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Effect of inoculum levels and final pH values on the antioxidant properties of black glutinous rice solution fermented by *Lactobacillus bulgaricus*

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Abstract

Black glutinous rice is known to have an antioxidant property due to the presence of phenolic compounds and anthocyanin. In this study, the antioxidant properties of black glutinous rice solution fermented by *Lactobacillus bulgaricus* were determined at different final pH values. Black glutinous rice grain was milled to become powder with the size of less than 595 µm, soaked in distilled water at a ratio of 1:5 for 30 min at room temperature and heated at 95±1ºC for 30 min. The heated rice solution that had a pH of 5.90±0.00 was aseptically inoculated with *Lb. bulgaricus* at a level of 10^4 to 10^7 cfu/ml and incubated at 41±1ºC until the pH of the fermented rice solution reached a value of 4.0, 4.5 or 5.0. During the fermentation time, the number of *Lb. bulgaricus* increased between 1.33 and 4.33 log cfu/ml. Both parameters of lactobacilli inoculation levels and final pH values of the fermented rice solution affected the antioxidant properties of the final rice product. At a final pH of 4.5, the level of anthocyanin improved from 15.43±1.05 to 23.15±0.16 mg/g when the inoculation levels of *Lb. bulgaricus* increased from 4.0 to 7.0 log cfu/ml. A similar finding was also found for phenolic compounds of the fermented rice solution at a final pH of 4.0. On the other hand, the content of phytic acid decreased from 17.92±2.19 to 5.59±2.69 µg phytic acid equivalents/ml in the fermented rice solution as the inoculation levels of the lactobacilli increased, irrespectively to the final pH value. The highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was determined in the fermented rice solution added with the lowest inoculation level of *Lb. bulgaricus* when the final pH values of rice solution were 4.0 and 4.5. A combination of an inoculation level of 7.0 log cfu/ml *Lb. bulgaricus* and a final pH of 4.5 produced a fermented rice solution with the highest anthocyanin (23.15±0.16 mg/g) and phenolic contents (114.93±1.66 µg gallic acid equivalents/g) and the lowest phytic acid of 5.59±2.69 µg phytic acid equivalents/ml.

Introduction

Black rice is one type of pigmented rice that is often mixed with white rice prior to cooking to enhance the flavor, color and nutritional value of the final product. The rice, which is a major crop in the South Asia and Mainland China, is known as an enriched rice with medicinal effects (Ryu *et al*., 1998; Min *et al*., 2010). This is partly due to a relatively high level of anthocyanin, mainly cyanidin 3-glucoside and peonidin 3-glucoside (Ryu *et al*., 1998), in the pericarp layer, which gives the dark purple color to the rice (Kristamtini *et al*., 2012). Beside anthocyanin, colored rice also contains acetylated procyanidin and other phenolic compounds that exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, antimicrobial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Yawadio *et al*., 2007; Vichapong *et al*., 2010). For anthocyanin pigments, it has been reported to be highly effective in reducing cholesterol levels in the human body (Lee *et al*., 2008) and exerted an inhibitory effect of cell invasion on various cancer cells (Chen *et al*., 2006). The presence of these phytochemical compounds in black rice caused the rice to be included as one of the potent functional food (Tananuwong and Tewaruth 2010).

Lactic acid bacteria are Gram positive, aerotolerant, catalase negative microorganisms that are widely distributed in nature (Campos *et al*., 2009; Maqsood *et al*., 2013). They are widely used in food fermentation as starter cultures due to their capability to produce different organic acids, mainly acetate and lactate, from degradation of raw material components, causing pH reduction (Wu *et al*., 2011; Tabasco *et al*., 2014). *Lactobacillus* spp. is part of the lactic acid bacteria that can be found in human gastrointestinal tract and is recognized as generally regarded as safe (GRAS) (Osuntoki and Korie, 2010; Ren *et al*., 2014). The bacteria were reported to have some

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beneficial activities, including immunomodulatory, antiallergenic, antimicrobial, antihypertensive and antitumourigenic effects, and possess antioxidant activities (Osuntoki and Korie, 2010). Regarding the antioxidant activities of lactobacilli, Kim *et al.* (2005) informed that *Lb. bulgaricus* LB207 had the highest antioxidant activity for linoleic acid peroxidation inhibition (81.3%) compared with *Lactobacillus acidophilus* LA5, *Lactobacillus casei* 01, *Lb. acidophilus* LA100 and *Lactobacillus rhamnosus* GG744 using *in vitro* assays. Saide and Gilliland (2005) also showed that *Lactobacillus delbrueckii* subsp. *bulgaricus* produced antioxidative activities when grown in de Man Rogosa Sharpe (MRS) broth supplemented with 0.5% 2,3,5 triphenyl tetrazolium chloride (TTC). In addition, the antioxidant activity of lactobacilli was correlated with its fermentation time. The work of Pistarino e*t al.* (2013) demonstrated that during 100 days fermentation of Taggiasca black olives by *Lactobacillus plantarum* at temperatures of 23-37ºC, the pH of the brine was continuously reduced, while the content of the phenolic content in the brine was constantly increased up to 40 to 70 days of fermentation, depending on the fermentation temperature, and followed by a decrease in its phenolic content at prolonged fermentation time. A similar finding was described by Ben Othman *et al.* (2009), who found that the phenolic content of flesh Chétoui olives was decreased during spontaneous and controlled olives fermentations at ambient temperature for 70 days, while the brine phenolic content was increased. These workers exhibited that the antioxidant activity and pH values of the flesh olives were also reduced during the fermentation period. Although the antioxidant activity of some lactic acid bacteria had been assessed in fermented soymilk-tea beverage (Zhao and Shah, 2014) and drinking yogurts with berry polyphenols (Sun-Waterhouse *et al.*, 2013), there was not any report that investigated the effect of lactobacilli fermentation on the antioxidant capacity of black glutinous rice solution. Therefore, the aim of this project was to evaluate the effect of *Lb. bulgaricus* inoculation levels and final pH values on the antioxidant properties of fermented black glutinous rice solution.

Materials and Methods

Preparation of black glutinous rice solution

Black glutinous rice (*Oryza sativa* L.) variety Lem Hua was milled using a blender (National, Thailand), sieved with a metal strainer of 30 mesh (595 µm), packed in polyethylene bags and kept at 4ºC. On the day of the experiment, the rice powder was soaked with distilled water at a ratio of 1:5 for 30 min at room temperature, boiled at 95±1ºC for 30 min in a water bath (Memmert, Germany) (Chiangpha, 2012) and immediately cooled down to the fermentation temperature.

Culture preparation and fermentation of black glutinous rice solution

Lb. bulgaricus was separated from thermophilic yoghurt cultures (Yo-Flex® Chr Hansen, Denmark) using MRS medium (Merck, Germany) at pH 5.4 using acetic acid (Merck, Germany) (Zare *et al.*, 2011). The isolated culture was confirmed with Gram staining (Ng *et al*., 2011). To be used in the rice experiment, the isolated *Lb. bulgaricus* was cultured in fresh 10 ml MRS broth and incubated at 37±1ºC for 24 h. The lactobacilli cells were collected by centrifugation at 10,000 rpm (12,100 x g) for 10 min at 4ºC (Hermle, United States) and washed twice with Maximum Recovery Diluent (Oxoid, England) (Sohail *et al.*, 2012). The young cultures of *Lb. bulgaricus* in MRS broth after 24 h incubation at 37ºC were approximately 8.0 log cfu/ml. The cells of *Lb. bulgaricus* were then inoculated into the black glutinous rice solution at initial inoculation levels between 4.0 and 7.0 log cfu/ml. After thoroughly mixed the culture with the rice solution in sterile glass bottles, the solution was incubated aerobically at 41±1ºC (Memmert, Germany) until the pH of the solution reached a value of 4.00±0.08, 4.50±0.05 or 5.00±0.05. The pH value of black glutinous rice solution before a fermentation process was 5.90±0.00. The fermentation time of different inoculum levels was between 18 and 29 h. After the final pH was reached, the fermented rice solution was kept at 4ºC for further analyses. Each treatment was carried out in triplicate.

Determination of color

Color measurement of black glutinous rice solution was performed using a colorimeter (Minolta Chroma Meter, Japan). The instrument was calibrated with a white tile (Y=86.4, X=0.3177 and y=0.3354). The Hunter L^*, a^* and b^* scales gave a measurement of color in units of approximate visual uniformity throughout a liquid. The L^* value represented the color lightness, the a^* value characterized the greenness and redness and the b^* value measured the blueness and yellowness (AOAC method no 46.1.12; AOAC, 2000).

Measurement of pH, total titratable acidity, total soluble solid and moisture contents

The pH of black glutinous rice solution was

measured by using a digital pH meter (Ezodo 6011, Taiwan). Total titratable acidity was determined by applying an AOAC method no 11.1.04 (AOAC, 2000). The total acidity of black glutinous rice solution was calculated using Eq.1.

Total acidity (% as lactic acid) = amount of 0.1 M NaOH (ml) x 100 x 0.009/ amount of sample (ml) (1)

Total soluble solid of black glutinous rice solution was examined by a hand refractometer (Atago, Japan). Moisture content of the rice solution was analyzed by transferring 2-3 g of black glutinous rice solution into a moisture can with a tight-fit cover that had been weighted. The moisture can was placed loosely in a hot air oven (Memmert, Germany) at 100±2ºC overnight. On the following day, the can cover was placed tightly into the moisture can, removed them from the oven, cooled in an active desiccator and weighted. The loss of weight of the rice solution was expressed as moisture content of the black glutinous rice solution (AOAC, 2000).

Determination of phytic acid

Phytic acid content in the black glutinous rice solution was determined by a colorimetric method described by Kong and Lee (2010) with some modifications. The phytic acid was extracted with 20 ml of 0.2 N HCl (RCI Labscan, Thailand) by shaking 150 mg of black glutinous rice solution at 200 rpm for 4 h at room temperature, followed by centrifugation at 3000 rpm (2300 x g) for 20 min (Hermie, Germany). The supernatant was used for the phytic acid determination. An amount of 500 µl supernatant was precipitated with 1 ml of ferric solution (Loba Chemie, India). The mixture was boiled at 100ºC for 30 min in a water bath (Memmert, Germany). After cooling, 1 ml of the supernatant was used to determine the phytic acid content in the sample concentration of the solution using 1.25 ml 2,2-bipyridine solution (Loba Chemie, India). After incubation for 1 min at room temperature, the absorbance of the mixture was measured at 519 nm (Genesys 10 UV Scanning, Thermo Spectronic, United Stated). The results were expressed as µg phytic acid equivalents per 1 ml of sample.

Determination of total phenolic content

Total phenolics were evaluated using a spectrophotometric method with Folin-Ciocalteu's phenol reagent (Tananuwong and Tewaruth 2010; Thammapat *et al*., 2015). In brief, a 2 g of black glutinous rice solution was mixed with 20 ml of 80% methanol (Merck, Germany) and hold for 30 min at room temperature. The solution was then centrifuged at 6000 rpm (3820 x g) for 20 min at room temperature (Hermie, Germany). An amount of 1 ml of supernatant was mixed with 5 ml of Folin-Ciocalteu's phenol reagent (Lobachemie, India) at room temperature and shaken for 8 min. After 8 min, 4 ml of a 7.5% sodium carbonate (Na_2CO_3) solution (QReC, New Zealand) was added and properly mixed. Following 2 h incubation at room temperature, the absorbance of the solution was read against the prepared blank (the solution without any sample) at 765 nm (Thermo Spectronic, United Stated). The standard curve for total phenolics was made using gallic acid standard solution (100-800 µg/g) (Sigma, China) under the same procedure as above. The result of standard curve was y = 0.0073 X, where y was absorbance at 765 nm and X was amount of gallic acid.

Determination of anthocyanin

Anthocyanin content in the sample was determined according to the procedure described by Sompong *et al.* (2011) with some modifications. Anthocyanins were extracted with acidified methanol (methanol (Merck, Germany) and 1 M HCl (RCI Labscan, Thailand) at a ratio of 85:15, v/v) with a solvent to sample ratio of 1:10. The mixture was kept in the dark at 4ºC for 24 h. On the following day, the mixture was filtered with a Whatman filter paper no.1 (Whatman, England) and the filtrate was measured for its absorbance at 535 nm by a spectrophotometer (Thermo Spectronic, United Stated). The amount of anthocyanin in the sample was calculated based on Eqs. 2 and 3.

$$\text{Total absorbance} = \frac{\text{absorbance x final volume x 100}}{\text{weight}} \quad (2)$$

$$\text{Total anthocyanin content (mg/g)} = \frac{\text{Total absorbance}}{98.2} \quad (3)$$

Determination of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

DPPH radical-scavenging ability of black glutinous rice solution extracts was evaluated according to the procedure reported by Aruoma *et al.* (1997). A reaction mixture was prepared by mixing 1 ml DPPH working solution (0.0168 g of DPPH (Sigma-Aldrich, Germany) in 50 ml ethanol (Merck, Germany)) and 1 g black glutinous rice solution extract. The mixture was shaken and incubated for 1 h in the dark at room temperature. The absorbance of the mixture was read at 517 nm relative to the control containing DPPH solution without sample and blank sample, which had distilled water, using

a spectrophotometer (Thermo Spectronic, United Stated). The DPPH radical scavenging activity was calculated according to Eq. 4.

$$\text{DPPH (\%)} = \frac{[(A_{control} - A_{sample})]}{A_{control}} \times 100 \quad (4)$$

Statistical analysis

Collected data were statistically analyzed by Factorial in Completely Randomized Design in Analysis of Variance with 2 factors of inoculum levels (4 levels) and final pH values (3 levels) using SPSS version 17.0. Differences between treatment means were determined by Duncan's New Multiple Range Test. Statistical significant was declared at $p < 0.05$.

Results and Discussion

Fermentation time of black glutinous rice solution by *Lb. bulgaricus* at 41±1ºC was between 18 and 29 h (data not shown), affected by inoculum levels of the lactic acid bacterium and final pH of the rice solution. A longer fermentation time of the rice solution than the normal fermentation time of yoghurt from cow milk could be affected by a low protein content of the rice solution, which was 0.14±0.03% (Chiangpha, 2012) and the absence of lactose in the solution. Tabasco *et al.* (2014) reported that *Lb. bulgaricus* had a higher preference towards lactose compared with glucose substrate. do Espirito-Santo *et al.* (2014) that fermented gruels made from rice at 10g/100 g using a single culture of *Lactobacillus fermentum* Ogi E1, *Lb. plantarum* A6, *Lb. acidophilus* L10, *Lb. casei* L26 and *Bifidobacterium animalis* subsp. *lactis* B94 needed a 24 h fermentation time at 40ºC to reach a pH between 4.6 to 5.3. After the fermentation period, the black glutinous rice solution contained *Lb. bulgaricus* in the range of 6.0 to 9.0 log cfu/ml affected by the initial inoculum level (data not shown), indicating that the growth of the bacterium induced changing in the physicochemical and phytochemical properties of the rice solution.

L^* value of the black glutinous rice solution was in the range of 22.12 to 22.47, showing the fermented rice solution did not have a light color, while the b^* value of the rice solution was between 1.40 and 1.64, indicating a slight yellow color intensity in the rice sample (Table 1). For the a^* value, it was observed that the color value had a tendency to be increased at higher inoculation levels of the starter culture. This might be affected by higher number of the lactobacilli causing more microbial activities. It could also be noticed that at *Lb. bulgaricus* inoculum levels of 5.0 log cfu/ml or higher, there was more anthocyanin in the black glutinous rice solution (Table 2).

The chemical and phytochemical properties of black glutinous rice solution can be seen in Table 2. Total titrable acidity of the rice solution was in the range of 0.09 to 0.15% as lactic acid. This value was much lower than the normal acidity of yogurt from cow milk, which had been reported to be 0.8% lactic acid (Dave and Shah, 1997). This could be affected by the low protein content in the fermented black glutinous rice solution, causing the rice solution to have a low buffering capacity. The fermented black glutinous rice solution contained total soluble solids between 8.0 and 14.0%Brix and moisture contents in the range of 82.74 to 88.03%. Although there was not any obvious effect of the final pH of the black glutinous rice on these chemical values, it could be observed that at higher inoculum levels, the amount of total soluble solids of the rice solution was increased and the moisture content was decreased.

For the antioxidant properties of black glutinous rice solution, the amount of anthocyanin, phenolic content, phytic acid and DPPH radical scavenging activity of the rice sample was analyzed (Table 2). The amount of anthocyanin in the rice solution was slightly varied affected by different inoculum levels and final pH values of the rice sample. The highest anthocyanin content of 26.29±2.99 mg/g was found in the black glutinous rice solution fermented with 7.0 log cfu/ml *Lb. bulgaricus* with a final pH value of 5.0. At the end of the fermentation time, this rice solution also had an increase in the number of *Lb. bulgaricus* to become 9.58 log cfu/ml. Hur *et al.* (2014) reviewed that the anthocyanin contents of black beans were enhanced after fermentation by filamentous fungi. There was a possibility that fermentation of *Lb. bulgaricus* in the rice solution might release more of the anthocyanin compound from rice particles. Since the stability of anthocyanin was enhanced at low pH values (Hur *et al.*, 2014), fermentation of the rice solution might also improve the anthocyanin stability in the final product.

The phenolic content in the black glutinous rice solution was significantly affected by the inoculum levels of *Lb. bulgaricus* (Table 2). The highest phenolic content in the rice sample was observed in the rice fermented with 7.0 log cfu/ml *Lb. bulgaricus*. A study about drinking yogurts with berry polyphenols by Sun-Waterhouse *et al.* (2013) showed that an addition of polyphenols before yogurt fermentation produced about 4 times as much as total extractable polyphenol compared with those added with polyphenols after fermentation. These authors suggested that the yogurt cultures of *Streptococcus*

Table 1. Color values of black glutinous rice solution fermented by different inoculum levels of *Lactobacillus bulgaricus* and final pH values

Lb. bulgaricus inoculation level (log cfu/ml)	Final pH	Color values		
		L*	a*	b*
4.0	4.0	22.14±0.21^{e}	6.88±0.26^{cde}	1.40±0.03^{c}
	4.5	22.38±0.06^{ab}	6.64±0.11^{e}	1.42±0.02^{c}
	5.0	22.47±0.04^{a}	6.78±0.13^{de}	1.40±0.03^{c}
5.0	4.0	22.39±0.03^{ab}	7.13±0.36^{abcd}	1.43±0.04^{bc}
	4.5	22.12±0.03^{e}	6.73±0.09^{de}	1.49±0.03^{bc}
	5.0	22.17±0.03^{de}	7.29±0.12^{abc}	1.63±0.01^{a}
6.0	4.0	22.29±0.04^{bcd}	7.46±0.04^{ab}	1.64±0.03^{a}
	4.5	22.33±0.07^{bc}	7.06±0.18^{bcde}	1.46±0.03^{bc}
	5.0	22.14±0.02^{e}	6.86±0.02^{cde}	1.50±0.02^{bc}
7.0	4.0	22.31±0.01^{bc}	7.54±0.05^{a}	1.53±0.02^{b}
	4.5	22.13±0.04^{e}	7.28±0.62^{abc}	1.40±1.74^{c}
	5.0	22.21±0.03^{cde}	7.11±0.04^{abcd}	1.48±0.02^{bc}

Data was mean ± standard deviation from three replicate experiments.
a-e Different letters within a column indicated significantly different treatment at $p < 0.05$.

Table 2. Total acidity (% as lactic acid), total soluble solid (% Brix), moisture content (%) and antioxidant properties of black glutinous rice solution fermented by different inoculum levels of *Lactobacillus bulgaricus* and final pH values

Lb. bulgaricus inoculation level (log cfu/ml)	Final pH	Total acidity (% as lactic acid)	Total soluble solid (%Brix)	Moisture content (%)	Anthocyanin content (mg/g)	Phenolic content (µg GAE*/g)	DPPH radical scavenging activity (%)	Phytic acid content (µg PAE*/ml)
4.0	4.0	0.12±0.02^{cd}	9.00±0.00^{d}	87.26±0.36^{ab}	15.15±3.11^{e}	66.67±2.81^{f}	61.82±4.52^{ab}	17.02±0.71^{a}
	4.5	0.12±0.01^{cd}	9.00±0.00^{d}	87.95±0.28^{a}	15.43±1.05^{e}	67.26±3.13^{f}	62.73±1.60^{a}	17.92±2.19^{a}
	5.0	0.15±0.01^{ab}	10.00±0.00^{c}	87.14±0.03^{abc}	17.27±3.96^{cde}	78.45±2.33^{de}	42.90±0.30^{cd}	16.83±1.45^{a}
5.0	4.0	0.13±0.00^{abc}	10.00±0.00^{c}	87.51±0.08^{ab}	20.45±2.37^{bc}	90.50±3.64^{c}	40.77±4.23^{cd}	14.58±1.18^{ab}
	4.5	0.15±0.01^{a}	8.00±0.00^{e}	88.03±0.14^{a}	16.12±1.12^{de}	61.78±2.97^{g}	42.72±5.57^{cd}	13.07±2.59^{abc}
	5.0	0.13±0.02^{cd}	11.00±0.00^{b}	86.29±0.21^{c}	15.51±1.20^{e}	83.01±3.96^{d}	39.40±4.05^{d}	9.70±0.23^{bcd}
6.0	4.0	0.12±0.02^{cd}	10.00±0.00^{c}	86.75±0.04^{bc}	15.05±3.68^{e}	98.45±1.30^{b}	43.00±5.79^{cd}	5.67±4.55^{d}
	4.5	0.12±0.01^{cd}	10.00±0.00^{c}	87.12±0.04^{abc}	17.55±1.51^{cde}	75.21±3.78^{e}	45.18±1.43^{cd}	9.27±4.42^{bcd}
	5.0	0.13±0.01^{bcd}	14.00±0.00^{a}	82.74±1.64^{e}	20.57±1.27^{bc}	58.45±1.85^{g}	55.32±1.18^{b}	8.92±4.98^{cd}
7.0	4.0	0.12±0.01^{cd}	11.00±0.00^{b}	86.32±0.06^{c}	20.03±1.11^{bcd}	110.87±2.64^{a}	44.54±4.80^{cd}	8.22±1.25^{cd}
	4.5	0.09±0.00^{e}	14.00±0.00^{a}	84.62±0.15^{d}	23.15±0.16^{ab}	114.93±1.66^{a}	48.46±6.96^{c}	5.59±2.69^{d}
	5.0	0.11±0.00^{de}	14.00±0.00^{a}	82.90±0.13^{e}	26.29±2.99^{a}	99.73±1.66^{b}	39.03±1.23^{d}	9.54±3.10^{bcd}

Data was mean ± standard deviation from three replicate experiments.
$^{a-g}$ Different letters within a column indicated significantly different treatment at $p < 0.05$.
*GAE: Gallic acid equivalents; **PAE: Phytic acid equivalents.

thermophilus and *Lb. bulgaricus* might break down the polyphenols into smaller polyphenol forms during fermentation. Hur *et al.* (2014) also reported that fermentation could improve the amount of phenolic compounds due to a microbial hydrolysis reaction.

DPPH assay was a simple method that gave information regarding the radical scavenging activity of the antioxidant substances present in sample (Najgebauer-Lejko *et al.*, 2011). During assay, the colored DPPH radical was reduced to non-radical DPPH-H in the presence of an antioxidant or a hydrogen donor (Di Cagno *et al*., 2011). The highest DPPH scavenging activity of the black glutinous rice solution was found in the rice solution fermented with 4.0 log cfu/ml *Lb. bulgaricus*. Although phenolics were the major contributors to DPPH

radical scavenging activity (Zhao and Shah, 2014), microbial fermentation could lead to deactivation of bioactive compounds or activation of inactive compounds, for example hydrolysis of glycosides into their aglycones with higher potential for radical scavenging, or breakdown of procyanidins to flavan-3-ols or to small phenolic acids (Sun-Waterhouse *et al.*, 2013).

Phytic acid (phytate) is a chemical compound that possesses anticarcinogenic and antioxidant capacities (Lai *et al*., 2013). However, the compound is generally considered as an antinutritional component due to the capability of phytate to form complexes with metal ions and protein, preventing optimal mineral absorption in the intestine and reducing their bioavailability (Corsetti and Settani, 2007; Lai *et al*., 2013). Therefore, a lower amount of phytic acid in the black glutinous rice solution was desirable and this was found in the rice sample fermented with 7.0 log cfu/ml *Lb. bulgaricus* and had a final pH value of 4.5. Corsetti and Settani (2007) reviewed that phytases, phytic acid hydrolyzing enzymes, were produced by a multitude of microorganisms, including yeasts and sourdough lactic acid bacteria. Raghavendra and Halami (2009) also found that forty lactic acid bacteria isolates from different sources (chicken and fish intestinal source, raw milk, cow dung and cucumber) could degrade calcium phytate in modified MRS agar, while in addition, two strains of *Pediococcus pentosaceus* were able to degrade sodium phytate.

Conclusion

Results in this study clearly demonstrated that the inoculum levels of *Lb. bulgaricus* had a significant role affecting the antioxidant properties of the black glutinous rice solution. At higher inoculum levels of the lactobacilli, the anthocyanin and phenolic contents of the rice solution were enhanced, while the phytic acid was decreased. This suggested that doing fermentation for food containing phenolic compounds needed a suitable inoculum level to maintain and/or improve its functional properties. Since the capability to metabolize phenolic compounds was strain- or species-dependent (Filannino *et al*., 2015), other species of lactic acid bacteria needed to be evaluated to determine the optimal species to be used in the fermentation of black glutinous rice solution.

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