



## QUALITY COMPARISON BETWEEN YOGHURTS PREPARED WITH INFUSION OF *LACTOBACILLUS SPS.* AGAINST COMMERCIALLY AVAILABLE YOGHURT

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Received: 06-04-2022; Accepted: 08-06-2022; Published: 30-06-2022

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### ABSTRACT

In the present study, physicochemical characteristics of prepared flavoured yoghurt in comparison with the market yoghurt after storage at 5°C for 7 days was studied. More specifically, morphological and biochemical tests were performed for identification of probiotic *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* isolated from curd. Furthermore, two different types of yoghurts were produced using the probiotic bacteria *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* in combination with 15% w/v strawberry pulp. During storage of yoghurts, several physicochemical characteristics were monitored such as pH, fat, carbohydrate, protein, total solids and moisture content along with antioxidant property and shelf-life and compared with the commercially available strawberry flavoured yoghurt infused with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The pH value of the yoghurt prepared was slightly less acidic than the market yoghurt and had more moisture and protein content and less total solids. No such differences were observed in shelf-life of both yoghurts (prepared and marketed). The carbohydrate evaluation of the produced yoghurt revealed the superiority of yoghurt for diabetic population with high antioxidant concentration.

**Keywords:** Yoghurt, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, probiotic.

### 1. INTRODUCTION

Over the past few decades, probiotics and prebiotics have indeed gained a lot of attention for its beneficial health effects and various microbiota management tools have been developed and commercialised for this purpose. Fermented dairy products such as- yoghurt, cheese have been considered useful vehicles for delivering probiotic bacteria. Most probiotics belongs to the genera - *Lactobacillus* and *Bifidobacterium*. Some of the potential health benefits of probiotic bacteria include improved digestibility, immune modulation, prevention of allergy, improved lactose utilization, antagonistic action towards enteric pathogens, anti-carcinogenic effect and hypo-cholesterolemic effect [1, 2].

*Lactobacillus acidophilus*, *L. casei*, *L. paracasei* and *Bifidobacterium sps.* are predominantly used in yoghurt production [3]. Fermented milk products contain whey proteins which exhibit numerous biological effects related to digestion functions and anti-carcinogenic activities [4]. Some probiotic strains can successfully autonomously grow in milk, but others need growth

stimulants. For this reason, it is common to use probiotic bacteria as standard starter cultures as in yoghurt. Most *Bifidobacterium sps.* cannot ferment milk solely as they require low redox potential and peptides produced from the breakdown of casein protein present in milk. Moreover, when co-cultured with lactobacilli, they become inhibited as the pH drops [5]. Several factors affect the viability of probiotics such as strain characteristics, food matrix, pH, temperature, and accompanying microbes [6]. The combined use of two or more probiotic species is common in commercial probiotic foods, as these strains are believed to act synergistically on each other. Thus, the strategy is to use yoghurt bacteria (e.g. *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) as the main starter culture to decrease fermentation time and probiotic bacteria (e.g. *Lactobacillus acidophilus*) as an adjunct starter. *Lactobacillus acidophilus* is added to the yoghurt to enhance its probiotic value [7]. Therefore, the objective of this study is to investigate the effect of strawberry pulp while monitoring and comparing various physicochemical

parameters of both yoghurts (laboratory-made and commercialised) with different mixture of lactic acid bacteria (LAB).

## 2. MATERIAL AND METHODS

### 2.1. Material

Cow's milk and the curd made from cow milk were obtained from local dairy farm (Noida). Strawberry (*Fragaria ananassa*) fruit were obtained from local market in Noida. Experimental yoghurts were prepared in the laboratory of Rapture Biotech International Pvt. Ltd. (Noida). Commercialised Epigamia Greek yoghurt (strawberry flavoured) infused with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Nestle skim milk powder were obtained from local grocery store, Noida.

### 2.2. Isolation and identification of *Lactobacillus* sps.

Serially diluted samples of the fermented milk product (curd) were inoculated on to De Mann Rogosa Sharpe (MRS) agar media [8] aseptically and incubated at 37°C for 24-48 hours. Colonies that appeared on the agar plates with clear zones were selected and purified by streaking twice on MRS agar [9], and incubated at 37°C for 48h. Preliminary tests of 2 colonies were identified as *Lactobacillus* sps. by following morphological and biochemical methods [10]. They were examined microscopically for Gram-staining and catalase production. The Gram positive, catalase-negative rods cultures were classified as lactic acid bacteria [11]. In addition, selected strains were tested for growth at 10°C for 10 days, 45°C for 48h and further classified as mesophilic lactobacilli. The pure isolates were stored at 5°C on MRS agar slant and sub-cultured every 15 days.

### 2.3. Fruit pulp preparation

Fruit pulp was extracted as described in the literature [12] with a slight modification. Fresh ripe fruit (strawberry) were gently washed under tap water, peeled with the help of a knife aseptically in the laminar air flow cabinet and then were subjected to pulp extraction with mortar and pestle. The prepared fruit homogenates were filled into jars and heated at 60°C for 15 min.

### 2.4. Yoghurt preparation

Yoghurt was prepared with some modifications to the protocol in literature [13]. Cow's milk was used (50 ml) for yogurt production and to increase solids of milk, 1g skim milk powder was added. The mix was heated to 60°C and homogenized for 15 minutes. The mix was

then allowed to cool down to 45°C. Pasteurized cow's milk was divided into two parts (each 25 ml). To both the flasks containing milk sample, 15% of fruit pulp was added. Then, the first flask was inoculated with isolated bacterial culture of (S1) *Lactobacillus acidophilus* and the second with (S2) *Lactobacillus bulgaricus* each at 2.5 % inoculation level. The inoculated samples were incubated at 43°C until pH reaches 4.7. After complete coagulation, yoghurts were stored in the refrigerator at 5°C for 7 days and examined after 7 days of storage.

## 2.5. Physicochemical analysis

### 2.5.1. Determination of crude fat content

The fat content was determined as in the literature [14] with some modifications. Briefly, to 1 ml of yoghurt sample, 500 µl of concentrated HCl was added and shaken vigorously for 1 minute. The mixture formed was heated at 60°C for 10 minutes. After cooling it down for 10 minutes, the mixture was subjected to 2 mL of 95% ethanol. This was then followed by addition of 2 ml of petroleum ether and shaken vigorously to mix well. The mixture was then left to stand for 24 hours of incubation. Three petriplates were taken and weighed individually. The sample was then centrifuged for 15 minutes and the supernatant was collected and transferred to the petriplates respectively, followed by weighing the samples. Then the supernatant was exposed to 60°C for 1 hour. After the extract was dried to a constant weight, the total fat content was determined gravimetrically. The percentage of fat was calculated by the following formula:

$$\text{Fat \%} = \left\{ \frac{\text{Final value} - \text{Initial value}}{\text{Weight of sample}} \right\} \times 100$$

### 2.5.2. Determination of crude Protein content

The crude protein was determined by the Lowry's method with some modifications [15]. The assay was carried out by preparing a series of dilutions of tannic acid 1mg/ml, in the same buffer containing the unknowns, to give concentrations of 30 to 240µg/ml (0.03 to 0.24 mg/ml) to 1 ml with H<sub>2</sub>O and adding 4.5 ml of Solution 1 (24 ml of Reagent 1- 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1N NaOH -0.5 g Na<sub>2</sub>CO<sub>3</sub> and 0.1 g of NaOH in 25 ml of distilled water, 500 µl of Reagent 2-1% KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·4H<sub>2</sub>O (potassium sodium tartarate) in distilled water-0.05 g in 5 ml of water, 500 µl of Reagent 3- 0.5% CuSO<sub>4</sub>·5H<sub>2</sub>O in distilled water-0.05 g in 10 ml of water.) before incubation for 10 min at room temperature. Following this, 500 µl of Solution 2 (1 part Folin-Ciocalteu phenol reagent [2 N]:1 part water = 2 ml

of FC reagent and 2 ml of distilled water) was added and left for 30 min. at room temperature. Absorbance was read at 660 nm and a standard curve was made of tannic acid (stock solution 1mg/ml).

### 2.5.3. Determination of reducing sugars content

Reducing sugars were determined using DNS method as described [16]. A series of dilutions were prepared of dextrose solution (10mg/ml) in the same buffer containing the sample, to make volume of 3 ml with H<sub>2</sub>O. Then, 2mL DNS reagent (Reagent A- Potassium sodium tartrate (60%):45 g of Potassium sodium tartrate in 75 ml of H<sub>2</sub>O. Reagent B- 3,5-DNS solution (5%):1.5 g of DNS reagent in 30 ml of 2MNaOH) was prepared freshly and mixed properly in each test tube. Following this, incubation for 5 min. at room temperature was done. Finally, absorbance was measured at 540 nm.

### 2.5.4. Determination of pH

The pH of yoghurt samples was measured using the electric digital pH meter after calibration with standardized pH buffer solutions 4.0 and 7.0 prior to the analysis.

### 2.5.5. DPPH radical scavenging activity

The determination of antioxidant activity through DPPH scavenging system was obtained according to the method in literature [17] with some modifications. Stock solution was prepared by dissolving 24 mg DPPH in 100 ml methanol and kept at -20°C until used. About 100 µl strawberry extracts and 100 µlyoghurt samples (S1, S2, and market yoghurt) with 2.9 mlDPPH solution was mixed to make up the volume 3 ml. The mixtures were kept for scavenging reaction in the dark for 30 min. at room temperature. Absorbance was read at 517 nm and the percentage of DPPH scavenging activity was determined as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / (A_{\text{blank}})] \times 100$$

where, A is the absorbance.

### 2.5.6. Determining Moisture %

Percentage of moisture was calculated as described in the literature [18] with a slight modification. 2 g of all the 3 yoghurt samples were taken in petridishes and weights of petridishes were recorded with and without samples respectively. The samples were subjected to heat treatment at 60°C for 1:30 hr in oven and weight was again analyzed after this time duration.

The percentage moisture content was calculated by the following formula:

$$\text{Moisture \%} = [(Total\ weight - Dried\ weight) / (Total\ weight)] \times 100$$

### 2.5.7. Determining total solids

The total solids were obtained from moisture content analysis as described in literature [19]. The obtained residue weight from moisture content was determined and expressed as percentage total solids by the relation: Total solids present in yogurt= 100- Moisture%

### 2.5.8. Microbial analysis

Brilliant green agar (BGA) medium was used to determine the presence of pathogenic bacteria *Salmonella* sps. Except *Salmonella typhi* and *Salmonella paratyphi*.

## 3. RESULTS AND DISCUSSION

### 3.1. Identification of *Lactobacillus* sps.

Two cultures were isolated and selected from the curd as lactic acid bacteria. The first screening revealed the presence of rods which were further identified using classical biochemical techniques as described in the literature [10]. Tentatively identified Lactic acid bacteria strains were allocated to 2 groups namely, S1 and S2 as shown in Table 1 and Table 2. *L. acidophilus* strains represented group S1 (Image 1) while *L. bulgaricus* was the lactobacilli species in group S2 (Image 2), respectively.

### 3.2. Biochemical evaluation of Strawberry pulp (15%)

The strawberry fruit used to prepare yoghurt was analysed for its carbohydrate, protein, and antioxidant content as shown in Table 3. Generally, fruits contain minimal level of fat. So, the addition of strawberry pulp might have increased the fat % slightly in the yoghurt. Although, the protein content in strawberry pulp was significant (0.108 mg/ml). The presence of reducing sugar in strawberry (0.115 mg/ml) may have increased the carbohydrate content of the yoghurt which was still very less as compared to its counterpart. The antioxidant value of the strawberry was significantly high (78.8%) which is why it is considered as a rich source of antioxidant.

### 3.3. Biochemical evaluation of yoghurts

The pH values of 2 yoghurts (S1, S2) ranged from 4.5 to 5.0 after the end of fermentation, decreasing further during the storage period. An important characteristic of a probiotic is its survival at low pH [20]. Highest acidity

4.0-4.5 was recorded in market yoghurt just after the retail purchase. The reason for increased acidity was that when liquid whey is removed, the resulting yoghurt has thicker consistency and has a more tart taste (more acidic) than unstrained yogurt [21]. Similarly, the commercialised yoghurt has lower moisture content (11.52%) than the S1 and S2 yoghurts (18.4 % each) as presented in table 4.

According to the data in table 4, total soluble solids (TSS) percent in prepared samples S1 and S2 are 81.6% and 81.7% respectively, lower than the market yoghurt (88.4%). Increasing milk total solids from 16 to 23 g/100 g enhanced the growth of *Lactobacillus bulgaricus* [22]. So, high TSS might aid in improving the number of lactobacilli in the yoghurts. The moisture content of fermented milk product is measured for shelf-life, lower the moisture content maximum the shelf-life. The moisture content of the samples (S1 and S2) is higher (18.4% each) than market fruit yoghurt (11.5%). Since moisture content and shelf-life are inversely proportional, market yoghurt should have higher shelf-life.

Chemical composition (fat, protein and carbohydrate) of samples S1 and S2 is shown in Table 4. Because of the higher proportion of fat (4%, 5%) and protein (0.187 mg/ml, 0.280 mg/ml) and lower proportions of carbohydrates (5.51 mg/ml, 5.96 mg/ml) in the laboratory samples, prepared strawberry flavored yoghurt has more nutritional values in comparison with market yoghurt (2% fat, 0.145 mg/ml protein and 9.60 mg/ml carbohydrates content). Fat content influence flavours and thus, the taste of the product. Furthermore, the digestibility of fat is improved during fermentation and in the present study prepared strawberry yoghurt have higher fat content which will have good digestibility compared with the market yoghurt. More than 3.25% of fat content in the yoghurt samples should be labelled as fat containing yoghurt [23]. Additionally, the probiotic prepared fruit yoghurt has lower lactose content which can be tolerated by lactose intolerant people and thus, will improve digestibility and boost immunity with its well-known probiotic effects.

Overall, the highest value for the antioxidant activity presents the yoghurt (S1) prepared with *Lactobacillus acidophilus* (66.10%) and the yoghurt (S2) with the starter culture of *Lactobacillus bulgaricus* (57.74%) whereas the market yoghurt was recorded with the lowest value (19.45%). Additionally, the concentration of antioxidants in market yoghurt was measured after many days of fermentation in comparison to the samples (S1 and S2) which recorded the highest values right after the

fermentation. The difference of the values for the antioxidant activity of samples may also be dependent on the conditions in the laboratory during the experiments- such as sensitivity of instruments used, the sanitary conditions in the laboratory and the conditions under which the fermentation takes place.

The shelf-life of the yoghurts were evaluated and the results showed no growth of *Salmonella sps.* in all the three samples.

**Table 1: Morphology characterization of isolated Lactobacilli bacteria**

S. No	Colony Morphology	Result
1.	Motility	Non- motile
2.	Gram's reaction	+ve
3.	Cell shape	rod
4.	Pigment	Cream white
5.	Spores	-ve
6.	Surface	mucoid
7.	Elevation	flat

**Table 2: Biochemical tests of isolated Lactobacilli bacteria**

S. No.	Biochemical tests	Bacteria S1	Bacteria S2
1.	Starch hydrolysis test	+ve	+ve
2.	Caesin test	+ve	+ve
3.	Catalase	-ve	-ve
4.	Citrate test	-ve	-ve
5.	Urease test	-ve	+ve
6.	Dextrose test	+ve	+ve
7.	Sucrose test	+ve	-ve
8.	Lactose test	+ve	+ve
9.	Maltose test	+ve	-ve
10.	D-Mannitol test	+ve	-ve
11.	Methyl red test	-ve	-ve
12.	Voges Proskauer test	-ve	-ve

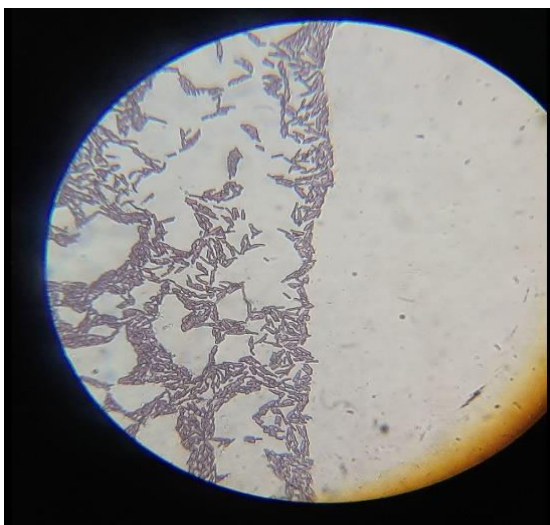
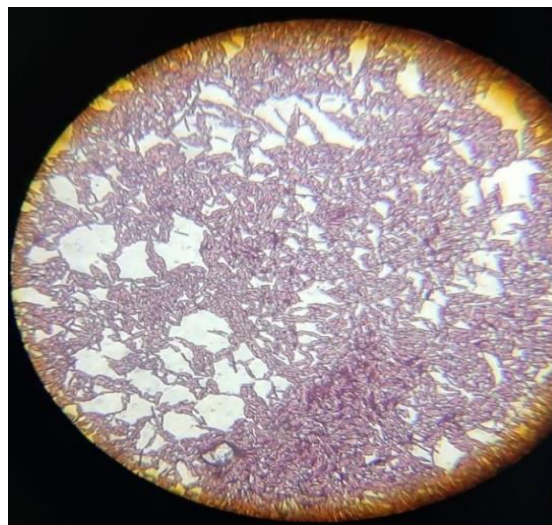
-ve= negative, +ve= positive

**Table 3: Summary of biochemical evaluation of strawberry sample**

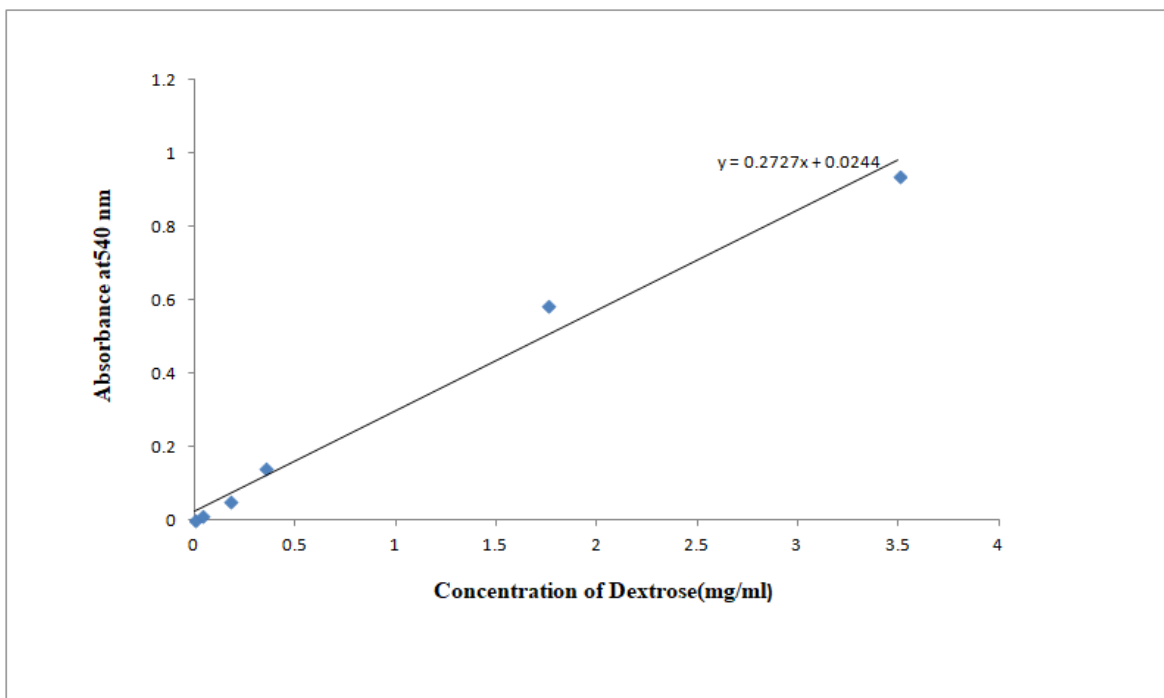
Chemical parameters	Strawberry fruit
Protein (mg/ml)	0.108
Reducing sugar (mg/ml)	0.115
Antioxidant (%)	78.8

**Table 4: Summary of the result of Biochemical evaluation of different types of yoghurt samples**

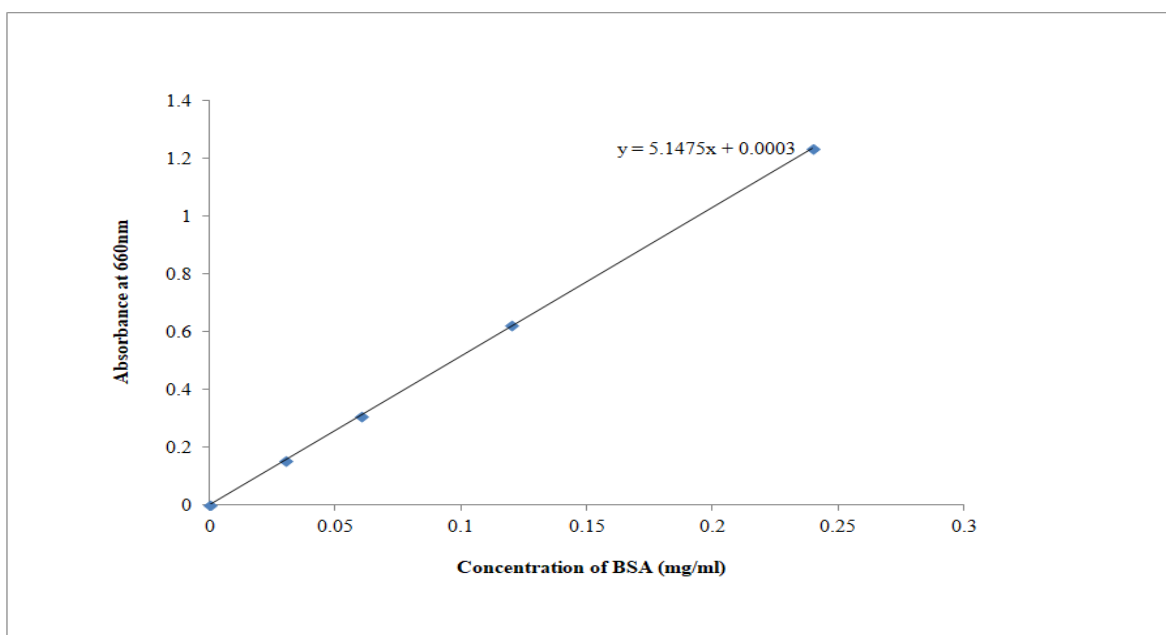
Chemical parameters	Yoghurt S1	Yoghurt S2	Market yoghurt
pH	4.5-5.0	4.5-5.0	4.0-4.5
TSS (%)	81.6	81.7	88.4
Moisture (%)	18.4	18.2	11.5
Fat (%)	4	5	2
Protein (mg/ml)	0.187	0.280	0.145
Reducing sugar (mg/ml)	5.51	5.96	9.60
Antioxidant (%)	66.10	57.74	19.45

**Image 1- *Lactobacillus acidophilus*****Image 2- *Lactobacillus bulgaricus***

S1 and S2 tentatively identified as *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, respectively.



**Graph 1: Calibration curve for standard (Glucose) graph of carbohydrates at different concentration based on spectrophotometrically assayed at 540nm**



**Graph 2: Calibration curve for standard (BSA) graph of Protein at different concentration based on spectrophotometrically assayed at 660 nm**

#### 4. CONCLUSION

Present findings revealed that there were differences in physical and chemical properties of prepared strawberry fruit yoghurt compared to commercialised strawberry fruit yoghurt. The strawberry fruit additive (15% w/v) results in boosting the antioxidant effect in the laboratory prepared fresh yoghurt and its good nutritional value (low carbohydrate and high protein content) makes it more suitable for diabetic people. It is known that certain Lactobacilli species adhere to the gut mucosal surface and in a certain way inhibit the attachment of gram-negative bacteria [24]. This makes yoghurts infused with *Lactobacillus* spp. an excellent healthy choice. In addition, absence of foodborne pathogen in the yoghurt indicates that probiotic properties in yoghurt can be enhanced by using a combination of *Lactobacillus* spp. as commercial starter culture which have increased shelf life and can also be used by lactose sensitive patients. Given the enormous opportunities that exist in the use of LABs as probiotics, the future is indeed promising.

#### Conflicts of interest

None declared

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