Effects of Extraction Methods on Phytochemicals of Rice Bran Oils Produced from Colored Rice

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Abstract: Rice bran oil (RBO) especially from colored rice is rich in phytochemicals and has become popular in food, cosmetic, nutraceutical and pharmaceutical applications owing to its offering health benefits. This study determined the contents of phytochemicals including oryzanols, phytosterols, tocopherols (Toc) and tocotrienols (T3) in RBOs extracted using different methods namely cold-press extraction (CPE), solvent extraction (SE) and supercritical CO2 extraction (SC-CO2). Two colored rice, Red Jasmine rice (RJM, red rice) and Hom-nin rice (HN, black rice), were studied in comparison with the popular Thai fragrant rice Khao Dawk Mali 105 (KDML 105, white rice). RBOs were found to be the rich source of oryzanols, phytosterols, Toc and T3. Rice varieties had a greater effect on the phytochemicals concentrations than extraction methods. HN rice showed the significantly highest concentration of all phytochemicals, followed by RJM and KDML 105 rice, indicating that colored rice contained high concentration of phytochemicals in the oil than non-colored rice. The RBO samples extracted by the CPE method had a greater concentration of the phytochemicals than those extracted by the SC-CO2 and SE methods, respectively. In terms of phytochemical contents, HN rice extracted using CPE method was found to be the best.

Key words: rice bran oil, oryzanol, phytosterol, tocopherol, tocotrienol

1 Introduction

Colored or pigmented rice is the unpolished rice that its bran layer contains red, purple, or black pigments whereas normal brown rice looks yellowish. Colored rice has been reported as an excellent source of natural phytochemicals including phenolic acids, anthocyanins, proanthocyanidins, oryzanol, phytosterol, tocotrienol and tocopherol¹-³. They have gained more attention for researchers as well as consumers due to its antioxidative ability that is beneficial to health. After a positive relationship between the lower incidence of cancers and coronary heart diseases in Asian populations and rice consumption was proposed⁴, a boost in research interest in rice antioxidants was observed. As a result, the research output on rice antioxidants increased rapidly. Currently, rice is the most studied cereal in animal and human clinical trials and in food fortification⁵. This trend is likely to increase in the near future as Europe, South America, and Africa are also becoming interested in the antioxidant potentials of their rice varieties⁶.

Rice bran oil (RBO) contains high amount of γ-oryzanol, vitamin E in both tocopherols and tocotrienols, and phytosterols⁶-⁸. These compounds have high capacity of antioxidants, serve to eliminate free radicals, prevent free radicals reaction with biomolecules causing damages to the body, and prevent certain diseases such as cardiovascular diseases and some cancers⁹-¹¹. However, processing steps involved in the production of RBOs affected the phytochemical contents and antioxidant properties¹²,¹⁰. Generally, the processing of RBOs starts from stabilization of rice bran. The stabilized rice bran was
then extracted for crude RBOs. The crude oils were then refined by a number of refining steps. Various commercial efforts to extract the oil have been made. RBO with low free-fatty acid (FFA) content can be extracted with hexane from extrusion stabilized bran. Recently, supercritical fluid extraction technique was evaluated as an alternative process for reducing the FFA content and minimizing the phytosterol loss of RBO during the process. This extraction method is nontoxic, nonflammable, and simple in operation when compared with traditional extraction using solvents. Moreover, the cold-pressing procedure involves neither heat nor chemical treatments, and it is becoming an interesting substitute for conventional practices because of consumers’ desire for natural and safe food products.

Cold pressing is simple, ecological and does not require much energy. The disadvantage of this process is low productivity and difficulties in obtaining a product of constant quality. As extraction is the most important step for RBO processing and various techniques are available, this paper aimed to determine the effects of cold-press extraction (CPE), solvent extraction (SE) and supercritical CO$_2$ extraction (SC-CO$_2$) on the phytochemical contents of red and black rice in comparison with normal brown rice. Phytochemicals which are high in RBOs including γ-oryzanol, phytosterols (specific phytochemicals that resemble cholesterol in structure but are found exclusively in plants) and vitamin E are determined in this study.

2 Materials and Methods

2.1 Sample preparation

Two colored rice, Red Jasmine rice (RJM, red rice) and Hom-nin rice (HN, black rice), were studied in comparison with the popular Thai fragrant rice Khao Dawk Mali 105 (KDMI 105, white rice). They were grown and harvested in Phichit Province of Thailand during December 2014 to February 2015. The paddy was dried to a moisture content of 13 g/100 g dry matter, milled and polished to obtain the bran which was instantaneously vacuum-packed in aluminum foil bags and stored at -20°C until further uses. The brans have not been stabilized as the frozen condition during storage could minimize all enzyme activities and deteriorations.

2.2 Extractions and refining processes

For 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.

For SE, the extraction process was conducted in the laboratory at room temperature. The ratio of rice bran:hexane 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.

For SC-CO$_2$, a pilot-scale SC-CO$_2$ extractor was employed using the following conditions; temperature of 60°C, pressure of 30 MPa, CO$_2$ flow rate of 55 L/h and extraction time for 4 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.

Crude RBO samples extracted from all three methods were refined using the method described earlier. Briefly, the refining steps involved dewatering by centrifugation at 6,000 rpm for 15 min, degumming by washing with hot water (80°C) and H$_2$PO$_4$ solution, neutralization of the oils by washing with hot water (80°C) and centrifugation at 6,000 rpm for 15 min until pH 7 was reached, bleaching by adding 7% bleaching earth and 0.7% activated carbon at 120°C under vacuum for 30 min, and finally deodorizing by steaming the oil at 220°C under vacuum for 60 min.

2.3 Determination of γ-oryzanol

The concentration of γ-oryzanol which consists of cycloartenol furulate (CAFA), 24-methylene cycloartenol furulate (24MCAFA), campesteryl furulate (CampFA) and β-sitosteryl furulate (SitoFA) was determined by a HPLC-MS/MS technique following the method described earlier. Approximately 100 mg of refined RBO was dissolved in 2 mL of isopropyl alcohol. Then 10 µL of sample stock solution was diluted with isopropyl alcohol to make a final volume of 1,000 µL. The sample solution was filtered with 0.2 µm syringe filter and then 10 µL of the prepared solution was injected to HPLC-MS/MS using ODS column. The methanol was used as a mobile phase with a flow rate of 1.0 mL/min. All components of γ-oryzanol were detected in atmospheric pressure chemical ionization mode. The concentrations of individual component of γ-oryzanol in the refined RBO samples were determined using calibration curves of standard. The analysis time was about 25 min. The peaks were sorted as CAFA at 14.5 min, 24MCAFA at 15.6 min, CampFA at 17.3 min and SitoFA at 19.0 min.

2.4 Determination of phytosterols

The concentration of phytosterol including campesterol, stigmasterol and sitosterol was determined by GC technique as described elsewhere. Each 0.1 g of RBO samples was weighed into a test tube with screw cup. The internal standard, 5α-cholestanol, was added to the sample in the test tube before saponification. The sample was saponified with 2.5 mL of 2 M KOH in ethanol solution and then heated at 60°C for 1 h. Then 2 mL of saturated NaCl and 3 mL of hexane were added to the reaction mixture, mixed and centrifuged at 3,000 rpm for 10 min to separate the
layers. The hexane phase was transferred into another test tube and it was extracted with 3 mL of hexane again. The combined hexane phases were evaporated to dryness. Then 0.3 mL of bis(trimethylsilyl)-trifluoracetamid containing 1% trimethylchlorosilane (BSTFA-TMCS) was added to the sample in the test tube and heated at 70°C for 15 min. The BSTFA-TMCS was evaporated to dryness under nitrogen stream and 1 mL of chloroform was added. The sample was injected to the GC (GL Sciences Inc., Tokyo, Japan) with ZB-5MS column (Phenomenex, USA, 30 m × 0.25 mm, thickness 0.25 µm) and FID detector. The concentrations of Toc and T3 were calculated from the peak area of the multiple standards.

2.5 Determination of α-, β-, γ-, δ- tocopherols and –tocotrienols

The concentrations of α-, β-, γ-, δ-tocopherols (Toc) and tocotrienols (T3) in RBOs were determined by a HPLC-MS/MS technique following the method described earlier[24,25]. Approximately 100 mg of refined RBO was dissolved in 2 mL of isopropyl alcohol. Then 50 µL of the solution was further diluted with isopropyl alcohol to make a final volume of 1,000 µL. The prepared solution was filtered with 0.2 µm syringe filter and then 20 µL of the prepared solution was injected to HPLC-MS/MS (4000 QTRAP, AB SCIEX, Tokyo, Japan). The mixture was performed at 40°C using a silica column (ZORBAX Rx-SIL, 4.6 × 250 mm; Agilent, Palo Alto, CA). The mixture of hexane:1,4-dioxane:2-propanol at 100:4:0.5 v/v/v was used as a mobile phase with a flow rate of 1.0 mL/min. The Toc and T3 were detected in atmospheric pressure chemical ionization mode. All vitamin E derivatives were successfully separated without peak overlapping, and the analytical time was about 18 min. The peaks were sorted as α-Toc at 6.8 min, α-T3 at 7.8 min, β-Toc at 9.1 min, γ-Toc at 9.8 min, β-T3 at 10.9 min, γ-T3 at 11.6 min, δ-Toc at 13.5 min and δ-T3 at 16.1 min. The concentrations of Toc and T3 were calculated from the peak area of the multiple standards.

2.6 Statistical analysis

All values are expressed as means ± standard deviations. Statistical analysis of data was performed using one-way ANOVA. Mean comparison was carried out using Duncan’s multiple range test. Differences were considered to be statistically significant when p < 0.05.

3 Results and Discussion

3.1 γ-Oryzanol

The concentrations of γ-oryzanol components (CAFA, 24MCAFA, CampFA and SitoFA) in RBO samples are shown in Table 1. Examples of the chromatograms (taken from HN rice variety extracted by the CPE) are illustrated in Fig. 1. Total γ-oryzanol content was found to be approximately 163-544 mg/100 g oil. In general, CampFA was the main component of γ-oryzanol, followed by CAFA, 24MCAFA and SitoFA, respectively. With regards to the varieties, HN contained significantly higher (p ≤ 0.05) total γ-oryzanol content than KDML 105 and CJM when extracted by the CPE and SE methods. However, its total γ-oryzanol content was not significantly different (p > 0.05) to CJM when extracted by SC-CO2 method. KDML 105 and CJM had no significant different (p > 0.05) in total γ-oryzanol content for all extractions except SE method. The results suggested that HN was the potential source of total γ-oryzanol. With regard to extraction methods, no significant difference (p

| RBO samples       | CAFA (mg/100 g oil) | 24MCAFA (mg/100 g oil) | Camp FA (mg/100 g oil)  | Sito FA (mg/100 g oil)  | Total (mg/100 g oil) |
|-------------------|---------------------|------------------------|-------------------------|-------------------------|----------------------|
| KDML105 (CPE)    | 89.99 ± 5.9a       | 75.16 ± 5.36abc       | 149.88 ± 9.74abc       | 11.59 ± 1.88ab        | 326.62 ± 7.58ab     |
| RJM (CPE)        | 75.04 ± 8.28d      | 76.09 ± 6.82abc       | 114.32 ± 7.33abc       | 9.63 ± 1.69ab         | 275.09 ± 6.05ab     |
| HN (CPE)         | 167.03 ± 2.53a     | 99.23 ± 6.23a         | 264.51 ± 5.69a         | 13.09 ± 2.37a         | 453.78 ± 7.41a      |
| KDML105 (SE)     | 88.78 ± 7.98e      | 85.21 ± 9.48abc       | 146.53 ± 5.10bc        | 12.15 ± 2.07e         | 332.67 ± 7.15e      |
| RJM (SE)         | 42.25 ± 6.51f      | 49.62 ± 3.79a         | 64.84 ± 9.30a          | 6.23 ± 0.44a          | 162.94 ± 5.11d      |
| HN (SE)          | 171.26 ± 1.75c     | 90.13 ± 6.65ab        | 268.22 ± 6.20a         | 13.18 ± 3.33a         | 542.79 ± 6.60a      |
| KDML105 (SC-CO2) | 57.61 ± 3.50g      | 54.77 ± 5.88abc       | 94.25 ± 5.71c          | 8.56 ± 2.47ab         | 215.19 ± 5.56d      |
| RJM (SC-CO2)     | 83.27 ± 4.65d      | 64.31 ± 6.05abc       | 118.34 ± 6.28abc       | 9.50 ± 0.83ab         | 275.42 ± 5.44d      |
| HM (SC-CO2)      | 139.48 ± 6.12b     | 67.84 ± 4.46abc       | 190.92 ± 3.66abc       | 9.89 ± 1.92ab         | 408.13 ± 6.16ab     |

Means with different letters within a column are significantly different (p ≤ 0.05).

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO2: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.
CAFA content in RBO samples extracted from all extraction methods on total \( \gamma \)-oryzanol content. Within the same rice variety, there was no significant difference for all extractions. Within the same rice variety, RBO samples were significantly higher in CAFA content of HN, although not statistically significant to other rice varieties for all extractions. This is understandable as different conditions were used between this paper and the mentioned one. Oryzanol contents were reported to be severely affected by alkali conditions. It has been reported that 83–95% of oryzanol from its original content was lost during alkali refining. Recently, SC-CO\(_2\) extraction of lipids has received much attention as an alternative to organic solvent extraction and has been shown to be an ideal method for extracting certain lipids. Most studies for the use of SC-CO\(_2\) in lipid extraction have focused on the yield of extractable material. However, each compound possesses a unique extractability under different conditions of supercritical fluid extraction. In addition, current publications indicated high phytochemicals including oryzanol in colored rice especially black rice, similar to HN in this study. More research is needed in this area.

### 3.2 Phyto sterols

The concentrations of phyto sterols including campsterol, stigmasterol and \( \beta \)-sitosterol are shown in Table 2. In addition, Fig. 2 shows the example of chromatograms, taken from HN rice extracted by the CPE method. For RBOs extracted by all methods, \( \beta \)-sitosterol was more prevalent than campsterol and stigmasterol. The concentrations of total phyto sterols ranged from 8.78-11.43 mg/g oil. RBOs from HN rice extracted by CPE and SC-CO\(_2\) methods had the highest (\( p \leq 0.05 \)) total phyto sterol content whereas RBOs from KDML 105 rice extracted by SC-CO\(_2\) method showed the lowest (\( p \leq 0.05 \)) value.

The campesterol contents of HN’s RBOs were significantly higher (\( p \leq 0.05 \)) than other rice varieties for all extraction methods. For each rice variety, there was significant difference of campesterol contents in RBOs extracted using different extraction methods. However, the stigmasterol content of RBOs from HN rice extracted from all methods did not show any significant difference (\( p > 0.05 \)). The RBO samples from HN rice had significantly higher (\( p \leq 0.05 \)) stigmasterol content than other varieties, except RJM extracted by SC-CO\(_2\) method. No significant difference (\( p > 0.05 \)) in the stigmasterol content was observed for KDML 105 and RJM extracted by CPE and SE. In terms of \( \beta \)-sitosterol content, RBOs from HN rice extracted by SC-CO\(_2\) method was the highest (\( p \leq 0.05 \)) while those of KDML 105 and RJM extracted by SC-CO\(_2\) method were the lowest (\( p \leq 0.05 \)). However, each compound possesses a unique extractability under different conditions of supercritical fluid extraction. In addition, current publications indicated high phytochemicals including oryzanol in colored rice especially black rice, similar to HN in this study. More research is needed in this area.

![Chromatograms of \( \gamma \)-oryzanol](image-url)

**Fig. 1** Chromatograms of \( \gamma \)-oryzanol detected in rice bran oils obtained from Hom-nin rice extracted by the cold-pressed extraction method.

> 0.05) was found in total \( \gamma \)-oryzanol content for all extraction methods within each rice variety except HN’s RBO extracted by SC-CO\(_2\) method, indicating not much effect of extraction methods on total \( \gamma \)-oryzanol content.

In terms of the components, CAFA contents of HN’s RBO samples were significantly higher (\( p \leq 0.05 \)) than other rice varieties for all extractions. Within the same rice variety, there was no significant difference (\( p > 0.05 \)) of CAFA content in RBO samples extracted from all extraction methods, except that HN from SE had significantly higher (\( p \leq 0.05 \)) CAFA content than those obtained from SC-CO\(_2\) method. The results suggested that rice varieties contributed to the CAFA content in RBOs. For 24MCAFA, the values ranged from 49.62 to 99.23 mg/100 g oil. HN’s RBO generally showed high contents of 24MCAFA, although not statistically significant (\( p > 0.05 \)) to all other samples.

For SitoFA, there was no significant difference (\( p > 0.05 \)) in SitoFA content of all RBO samples from all rice varieties and extraction methods, except that HN’s RBO sample obtained by SE method had significantly higher (\( p > 0.05 \)) SitoFA content than that of RJM.

In addition, CampFA content of HN’s RBO sample was significantly higher (\( p \leq 0.05 \)) than other rice varieties for CPE and SE methods. There was no significant difference (\( p > 0.05 \)) in CampFA content of RJM and KDML 105 in all extraction methods. Within the same rice variety, there was no significant difference (\( p > 0.05 \)) in CampFA content of RBO samples extracted by all extraction methods.

In this study, rice varieties were found to have the pronounced effects on phytochemicals than extraction methods. The contents of oryzanol components were different depending on rice varieties. Generally, 24MCAFA and CampFA were found to be the major ferulates which are in agreement with previously reported literature.

This study found that extraction methods had less effect than rice varieties on the contents of oryzanol. However, previous work has reported that SC-CO\(_2\) extraction provided tremendously higher yield of \( \gamma \)-oryzanol than solvent extractions. This is understandable as different conditions were used between this paper and the mentioned one. Oryzanol contents were reported to be severely affected by alkali conditions. It has been reported that 83–95% of oryzanol from its original content was lost during alkali refining. Recently, SC-CO\(_2\) extraction of lipids has received much attention as an alternative to organic solvent extraction and has been shown to be an ideal method for extracting certain lipids. Most studies for the use of SC-CO\(_2\) in lipid extraction have focused on the yield of extractable material. However, each compound possesses a unique extractability under different conditions of supercritical fluid extraction. In addition, current publications indicated high phytochemicals including oryzanol in colored rice especially black rice, similar to HN in this study. More research is needed in this area.
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Table 2  Phytosterol contents of refined RBOs from three rice varieties extracted using different extraction methods.

| RBO samples     | Campesterol (mg/ g RBO) | Stigmasterol (mg/ g RBO) | β-sitosterol (mg/ g RBO) | Total phytosterols (mg/ g RBO) |
|-----------------|--------------------------|--------------------------|--------------------------|-------------------------------|
| KDML105 (CPE)  | 2.59 ± 0.10c             | 1.87 ± 0.05bc            | 5.46 ± 0.14c             | 9.93 ± 0.30d                  |
| RJM (CPE)      | 2.57 ± 0.01e             | 1.81 ± 0.03ed            | 5.78 ± 0.05e             | 10.17 ± 0.10ed                |
| HN (CPE)       | 3.52 ± 0.03a             | 2.04 ± 0.01a             | 5.86 ± 0.04a             | 11.43 ± 0.00a                 |
| KDM105 (SE)    | 2.75 ± 0.02d             | 1.90 ± 0.03b             | 5.67 ± 0.05bc            | 10.32 ± 0.06bc                |
| RJM (SE)       | 2.47 ± 0.05d             | 1.81 ± 0.00ed            | 5.70 ± 0.14b             | 9.99 ± 0.20ed                 |
| HN (SE)        | 3.33 ± 0.03b             | 1.99 ± 0.00e             | 5.19 ± 0.03e             | 10.51 ± 0.06b                 |
| KDM105 (SC-CO₂) | 2.03 ± 0.06d            | 1.77 ± 0.02d             | 4.97 ± 0.19e             | 8.78 ± 0.28c                  |
| RJM (SC-CO₂)   | 2.43 ± 0.02d             | 1.99 ± 0.00e             | 4.98 ± 0.17e             | 9.41 ± 0.19e                  |
| HN (SC-CO₂)    | 2.91 ± 0.08c             | 2.05 ± 0.07a             | 6.25 ± 0.10c             | 11.21 ± 0.25c                 |

Means with different letters within a column are significantly different (p ≤ 0.05).

CPE: Cold-press extraction; SE: Solvent extraction; SC-CO₂: Supercritical carbon dioxide extraction; KDML 105: Khao Dawk Mali 105; RJM: Red Jasmine; HN: Hom-nin.

As γ-oryzanol is a mixture of ferulic acid esters of triterpene alcohols and sterols, various phytosterols in RBOs have been reported[27]. This study confirms the occurrence of major phytosterols, campesterol, stigmasterol and β-sitosterol, found in RBOs. Current researches focus on two aspects in this area, health benefits and analytical techniques. Influences of rice varieties and effects of processing steps on the contents of phytosterols were mostly studied as major bioactive compounds such as oryzanol and tocopherol. Very limited published papers that investigate deeply to each component are available.

3.3 Tocopherols (Toc) and tocotrienols (T3)

The chromatograms of Toc and T3, taken from HN rice extracted by CPE method is shown in Fig. 3. The contents of eight vitamin E homologues in RBOs extracted from different methods are shown in Table 3. Among vitamin E homologues, γ-T3 was the predominant while β-T3 was not detected in any RBO sample. The total Toc and T3 contents of HN’s RBO samples extracted by all methods were significantly higher (p ≤ 0.05) than those of KDML 105 and RJM, indicating the effect of varieties on total Toc and T3 contents. The HN’s RBO sample extracted by CPE had significantly higher (p ≤ 0.05) total Toc and T3 content than

Fig. 2 Chromatograms of phytosterols detected in rice bran oils obtained from Hom-nin rice extracted by the cold-pressed extraction method.

Fig. 3 Chromatograms of tocopherols and tocotrienols detected in rice bran oils obtained from Hom-nin rice extracted by the cold-pressed extraction method.

\( p > 0.05 \) in β-sitosterol content.

Means with different letters within a column are significantly different (p ≤ 0.05).
SC-CO$_2$ and SE methods, respectively, indicating that CPE preserved total Toc and T3 contents in the samples. In terms of vitamin E derivatives, this paper successfully separated all derivatives without peak overlapping. In general, HN’s RBO samples had significantly higher ($p \leq 0.05$) $\alpha$-Toc, $\beta$-Toc and $\delta$-Toc contents than other rice varieties for all extraction methods. Comparing within the HN variety, the sample extracted by CPE had significantly higher ($p \leq 0.05$) $\alpha$-Toc, $\beta$-Toc and $\delta$-Toc contents than SC-CO$_2$ and SE methods. For $\gamma$-Toc, HN’s RBO samples also exhibited higher $\gamma$-Toc content than other rice varieties. The RBO samples extracted from HN and RJM rice using CPE method showed the highest $\gamma$-Toc content, followed by SC-CO$_2$ and SE methods respectively. It can be concluded that HN rice and CPE method provided RBO samples with the highest ($p \leq 0.05$) concentration of four derivatives of Toc.

For T3 derivatives, $\alpha$-T3 could be detected in HN samples using all extraction methods. While in RJM, it could be detected only by using CPE and SC-CO$_2$ methods. For KDML 105, it could only be found by the SE method. In HN’s RBO sample, the CPE method provided the highest ($p \leq 0.05$) $\alpha$-T3 content, followed by SC-CO$_2$ and SE methods, respectively. In contrast with $\beta$-T3, it was not detected in any samples in this study. For $\gamma$-T3, significant differences ($p \leq 0.05$) of contents were found in samples extracted from three rice varieties using all extraction methods. The RBO sample obtained from HN rice extracted by SC-CO$_2$ method showed the highest $\gamma$-T3 content. Similar to $\delta$-T3, HN’s RBO samples had significantly higher ($p \leq 0.05$) $\delta$-T3 content than other rice varieties for all extraction methods. In addition, when considering the extraction methods, SC-CO$_2$ method provided the highest $\delta$-T3 content. Therefore, it can be summarized that HN rice had the highest $\delta$-T3 content than RJM and KDML. For $\alpha$-T3, CPE seemed to be the most appropriate extraction method while for $\gamma$-T3 and $\delta$-T3, SC-CO$_2$ seemed to be the best extraction method.

It is now well established that vitamin E refers to eight different isomers that belong to two categories, four saturated analogues ($\alpha$, $\beta$, $\gamma$, and $\delta$) called Toc and four unsaturated analogues referred to as T3. While the Toc has been investigated extensively, little is known about the T3. There is some evidence, however, that T3 may be superior in its biological properties, and that its anti-inflammatory and antioxidant activities could prevent cancer, diabetes, cardiovascular and neurodegenerative diseases. RBOs were described as one of the richest sources of Toc and T3$^{[33]}$. Quantities of Toc and T3 were found to be varied according to the origin of the rice bran$^{[24]}$. In this study, all forms of Toc and T3 except $\beta$-T3 were detected in RBOs extracted using different methods. Generally, HN’s RBO extracted using CPE were found to be the best in preserving both Toc and T3. Mild condition of CPE could limit the loss of vitamin E. HN’s RBO is from black rice variety. Currently, it has been reported that black rice provided higher bioactive compounds than other pigmented rice$^{[32]}$. Thought, some reports suggested that vitamin E and $\gamma$-oryzanol were not associated with bran color$^{[21]}$. Research on this issue is continuing.

### 4 Conclusion

Rice varieties were found to have the pronounce effects than extraction methods in terms of phytochemical quantities in RBOs. Regardless of the extraction methods, colored rice especially black rice exhibited considerably higher contents of phytochemicals as evidenced by oryzanol, phytoestrogens, Toc and T3 than those from non-color rice varieties. RBO is difficult to extract and therefore extraction is the most important process for RBO production. Solvent
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extraction and modern super critical CO₂ extraction although provide higher yields but affect phytochemical contents. Mild and minimal processing like cold-press extraction is the best in terms of phytochemical retentions.

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