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Product Development of Sweet Fermented Rice (Khao-Mak) from Germinated Native Black Glutinous Rice

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Product development of sweet fermented glutinous rice (Khao-Mak) of germinated native black glutinous rice was determined. Experiments were designed by using Randomized Complete Block Design (RCBD) with composed of 6 treatments. There are the ratio of glutinous rice and germinated native black glutinous rice 100:0,80 :20,60 :40,40 :60,20 : 80 and 0:100, respectively. Sensory evaluation was determined by using 9-point hedonic scale. The results were found that most of consumer liked treatment 2 (glutinous rice : germinated native black glutinous rice; 80:20) with the highest score 8.10 (like) and significantly different from another treatments ($p \leq 0.05$). Then change in chemical characteristic (pH, total soluble solid, % alcohol and anthocyanin content) during fermentation were studied. The results exhibited that the similar trends was found in all of treatments. The physical, chemical and microbiological characteristic of final product were L* a* b* (12.44, 11.81 , 28.72), chemical composition; moister, ash, lipid, protein, fiber and carbohydrate content were 55.86, 0.29, 0.35, 3.11, 1.59 and 38.80 (%wb), respectively, total soluble solid (5.23 ◦ brix), pH (4.34), alcohol content (1.15 %), the total plate count (8.00 $x10^3$ cfu/g), yeast and molds count (5.30 x 10^3 cfu/g) and *E. coli* were in the community standard product of Thailand. Finally, the free radical scavenging capacity were assay by DPPH method shown that IC_{50} value had significantly higher three fold than control (59.70 ± 1.28 and 199.38 ± 2.59 mg/ml, respectively) ($p \leq 0.05$). In addition, γ-aminobutyric acid (GABA) content were found 6.53 mg/100 g. In conclusion, the Khao-Mak from germinated black native glutinous rice from this research could be a new healthy product in the future.

Keywords: sweet fermented rice (Khao-Mak), germinated native black rice, radical scavenging capacity, γ-aminobutyric acid (GABA)

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Introduction

Rice (*Oryza sativa* L.) is the principal staple food for more than half of the world's population involing Thailand (Chanta *et al.,* 2014). However, nowadays, since widespread of western culture in Thailand making the trend of rice products consumption have been reduced. Especially, thai traditional fermented product have been ignored for young generation. Although, many product have been found to health care benefite.

Sweet fermented glutinous rice "Khao-Mak" are known for their health benefits. Khao-Mak is sweetened glutinous rice fermented to give the special taste and flavour of alcohol and lactic acid (Tanasupawat and Komagata, 1995). It is a kind of food brewed with cooked glutinous rice and Look-Pang (tradition fermentation starter). Look-Pang is a semicircle starch ball which contains mold (*Aspergillus* species, *Rhizopus* species and *Mucor* species) and yeast (*Saccharomyces cerevisiae* and *Candida* species) inoculum in rice flour mixed with herbs such as pepper, garlic and galangal as antibacterial agent (Ministry of Industry, 2003; Tochumpa, *et al.*, 2011; Manosroi *et al*., 2011). Enzyme (α-amylase, glucoamylase) from the mold hydrolyze starch in the rice to sugars, which are partially fermented to alcohol by the yeast. Organic acid such as lactic acid are also produced (Lotong, 1992). The product is packed in banana leaves or plastic cup and left to ferment for 2-3 days at ambient temperature (Ministry of Industry, 2003). This fermented product has varies characters such as lump of cooked glutinous rice, soft texture, pale white color, succulent grain, sweet taste and a little alcohol flavor (Wongpiyachon,1995). Most of the time, Khao-Mak is a traditional fermented rice product in many part of Thailand. Normally, it is well known for a sweet dessert of Thailand. They are produced in family or the community level. In thai folklore wisdom, Khao-Mak has been considered to promote the growth development of malnutritioned children, active bacterial activity, and used as a dietary supplement (Tongyai *et al.*, 2011; Manosroi *et al*., 2011). This is a probiotic which consists of live microorganisms and can have a health benefit on the host when administered in adequate amounts. Since, the fermentation process of Khao-Mak has used mold and yeast such as *Saccharomyces cerevisiae* strain which had been approved to be probiotics (De Llanos *et al*., 2006). It also increases and maintains a heathy bacteria gut flora by providing and increasing amount of food for these bacteria (Tongyai *et al.,* 2012).

In fact, Thai farmer grown rice in many area of Thailand, also in Chanthaburi provine. Native black rice (Khaohom Maephayatongdam) is the traditional rice varities plant in Amphur Kao Kitchakut, Chanthaburi province. The pigment of this rice is back. In pigment rice, there is a natural colorant ; anthocyanin. Which is reported to posses a free radical scavenging activity (Oki *et al.*, 2002). In addition, black rice contains more nutritional components such as dietary fibers, phytic acid, vitamin E and vitamin B, than the ordinary milled rice (Banchuen *et al*., 2010). Moreover, Sangkitikomon *et al.* (2008) reported that anthocyanin from black rice was found higher antioxidant activity than red rice and rice berry. From the nutional point of view, black rice is the most famous one and generally used as an ingredient is snack and desserts (Tananuwong and Tewaruth, 2010). Germinated black rice offers considerable more benefit. Especially, it had an increasing in γ-aminobutyric acid (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 the spinal cord of mammals. This substance can lower hypertension, promote the sleepiness and has the benefit for human health (Okada *et al*., 2000). Additionally, the germination of black rice frees it bound minerals, making them more absorbable by the body and the rice tendered and testier (Kayahara, 2004).

Traditionally, Khao-Mak usually make from glutinous rice (Manosroi *et al*., 2011), or mixed with cooked black glutinous rice (Tongyai *et al.*, 2012). Since, black glutinous rice is a rich source of phytochemicals such as anthocyanin. On the other hand, no information of produce Khao-Mak from germinated native black glutinous rice was reported. Therefore, the objective of this study were to develop of Khao-Mak from germinated native black glutinous rice, change in chemical properties during fermentation were investigated. Then the best treatment was selected to determine physical properties and microbiological characteristic. Finally, antioxidant activity and γ-aminobutyric acid (GABA) content were determined.

Materials and methods

Materials

Glutinous rice and Look-Pang were all purchased from a local market at Krating market in Amphur Kao Kitchakut. Native black glutinous rice was purchased from a local farmer and transported to the laboratory.

Germinated native black glutinous rice Preparation

Native black glutinous rice was prepared by the method of Panyanak *et al.* (2010). Brifty, it was selected and soaked with water at the ratio rice and water ; 1: 5 for 6 hours in the liter bottle. The water was drained and incubated for 24 hours. The germination were stop by steaming 15 minutes and dried by using hot air oven at 60 ℃ for 4 hours.

Khao-Mak Preparation

Glutinous rice (500 g) was soaked with water for 6 hours and then steamed for 45 minute. The sample was cool down and washed with water. For black native glutinous rice was prepared as the method of Tongyai *et al.* (2012). Then, black native glutinous rice were mixed with glutinous as the various ratio such as 100:0, 80: 20, 60:40, 40:60, 20:80 and 0:100, respectively. The cooked rice was mixed with Look-Pang 0.2 % w/w of sample, mixed with 80 g sugar and fermented in the plastic cup at room temperature for 7 days.

Product development of Khao-Mak

The optimum quantity of germinated native black glutinous rice for Khao-Mak production was studied by using Randomized Complete Block Design (RCBD) which is composed of the ratio of glutinous rice and germinated native black glutinous rice such as

100:0, 80: 20, 60:40, 40:60, 20:80 and 0:100, respectively. Then the change of chemical characteristic during fermentation and sensory evalution were done as describe below.

Change in chemical characteristic determination

Six treatments of germinated native back rice Khao-Mak were prepared as described previously , taken at day 0, 1, 2, 3, 5 and 7 for analyses. Then, the total soluble solid was determined by using density meter (DMA 4100, Anton Paar, German), pH by using pH-meter (Subtex, Taiwan), alcohol by density meter (DMA 4100, Anton Paar, German), total anthocyanin content was determined by the method of Lee *et al.* (2005). Brifely, the sample were mixed with 1N HCl with the ratio 1:10. The solution were adjusted pH to 1 with 4 N HCl and shaking at 100 rpm for 24 hours at room temperature. The supernatant were centrifuged at 3,461 rpm 10 minute and the absorbance were determined at 535 nm. The total anthocyanin content were calculated as equation:

total anthocyanin content (mg/l) = $(A \times MW \times DF \times 10^3)/(\varepsilon \times l)$

A = (A520-A700)pH1- (A520- A700) pH4.5
MW = 499.2 g/mol (cyanidine-3-glucoside)
DF = dilution factor
ε= molar extinction coefficient 26,900 (l.mol-1.cm-1) (cyanidine-3-glucoside)
l = the width of cuvettes (cm)

Sensory evalution

Six treatments of germinated native black glutinous rice Khao-Mak for 3 day fermentation were sensory evalution with 50 untrained panelists from the staff and students of Department of Product development and Management Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi campus. They evaluated the sample using a nine point hedonic scals ranging from 1(extremely disliked) to 9(extremely liked) (Watts *et al.*, 1989). Each panelist evaluated the samples for color, flavor, taste, texture and overall acceptability. Then the best treatment was selected to evaluate for the physical, chemical characteristic, microbiological characteristic, antioxidant activity and γ-amino butyric acid content (GABA), respectively.

Physical characteristic determination

The final fermented Khao-Mak were conducted for colure by using a Color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with standard plate. Color measurement were expressed in L* indicates the lightness on a 0 to 100 scale from black to white. a* (+,-) are indicates the redness or greeness,respectively. b* (+,-) are indicates yellowness and buleness, respectively.

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Microbiological characteristic determination

The final fermented Khao-Mak was examined total microorganism , mold and yeast by using total plate count on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA), respectively. Moreover, *Escherichai coli* (*E. coli*)were sent to analyze by Institute of Food Research and Product Development (IFRPD), Kasetsart university, Bangkok, Thailand.

DPPH radical scavenging activity determination

The free radical scavenging activity was determined by the method of Zhu *et al.* (2006) of Kasetsart Agricultural and Agro-Industril Product Improvement Institute (KAPI), Bangkok, Thailand. Briefly, one gram of sample were extracted with 10 ml ethanol. The solution were separated by centrifugation at 6,000 rpm. The obtained supernatant were tested by mixing with ethanol at the various concentration 10, 20, 30, 40 and 50 µg/ml. The sample (1 ml) were mixed with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 95 % ethanol (1ml) and incubated in dark condition for 30 minute. The absorbance were determined by using spectrophotometer at 517 nm. Vitamin C (L-ascorbic acid), Vitamin E (Tocopherol) and BHT (Butylated hydroxytoluen) were used in the reference standard conpound. The percentage of radical scavenging activity were calculated as 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 ploting inhibition percentage againt sample concentration.

γ-amino butyric acid content (GABA) determination

GABA content were sent to analyze by Institute of Food Research and Product Development (IFRPD), Kasetsart university, Bangkok, Thailand.

Data analysis

Properties analysis were carried out in three replicates. The data were subjected to Analysis of Variance (ANOVA) ($p \leq 0.05$) (Steel *et al*., 1997). Mean with significant differences were separated by Duncan's Multiple Range Test (DMRT) using computer software .

Results and discussion

Sensory evaluation of germinated native black glutinous rice Khao-Mak: Table 1 presents the sensory score associate with Khao-Mak made from the various ratio of glutinous rice and germinated native black glutinous rice by using Randomized Complete Block Design (RCBD) as mention above. The mean sensory scores of 6 treatments differed significantly ($p \leq 0.05$) in color, aroma, taste, texture and acceptability. The color, aroma, taste and general acceptability all ranged between nearly like slightly and like. Treatment 2 (glutinous rice *:* germinated native black glutinous rice ; 80:20) was most generally accepted among the samples and significantly different ($p \leq 0.05$) from other treatments. This could be because this treatment was accurated the needs of consumers. Then all treatments were determined of chemical characteristic changing in during fermentation as shown in Figure 1-4.

Table 1. Mean sensory scores of Khao-Mak produced from germinated native black glutinous rice

Treatments	glutinous rice : germinated native black glutinous rice	Parameter				
		Color	Aroma	Taste	Texture	Acceptability
1	100:0	7.60^{a}	7.84^{a}	7.92^{a}	7.68^{a}	7.14^{b}
2	80:20	6.92^{b}	8.00^{a}	7.36^{a}	7.66^{a}	8.10^{a}
3	60:40	6.62^{b}	6.70^{b}	6.94^{bc}	6.28^{b}	6.80^{bc}
4	40:60	6.48^{b}	6.64^{b}	6.44^{c}	5.76^{bc}	6.24^{cd}
5	20:80	6.50^{b}	6.30^{b}	5.70^{d}	5.42^{c}	5.90^{d}
6	0:100	6.40^{b}	6.08^{b}	5.58^{d}	5.24^{c}	5.56^{d}

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's Multiple Range test.

Change in pH of Khao-Mak produced from germinated native black rice during 7 day fermentation indicating the progress of fermentation process is shown in Figure 1. Every treatments were exhibited similar trend. At 0 day of fermentation, the pH of Khao-Mak were found approximately 6.00. After the fermentation start, the pH of Khao-Mak were declined sharply to approximately 4.00 at day 2 of fermentation. Because, yeast and mold could play an important role for fermentation system which ferments monosaccharide (sugar) to ethyl alcohol and acids (Manosroi *et al*., 2011). Therefore, the pH could be decreased.

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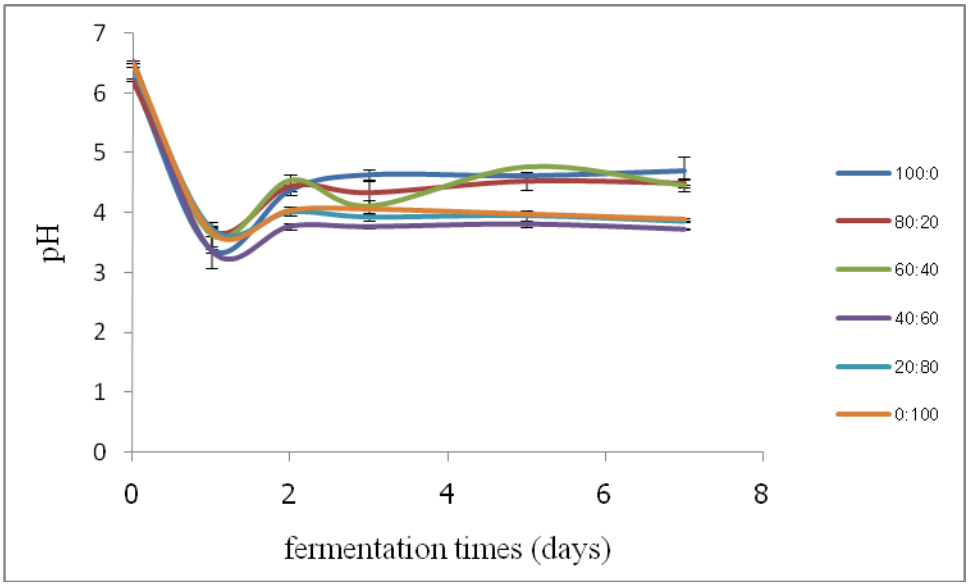

Figure 1 Change in pH of Khao-Mak produced from germinated native black glutinous rice during fermentation for 7 days. Bars represent standard deviation from triplicate determination.

Change in total soluble solid of Khao-Mak produced from germinated native black rice during 7 day fermentation indicating the progress of fermentation process is shown in Figure 2. Every treatments were exhibited similar trend. At 0 day of fermentation, the total soluble solid of Khao-Mak were found at zero. After the fermentation start, the total soluble solid of Khao-Mak were increased dramatically to approximately 7 ◦brix in the treatment of the ratio glutinous rice : germinated native black glutinous rice 80: 20, 60:40, 20:80 and 0:10, respectively. Because, mold could hydrolyzed starch to sugar. Therefore, the sugar could be increased. Our result agreed with Tongyai *et al.* (2012) who studied in the topic of the change in physicochemical and sensory characteristic during fermentation of Khao-Mak from black glutinous rice. Nevertheless, at day 4-7 of fermentation the total soluble solid of Khao-Mak in control and the treatment of the ratio glutinous rice : germinated native black glutinous rice 40: 60 were fluctuated.

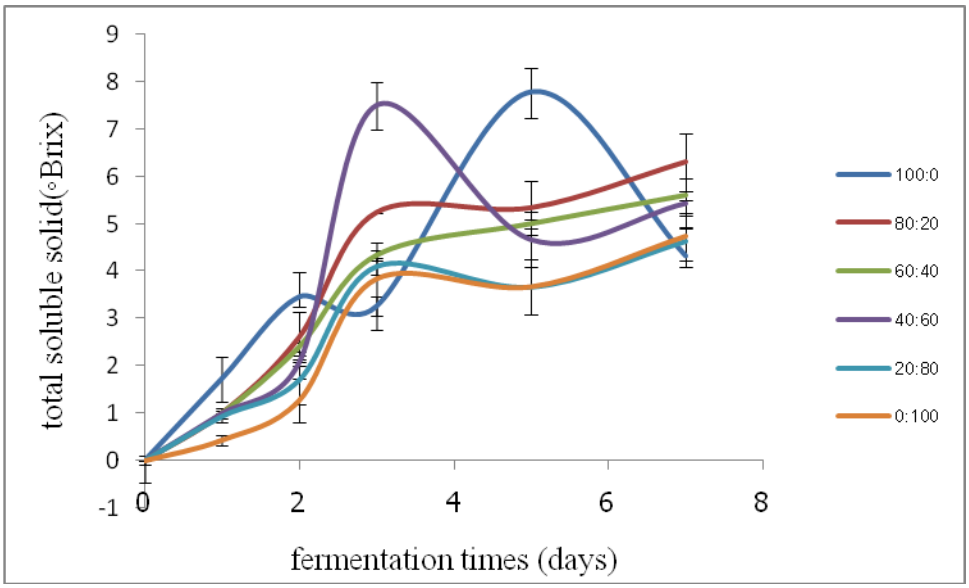

Figure 2 Change in the percentage of total soluble solid of Khao-Mak produced from germinated native black glutinous rice during fermentation for 7 days. Bars represent standard deviation from triplicate determination.

Change in ethyl alcohol content of Khao-Mak produced from germinated native black rice during 7 day fermentation indicating the progress of fermentation process is shown in Figure 3. Every treatments were exhibited similar trend. The high ethyl alcohol content was found in control fermented sample. At 0 day of fermentation, the ethyl alcohol content of Khao-Mak were found at zero. After the fermentation start, the ethyl alcohol content of Khao-Mak were significantly increased to approximately 0.50-3.00 % at day 3 fermentation and then remain the same at the end of fermentation. Because, yeast could play an important role for fermentation system which ferments monosaccharide (sugar) to ethyl alcohol. Therefore, the ethyl alcohol could be increased and then probably volatile in the end of fermentation.

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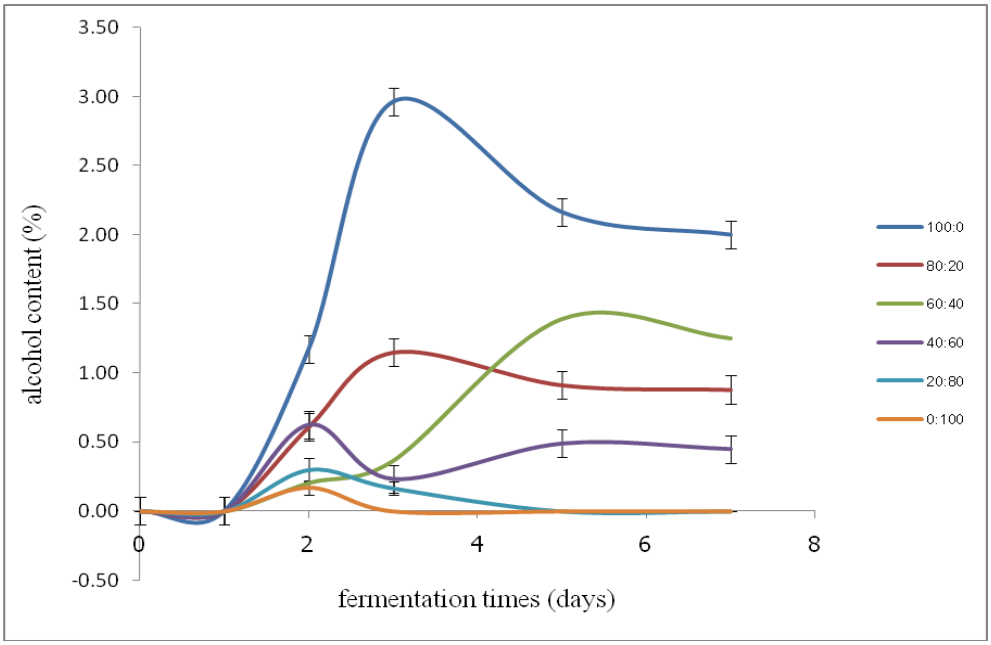

Figure 3 Change in alcohol content of Khao-Mak produced from germinated native black glutinous rice during fermentation for 7 days. Bars represent standard deviation from triplicate determination.

Change in total anthocyanin content of Khao-Mak produced from germinated native black glutinous rice during 7 day fermentation indicating the progress of fermentation process is shown in Figure 4. Every treatments were exhibited similar trend. At 0 day of fermentation, the total anthocyanin content of Khao-Mak were found at zero. After the fermentation start, the total anthocyanin content of Khao-Mak were reach a high at day 1 fermentation, then fall at day 3 fermentation, finally dramatically increased again in the end of fermentation. This could be because the effected of acid content from the fermentation. Kong *et al.* (2003) reported that acid or weak acid cause partial or total hydrolyzed anthocyanin molecule. Generally, the most widespread anthocyanin from fruit, vegetable and plants is cyaniding-3-glucoside. Therefore, the total anthocyanin content could be decreased for 3 day fermentation.

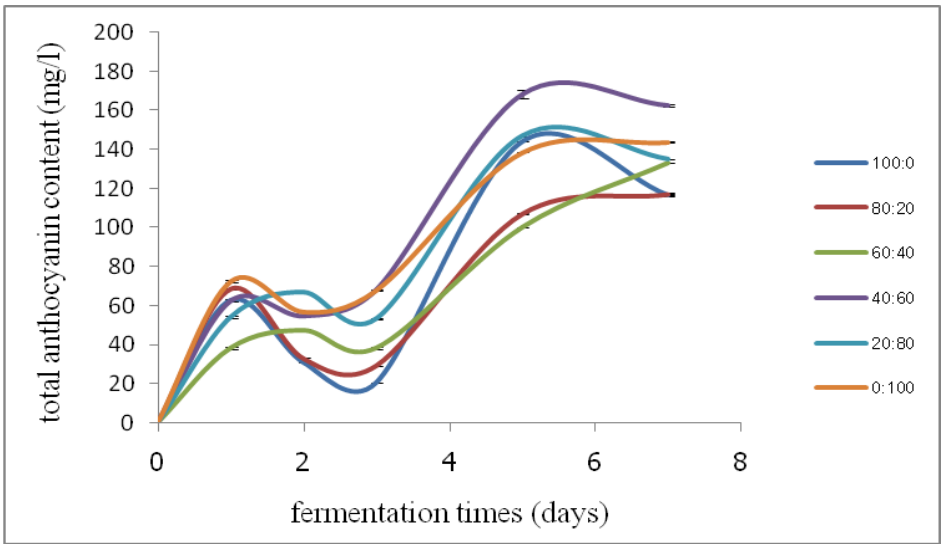

Figure 4 Change in total anthocyanin content of Khao-Mak produced from germinated native black glutinous rice during fermentation for 7 days. Bars represent standard deviation from triplicate determination.

Physical properties of Khao-Mak from germinated native black glutinous rice: The level of L*(lightness), a*(redness/greeness) and b* (yellowness/buleness) of the selected treatment were found 12.44±0.06, 11.81±0.16 and 28.72±0.26, respectively. The obtained color of Khao-Mak was black and white. After fermentation was completed, the color of Khao-Mak tended to be laver which is the effect from anthocyanin pigment in black glutinous rice.

Chemical composition of germinated native black rice Khao-Mak were presented in Table 2: the level of moisture content, ash, lipid, protein, crud fiber, carbohydrate, total soluble solid, pH, ethyl alcohol and total anthocyanin content were 55.86±0.50 (%wb), 0.29±0.49 (%wb), 0.35±0.48 (%wb), 3.11±0.50 (%wb), 1.59±0.25(%wb), 38.80±0.50 (%wb), 5.23±0.50 °Brix, 4.34±0.20, 1.15±0.5 (%) and 29.16±0.01 (mg/l), respectively. Form our results propose that this fermented sample could be served as a source of crude fiber, protein and carbohydrate. Moreover, lipid content were exhibited low amount in this sample. For ethyl alcohol content, sample contained 1.15 % which was higher than standard ethyl alcohol content for fermented Khao-Mak as determined by Thai community product standard (Ministry of Industry, 2003). In general, the ethyl alcohol content of Khao-Mak must less than 0.5 % by weight (Ministry of Industry, 2003). However, another research also found that Khao-Mak produced from black glutinous rice had high ethyl alcohol content too (2.28 %) (Tongyai, *et al.* 2012). Ethyl alcohol formation might be resulted from metabolized products from sugar by yeast and contributed to flavor of the products. For pH, the level of

acidity of fermented sample still in the standard acidity content for fermented Khao-Mak as determined by Thai community product standard (Ministry of Industry, 2003). Generally, the acidity value could be between 4 to 4.5. For the total anthocyanin content, our fermented sample tended to be purple after fermentation completed. This color could be obtained from germinated native black glutinous rice.

Table 2. Chemical characteristic of Khao-Mak produced from germinated native black glutinous rice

Sample	Composition									
	Moisture	Ash	Lipid	Protein	Crude fiber	carbohydrate	Total soluble solid	pH	Ethy l alcohol	Anthocya nin content
Khao-Mak	55.86 ±0.50	0.29± 0.49	0.35 ±0.48	3.11 ±0.50	1.59 ±0.25	38.80 ±0.50	5.23 ±0.50	4.34 ±0.20	1.15 ±0.50	29.16 ±0.01

Each data represents mean of three replications with standard errer.

Microbiological characteristic of Khao-Mak from germinated native black glutinous rice were presented in Table 3. The viable cell count, yeast and mold count of fermented sample were exhibited 8.00×10^3 , 5.30×10^3 cfu/g . Our results similar with Tanasuwapat (1995) reported that Khao-Mak could be the bacterial count are between 1.20 x 10 to 4.30×10^{10} cfu/g. In addition, *E. coli* was also found lower 3 by using MPN method. This number still in the standard *E. coli* content for fermented Khao-Mak as determined by Thai community product standard (Ministry of Industry, 2003).

Table 3. Microbiological characteristic of Khao-Mak produced from germinated native black glutinous rice

Sample	Parameter		
	Total plate count (cfu/g)	Yeast and mold count (cfu/g)	*E. coli* (MPN)
Khao-Mak	8.00×10^3	5.30×10^3	< 3

In addition, the antioxidative capacity of Khao-Mak produced from germinated native black glutinous rice was determined the free radical scavenging test using DPPH solution as present in Figure 5. The resulted found that our fermented Khao-Mak had the

strongest radical scavenging activity (IC_{50} 59.7 mg/ml) and significantly higher than control three fold (IC_{50} 199.37 mg/ml) ($p \leq 0.5$). Since, IC_{50} is the concentration of sample and standard required to inhibitory 50 % tyrosinase. Our result agreed with Oki *et al.* (2002) and Sangkitikomon *et al.* (2008), who reported that anthocyanin from black rice was found higher antioxidant activity than other pigment rice.

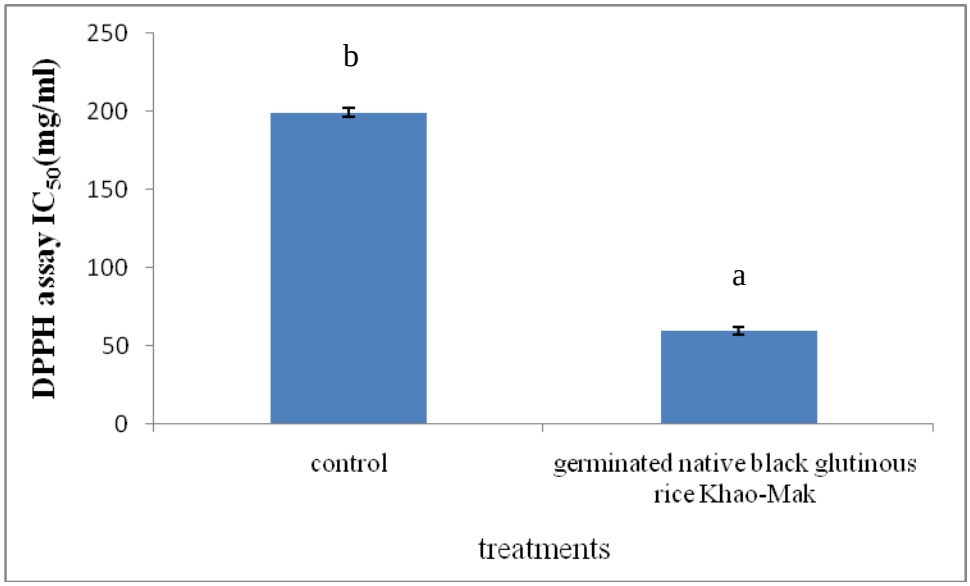

Figure 5 Antioxidant activity of Khao-Mak produced from germinated native black glutinous rice during fermentation for 3 days. Mean with different letters are statistically different ($p \leq 0.05$) according to t-test.

However, GABA content of Khao-Mak produced from germinated native black glutinous rice was exhibited in Figure 6. Khao-Mak produced from germinated native black glutinous rice was found 6.53 mg/100g. This value was still slightly lower than control fermented Khao-Mak (7.32 mg/100g). Nevertheless, GABA content evaluation could be required for repeating the nutrition

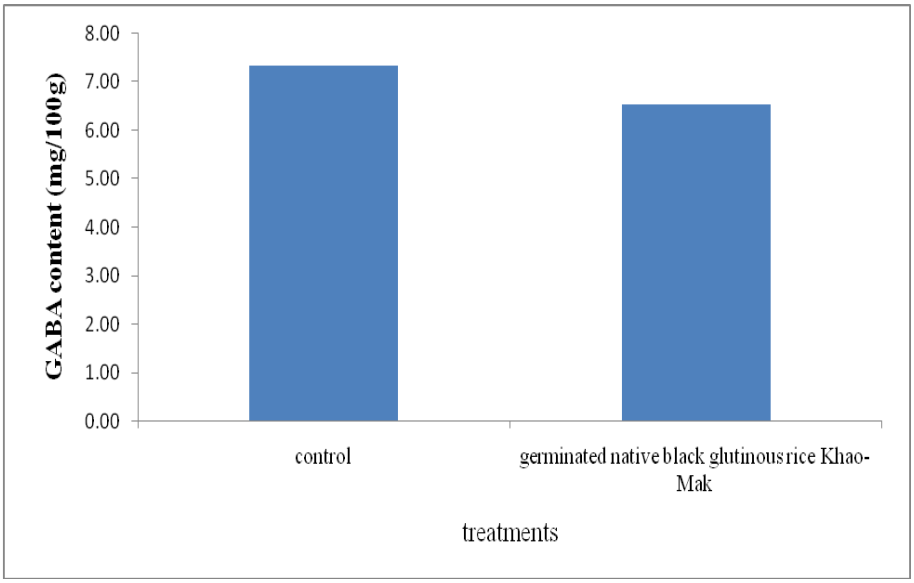

Figure 6 γ-aminobutyric acid (GABA) content of Khao-Mak produced from germinated native black glutinous rice during fermentation for 3 days

Conclusion

The obtained results showed that the optimum ratio of glutinous rice : germinated native black glutinous rice for Khao-Mak production were 80:20. This treatment was the high acceptable from the sensory evalution. Physical, chemical and microbiological properties of final product were found that the L*, a* and b* value were 12.44, 11.81, and 28.72, respectively. The color of fermented Khao-Mak tended to be laver after fermentation completed. Chemical characteristic were propose that this fermented sample could be served as a source of crude fiber, protein and carbohydrate. Moreover, lipid content were exhibited low amount in this sample. The level of total plate count, yeast and mold count and the number of *E. coli* were in the community standard product of Thailand. Interestingly, high antioxidant capacity activity was found in our fermented Khao-Mak. Although, the GABA content slightly lower than control. In conclusion, this research is good preliminary study on the new fermented product development. However, GABA content determination could be required for confirm the nutrition.

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