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FOOD MICROBIOLOGY – RESEARCH PAPER

In vitro gastrointestinal resistance of *Lactobacillus acidophilus* in some dairy products

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Abstract

In this study, different dairy products such as ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk were manufactured by using either *Lactobacillus acidophilus* DSM 20,079 or *Lactobacillus acidophilus* NCFM. The counts of *L. acidophilus* in the samples on days 1, 15, and 30 of the storage were determined. Additionally, the samples contained *L. acidophilus* were passed through a dynamic gastrointestinal model designed in laboratory conditions to compare the protective effect of different dairy products on viability of *L. acidophilus* against stress factors of the gastrointestinal model. The counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the samples decreased by between 0.04 and 0.37 log units and by between 0.11 and 0.27 log units, respectively, within 30 days of storage. During the passage through the gastrointestinal model, the highest percentage reduction in the counts of *L. acidophilus* was determined in yoghurt followed by fermented acidophilus milk, white pickled cheese, and ice cream, respectively. The reduction in the counts of *L. acidophilus* in the samples during the passage through the model increased with extension of storage time. The results of this study showed that the reduction in the counts *L. acidophilus* in the samples during the passage through the model was influenced significantly by the matrix of the dairy product and storage period.

Keywords Ice cream · Yoghurt · White pickled cheese · Fermented acidophilus · *Lactobacillus acidophilus* · Dynamic gastrointestinal model

Introduction

An increased demand on the probiotic microorganisms occurred due to increase of the resistance of pathogens against antibiotics and tendency of consumers for functional foods instead of pharmaceuticals [1]. Fermented dairy products with probiotic microorganisms are recognized as functional foods due to their health-promoting properties on the consumer's body [2]. The uptake of many of probiotic microorganisms occurs via fermented dairy products, which are considered among the most important probiotic carrier foods [3].

The viability of probiotic microorganism in the fermented dairy products is influenced by various factors such as chemical composition of the fermentation environment (source of carbohydrate), pH value after fermentation, total solid content of milk, the usage of nutrients, growth supporters or preventers, type of bacterial strain, interaction between the strains, sugar concentration (osmotic pressure), dissolved oxygen, level of inoculation, temperature of incubation, time of fermentation, and storage conditions [4]. Probiotic microorganisms have to survive during the processing as well as during the passage through the gastrointestinal system [5, 6]. The resistances to stomach acid and bile salts are two essential properties for the survival of probiotic microorganisms in the human gastrointestinal tract. Various in vivo and in vitro studies have been carried out to determine the viability of probiotic microorganisms in the gastrointestinal conditions [7–9]. Determination of probiotic properties of microorganisms used in the production of foods and assessment of the factors affecting the viability of probiotic

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microorganisms during transit through gastrointestinal system via in vivo studies are very complex [10, 11]. Compared with in vivo studies, in vitro studies are easy, fast, and reliable, and can be used without ethical constraints. There are two types of in vitro gastrointestinal models simulating gastrointestinal digestive system, namely dynamic and static types. In dynamic models, physical and mechanical processes and temporal changes occurring in vivo conditions can be simulated. In static models, physical processes that occur in vivo conditions such as mixing, shear, and hydration are not accounted and digestion products remain mostly immobile [12, 13].

Several strategies have been suggested to protect probiotic microorganisms against gastrointestinal conditions, such as stress adaptation, use of prebiotics, microencapsulation, two-step fermentation application, and use of oxygen impermeable containers as well as selection of appropriate carrier food matrix [5, 6]. The food matrix has an important role for the gastrointestinal resistance of probiotic microorganisms [14]. Sanders and Marco [15] reported that probiotic microorganisms survive better in dairy products such as drinking milk, cheese, and yoghurt compared with either saline or buffer solutions during exposure to gastrointestinal conditions.

Although various studies on the use of probiotic bacteria in the manufacture of dairy products have been carried out, as far as we know, no study has been conducted that compares the viability of the same probiotic bacteria used in the production of different dairy products during passage through the dynamic in vitro gastrointestinal model. The aim of the present study was to determine the viability of two strains of *L. acidophilus* used in the production of different dairy products, such as ice cream, yoghurt, and white pickled cheese as well as fermented acidophilus milk and to assess the viability of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in the yoghurt samples and the viability of total mesophilic aerobic bacteria and total *Lactococcus* spp. in the white pickled cheese samples during storage for 30 days and after passage through the dynamic in vitro gastrointestinal model. Furthermore, the effect of strain type of *L. acidophilus* used in the production of the different dairy products on the survival of *L. acidophilus* was investigated.

Materials and methods

Bacterial strains

In this work, two well-documented probiotic strains, *L. acidophilus* DSM 20,079 and *L. acidophilus* NCFM [16, 17], were used. *L. acidophilus* DSM 20,079 (Leibniz Institute DSMZ, Braunschweig, Germany) and *L. acidophilus* NCFM (Howaru® Dophilus) were kindly provided by the Technical University of Munich (Germany) and obtained from Danisco A/S (Copenhagen, Denmark), respectively. The cheese starter culture (R-704-DVS) containing mainly *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*, and yoghurt starter culture (CH-1 Yo-Flex) containing *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were purchased from Chr. Hansen A/S (Horsholm, Denmark).

Manufacture of the dairy products

Raw cow's milk (pH value of 6.7 ± 0.1, titratable acidity value of $0.1 \pm 0.0\%$, total solid content of $11.8 \pm 0.1\%$, protein content of $3.2 \pm 0.2\%$, fat content of $2.8 \pm 0.3\%$, and ash content of $1.1 \pm 0.1\%$) used in this study was purchased from the Dairy Processing Unit of the Faculty of Agriculture at Akdeniz University. Figure 1 illustrates the production lines for the manufacture of the dairy products as ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk containing *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM, indicating the inoculation steps of *L. acidophilus*. Ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk were produced in laboratory scale. All productions were made in duplicate.

Ice cream

The ice cream was produced according to the method of Ergin et al. [18]. In 2.5 kg of ice cream formulation, 269 g skim milk powder, 450 g sucrose, 86 g butter, 14 g stabilizer, and 1681 g water were used. Ice cream mix composition was 3% (w/w) fat, 10% (w/w) non-fat milk solids, 18% (w/w) sugar, 0.5% (w/w) stabilizer, and 68.5% (w/w) water. The mixture was heated at 75 °C for 5 min and cooled to 4 °C. The mixture was homogenized using a mechanical mixer (Bosch, Mixxo Quattro MSM 7700, Jesenice, Slovenia) during the heat treatment. The cooled mix was ripened overnight at 4 °C. After the ripening period, the temperature of the mix was adjusted to 37 °C and the mix was inoculated with *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM so as to obtain a final concentration of at least 10^8 cfu (colony-forming unit) *L. acidophilus* per gram of the ice cream. The mix was fermented at 37 °C until a pH of 5.5 was reached. The fermentation was ended by cooling the mix to 4 °C, followed by freezing. The ice cream mixes were frozen in a batch type freezer (M10C, Mehen Food Machine Manufacture Co. Ltd., Nanjing, China) with a 10 kg capacity and without air incorporation. The ice cream samples were packaged in 300 mL glass cups with lids and stored at − 20 °C for 30 days.

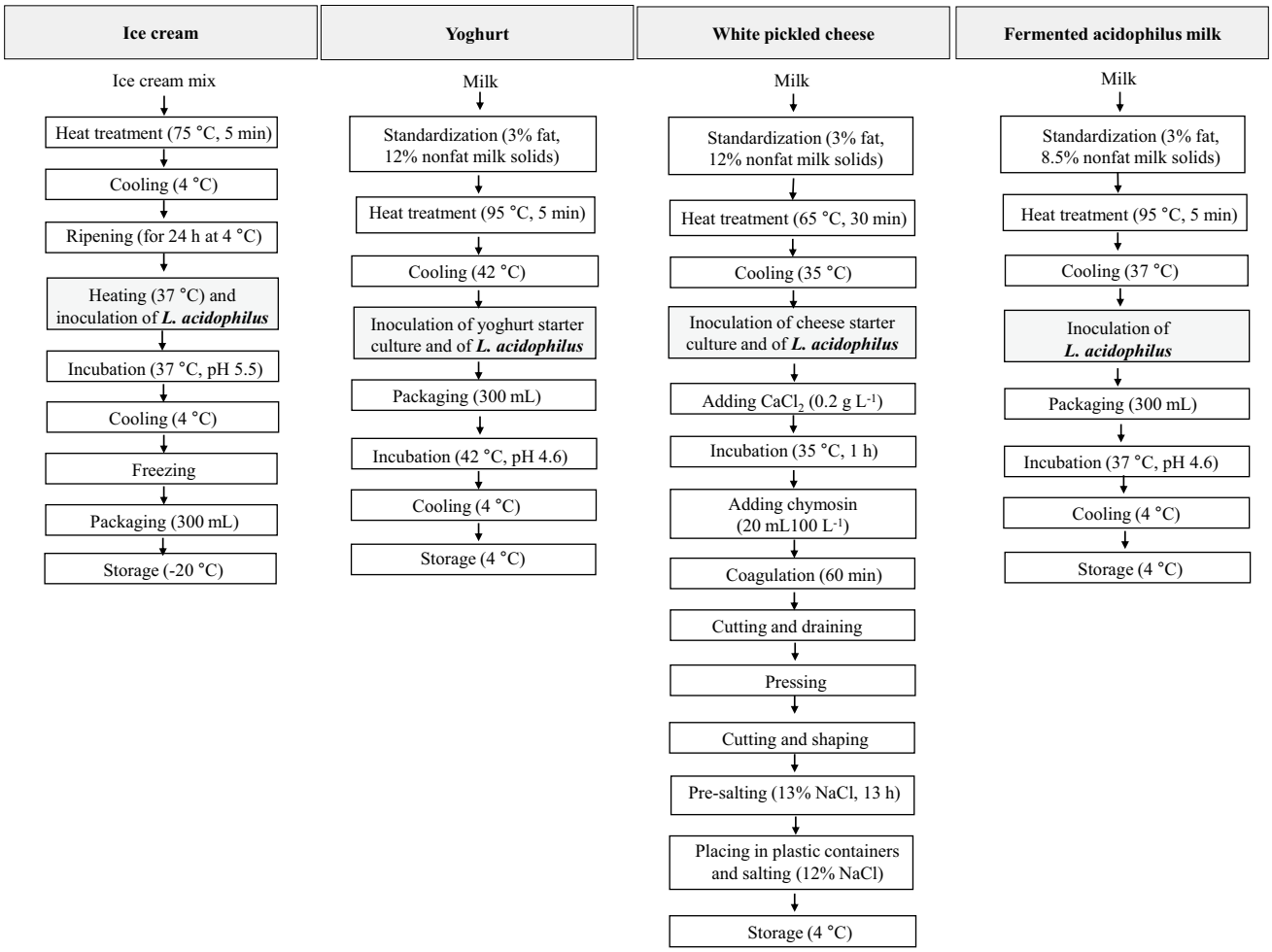

Fig. 1 Production lines for the manufacture of ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk containing *L. acidophilus*, indicating the inoculation steps of *L. acidophilus*

Yoghurt

Yoghurt production was performed as described by [19]. The milk was standardized to have 3% (w/w) fat and 12% (w/w) non-fat milk solids. After heating at 95 °C for 5 min, it was cooled to 42 °C. The milk was inoculated with the yoghurt starter culture at the ratio of 0.03 g L^{-1}, and then *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM inoculated into the milk to achieve a concentration of at least 10^8 cfu *L. acidophilus* per gram of the yoghurt. The inoculated milk was filled into 300 mL glass cups with lids and incubated at 42 °C until a pH of 4.6 was achieved. After cooling, the yoghurt samples were stored at 4 °C for 30 days.

White pickled cheese

White pickled cheese samples were manufactured according to the method of Kasımoğlu, Göncüoğlu, and Akgün [20] with some modifications. After standardization of milk to 3% (w/w) fat and 12% (w/w) non-fat milk solids, heat treatment was applied to milk at 65 °C for 30 min, and then it was cooled to 35 °C. The milk was inoculated with the cheese starter culture at 0.04 g L^{-1}, and then *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM inoculated into the milk so as to obtain a final concentration of at least 10^8 cfu *L. acidophilus* per gram of the white pickled cheese. After inoculation, $CaCl_2$ (0.2 g L^{-1}) was added to the milk and the milk was incubated at 35 °C for 1 h. Then, chymosin was added to the milk at a level sufficient to coagulate the milk in 60 min (20 mL 100 L^{-1}; Mayasan A.S., Istanbul, Turkey). The coagulum was cut into cubes (1 cm^3) and held for 15 min for whey separation. The curds were transferred into perforated molds lined with cheesecloth for further drainage of whey.

and pressed (7 kg weight for 10 L^{-1} milk) for about 15 h at 20 °C. Then, the curd was cut into cubic pieces (7 × 7 × 7 cm^3) to shape and these shaped curds were placed into brine (13%, w/v, NaCl) at 20 °C for about 13 h. After brine-salting,

cheeses were placed in plastic containers. The plastic containers were filled with brine (12%, w/v, NaCl), closed, and then stored at 4 °C for 30 days.

Fermented acidophilus milk

Fermented acidophilus milk production was performed according to method of Božanić et al. [21], with minor modifications. After standardization of milk to 3% (w/w) fat and 8.5% (w/w) non-fat milk solids, it was heated at 95 °C for 5 min and then cooled to 37 °C. The milk was inoculated with *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM to achieve a concentration of at least 10^8 cfu *L. acidophilus* per milliliter of the fermented acidophilus milk. The inoculated milk was filled into 300 mL glass cups with lids and incubated at 37 °C until a pH of 4.6 reached. The samples were stored at 4 °C for 30 days.

Design of dynamic gastrointestinal model

A dynamic gastrointestinal model to simulate the physiological condition characteristics of human upper gastrointestinal tract consists of three main parts in order to simulate the mouth, stomach, and small intestine (Fig. 2a). For

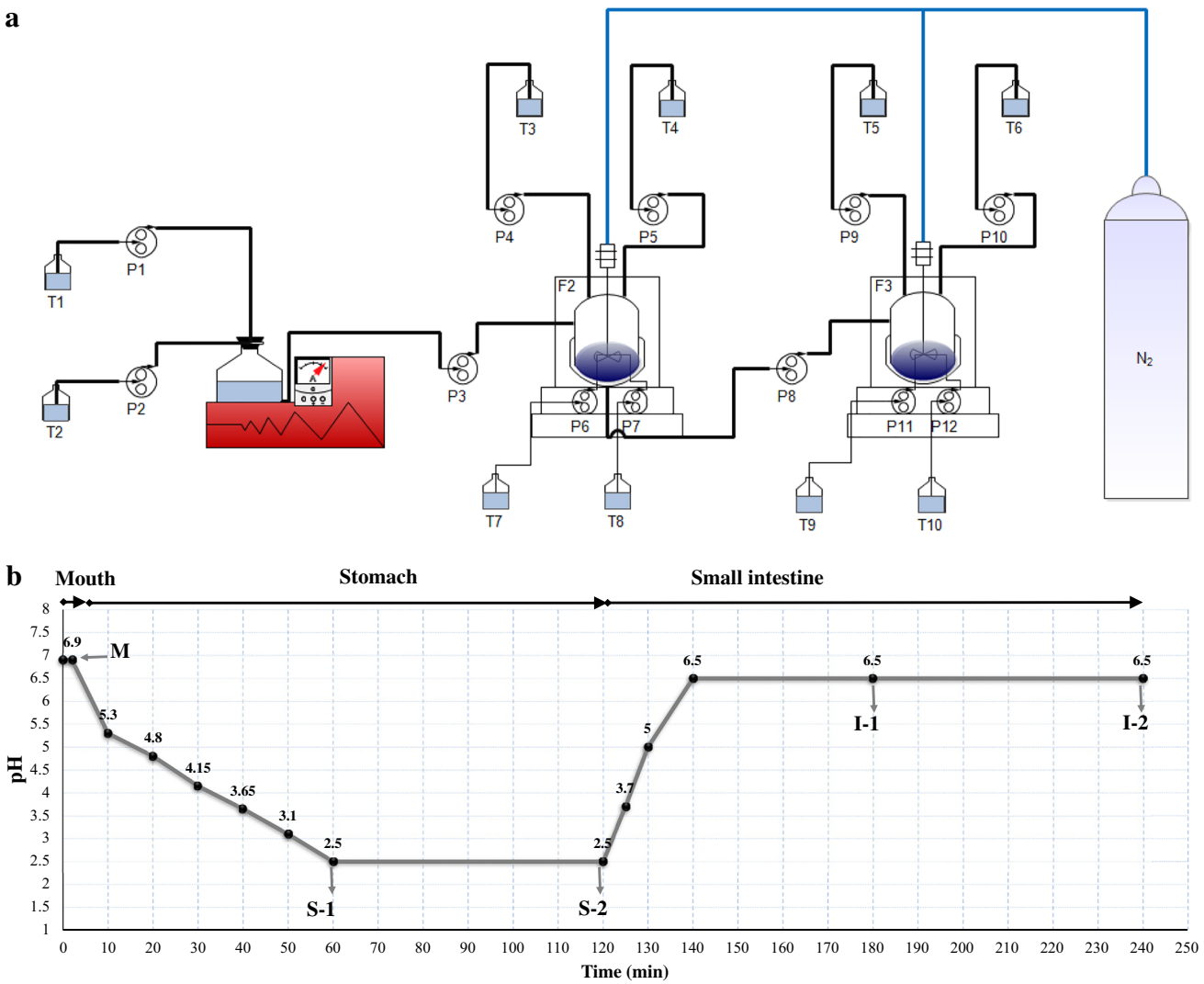

Fig. 2 Dynamic in vitro gastrointestinal model to simulate the physiological conditions of human upper gastrointestinal tract. T1: 40% NaOH solution (to adjust pH to 6.9); T2: saliva solution; T3: 1 M HCl solution (to reduce pH from 6.9 to 2.5); T4: stomach solution; T5: 1 M $NaHCO_3$ solution (to increase pH from 2.5 to 6.5); T6: small intestine solution; T7 and T9: 1 M HCl solution (to keep pH values constant at 2.5); T8 and T10: 1 M $NaHCO_3$ solution (to keep pH values constant at 6.5); F1: mouth part of the model; F2: stomach part of the model; F3: small intestine part of the model; N_2: nitrogen; P (1–12): peristaltic pumps (**a**). Sampling positions in the dynamic gastrointestinal model. M: end of mouth; S-1: stomach after 1 h digestion; S-2: stomach after 2 h digestion; I-1: small intestine after 1 h; I-2: small intestine after 2 h (**b**)

the part representing the mouth, a 750 mL bottle placed into the temperature-controlled water bath (JS Research Inc., JSRC-22(C)(CL), Chungcheongnam-do, Korea) and for the parts representing stomach and small intestine two temperature-controlled glass bioreactors (New Brunswick Scientific Co., BF-115, New Jersey, USA, and Electrolab Biotech Ltd., FerMac 320 Bioreactor, Tewkesbury, UK, respectively) have been used. The working volume of each bioreactor was 3.0 L. The bottle and bioreactors were autoclaved at 121 °C for 15 min before use. The temperatures of the water bath and bioreactors were kept constant at 37.0 ± 0.1 °C. In the bioreactors, pH value and mixing speed were controlled, and anaerobic conditions were maintained by purging with nitrogen during experiments. Mucin from porcine stomach (M2378, Sigma-Aldrich Co., St. Louis, USA) and α-amylase from porcine pancreas (A3176, Sigma-Aldrich Co., St. Louis, USA) to simulate saliva fluid, mucin and pepsin from porcine gastric mucosa (P700, Sigma-Aldrich Co., St. Louis, USA) to simulate stomach solution, and pancreatin from porcine pancreas (P7545, Sigma-Aldrich Co., St. Louis, USA) and bile salt mixture (B3426, Sigma-Aldrich Co., St. Louis, USA) to simulate small intestine solution were used in the model. All solutions of enzymes were freshly prepared. Furthermore, the time spent in the gastrointestinal model was controlled and arranged as follows: 2 min in the mouth part, 2 h in the stomach part, and 2 h in the small intestine part (Fig. 2b). In the model, total twelve peristaltic pumps were used in order to pump the simulated digestion solutions, control pH values, and as well as transfer the sample, which was exposed to the processes in the model, from mouth to stomach and from stomach to small intestine. The flow rates of these pumps were determined in preliminary experiments. Four of these pumps served for the flow of $NaHCO_3$ (1 M) and HCl (1 M) to arrange the pH values in the stomach and small intestine parts of the model. Approximately 300 g of yoghurt, ice cream, and fermented acidophilus milk samples containing 10^8 cfu g^{-1} or mL^{-1} of *L. acidophilus* and 100 g of white pickled cheese samples containing 10^8 cfu g^{-1} of *L. acidophilus* were used in the experiments in the dynamic gastrointestinal model. The yoghurt, ice cream, or fermented acidophilus milk samples were mixed with sterile water at room temperature at a ratio of 1:1 (w/v or v/v), while the white pickled cheese sample was mixed with sterile water at room temperature at a ratio of 1:5 (w/v). The sample-water mixture was homogenized using a stomacher (Laboratory blender stomacher 80, Seward Medical, London, UK) for 2 min. Digestion conditions were modified from gastrointestinal models previously developed by Madureira et al. [22],

Table 1 Description and preparation of the dynamic in vitro model system

Intestinal segment	Description	Preparation	pH	Retention time (min)	Volume (mL)
Mouth	Chewing	300 g of yoghurt, ice cream and fermented acidophilus milk samples were mixed with 300 g of sterile water at 37 °C, 100 g of white pickled cheese samples were mixed with 500 g of sterile water at 37 °C, and the samples were homogenized		-	-
	Simulated saliva solution	2 g L^{-1} α-amylase and 1 g L^{-1} mucin were dissolved in sterile water. The simulated saliva solution (0.05 mL g^{-1} sample) was added to the mouth part (5 mL min^{-1})	6.9	2	10
Stomach	Simulated stomach solution	25 g L^{-1} pepsin and 23 g L^{-1} mucin were dissolved in sterile stomach buffer solution*. The sample exposed to digestion in the mouth part was fed to the reactor simulating the stomach (100 mL min^{-1}). The simulated stomach solution was fed to the stomach part (0.25 mL min^{-1})	6.9→2.5	120	15
Small intestine	Simulated small intestine solution	1 g L^{-1} pancreatin and 12 g L^{-1} mucin were dissolved in sterile small intestine buffer solution**. The sample exposed to digestion in the stomach part was fed to the reactor simulating the small intestine (100 mL min^{-1}). The simulated intestine solution was fed to the intestine part (3 mL min^{-1})	2.5→6.5	120	60

*Stomach buffer solution: 2.2 g L^{-1} KCl, 6.2 g L^{-1} NaCl, 1.2 g L^{-1} $NaHCO_3$, 0.22 g L^{-1} $CaCl_2$

**Small intestine buffer solution: 0.6 g L^{-1} KCl, 5.0 g L^{-1} NaCl, 0.25 g L^{-1} $CaCl_2$

Marteau et al. [23], and Sumeri et al. [10] (Table 1). The homogenized mixture was added into 750 mL of sterile bottle placed into the water bath to perform the study of the mouth part of the model. After introducing the mixture into the mouth part, the pH of the mixture in the bottle was adjusted to 6.9 with 40% NaOH solution. Then, the saliva solution was added to the bottle containing the mixture at a flow rate of 5 mL min^{-1}. One hundred milliliters of 0.01 M HCl was added into the first bioreactor called stomach part of the model to imitate the empty stomach. Then, the contents of the bottle representing the mouth part of the model were pumped at 100 mL min^{-1} into the stomach part containing HCl. Then, the stomach solution was added to the bioreactor at a flow rate of 0.25 mL min^{-1}. In the process of adding the stomach solution completed about 60 min, the pH value of the contents of the stomach part was gradually reduced to 2.5. After completion of the addition of the stomach solution, the pH value of the contents of the stomach part was kept at 2.5 for 60 min. Thereafter, the contents of the bioreactor representing the stomach part of the model were pumped at 100 mL min^{-1} into the second bioreactor called small intestine part of the model. Then, the small intestine solution was added to the bioreactor at a flow rate of 3 mL min^{-1}. In the process of adding the small intestine solution completed about 20 min, the pH value of the contents of the small intestine part was gradually increased to 6.5. After completion of the addition of the small intestine solution, the pH value of the contents of the small intestine part was kept at 6.5 for 100 min. Time-dependent pH changes in the contents of the parts of the model are shown in Fig. 2b. At three different times of storage, after the passage of the dairy products containing *L. acidophilus* through the dynamic gastrointestinal model, the counts of *L. acidophilus* in all dairy products, the counts of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in yoghurt samples, and the counts of total mesophilic aerobic bacteria and total *Lactococcus* spp. in white pickled cheese samples were determined. The experiments performed with the dynamic in vitro gastrointestinal model were replicated in duplicate.

Analysis of the dairy products

Physicochemical analysis

Total solid content (%), fat content (%), protein content (%), and ash content (%) of the samples and the raw milk used for the manufacture of the dairy products were determined using gravimetric, Gerber, Kjeldahl, and gravimetric methods, respectively [24]. Percentage of titratable acidity for the samples was measured by the method of Bradley et al. [25]. The pH values of the milk and the samples were measured using a pH meter (Orion 2-Star, Thermo Scientific, Bremen, Germany).

Microbiological analysis

The microbiological analysis of the ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples taken from the dynamic gastrointestinal model was conducted on days 1, 15, and 30 of the storage of the samples. The pour plate technique was used.

Microbiological analysis of ice cream samples *L. acidophilus* counts were determined after frozen storage at − 20 °C. Serial dilutions of the ice cream samples (1 g) were made in 9 mL ringer solution (1/4). One milliliter aliquot dilutions were poured onto plates of the MRS agar (Merck KGaA, Darmstadt, Germany). All plates were incubated anaerobically at 37 °C for 72 h and the results were expressed as colony-forming units per gram of the sample [26].

Microbiological analysis of yoghurt samples *L. acidophilus* counts in the yoghurt samples were determined using MRS agar with bromocresol green and clindamycin (MRS-BC agar). The plates were incubated anaerobically at 37 °C for 72 h [2]. For the determination of the counts of *L. delbrueckii* subsp. *bulgaricus*, MRS agar was used and the plates were incubated at 45 °C for 72 h [25]. The counts of *S. thermophilus* were conducted using M17 agar (Merck KGaA, Darmstadt, Germany) containing 1% (w/v) lactose after incubation at 45 °C for 24 h [27].

Microbiological analysis of white pickled cheese samples The counts of total mesophilic aerobic bacteria were performed using Plate Count Agar (Merck KGaA, Darmstadt, Germany) and total *Lactococcus* spp. were enumerated on M17 agar according to the methods of Evrendilek et al. [28] and IDF [29], respectively. MRS-sorbitol agar was used for the selective enumeration of *L. acidophilus* according to the method described by Ong et al. [30]. The agar was prepared by adding 10 mL of sterile solutions (10% (w/v), membrane filtered) of sorbitol (Sigma-Aldrich Co., St. Louis, USA) to 90 mL of molten MRS agar just before pouring. The plates were incubated anaerobically at 37 °C for 72 h.

Microbiological analysis of fermented acidophilus milk samples *L. acidophilus* counts were performed using MRS agar with anaerobic incubation at 37 °C for 72 h [31].

The percentage of reduction in the counts of *L. acidophilus* in the dynamic in vitro gastrointestinal model .

The percentage reduction in the counts of *L. acidophilus* in the 1-, 15-, and 30-day stored samples during the passage

through each part of the dynamic in vitro gastrointestinal model was calculated according to the slightly modified method of Kos et al. [32] using the equation given below:

$$R(\%) = 100 - \left[\frac{\log cfuN}{\log cfuN_0} \times 100\right]$$

where N_0 is the count of *L. acidophilus* in the 1-, 15-, and 30-day stored samples before exposure to each part of the gastrointestinal model and N is the count of *L. acidophilus* in the 1-, 15-, and 30-day stored samples exposed to each part of the gastrointestinal model.

Statistical analyses

In this study, all measurements were carried out in duplicate. All statistical analyses were performed using SAS Statistical Software (release for Windows, SAS Institute Inc., Cary, NC, USA). A two-factor ANOVA was conducted to determine the effects of gastrointestinal model conditions and storage period on the count of *L. acidophilus* DSM 20,079 and *L. acidophilus* NCFM in all samples. The Duncan's multiple range test was used to detect differences among the means.

Results and discussion

Physicochemical properties

The mean total solids, protein, fat and ash contents, and titratable acidity and pH values of the ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples manufactured by using *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM are presented in Table 2. The chemical composition of the samples in the present study was generally similar to that found by Ranadheera et al. [33] for the ice cream, by Ribeiro et al. [34] for the yoghurt, by Kasımoğlu et al. [20] for the white pickled cheese, and by Akpınar [35] for the fermented acidophilus milk. The titratable acidity values of the samples investigated in this study increased with 30-day storage period, while the pH values of the samples decreased during storage. These agree with the results reported by Afzaal et al. [36] for ice cream, by Moschopoulou et al. [37] for yoghurt, by Kılıç et al. [38]

Table 2 Physicochemical properties of the ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples produced by using *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM after storage

Analysis	*L. acidophilus*	Storage period	Ice cream	Yoghurt	Cheese	Fermented acidophilus milk
Total solids (%)	DSM 20,079	1-day	29.1 ± 0.3	15.2 ± 0.2	41.4 ± 0.1	11.5 ± 0.1
	NCFM	1-day	29.2 ± 0.4	15.1 ± 0.1	41.2 ± 0.1	11.5 ± 0.1
Fat (%)	DSM 20,079	1-day	2.9 ± 0.2	3.0 ± 0.1	16.7 ± 0.1	3.0 ± 0.1
	NCFM	1-day	2.9 ± 0.1	3.0 ± 0.1	16.8 ± 0.1	3.0 ± 0.1
Protein (%)	DSM 20,079	1-day	3.4 ± 0.1	4.2 ± 0.1	17.9 ± 0.1	3.5 ± 0.1
	NCFM	1-day	3.5 ± 0.1	4.1 ± 0.1	17.7 ± 0.6	3.4 ± 0.1
Ash (%)	DSM 20,079	1-day	0.8 ± 0.1	1.0 ± 0.1	2.7 ± 0.1	1.0 ± 0.1
	NCFM	1-day	0.8 ± 0.1	1.0 ± 0.1	2.7 ± 0.1	1.0 ± 0.1
Titratable acidity (%)	DSM 20,079	1-day	0.33 ± 0.01b*	1.05 ± 0.07c	0.26 ± 0.05a	0.91 ± 0.01b
	DSM 20,079	15-day	0.35 ± 0.01ab	1.25 ± 0.15b	0.29 ± 0.01a	0.98 ± 0.01ab
	DSM 20,079	30-day	0.38 ± 0.02a	1.44 ± 0.04a	0.31 ± 0.04a	1.03 ± 0.03a
	NCFM	1-day	0.34 ± 0.01a	1.06 ± 0.09c	0.25 ± 0.01a	0.93 ± 0.01b
	NCFM	15-day	0.35 ± 0.03a	1.38 ± 0.11b	0.26 ± 0.03a	1.07 ± 0.02a
	NCFM	30-day	0.36 ± 0.01a	1.53 ± 0.06a	0.30 ± 0.01a	1.10 ± 0.01a
pH	DSM 20,079	1-day	5.52 ± 0.01a	4.45 ± 0.03a	5.86 ± 0.01a	4.58 ± 0.01a
	DSM 20,079	15-day	5.50 ± 0.01a	4.20 ± 0.02b	5.81 ± 0.01ab	4.49 ± 0.02b
	DSM 20,079	30-day	5.48 ± 0.01a	4.01 ± 0.03c	5.78 ± 0.01b	4.43 ± 0.01b
	NCFM	1-day	5.51 ± 0.01a	4.47 ± 0.01a	5.90 ± 0.01a	4.55 ± 0.01a
	NCFM	15-day	5.50 ± 0.01a	4.28 ± 0.05b	5.87 ± 0.01ab	4.46 ± 0.01b
	NCFM	30-day	5.47 ± 0.01a	4.06 ± 0.01c	5.84 ± 0.01b	4.37 ± 0.03c

Values (mean ± standard deviation) of total solids, fat, protein and ash contents, and titratable acidity and pH values of the samples. *For the titratable acidity and pH values of the each sample manufactured by using same strain of *L. acidophilus*, means with different letters show the difference in the same column ($P < 0.05$)

for cheese, and by Junaid et al. [39] for fermented acidophilus milk. During 30-day storage, the decreases in the pH values of yoghurt, cheese, and fermented asidophilus milk produced by both strains of *L. acidophilus* and the increases in the titratable acidity values of yoghurt and fermented asidophilus milk produced by both strains of *L. acidophilus* and of ice cream produced by *L. acidophilus* DSM 20,079 were significant. The increase in titratable acidity and decrease in pH during storage are most probably due to formation of lactic acid from lactose by lactic acid bacteria [40].

Microbiological properties of the dairy products during storage

The survival of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples on the 1st, 15th, and 30th days of the storage is illustrated in Fig. 3 and Fig. 4, respectively.

The counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the ice cream samples stored 1 day at − 20 °C were approximately 8.9 log cfu g^{-1}. At the end of the 30-day storage period, the counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the ice cream samples decreased about 0.4 and 0.2 log units, respectively. The incorporation of oxygen into the ice cream mix may reduce the count of probiotic bacteria in the ice cream. The freezing process may have influenced the number of probiotic bacteria in the ice cream, because batch freezing, used in the present study, is less efficient for incorporating air as compared to continuous freezing [41]. Another explanation for this finding may be the stress adaptation of the strains of *L. acidophilus* used in this study. The fermentation of ice cream mix may provide protection to the probiotic bacteria in the ice cream against cold stress, since the resistance of probiotic bacteria in the ice cream to cold stress may be improved by applying acid stress caused by the fermentation of ice cream mix [18].

In the present study, the viable counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the yoghurt

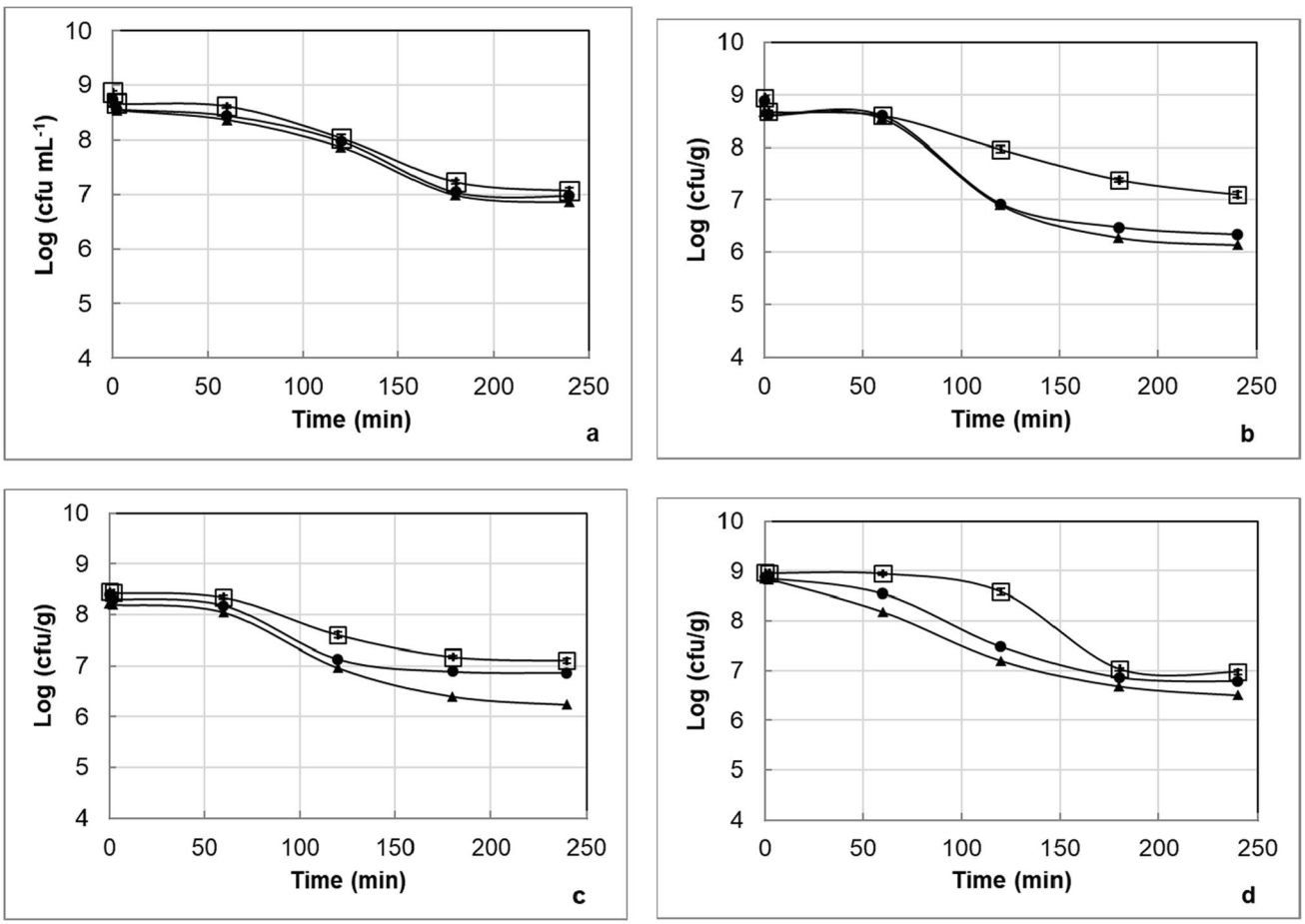

Fig. 3 The counts of *L. acidophilus* in the ice cream (**a**), yoghurt (**b**), white pickled cheese (**c**), and fermented acidophilus milk (**d**) samples produced with *L. acidophilus* DSM 20,079 on the 1st (white square), 15th (black circle), and 30th (black triangle) days of the storage during the passage through the dynamic in vitro gastrointestinal model

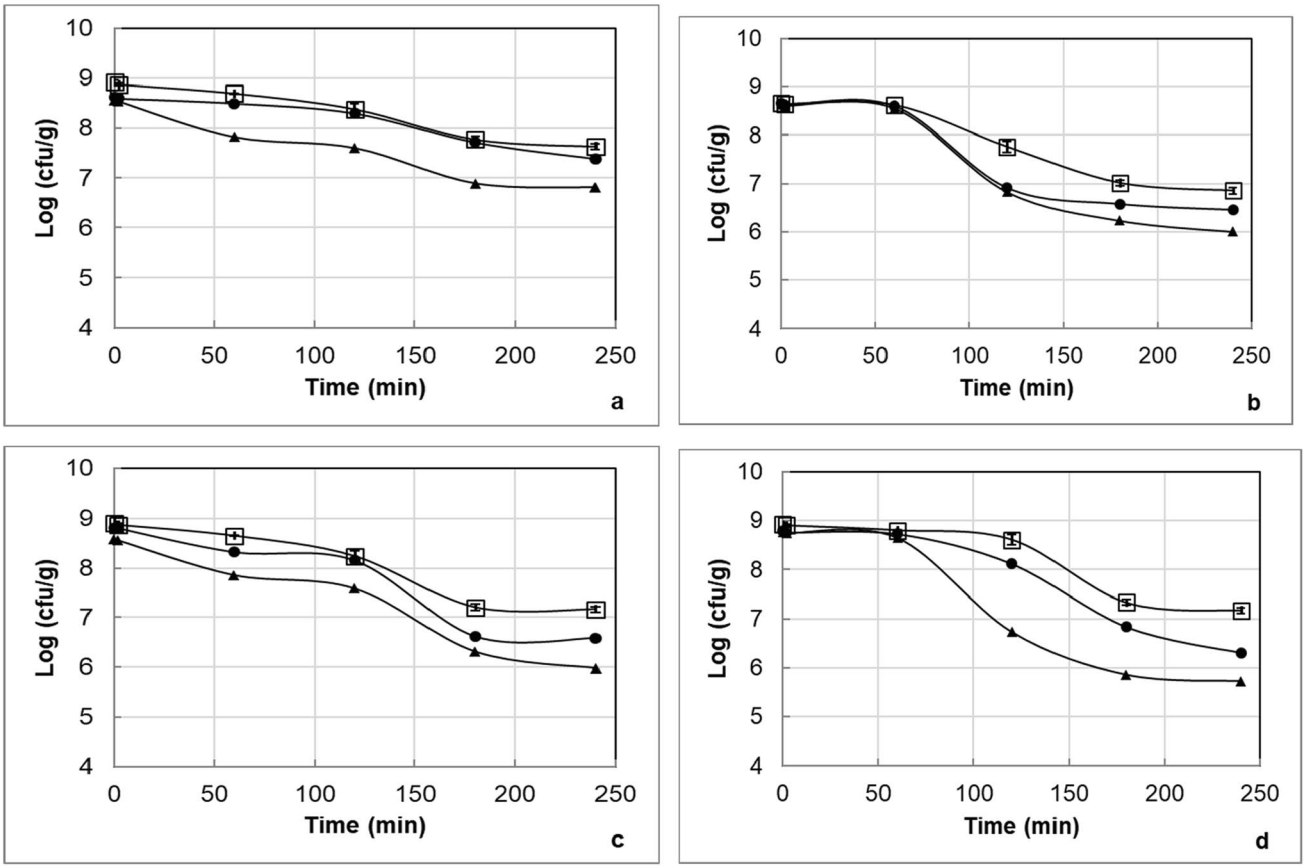

Fig. 4 The counts of *L. acidophilus* in the ice cream (**a**), yoghurt (**b**), white pickled cheese (**c**), and fermented acidophilus milk (**d**) samples produced with *L. acidophilus* NCFM on the 1st (white square), 15th (black circle), and 30th (black triangle) days of the storage during the passage through the dynamic in vitro gastrointestinal model

samples stored 1 day at 4 °C were 8.66 and 8.94 log cfu g^{-1}, respectively. The viable counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the yoghurt samples decreased about 0.04 and 0.27 log units, respectively, within 30 days of the storage. Geraldi et al. [42] and Ribeiro et al. [34] reported that the number of *L. acidophilus* in probiotic yoghurt decreased from 8.18 to 7.28 log cfu g^{-1} and from 8.74 to 7.64 log cfu mL^{-1}, respectively, during 30 days storage at about 4 °C. Nighswonger et al. [43] reported that some strains of *L. acidophilus* in yoghurt lost viability during storage at 7 °C for 28 days, while others maintained at near constant levels in yoghurt during the storage. However, Ng et al. [44] found that the count of *L. acidophilus* NCFM in yoghurt reduced by 4.6 logs during 28-day storage at 4 °C. They reported that *L. acidophilus* NCFM could be adversely affected from the elevated level of hydrogen peroxide produced by *L. delbrueckii* ssp. *bulgaricus* when grown in co-culture with the yoghurt starter culture. The contrasting findings regarding the viability of *L. acidophilus* NCFM in yoghurt during storage between the present study and the study of Ng et al. [44] may be due to the difference in strains of yoghurt starter bacteria used in the production of yoghurt. The survival of the probiotic bacteria may sometimes be threatened by the metabolic activities, e.g., productions of lactic acid and hydrogen peroxide, of yoghurt starter bacteria during incubation of milk and storage of yoghurt [6].

The counts of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the white pickled cheese samples remained at a constant level of 8 log cfu g^{-1} during the 30 days of the storage at 4 °C. Kılıç et al. [38] reported that the viability of probiotic bacteria was satisfactory in white pickled cheese even at the end of storage periods. Kasımoğlu et al. [20] manufactured probiotic white pickled cheese samples with *L. acidophilus* and found the numbers of *L. acidophilus* in the 30-day stored samples were greater than 7.0 log cfu g^{-1}.

The numbers of *L. acidophilus* NCFM and *L. acidophilus* DSM 20,079 in the fermented acidophilus milk samples stored 1 day at 4 °C were 8.92 and 8.96 log cfu mL^{-1}, respectively, and these counts remained stable up until the end of the storage period (30 days). Božanić et al. [21] reported fermented acidophilus cow's milk contained, after 30 days of storage, over 7.5 log cfu mL^{-1} of viable *L. acidophilus*. pH value is a critical factor for the survival of *L. acidophilus* in acidophilus milk, with a decrease in pH value of

acidophilus milk less than 4.5 reported to affect the viability of *L. acidophilus* [45]. Since the pH value of the fermented acidophilus milk samples containing *Lb. acidophilus* was around 4.5 during the 30-day storage period in this study, the viability of *Lb. acidophilus* in the fermented acidophilus milk was not negatively affected by the pH value.

The differences in strains of *L. acidophilus* and *L. acidophilus*-carrying dairy products, such as ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk, did not affect the viability of *L. acidophilus* in the product during the storage. A longer storage time of the dairy products samples resulted in slight decrease in the survival of the strains of *L. acidophilus*. In order to achieve the health benefits of probiotic bacteria for humans, the minimum viable count of probiotic bacteria should be ≥ 6 log cfu g^{-1} or mL^{-1} in the product up to the expiry date [18]. However, the numbers of viable *L. acidophilus* in all dairy products investigated in this study were equal or above 8 log cfu g^{-1} or mL^{-1} at the end of 30-day storage period.

The effects of the storage period on the counts of *L. acidophilus* in the samples during passage through the dynamic in vitro gastrointestinal model are given in Table 3. The statistical analysis showed that the effects of gastrointestinal model conditions and storage period on the counts of *L. acidophilus* DSM 20,079 and *L. acidophilus* NCFM in the samples were significant ($P < 0.001$). The counts of *L. acidophilus* NCFM in the ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples decreased 1.40, 2.22, 2.16, and 2.41 log units at the end of the small intestine part of the model, respectively, according to the number of *L. acidophilus* NCFM in the samples at the end of the mouth part of the model, while the counts of *L. acidophilus* DSM 20,079 in the ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples decreased 1.63, 2.11, 1.57, and 2.12 log units, respectively. A decrease in the counts of the strains of *L. acidophilus* was recorded in the samples during the passage through the model (Figs. 3 and 4). The decrease in the viable counts of *L. acidophilus* in the samples during the passage through the model increased significantly for all products with extension of storage time, as shown in Table 3.

The effects of dairy products, gastrointestinal model conditions, and storage period on the percent reduction of *L. acidophilus* counts in the samples are given in Table 4. The results showed that reduction of viable *L. acidophilus* counts was influenced significantly ($P < 0.05$) by the type of dairy products, gastrointestinal model conditions, and storage period. The highest decrease in the counts of *L. acidophilus* was determined in yoghurt sample followed by fermented acidophilus milk, white pickled cheese, and ice cream, respectively. However, the differences in the percentage reduction in counts of *L. acidophilus* NCFM between yoghurt and fermented acidophilus milk and in the percentage reduction in counts of *L. acidophilus* DSM 20,079

Table 3 Effects of the storage period on the counts of *L. acidophilus* in the samples during passage through the dynamic in vitro gastrointestinal model

	Count of *L. acidophilus* NCFM (log cfu g^{-1} or mL^{-1})				Count of *L. acidophilus* DSM 20,079 (log cfu g^{-1} or mL^{-1})			
	Ice cream	Yoghurt	White pickled cheese	Fermented acidophilus milk	Ice cream	Yoghurt	White pickled cheese	Fermented acidophilus milk
Gastrointestinal model conditions	***	***	***	***	***	***	***	***
End of mouth	8.67 ± 0.07 a*	8.66 ± 0.03 a	8.74 ± 0.14 a	8.81 ± 0.03 a	8.60 ± 0.02 a*	8.64 ± 0.01 a	8.31 ± 0.04 a	8.88 ± 0.02 a
Stomach after 1 h	8.34 ± 0.17 b	8.60 ± 0.02 a	8.28 ± 0.16 b	8.73 ± 0.03 b	8.48 ± 0.05 b	8.59 ± 0.02 a	8.18 ± 0.06 b	8.56 ± 0.14 b
Stomach after 2 h	8.09 ± 0.16 c	7.17 ± 0.19 b	8.00 ± 0.13 c	7.82 ± 0.36 c	7.96 ± 0.03 c	7.26 ± 0.22 b	7.23 ± 0.12 c	7.76 ± 0.27 c
Small intestine after 1 h	7.46 ± 0.18 d	6.61 ± 0.15 c	6.71 ± 0.17 d	6.67 ± 0.28 d	7.09 ± 0.05 d	6.71 ± 0.21 c	6.82 ± 0.14 d	6.86 ± 0.06 d
Small intestine after 2 h	7.27 ± 0.15 e	6.44 ± 0.16 d	6.58 ± 0.22 d	6.40 ± 0.27 e	6.97 ± 0.04 e	6.53 ± 0.19 d	6.74 ± 0.16 e	6.76 ± 0.09 e
Storage period (days)	***	***	***	***	***	***	***	***
1	8.27 ± 0.17 a	7.88 ± 0.25 a	8.03 ± 0.24 a	8.16 ± 0.25 a	7.93 ± 0.02 a	7.95 ± 0.21 a	7.73 ± 0.19 a	8.09 ± 0.30 a
15	8.10 ± 0.16 b	7.44 ± 0.33 b	7.69 ± 0.31 b	7.75 ± 0.34 b	7.80 ± 0.23 b	7.39 ± 0.34 b	7.47 ± 0.21 b	7.71 ± 0.28 b
30	7.53 ± 0.21 c	7.25 ± 0.38 c	7.26 ± 0.33 c	7.14 ± 0.44 c	7.73 ± 0.23 c	7.30 ± 0.36 c	7.17 ± 0.27 c	7.48 ± 0.30 c

Values are expressed mean ± standard deviation

*Values with different letters show the difference in the same column ($P < 0.05$), ***$P < 0.001$

Table 4 Effects of dairy products, gastrointestinal model conditions, and storage period on the reduction of *L. acidophilus* counts in the samples

	Reduction of *L. acidophilus* NCFM counts (%)	Reduction of *L. acidophilus* DSM 20,079 counts (%)
Dairy products		
Ice cream	8.47 ± 1.25c*	10.79 ± 1.54c *
Yoghurt	13.38 ± 2.13a	14.56 ± 2.02a
White pickled cheese	12.62 ± 1.94b	10.88 ± 1.45c
Fermented acidophilus milk	13.00 ± 2.36ab	12.74 ± 1.90b
Gastrointestinal model conditions		
End of mouth	0.25 ± 0.15e	1.23 ± 0.23e
Stomach after 1 h	2.87 ± 0.62d	2.97 ± 0.37d
Stomach after 2 h	11.08 ± 1.35c	13.33 ± 1.07c
Small intestine after 1 h	21.47 ± 1.27b	21.14 ± 0.77b
Small intestine after 2 h	23.66 ± 1.30a	22.54 ± 0.81a
Storage period (days)		
1	8.95 ± 1.27c	10.01 ± 1.29c
15	11.21 ± 1.67b	12.93 ± 1.53b
30	15.44 ± 1.98a	13.78 ± 1.67a

*Different superscript letters after values indicate significant differences using Duncan's multiple range test ($P < 0.05$). Values are expressed mean ± standard deviation

between white pickled cheese and ice cream were not significant ($P > 0.05$). When the viabilities of the two strains of *L. acidophilus* in the samples were taken in consideration, *L. acidophilus* in the ice cream samples was more resistant to the harsh conditions of the dynamic in vitro gastrointestinal model, while *L. acidophilus* in the yoghurt samples was less resistant to the same conditions of the model. Furthermore, during the passage of the dairy products containing *L. acidophilus* through the parts of the model and prolonged storage, the reduction of viable counts of *L. acidophilus* in the samples increased (Table 4).

Kailasapathy [46] reported that yoghurt was not suitable for use as probiotics carrier food, due to post acidification that occurred in yoghurt storage resulting in major cell death of probiotic bacteria. Sharp et al. [47] showed that *Lactobacillus casei* 334e in yoghurt had lower resistance than that in cheese to acid stress (pH 2). A possible reason for this was explained by the authors that lower pH of yoghurt (pH 4.3) might be the reason for the sublethal damage to *L. casei* 334e in yoghurt during storage compared with low-fat cheese (pH 5.1). Ranadheera et al. [48] evaluated effect of carrier food type (goat's milk ice cream, plain yoghurt, and fruit yoghurt) on in vitro gastrointestinal survival of *Lactobacillus acidophilus* LA-5, and ice cream was found to improve the bile tolerance of the *L. acidophilus* LA-5 compared to plain yoghurt and fruit yoghurt.

Among food products, ice cream is known to be an advantageous vehicle to deliver probiotic bacteria to human body since it has relatively high pH values (> 5.5) and high total solid contents providing protection for probiotic cells [49, 50]. Moreover, metabolizable sugars can protect the probiotic bacteria. Corcoran et al. [51] reported that the presence of 19.4 mM glucose resulted in up to 6 log enhanced survival of *Lactobacillus rhamnosus* GG in simulated gastric juice at pH 2.0 following 90 min of exposure as compared to the control without glucose. The authors indicated that in acid conditions, glucose provides ATP to F_0F_1-ATPase via glycolysis and allows protons to be removed from the cell thereby enhancing probiotic survival. In our study, the lowest decrease in the viability of *L. acidophilus* in ice cream samples after exposure to the dynamic in vitro gastrointestinal model could depend mainly on milk proteins, fat and lactose contents and relatively high pH values of ice cream samples [52]. Cheese has also a relatively high pH values, solid consistency (high fat and protein contents) compared to other dairy products and good vehicles for probiotics like ice cream [53, 54]. However, the high salt concentration in cheese could be a potential problem for viability of probiotics during long shelf life of cheese and after passage through gastrointestinal tract [55]. Moreover, the results of this study showed that although a decrease in the numbers of *L. acidophilus* in the dairy products was observed, *L. acidophilus* still survived ($\geq 10^6$ cfu g^{-1}) in the dynamic in vitro gastrointestinal model, except for the white pickled cheese and fermented acidophilus milk samples produced by using *L. acidophilus* NCFM only on the 30th days of storage (Figs. 3 and 4).

In Fig. 5a–d , the changes in the counts of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are illustrated for the yoghurt samples containing *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM within 30 days of storage during the passage through the dynamic in vitro gastrointestinal

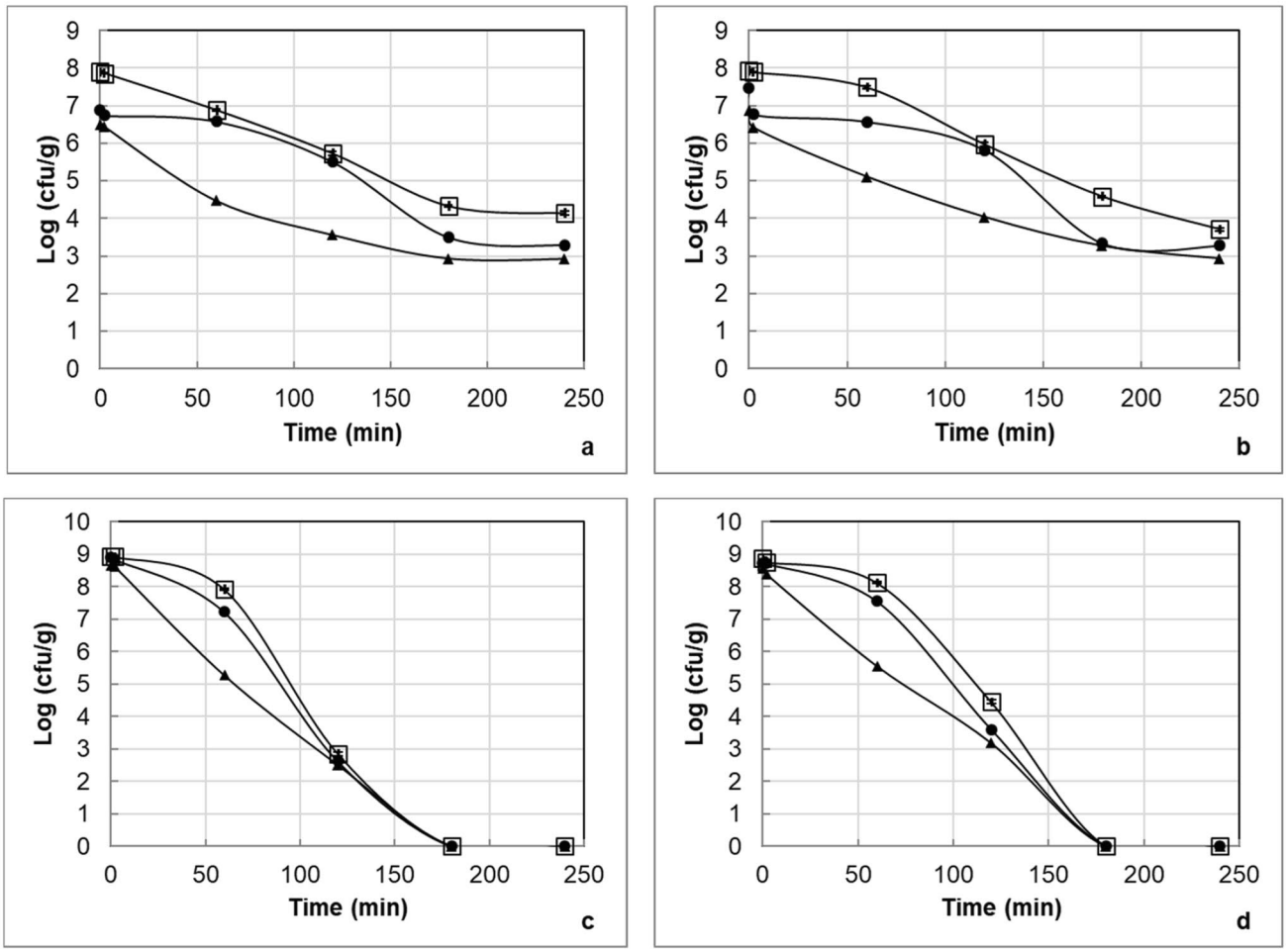

Fig. 5 The counts of *L. delbrueckii* subsp. *bulgaricus* in the yoghurt samples produced with *L. acidophilus* DSM 20,079 (**a**) or *L. acidophilus* NCFM (**b**) and in the counts of *S. thermophilus* in the yoghurt samples produced with *L. acidophilus* DSM 20,079 (**c**) or *L. acidophilus* NCFM (**d**) on the 1st (white square), 15th (black circle), and 30th (black triangle) days of the storage during the passage through the dynamic in vitro gastrointestinal model

model. In the yoghurt sample, a decrease for *L. delbrueckii* subsp. *bulgaricus* was observed; however, the decrease in the count of *S. thermophilus* was much pronounced and after 180 min of the passage through the dynamic in vitro gastrointestinal model no viable counts of *S. thermophilus* could be recorded. During 30-day storage, the viable counts of *L. delbrueckii* subsp. *bulgaricus* in the yoghurt samples containing *L. acidophilus* DSM 20,079 and *L. acidophilus* NCFM decreased in the range of 3.6–3.8 log units and 4.0 and 4.2 log units, respectively, after the passage through the dynamic in vitro gastrointestinal model. According to these findings, it is possible to say that the strain of *L. delbrueckii* subsp. *bulgaricus* used in the production of yoghurt is more resistant than the strain of *S. thermophilus* used in the production of yoghurt to the harsh conditions of the dynamic in vitro gastrointestinal model. Hernández-Galán et al. [56] did not observe any protective effect of the dairy matrices (skimmed milk, whole milk, rennet gel from skimmed milk, and rennet gel from whole milk) on survival of *Streptococcus thermophilus* TIL 257 during dynamic in vitro digestion. Furthermore, García-Hernández et al. [57] found that *Lactobacillus delbrueckii* subsp. *bulgaricus* CECT 4005 T and *Streptococcus thermophilus* CECT 801 in yoghurt were more sensitive to gastric juice than intestinal juice because of their high ability to resist intestinal conditions. Pacheco et al. [58] reported that *Lactobacillus delbrueckii* subsp. *bulgaricus* NRRL-734 could survive in a high number under simulated gastrointestinal conditions when it was consumed together with food with a viscous consistency because of slowed diffusion processes and less interaction between gastrointestinal conditions and *Lactobacillus delbrueckii* subsp. *bulgaricus* cells.

In Fig. 6a–d , the changes in the counts of total mesophilic aerobic bacteria and total *Lactococcus* spp. are shown for the white pickled cheese samples containing *L. acidophilus* DSM 20,079 or *L. acidophilus* NCFM within

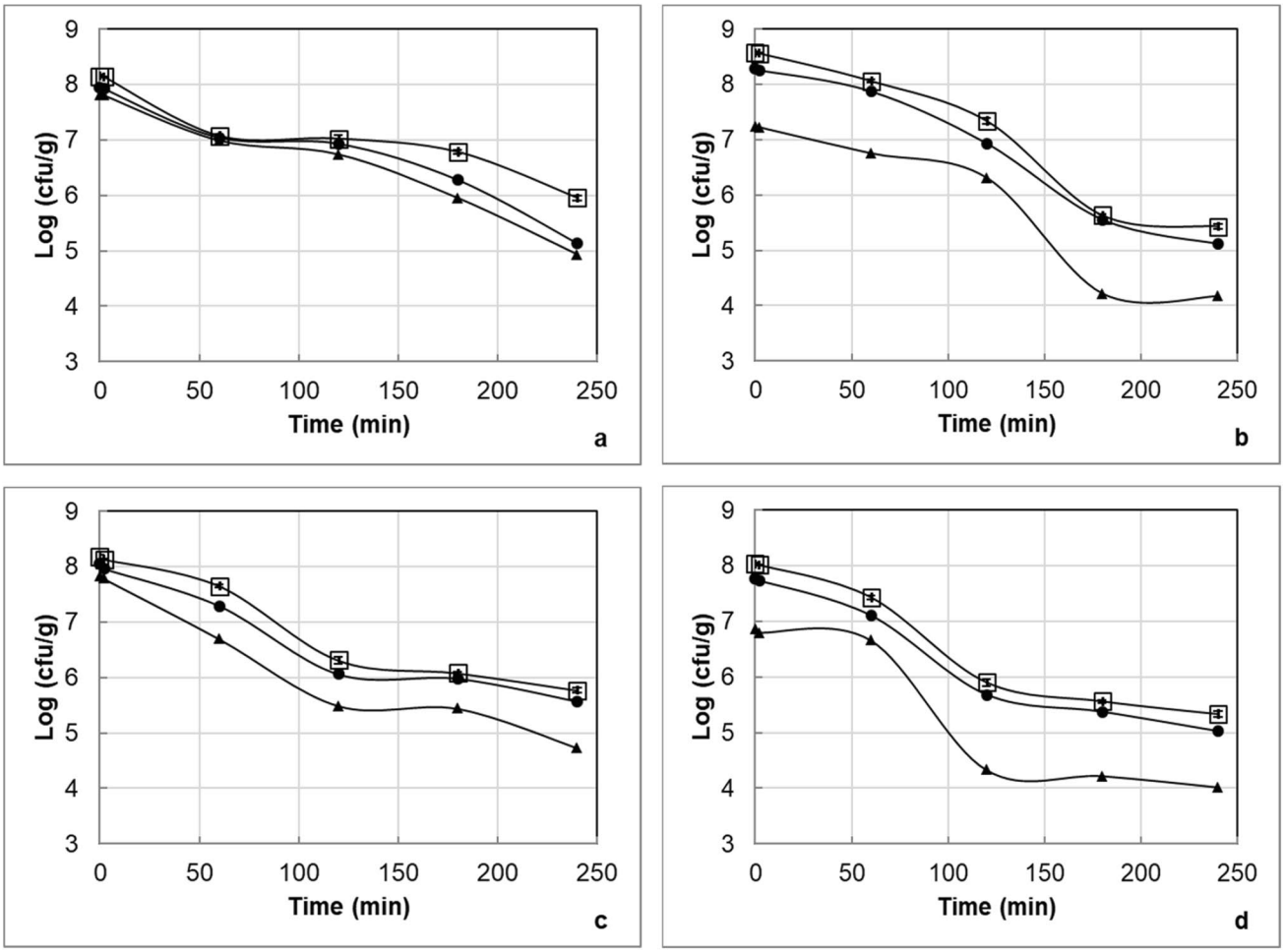

Fig. 6 The counts of total mesophilic aerobic bacteria in the white pickled cheese samples produced with *L. acidophilus* DSM 20,079 (**a**) or *L. acidophilus* NCFM (**b**) and in the counts of total *Lactococcus* spp. in the white pickled cheese samples produced with *L. acidophilus* DSM 20,079 (**c**) or *L. acidophilus* NCFM (**d**) on the 1st (white square), 15th (black circle), and 30th (black triangle) days of the storage during the passage through the dynamic in vitro gastrointestinal model

30 days of storage during the passage through the dynamic in vitro gastrointestinal model. About three-log reduction in the counts of total mesophilic aerobic bacteria and total *Lactococcus* spp. in the white pickled cheese samples was detected after exposure of the samples to the gastrointestinal model for approximately 4 h. The decrease in the counts of total mesophilic aerobic bacteria and total *Lactococcus* spp. in the white pickled cheese samples during the passage through the model increased significantly with extension of storage time. The counts of total mesophilic aerobic bacteria and total *Lactococcus* spp. in the cheese samples stored 30 day at 4 °C ranged between 4.2–4.9 log cfu g^{-1} and 4.0–4.7 log cfu g^{-1}, respectively. Our study was generally in agreement with the studies by Sumeri et al. [59] and Adouard et al. [60] who reported that the resistance of cheese bacteria against gastrointestinal stresses varied depending on their species, genotypes, and physiological states.

Conclusion

The dynamic gastrointestinal model designed in this study can effectively be used for comparative survival researches of probiotic bacteria in dairy products. The results obtained in the present study showed that there was no difference among ice cream, yoghurt, white pickled cheese, and fermented acidophilus milk samples in terms of carrying food of *L. acidophilus* if the gastrointestinal model part of the study was not considered. The matrix of the dairy product and storage period has significant effects on the survival of *L. acidophilus* during passage through the dynamic in vitro gastrointestinal model. This study demonstrates that among the examined dairy products, ice cream is the most protective product under in vitro gastrointestinal digestion conditions concerning the survival of *L. acidophilus*. The importance of the food matrix containing probiotic bacteria with regard to their survival during

the passage through the gastrointestinal tract should be studied more extensively.

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Declarations

Conflict of interest The authors declare no competing interests.

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