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Full Paper

Assessment of Physicochemical and Microbiological Parameters of Plain Set Yoghurt Sold in Colombo, Sri Lanka

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Abstract

Yoghurt is a popular fermented dairy product. The assessment of quality factors of yoghurt is essential to ensure that a safe product is supplied to the consumer. This study aimed to investigate the quality characteristics of marketed, high demand plain set yoghurts during refrigerated storage in Colombo, Sri Lanka. Four yoghurt brands were selected based on a preliminary survey and examined for physicochemical and microbiological attributes. Standard tests from SLS standard SLS: 824:1989 were adopted to analyze yoghurts on days 4, 7, 14, 21, and 28 of their refrigerated storage. The findings were compared against the control which had the SLS standard. The results revealed that the microbiological parameters; total aerobic count, and lactic acid bacterial count were within acceptable limits for all tested yoghurt brands. However, yeast and mold counts were higher than the control. Coliforms were not detected in all tested brands. A decrease in moisture content and pH were observed during storage. A rise in titratable acidity was observed in the samples compared to the control ($P<0.05$). The syneresis effect of yoghurts showed a non-significant increase during the storage period ($P<0.05$). The protein and fat content of all yoghurt samples varied from the values labeled on the yoghurt. The findings of the present study revealed that the quality characteristics of yoghurt such as the yeast and mold count, moisture content, pH, total titratable acidity, syneresis, fat and protein content are affected by storage conditions. Therefore, manufacturers should focus more on producing yoghurts with physicochemical and microbiological quality and maintaining quality during storage until they are consumed.

Keywords: microbiological, physicochemical, quality, storage, yoghurt

Introduction

Consumption of fermented dairy products dates to antiquity. Fermentation of dairy products not only extends the period of preservation; but also enhances the organoleptic properties [1]. Although there are ample fermented dairy products available, yoghurt is one of those products which has gained worldwide distribution [2].

The word "yogurt" comes from the Turkish verb "yoğurmak," to knead or mold [3]. The production of yoghurt involves milk fermentation with added starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. In addition, *Lactobacillus acidophilus* and *Bifidobacterium bifidus* are often added as probiotic cultures [4]. These bacteria convert lactose to lactic acid which gives yoghurt its characteristic flavor and aroma. A significant number of lactic acid bacteria, their activity, and viability should be preserved in the yoghurt until it reaches the consumer [5].

Yoghurt is a popular dairy product among all age groups. The popularity of yoghurts has increased the competition in the market. Thus, diverse types and varieties of yoghurt such as set yoghurt, stirred yoghurt, drinking yoghurt, flavored yoghurt, and frozen yoghurt are popular in the local and international markets. The categorization of yoghurt is based on its chemical and physical attributes, its flavor, and the characteristics of post-incubation processing [6]. However, the yoghurts may differ among producers by the source of the milk used, added starter cultures, the method of production, and the preservation methods [5]. Therefore, the quality of yoghurt differs from one manufacturer to another in the local market.

Yoghurt has achieved its reputation as a healthy food both due to its nutritional content and potential health benefits. A study suggests that there has been a change in the food consumption pattern in Sri Lanka over the years. Specifically, there has been an increase in the calorie supply from milk products including yoghurt by 2% from 1985 to 2009 [7]. This may be due to several factors, such as changes in dietary preferences, availability, and accessibility of these food products in the market, and changes in the overall lifestyle in Sri Lanka. Also, a survey made on household income and expenditure in Sri Lanka states that 8.2% of their total income is spent on milk and milk products [8]. Moreover, yoghurt has become a popular dessert among Sri Lankans too. Therefore, it is crucial that the quality of the yoghurt should be preserved.

There is an increasing trend of producing fortified food products. Yoghurts are often fortified with probiotics, calcium, B vitamins, and iron [9]. The consumption of yoghurt provides tremendous health benefits by improving gut function, enhancing immunity, and lessening lactose intolerance, constipation, diarrheal diseases, colon cancer, allergic reactions, and acting against inflammatory bowel diseases [10]. However, Milk, which is an important ingredient in yoghurt production is highly prone to pathogenic contaminations. A survey carried out on yoghurt in Sri Lanka reported that 10.71% of yoghurts were contaminated with *Listeria monocytogenes* [11]. Low-quality milk, poor hygienic practices used in milk processing, and the use of "wild type" starter cultures result in poor-graded yoghurt [12]. A reliability analysis carried out on yoghurt production line by Tsarouhas and Arvanitoyannis (2014) observed that the automated yoghurt production line had a failure every three hours of operation, thus the machines should be maintained properly to avoid any kind of quality-related losses, productivity, and safety issues [13].

Yoghurt is a nutritional product that has copious amounts of nutritional benefits which also influences the health and well-being of the general public. To ensure health security of the Sri Lankan population, yoghurts in local market are recommended to be produced according to the established standards given by Sri Lanka standard institution [14, 15]. This is crucial to ensure its positive impact on health and microbial safety, as well as to meet consumer expectations and preferences [16]. Once the final product is

distributed and until the product reaches the consumer, proper storage conditions should be maintained which in turn affects the shelf life of yoghurt [17]. Changes in storage conditions could affect the quality parameters of yoghurt. However, the information available on changes in quality attributes during the storage of yoghurt in Sri Lanka is insufficient [14]. Therefore, it is important to investigate the physicochemical and microbiological changes that take place during refrigerated storage of yoghurts to address the existing knowledge gap.

Materials and Methods

Sample Collection

A preliminary survey was conducted in different convenience stores of selected highly populated areas in Colombo to determine the yoghurt brands with higher consumer preferences. The identified brands were named Y1, Y2, Y3, and Y4. The Y4 brand that holds the Sri Lanka standard (SLS) was considered as the positive control. All yoghurt samples were collected in an ice box maintained at 4 °C and transported to the research laboratory at the University of Sri Jayewardenepura. The yoghurts were observed for packaging conditions and nutritional labeling prior to physicochemical testing. The yoghurt samples from each brand exhibited a production date difference ranging from 0 to 4 days, with the analysis commencing on the 4th day onward. Samples were analyzed in triplicates for the physicochemical and microbiological parameters evaluation.

Physicochemical Analysis

Moisture Content

The moisture content was determined by adopting the method described by Bibiana et al., (2014) [18]. Oven-dried clean crucibles with lids were weighed (W1), then 2 g ± 0.05 yoghurt samples were transferred into the crucibles, weighed (W2), and oven-dried without the lid at 100 ± 5 °C for 3 hours. The crucibles were covered with the lids and kept in a desiccator to cool down to room temperature and weighed again (W3). Readings were taken at a constant weight. A blank test was carried out with an empty crucible. Moisture content was determined using the following formula.

$$\text{Moisture \% by mass} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

pH Value

pH measurements were obtained using a pH meter using calibrated and standardized buffer solutions pH 4 and pH 7. The yoghurt samples were brought to room temperature before pH measurements were taken. Yoghurt samples were prepared by dissolving 10 ± 0.05 g of the yoghurt in 100 ml of distilled water. Samples were homogenized and measurements were taken using a pH meter at 27 °C.

Total Titratable Acidity

The titrimetric method was used to determine the amount of acid present in the yoghurt. For the analysis, the yoghurt samples were titrated with freshly prepared 0.1 N NaOH (Sigma Aldrich, USA) solution that was prepared by dissolving 2.0 g of NaOH pellets in 500 ml of distilled water. Yoghurt samples were diluted to 1:10 dilution by dissolving 10 ± 0.05 g of yoghurt in 100 ml of distilled water and homogenized using a homogenizer. A volume of 25 ml of the sample was pipetted out into a clean conical flask and 2-3 drops of phenolphthalein indicator were added to it. Then the sample was diluted against 0.1 N NaOH solution. The endpoint of the titration was determined by a colour change to a permanent faint pink. The percentage of titratable acidity was calculated as,

$$\text{Titratable acidity \%} = \frac{\text{Volume of 0.1 N NaOH} \times 0.9}{\text{Mass of the sample}}$$

0.9 – Is the conversion factor of lactic acid (Bibiana et al. [18].

Syneresis Effect

The syneresis effect of yoghurt samples were measured by filtering 10 ± 0.05 g of yoghurt samples into graduated cylinders using a muslin cloth-lined funnel for 20 minutes. The yoghurt samples were brought back to room temperature before being used to measure the drained-out whey. Readings were taken and recorded [19].

Fat Content

Fat determination was performed using Mojonnier-type fat-extraction method adopted by the SLS standard [15]. For this, 1.5 ± 0.05 g of the yoghurt samples were weighed and dissolved in 10 ml of preheated distilled water at 65 ± 5 °C and the samples were homogenized for 10-15 seconds using a homogenizer. This was transferred into Mojonnier-type fat-extraction flask. Then, 2 ml of ammonia solution (mass fraction of NH3 of ~25 %) was added and shaken thoroughly. This mixture was heated in a water bath at 65 ± 5 °C for 15 to 20 minutes. Then, it was allowed to cool down, and three drops of phenolphthalein were added. Afterward, 10 ml of ethyl alcohol (95%) was added and mixed well. This was followed by the addition of 25 ml of absolute diethyl ether (Sigma Aldrich, USA) and shaken gently for 30 seconds. Next, 25 ml of absolute petroleum ether (Sigma Aldrich, USA) was added and shaken well for 30 seconds. Finally, the samples were allowed to settle into clear separate layers for 30 minutes. After the separation was achieved, the supernatant was carefully decanted into a pre-weighed clean beaker. The rest of the mixture was used in the second extraction. A blank test was carried out without the sample with the same procedure and the reagents. The dried fat extract that was weighed to a constant weight was expressed as the percentage of fat per weight.

$$\text{Fat \% by mass} = \frac{\text{Weight of the extracted fat}}{\text{Weight of the sample}} \times 100\%$$

Protein Content

Protein contents were determined using Bradford dye-binding method [20]. For this, the standard protein curve was prepared using Bovine serum albumin (Sigma Aldrich, USA) in the concentration range of 5 – 25 µg / ml. Distilled water was added to make the final volume 100 µl. Then 3.9 ml of Bradford reagent (Sigma Aldrich, USA) was added, and the samples were vortexed at x1 speed for 2-3 seconds. The tubes were wrapped in aluminum foil and incubated for 10 minutes to equilibrate at room temperature. At the end of the incubation period, the absorbance was measured using a spectrophotometer (CT – 8200) at 595 nm. The Bradford reagent containing a total volume of 4 ml without the protein sample was used as the blank.

For the determination of the protein present in yoghurt samples, they were diluted with distilled water to meet the range of proteins that could be determined by the Bradford reagent. Then the absorbance was measured using a spectrophotometer at the wavelength of 595 nm. Using the standard protein curve the concentrations of the unknown samples were determined [20].

Microbiological Analysis

Enumeration of Total Aerobic Bacteria

The total aerobic bacterial count was determined on nutrient agar using the spread plate method with tenfold dilution up to 10^{-4}. The plates were aerobically incubated for 24 hours at 37 °C. Colonies that developed on the plate after the incubation period were counted and records were made accordingly [21].

Enumeration of Lactic Acid Bacteria (LAB)

Lactic acid bacterial count was determined using De Man Rogosa Sharpe Agar (MRS agar) and the spread plate method with tenfold dilution up to 10^{-4}. The culture plates were aerobically incubated for 48 hours at 37 °C. Colony count was taken and recorded [22].

Enumeration of Yeasts and Molds

The yeast and mold count were determined using Yeast Malt Agar by spread plate technique. The culture plates were incubated for 24 hours at 37 °C. Colonies that appeared on the plate after incubation were counted manually and records were made [23].

Enumeration of Coliform Bacteria

The Most Probable Number technique was used for the detection and enumeration of total coliforms. A weight of 10 ± 0.05 g of the yoghurt sample was added into 90 ml of sterile distilled water and mixed well. From the mixture 10.0 ml was transferred into the double-strength lactose broth and 1.0 ml of the samples

were added to the next set of three test tubes (single strength). A volume of 0.1 ml was transferred to the third set of test tubes, and all were incubated at 37 °C for 24-48 hours and observations were taken. For this, *Escherichia coli* was used as the positive control and *Bacillus sp.* was used as the negative control [24].

Statistical Analysis

All data were statistically analysed using Minitab 17.1.0. One-way analysis of variance (ANOVA) was used for the analysis. The Turkey pairwise comparison was used to group the mean values at 95% confidence intervals. The interaction plot was used to graph the parameters tested and the significant differences were determined at p-value (0.05).

Results and Discussion

Preliminary Survey; Questionnaire Evaluation

Among all marketed plain set yoghurts, four brands were selected for the present study based on customer preference. Figure 1 shows the dissemination of preference for plain set yoghurt brands obtained after the questionnaire evaluation regardless of age group and gender.

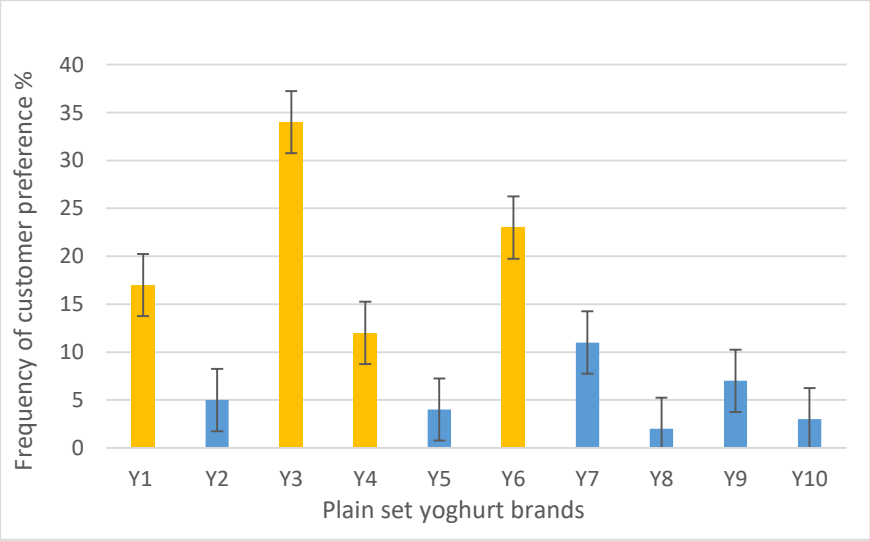

Figure 1. The percentage of customer preference for various brands of plain set yoghurt available in the market. Orange bars indicate the highly preferred four brands while blue bars indicate the least preferred yoghurt brands.

The most customer-preferred yoghurt brands in their descending order are brand 3, brand 6, brand 1, and brand 4 and the percentage values are 34, 23, 17, and 12% respectively. These four yoghurt brands were used for further studies. They were named Y1, Y2, Y3, and Y4 respectively, and out of these four brands, brand Y4 had the SLS standard which was considered the positive control for the present study.

Physicochemical Analysis

Moisture Content

The moisture content ranged from 74.98 to 78.73% throughout the storage period of all brands under study (Figure 2). A decrease in moisture content was observed in the brands Y2 and Y3 after the 7th day of storage compared to the control. The consistency of yogurt is influenced by both total solids and moisture content. When the moisture content of yogurt is decreased it elevates the total solids in the product. This interplay between moisture and total solids is a key aspect of yogurt production [25].

Moisture contents of samples (Y1, Y2, and Y3) were significantly lower than the control during storage ($P<0.05$). According to Ndife et al., (2014), most of the commercially available yoghurts contain 80-86% moisture [26]. Hassan and Amjad reported a similar value for moisture content (86.05%) [27]. However, our findings had lower values with a maximum of 78.73%. Literature indicates that an increase in moisture content affects the susceptibility to microbial contaminations and quick disintegration and perishing [28].

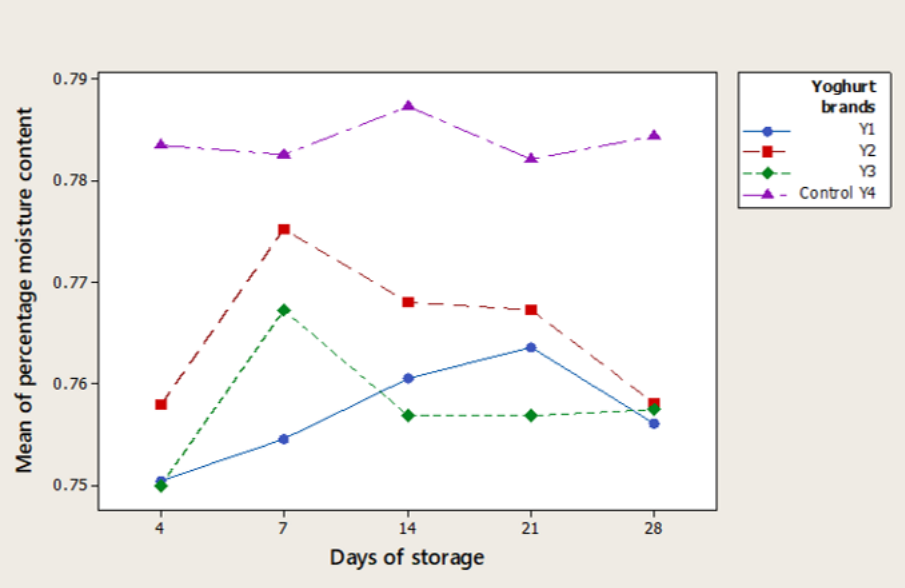

Figure 2. The changes in the moisture percentage of different brands of plain set yoghurts during refrigerated storage of 4-28 days.

pH Value

The pH ranged between 3.89 ± 0.03, and 4.48 ± 0.01 (Figure 3) almost within the values mentioned by SLS 824:2018 (pH 4.5) [29]. The result of pH indicates that the Y1 brand has the highest average pH value 4.48 ± 0.01 in its initial days and the control had the lowest average pH value 3.89 ± 0.03. The pH values of tested yoghurts were in accordance with the value (4.6 or lesser) mentioned by Olugbuyiro and Oseh [30]. However, during storage, the pH declined significantly in Y1 after the 21st day. A similar trend was observed by Hemamali et al., (2016) and Sivasankari et al., (2017) where the pH of yoghurts dropped during later stages of refrigerated storage [16, 31].

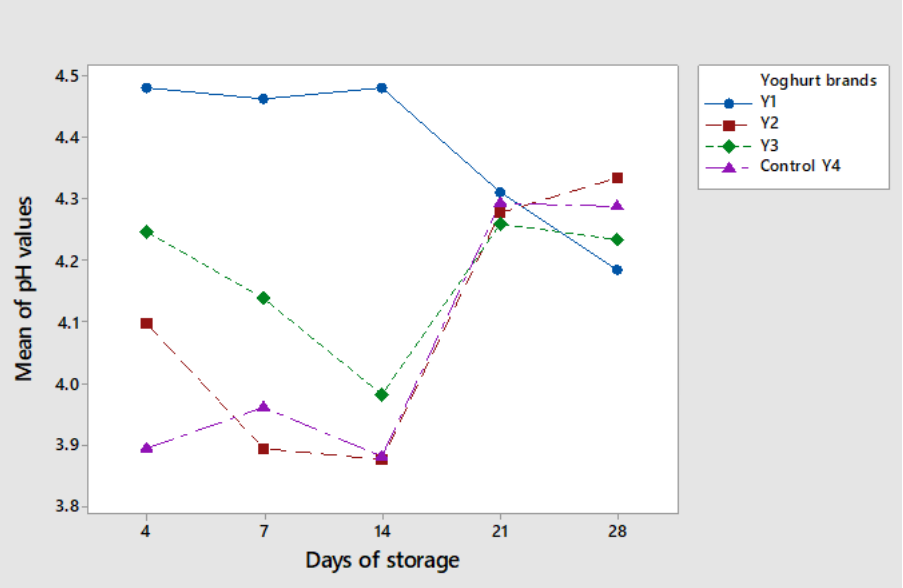

Figure 3. Variation in pH values of different brands of plain set yoghurts during refrigerated storage of 4-28 days.

However, the variability of pH values could be linked to sample preparation methods and the type of starter cultures used [32]. Production of lactic acid from lactose during lactic acid fermentation causes a reduction in the pH. The starter culture used in the production process determines the rate of acidification and this results in the variation of pH throughout its storage time. Therefore, this could ultimately affect the quality of yoghurt [27]. Besides, the composition of the yoghurts and the availability of nutrients could also affect the pH value during their storage period [33].

Total Titratable Acidity

A gradual increase in titratable acidity was evident in every brand during their storage period (Figure 4). Brand Y1 resulted in the highest titratable acidity while the control had the lowest titratable acidity. A gradual increase in titratable acidity was observed in every brand during their storage period. Moreover, the titratable acidity of Y1, Y2, and Y3 was significantly higher compared to the control ($P<0.05$). The Y1, Y2, and Y3 had higher titratable acidity than the value (0.6%) specified by the SLS 824: Part 2. The control sample had a close acidity value to the specified requirement. Furthermore, this value agreed with the value obtained by Hassan and Amjad (2010) while other test yoghurts had higher acidity values.

Many previous studies indicated that the titratable acidity and pH values of yoghurt brands showed a counter-relation [34, 35]. The gradual increase in titratable acidity observed during the storage period might be due to the amount of lactic acid bacteria present in the samples [36]. Acidity in yoghurt results from the fermentation of lactose to lactic acid and post-acidification during their refrigerated storage. The milk quality, the starter cultures used and the incubation temperature on the storage days determine the level of acidification [37].

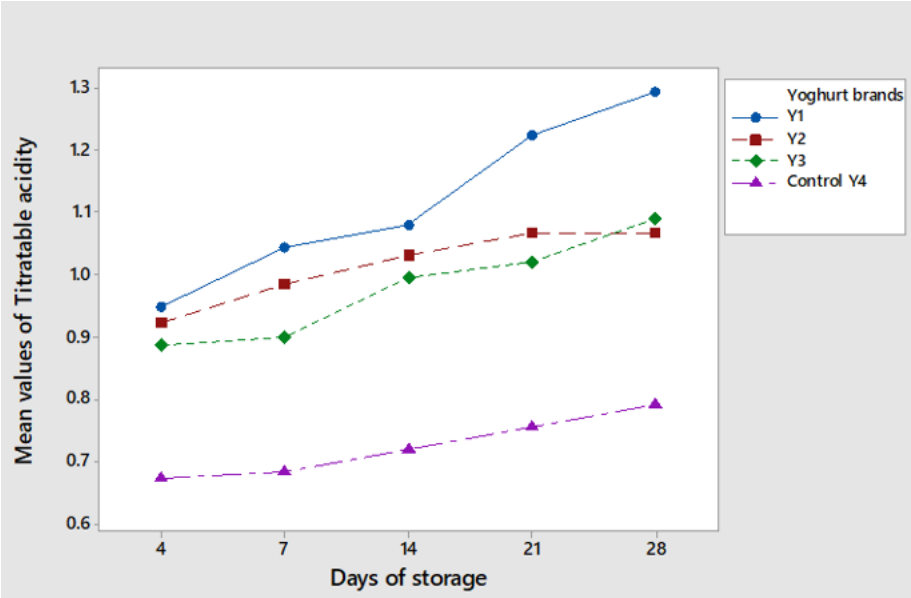

Figure 4. Variation in titratable acidity values of different brands of plain set yoghurts during refrigerated storage of 4-28 days.

Syneresis

The whey content of yoghurts exhibited a rising tendency during the storage period (Table 1). But, on the 28th day, a decline in collected whey was recorded in both Y1 and Y2 brands. Syneresis of yoghurt differed significantly during storage ($P<0.05$). A similar experiment carried out by Hemamali et al., (2016), observed an increase in the drained-out volume of whey during the storage period. The reduction in the quality of physical properties causes a higher volume ordained-out whey [19].

Table 1. Means of drained whey (syneresis) of tested yoghurt brands during refrigerated storage from 4-28 days.

Syneresis (ml / 10 g)	**Storage period**				
	4th day	**7th day**	**14th day**	**21st day**	**28th day**
Brand Y1	$2.0^{Ba}(\pm0.1)$	$2.2^{Ba}(\pm0.3)$	$2.9^{Aa}(\pm0.2)$	$3.0^{Aa}(\pm0.2)$	$2.9^{Aa}(\pm0.1)$
Brand Y2	$1.9^{Ba}(\pm0.1)$	$2.3^{ABa}(\pm0.1)$	$2.5^{Aba}(\pm0.3)$	$2.7^{Aa}(\pm0.3)$	$2.2^{Abb}(\pm0.2)$
Brand Y3	$2.2^{Ba}(\pm0.2)$	$2.2^{Ba}(\pm0.2)$	$2.6^{ABa}(\pm0.1)$	$3.0^{Aa}(\pm0.2)$	$3.0^{Aa}(\pm0.3)$
Brand Y4	$1.7^{Ca}(\pm0.3)$	$2.0^{BCa}(\pm0.3)$	$2.7^{Aba}(\pm0.3)$	$2.7^{Aba}(\pm0.3)$	$3.0^{Aa}(\pm0.2)$

Values are an average of triplicate observations; n=3 (± SD); Values followed by similar capital superscript in a row do not differ significantly ($P<0.05$) and values followed by similar superscript in a column do not differ significantly ($P<0.05$).

Protein Content

Results indicated that there was no significant difference in protein content among test yoghurt brands and the control (P>0.05). The lowest protein content was recorded in Y3 with a mean value of 0.7 ± 0.01 and the calculated total protein content of the yoghurt pack was 2.611 g. This value (2.611 g) was much lower compared to the actual protein content (3.15 g) given on the label of the yoghurt container. Y1 contained 0.8 ± 0.02 protein and a total protein content of 2.937 g and Y2 had 0.8 ± 0.02 and 3.435 g respectively. The given protein content of the Y1 was in line with the value given. Y2 had slightly higher protein content than the value stipulated in the nutritional label (3 g). The highest protein content was observed in the control (Y4) containing a mean value of 0.8 ± 0.02 and a calculated total protein content of 3.396 g which was slightly lower than indicated in the label (3.5 g). De Silva and Rathnayaka [19] reported that the average protein content present in set yoghurt samples was 4%. The results were in line with Deb and Seth (2014).

The protein present in milk could encounter hydrolysis by the lactic acid bacteria which breakdown proteins into their constituents such as amino acids and short peptides using proteinase enzymes. This causes a reduction in the protein level during their storage period [38]. Moreover, deviation from the actual values might be due to the interferences with other substances present in the sample or the nature of proteins [39]. Further, an increase in the protein content could result in the addition of skim milk powder during the production process [32].

Table 2. The labeled protein contents and calculated protein contents on the selected marketed yoghurt brands. Y1, Y2, and Y3 are test samples. Y4 is the positive control with the SLS Standard.

Samples	Protein content / g	Mean protein content
Y1	2.9	$0.8^{a}(\pm 0.02)$
Y2	3	$0.8^{a}(\pm 0.02)$
Y3	3.15	$0.7^{a}(\pm 0.01)$
Y4	3.5	$0.8^{a}(\pm 0.02)$

Values are an average of triplicate observations; n=3 (± SD). Values followed by similar superscript in a column do not differ significantly (P <0.05).

Fat Content

The fat content of yoghurt is important in improving its consistency [18]. According to SLS: 824: Part 2 [15], yoghurt should contain a minimum of 3% milk fat by mass. Y3 contained the highest fat content of 3.1 ± 0.3 while the control had the lowest value (2.3). Though the fat contents of Y1, Y2, and the control Y4 were lower (2.7 ± 0.3, 2.7 ± 0.2, and 2.3 of fat respectively) than the SLS standard (minimum 3%), the test brand Y3 (3.1 ± 0.3) complied with the standard. As enlisted in Table 3 below, there is no significant difference in fat content among the yoghurt brands tested (P>0.05). The results obtained were in accordance with the findings of Olugbuyiro and Oseh (2011) while lower fat percentages were reported by Omola et al., (2015) [40]. De Silva and Rathnayaka (2014) reported comparatively higher fat percentages than those of the present study but remained in line with the SLS standards given for yoghurts.

Table 3. Variations of fat percentage by mass values tested in marketed yoghurts.

Values are an average of triplicate observations; n=3 (± SD). Values followed by similar superscript in a column do not differ significantly (P <0.05).

Yoghurt samples	Mean fat (%)
Y1	$2.7^{a}(\pm 0.3)$
Y2	$2.7^{a}(\pm 0.2)$
Y3	$3.1^{a}(\pm 0.3)$
Y4	$2.3^{a}(\pm 0.0)$

Microbiological Analysis

Enumeration of Total Aerobic Bacteria:

All the total aerobic bacterial counts of yoghurt samples recorded in the study were within specifications when compared to the standard values (10^{8} CFU/g). Total aerobic counts of tested yoghurt brands had an increase until the 14th day of storage and decreased gradually thereafter. Similar results were obtained by Eissa et al., (2011) indicating that the elevated acidity levels during the storage would be the cause of decreased bacterial count in the later stages of storage [41]. A rapid decrease in the total aerobic bacterial count was observed on the 28th day of the control brand and this was similar to the results obtained by Mihoubi et al., (2017) confirming the effect of post-acidification which affects the viability of lactic acid bacteria [42]. There was no significant difference (P<0.05) of total aerobic bacteria among all samples of the 4th, 7th, and 14th days of
storage but a significant difference (P<0.05) was observed on the 21st and 28th days of storage.

Table 4. Variations in the total aerobic bacterial count of marketed yoghurts tested during refrigerated storage from 4-28 days. Values are an average of triplicate observations; n=3 (± SD). Values followed by similar capital superscript in a row do not differ significantly (P<0.05) and values followed by similar simple superscript in a column do not differ significantly (P <0.05).

Yoghurt Brands	Total aerobic bacteria (log 10 CFU/ml)×10^{8}				
	4th day	7th day	14th day	21st day	28th day
Y1	$7.6^{Aa}(\pm 0.0)$	$7.6^{Aa}(\pm 0.0)$	$7.7^{Aa}(\pm 0.1)$	$7.0^{Ba}(\pm 0.2)$	$6.1^{Ca}(\pm 0.1)$
Y2	$7.5^{Aa}(\pm 0.0)$	$7.5^{Aa}(\pm 0.0)$	$7.4^{Aa}(\pm 0.0)$	$6.7^{Ba}(\pm 0.2)$	$5.9^{Ca}(\pm 0.2)$
Y3	$7.5^{Aa}(\pm 0.1)$	$7.5^{Aa}(\pm 0.1)$	$7.5^{Aa}(\pm 0.1)$	$6.8^{Ba}(\pm 0.2)$	$6.0^{Ca}(\pm 0.2)$
Y4	$7.5^{Aa}(\pm 0.2)$	$7.1^{Ab}(\pm 0.1)$	$7.4^{Aa}(\pm 0.5)$	$7.0^{Aa}(\pm 0.1)$	$2.5^{Ba}(\pm 3.5)$

Enumeration of Lactic Acid Bacteria

Variations in the lactic acid bacterial count were also observed over the storage period (Table 5).

Table 5. Variations in lactic acid bacterial count of marketed yoghurts tested during refrigerated storage from 4-28 days.

Yoghurt Brands	Lactic Acid Bacteria (log 10 CFU/ml)×10⁸				
	4th day	7th day	14th day	21st day	28th day
Y1	7.5^{Aa}(±0.0)	7.6^{Aa}(±0.0)	7.8^{Aa} (±0.1)	7.0^{Ba}(±0.1)	6.2^{Cab}(±0.3)
Y2	7.4^{Aa}(±0.2)	7.3^{Ab}(±0.0)	7.0^{ABb}(±0.1)	6.6^{Ba}(±0.2)	5.7^{Cb}(±0.4)
Y3	7.2^{Ab}(±0.0)	7.2^{Abc}(±0.0)	7.3^{Aab}(±0.1)	6.6^{Ba}(±0.1)	6.0^{Cab}(±0.1)
Y4	7.6^{Aa}(±0.0)	7.1^{ABc}(±0.1)	7.0^{Abb}(±0.3)	6.5^{Ba}(±0.4)	6.8^{Aba}(±0.3)

Values are an average of triplicate observations; n=3 (± SD). Values followed by similar capital superscript in a row do not differ significantly (P<0.05) and values followed by similar simple superscript in a column do not differ significantly (P <0.05).

Initially, the counts were augmented specifying them as the predominant organisms in yoghurt. Then, a decline was observed after the 14th day. The test yoghurt brands, and the control brand were within SLS specifications. Higher lactic acid bacterial count is due to the fermentation of lactose which is the main energy supplementing source [26]. Microbial hydrolysis of yoghurts during storage is the key factor that affects the overall quality of yoghurt [43]. Lactic acid bacterial counts of test yoghurt brands Y1 and Y3 did not show any significant difference (P<0.05) up to 14th day but those brands showed significant differences (P<0.05) among different storage days.

Enumeration of Yeasts and Molds

A significant difference in yeast and mold counts was observed among different storage days of test brands. Until the 14th day yeast and mold counts showed a drop while it increased in the final two storage days in test brands. These counts were much higher in the test samples than the microbiological limits stated by SLS: Part 2 [15], (Yeast counts <1000 per gram and molds < 1 per gram). Higher yeast and mold counts could be a result of improper pasteurization [44]. Moreover, this could be due to reduced oxygen levels along with fermentation and the obvious increase in acidity [41].

Table 6. Variations in yeast and mold counts of marketed yoghurts tested during refrigerated storage from 4-28 days.

Yoghurt Brands	Yeasts and Molds (log 10 CFU/ml)×10⁸				
	4th day	7th day	14th day	21st day	28th day
Y1	7.0^{Ba}(±0.1)	7.1^{ABa}(±0.1)	6.1^{Da}(±0.1)	6.7^{Ca}(±0.0)	7.3^{Aa}(±0.1)
Y2	7.2^{Aa}(±0.1)	7.2^{Aa}(±0.1)	5.7^{Ca}(±0.4)	6.5^{Ba}(±0.0)	7.1^{Aa}(±0.1)
Y3	7.1^{ABa}(±0.1)	7.1^{ABa}(±0.1)	5.6^{Ca}(±0.5)	6.6^{Ba}(±0.2)	7.4^{Aa}(±0.1)
Y4	Abs	Abs	Abs	Abs	Abs

Abs: Yeast and mold counts were not recorded

Values are an average of triplicate observations; n=3 (± SD). Values followed by similar capital superscript in a row do not differ significantly (P<0.05) and values followed by similar simple superscript in a column do not differ significantly (P <0.05).

Enumeration of Coliform Bacteria

Enumeration of coliform bacteria is a hygienic indication. The samples that were tested did not contain any coliform bacteria (Table 7).

Table 7. Total coliform count in yoghurt samples on the selected marketed yoghurt brands. Y1, Y2, and Y3 are test samples. Y4 is the positive control with the SLS Standard.

Yoghurt brands	Tested number of samples	Number of Coliform positive sample
Y1	3	Nil
Y2	3	Nil
Y3	3	Nil
Y4	3	Nil

Nil: Coliforms not recorded

The results ensure that samples didn't have any fecal contamination during the production process [42]. Unhygienic practices during processing, samples handling, manufacturing process and improper storage practices could result in contamination of products with coliform bacteria [32]. Coliform bacteria contamination has been recorded even in branded yoghurt samples due to careless handling [45]. The presence of coliforms in commercial yoghurts has also been recorded by Tarakçi and Küçüköner [46].

Conclusion

Based on the results obtained in the present study, it was evident that many yoghurts sold in the market did not comply with the SLS standards. The physicochemical parameters of test yoghurts such as titratable acidity, and syneresis had higher values while moisture, protein, and fat had relatively lower values than the SLS standards. But the SLS-certified brand contained similar values stipulated in the standard (SLS specified values are as titratable acidity 0.6%, pH 4.5, Moisture 81%, Fat Min 3%, other values were compared with the SLS certified control). In terms of microbiological quality, yeasts and mold counts in tested yoghurts were higher than the accepted limits (standard yeast and mold count <1000/g, molds < 1/g). Variations in the titratable acidity, pH, syneresis, protein, fat and yeast and mold count during storage clearly showed that the yoghurts are affected by the storage conditions. Also, the labeling of yoghurts should be improved to precisely represent the contents of the yoghurt. Therefore, manufacturers should emphasize more on the quality characteristics of yoghurt and should pay more attention to getting the product certification and meeting the quality standards. Nutrient-rich delicate products like yoghurts need quality control during processing and storage as insufficient quality processing methods and unhygienic practices may raise concerns on the health of the consumers.

Conflicts of Interest

No conflicts of interest exist.

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