# The Influence of Lotus Root Extract on *Lactobacillus casei* subsp. *rhamnosus* Growth in Fermented Milk

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**Abstract**

The infant intestinal tract is essentially sterile part and experiences a period of steady colonization over the following weeks and months as it is exposed to microorganisms from the environment. It is generally accepted that these microorganisms have a major impact on the overall development and function of the gastrointestinal mucosa and immune system. This research aims at prebiotic extraction from lotus root which is a plant in the local area. The study found that prebiotic extracted from lotus root gave the prebiotic concentration of 1,748 μg/ml. The optimum conditions for prebiotic extraction consisted of the extraction ratio, temperature and soaking time of 1:30 (w/v), 85 $^{o}$C and 30 min, respectively. The growth of *Lactobacillus casei* subsp. *rhamnosus* in fermented milk supplemented with lotus root extract was investigated. Result showed that the fermented milk with prebiotic extract was able to promote growth of *L. casei* subsp. *rhamnosus*.

**Keywords:** Lotus root, Prebiotic, Probiotic, Fermented milk, Extracts

## Introduction

Nowadays, people play much attention on health care by healthy food consumption that contains prebiotic. It is an alternative for anyone who takes an interest in health (Fuller and Gibson, 1997). Probiotic is beneficial microorganism in human digestive system. They cause a several positive effect in body such as reducing the amount of pathogenic microorganisms and reduce the risk of colon cancer (Niness, 1999).

Moreover, body is also able to absorb more available calcium in condition of calcium deficiency which is cause of bone decay reduction. Finally, probiotic could well increase immunity in the body (Fuller, 1989).

The increasing level of bifidobacteria and lactobacilli should be regarded as effective prebiotic condition; prebiotic substances are able to display several characteristics. Firstly, they should be neither hydrolysis nor absorption of prebiotic substances in the upper part of the gastrointestinal tract. Secondly, the compound should be fermented selectively by the gut flora. Thirdly, it should stimulate the growth and/or activity of a limited number of beneficial bacteria in the colon in such a way that the composition of the intestinal microorganism is altered towards a healthier one and, as such, induces effects beneficial to health (Gibson and Roberfroid, 1995).

In a recent review of the evidence for the prebiotic nature of several compounds, inulin and oligofructose were confirmed as the most extensively studied prebiotic compounds with major prebiotic efficacy (Kaplan and Hutkins, 2000).

*Lactobacillus casei* subsp. *rhamnosus* has been successfully exploited commercially as a pharmaceutical product for more than 20 years. Its beneficial effects include treatment and prevention of nonorganic diarrhea (Barrangou et al., 2003), that recently showed *in vitro* that this strain has probiotic activities such as the ability to adhere to intestinal cells and antibacterial activity against several pathogens.

Previous study determined the ability of probiotic group to survive passage through the gastrointestinal tract and to evaluate the persistence of the strain after discontinuation of its administration. The minimum effective dose is not precisely known, but the usual recommended oral administration is in excess of $10^9$ CFU/day (Reddy, 1999).

This study recognizes the importance of prebiotic substances produced from natural raw materials that are used locally to reduce cost of production and adding value to existing local plants. In experiment, lotus root was extracted and measured the prebiotic substances. Extract substances were then studied for their effect on *L. casei* subsp. *rhamnosus* growth and survival in fermented milk.

## Materials and methods

### Raw material preparation

Fresh lotus root was washed and sliced crosswise into small pieces. They were dried by hot air oven at 70 $^{o}$C for 24 h. Dry samples were then ground and sieving through a sieve with a pore size of 75 microns. Dry powder samples were stored and used in the next step.

### The lotus root powder extraction

Two treatments of lotus root extract were conducted at extraction ratio of 1 : 30 and 1 : 50 (1 g lotus root powder : 30 ml distilled water and 1 g lotus root powder : 50 ml distilled water, respectively). The suspension was treated at the temperature of 85 $^{o}$C for 30 min and was then centrifuged at 5,000 rpm. The supernatant was sterilized at 121 $^{o}$C for 5 minutes and stored in the refrigerator at 4 $^{o}$C (Figure 1).


**Figure1.** Lotus root extract were conducted at extraction ratio of 1 : 30 and 1 : 50

**Quantification of prebiotic**

The lotus root extract was analyzed to measure prebiotic substances using following equation. (Santad et al., 2011)

Prebiotic substances = Total sugar – Reducing sugar (μg/ml)

**The growth of probiotic bacteria**

The fermented milk was prepared by mixing milk powder (12 g) with 88 ml of lotus root extract. The solution was then pasteurized at 63 $^{\circ}$C for 15 min and inoculated with 2% (v/v) *L. casei* subsp. *rhamnosus.* After that, fermented milk was incubated at 37 $^{\circ}$C for 48 h and sample was collected every 2 h for 24 h. The last collection was carried out at 48 h. The growth numbers of *L. casei* subsp*. rhamnosus* in each sample was measured by pour plate method. After incubating at 37 $^{\circ}$C for 48 h, the survival numbers of probiotic bacteria in fermented milk stored at 4 $^{\circ}$C was also studied. The sample was collected every 7 days for 21 days.

**Statistical Analysis**

All data analysis of *in vitro* experiments was conducted using analysis of variance (ANOVA) in SPSS software (version 11.5) with three replicates. For all experiments, the Scheffe' test method of SPSS was used for multiple comparisons between means. Alpha levels of 0.05 were used as the critical levels of significance.

**Results**

The results showed the amount of prebiotic in lotus root from two treatments with extraction ratio of 1:30 and 1:50 were 1,748 μg/ml and 1,188 μg/ml, respectively (Table 1). The latter stimulated the bacterial growths lower than the former (data not shown), and therefore the fermented milk should be prepared from lotus root extract conducted at extraction ratio of 1 : 30.

**Table 1.** The amount of prebiotic extracts from lotus root

| Prebiotic substances from extraction ratio (Total sugar – Reducing sugar, μg/ml) | |
|---|---|
| 1 : 30 | 1 : 50 |
| 1,748 | 1,188 |

The fermented milk supplemented with lotus root extract promoted the growth of *L. casei* subsp. *rhamnosus* higher than a control (without the extract), indicating that lotus root extract was able to display major prebiotic efficacy for lactobacilli. The maximum cell number was obtained at 8.7 $\times 10^{9}$ CFU/ml (Figure 2).

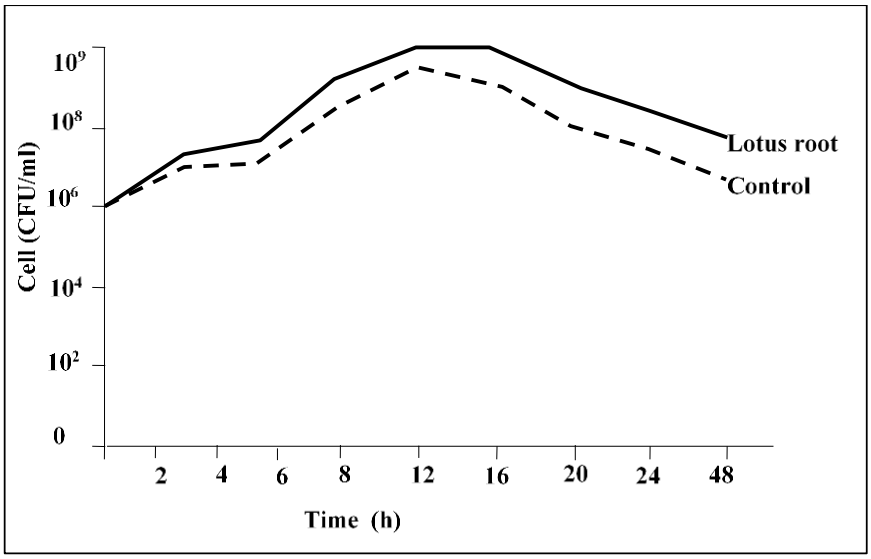


**Figure 2.** Effect of lotus root extracts on growth of *L. casei subsp. rhamnosus* (CFU/ml) in the fermented milk. Control was fermented milk without the extract.

During refrigerated storage for 3 weeks, probiotic survival was reduced, however a higher viable cell number was observed in fermented milk supplemented with lotus root extract in comparison with control (Figure 3). After 48 h fermentation, prebiotic substances significantly decreased, resulting from the growth of probiotic bacteria in fermented milk

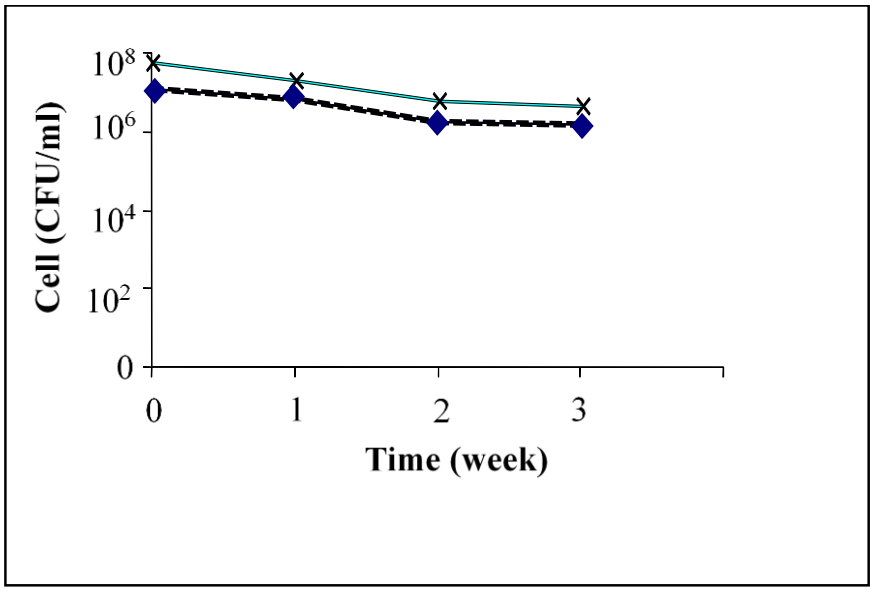


**Figure 3.** The survival of *L. casei* subsp. *rhamnosus* in fermented milk stored at 4 °C for 3 weeks: **( × )** lotus root, ( ◆ ) control.

**Discussion**

Lotus root extract with a ratio of 1:30 could promote a higher growth of *L. casei* subsp. *rhamnosus* in fermented milk than a control. The maximum cell number ($8.7 \times 10^9$ CFU/ml.) suggested that prebiotic substances properly stimulated the growth and survival of probiotic. It has been reported that lactobacilli could produce fructosidases which was able to hydrolyze fructose moieties and metabolize inulin-type fructans efficiently (Gibson et al., 1995). Several lines of evidence show that the appropriate strains of lactic acid bacteria and other lactobacilli in fermented milk products, can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach (Majamaa et al.,1997).

The previous study confirmed the better ability of bifidobacteria populations in prebiotic substances fermentation when compared to glucose (Wollowski, Rechkemmer and Pool-Zobel, 2011). The optimal treatment of lotus root could display major prebiotic efficacy for *L. casei* subsp. *rhamnosus*.

The unique characteristic of probiotic for inulin and oligofructose utilization was described by the presence of an inducible β – fructofuranosidase. This enzyme is able to hydrolyse the β 2–1 glycosidic linkages between the fructose moieties (Mohd et al., 2013). This ability of prebiotic is important for growth and survival of probiotic in fermented milk. Therefore, lotus root extract might be further used in making fermented dairy products for health. Because lactose intolerance effects of the population worldwide, consumption of these products may be a good way to incorporate dairy products and their accompanying nutrients into the diets of lactose intolerant individuals (Sanders, 1999).

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