yoghurt without probiotic showed low value of total solids (15.80±1.93%) and yoghurt with 0.1% probiotic showed low value (0.62±0.02%) of titratable acidity yoghurt with 0.4 % probiotic showed low value of reducing sugar, total sugar and pH. During the storage period, dry matter, ash and titratable acidity increased with increasing concentration of probiotic in the yoghurt. Reducing sugar, total sugar and pH decreased with increasing concentration of probiotic. The high numbers of bacterial colony forming unit was showed during second of weeks of storage than fourth week of storage, which is lower than those without probiotic yoghurt. The sensory evaluation of the produced yogurts revealed the superiority of yogurts with 0.3% probiotic was highly preferred by consumers for their taste and overall acceptability comparison with the other types of yogurt. Finally, yogurts with 0.3% probiotic were more suitable for the consumption.

Keywords: Cow milk, Yoghurt, Probiotic, Nutritional parameters, Sensory attributes

Introduction

Fermented milk products are products that can be produced via fermentation of lactose by lactic acid bacteria. When look around the worldwide, various dairy products which are different in name but similar in content can be found and those products are an important part of human diet (Nilsson *et al.*, 2006; Hugenholtz, 2013; Yerlikaya, 2014). Fermented dairy products are usually produced by using lactic acid bacteria and yeasts (Ozer and Kirmaci, 2010). Fermented milks have long been used as the main vehicles for probiotic strains. Less frequently, cheeses have been used for incorporation of probiotic microorganisms, but they may offer a number of advantages compared with fermented milks (Minervini *et al.*, 2012). Cheese has higher pH, more solid consistency, and relatively higher fat content compared with fermented milks such as yoghurt (Karimi *et al.*, 2012).

Probiotics are defined as "live microorganisms which are believed to exert beneficial health effects in the host by modulating the intestinal microflora (Donkora et al., 2006). The health benefits of consuming probiotics are immune system modulation (Schlee et al., 2008), improvement of lactose intolerance (De Vrese et al., 2001), cholesterol assimilation Shah. 2005), (Liong and and antimutagenic properties (Choi et al., 2005). In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including allergies, hepatic disease, cancer, helicobacter pylori infections, urinary tract infections and hyperlipidemia (Ejtahed et al., 2011). Probiotic microorganisms are common to be ingested through dairy products, mainly fermented milk products. Bifidobacterium spp. and Lactobacillus acidophilus are by far the most important probiotics regularly added to the fermented milk (Ferdousi et al., 2013). In recent years' foods that can be shown to provide health benefits have attracted consumer interest. These foods should also fit into current lifestyles, such as good flavor and convenience with an acceptable price. Such products are commonly referred to probiotic-containing products, especially yogurt.

Yoghurt is a semi solid fermented milk product, which have highly nutritious protein-rich product obtained bv fermentation of milk with S. thermophilus and L. bulgaricus. The product is highly acceptable to consumers because of its flavour and aroma, mainly attributed to acetaldehyde, and its texture (Kumar and Mishra, 2004). Nowadays, the technology of yogurt making has become more advanced, which delivers more functional health benefits as well as application of strict hygiene control along with a variety of yogurt types to suit individual tastes. At present, incorporation of probiotics like Bifidobacterium spp is a common practice in yogurt manufacture to improve the beneficial effects to the human health. Therefore, the objective of present study was to evaluate the effects of addition of probiotics (Bifidobacterium spp) on

physico-chemical parameters and sensory attributes of yoghurt during the storage.

Material and Methods

Raw material

Milk was collected from Livestock farm, Eastern University, Sri Lanka throughout the study period.

Starter culture and probiotic

starter The freeze-dried commercial voghurt culture (DVS, CHR HANSEN, composed of Streptococcus Denmark) Lactobacillus thermophilus (St) and delbrueckii subsp. bulgaricus (Lb) and probiotic cultures: Bifidobacterium animalis subsp. (Freeze dried direct vat set CHR HANSEN, Québec, Canada) were used in this study

Culture preparation and activation

a. Commercial starter culture

Commercial starter culture was prepared by adding commercial starter (0.33 g) in to one liter of sterilized skim milk (1.5% fat) and stored as 100 ml aliquots in erlene meyer flsk at frozen temperature (-20 °C) the cultures were thawed, activated and used as starter culture for the yoghurt preparation. Each 1 Lit of pasteurized milk was inoculated with 10 mL of commercial starter culture according to the manufacturer's instructions given for yoghurt production.

b. Probiotic culture

Similarly, probiotic (*Bifidobacterium*) culture was prepared by using 250 mL of sterilized skim milk (1.5% fat) and probiotic (0.083 g) culture. Prepared culture was stored as 50 mL aliquots into to erlene – meyer flasks and freezed at -20 °C. Before fermentation, the cultures in the to erlene – meyer flasks were thawed, activated and used as a probiotic culture for the yoghurt preparation. Probiotic (*Bifidobacterium*) culture was prepared in different concentration levels including 0%, 0.1%, 0.2%, 0.3% and 0.4% for yoghurt production

Yoghurt production

Milks were standardized (2.5% milk fat) by cream separator and standardized milk was pasteurized at 65 °C for 30 min and cooled to 37 °C. The pasteurised milk was inoculated with a commercial yoghurt culture (as described in commercial starter culture) and probiotic (*Bifidobacterium*) culture was substituted at different concentrations levels (0%, 0.1%, 0.2%, 0.3% and 0.4%) as described by Manjula *et al.* (2012). Aliquots of inoculated milk (125mL) were poured into plastic yoghurt cup, which were covered with lids and incubated at 42 °C 12 hrs. The plastic yoghurt cups were stored at 4 °C then samples were collected at Day 1, Week 1, 2, 3 and 4 interval for analyzing of physico-chemical parameters and sensory attributes.

Results and Discussion

Table 1. Physical and chemical parameters of yoghurts made from different concentration
of probiotic at day one

Parameters			Treatments		
	T1	T2	T3	T4	T5
Total solids	12.03±0.25 ^b	12.20±1.39 ^b	14.53±0.83 ^{ab}	15.47±2.44ª	16.87±0.21ª
Ash	0.60 ± 0.00^{a}	0.60 ± 0.06^{a}	61.±0.06 ^a	0.61 ± 0.10^{a}	0.61±0.12 ^a
Fat	2.91±0.10 ^a	2.92±0.21ª	2.94±0.10 ^a	2.96±0.12ª	2.96 ± 0.06^{a}
Reducing sugar	2.40 ± 0.04^{a}	2.37±0.07 ^a	2.36±0.03ª	2.35±0.07ª	2.33±0.05ª
Total sugar	13.53±0.84ª	13.17±0.06ª	13.10±0.26 ^a	13.07±0.12ª	13.03±0.06ª
Titratable acidity	0.55 ± 0.02^{a}	0.55±0.06ª	0.54 ± 0.04^{a}	0.54±0.04ª	0.59 ± 0.02^{a}
pН	4.70 ± 0.01^{a}	4.68 ± 0.01^{a}	4.66±0.01 ^b	4.64±0.02c ^b	4.63±0.01°

Values are means \pm standard deviations of triplicate determination. Mean with the same letters are not significantly different at (p< 0.05). T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt

At day 1, syneresis was higher in probiotic added yogurt than without probiotic yoghurt after 1/2 hr and 2 hrs. While 0.4% probiotic added yoghurt showed highest average value than other types of yoghurt (Table 2). Yoghurt with the higher probiotic had the highest level of syneresis, it may be differentiations in metabolic activities of starter cultures which cause poor consistency of the coagulum of milk and its inability to retain serum (Yilmaz-Ersan and Kurdal, 2014).

Table 1. Syneresis of yoghurt

Treatment						
Syneresis	T1	T2	T3	T4	T5	
After 1/2 hr	36.77±0.64 ^b	36.93±3.37♭	37.13±1.65 ^b	37.20±0.36 ^b	39.60±1.91ª	
	37.70+1.32 ^b	39 23+0 59ab	39.30+2.59ab	39.93+2.15 ^{ab}	40.73+2.05ª	

Values are means \pm standard deviations of triplicate determination. Mean with the same letters are not significantly different at (p< 0.05). T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt.

Total solids, Ash and fat contents of yoghurts made from different concentration of probiotic during storage

Table 3 shows that total solids content of probiotic yoghurt ranged from 12.80% to 21.87% during 4 weeks of storage. At the end of the storage period, 0.4% probiotic added yoghurt showed highest (p<0.05) value of total solids content than yoghurt without probiotic. The increasing trend of total solids content was observed with storage period. The increase in total solids contents could be due to loss of moisture and drain out whey from the yoghurt. These results were coincided with findings of Hassan and Amjad, (2010). Very minute

changes were observed in ash content in all yoghurt samples within the 4 weeks of storage period. The insignificant increase in ash contents was because of the loss of CO₂ and water during charring of yoghurt samples. The end of storage average ash contents of without probiotic voghurt and added yoghurt were 0.4% probiotic 0.73±0.46% and 0.76±0.20%, respectively. The results are in agreement with the findings of Hassan and Amjad, (2010) who reported the ash value of probiotic yoghurt as within the range. Fat content were relatively constant during storage, which suggests that probiotic did not affect significantly

Trt	Parameters	Week 1	Week 2	Week 3	Week 4
T1	Total solids (%)	12.80±0.53 ^e	13.00±1.39 ^e	14.33±1.50 ^{ed}	15.80±1.93 ^{bcde}
	Ash (%)	0.60 ± 0.12^{b}	0.65 ± 0.31^{b}	0.69 ± 0.35^{a}	0.73 ± 0.46^{a}
	Fat (%)	2.93±0.06ª	2.93±0.15 ^a	2.93±0.15 ^a	2.92±0.00 ^a
T2	Total solids (%)	12.93±1.33 ^e	14.80±1.11 ^{cde}	15.53±0.31 ^{bcde}	16.53±2.73 ^{bcd}
	Ash (%)	0.61 ± 0.12^{b}	0.66 ± 0.31^{b}	0.70 ± 0.12^{a}	0.74 ± 0.20^{a}
	Fat (%)	2.94 ± 0.15^{a}	2.93±0.06 ^a	2.92±0.12 ^a	2.92±0.12 ^a
T3	Total solids (%)	14.60 ± 0.60^{de}	15.20±1.25 ^{bcde}	16.00±1.06 ^{bcde}	17.87±1.33 ^{cb}
	Ash (%)	$0.62 \pm 0.12^{\circ}$	0.67 ± 0.31^{b}	0.71 ± 0.12^{ab}	0.75 ± 0.20^{a}
	Fat (%)	2.95±0.15ª	2.95±0.06 ^a	2.94±0.06 ^a	2.93±0.10 ^a
T4	Total solids (%)	15.93±2.34 ^{bcde}	16.33±1.40 ^{bcd}	17.40±0.40 ^{bcd}	21.47±3.11 ^a
	Ash (%)	$0.63 \pm 0.12^{\circ}$	0.67 ± 0.31^{b}	0.70 ± 0.12^{ab}	0.75 ± 0.20^{a}
	Fat (%)	2.97±0.15ª	2.97±0.15 ^a	2.97±0.06 ^a	2.95±0.10 ^a
T5	Total solids (%)	17.10±2.10 ^{bcd}	17.83±0.78 ^{bc}	18.20±2.62 ^b	21.87±1.40 ^a
	Ash (%)	$0.64 \pm 0.12^{\circ}$	0.68 ± 0.31^{b}	0.72 ± 0.12^{ab}	0.76 ± 0.20^{a}
	Fat (%)	2.98±0.15ª	2.97±0.12 ^a	2.97 ± 0.15^{a}	2.96 ± 0.17^{a}

Table 3. Changes of total solids, ash and fat content in yoghurt during storage

Values are means \pm standard deviations of triplicate determination. Mean with the same letters are not significantly different at (p< 0.05). T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt.

Reducing sugar and total sugar in yoghurt during the storage period

The result showed that reducing sugar and total sugar was (P < 0.05) decreased throughout the storage period (Table 4). At the end of the storage period 0.4% probiotic added yoghurt showed lowest value of reducing sugar (2.05±0.01) than yoghurt without probiotic (2.27±0.00). It might be due to the conversion of lactose into lactic acid with time of storage by

lactic acid bacteria. These results were coincided with findings of Metry and Owayss, (2009). Similarly, during the storage period 0.4% probiotic added voghurt showed lowest value of total sugar compare to other all types of yoghurt This may be due to samples. the concentration of probiotic hydrolyzed the polysaccharides oxidation of sugars present yoghurt. in

Trt	Parameters	Week 1	Week 2	Week 3	Week 4
T1	Reducing sugar (%)	2.43±0.04 ^a	2.35±0.04 ^b	2.22 ± 0.03^{efgh}	2.17 ± 0.02^{hij}
	Total sugar (%)	13.33±0.12 ^a	13.11 ± 0.10^{ab}	11.40 ± 0.10^{e}	10.72 ± 0.21 gh
T2	Reducing sugar (%)	2.30±0.05 ^{bc}	2.25±0.01 ^{cdef}	2.22 ± 0.02^{efgh}	2.15±0.04 ^{ij}
	Total sugar (%)	13.00 ± 0.10^{bc}	12.95 ± 0.15^{bc}	11.11 ± 0.13^{f}	10.50 ± 0.24^{hi}
T3	Reducing sugar (%)	2.29±0.02 ^{cd}	2.24 ± 0.03^{def}	$2.21\pm0.00^{\text{efgh}}$	2.12±0.00jk
	Total sugar (%)	12.93±0.03bc	12.82±0.06 ^c	11.07 ± 0.12^{f}	10.42 ± 0.38^{i}
T4	Reducing sugar (%)	2.29±0.09 ^{cd}	2.24 ± 0.02^{defg}	$2.20 \pm 0.01^{\text{fghi}}$	2.08 ± 0.01 ^{kl}
	Total sugar(%)	12.91 ± 0.04^{bc}	12.75±0.06 ^c	10.98 ± 0.03^{fg}	10.34 ± 0.12^{i}
T5	Reducing sugar (%)	2.27±0.00 ^{cde}	2.23±0.05 ^{defgh}	2.18±0.03ghij	2.05±0.011
	Total sugar (%)	$12.83 \pm 0.02 bc$	11.69±0.08 ^d	$10.87{\pm}0.16^{\rm fg}$	10.32 ± 0.23^{i}

Table 4. Changes of reducing sugar and total sugar in yoghurt during storage

Values are means \pm standard deviations of triplicate determination. Mean with the same letters are not significantly different at (p< 0.05). T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt.

pH and titratable acidity in yoghurt during the storage period

pH and titratable acidity presented the same trend in all types of yogurt groups during storage period (Table 5). During storage, all yoghurt samples showed continued decreased in pH and end of storage, average pH value of without probiotic yoghurt and 0.4% probiotic were 4.38 and 4.15, added yoghurt respectively. The reduction in pH can be due to the breakdown of lactose into lactic acid. Probiotic and starter cultures yielded a different pH profile with the passage of time. The lag time for pH decreases during storage and this reflected the acidification rate of the culture involved. These results

agree with results reported by Behrad et al. (2009), who mentioned that the pH for all yoghurts reduced from the initial values of 4.5 to 4.09 at 28 days of storage

The average acidity of without probiotic yoghurt was 0.65% and 0.4% probiotic added yoghurt was 0.67%. The results showed that acidity tends to increase in all types of yoghurt during storage period. The changes in titrable acidity of yoghurt could be a fermentation process by microorganism and degradation of lactose into lactic acid. These results are in agreement with the results of Hassan, A. and Amjad, (2010).

Trt	Parameters	Week 1	Week 2	Week 3	Week 4
T1	pН	4.68 ± 0.01^{a}	4.60±0.01 ^b	4.44 ± 0.03^{e}	4.38 ± 0.01 ^{hi}
	Titratable acidity (%)	0.56 ± 0.02^{ab}	0.60 ± 0.02^{ab}	0.62 ± 0.02^{ab}	0.65 ± 0.00^{ab}
T2	pН	4.62±0.01 ^b	4.58±0.01°	4.40 ± 0.01 fg	4.28 ± 0.0^{1k}
	Titratable acidity (%)	0.58 ± 0.07^{b}	0.59 ± 0.04^{ab}	0.61 ± 0.04 ab	0.62 ± 0.02^{ab}
Т3	pН	4.60 ± 0.01^{b}	4.56±0.01 ^c	4.39 ± 0.01 gh	4.22 ± 0.01^{1}
	Titratableacidity (%)	0.59 ± 0.06^{ab}	0.60 ± 0.06^{ab}	0.62 ± 0.06^{ab}	0.65 ± 0.04^{ab}
T4	pН	4.58±0.01°	4.53 ± 0.02^{d}	4.36 ± 0.01^{i}	4.20 ± 0.01^{1}
	Titratable acidity (%)	0.58 ± 0.07^{b}	0.59 ± 0.04^{ab}	0.64 ± 0.04^{ab}	0.65 ± 0.07^{ab}
T5	pН	4.52 ± 0.02^{d}	4.42 ± 0.01^{ef}	4.31±0.02 ^j	4.15±0.04 ^m
_	Titratableacidity (%)	0.61 ± 0.00^{ab}	0.62 ± 0.02^{ab}	0.65 ± 0.04 ab	0.67 ± 0.04^{a}

Table 5. Changes of pH and titratable acidity in yoghurt during storage

Values are means \pm standard deviations of triplicate determination. Mean with the same letters are not significantly different at (p< 0.05). T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt.

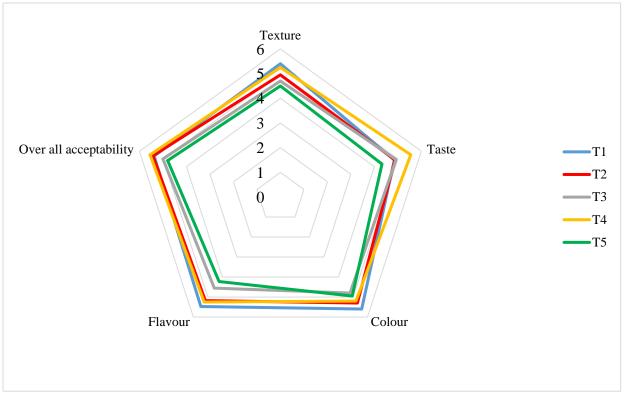
Microbial analysis of yoghurt during storage

The bacterial analysis of the yogurts showed that the decreasing colony forming unit during storage period at 4°C. After two weeks of storage without probiotic yogurt and 0.4% probiotic added yoghurt showed 5.76*105 CFU/ml and 8.13*105 CFU/ml colony forming unit, respectively. After 4 weeks of storage, in all cases the bacterial colony forming units were declined. At 4th weeks storage without probiotic yoghurt and 0.4% probiotic added yoghurt showed (4.13*10⁵ CFU/ml) 5.76*10⁵ colonies forming unit, and respectively. That because the growth rate of bacteria depends on the amount of lactic acid produced, and thus on the pH. The bacteria grow faster at higher pH value. Therefore, at 4 weeks of storage, the amount of lactic acid increased and pH decreased that affected to the decrease of total bacteria in yogurt product (Sabbah et al., 2009).

Sensory evaluation

Organoleptic evaluation was carried out to assess organoleptic and the quality characteristic of yoghurts prepared from different concentrations of probiotic. The panelist from various groups were served with the samples to evaluate certain attributes. The results of sensory evaluation of yoghurt on the basis of texture, taste, colour, flavour, overall acceptability are summarized in Fig.. 1. All mean scores for the different sensory attributes of the all yoghurts were within the commercially acceptable range (4-9 scores) recommended for yoghurt by the nine points scheme (Resurreccion, 1998). At Day 1, results showed that there were no significant differences (P > 0.05) in all attributes for different types yoghurt.

During storage, sensory attributes of the yoghurt sample were observed that without probiotic yoghurt for texture, colour and flavor were mostly preferred by On the other hand, sensory panelist. attributes like taste and overall acceptability were high in 0.3% probiotic added yoghurt while texture, colour and flavor were preferred by yoghurt made from without probiotic yoghurt. In general, all sensory attribute scores of samples increased during storage of up to 2 weeks and thereafter decreased for all attributes. This could be associated with development of acidity and decreases in acetaldehyde contents.



T1- yoghurt without probiotic T2- 0.1% probiotic added yoghurt T3- 0.2% probiotic added yoghurt T4- 0.3% probiotic added yoghurt T5- 0.4% probiotic added yoghurt

Fig. 1: Variation in sensory attributes during storage period

Conclusion

The study revealed that different concentration of probiotic added yoghurt had no significant effects on the ash, fat, reducing sugar, total sugar and titratable acidity at day 1 but total solids, pH and syneresis were gradually increased with increasing concentration of probitics.

During the storage period, total solids and titratable acidity were increasing while total sugar, reducing sugar and pH were decreased. At the end of storage 0.4% probiotic added yoghurt shows higher value of total solids and titratable acidity contents. On the other hand, yoghurt without probiotic shows higher value for reducing sugar, total sugar and pH. Sensory attributes, such as texture, taste, flavour, colour and overall acceptability, varied among the different types of yoghurt. Finally, taste and overall acceptability were preferred by panelist yoghurt made from 0.3% added probiotic

yoghurt while texture, colour and flavor were preferred by yoghurt made from without probiotic yoghurt. Finally, yogurts with 0.3% probiotic were more suitable for the consumption.

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