Influence of Prebiotics and Bacteriocin on Yogurt Quality

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Abstract

This study determined the quality of yogurt fortified with 2% inulin, 2% Fructo-oligo-saccharides (FOS), and Lactobacillus acidophilus with their bacteriocins. The results indicated that coagulation time increased by 3.29–22.75% in yogurts prepared with Lactobacillus acidophilus and their bacteriocins compared to the control also titratable acidity increased gradually in all groups during storage. The sensorial properties showed the superiority of inulin, FOS, and Lactobacillus acidophilus bacteriocin groups. *Lactobacillus bulgaricus*, *Streptococcus thermophiles*, and *Lactobacillus acidophilus* count increased in the treatments compared to the control group incorporated with an extended shelf life to 39 days of storage in the treatments containing lactobacillus acidophilus bacteriocin. Coliforms, Moulds, and yeasts did not detect in the group containing 2% inulin, 2% FOS, and lactobacillus acidophilus bacteriocin for 21 days of refrigerated storage. This study proved that 2% inulin, 2% FOS, and Lactobacillus acidophilus bacteriocin fortification extended the shelf life for 6 weeks.

Novelty Impact Statement

This work provides extending yogurt shelflife to 6 weeks compared with the conventional time of 14 days, also enhances the sensorial properties by adding (2%) of inulin and FOS plays the double effect as a texture enhancer prebiotic reflecting on the consumer's health and satisfaction.

1. Introduction

Yogurt considers the most common fermented dairy product widely consumed worldwide because of its high nutritional and therapeutic value. In addition, yogurt represents an excellent carrier of probiotic microorganisms (Pandey and Mishra, 2015; Shah, 2017). Probiotics are living microorganisms that provide superior health benefits to the host when taken in specific quantities and should be present in the products in specific viable counts and stay alive during shelf life. It is recommended that the minimum dose of probiotics to keep its therapeutic properties should be not less than 6 log CFU/g (Konar et al., 2018). As food demand rises, bio-preservation becomes increasingly essential; lactic acid bacteria were mainly used in fermented dairy products as starter cultures, producing an antimicrobial substance known as bacteriocin. Several lactic acid bacteria are used as probiotics, including *Lactobacillus lactis, Lactobacillus fermentum, Lactobacillus acidophilus and Lactobacillus reuteri* (Giraffa et al., 2010). They produce various metabolites such as organic acids, hydrogen peroxide, antifungal compounds, and bacteriocins (Ogueke et al., 2014; Anjum et al., 2014). Bacteriocins are ribosomal synthesized, extracellular bioactive peptides that have a bactericidal or bacteriostatic effect against many foodborne pathogens and spoilage bacteria in food (Hassan et al. 2020). The combination of probiotics and prebiotics will enhance the beneficial effects, and their inclusion in yogurt will produce a synbiotic food that can improve some disorders via stimulating antioxidant capacity and enzymes activities (Kleniewska et al. 2016; Qian et al. 2020). Prebiotics are non-digestible food compounds that beneficially affect the host by selectively stimulating probiotics' growth and/or activity. Fructo-oligo-saccharides (FOS) and inulin are among the most known prebiotic compounds (Fazilaha et al., 2018). Peshev and Van Den Ende

(2014) found that FOS and inulin help to improve the bioavailability of various essential minerals such as calcium, magnesium, and iron absorption from the diet, decreasing blood lipid concentrations, lowering blood cholesterol and triacylglycerol (Miremadi et al. 2016). Furthermore, inulin facilitates the digestion of high protein diets, retards fat absorption, provides roughage preventing constipation, remains in the digestive tract, provides satiety without carrying extra calories, and decreases the incidence of colon cancer (Aryana et al. 2007). They stimulate the immune system by producing IgA and modulation cytokine, and enhancing host defenses (Lomax et al., 2009). Aside from the prebiotic activity, they also act to replace fats and sugars and stabilize emulsion and foam without altering the structure and sensory properties of the product (Srisuvor et al., 2013; Gonzalez-Herrera et al., 2015). Because of the increased consumer demand for natural preservatives, this study aimed to investigate the effects of fortification with inulin, Fructo-Oligo-Saccharides, and *Lactobacillus acidophilus* and their bacteriocin on the shelf-life and quality of yogurt.

2. Materials And Methods

2.1. Materials

Fresh raw cow and buffalo milk were obtained from the herd of the Faculty of Veterinary Medicine, Menofia University, Egypt. Lyophilized strain of *Lactobacillus acidophilus* DSMZ 20079, *Lactobacillus bulgaricus* (*Lb. bulgaricus*), and *Streptococcus thermophilus* were obtained from Cairo-MIRCEN (Microbiological Resource Center) Faculty of Agriculture, Ain Shams University, Cairo, Egypt. MRS broth (De Man, Rogosa, and Sharp) was obtained from Biolife, Italy. Inulin was obtained from Baolingbao Biology Co., Ltd., Yucheng City, Shandong, China. Fructo-oligo-saccharides (FOS) (Orafti®P95) were obtained from Belgium.

2.2. Methods

2.2.1. Activation of lactobacillus strain

Lyophilized strain of *Lactobacillus* (Lb) *acidophilus* DSMZ 20079 was activated on MRS broth at 37°C for 24 hrs (Okuro et al., 2013).

2.2.2. Bacteriocin Preparation

The activated cultures from each strain were separately inoculated on MRS broth (1 L) under aseptic conditions and incubated at 37°C for16 hrs. After incubation, cultures were heated in a water bath at 100°C for 5 min to remove H2O2, and the cells were harvested by two successive centrifugations at 10,000 rpm for 20 min at 4ºC. The supernatants were collected and the pH neutralized to 7 by 1N NaOH. The extract was then sterilized using a single sheet 0.45 µm pore size Seitz filter to obtain cell-free crude bacteriocin (da Silva Sabo et al., 2015).

2.2.3. Yogurt Manufacture

Lyophilized mixed starter cultures containing *Lactobacillus bulgaricus* (*Lb. bulgaricus*) and *Streptococcus thermophilus* (1:1) were added to sterile 11% reconstituted skimmed milk powder and incubated at 37°C for 24 hrs. The active cultures were kept in the refrigerator used within 24 hrs and mixed at the percentage (1: 1). Milk fat was separated using a separator to obtain skimmed milk.

The total volume of skim milk was divided into 5 groups (2 L of each), two probiotics, and two bacteriocins yogurt samples supplemented with 2% inulin + 2% FOS then mixed well (Akalin and Erişir, 2008; Oliveira et al., 2011). The skim milk was heated to 85°C for 30 min and immediately cooled to 45°C, then inoculated by the activated starter cultures (*Lb. bulgaricus* and *Streptococcus thermophilus*) and divided to produce several groups as follows:

C: 2% yogurt starter cultures 1:1 (control).

T2: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T4: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T5: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

Samples from each group were then mixed, put into sterile cups (100 ml), and incubated at 42°C until curd formation, then kept in a refrigerator at 4°C.

2.2.4. Yogurt Analysis

2.2.4.1. Coagulation time and Titratable acidity determination

The coagulation time for each yogurt group was calculated from incubating the samples in an incubator until the formation of curd (Hassan and Amjad, 2010). Titratable acidity (as % of lactic acid) was determined using the method described in AOAC (2000). The yogurt preparation and examination were repeated three times.

2.2.4.2. Microbiological Evaluation

Bacterial counts were obtained after serial dilution (APHA, 2001). *Lb. acidophilus, Lb. bulgaricus* and *Streptococcus thermophiles* were enumerated using the pouring plate method (Kodaka et al., 2005). Enumerations of *Lb. acidophilus* was carried out in MRS agar under the anaerobic condition at 37°C for

48 hrs. *Lb. bulgaricus* was counted in MRS agar (pH 5.4) under aerobic incubation at 37°C for 48 hrs, and *Streptococcus thermophilus* was counted on M17 agar at 42°C for 48 hrs (Santo et al., 2012). Sabouraud Dextrose agar medium supplemented with chloramphenicol (0.01%) was used for the determination of total mold and yeast counts after incubation at 25°C for 5–7 days (IDF, 1990).

2.2.4.3. Sensory Evaluation

The sensory evaluation of the yogurt samples was carried out according to Mehanna et al. (2000). The scores used were 60 points for flavor, 30 points for body and texture, and 10 points for appearance, with an overall score of 100.

2.3. Statistical Analysis

Statistical analyses were performed using the one-way analysis of variance in SPSS 16.0. The results were considered significantly different, with $p < 0.05$ as Clarke and Kempson (1997) described.

3. Results And Discussion

3.1. Coagulation time and Titratable acidity

Coagulation of milk appears as a result of the precipitation of milk protein (casein) in acidic conditions at a pH of around 4.6 (Lee and Lucey, 2010). In Table (1), the treatment T4 which prepared by 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS has the longest coagulation time (4 hr:10 min) with increasing percentage by 22.75% followed by treatment (T3) prepared by 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin (4 hrs: 5 min) increasing percentage by 21.25%, than treatment (T2) prepared by 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS (3hr :50 min) increasing percentage by 4.79%. The lowest coagulation time was recorded for treatment (T1) which was prepared by 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* (3 hr: 45 min) with an increasing percentage of 3.29% compared to control treatment (coagulation time of control was 3.29). The variation in the coagulation time may be attributed to the antibacterial activity of probiotic bacteria and prebiotics (inulin and FOS) on the starter culture of yogurt and subsequent acid production (Akabanda et al., 2014).

Table 1
changes in coagulation time among different groups of yoghurt

Yogurt samples	Coagulation time	
	(Hr : min)	Increase (%)
C	3:34	0.00
T1	3:45	3.29
T 2	3:50	4.79
T 3	4:05	21.25
T 4	4:10	22.75

C: 2% yogurt starter cultures 1:1 (control).
T1: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T2: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T4: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

During refrigerated storage (4ºC), the titratable acidity of yogurt samples in all treatment groups increased gradually as the storage period progressed (Table 2). The addition of inulin and (FOS) insignificantly affected the titratable acidity of yogurt samples. On the other hand, the addition of inulin and FOS increased the shelf life to 39 days in group 4 which was prepared by 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS compared to control treatment (C) which spoiled at 21 day of refrigerated storage. Treatment T2 which prepared by 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS and T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin spoiled at 32 days of refrigerated storage. While treatment T1: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* was spoiled at 28 days of refrigerated storage. EOSQ (2005) stated that the titratable acidity of yogurt should not increase by 1.50%. Guven et al. (2005) found that yogurt's titratable acidity was not affected by the addition of different ratios of inulin, and it was increased during storage.

Table 2
Titratable acidity (% lactic acid) in yoghurt samples during storage

Storage (Day)	Titratable acidity (Mean ± S. E*)				
	C	T_1	T_2	T_3	T_4
Zero	0.84 ± 0.02^{A}	0.80 ± 0.04^{A}	0.79 ± 0.01^{B}	0.75 ± 0.03^{B}	0.72 ± 0.01^{C}
7	0.95 ± 0.03^{A}	0.97 ± 0.03^{A}	0.95 ± 0.03^{A}	0.83 ± 0.02^{B}	0.79 ± 0.01^{C}
14	1.23 ± 0.03^{A}	1.10 ± 0.02^{B}	1.06 ± 0.05^{BC}	0.90 ± 0.01^{C}	0.85 ± 0.00^{D}
21	S	1.16 ± 0.03^{A}	1.14 ± 0.02^{A}	1.06 ± 0.03^{B}	0.94 ± 0.02^{C}
28	S	S	1.22 ± 0.03^{A}	1.17 ± 0.03^{B}	1.08 ± 0.02^{C}
32	S	S	1.34 ± 0.03^{A}	1.26 ± 0.05^{B}	1.18 ± 0.3^{C}
39	S	S	S	S	1.20 ± 0.5^{A}
C: 2% yoghurt starter cultures 1:1 (control).					
T1: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus*.					
T2: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.					
T3: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.					
T4: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.					
S: spoilage					
*The values indicated are the mean ± S.E (n = 3). Values in the same raw denoted by different letters (^{ABCD}) differ significantly ($p < 0.05$) from each other.					

3.2. Sensory Evaluation

Yogurt products are beginning increasingly popular throughout the world (Aryana and Olson 2017). In addition to their health benefits, the texture of the product plays an important role in the consumer's acceptance of the product (Vital et al., 2015). The addition of inulin and FOS improved the sensory properties of the resultant yogurt samples. The result revealed that treatment (T2), 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS and treatment (T4), 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS) were the most highest accepted groups regarding to sensory evaluation (Figs. 1, 2 and 3). Our results are in line with those of Srisu et al. (2013), who reported that the addition of prebiotic could improve the physical and sensory properties of the yogurt. On the other hand, Cruz et al. (2010) explored that the addition of inulin may cause changes in yogurt quality attributes due to interactions between the functional ingredient and food matrix components. Inulin may provide yogurt's creamy mouthfeel and sweet taste (Donkor et al., 2007). The use of inulin in yogurt production as carbohydrate fat substitutes can improve the perception of color (Brennan et al., 2008).

3.3. Microbiological Quality

Table (3) shows the growth of *Lactobacillus delbrueckii sub spp bulgaricus* throughout the storage period in different groups. There is a decline in the growth rate of *Lactobacillus delbrueckii sub spp bulgaricus* in all examined samples. Treatment that contain inulin including treatment T2: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS and treatment T4: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS have the highest count at the end of the storage period. The *Lactobacillus delbrueckii sub spp bulgaricus* in treatment (T2) count was 9.27 ± 0.12 CFU/g at 32 days of refrigerated storage, while treatment (T4), the count was 9.52 ± 0.04 CFU/g at 39 days of refrigerated storage.

Table 3
Survival of *Lactobacillus delbrueckii sub spp bulgaricus* ($\log_{10}$ cfu/g) in different yogurt groups during storage

Storage (Day)	Treatments				
	C	T1	T2	T3	T4
Zero	12.75 ± 0.15^{A}	12.75 ± 0.03^{A}	12.65 ± 0.05^{B}	12.73 ± 0.07^{A}	12.71 ± 0.02^{A}
7	10.58 ± 0.04^{D}	11.35 ± 0.01^{C}	12.33 ± 0.05^{B}	12.15 ± 0.11^{B}	12.47 ± 0.09^{A}
14	9.00 ± 0.02^{D}	10.40 ± 0.02^{D}	11.86 ± 0.1^{C}	11.53 ± 0.11^{B}	12.31 ± 0.03^{A}
21	S	9.22 ± 0.03^{D}	11.13 ± 0.09^{B}	10.75 ± 0.06^{C}	12.14 ± 0.05^{A}
28	S	S	10.35 ± 0.04^{B}	10.52 ± 0.04^{B}	11.24 ± 0.05^{A}
32	S	S	9.27 ± 0.12^{C}	10.12 ± 0.03^{B}	10.53 ± 0.11^{A}
39	S	S	S	S	9.52 ± 0.04^{A}

C: 2% yogurt starter cultures 1:1 (control).
T1: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T2: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T4: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

S: spoilage

*The values indicated are the mean ± S.E (n = 3). Values in the same raw denoted by different letters (^{ABCD}) differ significantly ($p < 0.05$) from each other.

At the same time, the growth *of Streptococcus thermophiles* was also declined throughout the refrigerated storage. However, groups containing inulin and FOS have the highest count of *Streptococcus thermophiles*.

The count of *Streptococcus thermophiles* treatment (T2) was 7.65 ± 0.04 CFU/g at 32 days of refrigerated storage, and in treatment (T4), the count was 7.72 ± 0.04 CFU/g at 39 days of refrigerated storage (Table 4). These results agree with those obtained by EL-Nagar et al. (2002), and Akalin et al. (2007) mentioned that inulin could favor the growth and viability of lactic acid bacteria during fermentation or refrigerated storage. Furthermore, inulin concentrations were found to be perfect for stimulating growth and retaining the viability of probiotic cultures in fermented milk (Akalin et al., 2007; Aryana et al., 2007).

Table 4
Survival of *Streptococcus thermophilus* ($\log_{10}$ cfu/g) in different yoghurt groups during storage

Storage (Day)	**Treatments**				
	C	**T1**	**T2**	**T3**	**T4**
Zero	9.77 ± 0.05^{A}	9.54 ± 0.03^{A}	9.76 ± 0.03^{A}	9.62 ± 0.01^{A}	9.73 ± 0.02^{A}
7	8.50 ± 0.05^{C}	9.11 ± 0.03^{AB}	9.24 ± 0.01^{B}	9.35 ± 0.02^{B}	9.55 ± 0.01^{A}
14	7.16 ± 0.06^{D}	8.16 ± 0.03^{C}	9.01 ± 0.01^{A}	8.43 ± 0.01^{B}	9.00 ± 0.01^{A}
21	S	7.24 ± 0.05^{C}	8.87 ± 0.03^{A}	8.01 ± 0.03^{B}	8.73 ± 0.03^{A}
28	S	S	7.98 ± 0.03^{B}	7.68 ± 0.04^{C}	8.15 ± 0.04^{A}
32	S	S	7.65 ± 0.04^{B}	6.63 ± 0.04^{C}	7.78 ± 0.04^{A}
39	S	S	S	S	7.72 ± 0.04^{A}

C: 2% yogurt starter cultures 1:1 (control).
T1: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T2: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T4: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

S: spoilage

*The values indicated are the mean ± S.E (n = 3). Values in the same raw denoted by different letters (^{ABCD}) differ significantly ($p < 0.05$) from each other.

Table 5 shows the mean *Lb. acidophilus* counts for the yogurt samples examined. *Lb.acidophilus* counts declined throughout the storage period in T1 (yogurt with *Lb. acidophilus*) and T2 (yogurt with *Lb. acidophilus*, 2% inulin, and 2%FOS) with the final counts of *Lb. acidophilus* was 7.54 ± 0.26 CFU/g at day 21 and 7.32 ± 0.26 CFU/g at day 32 of storage in T1 and T2, respectively. The addition of inulin effectively increased the *Lb. acidophilus* mean count when compared to the group that contains *Lb. acidophilus* without inulin. These results are in agreement with those obtained by Donkor et al. (2007c), Buriti et al. (2010), Hernandez-Hernandez et al. (2012) they found that prebiotic ingredients such as inulin and FOS may exert a protective effect and improve the survival and activity of probiotic bacteria during storage of probiotic food products as well as during the passage through the GIT.

Table 5
Survival of *Lactobacillus acidophilus* ($\log_{10}$ cfu/g) in different yogurt groups during storage

Storage (Day)	**Treatments**	
	T1	**T2**
Zero	10.49 ± 0.11^{A}	11.65 ± 0.08^{A}
7	9.68 ± 0.06^{B}	11.18 ± 0.06^{AB}
14	8.43 ± 0.06^{C}	9.43 ± 0.18^{B}
21	7.54 ± 0.26^{D}	8.84 ± 0.26^{C}
28	S	7.52 ± 0.26^{D}
32	S	7.32 ± 0.26^{D}
39	S	S

C: 2% yoghurt starter cultures 1:1 (control).
T1: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T2: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T3: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T4: 1% yoghurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

S: spoilage

*The values indicated are the mean ± S.E (n = 3). Values in the same column denoted by different letters (^{ABCD}) differ significantly ($p < 0.05$) from each other.

The viability of probiotics was reported to be affected by many factors such as storage time, oxygen content, and fluctuation in temperature, low pH, reduced water activity, and high concentration of salutes (Carvalho et al., 2004 and Capela et al., 2006).

Our study observed that the addition of inulin to probiotic yogurt enhances the growth of *S. thermophiles*, *L. bulgaricus*, and *L. acidophilus* until the end of the storage period compared with groups without inulin. The increase in probiotic counts of yogurt may be attributed to the action of inulin as a prebiotic substance. Akın et al. (2007), Aryana et al. (2007), Sendra et al. (2008) attributed the increase in probiotic counts to the ability of probiotics and yogurt starter cultures to utilize inulin. Similarly, Donkor et al. (2007) showed that chicory-based inulin was a preferred carbon source for probiotic bacteria by increasing the growth performance and maintaining viability during cold storage. Acidity is one of the most critical factors that affect the viability of *S. thermophiles*, *L. bulgaricus*, and *L. acidophilus* (Dave and Shah, 1997). Oligosaccharides as (FOS) act as substrate for the growth of LAB and inhibition of colonic cancer cells growth and putrefactive or pathogenic bacteria present in the colon through the production of short chain fatty acids (SCFA). In addition, strengthening of the gut mucosal barrier, modification of gut microflora, prevention proliferation of pathogen and adherence to intestinal mucosa, and transformation of bacterial enzyme activity (Dma et al., 2009). Rousseau et al. (2005) reported that FOS could stimulate the growth of the beneficial strains but not pathogenic one. The inhibitory effect against pathogenic bacteria is usually as a result to reduction in pH results from acid production, secretion of hydrogen peroxide, and release of natural antibiotics (bacteriocin) from beneficial microflora selectively stimulated by various prebiotics (Manning et al. 2004).

In our study, (T4) yogurt samples appear to be the group that can resist spoilage with yeasts and molds (Table 6). According to EOSQ (2005) molds and yeasts, the count must not exceed 10 cfu/g in yogurt. T4 was in a permissible limit until 28 days of storage with a mean count was 1.00 ± 0.03 CFU/g, while T2 was within the permissible limit until 14 days of storage. T3 was within the permissible limit until 21 days of storage, with a mean count was 1.00 ± 0.05 CFU/g. T1 was within the permissible limit until 14 days of storage, with a mean count was 1.00 ± 0.01. At the same time, the control group, yeast, and mold were not detected in zero day only. These findings are consistent with the findings obtained by Abee et al. (1995), who mentioned that LAB produces a proteinaceous antimicrobial substance known as bacteriocins that generally act through inactivation of enzymes, depolarization of the target cell membrane, or inhibition of the formation of the cell wall of pathogenic microflora including bacteria, mold, and yeast. Probiotic bacteria also produce metabolic substances in varying quantities that may exert their antimicrobial effect by interfering with maintaining the cytoplasmic membrane, inhibiting active transport, and hindering various metabolic functions. Surajit et al. (2016) reported that inulin has antimicrobial activity against pathogenic microorganisms.

Magnusson and Schnürer (2001) and Rouse et al. (2008) mentioned that lactic acid bacteria produced antifungal metabolites that are proteinaceous. *Lactobacillus acidophilus* has the most significant activity against *Aspergillus flavus* and *Aspergillus parasiticus*. In addition, *Lactobacillus acidophilus* has been shown to reduce aflatoxin production during 30 days of storage at room temperature in maize kernels (Elsanhoty, 2008). The antifungal activity appears to be more assertive at lower pH ranges (Rouse et al., 2008); this may explain the antifungal activity of *Lb. acidophilus* bacteriocin in acidic yogurt media. Batish et al. (1990) and Stiles et al. (2002) reported that *Lb. acidophilus* has antifungal activity.

Coliforms counts were not detected in any of the yogurt groups samples on either the first day or during the storage period; this may be attributed to the good hygienic conditions during the preparation and storage of yogurts.

Table 6
Yeasts and moulds count (log $_{10}$ cfu/g) in different yogurt groups during storage

Treatments	**Storage (Day)**						
	Zero	**7**	**14**	**21**	**28**	**32**	**39**
C	ND	2.15 ± 0.01^{B}	3.64 ± 0.04 ^{A}	S	S	S	S
T1	ND	ND	1.00 ± 0.01 ^{B}	2.36 ± 0.03 ^{A}	S	S	S
T2	ND	ND	ND	1.15 ± 0.02^{C}	1.87 ± 0.01^{B}	2.16 ± 0.03^{A}	S
T3	ND	ND	ND	1.00 ± 0.05 ^{C}	1.35 ± 0.05 ^{B}	2.14 ± 0.06 ^{A}	S
T4	ND	ND	ND	ND	1.00 ± 0.03 ^{C}	1.85 ± 0.01^{B}	2.01 ± 0.05 ^{C}

C: 2% yogurt starter cultures 1:1 (control).
T1: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus*.

T2: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* + 2% inulin + 2% FOS.

T3: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin.

T4: 1% yogurt starter cultures 1:1 + 1% *Lb. acidophilus* bacteriocin + 2% inulin + 2% FOS.

S: spoilage

ND: Not Detected

*The values indicated are the mean ± S.E (n = 3). Values in the same raw denoted by different letters (^{ABC}) differ significantly ($p < 0.05$) from each other.

Declarations

Conflict of interest

Theauthorshavedeclared no conflicts of interestforthisarticle.

Authors' Contribution

H.H and M.S contributed to conceptualization, methodology, formal analysis. H.H, M.S. and H. S. A. contributed to resources and data analysis. H.S. A. contributed to writing original draft preparation, reviewing and editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to participate

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Availability of Data and Material

Please contact the corresponding author for any details about the data and materials.

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References

1. Abee T, Krockel L, Hill C. (1995). Bacteriocins: Modes of action and potentials in food preservation and control of food poisoning. International Journal of Food Microbiology 28, 169-185. doi:doi.org/10.1016/0168-1605(95)00055-0
2. Akabanda F., Owusu-Kwarteng, J., Tano-Debrah, K., Parkouda, C. and Jespersen, L. (2014). The use of lactic acid bacteria starter culture in the production of Nunu, a spontaneously fermented milk product in Ghana. International Journal of Food Science, 2014, 1-11.doi:doi.org/10.1155/2014/721067
3. Akalin, A. S., Gönç, S., Unal, G., Fenderya, S. (2007). Effects of fructooligosaccharide and whey protein concentrate on the viability of lactic culture in reduced-fat probiotic yogurt during storage. Journal of Food Science, 72, 222-227. doi: doi.org/10.1111/j.1750-3841.2007.00436.x
4. Akalin, A.S. and Erişir, D. (2008). Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. Journal of Food Science, 73, M184-188.doi:doi.org/10.1111/j.1750-3841.2008.00728.x
5. Akin, M. B., Akin, M. S., Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chemistry, 104, 93-99. doi: doi.org/10.1016/j.foodchem.2006.11.030
6. Anjum, N., Maqsood, S., Masud, T., Ahmad, A., Sohail, A. and Momin, A. (2014). *Lactobacillusacidophilus*: Characterization of the Species and Application in Food Production. Critical Reviews in Food Science and Nutrition, 54, 1241-51.doi: doi.org/10.1080/10408398.2011.621169
7. AOAC (Association of Official Analytical chemists) (2000). Official methods of analysis (17th Eds.) AOAC International, Washington, D.C., USA.
8. APHA (American Public Health Association) (2001). Compendium Methods for the Microbiological Examination of Foods (4th Eds.) APHA, Washington D.C., USA.
9. Aryana, K.J., Plauche, S., Rao, R. M., Mcgrew, P. and Shah, N.P. (2007). Fat free plain yogurt manufactured with inulins of various chain lengths and *Lactobacillus acidophilus*. Journal of Food Science, 72, M79–84.doi: doi.org/10.1111/j.1750-3841.2007.00302.x
10. Batish, V.K., Lai, R. and Grover, S. (1990). Studies on environmental and nutritional factors on production of antifungal substance by *Lactobacillus acidophilus* R. Food Microbiology 7, 199-206.doi: doi.org/10.1016/0740-0020(90)90025-D
11. Brennan, C. S., Tudorica, C. M. (2008). Carbohydrate-based fat replaces in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilization of barley beta-glucan, guar gum and inulin. International Journal of Food Science and Technology, 43, 824-833.doi: doi.org/10.1111/j.1365-2621.2007.01522.x
12. Buriti, F.C.A., Castro, I.A., Saad, S.M.I. (2010). Viability of Lactobacillus acidophilus in synbiotic guava mousses and its survival under in vitro simulated gastro- intestinal conditions. International Journal of Food Microbiology 137, 121-129. doi: doi.org/10.1016/j.ijfoodmicro.2009.11.030
13. Capela, P., Hay, T. and Shah, N. (2006). Effect of cryoprotectants, prebiotics, and microencapsulation on the survival of probiotic organisms in yogurt and freeze-dried yogurt. Food Research International,

39, 203–211.doi: doi.org/10.1016/j.foodres.2005.07.007

14. Carvalho, A., Silva, J., Ho, P., Teixeira, P., Malcata, F., Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria, International Dairy Journal, 14, 835–847.doi: doi.org/10.1016/j.idairyj.2004.02.001
15. Clarke, G.M. and Kempson, R.E. (1997). Introduction to the design and analysis of experiments. Arnold, a Member of the Holder Headline Group, 1st Ed. London, UK.
16. Cruz, A. G., Walter, E.H.M., Mortazavian, A.M., Granato,D., Faria,J. A.F., Bolini, H.M.A. (2010). Sensory analysis: relevance for prebiotic, probiotic, and synbiotic product development. Comprehensive Reviews in Food Science and Food Safety, 9, 358-373.doi: doi.org/10.1111/j.1541-4337.2010.00115.x
17. Dave, R.I. and Shah, N.P. (1997): Viability of yogurt and probiotic bacteria in yogurts made from commercial starter cultures. International Dairy Journal, 7, 31-41.doi: doi.org/10.1016/S0958-6946(96)00046-5
18. Donkor, O.N., Henriksson, A., Vasiljevic, T. and Shah, N.P. (2007). Rheological properties and sensory characteristics of set-type soy yogurt. Journal of Agriculture Food Chemistry, 55, 9868–9876. doi: doi.org/10.1021/jf071050r
19. Donkor, O.N., Nilmini, S.L.I., Stolic, P., Vasilgevic, T., Shah, N.P. (2007). Survival and activity of selected probiotic organism in set– type yogurt during cold storage. International Dairy Journal, 17, 92–151.doi: doi.org/10.1016/j.idairyj.2006.08.006
20. EL-Nagar, C. G. Clowes, G., Tudorică, C.M., Kuri, V., Brennan, C.S. (2002). Rheological quality and stability of yog- ice cream with added inulin. International Journal of Dairy Technology, 55, 89-93. doi: doi.org/10.1046/j.1471-0307.2002.00042.x
21. Elsanhoty, R.M. (2008). Screening of some lactobacillus strains for their antifungal activities against aflatoxin-producing Aspergilla in vitro and maize. Journal of Food Agriculture and Environment, 6, 35-40.
22. EOSQ (Egyptian Organization Standardization and Quality) (2005). Egyptian Organization for Standardization and Quality No. 1000.
23. Fazilaha, F.N., Ariff, B.A., Khayat, E.M, Rios-Solis, L. and Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. Journal of Functional Foods, 48, 387–399.doi: doi.org/10.1016/j.jff.2018.07.039
24. Giraffa, G., Chanishvili, N. and Widyastuti, Y. (2010). Importance of lactobacilli in food and feed biotechnology. Research in Microbiology, 161, 1-8.doi: doi.org/10.1016/j.resmic.2010.03.001
25. Gonzalez-Herrera, M.S., Rodriguez Herrera, R., Lopez, M.G., Rutiaga, O.M., Aguilar, C.N., Esquivel, J.C.C. andMartinez, L.A.O. (2015). Inulin in food products: prebiotic and functional ingredient. British Food Journal, 117, 371-387.doi:doi.org/10.1108/BFJ-09-2013-0238
26. Guven, M.; Yasar, K.; Karaca, O.B. and Hayaloglu, A.A. (2005). The effect of inulin as a fat replacer on the quality of set type low fat yogurt International journal of dairy Technology, 5,180-184.doi:doi.org/10.1111/j.1471-0307.2005.00210.x

27. Hassan, A. and Amjad, I. (2010).Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage. African Journal of Microbiology, 4, 022-026.
28. Hassan, M. U., Nayab, H., Rehman, T.U., Williamson, M.P., Haq, H. U., Shafi, N. and Shafique, F. (2020). Characterisation of Bacteriocins Produced by *Lactobacillus* spp. Isolated from the Traditional Pakistani Yoghurt and Their Antimicrobial Activity against Common Foodborne Pathogens. BioMed Research International, 2020, doi: doi.org/10.1155/2020/8281623.
29. Hernandez-Hernandez, O., Muthaiyan, A., Moreno, F.J., Montilla, A., Sanz, M.L. and Ricke, S.C. (2012). Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*. Food Microbiology, 30, 355-361. doi: doi.org/10.1016/j.fm.2011.12.022
30. IDF (International Dairy Federation) (1990): Milk and Milk Products: Enumeration of Yeast and Molds IDF: 94B.
31. Kleniewska, P., Hoffmann, A., Pniewska, E. and Pawliczak, R. (2016). The influence of probiotic *Lactobacillus casei* in combination with prebiotic inulin on the antioxidant capacity of human plasma. Journal of Oxidative Medicine and Cellular Longevity, 2016, 1–11.doi: doi.org/10.1155/2016/1340903
32. Kodaka, H., Mizuochi, S., Teramura, H. and Nirazuka, T. (2005). Comparison of the compact dry TC method with the standard pour plate method (AOAC Official Method 966.23) for determining aerobic colony counts in food samples: performance-tested method SM. Journal of AOAC International, 88, 17021713.doi: doi.org/10.1093/jaoac/88.6.1702
33. Konar, N., Palabiyikb, I. and Toker O. S. (2018). Conventional and sugarfree probiotic white chocolate: Effect of inulin DP on various quality properties and viability of probiotics. Journal of Functional Foods, 43, 206–213.doi: doi.org/10.1016/j.jff.2018.02.016
34. Lee, W.J., Lucey, J.A., (2010). Formation and physical properties of yogurt. Journal of Animal Science, 23, 1127–1136.doi: doi.org/10.5713/ajas.2010.r.05
35. Lomax, A.R. and Calder, P.C. (2009): Prebiotics, immune function, infection, and inflammation: A review of the evidence. British Journal of Nutrition, 101, 633-658. doi: doi.org/10.1017/S0007114508055608
36. Magnusson, J. and Schnürer, J. (2001). *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad- spectrum proteinaceous antifungal compound. Applied and Environmental Microbiology, 67, 1-5. doi: doi.org/10.1128/AEM.67.1.1-5.2001
37. Manning, T.S. and Gibson G.R. (2004): Prebiotics. Best Practice Research Clinical Gastroenterology, 18: 287-298.
38. Mehanna, N.M., Sahel, T.M., Mehanna, A.S. and El- Asfory, S.M.A. (2000). The quality of low-calorie buffalo Zabady. Egyptian Journal of Dairy Science, 28, 59-71.
39. Miremadi, F., Sherkat, F. and Stojanovska, L. (2016). Hypocholesterolaemic effect and anti-hypertensive properties of probiotics and prebiotics: A review. Journal of Functional Foods, 25, 497–510.doi: doi.org/10.1016/j.jff.2016.06.016

40. Nighswonger, B.D., Brashears, M.M. and Gilliland, S.E. (1996). Viability of *Lactobacillus acidophilus* and *Lactobacillus casei* in fermented milk products during refrigerated storage. Journal of Dairy Science, 79, 212-219.doi: doi.org/10.3168/jds.S0022-0302(96)76353-1
41. Ogueke C.C., Owuamanam, C.I., Olawuni, I.A., Iwouno, J.O. and Peter-Ikechukwu, A.I. (2014). Addition of Inulin and Manganese Chloride Increased Antibacterial Metabolites in Yoghurt. Pakistan Journal of Nutrition, 13, 12-16.
42. Okuro, P.K., Thomazini, M., Balieiro, J.C.C., Liberal, R.D.C.O. and Favaro-Trindade, C.S. (2013). Co-encapsulation of *Lactobacillus acidophilus* with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. Food Research International, 53, 96-103.doi: doi.org/10.1016/j.foodres.2013.03.042
43. Oliveira, R.P., Perego, P., De Oliveira, M.N. and Converti, A. (2011). Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. LWT - Food Science and Technology, 44, 520-523. doi: https://doi.org/10.1016/j.lwt.2010.08.024
44. Pandey, S.M. and Mishra H.N. (2015). Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology. LWT-Food Science and Technology,62, 458-467.doi: doi.org/10.1016/j.lwt.2014.12.003
45. Peshev, D. and Van Den Ende, W (2014). Fructans: Prebiotics and immunomodulators. Journal of Functional Foods, 8, 348–357. Doi: https://doi.org/10.1016/j.jff.2014.04.005
46. Qian, Z., Zhao, D., Yin, Y., Zhu, H. and Chen, D. (2020). Antibacterial Activity of Lactobacillus Strains Isolated from Mongolian Yogurt against Gardnerellavaginalis. BioMedResearch International, 3548618. doi: doi.org/10.1155/2020/3548618
47. Rouse, S., Harnett, D., Vaughan, A. and van Sinderen, D. (2008). Lactic acid bacteria with potential to eliminate fungal spoilage in foods. Journal of Applied Microbiology, 104, 915-923.doi:10.1111/j.1365-2672.2007.03619.x
48. Rousseau V, Lepargneur JP, Roques C, Remaud-Simeon M, Paul F. (2005). Prebiotic effects of oligosaccharides on selected virginal lactobacilli and pathogenic microorganisms. Clinical Microbiology, 11, 145-153. doi: doi.org/10.1016/j.anaerobe.2004.12.002
49. Sabrina da Silva Sabo, S., Converti, A., Todorov, S.D., Dominguez, J.M. and Oliveira, R.P. (2015). Effect of inulin on growth and bacteriocin production by *Lactobacillus plantarum* in stationary and shaken cultures. International journal of food science and technology, 50, 864-870.doi:doi.org/10.1111/ijfs.12711
50. Santo, A.P.E., Cartolano, N.S., Silva, T.F., Soares, A.S.M.F., Gioielli, L.A., Perego, P., Converti, A. and Oliveira, M.N. (2012). Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. International Journal of Food Microbiology, 154, 135-144.doi: doi.org/10.1016/j.ijfoodmicro.2011.12.025
51. Saulnier DMA, Spinler JK, Gibson GR, Versalovic J. (2009). Mechanisms of probiosis and prebiosis: Considerations for enhanced functional foods. Current Opinion Biotechnology, 20, 135-141. doi: doi.org/10.1016/j.copbio.2009.01.002

52. Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E., Pérez-Alvarez, J.A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiology, 25, 13–21.doi: doi.org/10.1016/j.fm.2007.09.003
53. Shah, N. P. (2017): Yogurt in health and disease prevention. London, UK: Academic Press.
54. Srisuvor, N., Chinprahast, N., Prakitchai, C.,Suwanna, W., and Subhimaros (2013). Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana purée. Food science and technology, 51, 30-36.doi: doi.org/10.1016/j.lwt.2012.10.018
55. Srisuvor, N., Chinprahast, N., Prakitchaiwattana, C. and Subhimaros, S. (2013). Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana puree. LWT - Food Science and Technology, 51, 30-36. doi: doi.org/10.1016/j.lwt.2012.10.018
56. Stiles, J., Penkar, S., Plockova, N., Chumchalova, J. and Bullerman, L.B. (2002). Antifungal activity of sodium acetate and *Lactobacillus rhamnosus*. Journal of Food Protection, 65, 1188-1191. doi: doi.org/10.4315/0362-028X-65.7.1188
57. Surajit, M. Dev, P. A. and Subrota, H. (2016). Effect of inulin on growth and antimicrobial activity of Lactobacillus spp. International Journal of Fermented Foods, 5, 47-52.
58. Vital, A., Goto, P., Hanai, L., Gomes, S., de Abreu, B., Nakamura, C. and Matumoto, P. (2015). Microbiological, functional and rheological properties of low fat yogurt supplemented with Pleurotusostreatus aqueous extract. LWT - Food Science and Technology, 64, 1028–1035. doi: doi.org/10.1016/j.lwt.2015.07.003

Figures

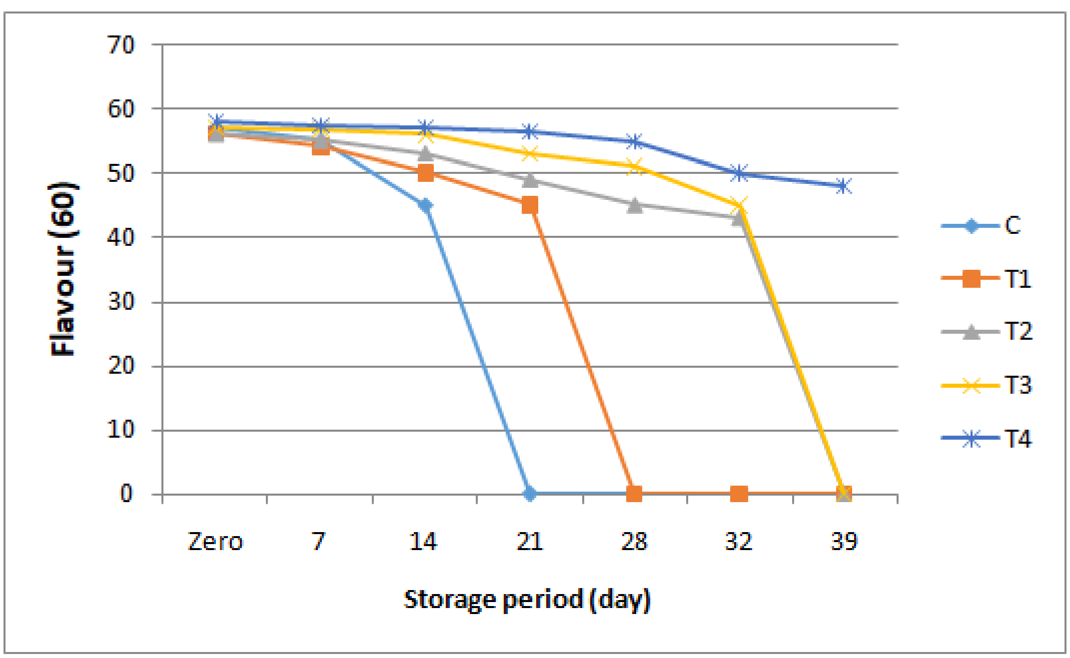

Figure 1

Flavour scores in the examined yoghurt groups throughout their refrigerated storage.

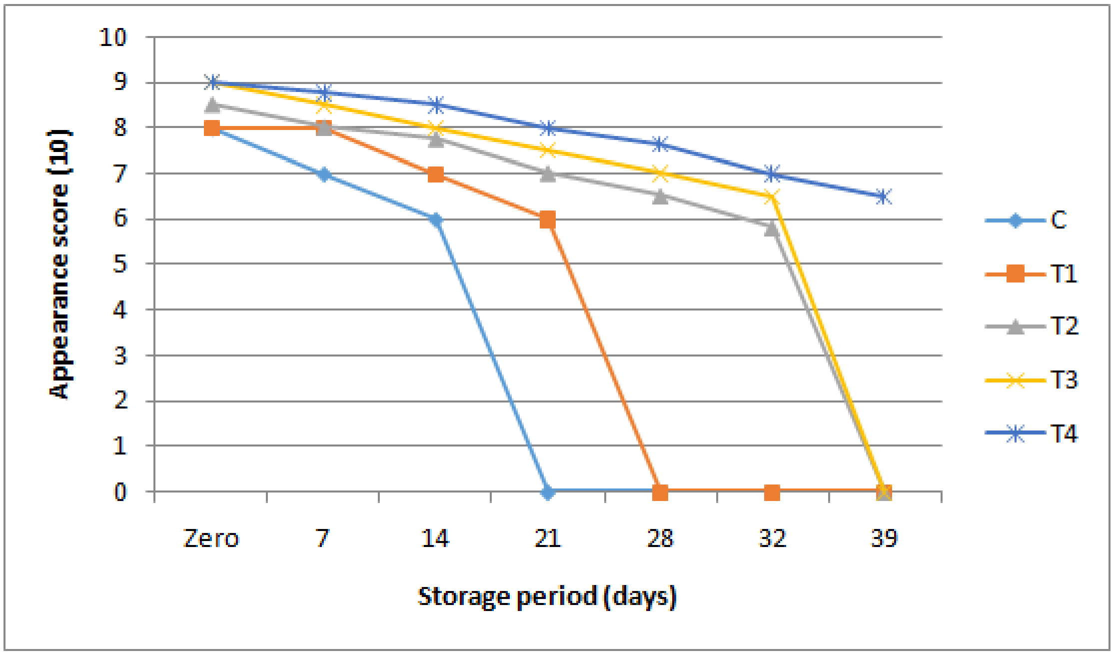

Figure 2

Appearance scores in the examined yoghurt groups throughout their refrigerated storage.

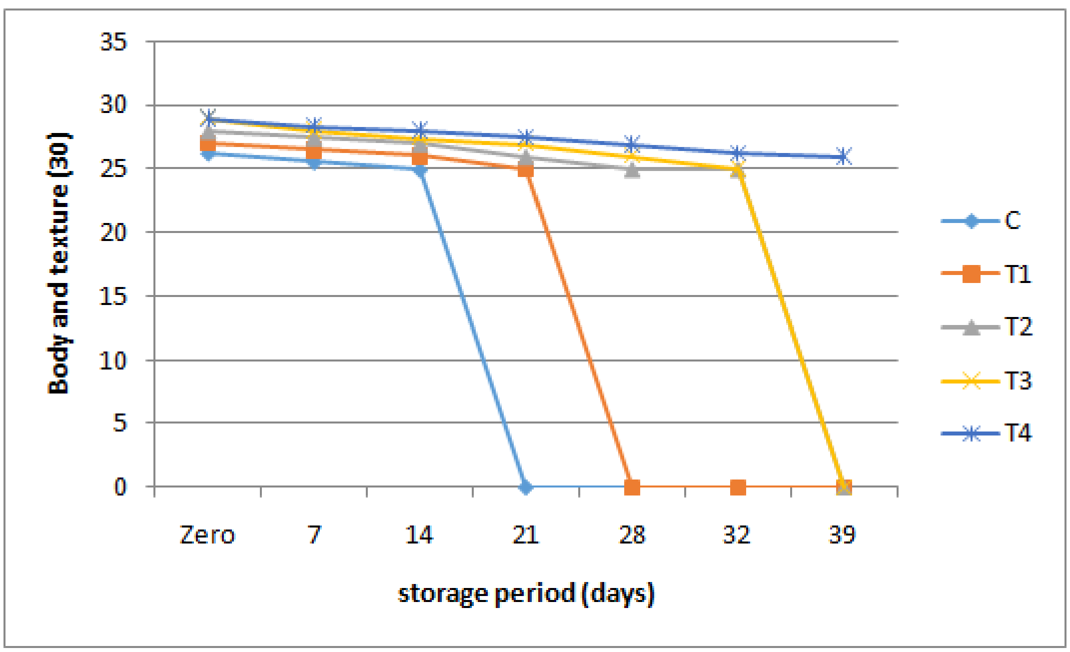

Figure 3

Body and Texture scores in the examined yoghurt groups throughout their refrigerated storage.