



Germinated Sang Yod Brown Rice as a Novel Prebiotic for Synbiotic Dietary Supplement Development

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Abstract: This study aimed to investigate the prebiotic properties of germinated Sang Yod brown rice in supporting the growth of the probiotic bacteria *Lactobacillus pentosus* GP6. Germinated brown rice was dried, ground into powder, and mixed with De Man Rogosa and Sharpe (MRS) broth at 1%, 3%, and 5%. Bacterial cells were counted at 0, 3, 6, 9, 12, and 24 h after incubation at 37°C. The results showed that the concentration of germinated Sang Yod brown rice powder that exhibited the highest bacterial growth was 3% (10.97 log cfu/ml), followed by 5% (10.85 log cfu/ml) and 1% (10.42 log cfu/ml), respectively. The 3% germinated Sang Yod brown rice powder produced a synbiotic dietary supplement with *L. pentosus* GP6. The results showed that *L. pentosus* GP6 survived with 10.00-10.30 log cfu/ml cell numbers at 4°C for 6 months. This study demonstrated that germinated Sang Yod brown rice could be implemented as a prebiotic and component of synbiotic dietary supplement products.

Keywords: Germinated Sang Yod brown rice; Prebiotic; Synbiotic dietary supplement

1. Introduction

Consumers increasingly demand healthy and safe foods like functional foods to improve overall health and well-being and reduce the risk of specific diseases [1]. Prebiotics are one of the fastest-growing sectors within functional foods. Many food products can consume these compounds as supplements or functional ingredients [2]. Prebiotics are substrates selectively utilized by probiotics, conferring a health benefit. Probiotics are "harmless live normal flora/microorganisms dietary food supplements which provide a health benefit on the host when administered in adequate amounts, and it also leads to having nutritional advantages" [3]. Different prebiotics will stimulate the growth of indigenous gut bacteria. Prebiotics have enormous potential for modifying gut microbiota. These substances are not digested in the stomach. Consequently, they reach the colon, selectively fermented by potentially beneficial bacteria [4]. Fruit, vegetables, cereals, and other edible plants are sources of carbohydrates constituting potential prebiotics. The following may be mentioned as such potential sources: bananas, corn cob, sweet potato, barley, wheat, and rice [5-11].

Rice is one of the basic components of the daily diet for nearly half of the world's population. Nutritionally, it is an important source of carbohydrates, protein, iron, calcium, thiamine, riboflavin, and niacin [12]. Rice, especially non-white rice, is considered a good source of prebiotic compounds. The Sang Yod red rice (*Oryza sativa*, L., var. indica) is a unique red rice, initially planted in Phatthalung, a



province in the south of Thailand, for a hundred years. It has been proposed as the protected rice variety under the law and registered as a good associated with geographical indications called Sang Yod rice of Muang Phatthalung since 2006 [13]. Sang Yod rice contains minerals, vitamin B complex, and bioactive compounds. The germinated brown rice contains biofunctional components like dietary fibers, phytic acids, vitamins, and γ -aminobutyric acid (GABA) [14,15]. Prebiotics are called dietary carbohydrates and soluble fiber that can stimulate the growth of potentially beneficial bacteria [16].

Finding optimal use of dietary fibers becomes increasingly imperative. Germinated rice is also a source of resistant starch, but its prebiotic properties have not been evaluated directly. In this respect, the exploration of the prebiotic effect of germinated brown rice and its application for the production of the synbiotic dietary supplement is lacking. Therefore, we investigate the prebiotic properties of germinated Sang Yod brown rice to support the growth of probiotic bacteria and the production of synbiotic dietary supplements.

2. Materials and Methods

2.1 Preparation of germinated Sang Yod brown rice

Sang Yod brown rice was obtained from a local polishing mill in Phatthalung, in southern Thailand. The germinated Sang Yod brown rice was prepared by washing brown rice with water and repeated twice with water. The washed brown rice was soaked with water at room temperature (28-30 °C) for 4 h. The soaked brown rice was put in a container for germination. The germination process was performed at 35 °C for 36-40 h. The germinated brown rice was washed with water and used for further experiments.

2.2 Preparation of germinated Sang Yod brown rice powder

Germinated Sang Yod brown rice was dried in a hot air oven at 60 °C for 13 h (the final moisture content was 5%), then ground with a blender to make a fine powder. All samples were finely ground to obtain the 100-mesh powder. The samples were stored at -20 °C and used for further experiments.

2.3 Preparation of probiotic microorganisms

The probiotic microorganism used in the experiment, *Lactobacillus pentosus* GP6, was cultured in De Man Rogosa and Sharpe (MRS) broth at 37°C in an anaerobic condition for 48 h [17]. This bacterial culture was adjusted to 0.5 McFarland standard (microbial concentration was approximately 1.5×10^8 cfu/ml) with 0.85% normal saline solution (NSS). The bacterial suspension was diluted with MRS broth to obtain an initial microbial concentration of approximately 1×10^6 cfu/ml for further experiments.

2.4 Study on prebiotic properties

The prepared germinated Sang Yod brown rice powder was mixed in the MRS broth by adding the powder at 1, 3, and 5% (w/v) concentration. The sample without the germinated Sang Yod brown rice powder was used as a control sample. All samples were autoclaved at 121 °C for 15 min. *L. pentosus* GP6 (5%) was added to each sample and incubated at 37 °C under anaerobic conditions. Throughout the incubation period, samples were collected at 0, 3, 6, 9, 12, and 24 h to count microbial cells.

2.5 Prebiotic index

Prebiotic index (I_{preb}) is the ratio of probiotic growth in the prebiotic to probiotic growth in a control (0% prebiotic). A prebiotic index higher than 1 means that tested carbohydrates positively affect the probiotic growth. If the prebiotic index is near 1, it indicates a low effectiveness of the tested carbohydrate. The prebiotic index was calculated using the equation: $\log \text{cfu/ml}$ of probiotic in tested carbohydrate / $\log \text{cfu/ml}$ of probiotic in control.

2.6 Statistical analysis

All experiments were conducted in triplicate and the results were expressed as mean $\pm$ SD. The statistical examination of the data was performed using the SPSS version 16.0 program. The probiotic growth

data (24 h of incubation) at different concentrations of Sang Yod germinated brown rice powder was compared to the control (0% prebiotic) using an analysis of the variance (ANOVA) test. These means were compared using the Duncan two-way Test at a 95 percent confidence level.

3. Results and Discussion

3.1 Germinated Sang Yod brown rice powder

This study used germinated Sang Yod brown rice powder to study prebiotic properties. The yield of germinated Sang Yod brown rice soaked with water for 4 h following incubation at 35 °C was 85.70%. The germinated Sang Yod brown rice was dried at 60 °C, homogenized, and sifted through a 100-mesh sieve to generate the germinated Sang Yod brown rice powder (Figure 1). The mass of powder was 35.31 g or a yield of 8.31%. Germination can alter brown rice's physicochemical, nutritional, and nutraceutical value. These changes in brown rice characteristics during germination are valuable information for developing functional rice-based food products [18].



Figure 1. Germinated Sang Yod brown rice powder.

3.2 Prebiotic property of Germinated Sang Yod brown rice powder

In this study, we examined the prebiotic capacity of germinated Sang Yod brown rice to increase the growth of the probiotic strain. The growth promotion of *L. pentosus* GP6 by germinated Sang Yod brown rice is shown in Figure 2. The result found that all the concentrations of germinated Sang Yod brown rice powder increased the growth of *L. pentosus* GP6 compared to the control group (MRS broth without the germinated Sang Yod brown rice powder). The concentration of germinated Sang Yod brown rice powder that exhibited the highest bacterial growth was 3% (10.97 log cfu/ml) followed by 5% (10.85 log cfu/ml) and 1% (10.42 log cfu/ml), respectively. Based on these experimental results, the germinated Sang Yod brown rice expressed prebiotic properties. This finding was consistent with the previous study [19].

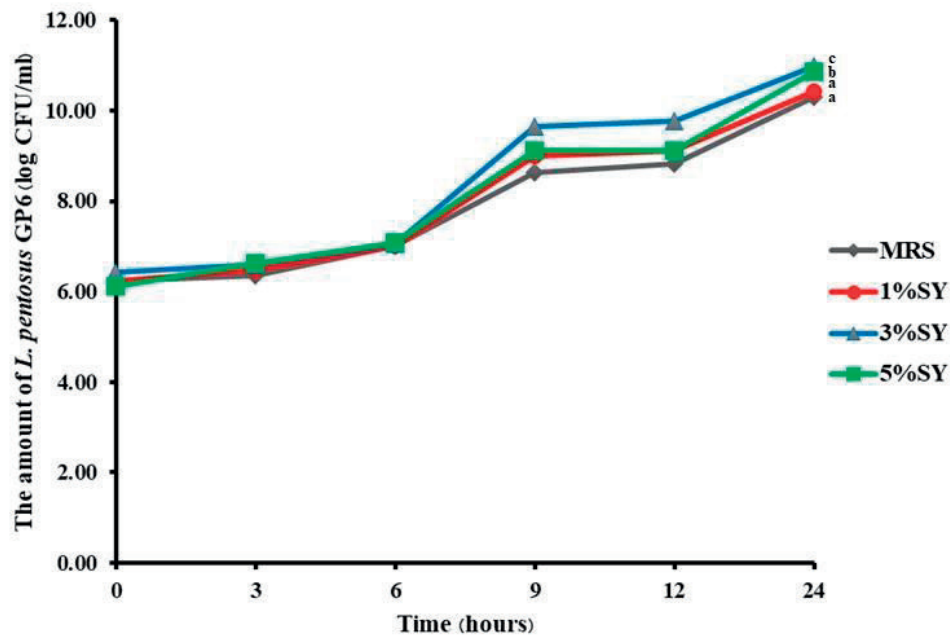


Figure 2. The growth of *L. pentosus* GP6 in MRS broth mixed with germinated Sang Yod brown rice powder at concentrations of 1, 3, and 5%, for which the various letters showed a significant mean difference ($p < 0.05$) in each added concentration of germinated Sang Yod brown rice powder.

The results obtained in this study showed that the probiotics could grow using the prebiotic as a carbon source. Several quantitative approaches have been devised to determine the functional activity of prebiotics under in vitro fermentation conditions. The previous research reported maximum *Lactobacillus plantarum* TISTR 875 growth in germinated Hang rice (3 and 5% w/v) of 4.5 log cfu/ml and 4.10 log cfu/ml, respectively [20]. Banana starch from Kluai Namwa Luang possessed the significantly highest probiotic growth promotion ability [21]. Moreover, to evaluate the prebiotic capacity of three commercial prebiotics, i.e., Frutafit, Oligomate 55, and lactulose, mostly probiotic bacteria isolated from commercial products grew faster on Frutafit and Oligomate 55 than on lactulose [22]. Another study reported that the fermentation of instant Sang-Yod rice porridge mixed with isomaltooligosaccharides produced from rice starch showed a good preference for prebiotic properties [23]. The growth and viability of *Lactobacillus rhamnosus* GR-1 in fermented rice pudding samples supplemented with one type of prebiotic were investigated. The results showed that adding short-chain inulin, long-chain inulin, and oat had no adverse supplementation effects on the viability of *L. rhamnosus* GR-1. There was the potential for producing a novel functional food [24].

The number of bacterial cells at all concentrations of germinated Sang Yod brown rice powder was higher than that of probiotics at the minimum therapeutic dose (at least 10^6 cfu/ml or 6 log cfu/ml [25-26]. However, germinated Sang Yod brown rice powder added to MRS broth at concentrations of 1, 3 and 5%, respectively, after incubation at 37 °C for 24 h, *L. pentosus* GP6 did not differ in the early fermentation period compared to the control. Inoculation growth was inversely proportional to the amount of germinated Sang Yod brown rice powder used. This may be due to the addition of germinated Sang Yod brown rice powder, causing an increase in the amount of nutrients or the concentration of the solution. The viscosity of the food, especially macromolecules such as biopolymers, increases the viscosity of the food, which may impede its transmission into cells. This causes the cells to take time to adapt early. However, over time, the macromolecule compounds were degraded and promoted inoculation thus at the end of the fermentation process, the count in MRS broth mixed with germinated Sang Yod brown rice powder was higher than in MRS broth control unit at 24 h of curing. In addition, the different concentrations of germinated Sang Yod brown rice powder showed a significantly different effect on the growth promotion of *L. pentosus* GP6 ($p < 0.05$). Thus, 3% of germinated Sang Yod brown rice powder was used as a prebiotic to produce a synbiotic dietary supplement product.

3.3 Production of synbiotic dietary supplements

The synergistic combination of probiotics and prebiotics leads to the formation of synbiotics. Synbiotics are the future-proof solution for novel and functional food [27]. Creating appropriate combinations of probiotic strains and nutraceutical mixtures remains key to developing multi-benefits synbiotic products because little information is known on nutraceuticals' effect on probiotics' viability [28]. In this study, we used 3% of germinated Sang Yod brown rice powder as the component of a synbiotic dietary supplement product. This product examined the amount of bacterial cells during storage conditions at 4 °C for 6 months. The result showed that *L. pentosus* GP6 survived and grew from 10.00-10.30 log cfu/ml (Figure 3). Moreover, the survival bacteria cells exhibited more than 10.00 log cfu/ml, or 97.09% survival rates compared with the amount of *L. pentosus* GP6 cells at the start period.

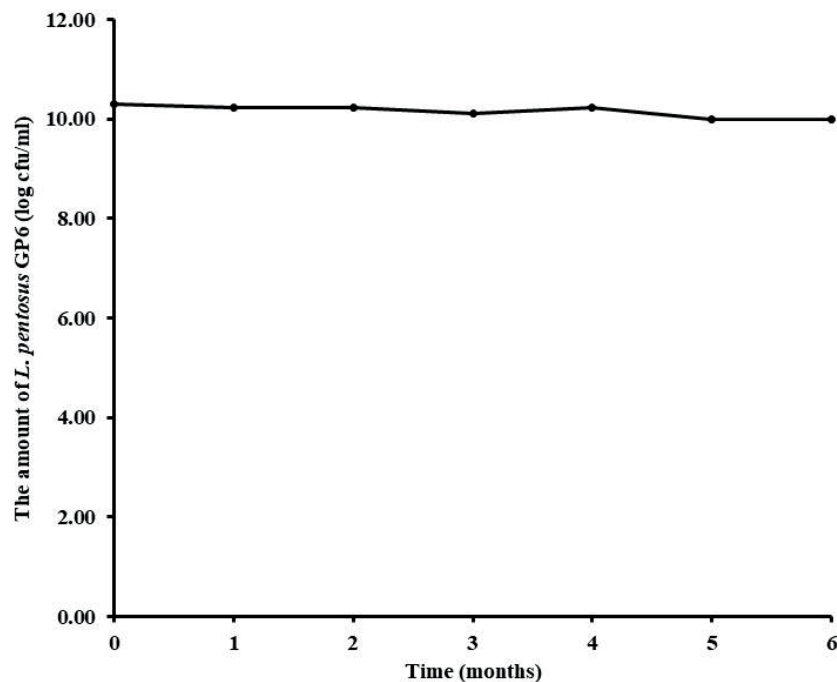


Figure 3. The amount of bacterial cells in synbiotic dietary supplement product during storage at 4 °C for 6 months.

4. Conclusions

The present study deals with the prebiotic property evaluation of germinated Sang Yod brown rice to support the growth of *L. pentosus* GP6 and the production of synbiotic dietary supplements. The germinated Sang Yod brown rice powder increased the growth of *L. pentosus* GP6; thus, germinated Sang Yod brown rice exhibited prebiotic properties. The 3% germinated Sang Yod brown rice powder was also used as a prebiotic to produce a synbiotic dietary supplement in conjunction with the probiotic *L. pentosus* GP6 strain.

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Conflicts of Interest: The author declares no conflict of interest.

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