Food and Bioprocess Technology
https://doi.org/10.1007/s11947-022-02985-z

RESEARCH

Enhancing Functional Properties of Fermented Rice Cake by Using Germinated Black Glutinous Rice, Probiotic Yeast, and Enzyme Technology

Benjamas Cheirsilp[1] · Waraporn Mekpan[1] · Nantikarn Sae-ear[1] · Asma Billateh[1] · Sawai Boukaew[2]

Received: 11 October 2022 / Accepted: 22 December 2022

Abstract
The fermented rice cake is a unique traditional product of Thailand called "Khao-Maak," which is usually made from white or black glutinous rice. Because the germinated black glutinous rice contains high levels of antioxidants and γ-aminobutyric acid (GABA), as well as epidermis with prebiotic potential, it was used in this study to increase the health benefits and functional values of Khao-Maak. Moreover, the probiotic yeast, *Saccharomyces boulardii*, was added during fermentation to ensure the synbiotic characteristics. In vitro gastrointestinal tests showed that the developed product had a higher yeast survival rate (97.10 ± 0.87%) compared to the control (< 70%). The prebiotic activity score (PAS) also showed a positive value of 1.81 ± 0.15, indicating its prebiotic characteristics. However, as the germinated rice is hard to digest, its use slowed down the fermentation process. Therefore, the cellulase and amylase each were added at 10 U/g to increase the fermentation rate while resulting in an acceptable score from the consumers. The functional value analysis showed that the developed product contained antioxidants such as anthocyanins, flavonoids, and GABA, each not less than 30 mg per 100 g-product. The shelf life study revealed that the product could be stored at 4 °C for 10 days while maintaining functional values within product specifications and meeting community product standards. This study demonstrated the possibility of increasing health benefits and functional values of rice products, as well as the effective use of food-grade enzymes to improve the productivity and quality of fermented foods.

Keywords Fermented rice cake · Synbiotic · Germinated brown rice · GABA · Enzymes

Introduction

Khao-Maak is traditional rice cake fermented by a microbial starter containing fungi and yeasts. During rice fermentation, the fungi, mainly *Aspergillus* species, *Rhizopus* species, and *Mucor* species, produce amylases that hydrolyze starch into sugars, and these sugars are partially fermented into alcohol by the yeasts (*Saccharomyces* species and *Candida* species). Organic compounds such as esters and organic acids are produced along with this fermentation process (Mongkontanawat & Lertnimitmongkol, 2015; Rakmai et al., 2019). This fermented rice cake gives soft texture and sweet taste with a little alcoholic and aromatic ester flavor. It has been reported that the fermentation process can induce hydrolysis of protein and carbohydrates and give high levels of small molecules-peptides and polysaccharides (Korkmaz et al., 2020) and organic acids (Liu et al., 2017) and may also have other special functional properties. It has also been considered a dietary supplement to promote the growth development of malnourished children (Manosroi et al., 2011). Some of them have been reported to contain live microorganisms that can promote health benefits for the host and can be called probiotics (Tinrat et al., 2018). It is also used as a dietary supplement to increase and maintain healthy gut flora by providing food for bacteria (Tongyai et al., 2012).

The quality and nutritional value of the fermented products depends on the variations of rice, and starter. Unlike white glutinous rice, brown glutinous rice retains the exterior bran layer, endosperm, and embryo. The weight of the epidermis accounts for 6–11% of the total brown rice, and it

✉ Benjamas Cheirsilp
benjamas.che@psu.ac.th

1 International Program of Biotechnology, Center of Excellence in Innovative Biotechnology for Sustainable Utilization of Bioresources, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

2 College of Innovation and Management, Songkhla Rajabhat University, Songkhla 90000, Thailand

Published online: 06 January 2023

mostly contains protein, dietary fiber, and minerals that show important physiological regulatory effects on the human body (Kuo et al., 2019; Wang et al., 2019). In addition, black glutinous rice also contains higher antioxidants and bioactive compounds such as anthocyanins, γ-oryzanol, and phenolic compounds than white rice (Pornputtapitak et al., 2018). The antioxidant ability of anthocyanins depends on the basic structural orientation of the compound. The ring orientation will determine the ease by which a hydrogen atom from a hydroxyl group can be donated to a free radical as well as the capacity of the anthocyanin to support an unpaired electron (Miguel, 2011). Phenolic compounds, which naturally exist in the bran layer, can inhibit the activity of the angiotensin-converting enzymes (Ramos-Ruiz et al., 2018). During germination, biochemical processes take place, carbohydrates are converted to oligosaccharides and reducing sugars, and grain proteins are digested into amino acids, peptides, and also in an accumulation of many nutrients such as gamma oryzanol, tocopherol, tocotrienol, and γ-aminobutyric acid (GABA) (Karladee & Suriyong, 2012). GABA is a four-carbon non-protein amino acid, produced primarily by the decarboxylation of L-glutamic acid, catalyzed by the enzyme glutamate decarboxylase during the germination process of brown rice. GABA is a neurotransmitter in the brain and spinal cord of mammals and induces hypotensive, diuretic, and tranquilizing effects. Additionally, germinated brown rice extracts containing GABA are also used as medication to ameliorate blood flow in the brain, to inhibit cancer-cell proliferation, and for other beneficial health effects (Karladee & Suriyong, 2012). The germination of black rice also frees its bound minerals, making them more absorbable by the body and giving tendered and tastier rice (Tian et al., 2004). It has been reported that GABA may exert its antioxidant activity by regulating major inflammatory and cellular immunologic activities (Zhu et al., 2019).

Probiotics are able to grow and survive under unfavorable conditions of the human digestive track (e.g., digestive enzymes, pancreatic juice, and low pH) and contribute to the health of the host environment by regulating microbiota as well as exerting biological functions; some also adhere to gut epithelial cells' mucus. Multiple mechanisms (modulation of the normal microbiome of the gut, antagonism against pathogens, adhesion to the mucus, immune modulation, and trophic effects on the gastrointestinal tract) have been proposed for the probiotic action of *Saccharomyces boulardii* (Staniszewski & Kordowska-Wiater, 2021). *S. boulardii* (trademark BIOFLOR®) is a commercial yeast, approved by the Food and Drug Administration, Ministry of Health for use in food. Probiotic products must contain at least 10^6 colony forming unit (CFU) of viable microorganisms per 1 g of food. Interestingly, the fiber covering germinated brown rice also has prebiotic properties that can promote the growth of probiotic microorganisms (Zhu et al., 2018). The products that contain both probiotics and prebiotics are called "synbiotic products," which is another option to increase product value and competitiveness for food and agricultural products. Synbiotics are now also recognized as being more effective in preventing colon cancer than probiotics or prebiotics alone (Chong, 2014).

It should be noted that the germinated brown rice is difficult to ferment by fungi and yeast due to its hard seed coat, which causes a slower fermentation process than normal rice. The solution can be achieved by soaking the rice in water before steaming and taking longer time to soak, steam, and ferment. On the other hand, taking a long time to soak and ferment will cause the rice to spoil quickly, namely, easy to contaminate. Enzymes have usually been used to improve the quality of starchy foods (including bread), accelerate the fermentation process (Rakmai et al., 2019), and prolong their shelf-lives in different storage conditions (Meng & Kim, 2020). In this study, to ensure the probiotic function, the rice cake was added with the commercial probiotic yeast *Saccharomyces boulardii* approved by the Food and Drug Administration Ministry of Health which was declared for use in food. Probiotic products must contain at least 10^6 CFU of viable microorganisms per 1 g of food. In addition to the development of probiotic products, this study also aimed to increase the nutritional values of Khao-Maak by using germinated black glutinous rice and improve the fermentation process as well as the characteristics and nutritional values by applying commercial food-grade enzymes.

Materials and Methods

Materials

Brown black glutinous rice (*Oryza sativa* Linn.) and rice cake starter were purchased from a local farmer. The rice cake starter contained fungi and yeasts in the range of 10^4–10^5 CFU/g. Probiotic yeast used in this study was *S. boulardii*, which was purchased from BIOCODEX, France. The probiotic yeast contained viable cells of 10^3–10^4 cells/mg. Food-grade cellulase and amylase were purchased from iKnowZyme, Thailand.

Germination of Brown Black Glutinous Rice and Rice Cake Fermentation

The method for preparing Khao-Maak in the laboratory was adapted from a local manufacturer, with some modifications. Brown black glutinous rice was prepared by the method of Panyanak et al. (2010). Briefly, it was soaked in water at the ratio of 1:5 for 6 h, the water was drained, and the rice was incubated for 18–24 h. The germinated rice was cooked using steam to gelatinize the starch and then cooled to room temperature prior to brewing with a starter (0.2% w/w). The mixture of cooked rice and starter was then fermented at room temperature for 36–48 h to obtain the desired Khao-Maak characteristics such as lumps of cooked glutinous rice with soft texture, succulent grains, and sweet taste with little alcohol flavor. The sample was taken to determine sugar content, ethanol, ester, anthocyanin, GABA, and antioxidant activity.

Inoculation of Probiotic Yeast

The probiotic yeast was inoculated onto cooked rice after the addition of starter and the fermentation was performed as mentioned above. The effect of yeast inoculum size on rice cake fermentation was studied using various yeast inoculum sizes of 10^2, 10^3, and 10^4 cells/100 g-cooked rice (Rakmai et al., 2019). The sample was taken and analyzed for total yeast cells.

Cellulase and Amylase Addition Affects Rice Cake Fermentation

Food-grade cellulase, amylase, and their combinations were added together with the starter. Time courses of pH, sugar concentration, and acids were compared. The effect of enzyme loading was studied using various enzyme concentrations of 10, 20, and 30 U/g-rice (Cheirsilp & Umsakul, 2008; Rakmai et al., 2019).

Survival of Yeast In Vitro Gastrointestinal Condition

The survival of yeasts in simulated gastrointestinal juice was evaluated following the method of Fratianni et al. (2014). Artificial gastric juice (containing 0.05 M phosphate buffer, 1% NaCl and 3 mg/mL pepsin added with 1 M HCl to adjust the pH to 2.5 and 3.0) and artificial intestinal juice (containing 0.3% w/v bile salts and 1 mg/mL pancreatin at pH 8.0) were used. Both solutions were sterile filtered (0.22 mm, Millipore SpA, Milano, Italy). Samples of yeast in fermented rice cake and yeast in culture medium were each incubated in artificial gastric juice for 2 and 4 h at 37 °C and subsequently in intestinal juice for 4 h at 37 °C. Cell viability (colony forming units/ml) was determined by culturing cells on agar plates before and after gastrointestinal transit simulation. Free yeast grown in potato dextrose broth and equally treated was used as a control.

Analytical Methods

Chemical Characteristic Determination

Fermented rice cake was analyzed for total soluble solids using a brix refractometer (SDB-032, Fzcenter, China) and pH using a pH-meter (F20, Mettler Toledo, Switzerland). The alcohol (ethanol) and aroma ester (ethyl ethanoate) concentrations were determined by using gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector (FID) and Stabilwax column (Noomtim & Cheirsilp, 2011). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the method of Pedro et al. (2016). Briefly, one gram of the sample was extracted with 10 mL of ethanol. The solution was separated by centrifugation at 4000 g for 10 min. The sample (1 mL) was mixed with 0.1 mM DPPH solution in 95% ethanol (1 mL) and incubated in dark condition for 30 min. The absorbance was determined at 517 nm. The ability of extracts to scavenge the DPPH was calculated. For the GABA determination, 0.1 mL of the sample was added with 0.2 mL of 0.2 M borate buffer and 1.0 mL of 6% phenol reagent. The solution was mixed thoroughly and cooled in a cooling bath for 5 min. Next, 0.4 mL of 10–15% NaOCl was added, and the solution was shaken vigorously for 1 min and again cooled in a cooling bath for 5 min. Finally, the solution was boiled in a water bath (100 °C) for 10 min and allowed to cool. Absorbance was determined by spectrophotometry at a wavelength of 630 nm, with ethanol 2.0 mL as a blank. GABA content was quantified by comparing these with the standard GABA content curve. Total anthocyanins and flavonoids were extracted from one gram of finely ground black rice using a mixture of ethanol and citric acid at 1.0 M in the ratio of 80:20. After extraction, the extractant was filtered, and the residue and filter paper were rinsed using the same solvent until total volume of 50 mL was reached. The total anthocyanin and flavonoid contents were calculated following the method of Pedro et al. (2016).

Microbiological Characteristic Determination

Total microorganisms, mold, and yeast in the final fermented rice cake were counted as CFU/g-sample. Briefly, a well-homogenized sample was appropriately diluted and aseptically transferred into sterile Plate Count Agar (PCA) plates. The inverted plate was incubated for 48 h, and total viable cells were counted. Potato dextrose agar (PDA) was used for mold and yeast enumeration. The appropriately diluted samples were aseptically transferred into PDA plates. The plates were then incubated for 48–72 h and total colonies were counted.

Sensory Evaluation

Thirty untrained adults were recruited through convenience sampling and asked to evaluate the appearance, sweetness, flavor, texture, and likeliness of the fermented rice cake on a 9-point hedonic scale (1 = extremely disliked, 5 = neutral, and 9 = extremely liked). All samples (30 mL) were served cold in uniform plastic cups labelled with random 3-digit codes and presented in a randomized order across the participants. The flavored fermented rice cake product with the best hedonic liking scores was selected for the subsequent analysis.

Nutritive Value Analysis

The nutritive values of fermented rice cake products were determined using the standard protocols of the Association of Official Analytical Chemists (AOAC, 2005). In brief, total nitrogen was analyzed via the Kjeldahl method using digestion and distillation units (FOSS Analytical, Denmark), and the protein content was calculated using a conversion factor of 6.25. The total fat content was determined using a Soxhlet apparatus (EV6 ALL/16 No. 10–0012, Gerhardt, Germany). By subtracting the contents of the abovementioned nutrients from 100, the total carbohydrate content (including dietary fiber) was determined. The energy was calculated by using multiplication factors: 4 for carbohydrates and protein, and 9 for total fat (kcal per gram). The prebiotic activity score (PAS) was developed to assess the substrate's selectivity in promoting the growth of intestinal bacteria associated with both health and pathogenic bacteria (Huebner et al., 2007). The color of samples was measured as L* a* b* in the mode of International Commission on Illumination (CIE) by ColorFlex EZ Spectrophotometer (ColorFlex 45/0, HunterLab, USA).

Storage Stability Test

Fermented rice cake products were produced and transferred to sterilized glass bottles with a sealed screw cap. The total volume of the product was 100 g. The products were cold stored in a refrigerator (4 °C) for 4 weeks. During the storage period, the samples were aseptically taken for periodic determination of total yeast cells.

Statistical Analysis All experiments were conducted at least in triplicate, and the results were presented as the mean ± standard deviation with significant digits when appropriate. Statistical analysis was performed using one way ANOVA and Duncan's multiple range tests. When $P < 0.05$, the values are considered significantly different. All statistical data was conducted using SPSS software.

Results and Discussion

Rice Cake Fermentation Using Germinated Black Glutinous Rice and Probiotic Yeast

In this study, brown black glutinous rice post-harvested for not more than 6 months was used. The germinated rice with 0.5–1.0 mm root was used as it contains the highest nutrition (Patil & Khan, 2011). The GABA content in the germinated rice was 36.49 ± 1.36 mg/100 g-rice, which was much higher than that in the non-germinated rice (0.82 ± 0.12 mg/100 g-rice). In addition to GABA, brown rice also contains dietary fiber that has been reported as

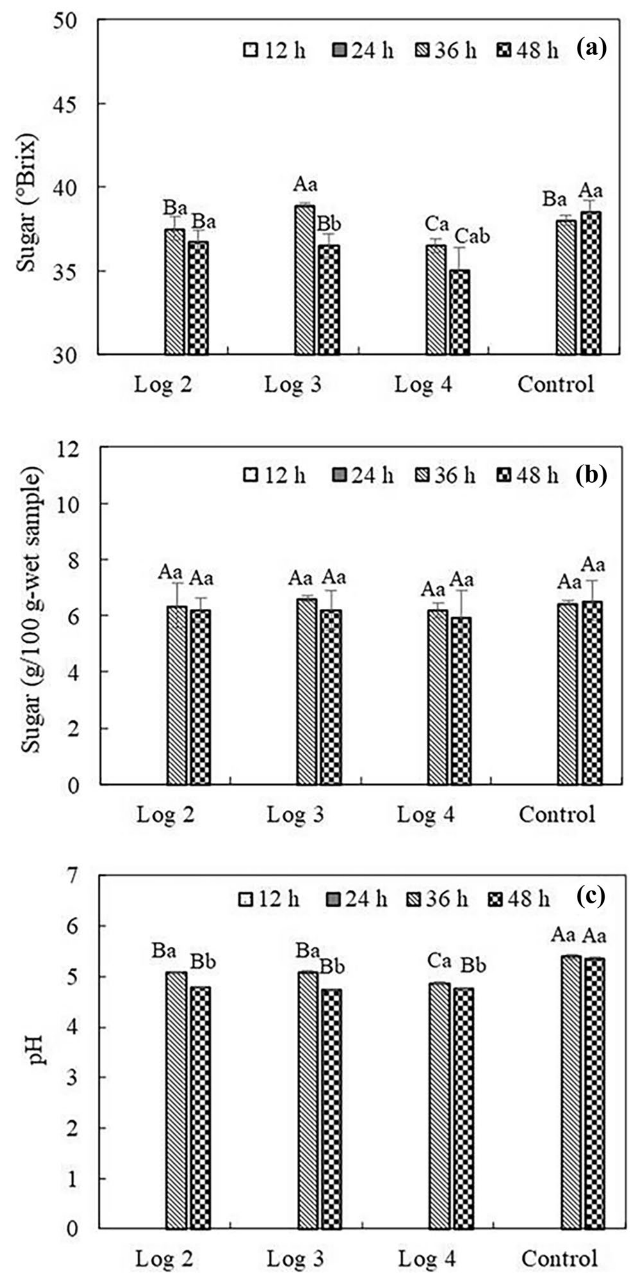

Fig. 1 Effect of probiotic yeast addition on sugar and pH of the fermented rice cake using germinated black glutinous rice. Log 2, Log 3, and Log 4 represent rice cake fermentation using rice cake starter and yeast inoculum at 10^2, 10^3, and $10.^4$ cells/100-g rice, respectively. Control represents rice cake fermentation using only rice cake starter. Data are means with standard deviation ($n = 3$). Different uppercase and lowercase letters on the bar indicate significant differences between conditions and incubation time, respectively ($P < 0.05$)

Table 1 Effect of probiotic yeast addition on characteristics of fermented rice cake after fermentation for 48 h

Probiotic yeast (cells/100 g)	Ethanol (%)	Ester (mg/100 g)	Antioxidant activity (%)	Anthocyanin (mg/100 g)	Flavonoid (mg/100 g)	GABA (mg/100 g)
Control	0.096 ± 0.13^{d}	11.40 ± 0.74^{a}	72.08 ± 2.66^{a}	42.98 ± 2.04^{a}	29.34 ± 0.13^{b}	32.88 ± 4.86^{a}
10^2	0.128 ± 0.04^{c}	11.58 ± 0.65^{a}	70.56 ± 0.30^{a}	40.26 ± 3.06^{a}	28.46 ± 1.57^{b}	30.90 ± 3.36^{a}
10^3	0.153 ± 0.08^{b}	11.99 ± 2.16^{a}	73.17 ± 2.80^{a}	41.50 ± 3.77^{a}	31.46 ± 0.13^{a}	28.26 ± 1.40^{a}
10^4	0.177 ± 0.11^{a}	10.58 ± 0.20^{a}	71.89 ± 3.53^{a}	42.95 ± 1.39^{a}	33.83 ± 1.58^{a}	28.00 ± 1.31^{a}

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

prebiotic (Nealon et al., 2017). The germinated rice was fermented with the addition of rice cake starter and probiotic yeast as shown in Fig. 1. During the first 24 h of fermentation, there was no liquid that could be squeezed from the sample. The sugar content and pH of the liquid was measured at 36 h and 48 h. The addition of probiotic yeast gave slightly lower sugar content and pH, possibly because the added yeast consumed more sugar and produced more acids. During rice fermentation by rice cake starter, the starch content was hydrolyzed by the saccharifying enzymes secreted by the fungi, and the sugar content increased accordingly. Table 1 shows the characteristics of fermented rice cake after fermentation for 48 h. The ethanol content of the fermented rice cake increased with increasing the amount of yeast inoculum. There was no significant effect on antioxidant activity, anthocyanin, or GABA content. Only the flavonoid content increased as the yeast inoculum was increased to 10^3 cells/100 g-rice. It has been reported that the phenol in the food matrix is more easily extracted by the action of microorganisms and enzymes. Moreover, the organic acids produced during fermentation may affect the permeability of the cell membrane and promote the bound phenolic release of brown rice (Chupeerach et al., 2021). Antioxidants in the GABA-contained product could benefit the prevention of oxidative stress-related disorders such as cancers, inflammation, autoimmunity, neurodegenerative diseases, and cardiovascular diseases (Kittibunchakul et al., 2021).

The synbiotic fermented rice cake offering substantial health benefits was successfully developed in this study. The results showed that the average scores of the appearance, sweetness, flavor, texture, and likeliness for all synbiotic fermented rice cake were higher than 5 (neutral) (Table 2), thus indicating positive consumer responses to the sensory attributes of the products. When comparing the total yeast cell count, there were no viable yeast cells detected in the control, while the fermented rice cake with probiotic yeast at inoculum sizes of 10^3 and 10^4 cells/100 g-rice gave comparable cell counts of log 8.38 ± 0.05 and log 8.22 ± 0.06 cells/g, which were significantly higher than that of 10^2 cells/100 g-rice (log 7.98 ± 0.19 cells/g). Therefore, the fermented rice cake inoculated with 10^3 probiotic yeast cells/100 g-rice was chosen for further study.

Survival of Yeast In Vitro Gastrointestinal Environment

Figure 2 shows the survival of probiotic yeast in simulated gastrointestinal environment for 2 and 4 h. A dramatic loss of viability was exhibited by the control after the simulated gastrointestinal passage, while the probiotic yeast in the synbiotic fermented rice cake could endure the negative effects of the gastrointestinal environment better than the control. After gastric treatment for 2 h, the survival rate of probiotic yeast in the control was $84.7 \pm 0.86\%$ while that in the fermented rice cake was $97.10 \pm 0.87\%$. After 4 h of gastric treatment, there was no significant difference in the survival rate in both samples, which were $83.0 \pm 0.58\%$ and $84.8 \pm 0.54\%$, respectively. The survived probiotic yeast after gastric treatment for 2 h was exposed to pancreatic treatment for 2 and 4 h. After the pancreatic treatment for 2 h, the yeast in fermented rice cake exhibited a 100% survival rate. After the gastric and pancreatic treatments

Table 2 Sensory analysis of fermented rice cake using various probiotic yeast inoculum sizes

Probiotic yeast (cells/100 g)	Appearance	Sweetness	Flavor	Texture	Likeliness
Control	7.60 ± 0.81^{a}	6.93 ± 1.80^{a}	6.73 ± 1.41^{a}	6.63 ± 1.25^{a}	6.83 ± 1.46^{a}
10^2	6.73 ± 1.17^{a}	6.37 ± 5.97^{a}	6.60 ± 1.28^{a}	6.67 ± 1.40^{a}	6.60 ± 1.43^{a}
10^3	7.37 ± 0.93^{a}	6.13 ± 2.00^{a}	6.37 ± 1.75^{a}	6.53 ± 1.50^{a}	6.37 ± 1.59^{a}
10^4	7.60 ± 0.67^{a}	6.23 ± 1.68^{a}	5.93 ± 2.16^{a}	6.20 ± 1.77^{a}	6.10 ± 1.99^{a}

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

each for 2 h, the yeast in fermented rice cake exhibited 6.30×10^6 CFU/g, a value markedly higher than that of the control, which was only 5.77×10^6 CFU/g. Therefore, the overall survival rate after the gastric and pancreatic treatments each for 2 h was as high as $97.10 \pm 0.87\%$ while that of the control was only $69.72 \pm 0.49\%$. These data support the prebiotic property of the fermented rice cake in which probiotic yeast, *S. boulardii*, exhibited a better survival rate when compared with the control. The presence of prebiotics may retard or limit the influx of acidic fluids into the yeast cells, thereby protecting them during their passage through the gastrointestinal tract as well as against bile attacks. Therefore, the fermented rice cake developed in this study, which contained both probiotics and prebiotics, can be defined as the synbiotic product.

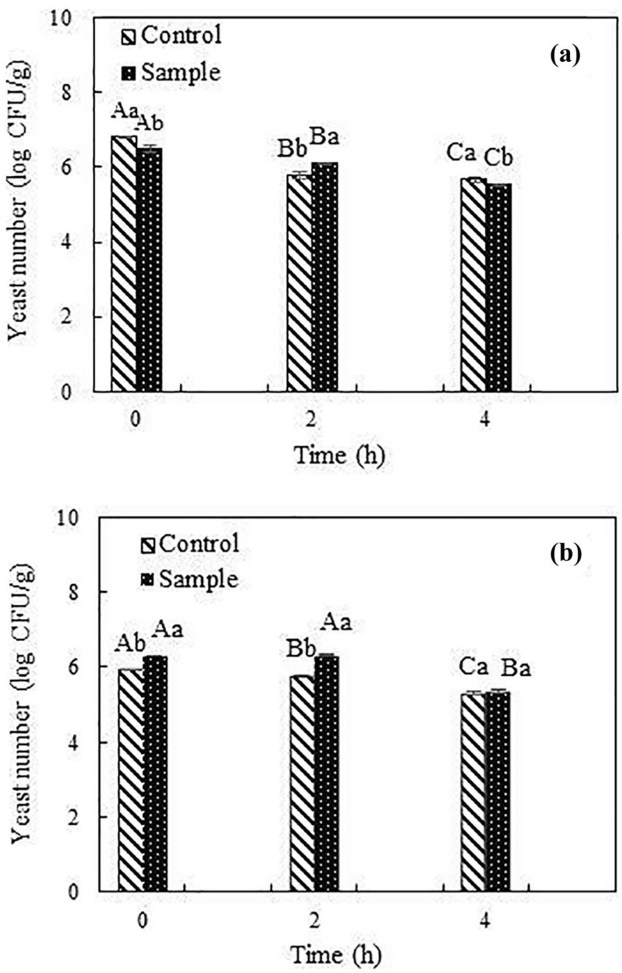

Fig. 2 Survival of probiotic yeast in vitro gastrointestinal condition. Yeast in fermented rice cake (sample) and yeast in culture medium (control) each was incubated in artificial gastric juice for 2 and 4 h at 37 °C **a** and subsequently in intestinal juice for 2 and 4 h at 37 °C **b**. Data are means with standard deviation ($n=3$). Different uppercase and lowercase letters on the bar indicate significant differences between incubation time and conditions, respectively ($P < 0.05$)

Effect of Cellulase and Amylase Addition

The starch-hydrolyzing enzyme (amylase, A), cellulose-hydrolyzing enzyme (cellulase, C), and their combinations were added together with the rice cake starter and probiotic yeast to increase the sweetness and accelerate the fermentation process of the synbiotic rice cake. Figure 3 depicts the effects of enzyme addition on the sugar and pH of fermented

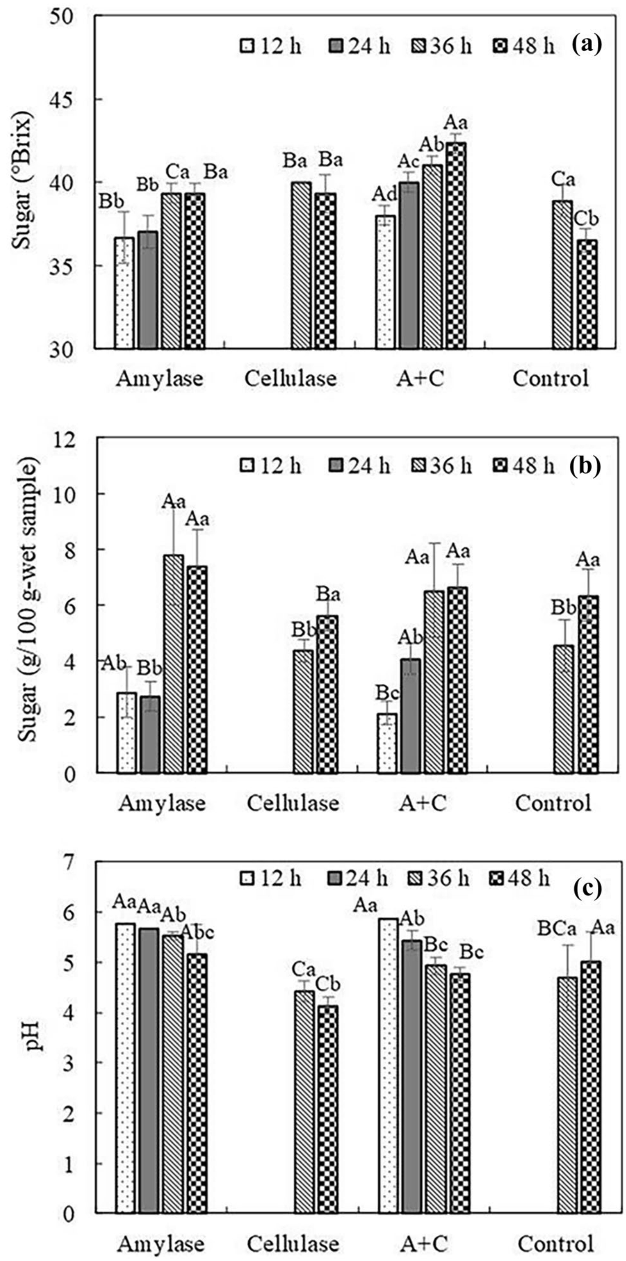

Fig. 3 Effect of amylase (**A**), cellulase (**C**) and their combination (**A + C**) on rice cake fermentation. Each was added at 20 U/g-rice. Data are means with standard deviation ($n=3$). Different uppercase and lowercase letters on the bar indicate significant differences between conditions and incubation time, respectively ($P < 0.05$)

Table 3 Effect of amylase (A) and cellulase (C) addition on characteristics of fermented rice cake after fermentation for 48 h

Sample	Ethanol (%)	Ester (mg/100 g)	Antioxidant activity (%)	Anthocyanin (mg/100 g)	Flavonoid (mg/100 g)	GABA (mg/100 g)
Control	0.153 ± 0.080^{ab}	11.99 ± 2.16^{ab}	87.94 ± 4.70^{b}	41.68 ± 4.04^{a}	34.79 ± 0.94^{a}	28.26 ± 1.40^{a}
A 20 U/g	0.174 ± 0.039^{ab}	12.14 ± 2.72^{ab}	91.69 ± 4.20^{ab}	41.84 ± 3.86^{a}	36.52 ± 2.07^{a}	28.95 ± 0.74^{a}
C 20 U/g	0.220 ± 0.071^{a}	13.39 ± 1.03^{a}	93.75 ± 3.63^{a}	40.01 ± 2.98^{a}	37.59 ± 2.26^{a}	28.96 ± 2.33^{a}
A + C each 20 U/g	0.233 ± 0.044^{a}	9.61 ± 1.17^{b}	95.72 ± 0.48^{a}	41.22 ± 4.48^{a}	37.39 ± 0.47^{a}	28.42 ± 1.44^{a}

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

rice cake. The addition of amylase and cellulase could accelerate the hydrolysis of rice and release sugar higher than the control. Interestingly, their combinations accelerated the hydrolysis of starch and cellulose the most. According to the Thai community product standard (Ministry of Industry, 2003), for fermented rice cake, the product should contain sugar in terms of total soluble solids higher than 40°Brix. These results show that when applying enzyme technology, it was possible to develop the fermented rice cake using germinated black glutinous rice. It should also be noted that the pH in the fermented rice cake decreased faster. This could be because the addition of enzymes improved the hydrolysis rate of starch to sugar and promoted probiotic yeast to grow and produce more acids. Another possible reason might be the release of acidic phenol content in the bran of brown rice during fermentation (Manosroi et al., 2011). The addition of amylase during rice cake fermentation has been reported by Rakmai et al. (2019), who found that when the commercial amylase was added, the sugar content in the product increased rapidly, and the addition of higher amount of amylase led to higher content of sugar and sweeter taste of the product.

The ethanol production increased with the addition of amylase, cellulase, and their combinations indicating the better activity of the yeast (Table 3). Interestingly, the antioxidant activity also increased with the addition of enzymes. This could also be due to the action of enzymes on the permeability of the cell membrane and promoting the bound phenolic release of brown rice (Chupeerach et al., 2021). There was no significant difference in anthocyanin, flavonoid, and GABA content. Table 4 shows the sensory analysis of fermented rice cake added with enzymes. There was no significant difference in the characteristics of the fermented rice cake added with different enzymes.

Effect of Enzyme Loadings

The combination of amylase and cellulase was used to accelerate the rice cake fermentation process and increase the antioxidant activity. The effect of enzyme loadings was studied by adding each enzyme at 10, 20, and 30 U/g (Fig. 4). The sugar content in total soluble solid form increased faster with increasing enzyme loadings. As the product should contain sugar in terms of total soluble solids higher than 40°Brix, the minimum requirements for enzyme loading and fermentation could be set at 10 U/g-rice and 36 h, respectively, as shown in Fig. 4. Therefore, it could be suggested that with the addition of enzymes, the fermentation rate could be increased. The faster fermentation rate, the safer from other bacterial contamination. However, other characteristics and sensory analysis should be considered as well. The results of characteristics and sensory analysis after fermentation for 36 h and 48 h are shown in Tables 5 and 6, respectively. The use of higher enzyme loading did not significantly increase ethanol and ester production. The antioxidant activity increased with the addition of enzymes, but increasing enzyme loading higher than 10 U/g did not further increase the antioxidant activity. There was no significant difference in the anthocyanin, flavonoid, and GABA contents in the fermented rice cake with and without enzyme addition and also those in the 36-h and 48-h fermented rice cake (Fig 5).

Table 6 shows the sensory analysis of fermented rice cake added with enzymes. There was no significant difference in the characteristics of the fermented rice cake added with

Table 4 Sensory analysis of fermented rice cake adding with amylase (A) and cellulase (C) after fermentation for 48 h

Treatment	Appearance	Sweetness	Flavor	Texture	Likeliness
Control	6.47 ± 1.41^{a}	5.93 ± 1.78^{a}	6.33 ± 1.81^{a}	5.91 ± 2.04^{a}	6.10 ± 1.69^{a}
A 20 U/g-rice	6.37 ± 1.45^{a}	5.53 ± 1.61^{a}	6.00 ± 1.26^{a}	6.34 ± 1.91^{a}	5.60 ± 1.38^{a}
C 20 U/g-rice	5.97 ± 1.75^{a}	5.17 ± 2.18^{a}	5.73 ± 1.76^{a}	6.97 ± 1.94^{a}	5.23 ± 2.06^{a}
A + C (each 20 U/g-rice)	6.33 ± 1.60^{a}	5.63 ± 2.01^{a}	5.87 ± 1.68^{a}	6.03 ± 1.83^{a}	5.47 ± 1.76^{a}

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

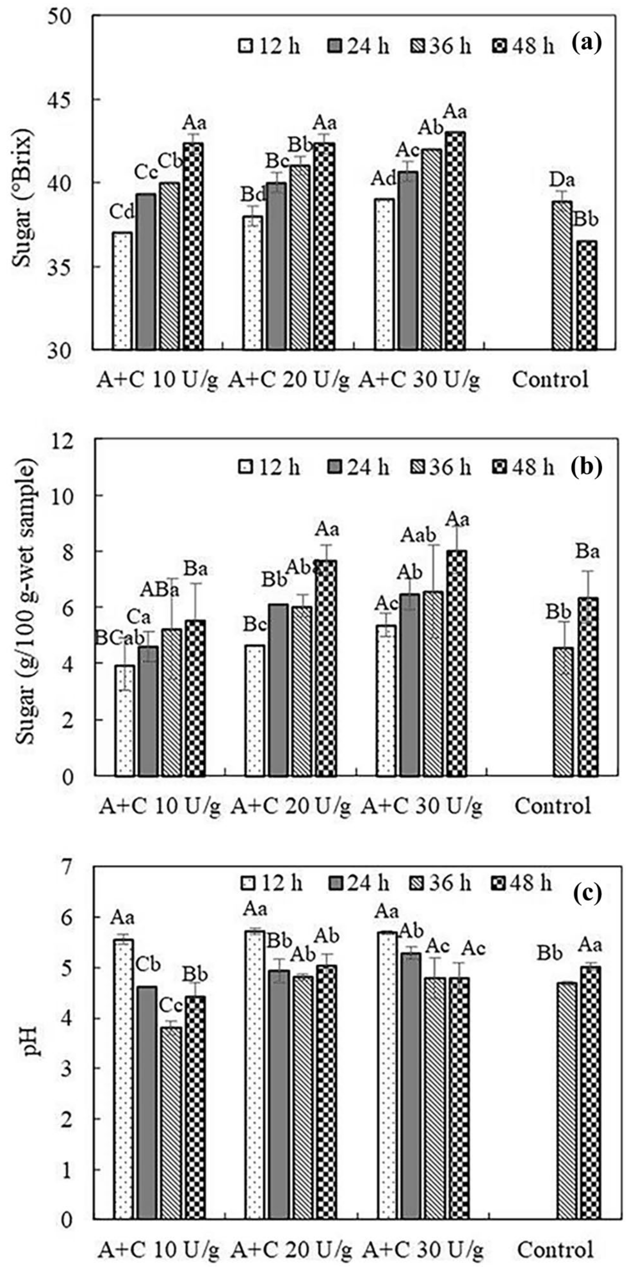

Fig. 4 Effect of enzyme loadings on rice cake fermentation. Amylase (**A**), cellulase (**C**), and their combination (**A + C**), each was added at 10, 20, and 30 U/g-rice. Control: the rice cake fermentation without enzyme addition. Data are means with standard deviation ($n=3$). Different uppercase and lowercase letters on the bar indicate significant differences between conditions and incubation time, respectively ($P<0.05$)

different enzyme loadings. It was found that the appearance and sweetness acceptance scores of all experimental sets were not statistically significantly different. However, the overall scores on flavor, texture, and likeliness of the product using a combination of amylase and cellulase at 20 and 30 U/g-rice were lower than those using 10 U/g-rice. It should also be noted that the fermentation time of 48 h was too long when enzymes were added. Therefore, the enzyme loading of 10 U/g-rice and fermentation time of 36 h were selected.

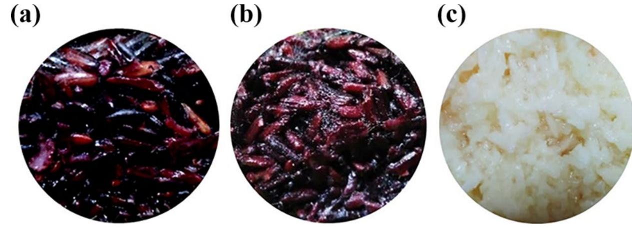

Fig. 5 Fermented rice cake using germinated black glutinous rice (**a**), black glutinous rice (**b**), and white glutinous rice (**c**)

Nutritive Analysis of Synbiotic Fermented Rice Cake

To assure the quality of the synbiotic fermented rice cake product, the specifications of raw materials used are defined as follows: brown rice raw material post-harvested for not more than 6 months, rice cake starter contains fungi and yeasts in the range of 10^4–10^5 CFU/g, and probiotic yeast contains viable cells of 10^3–10^4 cells/mg. After the fermentation for 36 h, the nutritional values in 100 g of synbiotic fermented rice cake product include total protein, carbohydrate, fat, sugar, and sodium at 5.29 g, 37.96 g, 2.28 g, 9.45 g, and 73.54 mg, respectively. The total energy content was 190 kcal per 100 g. These results show that the developed synbiotic fermented rice cake could be served as a source of protein and carbohydrates. It has also been reported that the use of germinated black glutinous rice makes the product contain higher functional values than the use of white glutinous rice. This is because it contains antioxidants such as anthocyanins, flavonoids, GABA, and prebiotics. The addition of probiotic yeast and their viable cell count of log 6 cells/g also make the product have synbiotic properties. In addition, brown glutinous rice was also found to provide more protein and amino acids than white rice (Frei & Becker, 2004). The specifications of synthetic fermented rice cake products are as follows: sugar content of 40°Brix, ethanol content of 0.137%, and pH of 4–4.5, those meeting the standard sugar content (not less than 40°Brix), ethyl alcohol (< 0.5%), and pH (4–4.5) for Thai community product standards (Ministry of Industry, 2003). Ethyl alcohol formation results from metabolized products from sugars by yeast and contributes to the flavor of the products. The antioxidant activity, total anthocyanin, flavonoids, and GABA were 95.51 ± 0.15%, 41.43 ± 2.72 mg/100 g, 31.55 ± 1.54 mg/100 g, and 31.19 ± 1.21 mg/100 g, respectively. The *E. coli* content also meets the standard *E. coli* content (not found).

Table 5 Effect of amylase and cellulase (A + C) loading on characteristics of fermented rice cake

Enzyme loading	Ethanol (%)	Ester (mg/100 g)	Antioxidant activity (%)	Anthocyanin (mg/100 g)	Flavonoid (mg/100 g)	GABA (mg/100 g)
After fermentation for 36 h						
Control	0.152 ± 0.005^{b}	10.10 ± 0.49^{a}	81.72 ± 1.92^{c}	40.35 ± 0.07^{a}	33.12 ± 0.31^{a}	33.08 ± 1.06^{a}
A + C each 10 U/g	0.139 ± 0.033^{b}	11.56 ± 0.51^{a}	95.51 ± 0.15^{a}	41.43 ± 2.72^{a}	31.55 ± 1.54^{a}	31.19 ± 1.21^{a}
A + C each 20 U/g	0.129 ± 0.002^{bc}	9.58 ± 1.96^{a}	95.82 ± 0.30^{a}	40.50 ± 4.78^{a}	29.16 ± 5.66^{a}	29.55 ± 1.21^{ab}
A + C each 30 U/g	0.116 ± 0.014^{c}	8.63 ± 2.31^{a}	95.41 ± 0.59^{a}	39.30 ± 1.47^{a}	32.51 ± 0.43^{a}	32.97 ± 2.42^{a}
After fermentation for 48 h						
Control	0.138 ± 0.021^{b}	9.15 ± 0.21^{b}	93.84 ± 0.44^{b}	39.23 ± 1.25^{a}	29.89 ± 1.91^{a}	28.28 ± 1.03^{ab}
A + C each 10 U/g	0.137 ± 0.002^{b}	12.50 ± 0.22^{a}	93.63 ± 0.15^{b}	40.33 ± 3.60^{a}	31.59 ± 2.34^{a}	30.62 ± 1.82^{ab}
A + C each 20 U/g	0.187 ± 0.008^{a}	10.86 ± 1.24^{a}	95.09 ± 0.44^{a}	40.24 ± 4.12^{a}	31.98 ± 2.28^{a}	30.69 ± 3.48^{ab}
A + C each 30 U/g	0.115 ± 0.001^{c}	12.40 ± 0.24^{a}	95.72 ± 0.15^{a}	36.65 ± 0.96^{ab}	31.38 ± 1.54^{a}	33.30 ± 2.88^{a}

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

Storage Stability Test

The synbiotic fermented rice cake was kept at 4 °C for 4 weeks. The change of viable yeast cells (log cells/g), sugar (°Brix), pH, ester, and ethanol as well as functional values during storage are shown in Table 7. By storing the product for more than 10 days, the viable yeast cells reduced to less than 6 log CFU/g, and by 4 weeks of storage, the viable yeast cells was only 3.59 ± 0.01 log CFU/g. The viability of probiotics was strain-dependent and strongly influenced by the nature and quality of the carrier-matrix (Nguyen et al., 2019). On the other hand, the antioxidant activity increased when the product was kept longer, while anthocyanin and flavonoid contents reduced significantly. The amount of GABA, ethanol, and ester did not significantly change indicating that a low storage temperature and acidic pH of the products could retain the GABA stability and volatile compounds like ethanol and ester.

Color properties and hardness of the product were also evaluated as shown Table 7. L* values ranged from 5 to 9, a* values were positive in the range of 7–10, and b* values were also positive in the range of 2–6. As black glutinous rice contains anthocyanin pigments, the product was in dark purple color. The hardness values were not significantly different during the 4-week storage. According to the standard for probiotic products in Thailand, the shelf life of the synbiotic fermented rice cake was defined at 10 days, which still contained viable probiotic yeast not less than 6 log CFU/g and without significant degradation of properties. The synbiotic fermented rice cake had the positive prebiotic activity score of 1.81 ± 0.15 (Table 7). This was likely because of the presence of dietary fiber and polyphenol of brown glutinous rice. It has been considered prebiotics which can stimulate probiotics growth and survival (Sawangwan & Saman, 2016). During storage for 2 and 4 weeks, the prebiotic activity score increased up to 2.52 ± 0.16 and 3.00 ± 0.05, respectively. It has been reported that when large molecules in rice, i.e., anthocyanin, are hydrolyzed the prebiotic activity increased (Zhu et al., 2018).

Table 6 Sensory analysis of fermented rice cake adding with various enzyme loadings

Treatment	Appearance	Sweetness	Flavor	Texture	Likeliness
After fermentation for 36 h					
Control	6.57 ± 1.63^{a}	7.23 ± 1.14^{a}	6.87 ± 1.25^{a}	6.27 ± 1.86^{a}	6.57 ± 1.63^{a}
A + C each 10 U/g	7.13 ± 1.61^{a}	7.47 ± 1.50^{a}	6.30 ± 1.64^{a}	6.20 ± 1.69^{a}	6.10 ± 1.83^{a}
A + C each 20 U/g	6.43 ± 1.57^{a}	6.77 ± 1.38^{a}	5.07 ± 2.13^{ab}	4.00 ± 2.02^{b}	4.17 ± 1.95^{bc}
A + C each 30 U/g	6.70 ± 1.53^{a}	6.83 ± 1.12^{a}	5.33 ± 1.88^{ab}	4.10 ± 1.95^{b}	4.10 ± 1.84^{bc}
After fermentation for 48 h					
Control	6.53 ± 1.80^{a}	6.97 ± 1.37^{a}	6.23 ± 1.99^{a}	6.53 ± 1.83^{a}	6.63 ± 1.75^{a}
A + C each 10 U/g	6.60 ± 1.59^{a}	6.97 ± 1.56^{a}	5.30 ± 2.12^{ab}	4.93 ± 1.68^{b}	5.30 ± 1.56^{b}
A + C each 20 U/g	6.97 ± 1.25^{a}	7.03 ± 1.19^{a}	5.30 ± 1.91^{ab}	4.53 ± 1.94^{b}	4.80 ± 1.83^{bc}
A + C each 30 U/g	7.00 ± 1.34^{a}	7.10 ± 1.37^{a}	5.33 ± 1.90^{ab}	4.60 ± 2.30^{b}	4.60 ± 2.18^{bc}

A amylase, C cellulase. Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

Table 7 Storage stability of the synbiotic fermented rice cake

Storage period	Probiotic yeast (log cells/g)	Sugar (°Brix)	pH	Ester (mg/100 g)	Ethanol (%)
0 day	7.45 ± 0.02^{b}	41.00 ± 0.00^{a}	4.88 ± 0.05^{a}	0.137 ± 0.02^{a}	14.52 ± 2.28^{a}
7 days	8.15 ± 0.04^{a}	41.00 ± 0.00^{a}	4.77 ± 0.04^{ab}	0.147 ± 0.02^{a}	11.79 ± 1.32^{a}
10 days	7.11 ± 0.02^{c}	40.00 ± 0.00^{a}	4.68 ± 0.05^{b}	0.139 ± 0.02^{a}	13.02 ± 2.60^{a}
14 days	5.22 ± 0.08^{d}	40.50 ± 0.71^{a}	4.56 ± 0.01^{c}	0.119 ± 0.05^{a}	12.94 ± 0.55^{a}
28 days	3.59 ± 0.01^{e}	40.00 ± 0.00^{a}	4.23 ± 0.02^{d}	0.124 ± 0.02^{a}	13.00 ± 0.83^{a}
Storage period	Antioxidant activity (%)	Anthocyanin (mg/100 g)	Flavonoid (mg/100 g)	GABA(mg/100 g)	
0 day	92.07 ± 1.03^{a}	35.79 ± 0.09^{a}	36.44 ± 0.28^{a}	34.33 ± 2.61^{a}	
7 days	90.71 ± 0.74^{a}	31.56 ± 0.14^{b}	37.80 ± 1.32^{a}	33.74 ± 1.03^{a}	
10 days	90.81 ± 0.30^{a}	31.69 ± 0.99^{b}	37.12 ± 0.47^{a}	31.96 ± 2.80^{a}	
14 days	90.50 ± 0.15^{a}	29.39 ± 0.57^{b}	36.99 ± 0.85^{a}	31.76 ± 0.28^{a}	
28 days	88.00 ± 0.15^{b}	25.13 ± 0.47^{c}	31.99 ± 0.85^{b}	31.03 ± 0.37^{a}	
Storage period	Prebiotics activity score (PAS)	Color value			Hardness (g)
		L*	a*	b*	
0 day	1.81 ± 0.15^{c}	9.63 ± 0.41^{a}	10.86 ± 0.78^{a}	5.46 ± 0.05^{a}	11113.57 ± 2165.32^{a}
7 days	ND	5.55 ± 0.12^{d}	10.61 ± 0.42^{a}	4.10 ± 0.88^{bc}	12943.15 ± 3293.64^{a}
10 days	ND	8.49 ± 0.10^{b}	7.83 ± 0.40^{b}	5.36 ± 0.07^{ab}	12943.15 ± 3293.64^{a}
14 days	2.52 ± 0.16^{b}	5.39 ± 0.23^{d}	7.97 ± 0.44^{b}	2.86 ± 0.06^{c}	13393.09 ± 1921.73^{a}
28 days	3.00 ± 0.05^{a}	7.62 ± 0.08^{c}	10.74 ± 0.73^{a}	5.74 ± 0.13^{a}	11821.56 ± 2774.17^{a}

ND, not determined. Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

Comparison with Commercial Fermented Rice Cake Products

When comparing the soluble solids and ethanol content of the three products, the product using white glutinous rice contained the highest amount of 47.67 ± 0.58°Brix and 0.37 ± 0.04%, respectively, while those of the product using black glutinous rice and the synbiotic product developed in this study were not significantly different (Table 8). This was possibly because the white glutinous rice was easier to digest by the fungal enzymes than the black glutinous rice and germinated black glutinous rice. This made it have higher sugar content, and the yeast produced slightly more ethanol. In terms of ester content, only synbiotics from germinated black glutinous rice showed an ester content of 4.79 ± 0.86 mg/100 g of rice. When comparing the antioxidant activity, the synbiotic product developed in this study had the highest activity of 91.44 ± 0.59%, followed by the product using black glutinous rice. The product using white glutinous rice had the lowest activity of 53.76 ± 2.21%. The anthocyanin content was found only in the product using black glutinous rice and the synbiotic product developed in this study in the range of 34–37 mg per 100 g of rice. The flavonoid content in the product using black glutinous rice and the synbiotic product developed in this study were also much higher than the product using white glutinous rice in the range of 31–35 mg/100 g of rice. The GABA content of the synbiotic product was highest at 34.93 ± 2.71 mg/100 g

Table 8 Characteristics of fermented rice cake developed in this study compared with those using black glutinous rice and white glutinous rice

Fermented rice cake	Sweetness (°Brix)	Ethanol (%)	Ester (mg/100 g)	Antioxidant activity (%)	Anthocyanin (mg/100 g)	Flavonoid (mg/100 g)	GABA (mg/100 g)	Prebiotic activity score
Synbiotic product in this study	37.00 ± 0.00^{b}	0.30 ± 0.02^{b}	4.79 ± 0.86	91.44 ± 0.59^{a}	34.32 ± 1.74^{b}	35.70 ± 1.02^{a}	34.93 ± 2.71^{a}	1.29 ± 0.04^{a}
Black glutinous rice	36.33 ± 1.15^{b}	0.28 ± 0.01^{b}	nd	81.32 ± 1.92^{b}	37.96 ± 0.08^{a}	31.64 ± 0.04^{b}	1.12 ± 0.09^{b}	0.90 ± 0.04^{b}
White glutinous rice	47.67 ± 0.58^{a}	0.37 ± 0.04^{a}	nd	53.76 ± 2.21^{c}	nd	2.57 ± 0.06^{c}	0.59 ± 0.09^{b}	0.91 ± 0.11^{b}

ND, not detected. Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

Table 9 Sensory analysis of fermented rice cake developed in this study compared with those using black glutinous rice and white glutinous rice

Fermented rice cake	Appearance	Sweetness	Flavor	Texture	Likeliness
Synbiotic product in this study	7.73 ± 0.83^a	7.73 ± 0.87^a	7.67 ± 0.96^a	7.50 ± 1.07^a	7.33 ± 1.09^a
Black glutinous rice	7.87 ± 0.86^a	8.00 ± 0.64^a	7.97 ± 0.76^a	7.97 ± 0.89^a	7.97 ± 0.85^a
White glutinous rice	7.00 ± 1.70^a	7.23 ± 1.48^a	7.43 ± 1.36^a	7.37 ± 1.19^a	7.47 ± 1.28^a

Values are means ± standard deviation ($n = 3$). Different superscript letters indicate significant differences between conditions in the same group ($P < 0.05$)

of rice, while those of the products using black glutinous rice and glutinous rice were only 1.12 ± 0.09 and 0.59 ± 0.09 mg/100 g of rice, respectively. The prebiotic activity score of the synbiotic product was also higher than the other two products.

This study has shown that the technique developed in this study was effective for enhancing not only the productivity but also the health benefits and functional values of the fermented rice cake. In addition, the sensory analysis showed that the synbiotic product received scores on appearance, sweetness, flavor, and texture slightly higher than the product using white glutinous rice (Table 9). It could be concluded that there was no significant difference in the overall likeliness score for the three products. But when they choose to take the synbiotic product developed in this study, they receive higher functional values including anthocyanin, flavonoid, and GABA, and also higher health benefits as it contains both probiotic yeast and prebiotics.

Conclusions

The fermented rice cake using germinated black glutinous rice was successfully developed in this study. The health benefits and functional values of fermented rice cake were much improved as germinated black glutinous rice contains prebiotics, antioxidants, and γ-aminobutyric acid at much higher levels than white rice. The probiotic yeast was able to grow during rice cake fermentation and reach the maximum value that meets the standard for probiotic products. The superior characteristics of the developed products were demonstrated by the survival of yeast cells during in vitro gastrointestinal environment, prebiotic activity score, and sensory analysis. This study also showed the effectiveness of the food-grade cellulase and amylase to improve the fermentation rate and quality of the fermented rice cake. These strategies may also be applicable to other fermented products.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11947-022-02985-z.

Author Contribution Benjamas Cheirsilp: conceptualization, methodology, funding acquisition, writing-original draft preparation and review and editing. Waraporn Mekpan: investigation, data curation. Nantikarn Sae-ear: investigation, data curation. Asma Billateh: investigation, data curation. Sawai Boukaew: writing-review and editing.

Funding This research work was financially supported by the Agricultural Research Development Agency (Public Organization) under Grant No. PRP6405030430 and partially supported by Thailand Toray Science Foundation (TTSF).

Data Availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest The authors declare no competing interests.

References

AOAC. (2005). In W. Horwitz, G. W. Latimer (Eds.). *Official methods of analysis of AOAC international* (18th ed.). Rockville, MD: AOAC International.

Cheirsilp, B., & Umsakul, K. (2008). Processing of banana-based wine product using pectinase and α-amylase. *Journal of Food Processing and Engineering, 31*, 78–90.

Chong, E. S. L. (2014). A potential role of probiotics in colorectal cancer prevention: Review of possible mechanisms of action. *World Journal of Microbiology and Biotechnology, 30*(2), 351–374.

Chupeerach, C., Aursalung, A., Watcharachaisoponsiri, T., Whanmek, K., Thiyajai, P., Yosphan, K., Sritalahareuthai, V., Sahasakul, Y., Santivarangkna, C., & Suttisansanee, U. (2021). The effect of steaming and fermentation on nutritive values, antioxidant activities, and inhibitory properties of tea leaves. *Foods, 10*(1), 117.

Fratianni, F., Cardinale, F., Russo, I., Iuliano, C., Tremonte, P., Coppola, R., & Nazzaro, F. J. (2014). Ability of synbiotic encapsulated *Saccharomyces cerevisiae boulardii* to grow in berry juice and to survive under simulated gastrointestinal conditions. *Journal of Microencapsulation, 31*(3), 299–305.

Frei, M., & Becker, K. (2004). On rice, biodiversity & nutrients. *University of Hohenheim Stuttgart*. Available online: http://www.greenpeaceweb.org/gmo/nutrients.pdf.

Huebner, J., Wehling, R. L., & Hutkins, R. W. (2007). Functional activity of commercial prebiotics. *International Dairy Journal, 17*(7), 770–775.

Karladee, D., & Suriyong, S. (2012). γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination. *Science Asia, 38*, 13–17.

Kittibunchakul, S., Yuthaworawit, N., Whanmek, K., Suttisansanee, U., & Santivarangkna, C. (2021). Health beneficial properties of a novel plant-based probiotic drink produced by fermentation of brown rice milk with GABA-producing *Lactobacillus pentosus* isolated from Thai pickled weed. *Journal of Functional Foods, 86*, 104710.

Korkmaz, A., Atasoy, A. F., & Hayaloglu, A. A. (2020). Changes in volatile compounds, sugars and organic acids of different spices of peppers (*Capsicum annuum* L.) during storage. *Food Chemistry, 311*.

Kuo, C.-H., Shieh, C.-J., Huang, S.-M., Wang, H.-M.D., & Huang, C.-Y. (2019). The effect of extrusion puffing on the physicochemical properties of brown rice used for saccharification and Chinese rice wine fermentation. *Food Hydrocolloids, 94*, 363–370.

Liu, Q., Wang, L., Hu, J., Miao, Y., Wu, Z., & Li, J. (2017). Main organic acids in rice wine and beer determined by capillary electrophoresis with indirect UV detection using 2,4-dihydroxybenzoic acid as chromophore. *Food Analytical Methods, 10*(1), 111–117.

Manosroi, A., Ruksiriwanich, W., Kietthankorn, B., Manosroi, W., & Manosroi, J. (2011). Relationship between biological activities and biological activities and bioactive compound in the fermented rice sap. *Food Research International, 44*, 2757–2765.

Meng, L. W., & Kim, S. M. (2020). Effects of different carbohydrases on the physicochemical properties of rice flour, and the quality characteristics of fermented rice cake. *Food Science and Biotechnology, 29*(4), 503–512.

Miguel, M. G. (2011). Anthocyanins: Antioxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science, 1*(6), 7–15.

Ministry of Industry. (2003). Community product standard of sweet fermented glutinous rice. Document of CPS at 162/2003. Agro product standard office, Bangkok.

Mongkontanawat, N., & Lertnimitmongkol, W. (2015). Product development of sweet fermented rice (Khao-Maak) from germinated native black glutinous rice. *International Journal of Agricultural Technology, 11*(2), 501–515.

Nealon, N. J., Worcester, C. R., & Ryan, E. P. (2017). *Lactobacillus paracasei* metabolism of rice bran reveals metabolome associated with *Salmonella Typhimurium* growth reduction. *Journal of Applied Microbiology, 122*, 1639–1656.

Nguyen, B. T., Bujna, E., Fekete, N., Tran, A. T. M., Rezessy-Szabo, J. M., Prasad, R., & Nguyen, Q. D. (2019). Probiotic beverage from pineapple juice fermented with *Lactobacillus* and *Bifidobacterium* strains. *Frontiers in Nutrition, 6,* Article 54.

Noomtim, P., & Cheirsilp, B. (2011). Production of butanol from palm empty fruit bunches hydrolyzate by *Clostridium acetobutylicum*. *Energy Procedia, 9*, 140–146.

Panyanak, P., Suwanketnikom, S., Tonhang, S., & Siripoonwiwat, W. (2010). Correlations between seed characteristics, seed germination and γ-aminobutyric acid (GABA) content of 14 rice cultivars. *Thai Journal of Botany, 2*, 97–113.

Patil, S., & Khan, Md. (2011). Germinated brown rice as a value added rice product: A review. *Journal of Food Science and Technology, 48*, 661–667.

Pedro, A. C., Granato, D., & Rosso, N. D. (2016). Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. *Food Chemistry, 191*, 12–20.

Pornputtapitak, W., Pantakitcharoenkul, J., Panpakdee, R., Teeranachaideekul, V., & Sinchaipanid, N. (2018). Development of γ-oryzanol rich extract from Leum Pua glutinous rice bran loaded nanostructured lipid carriers for topical delivery. *Journal of Oleo Science, 67*(2), 125–133.

Rakmai, J., Cheirsilp, B., & Srinuanpan, S. (2019). Designation of rice cake starters for fermented rice products with desired characteristics and fast fermentation. *Journal of Food Science and Technology, 56*, 3014–3022.

Ramos-Ruiz, R., Poirot, E., & Flores-Mosquera, M. (2018). GABA, a non-protein amino acid ubiquitous in food matrices. *Cogent Food & Agriculture, 4*(1).

Sawangwan, T., & Saman, P. (2016). Shelf life enhancement of isomalto-oligosaccharide from glutinous rice syrup with prebiotic properties. *International Journal of Probiotics, 11*(2), 77–84.

Staniszewski, A., & Kordowska-Wiater, M. (2021). Probiotic and potentially probiotic yeasts—characteristics and food application. *Foods, 10*, 1306.

Tian, S., Nakamura, K., & Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agricultural and Food Chemistry, 52*(15), 4808–4813.

Tinrat, S., Khuntayaporn, P., Thirapanmethee, K., & Chomnawang, M. T. (2018). *In vitro* assessment of *Enterococcus faecalis* MTC 1032 as the potential probiotic in food supplements. *Journal of Food Science and Technology, 55*, 2384–2394.

Tongyai, A., Riebroy, S., Maneerat, S., Siriwong, N., & Chulakarungka, S. (2012). *Changes in physicochemical and sensory characteristics during fermentation of Khaow-Maak from black glutinous rice*. Kasetsart University.

Wang, J., Zhu, L., Zhang, W., & Wei, Z. (2019). Application of the voltammetric electronic tongue based on nanocomposite modified electrodes for identifying rice wines of different geographical origins. *Analytica Chimica Acta, 1050*, 60–70.

Zhu, Y., Sun, H., He, S., Lou, Q., Yu, M., Tang, M. & Tu, L. (2018). Metabolism and prebiotics activity of anthocyanins from black rice (*Oryza sativa* L.) *in vitro*. *PLoS One, 13*(4), e019575.

Zhu, Z., Shi, Z., Xie, C., Gong, W., Hu, Z., & Peng, Y. (2019). A novel mechanism of Gamma-aminobutyric acid (GABA) protecting human umbilical vein endothelial cells (HUVECs) against H_2O_2-induced oxidative injury. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 217*, 68–75.