

# Non-fermented Synbiotic Drink Based on Lactic Cheese Whey Which Incorporates *Lactobacillus rhamnosus* GG and *Lactobacillus paracasei*

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## Abstract

The use of acid whey in food formulations is one way to reduce the environmental problems associated with its disposal. In the present study, a new formulation of a drinking dessert was prepared using Lactic cheese whey, milk, xanthan gum at 4 levels (0, 0.1, 0.2 and 0.3%), resistant corn starch at 4 levels (0, 0.5, 1 and 1.5%), cocoa powder and sugar. Samples containing starch and gum had higher viscosity and were completely stable, with no syneresis and sedimentation after a month of storage at 4 °C. Samples containing 0.3% xanthan gum and 1% corn starch were considered as the desired drink based on sensory analysis. Study of the optimal flow behavior indicated that the drinking dessert is a non-Newtonian pseudoplastic fluid, and the Herschel-Bulkily model was the best model to describe the flow behavior. The pH of the synbiotic dessert containing *L. GG* was almost constant after 7 days of storage at 4 °C, while the pH of samples containing *L. paracasei* decreased by 0.7. The population of both probiotic bacteria decreased during storage time at 4 °C. The rate of decrease was higher for *L. paracasei* than *L. GG*. However, both contained  $>10^6$  CFU mL<sup>-1</sup>, which is necessary for the health benefits of probiotic bacteria.

**Keywords:** Lactic cheese whey (LCW); Drinking dessert; Synbiotic; *Lactobacillus rhamnosus* GG, *Lactobacillus paracasei*

## 1 Introduction

Whey is a by-product of cheese making in the dairy industry, which is obtained after the removal of casein from milk (Smithers, 2008). The amount of organic nutrients in whey is remarkable, and is responsible for its high Biological oxygen demand (BOD) and Chemical oxygen demand (COD). Owing to this fact, disposal of whey is expensive (Abdolmaleki, Mazaheri Asadi, & Jahadi, 2010; Smithers, 2008). Lactic cheese (LC) is widely consumed in Iran, and it is made by coagulation of milk using high acidity yogurt, usually more than 120 °D, and/or or-

ganic acids such as citric, lactic or acetic acids. A large amount of lactic cheese whey (LCW) is produced as a by-product of LC production. Drying of LCW to produce a powder is problematic due to its undesirable taste and acidity. Thus, a considerable amount of LCW is directly used in food formulation (Zadow, 1992).

There is an increasing use of probiotic bacteria and prebiotic carbohydrates in fermented and non-fermented dairy foods. Milk and whey are used to make functional foods, which due to their high nutritional value are good choices for human consumption and as carriers for probiotic bac-

teria. For example, sweet and acid whey were studied to make a beverage in a fermented or non-fermented form (Abdolmaleki et al., 2010; Djurić, Carić, Milanović, Tekić, & Panić, 2004). The sour taste of fermented products is unpleasant for some people, so the production of non-fermented probiotic products would broaden the range of people interested in consuming probiotic products. One of the few studies carried out on non-fermented probiotic beverages is the production of an orange probiotic beverage using *Lactobacillus acidophilus* (Khamirian, Jooyandeh, Hesari, & Barzegar, 2016).

A dairy drinking dessert is a new type of beverage that, in addition to near neutral pH, usually has a high viscosity, opaque appearance and complete stability over the shelf-life (Beecher, Drake, Luck, & Foegeding, 2008). Addition of acid whey, at pH values below the natural pH of the milk (6.4-6.6), and starch may cause syneresis or sedimentation in the final product. Because texture, oral sensitivity and stability are three important factors affecting the consumer's opinion about a drinking dessert, selection of an effective hydrocolloid in an appropriate amount can play an important role in consumer's acceptance.

In this study, lactic cheese whey was used, a by-product of the cheese industry, to make a synbiotic drinking dessert which incorporated *L. rhamnosus* GG and *L. paracasei*, that had a complete stability and desirable sensory properties. Different physicochemical and microbial tests were also performed to determine the properties of this novel product.

## 2 Materials and Methods

### 2.1 Probiotic bacterial strain

*Lactobacillus rhamnosus* GG (ATCC53103) and *Lactobacillus paracasei* (L. casei 431) were obtained from Chr. Hansen (Denmark). 10 g Direct Vat Set (DVS) culture was added to 100mL 1.5% sterilized milk as a stock culture, and was frozen in liquid nitrogen and stored at -80 °C.

### 2.2 Determination of physicochemical properties of LCW

Physicochemical properties of LCW were analyzed using the following methods: Density using a lactodensimeter (Quevenne, Germany) (AOAC 925.225); pH by a digital pH meter (Knick model 766, Germany); acidity by a titration method with 0.1N NaOH in the presence of phenolphthalein as an indicator (AOAC 947.05); soluble solids using a digital refractometer (CETI, Belgium) (AOAC 923.12); dry matter by air drying in an oven (Nuve, Model FN120, Turkey) (AOAC 990.20); ash by incinerating in an electric furnace at 550 °C (Nabertherm, Germany) (AOAC 945.46); protein by the Kjeldahl method (Behr, Germany) using a factor of 6.38 (AOAC 991.23); and fat by the Gerber method (AOAC 2000.18). The lactose content of whey was determined by an HPLC system (Knauer, Germany), equipped with an analytical column from Eurokat (300 × 8 mm, 10 µm) and a refractive index detector. Sample preparation was performed as described by Chavez-Servin, Castellote, and Lopez-Sabater (2004). A standard curve was also prepared by HPLC analysis of different concentrations of pure lactose.

### 2.3 Preparation of dairy drinking dessert

In the formulation of this new dairy product, lactic cheese whey, milk, xanthan gum, resistant corn starch, sugar and cacao powder were used. The pre-tests were performed to obtain the proper proportion of milk and whey as the main components of the product. According to the primary tests, the best ratio for milk and whey in the mix was 1:1. Whey with Xanthan gum (Puratos, Belgium) at 4 levels (0, 0.1, 0.2 and 0.3%), resistant corn starch (Ingredion, USA) at 4 levels (0, 0.1, 0.2 and 0.3%), 5% sugar and 0.3% cocoa powder were mixed using a magnetic stirrer (Labtron, Iran). This mixture was sterilized at 110 °C for 10min. Then sterilized milk was added and the dessert mix was homogenized by a homogenizer (Heidolph, model D-91126, Germany) (10000 rpm for 2 minutes) which had been

Table 1: Formulation of 16 different treatments to prepare the drinking desserts

Treatments <sup>1</sup>	Xanthan gum (%)	Resistant corn starch (%)
1	0	0
2	0	0.5
3	0	1
4	0	1.5
5	0.1	0
6	0.1	0.5
7	0.1	1
8	0.1	1.5
9	0.2	0
10	0.2	0.5
11	0.2	1
12	0.2	1.5
13	0.3	0
14	0.3	0.5
15	0.3	1
16	0.3	1.5

<sup>1</sup>In all treatments, whey and milk were used in 1:1 ratio, cacao powder (0.3%) and sugar (5%)

sterilized using 70% alcohol. In this study, 16 treatments were used for preparation and testing of the desserts (Table 1).

## 2.4 Evaluation of storage stability

Syneresis and sedimentation were analyzed using a volumetric cylinder (Laurent & Boulenguer, 2003). 12 mL of each sample was poured into a volumetric cylinder and after one-week storage at 4 °C, the volume of sediment at the bottom of the tube and the volume of water in the upper part of the sample were measured in milliliters. Results were calculated using the following equations:

$$\text{syneresis}(\%) = \frac{V_s \times 100}{V_t} \quad (1)$$

$$\text{sedimentation}(\%) = \frac{V_p \times 100}{V_t} \quad (2)$$

where  $V_s$  is the supernatant volume,  $V_p$  is the sedimentation volume and  $V_t$  is the total volume of the sample in the tube.

## 2.5 Determination of viscosity

The viscosity of drinking desserts was measured by a viscometer (RV-DV II Brookfield, USA), equipped with a thermal circulator and using spindle NO. S00. All samples were subjected to a different shear rate and finally, depending on the texture of the sample, 15 rpm was selected. Therefore, 16 mL of the sample was poured into a special cylinder and the viscosity was read at 25 °C.

## 2.6 Rheological properties of optimal drinking dessert

Flow curves were obtained by increasing the speed of shearing from 15 rpm to 120 rpm. In order to determine the optimum flow behavior of the optimum sample of drinking dessert, and obtain the rheological parameters, three time-independent models were used: power-law ( $\tau = k\gamma^n$ ), Herschel-Bulkley ( $k\gamma^n + \tau$ ) and Bingham ( $\tau = \mu_p\gamma + \tau_0$ ) (Bhattacharya & Bhattacharya, 1994).

In these models,  $k$  is the consistency coefficient

(Pa.sn),  $n$  is the flow behaviour index,  $\gamma$  is the shear rate,  $\tau_0$  is the yield stress (Pa) and  $\mu_p$  is the Bingham plastic viscosity (Pa.s).

## 2.7 Sensory analysis

Samples that were completely stable during refrigerated storage were selected for sensory analysis. Samples were served in cups coded with three random alphabets. 15 trained panelists were asked to rate consistency, taste and mouthfeel on the basis of a 5-point hedonic scale (5- really good, 4- good, 3- normal, 2- bad, 1- really bad). The analysis was carried out in two stages. In the first stage, panelists were served samples in three groups of four (during three consecutive days), and for each group, the sample that had the highest score was selected for the second stage. Finally, among the three dessert samples, the sample with the highest score was selected as the desired dessert. It should be noted that due to the importance of mouthfeel for drinking desserts, the score of this parameter was weighted as 2 (Janhøj, Frøst, & Ipsen, 2008).

## 2.8 Preparation of synbiotic drinking dessert

After selection of the optimal formulation based on stability and sensory analysis, *L. rhamnosus* GG and *L. paracasei* culture were inoculated to the beverage individually at 4 °C. Beverage samples were stored in the refrigerator for a week and were analysed for pH, titratable acidity and probiotic viable counts just after production, and 1, 2, 4 and 7 days after storage.

## 2.9 Determination of pH and titratable acidity

pH was measured using a digital pH meter by direct immersion of the pH meter electrode in the sample.

Titratable acidity was assessed according to the method of Purwandari, Shah, and Vasiljevic (2007). 10mL of the sample was mixed with 10mL CO<sub>2</sub> free distilled water and the temperature was adjusted to 22 °C. The pH meter elec-

trode was placed in the sample and the pH was adjusted to 8.3 with 0.1N NaOH. The following equation was used to calculate the results and expressed as °D.

$$A = V \times 10 \quad (3)$$

A = acidity percentage, V = volume of sodium hydroxide consumed in milliliters

## 2.10 Enumeration of probiotics viable counts

The viability of *L. rhamnosus* GG and *L. paracasei* was evaluated by selective enumeration according to the method of Tharmaraj and Shah (2003). 10 mL of sample was diluted in 90 mL of saline solution (0.85 g NaCl 100mL<sup>-1</sup> distilled water) for the first dilution, and keep diluting with a 1:10 ratio until the appropriate dilution is reached. 1mL of appropriate dilutions were pour plated in MRS-Vancomycine agar and incubated under an aerobic condition at 37 °C for 72 hours and then two consecutive dilutions were counted. The following equation used to calculate the results (Cappuccino & Welsh, 2018)

$$N = \frac{\sum C_i}{V(n_1 + 0.1n_2)d} \quad (4)$$

where  $C$  is the sum of colonies on all plates counted,  $v$  is the volume applied to each plate,  $n_1$  is the number of plates counted at the first dilution,  $n_2$  is the number of plates counted at the second dilution and  $d$  is the dilution factor from which the first count was obtained.

## 2.11 Microbial quality control

Samples were analyzed for enumeration of coliforms, molds and yeasts immediately after production and 1, 2, 4 and 7 days after storage. The test for coliforms was performed according to the Iranian National Standard No. 11166, using Violet Red Bile Agar medium (Merck, Germany), and molds and yeasts according to the Iranian National Standard No. 10154, using Yeast Extract Glucose Chloramphenicol medium (Liofilchem, Italy).

## 2.12 Microbial quality control

Experiments were replicated three times following a completely randomized design using a factorial arrangement. All data were analyzed using the one-way ANOVA procedure, followed by Duncan multiple comparison tests, using SPSS version 23 (SPSS, USA). Probabilities of  $P < 0.05$  were considered significant.

## 3 Results and Discussions

### 3.1 Physicochemical properties of LCW

The physicochemical properties of LCW used in this study were as follows: pH ( $5.7 \pm 0.8$ ), acidity ( $17.5 \pm 0.44$  °D), density ( $1.0238 \pm 0$ ), dry matter ( $6.11 \pm 0.25\%$ ), fat ( $0.35 \pm 0.02\%$ ), protein ( $0.94 \pm 0.17\%$ ), lactose ( $4 \pm 0.01\%$ ) (Fig. 1), salts ( $0.46 \pm 0.02\%$ ) and ash ( $0.62 \pm 0.01\%$ ). Khamirian et al. (2016) found similar values for whey characteristics except for pH (6.63) and protein content (0.46) which were higher than those found in the present study. Differences in the process of cheese production can influence the whey characteristics. The difference in protein content could be due to the high efficiency of ultrafiltration in feta cheese production. The pH reported by Djurić et al. (2004) for acidic whey was 3.63, which could have been related to differences in type (lactic acid, acetic acid or citric acid) and amount of acid used in cheese production. Therefore, the composition of the main constituents of whey varies depending on the type of cheese and the type of milk used (Alsaed et al., 2013).

### 3.2 Effect of xanthan gum and resistant starch on syneresis and sedimentation

Addition of acid whey to a drinking dessert mix makes the milk protein network unstable and a clear layer of serum is formed in the beverage over time. On the other hand, the opinion of consumers is that the drinking dessert should be completely stable. Therefore, in the preparation of drinking desserts, hydrocolloids need to be added. Table 2 shows the values obtained for

syneresis and sedimentation of drinking desserts containing different levels of xanthan gum and resistant starch. In the control sample (without starch and xanthan gum), a significant syneresis (33.33%) was observed, which can be due to denaturation of casein micelles at a pH below milk pH (de Kruif, 1998). Other samples containing xanthan gum did not have syneresis after 30 days storage at 4 °C. This is due to the binding of hydrocolloids to the casein micelles, which do not allow casein micelles to aggregate (Syrbe, Bauer, & Klostermeyer, 1998). However, there was no significant difference between the mean percentage (%) syneresis and sedimentation in all samples containing different amounts of gum ( $P > 0.05$ ). This indicates that xanthan gum has a significant effect at the 0.1% level. In samples containing only starch, sedimentation was observed, which naturally could be due to starch sedimentation. Samples containing both starch and xanthan gum conferred a great stability, with no syneresis and sedimentation after 30 days of storage at 4 °C (Table 2), which demonstrates that a mix of resistant starch and gum is more effective in the production of a single-phase beverage. Many researchers have investigated the effect of different hydrocolloids on the stability of beverages containing whey (Janhøj et al., 2008; Laurent & Boulenguer, 2003; Mohammadi, Abbasi, & Hamidi, 2011). Paraskevopoulou et al. (2003) evaluated the effect of three polysaccharides (pectin, xanthan gum and guar gum) on the stability of whey-milk kefir. They showed that Xanthan gum was the most effective stabilizer at 0.2% level. Generally, polysaccharides contribute to the formation of a stable colloid system by increasing the viscosity of the aqueous phase and preventing particle movement (Parker, Gunning, Ng, & Robins, 1995).

### 3.3 Effect of xanthan gum and starch on the viscosity of drinking dessert

The viscosity of dairy desserts is primarily affected by the type and concentration of thickening agents, especially polysaccharide hydrocolloids such as gums and starch. Figure. 2 shows the viscosity analysis of the desserts. There

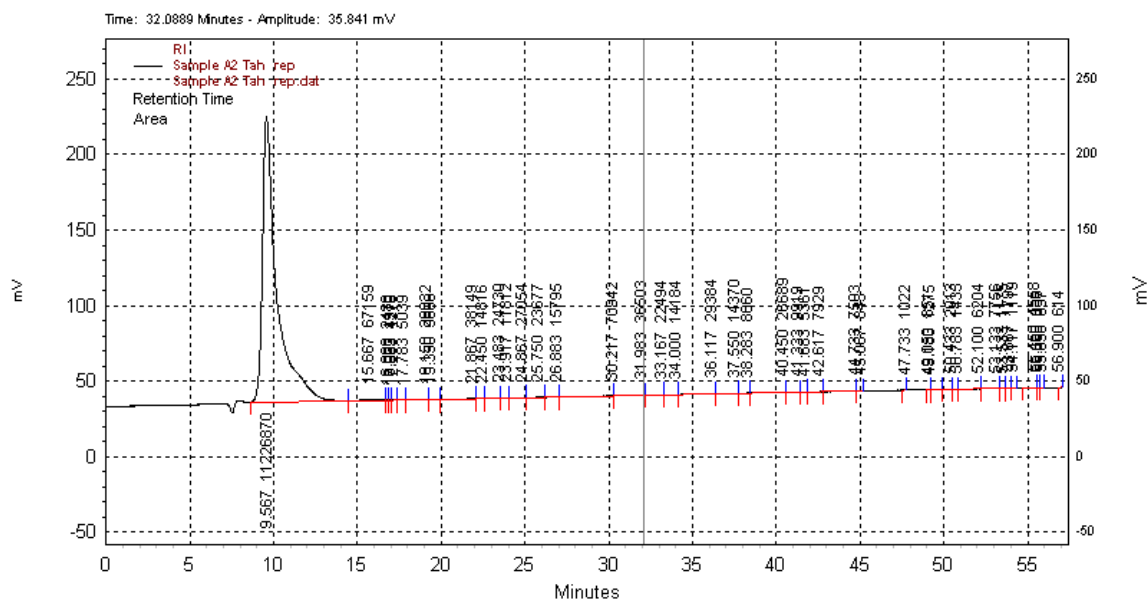


Figure 1: Chromatogram of whey lactose analysis by the HPLC-RI method. Peaks: 1, Lactose

Table 2: Effect of different amounts of gum and starch on sedimentation, syneresis and viscosity

Treatment	Sedimentation (%)	Syneresis (%)
1	0±0 <sup>c</sup>	33.33±4.8 <sup>a</sup>
2	61.11±7.11 <sup>a</sup>	0±0 <sup>b</sup>
3	58.32±5.3 <sup>b</sup>	0±0 <sup>b</sup>
4	50±0 <sup>b</sup>	0±0 <sup>b</sup>
5-16	0±0 <sup>c</sup>	0±0 <sup>b</sup>

<sup>a-c</sup> Data with different superscripts are significantly different ( $p < 0.05$ ) according to Duncan's comparison test.

The results are expressed as mean values ± standard error (n=3)

Table 3: Overall scores for different samples in sensory analysis. Stage 1

Sample no.	Overall score (out 20)	Sample no.	Overall score (out 20)	Sample no.	Overall score (out 20)
5	8.81±0.41 <sup>b</sup>	9	13.25±0.84 <sup>a</sup>	13	12.87±1.03 <sup>a</sup>
6	9.68±0.59 <sup>b</sup>	10	13.5±0.85 <sup>a</sup>	14	14.37±0.74 <sup>a</sup>
7	10.87±0.9 <sup>ab</sup>	11	14.31±0.89 <sup>a</sup>	15	14.43±0.88 <sup>a</sup>
8	12.43±0.87 <sup>a</sup>	12	11.06±0.79 <sup>a</sup>	16	14.43±1.08 <sup>a</sup>

<sup>a-b</sup> Mean values with different superscripts are significantly different ( $p < 0.05$ ) according to Duncan's comparison test

The results are expressed as mean values ± standard error (n = 3)

Table 4: Rheological parameters obtained using power law models for the best drinking dessert

Model	$n$	RMSE	SSE	R <sup>2</sup>	$K$	$\tau_0$	$\mu$
Power law	0.298	0.395	2.34	0.998	10.11	-	-
Hershel-Bulkley	0.23	0.396	2.2	0.998	10.11	3.68	-
Bingham	-	9.146	1255	0.953	-	-119.2	4.41

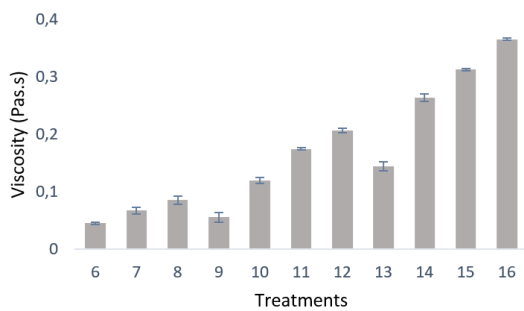


Figure 2: Effect of different percentages of Xanthan gum and resistant corn starch on viscosity of drinking dessert. Results for viscosity of samples 1 to 5 have not been reported, because they had a torque less than 10.

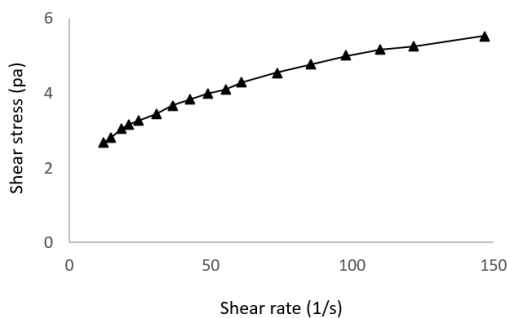


Figure 3: Flow curve of shear stress versus shear rate for the best drinking dessert

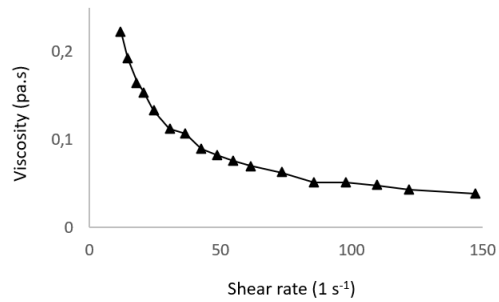


Figure 4: Apparent viscosity versus shear rate for the optimum drinking dessert

was a significant difference between the viscosity of samples for different treatments. With constant gum content and increasing starch content, the viscosity increased and has a maximum at 0.3% xanthan gum and 1.5% resistant starch. However, gum and starch in combination had a greater impact on the viscosity than their individual usage (Fig. 2), as a result of hydrocolloids in the continuous phase and starch in the dispersed phase. Sikora, Kowalski, and Tomasik (2008) studied the effect of starch and xanthan gum on viscosity and found that gum and starch individually form two separate phases, but when combined, they form one phase that increases viscosity. Wei, Wang, and Wu (2001) also obtained the same results when using gums and corn starch.

### 3.4 Sensory analysis

There were no significant differences between the samples in each group ( $P > 0.05$ ), except for group number 3. The dessert containing 0.1% gum and 1.5% starch had the highest overall



acceptability in the first group. In the second group, due to the increase in gum concentration and the desired consistency, the desirable samples were those containing 0.2% gum and 1% starch. In the third group, samples containing 0.3% gum, 1.5% starch and 0.3% gum and 1% starch had the same score, however, the dessert formulation containing 1% starch and 0.3% gum was selected because of lower costs of preparation as a result of less starch. Thus, samples containing 0.1% gum and 1.5% starch, 0.2% gum and 1.5% starch, 0.3% gum and 1% starch were selected for the second stage of sensory analysis (Table 3).

In second stage, samples number 8, 11 and 15 gained overall scores of  $13.37 \pm 3.9$ ,  $15.6 \pm 3.2$  and  $16.93 \pm 3.3$  respectively. When comparing the three desserts with respect to overall acceptability, there was no significant difference. Nevertheless, the dessert containing 0.3% gum and 0.1% starch had the highest score and finally was considered as a best one to prepare the synbiotic dairy dessert.

### 3.5 Rheological properties of optimal drinking dessert

The study of viscosity and flow behavior of fluids is necessary for the design and engineering of equipment and systems, such as pumps, mixers and tubes (Augusto, Cristianini, & Ibarz, 2012). The rheological properties of dairy desserts mainly depend on the amount of milk fat, the type and concentration of starch and hydrocolloids and their interactions (Torres, Tárrega, & Costell, 2010). Figures 3 and 4 respectively show shear stress ( $\delta$ ) versus shear rate ( $\gamma$ ) and apparent viscosity ( $\eta$ ) versus shear rate for the optimal drinking dessert containing 0.3% xanthan gum and 1% resistant corn starch. With respect to shear stress, the increase in shear rate was not linear. By increasing the shear rate, the apparent viscosity of the sample decreased and shear stress increased, indicating that the dairy dessert had a shear-thinning behaviour. At the lower shear rate, due to the irregular molecular arrangement, the viscosity is high, while with increasing shear rate, the number of molecules that orient to one side is greater and hence the viscos-

ity decreases (Mahdian, Mehraban, Karazhian, & Vaghei, 2014). Such behaviour has already been reported in dairy desserts (Bayarri, Chuliá, & Costell, 2010; González-Tomás, Bayarri, Coll-Marqués, & Costell, 2009). Many research studies have focused on the effect of hydrocolloids to increase the shear-thinning behaviour of foods (Panaras, Moatsou, Yanniotis, & Mandala, 2011). Because of the increasing viscosity of the serum phase, a condensed polysaccharide network is formed, which is very susceptible to shear rate (Panaras et al., 2011). Xanthan gum is used in dairy products as a semi-solid compound with a gel network which is very similar to a thick three-dimensional gel network. The gel formed by xanthan gum flows freely; therefore, it is extremely shear thinning (Sworn, 2009). The relatively low viscosity, at high shear rate, makes the food containing xanthan gum easy to mix, pour and swallow. Some features, such as inducing high viscosity at low levels and lack of gel formation, make xanthan gum a convenient viscosity control compound as well as a thickening agent, stabilizer and emulsifier in dairy products (Kang & Pettit, 1993).

According to the flow behaviour index ( $n < 1$ ), the drinking dessert is a non-Newtonian fluid. Although many food fluids have Newtonian behaviour, some liquids and semi-solids have non-Newtonian behaviour. The pseudoplastic behaviour found in this research, has also been reported for frozen soy yogurt containing beta-glucan and modified starch (Rezaei, Khomeiri, Kashaninejad, Aalami, & Mazaheri-Tehrani, 2017), frozen yogurt containing inulin (Rezaei, Khomeiri, Aalami, & Kashaninejad, 2014), ice cream containing xanthan gum (Toker, Dogan, Canyılmaz, Ersöz, & Kaya, 2013) and dairy dessert based on starch containing inulin (Torres et al., 2010).

Among the models used to determine the flow behaviour of the best drinking dessert, both the power law and Herschel-Bulkley models could be identified as the best because of the high similarity between  $R^2$  and RMSE (Table 4).

According to the Herschel-Bulkley model, the consistency coefficient ( $K$ ) was 10.11. This is higher than  $K$  value reported for frozen soy yogurt containing 1% resistant starch (Rezaei et al., 2017) ( $K=0.985$ ) and lower than dairy dessert



starch based (2.5%) enriched with 7.5% inulin (K=11.81) (Torres et al., 2010) and frozen yogurt containing 1% inulin (K=15.45) (Rezaei et al., 2014). The consistency of our best drinking dessert was favourable to allow easy drinking with a straw and provide a proper mouthfeel, with acceptable thickness.

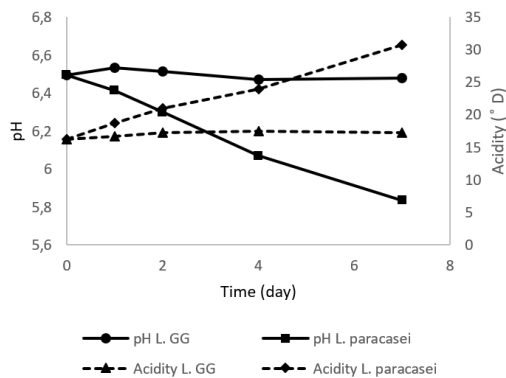


Figure 5: Changes in pH and acidity for both types of synbiotic drinking dessert at 4 °C

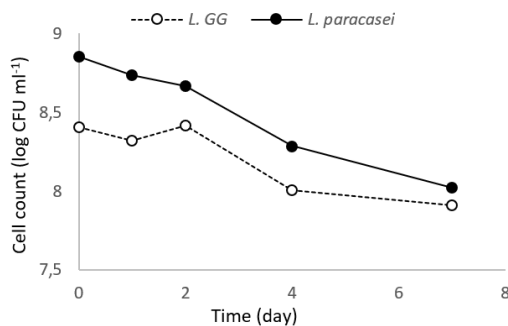


Figure 6: Changes in viable cell counts of probiotic bacteria at 4 °C

### 3.6 Changes in pH and acidity at refrigerator temperature

As shown in Figure 5, the pH of the refrigerated dessert containing *L. rhamnosus* GG was constant ( $P > 0.05$ ) as *L. rhamnosus* GG is not ca-

pable of fermenting lactose and casein because of alterations in the nature of anti-terminator (*lac T*) and 6-phospho- $\beta$ -galactosidase (*lac G*) genes (Kankainen et al., 2009). Therefore, the presence of simple sugars, such as glucose and fructose, and proteins, such as whey protein and amino acids as nitrogen sources, are essential for growth and survival of this bacterium. Karlton-Senaye and Ibrahim (2013) also reported that *L. rhamnosus* GG slowly reduces the pH of a dairy dessert during a month under refrigerated storage.

The pH of the synbiotic drinking dessert containing *L. paracasei* decreased and its differences were significant after a week. As no yeasts and molds were detected during one-week storage, decreasing the pH could have been due to probiotic bacteria activity. Probiotic bacteria have enzymatic activity of  $\beta$ -galactosidase,  $\beta$ -glucosidase and  $\alpha$ -glucosidase, which are inactive in most probiotic strains at refrigerator temperature but some strains could have  $\beta$ -galactosidase activity even at refrigerator temperature (Lipovová, Spiwok, Mala, Králová, & Russell, 2002). Lipovová et al. (2002) studied the activity of the  $\beta$ -galactosidase enzyme in some lactic acid bacteria, and the results showed that some *L. paracasei* strains at refrigerator temperature are able to ferment lactose, albeit slowly. As shown in Figure 5, the acidity of the samples containing *L. paracasei* increased and decreased the pH. But the acidity of the sample containing *L. rhamnosus* GG was relatively constant and significant changes were not observed ( $P > 0.05$ ). Similar results were obtained by Mani-López, Palou, and López-Malo (2014). They reported that the acidity of yogurt containing probiotic bacteria *L. casei*, *L. reuteri* and *L. acidophilus* increased at 4 °C temperature with respect to decreasing pH.

### 3.7 Cell count of probiotic bacteria

The population of probiotic strains were analyzed for 7 days of storage at 4 °C. In the whole 7 days, because of increasing acidity, the population of *L. rhamnosus* GG decreased 0.5 log CFU mL<sup>-1</sup> compared to the initial time of inoculation

and was significant. The population of *L. paracasei* decreased significantly in all 7 days up to 7.89 log CFU mL<sup>-1</sup>. (Fig. 6) The lower counts of *L. paracasei* than *L. rhamnosus* GG, could be due to the intense decrease in pH.

The viable counts of both probiotic bacteria remained above 10<sup>6</sup> CFU mL<sup>-1</sup> during the whole refrigerated storage period which is necessary for the health benefits (Shah, 2007). The results of the present study suggest that it is possible to produce a non-fermented synbiotic drinking dessert, incorporating *L. rhamnosus* GG, which is not able to reduce the pH at refrigerated temperature and is not sour.

Sarvari, Mortazavian, and Fazei (2014) studied the population of *L. animalis* and *L. acidophilus* at three different pH values (4.5, 4.7 and 4.9) in a mixture of probiotic yogurt. They reported a significant decrease in the population of the probiotic bacteria at lower pH as observed in this study. Vinderola, Bailo, and Reinheimer (2000) also examined the viability of two probiotic bacteria, *B. bifidum* BBI and *L. acidophilus* LAI in Argentinian yogurt during cold storage. Their results showed that the decrease in *B. bifidum* population was not significant at pH 6.5, 5.5, and 4.5, while the population of *L. acidophilus* significantly decreased at these pH values.

## 4 Conclusion

In this research, we tried to introduce a new formulation of a dairy drinking dessert as a non-fermented probiotic product using LCW. The results showed that the addition of both xanthan gum and corn starch have a positive effect on the viscosity of the samples and their complete stability during storage at refrigerated temperature. The drinking dessert is a non-Newtonian pseudoplastic fluid, where the Herschel-Bulkley model is the best to describe the behaviour of this food fluid. To produce a non-fermented probiotic product with a sweet taste, it is important to select a probiotic bacteria with the lowest possible activity at refrigerated temperature. *L. rhamnosus* GG was considered as a suitable species to produce a dairy dessert of non-fermented form

due to inactivity at refrigerated temperature.

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