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Initial enumeration and viability of probiotic strains in commercial yogurt products under refrigerated conditions

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ABSTRACT

Yogurt is one of the main food products that contain probiotic bacteria. Probiotic bacteria should be of not less than log 8 Colony-forming unit per ml (CFU/ml) in yogurt products to be effective as probiotic product. The objectives of this study were to investigate the initial enumeration and the viability of probiotic bacteria in the yogurt marketed in Saudi Arabia under refrigerated storage conditions. Twenty yogurt samples were collected and stored in 4°C, and then enumeration was conducted using the specific growth agar for the probiotic bacteria. In addition, pH measurements and water holding capacity were conducted. The results of this study showed that the initial enumeration of both *S. thermophilus* and *Lactobacillus delbrueckii* spp. Bulgaricus was higher than log 8 CFU/ml in all the commercial products studied. However, the initial enumeration of bifidobacteria was considerably low (0.8-4.5 log CFU/ml) in all the commercial products studied. Viability of both *S. thermophilus* and *Lactobacillus delbrueckii* spp in all commercial products slightly decreased during the 4-week refrigeration period. However, the viability of bifidobacteria in the commercial products was slightly decreased up to 3 weeks of recreation. In week 4, the viability of bifidobacteria in all the commercial products studied was drastically decreased. Different techniques should be adopted to improve the initial enumeration of bifidobacteria in commercial yogurt products and to improve its viability during storage conditions.

1. Introduction

Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Gilliland et al., 2001). One of the main products that contain probiotic bacteria is fermented dairy products. To be considered as probiotics, fermented dairy products should contain a minimum level of live and active cultures at the time of consumption. Studies proposed a minimum level of 8 log CFU/ml of the probiotic to confer the health benefits on the human (Shah, 2000). Nevertheless, the viability of probiotics in fermented dairy products is affected by several factors, such as oxygen and acid content (Talwalkar and Kailasapathy, 2004; Choi and Lim, 2019) and the presence of additives and thickening material (Antunes et al., 2005; Allgeyer et al., 2010). Many dairy products claim to have live cultures of probiotics. However, there are differences among the commercial brands in the species of probiotic bacteria, as well as the amount. A study by Ibrahim and Carr (2006) demonstrated that only 76% of the tested yogurt samples in North Carolina, USA, contained viable cultures. Most yogurt products studied by Ibrahim and Carr (2006) showed that viable bifidobacteria cultures were below the level declared by the commercial label. This might lead to not having the suggested health claims, especially in the GI tract, for humans consuming yogurt products. Several studies showed that there are differences among the initial enumeration of probiotics among the commercial dairy products (Shah, 2000; Ibrahim and Carr, 2006; Chou et al., 2019) and viable counts of yogurt varied from different commercial products.

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It seems that the most critical period for the viability of bacteria in yogurt products is during the storage period (Gilliland et al., 2002; Ibrahim and Carr, 2006; Mortazavian et al., 2006; Damin et al., 2008; Naji et al., 2014). Since there are several factors that might affect the viability of probiotic bacteria in yogurt products at refrigerated conditions, there is a need to study the enumeration of bacteria in the local products in Saudi Arabia. The objectives of this study were to examine the initial enumeration of probiotic bacteria and to test the viability of probiotics in the commercial yogurt products of Saudi Arabia at refrigerated conditions (4°C). This will lead to further understanding of how to improve the viability of probiotics in yogurt products at refrigerated conditions, so they might have the putative health effect for humans.

2. Materials and methods

2.1. Sampling

Twenty yogurt product samples from three different local commercial brands were selected from the local market located in Ha'il, Saudi Arabia, to identify the different genera and viability of probiotic bacteria. Triplicate samples were taken from each product. In order to prevent bias toward a particular product, random codes were assigned for samples. Samples were stored at 4 ± 0.5°C during the duration of the study. The total number of products was 60.

2.2. Enumeration experiment

Enumeration of probiotic strains of bacteria was conducted using the specific growth agar according to the method adapted from Ibrahim and Carr (2006). Lactobacilli MRS (Difco, Detroit, MI, USA) and glucose M 17 (Difco) were used for enumeration of *Lactobacillus* and *Streptococcus* species, respectively. Modified BIM-25 was used for the enumeration of bifidobacteria (Ibrahim and Salameh, 2001). The inoculated plates were then incubated for 72 h at 37°C in anaerobe chambers, and colonies were counted using a Quebec colony counter (Fisher Scientific Hampton, New Hampshire, United States).

2.3. Measurement of pH and water holding capacity

The pH of the yogurt samples was measured using a pH meter (model MP120 Meter, Mettler Toledo, Switzerland). Water-holding capacity was determined according to the method of Isanga and Zhang (2009), with slight modifications. Yogurt samples (100 g) were centrifuged (10,000 rpm; Fisher Scientific Hampton, New Hampshire, United States) for 15 min at 4°C. A graduated cylinder was used to measure the amount of the collected supernatant. Water-holding capacity (WHC) was presented as the percentage of the weight of the total sample of yogurt minus the weight of supernatant divided by the weight of the total sample. This is presented in the following equation: [((weight of the total sample-weight of the supernatant)/ (weight of the total sample)) × 100].

2.4. Viability experiment

We studied the viability of probiotic bacteria in the refrigerated (4°C) yogurt samples at 0, 1, 2, 3, and 4 weeks. Yogurt samples were aseptically removed from the stored samples, and each sample was diluted by mixing 11 g of each sample into 99 ml of sterile 0.1% peptone water. Selective media, as mentioned before of the perspective yogurt probiotic cultures, were used for the enumeration of the probiotic bacteria in the stored yogurts samples.

3. Results and discussion

3.1. Results

The average initial bacterial count of the different probiotic bacteria in the three commercial yogurt brands in Saudi Arabia is shown in Fig. 1. The initial enumeration of both *S. thermophilus and L. delbrueckii* spp. bulgaricus were comparable (>8 log CFU/ml) in all brands. However, the initial enumeration of bifidobacteria in all studied brands was lower than the other two types of bacteria. Results of our study showed that the average initial enumeration of *S. thermophilius* in the yogurt samples of the local market of Saudi Arabia was 8.61 log CFU/ml (8.2-9.3) for brand 1; 8.1 log CFU/ml (7.1-8.9) for brand 2; and 8.23 log CFU/ml (8.1-8.6) for brand 3 (Table 1). The average initial enumeration of *L. delbrueckii* spp bulgaricus in the yogurt samples in Saudi Arabia was 8.2 log CFU/ml (7.4-8.6) for brand 1; 8.0 log CFU/ml (7.5-9.0) for brand 2; and 8.3 log CFU/ml (8.0-8.7) for brand 3. However, the results of this study showed that the average initial enumeration of bifidobacteria was 2.3 log CFU/ml (1.0-3.1), for brand 1; 2.6 log CFU/ml (0.8-4.5) for brand 2; and 4.3 log CFU/ml (4.1-4.5) for brand 3.

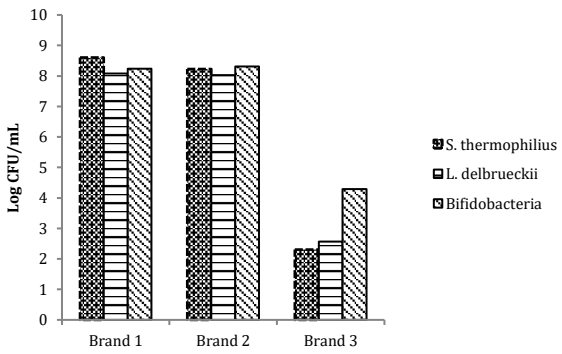

Fig. 1: Average Initial bacterial counts (log CFU/ml) of *L. delbrueckii* spp. bulgaricus, *S. thermophilus*, and bifidobacteria in three different commercial yogurt brands in Saudi Arabia

Table 1: Initial enumeration (log CFU/ml) of different probiotic bacteria in yogurt, pH and water-holding capacity

Type	*S. thermophilius*	*L. delbrueckii*	bifidobacteria	pH	WHC (%)
			Brand 1		
B1-Greek full fat	8.5	8.2	2.6	4.8	38.9
B1-Greek with strawberry	8.2	8.4	3.1	4.7	34.6
B1-Full fat	9.1	8.6	2.5	5.1	57.4
B1-Low fat	8.3	7.6	1.5	4.9	51.3
B1-Skimmed	8.4	8.6	1.0	4.3	42.3
B1-Full fat with cream	8.5	8.1	2.5	5.0	31.2
B1-Syrian	8.4	8.4	2.0	3.9	23.6
B1-Special	9.3	8.1	3.1	4.9	27.9
Average	8.6±0.4	8.2±0.4	2.3±0.7	4.7±0.4	38.4±11.6
			Brand 2		
2-ates	7.1	7.5	4.1	4.6	11.2
2-Full fat	8.6	8.5	4.5	5.0	42.6
2-Low fat	8.2	8.1	2.1	4.5	32.2
2-Skimme	7.6	8.5	0.8	4.9	24.1
2-Greek	8.9	8.9	1.9	4.1	18.5
2-Greek Strawerry	8.6	8.5	2.1	4.7	13.5
2-Greek mixe erries	7.8	7.1	2.7	3.9	15.4
2-Fruit Yoghurt	8.2	7.0	2.4	3.8	15.3
2-Peah an Apriot	7.7	8.1	2.7	4.5	18.7
Average	8.1±0.6	8.0±0.7	2.6±1.1	4.4±0.4	21.3±10.1
			Brand 3		
3- Whole milk	8.1	8.0	4.1	4.7	20.0
3-Low fat	8.4	8.7	4.5	4.9	21.5
Average	8.2±0.1	8.3±0.3	4.3±0.2	4.8±0.1	20.8±0.8

Our results showed that the average pH of yogurt products was 4.7 (3.9-5.1), for brand 1; 4.4 (3.8-5.0) for brand 2; and 4.76 (4.7-4.9) for brand 3 (Fig. 1). There were discrepancies in the results of water holding capacity among commercial brands and among the different flavors of the same brand. The average water holding capacity for yogurt products in Saudi Arabia was 38.40% (23.6-57.4%) for brand 1; 21.3% (11.2-42.6) for brand 2; and 20.8% (11.2-42.6) for brand 3. Average pH and WHC% of the three commercial brands are shown in Fig. 2.

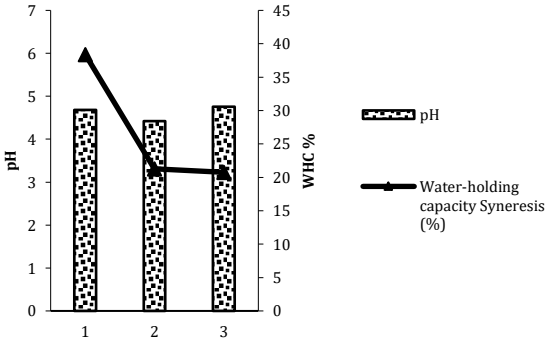

Fig. 2: Average pH and water holding capacity (WHC, %) of the three different commercial yogurt brands located in the local market of Saudi Arabia

The results of our study showed that there was a slight decrease in the viability of *S. thermophilus* in brand 1 of commercial yogurt products of Saudi Arabia at refrigerated conditions during the 4-week period (Fig. 3). Also, the enumeration of *S. thermophilus* in brand 1 showed a slight decrease from 8.5 log CFU/ml in week 1 to 8.2 log CFU/ml in week 4. In addition, there was a slight decrease in the viability of *L. delbrueckii* spp. bulgaricus from 8.1 log CFU/ml in week 1 to 7.7 log CFU/ml in week 4. The viability of bifidobacteria in the different yogurt products followed the same trend as in the previous two bacterial species up to week 3 of refrigeration. The viability of bifidobacteria in brand 1 showed a slight decrease from week 1 (2.3 log CFU/ml) to week 3 (2.2 log CFU/ml); however, the viability of bifidobacteria showed a drastic decrease (0.6 log CFU/ml) at week 4 (Fig. 3). For brand 2, there was a slight decrease in the viability of the different bacterial species of commercial products of yogurt, during the 4-week period, at refrigerated conditions (Fig. 4). However, the viability of bifidobacteria slightly decreased from 2.6 to 1.9 from week 1 to week 3, respectively. Interestingly, at week 4 of refrigeration, no bifidobacteria cultures (0.0 CFU/ml) were observed in the samples of brand 2. The same trend that was observed in brand 1 and 2, also observed in brand 3 for *S. thermophilus* and *L. delbrueckii* spp. bulgaricus (Fig. 5). There was a slight decrease in the viability of *S. thermophilius* from 8.1 to 8.0 log CFU/ml in the refrigerated samples of brand 3, and a decrease in the viability of *L. delbrueckii* spp. bulgaricus from 8.3 to 8.1 log CFU/ml, from week 1 to week 4 of refrigerated samples. For bifidobacteria, the slight decrease in the viability was shown from week 1 to week 3 (4.3 to 3.9 log CFU/ml, respectively). However, the viability of bifidobacteria in the refrigerated samples of brand 3 at week 4 was very low (0.3 log CFU/ml).

3.2. Discussions

Our results showed that the results of the initial enumeration for both *S. thermophilus* and *L. delbrueckii* spp. bulgaricus among the three different commercial brands of yogurt in Saudi Arabia were comparable. The initial enumeration for both the aforementioned species of bacteria was higher than 8 log CFU/ml, which is higher than the proposed bacterial count by Shah (2000) as the minimum level of the probiotic to confer the desired health benefits.

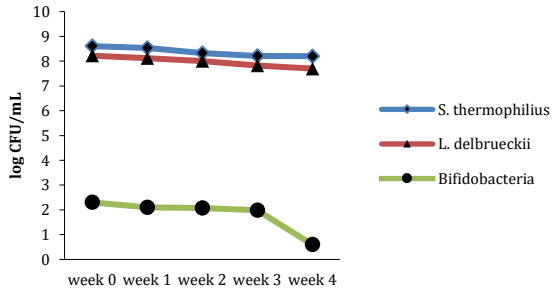

Fig. 3: Average viability of *S. thermophilus, L. delbrueckii* spp. bulgaricus, and bifidobacteria in brand 1 commercial product of yogurt located in the local market of Saudi Arabia, during 4 weeks of refrigerated storage at 4°C

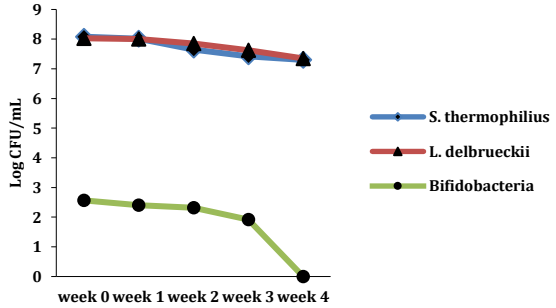

Fig. 4: Average viability of *S. thermophilus, L. delbrueckii* spp. bulgaricus, and bifidobacteria in brand 2 commercial product of yogurt located in the local market of Saudi Arabia, during 4 weeks of refrigerated storage at 4°C

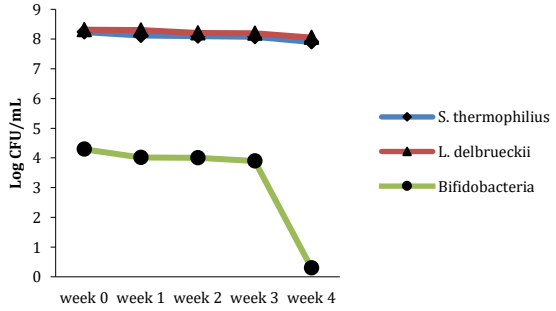

Fig. 5: Average viability of *S. thermophilus, L. delbrueckii* spp. bulgaricus, and bifidobacteria in brand 3 commercial product of yogurt located in the local market of Saudi Arabia, during 4 weeks of refrigerated storage at 4°C

However, the initial enumeration of bifidobacteria showed differences among the brands and within the same brand. The low initial enumeration of bifidobacteria in yogurt might suggest a difficulty in the survivability of bifidobacteria in commercial yogurt products. In addition, the striking differences among brands and within the same brand, in respect to the enumeration of bifidobacteria, might suggest the sensitivity of bifidobacteria to the type and condition of yogurt products, especially acidic pH conditions (Takahashi et al., 2004; Shafiee et al., 2010). Bifidobacteria is sensitive to the change in culture and would have low viability in yogurt products during refrigerated storage.

Therefore, special techniques should be adopted to maintain good viability of bifidobacteria in yogurt samples during refrigeration. This could be attained by the method of microencapsulation, as suggested by Adhikari et al. (2000). Another study by Adhikari et al. (2003) showed that microencapsulation of bifidobacteria in refrigerated yogurt samples would lead to a stable cell population. In comparison, the nonencapsulated bifidobacteria in the aforementioned study showed a drastic decrease in the cell population. Other studies suggested the use of the immobilization technique of bifidobacteria to overcome the acidic conditions in yogurt (Sun and Griffiths, 2000).

One study used wheat bran as a cell immobilizer to enhance the viability of probiotics (Terpou et al., 2017). Another method is used to entrap bifidobacteria in a matrix of poly (tetrafluoroethylene) (PTFE) fibrils (Hyde et al., 1991). Ibrahim and Carr (2006) suggested adding a higher level of the probiotic bacteria to the commercial products to solve the issue of low viability of probiotic products in yogurt. Dave and Shah (1997) showed that the addition of some nutrient ingredients such as cysteine, whey protein, and acid casein hydrolysates could improve the viability of probiotic bacteria in yogurt products. Other studies showed that the addition of prebiotics such as inulin, oat bet glucan, oligo-fructo saccharides, and plant extracts, would increase the viability of yogurt products (Lamoureux et al., 2002; Akin et al., 2007; Rosburg et al., 2010; Michael et al., 2010; Shima et al., 2012).

Our results indicated discrepancies in the results of pH and WHC in the yogurt products, which might be due to the effect of different flavors and additives such as thickening agents. This, in turn, would affect the viability of the different species of bacteria in yogurt (Antunes et al., 2005; Allgeyer et al., 2010). Our results indicated that both *S. thermophilus* and *L. delbrueckii* spp. bulgaricus could tolerate refrigerated conditions of yogurt regardless of the brand and flavor. However, bifidobacteria can tolerate refrigerated conditions of yogurt up to week 3 of refrigeration. In week 4, the viability of bifidobacteria decreased drastically in all brands and flavors studied.

4. Conclusion

Our results showed that the initial enumeration of both *S. thermophilus* and *L. delbrueckii* spp. bulgaricus in refrigerated yogurt samples was better than that of bifidobacteria. In addition, *S. thermophilus* and *L. delbrueckii* spp. bulgaricus could tolerate refrigerated conditions of yogurt during the study period (4 weeks). However, bifidobacteria can tolerate refrigerated conditions of yogurt up to week 3; after that, the viability of bifidobacteria deteriorated. Therefore, certain techniques should be used to extend the viability of bifidobacteria in refrigerated conditions of yogurt such as microencapsulation, immobilization or addition of prebiotics.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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