Subcritical Butane and Propane Extraction of Oil from Rice Bran

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Rice bran oil was extracted using hexane, as well as subcritical butane and propane. The quality of rice bran oils obtained using these three solvents was comparatively studied. The results showed that subcritical fluid extraction oil showed higher concentrations of health-conducive components (such as vitamin E, oryzanol, and phytosterol) compared with hexane-extracted oil. Extraction solvent had an important effect on the distribution of linoleic acid and oleic acid. The sterol compositions in rice bran oils were identified to be composed of ethyl iso-allocholeate, campesterol, stigmasterol, sitosterols, and cyclolanosts. NMR data showed that butane-extracted oil had the highest triacylglycerols (TAGs) content and that subcritical propane-extracted oil had the highest wax content among the oils extracted with these three extraction solvents. The oils extracted by subcritical butane and propane showed higher oxidative stabilities. These results indicate that subcritical fluid extraction technology could be a useful method for producing high-quality rice bran oils and could become widely used in industrial applications.

Keywords: Subcritical fluid extraction; Rice bran oil; NMR; Sterols

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INTRODUCTION

Rice bran is the cuticle between the rice grain and paddy husk obtained as a by-product of rice processing and is a rich source of fiber, oil, protein, and nutrients essential to life (Amarasinghe et al. 2009; Bakota et al. 2013). The major health-promoting components contained in rice bran oil are γ-oryzanol, phytosterols, tocopherols, and tocotrienols, and it is found that the concentrations of the health components in rice bran oil are appreciably greater as compared with most other vegetable oils (Liu et al. 2013). The tocopherol content of oil is also important for protecting lipids against autoxidation and thereby increasing their storage life (Afaf and Appelqvist 1996). The presence of oryzanol in rice bran oil has functions similar to that of vitamin E for improving growth, facilitating capillary growth in the skin, and promoting blood circulation, along with stimulating hormonal secretions (Mishra et al. 2012). Therefore, there has been increasing interest in the health and nutritional aspects of rice bran oil, as well as in its potential industrial application in recent years (Worasith et al. 2011).

Generally, the major traditional methods of plant oil extraction include conventional organic solvent extraction and expeller pressing methods (Liu et al. 2014). The drawback of the organic solvent extraction method is that the oil must be heated to distill it and contains residual solvents and that the oil is oxidatively unstable and easily subject to rancidity during the separation process (Liu et al. 2014). The oil yield of the
expeller pressing method is lower compared with organic solvent extraction. Supercritical fluid extraction is also attracting increasing attention because of advantages that include the high yield and quality of oil (Wang et al. 2011; Xia et al. 2011). During the supercritical fluid extraction process, both pressure and temperature can be controlled to modify solvent physical properties such as viscosity, diffusivity, and density. However, supercritical fluid extraction requires high pressure and is expensive, which limits its industrial application. Subcritical fluid extraction is performed at a lower pressure and temperature as compared with supercritical fluid extraction. Although various solvents (include hexane) have been used in subcritical fluid extraction, compressed gases (such as propane and butane) are used as subcritical fluid primarily because they require a low critical temperature and pressure and they are an colorless, clean solvents that leave no toxic residue in the product (Sparks et al. 2006; Liu et al. 2014). Subcritical propane and butane extraction has many advantages besides lower pressure and temperature, such as high selectivity, environmental compatibility, short extraction time, a one-step process from extraction to separation, and low residual solvent (Herrero et al. 2006). Several commercial processes have used propane and butane as extraction solvents, including forestry products and essential oils (Herrero et al. 2006; Liu et al. 2014). Rice bran oil is usually obtained by solvent extraction, and hexane is the extraction solvent most commonly used. However, hexane has a high volatility and is considered toxic to humans and animals at relatively low concentrations (Sparks et al. 2006). In addition, the expensive process of distillation is necessary to remove hexane residue completely if the defatted meal is to be used for animal feed. By comparison, subcritical fluid extraction is a continuous counter-current process, and the solvent is removed using a vacuum at a low temperature after extraction (Miao et al. 2013). Therefore, the subcritical fluid extraction process is safe and is not expected to damage the protein in meal during the oil extraction process because of very lower extraction and distillation temperatures.

The main objective of this investigation was to produce high-quality rice bran oils using subcritical butane and propane extraction. The rice bran oils obtained with subcritical fluid extraction were studied comparatively with the hexane-extracted oil. The physicochemical properties of rice bran oils obtained with various extraction solvents were comparatively analyzed. In addition, the chemical compositions of the various rice bran oils were analyzed by GC-MS and NMR.

**EXPERIMENTAL**

**Materials and Chemicals**

Rice bran, hexane, butane, and propane were obtained from Henan Yalinjie Biological Technology Co., Ltd. (Anyang, China). Other reagents were of analytical grade and directly used as purchased without further purification. The crude lipid contents in rice bran were determined by using the methods described by Baboli and Kordi (2010), and the result showed that the rice bran contained crude lipid at a level of 22.1%.

**Extraction of Oil**

Subcritical butane and propane extractions were performed in a flow circulatory extraction apparatus (CBE-5L, Yalinjie, Anyang, China). For a typical run, a 700-g
sample of rice bran was used. The extraction capacity was 5 L, and the butane and propane flow rates were 77 L/h. Approximately 2.5 L of fluid was pumped into the extractor from a 2.0-MPa pressurized bottle. Extraction pressure was maintained at a constant 0.5 MPa in the extractor, and the extractor and separators were jacketed to maintain constant temperatures of 40 and 45 °C, respectively. The rice bran oil was collected from the two separators every 30 min (three times), and the butane and propane were cooled and recycled into the extraction system. Hexane-oil was extracted from rice bran by Soxhlet for 3 h using hexane as the solvent (Zullaikah et al. 2009). Briefly, 50 g of rice bran and 400 mL of hexane were added to a Soxhlet extractor. The contents were rapidly heated to reflux for 1.5 h with vigorous stirring. After cooling, the contents were filtered and the hexane was evaporated to dryness under reduced pressure at 70 °C. The extraction yield was calculated with the following formula,

\[
Y_{oil} = \frac{(Y - Y_r)}{Y} \times 100\% \tag{1}
\]

where \(Y_{oil}\) is the extraction yield (%), \(Y\) is the total oil in rice bran, and \(Y_r\) is the oil in extracted residue.

**Fatty Acid Composition of Oil and Analysis of Chemical Properties**

The fatty acid composition of rice bran oil was determined by GC-MS. Before analysis, the fatty acids were derivatized into methyl esters according to the method published by Ichihara et al. (1996). The fatty acid methyl esters were determined on an Agilent GC-MS (7890A/5978, USA) with a HP-5 capillary. The temperatures of column, injector, and detector were set at 180, 210, and 230 °C, respectively. The flow rate of carrier gas \(H_2\) with a split ratio of 1:20 was set at 1 mL/min. The injection size was 2 μL. The fatty acids were identified by comparing mass spectra with the National Institute of Standards and Technology (NIST) 2005 library data. The mass range scanned was from 30 to 500 amu in electron-impact (70 eV) mode. The chemical properties (FFA, acid value, and peroxide value) were determined by using the methods described by Baboli and Kordi (2010).

**Analysis of Tocopherol, Tocotrienol, Oryzanol, and Phytosterol**

*Analysis of tocopherol and tocotrienol*

Tocopherol and tocotrienol were analyzed by an HPLC system (Waters e2695, USA) consisting of a pump, an autosampler, and a fluorescence detector (2475 FLR Detector) interfaced with a computer. A Waters Spherisorb column (250×4.6 mm, 5 μm) was used with hexane/isopropanol (99/1, v/v) as a mobile phase. The system was operated isocratically at a flow rate of 0.8 mL/min. Separations were carried out at 40 °C with the fluorescence detector excitation and emission wavelengths set at 298 and 325 nm, respectively. The injection size was 5-μL samples.

*Analysis of oryzanol*

Oryzanol content in the sample was determined spectrophotometrically. The sample (2.0 to 3.0 g) was first dissolved in chloroform in a 25-mL volumetric flask, and then 1 mL of the solution was dissolved in ethanol in a 50-mL volumetric flask. The absorptions at multiple wavelengths (200 to 400 nm) were measured. The calibration curve was obtained with pure oryzanol in a concentration range of 8 to 18 μg/mL. In this concentration range, the absorption obeys Beer’s law and the calibration curve at \(\lambda=327.6\)
was a straight line passing through the origin ($R^2=0.9999$), where the slope represents the specific extinction coefficient [$E(327\text{ nm})=(29.35x-0.1831)\text{ g/L/cm}$].

**Analysis of phytosterol**

Phytosterol content in the sample was also determined spectrophotometrically. The calibration curve was obtained with pure phytosterol in a concentration range of 136 to 546 $\mu$g/mL. The straight line passing through the origin ($R^2=0.9985$) was obtained at the absorption peak of $\lambda=441$ nm, and the specific extinction coefficient was $E(327\text{ nm})=(682.68x+0.7047)\text{ g/L/cm}$. A 3-g sample was weighed and saponified with 2 N ethanolic KOH at 90 $^\circ$C for 2 h, with periodic vortex mixing to ensure complete saponification. Unsaponifiable materials were extracted with hexane three times. Hexane fractions were combined, and the solvent was evaporated at 50 $^\circ$C. The unsaponifiable materials were dissolved in ethanol, and absorptions at multiple wavelengths (200 to 400 nm) were measured. The qualitative identification of phytosterol was also confirmed by GC-MS analysis performed on an Agilent GC-MS (7890A/5978, USA) equipped with an HP-5 capillary column. Before analysis, trimethylsilyl derivatives of the phytosterols were created by adding 100 $\mu$L of N,O-bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane and heating at 60 $^\circ$C for 1 h. The GC was programmed at 250 $^\circ$C for 2 min and then increased at 10 $^\circ$C/min to 270 $^\circ$C for 30 min. The velocity of the carrier gas, He, was 1 mL/min, and the injection size was 0.1 $\mu$L. Triplicates were prepared for all of above analysis.

**NMR Analysis**

The rice bran wax was determined by NMR as described previously (Bakota et al. 2013) with some modifications. $^1$H NMR spectra were acquired in DMSO-d6 as solvent on a Bruker (Germany) Advance digital spectrometer operating at 400 MHz. The NMR tube was heated at 60 $^\circ$C before measurement to dissolve all components. The triacylglycerol (TAG) backbone peak and the wax ester peak at 3.96 to 4.06 ppm for two protons (triplet) and at 4.06 to 4.18 ppm for two protons (quarter) were compared by obtaining the molar ratio between TAG and wax ester. SpinWorks 3.1.7 software was used for integration of the peak areas.

**Oxidative Stability Index (OSI)**

The oxidative stability of rice bran oil was analyzed according to the Rancimat method using Metrohm 743 Rancimat equipment (Herisau, Switzerland) as described previously (Serrano et al. 2013) with some modifications. For the measurement of OSI, 3 g of the rice bran oil was placed in the heating block at 130 $^\circ$C; the air flow rate was 20 L/h, and volatile products were discharged in a flask containing 50 mL of distilled water, where conductivity changes were measured. The time corresponding to the inflection point in the oxidation curve is the induction period (IP).

**Statistical Analysis**

Microsoft Excel 2007 was used to calculate mean, standard deviation, and variance. Statistical significance was set at a level of $p < 0.05$. The data represent the mean ± standard deviation of three independent experiments.
RESULTS AND DISCUSSION

Properties of Rice Bran Oils and Extraction Yields

The main objective of this investigation was to characterize the rice bran oil extracted using butane and propane compared with that obtained with hexane. Table 1 shows acid and peroxide values of the various extraction oils, which are very important because they show some information about the composition and application of rice bran oil. As shown in Table 1, the acid values of rice bran oils extracted using butane and hexane were 27.61 ± 0.05 and 27.23 ± 0.09 KOH mg/g, respectively, which was lower than the propane-extracted oil (36.44 ± 0.10 KOH mg/g). Free fatty acid (FFA) content is one of the most important parameter features determining the quality of rice bran oil. Rice bran oil with total FFA contents of less than 10% are economically suitable for edible oil production (Tao et al. 1993). The high values of FFAs in the oils may be traced to rice bran lipase enzyme activities after milling. The results showed that there was a higher percentage of FFAs (40.77 ± 0.10%) in rice bran oil extracted by propane extraction compared with butane and hexane-extracted oils. There was a difference in extraction kinetics between total lipid and FFA from rice bran, and the FFAs were extracted at faster rate than the TAGs (Balachandran et al. 2008). The relative difference among the extraction of FFA from rice bran may be explained by differences in solubility among pure oleic acid and triolein (Brunetti et al. 1989). The FFA concentration in rice bran oil extracted using subcritical propane was 10.0% higher compared with the other two extraction methods. From an economic perspective, more concentrated FFAs in rice bran oil would increase the cost of refining. Peroxides were the foremost initial reaction products of lipid oxidation and were responsible for primary oxidation (Debnath et al. 2012). Table 1 shows that the peroxide values of hexane-extracted oil were significantly higher than those of rice bran oils extracted by subcritical butane and propane. This could be attributed to the following: (1) higher extraction temperature compared with subcritical butane and propane extraction processes; and (2) low content of antioxidants such as vitamin E in hexane-extracted oil. The results indicate that subcritical butane and propane processes could be a useful method for producing high-quality rice bran oils. For the three extraction solvents, the same extraction times were used, as described in the experimental section. As shown from Table 1, the yields of rice bran oil extracted by butane, propane, and hexane were 89.11 ± 0.41%, 91.42 ± 0.31%, and 67.73 ± 0.37%, respectively. Among the three solvents, subcritical butane and propane had better extraction efficiencies as compared with hexane extraction process.

Table 1. FFA Content, Acid Value, Peroxide Value, and Extraction Yields of Rice Bran Oils Extracted by Different Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Yield (%)</th>
<th>FFA content (wt%)</th>
<th>Acid value (KOH mg/g)</th>
<th>Peroxide value (mequiv O2/kg oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butane</td>
<td>89.11 ± 0.41</td>
<td>30.52 ± 0.05</td>
<td>27.61 ± 0.05</td>
<td>10.31 ± 0.05</td>
</tr>
<tr>
<td>Propane</td>
<td>91.42 ± 0.31</td>
<td>40.77 ± 0.10</td>
<td>36.44 ± 0.10</td>
<td>10.13 ± 0.07</td>
</tr>
<tr>
<td>Hexane</td>
<td>67.73 ± 0.37</td>
<td>30.86 ± 0.09</td>
<td>27.23 ± 0.09</td>
<td>362.36 ± 0.33</td>
</tr>
</tbody>
</table>

Analysis of Tocopherol, Tocotrienol, Oryzanol, and Phytosterol

The contents of tocopherol, tocotrienol, oryzanol, and phytosterol in rice bran oils extracted by various solvents are presented in Table 2. As shown, the total vitamin E
contents in the various extraction rice bran oils were 81.61±0.02 (butane), 75.68±0.03 (propane), and 8.60±0.02 (hexane) mg.100g⁻¹, respectively. Comparatively, the content of tocopherols and phytosterols in rice bran oils extracted by subcritical butane and propane were higher compared with hexane-extracted oil. As compared with the extraction of vitamin E and phytosterol, phytosterol, and free fatty acid, oryzanol was the most difficult component to extract from rice bran (Balachandran et al. 2008). The molecular weight of oryzanol is lower than triglyceride. However, its recovery yield was much lower compared with that of total rice bran oil, which could be because of its more voluminous and rigid polycyclic structure or linkage with other components of the rice bran matrix (Shen et al. 1996). As can be seen from Table 2, the content of oryzanol in rice bran oils extracted by hexane, subcritical butane, and propane were 1.60±0.02%, 1.77±0.01%, and 1.45±0.01%, respectively. Consequently, considering oryzanol content in extraction oil, the extraction efficiency of the three solvents was as follows: butane>hexane>propane. Because phytosterols are a hypocholesteremic agent, maximizing their recovery has a great bearing on process development (Balachandran et al. 2008).

The spectrophotometer was used to quantify phytosterols in rice bran oils extracted by hexane, subcritical butane, and propane. For the three solvent extraction methods, a notable difference in phytosterol content was also observed. The contents of phytosterols in rice bran oils extracted by butane, propane, and hexane were 2.08±0.02%, 2.01±0.02%, and 1.79±0.01%, respectively. The different micronutrient contents in various rice bran oils extracted by various solvents was caused by the possibility of modifying the selectivity of the extraction of the different compounds by various solvent extractions. Considering the quality of various extracted oils, the major health components (such as vitamin E, oryzanol, and phytosterols) contained in rice bran oils extracted by subcritical butane and propane were higher compared with hexane-extracted oil.

Table 2. Vitamin E and Phytosterol Compounds of Rice Bran Oils Extracted by Various Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Tocopherol and tocotrienol (mg.100g⁻¹)</th>
<th>Oryzanol (wt%)</th>
<th>Phytosterol (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butane</td>
<td>81.61 ± 0.02</td>
<td>1.77 ± 0.01</td>
<td>2.08 ± 0.02</td>
</tr>
<tr>
<td>Propane</td>
<td>75.68 ± 0.03</td>
<td>1.45 ± 0.01</td>
<td>2.01 ± 0.02</td>
</tr>
<tr>
<td>Hexane</td>
<td>8.60 ± 0.02</td>
<td>1.60 ± 0.02</td>
<td>1.79 ± 0.01</td>
</tr>
</tbody>
</table>

The sterol composition of rice bran oils extracted with various solvents was also investigated using GC-MS analysis. The assignment of the peaks was conducted by computer matching of the mass spectra with the NIST 2005 library. The possible sterol names and GC peak areas are given in Table 3. The percentage values (by integration from total ion chromatogram) indicate the proportions of sterol compounds and do not represent the actual concentration of these compounds. As shown in Table 3, these rice bran oils contained very high concentrations of cycloartenol and 24-methylene-cycloartenol, which made up over 36% of the total phytosterols. Meanwhile, all the extracted rice bran oils were rich in sitosterols. The sterol compositions were identified to be composed of ethyl iso-allocholate, campesterol, stigmasterol, sitosterols, and cyclolanosts. However, extraction solvent had an important effect on the distribution
of various sterols in rice bran oils. In conclusion, it is feasible to obtain rice bran oil with better nutritional properties using subcritical butane and propane compared with hexane extraction.

**Table 3. Phytosterol Compositions in Rice Bran Oils Extracted by Various Solvents**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Phytosterol Compositions</th>
<th>Hexane</th>
<th>Butane</th>
<th>Propane</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.3</td>
<td>Ethyl iso-allocholate</td>
<td>4.8±1.13</td>
<td>4.7±0.42</td>
<td>2.1±0.31</td>
</tr>
<tr>
<td>15.5</td>
<td>Campesterol</td>
<td>12.6±0.92</td>
<td>10.0±0.67</td>
<td>8.4±0.72</td>
</tr>
<tr>
<td>16.4</td>
<td>Stigmasterol</td>
<td>5.9±0.85</td>
<td>7.2±0.73</td>
<td>14.4±0.42</td>
</tr>
<tr>
<td>18.4</td>
<td>γ-Sitosterol</td>
<td>23.2±1.21</td>
<td>19.5±0.51</td>
<td>17.2±1.02</td>
</tr>
<tr>
<td>19.5</td>
<td>β-Sitosterol</td>
<td>9.1±0.73</td>
<td>12.1±0.63</td>
<td>21.0±1.37</td>
</tr>
<tr>
<td>21.3</td>
<td>Cycloartenol</td>
<td>11.2±1.06</td>
<td>11.8±1.44</td>
<td>11.7±0.72</td>
</tr>
<tr>
<td>23.9</td>
<td>24-methylenecyclolanost</td>
<td>32.9±1.09</td>
<td>34.3±1.02</td>
<td>25.0±0.81</td>
</tr>
</tbody>
</table>

**Composition of Rice Bran Oils**

The percentage values (by integration from total ion chromatogram) indicated the proportions of individual compounds in the rice bran oils but did not present the actual concentrations. The fatty acid compositions identified are shown in Table 3. As shown, all the rice bran oils were rich in unsaturated fatty acids (such as linoleic acid and oleic acid) and relatively low in saturated fatty acids (such as palmitic acid, myristic acid, octadecanoic acid, and so on). The major fatty acid compositions of these rice bran oils were linoleic acid, oleic acid, and palmitic acid, and the concentrations of these major fatty acids were between 93.59 and 94.30%.

**Table 4. Fatty Acid Compositions of Rice Bran Oils Extracted by Different Solvents**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fatty Acid Compositions</th>
<th>Chain</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Propane</td>
</tr>
<tr>
<td>11.98</td>
<td>Myristic acid</td>
<td>C14:0</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td>15.51</td>
<td>Hexadecenoic acid</td>
<td>C16:1</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>16.01</td>
<td>Palmitic acid</td>
<td>C16:0</td>
<td>17.33±0.03</td>
</tr>
<tr>
<td>19.30</td>
<td>Linoleic acid</td>
<td>C18:2</td>
<td>40.98±1.22</td>
</tr>
<tr>
<td>19.45</td>
<td>Oleic acid</td>
<td>C18:1</td>
<td>35.28±2.02</td>
</tr>
<tr>
<td>19.75</td>
<td>Octadecanoic acid</td>
<td>C18:0</td>
<td>2.04±0.12</td>
</tr>
<tr>
<td>23.26</td>
<td>Eicosenoic acid</td>
<td>C20:1</td>
<td>0.70±0.08</td>
</tr>
<tr>
<td>23.78</td>
<td>Eicosanoic acid</td>
<td>C20:0</td>
<td>0.97±0.12</td>
</tr>
<tr>
<td>26.96</td>
<td>Docosanoic acid</td>
<td>C22:0</td>
<td>0.52±0.10</td>
</tr>
<tr>
<td>29.37</td>
<td>Tetracosanoic acid</td>
<td>C24:0</td>
<td>0.92±0.06</td>
</tr>
</tbody>
</table>

“-” indicates either absent or not detected.

It can be inferred that the fatty acid profile in the rice bran oils presents a good balance in terms of the saturated-to-unsaturated fatty acids ratio. Saturated fatty acid consumption, mainly palmitic and myristic acids, is directly related to the risk of cardiovascular diseases (Oliveira et al. 2012). These fatty acids with 12-16 carbons have an important effect on raising the low-density lipoprotein cholesterol level (Oliveira et al. 2012). However, diets should provide an adequate supply of polyunsaturated fatty acids and oleic acid (Oliveira et al. 2012). However, extraction solvents had an important effect
on the distribution of linoleic acid and oleic acid in rice bran oils. As shown in Table 3, the maximum oleic acid content and minimum linoleic acid content were found in the rice bran oil extracted using hexane solvent as compared with subcritical propane and butane extraction. These results indicate that subcritical propane and butane were propitious to the selectivity of linoleic acid.

Fatty acid analysis could not measure the content of rice bran wax in the oils. Therefore, $^1$H NMR spectroscopy was employed to investigate the compound of rice bran wax, and the spectra are shown in Fig. 1. The TAGs and rice bran wax observed in the NMR spectra were conducted by comparison with literature data. The chemical shifts of 3.95 to 4.00 ppm and 4.00 to 4.29 ppm were caused by the TAGs and rice bran wax, respectively. The NMR data suggested that the two principal components of the rice bran oils were TAGs and rice bran wax. The rice bran oil extracted using subcritical propane contained the highest content of rice bran wax and the lowest content of rice bran TAGs, while the hexane-extracted oil contained relatively lower content of rice bran wax but higher content of TAGs. Comparatively, the butane-extracted oil showed the highest TAGs ratio compared with that of the propane and hexane-extracted oils.

**Oxidative Stabilities**

Rice bran oil was unstable and its triglycerides tend to be hydrolyzed into glycerol and free fatty acids during storage (Lakkakula et al. 2004). To investigate the effect of various solvent extraction processes on the oxidative stabilities of rice bran oils, the OSI at 130 °C of the three rice bran oils were measured. The rice bran oils extracted by hexane and subcritical butane and propane had OSIs of 0.52, 5.02, and 3.36 h.
respectively (figure not shown in this paper). This result demonstrates the instability of hexane-extracted oil. Generally, it is widely agreed that the relative antioxidant activity of the tocopherols is in the order $\alpha > \beta > \gamma > \delta$. According to the results of vitamin E analysis, the contents of tocopherol and phytosterol in rice bran oils extracted by subcritical butane and propane were higher compared with hexane-extracted oil (Table 2). Therefore, subcritical butane and propane-extracted oils showed even better oxidative stabilities compared with hexane-extracted oil because they were relatively rich in vitamin E.

CONCLUSIONS

1. Butane and propane-extraction oils showed higher concentrations of health-promoting components (such as vitamin E, oryzanol, and phytosterols) than hexane-extracted oil.
2. Extraction solvent had an important effect on the distribution of linoleic acid and oleic acid.
3. Butane-extracted oil had the highest triacylglycerol (TAG) contents, and subcritical propane-extracted oil had the highest wax content among the oils extracted using these three extraction methods.
4. The oils extracted by subcritical butane and propane showed higher oxidative stabilities.

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