Physicochemical, microbiological and sensory properties of probiotic drinking yoghurt developed with goat milk

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Abstract- The present study was carried out to develop a drinking yoghurt made with goat milk using thermophilic yoghurt culture (YC-X11), *Bifidobacterioum animalis* subsp. *Lactis* (BB-12) and *Lactobacillus acidophilus* (LA-5) and to evaluate physicochemical, microbiological and sensory properties. Three inoculation levels (0.2 gL^{-1}, 0.3 gL^{-1} and 0.4 gL^{-1}) of traditional yoghurt cultures and two probiotic cultures were separately used to develop drinking yoghurt. Drinking yoghurts were stored at 4 ^{0}C for 21 days and analyzed for microbial counts, titratable acidity and pH on 0, 7, 14 and 21 days of storage. Sensory evaluation was done within four-day interval from day 1 to day 14 with 30 untrained panelists using five point hedonic scales. Titratable acidity and pH were significantly different ($P < 0.05$) with added cultures and culture levels. There was an interactive effect of treatment and storage time on pH for the developed product ($P < 0.05$) and developed product was within the acceptable range up to 14 days of storage at 4 ^{0}C. Ash, dry matter, fat contents, brix value and density did not significantly differ ($P > 0.05$) with cultures and culture levels. Yoghurt made with LA-5 received the highest overall acceptability. The developed products were negative for pathogenic microbes up to 21 days of storage. In conclusion, 0.3 gL^{-1} inoculation level of LA-5 could be used to develop the probiotic drinking yoghurt using goat's milk with desired physiochemical, microbiological and sensory properties.

Index Terms- Drinking yoghurt, Goat's milk, Probiotic culture

I. INTRODUCTION

Goat milk is considered as a good source of fatty acids, proteins and minerals. In addition, it has been reported to be a functional food due to its high digestibility and reported nutritional properties. Furthermore, goat milk is recommended for individuals who are allergic to cow milk [1]. The basic nutrient composition of goat milk resembles cow milk, where both milks contain substantially higher protein and ash, but lower lactose content than human milk. However, goat milk differs from cow milk having better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition [2]. It has some technological advantages in comparison to cow's milk, such as smaller size of fat globules which provides a smoother texture in derived products, lower amounts of α s1-casein, resulting softer gel products, higher water holding capacity and a lower viscosity [3]. The flavor of goat milk is more intense in comparison to cow milk, which can restrict the acceptance of its derivatives by consumers [4].

Yoghurt is a dairy product made by adding live, active bacterial cultures to milk that cause microbial fermentation. Different types of yoghurt are available in the world such as traditional yoghurt, greek yoghurt, probiotic yoghurt, organic yoghurt, non-dairy yoghurt and drinking yoghurt. Main starter cultures used for the yoghurt production are thermophilic culture which are made with the 1:1 ratio of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* and in general they are used at 1:1 ratio [5]. Probiotic bacteria are defined as "live microorganisms when administrated in adequate amounts can provide a health benefit on the host" (FAO/WHO, 2001). They can be used in single or mixed culture that have a beneficial health effects on the host. They have been used in both food and non-food applications. In dairy industry, lactic acid bacteria are widely used as probiotic bacteria. In addition, some bifidobacteria and yeast have been incorporated as probiotic organisms in various dairy products [6].

Probiotic bacterial species have been reported to improve physicochemical and sensory properties of different dairy products [7, 8]. *Lactobacillus acidophilus* and strains of *Bifidobacterium* spp. have been well researched with cow milk though there are limited published data on application of these probiotics in drinking yoghurt made with goat's milk. Therefore, this study was conducted to evaluate the effects of inoculation level of *Bifidobacterium animalis* subsp. *Lactis* (BB-12) and *Lactobacillus acidophilus* (LA-5) on physicochemical, microbiological and sensory properties of probiotic drinking yoghurt developed with goat milk.

II. MATERIALS AND METHODS

Identification of suitable incubation time for the development of drinking yoghurt made with goat milk

Bacterial cultures were provided by CHR Hansen Division, J.L. Morison Son and Jones (Ceylon) PLC. Goat milk was pasteurized at 95 ^{o}C for 5 min. and inoculated at 37 ^{o}C with thermophilic yoghurt culture (YC-X11), *Bifidobacterium animalis* subspp. *lactis* (BB-12) and *Lactobacillus acidophilus* (LA-5) starter cultures in three inoculation levels (2.0 gL^{-1}, 3.0 gL^{-1} and 4.0 gL^{-1}) respectively, for a sample size of 500 mL. The inoculated samples were incubated at 37±1 ^{o}C for about 4 hours. The pH changes of inoculated milk samples were measured at 1 hour interval during the incubation. The time required to reach pH of 4.5 was recorded.

Preparation of drinking yoghurt made with goat milk

Fresh goat milk was obtained from N.S.D. Agro Farm, Galewela, Sri Lanka. Drinking yoghurt made with goat milk was

prepared using a standard procedure at Dairy Processing Unit, Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka. Three different starter cultures (YC-X11, BB-12 and LA-5) and three inoculation levels (2 gL^{-1}, 3 gL^{-1} and 4 gL^{-1}) were used to prepare the drinking yoghurt. The prepared samples were stored in a refrigerator at 4 ^{o}C until used.

Physicochemical analysis of drinking yogurt made with goat milk

The pH of the samples was measured weekly during storage period up to 21 days using a pH meter (sensION+PH1, Hach, USA). Acidity was estimated by titration with 0.1% NaOH. Total solids, total soluble solids (brix value), specific gravity, crude protein, ash and fat of the developed yoghurt were estimated according to AOAC procedure [9].

Microbiological analysis

Prepared drinking yoghurt samples were analyzed for desirable (Lactic bacteria) and undesirable (*E-coli*, yeast and mould) microorganisms weekly using pour plate methods. Required amount of culture media (Lactobacillus MRS for lactic acid bacteria, PDA for yeast and mould, EMB agar for E-coli) was taken and sterilized by autoclaving at 121 ^{0}C for 15 min. One milliliter of drinking yoghurt was mixed with 9 mL sterilized distill water to make the 10^{-1} dilution and repeated until 10^{-5} dilution. One milliliter samples of drinking yoghurt (dilution factor =10^{-5}) was transferred in to the Petri dishes and 15 mL of prepared media was poured and allowed to solidify at ambient temperature. Then, dishes were transferred in to the incubator (For lactic acid bacteria, at 37±1 ^{0}C for 48 hours and E-coli, yeast and mould, at normal room temperature for 48 hours). Colony counter was used for the enumeration of microorganisms.

Sensory evaluation of developed drinking yoghurt

Sensory evaluations were carried out at 4 days intervals during storage period of 14 days to identify the best product. A total of 30 untrained panelists from Faculty of Agriculture, Rajarata University of Sri Lanka, Sri Lanka were used in the analysis. Yoghurt samples were served in randomized order in plastic cartoons coded with three random digits. The panelists were requested to evaluate the sensory properties including colour, odour, texture, and overall acceptability according to a five point hedonic scale.

Statistical Analysis

Parametric data were analyzed by one way Analysis of Variance (ANOVA) in Statistical Analysis Software ver. 9.0 [10]. Means were separated by Tukey's Studentized Range Test (TSRT). Sensory data were analyzed by Friedman non-parametric test in MINITAB 15 with 95% confidence interval. Microbial data were presented by comparing with SLS 824: part 2: 1989 yoghurt standards. Statistical significance was declared at $P < 0.05$.

III. RESULTS AND DISCUSSION

Optimum incubation time required for producing goat drinking yoghurt

Table 01 shows time required to reach pH 4.5 by different cultures and culture levels. The culture combination and level of inoculation had a significant effect on the rate of acidification. Yoghurt cultures showed the fastest growth and showed a high acidification rate than probiotic cultures. Thermophilic yoghurt culture reached pH 4.5 in 6 hr. whereas probiotic cultures spent 18 hr. to reach the same pH level. The observed longer acidification period with probiotic is related to the poor growth of probiotic bacteria in goat milk.

Table 01: Incubation time required for producing goat drinking yoghurt with Different Cultures and Culture Levels

Treatments		Optimum incubation (hr.)
Cultures	**Culture levels (gL^{-1})**	
YC-X11	0.2	6
	0.3	6
	0.4	6
BB-12	0.2	18
	0.3	18
	0.4	18
LA-5	0.2	18
	0.3	18
	0.4	18

Determination of time duration to reach pH 4.5 is important to identify the coagulum breaking point. The transformation of lactose in to lactic acid is the reason to reduce pH value up to 4.5, which is responsible pH to form casein coagulum which is called as iso electric point of milk casein. At pH of 4.5, negatively charged casein micelles neutralized by positive charges. Neutralized casein micelles accumulate in to the sole and form coagulum [11]. Therefore, incubation should be stopped before developing the pH value more than 4.5.

Nutritional composition of developed goat drinking yoghurt

Fat, protein, ash and dry matter contents of drinking yoghurt prepared with different cultures and culture levels were not significantly different (Table 02, $P > 0.05$)**.** Therefore, three cultures that were used, might not involve in the nutritional composition changes in prepared drinking yoghurt from goat milk. Jenness [12] reported that difference in animal species, breed, weather, type of feed, age of animal, stage of lactation etc. are the major reasons of changing composition of raw milk and those changes may affect on the nutritional composition of prepared drinking yoghurt.

Table 02: Fat, protein, ash and dry matter (DM) contents in the drinking yoghurts made with goat milk

Treatments		Nutritional properties			
Cultures	**Culture levels (gL^{-1})**	**Fat (%)**	**Protein (%)**	**Ash (%)**	**DM (%)**
YC-X11	0.2	1.45	3.86	0.52	24.33
	0.3	1.24	4.08	0.52	24.09
	0.4	1.39	4.75	0.47	23.67
BB-12	0.2	1.10	4.08	0.48	23.35
	0.3	0.84	4.10	0.50	23.14
	0.4	0.97	4.65	0.44	24.03
LA-5	0.2	1.16	4.61	0.51	23.87
	0.3	1.31	4.15	0.49	23.71
	0.4	1.42	4.03	0.48	24.11
SE		0.24	0.55	0.03	0.84

Physicochemical properties of developed goat milk drinking yoghurts

Brix value and specific gravity in developed drinking yoghurts prepared with different cultures and culture levels were not significantly different ($P > 0.05$). The pH value in each product was significantly different ($P < 0.05$) among the treatments with storage (Figure 01). During the storage period, pH of yoghurt gradually decreased in the treatments may be due to post acidification. pH of yoghurt can be decreased due to accumulation of lactic acid which has been produced by lactic acid bacteria and other living microorganisms during storage [13].

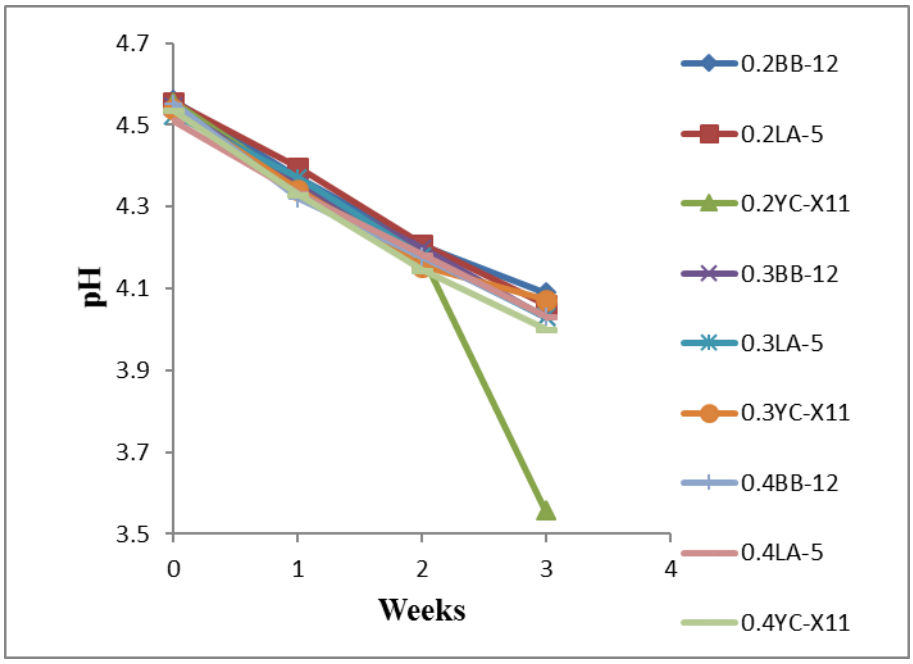

Figure 01: Changes of pH in developed goat drinking yoghurts with storage

Titratable acidity was significantly different ($P < 0.05$) among the treatments and increased during the storage (Figure 02). Muhammad, Abubakar [14] also observed that, gradual increase in titratable acidity in cow milk yoghurts during storage period, due to the microbial activity [13].

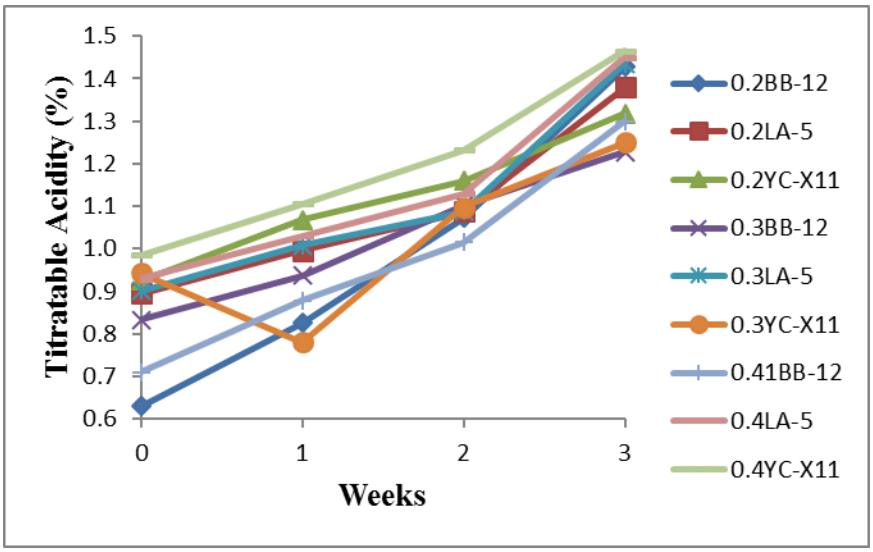

Figure 02: Change in titratable acidity of developed goat drinking yoghurts with storage time

Microbiological analysis of developed goat drinking yoghurts

Lactic acid bacterial counts were significantly ($P < 0.05$) different among the treatments. Yoghurt prepared with 0.2 gL^{-1} *Lactobacillus acidophilus* had the highest microbial counts at initial stage of production and yoghurt prepared with 0.4 gL^{-1} Thermophilic yoghurt culture had the lowest microbial counts (Figure 03). Microbial counts were reduced with the storage time. Normal lactic acid bacteria (Probiotic bacteria) requires 37±1 ^{0}C for their optimum growth and survivability, but refrigerator maintains at 4 ^{0}C may be the reason to retard the growth rate and amount of microbial count with storage time [5].

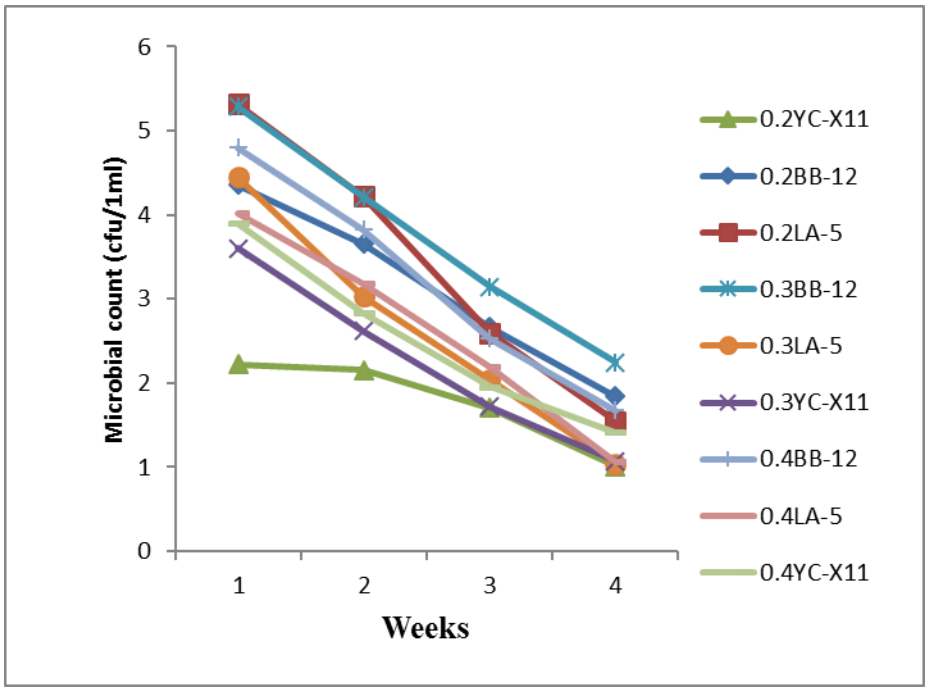

Figure 03: Change in microbial counts in developed goat milk drinking yoghurts with storage time

Yeast and mould counts in drinking yoghurt increased with the time of storage. *E-coli* counts were zero for all treatments during storage period of 21 days, probably due to good hygienic practices adapted during manufacturing process. According to

the Sri Lanka Standards, E-coli, yeast and mould counts were within the acceptable level in the developed drinking yoghurt during the four weeks of storage.

Sensory qualities of developed goat drinking yoghurt

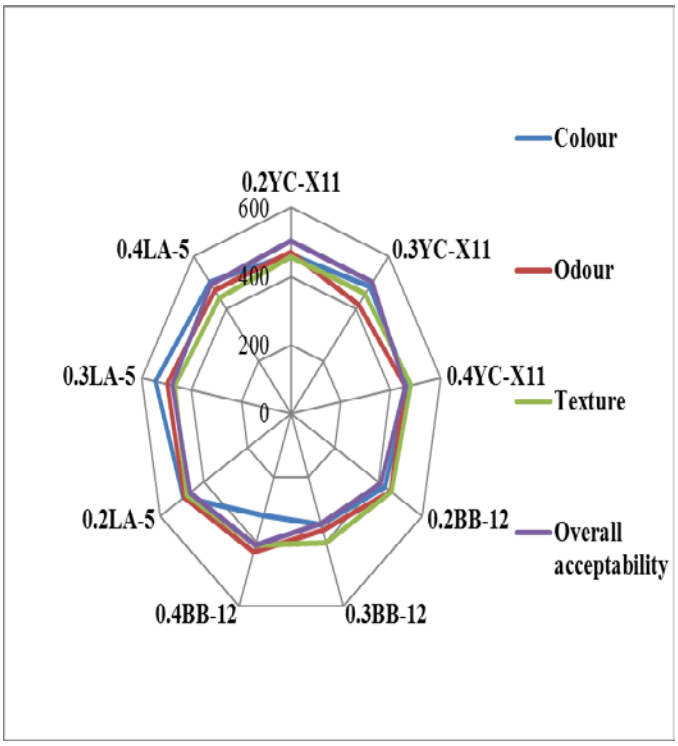

Figure 04: Sensory evaluation analysis of goat drinking yoghurt

The results of sensory tests for the colour, odour, texture and overall acceptability of developed drinking yoghurt are shown in Figure 04. The panelists were able to differentiate a significant difference ($P < 0.05$) for colour, odour and overall acceptability among different yoghurt types. A higher score for colour (545.5) and odour (498) was observed in drinking yoghurt developed from *Lactobacillus acidophilus* at 0.3 gL^{-1} culture level. However, texture did not differ significantly among developed drinking yoghurt ($P = 0.05$). The highest overall acceptability (503) was recorded in drinking yoghurt developed from regular yoghurt culture at 0.2 gL^{-1} culture level followed by *Lactobacillus acidophilus* at 0.3 gL^{-1} culture level (501). Type of starter culture and level of inoculation had an influence on the sensory properties of developed yoghurt samples. Drinking yoghurt developed from goat milk with starter culture, *Lactobacillus acidophilus* and 0.3 gL^{-1} culture level showed the best sensory qualities even though overall acceptability was not observed.

Shelf life of developed goat drinking yoghurt

Shelf life of developed drinking yoghurt was 14 days according to the pH variation. According to the yoghurt standard SLS 824 : part 2 : 1989, pH value should not be more than 4.2 in yoghurt. After the 14 days of production, pH value was not within the acceptable range. However, undesirable microorganism counts were within the acceptable range.

IV. CONCLUSIONS

Three cultures; thermophilic yoghurt culture YC-X11, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 and three culture levels; 0.2 gL^{-1}, 0.3 gL^{-1} and 0.4 gL^{-1} can be used to develop a goat milk drinking yoghurt with acceptable sensory and nutritional qualities. Drinking yoghurt prepared with 0.3 gL^{-1} *Lactobacillus acidophilus* culture shows the best sensory qualities. The shelf life of the final product is acceptable under normal refrigerated condition for about 14 days without any quality deterioration.

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