Received: 19 April 2019 | Revised: 14 January 2020 | Accepted: 13 February 2020

DOI: 10.1111/jfpp.14442

ORIGINAL ARTICLE

Journal of Food Processing and Preservation | Institute of Food Science + Technology | ifst | WILEY

Effects of germinated and nongerminated rice grains on storage stability of pressurized purple rice beverages with *Lactobacillus casei* 01 supplement

Sasitorn Baipong[1] | Arunee Apichartsrangkoon[1] | Srivilai Worametrachanon[1] | Autchara Tiampakdee[1] | Sujinda Sriwattana[1] | Yuthana Phimolsiripol[1] | Danchai Kreungngern[2] | Panlop Sintuya[3]

[1]Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand

[2]Division of Food Science and Technology, Faculty of Science and Technology, Kamphaeng Phet Rajabhat University, Kamphaeng Phet, Thailand

[3]Institute of Product Quality and Standardization, Maejo University, Chiang Mai, Thailand

Correspondence
Arunee Apichartsrangkoon, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand.
Email: arunee.a@cmu.ac.th

Funding information
Office of Research Administration, Chiang Mai University

Abstract

Changes of phytochemicals and probiotic in the pressurized purple rice beverages on storage were compared with heated products. Germinated or nongerminated purple rice were used to produce the beverages which were subjected to either pressurization at 500 MPa for 20 min at 25°C or heat at 95°C for 20 min. Subsequently, encapsulated *Lactobacillus casei* 01 was aseptically added to the beverages which were stored at 4°C for 4 weeks. It was found that germination led to reduction of anthocyanins, phenolic compounds and antioxidant capacity, but enhanced GABA and γ-oryzanol contents. Pressurized beverages were lighter in color than thermally treated ones; however, their color changed faster than the heated samples during storage. Phytochemicals such as anthocyanins, phenolics, and DPPH radical inhibitors in both pressurized (germinated and nongerminated) beverages had the same trends of decreasing, but GABA and γ-oryzanol were stable on storage. *L. casei* 01 remained at about 8 log CFU/ml.

Practical applications

Compared to ordinary rice, purple rice contains higher contents of phytochemicals such as anthocyanins and phenolic compounds. In particular, germinated grains have high concentrations of health promoting aminobutyric acid and γ-oryzanol. To enrich its health benefits, an encapsulated probiotic can be added to the processed beverages and pressurization is a promising process to preserve these phytochemicals. Currently, pressure treatment, though relatively expensive, is becoming more widely used in food manufacturing. Since its benefits in retaining important nutrients and, as this study shows, phytochemicals are significant, and justify the increased costs that health conscious consumers will pay. The increasing popularity of functional foods suggest the processes researched in this study could be easily taken up and exploited in the market place to produce high-quality purple rice products.

1 | INTRODUCTION

In the past few years, pigmented rice such as purple, brown, and red rice has gained popularity due to the presence of anthocyanins, phenolic compounds, γ-oryzanol, vitamin E, γ-amino butyric acid (GABA), and other antioxidative constituents (Lee et al., 2007). Polyphenols, GABA, and γ-oryzanol in purple rice could effectively lower blood cholesterol, promote anticarcinogenic, and anti-inflammatory activities (Cho et al., 2012). Therefore, it is worth producing functional soft drink from purple rice, but their bioactive components undergo degradation on storage. Andrés, Villanuev, and Tenorio (2016a) pressurized (at 450 MPa/3 min/20°C) fruit smoothies and stored them at 4°C for 45 days. They found decreases in ascorbic acid, total phenol, and antioxidant activities measurer as ferric reducing antioxidant power (FRAP) and diphenyl-2-picrylhydrazyl (DPPH) radical inhibition by 32%, 12%, 41%, and 19%, respectively, while those pressurized at 600 MPa lost 36.6%, 11.2%, 60.4%, and 34.5% respectively. Similarly, Chaikham, Chunthanom, and Apichartsrangkoon (2013) also found some degradation of ascorbic acid, total phenol, and antioxidant activities as FRAP in pressurized pennywort juices on storage at 4°C for 4 months, while asiaticoside, madecassoside, and β-carotene were relatively stable. Andrés, Mateo-Vivaracho, Guillamon, Villanueva, and Tenorio (2016b) found that ascorbic acid in pressurized (at 550 or 650 MPa) soy smoothies diminished by 43% on storage at 4°C for 45 days.

Apart from bioactive components, the beverages could also be aseptically fortified with probiotics such as lactic acid bacteria to enrich its health-promoting potential. Normally, probiotics such as *Lactobacillus casei* 01 can reside and propagate in the human colons as well as control microbiome by balancing the growth of different beneficial and harmful bacteria (Worametrachanon et al., 2014). They also assist in synthesis of essential components such as short chain fatty acids for utilization in different human organs (Chaikham, Apichartsrangkoon, Worametrachanon, & Van de Wiele, 2016). On storing foods, probiotics could also be degraded by several intrinsic factors such as an accumulation of toxic substances to them or in the depletion of nutrients. Chaikham and Apichartsrangkoon (2012a) noticed that *L. casei* 01 in pressurized longan juices reduced from 9 to 6 log CFU/ml during storage at 4°C for 4 weeks. Moreover, Costa, Júnior, Rosa, Caliari, and Pimentel (2017) fermented mixed extract of soy and rice byproduct beverage with *L. acidophilus* and *Bifidobacterium* spp. then stored them at 5°C for 4 weeks. It was found that the products had a 28-day shelf life, but the probiotic effect lasted only 14 days, which were the effects of pH drop and some reduction of the probiotics. Previously, Allgeyer, Miller, and Lee (2010) demonstrated that drinking yogurts with added *Bifidobacterium lactis* Bb-12 and *L. acidophilus* LA-5 including prebiotics showed a decrease in viable cells of 1–3 log CFU/ml on storage in a refrigerator for 30 days, while those with added polydextrose exhibited higher survival counts. In addition, Angelov, Gotcheva, Kuncheva, and Hristozova (2006) found that in oat-based probiotic beverage, stored at 5°C for 24 days, the viable cells were reduced by 1 log cycle, while acidity and pH remained within the desired ranges for 21 days.

It is well recognized that high hydrostatic pressure preserves food nutrients better than thermal treatments. In this study, high pressure of 500 MPa at 25°C for 20 min was used to process either nongerminated or germinated purple rice beverages. Normally, purple rice contains several bioactive components especially after germination, but these phytochemicals might degrade on storage. Hence, it was of interest to examine how the phytochemicals in such beverages were altered in cold storage condition. To enhance the health-promoting effects, an encapsulated probiotic *L. casei* 01 was aseptically added prior to storing at 4°C for 4 weeks.

2 | MATERIALS AND METHODS

2.1 | Preparation of pressurized and heated purple rice beverages

Purple-glutinous rice grains (*Oryza sativa* Linn.) were germinated following the procedure described by Worametrachanon et al. (2014). Unpolished rice grains were soaked in drinking water at room temperature for 12 hr, then drained and incubated at 30°C/24 hr for germination. The germinated and nongerminated rice grains were dehydrated using a hot-air drier and ground to powder. Both rice powders were blended with 12-folds of drinking water and heated at 70°C for 3 min to reach the pregelatinized state. Subsequently, 100 ml of heated gel was pressurized at 500 MPa with 25°C for 20 min using "Food Lab" 900 high-pressure rig (Stansted Fluid Power, UK). For thermal treatment, both the germinated and nongerminated rice beverages were heated at 95°C for 20 min to reach the state of completely gelatinization. The pressurized and heated rice beverages were each mixed with 10 g of encapsulated *L. casei* 01 (around 9 log CFU/ml) under aseptic condition. Prior to incorporation, the beads were washed twice with boiled drinking water. After that, the rice beverages were packed and stored at 4°C for 4 weeks. Each sample was analyzed weekly for physicochemical and microbiological properties.

2.2 | Encapsulation of *L. casei* 01

Activated *L. casei* 01 (FD-DVS nu-trish Chr. Hansen, Denmark) was encapsulated by an emulsion technique as described by Sheu and Marshall (1993); Sultana et al. (2000) with some modifications. A volume of 40 ml of 2% (w/v) sterile sodium alginate solution (Sigma-Aldrich, UK), 1% (w/v) hi-maize starch (National Starch and Chemical Co., Ltd., Thailand), and 10 ml of activated culture were mixed thoroughly and added into a mixture of 200 ml soybean oil containing 0.2% (v/v) Tween 80 (Sigma, Germany), then stirred for 20 min. Subsequently, 200 ml of 0.1 M $CaCl_2$ (O.V. Chemical & Supply, Thailand) was gently added into the mixture. The alginate beads formed, average size of 30–70 μm, were then washed twice with 0.85% (w/v) saline containing 5% (v/v) glycerol.

2.3 | Color measurement

Color parameters, L, a^*, and b^* were measured by a colorimeter (model Color Quest XE, Hunter Lab, USA). The collected data were used to calculate the Browning index (*BI*) (Chaikham & Apichartsrangkoon, 2012b).

2.4 | Determination of total anthocyanins

Total anthocyanins were determined by pH differential method following the procedure described by Hosseinian Li and Beta (2008). A volume of 10 ml rice beverage was centrifuged at 1,000 *g* for 10 min (Hettich; model Rotina 46R, Germany). The clear supernatant was then adjusted with either potassium chloride buffer to pH 1.0 or sodium acetate buffer to pH 4.5. The absorbance of each solution was then measured at λ_{max} 520 and 700 nm, respectively, using a UV/VIS Spectrophotometer (Perkin Elmer, Lambda 20, Massachusetts, USA). The concentration of anthocyanin was then calculated and expressed as mg cyanidin 3-glucoside equivalent per 100 ml sample (mg/100 ml) (Worametrachanon et al., 2014).

2.5 | Determination of γ-oryzanol

The γ-oryzanol content was analyzed as described by Iqbal, Bhanger, and Anwar (2005). Briefly, 2 g dehydrated rice beverage was extracted with 150 ml dichloromethane (RCI-Labscan, Thailand). The clear liquid was then used for high-performance liquid chromatography (HPLC) assay. A volume of 10 μl extract was injected into a HPLC system (Shimadzu LC-10AD; Shimadzu, Japan) equipped with an ultra-aqueous C18 column (Restek, USA) and a diode array detector operated at λ_{max} 330 nm. The mobile phase consisted of ratio of methanol:acetonitrile: dichloromethane:acetic acid (50:44:3:3) with a flow rate of 1.4 ml/min. Peak area of the assayed γ-oryzanol was recorded, and its content was calculated based on the peak area of standard γ-oryzanol. Samples of their chromatograms are shown in Figure 1.

2.6 | Determination of γ-aminobutyric acid (GABA)

Gamma-aminobutyric acid was determined following the method described by Dajanta, Apichartsrangkoon, Chukeatirote, and Frazier (2011). A volume of 10 ml rice beverage was centrifuged at 1,000 *g* for 10 min (Hettich; model Rotina 46R, Germany). The supernatant was then filtered through a 0.20 μm nylon filter (Vertical, Thailand) and the filtrate was used for HPLC analysis. An aliquot of 10 μl extract was injected into HPLC system as described for γ-oryzanol. In this analysis, a fluorescence detector (Shimadzu RF-10AXL; Shimadzu, Japan) was used, at 263 nm excitation and 313 nm emission. In the gradient elution, the mobile phase consisted of solution A containing 20 mM ammonium dihydrogen orthophosphate in 15% (v/v) methanol and solution B, 90% (v/v) acetonitrile in water. The flow rate of the eluent was 1 ml/min. Peak area of the assayed GABA was recorded, and the content calculated based on the peak area of standard GABA. Samples of their chromatograms are shown in Figure 2.

2.7 | Determination of total phenolic compounds

Total phenolic compounds were determined following the method described by Moongngarm and Saetung (2010). Thus, 10 ml rice beverage was centrifuged at 1,000 *g* for 10 min. An aliquot of 0.2 ml supernatant was added to a mixture of 0.8 ml Folin–Ciocalteu phenol reagent, 2 ml of 7.5% (w/v) sodium carbonate and 7 ml deionized water. The mixed solution was then kept in a dark place for 2 hr, then the absorbance was measured at λ_{max} 765 nm. Total phenolics were expressed as mg gallic acid equivalent per 100 ml of sample (mg/100 ml).

2.8 | Determination of antioxidant capacity

Antioxidant capacity as 2,2-diphenyl-1-picrylthydrazyl (DPPH) radical scavenging activity was determined as described by Bobinaite,

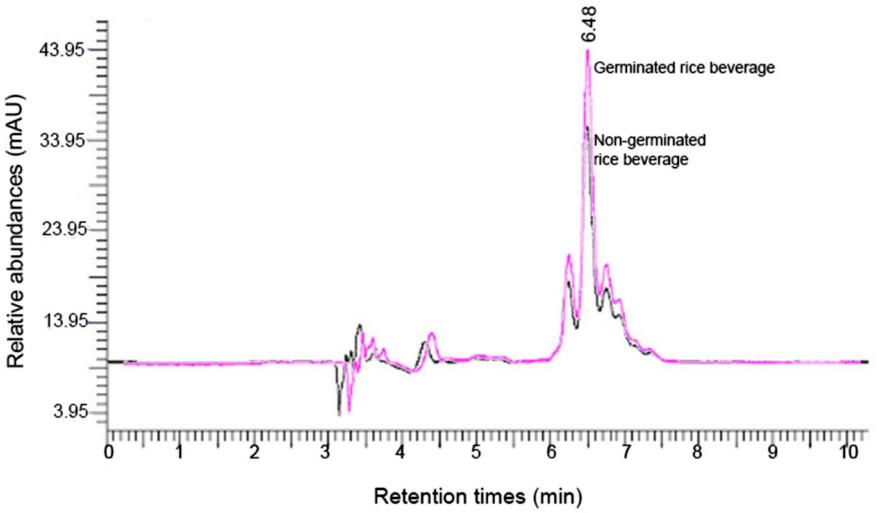

FIGURE 1 Chromatograms of γ-oryzanol in pressurized germinated and nongerminated purple rice beverages at the initial state

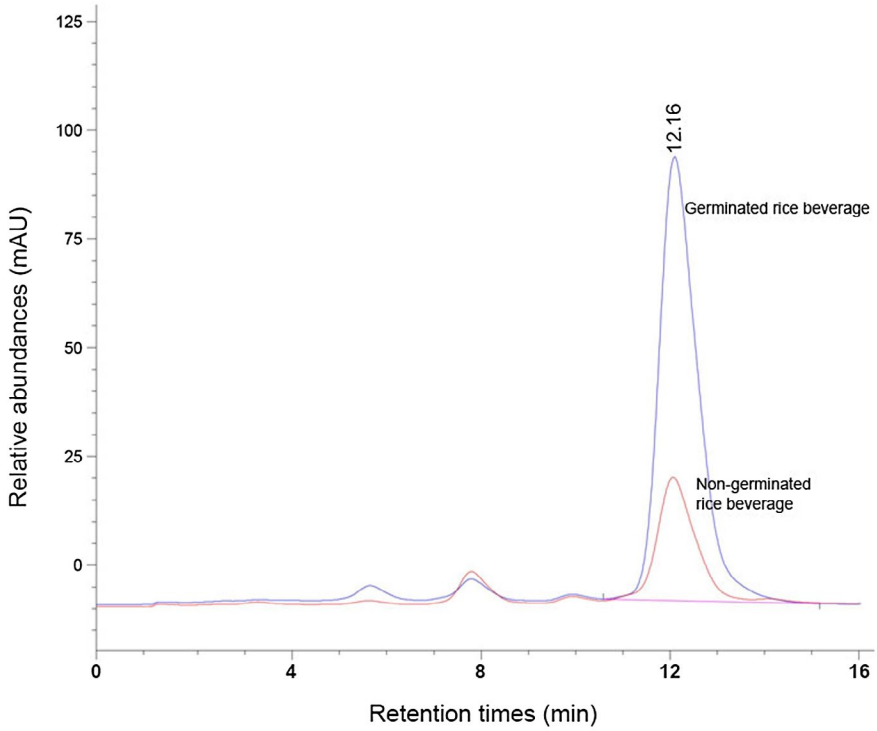

FIGURE 2 Chromatograms of GABA in pressurized germinated and nongerminated purple rice beverages at the initial state

Viskelis, and Venskutonis (2012). Briefly, 10 ml rice beverage were centrifuged at 1,000 *g* for 10 min. A volume of 0.1 ml aliquot was mixed with 3.9 ml of 6×10^{-5} M DPPH radical methanol solution. Subsequently, an absorbance of the solution was measured at λ_{max} 515 nm and percentage of DPPH radical inhibition was calculated as described by Worametrachanon et al. (2014).

2.9 | Microbiological assessments

Microbiological assessments were performed following the method of the U.S. Food and Drug Administration (U.S. Food & Drug Administration, 2001). Various beverages dilutions were plated on different media as follows: MRS for *L. casei* 01, PCA for total bacteria and PDA for yeasts and molds. MRS was supplemented with cysteine to promote the recovery of injured cells of lactic acid bacteria (Vinderola, Reinheimer, & Salminen, 2019). PCA was used to assess some contaminants from the rice beverages. All plated media were enumerated by incubating at 37°C except PDA at 25°C for 24 hr.

2.10 | Statistical analysis

The experimental design consisted of four treatments, namely pressurized germinated beverage, pressurized nongerminated beverage, heated germinated beverage, and heated nongerminated beverage. Each treatment was varied with five storage times: initial state, weeks 1, 2, 3, and 4. The statistical analysis was one-way ANOVA with triplication, using SPSS Version 11.5 for windows (SPSS Inc., USA) and comparisons of significant differences among means were done by Duncan's multiple range tests ($p \leq .05$).

3 | RESULTS AND DISCUSSION

3.1 | Color alteration during storage

Table 1 shows that *L* (lightness) parameters of most beverages significantly declines ($p < .05$) from the second week onward. Accordingly, heated germinated rice beverages showed the highest reduction rate indicated by the slope (0.15). Overall, the germinated beverages of both processes displayed significantly higher ($p < .05$) *L* parameters or lighter color than the nongerminated beverages, which were also perceptible. These changes might be due to loss of anthocyanins during germination through biochemical and physiological processes (Yang et al., 2007). The contents of the corresponding anthocyanins in the germinated rice beverages were lower than those in the nongerminated samples (Table 2). On the other hand, most a^* (redness) and b^*(yellow) parameters significantly increased ($p < .05$) from the third week of storage (Table 1). Heating significantly increased ($p < .05$) all parameters especially b^*, which could be associated with Maillard browning.

The increasing a^* parameters (0.13) during storage were similar among treatment groups, whereas the increase in b^* parameters for pressurized samples (0.07–0.09) was slightly lower than for the heated batches (0.08–0.10) (Table 1). Interestingly, the increases in the a^* parameters were much higher than those of the b^* parameters, suggesting that the redness (a^*) due to anthocyanins changes were faster than the yellowness (b^*) changes due to Maillard reactions.

TABLE 1 Color parameters of processed purple rice beverages during storage at 4°C for 4 weeks

Color parameters	Rice beverages	Storage time (weeks)					Slopes of storage time
		Initial state	1	2	3	4	
L	HPG	30.99 ± 0.14^{aA}	30.91 ± 0.06^{aA}	30.60 ± 0.01^{bA}	29.83 ± 0.41^{cA}	29.60 ± 0.09^{cA}	−0.12
	HG	30.16 ± 0.18^{abB}	30.26 ± 0.08^{aB}	30.05 ± 0.04^{bB}	29.81 ± 0.06^{cA}	29.66 ± 0.11^{cA}	−0.15
	HPNG	29.96 ± 0.20^{aB}	29.84 ± 0.11^{abC}	29.77 ± 0.19^{bC}	29.52 ± 0.16^{bAB}	28.47 ± 0.18^{cB}	−0.13
	HNG	28.71 ± 0.14^{aC}	28.69 ± 0.09^{aD}	28.78 ± 0.21^{aD}	28.57 ± 0.05^{aC}	28.27 ± 0.14^{bB}	−0.10
*a**	HPG	5.55 ± 0.06^{cC}	5.86 ± 0.06^{bB}	5.95 ± 0.26^{abC}	6.06 ± 0.28^{aB}	6.14 ± 0.11^{aC}	0.14
	HG	6.61 ± 0.08^{cB}	6.97 ± 0.06^{bA}	6.98 ± 0.07^{bAB}	7.10 ± 0.15^{abA}	7.21 ± 0.10^{aB}	0.13
	HPNG	6.70 ± 0.11^{bB}	6.82 ± 0.11^{bA}	7.27 ± 0.27^{aA}	7.22 ± 0.26^{aA}	7.15 ± 0.17^{aB}	0.13
	HNG	7.15 ± 0.07^{cA}	6.88 ± 0.09^{dA}	7.37 ± 0.06^{bA}	7.33 ± 0.17^{abA}	7.56 ± 0.12^{aA}	0.13
*b**	HPG	0.47 ± 0.01^{bD}	0.58 ± 0.10^{abC}	0.64 ± 0.20^{aC}	0.66 ± 0.10^{aD}	0.76 ± 0.09^{aC}	0.07
	HG	2.19 ± 0.02^{cB}	2.35 ± 0.04^{bB}	2.34 ± 0.03^{bB}	2.44 ± 0.04^{aB}	2.53 ± 0.08^{aB}	0.08
	HPNG	0.57 ± 0.04^{cC}	0.62 ± 0.05^{bcC}	0.71 ± 0.04^{bC}	0.92 ± 0.06^{aC}	0.86 ± 0.09^{aC}	0.09
	HNG	2.57 ± 0.02^{cA}	2.66 ± 0.06^{bA}	2.75 ± 0.03^{bA}	2.87 ± 0.08^{aA}	2.98 ± 0.09^{aA}	0.10
Browning Indices (*BI*)	HPG	13.84 ± 0.10^{cD}	14.92 ± 0.27^{bD}	15.46 ± 0.68^{bD}	16.17 ± 0.38^{aD}	16.83 ± 0.53^{aD}	0.72
	HG	22.55 ± 0.18^{cB}	23.86 ± 0.20^{bB}	24.01 ± 0.48^{bB}	24.84 ± 0.70^{aB}	25.55 ± 0.60^{aB}	0.70
	HPNG	17.21 ± 0.29^{cC}	17.72 ± 0.46^{cC}	19.07 ± 0.33^{bC}	19.88 ± 0.41^{aC}	20.18 ± 0.20^{aC}	0.81
	HNG	26.41 ± 0.34^{bA}	26.14 ± 0.10^{bA}	27.56 ± 0.47^{bA}	28.15 ± 0.63^{bA}	29.41 ± 0.36^{aA}	0.75

Notes: The means ± *SD* in the same row or column with the same small or capital letters are not significantly different ($p > .05$). Each data point is the average of triplication.

Abbreviations: HG, Heated germinated beverage; HNG, Heated nongerminated beverage; HPG, Pressurized germinated beverage; HPNG, Pressurized nongerminated beverage.

Change in anthocyanins during storage is probably due to derivatization of anthocyanins by condensation reactions to form polymeric pigments (Zhang, Li, & Fan, 2019).

Most Browning indices (*BI*) for all treatments were significantly elevated ($p < .05$) from the first week onward. Overall, pressurized germinated beverages had the lightest color or the lowest *BI*, while heated nongerminated beverages showed the highest *BI* (Table 1). Thus, pressurization yielded lighter colored products than heat did; however, the color of the pressurized beverages changed faster than those heated batches on storage. This could be due to greater oxygen being absorbed in the pressurized samples, accelerating oxidation of phytochemicals, thus triggering high reduction rates on storage. Similarly, Keenan et al. (2010) found *L* and *a** parameters of pressurized (450 MPa/5 min) fruit smoothies changing faster than those of heated (70°C/10 min) samples on storage at 4°C for 30 days.

3.1.1 | Alteration of phytochemicals on storage

Table 2 demonstrates that the onset of losing anthocyanins by ~16%–19% is notable ($p < .05$) at the second week and reduces by 40% at the fourth week. Anthocyanins in the nongerminated rice beverages from both processes had slightly higher reduction rate than those germinated beverages depicted by the slopes, presumably due to greater derivatization of anthocyanins in the nongerminated batches during storage (Zhang et al., 2019). Overall, germination triggered significant loss ($p < .05$) of anthocyanins, irrespective of processing techniques. Apart from loss of anthocyanins, phenolic compounds and DPPH radical inhibition were also decreased in the germinated batches, possibly due to the enzymic hydrolysis of phenolic compounds during germination (Chinma, Anuonye, Simon, Ohiare, & Danbab, 2015). Tian, Nakamura, Cui, and Kayahara (2005) also found some decrease of 6′-O-feruloylsucrose and 6′-*O*-sinapoylsucrose on treating brown rice for 24 hr germination. Upon storage, DPPH radical inhibition and total phenolic compounds in this investigation significantly ($p < .05$) reduced during the first 2 weeks of storage, and decreased further 28–31 and 16%–24%, respectively, at the end of storage, presumably due to oxidation of phenols into quinones and further polymerization (Li, Guo, & Wang, 2008). Another reason for loss of anthocyanins and phenolic compounds during storage might be associated with condensation between anthocyanins and phenols (Cao et al., 2012) as well as polymerization of phenols with proteins (Cao, Xu, Liao, Hu, & Zhang, 2011). In addition, Aqil et al. (2014) found that phenolic compounds and anthocyanins contributed significantly to radical scavenging capacity. Liu, Zhang, You, Guo, and Chang (2018) also found high correlation (with $r > 0.98$) between DPPH and total phenols as well as anthocyanins in the assessment of hawthorn wine.

Regarding the effects of processing, pressurization significantly ($p < .05$) enhanced phenolic contents and their activities compared with thermal processing. This to be expected since pressure has less effect on small molecules like phenols than heat. Also pressure changes the distribution and aggregation of bioactive compounds,

TABLE 2 Changes of phytochemicals and pH of processed purple rice beverages during storage at 4°C for 4 weeks

Phytochemicals	Rice beverages	Storage time (weeks)					Slopes of storage time
		Initial state	1	2	3	4	
Anthocyanin (mg/100 ml)	HPG	2.04 ± 0.12^{aB}	1.92 ± 0.11^{aB}	1.71 ± 0.08^{bA}	1.47 ± 0.12^{cB}	1.24 ± 0.03^{dAB}	−0.21
		(100%)	(5.77%)	(16.35%)	(27.88%)	(39.42%)	
	HG	1.93 ± 0.09^{aB}	1.80 ± 0.17^{aB}	1.57 ± 0.13^{abAB}	1.42 ± 0.09^{bB}	1.13 ± 0.10^{cB}	−0.20
		(100%)	(6.74%)	(18.65%)	(26.42%)	(41.45%)	
	HPNG	2.26 ± 0.04^{aA}	2.11 ± 0.05^{bA}	1.90 ± 0.17^{bA}	1.68 ± 0.03^{cA}	1.40 ± 0.16^{dA}	−0.22
		(100%)	(7.14%)	(15.87%)	(25.40%)	(38.10%)	
	HNG	2.29 ± 0.07^{aA}	2.14 ± 0.13^{aA}	1.89 ± 0.14^{abA}	1.53 ± 0.07^{cB}	1.38 ± 0.12^{cA}	−0.24
		(100%)	(6.55%)	(17.47%)	(33.19%)	(39.74%)	
Total phenolic contents (mg/100 ml)	HPG	29.84 ± 2.11^{aC}	28.31 ± 0.88^{aC}	27.74 ± 0.86^{aC}	26.19 ± 1.20^{bC}	24.42 ± 1.47^{bC}	−1.30
		(100%)	(5.13%)	(7.04%)	(12.23%)	(18.16%)	
	HG	24.35 ± 1.08^{aD}	22.76 ± 1.29^{abD}	21.52 ± 0.39^{bD}	20.90 ± 0.96^{bcD}	18.53 ± 1.58^{cD}	−1.35
		(100%)	(6.53%)	(11.62%)	(14.17%)	(23.90%)	
	HPNG	49.21 ± 0.76^{aA}	47.68 ± 1.43^{abA}	45.90 ± 0.53^{bA}	43.62 ± 1.68^{cA}	41.06 ± 2.01^{cA}	−2.04
		(100%)	(3.11%)	(6.73%)	(11.36%)	(16.56%)	
	HNG	37.90 ± 0.93^{aB}	36.03 ± 0.65^{bB}	35.36 ± 1.27^{bB}	33.14 ± 0.82^{cB}	31.69 ± 1.62^{cB}	−1.53
		(100%)	(4.93%)	(6.70%)	(12.56%)	(16.38%)	
DPPH radical scavenging activities (% inhibition)	HPG	13.94 ± 0.45^{aC}	12.09 ± 0.17^{bC}	11.34 ± 0.44^{cC}	10.52 ± 0.31^{dC}	9.63 ± 0.29^{eC}	−1.02
		(100%)	(13.27%)	(18.65%)	(24.53%)	(30.92%)	
	HG	10.83 ± 0.40^{aD}	10.04 ± 0.12^{bD}	9.01 ± 0.17^{cD}	8.24 ± 0.16^{dD}	7.48 ± 0.24^{eD}	−0.85
		(100%)	(7.29%)	(16.80%)	(23.92%)	(30.93%)	
	HPNG	27.11 ± 0.17^{aA}	23.90 ± 0.27^{bA}	21.61 ± 0.40^{cA}	20.39 ± 0.11^{dA}	18.90 ± 0.25^{eA}	−2.00
		(100%)	(11.84%)	(20.29%)	(24.79%)	(30.28%)	
	HNG	19.62 ± 0.28^{aB}	17.76 ± 0.33^{bB}	15.37 ± 0.38^{cB}	14.95 ± 0.34^{dB}	14.12 ± 0.71^{dB}	−1.38
		(100%)	(9.48%)	(21.66%)	(23.80%)	(28.03%)	
GABA (mg/100 ml)	HPG	13.37 ± 0.22^{aA}	13.19 ± 0.10^{aA}	12.63 ± 0.23^{bA}	12.09 ± 0.14^{cA}	11.68 ± 0.29^{dA}	−0.45
		(100%)	(1.35%)	(5.53%)	(9.57%)	(12.64%)	
	HG	13.48 ± 0.26^{aA}	13.21 ± 0.15^{aA}	12.67 ± 0.31^{bA}	11.81 ± 0.16^{cA}	11.37 ± 0.12^{dA}	−0.56
		(100%)	(2.00%)	(6.01%)	(12.39%)	(15.65%)	
	HPNG	5.97 ± 0.19^{aB}	5.88 ± 0.13^{aB}	5.63 ± 0.18^{abB}	5.35 ± 0.30^{bB}	5.16 ± 0.21^{cB}	−0.22
		(100%)	(1.51%)	(5.70%)	(10.38%)	(13.56%)	
	HNG	3.56 ± 0.11^{aC}	3.48 ± 0.29^{abC}	3.36 ± 0.14^{abC}	3.22 ± 0.27^{abC}	3.15 ± 0.07^{bC}	0.11
		(100%)	(2.25%)	(5.62%)	(9.55%)	(11.52%)	
γ-oryzanol (mg/100 ml)	HPG	3.63 ± 0.24^{aA}	3.49 ± 0.17^{aA}	3.30 ± 0.26^{abA}	3.16 ± 0.13^{bA}	2.98 ± 0.19^{bA}	−0.16
		(100%)	(3.86%)	(9.09%)	(12.95%)	(17.90%)	
	HG	2.51 ± 0.12^{aB}	2.44 ± 0.13^{abB}	2.27 ± 0.16^{abB}	2.19 ± 0.17^{bB}	2.02 ± 0.13^{bB}	−0.12
		(100%)	(2.79%)	(9.56%)	(12.75%)	(19.52%)	
	HPNG	2.77 ± 0.12^{aB}	2.56 ± 0.11^{aB}	2.44 ± 0.22^{abB}	2.38 ± 0.23^{abB}	2.21 ± 0.21^{bB}	−0.13
		(100%)	(7.58%)	(11.91%)	(14.08%)	(20.22%)	
	HNG	1.58 ± 0.14^{aC}	1.50 ± 0.18^{abC}	1.42 ± 0.12^{abC}	1.35 ± 0.11^{abC}	1.21 ± 0.19^{bC}	−0.09
		(100%)	(5.06%)	(10.13%)	(14.56%)	(23.42%)	
pH	HPG	6.47 ± 0.06^{aA}	6.40 ± 0.06^{abA}	6.40 ± 0.02^{aA}	6.33 ± 0.03^{bA}	6.20 ± 0.04^{cB}	−0.06
	HG	6.45 ± 0.04^{aA}	6.42 ± 0.03^{aA}	6.35 ± 0.04^{abA}	6.30 ± 0.04^{bA}	6.17 ± 0.07^{cB}	−0.07
	HPNG	6.50 ± 0.02^{aA}	6.45 ± 0.02^{bA}	6.43 ± 0.05^{abA}	6.39 ± 0.08^{bA}	6.30 ± 0.06^{bAB}	−0.04
	HNG	6.49 ± 0.07^{aA}	6.43 ± 0.07^{abA}	6.41 ± 0.06^{abA}	6.34 ± 0.04^{bA}	6.32 ± 0.03^{bA}	−0.04

Notes: Variables in the parenthesis are the percentage of losing each phytochemical from its initial weeks. The means ± *SD* in the same row or column with the same small or capital letters are not significantly different ($p > .05$). Each data point is the average of triplication.

Abbreviations: HPG, pressurized germinated beverage; HG, heated germinated beverage; HPNG, pressurized nongerminated beverage; HNG, heated nongerminated beverage; ns, nonsignificant difference.

and increases in the rate of mass transfer, which induces solvent penetration into the cells by disrupting the cell walls and hydrophobic bonds in the cell membrane. This leads to high permeability and releases of phytochemicals (Andrés, Villanuev, et al., 2016). However, these phytochemicals underwent further degradation on storage, which was faster in the pressurized compared with the heated batches. This could be associated with oxidation and catalysis of the phenols by residual peroxidase (Terefe, Delon, & Versteeg, 2017) in the pressurized batches, whereas most enzymes were denatured by heat, triggering less effect on the heated batched (Phunchaisri & Apichartsrangkoon, 2005). Similarly, Keenan et al. (2010) found that, total phenols and antioxidant capacity (DPPH) in pressurized fruit smoothies decreased by 33.9% and 29.4%, respectively, upon storage at 4°C for 30 days. Later, Chaikham, Apichartsrangkoon, and Seesuriyachan (2014) pressurized longan juice at 500 MPa/25°C/30 min and found that total phenols and antioxidant activity (DPPH assay) decreased by 28% and 37%, respectively, on storage at 4°C for 4 weeks. Decrease in anthocyanins and total phenols by 29.76% and 16.22%, respectively, was also found in pressurized (at 600 MPa/4 min) cloudy strawberry juices after storage at 4°C for 6 month (Cao et al., 2012).

It was not unexpected that GABA in the germinated beverages was 2–3 folds higher than in the nongerminated batches (Table 2). The reduction was notable ($p < .05$) by the second week, the germinated samples showed higher reduction rate than the nongerminated batches. However, in comparison with other phytochemicals, little loss of GABA, 12%–16% at the end of storage was observed. This affirms that GABA is relatively stable on storage. Srisang, Varanyanond, Soponronnarit, and Prachayawarakorn (2011) found that GABA content in germinated brown rice was resistant to drying temperature <130°C, because GABA melted at 203°C. Thuwapanichayanana, Yoosabaia, Jaisuta, Soponronnaritb, and Prachayawa (2015) also found that heat increased GABA content in germinated rice.

Gama-oryzanol was notably decreased ($p < .05$) from the third week especially in the germinated beverages (Table 2). Although γ-oryzanol decreased 18%–23% by the fourth week, it had the least loss on storage (indicated by the slopes) compared with other phytochemicals suggesting that γ-oryzanol is also relatively stable on storage. Pascual et al. (2013) stated that brown rice packed in a polyethylene pouches lost <20% γ-oryzanol during storage at 25 ± 5°C for 6 months.

3.2 | Microbiological alteration on storage

Product pH had great impact on the survivability of *L. casei* 01. Table 2 illustrates that pH of most products significantly drops ($p < .05$) from the third week onward. The pH drop for all treatments from the initial to the final states was in the range of 0.17–0.28 with low reduction rate. On the other hand, slight increase of *L. casei* 01in all treatments at the final state of storage was also observed (Figure 3); however, the increase of these bacteria were slower than the pH drops (evident by slopes). This might be due to the synthesized products of *L. casei* 01 and other bacteria. The standard plate counts showed similar trends of increase of bacteria, for which the colonies were very tiny at the first 2 weeks, presumably being the colonies of *L. casei* 01. However, some bigger colonies appeared at the final state of storage, which could be an existence of other bacteria apart from *L. casei* 01. These bacteria should originate from the rice beverages and reactivate during storage. It is inevitable that pressurized or pasteurized products tend to be degraded by microbes on prolonged keeping. Nevertheless, yeasts and molds were not detected throughout the storage period. In addition, the retention of 8 log CFU/ml *L. casei* 01 in the products complied with the daily dose requirement for probiotic supplemented foods (Jaiswal & Abu-Ghannam, 2013). Similar results were obtained by Jaiswal and Abu-Ghannam (2013) who pasteurized cabbage juice and stored at

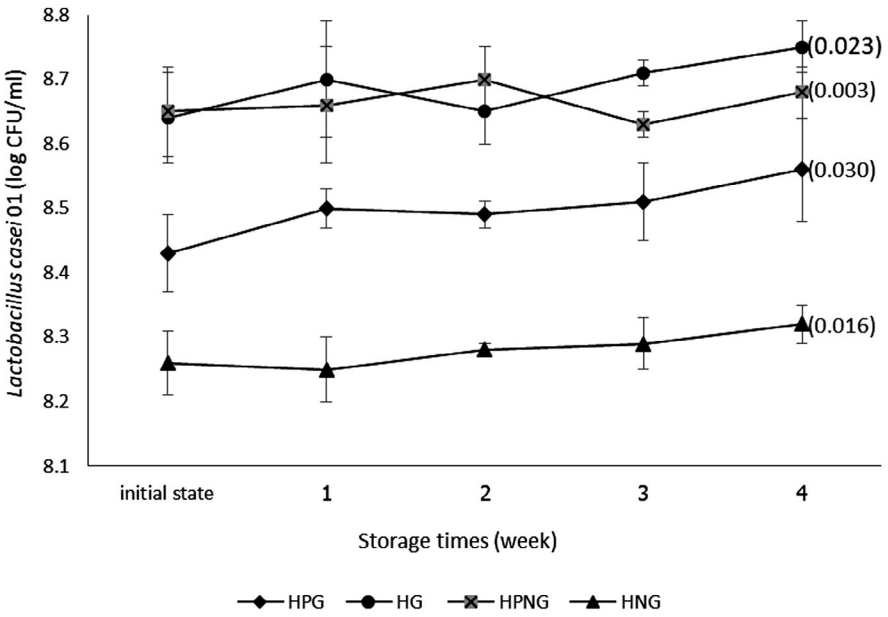

FIGURE 3 Changes of lactic acid bacteria of processed purple rice beverages during storage at 4°C for 4 weeks

4°C for 30 days, found increases of *L. plantarum*, *L. rhamnosus*, and *L. brevis* about 1 log CFU/ml at the end of storage. Zheng et al. (2014) noticed that *L. casei* in pressurized lychee juice slightly augmented with the retention of 8 log CFU/ml on storage at 4°C for 4 weeks.

4 | CONCLUSIONS

Germination induced loss of anthocyanins, phenolic compounds, and their DPPH activities, while GABA and γ-oryzanol contents were increased. Despite of the pressurized rice beverages being lighter in color than the heated ones, their color changed faster on storage. Pressurization preserved more phytochemicals than heat did. Upon storage at 4°C for 4 weeks, phytochemicals such as anthocyanins, phenolic compounds, and DPPH radical inhibition in most rice beverages significantly decreased, whereas *L. casei* 01 in all treatments were slightly increased with the retention of 8 log CFU/ml. On the other hand, γ-oryzanol and GABA were relatively stable throughout the storage. Some future prospects of this research, using different type of cereals or fortifying with other probiotics or survey for consumer acceptability might be worthy of further study.

ACKNOWLEDGMENTS

This research was supported by Office of Research Administration, Chiang Mai University for whose effort was greatly appreciated.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Arunee Apichartsrangkoon https://orcid.org/0000-0002-5160-0538

REFERENCES

Allgeyer, L. C., Miller, M. J., & Lee, S. Y. (2010). Sensory and microbiological quality of yogurt beverages with prebiotics and probiotics. *Journal of Dairy Science*, *93*, 4471–4479. https://doi.org/10.3168/jds.2009-2582

Andrés, V., Mateo-Vivaracho, L., Guillamon, E., Villanueva, M. J., & Tenorio, M. D. (2016b). High hydrostatic pressure treatment and storage of soy-smoothies: Colour, bioactive compounds and antioxidant capacity. *Lwt—food Science and Technology*, *69*, 123–130. https://doi.org/10.1016/j.lwt.2016.01

Andrés, V., Villanuev, M. J., & Tenorio, M. D. (2016a). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry*, *192*, 328–335. https://doi.org/10.1016/j.foodchem.2015.07.031

Angelov, A., Gotcheva, V., Kuncheva, R., & Hristozova, T. (2006). Development of a new oat-based probiotic beverage. *International Journal of Food Microbiology*, *112*, 75–80. https://doi.org/10.1016/j.ijfoodmicro.2006.05.015

Aqil, F., Munagala, R., Kash, J., Thwisha, J., Gupta, J. R. C., & Singh, I. P. (2014). The Indian Blackberry (Jamun), Antioxidant Capacity, and Cancer Protection. In *Cancer: Oxidative stress and dietary antioxidants* (pp. 101–113). New York, NY: Academic Press. https://doi.org/10.1016/B978-0-12-405205-5.00010-6

Bobinaite, R., Viskelis, P., & Venskutonis, P. R. (2012). Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars. *Food Chemistry*, *137*(11), 456–462. https://doi.org/10.1016/j.foodchem.2011.11.137

Cao, X., Bi, X., Huang, W., Wu, J., Hu, X., & Liao, X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science and Emerging Technology*, *16*, 181–190. https://doi.org/10.1016/j.ifset.2012.05.008

Cao, X. M., Xu, W. W., Liao, X. J., Hu, X. S., & Zhang, Y. (2011). Effect of high pressure processing steps on antioxidants and antioxidant activity in strawberry juice drinks. *Food and Nutrition in China*, *17*, 30–35. Retrieved from en.cnki.com.cn/article_en/cjfdtotal-zgwy201110008.htm

Chaikham, P., & Apichartsrangkoon, A. (2012a). Comparison of bioactive components in pressurized and pasteurized longan juices fortified with encapsulated *Lactobacillus casei* 01. *High Pressure Research*, *32*(2), 316–322. https://doi.org/10.1080/08957959.2012.665052

Chaikham, P., & Apichartsrangkoon, A. (2012b). Comparison of dynamic viscoelastic and physicochemical properties of pressurised and pasteurized longan juices with xanthan addition. *Food Chemistry*, *134*, 194–2200. https://doi.org/10.1016/j.foodchem.2012.04.056

Chaikham, P., Apichartsrangkoon, A., & Seesuriyachan, P. (2014). Physical and biochemical qualities of pressurized and pasteurized longan juices upon storage. *Emirate Journal of Food and Agriculture*, *26*(3), 218–228. https://doi.org/10.9755/ejfa.v26i2.15508

Chaikham, P., Apichartsrangkoon, A., Worametrachanon, S., & Van de Wiele, T. (2016). Impact of encapsulated *Lactobacillus casei* 01 along with pasteurized purple-rice drinks on modulating colon microbiome using a digestive model. *International Journal of Food Engineering*, *12*(7), 637–646. https://doi.org/10.1515/ijfe-2016-0008

Chaikham, P., Chunthanom, P., & Apichartsrangkoon, A. (2013). Storage stability of pennywort extract as affected by high pressure and thermal processing. *International Food Research Journal*, *20*(6), 3069–3076. ifrj.upm.edu.my

Chinma, C. E., Anuonye, J. C., Simon, O. C., Ohiare, R. O., & Danbab, N. (2015). Effect of germination on the physicochemical and antioxidant characteristics of rice flour from three rice varieties from Nigeria. *Food Chemistry*, *185*(15), 454–458. https://doi.org/10.1016/j.foodchem.2015.04.010

Cho, J. Y., Lee, H. J., Kim, G. A., Kim, G. D., Lee, Y. S., Shin, S. C., … Moon, J. H. (2012). Quantitative analyses of individual γ-oryzanol (steryl ferulates) in conventional and organic brown rice (*Oryza sativa* L.). *Journal of Cereal Science*, *55*(3), 337–343. https://doi.org/10.1016/j.jcs.2012.01.005

Costa, K. K. F. D., Júnior, M. S. S., Rosa, S. I. R., Caliari, M., & Pimentel, T. C. (2017). Changes of probiotic fermented beverage obtained from soy and rice byproducts during cold storage. *LWT-Food Science and Technology*, *78*, 23–30. https://doi.org/10.1016/j.lwt.2016.12.017

Dajanta, K., Apichartsrangkoon, A., Chukeatirote, E., & Frazier, R. A. (2011). Free-amino acid profiles of *thua nao*, a Thai fermented soybean. *Food Chemistry*, *125*, 342–347. https://doi.org/10.1016/j.foodchem.2010.09.002

Hosseinian, F. S., Li, W., & Beta, T. (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry*, *109*, 916–924. https://doi.org/10.1016/j.foodchem.2007.12.083

Iqbal, S., Bhanger, M. I., & Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chemistry*, *93*, 265–272. https://doi.org/10.1016/j.foodchem.2004.09.024

Jaiswal, A. K., & Abu-Ghannam, N. (2013). Kinetic studies for the preparation of probiotic cabbage juice: Impact on phytochemicals and bioactivity. *Industrial Crop Production*, *50*, 212–218. https://doi.org/10.1016/j.indcrop.2013.07.028

Keenan, D. F., Brunton, N. P., Gormley, T. R., Butler, F., Tiwari, B. K., & Patras, A. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science and Emerging Technology*, *11*(4), 551–556. https://doi.org/10.1016/j.ifset.2010.07.003

Lee, Y. R., Kim, J. Y., Woo, K. S., Hwang, I. G., Kim, K. H., Kim, J. H. … Jeong, H. S. (2007). Changes in the chemical and functional components of Korean rough rice before and after germination. *Food Science and Biotechnology*, *16*, 1006–1010. Retrieved from https://lib.rda.go.kr/newlib/adlib_en/index.html

Li, H., Guo, A., & Wang, H. (2008). Review: Mechanisms of oxidative browning of wine. *Food Chemistry*, *108*, 1–13. https://doi.org/10.1016/j.foodchem.2007.10.065

Liu, S., Zhang, X., You, L., Guo, Z., & Chang, X. (2018). Changes in anthocyanin profile, color, and antioxidant capacity of hawthorn wine (*Crataegus pinnatifida*) during storage by pretreatments. *Lwt—food Science and Technology*, *95*, 179–186. https://doi.org/10.1016/j.lwt.2018.04.093

Moongngarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chemistry*, *122*(3), 782–788. https://doi.org/10.1016/j.foodchem.2010.03.053

Pascual, C. S. C. I., Massaretto, I. L., Kawassaki, F., Barros, R. M. C., Noldin, J. A., & Marquez, U. M. L. (2013). Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols and γ-oryzanol in brown rice (*Oryza sativa* L.). *Food Research International*, *50*(2), 676–681. https://doi.org/10.1016/j.foodres.2011.07.013

Phunchaisri, C., & Apichartsrangkoon, A. (2005). Effect of ultra-high pressure on biochemical and physical modification of Lychee (*Litchi chinensis* Sonn.). *Food Chemistry*, *93*, 57–64. https://doi.org/10.1016/j.foodchem.2004.10.001

Sheu, T. Y., & Marshall, R. T. (1993). Microencapsulation of lactobacilli in calcium alginate gels. *Journal of Food Science*, *54*, 557–561. https://doi.org/10.1111/j.1365-2621.1993.tb04323.x

Srisang, N., Varanyanond, W., Soponronnarit, S., & Prachayawarakorn, S. (2011). Effects of heating media and operating conditions on drying kinetics and quality of germinated brown rice. *Journal of Food Engineer*, *107*, 385–392. https://doi.org/10.1016/j.jfoodeng.2011.06.030

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology*, *62*(1–2), 47–55. https://doi.org/10.1016/S0168-1605(00)00380-9

Terefe, N. S., Delon, A., & Versteeg, C. (2017). Thermal and high pressure inactivation kinetics of blueberry peroxidase. *Food Chemistry*, *232*, 820–826. https://doi.org/10.1016/j.foodchem.2017.04.081

Thuwapanichayanana, R., Yoosabaia, U., Jaisuta, D., Soponronnaritb, S., & Prachayawarakorn, S. (2015). Enhancement of γ-aminobutyric acid in germinated paddy by soaking in combination with anaerobic and fluidized bed heat treatment. *Food and Bioproducts Processing*, *95*, 55–62. https://doi.org/10.1016/j.fbp.2015.03.010

Tian, S., Nakamura, K., Cui, T., & Kayahara, H. (2005). High-performance liquid chromatographic determination of phenolic compounds in rice. *Journal of Chromatography A*, *1063*, 121–128. https://doi.org/10.1016/j.chroma.2004.11.075

U.S. Food and Drug Administration (U.S. FDA). (2001). *Bacteriological Analytical Manual (BAM)*. Concord, NH: Department of Health and Human Services. Retrieved from fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm

Vinderola, G., Reinheimer, J., & Salminen, S. (2019). The enumeration of probiotic issues: From unavailable standardized culture media to a recommended procedure. *International Dairy Journal*, *96*, 58–65. https://doi.org/10.1016/j.idairyj.2019.04.010

Worametrachanon, S., Apichartsrangkoon, A., Chaikham, P., Van den Abbeele, P., Van de Wiele, T., & Wirjantoro, T. I. (2014). Effect of encapsulated *Lactobacillus casei* 01 along with pressurized-purple-rice beverages on colonizing the colon in the digestive model. *Applied Microbiology and Biotechnology*, *98*, 5241–5250. https://doi.org/10.1007/s00253-014-5624-8

Yang, P., Li, X., Wang, X., Chen, H., Chen, F., & Shen, S. (2007). Proteomic analysis of rice (*Oryza sativa*) seeds during germination. *Proteomics*, *7*(18), 3358–3368. https://doi.org/10.1002/pmic.200700207

Zhang, Z., Li, J., & Fan, L. (2019). Evaluation of the composition of Chinese bayberry wine and its effects on the color changes during storage. *Food Chemistry*, *276*, 451–457. https://doi.org/10.1016/j.foodchem.2018.10.054

Zheng, X., Yu, Y., Xiao, G., Xu, Y., Wu, J., Tang, D., & Zhang, Y. (2014). Comparing product stability of probiotic beverages using litchi juice treated by high hydrostatic pressure and heat as substrates. *Innovative Food Science and Emerging Technology*, *23*, 61–67. https://doi.org/10.1016/j.ifset.2014.01.013

How to cite this article: Baipong S, Apichartsrangkoon A, Worametrachanon S, et al. Effects of germinated and nongerminated rice grains on storage stability of pressurized purple rice beverages with *Lactobacillus casei* 01 supplement. *J Food Process Preserv*. 2020;00:e14442. https://doi.org/10.1111/jfpp.14442