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☑ **ตีพิมพ์ในวารสารทางวิชาการ**
ชื่อเรื่องบทความ.....
.....
ภาษาอังกฤษ Total Phenolics, Flavonoids, Anthocyanins and Antioxidant Activities of Khaow-Mak Extracts from Various Colored Rice
ชื่อวารสาร Journal of Food Health and Bioenvironment Science ○ ระดับนานาชาติ ✓ ระดับชาติ
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เป็นวารสารของประเทศ ไทย ปีที่ 13 ฉบับที่ 1 หน้าที่ 10-18

☐ **นำเสนอในที่ประชุมทางวิชาการที่มีรายงานการประชุมฉบับสมบูรณ์ (Proceedings) (เป็นรวมเล่มบทความฉบับเต็มไม่ใช่บทคัดย่อ)**
ชื่อเรื่องบทความ.....
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ภาษาอังกฤษ .....
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ชื่องานประชุมทางวิชาการ.....
วัน/เดือน/ปี ที่จัดประชุม.....หน่วยงานที่จัดประชุม.....
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เป็นการประชุมทางวิชาการ ○ **ระดับนานาชาติ** ○ **ระดับชาติ** ○ **อื่นๆ** .....
การนำเสนอ ○ **แบบบรรยาย (Oral Presentation)** ○ **แบบโปสเตอร์ (Poster Presentation)**

☐ **การเผยแพร่ในรูปแบบอื่นๆ**.....
.....

ข้าพเจ้าขอรับรองว่า ได้ดำเนินการตามข้อความข้างต้นแล้ว และได้แนบหลักฐานการเผยแพร่ที่ได้รับการตรวจสอบความถูกต้องมาด้วยแล้ว

ลงชื่อ.....
(ดวงเดือน วัฒฐานจักร์)
นักศึกษา
วันที่ 10 เดือน ส.ค. 2564 พ.ศ. .....

โปรดพลิก ⇨

คำรับรอง

ขอรับรองว่า (นาย/นาง/นางสาว) ลงเพื่อน วิฤฎานุโกษ์

ได้เผยแพร่ผลงานวิทยานิพนธ์/การค้นคว้าอิสระ ตามรายละเอียดที่ระบุข้างต้นเรียบร้อยแล้ว และได้ตรวจสอบความถูกต้องเรียบร้อยแล้ว เห็นสมควรเสนอขอส่งเอกสารการเผยแพร่บทความวิจัยได้

ลงชื่อ..... (รศ.ดร.ศศมล ผาสุข) อาจารย์ที่ปรึกษาวิทยานิพนธ์/การค้นคว้าอิสระ วันที่..... เดือน 10 ส.ค. 2564 พ.ศ.....	ลงชื่อ..... (รศ.ดร.ศศมล ผาสุข) ประธานคณะกรรมการประจำหลักสูตร วันที่..... เดือน 10 ส.ค. 2564 พ.ศ.....

ให้นักศึกษาแนบเอกสารตามประเภทการเผยแพร่บทความวิจัยให้ครบถ้วน แล้วนำเอกสารส่งที่บัณฑิตวิทยาลัย

ประเภทตีพิมพ์ในวารสารทางวิชาการ

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☑ ครบ

☐ ไม่ครบ

ประเภทนำเสนอผลงานในที่ประชุมวิชาการ

สิ่งที่แนบมาด้วย

1. แบบเสนอขอรับการส่งเอกสารการตีพิมพ์ (มรว.บ.11)
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(ผศ.ดร.สิตา โชคกุล)

ลงชื่อ..... ผศ.ดร.ดนุชา สลีวงศ์ (รองคณบดีบัณฑิตวิทยาลัย) วันที่ 11 เดือน ส.ค. พ.ศ. 64	ลงชื่อ..... รศ.ดร.กันต์ฤทัย คลังพหล (คณบดีบัณฑิตวิทยาลัย) วันที่ 16 ส.ค. 2564 เดือน..... พ.ศ.....

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April 1, 2020

To Miss Duangduan Wattanuruk

This is to certify that, Miss Duangduan Wattanuruk from Science Education Department, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani, Thailand is an author of the paper entitled: **"Total Phenolic, Flavonoids, Anthocyanins and Antioxidant Activities of Khaow-Mak Extracts from Various Colored Rice".** That is currently accepted for publication in the incomimg Issue of Journal of Food Health and Bioenvironmental Science Vol.13 (January-April, 2020) No.1.

Sincerely Yours,

(Assist. Prof. Dr. Yutthaya Yuyen)
Journal of Food Health and Bioenvironmental Science Editor-in-Chief
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Total Phenolics, Flavonoids, Anthocyanins and Antioxidant Activities of Khaow-Mak Extracts from Various Colored Rice

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Abstract

Khaow-Mak is fermented food of rice, which contains a lot of nutrients and antioxidant bioactive compounds. Generally, Khaow-Mak is fermented from cooked white glutinous rice. However, it can be fermented with colored rice (black, purple and red pericarp colored grains) in order to increase bioactive compounds and antioxidant performance. The study was conducted to investigate the chemical composition of Khaow-Mak extracts, total phenolic, flavonoid and anthocyanin contents. The antioxidant activities were evaluated by using the scavenging towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The colored rice was collected from 16 local colored rice (red, black and purple) around Thailand. The cooked rice was fermented with a starter (Look Pang) at room temperature for 5 days. Fermented rice samples were extracted with 95% ethanol for 24 hours. Dried crude extracts were obtained using a rotary evaporator at 45°C. The results showed that the content of bioactive compounds of all colored rice were increased after fermenting time. Leum Phua glutinous rice had the highest contents of total phenolic (45.66±0.01 mgGAE/g) flavonoid contents (39.35±0.07 mgRE/g) and anthocyanin contents (3.37 ± 0.21 mg/g). In addition, Leum Phua glutinous rice showed the hightest antioxidant activity of DPPH (EC_{50} 0.364±0.02 mg/ml) and FRAP (28.018±0.01 mgFe(II)/g. These results potentially support the use of local rice extracts as the active raw material of functional food and/or cosmetics.

Introduction

Colored rice (pigmented rice) is a kind of brown rice obtained by removal of husk. Pigmented rice is distinguished by the rice grain having red brown or dark purple color in its covering layers. Pigments, which are located in the aleurone layer of rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids (Yawadio et al., 2007). Flavonoids, the major class of phenolic compounds in plants, can be divided into different classes, being the anthocyanidins the most common. Generally, the anthocyanidins are bound to glycosides, which are called anthocyanins (Kong et al., 2003). The phenolic compounds have been found as a major active component

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for antioxidation (Iqbal et al., 2005). Several compounds have already been identified in this cereal, mainly phenolic acids and anthocyanins (Oki et al., 2002).The anthocyanin plays an important role in antioxidant (Sutharut & Sudarat, 2012) which is natural phenolic pigments that was reported to scavenge free radicals such as superoxide ($O_2^{\cdot-}$), singlet oxygen ($^{\cdot}O_2$), peroxide ($ROO^{\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) (Wang & Jiao, 2000b). Colored rice is reported as a potent source of phenolic compounds (polyphenols) which has greater amount comparing with white rice and it contains a lot of nutritional advantages over white rice (Vichapong et al., 2010). The total polyphenols content in rice exist in the soluble form representing 40% in light brown rice grains and around 81% in red and black pericarp color grains. (Mira et al., 2009). In addition, the pigmented rice has higher DPPH radical-scavenging activity than white rice due to the polymeric procyanidins which are the major component for antioxidant (Oki et al., 2002).

Fermented sweet rice, which is called Khaow-Mak, is known as one of the famous traditional food from Thai folk wisdom in Thailand produced from the process of fermentation using microorganisms. The traditional starter culture (Look Pang) contains yeast, mold and herbs, which is used for fermenting cooked white glutinous rice (Manosroi et al., 2011). Enzymes from the molds hydrolyze starch in the rice which turns into sugars, which are partially fermented into alcohol by the yeast. Organic acids (e.g. lactic acid) are also produced (Lotong, 1992). Black glutinous rice is sometimes substituted for white glutinous rice to produce Khao-Mak since it is a rich source of phytochemicals such as anthocyanins (Sompong et al., 2011). Khaow-Mak has been documented as a rich source of probiotics and bioactive compounds, offering various food properties that influence human health. Several studies showed that the fermentation can increase bioactive compounds, such as polyphenolics, flavonoids, phytic acid, γ-oryzanol and vitamin E. Besides, the degradation of antinutritional compounds to phytic acid was also found in antioxidative activity. The fermentation can lead to the improved nutritional quality of food (Zhai et al., 2015; Plaitho et al., 2013; Michela et al., 2019). Rice (*Oryza sativa* L.) grain has been reported to contain several groups of antioxidants, including phenolic compounds, flavonoid, and anthocyanin (Iqbal et al., 2005). These compounds have been especially rich in pigmented rice (black or red pericarp) (Kehrer, 1993). Antioxidants are defined as organic molecules that promote health by protecting the body's cells from damage caused by free radicals and reactive oxygen species that may otherwise exert harmful metabolic effects. It has been widely accepted that an excess of generation of free radicals leading to oxidative damage, which are responsible for the age-related damage at cellular and tissue levels (Fusco et al., 2007). Thus, a balance between oxidant and antioxidant is necessary in order to reduce the rate of formation of aging changes and disease pathogenesis (Rohrer & Siebenmorgen, 2004).

In this study, Khaow-Mak was produced from 16 varieties of rice. The aims are to evaluated the concentration of bioactive compounds, total phenolics, flavonoids and anthocyanins content. The total antioxidant capacity determined by the DPPH method compared with those determined by FRAP method. In addition, the comparison of bioactive compounds and antioxidant activities from various colored Khaow-Mak extracts between before and after fermentation. The extracts with antioxidant activity are fundamental to the development of health and beauty products further, which can be applied to the active ingredient in cosmetic, food and other industries.

Materials and methods

1. Preparation of crude extracts from Khaow-Mak

The glutinous rice of Leum Phua rice (Tak province), Khao Kam (Chiang Rai province), Khao Kam Doi (Phayao province), Khaoneow Damhmo (Phatthalung province), Khaoneow Dam (Buri Ram province) and Khaoneow Dang (Loei province). The nonglutinous rice of Hommali Dang rice (Saraburi province), Hom Nin rice (Lopburi province), Rice Berry rice (Pathum Thani province), Homnin-Jakkapat rice (Ubon Ratchathani province), Sang Yod rice (Phatthalung province), Mali Nin rice (Surin province), Niang Guang rice (Buri Ram province), Tubtim Chumphae rice (Khon Kaen province), Homdam Sutabut rice (Chiang Rai province) and Hommali Dam rice(Chiang Rai province), all of these are rice varieties used for experiment. The rice was soaked with water for 6 hours. It was mixed with distilled water (1:3 w/v) and cooked with the ordinary rice cooker. Cooked rice was cooled at room temperature and fermented with 0.5% Look-Pang (0.5g/100 g of raw rice) at room temperature for 5 days in a glass container. The fermented rice was dried in the oven at 60°C for 24 hours. Dried rice samples were extracted with 95%

ethanol under stirring in a shaker at 120 rpm for 24 hours. The ethanol extracts were separated in the centrifuge at 6,000 rpm for 10 min and were filtered through a paper filter (Whatman No.1). The remaining wastes were reprocessed by the same methods and the extracts which were combined well. The extracts were transferred to a flat-bottomed flask. The solvents were evaporated by a rotary evaporator at 45°C until dry samples. All crude Khaow-Mak extracts were stored at -10°C in storage vials for determination of bioactive compounds and antioxidant activities (Plaitho, 2016).

2. Total phenolic content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method with some modification (Iqbal et al., 2005). 20 g of Khaow-Mak extracts was diluted with 99.99% ethanol. Then, 100 µl of diluted extracts in 8.4 ml distilled water was mixed with 500 µl of freshly prepared diluted Folin–Ciocalteu reagent (0.2 N). After 1 min, 1 ml of 20% sodium carbonate was added. Mixtures were incubated at room temperature for 2 hours in the dark. The absorbance at 760 nm was measured by spectrophotometer. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample (mg GAE/g dried extract).

3. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was assayed as described by Shen et al. (2009) with minor modifications using rutin as a standard. 10 mg of Khaow-Mak extracts was diluted with 80% ethanol. Then, 1 ml of the extracted samples were put in a 10 ml volumetric flask containing 4 ml of distilled water and mixed with 0.3 ml 5% $NaNO_2$ solutions. After 6 min, 0.3 ml 10% $AlCl_3.6H_2O$ solution was added. Al $(NO_3)_3$ was added to the flask for another 6 min reaction. After another 6 min, 2 ml 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at 510 nm. Qualification was done using the Rutin as standard and the results was expressed as milligrams of rutin equivalent (mg RE) per gram of the sample (mg RE/g dried extract).

4. Determination of Total Anthocyanin Content

The total anthocyanin content (TAC) was determined by the pH-differential method which bases on the structural changes in chemical forms of anthocyanin and absorbance measurements at pH 1.0 and 4.5. (Giusti & Wrolstad, 2001). 10 mg of Khaow-Mak extracts was diluted with 80% ethanol. Then, 1 ml of Khaow-Mak extracts solution into 10 ml volumetric flask for preparing two dilutions of the sample, one adjust volume with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, diluting each. Let these dilutions equilibrate for 15 min. Measure the absorbance of each dilution at the 510 and 700 nm (to correct for haze), against a blank cell filled with distilled water. All measurements should be made between 15 min and 1 hr after sample preparation, since longer standing times tend to increase observed readings. Absorbance readings are made against water blanks. The samples to be measured should be clear and contain no haze or sediments; however, some colloidal materials may be suspended in the sample, causing scattering of light and a cloudy appearance (haze). This scattering of light needs to be corrected for by reading at a wavelength where no absorbance of the sample occurs, i.e., 700 nm. Calculate the absorbance of the diluted sample (A) (Sutharut & Sudarat, 2012) as follows:

$$A = (A510 - A700)\ pH\ 1.0 - (A510 - A700)\ pH\ 4.5$$

Calculate the monomeric anthocyanin pigment concentration in the original sample using the following formula:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

and it was converted to mg of total anthocyanin content /100 g sample. Where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity, calculate pigment content as cyanidin-3-glucoside, where MW = 449.2 and ε= 26,900

5. DPPH radical scavenging activity

The DPPH free radical scavenging activity was carried out according to Fukumoto & Mazza (2000) with some modifications. 0.02 g of Khaow-Mak extracts was diluted with 40 ml of 99.99% ethanol. A series of concentrations of the extract sample at 31.25, 62.50, 125, 250 and 500 mg/ml was prepared. Briefly, 1 ml of each extract was allowed to react with 2 ml of 0.1 mmol/l DPPH solution for 30 min in the dark before the absorbance was read at 517 nm. The radical scavenging activity was calculated as

$$\%\ \text{Inhibition} = [(AB - AA)/AB] \times 100$$

where AA was the absorption of tested extract solution and AB was the absorption of blank sample.

The sample concentration providing 50% effective concentration (EC_{50}) was calculated from the graph ploting inhibition percentage againt sample concentration.

6. Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay based on the reduction of the Fe(III)-TPTZ complex to the ferrous form was performed according to methods described by Griffin & Bhagooli (2004). Briefly, freshly prepared FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH 3.6) and 10 mM TPTZ solution prepared in 40 mM HCl and 20 mM ferric chloride ($FeCl_3$) at a ratio of 10:1:1 (v/v/v). The 200 µl of Khaow-Mak extracts was mixed with 1.3 ml of the FRAP reagent and after 30 min of incubation at 37°C, absorption was measured at 600 using a spectrophotometer. Aqueous or methanolic solutions of known Fe(II) concentration are used for calibration of the FRAP assay. FRAP values, expressed as mg Fe(II) equivalent/g dried extract (mg Fe(II)/g dried extract).

7. Statistical analysis

All treatments and determinations were implemented in triplicate and the data are expressed as the mean ± standard deviation. One-way analysis of variance followed by Duncan's multiple range tests and T-test were employed for analyzing the variance ($p < 0.05$) of the data.

Results and discussion

Total phenolic content (TPC) of Khaow-Mak produced from colored rice is shown Table 1. The results were found that TPC of Khaow-Mak from colored rice at after fermentation was found higher than before fermentation. Moreover, TPC from black rice and purple rice was found higher than red rice. The TPC of crude Khaow-Mak rice extracts was not clearly differentiated among the black and purple rice varieties with a range of 21.94-45.66 and 21.36-36.02 mg GAE/g, respectively; whereas the red rice varieties showed the lowest of TPC content (19.30-21.03 mg GAE/g). Khaow- Mak produced from Leum Phua glutinous rice had the highest TPC 45.66±0.01 mg GAE /g.The type and concentration of polyphenols in the rice grain vary among genotypes and are related mainly to the pericarp color. Normally, grains with purple and black pericarp colors have a higher concentration of phenolic compounds compared to red pericarp color (Tian et al., 2004; Zhou et al., 2004). Sadabpod et al. (2010) also reported that total phenolic contents of fermented Hom Nil rice and black glutinous rice were higher than those of both raw rice and cooked rice. Similar to other cereal grains, the phenolic compounds in rice exist in the soluble and insoluble (bound) form. However, the grains with red and black pericarp colors were observed higher concentrations of total soluble phenolic compounds (Melissa et al., 2013).

The total flavonoid content (TFC) of the rice samples followed a similar trend to that of TPC. The TFC of fermented rice was higher than that of its corresponding unfermented one (Table 1). Moreover, TFC from black rice and purple rice was found higher than red rice. The highest total flavonoid content (39.35±0.07 mgRE/g) belonged to Leum Phua glutinous rice fermented and the high levels of TFC was also found in other black rice samples. The TFC of crude Khaow-Mak rice extracts from the black, purple and red rice varieties with a range of 13.38-39.35, 16.47-28.03 and 15.30-20.37 mgRE/g respectively. Anthocyanin is well known as the predominant flavonoid in pigmented rice (Kim et al., 2010).

Total anthocyanin content (TAC) of Khaow Mak produced from colored rice is shown Table 1. After the fermentation, the total anthocyanin content of Khaow-Mak were high at day 5 fermentation. Every treatment of rice varieties exhibited a similar trend. Moreover, the total anthocyanin content from black rice and purple rice was found higher than red rice with a range of 1.82-3.37, 2.38-3.27 and 1.32-1.89 mg/g respectively. The TAC of colored rice crude extracts was prominent in the black rice varieties, followed by the red rice varieties. Khaow-Mak produced from Leum Phua glutinous rice had the highest anthocyanin contents 3.37 ± 0.21 mg/g. Mongkontanawat & Lertnimitmongkol (2015) reported that total anthocyanin content of Khaow-Mak fell at day 3 fermentation because acid or weak acid cause partial or total hydrolyzed anthocyanin molecule, finally it dramatically increased again in the end of fermentation. The color of fermented rice (red, black) could be obtained from anthocyanin. Generally, the most widespread anthocyanin from fruit, vegetable and plants is cyaniding-3-glucoside. Abdel-Aal et al. (2006) reported that cyanidin-3-glucoside and peonidin-3-glucoside were identified as two major anthocyanins in pigmented rice, especially black rice.

The DPPH radical-scavenging ability is frequently used to evaluate the hydrogen donating of the antioxidants and the results are expressed as EC_{50} values, indicating

Table 1 Total phenolic, Flavonoid contents and Antrocyanin contents of Khaow-Mak extracts from colored rice

Sample (Color rice varieties)	Extraction Yield (%)	Phenolic (mg GAE /g)		Flavonoid (mgRE/g)		Anthocyanin mg/g	
		Before fermentation	After fermentaion	Before fermentaion	After fermentaion	Before fermentaion	After fermentaion
Glutinous rice							
Leum Phua rice^{B}	17.41^{i}	37.45±0.01^{a}	45.66±0.01^{a*}	35.64±0.01^{f}	39.35±0.07^{a*}	2.48±0.35^{f}	3.37 ± 0.21^{a*}
Khaoneow Damhmo^{B}	20.28^{a}	35.68±0.01^{b}	42.37±0.02^{b*}	28.21±0.05^{a}	36.02±0.03^{b*}	2.52±0.12^{e}	3.33±0.21^{b*}
Khaoneow Dam^{B}	19.25^{c}	29.65±0.01^{c}	35.42±0.01^{d*}	21.89±0.05^{b}	24.57±0.02^{g*}	2.64±0.29^{d}	3.31±0.39^{c*}
Khao Kam Doi^{P}	17.87^{g}	20.67±0.02^{f}	27.78±0.01^{f*}	15.55±0.03^{f}	26.47±0.03^{e*}	2.27±0.51^{i}	2.97±0.41^{e*}
Khow Kam^{P}	18.42^{e}	17.40±0.01^{j}	21.36±0.01^{k*}	15.50±0.07^{g}	16.47±0.03^{k*}	2.32±0.30^{h}	2.38±0.23^{i*}
Khaoneow Dang^{R}	15.52^{k}	15.60±0.01^{l}	19.50±0.01^{o*}	13.38±0.03^{i}	15.30±0.02^{n*}	1.24±0.21^{n}	1.32±0.31^{o*}
Non-glutinous rice							
Mali Nin rice^{P}	19.55^{a}	22.64±0.02^{e}	36.02±0.03^{c*}	20.21±0.11^{c}	28.03±0.03^{c*}	3.12±0.12^{a}	3.27±0.35^{d*}
Hom Nin rice^{P}	18.41^{e}	25.25±0.02^{d}	32.80±0.04^{e*}	17.75±0.07^{d}	27.67±0.08^{d*}	2.87±0.12^{b}	2.88±0.66^{g*}
Homnin-Jakkapat rice^{P}	19.23^{c}	20.27±0.44^{g}	23.72±0.02^{g*}	17.25±0.02^{e}	24.12±0.01^{h*}	2.38±0.23^{g}	2.94±0.21^{f*}
Rice Berry rice^{P}	20.28^{a}	18.74±0.02^{h}	22.84±0.02^{h*}	15.54±0.56^{f}	25.12±0.05^{f*}	2.22±0.12^{j}	2.95±0.71^{f*}
Hommali Dam rice^{B}	18.96^{d}	16.65±0.02^{k}	22.61±0.05^{i*}	14.40±0.05^{h}	24.61±0.02^{g*}	2.51±0.06^{c}	2.63±0.78^{h*}
Homdam Sutabut rice^{B}	17.96^{f}	18.20±0.05^{i}	21.94±0.01^{j*}	13.38±0.03^{i}	16.50±0.01^{k*}	1.57±0.12^{k}	1.82±0.34^{l*}
Hommali Dang rice^{R}	15.82^{j}	15.50±0.02^{n}	21.03±0.02^{l*}	14.25±0.01^{h}	20.37±0.02^{i*}	1.32±0.21^{l}	1.37±0.56^{n*}
Sang Yod rice^{R}	15.22^{m}	15.55±0.01^{m}	20.27±0.02^{n*}	13.33±0.63^{i}	18.03±0.02^{j*}	2.68±0.08^{c}	1.87±0.11^{k*}
Niang Guang rice^{R}	17.48^{h}	14.44±0.01^{q}	20.57±0.01^{m*}	14.28±0.02^{h}	16.27±0.06^{l*}	1.26±0.21^{m}	1.45±0.36^{m*}
Tubtim Chumphae rice^{R}	15.27^{l}	14.36±0.23^{p}	19.30±0.01^{p*}	12.28±0.05^{j}	15.44±0.02^{m*}	1.18±0.54^{o}	1.89±0.53^{j*}

Remark: B=black rice, P=purple rice, R= red rice
Mean values for each parameter followed by a different letter within each column are significantly different ($p \leq 0.05$) according to Duncan's Multiple Range test, * Means within each row between before and after fermentation are significantly different ($p \leq 0.05$) according to T-test.

the concentration of antioxidant that caused the decrease of DPPH radicals to half of its initial concentration. Therefore, the lower of EC_{50} value provides higher antioxidant efficiency. The antioxidative capacity of Khaow-Mak produced from colored rice was determined the free radical scavenging test using DPPH solution as present in Table 2. The EC_{50} values for the fermented black and purple rice varieties varied from 0.364 to 1.032 and 0.655 to 0.901 mg/ml respectively, while the fermented red rice varieties were in the range of 1.129-1.985 mg/ml.The lowest EC_{50} value was found in Leum Phua glutinous rice, corresponded to the highest content of TPC, TFC and TAC content that observed in this sample. Khaow-Mak produced from Leum Phua glutinous rice gave the strongest free radical scavenging activity with the EC_{50} value of 0.364±0.02 mg/ml. It was found that Khaow-Mak after fermentation had the strongest radical scavenging; higher than before fermentation. Moreover, the free radical scavenging activity from black rice and purple rice was found higher than red rice. Sangkitikomon et al. (2008) suggested that black rice's anthocyanin performs higher antioxidant activity than red rice and other rice varieties. In addition, total antioxidant capacity was determined by ferric reducing antioxidant power (FRAP). The FRAP activity in the phenolic extracts is related to the level of phenolic compounds. It is simple, fast and reproducible (Wong et al., 2006). It measures the ferric to ferrous reduction

in presence of antioxidants, which are effective as secondary antioxidants because they reduce the redox potential. It was noted that fermented colored rice had higher reducing abilities than that of unfermented rice of the same variety. The fermented black and purple rice varieties were not clearly differentiated in terms of FRAP values, whereas the lowest of FRAP value was observed from the fermented red rice varietie. The FRAP value of fermented black rice and purple rice was found higher than red rice with a range of 15.204-28.018, 14.136-20.588 and 7.948-14.667 mgFe(II)/g respectively.The fermented Leum Phua glutinous rice also presented the greatest FRAP value (28.018±0.01 mgFe(II)/g).

Table 2 Antioxidant performance of Khaow-Mak extracts from colored rice

Sample (Color rice varieties)	DPPH assay (EC_{50}) mg/ml		FRAP assay mgFe(II)/g	
	Before fermentation	After fermentaion	Before fermentaion	After fermentaion
Glutinous rice				
Leum Phua rice^{B}	1.215±0.06^{k}	0.364±0.02^{p*}	536.18±0.01^{c}	28.018±0.01^{a*}
Khaoneow Damhmo^{B}	1.362±0.01^{i}	0.432±0.03^{o*}	236.22±0.02^{a}	16.775±0.04^{f*}
Khaoneow Dam^{B}	1.236±0.02^{j}	0.502±0.02^{n*}	536.14±0.06^{i}	15.997±0.09^{g*}
Khao Kam Doi^{P}	1.563±0.02^{b}	0.858±0.05^{j*}	988.16±0.02^{f}	17.343±0.01^{e*}
Khow Kam^{P}	1.362±0.01^{i}	0.924±0.02^{g*}	633.11±0.02^{k}	14.136±0.05^{i*}
Khaoneow Dang^{R}	2.623±0.01^{b}	1.825±0.02^{c*}	858.6±0.02^{n}	10.582±0.02^{m*}
Non-glutinous rice				
Mali Nin rice^{P}	1.653±0.01^{g}	0.655±0.02^{m*}	19.869±0.02^{c}	19.127±0.02^{c*}
Hom Nin rice^{P}	0.956±0.07^{m}	0.852±0.02^{k*}	14.536±0.02^{i}	17.862±0.01^{d*}
Homnin-Jakkapat rice^{P}	0.985±0.01^{l}	0.842±0.01^{k*}	19.786±0.06^{d}	20.588±0.09^{b*}
Rice Berry rice^{P}	0.952±0.01^{n}	0.901±0.01^{k*}	20.436±0.05^{b}	15.895±0.01^{h*}
Hommali Dam rice^{B}	0.945±0.02^{o}	0.894±0.02^{k*}	15.697±0.01^{g}	15.424±0.02^{i*}
Homdam Sutabut rice^{B}	2.336±0.01^{e}	1.032±0.01^{f*}	15.063±0.05^{h}	15.204±0.09^{i*}
Hommali Dang rice^{R}	2.384±0.01^{d}	1.129±0.01^{e*}	9.763±0.05^{m}	14.667±0.02^{k*}
Sang Yod rice^{R}	2.653±0.01^{a}	1.635±0.02^{d*}	12.663±0.02^{j}	10.017±0.06^{n*}
Niang Guang rice^{R}	2.269±0.02^{f}	1.963±0.01^{b*}	5.368±0.08^{o}	8.497±0.06^{o*}
Tubtim Chumphae rice^{R}	2.542±0.25^{c}	1.985±0.01^{a*}	9.869±0.07^{l}	7.948±0.06^{p*}

Remark: B=black rice, P=purple rice R= red rice
Mean values for each parameter followed by a different letter within each column are significantly different ($p \leq 0.05$) according to Duncan's Multiple Range test, * Means within each row between before and after fermentation are significantly different ($p \leq 0.05$) according to T-test.

Our result agreed with Oki et al. (2002) who reported that anthocyanin from black rice and purple rice was found higher antioxidant activity than other pigmented rice. In addition, Researchers have demonstrated a positive correlation between the concentration of phenolic compounds and the antioxidant activity (Zhang et al., 2006). This might be due to the higher content of total phenolic compounds, anthocyanins and antioxidant activities in fermented rice probably because of the catalytic action of enzymes produced by the starter organisms in Look-Pang such as *S. cerevisiae, Aspergillus* spp. and *Rhizopus* spp. during fermentation which are capable of hydrolyzing glucosides of the inactive components to the active. Therefore, the action of enzyme such as beta-glucosidase produced by the starter organism during fermentation might be an important factor contributing to the increase of phenolic and anthocyanin contents of fermented rice (Plaitho et al., 2013). Anthocyanins are commonly a group of pigments found in pigmented rice such as purple, black and red rices. These compounds provide many biological properties such as scavenging free radicals.

Wang & Jiao (2000a) the researchers, reported that Thai pigmented rice such as black glutinous rice and Hom Mali Daeng had higher phenolic compounds, total flavonoid and antioxidant activity than normal white staple rice. Moreover, Pramai & Jiamyangyuen (2016) reported that total phenolic and flavonoid contents were the highest in the black rice followed by red rice and antioxidant capacities were predominant in pigmented varieties. Black rice grown in mountainous area presented the highest antioxidant activity compared to the other growing locations. From our experiment found that fermented Leum Phua glutinous rice had the highest contents of total phenolic, flavonoid, anthocyanin contents and showed the hightest antioxidant performance. It can be grown only once a year during rainy season in mountainous area and able to survive in high levels of water around Northern Thailand such as, Tak, Phitsanulok, Chiang Rai, and Phetchabun province. Luem Pua is one of the aromatic and indigenous black (dark purple) sticky rice, enriched with flavonoids, especially anthocyanins, and have total antioxidant higher than other black rices (Suwannalert & Rattanachitthawat, 2011; Wang & Shu, 2007; Boonsit et al., 2010). In experiment of Nakornriab, (2018) suggests that phenolic compounds are the major contributors to the antioxidant activities of brown rice. In addition, germinated brown rice is a potential source

of antioxidative and phytochemicals.

Glutinous rice differs from the non-glutinous rice mainly in having low (<5%) or almost no amylose in its starch but basically high in amylopectin. In general, the amylose content of rice starch varies from 0-2% in glutinous. Non-glutinous rice has higher amylose content but less sticky texture than glutinous rice (Setyaningsih et al., 2015). In this study, the level of total phenolics in glutinous rice was higher than its non-glutinous variety. The rate of enzymatic hydrolysis of the polymeric materials softens the rice kernels was fastest in glutinous rice because amylopectin content plays a major role in water hydration as it absorbs water faster than amylose. However, the level of phenolic compounds in different varieties of rice grains may diverge in phenolics concentrations. This discrepancy reveals that one would also expect changes due to the differences on their type of starch that have been distinguished as glutinous and non-glutinous variety (Mi-Young et al., 2010). In contrast, our result not agreed with Setyaningsih et al. (2015) who reported that the composition of phenolic compounds are noticeably different between glutinous and non-glutinous rice grains. The level of total phenolics in non-glutinous rice was higher than its glutinous variety. Hence, higher amylose content exhibits relatively higher amount of antioxidant compounds. The difference of phenolics content between black glutinous and black non-glutinous rice was not as impressive as if compared to the non-pigmented rice. Thus, in this particular case, phenolics concentration in rice appears to be strongly related to the pigment of rice in their bran. Both black pigmented glutinous and non-glutinous rice grains were produced without a bran removal process called polishing. Hence, as the phenolic compounds are mainly associated with the pericarp in the grain, the milling process to produce polished grain reduces the level of these compounds in the grain.

Conclusion

Various rice, including glutinous rice (black, purple and red) and non-glutinous rice (black, purple and red) were fermented for total phenolic, flavonoid, and anthocyanin contents analysis. The results showed that the content of bioactive compounds of all rice colors increased after fermention. Moreover, Khaow Mak produced from black and purple colored rice gave the content higher than red colored rice. The obtained results showed that each sample exhibited higher than that of the unfermented one of the same variety. However, Leum Phua glutinous rice had the highest contents of total phenolic (45.66±0.01 mgGAE/g), flavonoid contents (39.35±0.07 mgRE/g) and anthocyanin contents (3.37 ± 0.21 mg/g). In addition, Leum Phua glutinous rice showed the hightest antioxidant activity, including 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (EC_{50} 0.364±0.02 mg/ml) and ferric reducing antioxidant power (FRAP) (28.018±0.01 mgFe (II)/g).

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