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Physico-Chemical, Microbiological and Sensory Characteristics of Yogurt as Affected by Added Lactose

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Abstract

Enrichment of yogurt with lactose addition may increase the growth of the yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and enhance yogurt physico-chemical and sensory attributes. The objectives of this study were to determine the influence of added lactose on the 1) physico-chemical characteristics, including the final lactose content of yogurt, during its shelf life; 2) growth of the yogurt starter culture over yogurt shelf life and 3) the sensory attributes of yogurt. Fat free plain set-type yogurt was manufactured using 0%, 1%, 3% and 5% w/w added lactose to accomplish objectives 1 and 2. For objective 3, a blueberry yogurt was manufactured using the same lactose levels. Analyses for plain set-type yogurt were conducted at 7 days intervals during 35 days of storage. Sensory evaluation was conducted on flavored yogurt three days after its manufacture. Data were analyzed using Proc Mixed model of SAS® 9.3 program. Significant differences between means were analyzed at $\alpha = 0.05$ using Tukey adjustment. Lactose addition influenced some of the yogurt characteristics in a positive manner. Lactose contents of yogurts with lactose added at 1%, 3% and 5% stayed higher in that proportion than control throughout the 35 days of storage. Yogurts containing 5% w/w added lactose had the lowest pH. Yogurts containing 5% w/w added lactose had significantly the highest syneresis values compared to 0%, 1% and 3% w/w added lactose during storage period at day 7 and from day 21 onwards. Use of 5% w/w added lactose resulted in significantly higher counts of *Streptococcus thermophilus* compared to control and this bacterial survival was the highest for 1% w/w added lactose compared to the rest. The overall liking scores indicated that yogurts containing added lactose were preferred over control. For taste, sourness and sweetness samples containing added lactose had higher scores than control. The consumer acceptability of yogurts increased as lactose addition increased. The acceptability of yogurts and purchase intent frequency scores markedly increased with the addition of lactose.

Keywords

Added Lactose, Yogurt, Probiotic Properties, Starter Culture, Lactic Acid Bacteria

1. Introduction

Yogurt is a popular dairy product in the US and globally. The US Code of Federal Regulation describes yogurt as a cultured food that contains the lactic acid-producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [1]. Yogurt starter cultures use lactose as a source of energy, fermenting it to lactic acid. These strains have a synergistic effect of one over the other. Initially *Streptococcus thermophilus* grows faster than *Lactobacillus bulgaricus* and releases lactic acid creating an acidic environment that favors the growth *of Lactobacillus bulgaricus*. *Streptococcus thermophilus* also produces formic acid and carbon dioxide (CO_2) which stimulate the growth of *Lactobacillus bulgaricus*. The later has a high proteinase activity to produce peptides that are utilized by *Streptococcus thermophilus* which acts on the peptides and releases free amino acids that are utilized by both microorganisms [2]. Yogurt starter culture is considered to have probiotic properties and yogurt is a fermented food matrix desirable for delivery of probiotics [3].

Lactose also known as "milk sugar" is a disaccharide carbohydrate, composed of two monosaccharide components: D-glucose and D-galactose [4]. Lactose is the most abundant constituent and main carbohydrate in bovine milk, crucial in the lactic acid fermentation of yogurt and other acid-coagulated dairy products [5]. It can be used as sweetener in low-calorie products. β-galactosidase is added to lactose into glucose and galactose, which are much sweeter than lactose itself [6]. Lactose is important for the metabolic activities of lactic acid bacteria and has a beneficial role in the manufacture of fermented dairy products. The amount of sugar added to yogurt milk should not exceed 9% because it may inhibit culture growth [7]. Lactic acid is one of the flavor compounds in yogurt. Lactose utilization is the primary function of lactic acid bacteria used in industrial dairy fermentations [8]. Lactic acid bacteria convert lactose into lactic acid, which reacts with proteins in the milk, causing them to precipitate at pH 4.6, and make the milk creamier. The lactic acid has a sour taste, which causes a change in flavor of the fermented product, e.g. yogurt and cheese.

The pH is an important quality attribute of yogurt as it defines the endpoint of fermentation process [9]. Lactose is converted to lactic acid by the fermentation process conducted by lactic acid bacteria, which drops milk pH from 6.7 to 4.2 -

4.6 [10]. When the pH end point of 4.5 is achieved, the yogurt mixture is cooled to slow the reaction. Incorrect pH levels can lead to excessive free whey production and excess or insufficient tartness [11]. Titratable acidity is the total acid concentration in a food and is an important predictor of how organic acids influence flavor [12]. Yogurt has a titratable acidity of not less than 0.9%, expressed as lactic acid [2].

Viscosity of yogurt is greatly influenced by the total solids content of milk to be used, especially the protein content [6]. Rotational viscometers, such as the Brookfield viscometer, are often used to describe the flow behavior of yogurts [6]. Syneresis or spontaneous whey separation on the surface of set yogurt is regarded as a defect [13]. This problem can be reduced by increasing the milk solids content to approximately 15% [14] [15]. Homogenization improves the consistency and viscosity of yogurt, thus a greater stability to syneresis can be obtained [14] [16] [17]. However, [18] reported that homogenization has an adverse impact on yogurt with a lower fat content; it increases syneresis reducing water holding capacity due to empty spaces between casein matrices, and lack of native milk-fat globule membrane (FGM). Nonfat yogurt is normally low in total solids (10% to 12%) and consequently suffers from whey separation or syneresis [19]. Reference [20] developed a method for the measurement of spontaneous whey separation in set type yogurt called the siphon method. This method determines the level of spontaneous whey separated on the surface of gels. By comparing three methods (drainage, centrifugation and siphon), the siphon method would be more appropriate in the determination of spontaneous whey separation level on the surface of set yogurt [13].

Lactose is an important energy source for yogurt culture bacteria. How added lactose would influence the characteristics of this important dairy product is not clearly understood. The aims of this study were to determine the influence of added lactose at various concentrations on the final lactose content, physicochemical, microbiological and sensory characteristics of yogurt.

2. Materials and Methods

2.1. Yogurt Manufacture

Two types of yogurt were manufactured. The first type was lactose added "plain" set yogurt. The second type was lactose added blueberry yogurt for the sensory study. Plain set-type yogurt was manufactured according to earlier published methods [21] [22] [23]. Briefly, the yogurt mixes contained skim milk and added lactose at 0%, 1%, 3% and 5% w/w. The yogurt mixes were preheated to 60˚C then homogenized in a two-stage homogenizer (Type: 300 DJP4 2PS, Gaulin, Manton-Gaulin MFG Co Inc., Everett, MA, USA) at 13.8 MPa for the first stage and 3.45 MPa for the second stage and later pasteurized at 85˚C for 30 minutes. Yogurt mixes were tempered to 40˚C and inoculated. Freshly thawed frozen yogurt starter culture concentrate of *Streptococcus thermophilus* (ST-M5) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB-12) (CH-3, yogurt culture, Chr. Hansen's Laboratory, Milwaukee, WI, USA) was added at 0.75 mL per 3.78 L of mix for each bacterial strain, 7.56 L of mix per treatment was used. After mixing, the yogurt mixes were poured into previously labeled 340 g plastic cups.

The inoculated mixes were incubated at 40˚C until pH reached 4.65 ± 0.1 to obtain a set-type yogurt and transferred to the cooler at 4˚C for refrigeration until further analyses. For the sensory evaluation blueberry flavored yogurt with the same lactose treatments (0%, 1%, 3% and 5% w/w added lactose) were manufactured. The manufacture process was the same with the exception that 15% w/w blueberry puree was incorporated after plain yogurt manufacture and refrigerated at 4˚C. Product manufacture was replicated 3 times.

2.2. Lactose Content

Lactose concentration of yogurts was determined using the Lactose/ D-Galactose determination kit [24]. Briefly, 1 g of sample was weighed into a 100 mL volumetric flask. Then 60 mL of distilled water was added and flasks were incubated for 15 minutes at 70˚C. Samples were shaken from time to time. For clarification, 5 mL of Carrez-I-solution (3.60 g potassium hexacyanoferrate (II), 5 mL of Carrez-II-solution (7.20 g of zinc sulfate/100 mL) and 10 mL of NaOH (0.1 M) were added and mixed after each addition. The sample solution was adjusted to 20˚C - 25˚C and filled up to 100mL with distilled water, then filtered using a 12.5 cm Whatman® filter paper. The clear solution was used for the assay. Absorbance measurements were taken at a wavelength of 340 nm using an Evolution 100 spectrophotometer (Thermo Scientific, Waltham, Massachusetts). Lactose concentrations were measured every 7th day over the 35 days of storage for each of the 3 replications.

2.3. pH

The pH of the yogurts was determined using the Oysters Series pH meter (Extech Instruments, Waltham, MA). The instrument was calibrated using commercial pH 4.00 and 7.00 buffers (Fisher Scientific, Fair Lawn, NJ) and instrument's temperature was adjusted to the sample's temperature of 8˚C ± 2˚C before reading. Two measurements were taken per replication for each of the 3 replications.

2.4. Titratable Acidity

The titratable acidity (TA) was determined by weighing 9 g of yogurt to which 5 drops of phenolphthalein indicator solution were added. Samples were titrated with 0.1 N NaOH as until color changed to rose pink that persisted for 30 seconds.

2.5. Apparent Viscosity

Apparent viscosities were measured using a Brookfield DV II + viscometer (Brookfield Engineering Lab Inc., Stoughton, MA, USA) with a helipath stand at

10˚C ± 2˚C. A T bar B spindle was set to 10 rpm to obtain a torque between 10% - 90%. The T bar B spindle was inserted in the sample at a constant depth of 2 cm from the top level of the sample container. The helipath was set in downward motion to cut new circular layers at increasing depths of the sample. Sample's container geometry was 4.55" top diameter, 3.25" bottom diameter and 2.45" height with 340 g capacity. The data were gathered using the Wingather® software (Brookfield Engineering Lab Inc., Stoughton, MA, USA). The viscosity measurements were continuous over 33 seconds required to collect one hundred data points averaged per sample per replication.

2.6. Syneresis

Syneresis was determined with the method described by [13] with slight modifications. The 300 mL of yogurt mix was poured into plastic cups prior to incubation during yogurt manufacture. The cups of set yogurt were kept at an angle of 45˚ and spontaneous whey was collected at the side of the cup with a pipette. Amount of whey in mL was measured at 22˚C. The yogurt gel was allowed to stand for 1 minute and any further surface whey was pipetted and total whey release in mL was measured.

2.7. Growth

Growths of *Lactobacillus delbrueckii* ssp. *bulgaric*us (LB-12) and *Streptococcus thermophilus* (ST-M5) were determined using the pour plate technique with serial dilutions of yogurt samples. Yogurts were sampled at days 1, 7, 14, 21, 28 and 35 of storage period. The yogurt in the cup was agitated and 1 g of yogurt was pipetted from the center of the cup into a sterile bottle containing 99 mL of sterile 0.1% peptone water (Difco, Detroit, MI, USA). Contents in bottle were agitated to prepare serial dilutions and plated on 5.2 modified pH *Lactobacilli* MRS agar for *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* agar for *Streptococcus thermophilus* ST-M5. Pour plates were incubated anaerobically at 43˚C for 72 hours for *Lactobacillus bulgaricus* LB-12 [25] and aerobically at 37˚C for 24 hours for *Streptococcus thermophilus* ST-M5 [26] and colonies were counted.

2.8. Coliform Counts

The blueberry yogurt was tested for coliforms using petrifilms (3M®, St. Paul, MN) which contain violet red bile agar (VRBA). The procedure was performed by weighing 11 g of yogurt samples in 99 mL of sterile 0.1% peptone water (Difco, Detroit, MI, USA). Contents in bottle were agitated and serial dilutions were prepared. Aliquots of 1mL were taken from dilutions 10-1 and plated in duplicate for control and added lactose samples. Previously labeled petrifilms were incubated aerobically at 32˚C for 24 hours.

2.9. Sensory Study

The sensory study was approved by the LSU Institutional Review Board with the

IRB exemption number of HE13-6. Blueberry yogurt containing the four treatments (0%, 1%, 3% and 5% w/w added lactose) was poured into 2.5 oz. previously labeled shuffle cups. A 3-digits random number code was used to label the cups. Consumer acceptance study was performed with 100 consumers of yogurt. One cup per treatment, that is four cups in total, was given to each consumer along with the evaluation questionnaire which consisted of a 9-points rating scale (1 = Dislike extremely, 9 = Like extremely), with acceptability and purchase intent questions (yes/no). Panelists were asked to evaluate each yogurt sample for the following attributes: Appearance, Color, Aroma, Taste, Sourness, Sweetness, Thickness, Graininess and Overall like.

2.10. Statistical Analysis

The *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5 counts were converted to log 10 scale before analyzing the data by SAS. Data were analyzed using Proc Mixed of the SAS® 9.3 program. Differences of Least Square Means were used to determine significant differences at $P < 0.05$ for main effects (lactose concentration and time) and interaction effects (lactose concentration*time). Sensory data are presented as mean ± standard deviation (SD) of the means. Significant differences between means were analyzed at $\alpha = 0.05$ using Tukey adjustment to determine the best treatment. All experiments were replicated three times.

3. Results and Discussion

3.1. Lactose Content

Lactose content of yogurts as influenced by lactose addition over storage of 35 days is shown in Figure 1. Treatment*day interaction effect was not significant

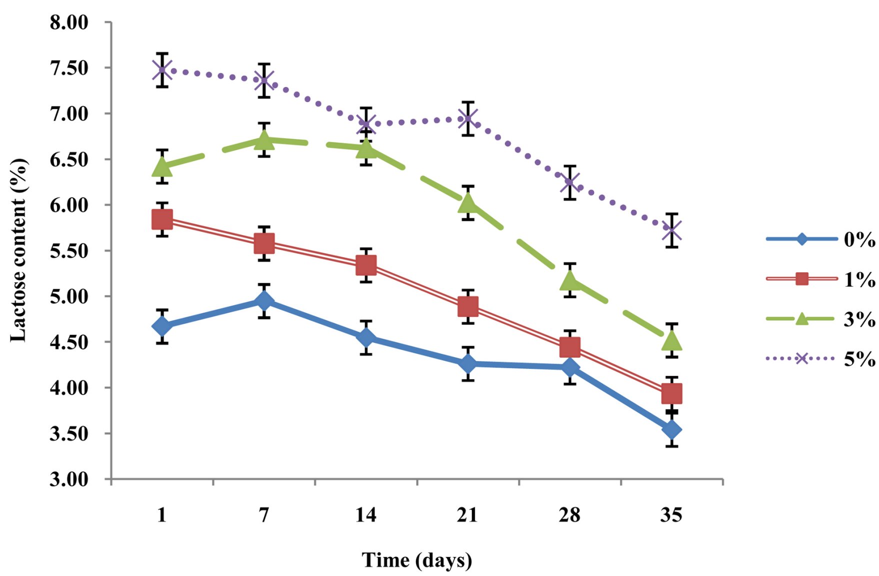

Figure 1. Lactose content of yogurts as influenced by lactose addition over storage period of 35 days, means + SE.

(P > 0.05) while treatment effect and day effect were significant (P < 0.05) (Table 1). Lactose content decreased for all treatments over time (Figure 1). Yogurts with lactose added at 1%, 3%, 5% stayed in that proportion higher than control throughout the 35 days of storage (Figure 1).

As expected, treatment containing 5% w/w added lactose had the highest lactose content (Table 2). The highest lactose content was observed at days 1 and 7 of storage period (Table 3). Also, as expected lactose content steadily decreases from day 21 onwards over the rest of the storage time (Table 3). Reference [27] found a reduction in lactose concentration of yogurt after storage period of 7 days. They attributed the loss of lactose to the fact that lactic acid bacteria not only produce lactic acid from lactose but also flavor compounds and polysaccharides during storage consuming more of this molecule.

Table 1. Probability > F Value (Pr > F) for lactose content (LC), pH, titratable acidity (TA), apparent viscosity (AV), syneresis, *Streptococcus thermophilus* ST-M5 counts (ST) and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts (LB) in yogurts containing 0%, 1%, 3% and 5% w/w of added lactose over storage period of 35 days.

Effect	LC	pH	TA	AV	Syneresis	ST	LB
Treatment	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	0.0225	0.5903
Day	<0.0001	<0.0001	<0.0001	0.6735	<0.0001	<0.0001	<0.0001
Treatment*Day	0.0970	0.9988	<0.0001	0.8809	<0.0001	0.0859	0.1000

Table 2. Least square means for lactose content of yogurts as influenced by added lactose concentrations.

Added lactose concentration (%)	Lactose content
	LS means
Control (0)	4.37^{d}
One	5.01^{c}
Three	5.92^{b}
Five	6.77^{a}

$^{a-d}$LS means with the same letter does not indicate significant difference (P < 0.05).

Table 3. Least square means for lactose content of yogurts as influenced by the storage period of 35 days.

Storage period (days)	Lactose content
	LS means
1	6.11^{a}
7	6.16^{a}
14	5.85^{ab}
21	5.53^{b}
28	5.03^{c}
35	4.42^{d}

$^{a-d}$LS means with the same letter does not indicate significant difference (P < 0.05).

3.2. pH

The pH of yogurts as influenced by added lactose concentration over storage of 35 days is shown in **Figure 2**. Treatment*day interaction effect was not significant ($P > 0.05$) while treatment effect and day effect were significant ($P < 0.05$) (**Table 1**). The pH values decreased for all treatments at day 35 compared to day 1 (**Figure 2**). According to [28] a decrease in pH during storage is expected as a result of the metabolic activity of starter cultures. Yogurts containing 5% w/w added lactose had the lowest pH (**Table 4**). The more lactose present, the higher the production of lactic acid by the starter cultures. According to [29] at high pH most of the lactic acid is formed due to a growth-associated mechanism and the growth curve has a short stationary growth phase. On the contrary, at low pH most of the lactic acid produced is non-growth-associated [29]. The lowest pH values were obtained from day 14 onwards (**Table 5**).

3.3. Titratable Acidity

The TA of yogurts as influenced by added lactose concentration over storage of

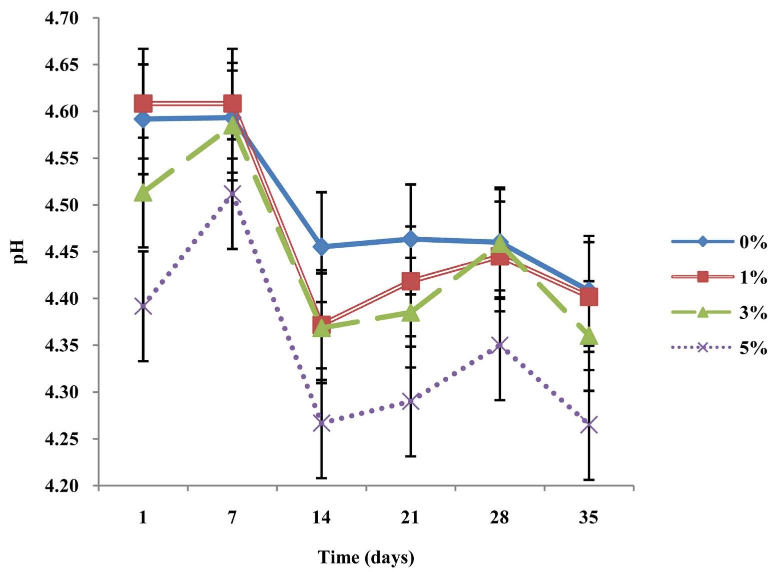

Figure 2. pH of yogurts as influenced by added lactose levels over storage period of 35 days, means + SE.

Table 4. Least square means for pH of yogurts as influenced by added lactose concentrations.

Added lactose concentration (%)	pH
	LS means
Control (0)	4.50[a]
One	4.48[a]
Three	4.45[a]
Five	4.35[b]

[a-b]LS means with the same letter does not indicate significant difference ($P < 0.05$).

Table 5. Least square means for pH of yogurts as influenced by the storage period of 35 days.

Storage period (days)	pH
	LS means
1	4.53[ab]
7	4.58[a]
14	4.37[c]
21	4.39[c]
28	4.43[bc]
35	4.36[c]

[a-c]LS means with the same letter does not indicate significant difference (P < 0.05).

35 days is shown in **Figure 3**. Treatment*day interaction effect, treatment effect and day effect were significant (P < 0.05) (**Table 1**).

In general, yogurts containing 1% and 3% w/w lactose had higher TA at day 35 compared to days 1 and 7 (**Table 6**, **Figure 3**). Yogurts with 3% and 5% w/w added lactose had lower TA values compared to 0% and 1% w/w added lactose, except at day 7 for 3% w/w added lactose (**Table 6**). This phenomenon can be explained through the fact that whey separation caused by lactose hydrolysis leads to slow rate of acid production in yogurt [30]. As lactose is hydrolyzed by lactic acid bacteria the amount of lactic acid production increased. This behavior may be due to the availability of more quantity of easily fermentable sugar (glucose) which is required for the faster growth of starters [31] [32] [33]. Reference [34] reported that changes in titratable acidity do not necessarily have an effect on pH values.

3.4. Apparent Viscosity

The apparent viscosity of yogurts as influenced by lactose addition over storage of 35 days is shown in **Figure 4**. Treatment*day interaction effect and day effect were not significant (P > 0.05) while treatment effect was significant (P < 0.05) (**Table 1**). Yogurts containing 5% w/w added lactose had the lowest apparent viscosity values compared to 0%, 1% and 3% w/w added lactose (**Table 7**). This was because of the higher amount of whey released. Weaker body and texture of yogurt may be due to higher amount of whey separation which reduced the viscosity when lactose is hydrolyzed [35] [36] [37].

3.5. Syneresis

The syneresis of yogurts as influenced by lactose addition over storage of 35 days is shown in **Figure 5**. Treatment*day interaction effect, treatment effect and day effect were significant (P < 0.05) (**Table 1**). Yogurts containing 5% w/w added lactose had significantly the highest syneresis values compared to 0%, 1% and 3% w/w added lactose during storage period at day 7 and from day 21 onwards (**Table 8**). Reference [30] reported that as the degree of lactose hydrolysis in-

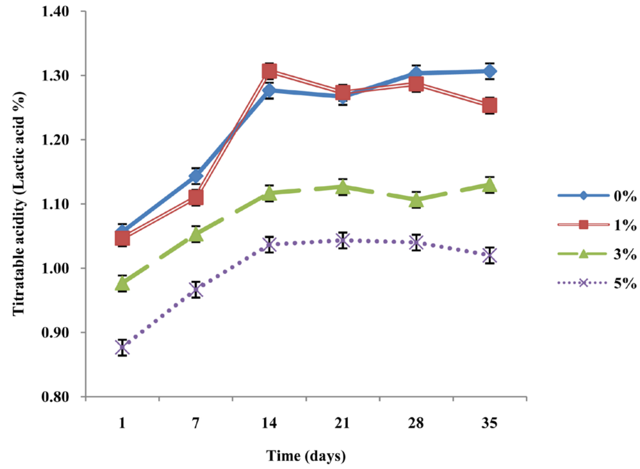

Figure 3. Titratable acidity of yogurts as influenced by added lactose levels over storage period of 35 days, means + SE.

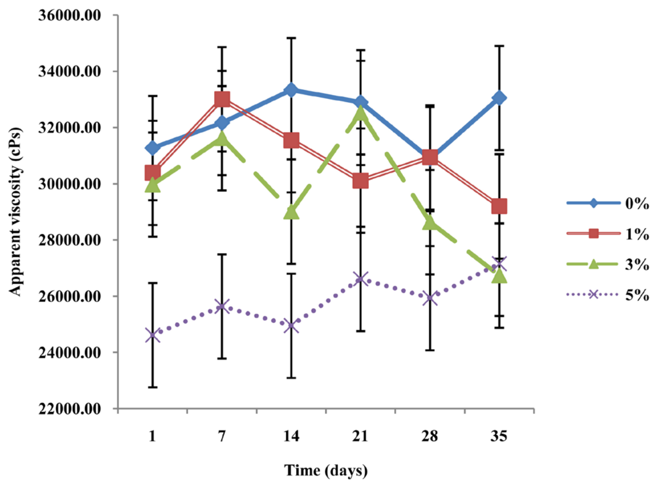

Figure 4. Apparent viscosity of yogurts as influenced by added lactose levels over storage period of 35 days, means + SE.

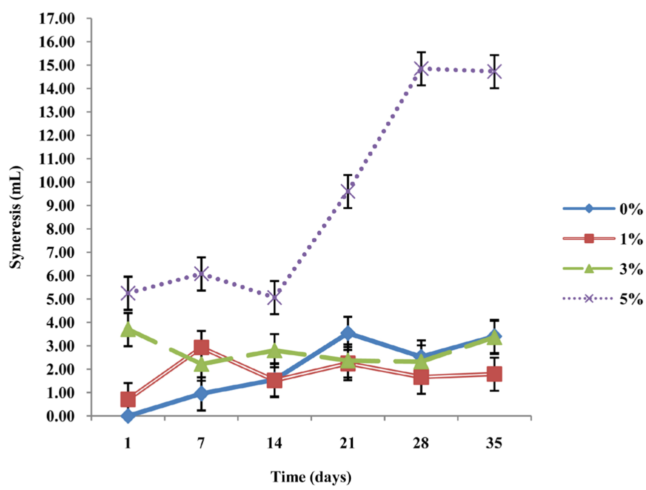

Figure 5. Syneresis of yogurts as influenced by added lactose levels over storage period of 35 days, means + SE.

Table 6. Least square means for titratable acidity of yogurts as influenced by added lactose concentrations over storage period of 35 days.

Added lactose concentration (%)	Titratable acidity					
	Time (days)					
	1	7	14	21	28	35
Control (0)	1.06^{CDEF}	1.14^{B}	1.28^{A}	1.27^{A}	1.30^{A}	1.31^{A}
One	1.05^{DEF}	1.11^{BCD}	1.31^{A}	1.27^{A}	1.29^{A}	1.25^{A}
Three	0.98^{GH}	1.05^{CDEF}	1.12^{BC}	1.13^{B}	1.11^{BCDE}	1.13^{B}
Five	0.88^{I}	0.97^{H}	1.04^{FG}	1.04^{DEFG}	1.04^{EFG}	1.02^{FGH}

^{ABCDEFGH}LS means with the same letter does not indicate significant difference ($P < 0.05$).

Table 7. Least square means for apparent viscosity of yogurts as influenced by added lactose concentrations.

Added lactose concentration (%)	Apparent viscosity
	LS means
Control (0)	$32{,}267.00^{a}$
One	$30{,}866.00^{a}$
Three	$29{,}751.00^{a}$
Five	$25{,}820.00^{b}$

$^{a-b}$LS means with the same letter does not indicate significant difference ($P < 0.05$).

Table 8. Least square means for syneresis of yogurts as influenced by added lactose concentrations over storage period of 35 days.

Added lactose concentration (%)	Syneresis					
	Time (days)					
	1	7	14	21	28	35
Control (0)	0.00^{E}	0.95^{E}	1.54^{DE}	3.54^{CDE}	2.53^{CDE}	3.40^{CDE}
One	0.70^{E}	2.93^{CDE}	1.52^{DE}	2.24^{CDE}	1.66^{DE}	1.79^{DE}
Three	3.70^{CDE}	2.22^{DE}	2.80^{CDE}	2.36^{CDE}	2.32^{CDE}	3.36^{CDE}
Five	5.25^{CD}	6.08^{BC}	5.06^{CD}	9.60^{B}	14.85^{A}	14.73^{A}

^{ABCDE}LS means with the same letter does not indicate significant difference ($P < 0.05$).

creased the amount of whey separation increased ($P < 0.05$).

3.6. Growth

3.6.1. *Streptococcus thermophilus* ST-M5

The growth of *Streptococcus thermophilus* ST-M5 as influenced by added lactose concentration over storage of 35 days is shown in **Figure 6**. Treatment*day interaction effect was not significant ($P > 0.05$) while the treatment effect and day effect were significant ($P < 0.05$) (**Table 1**). In general, upon addition of lactose there was an increase in viable counts at day 35 compared to day 1 (**Figure 6**). Reference [38] found that the counts of *S. thermophilus* and *L. bulgaricus* decreased after 12 h of storage due to a reduction of lactose content in

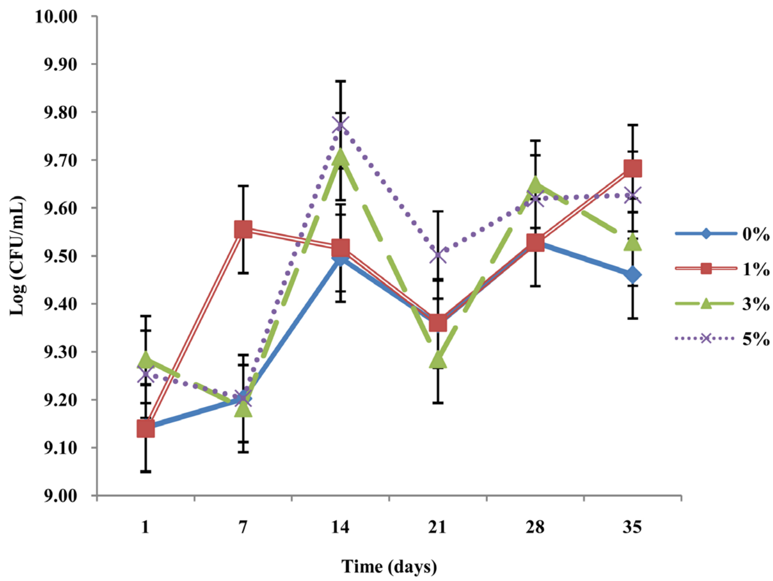

Figure 6. Growth of *Streptococcus thermophilus* ST-M5 as influenced by added lactose concentration over storage period of 35 days, means + SE.

yogurt. Use of 5% w/w added lactose resulted in significant higher counts compared to control (**Table 9**). Reference [39] studied the effect of 2% lactose on probiotic bacteria in soymilk. Counts of soymilk containing lactose were significantly higher than soymilk with the absence of lactose (8.13 log CFU/mL and 6.36 log CFU/mL respectively). They stated that probiotic bacteria are traditionally grown in lactose rich dairy foods such as yogurt; hence the growth is better in the presence of this carbohydrate. The highest counts of *Streptococcus thermophilus* ST-M5 were observed at days 14 and 28 of storage (**Table 10**). At day 21, there was a significant decrease in counts compared to days 14 and 28 (**Table 10**). However, the counts stayed within the same log CFU/mL (**Figure 6**). Studies have shown that *Streptococcus thermophilus* survive well in yogurt throughout the shelf life [40] [41] [42]. Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 was obtained by subtracting log CFU/mL of day 1 from day 35 of storage. A high number indicates higher bacterial growth. The bacteria survival was higher for lactose added samples compared to control. The bacterial survival was the highest for 1% w/w added lactose compared to the rest (**Table 11**).

3.6.2. *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12

The growth characteristics of *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration over storage of 35 days is shown in **Figure 7**. Treatment*day interaction effect and treatment effect were not significant ($P > 0.05$). The day effect was significant ($P < 0.05$) (**Table 1**). Viable counts of *Lactobacillus bulgaricus* LB-12 decreased for all treatments over storage period (**Figure 7**). This behavior agrees with the results reported by [29] who found that when fermentations were carried out with 60 g/liter of lactose in the medium, specific growth rates for *Lactobacillus delbrueckii* ssp. *bulgaricus* increased

Table 9. Least square means (Log CFU/mL) for growth of *Streptococcus thermophilus* ST-M5 as influenced by added lactose concentrations.

Added lactose concentration (%)	*Streptococcus thermophilus* ST-M5
	LS means
Control (0)	9.33[b]
One	9.46[ab]
Three	9.44[ab]
Five	9.49[a]

[a-b]LS means with the same letter does not indicate significant difference (P < 0.05).

Table 10. Least square means (Log CFU/mL) for growth of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by the storage period of 35 days.

Storage period (days)	*Streptococcus thermophilus* ST-M5	*Lactobacillus bulgaricus* LB-12
	LS means	
1	9.20[c]	8.38[a]
7	9.28[c]	8.19[bc]
14	9.62[a]	8.38[a]
21	9.38[bc]	8.18[c]
28	9.58[a]	7.21[d]
35	9.53[ab]	6.34[e]

[a-d]LS means with the same letter does not indicate significant difference (P < 0.05).

Table 11. Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration.

Added lactose concentration (%)	*Streptococcus thermophilus* ST-M5	*Lactobacillus bulgaricus* LB-12
	Log CFU/mL	
Control (0)	0.15	1.65
One	0.54	2.31
Three	0.25	2.11
Five	0.38	2.09

to a maximum and then decreased. Reference [43] reported that the growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* declined from 7 to 6 log CFU/g in yogurt during storage period of 28 days. These findings might also be explained by the competitive and growth advantage that *S. thermophilus* shows over *L. bulgaricus* [44] [45]. The highest counts of *Lactobacillus bulgaricus* LB-12 were observed at days 1 and 14 of storage (**Table 10**). Mean log difference in the viable counts of *Lactobacillus bulgaricus* LB-12 was obtained by subtracting log CFU/mL of day 35 from day 1 of storage. A low number indicates less bacterial death. The bacterial death was the lowest for control compared to the rest (**Table 11**).

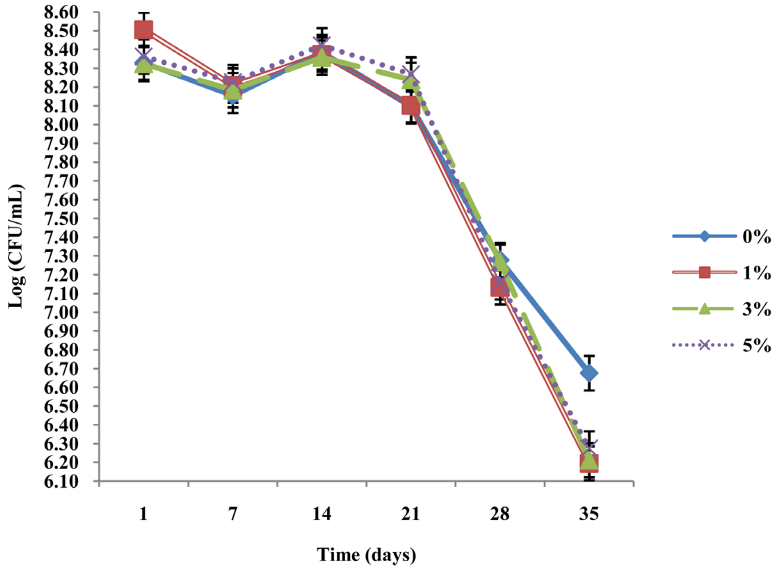

Figure 7. Growth of *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration over storage period of 35 days. means + SE.

3.7. Coliform Counts

There were no detectable coliforms in all samples.

3.8. Sensory Study

Means for all tested attributes (appearance, color, aroma, taste, sourness, sweetness, thickness, graininess, and overall liking) are shown in Figure 8. The probabilities for fixed effect of sensory attributes are shown in Table 12. Samples containing 1% w/w added lactose had higher scores for thickness compared to 5% w/w added lactose, which might be explained by increased syneresis in added lactose samples. Control samples had lower scores for graininess compared to 5% w/w added lactose (Table 13), possibly due to difference in total solids content.

The overall liking scores indicated that samples containing added lactose were preferred over control (Table 13). Lactose addition did not appear to significantly (P > 0.05) impact appearance and color of yogurts (Table 12). Similar results were reported by [30] who studied lactose hydrolysis on enzymatically hydrolyzed yogurts. Aroma and taste are important sensory characteristics of yogurt [46]. Control had higher aroma scores compared to 1% and 3% w/w added lactose (Table 13). For taste, sourness and sweetness samples containing added lactose had higher scores than control (Table 13). Yogurt acceptability frequency values are shown in Figure 9(a). Added lactose yogurts had a greater acceptability compared to control yogurts. The consumer acceptability of yogurts increased as lactose addition increased. Yogurts containing 5% w/w added lactose led to the highest acceptability (88%) compared to control (70%). This is probably due to a better palatability given by the sweetness of lactose. According to [30] when lactose is hydrolyzed in yogurt it resulted in increased digestibility, sweeter

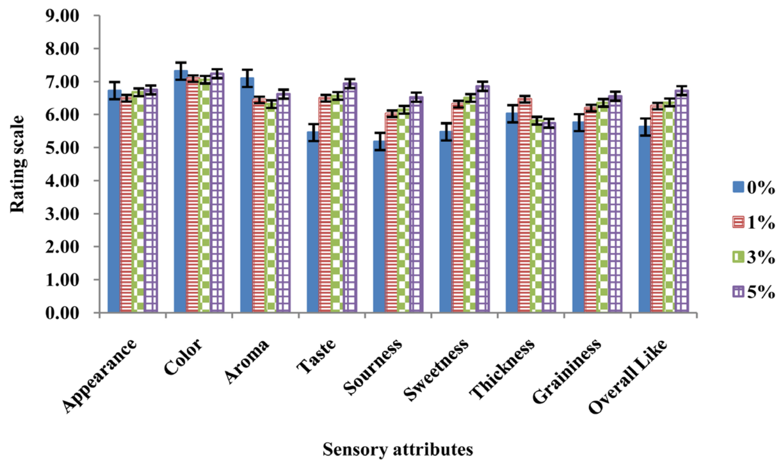

Figure 8. Means + SE for sensory attributes of blueberry yogurt as influenced by lactose addition.

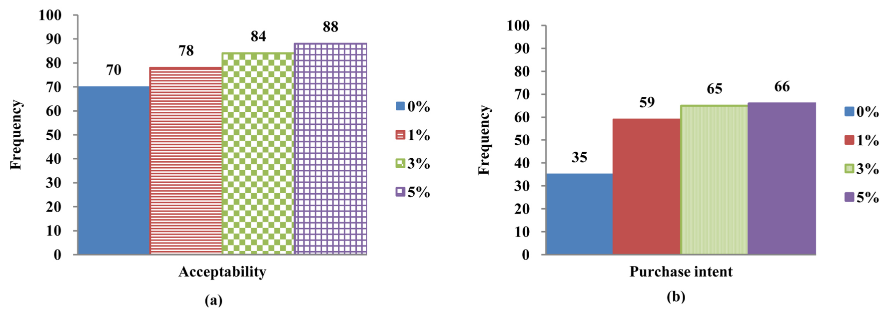

Figure 9. Frequency for (a) acceptability and (b) purchase intent of blueberry yogurt as influenced by lactose addition.

Table 12. Probability > F Value (Pr > F) for fixed effect of sensory attributes of yogurts containing 0%, 1%, 3% and 5% w/w added lactose.

Sensory attributes	Treatment effect
Appearance	0.6258
Color	0.3587
Aroma	0.0011
Taste	<0.0001
Sourness	<0.0001
Sweetness	<0.0001
Thickness	0.0217
Graininess	0.0095
Overall Like	0.0001

taste, and better mouth feel. Yogurt purchase intent frequency values are shown in Figure 9(b). Added lactose yogurts had greater purchase intent values compared to control yogurts. Purchase intent increased as lactose addition increased. Yogurts containing 5% w/w added lactose led to higher purchase intent (66%) compared to control (35%). Reference [47] reported that sweetness is one of the

Table 13. Means ± SD for sensory properties of yogurts as influenced by added lactose concentrations.

Sensory attributes	Added lactose concentration (%)			
	Control (0)	One	Three	Five
Appearance	$6.73^{a} \pm 1.48$	$6.50^{a} \pm 1.56$	$6.68^{a} \pm 1.48$	$6.75^{a} \pm 1.43$
Color	$7.32^{a} \pm 1.14$	$7.09^{a} \pm 1.26$	$7.06^{a} \pm 1.14$	$7.24^{a} \pm 1.22$
Aroma	$7.10^{a} \pm 1.46$	$6.45^{b} \pm 1.40$	$6.32^{b} \pm 1.61$	$6.62^{ab} \pm 1.35$
Taste	$5.46^{b} \pm 1.93$	$6.50^{a} \pm 1.81$	$6.57^{a} \pm 1.55$	$6.94^{a} \pm 1.43$
Sourness	$5.19^{b} \pm 1.93$	$6.03^{a} \pm 1.70$	$6.15^{a} \pm 1.58$	$6.53^{a} \pm 1.61$
Sweetness	$5.48^{b} \pm 1.79$	$6.32^{a} \pm 1.63$	$6.51^{a} \pm 1.49$	$6.86^{a} \pm 1.33$
Thickness	$6.03^{ab} \pm 1.84$	$6.47^{a} \pm 1.71$	$5.82^{ab} \pm 1.84$	$5.74^{b} \pm 1.85$
Graininess	$5.76^{b} \pm 1.81$	$6.20^{ab} \pm 1.68$	$6.36^{ab} \pm 1.79$	$6.56^{a} \pm 1.63$
Overall Like	$5.63^{b} \pm 1.85$	$6.26^{ab} \pm 1.85$	$6.37^{a} \pm 1.70$	$6.73^{a} \pm 1.53$

$^{a-b}$Means with the same letter does not indicate significant difference ($P < 0.05$).

main traits for flavored yogurts.

4. Conclusion

Results indicated that added lactose had a positive effect on yogurt characteristics. Growth of *Streptococcus thermophilus* ST-M5 was significantly increased by 5% w/w lactose addition in yogurt. Lactose addition did not have a significant effect on growth of *Lactobacillus bulgaricus* LB-12 in yogurt. Treatments containing 5% w/w added lactose showed the highest lactose content during storage period and the lowest pH. Treatment containing 5% added lactose showed the lowest viscosity values compared to the remaining yogurts; and the highest syneresis values over storage period of 35 days. The amount of added lactose had no effect on appearance and color of blueberry yogurt. Scores for aroma were higher for control and 5% w/w added lactose. Samples containing added lactose showed higher scores for taste, sourness and sweetness compared to control. Lactose addition contributed to higher scores for overall liking. The acceptability of yogurts and purchase intent frequency scores markedly increased with the addition of lactose.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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