



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

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ABSTRACT

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 Vocablo models. Vocablo 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.

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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, Spettoli, & 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 Jelčić (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-capped 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 Vocablo model (Eq. (3));

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

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

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

where τ is shear stress (Pa) and τ_0 is yield stress (Pa) while n is flow behavior index, K is consistency coefficient (Pa sⁿ) 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).

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