



## Functional Probiotic Yoghurt Production with Pomegranate (*Punica granatum* L.) Juice Concentrate Fortification

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### Authors' contributions

This work was carried out in collaboration between both authors. Author NK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GK and NK managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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### ABSTRACT

In this study, probiotic yoghurts were produced with cow's milk with 13% dry matter standardized concentration by 3% skimmed milk powder addition and pomegranate (*Punica granatum* L.) juice concentrate (PGJC) fortification at different ratios (1% v/v and 2% v/v) One sample was produced as plain yoghurt (PY). The samples were stored at +4°C±1 for 14 days. Physicochemical, microbiological, sensory properties as well as total phenolic content (TFC) were analyzed at the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> and the 14<sup>th</sup> days of the storage. In the study, some physicochemical properties and TFC levels of pomegranate juice (PGJ) and PGJC were analyzed at the 0<sup>th</sup> day of the storage. It was determined that, with PGJC fortification, physicochemical, rheological properties of yoghurt improved and TFC levels increased. PGJC fortification had no negative effect on the sensory properties of the samples, however it significantly affected the color property of the 2% (w/v) level

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fortification. The relation between PGJC fortification and viability and numerical increase of probiotics were significant ( $p < 0.05$ ). The increase in PGJC level improved the mentioned parameters. In the study, the relation between PGJC fortification and the physicochemical, microbiological, rheological, sensory properties and the increase in TFC levels of yoghurts were significant. Conclusively, 1% (w/v) and 2% (w/v) PGJC fortification improved the functional properties of yoghurt samples.

*Keywords: Probiotic; yoghurt; pomegranate juice fortification; total phenolic content; Punica granatum L.*

## 1. INTRODUCTION

Probiotics are live non-pathogenic micro-organisms which provide numerous health benefits on the human health when consumed in adequate amounts due to their effects in the intestinal flora [1]. These effects can be listed as promoting digestion and immune systems, reduction of blood cholesterol levels, cardiovascular disease, protection against osteoporosis and urinary infections and anti-mutagenic and anti-carcinogenic effects.

Fermented dairy products are ideal food matrix for promoting probiotics and increasing their viability. An important technological property of a probiotic culture is the viability during process and storage. Factors including probiotic species, incubation level, incubation temperature, final incubation pH, the presence of hydrogen peroxide and oxygen, metabolite concentration, the presence of other microorganisms, medium lactic and acetic acid levels and storage temperature are affective in the viability of probiotics in the food [2,3]. However, the viability of probiotics in the acidic environment vary depending on the species [4]. The relation between the viability of probiotics and the probiotic species during the storage of the products are significant [5].

There is a novel trend for using fruits, cereals and especially fruit juices with high phenolic content in probiotic yoghurt production in order to promote the development of probiotics and preserve their viability [2]. The level of using of non-dairy ingredients (fruits, vegetables, fruit juices, pulps and mashes) in yoghurt production is limited to 50% maximum (m/m). There are many studies on fruit-food matrix which have positive effects on the viability and activity of probiotics. In these studies, it was reported different fruit juice and concentrates were used in yoghurt production and some of them were affective in the preservation of the viability of probiotics [6,7,8]. The effect of fruit juice

fortification of the viability of probiotics vary depending on some factors including culture preparation method, fruit juice concentration, culture ratio, storage temperature and oxygen levels [7]. Today, Greek type commercial yoghurt with pomegranate juice (PGJ) concentrate fortification is produced in USA and UK [9]. Also, it was reported that PGJ fortification had a positive effect on the viability of probiotics including *Lactobacillus plantarum*, *Lactobacillus delbruekii*, *Lactobacillus paracasei*, *Lactobacillus acidophilus* in non-dairy probiotic beverages [10].

Pomegranate juice obtained from pomegranate (PG, *Punica granatum L.*) of Punicaceae familia contains high levels of antioxidant polyphenols and phenolic acids (ellagic acid (EA), caffeic acid and chlorogenic acid) [11]. Antioxidant polyphenols are known as ellagic tannins (= hydrolysable phenolics) (gallic acid and ellagic acid) [12] and anthocyanins (such as delphinidin, cyanide and pelargonidin) [13]. Chlorogenic and caffeic acid in PGJ composition have anti-carcinogenic, anti-tumor and antioxidant activities [14]. The antioxidant property of PGJ is attributed to polyphenol and punicalagine which is a water-soluble basic ellagitanine. PGJ and pomegranate seed extracts have 2-3 times more antioxidant effects compared to red grapes and green tea. The acidity of pomegranate gradually decreases during ripening, whereas total sugar content increases and lead to color changes in anthocyanin pigments [14].

In this study, probiotic yoghurts were produced with cow's milk with 13% dry matter standardized concentration by 3% skimmed milk powder addition and pomegranate juice concentrate (PGJC) fortification at different ratios (1% v/v and 2% v/v). Yoghurt samples were stored for 14 days at  $+4^{\circ}\text{C} \pm 1$ , and physicochemical, rheological, total phenolic content, microbiological and sensory analysis were conducted on the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days of the storage.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Raw cow's milk (CoM) used in the study was obtained from Ege University Department of Animal Science, Hicaz type pomegranate (*Punica granatum* L.) used in the production of pomegranate juice concentrate (PGJC) was obtained from a local producer in, Mersin (Turkey), skimmed milk powder (SMP) was obtained from Pinar Sut Inc. (Turkey), probiotic yoghurt culture YO-MIX 205 (*Streptococcus thermophilus* + *Lactobacillus bulgaricus*, + *Lactobacillus acidophilus*) (Danisco-FRANCE) and freeze-dried yoghurt culture BIFI (*Bifidobacterium spp.*) was obtained from CSL laboratories (Strade per Merlino, 3- 26839, Italy). Probiotic yoghurt production was conducted in Ege University Dairy Technology Pilot Plants.

### 2.2 Pomegranate Juice Concentrate (PGJC) Production

In PGJ production, pomegranates were washed with water, separated to their pieces and pressed in press (Bucher-Guyer, Niederweningen, Switzerland) under 1.2-1.8 bar for 5 minutes (as the press pressure causes an extreme bitter taste in pomegranate juice, a moderate pressure was applied in the research) and the juice was clarified. In the clarification of PGJ, 0.3 g/L (Sigma-Aldrich) gelatin which was determined as a result of preliminary trials was added to the pomegranate juice and waited for 15 minutes. Then 0.3 g/L bentonite was added (Sigma-Aldrich) and kept at water bath at 50°C for 45 minutes. Clarification process was applied to the pomegranate juice and cooled to room temperature. Pomegranate juice was filtered (40x40 cm, pore size 20 Mikron) through a filtration system consisting of gauze and filter paper and separated from the sediments. Then it was concentrated (PGJC) to 55° Brix value in a laboratory type rotary evaporator (SCILOGEX RE 100-Pro/20 - 280 rpm) at 85±1°C and stored at +4°C±1. Total dry matter, water soluble dry matter (°Brix), pH, titratable acidity and total phenolic content (TFC) (methods are given in section 2.4) of PGJ and PGJC were determined on the 0th day of the storage.

### 2.3 Probiotic Yoghurt (PY) Production

In this study, probiotic yoghurts were produced with cow's milk with 13% dry matter standardized concentration by 3% skimmed milk powder

addition and pomegranate (*Punica granatum* L.) juice concentrate (PGJC) fortification at different ratios (1% v/v (PY<sub>x</sub>) and 2% v/v (PY<sub>y</sub>) and 5% starter culture (*S. thermophilus* + *L. bulgaricus*+ *L. acidophilus* and *Bifidobacterium spp.*) mixture . Cow's milk was divided into 3 batches in yoghurt production. PGJC was added before pasteurization in order to maintain the degradation of anthocyanins [15] and make a lesser effect on the color properties of yoghurt samples. Accordingly, the 1st batch was the plain batch while the 2<sup>nd</sup> batch was fortified with only 1% (w/v) PGJC (PY<sub>x</sub>) and the 3rd batch was fortified with %2 (w/v) PGJC (PY<sub>y</sub>). The batches were then homogenized with Ultra Turrax Blender (at 1200 rpm for 40 seconds) (IKA, Merc, Germany) and pasteurized at 85°C for 20 minutes. Then the samples were cooled to 42-43°C and inoculated with 5% (v/v) starter culture. The samples were distributed to plastic cups (200 g) and left to incubation. The incubation was ended at 4.60 pH (5 hours) and PY, PY<sub>x</sub> and PY<sub>y</sub> probiotic yoghurt samples were obtained. Samples were stored for 14 days at 4°C±1, and physicochemical, rheological, color, microbiological and sensory analysis were conducted on the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days of the storage. Total phenolic contents were determined at the 5<sup>th</sup>, 12<sup>th</sup>, 24<sup>th</sup>, 48<sup>th</sup>, 72<sup>nd</sup> hours and at the 14<sup>th</sup> day of the storage.

### 2.4 Physical-chemical Analyses

Yoghurt and raw cow milk (CoM) dry matter and ash content measurements was performed according to the gravimetric method with Binder ED-53 equipment and Protherm PFL 110/6 equipment , fat was determined according to the Gerber method, titratable acidity was determined as lactic acid %, pH was measured with a SS-3 Zeromatic pH meter (Beckman Instruments Inc., California, USA), protein content was analyzed by the Kjeldahl method [16], lactose levels were measured with an Atago Polax x 2L (Japan) polarimeter [17], serum separation was analyzed according to [18], texture analysis was performed with a Brookfield CT3 4500 Texture Analyzer (USA/ Shape Cylinder; target 10 mm; test speed 1 mm/s), color measurements were performed with a Hunter color and color difference measuring device (Model D25A-9) (after the zero calibration and adjustments were done according to a white plate (L=95.4, a=-1.3, b=2.1)), and viscosity levels were measured with a Brookfield Digital Viscometer (Model DV-II+PRO, USA) [180 rpm, 10°C, in CaM and yoghurt samples LV2 spindle (23.47 g), between 13-42% Torque]

as cP [19]. Water soluble dry matter value (°Brix) of the pomegranate juice concentrate was measured using a table-type Abbe refractometer at 20°C [20].

## 2.5 Total Phenolics (TP)

Total phenolic (TP) levels of yoghurt samples were measured by spectrophotometer (Optima SP-300, Japan) at 720 nm according to the Folin-Ciocalteus method and determined as "mg gallic acid equivalent (GAE) L<sup>-1</sup>" [21]. TP analyses were replicated three times for each yoghurt sample. First, gallic acid stock solution at (500 mg L<sup>-1</sup> concentration) was prepared. Then, solutions were prepared by adding appropriate amounts of the stock solution (1, 2, 4, 6 or 8 mL). Absorbance values were read at 720 nm, linear regression analysis was applied and a gallic acid standard curve and the equation describing the curve were obtained (Fig. 1). Absorbance values of yoghurt samples were read at 720 nm, these values were calculated by placing in the equation describing the standard curve.

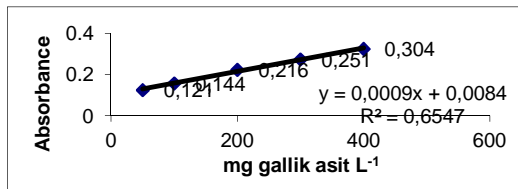


Fig. 1. Gallic acid standard curve and equation

## 2.6 Microbiologic Analyses

Starter culture counts of the yoghurt samples were performed according to International Dairy Federation standard method [22,23]. *L. acidophilus* and *Bifidobacterium spp.* counts were determined according to International Dairy Federation standard methods [24,25].

## 2.7 Sensory Evaluation

The sensory evaluation of yoghurts was performed by consumer acceptance test [26] based on the appearance, texture, flavor, aroma, and overall impression of the product, using a 9-point hedonic scale (1-disliked extremely; 9-liked extremely). The sensory evaluation was made by a panel of nine individuals. The sensory evaluation of the yoghurt samples was performed after 1 and 14 days of refrigerated storage.

## 2.8 Statistical Analysis

Samples were examined with 3 parallels and 2 repetitions. SPSS version 15 (IBM SPSS Statistics) statistical analysis package software was used for analyses. Significance according to analysis of variance (ANOVA) was tested according to the Duncan multiple comparison test at  $p < 0.05$  level.

## 3. RESULTS AND DISCUSSION

In the study, in CoM, dry matter was determined 10.16%, fat 2.90%, protein 3.04%, lactose 2.92%, ash 1.68%, lactic acid 0.130%, pH 6.53, and viscosity was 2.74 cp (20°C). Total dry matter content of PGJ at the 0th day was determined as 14.70% while water soluble dry matter was 15.12°Brix, pH was 3.33, titratable acidity was 1.320% and TPC was 2239.21 mg GAE L<sup>-1</sup>. It is known that pH values may vary in pomegranate juice. This change in pH values is associated with the application process and the varying amount of buffer in the composition of pomegranate juice. Vardin and Fenercioğlu [27] reported that TPC varied depending on the species, ripening and pressing method. Total dry matter in PGJC on the 0th day was %62.24, while water soluble dry matter was 55°Brix, pH was 3.08, titratable acidity was 7.21%g<sup>-100ml</sup> and TPC was determined as 18133.47 mg GAE L<sup>-1</sup>. With condensing the fresh pomegranate juice, dry matter, water soluble dry matter, titratable acidity and TPC values increased whereas pH decreased. The relation between the condensation process and the parameters were found to be significant ( $p < 0.05$ ). Physicochemical properties of probiotic yoghurts produced from CoM with increased dry matter with 3% SMP (w/v) addition are given Table 1.

Lactic acid % (LA%) value increased during storage. The increase in acidity in PY<sub>Y</sub> and PY<sub>X</sub> between the 1<sup>st</sup> and the 14<sup>th</sup> days was higher than that in PY sample. The increase in PY<sub>Y</sub> was higher than that in PY<sub>X</sub>. The relation between the increase in acidity and fruit concentrate during storage was significant ( $p < 0.05$ ). The increase in acidity in yoghurts fortified with PGJC was associated with high levels of total sugar content (glucose + fructose + maltose) in PGJ [28]. Lactic acid bacteria showed a better growth especially in the presence of glucose and some other sugars (saccharose, maltose) and the increase in acidity was higher [29].

**Table 1. Physicochemical properties of PY, PY<sub>x</sub> and PY<sub>y</sub> (n=3)**

	Time storage	PY	PY <sub>x</sub>	PY <sub>y</sub>
Dry matter (%)	1 <sup>st</sup> day	13.26±0.05 <sup>aA</sup>	14.33±1.06 <sup>aB</sup>	15.85±1.14 <sup>aC</sup>
	5 <sup>th</sup> day	13.15±0.72 <sup>aA</sup>	14.20±1.40 <sup>aB</sup>	15.72±1.10 <sup>aC</sup>
	10 <sup>th</sup> day	12.21±0.21 <sup>aA</sup>	13.51±0.15 <sup>aB</sup>	15.70±1.16 <sup>aC</sup>
	14 <sup>th</sup> day	12.05±2.47 <sup>aA</sup>	13.19±1.12 <sup>aB</sup>	15.68±1.01 <sup>aC</sup>
Viscosity (cP)	1 <sup>st</sup> day	862±1.09 <sup>aA</sup>	1157±3.89 <sup>aB</sup>	1356±4.31 <sup>aC</sup>
	5 <sup>th</sup> day	1226±2.19 <sup>bA</sup>	1289±5.16 <sup>bB</sup>	1445±5.28 <sup>aC</sup>
	10 <sup>th</sup> day	2387±1.25 <sup>bA</sup>	2516±5.06 <sup>bB</sup>	2647±4.42 <sup>bC</sup>
	14 <sup>th</sup> day	2654±3.51 <sup>bA</sup>	2717±4.89 <sup>bB</sup>	2916±7.04 <sup>bC</sup>
Serum separation (%)	1 <sup>st</sup> day	6.21±1.16 <sup>aA</sup>	5.41±1.27 <sup>aB</sup>	3.27±0.12 <sup>aC</sup>
	5 <sup>th</sup> day	11.06±0.07 <sup>aA</sup>	10.28±0.52 <sup>aB</sup>	4.67±0.06 <sup>aC</sup>
	10 <sup>th</sup> day	16.09±2.76 <sup>aA</sup>	13.08±0.19 <sup>aB</sup>	6.76±0.03 <sup>aC</sup>
	14 <sup>th</sup> day	18.25±0.90 <sup>aA</sup>	15.23±0.54 <sup>aB</sup>	7.02±0.02 <sup>aC</sup>
pH	1 <sup>st</sup> day	4.51±0.28 <sup>aA</sup>	4.47±1.06 <sup>aB</sup>	4.40±0.52 <sup>aC</sup>
	5 <sup>th</sup> day	4.44±0.39 <sup>aA</sup>	4.39±1.02 <sup>aB</sup>	4.32±0.17 <sup>bC</sup>
	10 <sup>th</sup> day	4.25±0.84 <sup>aA</sup>	4.21±0.69 <sup>aB</sup>	4.19±0.67 <sup>bC</sup>
	14 <sup>th</sup> day	4.18±0.09 <sup>bA</sup>	4.08±0.74 <sup>bB</sup>	4.02±0.73 <sup>bC</sup>
Titration acidity (LA %)	1 <sup>st</sup> day	0.905±0.17 <sup>aA</sup>	0.978±0.22 <sup>aB</sup>	1.011±0.19 <sup>aC</sup>
	5 <sup>th</sup> day	1.022±0.06 <sup>aA</sup>	1.051±0.11 <sup>aB</sup>	1.077±0.09 <sup>bC</sup>
	10 <sup>th</sup> day	1.102±0.02 <sup>bA</sup>	1.109±0.05 <sup>bB</sup>	1.116±0.08 <sup>bC</sup>
	14 <sup>th</sup> day	1.113±0.07 <sup>bA</sup>	1.121±0.02 <sup>bB</sup>	1.141±0.05 <sup>bC</sup>
Fat (%)	1 <sup>st</sup> day	2.84±1.16 <sup>aA</sup>	2.86±0.40 <sup>aB</sup>	2.88±0.19 <sup>aC</sup>
	5 <sup>th</sup> day	2.82±2.17 <sup>aA</sup>	2.83±0.19 <sup>aB</sup>	2.85±0.20 <sup>aC</sup>
	10 <sup>th</sup> day	2.79±0.13 <sup>aA</sup>	2.80±0.20 <sup>aB</sup>	2.83±0.41 <sup>aC</sup>
	14 <sup>th</sup> day	2.75±1.42 <sup>aA</sup>	2.74±0.31 <sup>aB</sup>	2.79±0.29 <sup>aC</sup>
Protein (%)	1 <sup>st</sup> day	3.06±0.58 <sup>aA</sup>	3.05±0.84 <sup>aA</sup>	3.01±0.68 <sup>aB</sup>
	5 <sup>th</sup> day	3.02±0.67 <sup>aA</sup>	3.01±0.56 <sup>aA</sup>	2.84±0.83 <sup>aB</sup>
	10 <sup>th</sup> day	3.00±0.36 <sup>bA</sup>	2.99±0.74 <sup>aA</sup>	2.77±0.79 <sup>aB</sup>
	14 <sup>th</sup> day	2.97±0.58 <sup>bA</sup>	2.94±0.71 <sup>bA</sup>	2.65±0.59 <sup>bB</sup>
Lactose (%)	1 <sup>st</sup> day	2.52±1.47 <sup>aA</sup>	2.33±0.22 <sup>aA</sup>	2.27±0.61 <sup>aB</sup>
	5 <sup>th</sup> day	2.36±0.11 <sup>aA</sup>	2.11±0.88 <sup>aA</sup>	2.06±0.72 <sup>aB</sup>
	10 <sup>th</sup> day	2.24±0.37 <sup>aA</sup>	1.74±0.15 <sup>aA</sup>	1.53±0.24 <sup>bB</sup>
	14 <sup>th</sup> day	2.18±0.49 <sup>bA</sup>	1.48±0.17 <sup>bA</sup>	1.21±0.21 <sup>bB</sup>
Ash (%)	1 <sup>st</sup> day	1.62±0.28 <sup>aA</sup>	1.75±0.19 <sup>aA</sup>	1.81±0.29 <sup>aA</sup>
	5 <sup>th</sup> day	1.31±0.96 <sup>aA</sup>	1.33±0.29 <sup>aA</sup>	1.28±0.04 <sup>aA</sup>
	10 <sup>th</sup> day	1.08±0.75 <sup>aA</sup>	1.11±0.22 <sup>aA</sup>	1.16±0.27 <sup>aA</sup>
	14 <sup>th</sup> day	1.02±0.58 <sup>aA</sup>	1.06±0.17 <sup>aA</sup>	1.11±0.12 <sup>aA</sup>
Hardness(g)	1 <sup>st</sup> day	14.11±1.56 <sup>aA</sup>	16.0±1.89 <sup>aB</sup>	19±1.33 <sup>aC</sup>
	5 <sup>th</sup> day	16.47±1.47 <sup>bA</sup>	17.72±2.47 <sup>bB</sup>	23±3.42 <sup>bC</sup>
	10 <sup>th</sup> day	18.56±1.73 <sup>cA</sup>	19.81±1.87 <sup>cB</sup>	27.2±3.10 <sup>cC</sup>
	14 <sup>th</sup> day	21.41±1.49 <sup>dA</sup>	23.29±3.05 <sup>dB</sup>	29.1±2.23 <sup>dC</sup>

<sup>a, b, c</sup>: The differences between the values in the same column are statistically significant ( $p < 0.05$ )

<sup>A, B, C, D</sup>: The differences between the values in the same line are statistically significant ( $p < 0.05$ )

Dry matter decreased between the 1<sup>st</sup> and the 14<sup>th</sup> days. The highest decrease was determined as PY, PY<sub>x</sub> and PY<sub>y</sub> respectively. The relation between the level of fruit concentrate used in the yoghurt production and dry matter was significant ( $p < 0.05$ ).

Fat levels decreased in all samples during storage. The highest decreases were determined in PY, PY<sub>x</sub> and PY samples respectively. The fat

levels in PY<sub>y</sub> and PY<sub>x</sub> were higher compared to that determined in PY during storage. This was associated with 0.9% fat content found in the composition of PGJ [30]. A significant relationship was found between fat levels and fruit concentrate levels ( $p < 0.05$ ).

Protein and lactose decrease during storage. The highest decrease in protein and lactose levels determined between the 1<sup>st</sup> and the

14<sup>th</sup> days were sorted as PY<sub>Y</sub>, PY<sub>X</sub> and PY, respectively ( $p > 0.05$ ). Ash% values decreased during storage, and the decrease rate in ash values from highest to lowest was PY, PY<sub>X</sub> and PY<sub>Y</sub> respectively ( $p > 0.05$ ).

### 3.1 Rheological Properties

Coagulum stability (hardness) increased during storage and the effect of storage was significant ( $p < 0.05$ ). Serum separation was low in PY<sub>Y</sub> during storage. Serum separation and the decrease in dry matter in PY<sub>X</sub> was lower than that in PY. The relation between the increase in acidity and the serum separation was found to be significant ( $p < 0.05$ ). The relation between viscosity and fruit concentrate level, and the increase in acidity were found to be significant ( $p < 0.05$ ). Viscosity increased in all samples during storage, the increase rates from highest to lowest were PY<sub>Y</sub>, PY<sub>X</sub> and PY, respectively. It was reported that the viscosity increased with the increase in acidity and the prolongation of cold storage [31]. Rheological properties of the curd in yoghurt develop depending on the milk composition (casein and whey proteins), applied temperature, pH, soluble Ca ++ ratio and other factors. The increase in acidity increases the interaction between serum proteins and casein micelles, decreases the serum separation, makes the calcium more soluble and consequently increases the viscosity [32]. Rheological properties determined in PY<sub>Y</sub> were better than those in PY<sub>X</sub> and PY while rheological properties of PY<sub>X</sub> was more acceptable compared to those of PY.

### 3.2 Total Phenolics (TF)

The relation between TPC and storage period, serum separation, protein and fruit concentrate was significant ( $p < 0.05$ ). TPC value was 2239.21 mg GAE L<sup>-1</sup> in PGJ while it was 18133.47 mg GAE L<sup>-1</sup> in PGJC. However, TPC decreased by the end of the incubation (5th hour) in PY<sub>Y</sub> and PY<sub>X</sub> samples. TPC values of PY<sub>Y</sub> and PY<sub>X</sub> were determined as 10428 mg GAEL<sup>-1</sup> and 7457 mg GAEL<sup>-1</sup>, respectively. TPC levels increased until the 7<sup>th</sup> hour in both samples significantly. This increase continued until the 14<sup>th</sup> day of the storage at low levels. TPC values of PY<sub>Y</sub> at the 72<sup>nd</sup> hour and the 14<sup>th</sup> day were 11012 mg GAEL<sup>-1</sup> and 11183 mg GAEL<sup>-1</sup>, respectively. Additionally, TPC values of PY<sub>X</sub> at the 7<sup>th</sup> hour and the 14<sup>th</sup> day were 7562 mg GAE L<sup>-1</sup> and 7603 mg GAEL<sup>-1</sup>, respectively. The increase in TPC at the 72<sup>nd</sup> hour and the 14<sup>th</sup> day in PY<sub>Y</sub> was

significantly higher than that in PY<sub>X</sub>. The increase in TPC levels in PY<sub>Y</sub> was associated with the level of fruit concentrate (2% w/v) added to the milk. The relation between the level of fruit concentrate and the increase in TPC levels in yoghurt samples during storage was significant ( $p < 0.05$ ). Additionally, the increase in TPC during storage was associated with the complex structure formed as a result of the interaction between phenolic and protein [33]. Hydroxycinnamic acids with low molecular weight (including caffeic, ferulic, coumaric acid) and condensed phenols (catechin and derivatives) are high in PGJ [34]. Especially condense phenols are capable of forming stronger bonds with proteins. As a result of protein-phenolic interaction, a complex structure forms at a pH close to the isoelectric point [35]. Consequently, a large part of the protein precipitates and a small part is left dissolved [36]. It was reported that precipitated protein-phenol complexes have a more effective antioxidant activity compared to dissolved complexes [37]. As a result of the interaction between hydrophobic regions of proteins and the aromatic rings of the condensed tannins, nucleophilic groups of proteins (SH, OH NH<sub>2</sub>) and condensed phenolic groups concentrate and TPC increase during storage.

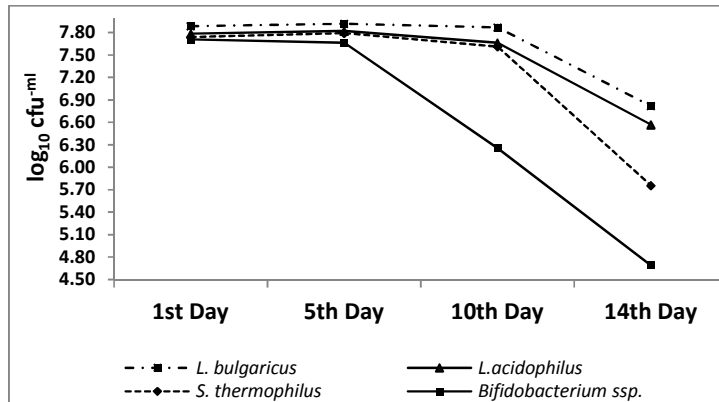
### 3.3 Microbiological Properties

Changes in *L. bulgaricus*, *S. thermophilus*, *L. acidophilus* and *Bifidobacterium spp.* levels in PY samples are given in Fig. 2.

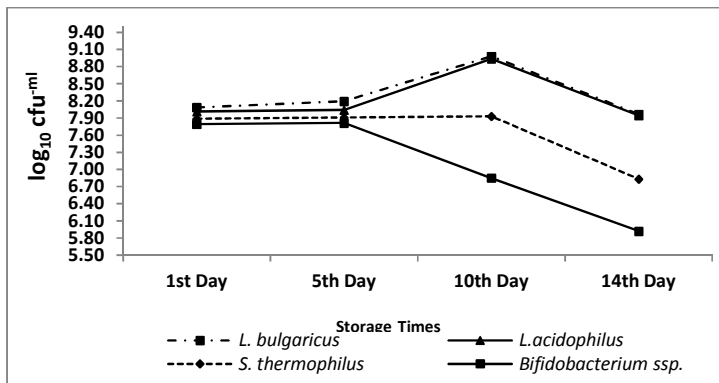
In PY samples, development and viability of starter cultures improved as the PGJC levels increased. Indeed, probiotic content in PY<sub>Y</sub> during storage was higher compared to that in PY<sub>X</sub>. The relation between fruit concentrate level and the probiotic levels was significant ( $p < 0.05$ ). Serum separation decreased during storage as the level of PGJC increased. This also had a positive effect on the development and viability of probiotics. It was reported that serum separation decreased as the fiber ratio in yoghurt production increased [38]. It was also reported that low serum separation helped the preservation of the symbiotic relationship between the starter cultures and the viability [39]. In the study, the samples with the highest probiotic contents were sorted as PY<sub>Y</sub>, PY<sub>X</sub> and PY. Probiotic development was at the lowest levels in PY sample which was the sample with the highest serum separation. Our study results was compatible with the studies reporting that starter cultures show a better development in the

presence of some sugars (such as glucose, maltose) [29], and that the relation between this development and the fruit concentrate levels was significant [7,40,41]. It was reported that pomegranate juice contains high levels of glucose and fructose and lower levels of maltose

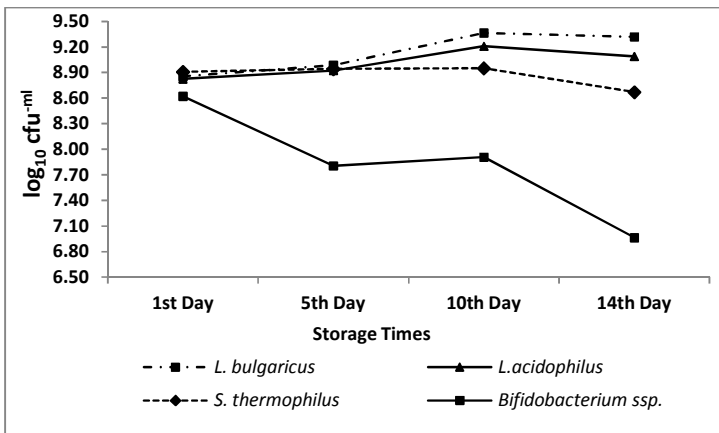
[28]. With the increase in starter culture levels, the relation between fat content and cold storage was significant. Ranadheera et al. [42] reported that the high fat content was affective in the preservation of the viability of probiotic microorganisms.



(a)



(b)



(c)

Fig. 2. *L. bulgaricus*, *L. acidophilus*, *Bifidobacterium spp.* and *S. thermophilus* counts in PY (a); PY<sub>x</sub> (b) and PY<sub>y</sub> (c) samples during storage

In general, probiotics in PY, PY<sub>Y</sub> and PY<sub>X</sub> increased from the 1<sup>st</sup> day of the storage. The highest increase in PY<sub>Y</sub> and PY<sub>X</sub> was determined at the 10<sup>th</sup> day while it was determined at the 5<sup>th</sup> day in PY. *Bifidobacterium spp.* level in PY decreased to 7.66 Log<sub>10</sub> cfu<sup>-ml</sup> at the 5<sup>th</sup> day. Probiotic levels decreased after the 10<sup>th</sup> day in PY<sub>Y</sub> and PY<sub>X</sub> and after the 5<sup>th</sup> day in PY. The highest decrease was determined in PY, PY<sub>X</sub> and PY<sub>Y</sub> samples, respectively. The increase in PY<sub>Y</sub> was higher than that in PY<sub>X</sub>, whereas the decrease was lower. The increase in probiotics levels in PY was lower than those in PY<sub>Y</sub> and PY<sub>X</sub>, however the decrease was higher. Probiotics with the highest viability were sorted as *L. acidophilus* and *Bifidobacterium spp.* Microorganism level at the 1<sup>st</sup> day in PY was 7 Log<sub>10</sub>cfu<sup>-ml</sup>, while it was 8 Log<sub>10</sub>cfu<sup>-m</sup> in PY<sub>Y</sub>. *L. bulgaricus*, *L. acidophilus* levels in PY<sub>X</sub> was 8Log<sub>10</sub>cfu<sup>-ml</sup>, while *S. thermophilus* and *Bifidobacterium spp.* level was 7Log<sub>10</sub>cfu<sup>-ml</sup>. The level of probiotics in PY<sub>X</sub> was higher compared to that of PY. *Bifidobacterium spp.* levels in the final products were low in PY (4.69 Log<sub>10</sub> cfu<sup>-ml</sup>) and PY<sub>X</sub> (5.92 Log<sub>10</sub> cfu<sup>-ml</sup>).

### 3.4 Sensory Evaluation

In the sensory evaluation conducted during storage, PY<sub>Y</sub> and PY<sub>X</sub> samples were more appreciated in terms of textural properties (including structure-consistency, appearance and color) compared to those of PY. In general, PY<sub>Y</sub> received higher structure-consistency scores compared to PY<sub>X</sub>. This was associated with low serum separation values, the increase in dry matter, viscosity and hardness during storage. The relation between the increase in storage period and structure-consistency was found to be significant (p<0.05). PGJC fortification provided an increasing taste and aroma to PY<sub>Y</sub> and PY<sub>X</sub> samples during storage and this was well appreciated by the panelists. The differences between aroma and taste properties of samples containing PGJC were not significant at the 1<sup>st</sup> day of the storage. However taste and aroma was more perceptible in PY<sub>Y</sub> sample. It was thought that high fat levels determined in samples fortified with PGJC during storage had an effect on taste. Fat presence was more felt with pomegranate concentrate level. Additionally, color change was observed in PY<sub>Y</sub> and PY<sub>X</sub> samples respectively with PGJC fortification. The color change was regarded as 'interesting' by the panelists. The change in PY<sub>X</sub> was reported to be more acceptable. The effect of PGJC on color was observed in all samples. However the

differences in color became more evident in the further days of the storage.

### 4. CONCLUSION

In this study, 1% (w/v) and 2% (w/v) PGJC fortification used in the yoghurt production improved the textural, sensory and microbiological properties compared to those of PY. Higher levels of PGJC fortification improved these properties. The viability of probiotics in the final product increased with PGJC fortification. TPC levels also increased with PGJC fortification. PGC fortification increased the concentration of yoghurts and changed the color of the samples. PY<sub>X</sub> sample received higher sensory scores in terms of these properties. However, this change was found acceptable by the panelists. Conclusively, it was determined that probiotic yoghurt production with increased functionality is possible.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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