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Yoghurt Production from Germinated Native Black Rice (Maepayatong Dum Rice)

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Abstract The incubation period of 12, 24, 36, 48, 60, and h72 for germination on the quantity of γ-amino butyric acid of native black rice (Maepayatong dum rice) were conducted. The result found that the 48h of germination time had significantly ($p \leq 0.05$) the highest amount of γ-amino butyric acid (27.99 ± 1.44 mg/100 g). This treatment was therefore selected to further determine the product development of germinated native black rice yoghurt. Changes in physical and chemical properties during fermentation under controlled conditions (42 ℃) at 0h, 2h, 4h, 6h and 8h fermentation were evaluated. The result found that change in the amount of titratable acidity expressed as lactic acid and apparent viscosity of germinated black rice yoghurt were increased at the end of 8h fermentation. On the other hand, the consistency, pH, and total soluble solid were slightly decreased and the lowest at the end of 8h fermentation. From sensory evaluation, the germinated native black rice yoghurt fermentation by 20% w/w of Revon starter) had the highest overall acceptability (6.88: moderately like). It was then chosen to study on the effect of shelf life on yoghurt properties every week until 4 weeks of storage. The consistency, pH, and viable cell count were slightly decreased; while apparent viscosity, lightness, redness and lactic acid were increased when the shelf life was longer. Interestingly, the free radical scavenging capacity assayed by DPPH method showed that IC_{50} values had significantly ($p \leq 0.05$) higher than the control (86.92 ±1.21 and 96.61 ±1.25 mg/ml, respectively). Although, the amount of γ-aminobutyric acid (GABA) content exhibited significantly ($p \leq 0.05$) two fold lower than the control (0.99 ±0.03 and 1.80 ±0.07 mg/100g, respectively). It concluded that the research finding could increase the value addition of native black rice and should be guided to develop the other healthy food in the future.

Keywords: yoghurt, germinated native black rice, probiotic,γ-aminobutyric acid (GABA)

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Introduction

Yoghurt is a healthy food due to the beneficial aspects of its high protein and calcium contents (McKinley, 2005). This product is fermented by lactic acid bacteria with is probiotic microorganism. Probiotics have several beneficial effects on human health. Lactic acid bacteria have been proven to exert health-promoting activities such as adjustment of the immune response to a desired level, enhancement of resistance against pathogens, and reduction of blood cholesterol levels (Karovicova and Kohajdova, 2003). Normally, yoghurts are produced using milk as a raw material. Available probiotic products on the market are usually dairy-based. However, there are consumers who suffer lactose intolerance and allergies due to milk proteins (Sharma and Misha, 2008). In addition, with an increase in vegetarian consumers throughout developed countries, there is also a demand for vegetarian probiotic products.

Rice (*Oryza sativa* L.) is the principal staple food for more than half of the world's population involving Thailand (Banchuen *et al.,* 2010). In fact, Thai farmers grow rice in many areas of Thailand, including Chanthaburi province. Native black rice (Maepayatong dum rice) is the traditional rice varieties in the district of Kao Kitchakut, Chanthaburi province. The pigment of this rice is black. In rice pigmentation, anthocyanin is reported to possess a free radical scavenging activity (Sangkitikomon *et al.,* 2008). In addition, black rice contains more nutritional components such as dietary fibers, phytic acid, vitamins E and B than the ordinary milled rice (Tananuwong and Tewaruth, 2010). Moreover, Sangkitikomon *et al*. (2008) reported that anthocyanin from black rice has higher antioxidant activity than red rice and rice berry. For this reason, black rice is the famous ingredient used in snacks and desserts (Tananuwong and Tewaruth, 2010). Germinated black rice offers more considerable benefit. Especially it has an increasing GABA, dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor. GABA is a neurotransmitter in the brain and in the spinal cord of mammals. This substance can lower hypertension, promote sleepiness, and has benefit for human health (Okada, *et al.*, 2000). Additionally, the germination of black rice releases its bound minerals which makes the rice more absorbable by the body, tender and tastier (Karahara, 2004).

Since, this rice has been reported to have high antioxidant activity (Sangkitikomon *et al.,* 2008), there was no information reported to produce non-diary probiotic yoghurt from germinated native black rice. Therefore, the aim of this study was to investigate the effect of incubation time to germination percentage, length of roots, moisture content, and γ-aminobutyric acid (GABA) content;.the highest GABA content treatment was opted to evaluate product development of yoghurt production; and finally, antioxidant activity and GABA content were compared between un-fermented sample and rice yoghurt.

Materials and methods

Materials

Native black rice (Maepayatong dum rice) was purchased from a local farmer in the district of Kao Kitchakut, Chanthaburi province and then transported to the laboratory.

Germinated native black rice preparation

Germinated native black rice was prepared according to modified methods described by Panyanak *et al.* (2010). Briefly, the samples were selected and soaked in water at the ratio of rice and water (1:10) at 40 ℃ for 6 hours in the tray. Then, the water was drained. The samples of rice were incubated in various durations: 0, 12, 24, 36, and 48 hours. The germination was stopped by drying using a hot air oven, at 55 ℃ for 4.5 hours. Then, the obtained germinated native black rice was stored at room temperature (37 ℃) and monitored for germination percentage, length of roots, moisture content, and GABA content, respectively.

Moisture content determination

Moisture content evaluation was performed using the Official method of AOAC (1990). Germinated native black rice sample (3 g) was used in the assessment.

γ-Aminobutyric acid (GABA)determination

GABA content was sent to analyze by the Institute of Food Research and Product Development (IFRPD) at Kasetsart University in Bangkok, Thailand. A sample of 2.5 g was added to 18 ml of distilled water and 2 ml of 3% sulfosalicylic acid, respectively. Then, the mixture was stirred for 30 minutes and centrifuged. The supernatant (0.1 ml) was mixed with 0.1 ml of $NaHCO_3$ and 0.40 ml of debsyl-C and these solutions were mixed together. The solution was placed in water bath at 70 ℃ for 10 minutes. The obtained solution was then mixed with 0.25 ml of ethanol and 0.25 ml of 0.025 M KH_2PO_2. The γ-aminobutyric acid (GABA) was evaluated using a high performance liquid chromatography (HPLC) (HPLC-UV detector: agilent, 1,200 serice; column:supercosil LC-DABS, 15 cm x 4.6 cm,3 um; flow rate 1 ml/min; mobile phase: gradient 80 % CH_3COONa pH 6.80, acetonitrile inject volume 5 ul; column temperature 40 °C, detector: uv 465 nm and standard GABA ≥ 99 %) (Tadashi *et al*., 1998).

Strain and Culture

Two type starter cultures such as YC 380 and Revon were purchased from Chr.Hansen, Denmark and local convenicece store in Thailand, respectively. For YC380, the culture was grown at 37 ℃ for 24 hours in rice milk (rice milk 92 %w/w, sucrose 3 %w/w and lactose 5 % w/w) was used as inoculums.

Production of rice yoghurt

Rice yoghurt was prepared. The previous high GABA content germinated native black rice was cooked in a rice cooker with a ratio of 1:2 (rice and water). The cooked rice was blended with water using a blender with a ratio of 1:2 (rice and water) and then combined and filtered by a straining cloth. The germinated native black rice milk was then added with 3% (w/v) sugar and 5% (w/v) lactose. The rice media were prepared in the small polypropylene container (50 ml) which were pasteurized at 80 ℃ for 10 minutes. Experimental design used was factorial in randomized completely block design (RCBD) for sensory evaluation and factorial in completely randomized design (CRD) for properties determination. The starter cultures were added per various type and concentration as presented in Table1.

Table 1. Nine treatments of germinated native black rice yoghurt

Treatments	**Type and concentration (%w/w)**	
	YC380	**Revon**
1(Control)	-	-
2	10	-
3	20	-
4	-	10
5	-	20
6	10	10
7	10	20
8	20	10
9	20	20

Then, the samples were incubated at 42℃ for 8 hours and were determined for sensory evaluation. Change in chemical and microbiological of the fermented yoghurt was monitored every 2 hours until 8 hours.

Chemical and Physical properties Analyses

Samples were taken at 2 hours intervals until 8 hours for chemical and physical analysis. The pH was measured with a pH meter (Subtex, Taiwan). Total acidity expressed as percent lactic acid was determined by titrating with 0.02 N NaOH to pH 8.2. Total soluble solid was analyzed by hand refractometer (Atago, Japan). The color of the samples was analyzed using a color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. Color measurement was expressed in L*(lightness), a* (red-green component)) and b* (yellow-blue component) where L* refers to the lightness on a 0 to 100 scale from black to white, while b* (+,-) refers to the yellowness or blueness, respectively. For apparent viscosity and the test sample (60g at 37℃) were evaluated using a Bookfield. The consistency was determined using a Bostwick consistometer.

Sensory Evaluation

The non-diary probiotic germinated native black rice yoghurt fermentation for 8 hours at 42 ℃ was sensory evaluated with 50 untrained panelists from the staff and students of the Department of Product Development and Management Technology at Rajamangala University of Technology Tawan-ok Chanthaburi campus. The panelists evaluated the samples using a nine-point hedonic scales ranging from 1(extremely disliked) to 9(extremely liked) (Watts *et al.*, 1989). Each panelist evaluated the samples for colour, aroma, taste, texture, and overall acceptability.

Effect of cold storage on the properties of the probiotic native black rice yoghurt

After 8h of fermentation at 42℃, the fermented samples (50 g) were stored at 4° ℃ for 4 weeks. Samples were taken at weekly intervals. Then, the fermented samples were analyzed for its physical, chemical, and microbiological properties. Regarding physical property, the samples were monitored for apparent viscosity, consistency, and colour as described previously. For chemical property, the yoghurt samples were evaluated for its total soluble solid, pH, and titratable acidity (lactic acid). For the microbiological, viable cell counts (log CFU/ml) were evaluated by the standard plate count method with lactobacilli MRS medium after 48h inoculation at 37 ℃ and expressed as colony forming unit (CFU).

DPPH radical scavenging activity determination

The free radical scavenging activity was sent to analyze by the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) in Bangkok, Thailand. DPPH radical scavenging activity procedure was performed according to the methods described by Zhu *et al.* (2006)**.** Briefly, one gram of sample was extracted with 10 ml ethanol. The solution was separated by centrifugation at 6,000 rpm. The obtained supernatant was tested by mixing with ethanol at various concentrations of 10, 20, 30, 40, and 50 μg/ml. The sample (1 ml) was mixed with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 95% ethanol (1ml) and incubated in dark condition for 30 minutes. The absorbance was determined using a spectrophotometer at 517 nm. Vitamin C (L-ascorbic acid), Vitamin E (Tocopherol), and BHT (Butylated Hydroxyl Toluen) were used in the reference standard compound. The percentage of radical scavenging activity was calculated with the following equation:

DPPH radical scavenging activity (%) = $[(A_0 – A_1)/ A_0] \times 100$

A_0 = the absorbance of control reaction

A_1 = the absorbance of test compound

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against the sample concentration.

Bacterial cell behaviour

The final germinated native black rice yoghurt were sampled from the culture and were photographed with a scanning electron microscopy (SEM) (JEOL, model JSM-MEDEL jsm-5410LV, Japan) at a magnification of 5,000x and 50,000x to capture images for the lactic acid bacterial cell behaviour grown under the culture condition.

Data analysis

Analysis of the above mentioned properties were carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$). Mean with significant differences was separated by Duncan’s multiple range test (DMRT) using computer software.

Results

Effect of incubation time on germination percentage, length of roots, moisture content and GA BA content

The effect of incubation time on germination percentage, length of roots, content is shown in Table1. Both germination percentage and length of roots increased dramatically when the incubation time was longer. Significantly highest %germination and length of roots significantly increased at 48 hours of incubation (96.10 ± 1.65 % and 2.90 ± 0.00 mm, respectively). However, moister content decreased when dried by hot air oven at 55 ℃ for 4.5 hours in all of the treatments comparing with control (0 h incubation). The effect of incubation time on GABA content is represented in Figure 1. Significantly highest GABA content was found when the incubation time was at 48 hours (27.99 ± 1.44 mg/100g). Therefore, GABA rice with incubation time of 48 hours was selected to determine further product development of yoghurt production.

Table 2. Effect of incubation time on germination percentage, length of roots and moisture content of germinated native black rice

Incubation time(hour)	%Germination	Length of roots (mm)	Moisture content (%w/w)
0	0.00±0.00^{c}	0.00±0.00^{d}	11.24±0.49^{a}
12	0.00±0.00^{c}	0.00±0.00^{d}	9.59±0.16^{b}
24	94.00±1.08^{b}	1.50±0.00^{c}	9.50±0.26^{b}
36	95.00±1.22^{ab}	2.08±0.05^{b}	9.52±0.24^{b}
48	96.10±1.65^{a}	2.90±0.00^{a}	9.55±0.16^{b}

Mean values ± standard deviation (n = 3) with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test (DMRT).

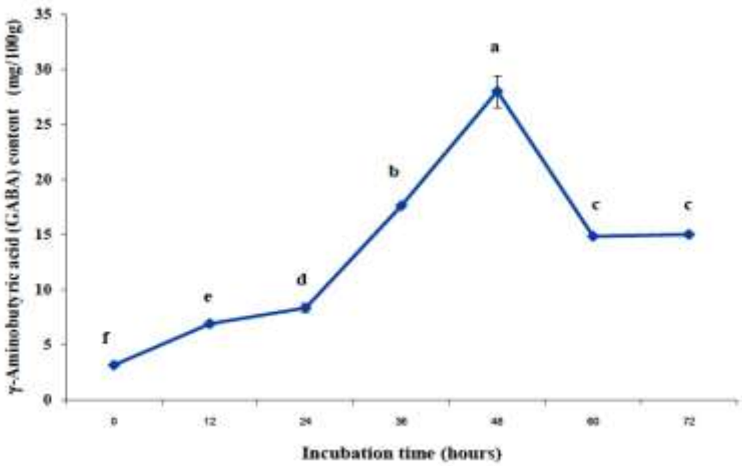

Figure 1. Effect of incubation time on γ-aminobutyric acid (GABA) content of germinated native black rice. Bars represent standard deviation from duplicate determination.

Subsequently, the GABA rice was studied for product development of yoghurt. Nine treatments of germinated native black rice yoghurt were fermented with starter at 42 ℃ until 8 hours. Change in physical properties, and chemical properties during the fermentation determined every 2 hour until 8 hour can be seen in Figures 2-4, respectively.

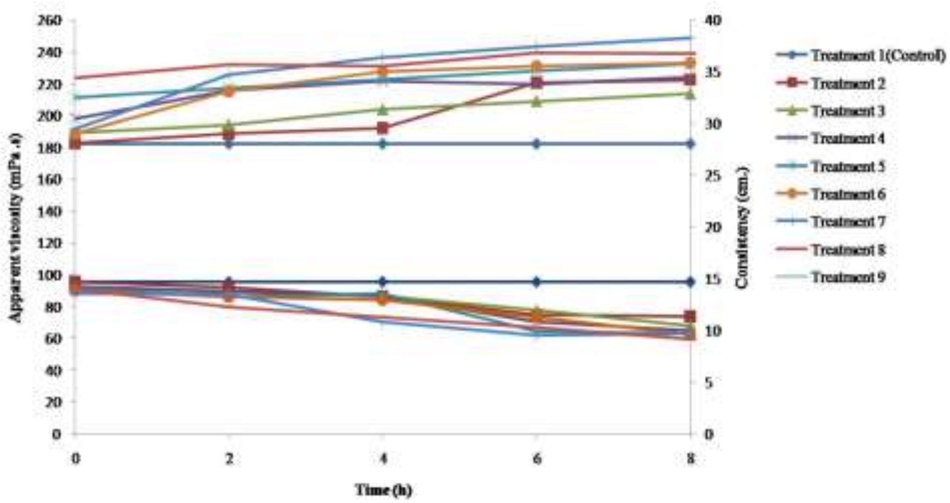

Figure 2. Apparent viscosity and consistency of yoghurt produced from germinated native black rice during fermentation for 8 hours at 42 ℃.

It showed no interaction between the type and concentration of yoghurt starter for change of the properties during fermentation were observed. Change in apparent viscosity and consistency of germinated native black rice yoghurt during 8h fermentation at 42 ℃ indicating the progress of fermentation process is shown in Figure 2. The samples were collected at 2 hours intervals until 8 hours and the data were exhibited by triplicate determinatation ± standard deviation. All treatments exhibited similar results when compared to the control. The apparent viscosity increased, while consistency decreased significantly different ($p \leq 0.05$). At the initial stage of fermentation, the apparent viscosity of rice yoghurt was found to be approximately 180- 225 mPa.s. After the start of fermentation, the apparent viscosity of rice yoghurt slightly increased. In contrast, the consistency of rice yoghurt exhibited approximately 14 cm at the initial stage of fermentation. After the start of fermentation, the consistency of rice yoghurt slightly decreased.

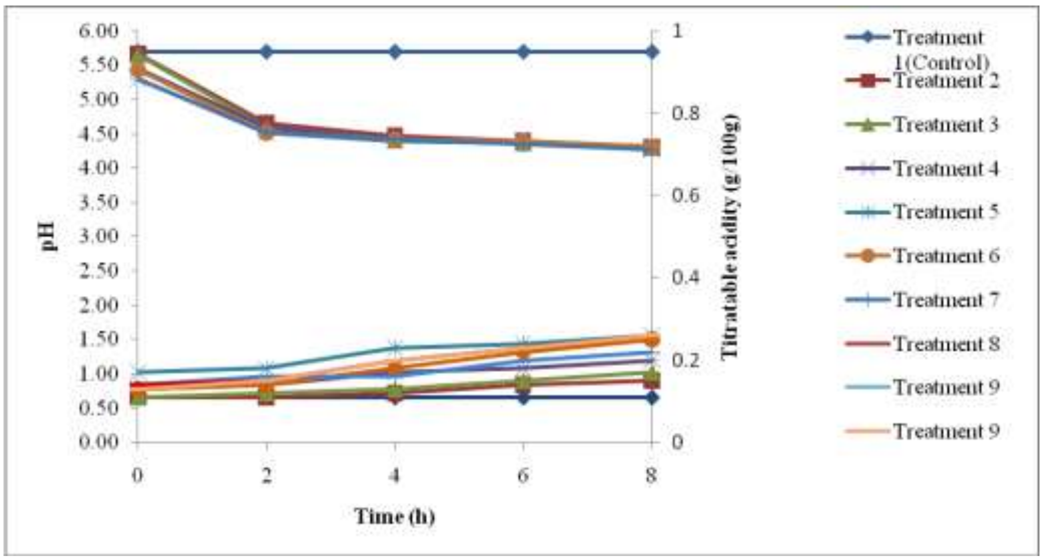

Figure 3. pH and titratable acidity of yoghurt produced from germinated native black rice during fermentation for 8 hours at 42 ℃.

Changes in pH and titratable acidity (lactic acid) were compared in all of the treatments during fermentation (Fig. 3). The initial stage of fermentation and pH of rice yoghurt were found to be approximately 5.50 when compared to the control. After fermentation started, the pH of rice yoghurt dramatically decreased in all treatments. However, the level of titratable acidity slightly increased. At the initial stage of fermentation, the numbers of acidity of rice yoghurt were found to be approximately 0.10 g/100g. After the start of fermentation, the numbers of acidity of rice yoghurt slightly increased around 0.20 g/100g in all of the treatments. Moreover, the total soluble solid was slightly decreased in the end of fermentation in all of the as shown in Figure 4.

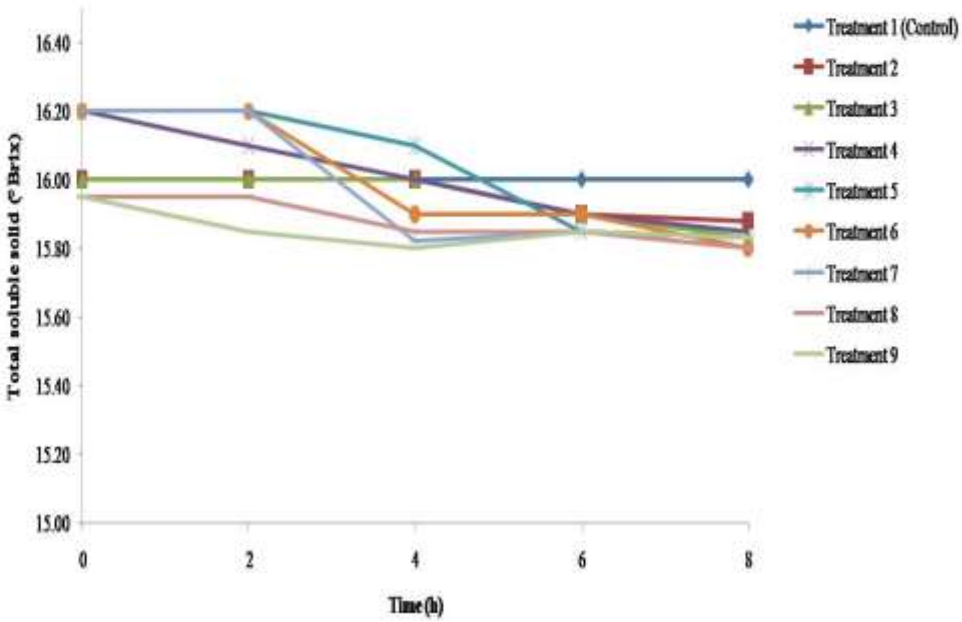

Figure 4. Total luble solid of yoghurt produced from germinated native black rice during fermentation for 8 hours at 42 ℃.

Regarding the sensory evaluation, it was found that treatment 5 (fermented with 20%w/w Revon) was the highest acceptable for overall liking as presented in Table 3 with the score of 6.88 ± 1.26 (moderately like). On the other hand, it was not significantly different from treatments 6, 7, 8, and 9.

Table 3. Mean sensory scores of germinated native black rice yoghurt

Treatments	Preference scores (Mean ± SD)				
	Colour	Aroma	Taste	Texture	Overall liking
1	n.d.	n.d.	n.d.	n.d.	n.d.
2	4.82 ± 1.71^{b}	5.10 ± 1.36^{c}	4.48 ± 1.57^{c}	4.84 ± 1.54^{b}	4.84 ± 1.52^{c}
3	5.00 ± 1.91^{b}	4.94 ± 1.58^{c}	4.66 ± 1.57^{c}	4.62 ± 1.65^{b}	4.84 ± 1.46^{c}
4	6.50 ± 1.16^{a}	6.04 ± 1.19^{b}	6.40 ± 1.28^{ab}	6.34 ± 1.22^{a}	6.26 ± 1.12^{b}
5	7.08 ± 0.99^{a}	6.82 ± 1.40^{a}	6.90 ± 1.37^{a}	6.68 ± 1.43^{a}	6.88 ± 1.26^{a}
6	6.56 ± 1.09^{a}	6.16 ± 1.22^{ab}	6.64 ± 1.14^{ab}	6.48 ± 1.25^{a}	6.40 ± 1.03^{ab}
7	6.76 ± 1.12^{a}	6.34 ± 1.33^{ab}	6.42 ± 1.30^{ab}	6.40 ± 1.34^{a}	6.54 ± 1.23^{ab}
8	6.88 ± 1.35^{a}	6.30 ± 1.37^{ab}	6.46 ± 1.09^{ab}	6.50 ± 1.27^{a}	6.54 ± 1.03^{ab}
9	7.06 ± 1.04^{a}	6.28 ± 1.28^{b}	6.30 ± 1.34^{b}	6.80 ± 0.88^{a}	5.58 ± 1.07^{ab}

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test. n.d.,not determined.

Consequently, this treatment was selected to evaluate influence of cold storage on physical properties, chemical properties, and microbiolgical properties when the shelf life was longer. The apparent viscosity, lightness(L*), redness (a*) and acidity increased,while consistency , pH, and viable cell count decreased (Tables 4,5). At the initial stage of storage, apparent viscosity, lightness (L*), redness (a*) and acidity exhibited 232.50±1.27 mPa.s , 40.68±0.10%, 6.99±0.12% and 0.26±0.01%, respectively. At the end of storage, the level of the datas increased to 960.70±8.34 mPa.s, 41.45±0.07%, 7.14±0.10%, 0.70±0.07%, respectively. In contrast, consistency, pH, and viable cell count were found to be at 8.83 ±0.2 cm., 4.28±0.02 and 6.34±0.02 log CFU/g at the initial stage of storaged then the numbers of data were decreased to 3.42±0.20 cm., 3.77±0.02, and 4.54±0.06 log CFU/g, respectively.

Table 4. Effect of cold storage on the physical properties of germinated native black rice yoghurt

Time (weeks)	Apparent viscosity (mPa.s)	Consistency (cm.)	colour		
			L*	a*	b^{*ns}
0	232.50 ±1.27^{e}	8.83 ±0.26^{a}	40.68±0.10^{d}	6.99±0.12^{c}	5.04±0.10
1	606.70 ±7.78^{d}	8.67 ± 0.26^{a}	40.78±0.05^{c}	7.03±0.09^{bc}	5.19±0.04
2	680.80 ±9.26^{d}	7.42±0.78^{b}	40.85±0.06^{c}	7.17±0.03^{a}	5.13±0.20
3	818.90 ±7.92^{d}	3.92±0.58^{c}	41.13±0.04^{b}	7.06±0.24^{bc}	5.11±0.27
4	960.70 ±8.34^{d}	3.42±0.20^{d}	41.45±0.07^{a}	7.14±0.10^{ab}	5.06±0.13

Table 5. Effect of cold storage on the chemical properties and microbiological properties of germinated native black rice yoghurt

Time (weeks)	Total soluble solid (°Brix)^{ns}	pH	Acidity (%lactic acid)	Viable plate count (log CFU/g)
0	15.80±0.05	4.28±0.02^{a}	0.26±0.01^{e}	6.34±0.02^{c}
1	15.80±0.03	4.21±0.01^{b}	0.29±0.05^{d}	6.66±0.04^{b}
2	15.80±0.04	4.17±0.01^{c}	0.38±0.02^{c}	6.93±0.05^{a}
3	15.80±0.03	3.82±0.00^{d}	0.49±0.03^{b}	6.13±0.02^{d}
4	15.80±0.00	3.77±0.02^{e}	0.70±0.07^{a}	4.54±0.06^{e}

Finally, the final fermented yoghurt was compared with the control; the antioxidant activity of germinated black rice yoghurt exhibited higher than that control with significantly difference ($p \leq 0.05$). The number of IC50 was 86.92±1.21 and 96.61 ±1.25 mg/ml, respectively. However, GABA content of germinated black rice yoghurt was lower than that of the control. The number of GABA content was 0.99 ± 0.03 and 1.80 ±0.07mg/100g, respectively.

Table 6. Antioxidant activity and γ-aminobutyric acid (GABA) content of germinated native black rice yoghurt

Treatments	DPPH assay IC50 (mg/ml)	GABA content (mg/100g)
Control	96.61 ±1.25^{b}	1.80 ±0.07^{a}
Germinated black rice yoghurt	86.92±1.21^{a}	0.99 ± 0.03^{b}

Mean with different letters are statistically different ($p \leq 0.05$) according to T-test.

From scanning electron microscope, it was found that the lactic acid bacteria lived by mixing in the germinated native black rice as shown in Figure 5. The pictures showed that germinated native black rice could be a good raw material for lactic acid fermentation.

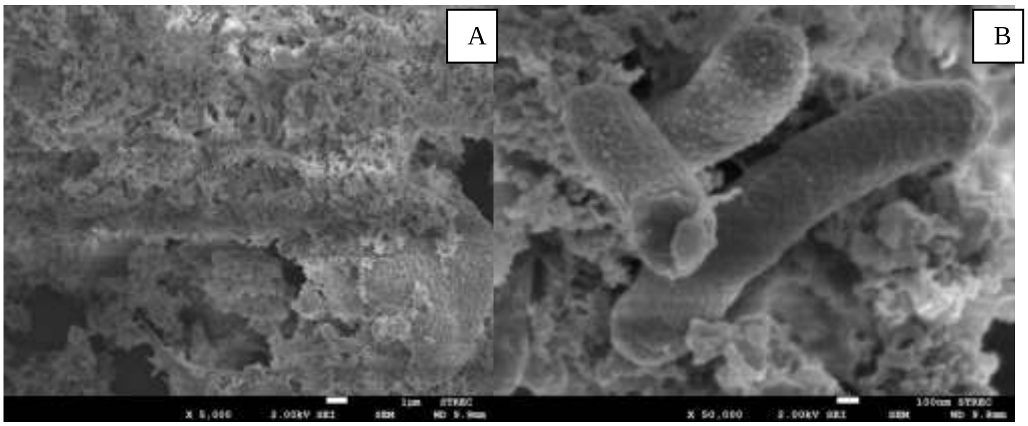

Figure 5. The electron micrograph of Revon starter in germinated native black rice yoghurt at magnification 5,000X (A) and 50,000X (B)

Discussion

As shown in Table 1, both germination percentage and length of roots increased dramatically when the incubation time was longer. This result agreed with Pongpipatpong *et al.* (2013), they found that longer incubation time also increased germination percentage. For the moisture content, the datas were found lower than that of control in all of treatments. This could be because control sample was the originally moisture content of rice. Regarding of γ-aminobutyric acid black (GABA) content, the level of GABA content trended to be drammatically signicicantly ($p \leq 0.05$) increased and significantly highest the level of GABA content was found when incubation time of 48 hours (27.99 ± 1.44 mg/100g). Therefore, the GABA content was then determined at the incubation time longer for confirmation as represented in Figure 1. However, the numbers of GABA content of 60 and 72 hours was significantly ($p \leq 0.05$) lower when than that the incubation time of 48 hours. So, this treatment was selected to determine further product development of yoghurt production. After the fermentation start, changes in chemical and physical properties were evaluated. The change in the amount of titratable acidity expressed as lactic acid and apparent viscosity of germinated black rice yoghurt were increased at the end of fermentation (8h). However, the consistency, pH and total soluble solid were slightly decreased and the lowest at the end of fermentation (8h). It may due to the lactic acid bacteria had metabolized glucose the pH was then decreased and the total soluble solid slightly decreased in the end of fermentation in all of the treatments (Lee and Lucey, 2010). From sensory evaluation, treatment 5 (germinated native black rice yoghurt fermented by 20% w/w of Revon starter) had the highest overall acceptability (moderately like). Then, this treatment was selected to study on the effect of shelf life on yoghurt properties every week until the 4 weeks of storage. The consistency, pH, and viable cell count were slightly decreased; while viscosity, lightness, redness (a*) and lactic acid were increased when the shelf life was longer. This could be explained that when the shelf life was prolonged, the metabolism of lactic acid bacteria resulting in more amount of acid to produce. For this reason, when the pH decreased, then the effect to decrease of consistency. According to the high acid condition and lack of food, the number of lactic acid bacteria was dramatically decreased approximately by 2 log cycle at the 4 weeks of storage (Lee and Lucey, 2010). Finally, the final fermented yoghurt properties was compared with the control; the antioxidant activity of germinated black rice yoghurt exhibited higher than that control with significantly difference ($p \leq 0.05$). However, GABA content of germinated black rice yoghurt was lower than that of the control. The result was agree with our previously research, the level of GABA content of germinated native black rice (Kowmak) was also twice lower than that control (Mongkontanawat and Lertnimitmongkol, 2015). As previously reported, the GABA content of germinated brown rice did not change after pasteurization by steaming (Komatsuzuki *et al*, 2005; Anawachkul and Jiamyangyuen, 2009). With the results of the study, it could be confirmed that the effect of GABA content could be due to a lot of reasons. One reason could be that the metabolized acid or enzyme destroyed the GABA molecule. Nevertheless, this reason could be confirmed in the further study.

Conclusion

The results showed that 48h of germination had significantly ($p \leq 0.05$) the highest amount of γ-amino butyric acid. Therefore, this treatment was selected to further investigate on product development of germinated native black rice yoghurt. The change in the amount of titratable acidity expressed as lactic acid and apparent viscosity of germinated black rice yoghurt were increased at the end of fermentation (8h). On the other hand, the consistency, pH and total soluble solid were slightly decreased and the lowest at the end of fermentation (8h). From sensory evaluation, treatment 5 (germinated native black rice yoghurt fermented by 20% w/w of Revon starter) had the highest overall acceptability (moderately like). Consequently, this treatment was selected to study on the effect of shelf life on yoghurt properties every week until the 4 weeks of storage. The consistency, pH, and viable cell count were slightly decreased; while viscosity, lightness, redness (a*) and lactic acid were increased when the shelf life was longer. Although, the amount of γ-aminobutyric acid (GABA) content exhibited significantly ($p \leq 0.05$) two fold lower than that of control, the consumers still recived enough probiotic microorganism. Interestingly, the free radical scavenging capacity was assayed by DPPH method shown that IC_{50} values had significantly ($p \leq 0.05$) higher than that of control. In summary, fermented germinated native black rice yoghurt could be used as a good raw material for lactic acid fermentation, and the product could serve as a healthy food for vegetarians and milk allergy consumers. However, the taste could be improved in order to increase the acceptabitity of the product.

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