Development of probiotic yoghurt mixed with roselle syrup

*Pummarat Lawin[1] and Teeraporn Kongbangkerd[1]**

Abstract

Yoghurt mixed with probiotic *Lactobacillus casei* and roselle syrup at concentration of 5 and 10% were produced. Counts of yoghurt bacteria and probiotic bacteria increased during fermentation at 43°C for 12 hours indicated that the bacterial starters had no effect on the growth of *L. casei.* The roselle syrup mixed yoghurts with probiotic were subjected to determine their properties during storage up to 21 days at 4°C. The survival of starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophillus*) and probiotic *L. casei*, viscosity, syneresis, color values, pH, acidity and antioxidant activity were monitored. The results showed that the viscosity, syneresis and antioxidant activity decreased while the storage time had no effect on pH and the color values were not significantly changed. The survival of *L. bulgaricus*, *S. thermophillus* and *L. casei* decreased throughout the storage period but different levels of roselle syrup had no effect on the growth of probiotic *L. casei* which 8-9 log cfu/g was obtained until day 21, ensured that the yoghurt could be claimed for human health. The yeast and mold content was <100 cfu/g. The sensory scores of the 10% roselle syrup mixed yoghurt with probiotic *L. casei* was significantly higher than that of the 5% ($p<0.05$) and the liking score ranged in the medium-like.

Keywords : yoghurt, roselle syrup, probiotic

[1] *Department of Agro-Industry, Natural Resources and Environment, Naresuan University, T. Tha-poh, A. Muang, Phitsanulok 65000, Thailand.*
** Corresponding author, e-mail: teerapornk@nu.ac.th*

Introduction

Probiotic have been known for microorganisms that should be viable and reach their site of action alive and in some cases, even components of bacteria can have positive health effects (immune stimulation) (Ouwehand, et al., 1999). Several studies have reported the effects of probiotic strains on human i.e. lowering serum cholesterol, increasing the bioavailability of vitamins and minerals, binding of mutagens, increasing immune response, relieving of constipation and also inactivating pathogens by production of antimicrobial substances. Currently, there are many new probiotic products which have been created and are commercialized. However, the challenge is to ensure the survival and activity of the orally ingested microorganisms within the environment of the human digestive tract. Roselle (*Hibiscus sabdariffa* L.) is one of the commonly used herbs in Thailand and several studies reported the potent antioxidant effect of its aqueous extract of the dried calyx. Therefore, the objective of this research was to develop yoghurt mixed with probiotic *L. casei* and roselle syrup and to study their properties which could be an alternative functional food for the consumers.

Materials and Methods

Starter cultures, probiotic culture and cow milk

Mixed powdered cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophillus* (YC-380) and probiotic *Lactobacillus casei* (LC-01) were obtained from Chr. Hansen, Denmark. Fresh pasteurized milk was obtained from the local department store in Phitsanulok, Thailand.

Roselle syrup preparation

Fifty grams of dried calyx roselles (*Hibiscus sabdariffa* L.) were extracted with 500 mL drinking water and then heated until boiling and boiled for 5 min. The extract was filtered and 235 g of white sugar was added and boiled for 20 min. The calyx residue was separated and the syrup was cooled down to room temperature and thoroughly mixed with the prepared set yoghurt.

Yoghurt preparation

Six liters of pasteurized milk was mixed with 3% (w/v) milk powder and 0.4% (w/v) gelatin, heated at 60-65^{o}C for 25 min and homogenized. The prepared milk was pasteurized at 85^{o}C for 10 min and let it cool down to about 43^{o}C and mixed with 0.132 g of starter cultures and 0.6 g of probiotic *L. casei* following the company bulletins. The inoculated milk was incubated at 43^{o}C for 10-12 hr or until the pH was 4.2 - 4.5. When the set yoghurt was obtained, it was chilled at 4^{o}C and subjected to add roselle syrup at different levels (0, 5 and 10% (w/v)). The yoghurts mixed with roselle syrup were determined their properties along the storage period at 4^{o}C.

Determination of physical, chemical and microbiological of the yoghurt

The Hunterlab DP900 was used for color measurement and expressed in L*, a* and b* values. The viscosity was determined by DV-III Brookfield viscometer. The % syneresis was assayed following Supavititpatana (2008). The pH and acidity (as %lactic acid) were monitored using Consort C-830 pH meter and titration with 0.1 N NaOH (AOAC, 1998), respectively. The antioxidant activity was monitored using the DPPH scavenging assay as described by Turkmen et al (2005). *L. bulgaricus, S. thermophilus* and *L. casei* were counted on MRS agar (incubated at 37^{o}C, 3 days), M-17 agar (incubated at 43^{o}C, 3 days), and MRS-Im agar with

glucose (incubated at 20°C, 6 days), respectively. Yeast and mold count was performed on Rose bengal agar and incubated at 25°C for 5 days. The microbial counts were expressed in log cfu/g.

Sensory evaluation of the yoghurt

The probiotic yoghurt mixed with roselle syrup with different levels were evaluated for the sensory acceptance in their color, flavor, odor, texture, appearance and overall acceptance by using 9-point hedonic scale with 30 untrained panelists.

Statistical analyses

The mean differences of 4 replication data were analysed by Duncan's Multiple Range Test.

Results and Discussion

During fermentation of the yoghurt, it was found that the growth of *L. bulgaricus, S. thermophilus* and probiotic *L. casei* in pasteurized milk at 43°C increased with increasing storage time. After 12 hr, the counts were >9 cfu/g, the pH was between 4.2 – 4.5 and the acidity was about 1.2% (data not shown). Therefore, the yoghurt was ready and was chilled to slow down the fermentation and thoroughly mixed with roselle syrup at different levels (0, 5 and 10%) and stored at 4°C. During 21d of storage, the pH of the probiotic yoghurts mixed with roselle syrup ranged between 4.1 - 4.3 and slightly higher than that of the control. The acidity was between 1.25 – 1.48% and the color values (L*, a* and b*) were slightly changed (data not shown). The syneresis, viscosity and antioxidant activity of the probiotic yoghurt mixed with roselle syrups are shown in Figure 1, 2 and 3, respectively.

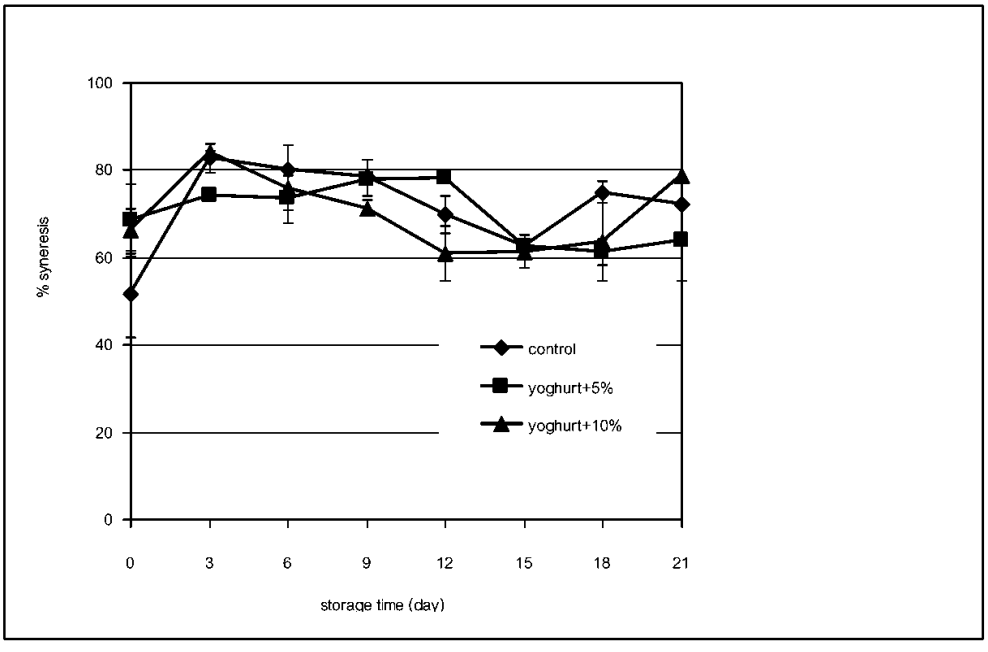

Figure 1. The syneresis of the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

The syneresis of the probiotic yoghurts with different levels of roselle syrup ranged between 51.70 – 84.11%. During the first 3d storage, the syneresis of probiotic yoghurt which contained no syrup significantly increased. This might be due to its initial pH which was a bit higher compared to the other two yoghurts and the growth could be better. The addition of roselle syrup into the probiotic yoghurt also affected the viscosity of the yoghurt as shown in Figure 2. The increase of viscosity of the syrup mixed yoghurts was due to the additional soluble solids especially sucrose and it tended to decrease with increasing time as the cultures have grown and started to produce proteolytic enzymes (Shihata & Shah, 2002). The antioxidant activity of probiotic yoghurt with syrups was shown in Figure 3. The antioxidant activity of the yoghurts increased as the syrup content increased and then contained more anthocyanin, the antioxidant agent. However, the activity gradually disappeared after 6d storage due to the loss of anthocyanin stability as supported by the data from Panichajakul et al. (1988). They reported the decrease of anthocyanin content of *Hisbicus sabdariffa* L. up to 42.8% after keeping the extract at 20°C for 13 weeks. The growth of *L. bulgaricus*, *S. thermophilus* and probiotic *L. casei* in the yoghurts mixed with roselle syrups during storage at 4°C are shown in Figure 4, 5 and 6, respectively.

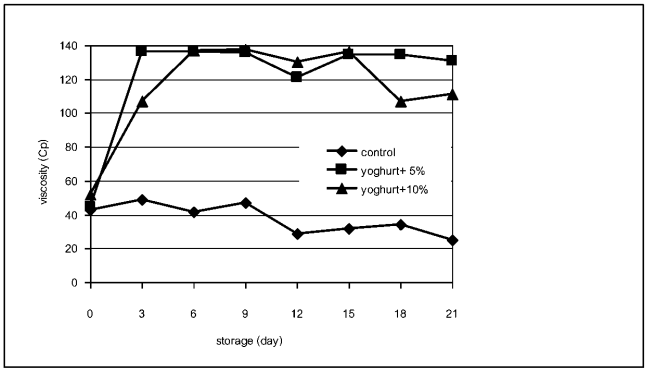

Figure 2. The viscosity of the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

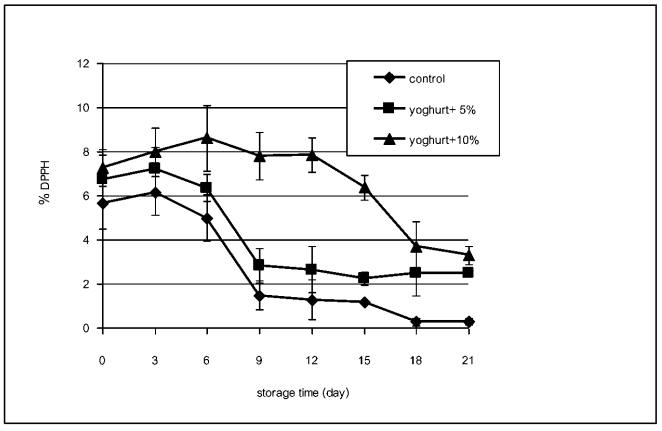

Figure 3. The antioxidant activity of the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

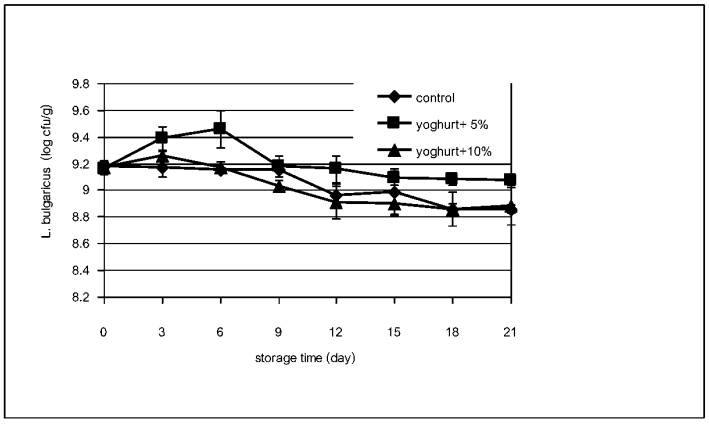

Figure 4. Growth of *L. bulgaricus* in the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

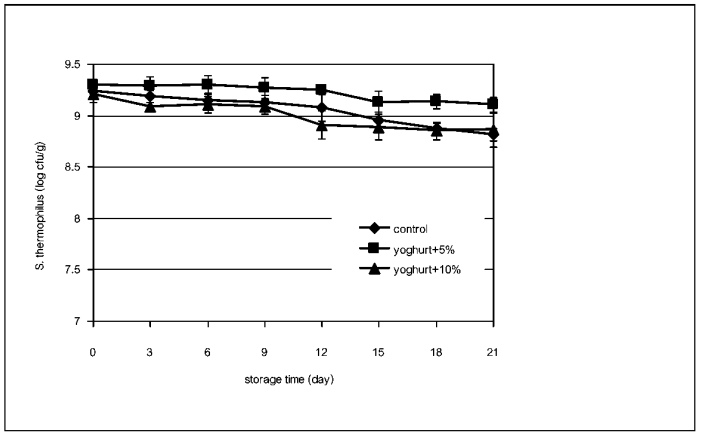

Figure 5. Growth of *S. thermophilus* in the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

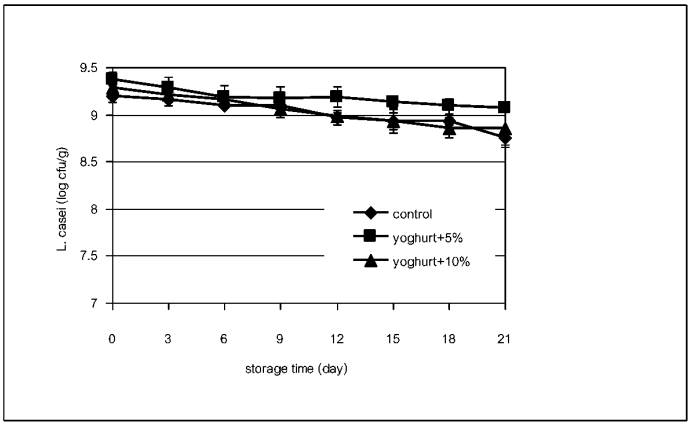

Figure 6. Growth of probiotic *L. casei* in the probiotic yoghurt mixed with different levels of roselle syrup during storage at 4°C.

The counts of starter cultures and *L. casei* during storage at 4°C for 21d ranged between 8.75 – 9.38 log cfu/g. Lee & Salminen (1995) reported the number of probiotics that >5 log cfu/g of the final product could be claimed to exert human health hence the probiotic yoghurt mixed with roselle syrups could be considered the health product. The yeast and mold counts of the yoghurts were < 100 cfu/g during storage time and were within the standard of The Ministry of Public Health (data not shown). The sensory evaluation showed that the scores of probiotic yoghurt mixed with 10% roselle syrup was significantly higher than that mixed with 5% ($p<0.05$) and ranged in the medium-like.

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