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ORIGINAL PAPER

Investigating the effect of addition of probiotic microorganisms (bacteria or yeast) to yoghurt on the viability and volatile aromatic profiles

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Abstract

Aroma compounds are key components of food, and the food industry places a high priority on the enhancement of these chemicals. *Streptococcus thermophilus* (ST), *Lactobacillus bulgaricus* (LB), *Limosilactobacillus reuteri* (LR), *Lactobacillus acidophilus* (LA-5), *Bifidobacterium bifidum* (BB-12) and *Saccharomyces boulardii* (SB) were mixed starter cultures used in this study to improve the flavor of the yoghurt. To make the yoghurt, four starter mixtures were used, with the presence of the common yoghurt starter (CYS) as a control sample: CYS + LR, CYS + LA-5, CYS + BB-12, and CYS + SB. The pH, total acidity, and starter culture viability of yoghurt samples were then estimated over the course of 21 days of storage, while aroma compounds and sensory evaluation were determined on the first day of storage. The pH and total acidity of the yoghurt samples ranged between 4.2 and 4.5 and 0.84–0.93% on the first day to storage respectively, according to the results. The vaiable count of probioticbacteria were 8.1 (LR), 9.3 (LA-5), and 7.9 (BB-12) log CFU/g while it was 6.9 CFU/g for the probiotic yeast at the end of storage period. Furthermore, yoghurt samples contained a total of 47 volatile compounds. Esters and alcohols had the greatest impact on the formation of volatile compounds in fermentations containing various probiotic microorganisms and yoghurt starter. The mixture of aroma compounds produced therefore in the current study would be useful for selecting starter cultures that could serve as significant resources in the development of novel fermented milks.

Keywords Yoghurt · Aroma · Starter culture · Probiotic bacteria · Probiotic yeast

Introduction

The addition of starter cultures causes the lactose in milk to be converted into lactic acid, which then leads to the production of a wide variety of cultured dairy products. In ealier era when nobody knew anything about bacteriology, people used trace amounts of sour milk as starter cultures to inoculate fresh milk. People living in many parts of the world, especially in hot temperatures, desired to consume a variety of fermented milk products because the high acidity of these products kept them safe by eliminating harmful microorganisms [1]. This was especially true for people living in hotter climates. Probiotic yoghurt products contribute significantly to human health by delivering natural food components, enhancing gut microbiota with probiotic strains and other lactic acid bacteria (LAB), and supplying natural food components. Inhibition of pathogens (such as

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Salmonella, *Yersinia*, and *Helicobacter* species), activation of the immune system, and enhanced absorption of lactose and essential minerals may be achieved through consumption of probiotic yoghurt [2].

The flavor components that contribute to the overall flavor of yoghurt products can be categorized into the following four groups: non-volatile acids (such as lactic or pyruvic), volatile acids (such as butyric or acetic), carbonyl compounds (such as acetaldehyde or diacetyl), and miscellaneous chemical substances (amino acids or chemicals created by heat degradation). Monitoring bacterial growth and activity requires quantitative detection of organic acid production during the fermentation process [3]. In a previous study, Serra et al. [4] discovered that yoghurts made from milk that was homogenized at 200 MPa at 30 or 40 °C have varied profiles, in addition to a significant decrease in the number of lactobacilli counts. During the storage period, there was found to be very little variation in the yoghurt's flavor components or its counts of bacteria. Flavor components, which are one of the most important taste compounds in yoghurt, are a crucial factor in determining whether or not customers will accept the product in a variety of different contexts [5]. It was discovered that the flavor of the yoghurt was subpar when the level of acetaldehyde was lower than 4.0 parts per million (ppm), which is considered to be an inadequate quantity. On the other hand, the flavor of the yoghurt was exceptional when the level of acetaldehyde was 8.0 ppm or higher [6].

It has been demonstrated that the combination of both the strains *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as starters is essential for manufacturing yoghurt at industrial level with respect to quality aspects like development of body-texture, aroma, flavour and overall acceptance than starter containing a single species of bacteria. Therefore, choosing the appropriate bacterial strains and combining them is essential for the production of yoghurt-flavored bases of the highest possible quality [7]. The objective of this research was to investigate how the addition of probiotic microorganisms (bacteria or yeast) to yoghurt starter affected the viability, total acidity, and volatile aromatic profiles of the microbes present in yoghurt both after the product had been manufactured and while it was being stored. This study may help determine which starter is the most effective when it comes to producing yoghurt-flavored bases.

Materials and methods

Microorganisms and materials

Streptococcus thermophilus (ST) and *Lactobacillus bulgaricus* (LB) were both found in the common yoghurt starter (CYS) that was supplied by the Italian company SACCO. In the past, *Limosilactobacillus reuteri* (LR) [8] was isolated from the feces of human infants in the city of Basrah in Iraq. The Chr. Hansen Laboratory (Denmark) was the source for both the *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium bifidum* (BB-12) strains. The Swanson Lab (Australia) provided the *Saccharomyces boulardii* ATCC MYA-796™ (SB) strain. The composition of the pasteurized cow milk that was purchased from the market in Basra city was determined using lacto-flash dairy constituent analyzers (Germany). Accordingly, the milk had 3.7% fat, 3.5% protein, 4.0% lactose, 0.16% ash, 7.7% nonfat solids, and a pH of 6.8.

Yoghurt production

For manufacturing of yoghurt a method of Mani-López et al. (2014) was followed with minor modifications.Brefly, the procedures shown in Fig. 1 were followed in order to prepare five yoghurt-flavored bases: three for the mixed starter yoghurt with probiotic bacteria fermented group, one for the mixed starter yoghurt with probiotic yeast fermented group, and one control sample. For this purpose, three samples were prepared, including (1) 2 mL of CYS with 1 mL of LR, (2) 2 mL of CYS with 1 mL of LA-5, and (3) 2 mL of CYS with 1 mL of BB-12 cultures. Each of these samples contained CYS with 1.0×10^8 CFU/mL and LR with 3.2×10^8 CFU/mL (1), CYS with 1.0×10^8 CFU/mL and LA-5 with 3.7×10^8 CFU/mL (2), and CYS with 1.0×10^8 CFU/mL and BB-12 with 4.1×10^8 CFU/mL) (3). The preparation of the fourth sample involved adding 1 mL of SB to 2 mL of CYS, which resulted in the presence of yoghurt starter (1.0×10^8 CFU/mL) and SB (5.3×10^6 CFU/mL). The preparation of the control sample consisted solely of adding 3 mL of yoghurt starter. After being heated at a temperature of 90 °C for 15 min, the inoculated milk was placed into containers (150 mL) and incubated at a temperature of 37 °C for 5–6 h until the milk curd reached. After that, the yoghurt surfaces were covered with circular prepared layers and chilled overnight at a temperature of 4 °C [9].

pH values and total acidity

The pH levels were measured with a pH-330i meter (WTW GmbH, Germany) in order to get accurate results. Titration with 0.5 N NaOH using 1% phenolphthalein as an indicator

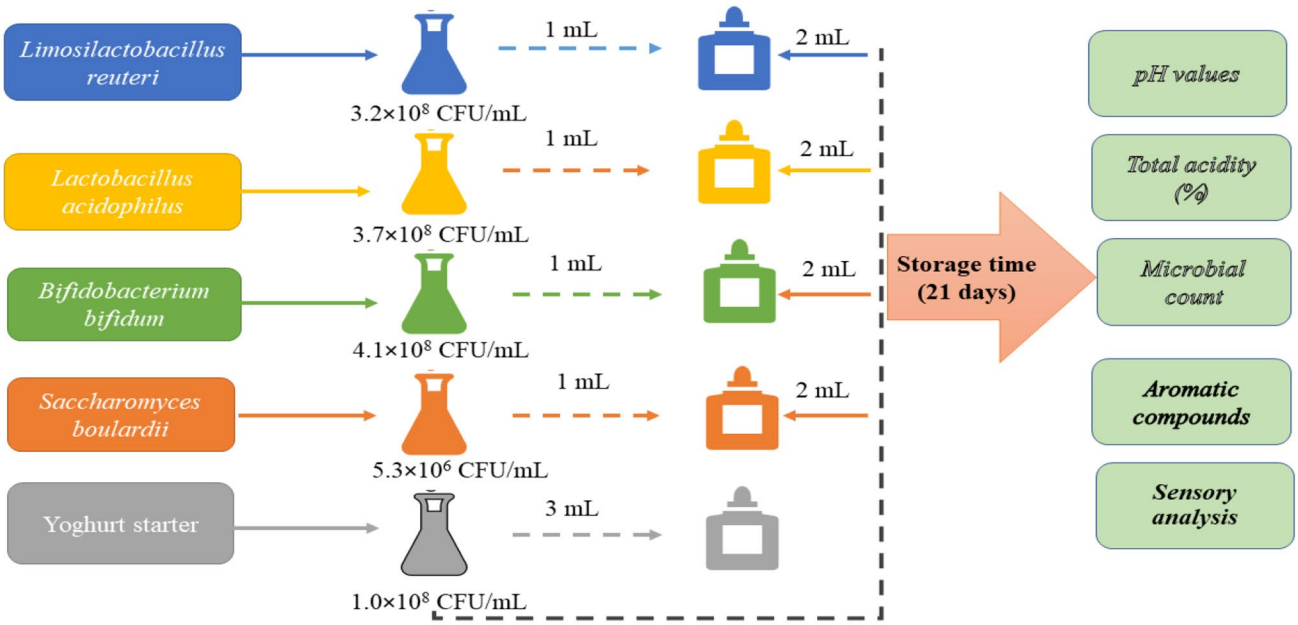

Fig. 1 A summarized scheme for the preparation of five yoghurt samples for examination, using a variety of different types of probiotic starters

was used to calculate the total acidity (percentage of lactic acid by weight) during storage time at 1, 7, and 21 days [10].

Enumeration of starter microorganisms

Yoghurt sample was taken after fermentation and while it was cold stored at 4 ± 2 °C. The samples were analyzed as quickly as possible, within 24 h. At 1, 7, 14, and 21 days after storage, the counts of microorganisms were determined by using selective culture media. Peptone water containing 0.1% peptone was utilized for the purpose of diluting samples in preparation for microbiological examination. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were quantified using the method of Delgado-Fernández and coworkers [11]. *Streptococcus thermophilus* was grown for a total of three days on M17 agar (Hopebio, China), which had 1% (w/v) of lactose (M17-lactose) in it. Pour plating was used to spread appropriate dilutions of *Lactobacillus bulgaricus* on MRS-fructose fermentation broth (Hi-media, India), which was devoid of both meat extract and glucose. Agar is included at a concentration of 1.5% (w/v), along with Tween-80 at a concentration of 0.25% (w/v), fructose at a concentration of 1% (w/v), and L-cysteine hydrochloride at 0.05% (w/v). *L. reuteri* was added to MRS-T agar (tetracycline 0.9 mg/mL, Sigma, Germany) before the plates were put into anaerobic jars and heated to 45 °C for three days and nights in order to count the number of *Limosilactobacillus reuteri*. After that, the MRS-T plates were put into an anaerobic environment for 48 h at 37 °C [12]. For the purpose of counting *Lactobacillus acidophilus*, which had been cultured aerobically at 37 °C for seventy-two hours [13], bile-MRS agar (bile salts 0.15% of w/v, Sigma, Germany) was used. LP-MRS agar (0.2 g % (w/v) lithium chloride and 0.3 g % (w/v) sodium propionate, BDH, USA) was used to count *Bifidobacterium bifidum*, which was cultured in anaerobic conditions at 37 °C for 72 h [13]. Counts of *Saccharomyces boulardii* were taken on chloramphenicol glucose yeast extract agar (Hi-media, India) after the fungus had been cultured at 30 °C for 48 h in an aerobic environment [9].

Identification of aromatic compounds

The SPME method and GCMS analysis were utilized in order to extract the aromatic compounds that were present in yoghurt samples. In order to conduct an analysis of aromatic chemicals, three grams of samples were loaded into a vial that had a capacity of 20 mL. To expedite the process of extracting the volatile components found in the yoghurt samples, the samples were heated to 80 °C and stirred for 10 min. After that, in order to extract volatile chemicals, a 65 m Divinylbenzene/Polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA, USA) was inserted into the vial and exposed to the headspace for 20 min at 80 °C. After inserting the fiber into the injection port and leaving it there for one minute at 260 °C with helium serving as the carrier gas and a column flow of 1.78 mL/minute, an analysis of the aromatic components was performed. After inserting the fiber, the temperature of the oven was maintained at 40 °C for 5 min, after which it was raised at a rate of 4 °C per

minute to 70 °C, where it was maintained for 4 min. After that, using a splitless mode, the temperature was brought up to 250 °C at a rate of 10 °C per minute for the next 5 min [14].

Sensory analysis

The University of Basrah's Department of Food Sciences provided the pool of candidates for the selection of the ten people with relevant experience and background knowledge who would serve as members of expert panel (evaluators) to carry out sensory analysis on samples of yoghurt prepared from a variety of starting cultures. During the sensory testing, random samples of coded yoghurt were evaluated based on their acceptability in terms of their taste, texture, color, and flavor. If there is a scale that goes from 1 to 5, with 1 being the bad, 2 being acceptable, 3 being good, 4 being very good, and 5 being excellent, after evaluating all three triplicates of each characteristic, the results were averaged to get a final score. Washing with water was done in between each sample, and the minimum amount of time that passed between each sample was several minutes [15].

Statistical analyses

The experiments were done in triplicate in three independent repetitions wheresoever prerequisite. The data was presented as a mean along with a standard deviation (±SD). The program GenStat 12.1 (VSN International Limited is registered in England and Wales) was used to perform an analysis of variance (ANOVA) and the test for the least significant difference ($p \leq 0.05$) in order to conduct multifactorial comparisons.

Results and discussion

pH values and total acidity

Figure 2 displays the pH levels as well as the total acidity (%) of five different samples of yoghurt. When the pH of milk reaches 4.6, the casein in the milk begins to precipitate, whereas the curdling of yoghurt is accomplished by lowering the pH in order to encourage micelle binding [16]. On the first day of storage, the pH of all of the samples was lower than 4.5, and there were significant differences in pH across the five groups (CYS, CYS + LR, CYS + LA-5, CYS + BB-12, and CYS + SB), while the total acidity (%) was (0.84, 0.91, 0.93, and 0.90 and 0.88%, respectively. The reduction in pH values and the increase in total acidity (%) in the yoghurt samples across the storage durations, with the latter reaching its peak after 21 days. The CYS + BB-12 sample had the lowest pH value and the highest total acidity (%) compared to the other yoghurt samples. CYS + BB-12 had a pH value of 3.6 and a total acidity that was 1.19%, respectively. The conversion of lactose into lactic acid can result in a drop in pH as well as an increase in acidity levels overall (%).The statistical analysis revealed that there were significant variations ($p \leq 0.05$) in the pH and total acidity values between the five treatments over the different storage durations. The pH values and total acidity of the starter culture shifted over the course of time as the culture was allowed to mature. A decrease in the lag time for pH and an increase in overall acidity take place during storage. These changes are a reflection of the acidification rate caused by the starter cultures that are involved. As a consequence of this, the proliferation of microbes can have an effect on the quality of yoghurt. Yoghurt made with the *Lactobacillus bulgaricus* strain had an average pH of 4.18, while yoghurt made with the *Lactobacillus acidophilus* strain had an average pH of 4.29 [17]. The lactic acid bacteria mentioned earlier are responsible for the production of over 400 volatile chemicals during the fermentation of milk. These chemicals (including lactic acid, acetaldehyde, diacetyl, acetone, 2-butanone, acetoin, etc.) are derived from the diverse chemical structure of milk. These volatile components and other non-volatile components are responsible for giving yoghurt its distinctive flavor, which is appreciated by consumers [18].

Enumeration of microorganisms

The outcomes of the microbiological examination of the yoghurt samples are presented in Fig. 3. On the first day of storage time at 4 °C, the log number of starter bacteria in all yoghurt samples ranged from 7.90 to 8.30 CFU/g for *Streptococcus thermophilus* and 8.51–9.51 CFU/g for *Lactobacillus bulgaricus*. These results were determined using the colony forming unit (CFU) method. While the log of numbers probiotic bacteria, LR, LA-5, and BB-12 were 8.1, 9.3 and 7.9 CFU/g, respectively. The log numbers of the colony forming units per gram of probiotic yeast (SB) was 6.9 CFU. All of the yoghurt samples experienced a gradual decline in the total number of viable probiotic microorganisms as the temperature was lowered. Following the period of time spent in storage (21 days), the log numbers of LB ranged from 7.2 to 8.2 CFU/g, while the log of ST ranged from 6.1 to 7.1 CFU/g across all of the yoghurt samples. LR, LA-5, BB-12, and SB all had log values of 7.1,7.5, and 6.6 and 6.3 CFU/g, respectively. The statistical difference ($p < 0.05$) was found by comparing probiotic organism viability (CFU/g) values at the beginning of storage time (first day) with probiotic organism viability results at the conclusion of storage time (last day). Both lactic acid

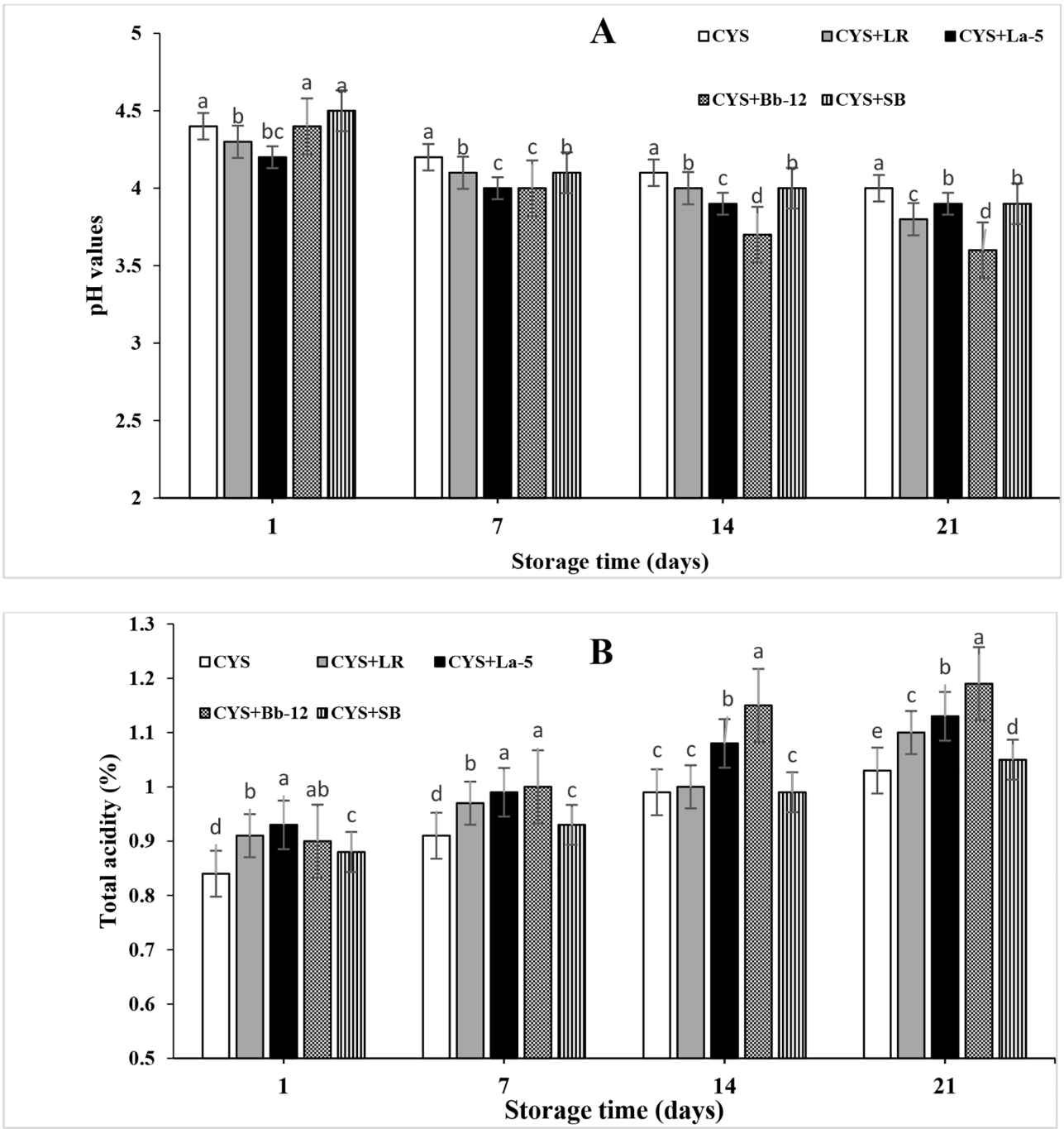

Fig. 2 Comparison of the pH values **(A)** and total acidity (%) **(B)** of yoghurt samples produced by a variety of starters. **Note**: The data shows the mean standard deviation SD (n = 3 × 5). The least significant difference test ($p < 0.05$) indicates that bars followed by the same letter for each treatment are not significantly different

bacteria and probiotic bacteria are susceptible to death when exposed to cold storage temperatures between 4 and 8 °C. In addition to the fact that the medium is more acidic as a result of the formation of organic acids by the bacteria while they are growing, the low temperature also has the effect of reducing the number of bacteria present. The temperature at which probiotics were kept had a important impact on their ability to survive, and storing them in a refrigerator resulted in a noticeable reduction in their viability. In addition, the type of probiotic strain had an effect on the likelihood of survival [19, 20]. The decreased pH was a reflection of the production and accumulation of organic acids such as lactic acid in the yoghurt samples caused by the reduced oxygen levels. This production and accumulation were reflected

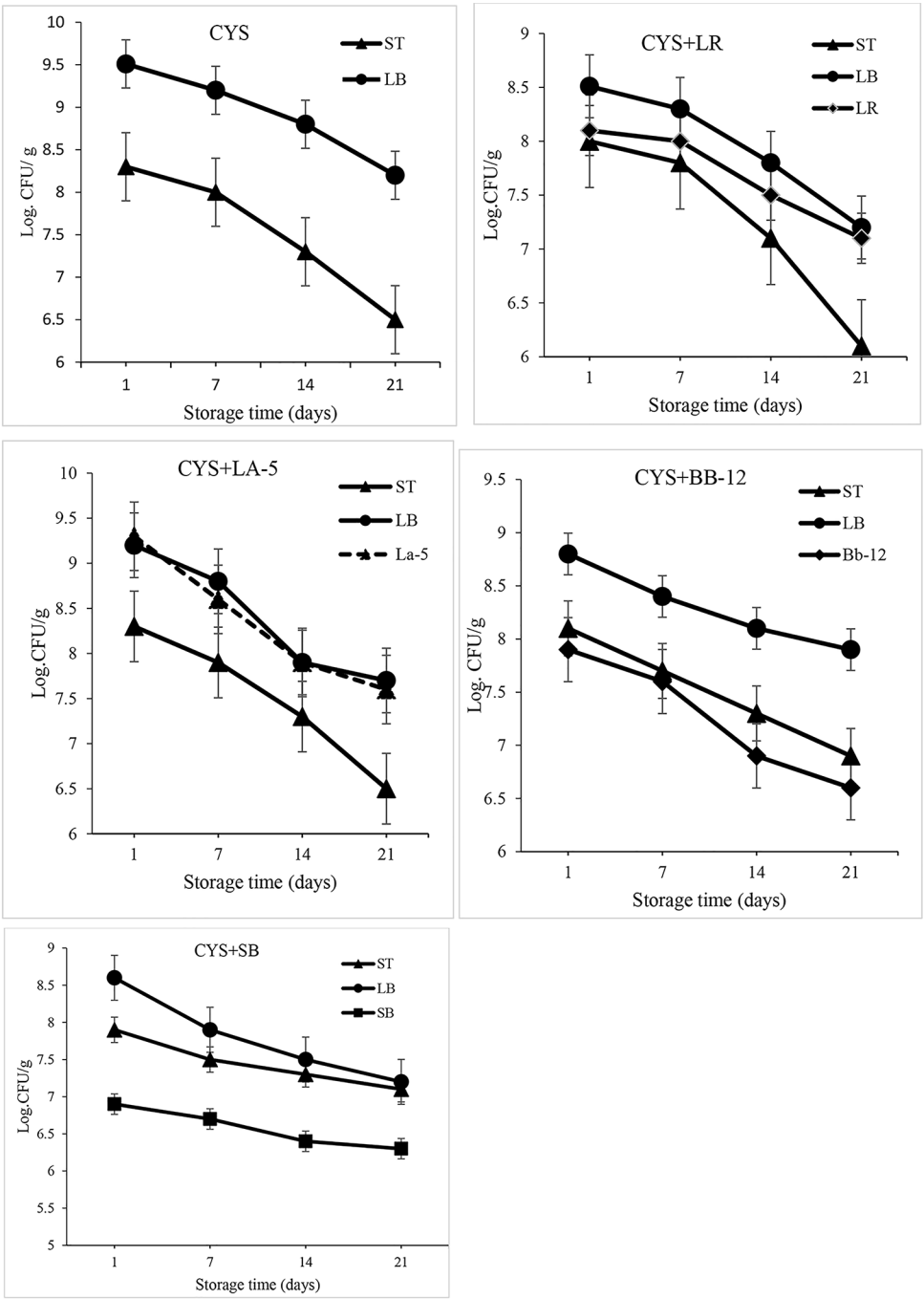

Fig. 3 Viable counts (log CFU/g) of starters bacteria in various treatments over the course of cold storage (21 days). [Abbreviations: CYS: yoghurt containing common starter; CYS + LR: yoghurt containing common starter plus *Limosilactobacillus reuteri*; CYS + LA-5: yoghurt containing common starter plus *Lactobacillus acidophilus*; CYS + BB-12: yoghurt containing common starter plus *Bifidobacterium bifidum*; CYS + SB: yoghurt containing common starter plus *Saccharomyces boulardii*], **Note**: The data represents the mean SD (n = 3). According to the least significant difference test ($p < 0.05$)

by the increase in the lactic acid bacteria count. Because lactic acid bacteria metabolize polysaccharides to produce organic acids and short-chain fatty acids, which resulted in a decrease in pH in growth media [21], the overflow was able to be reduced, which caused the indicator of low oxygen to become more apparent. This was a reflection of the fact that these bacteria cause the polysaccharides to be metabolized. To ensure that probiotic organisms exert their health benefits, their viability must be maintained until they reach the site of action (concentration of 10^6-10^7 CFU/mL or gm) and they must be able to withstand the harsh conditions of the gastrointestinal tract [22]. The results of the probiotic yeast numbers agreed with Homayouni-rad and co-workers [23], which reported that the counts of *Kluyveromyces marxianus* as probiotic yeast in yoghurt were 7.35 log CFU/g after 28 days of cold storage. This number is within the limit that is recommended by the International Dairy Federation (IDF). Despite the fact that the colony count of *Saccharomyces boulardii* in yoghurt must be greater than 6.0 log CFU/g in order for the organism to remain viable [24].

Aromatic compounds profiles by SPME-GC/MS

After one day of storage, the GC-MS profiles and concentrations of volatile components in each of the yoghurt samples are summarized in Table 1, which may be seen below. There was a total of 47 volatile compounds found in the yoghurt samples. These chemicals were divided into the following: 10 acids, 12 esters, 5 ketones, 6 aldehydes, 11 alcohols, and 3 alkanes. Both the metabolic process that occurs during the production of yoghurt and the activity of the starter culture contribute to the accumulation of these chemicals in the fermented milk product. In this line, the differences in the activity and type of the starting microorganisms might be utilized to explain the changes in the concentration of these compounds found in the different samples of yoghurt. During the process of metabolism, lactic acid bacteria produce volatile chemicals, which are crucial in the fragrance profile of various different foods. These compounds are impacted by a variety of conditions and play an important role in the aroma profile of these bacteria. The acetaldehyde molecule, which is the most important volatile ingredient in yoghurt products, is produced by the *Lactobacillus bulgaricus* bacteria [25]. In the fermentation of cream by *Lactococcus lactis* subsp. *lactis* [26], methyl ketone was the primary volatile compound. However, in this study, the use of mixed starters from microorganisms results in different metabolic compounds due to their metabolic pathways, and this contributes to an improvement in the flavor of the yoghurt as a whole. The volatiles that were found in our research were comparable to the volatiles that were found during the production of yoghurt by employing a variety of starter cultures and SPME-GCMS to analyze the product at different stages [27–29]. Acetaldehyde is a important component in yoghurt flavor components that give it fragrance, freshness, and a green aroma; nevertheless, it was not discovered in this investigation with the exception of the CYS sample. The CYS sample contained the highest concentration of acetaldehyde compared to the other yoghurt samples. This might be owing to the ability of the newly introduced bacteria to convert this chemical into other molecules such as ethanol and acetate. A number of studies have shown that adding other bacteria of lactic acid bacteria to yoghurt starting bacteria resulted in lesser acetaldehyde production or none at all [29].

The yoghurt samples were analyzed, and ten different fatty acids were found. Indicators that are suitable for use in the preservation of yoghurt products include fatty acids. The yoghurt that was made using a common starter combined with probiotic bacteria included a greater variety of fatty acid types than the yoghurt that was made using a common starter alone or the yoghurt that contained probiotic yeast. In addition, the low-fat yoghurts that had 1% buttermilk added to them enhanced the amount of essential volatile components such as acids, esters, aldehydes, ketones, and alcohols [30]. These components are responsible for the flowery and pungent flavor that yoghurts have. Distinct yoghurt starter cultures resulted in the production of yoghurt samples that contained a total of twelve different ester components. The percentages of esters compounds found in the yoghurt samples were as follows: 14.57% for CYS, 38.79% for CYS + LR, 28.48% for CYS + LA-5, 7.83% for CYS + BB-12, and 26.30% for CYS + SB. According to research that was conducted in the past, esters of chemicals like ethyl acetate have been shown to be present in yoghurt production [31, 32].

Acetoin, 2-heptanone, 2-nonanone, 8-nonen-2-one, and acetyl propionyl are the five ketones that were discovered in the produced yoghurt that was fermented by a mixed yoghurt starter that contained probiotic bacteria and yeast. Other ketones found in the yoghurt include acetoin. When it comes to the production of yoghurt, ketone compounds are one of the most prominent aroma molecules that are present at a low level. Oil oxidation, thermal breakdown, the degradation of proteins, and the microbial metabolism of polyunsaturated fatty acids are all potential routes for the production of ketones [33]. Acetaldehyde, 2-acrolein, nonanal, benzaldehyde, 2,5-dimethylbenzaldehyde, and phenyl butyraldehyde were six aldehydes that were found in the analyzed yoghurt samples. The ratio of aldehyde compounds was greatest in the sample that included CYS + BB-12. Yoghurt's naturally bright aroma is heightened by the addition of aldehydes [34]. A new trend that has emerged in the manufacturing of functional dairy products is the addition of probiotic bacteria to yoghurt. Generally speaking, the starter strains of the *Lactobacillus* and *Bifidobacterium* genera are the commercial probiotics that are utilized in the production of yoghurt. In addition to the purported health benefits they offer, they also have an effect on the flavor of the yoghurt when they are added to it. During the process of carbohydrate fermentation, alcohol molecules are a typical byproduct produced by yeasts as well as certain types of lactic acid bacteria. In the yoghurt samples, eleven different types of alcohol molecules were found. These were ethanol, 1-butanol, 2,2-dimethy-1-pentanol, 4-ethyl-1-octyn-3-ol, 1-hexanol, dimethyl cyclohexanol, cyclohexane propanol, decylene glycol, benzyl alcohol, phenethyl alcohol, and 2,3-pentanedione. The amounts of alcohol compounds that were found in the samples of CYS, CYS + LR, CYS + LA-5, CYS + BB-12, and CYS + SB were, respectively, 5.65%, 18.16%, 10.84%, 22.98%, and 41.39%. It is interesting to note that the alcohols that were formed the most were found in the sample that included CYS + SB since the starter of the yoghurt had probiotic yeast added to it. This had a beneficial impact on the overall flavor and taste of the yoghurt that

Table 1 Identified aroma compounds (%) in all yoghurt samples using the SPME-GCMS method

Aromatic compounds	CYS	CYS + LR	CYS + LA-5	CYS + BB-12	CYS + SB
Acids					
Acetic acid	6.10	10.03	16.04	16.72	0.40
Butyric acid	4.18	0.35	2.55	1.00	2.17
Caproic acid	10.44	2.14	2.44	1.37	ND
Hexanoic acid	ND	0.86	0.57	3.46	6.02
Octanoic acid	ND	0.46	2.39	ND	3.50
n-Decanoic acid	5.32	0.10	0.66	1.12	0.69
n-Hexadecanoic acid	23.20	5.00	10.02	1.93	0.65
Lauric acid	4.82	2.37	0.86	5.04	ND
Myristic acid	0.34	ND	ND	1.65	ND
Palmitic acid	ND	ND	1.95	5.94	1.02
Total	**56.94**	**21.31**	**37.48**	**38.23**	**14.45**
Esters					
Ethyl carbonate	4.79	0.11	13.40	0.56	5.33
Ethyl acetate	2.15	0.61	0.70	2.34	11.99
3-methylpentyl acetate	ND	ND	10.01	0.30	ND
3-propenyl heptanoate	ND	12.10	0.99	1.26	ND
2-heptanol acetate	ND	ND	0.37	0.50	1.81
2-octanol acetate	ND	9.10	0.63	0.66	ND
Heptyl acetate	ND	2.50	ND	0.17	0.68
Ethyl caprylate	5.22	11.20	ND	0.25	ND
Octyl acetate	2.41	ND	0.74	0.65	5.58
Ethyl caprate	ND	ND	1.64	0.51	ND
Decyl formate	ND	1.50	ND	0.26	ND
Ethyl laurate	ND	1.67	ND	0.37	0.91
Total	**14.57**	**38.79**	**28.48**	**7.83**	**26.30**
Ketones					
Acetoin	ND	5.16	ND	0.34	0.75
2-Heptanone	0.92	1.76	8.71	11.25	11.29
2-nonanone	0.18	0.24	1.42	1.77	1.57
8-nonen-2-one	0.59	ND	0.52	4.84	ND
Acetyl propionyl	ND	ND	9.11	2.18	0.99
Total	**1.69**	**7.16**	**19.76**	**20.38**	**14.60**
Aldehydes					
Acetaldehyde	2.45	ND	ND	ND	ND
2-acrolein	ND	0.15	ND	ND	1.88
Nonanal	3.66	0.07	0.25	0.27	0.32
Benzaldehyde	9.01	10.10	ND	7.36	0.30
2,5-dimethylbenzaldehyde	4.86	ND	ND	0.29	ND
Phenyl butyraldehyde	ND	ND	0.93	0.27	0.41
Total	**17.53**	**10.32**	**1.18**	**8.19**	**2.91**
Alcohols					
Ethanol	1.12	3.05	2.10	4.40	22.80
1-butanol	ND	0.22	ND	ND	2.29
2,2-dimethy-1-pentanol	ND	ND	ND	0.53	ND
4-ethyl-1-octyn-3-ol	ND	0.14	ND	ND	6.19
1-Hexanol	ND	ND	1.76	ND	3.76
Dimethyl cyclohexenol	4.14	2.58	0.50	ND	0.70
Cyclohexanepropanol	ND	0.21	ND	ND	ND
Decylene glycol	ND	ND	6.01	ND	ND
Benzyl alcohol	0.39	4.47	ND	ND	2.59
Phenethyl alcohol	ND	7.49	0.47	0.63	2.11
2,3-Pentanedione	ND	ND	ND	17.42	0.95
Total	**5.65**	**18.16**	**10.84**	**22.98**	**41.39**
Alkanes					

Table 1 (continued)

Aromatic compounds	CYS	CYS + LR	CYS + LA-5	CYS + BB-12	CYS + SB
Cyclobutane	2.66	2.13	1.69	0.42	0.16
3-hexene	ND	0.47	0.57	1.62	0.19
Cyclohexane	0.96	1.66	ND	0.35	ND
Total	**3.62**	**4.26**	**0.57**	**2 0.39**	**0.35**

Abbreviations: ND: Not detected; CYS: yoghurt containing common starter; CYS + LR: yoghurt containing common starter plus *Limosilactobacillus reuteri*; CYS + LA-5: yoghurt containing common starter plus *Lactobacillus acidophilus*; CYS + BB-12: yoghurt containing common starter plus *Bifidobacterium bifidum*; CYS + SB: yoghurt containing common starter plus *Saccharomyces boulardii*

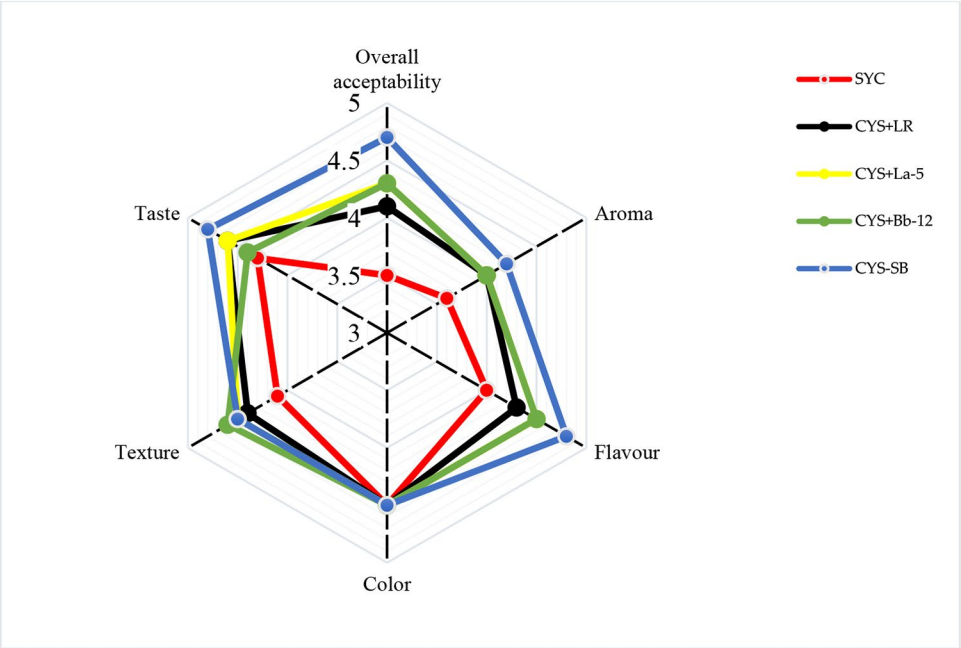

Fig. 4 Analysis of the sensory experiences to determine the mean values of the yoghurt's sensory qualities in the samples. [Abbreviations: CYS + LR: yoghurt containing common starter plus *Limosilactobacillus reuteri*; CYS + LA-5: yoghurt containing common starter plus *Lactobacillus acidophilus*; CYS + BB-12: yoghurt containing common starter plus *Bifidobacterium bifidum*; CYS + SB: yoghurt containing common starter plus *Saccharomyces boulardii*]

was made. All of the samples of yoghurt were found to have traces of three different alkanes: cyclobutane, 3-hexene, and cyclohexane. The percentages of alkane compounds in the samples were as follows: 3.62% for the CYS sample, 4.26% for the CYS + LR sample, 0.57% for the CYS + LA-5 sample, 2.39% for the CYS + BB-12 sample, and 0.35% for the CYS + SB sample. Consumers have a limited understanding of the hydrocarbons that are present in fermented dairy products since the influence of alkanes on the aroma of yoghurt is quite insignificant. Through the process of autoxidation, alkyl radicals produce alkane molecules with a high threshold [35]. Similar research was carried out by Xu and colleagues to investigate the influence of probiotic cultures on the quality of soy protein yogurt and to identify the aroma components that comprise the yogurt [36].

Sensory analysis of yoghurt samples

When compared to the control yoghurt (CYS), which had low degrees for each of these characteristics ($p < 0.05$), all four treated yoghurt samples showed very acceptable rates for overall acceptability, scent, flavor, color, texture, and taste. The control yoghurt had low degrees for each of these characteristics. On the one hand, there were not any significant changes ($p < 0.05$) in any of the scores pertaining to the samples' sensory qualities when comparing CYS + LR, CYS + LA-5, and CYS + BB-12. On the other hand, as compared to the CYS sample (Fig. 4), the aroma score was significantly higher when yoghurt samples contained *Limosilactobacillus reuteri*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*. This was shown by a significance level of $p < 0.05$ (Fig. 4). Every single one of the sample's sensory properties, with the exception of color, was significantly improved when it was fermented with probiotic yeast (CYS + SB), compared to the other samples. Yoghurt can be made from a variety of milks through the use of starter cultures such as lactic acid bacteria. These bacteria produce lactic acid products through the fermentation of lactose, along with other metabolites that contribute to the yoghurt's distinctive flavors and aromas [15]. Yoghurt can be stored in the refrigerator for up to a week after it has been made. It is possible that the fact that *Saccharomyces boulardii* was included in the yoghurt starter culture led to the production of 31 compounds that are responsible for the flavor. The

majority of these compounds are alcoholic compounds that impart a reviving flavor when consumed, and it is possible that this was the primary reason why this sample performed so much better in the sensory analysis. There has been a growth in the use of probiotic yeasts in dairy products due to the therapeutic capabilities that these yeasts possess as well as the metabolic products that they produce, both of which contribute to the overall improvement of the product [37–41].

Conclusions

The current investigation observed that the yoghurt samples containing probiotic cultures (both lactic acid bacteria and yeast) showed better viability (more than 6.0 log CFU/g) during storage (21 days) period. The combination of lactic acid bacteria and *Saccharomyces boulardii* significantly improved the physicochemical properties of the end product, particularly the total volatile organic acids. Further, it was concluded that esters and alcohols had the greatest impact on the formation of volatile compounds in yoghurt samples during fermentations depending on the presence of different probiotic microorganisms and normal yoghurt starters. Therefore, the mixture of aroma compounds produced in this study could be useful while selecting starter cultures that could serve as significant resources in the development of novel fermented milks. It is possible that further research on flavor enhancement will help to explain metabolic contributions, advance the production process, and improve the flavor of fermented foods such as yoghurt.

Authors' Contributions **AKN, STGA, and DKV** conceptualized and supervised the manuscript; **AKN, STGA, and DFA** developed the framework, conducted experimental work and analysis, tabulated experimental data, and drew the illustrations; **AKN, STGA, DFA, DKV, and ARP** compiled literature, wrote, interpreted, and corrected the original draft of the manuscript; and **DKV, ARP and SS** read, edited and revised the manuscript. The final submission of the work was reviewed by **DKV**, who made final comments and revisions to the manuscript. All authors critically evaluated and approved the final submission version of the work.

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Declarations

Conflict of interest The authors state that there is no conflict of interest.

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