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Physicochemical and Microbiological Properties of Stirred Bio-Yoghurt Manufactured from Sheep Milk

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ABSTRACT

Sheep milk is considered an important nutritional and health-promoting food source due to its high protein content and minerals. The current study aimed to ascertain the impact of probiotic bacteria (*Bifidobacterium longum*) on the physicochemical properties, bacterial evaluation and sensory assessment of stirred bio-yoghurt made from sheep milk. Stirred bio-yoghurt was prepared with various inoculation levels of *Bifi. longum* (3, 6, 9 and 12% for T1, T2, T3 and T4; respectively). According to the study's findings, the moisture content of all treatments steadily declined with increasing of storage periods at 4±1°C up to 15 days. The control samples had higher moisture than that of stirred bio-yoghurt made with various amounts of *Bifi. longum*. While the fresh samples had lower moisture that that of other treatments. The fat content of stirred bio-yoghurt as well as control samples found to decrease and significantly differences with increasing the storage periods at refrigerator temperature up to 15 days. In addition, the incorporation of *Bifi. longum* significantly raised protein and ash contents of stirred bio-yoghurt than that of control samples, and their contents were increase and significantly differences with increasing the storage periods at refrigerator temperature up to 15 days. There were astatically significant change in the syneresis of all moduli (treatment and control sample). Stirred bio-yoghurt treatments and the control samples had no coliform as well as mould and yeast levels in all treatments.

Keywords: Functional dairy food; Sheep milk; Stirred bio-yoghurt; *Bifidobacterium longum*.

INTRODUCTION

Recent research has demonstrated that, one of the probiotics' most significant functions is immunomodulatory activity. These investigations demonstrated that, certain probiotics' immunomodulatory effects may confer potential antiviral action (Hansen, 2005). Probiotics are live organisms that, when taken in sufficient amounts through food or medication, have positive effects on health (Novik & Savich, 2020). There are numerous local effects of the gut microbiota that help to maintain human health. These include the metabolism of indigestible foods, immune system stimulation, provision of colonisation resistance against gastrointestinal tract infections and maintenance of the intestinal barrier (Groves *et al*., 2020). In order to understand how the gut microbiota influences immune responses in the airways, most research on respiratory disease has concentrated on this relationship (Hauptmann & Schaible, 2016). The gut metabolome and host immune function changed in response to modifications in the composition of the gut microbiota (Corrêa-Oliveira *et al*., 2016). The antibacterial and antiviral qualities of bacterial species of lactic acid are well-established and have a crucial role in the treatment of gastrointestinal disorders (Di Cerbo *et al*., 2016; Sunmola *et al*., 2019). Numerous lactic acid bacterial species secrete different metabolites that boost the body's defences against viruses, including lactic acid, acetic acid, gamma-aminobutyric acid, and Planta ricin (Anwar *et al*., 2021). The human gastrointestinal system is thought to be home to about 400 different kinds of bacteria, and the colon's *predominant anaerobic flora*, *Bifidobacterium spp*. *B. adolescentis*, *B. bifidum*, *Bifi. longum subsp*. *infantis*, *B. breve* and *Bifi. longum* are the primary varieties found in the human colon (Singh *et al*., 2017). *Bifidobacteria* and other probiotic bacteria can be added to cultured dairy products to improve their nutrient content, according to a growing body of investigation as the functional food industry expands (Pulina *et al*., 2018). Yoghurt can be additionally supplemented with probiotic cultures of *Lactobacillus acidophilus* or *Bifidobacterium* to enhance its nutritional value. When *L. acidophilus* and *Str. thermophilus* were used to make yoghurt, the quality was noticeably higher than when *Lact. bulgaricus* and *Str. thermophiles* were used (Balthazar, Silva, et al., 2017). Numerous factors have been identified as having an impact on the probiotic culture in yoghurt's viability and customers' acceptance of it (Temerbayeva *et al*., 2018). Notably, not all probiotic strains can survive in challenging processing environments and during their path through the gastrointestinal system (Aryana & Olson, 2017). Sheep milk has a greater nutritional content and more nutrient than goat and cow milk due to its greater content of vital proteins, lipids, minerals and vitamins for human health as well as its higher caloric content (Balthazar, Pimentel, et al., 2017; Moatsou & Sakkas, 2019). Chemical composition of fresh sheep milk's fluctuates over time and between animals based on a few factors including; the animal's age, nutrition, the stage of lactation, parity, season, external temperature and udder

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infections. Because of the variations in the forage content of animals raised on the range, changing seasons have a significant impact on the fatty acid composition (Pecka-Kiełb *et al*., 2020). Therefore, the aim of the current study was to ascertain the impact of probiotic bacteria *Bifi. longum* treatment on the physicochemical characteristics, bacterial evaluation and sensory assessment of stirred bio-yoghurt made from sheep milk.

MATERIALS AND METHODS

Materials:

Fresh sheep milk (6.5% fat) was obtained from privet farms, Quena Governorate. Starter strains: *Bifi. longum* (ATCC 15707) was obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University. DVS thermophilic yoghurt strains 1:1 *Lact. bulgaricus* and *Str. thermophilus* (commercial Yoflex culture) were obtained from Copenhagen, Denmark's Christian Hansen.

Methods;

Manufacture of stirred bio-yogurt:

A process described by (Tamime & Robinson, 1999) was used to make probiotic-fermented sheep's milk, which was modified by (Hashim et al., 2009), as follows: Fresh sheep milk was heated to 45°C, 1.5% skim milk powder (SMP) and 0.4% gelatine were added with well stirring, continued heating to 85-90°C for 15 min, and then cooled to 42°C. Milk was inoculated with DVS thermophilic yoghurt starter, and the milk was divided into five portions, followed by the addition of *Bifi. longum* as follows:

Control (C) with no *Bifi. longum*.
T1 inoculated with 3% w/w of *Bifi. longum*.
T2 inoculated with 6% w/w of *Bifi. longum*.
T3 inoculated with 9%w/w of *Bifi. longum*.
T4 inoculated with 12% w/w *Bifi. longum*.

Milk was incubated at 42ºC until full coagulation; the probiotic yoghurt curd was refrigerated at 4°C overnight and stirred using the mixer; then bottled and stored at 4±1°C for 15 days. Probiotic yoghurt samples that were stored for fresh, 5, 10 and 15 days were analysed for physicochemical, rheological, microbiological, and sensory properties. The experiment was repeated three times.

Chemical composition:

pH values:

The pH values of stirred bio-yoghurt samples were measured according to the method of (Igbabul et al., 2014). In brief, 100 ml of distilled water was mixed with 10 g of stirred bio-yoghurt at room temperature; the mix was permitted to equilibrate. A pH meter (HANNA, Italy) was then used to figure out the values of pH.

Titratable acidity determination:

The procedure outlined by (Oladipo et al., 2014) was used to determine titratable acidity. 10 g of the sample were quickly mixed with 30 ml of distilled water. In the combined solution, a few drops of phenolphthalein indicator were administered. To ensure complete neutralisation, it was titrated against a standard 0.1N sodium hydroxide solution until a light pink colour persisted for between 10 and 15 seconds. The below equation was used to calculate the titratable acidity of lactic acid:

$$\text{Titratable acidity} = \frac{\text{Vol.NaoH used (ml)} \times 0.1\text{N NaoH} \times \text{millieqivalent factor} \times 100}{\text{mass of sample(g)} \times 1000}$$

Moisture determination:

5 gram was weighted in a clean and dry crucible. The specimen was dried in an oven set at 105°C until the dry substance's weight remained constant (Htay *et al*., 2020). The moisture content was calculated using the following equation:

$$\text{Moisture (\%)} = \frac{\text{Weight before drying} - \text{Oven dried weight}}{\text{Wt.of sample}} \times 100$$

Ash determination:

The previous crucible, which was used to determine the moisture content of the sample (5 g), was heated in the furnace at 550°C until the residue evenly turned white. Then cooled in the desiccator, it was weighed. Heating, chilling, and weighing were repeated until a steady weight was reached (Htay *et al*., 2020). The following equation was used to calculate the ash percentages:

$$\text{Ash (\%)} = \frac{\text{wt of residue}}{\text{wt of sample(g)}} \times 100$$

Protein determination:

Protein was determined by Kjeldhal (Htay *et al*., 2020).

$$\text{Protein (\%)} = \frac{((x-v)NA \times 0.014) \times 100}{w} \times 6.36$$

Where: 0.014 = Milliequivalent weight of the nitrogen,
w = Weight (g) of the sample,
v = volume (ml) of NaOH solution used in test,
x = volume (ml) of NaOH solution used in blank
NA = Molarity of NaOH solution

Syneresis determination:

By pouring 100 ml of yoghurt specimen onto filter paper (Whatman No. 1), it was possible to ascertain the yoghurt sample's susceptibility to syneresis (STS). The amount of whey that was gathered in the measuring cylinder after 6 hours of emptying was stated by (Ibrahim & Khalifa, 2015) calculating using the subsequent equation:

$$\text{STS (\%)} = [V1/\ V2] \times 100.$$

Where: V1: Whey volume collected, V2: Yogurt volume.

Fat Determination:

Fat was determined following the Gerber method (IDF, 1997).

Microbiological analysis:

Yoghurt samples were tested for microbiological analysis during fresh, 5, 10, and 15 days of storage at 4°C. MRS agar used to *Bacilli*, M17 used for *Cocci*, nutrient agar used for total count bacteria, MRS agar with 0.05 g l^{-1} cysteine hydrochloride monohydrate and lactose as supplemental materials used for *Bifidobacteria*, under anaerobic conditions .VERBA medium used for *Coliform* count for 24 h at 30ºC, YM medium used for Yeast and Mould at 25ºC for 48 h (Dave & Shah, 1996; Hansen, 2005; Moubasher et al., 2018; Richardson, 1985; Tharmaraj & Shah, 2003). All microbiological analysis were carried out three times.

Sensory evaluation:

With certain adjustments, the sensory assessment of stirred bio-yoghurt was also determined according to Hamdy *et al.* (Hamdy et al., 2021). Samples were given a total of 100 points after being scored based on their colour & appearance (15 points), flavour (45 points), acidity (10 points) and body & texture (30 points) at fresh and after 5, 10 and 15 days of storage periods at 4±1°C.

Statistical analysis:

SAS software (version 913) was used to conduct the statistical analyses. Analysis of variance (ANOVA) was used for data analysis. At level ≤ 0.05, Duncan's multiple comparison test was used for evaluating mean differences.

RESULTS AND DISCUSSION

Chemical analysis:

The data presented in Table (1) shows the chemical composition of stirred bio-yoghurt made with various amounts of *Bifi. longum* at fresh and during storage periods at 4±1°C for 15 days.

Moisture content:

The obtained data observed that, the moisture content of all treatments steadily declined with increasing of storage periods at 4±1°C up to 15 days. This may be due to the evaporation throughout storage (Mao et al., 2019). Moreover, the control samples had higher moisture than that of stirred bio-yoghurt made with various amounts of *Bifi. longum*. In addition, the fresh samples had lower moisture that that of other treatments. These results in agreement of those reported by Hashmi *et al*. (2011) and El-Shobery *et al*. (2012) and Salman *et al*. (2012), who stated that the control samples had lower total solids than that of stirred probiotic yoghurt.

Fat content:

The obtained data observed that, the fat content of all treatments steadily declined with increasing of storage periods at 4±1°C up to 15 days. The fat content of stirred bio-yoghurt as well as control samples found to decrease and significantly differences with increasing the storage periods at refrigerator temperature up to 15 days. This may be due to hydrolysis of fat with increasing the storage. Similar results were obtained by El-Shobery *et al*. (2012), Salman *et al*. (2012) and Amna *et al*. (2023). The largest proportions of fat content were found in treatments T1 and the lower were observed in T3. This may be due to the higher *Bifi. longum* in T3 than that of T1.

Protein content:

The incorporation of *Bifi. longum* significantly raised the protein content of stirred bio-yoghurt than that of control samples. In addition, the protein content of fresh samples was lower than that of other treatments. According to the current study, the protein contents in the fresh stirred bio-yoghurt treatments were 6.14, 6.18, 6.57 and 6.36% for T1, T2, T3 and T4; respectively.

The protein content of stirred bio-yoghurt as well as control samples found to increase and significantly differences with increasing the storage periods at refrigerator temperature up to 15 days. The reason for this increase was due to the ongoing decrease in the stirred yoghurt's moisture content over the storage period. In this investigation, there was a statistically considerable variation between the control and treatment groups ($p < 0.05$). These results are in agreement with those reported by El-Shobery *et al*. (2012). In addition, there were increase of protein content in stirred bio-yoghurt with the increasing of *Bifi. longum* up to 9%, and then decrease at 12%.

Table 1. Chemical composition of stirred bio-yoghurt **fortified with varying concentrations of *Bifi. longum* (mean ± SD) at fresh and after storage periods (5, 10 and 15 days).**

Parameter	Treatment	Storage period (day)			
		Fresh	5	10	15
Moisture %	Control	83.92±0.02^{a}	83.20±0.02^{a}	83.15±0.02^{a}	82.56±0.015^{b}
	T1	82.83±0.03^{a}	82.27±0.03^{a}	81.35±0.03^{b}	80.65±0.01^{c}
	T2	83.55±0.015^{a}	81.26±0.03^{b}	81.17±0.026^{b}	80.65±0.026^{c}
	T3	83.32±0.015^{a}	82.20±0.025^{b}	80.82±0.025^{c}	79.92±0.025^{d}
	T4	82.55±0.02^{a}	81.73±0.035^{b}	80.95±0.015^{c}	80.03±0.055^{c}
Fat %	Control	6.59±0.005^{a}	6.58±0.11^{a}	6.57±0.06^{a}	6.51±0.069^{a}
	T1	6.58±0.064^{a}	6.56±0.115^{a}	6.54±0.005^{a}	6.49±0.115^{a}
	T2	6.56±0.057^{a}	6.54±0.02^{a}	6.53±0.017^{a}	6.48±0.055^{a}
	T3	6.55±0.01^{a}	6.54±0.051^{a}	6.52±0.005^{a}	6.47±0.06^{a}
	T4	6.54±0.055^{a}	6.53±0.055^{b}	6.50±0.032^{b}	6.45±0.078^{c}
Protein %	Control	6.09±0.02^{a}	6.38±0.02^{a}	6.48±0.015^{a}	6.77±0.026^{a}
	T1	6.14±0.015^{a}	6.48±0.025^{b}	6.57±0.025^{b}	6.96±0.03^{c}
	T2	6.18±0.025^{a}	6.56±0.015^{a}	6.78±0.02^{b}	7.08±0.02^{c}
	T3	6.57±0.015^{a}	6.83±0.015^{a}	6.87±0.005^{a}	6.90±0.005^{a}
	T4	6.36±0.015^{a}	6.45±0.06^{a}	6.69±0.015^{a}	6.78±0.02^{a}
Ash %	Control	0.82±0.023^{a}	0.87±0.015^{b}	0.88±0.005^{c}	0.90±0.011^{d}
	T1	0.84±0.02^{a}	0.87±0.005^{b}	0.89±0.01^{c}	0.90±0.01^{d}
	T2	0.84±0.01^{a}	0.88±0.01^{b}	0.91±0.01^{c}	0.93±0.015^{d}
	T3	0.85±0.01^{a}	0.88±0.005^{b}	0.92±0.011^{c}	0.94±0.01^{d}
	T4	0.89±0.015^{a}	0.90±0.01^{b}	0.93±0.01^{b}	0.95±0.01^{c}

a-e Means with same superscripts across a row are not significantly different control=without *Bifi. longum*, T1=3% of *Bifi. longum*, T2=6% of *Bifi. longum*, T3=9% of *Bifi. longum* and T4=12% of *Bifi. longum*, Data was expressed as (mean ± standard deviation).

Ash:

Similarly, to the protein level, all stirred bio-yoghurt treatments showed an elevation in ash level up to 15 days of storage period was complete, with statistically considerable variations ($p < 0.05$).

The protein content of stirred bio-yoghurt as well as control samples found to increase and significantly differences with increasing the storage periods at refrigerator temperature up to 15 days. The reason for this increase was due to the ongoing decrease in the stirred bio-yoghurt's moisture content over the storage period. When the amounts of *Bifi. longum* rose correspondingly, the samples' amount of ash slightly rose. Comparing the stirred bio-yoghurt treatment samples to the control, the incorporation of *Bifi. longum* extracts has increased the amount of ash. In addition, there were increase of ash content in stirred bio-yoghurt with the increasing of *Bifi. longum* up to 9%, and then decrease at 12%.

pH:

After 15 days of preservation, the pH levels were low for both stirred bio-yoghurt and the control samples. In addition, the pH levels for the stirred bio-yoghurt were lower than those for the control samples (Fig 1). Additionally (Joung et al., 2016) discovered that all yoghurt treatments had a lower

pH level during the 14 days of preservation, and that this pH decline may have contributed to the change in lactose to lactic acid during preservation (Singh *et al*., 2011). For instance, the pH and lactic acid content of yoghurt produced with the probiotic bacteria *L. acidophilus*, *Bifidobacterium bifidum BB12*, and *L. paracasei subsp. casei* with *Lact. delbrueckii subsp*. *bulgaricus* are significantly affected (Bonczar et al., 2002; GÜLER-AKIN, 2005). Another study conducted by Vianna *et al*. (2017), there was no variation (P > 0.05) between the pH levels for probiotic yoghurts from 7th day of storage up to 28th day. Moreover, (Badahdah et al., 2019) stated that when the *Bifi. longum* BB536 strain was used in the fermentation process, the pH values for all types of beverages significantly (P<0.05) decreased when the fermentation period increased to 24 h and the initial pH of samples was 6.55, 5.52, 5.57 & 5.72 and decreased to 4.10, 3.57, 3.55 & 3.60 in fermented skim milk, Bukur, Balady and Local 46; respectively.

When *Bifi. longum* BB536 ferments sugar, it produces more acids during the fermentation process, including acetic and lactic acids, which is why the pH falls. According to a study by Hosseini *et al*. (2012), the pH range between 6.5 and 7.0 was ideal for *Bifidobacterium* growth. Whilst, Hosseini *et al*. (2012) revealed that the pH of malted-roasted beverages was higher (pH 4.01) than the pH of malt beverages made from barley and oats.

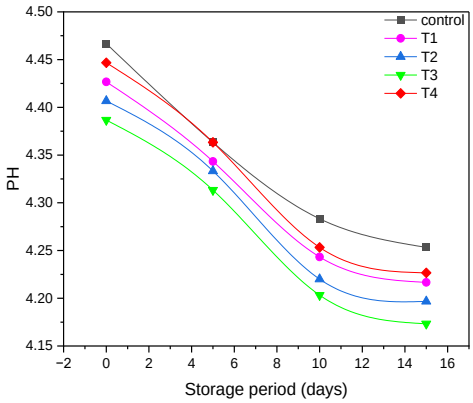

Fig. 1. pH values of stirred bio-yoghurt inoculated with varying levels of *Bifi. longum* (mean ± SD) throughout storage time (Fresh, 5, 10 and 15 days).

a-e. Means with same superscripts across a row are not significantly different.

The pH of the yoghurt in relation to the *Bifi. longum* fermentation period and inoculation concentration was shown by (Son et al., 2023), who found that the overall acidity rose as the amount of *Bifi. longum* in the mixture grew and the length of the fermentation process. Before fermentation, the total acidity was 0.10%, and eight h later, the control was 0.59%. Total acidity rose from 0.65 to 0.76% as *Bifi. longum* inoculation concentrations increased from 0.001 to 0.0015%.

The yogurt's pH dropped as fermenting time rose, where before fermentation, the pH was 6.67, the control group's pH was 4.69 eight hours later, and the pH values for *Bifi. longum* inoculation concentrations of 0.001, 0.00125, and 0.0015% were 4.54, 4.47 and 4.42; respectively.

According to the concentration of *Bifi. longum* inoculation and the length of fermentation, the pH of the yoghurt decreased, and the overall acidity increased, in line with the findings of Wolf *et al*. (2015). The trend for the lactic acid content and total acidity was the same. The lactic acid content rose as *Bifi. longum* concentrations increased, much like the overall acidity did. Lactic acid concentration in yoghurt produced by inoculating *Bifi. longum* is known to rise with longer fermentation times (Hadadji & Bensoltane, 2006), and this study showed the same outcome. This modification showed that, like the change in sugar, an increase in *Bifi. longum* concentration could successfully cause fermentation.

The titratable acidity levels in the treatment groups gradually increased as their levels increased in comparison to the control samples. No statistically significant changes between the *Bifi. longum* treatment groups and the control samples (p > 0.05) were found. It might be because *Bifi. longum* contains antimicrobial compounds that improve the likelihood of fermentation. Additionally, *Bifi. longum* contains bioactive components and is used effectively (Schöpping *et al*., 2021). Similar to the present investigation, Ali *et al*. (2021) found no statistically significant changes (p > 0.05) between the treatment groups and the control group for the titratable acidity level of *Bifi. longum*. The titration acidity investigation revealed that, the control samples had lower acidity than any of the treatment groupings of samples (Fig 2). This could be the source of the complete or partial inactivation of various microorganisms in the treatment groups that contained varying quantities of bacteria extracts, notably for Gram-positive bacteria, as a result of the bacteria absorption extracts that influence the cell surface (Mazloomi *et al*., 2011).

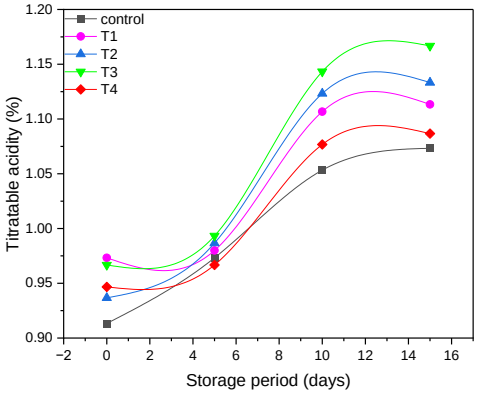

Fig. 2. Titratable acidity % of stirred bio-yoghurt inoculated with varying levels of *Bifi. longum* (mean ± SD) throughout storage time (Fresh, 5, 10, and 15 days).

a-e Means with same superscripts across a row are not significantly different.

Syneresis:

The data presented in Fig 3 shows the syneresis of stirred bio-yoghurt made with various amounts of *Bifi. longum* at fresh and during storage periods at 4±1°C for 15 days. It's obvious in the current study that, there is astatically significant change in the syneresis of all moduli (treatment and control sample), it's found a highest level of spontaneous syneresis (p<0.005) this conforms with (Vianna et al., 2017).

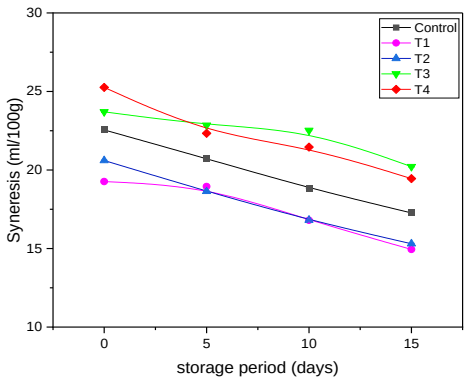

Fig. 3. Syneresis (ml/100g samples) of stirred bio-yoghurt inoculated with varying levels of *Bifi. longum* (mean ± SD) throughout storage time (Fresh, 5, 10, and 15 days).

a-e Means with same superscripts across a row are not significantly different.

Microbiological analysis:

The data presented in Fig 4 shows the microbiological analysis (total counts of bacteria, coliform bacteria, *Bifi. longum*, *Lactobacilli* bacteria, *Streptococci* bacteria and yeasts & moulds) of stirred bio-yoghurt made with various amounts of *Bifi. longum* at fresh and during storage periods at 4±1°C for 15 days.

For the control samples, in the current investigation observed that the total bacterial count increased from 6.37±0.02 log cfu/g in fresh samples to 8.10±0.09 log cfu/g after 15 days, whereas all of the control samples displayed a greater total bacterial count than that of treated samples (Fig 4 A). Moreover, (Ali et al., 2021) stated that for control samples, the total bacterial count increased from 6.18 CFU/g yoghurt in fresh samples to 8.16 CFU/g yoghurt after 14 days.

Regarding the *Bifi. longum* counts, the dada in Fig 4 B showed that the inoculation levels had no significant effect on among treatments. The counts of the starter culture bacteria (*Lactobacilli* and *Streptococci*) were negatively impacted by the inoculation size of the bacterial strain when stored (Figs C & D). In addition, the probiotic strain and starter culture did not exhibit identical stability.

Regarding the *Streptococci* counts, the dada in Fig 4 D showed that there were decreased in all treatments and control samples during storage times. Where the fresh samples in all treatments including control samples had the highest counts of *streptococci*, and the samples at the end of storage had the lowest counts of *streptococci*.

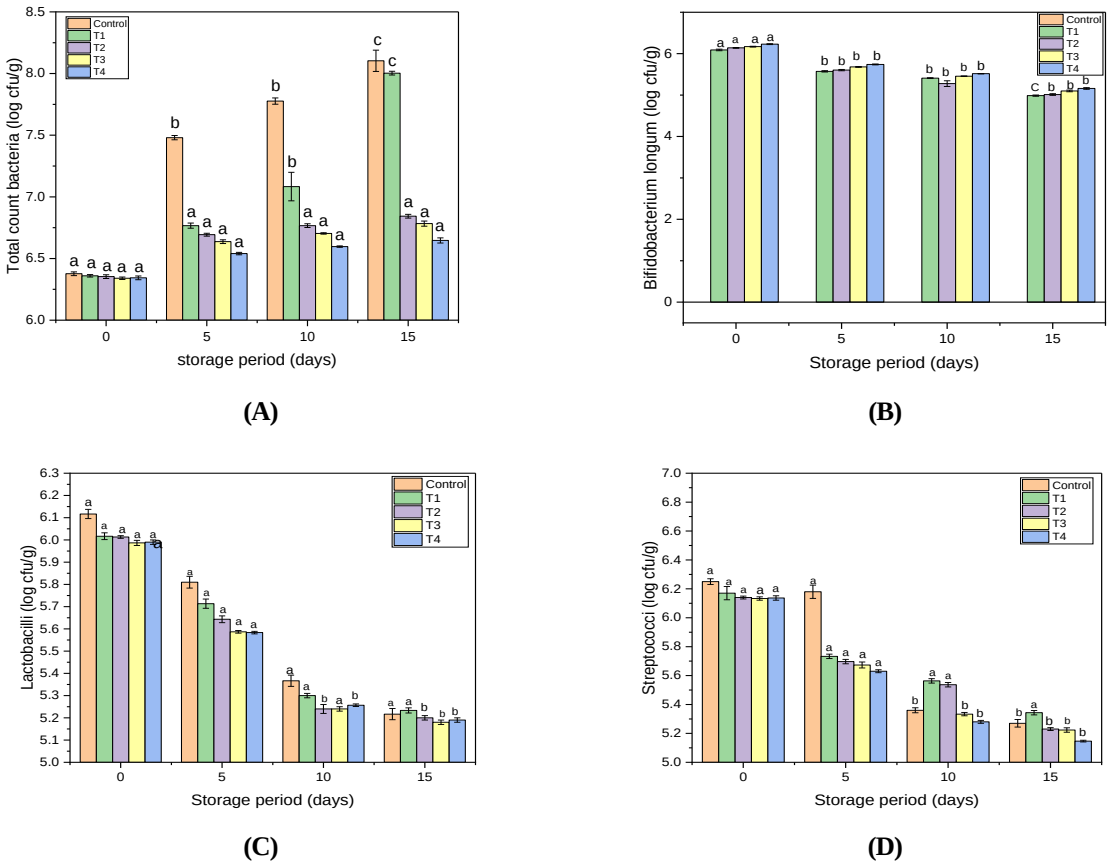

Fig. 4. Microbiological analysis of stirred bio-yoghurt fortified with varying concentrations of *Bifi. longum* (mean±SD) throughout preservation (0, 5, 10, and 15 days).

(A) Total bacterial count distribution among different treatments. **(B) *Bifi. longum* distribution among different treatments.**
(C) *Lactobacilli* distribution among different treatments. **(D) *Streptococci* content among different treatments.**
a-e Means with same superscripts across a row are not significantly different.

The current results revealed that, stirred bio-yoghurt treatments and the control samples had no coliform as well as yeast and mould count in all treatments. However, neither when fresh nor during periods of storage up to 15 days in any

treatment, coliform bacteria could be found, which may be related to how severely milk was heated and the protective effects of lactic acid bacteria (Karam-Allah *et al*., 2022). Moreover, Ali *et al*., (2021), shown that the inoculated strain had a favourable impact on the total amounts of yeasts and moulds because none of the tested samples underwent fungal development throughout storage periods of fresh and 7 days.

Sensory evaluation:

The presented data in Table 2 shows the sensory evaluation of stirred bio-yoghurt made with various amounts of *Bifi. longum* at fresh and during storage periods at 4±1°C for 15 days. The data showed that, the addition of the *Bifi. longum* strain had an impact on the flavour, body & texture, appearance, acidity and overall acceptance of the stirred bio-yoghurt samples. Furthermore, all the stirred bio-yoghurt that had been enriched with various inoculums size of the *Bifi. longum* strain received favourable ratings for flavour, body, texture and appearance.

The inoculation levels as well as the storage time had a significant effect on flavour scores, where treatment 2 at 5 days of storage and treatment 3 at 10 days of storage gained the highest score of flavour, while treatment 2 at 10 days gained the lowest score of flavour. Treatment 1 at the end of storage and treatment 4 at five days of storage gained the highest score of body and texture. Regarding the overall score, treatment 1, 2 and 3 stored at 15, 15 and 4 days gained the highest score, while treatment 1 and control samples stored at 10 and 5 days had the lowest score; respectively.

Table 2. Sensory evaluation of stirred bio-yoghurt **inoculated with varying levels of *Bifi. longum*.**

Characteristics	Treatment	Fresh	5 Days	10 Days	15 Days
Flavour (45)	Control	42.20±3.01^{a}	39.60±1.26^{b}	40.20±1.61^{a}	41.30±1.70^{a}
	T1	41.70±2.05^{a}	40.80±3.55^{a}	40.00±3.43^{a}	42.00±1.76^{b}
	T2	42.50±3.17^{a}	42.90±1.66^{a}	39.20±3.29^{b}	42.20±3.04^{a}
	T3	42.30±2.45^{a}	42.00±1.82^{a}	42.90±1.66^{a}	41.00±3.65^{a}
	T4	40.80±3.55^{a}	42.00±1.76^{b}	41.80±2.30^{b}	42.00±1.76^{b}
Body and texture (30)	Control	28.10±1.85^{a}	27.30±2.11^{a}	28.30±1.41^{a}	26.40±1.43^{b}
	T1	27.30±1.82^{a}	28.80±0.63^{a}	26.30±1.76^{b}	29.50±1.43^{a}
	T2	27.10±1.52^{a}	28.70±1.16^{b}	28.30±1.41^{b}	29.30±1.76^{b}
	T3	27.80±1.47^{a}	28.40±1.50^{b}	28.70±1.16^{b}	28.40±1.50^{b}
	T4	28.50±0.70^{a}	29.50±1.43^{b}	28.40±0.69^{a}	27.50±1.84^{c}
Appearance (15)	Control	13.40±0.52^{a}	13.80±0.42^{a}	13.80±0.92^{a}	14.10±0.88^{a}
	T1	14.00±0.81^{a}	14.40±1.26^{a}	14.00±0.47^{a}	14.30±0.67^{a}
	T2	13.80±0.63^{a}	14.80±0.42^{b}	14.00±0.66^{b}	13.60±0.69^{a}
	T3	13.70±0.82^{a}	14.80±0.42^{b}	14.80±0.42^{b}	14.80±0.42^{b}
	T4	14.80±0.42^{a}	14.30±0.67^{a}	14.30±1.05^{a}	14.70±0.48^{a}
Acidity (10)	Control	2.80±1.93^{a}	4.20±1.93^{b}	3.00±1.70^{c}	4.70±1.49^{b}
	T1	2.90±1.59^{a}	3.30±1.05^{b}	3.90±2.33^{b}	4.20±0.78^{b}
	T2	2.60±1.07^{a}	3.50±0.70^{b}	3.00±1.70^{b}	4.90±0.56^{c}
	T3	3.10±2.02^{a}	2.70±1.05^{b}	3.50±0.70^{a}	2.70±1.05^{b}
	T4	3.30±1.05^{a}	4.20±0.78^{b}	3.30±1.05^{a}	4.20±1.93^{b}
Overall acceptance (100)	Control	86.5	84.9	85.3	86.5
	T1	85.9	87.3	84.2	90.0
	T2	85.9	89.9	88.4	90.0
	T3	86.9	87.9	89.9	86.9
	T4	87.4	90.0	87.8	92.6

a-c Means with same superscripts across a row are not significantly different. Data was expressed as mean ± standard deviation.

CONCLUSIONS AND RECOMMENDATIONS

This research indicated that, fortifying stirred yoghurt with the *Bifi. longum* strains resulted in marginally elevated protein and ash levels. Additionally, the addition of the *Bifi. longum* strain had an impact on the flavour, body & texture, appearance, acidity and overall acceptance of the stirred bio-yoghurt samples. The inoculation levels as well as the storage time had a significant effect on flavour scores, where treatment 2 at 5 days of storage and treatment 3 at 10 days of storage gained the highest score of flavour, while treatment 2 at 10 days gained the lowest score of flavour. The overall score, treatment 1, 2 and 3 stored at 15, 15 and 4 days gained the highest score, while treatment 1 and control samples stored at 10 and 5 days had the lowest score: respectively. Combining *Bifi. longum* strain in probiotic-fermented milk led to enhanced nutritional content and functional qualities. As a result, this research looked at the characteristics of the *Bifi. longum* strain and recommended using it to make enriched fermented milk using a suitable, economical method.

REFERENCES

Ali, H. I., Dey, M., Alzubaidi, A. K., Alneamah, S. J. A., Altemimi, A. B., & Pratap-Singh, A. (2021). Effect of rosemary (Rosmarinus officinalis L.) supplementation on probiotic yoghurt: Physicochemical properties, microbial content, and sensory attributes. *Foods*, *10*(10), 2393.

Anwar, F., Altayb, H. N., Al-Abbasi, F. A., Al-Malki, A. L., Kamal, M. A., & Kumar, V. (2021). Antiviral effects of probiotic metabolites on COVID-19. *Journal of Biomolecular Structure and Dynamics*, *39*(11), 4175-4184.

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*(12), 9987-10013.

Badahdah, S. A., Kabeir, B. M., & Mustafa, S. E. (2019). Evaluation of physicochemical properties and growth of bifidobacterium longum BB536 during fermentation of barley beverages. *International Journal of Food and Nutritional Sciences*, *8*(1), 69.

Balthazar, C., Pimentel, T., Ferrão, L., Almada, C., Santillo, A., Albenzio, M., Mollakhalili, N., Mortazavian, A., Nascimento, J. S., & Silva, M. (2017). Sheep milk: Physicochemical characteristics and relevance for functional food development. *Comprehensive reviews in food science and food safety*, *16*(2), 247-262.

Balthazar, C., Silva, H. A., Vieira, A., Neto, R., Cappato, L., Coimbra, P., Moraes, J., Andrade, M., Calado, V., & Granato, D. (2017). Assessing the effects of different prebiotic dietary oligosaccharides in sheep milk ice cream. *Food Research International*, *91*, 38-46.

Bonczar, G., Wszołek, M., & Siuta, A. (2002). The effects of certain factors on the properties of yoghurt made from ewe's milk. *Food chemistry*, *79*(1), 85-91.

Corrêa-Oliveira, R., Fachi, J. L., Vieira, A., Sato, F. T., & Vinolo, M. A. R. (2016). Regulation of immune cell function by short-chain fatty acids. *Clinical & translational immunology*, *5*(4), e73.

Dave, R., & Shah, N. (1996). Evaluation of media for selective enumeration of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus, and bifidobacteria. *Journal of dairy science*, *79*(9), 1529-1536.

Di Cerbo, A., Palmieri, B., Aponte, M., Morales-Medina, J. C., & Iannitti, T. (2016). Mechanisms and therapeutic effectiveness of lactobacilli. *Journal of clinical pathology*, *69*(3), 187-203.

Groves, H. T., Higham, S. L., Moffatt, M. F., Cox, M. J., & Tregoning, J. S. (2020). Respiratory viral infection alters the gut microbiota by inducing inappetence. *MBio*, *11*(1), 10.1128/mbio. 03236-03219.

GÜLER-AKIN, M. B. (2005). The effects of different incubation temperatures on the acetaldehyde content and viable bacteria counts of bio-yogurt made from ewe's milk. *International Journal of Dairy Technology*, *58*(3), 174-179.

Hadadji, M., & Bensoltane, A. (2006). Growth and lactic acid production by Bifidobacterium longum and Lactobacillus acidophilus in goat's milk. *African journal of Biotechnology*, *5*(6), 505-509.

Hamdy, A. M., Ahmed, M. E., Mehta, D., Elfaruk, M. S., Hammam, A. R., & El-Derwy, Y. M. (2021). Enhancement of low-fat Feta cheese characteristics using probiotic bacteria. *Food Science & Nutrition*, *9*(1), 62-70.

Hansen, C. (2005). Lactobacillus acidophilus, Lactobacillus casei and Bifidobacteria in fermented milk products-guidelines. Method for counting probiotic bacteria. *Technical Bulletin F-6*, 1-8.

Hashim, I., Khalil, A., & Habib, H. (2009). Quality and acceptability of a set-type yogurt made from camel milk. *Journal of Dairy Science*, *92*(3), 857-862.

Hauptmann, M., & Schaible, U. E. (2016). Linking microbiota and respiratory disease. *FEBS letters*, *590*(21), 3721-3738.

Htay, Y. Y., Htwe, T. M. M., Moe, T. T., Win, K. K., Cho, T. T., & Phyu, S. (2020). *The Nutrients, Lactic Acid and Vitamin C contents containing in commercial Yogurts* MERAL Portal].

Ibrahim, A. H., & Khalifa, S. A. (2015). The effects of various stabilizers on physiochemical properties of camel's milk yoghurt. *Journal of American Science*, *11*(1), 15-24.

IDF, S. (1997). 152A, Milk and milk products, determination of fat content, general guidance on the use of butyrometric methods, Int. *Dairy Fed., Brussels, Belgium*.

Igbabul, B., Shember, J., & Amove, J. (2014). Physicochemical, microbiological and sensory evaluation of yoghurt sold in Makurdi metropolis. *African Journal of Food Science and Technology*, *5*(6), 129-135.

Joung, J. Y., Lee, J. Y., Ha, Y. S., Shin, Y. K., Kim, Y., Kim, S. H., & Oh, N. S. (2016). Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean journal for food science of animal resources*, *36*(1), 90.

Karam-Allah, A. A.-K., Abo-Zaid, E. M., Refae, M. M., Shaaban, H. A.-G., Saad, S. A., Hassanin, A. M., & El-Waseif, M. A. E.-M. (2022). Functional stirred Yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin, effect of lactoferrin on pathogenic bacteria and amino acids of buffalo, bovine colostrum and lactoferrin. *Egyptian Journal of Chemistry*, *65*(7), 583-594.

Mao, L., Pan, Q., Yuan, F., & Gao, Y. (2019). Formation of soy protein isolate-carrageenan complex coacervates for improved viability of Bifidobacterium longum during pasteurization and in vitro digestion. *Food chemistry*, *276*, 307-314.

Mazloomi, S., Shekarforoush, S., Ebrahimnejad, H., & Sajedianfard, J. (2011). Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yogurt. *Iranian Journal of Veterinary Research*, *12*(2), 93-98.

Moatsou, G., & Sakkas, L. (2019). Sheep milk components: Focus on nutritional advantages and biofunctional potential. *Small Ruminant Research*, *180*, 86-99.

Moubasher, A.-A., Abdel-Sater, M., & Soliman, Z. (2018). Yeasts and filamentous fungi associated with some dairy products in Egypt. *Journal de mycologie medicale*, *28*(1), 76-86.

Novik, G., & Savich, V. (2020). Beneficial microbiota. Probiotics and pharmaceutical products in functional nutrition and medicine. *Microbes and infection*, *22*(1), 8-18.

Oladipo, I., Atolagbe, O., & Adetiba, T. (2014). Nutritional evaluation and microbiological analysis of yoghurt produced from full cream milk, tiger-nut milk, skimmed milk and fresh cow milk. *Pensee*, *76*(4), 30-38.

Pecka-Kiełb, E., Kowalewska-Łuczak, I., Czerniawska-Piątkowska, E., & Zielak-Steciwko, A. E. (2020). Effects of single nucleotide polymorphisms in the SLC27A3 gene on the nutritional value of sheep milk. *Animals*, *10*(4), 562.

Pulina, G., Milán, M., Lavín, M., Theodoridis, A., Morin, E., Capote, J., Thomas, D., Francesconi, A., & Caja, G. (2018). Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *Journal of Dairy Science*, *101*(8), 6715-6729.

Richardson, G. H. (1985). Standard methods for the examination of dairy products.

Schöpping, M., Gaspar, P., Neves, A. R., Franzén, C. J., & Zeidan, A. A. (2021). Identifying the essential nutritional requirements of the probiotic bacteria Bifidobacterium animalis and Bifidobacterium longum through genome-scale modeling. *NPJ Systems Biology and Applications*, *7*(1), 47.

Singh, G., Kapoor, I. P. S., & Singh, P. (2011). Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. *Journal of Food Processing and Preservation*, *35*(6), 778-783.

Singh, R. K., Chang, H.-W., Yan, D., Lee, K. M., Ucmak, D., Wong, K., Abrouk, M., Farahnik, B., Nakamura, M., & Zhu, T. H. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of translational medicine*, *15*(1), 1-17.

Son, J. K., Jo, Y. J., Jung, Y. J., Lee, Y. R., Lee, J., & Jeong, H. S. (2023). Fermentation and Quality Characteristics of Yogurt Treated with Bifidobacterium longum. *Nutrients*, *15*(15), 3490.

Sunmola, A. A., Ogbole, O. O., Faleye, T. O., Adetoye, A., Adeniji, J. A., & Ayeni, F. A. (2019). Antiviral potentials of Lactobacillus plantarum, Lactobacillus amylovorus, and Enterococcus hirae against selected Enterovirus. *Folia Microbiologica*, *64*, 257-264.

Tamime, A., & Robinson, R. (1999). Yoghurt. Science and Technology. Cambridge, UK: Woodhead Publishing Limited England.

Temerbayeva, M., Rebezov, M., Okuskhanova, E., Zinina, O., Gorelik, O., Vagapova, O., Beginer, T., Gritsenko, S., Serikova, A., & Yessimbekov, Z. (2018). Development of yoghurt from combination of goat and cow milk. *Annual Research & Review in Biology*, 1-7.

Tharmaraj, N., & Shah, N. (2003). Selective enumeration of Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and propionibacteria. *Journal of dairy science*, *86*(7), 2288-2296.

Vianna, F. S., Canto, A. C., da Costa-Lima, B. R., Salim, A. P. A., Costa, M. P., Balthazar, C. F., Oliveira, B. R., Rachid, R. P., Franco, R. M., & Conte-Junior, C. A. (2017). Development of new probiotic yoghurt with a mixture of cow and sheep milk: effects on physicochemical, textural and sensory analysis. *Small Ruminant Research*, *149*, 154-162.

الخصائص الفيزيوكيميائية والميكروبيولوجية لمشروب الزبادي الحيوي المحضر من حليب الأغنام

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الملخص

يعتبر حليب الأغنام مصدرا غذائيا مهما ومعززا للصحة نظرا بسبب احتوائه على نسبة عالية من البروتين والمعادن، كان هدف الدراسة الحالية هو التأكد من تأثير بكتيريا البروبيوتيك (*Bifidobacterium longum*) على الخصائص الفيزيائية والكيميائية والتقييم البكتيري والتقييم الحسي لمشوب الزبادي الحيوي المصنوع من حليب الأغنام، تم تحضير مشروب الزبادي الحيوي بمستويات تلقيح مختلفة من البكتريا الحيوية (3، 6، 9، 12٪ للمعاملات (1، 2، 3، 4 على التوالي)، وتشير النتائج إلى تناقص المحتوى الرطوبي لجميع المعاملات بشكل مطرد مع زيادة فترات التخزين في الثلاجة حتى 15 يوما، وكانت عينات المقارنة أعلى من تلك الموجودة في مشروب الزبادي الحيوي المصنوع بكميات مختلفة من *Bifidobacterium longum*، بينما كانت العينات الطازجة أقل رطوبة من المعاملات الأخرى، كما تشير النتائج إلى تناقص نسبة الدهن في كلا من مشروب الزبادي الحيوي وكذلك عينات المراقبة بشكل كبير مع زيادة فترات التخزين في درجة حرارة الثلاجة حتى 15 يومًا، وكان هناك زيادة في كلا من البروتين والرماد في مشروب الزبادي الحيوي عنها في عينات المقارنة، وكانت القيمة تزداد بزيادة مدة التخزين في جميع المعاملات، وكانت كمية البروتين والرماد اكبر في العينات الطازجة عنها في باقي المعاملات، كان هناك تغير كبير بشكل إحصائي في التخلص من الشرش بين جميع المعاملات. لم تحتوي أي من المعاملات بكتريا القولون وكذلك العفن والخميرة في جميع المعاملات. حصلت معاملات مشروب الزبادي الحيوي الأولى والثانية والثالثة المخزنة لمدة 5 و١٠ و١٥ ايام على أعلى الدرجات، في حين حصلت عينات المعاملة الأولى وعينات المقارنة لمدة 10 و5 أيام على أدنى الدرجات؛ على التوالي.