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Changes on some quality characteristics of fermented soy milk beverage with added apple juice

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A B S T R A C T

The fermented soy milk beverage with added apple juice was produced by using *Lactobacillus acidophilus*. The numbers of *L. acidophilus*, rheological properties, acidity and pH values were determined both after the production and during the storage period of 21 days at 4 °C. In addition, sensory analyses of the beverages were done. The flow behavior of beverage was assessed by using three different rheological models, namely: Power Law, Herschel Bulkley and Vocadlo models. Vocadlo model was selected as the best model fitting the experimental data. The beverage was found to be a pseudoplastic fluid having the shear thinning nature. The results showed that *L. acidophilus* had good growth and viability in the beverage with or without apple juice. *L. acidophilus* counts were in the range of 8.73–9.11 log cfu/g after storage at 4 °C for 21 days, thus they met required number of viable bacteria for probiotic functional food. Results revealed that this beverage could be a good vehicle to deliver probiotic microorganisms to consumers and carry a potential to become a commercial product. It is thought that the results of this study could provide valuable information for the design of pumping systems for the fermented soy milk beverages.

1. Introduction

The concept of "probiotics" has attracted much attention with the emergence of antibiotic resistant bacteria and natural ways of suppressing pathogens (Tharmaraj & Shah, 2004). Soymilk is rich in high quality proteins and contains no cholesterol or lactose, and only small quantities of saturated fatty acids. It may be supplied to the lactase-deficient consumers (Scalabrini, Rossi, Spettioli, & Matteuzi, 1998). On the other hand, many people may find the taste of soymilk undesirable (Liu & Lin, 2000). Wang, Yu, and Chou (2002) indicated that the presence of indigestible oligosaccharides such as stachyose and raffinose and the raw bean flavor have limited the wide consumption of soymilk and other soybean products. Stachyose and raffinose are the principle oligosaccharides found in soymilk, and believed to cause flatulence in human after eating soybean foods. *Lactobacillus acidophilus* reduced the level of stachyose, raffinose and sucrose while it increased the content of fructose, glucose and galactose (Wang, Yu, Yang, & Chou, 2003). For these reasons, the fermentation of soybean products with lactic acid bacteria for the development of more digestible and palatable foods such as fermented soybean cheese, sour milk beverage, soybean yogurt has been studied extensively (Wang et al., 2002). Fermented soy products containing probiotics also has the potential of benefiting the elderly or extending the microgravity duty for the astronauts (Champagne, Green-Johnson, Raymond, Barrette, & Buckley, 2009).

It is important to maintain the viability of microorganisms in fermented food until the products are consumed. Shah, Lankaputhra, Britz, and Kyle (1995) reported that three of five commercial yogurt products contained 10^7–10^8 cfu/g *L. acidophilus*, whereas the other two yogurt samples contained $<10^5$ of *L. acidophilus* during the storage period of 5 weeks.

L. acidophilus demonstrates therapeutic values while it does not produce acetaldehyde giving the characteristic buttery flavor of regular yogurt. Since fermented acidophilus milk is tart and plain, fruit juices such as strawberry, apple, orange, grape, mango and pineapple are often used to improve the flavor of acidophilus milk products (Lee & Wong, 1998). Božanić, Lovković, and Jelicic (2011) also reported that the specific flavor of soymilk can be masked by the addition of sugar, aromas and fruit paste. In that way, an eligible probiotic and nutritionally improved product may be yielded.

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Rheology science investigates the flow and deformation of the substance. Liquid foods are classified as Newtonian or non-Newtonian depending on the relationship between shear rate and shear stress (Genc, Zorba, & Ova, 2002). The viscosity of foods has an important place in order to understand the structure of food, food processing and equipment, control of the food production (Krokida, Maroulis, & Saravacos, 2001). There have been a lot of recent studies to define the rheological properties of materials used in food industry (Gabsi, Trigui, Barrington, Helal, & Taherian, 2013; İçier, Bozkurt, & Gürbüz, 2008; Maskan & Göğüş, 2000; Prudencio, Prudencio, Gauche, Bareto, & Bordigno-Luiz, 2008). On the other hand, there is no enough information about rheological characteristics of fermented soy milk with added apple juice, according to the best of the authors' knowledge. Since fermented soy milk with added apple juice could be produced as commercial beverages, the information on its rheological properties is essential for its formulation and to design of fermented soy milk beverage production equipment, especially pumping and piping systems.

The aim of this study was to combine soymilk and apple juice at different concentrations to produce a new fermented soymilk drink, and also to determine the survival of *L. acidophilus* and changes on rheological properties throughout the storage period of 21 days.

2. Materials and methods

2.1. Microorganisms

Lyophilized probiotic culture of *L. acidophilus* LA-5 (Chr. Hansen's, Denmark) was used as the DVS (direct vat set) culture. *L. acidophilus* LA-5 was grown at 37 °C for 24 h and subcultured twice in soymilk. This culture was used as the working culture in fermented soy milk production.

2.2. Soy milk

Commercially produced long life soymilk (Alpro Comm. Va., Belgium) without any additives were used for the production of soymilk drink. According to supplier, the soymilk contained (in g/100 ml): 3.3 g protein, 0.2 g carbohydrates, 0.1 g total sugar, 1.8 g total fat, 0.6 g dietary fiber and 0.12 g calcium per 100 ml of soy milk.

2.3. Apple juice

Commercially sterilized apple juice (% 100 apple juice, Tat, Turkey) was used. According to supplier, the contents of protein, carbohydrates in apple juice were 0.18 g and 10.65 g per 100 ml, respectively.

2.4. Production of fermented soymilk drink

The apple juice was added to the soymilk at the concentrations of 15 or 25%. Soymilk without apple juice was used as a control. Preliminary experiments were performed to determine the optimal apple juice concentrations. The soymilk with or without added apple juice with a total weight of 140 g was placed to sterile screw-caped glass jars (210 cc in volume). The soymilk with or without added apple juice was inoculated with 10 g of subcultured *L. acidophilus* cultures in soy milk, in order to achieve the initial populations of 7 log cfu/ml (Santos, Libeck, & Schwan, 2014) and fermented for 24 h at 37 °C. The pH values of samples were determined during the fermentation process. Fermentations were conducted until reaching pH 4.6 for control samples (Božanić, Brletić, & Lovković, 2008). After the fermentation process, the fermented products were cooled to 4 °C, and stored at 4 °C for 21 days.

During the storage period, the numbers of *L. acidophilus*, mold and yeasts, pH, titratable acidity and rheological properties were examined during the storage period (1, 7, 14 and 21 days).

2.5. Determination of titratable acidity and pH

The titratable acidity was determined by titration with 0.1 N NaOH solution, and expressed as percent lactic acid (Wang et al., 2003). The pH of samples was measured using a pH meter (Nel Mod 821) during the storage period of 21 days. The measurements were performed in triplicate for each fermented soy milk sample.

2.6. Microbiological analysis

2.6.1. Enumeration of L. acidophilus

The numbers of *L. acidophilus* were determined using MRS Agar (de Man Rogosa Sharpe Agar, pH 5.4, Oxoid, Hampshire, UK). 10 g of sample was taken from each soymilk drink samples. Serial ten-fold dilutions were prepared in a solution of with 0.1% (w/v) bacto peptone. Appropriate dilutions were plated to MRS Agar plates and incubated at 37 °C for 3 days under microaerophilic conditions (Božanić et al., 2008). After the incubation period, the colonies were counted and the number of *L. acidophilus* was calculated.

2.6.2. Mold and yeast count

For the mold and yeast counts, serial ten-fold dilutions were plated to acidified Potato Dextrose Agar plates (PDA, pH 3.5, Merck, Darmstadt, Germany) and incubated at 25 °C for 5 days (Tournas, Stack, Mislivec, Koch, & Bandler, 1998). The colonies were counted after the incubation period and the number of mold and yeasts were calculated.

2.7. Rheological measurements

The rheological properties of fermented soy milk were measured using a controlled-viscometer (Brookfield DV-II + Pro Viscometer). The temperature was kept constant at 4 °C by using a circulating bath and pump (Masterflex L/S model 77250-62, Cole–Parmer, USA). By using small device adapter, the fluid flow in a dead zone and/or the formation of unstable flow was minimized. Rheological measurement was performed as a shear rate sweep by increasing the shear rate from 0 to 200 rpm. During measurements, shear rate, shear stress, apparent viscosity and tork (%) values were recorded. Experimental shear stress-shear rate measurements were fitted to selected rheological models to assess the flow behavior of the fermented soy milk. Three different rheological models were applied; Power Law Model (Eq. (1)), Herschel Bulkley model (Eq. (2)), and Vocadlo model (Eq. (3));

$$\tau = K \times \gamma^n \quad (1)$$

$$\tau = \tau_0 + K \times \gamma^n \quad (2)$$

$$\tau = \left(\tau_0^{1/n} + K \times \gamma\right)^n \quad (3)$$

where τ is shear stress (Pa) and τ_0 is yield stress (Pa) while n is flow behavior index, K is consistency coefficient (Pa s^n) and $\dot{\gamma}$ is the shear rate (1/s).

2.8. Sensory evaluation

Sensory evaluation of beverages was carried out on 1st day of storage at 4 °C. Sensory evaluation of the samples was made individually by using three-point ranking test (Altug & Elmaci, 2005).

Ranking test was applied to questions of sensory intensities. Three samples were tested with a panel of 20 untrained panelists. The panelists received the samples (coded with three digit numbers) in balanced, random order. Panelists ranked three samples in ascending order of any related property. Water was provided for mouth rinsing during the ranking of individual samples. Because the rank scale used was 1 = preferred least and 3 = preferred most, the highest rank sum meant that the product was the most preferred. The ranking data was analyzed by using the critical value tables (Lawless & Heymann, 2010; Newell & MacFarlane, 1987). For each property, the ranks were summed and the differences between the sums were compared to critical value in this table. The number where the two points cross was the critical value for the difference; in our case it was 15. If the difference of sum of their rank between each pair of samples was greater than the critical value, it was considered that the samples were significantly different in the related property or in overall acceptability (Marshall, 2006). On the other hand, to determine which samples were significantly different, the multiple comparison test (Duncan) method was also performed. Significant differences between fermented beverages having different apple juice concentrations were evaluated for each sensory property, individually.

Since this study involved taste or food quality evaluations, where the soy milk, apple juice and the culture used are wholesome products without additives, it has fallen into Exempt Category of IRB procedure (Anonymous, 2015).

2.9. Statistical evaluation

All the experiments were repeated for three different times, and duplicate samples were analyzed at each sampling time. Data were analyzed with the SPSS statistical package (ver. 15.0, Chicago). The significant difference was defined as $P < 0.05$. One way analysis of variance was used, and the significant differences were evaluated by the Duncan multiple range test.

The compatibility of the rheological models with experimental data was determined by using a non-linear regression analysis of statistical software package SPSS (ver. 15, Chicago). The regression coefficient (R^2), root mean square error (RMSE) (4) and chi-square (χ^2) (5) values were calculated. Duncan test was applied as a comparative statistical analysis. The statistical criteria of having highest R^2, lowest RMSE and lowest χ^2 were chosen for selection of best model for fitting.

$$\mathrm{RMSE} = \left[\frac{1}{N}\sum_{i=1}^{n}\left(KG_{theoric,i} - KG_{experimental,i}\right)^2\right]^{0.5} \quad (4)$$

$$\chi^2 = \frac{\sum_{i=1}^{n}\left(KG_{theoric,i} - KG_{experimental,i}\right)^2}{N-n} \quad (5)$$

where, KG; rheological data (experimental and predicted), i; observation values at i experiment; N, observation number; n, number of parameters in model.

3. Results and discussion

3.1. Titratable acidity and pH

The average values of titratable acidity and pH of fermented soy milk samples during storage were given in Table 1. There was no significant difference ($P > 0.05$) in acidity values for control samples during storage, while significant differences were obtained in samples containing 15 and 25% apple juice ($P < 0.05$). As seen on Table 1, the higher acidity was noted at all storage periods of the fermented soy milk beverage with 15% apple juice. Titratable acidity values of beverages with 15 or 25% apple juice increased during the storage period of 14 days. At the 21st day of storage, the titratable acidity values of these beverages decreased slightly. Other studies also observed that titratable acidity values of fermented beverages or yogurts decreased with prolonged storage time. Espírito Santo, Perego, Converti, and Oliveira (2012) also reported that titratable acidity of yogurt co-fermented *L. acidophilus* L10 was increased at the 14 days of storage and decreased after 28 days of storage. Donkor, Henriksson, Vasiljevic, and Shah (2007a) indicated that supplementation of soy milk with 1% raffinose and % 1 glucose significantly increased the production of lactic acid in probiotic soy yogurts during the storage period of 28 days. On the other hand, acetic acid production increased within the storage period of 21 days and decreased significantly at the 28 days of storage. Donkor, Henriksson, Vasiljevic, and Shah (2006) obtained that probiotic bacteria released greater amounts of amino acids with the amount of free NH_3 groups, suggesting that these probiotic strains were strongly proteolytic in the probiotic yogurt. They were observed that the proteolytic activity of test cultures, including *L. acidophilus,* increased linearly during the storage period of 28 days. *L. acidophilus* exhibited proteolytic activity with producing amino groups and peptides in fermented soy milk and showed increasing in proteolytic activity with storage time (Donkor, Henriksson, Vasiljevic, & Shah, 2007b).

In this study, the mean pH values of samples added with apple juice were lower than those of control samples (Table 1). Supplementation of soy milk with apple juice provided additional carbohydrates as energy sources led to increase in the metabolic activity of *L. acidophilus* which contributed to the decrease of pH. For each sample group, there was no significant difference between pH values determined at different storage periods. The variations in pH and titratable acidity were slight through the storage period of 21 days for control and apple juice containing beverages. Lin, Chiu, and Pan (2004) suggested that slight variations of pH and titratable acidity values of soy milk beverage may have been due to the buffering effect of solids such as soy proteins and sugar.

3.2. Survival of *L. acidophilus* throughout the storage period

The growth of *L. acidophilus* in soymilk after 24 h at 37 °C was about 8.73 log cfu/g (Table 2). Soymilk could support the growth of *L. acidophilus* without any need to add additional carbohydrate source. Similar to this study, Telang, Joshi, Sutar, and Thorat (2010) observed that the viable count of *L. acidophilus* in soymilk was 8.54 log cfu/ml after the fermentation period of 24 h at 37 °C. Božanić

Table 1
Titratable acidity and pH values of fermented soy milk with added apple juice during storage period.

Apple juice added	Storage (days)	Titratable acidity (%)	pH values
0%	1	0.760 ± 0.023	4.86 ± 0.08
	7	0.737 ± 0.032	5.17 ± 0.22
	14	0.757 ± 0.033	5.13 ± 0.29
	21	0.770 ± 0.042	5.03 ± 0.34
15%	1	1.417 ± 0.046	3.83 ± 0.02
	7	1.540 ± 0.036	3.96 ± 0.19
	14	1.667 ± 0.074	3.90 ± 0.06
	21	1.565 ± 0.010	3.86 ± 0.03
25%	1	1.189 ± 0.032	4.18 ± 0.25
	7	1.397 ± 0.094	4.05 ± 0.07
	14	1.413 ± 0.021	4.07 ± 0.23
	21	1.332 ± 0.153	4.09 ± 0.11

Table 2
Survival of *Lactobacillus acidophilus* in fermented soy milk beverage with or without apple juice during the storage period.

Storage time (days)	Populations of *L. acidophilus*[a] (log cfu/g)		
	0% Apple juice	15% Apple juice	25% Apple juice
1	8.73 (0.34) a	8.98 (0.09) a	9.08 (0.16) a
7	8.98 (0.35) a	9.11 (0.35) a	8.97 (0.08) a
14	9.04 (0.10) a	8.98 (0.23) a	8.96 (0.25) a
21	9.10 (0.22) a	8.98 (0.04) a	9.10 (0.04) a

Values that are not followed by the same letter are significantly different (P < 0.05).
[a] Values are the mean of three replicates and two parallel, standard deviations are given in parenthesis.

et al. (2008) also reported that *L. acidophilus* La5 showed growth in soymilk with the fermentation time reaching to 12–17 h.

In this study, the addition of apple juice to soymilk increased the number of *L. acidophilus* slightly of about 0.25 and 0.35 log units for the apple juice concentrations of 15 and 25%, respectively. On the other hand, the increase in the numbers of *L. acidophilus* for these samples were not significantly different from the control samples (P > 0.05). Božanić et al. (2008) reported that the addition of sugar (5% glucose or sucrose) to soymilk before fermentation did not influence the fermentation time and the number of *L. acidophilus*.

The survival of *L. acidophilus* in the fermented soymilk drinks during storage at 4 °C for 21 days was given in Table 2. No significant differences between the numbers of *L. acidophilus* during different storage days were obtained ($P > 0.05$). During the storage period of 21 days, the numbers of *L. acidophilus* were in the range of 8.73–9.11 log cfu/g. According to Turkish Food Codex Communique on Fermented Milk (No: 2009/25), acidophilus milk must contain at least a minimum of 6 log cfu/g total specific microorganisms (Anonymous, 2009). In our study, acidified soymilk beverage with or without added apple juice met this criterion with a minimum *L. acidophilus* count of about 8.73 log cfu/g. Other studies also confirmed the higher survival rates of probiotic bacteria in fermented soymilk products. Özbey, Topçu, and Saldamlı (2007) reported that the number of *L. acidophilus* in yogurts containing 15% soymilk increased significantly about 0.65 log cfu/g after 14 days of storage. Beasley, Tuorila, and Saris (2003) observed that *Lactococcus lactis* (LL3) survived at levels of over 8 log cfu/ml in the fermented soy milk products containing strawberry (3%) and glucose (2%) for 3 weeks. Wang et al. (2002) reported that regardless of whether or not sucrose (15 °Bx) was added, no marked changes in the numbers of *L. acidophilus* (about 8 log cfu/ml) were observed during 10 days of storage at 5 °C. Lin et al. (2004) also reported that supplementation of milk-soymilk mixture with different amounts of *Lycium chinense* Miller juice did not affect the growth of *Lactobacillus paracasei* subsp. paracasei and *Bifidobacterium longum*; the cell numbers for both starters were about the same. On the contrary to the findings of this study, it was indicated that, *L. acidophilus* survived poorly during 21 days of cold storage and the viable cell count was under the 10^6 cfu/ml as soon as after the first week of storage (Božanić et al., (2011).

Yeasts and mold growths were not detected (<10 cfu/g) during the storage period of 21 days at 4 °C for each sample. Similar to this study, Beasley et al. (2003) also reported that they could not detect the growth of yeast or mold in the fermented soymilk products (<10 cfu/ml).

The incubation temperatures of 37 °C (Wang et al., 2002) and 42–43 °C (Ashraf & Shah, 2011) were used for the fermented products with *L. acidophilus*. In the present study, fermentation temperature of 37 °C was used. Božanić et al. (2008) reported that 37 °C was more appropriated than 43 °C for all tested probiotic bacteria.

3.3. Rheological properties of fermented soy milk beverages

Most of the studies related to the fermented beverages have been focused on the fermentation process in terms of microbiota while the determination of rheological parameters and sensory properties can devote the additional quality characteristics of fermented foods.

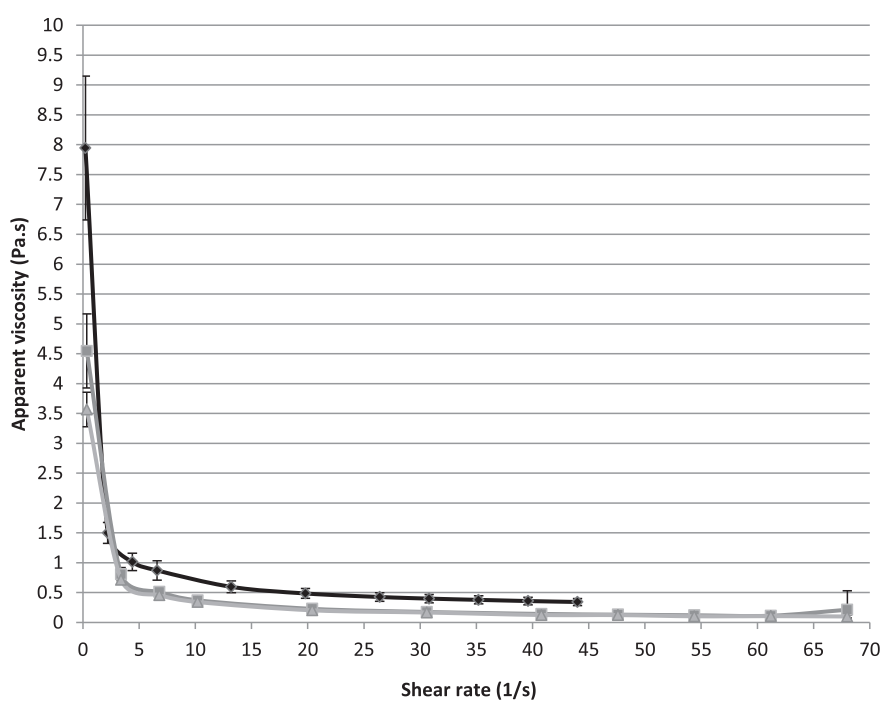

Fig. 1. Changes of apparent viscosity of fermented soy milk beverage depending on shear rate for different apple juice concentrations added (at 1st day of storage) —◆—0% —■—15% —▲—25%.

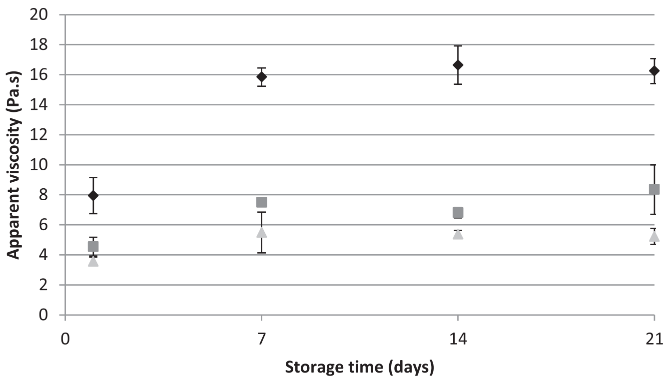

Fig. 2. Changes on apparent viscosity depending on apple juice concentration added during storage period ◆ 0% ■ 15% ▲ 25%.

In this study, the effect of the addition of apple juice to soy milk on rheological behavior of the fermented beverage was investigated. The empirical data obtained from the Brookfield viscometer were converted into viscosity functions (apparent viscosity and shear rate). It was found that the apparent viscosity decreased as the rate of shear increased (Fig. 1), and this behavior continued during storage (data not shown). It was found that the fermented soy milk beverage with added apple juice samples had non-Newtonian fluid (shear thinning or pseudoplastic) behavior. The apparent viscosity of the fermented soy milk beverages increased for the 7th days of the storage period, and then remained similar (Fig. 2) ($P < 0.05$). In addition, the control group had the highest apparent viscosity values during storage period at 4 °C. As the apple juice concentration increased the apparent viscosity of the fermented soy milk beverage decreased, and this behavior remained similar during storage (Fig. 2) ($P < 0.05$).

The relationships between shear stress and shear rate values for fermented soy milk beverages with added apple juice during storage were determined (Fig. 3). Rheological constants were predicted to describe this behavior by means of fitting different rheological models of Power law (Table 3), Herschel Bulkley (Table 4) and Vocadlo (Table 5) to experimental data.

The regression coefficients of Power Law model were lower than those of Herschel Bulkley and Vocadlo models. Furthermore, RMSE and $\chi 2$ values of Vocadlo model were lower than those of Herschel-

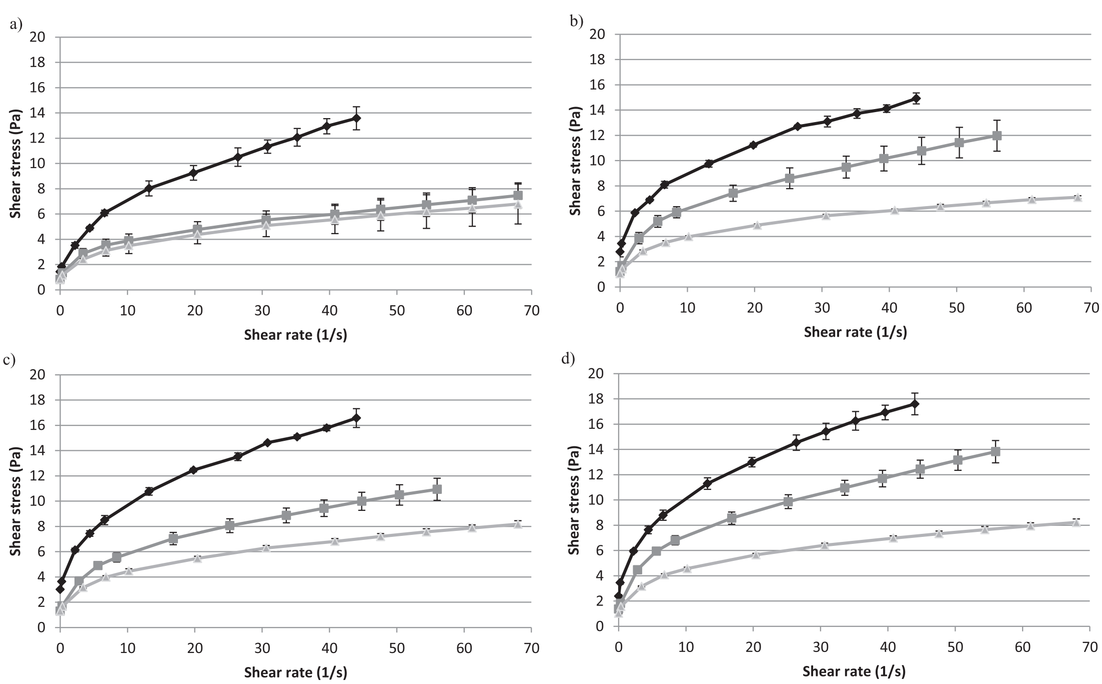

Fig. 3. Shear stress-shear rate data for fermented soy milk beverages during storage period at 4 °C; a) 1st day, b) 7th day, c) 14th day, d) 21st day ◆ 0% ■ 15% ▲ 25%.

Table 3
Statistical evaluation of the compatibility of the Power Law model with experimental shear stress-shear rate data.

Apple juice added (%)	Storage time (days)	K(Pa.s^n)	n	R^2	RMSE	χ^2
0	1	2.42 ± 0.23	0.49 ± 0.07	0.985 ± 0.004	6.57 ± 1.53	44.33 ± 22.79
	7	3.26 ± 0.74	0.47 ± 0.05	0.980 ± 0.007	9.16 ± 2.00	87.24 ± 36.52
	14	3.53 ± 0.37	0.49 ± 0.02	0.981 ± 0.003	10.88 ± 2.32	122.78 ± 52.64
	21	3.39 ± 0.30	0.52 ± 0.02	0.985 ± 0.002	10.40 ± 1.08	129.99 ± 66.95
15	1	1.80 ± 0.25	0.32 ± 0.01	0.985 ± 0.007	2.83 ± 0.39	8.16 ± 2.01
	7	2.70 ± 0.39	0.37 ± 0.01	0.987 ± 0.003	4.42 ± 0.77	21.41 ± 5.70
	14	2.23 ± 0.63	0.39 ± 0.04	0.988 ± 0.003	3.77 ± 0.61	12.82 ± 4.30
	21	3.56 ± 0.92	0.36 ± 0.03	0.983 ± 0.016	6.10 ± 2.96	23.20 ± 11.20
25	1	1.60 ± 0.15	0.34 ± 0.04	0.982 ± 0.005	2.79 ± 0.39	7.93 ± 2.25
	7	1.94 ± 0.67	0.30 ± 0.01	0.975 ± 0.005	3.97 ± 0.96	16.53 ± 7.75
	14	2.06 ± 0.18	0.31 ± 0.01	0.974 ± 0.004	3.87 ± 0.56	15.99 ± 4.71
	21	2.20 ± 0.15	0.34 ± 0.04	0.981 ± 0.006	4.06 ± 0.99	17.31 ± 8.77

Table 4
Statistical evaluation of the compatibility of the Herschel-Bulkley model with experimental shear stress-shear rate data.

Apple juice added (%)	Storage time (days)	K(Pa.s^n)	n	τ_0(Pa)	R^2	RMSE	χ^2
0	1	1.30 ± 0.37	0.63 ± 0.08	1.32 ± 0.32	0.997 ± 0.001	2.976 ± 1.289	10.34 ± 8.36
	7	1.87 ± 0.41	0.56 ± 0.09	2.39 ± 0.51	0.998 ± 0.002	2.578 ± 0.765	7.17 ± 4.24
	14	2.04 ± 0.41	0.58 ± 0.10	2.63 ± 0.51	0.999 ± 0.001	2.553 ± 0.678	6.95 ± 3.58
	21	2.14 ± 0.47	0.59 ± 0.10	2.09 ± 0.80	0.999 ± 0.001	2.800 ± 1.103	8.94 ± 6.62
15	1	1.20 ± 0.22	0.40 ± 0.01	0.74 ± 0.09	0.997 ± 0.001	1.312 ± 0.393	1.86 ± 0.99
	7	1.87 ± 0.21	0.44 ± 0.01	1.04 ± 0.30	0.997 ± 0.002	2.335 ± 0.581	6.08 ± 2.80
	14	1.34 ± 0.33	0.47 ± 0.04	0.78 ± 0.30	0.998 ± 0.001	1.679 ± 0.196	2.86 ± 0.66
	21	2.13 ± 0.07	0.44 ± 0.01	1.13 ± 0.17	0.997 ± 0.001	2.574 ± 0.284	6.70 ± 1.47
25	1	1.02 ± 0.11	0.42 ± 0.04	0.74 ± 0.09	0.998 ± 0.001	1.084 ± 0.234	1.22 ± 0.56
	7	1.21 ± 0.37	0.44 ± 0.08	0.93 ± 0.32	0.997 ± 0.001	1.474 ± 0.290	2.25 ± 0.91
	14	1.19 ± 0.10	0.41 ± 0.01	1.07 ± 0.16	0.998 ± 0.001	1.201 ± 0.140	2.34 ± 1.83
	21	1.21 ± 0.28	0.46 ± 0.06	0.81 ± 0.37	0.998 ± 0.001	1.145 ± 0.175	1.41 ± 0.56

Table 5
Statistical evaluation of the compatibility of the Vocadlo model with experimental shear stress-shear rate data.

Apple juice added (%)	Storage time (days)	K_v(Pa.s^n)	n_v	$\tau_{0,v}$(Pa)	R^2	RMSE	χ^2
0	1	7.31 ± 1.23	0.45 ± 0.02	1.43 ± 0.04	0.997 ± 0.001	0.263 ± 0.108	0.10 ± 0.07
	7	71.60 ± 4.73	0.34 ± 0.01	2.73 ± 0.39	0.999 ± 0.001	0.577 ± 0.438	0.61 ± 0.76
	14	60.93 ± 7.96	0.35 ± 0.01	3.07 ± 0.30	0.999 ± 0.001	0.397 ± 0.288	0.28 ± 0.33
	21	51.29 ± 5.36	0.37 ± 0.01	2.53 ± 0.10	0.999 ± 0.001	0.340 ± 0.203	0.19 ± 0.18
15	1	6.34 ± 2.16	0.33 ± 0.01	0.84 ± 0.02	0.997 ± 0.001	0.274 ± 0.114	0.11 ± 0.07
	7	12.49 ± 2.47	0.38 ± 0.01	1.29 ± 0.13	0.999 ± 0.001	0.235 ± 0.080	0.08 ± 0.05
	14	13.86 ± 2.53	0.36 ± 0.01	1.24 ± 0.05	0.999 ± 0.001	0.132 ± 0.026	0.02 ± 0.01
	21	17.54 ± 2.09	0.38 ± 0.01	1.29 ± 0.17	0.998 ± 0.001	0.238 ± 0.055	0.08 ± 0.04
25	1	3.71 ± 1.00	0.34 ± 0.04	0.83 ± 0.10	0.999 ± 0.001	0.358 ± 0.278	0.24 ± 0.29
	7	8.13 ± 1.38	0.31 ± 0.02	1.03 ± 0.08	0.997 ± 0.003	0.229 ± 0.166	0.09 ± 0.09
	14	9.96 ± 2.12	0.32 ± 0.01	1.28 ± 0.07	0.999 ± 0.001	0.099 ± 0.053	0.02 ± 0.02
	21	11.91 ± 1.22	0.31 ± 0.01	1.01 ± 0.06	1.000 ± 0.001	0.186 ± 0.145	0.07 ± 0.08

Bulkley model. Since Vocadlo model had higher regression coefficients and lowest error values (RMSE and $\chi 2$), it was selected as best model fitting the experimental data adequately (Table 5). Vocadlo model estimation was in the range of 0.73–3.37 Pa for yield stress, in the range of 0.29–0.47 for flow behavior index (n), and in the range of 2.71–76.33 for consistency coefficient (K_V; Pa.s^n) of fermented soy milk beverages with added apple juice. Since n values of fermented soy milk beverages with added apple juice were lower than 1, the fermented soy milk beverages with added apple juice were found as a pseudoplastic fluid nature. Similarly, the rheological studies on kefir, which is a type of fermented beverage, revealed that it behaves as a thixotropic fluid and flow curves showed that it has shear-thinning (pseudoplastic) properties (Glibowski & Kowalska, 2012).

It was concluded that the consistency of fermented soy milk beverage was affected from both the concentration of apple juice and the storage period ($P < 0.05$). As the apple juice concentration increased, the consistency coefficient of the fermented beverage decreased for the same storage period ($P < 0.05$). In addition, the consistency of all fermented soy milk beverages increased at the first week of the storage, and then remained similar.

Similarly, Gabsi et al. (2013) reported that rheological properties of date syrup changed with increasing of the syrup concentration. The apparent viscosity of the fruit purees (raspberry, strawberry, peach and prune) was affected by the increasing with sugar content while Ostwald Waele model and Herschel–Bulkley were suitable to define rheological properties of these fruit purees (Maceiras, Alvarez, & Cancela, 2007). Researchers have suggested the composition-related solutions to the quality-related problems of fermented beverages (Altay, Karbancıoğlu-Güler, Daskaya-Dikmen, & Heperkan, 2013). For example, Dogan (2011) suggested that increasing the milk solids may solve the textural problem in commercial manufacture of fermented dairy products. The whole ayran had pseudoplastic behavior whereas light ayran containing lower fat ratio exhibited nearly Newtonian behavior (Bayraktaroglu & Obuz, 2008).

3.4. Sensory properties of fermented soy milk beverages

In this study, the apple juice was added to soy milk at different concentrations to improve the acceptability of fermented soymilk drink. Researches have shown that fruit juices could be used for the production of fermented soy milk beverages with a wide range of concentrations from 1 to 50 % to improve sensory properties (Beasley et al., 2003; Daneshi, Ehsani, Razavi, & Labbafi, 2013; Dauda & Adegoke, 2014). In the present study, apple juice concentrations of lower than 15% was not suitable for sensory appeal,

Table 6
Sensory evaluation scores of fermented soy milk beverages having different apple juice concentrations determined at 1st day of the storage period.

Apple juice added (%)	Color	Consistency	Acidity	Apple flavor	Overall acceptance
0	27^a	52^a	24^a	25^a	38^a
15	39^a	32^b	57^b	39^a	36^a
25	54^b	36^b	39^a	56^b	46^b

^{a,b} in the same column with different superscripts are the significant differences ($P < 0.05$).

on the other hand increasing the concentration to lead to syneresis during the fermentation period (data not shown).

The results of the evaluation of rank sums by using critical tables (Newell & MacFarlane, 1987) were agreed with the results of the evaluation method using significant differences obtained by multiple comparison tests, except overall acceptability (Table 6). The consistency of control samples was evaluated as significantly higher than apple juice added samples ($P < 0.05$) while no difference was found between the beverages with 15% and 25% apple juice ($P > 0.05$). Panelists suggested that acidity of the control samples was lowest, and the beverage with 15% apple juice was more acidic. This promoted that the best proper medium for *L. acidophilus* was the soy milk with 15% apple juice. When apple flavor and color in the beverage was compared between the samples, members evaluated that the sample containing 25% apple juice had the best ($P < 0.05$).

The sums of ranks for overall acceptability were not significantly different for the evaluation method using the critical value tables ($P < 0.05$). The overall acceptability rank sums obtained by multiple comparison tests (Duncan) showed that the fermented soymilk beverages with 25% apple juice were preferred by panelists compared to with 15% apple juice and control beverages without apple juice ($P < 0.05$) (Table 6). The multiple comparison evaluation method could have distinguished the difference between overall acceptability in detail.

4. Conclusion

According to the results of this study, soy milk with or without added apple juice could support the growth of *L. acidophilus* and *L. acidophilus* could survive at higher numbers during the storage period of 21 days at 4 °C. The pH values of the fermented soy milk beverages with or without added apple juice changed little during the storage period of 21 days at 4 °C.

The fermented soy milk beverage with added apple juice samples was found to be having pseudoplastic shear-thinning nature. It was obtained that the addition of the apple juice decreased the consistency coefficient of the fermented beverage. The consistency of the control group increased up to the value of 71.60 ± 4.73 Pa.s^n (rheological measurements) in the 1st week of the storage at 4 °C. Similarly, the consistency of control samples was found as significantly higher than apple juice added samples in the sensory tests by the panelists.

Fermented soymilk beverage with apple juice presented good sensory acceptance and provided its microbiological stability for at least 21 days at 4 °C with acceptable survival rates of the probiotic bacteria. Since this fermented soy milk beverage is new and going to be possible commercial product, the data obtained in this study could serve valuable information for the design of pumping systems for the fermented soy milk beverages and development of novel fermented drinks.

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