

# Physicochemical and Antioxidant Properties of Rice Bran Oils Produced from Colored Rice Using Different Extraction Methods

Sukanya Mingyai<sup>1</sup>, Aikkarach Kettawan<sup>2</sup>, Khongsak Srikaeo<sup>3</sup> and Riantong Singanusong<sup>1, 4\*</sup>

<sup>1</sup> Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, THAILAND

<sup>2</sup> Institute of Nutrition, Mahidol University, Salaya, Phutthamonthon, Nakhon Pathom 73170, THAILAND

<sup>3</sup> Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Muang, Phitsanulok 65000, THAILAND

<sup>4</sup> Centre of Excellence in Fats and Oils, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, THAILAND

**Abstract:** This study investigated the physicochemical and antioxidant properties of rice bran oil (RBO) produced from the bran of three rice varieties; Khao Dawk Mali 105 (white rice), Red Jasmine rice (red rice) and Hom-nin rice (black rice) using three extraction methods including cold-press extraction (CPE), solvent extraction (SE) and supercritical CO<sub>2</sub> extraction (SC-CO<sub>2</sub>). Yields, color, acid value (AV), free fatty acid (FFA), peroxide value (PV), iodine value (IV), total phenolic compound (TPC),  $\gamma$ -oryzanol,  $\alpha$ -tocopherol and fatty acid profile were analyzed. It was found that the yields obtained from SE, SC-CO<sub>2</sub> and CPE extractions were 17.35–20.19%, 14.76–18.16% and 3.22–6.22%, respectively. The RBO from the bran of red and black rice samples exhibited high antioxidant activities. They also contained higher amount of  $\gamma$ -oryzanol and  $\alpha$ -tocopherol than those of white rice sample. In terms of extraction methods, SC-CO<sub>2</sub> provided better qualities of RBO as evidenced by their physicochemical and antioxidant properties. This study found that RBO produced from the bran of black rice samples using SC-CO<sub>2</sub> extraction method showed the best physicochemical and antioxidant properties.

**Key words:** rice bran oil, colored rice, extraction, physicochemical property, antioxidant property

## 1 Introduction

Rice (*Oryza sativa* L.) is the principle cereal consumed in Asia, and the primary staple for nearly half of the world's population. Recent observations suggested that pigmented or colored rice varieties may have beneficial effects in the human diet. Many researchers reported that they contained high amount of phytochemicals and exhibited antioxidant activity<sup>1–3</sup>. Rice paddy consists of the grain, husk, germ and bran. Rice bran comprises 12–23% of oil which is high in physiologically active compounds<sup>4–6</sup>. Rice bran oil (RBO) is one of the vegetable oils, which is high nutritious and health-beneficial. It contains  $\gamma$ -oryzanol and vitamin E (tocopherol and tocotrienol)<sup>7</sup>. These agents have the capacity of antioxidants, which are served to eliminate free radicals, prevent free radicals reaction with biomolecules causing damages to the body and also prevent certain dis-

eases such as cardiovascular diseases and some cancers<sup>8–10</sup>. Furthermore, published researches have well confirmed that RBO contains high vitamin E and  $\gamma$ -oryzanol content<sup>11</sup>. Currently, most of RBO industries usually use rice bran from whitish kernels or non-pigmented rice varieties for RBO production. There has been interest in using the colored rice brans as materials for RBO extraction. However, the research in this area is limited. Therefore, the objective of this study was to determine the physicochemical and antioxidant properties of RBO extracted from the bran of colored rice varieties using different methods in order to provide the information for RBO industry and for further research. Two varieties of Thai colored rice (Red Jasmine, RJM and Hom-nin, HN) were studied in comparison with widely consumed non-pigmented rice (Khao Dawk Mali 105, KDML 105).

\*Correspondence to: Riantong Singanusong, Centre of Excellence in Fats and Oils, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, THAILAND

E-mail: riantongs@nu.ac.th

Accepted February 24, 2017 (received for review January 23, 2017)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

## 2 Materials and methods

### 2.1 Rice bran samples

Three varieties of paddy (KDML 105, RJM and HN) were obtained from community enterprise in Phichit Province of Thailand. They were harvested between December 2014–February 2015. The paddy was dried to reach a moisture content of 13% by an oven. The dried paddy was milled and polished to obtain the bran. Their appearances are shown in Fig. 1. The obtained rice brans were vacuum-packed in aluminum foil bags and stored at  $-20^{\circ}\text{C}$  until further use. The initial oil content of rice bran was 15.64% for KDML 105, 16.84% for RJM and 12.62% for HN. One well-known brand of commercial refined RBO purchased from a supermarket was used for comparison of color to the samples used in this study.

### 2.2 Extraction of the rice bran oils

#### 2.2.1 Cold-press extraction (CPE)

The rice bran was fed continuously into the hopper of a 2-HP screw press extractor. The crude oil was forced through the slits along the barrel length. The compressed rice bran was simultaneously discharged through a choke at the end of the barrel.

#### 2.2.2 Solvent extraction (SE)

The extraction process was conducted in the laboratory. The ratio of rice bran:hexane used was 1:3 (w/v) and the extraction was performed for 3 h with a regular stirring. The bran was separated from the extract by filtration through a filter paper No. 4 under vacuum.

#### 2.2.3 Supercritical carbon dioxide extraction (SC-CO<sub>2</sub>)

The oil was extracted from rice bran using a SC-CO<sub>2</sub> extractor following this condition; temperature of  $60^{\circ}\text{C}$ , pressure of 30 MPa and CO<sub>2</sub> flow rate of 35 L/h. The rice bran sample was loaded into the extraction vessel, and filled with glass beads at the bottom and top of the cell. The cell was placed in the heating chamber to maintain the operating temperature<sup>12</sup>.

Yields (dry weight basis) of crude oils were calculated using equation (1):

$$\text{Yield (\%)} = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of rice bran sample (g)}} \times 100 \quad (1)$$

### 2.3 Refining of the rice bran oils

The refining process of RBO was carried out using the method as described by Pestana *et al.*<sup>13</sup>. Briefly, the refining processes included dewaxing (cooled the oil and removed the wax by centrifugation at 6,000 rpm for 15 min), degumming (washed the oil with hot water ( $80^{\circ}\text{C}$ ) with 85% H<sub>3</sub>PO<sub>4</sub> solution), neutralization (washed the oil using hot water ( $80^{\circ}\text{C}$ ) and centrifugation at 6,000 rpm for 15 min until pH 7 was reached), bleaching (7% bleaching earth and 0.7% activated carbon at  $120^{\circ}\text{C}$  under vacuum for 30 min) and deodorizing (steamed the oil at  $220^{\circ}\text{C}$  under vacuum for 60 min).

### 2.4 Physicochemical properties

The color of RBO was determined by Lovibond spectrophotometer (PFX-880L). Acid value (AV), free fatty acid (FFA), peroxide value (PV) and iodine value (IV) of the RBO were determined according to the AOCS methods<sup>14</sup>.

#### 2.4.1 Determination of total phenolic content

The total phenolic content (TPC) was measured using the Folin-Ciocalteu colorimetric method<sup>9</sup>. The reaction mixture contained 200  $\mu\text{L}$  of refined oils, 800  $\mu\text{L}$  of Folin-Ciocalteu reagent (diluted with water 1:10 v/v and freshly prepared) and 2 mL of 7.5% sodium carbonate. The final mixture was diluted to 7 mL with deionized water. The mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. Then the absorbance at 765 nm was measured using a UV-VIS spectrophotometer (Hitachi, U2900/2910, Japan). Gallic acid was used as a standard and the results were calculated as gallic acid equivalents (g/100 g) of oils.

#### 2.4.2 Determination of $\gamma$ -oryzanol

The  $\gamma$ -oryzanol was determined by RP-HPLC method<sup>9, 15, 16</sup>. Briefly, the refined oils (50 mg) were dissolved in methanol (3 mL) and mixed vigorously for 3 min at room temperature before filtering through a syringe filter with PTFE (0.2  $\mu\text{m}$ ). The RP-HPLC consisted of an Agilent 1100 series (USA), column oven equipped with Hypersil ODS (4.0  $\times$  250 mm, 5  $\mu\text{m}$ , Agilent Technologies, USA), and a variable wavelength UV-VIS detector (model G1379A) at 330 nm. The mixture of methanol:acetonitrile:dichloromethane:acetic acid at 50:44:3:3 v/v/v/v was used as a mobile phase with

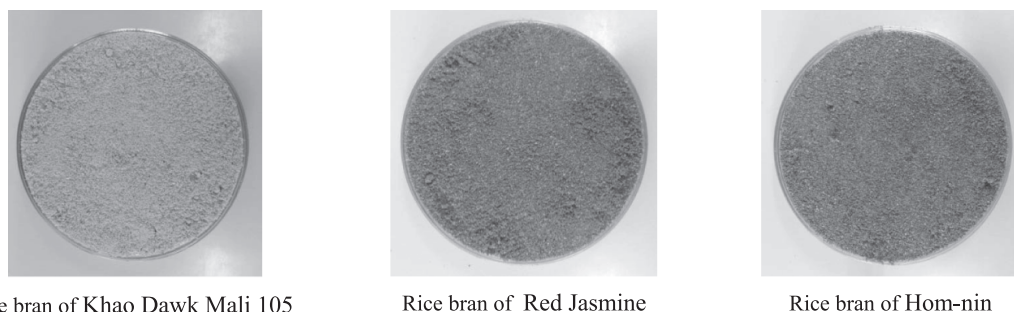


Fig. 1 The appearance of three rice bran varieties.

a flow rate of 1.0 mL/min. The content of total  $\gamma$ -oryzanol was calculated from the peak area of  $\gamma$ -oryzanol compared with standard  $\gamma$ -oryzanol.

#### 2.4.3 Determination of $\alpha$ -tocopherol

The  $\alpha$ -tocopherol of refined RBO was measured following the method of Speek *et al.*<sup>17)</sup> and AOAC<sup>18)</sup>. The oil sample (0.5 g) was diluted with n-heptane (10 mL) in the volumetric flask and filtered through syringe filters (0.45  $\mu$ m). The  $\alpha$ -tocopherol was separated on a RP-HPLC (Agilent 1100 series equipped with a Mightysil RP-18 GP column (4.6  $\times$  250 mm, 3  $\mu$ m, Kanto Chemical Co., Inc., Tokyo, Japan) and a FLD G1321A fluorescence detector operating with excitation and emission wavelengths of 290 and 330 nm, respectively. The mobile phase was n-heptane and 2% isopropyl alcohol with a flow rate of 1.0 mL/min. The peak areas of standard  $\alpha$ -tocopherol were used for calculating the  $\alpha$ -tocopherol contents.

#### 2.4.4 Determination of fatty acid composition

The fatty acid compositions were determined by GC following the methods described by AOAC<sup>18)</sup> and Jham *et al.*<sup>19)</sup>. Capillary GC (Agilent 6850 Series) equipped with a capillary column (DB-23 Agilent; 50% cyanopropyl-methylpolysiloxane; 60 m  $\times$  0.25 mm–0.25  $\mu$ m film) was used. The GC conditions operated at the initial temperature of 60°C for 2 min, then increased to 60°C–190°C at 10°C/min, 190°C–200°C at 0.5°C/min and at the final temperature of 200°C–240°C at 50°C/min with a total run time of 75 min. The flow rate of gas (Nitrogen) was 2 mL/min. A split ratio of 1:10 and an injection volume of 1  $\mu$ L were used. The fatty acid composition was obtained by comparison of the peak retention times with the respective fatty acids standards.

### 2.5 Antioxidant property

#### 2.5.1 DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging effects were determined according to the method of Brand-Williams *et al.*<sup>20)</sup>. The reaction mixture contained 2 mL DPPH solution (0.0394 g DPPH in 1 L methanol) and 300  $\mu$ L oil samples. The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance was determined at 517 nm by UV-VIS spectrophotometer (Hitachi, U2900/2910, Japan). The data were obtained and 50% inhibition ( $IC_{50}$ ) was calculated using Microsoft<sup>®</sup> EXCEL<sup>®</sup>.

#### 2.5.2 Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay which is based on the reduction of the Fe(III)-TPTZ complex was measured by a spectrophotometer<sup>21)</sup>. 150  $\mu$ L of oil samples was mixed with 3 mL of FRAP solution (25 mL; 300 mM acetate buffer (pH 3.6), 2.5 mL; 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl and 2.5 mL; 20 mM ferric chloride). The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance

was determined at 593 nm by UV-VIS spectrophotometer (Hitachi, U2900/2910, Japan). The Trolox was used as a standard.

#### 2.5.3 Oxygen radical absorbance capacity

The oxygen radical absorbance capacity (ORAC) assay was determined according to the method described by Ou *et al.*<sup>22)</sup>. Analyses were conducted in phosphate buffer pH 7.4 at 37°C. Peroxyl radical was generated using 2,2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. The fluorescence conditions were set at excitation of 493 nm and emission of 515 nm. The standard curve was the linear of Trolox. The results were expressed as mM TE/g fresh mass.

### 2.6 Statistical analysis

All the values are expressed as mean  $\pm$  SD. Statistical analysis of data was performed using one-way analysis of variance (ANOVA). Mean comparison was carried out using Duncan's multiple range test. Differences were considered to be statistically significant when  $p < 0.05$ . SPSS version 17.0 was used.

## 3 Results and Discussion

### 3.1 Yields

The yields of RBO from the bran of three rice varieties extracted using different extraction methods are shown in Fig. 2. The SE method provided higher yield than CPE and SC-CO<sub>2</sub> methods for about 75% and 10%, respectively. For all extraction methods, the bran from RJM rice produced more RBO than those from the other two varieties.

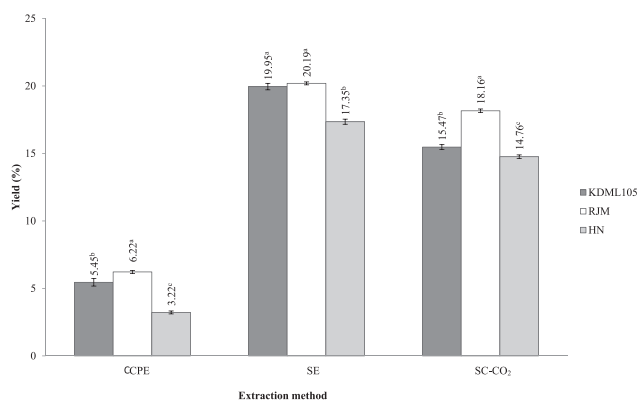


Fig. 2 Yield of RBO from three rice varieties extracted using different extraction methods. CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.

### 3.2 Physicochemical properties

The color of RBO samples obtained in this study is shown in Table 1. Color is the important characteristic for visual inspection of RBO. The color standard for refined oil as recommended by the CODEX standard<sup>23)</sup> is  $Y + 5R < 20$ . In this study, it was found that all the refined RBO samples complied with the CODEX standard. The color of commer-

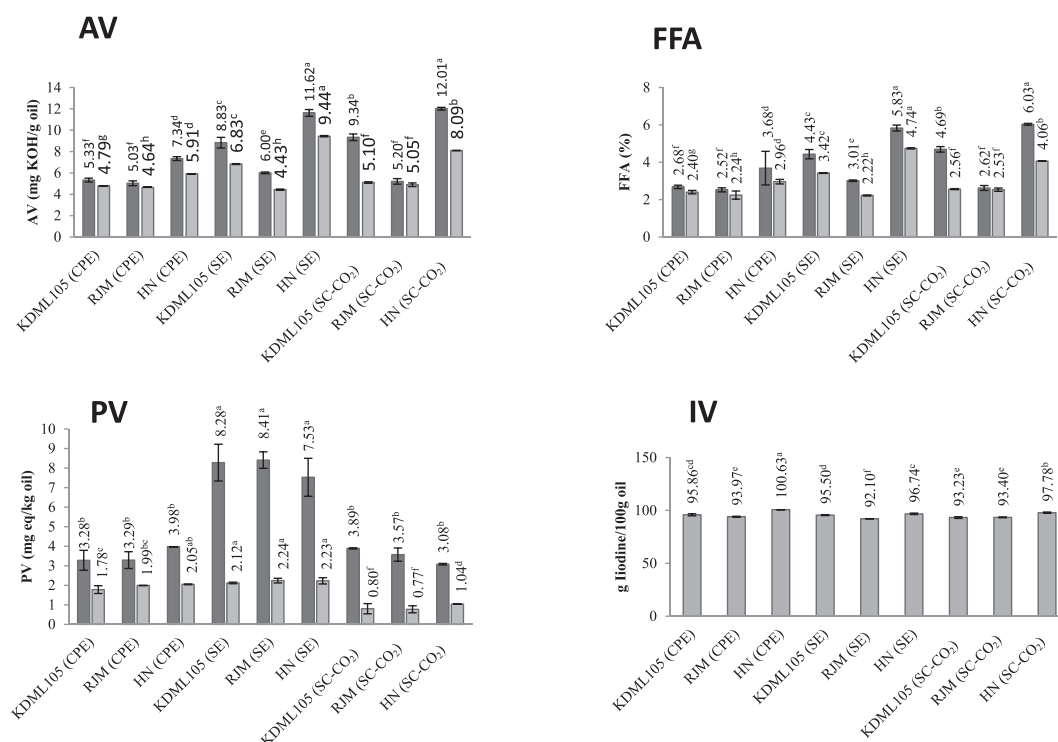
cial refined RBO was 10–11 which was similar to the values found in the RBO samples obtained from KDML 105 and RJM rice extracted by CPE, and KDML 105 extracted by SE. The RBO samples extracted by SC-CO<sub>2</sub> showed very low color values indicating the excellent color quality.

For AV, FFA, PV and IV, the results are show in Fig. 3. The black bars represent the mean values for crude oils

**Table 1** The color of RBO from three rice varieties extracted using different extraction methods.

Sample	Red (R)	Yellow (Y)	Blue (B)	Neutral (N)	Y+5R
KDML105 (CPE)	1.00	6.20	0.00	0.10	11.20
RJM (CPE)	1.00	5.10	0.00	0.10	10.10
HN (CPE)	1.50	9.60	0.00	0.10	17.10
KDML105 (SE)	1.00	5.40	0.00	0.10	10.40
RJM (SE)	1.00	4.70	0.00	0.10	9.70
HN (SE)	1.40	11.00	0.00	0.10	18.00
KDML105 (SC-CO <sub>2</sub> )	0.40	2.60	0.00	0.10	4.60
RJM (SC-CO <sub>2</sub> )	0.40	2.60	0.00	0.10	4.60
HN (SC-CO <sub>2</sub> )	0.60	3.80	0.00	0.10	6.80
Commercial oil	1.00	6.50	0.00	0.10	11.50

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.



**Fig. 3** Acid value (AV), Free fatty acid content (FFA), Peroxide value (PV) and Iodine value (IV) of RBO from three rice varieties extracted using different extraction methods. ■: black bar is crude RBO, □: grey bar is refined RBO. Bars with different letters on top are significantly different in retained percentage ( $p \leq 0.05$ ). CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.

while the grey bars represent the values for refined oils. The AV and FFA formed as a result of hydrolysis reaction of triglyceride to glycerol and FFA by lipase activity, which was increased rapidly after the milling process<sup>24</sup>. The PV content is the foremost initial reaction product of lipid oxidation and the IV is often used to determine the amount of unsaturation in RBO. The AV, FFA and PV of the crude oils were 5.03–12.01 mg KOH/g, 2.52–6.03% and 3.08–8.41 mg eq/kg oil, respectively while the AV, FFA and PV of the refined oils were 4.43–9.44 mg KOH/g oil, 2.22–4.74% and 0.77–2.24 mg eq/kg oil, respectively. The refining processes reduced the AV, FFA and PV values. This was occurred at deodorization step where FFA was removed and the oxidation reaction was prevented by heating<sup>25</sup>. The PV contents of both crude and refined oils were lower than the CODEX standard of fats and oils<sup>23</sup>. According to the CODEX standard, the maximum level of AV and PV of RBO are 0.5 mg KOH/g oil and 10 mg eq/kg oil, respectively. In addition, Tao, Rao and Liuzzo<sup>26</sup> suggested that the maximum level of FFA for RBO should be 5%. However, the RBO samples obtained in this study had the AV and FFA contents higher than the recommendations. This could influence by raw material quality as the bran did not stabilize before use. The bran quality deteriorated due to lipase activity<sup>24, 27</sup>. In terms of IV, the CODEX standard recommended between 90–105 g Iodine/100 g oil. The RBO samples in this study had the IV of 92.10–100.63 g Iodine/100 g oil which was conformed to the CODEX standard<sup>23</sup>.

The TPC, total  $\gamma$ -oryzanol and  $\alpha$ -tocopherol content found in RBO samples were 6.63–10.22 mg gallic acid/g oil, 119.75–281.95 mg/g oil and 0.37–1.84 mg/g oil, respectively (Table 2). All the phytochemical contents of the refined RBO samples were significantly different in both varieties and extraction methods ( $p \leq 0.05$ ). The RBO samples of

HN rice obtained by three extraction methods had the highest content of TPC,  $\gamma$ -oryzanol and  $\alpha$ -tocopherol. This result showed the pigmented rice contained higher antioxidants (TPC,  $\gamma$ -oryzanol and  $\alpha$ -tocopherol) concentrations than the non-pigmented rice which is in accordance with previously reported literatures<sup>28, 29</sup>. Data from Table 2 also showed that among pigmented rice, the black rice (HN) had higher antioxidants concentration than the red rice (RJM).

For extraction method, the RBO samples extracted by all three methods did not show a clear trend of TPC contents. However, the RBO sample extracted by the SE method showed the highest content of  $\gamma$ -oryzanol, followed by those extracted by the SC-CO<sub>2</sub> and CPE methods, respectively. This is because  $\gamma$ -oryzanol is soluble in organic solvents<sup>30</sup>. The RBO sample extracted by the CPE method had the highest  $\alpha$ -tocopherol, followed by those extracted by the SE and SC-CO<sub>2</sub> methods, respectively. The results illustrated that each extraction method was more suitable use for extraction of a particular compound, not all the compounds.

### 3.3 Fatty acid profile

The fatty acid profiles of the refined RBO samples are presented in Table 3. There were seven fatty acids found in the profiles including Tetradecanoic (Myristic, C14:0), Hexadecanoic (Palmitic, C16:0), *cis*-9-Hexadecenoic (Palmitoleic, C16:1 n7), Octadecanoic (Stearic, C18:0), *cis*-9-Octadecenoic (Oleic, C18:1 n9), *cis*-9,12-Octadecadienoic (Linoleic, C18:2 n6) and *cis*-9,12,15-Octadecatrienoic (Linolenic, C18:3 n3). The major saturated fatty acids found in the samples were myristic, palmitic and stearic acids with the concentration of 0.29–0.42, 17.87–20.57 and 2.18–2.65 g/100 g oil, respectively. The four unsaturated fatty acids found were palmitoleic, oleic, linoleic and linole-

**Table 2** Phytochemicals of RBO from three rice varieties extracted using different extraction methods.

Samples	TPC (mg gallic acid/g oil)	Total $\gamma$ -oryzanol (mg/g oil)	$\alpha$ -Tocopherol (mg/g oil)
KDML105 (CPE)	8.75 $\pm$ 0.10 <sup>c</sup>	106.90 $\pm$ 0.74 <sup>g</sup>	0.96 $\pm$ 0.01 <sup>c</sup>
RJM (CPE)	6.84 $\pm$ 0.09 <sup>f</sup>	124.89 $\pm$ 1.27 <sup>f</sup>	1.01 $\pm$ 0.01 <sup>c</sup>
HN (CPE)	9.26 $\pm$ 0.15 <sup>b</sup>	165.89 $\pm$ 0.07 <sup>e</sup>	1.84 $\pm$ 0.07 <sup>a</sup>
KDML105 (SE)	8.05 $\pm$ 0.12 <sup>d</sup>	120.20 $\pm$ 2.16 <sup>f</sup>	0.54 $\pm$ 0.01 <sup>f</sup>
RJM (SE)	7.33 $\pm$ 0.03 <sup>e</sup>	204.81 $\pm$ 0.26 <sup>c</sup>	0.97 $\pm$ 0.01 <sup>c</sup>
HN (SE)	9.28 $\pm$ 0.16 <sup>b</sup>	281.95 $\pm$ 2.13 <sup>a</sup>	1.10 $\pm$ 0.05 <sup>b</sup>
KDML105 (SC-CO <sub>2</sub> )	7.19 $\pm$ 0.06 <sup>e</sup>	119.75 $\pm$ 2.97 <sup>f</sup>	0.37 $\pm$ 0.01 <sup>g</sup>
RJM (SC-CO <sub>2</sub> )	6.63 $\pm$ 0.24 <sup>f</sup>	177.50 $\pm$ 1.37 <sup>d</sup>	0.69 $\pm$ 0.01 <sup>c</sup>
HN (SC-CO <sub>2</sub> )	10.22 $\pm$ 0.06 <sup>a</sup>	226.56 $\pm$ 2.10 <sup>b</sup>	0.81 $\pm$ 0.03 <sup>d</sup>

Means with different letters within a column are significantly different ( $p \leq 0.05$ ).

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.



**Table 3** Fatty acid composition (g/100 g) of refined RBO from three rice varieties extracted using different extraction methods.

Samples	Fatty acid composition (g/100 g oil)						
	C14:0	C16:0	C16:1 n7	C18:0	C18:1 n9	C18:2 n6	C18:3 n3
KDML105 (CPE)	0.31 ± 0.00 <sup>d</sup>	20.25 ± 0.05 <sup>c</sup>	0.21 ± 0.00 <sup>a</sup>	2.35 ± 0.00 <sup>c</sup>	43.14 ± 0.03 <sup>bc</sup>	28.42 ± 0.11 <sup>e</sup>	1.03 ± 0.01 <sup>e</sup>
RJM (CPE)	0.29 ± 0.00 <sup>e</sup>	20.51 ± 0.01 <sup>a</sup>	0.20 ± 0.00 <sup>b</sup>	2.39 ± 0.00 <sup>c</sup>	42.85 ± 0.07 <sup>c</sup>	28.31 ± 0.00 <sup>e</sup>	1.13 ± 0.01 <sup>b</sup>
HN (CPE)	0.38 ± 0.01 <sup>b</sup>	17.92 ± 0.09 <sup>e</sup>	0.17 ± 0.00 <sup>c</sup>	2.54 ± 0.07 <sup>b</sup>	41.51 ± 0.34 <sup>e</sup>	32.11 ± 0.38 <sup>a</sup>	1.03 ± 0.02 <sup>de</sup>
KDML105 (SE)	0.35 ± 0.01 <sup>c</sup>	20.09 ± 0.07 <sup>d</sup>	0.21 ± 0.00 <sup>a</sup>	2.35 ± 0.05 <sup>c</sup>	42.14 ± 0.20 <sup>d</sup>	29.39 ± 0.27 <sup>c</sup>	1.06 ± 0.02 <sup>cd</sup>
RJM (SE)	0.29 ± 0.01 <sup>e</sup>	20.57 ± 0.06 <sup>a</sup>	ND	2.49 ± 0.04 <sup>b</sup>	44.15 ± 0.06 <sup>a</sup>	27.03 ± 0.00 <sup>f</sup>	1.05 ± 0.00 <sup>cd</sup>
HN (SE)	0.42 ± 0.01 <sup>a</sup>	17.87 ± 0.01 <sup>e</sup>	ND	2.65 ± 0.02 <sup>a</sup>	42.24 ± 0.17 <sup>d</sup>	31.43 ± 0.19 <sup>b</sup>	0.97 ± 0.01 <sup>f</sup>
KDML105 (SC-CO <sub>2</sub> )	0.32 ± 0.01 <sup>d</sup>	20.09 ± 0.05 <sup>d</sup>	0.21 ± 0.00 <sup>a</sup>	2.18 ± 0.02 <sup>d</sup>	42.94 ± 0.12 <sup>bc</sup>	28.76 ± 0.07 <sup>d</sup>	1.08 ± 0.01 <sup>c</sup>
RJM (SC-CO <sub>2</sub> )	0.31 ± 0.00 <sup>d</sup>	20.35 ± 0.02 <sup>b</sup>	0.20 ± 0.00 <sup>b</sup>	2.24 ± 0.02 <sup>d</sup>	43.19 ± 0.11 <sup>b</sup>	28.11 ± 0.09 <sup>e</sup>	1.17 ± 0.01 <sup>a</sup>
HN (SC-CO <sub>2</sub> )	0.41 ± 0.00 <sup>a</sup>	17.56 ± 0.05 <sup>f</sup>	ND	2.69 ± 0.04 <sup>a</sup>	42.88 ± 0.18 <sup>bc</sup>	31.12 ± 0.15 <sup>b</sup>	0.93 ± 0.01 <sup>g</sup>

Means with different letters within a column are significantly different ( $p \leq 0.05$ ).

ND : Not detected.

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.

**Table 4** Antioxidant activity of refined RBO from three rice varieties extracted using different extraction methods.

Samples	DPPH IC <sub>50</sub> (mg/g oil)	FRAP (μM TE/g oil)	ORAC (μM TE/g oil)
KDML105 (CPE)	2.44 ± 0.00 <sup>b</sup>	38.18 ± 0.72 <sup>f</sup>	537.69 ± 5.30 <sup>g</sup>
RJM (CPE)	2.11 ± 0.04 <sup>d</sup>	71.72 ± 1.83 <sup>c</sup>	577.99 ± 9.26 <sup>c</sup>
HN (CPE)	1.27 ± 0.01 <sup>g</sup>	79.12 ± 1.01 <sup>b</sup>	640.22 ± 8.75 <sup>b</sup>
KDML105 (SE)	3.41 ± 0.02 <sup>a</sup>	39.60 ± 0.52 <sup>f</sup>	561.36 ± 8.77 <sup>f</sup>
RJM (SE)	2.28 ± 0.01 <sup>c</sup>	60.38 ± 0.80 <sup>d</sup>	622.78 ± 5.16 <sup>c</sup>
HN (SE)	1.71 ± 0.07 <sup>f</sup>	78.35 ± 4.06 <sup>b</sup>	690.10 ± 7.51 <sup>a</sup>
KDML105 (SC-CO <sub>2</sub> )	2.28 ± 0.00 <sup>c</sup>	51.78 ± 2.01 <sup>e</sup>	505.20 ± 9.75 <sup>i</sup>
RJM (SC-CO <sub>2</sub> )	1.85 ± 0.01 <sup>e</sup>	57.07 ± 0.34 <sup>d</sup>	523.91 ± 6.02 <sup>h</sup>
HN (SC-CO <sub>2</sub> )	0.93 ± 0.01 <sup>h</sup>	89.76 ± 4.64 <sup>a</sup>	596.03 ± 8.68 <sup>d</sup>

Means with different letters within a column are significantly different ( $p \leq 0.05$ ).

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO<sub>2</sub>: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.

nic acids with the concentration of 0–0.21, 41.51–44.15, 27.03–32.11 and 1.01–1.13 g/100 g oil, respectively. The fatty acid contents of all samples obtained from different extraction methods were significantly different ( $p \leq 0.05$ ). Palmitic, linoleic and oleic acids were the major fatty acids in the RBO samples<sup>31,32</sup>. The content of unsaturated fatty acids was higher than saturated fatty acids. The results of fatty acid profiles in this study are in accordance with Oluremi *et al.*<sup>33</sup>. High amount of unsaturated fatty acids in the RBO samples could promote nutritional and health benefits since unsaturated fatty acids could prevent diseases that associated with the cholesterol<sup>34,35</sup>.

### 3.4 Antioxidant activity

The antioxidant activity of the refined RBO samples

were evaluated using DPPH, FRAP and ORAC assays and the results are shown in **Table 4**. DPPH assay was determined through the hydrogen donation mechanism. The scavenging effects of all samples were significantly different in both varieties and extraction methods ( $p \leq 0.05$ ). DPPH IC<sub>50</sub> value represents the concentration that will inhibit 50% of a process. The RBO samples from HN rice extracted by three methods used the lowest concentration to inhibit 50% when compared to the RBO samples from KDML 105 and RJM rice. In addition, the RBO sample from HN rice extracted by SC-CO<sub>2</sub> provided the lowest IC<sub>50</sub> value, 0.93 mg/g oil. For FRAP assay, the antioxidant activity was 38.18–89.76 μM TE/g oil and the RBO sample produced from HN rice exhibited the highest value, followed by RJM and KDML 105 rice. The trend of antioxidant activ-

ity as observed by FRAP assay was similar to those observed by DPPH IC<sub>50</sub>. In terms of ORAC, the RBO sample from HN rice extracted using SE provided the best results, 690.10 µM TE/g oil. It can be concluded that the RBO samples from colored rice exhibited better antioxidant activity than those from white rice. This result is in agreement with previously reported by Fujita *et al.*<sup>36)</sup> and Sompong *et al.*<sup>37)</sup>. Antioxidant activity of each sample is different because each analysis method is sensitive to different antioxidative compounds<sup>38)</sup>.

#### 4 Conclusion

The RBO samples produced from the bran of three rice varieties including colored and non-colored (white) rice cultivars provided different physicochemical and antioxidant properties. It is confirmed in this study that colored rice (HN and RJM) provided better antioxidant property than white rice (KDML 105). Extraction methods were also found to influence the yields, physicochemical and antioxidant properties of RBO samples. The SC-CO<sub>2</sub> extraction provided the highest RBO yield. The RBO sample obtained from the bran of HN rice extracted by SC-CO<sub>2</sub> showed the highest antioxidant activity.

#### Acknowledgments

This study was financially supported by the Thailand Research Fund (TRF-RRI Grant No. PHD 56I0030). The cooperation from CEO Agrifood Co., Ltd. is greatly acknowledged.

#### Reference

- 1) Ryu, S.N.; Park, S.Z.; Ho, C.T. High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. *J. Food Drug Anal.* **6**, 729-736 (1998).
- 2) Boonsit, P.; Pongpiachan, P.; Julsrigival, S.; Karladee, D. Gamma oryzanol content in glutinous purple rice landrace varieties. *C.M.U. J. Nat. Sci.* **9**, 151-157 (2010).
- 3) Saenjum, C.; Chaivasut, C.; Chansakaow, S.; Suttajit, M.; Sirithunyulug, B. Antioxidant and anti-inflammatory activities of gamma-oryzanol rice extracts from Thai purple rice bran. *J. Med. Plants Res.* **6**, 1070-1077 (2012).
- 4) Yu, F.; Kim, S.H.; Kim, N.S.; Lee, J.H.; Bae, D.H.; Lee, K.T. Composition of solvent-fractionated rice bran oil. *J. Food Lipids* **13**, 286-297 (2006).
- 5) Kadam, M.; Bhowmick, D.N. HPLC analysis of rice bran oil. *J. Food Lipids* **13**, 354-361 (2006).
- 6) Gopala, K.A.G.; Khatoon, S.; Babylatha, R. Frying performance of processed rice bran oils. *J. Food Lipids* **12**, 1-11 (2005).
- 7) Reshma, M.V.; Saritha, S.S.; Balachandran, C.; Arumughan, C. Lipase catalyzed interesterification of palm stearin and rice bran oil blends for preparation of zero trans shortening with bioactive phytochemicals. *Biol. Tech.* **99**, 5011-5019 (2007).
- 8) Rit-Udom, S. The physicochemical properties and antioxidant activity of strawberry during storage at low temperatures. *Thesis of Master Degree, Naresuan University* (2004).
- 9) Iqbal, S.; Bhangar, M.I.; Anwar, F. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem.* **93**, 265-272 (2005).
- 10) Imsanguan, P.; Roaysubtawee, A.; Borirak, R.; Pong-amphai, S.; Douglas, S.; Douglas, P.L. Extraction of  $\alpha$ -tocopherol and  $\gamma$ -oryzanol from rice bran. *J. Food Sci. Technol.* **41**, 1417-1424 (2008).
- 11) Thanonkaew, A. Development process to enhance the quality of brown rice bran oil cold-pressed. Area based development research (ABC). Vol.3(5), May - June 2011 (2011).
- 12) Balachandran, C.; Mayamol, P.N.; Thomas, S.; Sukumar, D.; Sundaresan, A.; Arumughan, C. An ecofriendly approach to process rice bran for high quality rice bran oil using supercritical carbon dioxide for nutraceutical applications. *Bioresour. Technol.* **99**, 2905-2912 (2008).
- 13) Pestana, V.R.; Zambiasi, R.C.; Mendonça, C.R.B.; Bruscato, M.H.; Lerma-Garcia, M. J.; Ramis-Ramos, G. Quality changes and tocopherols and  $\gamma$ -oryzanol concentrations in RBO during the refining process. *J. Am. Oil Chem. Soc.* **85**, 113-119 (2008).
- 14) AOCS. Official methods and recommended practices of the American Oil Chemists' Society (Mehlenbacher, V.C.; Hopper, T.H.; Sallee, E.M.; Link, W.E.; Walker, R.O.; Walker, R.C. *et al.* eds.). 6th ed. American Oil Chemists' Society, Champaign (2009).
- 15) McBride, H.D.; Evans, D.G. Rapid voltammetric method for the estimation of tocopherols and antioxidants in oils and fats. *Anal. Chem.* **45**, 446-452 (1973).
- 16) Chen, M.H.; Bergman, C.J. A rapid procedure for analysing rice bran tocopherol, tocotrienol and  $\gamma$ -oryzanol contents. *J. Food Comp. Anal.* **18**, 139-151 (2005).
- 17) Speek, A.J.; Schrijver, J.; Scherurs, W.H.P. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. *J. Food Sci.* **50**, 121-124 (1985).
- 18) AOAC. Official methods of analysis. 18th ed. Association of Official Analytical Chemists, Washington, D.C. (2005).
- 19) Jham, G.N.; Teles, F.F.F.; Compos, L.G. Use of aqueous

- HCl/MeOH as esterification reagent for analysis of fatty acid derived from soybean lipids. *J. Am Oil Chem. Soc.* **59**, 132-133 (1982).
- 20) Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **28**, 25-30 (1995).
- 21) Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **239**, 70-76 (1996).
- 22) Ou, B.; Hampsch-Woodill, M.; Prior, R.L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agr. Food Chem.* **49**, 4619-4926 (2001).
- 23) CODEX STANDARD. CODEX standard for named vegetable oils. (1999).
- 24) Pourali, O.; Salak, A.F.; Yoshida, H.A. Rapid and eco-friendly treatment technique for rice bran oil stabilization and extraction under sub-critical water condition. *Proceed. World Congress on Eng. Comput. Sci. (WCECS)*. October 20-22, 2009. San Francisco, USA. 1, 978-988 (2009).
- 25) Bachari-Saleh, Z.; Ezzatpanah, H.; Aminafshar, M.; Safafar, H. The effect of refining process on the conjugated dienes in soybean oil. *J. Agr. Sci. Tech.* **15**, 1185-1193 (2013).
- 26) Tao, J.; Rao, R.; Liuzzo, J. Microwave heating for rice bran stabilization. *J. Microw. Power Electromagn. Energy* **28**, 156-164 (1993).
- 27) Malekian, F.; Rao, R. M.; Prinyawiwatkul, W.; Marshall, W.E.; Windhauser, M.; Ahmedna, M. Lipase and lipoxygenase activity, functionality and nutrient losses in rice bran during storage. Bull. No. 870. LSU Agricultural Center, Baton Rouge, LA, pp. 1-68 (2000).
- 28) Nam, S.H.; Chol, P.S.; Kang, M.Y.; Koh, H.J.; Kozukue, N.; Friedman, M. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. *Food Chem.* **94**, 613-620 (2006).
- 29) Yowadio, R.; Tanimori, S.; Morita, N. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. *Food Chem.* **101**, 1616-1625 (2007).
- 30) Orthofer, F.T. RBO: Healthy lipid source. *Food Technol.* **50**, 62-64 (1996).
- 31) Cicero, A.F.G.; Derosa, G. Rice bran and its main components: potential role in the management of coronary risk factors. *Curr. Top. Nutraceutical Res.* **3**, 29-46 (2005).
- 32) Rudzińska, M.; Hassanein, M.M.; Abdel-Razek, A.G.; Ratusz, K.; Siger, A. Blends of rapeseed oil with black cummin and rice bran oils for increasing the oxidative stability. *J. Food Sci. Technol.* **53**, 1055-1062 (2015).
- 33) Oluremi, O.L.; Solomon, A.O.; Saheed, A.A. Fatty acids, metal composition and physico-chemical parameters of Igbemo Ekiti rice bran oil. *J. Environ. Chem. Ecotoxicol.* **5**, 39-46 (2013).
- 34) Law, M. Dietary fat and adult diseases and the implications for childhood nutrition: An Epidemiologic approach. *Am. J. Clin. Nutr.* **72**, 1291-1296 (2000).
- 35) Choe, E. Effects and mechanism of minor compounds in oil on lipid oxidation. in *Food lipids* (Akoh, C.C.; Min, D.B. eds.). CRC Press, Taylor and Francis Group, Boca Raton FL, pp. 449-474 (2008).
- 36) Fujita, A.; Fujitake, H.; Kawakami, K.; Nomura, M. Antioxidant activity of colored rice bran obtained at different milling yields. *J. Oleo Sci.* **59**, 563-568 (2010).
- 37) Sompong, R.; Siebenhandl-Ehn, S.; Linsberger-Martin, G.; Berghofer, G.E. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem.* **124**, 132-140 (2011).
- 38) Aguilar-Garcia, C.; Gavino, G.; Baragaño-Mosqueda, M.; Hevia, P.; Gavino, V.C. Correlation of tocopherol, tocotrienol,  $\gamma$ -oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chem.* **102**, 1228-1232 (2007).