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Full Paper

Determination of prebiotic properties of rice bran extract

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This research investigated and compared the prebiotic properties of a rice bran extract obtained through commercial xylanase extraction in comparison with water extraction. Prebiotic properties were evaluated by probiotic growth stimulation (*Lacticaseibacillus casei* and *Lactiplantibacillus plantarum*) and gastrointestinal pathogen inhibition (*Bacillus cereus* and *Escherichia coli*). The rice bran extract obtained with xylanase (RB1) displayed significantly higher total polysaccharide and total reducing sugar contents than those obtained with water (RB2; $p<0.05$). After extraction for 30 min, RB1 exhibited the highest total polysaccharide and total reducing sugar contents. HPLC (high performance liquid chromatography) analysis revealed that RB1 primarily contained xylose, while RB2 contained less glucose and lacked other sugar derivatives. RB1 proved effective in stimulating the growth of *L. casei* and *L. plantarum*, surpassing even inulin (a commercial prebiotic). Furthermore, it demonstrated a high potential for inhibiting the growth of pathogenic *B. cereus* and *E. coli,* comparable to inulin. In contrast, RB2 exhibited lower inhibitory capacity against *B. cereus* and *E. coli*.

Key words: rice bran extract, prebiotic, probiotic stimulation, pathogenic inhibition, xylanase, functional food

INTRODUCTION

Nowadays, consumers are more interested in health care, especially functional foods and beverages that focus on providing health benefits aside from their nutritional value. Functional foods are rich in bioactive compounds that are naturally extracted or biosynthesized, promote good health, and reduce the risk of various diseases [1]. Prebiotics are one of the kinds of functional food that are receiving a lot of attention at present. They have growth-stimulating properties of probiotics that help inhibit pathogens in the digestive tract and promote other beneficial health effects on the host. Prebiotics are non-digestible oligosaccharides that are not absorbed in the upper gastrointestinal tract [2]. They are comprised of 2–10 sugar units with glycosidic bond linkages and are commonly found in natural sources and many food plants containing short-chain polysaccharides [3]. Fructooligosaccharide (FOS), mannooligosaccharide (MOS), galactooligosaccharide (GOS), and xylooligosaccharide (XOS) are examples of prebiotics commonly used as functional foods in the food industry [4].

Rice bran is an agricultural by-product obtained after the milling process that has been used for a variety of agricultural purposes, such as to produce rice bran oil, as an animal feed, and as an ingredient for cosmetic product [5]. Rice bran consists of high amounts of protein, fat, carbohydrates, vitamins, and bioactive compounds [6]. The health benefits of rice bran include helping reduce cholesterol in the blood, minimizing the risk of heart disease, and prohibiting gallstone formation, as well as others [7]. Several studies have documented rice bran extraction with various methods, such as solvent, Soxhlet, superfluid, and supercritical fluid extraction, to investigate its bioactive compounds [8–10]. However, these extraction methods are limited by their small yields and the low specificity of bioactive compounds. Therefore, there has been increased interest in enzymatic extraction of rice bran in recent years, considering the high content and specificity of bioactive compounds. Moreover, the reduction in energy consumption and short amount of time required for rice bran extraction are additional advantages.

This research highlighted the importance of rice bran and was intended to explore new sources of raw material for use in the synthesis of prebiotics. The objective was to investigate the prebiotic properties of a rice bran extract obtained with xylanase by examining probiotic growth stimulation and gastrointestinal pathogenic inhibition, including an analysis of sugar content and its composition. The findings could serve as additional preliminary information for further healthy food applications.

MATERIALS AND METHODS

Rice bran, xylanase, and microorganisms

Thai jasmine rice bran 105 was obtained from Prachinburi province, Thailand. A commercial crude xylanase, iKnowZyme

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XL (EC 3.2.1.8), was purchased from Reach Biotechnology, Co., Ltd. (Bangkok, Thailand). Probiotic strains (*Lacticaseibacillus casei* TISTR1463 and *Lactiplantibacillus plantarum* TISTR1465) and pathogenic strains (*Bacillus cereus* ATCC11778 and *Escherichia coli* ATCC25922) were cultivated in appropriate culture media: de Man, Rogosa and Sharpe (MRS), nutrient agar (NA), and nutrient broth (NB), respectively. All other chemicals used were analytical grade.

Rice bran extraction

Rice bran was dried at 105°C for overnight. Then 10 g of the dried rice bran was placed in an Erlenmeyer flask, distilled water was added to bring the total volume to 100 mL, and the sample was incubated at 90°C for 60 min. Incubated rice bran samples were cooled down for 15 min, and then 5 mL of crude xylanase (1,000 units/mL) or 5 mL of distilled water, as the control, was added. After incubation again for 30 and 60 min, each sample was divided into 5 mL aliquots, which were then centrifuged at 10,000 rpm at room temperature for 15 min. All supernatants were collected and kept at 4°C for further analysis.

Total polysaccharide determination

The total polysaccharide content was determined by the phenol sulfuric acid method described by DuBois *et al.* [11]. After appropriate dilution, 0.5 mL of the appropriately diluted sample was immediately mixed with 2.5 mL concentrated sulfuric acid (95% v/v) and 0.5 mL of 5% v/v phenol, heated at 90°C for 5 min, and then cooled to room temperature. The total polysaccharide content was analyzed by spectrophotometry at an absorbance of 490 nm. The glucose standard and blank were analyzed in the same way as the samples, with 0.5 mL glucose standard and 0.5 mL distilled water added instead of the sample, respectively.

Total reducing sugar determination

Total reducing sugar content was determined in rice bran extract samples by DNS assay according to the method of Miller [12]. After the rice bran extraction process, a 1 mL aliquot of the supernatant was mixed with 1 mL DNS reagent and incubated at 100°C for 5 min. After cooling to room temperature, the reducing sugar content was measured by monitoring the absorbance at 540 nm. A standard and blank were prepared in the same way as the analyzed sample by mixing with 1 mL glucose standard or 1 mL distilled water instead of the sample.

High-performance liquid chromatography analysis

The sugar component from the rice bran extraction was analyzed by high-performance liquid chromatography (HPLC) as previously described by Sawangwan *et al.* [13]. HPLC was performed with a Hypersil Gold™ Amino column (3 µm, 4 × 150 mm) at 35°C using an HPLC system equipped with a refractive index (RI) detector. The analysis was conducted using acetonitrile and distilled water at the ratio of 75:25 (v/v) as the mobile phase at a constant flow rate of 1.4 mL/min and with a sample injection volume of 20 µL. The samples were analyzed with a differential refractometer and then compared with all standard compounds (glucose, galactose, maltose, maltotriose, xylose, xylobiose, and xylotriose) for peak identification.

Probiotic stimulation

L. casei and *L. plantarum* were grown in MRS broth supplemented with each of the rice bran extract (1% v/v) and their growths were compared with those when they were grown with the same concentration of inulin (commercial prebiotic compound) as a positive control and distilled water as a negative control. The lactic acid bacteria were grown in the various substrate preparations at 37°C for 48 hr under anaerobic conditions in an anaerobic box. After incubation, the cultures were monitored by measuring the optical cell density with a spectrophotometer at 600 nm, adapting the procedures used by Sawangwan *et al.* [13] and Siragusa *et al.* [14]. The probiotic stimulation efficiency was calculated with the following equation:

$$Stimulation\ activity\ (\%) = \frac{Sample\ OD_{600} - Control\ OD_{600}}{Control\ OD_{600}} \times 100$$

Pathogenic bacteria inhibition

Pathogenic bacteria (*B. cereus* and *E. coli*) were cultured at 37°C in NB supplemented with rice bran extract samples and inulin at the same concentrations as above (1% v/v) for 24 hr. The growth of pathogens was compared with distilled water as a blank supplement. In accordance with Wongsiridetchai *et al.* [15], growth was estimated using the optical cell density reading at 600 nm and was calculated with the following equation:

$$Inhibition\ activity\ (\%) = \frac{Control\ OD_{600} - Sample\ OD_{600}}{Control\ OD_{600}} \times 100$$

Statistical analysis

One-way analysis of variance (ANOVA) was performed with IBM SPSS® Statistics for Windows, V.21 (IBM Corp., Armonk, NY, USA). Differences with a probability value of <0.05 were considered significant, and all data are reported as the mean ± SD.

RESULTS AND DISCUSSION

Rice bran samples were extracted with xylanase (RB1) or water (RB2). Extraction was performed for 30 min (RB1/30 and RB2/30) and 60 min (RB1/60 and RB2/60).

Total polysaccharide and total reducing sugar determination

The rice bran extracts were analyzed for the total polysaccharide and total reducing sugar contents by the phenol sulfuric and DNS methods, as described above. The results were derived by calculating the slope of the standard glucose from the optical densities of the rice bran extracts at an absorbance of 490 nm for total polysaccharide content and 540 nm for total reducing sugar content. As shown in Table 1 and Fig. 1, the samples of rice bran extracts obtained with the xylanase treatment (RB1) contained significantly higher ($p<0.05$) total polysaccharide and total reducing sugar contents than the rice bran extracts from water treatment (RB2). There was no difference in the total polysaccharide content between the 30 and 60 min extraction periods. The total polysaccharide contents of RB1 were 21.51 and 21.26 mg/mL at 30 and 60 min, respectively, while RB2 displayed lower values (2.34 and 2.07 mg/mL).

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Table 1. Total polysaccharide, reducing sugar, and non-reducing sugar contents of rice bran extract samples at different time points

Sample	Total polysaccharide (mg/mL)	Reducing sugar (mg/mL)	Non-reducing sugar (mg/mL)
RB1/30	$21.51^{a} \pm 0.50$	$4.21^{c} \pm 0.04$	$17.31^{e} \pm 0.54$
RB1/60	$21.26^{a} \pm 0.03$	$3.93^{c} \pm 0.01$	$17.32^{e} \pm 0.03$
RB2/30	$2.34^{b} \pm 0.01$	$0.81^{d} \pm 0.01$	$1.51^{f} \pm 0.01$
RB2/60	$2.07^{b} \pm 0.01$	$0.85^{d} \pm 0.01$	$1.22^{f} \pm 0.01$

[a–f] Means within the same column followed by different letters were significantly different ($p<0.05$).

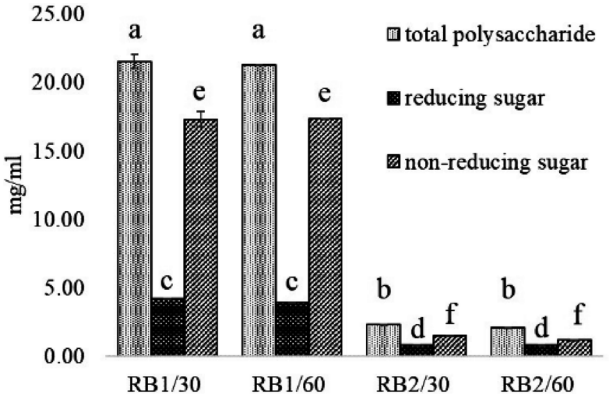

Fig. 1. Total polysaccharide, reducing sugar, and non-reducing sugar contents (mg/mL) of rice bran extract samples at different time points.

For total reducing sugar content, RB1 had values of 4.21 and 3.93 mg/mL at 30 and 60 min extraction, respectively. On the other hand, RB2 had total reducing sugar contents of only 0.81 and 0.85 mg/mL in the same extraction periods.

The difference between the total polysaccharide and total reducing sugar contents was calculated as the non-reducing sugar content, which was considered to represent oligosaccharides, and indicated a prebiotic property. According to a study conducted by Rugwong *et al.* [16], a prebiotic jackfruit extract was isolated and purified by the crystallization method. The non-reducing sugar content was calculated by subtracting the reducing sugar content from the total polysaccharide content. The highest percentage of non-reducing sugar (83.5%) was obtained with a mixing speed of 100 rpm at the optimal crystallizing temperature (58°C). Moreover, Kamollak *et al.* [17] investigated the effect of alkaline pretreatment on the hydrolysis of defatted rice bran using beta-1,4-endoxylanase to produce xylooligosaccharide and arabino-xylooligosaccharide. The oligosaccharides were assayed based on the non-reducing sugar content and quantified by high-performance anion-exchange chromatography with pulsed amperometry detection (HPAEC-PAD). Their study revealed that samples subjected to alkaline pretreatment displayed the highest oligosaccharide content, at 33.59 mg/g, whereas the control samples had an oligosaccharide content of only 14.15 mg/g with the same 24 hr incubation time.

Sugar analysis by HPLC

The sugar compositions of the rice bran extract samples obtained with xylanase (RB1) and water (RB2) are illustrated in Fig. 2. Of note, the RB1/30 chromatogram (Fig. 2A) indicated elevated levels of glucose and xylose, which are the major components of hemicellulose in rice bran, compared with RB2/30 (Fig. 2B). These findings are attributed to the enhanced hydrolysis activity induced by the xylanase in the RB1 sample, which led to a higher concentration of sugar in its extraction. However, the analysis could not identify additional sugars, such as galactose, maltose, maltotriose, xylobiose, and xylotriose, in the chromatogram. This inconsistency could be attributed to the relatively low concentrations of the rice bran extract samples. One potential approach to remedy this would be to concentrate all the rice bran extracts through evaporation before conducting the HPLC analysis. Nevertheless, the analysis of the sugar composition of the rice bran extracts took into consideration rice bran pretreatment using a variety of methods, including alkaline-acidic treatment and hot water and enzyme hydrolysis, as documented elsewhere in the literature [18, 19].

Determination of prebiotic properties

After analysis of sugar content and composition, the rice bran extract samples were evaluated for prebiotic properties based on the stimulation of probiotic growth and inhibition of gastrointestinal pathogens. RB1/30 and RB2/30 added individually to culture media were compared with inulin, which served as the positive control, and distilled water, which served as a blank for comparisons.

Probiotic stimulation

The growths of *L. casei* and *L. plantarum* cultivated in MRS media containing the rice bran extract samples (RB1/30 and RB2/30) were assessed in comparison with inulin and distilled water. Growth was measured after 48 hr using spectrophotometry at OD600. As depicted in Fig. 3, RB1/30 demonstrated the highest percentage of stimulation for both probiotics (43.10% for *L. casei* and 34.42% for *L. plantarum*) and was significantly different, at the $p=0.05$ level, compared with inulin. On the other hand, RB2/30 revealed lower percentages of growth-stimulating potential for *L. casei* and *L. plantarum*, at 31.17% and 23.71%, respectively. Interestingly, both rice bran extracts displayed effective levels of performance in stimulating probiotic growth that were comparable to that of inulin. These results support the results of a previous study conducted by Lin *et al.* [20], who investigated the growth stimulation of *Lactobacillus* spp. and *Bifidobacterium* spp. when cultivated with xylooligosaccharide (XOS) from a rice bran extract. Their study reported higher growth stimulation compared with another microorganism in the human gastrointestinal tract. Furthermore, our findings appear to be consistent with the findings of Hatami *et al.* [21], who showed that enrichment of a probiotic beverage with a rice bran extract maintained *L. casei* viability at $\geq 10^{6}$ CFU/mL for 14 days at 4°C.

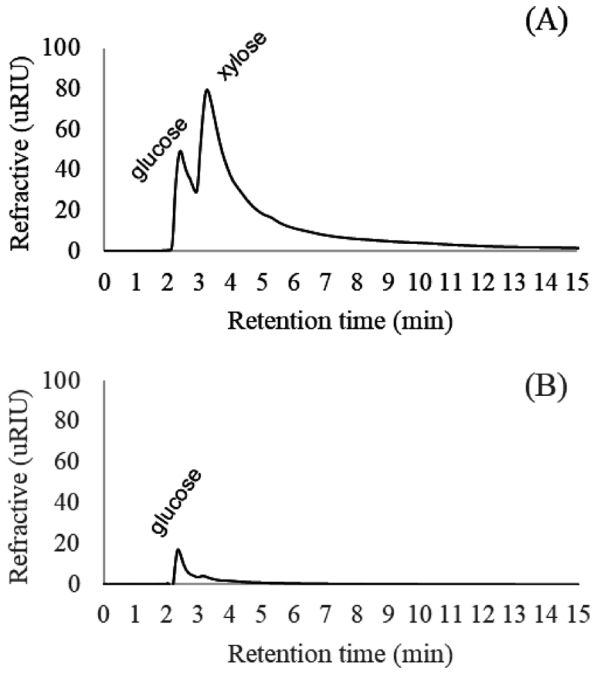

Fig. 2. High-performance liquid chromatography (HPLC) analysis of rice bran extract samples. (A) RB1/30, (B) RB2/30.

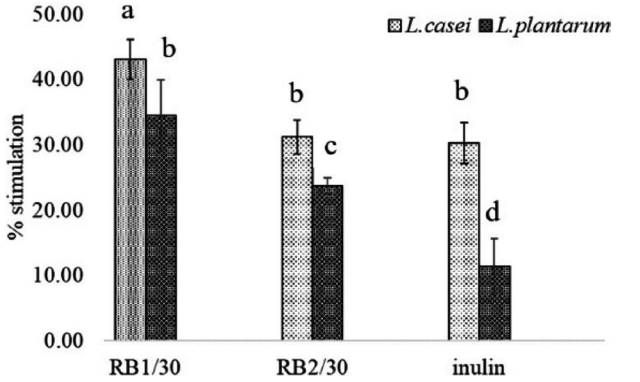

Fig. 3. Probiotic stimulation efficiency of rice bran extracts compared with inulin.

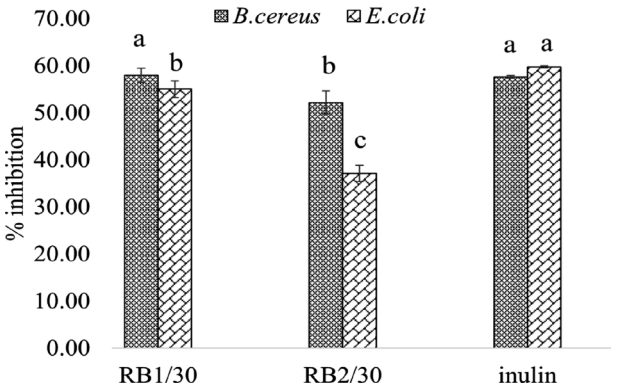

Fig. 4. Pathogenic inhibition efficiency of rice bran extracts compared with inulin.

The observed stimulation of probiotic growth could be attributed to the ability of XOS from the rice bran extract to promote the growth and survival of probiotics.

Pathogenic bacteria inhibition

The cultivation profiles of *B. cereus* and *E coli* cultivated in NB supplemented with the rice bran extract samples (RB1/30 and RB2/30) and inulin for 24 hr were determined at OD600 to investigate inhibition of the pathogenic bacteria. As illustrated in Fig. 4, all samples demonstrated effectiveness in inhibiting pathogenic bacteria, particularly *B. cereus*, for which the inhibition rates reached 57.97%, 52.16%, and 57.61% when cultivated with RB1/30, RB2/30, and inulin, respectively. However, regarding the inhibition of *E. coli*, the rice bran extract samples exhibited a lower efficacy compared with inulin. The inhibition rates were 55.03% (RB1/30) and 37.13% (RB2/30), whereas inulin demonstrated the highest inhibition at 59.78%, with significant differences at the p=0.05 level for all conditions. The experimental results could be further explained by the decrease in pH values within the range of 5.2–6.5 observed during pathogen cultivation supplemented with the rice bran extracts and inulin after 24 hr. This may have consequently led to the synthesis of organic acids occurred, causing the pH to decrease and thus become unsuitable for pathogenic growth, as mentioned elsewhere by Razmi *et al.* [22].

In a previous study, Yang *et al.* [23] reported that rice bran was able to reduce human rotavirus diarrhea in gnotobiotic pigs via the synergic effects between the probiotic growth of *L. rhamnosus* GG and *E. coli* Nissle when added to the culture media. This discovery also indicated that dietary rice bran enhanced the growth of both probiotics and protected against damage to intestinal epithelium during rotavirus infection. Additionally, there are several explanations for this outcome, such as the capability of bioactive compounds from rice bran to inhibit pathogens. Similarly, a study reported by Milutinović *et al.* [24] examined the properties of five extracts derived from medicinal plants (yarrow, St. John's wort/MAE, St. John's wort/EMA, winter savory, and willow gentian) with respect to their impact on the growth of probiotics (*L. rhamnosus*, *L. plantarum*, and *Saccharomyces boulardii*) and pathogenic microbes (*Enterococcus faecalis, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Listeria monocytogenes*, and *Candida albicans*). In their investigation, polyphenols were identified as active metabolites in the plant extracts, demonstrating a stimulative effect on probiotic growth while concurrently controlling pathogen expansion.

These experiments revealed that the rice bran extract obtained with xylanase (RB1) contains a higher concentration of oligosaccharide, serving as a prebiotic compound, compared with the one obtained with water (RB2), as evidenced by the sugar contents and HPLC analysis. The results from this study could suggest that the rice bran extract samples (RB1 and RB2) exhibited prebiotic properties, displaying effective stimulation of probiotics and inhibition of pathogens when compared with inulin. Further exploration of alternative residues and investigation into the long-term gastrointestinal effects would be of interest. In conclusion,

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this research will serve as a base for future studies of functional foods and provide a framework for utilizing agricultural residues and adding high value to them.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Banwo K, Olojede AO, Adesulu D, Adekemi TAD, Verma DK, Mamta T, Soubhagya T, Singh S, Patel AR, Gupta AK, *et al.* 2021. Functional importance of bioactive compounds of foods with potential health benefits: a review on recent trends. Food Biosci 43: 101320. [CrossRef]
2. Patel S, Goyal A. 2012. The current trends and future perspectives of prebiotics research: a review. 3 Biotech. 2: 115–125. [CrossRef]
3. Rezende ESV, Lima GC, Naves MMV. 2021. Dietary fibers as beneficial microbiota modulators: a proposed classification by prebiotic categories. Nutrition 89: 111217. [Medline] [CrossRef]
4. Ibrahim O. 2018. Functional oligosaccharide: chemicals structure, manufacturing, health benefits, applications and regulations. J Food Chem Nanotechnol. 4: 65–76. [CrossRef]
5. Punia S, Kumar M, Siroha AK, Purewal SS. 2021. Rice bran oil: emerging trends in extraction, health benefit, and its industrial application. Rice Sci 28: 217–232. [CrossRef]
6. Rigo LA, Pohlmann AR, Guterres SS, Ruver B, Ruy C. 2014. Chapter 23-rice bran oil: benefits to health and applications in pharmaceutical formulations. Wheat and rice in disease prevention and health. Academic Press, San Diego, pp. 311–322.
7. Sawangwan T, Porncharoennop C, Nimraksa H. 2021. Antioxidant compounds from rice bran fermentation by lactic acid bacteria. AIMS Agric Food. 6: 579–587.
8. Wongwaiwech D, Weerawatanakorn M, Tharatha S, Ho CT. 2019. Comparative study on amount of nutraceuticals in by-products from solvent and cold pressing methods of rice bran oil processing. Yao Wu Shi Pin Fen Xi 27: 71–82. [Medline]
9. Benito RO, Varona S, Sanz MT, Beltrán S. 2019. Valorization of rice bran: modified supercritical CO_2 extraction of bioactive compounds. J Ind Eng Chem 80: 273–282. [CrossRef]
10. Go A, Conag A, Cuizon D. 2016. Recovery of sugars and lipids from spent coffee grounds: a new approach. Waste Biomass Valoriz 7: 1047–1053.
11. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350–356. [CrossRef]
12. Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426–428. [CrossRef]
13. Sawangwan T, Wansanit W, Pattani L, Noysang C. 2018. Study of prebiotic properties from edible mushroom extraction. Agric Nat Resour (Bangk) 52: 519–524.
14. Siragusa S, Di Cagno R, Ercolini D, Minervini F, Gobbetti M, De Angelis M. 2009. Taxonomic structure and monitoring of the dominant population of lactic acid bacteria during wheat flour sourdough type I propagation using *Lactobacillus sanfranciscensis* starters. Appl Environ Microbiol 75: 1099–1109. [Medline] [CrossRef]
15. Wongsiridetchai C, Jonjaroen V, Sawangwan T, Charoenrat T, Chantorn S. 2021. Evaluation of prebiotic mannooligosaccharides obtained from spent coffee grounds for nutraceutical application. Lebensm Wiss Technol 148: 111717. [CrossRef]
16. Rugwong T, Chetpattananondh P, Prasertsit K. 2011. Separation of prebiotics compounds from extract of jackfruit. Proceeding in TIChE International Conference, 10–11th November, Hatyai, Thailand.
17. Kamollak J, Ladda SW, Pinthip R. 2018. Preparation and purification of oligosaccharides from commercially defatted rice bran. Proceeding in 56th Kasetsart University Annual Conference: Science and Genetic Engineering, Architecture and Engineering, Agro-Industry, Natural Resources and Environment, 30 January–2 February 2018, Bangkok, Thailand.
18. Jaichakan P, Thongsook T, Nakphaichit M, Wattanasiritham LS, Phongthai S, Pattarapisitporn A, Utama-ang N, Laokuldilok T, Klangpetch W. 2022. Xylobiose and xylotriose production from alkali soluble defatted rice bran arabinoxylan using endoxylanase from *Neocallimastix Partriciarum*. Stärke 74: 2100177. [CrossRef]
19. Chen B, Qiao Y, Wang X, Zhang Y, Fu L. 2023. Extraction, structural characterization, biological functions, and application of rice bran polysaccharides: a review. Foods 12: 639. [Medline] [CrossRef]
20. Lin SH, Chou LM, Chien YW, Chang JS, Lin CI. 2016. Prebiotic effects of xylooligosaccharides on the improvement of microbiota balance in human subjects. Gastroenterol Res Pract 2016: 5789232. [Medline] [CrossRef]
21. Hatami S, Tajabadi N, Massoud R, Sharifan A. 2021. Chemical and sensorial properties of probiotic beverage based on rice bran extract and honey. Biomass Convers Biorefin 13: 5151–5156. [CrossRef]
22. Razmi N, Lazouskaya M, Pajcin I, Petrovic B, Grahovac J, Simic M, Willander M, Nur O, Stojanovic GM. 2023. Monitoring the effect of pH on the growth of pathogenic bacteria using electrical impedance spectroscopy. Results Eng 20: 101425. [CrossRef]
23. Yang X, Twitchell E, Li G, Wen K, Weiss M, Kocher J, Lei S, Ramesh A, Ryan EP, Yuan L. 2015. High protective efficacy of rice bran against human rotavirus diarrhea via enhancing probiotic growth, gut barrier function, and innate immunity. Sci Rep 5: 15004. [Medline] [CrossRef]
24. Milutinović M, Dimitrijević-Branković S, Rajilić-Stojanović M. 2021. Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms. Front Nutr 8: 688843. [Medline] [CrossRef]

