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COMPARISON OF SUPERCRITICAL FLUID AND SOLVENT EXTRACTION METHODS IN EXTRACTING BIOACTIVE COMPOUNDS AND MINOR COMPONENTS OF RICE BRAN OIL

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ABSTRACT

(Comparing supercritical fluid and Soxhlet extraction systems (SFE and SE) for concentrating bioactive components of rice bran oil was studied. SFE was utilized to fractionate high oryzanol (HO) and low oryzanol (LO) RBOs. The tocopherols, tocotrienols, oryzanols and ferulic acid were measured utilizing HPLC, while, phytosterols and fatty acids were quantified utilizing GC. The results demonstrated there was a significant difference ($p < 0.05$) in unsaponifiable of HO compared with LO and Soxhlet oils. In neutral lipids and phospholipids classes, there were no significant differences ($p < 0.05$) between the three oils. On the other hand, in glycolipids class, there was a noteworthy distinction in oil concentrated utilizing Soxhlet compared with SFE technique. There were distinctive concentrations of tocopherols, tocotrienols, and oryzanols separated by the two methods. Oryzanol in RBO demonstrated a significant difference ($p < 0.05$) between diverse fractions of RBOs. Consequently, extraction by SFE impacted the sum, composition, and antioxidant of lipid in RBOs.)

1.Introduction

(Rice bran oil (otherwise called rice bran extract) is the oil extracted from the germ and inner husk of rice. It is remarkable for its high smoke point of 213 °C and its gentle flavor, making it suitable for high-temperature cooking routines, for example, blend frying and deep frying. It is well known as a cooking oil in a few Asian nations, including Japan and China (Orthoefer, 2005), and can be concentrated by diverse techniques for extraction. Solvent extraction is generally utilized anyway; it is much of the time utilizing toxic solvents. Then again; these solvents have been recognized as an air contaminant since it can respond with different pollutants to create ozone and photochemical oxidants (Tanzi et al. 2012). These days supercritical fluid extraction (SFE) system offers a finer system for extraction as it is more safe and free from solvent contamination (Lang and Wai, 2001). There are a few preferences in utilizing the SFE as a part of oil extraction. According to Rozzi and Singh (2002), supercritical fluids have higher diffusion coefficients and lower viscosity than liquid solvents. The nonappearance of a significant surface tension permits the fast penetration of supercritical liquid into pores,

thereby serving to improve the extraction productivity. By controlling the temperature and pressure, SFE can influence the dissolvability of the extracting fluid and subsequent control of the shifting arrangement of component in a sample (Tanaka and Takeshi, 2004). Many researchers used SFE for extraction of unconventional oils (Mariod et al. 2010; Chan et al. 2009; Louli et al. 2004).

RBO comprise of around 3.0 to 5.0% of unsaponifiable matter, which contain a unique complex of naturally occurring antioxidant components, for example, tocopherols, tocotrienols and oryzanol (Lloyd et al. 2000). Unsaponifiable matter incorporates higher aliphatic alcohols, sterols, pigments and hydrocarbons that often discovered broke up in fats and oils, and that can't be saponified by caustic alkalis but are soluble in the extraction solvents (Guthalugu et al. 2006).

Gamma–oryzanol is a mixture of sterol esters of ferulic acid consists of ferulate ester of a mixture 4, 4-dimethyl sterols consisting of cycloartenol, 24-methylenecycloartanol, campesterol, Δ- stigmasterol, Δ- campestenol, campestenol, β- sitosterol, sitostenol and Δ-sitostenol (Ash et al. 2011). RBO also contain a variety of fatty acids such as oleic, linoleic and palmitic (Amarasinghe et al. 2009).

Xu and Godber (2000) compared solvent extraction of rice bran to supercritical carbon dioxide extraction at 50°C and 68.9 MPa pressure for the extraction of γ -oryzanol. From their study, they found that SFE extract may contain up to four times higher γ -oryzanol (5.39 mg/g of rice bran) in content relative to solvent extraction. Perretti et al. (2003) studied various conditions, particularly pressure and temperature to extract oil from the products and by products of rice to increase the concentration of natural antioxidants (tocopherol, tocotrienol and oryzanol) in oil. They applied high pressure extraction of 5000psi (4hr), 7500 psi (2hr) and 10000 psi and temperature from 40ºC, 60ºC and 80ºC, respectively. They further found that the extraction conducted at 10000psi and 80ºC gave the highest extraction yield.

Sarmento et al. (2006) demonstrated the precipitation of rice bran oil with distinctive concentrations of tocopherol and tocotrienol in the first and second separators by utilizing SFE. They found that, the ideal conditions for the extraction of parboiled rice oil enhanced with tocopherol and tocotrienol was at 200bar and 40°c. At this condition the yield of tocopherol and tocotrienol were much higher which were 234.96mg/100g and 704.16mg/100.

RBO contain a high measure of unsaponifiable matter that possibly can anticipate chronic illnesses Kahlon et al. (1996) proposed that the cholesterol lowering activity of rice bran may be impacted by the level of unsaponifiable matter in the lipid portions of rice bran. Oryzanol is a mixture of sterol esters of ferulic acid, which have physiological properties, for example, having superoxide dismutase-like antioxidant activity and hypocholesterolemic impacts in humans (Visser et al. 2000). This study aims to determine the composition of RBOs extracted using supercritical fluid extraction and Soxhlet systems, and to determine and compare the resultant lipid classes of SFE and Soxhlet RBOs.

2. Materialsandmethods

2.1.Materials

2.1.1.Samples

Rice bran was obtained from Bernas Rice Mills, Sg. Tiram, Selangor, Malaysia. The rice bran sample was stabilized using oven at higher temperature for 3 minute before the extraction commenced.

2.1.2. Chemicals

All solvents (chloroform, acetone, methanol, hexane, ethanol, hydrochloric acid, ethyl acetate, acetonenitrile, isopropanol, acetic acid, pyridine, bis trimethylsiyl trifluoro acitamide (BSTFA) and isooctane) were of analytical and HPLC grade obtained from Fisher Scientific (Loughborough, LE, UK) and Merck (Darmstad, Germany).

Chemicals such as potassium hydroxide, sodium chloride and sodium methoxide were obtained from Sigma - Aldrich (St. Louis, MO, USA). Standard for unsaponifiable matter (tocopherol, tocotrienol, oryzanol and phytosterol) and neutral lipid were purchased from Sigma - Aldrich (St. Louis, MO, USA). The FAME reference standard was purchased from AccuStandard (New Haven, CT, USA)

2.1.3. Instruments

Supercritical fluid extraction system (Thar Technologies, Pittsburgh, PA, USA model SF2000), rotary evaporator (Buchi, Flawil, Switzerland), centrifuge machine, universal and micro type (Hettich Zentrifugen, Tuttlingen, Germany), ultrasonic water bath (HanShin, USA), analytical balance (Shimadzu, Nakagyo Ku, Kyoto, Japan model number). Each component of the lipid in rice bran oil was determined using an Agilent Technologies HPLC series 1200 (Santa Clara, CA, USA) and Thermo Fisher Scientific Triple Quad GCMS (St. Waltham, MA, USA).

2.2. Methods

2.2.1. Extraction of RBOs

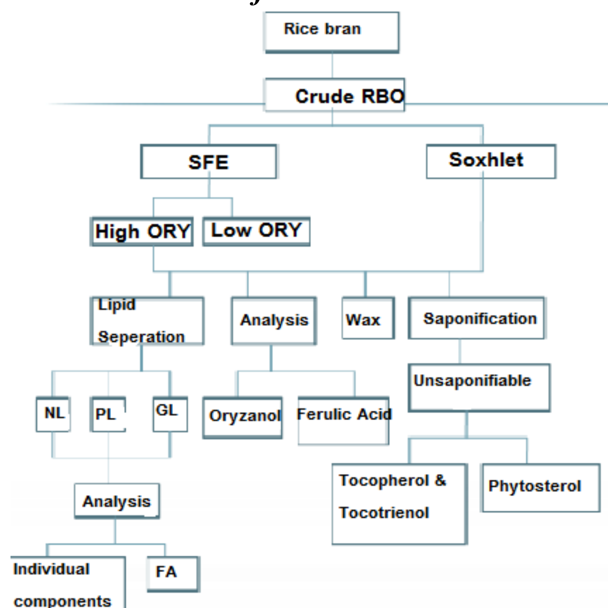

Figure 1. Layout of extracting bioactive compounds from rice bran using SFE and Soxhelt methods

2.2.2. SFE extraction

The high oryzanol RBO (HO) and low oryzanol RBO (LO) were concentrated by SFE utilizing distinctive weight and temperature (Fig.2). In a word, carbon dioxide (CO_2) from a cylinder was pressurized and fed into the solvent reservoir. This pressurized CO_2 was turned into the supercritical liquid when preheated in an equipped air-bath. After rice bran (180 g) was placed in the extractor, the supercritical liquid, then was streamed into the extractor and was blended with the rice bran. The extractor was joined with the collector vessel. The temperature and weight were controlled to obtain the SFE oil. After that the mixed solutions of RBO and supercritical liquid from the extractor were depressurized and the RBO was gathered (Kim et al. 2005). To get the LO from SFE weight of 600 bars and a temperature of 60°C were utilized. Meanwhile, to acquire the LO the pressure was 200 bars and temperature was 40°C. The flow rate and duration of time to concentrate HO and LO was comparative with 25g/min of flow rate and 150 mins duration time.

2.2.3. Soxhlet extraction

Utilizing the procedure described in the Official and Recommended Methods of AOCS (1998). Soxhlet extraction of RBO was done (Fig. 1). In short, 450 g of rice bran was weighed and put in extraction thimbles. The thimble was set in a Soxhlet extractor. Next, n-hexane was added into round flask, which had been joined with the extractor and condenser. Extraction methodology was begun when the solvent flow through the rice bran and extract the oil. After extraction was finished, n-hexane was removed under reduced pressure utilizing a rotary evaporator (Buchi, Flawil, Switzerland). Consequently, the Soxhlet RBO was set into a desiccator chamber for 1 h to absorb the moisture in the oil.

2.2.4. Saponification of rice bran oil

RBOs were saponified to get the unsaponifiable matter. This method is critical to examine sterol components in oils. 5.0g of RBOs sample was re-fluxed with 50 ml of

ethanolic potassium hydroxide (2m) for 1h. After the response, the sample was kept in overnight. At that point, the sample was mixed with 100 ml of distilled water to structure aqueous solution in a separation funnel. Next, the solution was washed with 100 ml of diethyl ether for 3 times this removes neutral lipid components. Hence the unsaponifiable matter was gotten after the removal of diethyl ether under diminished pressure (Canabate-Diaz et al. 2007). The weight and percentage of unsaponifiable matters were calculated

2.2.5. Separation of lipid classes

RBOs samples were separated according to the method described by Shin and Godber (1996). Column chromatography (25 mm i.d. X 30 cm) was used to separate the neutral lipid (NL), glycolipid (GL) and phospholipid (PL) fractions RBOs samples. A loading ratio of 3.0g oil: 30g of activated silica gel 70-230 Mesh (Merck, Darmstad, Germany) was used. NL was eluted first with 400 ml chloroform, GL with 600 ml acetone and PL with 400 ml methanol. Solvents were removed under reduced pressure and the yield of each fraction was determined gravimetrically.

2.2.6. Determination of wax

The waxy substance of RBOs was determined by utilizing the solvent dewaxing methodology. One gram of unrefined RBOs sample was weighed into a centrifuge tube, and one ml of chilled acetone was included in ratio 1:1. The solution (oil/acetone) was centrifuged at 4000rpm for 10 min in a universal centrifuge machine (Hettich Zentrifugen, Tuttlingen, Germany). The supernatant or the dewaxed oil was totally removed. The wax stayed at the base of the tube was washed with chilled actenone and centrifuged once more. The wax was gathered and the yield of wax was determined gravimetrically (Ramaswamy et al. 1980).

2.2.7. Analysis of neutral lipid (NL) of RBOs

The neutral lipid fraction that was collected from column chromatography was analyzed by HPLC using a C18 Supelco column (25 x 4.6mm, 5µ). Acetone/acetonitrile/methanol (50:50:10, v/v/v) was used as a mobile phase with an isocratic flow 0.5 ml/min. Column temperature was maintained at 30ºC and the components were detected using a refractive index detector (JASCO Model RI-2031, Easton, MD 21601, USA). Oleic acid, monolein, diolein and triolein were used as standards. The calibration curve of each standard was used to quantify the components such as monoaylglycerides (MAG), dicylglycerides (DAG), and triacyglycerides (TAG) and free fatty acid (FFA) in a neutral lipid fraction of RBOs samples (Shin and Godber, 1996).

2.2.8. Fatty acid analysis of RBOs

2.2.8.1 Preparation of fatty acid methyl ester (FAME)

An adjustment of the method depicted by Cert et al. (2000) was utilized. 25 mg of RBOs samples were weighed a test tube, and one ml of 0.2n of sodium methoxide in methanol was included. At that point, the example was warmed in a water bath (55ºC) for 20 minutes. After taken out from water bath, 1.0 ml of concentrated hydrochloric (HCl) acid was included and the incubation was continued in 40 minutes. Following 40 minutes the test tubes were taken out and 10 ml of 0.05m sodium chloride. The solution was mixed well and 2.5 ml of hexane were included. The upper layer of the solution was withdrawn and subjected to FAME analysis.

2.2.8.2. Analysis of fatty acid composition (FAME)

Analysis of FAME was carried out in the GCMS Triple Quad (St. Waltham, MA, USA) equipped with a splitless capillary inlet system with a TG-5MS column (15mm x 0.25mm id x 0.25µm, Agilent J&W DB-5ms Ultra Inert Capillary, Santa Clara CA 95051 USA) Others parameters involved in this analysis were as followed: injector temperature (250ºC); carrier gas (He); flow rate (1ml/min); oven temperature (50ºC hold for 1 minute until 280ºC and hold for another 5 minute). Fatty acid composition of RBOs was identified and quantified through the calibration curve of FAME standard

2.2.9. Analysis of unsaponifiable matters

Quantification of phytosterol

Sample preparation was carried out according to the method described by Toivo et al. (2000). The saponified RBOs sample was diluted with 10 ml pyridine. Then, 1.0 ppm concentration of RBOs samples was prepared by serial dilution. The sample was derivatized with a mixture of bistrimethylsiyl trifluoro acitamide (BSTFA) and isooctane (99:1). The sample solution was then heated at 60ºC before mixed with 500 µl hexane. Individual standards such as *β*-sitosterol, stigmasterol and stigmastanol were prepared using ethanol and used to determine the phytosterol content in the tested samples. Sterol components were separated using GCMS Triple Quad (St. Waltham, MA, USA) equipped with a splitless capillary inlet system with a TG-5MS column (15mm x 0.25mm id x 0.25µm). Others parameters involved in this analysis were as followed: injector temperature (250ºC); carrier gas (He); flow rate (1.0 ml/ min); oven temperature (50ºC and hold for 1 min until 300ºC and hold for another 5min. The sterol components of RBOs were quantified using the calibration curves for *β*-sitosterol, stigmasterol and stigmastanol standard.

2.2.10. Quantification of oryzanol by HPLC

The oryzanol content of RBO samples was analyzed as per technique created by Rogers et al. (1993) and utilizing reverse-phase HPLC. Oryzanol content was detected at 325 nm with Photodiode Array Detector (PDA). Oryzanols were differentiated with ODS (C18) Hypersil silica column (5µm X 250nm). The mobile phase comprised of acetonitrile/methanol/isopropanol with a ratio of 50:45:5 (v/v/v) and stream rate of 1 ml/ min. The sample was prepared by diluting it with ethanol before injection into the HPLC. A standard calibration curve of oryzanol standard was utilized to quantify the content of oryzanol in an oil sample.

2.2.11. Quantification of Ferulic acid

The evaluation of ferulic acid in RBOs samples was done as per method created by Zhou et al. (2004) and utilizing reversed phase HPLC. The components were differentiated by 2 ORBAX SB (C18) columns (150mm x 4.6mm, 5µm) and detected utilizing diode array detector at 280nm and with flow rate 1 ml/ min. The mobile phase was solvent A (water: acidic acid, 100:1, v/v) and solvent B (methanol: acetonitrile: acidic acids, 95:5:1.v/v/v). A standard calibration curve of ferulic acid standard was utilized to evaluate the content of ferulic acid in an oil sample.

2.2.12. Quantification of tocopherol

Tocopherols in RBOs were quantified utilizing an HPLC equipped with a UV detector and a 5 µm, 250 X 4.6 mm inertial NH_2 column. The mobile phase consisted of ethyl acetate/ hexane with ratio 30:70 (v/v). The flow rate was 1.0 ml/ min and increased slightly to 1.5 ml/ min for 5 min. The compound was at 290nm wavelength. The standard calibration curve of tocopherol standard was used to quantify the content of tocopherol in an oil sample.

2.2.13. Quantification of tocotrienol

Quantification of tocotrienol was completed as indicated by Shin and Godber (1994) technique and utilizing HPLC. Tocotrienol was detected utilizing fluorescence detector. Tocotrienol of the RBOs samples was detected at 330nm. A column that utilized in this system was Zorbax Nh2 (250 x 4.6 mm, 5µ) with a flow rate 0.5 ml/ min. The mobile phase comprised of hexane and isopropanol with proportion 99.5:0.5 (v:v). The standard calibration curve of tocotrienol standard was utilized to measure the substance of tocotrienol in oil test.

2.3.Statistical Analysis

The statistical analysis system SPSS 16 was used to perform statistical computations. Analysis of variance (ANOVA) accompanied using Tukey's multiple range tests and LSD were performed if a significant difference in the means of values at $p < 0.05$.

3.Results and discussions

TABLE 1. Lipid Composition of Rice Ban Oil Samples

		Sample (g/g)			
	Unsaponifiable matters	**Wax**	**Neutral Lipid**	**Glycolipid**	**Phospholipid**
HO	0.08±0.002[a]	0.17±0.10[a]	0.97 ±0.013[a]	0.03±0.01[a]	0.01±0.01[a]
LO	0.05±0.002[b]	0.18±0.001[a]	0.95±0.011[a]	0.03±0.000[a]	0.01±0.01[a]
Soxhlet	0.05±0.001[b]	0.16±0.063[a]	0.72±0.43[a]	0.01±0.003[b]	0.06±0.03[a]

a-b: The different letters within the same column indicate a significant difference ($p < 0.05$) Abb: HO – high oryzanol oil, LO – low oryzanol oil

3.1. Lipid composition of rice bran oils

Table 1 shows the estimation of lipid composition in RBO samples. This table showed that the estimated amount of unsaponifiable matter of Soxhlet RBO was about 0.048±0.001 g/g oil which represents about 4.8%. A previous study reported that the amount of unsaponifiable matter of crude rice bran oil was approximately 4.0% (Lloyd et al., 2000). The amount of unsaponifiable matter in HO (0.08±0.02 g/g oil) was estimated to be about two times higher ($p<0.05$) than the Soxhlet method (0.05±0.01 g/g oil). Meanwhile, the unsaponifiable matters in LO was 0.05±0.02 g. Extractions by SFE might have influenced the extraction composition in rice bran. As reported by Dunford and King (2000) fractionation of RBO using supercritical fluid carbon dioxide (SCC) can minimize the loss of unsaponifiable matter in samples. This is due to a closed SFE system that prevents oil from exposed to air. Exposure of RBOs to air for a longer time causes the degradation of antioxidant which is abundant in unsaponifiable matter (Duvernay et al. 2005). From Table 1, the wax content in three different types of oil extraction was different. The amount of wax estimated in HO and LO was 0.17±0.09 g/g oil and 0.18±0.01 g/g oil, respectively. Compared with Soxhlet RBO, amount of wax was 0.16±0.06 g/g oil slightly lower than RBOs extracted by SFE ($p\leq0. 05$). This was supported by Patel (2005) who found that RBO extracted using the SFE method was high in waxes.

As shown in Table 1, lipid classes in different types of oil extraction were different. In NL and PL classes, there were no significant differences in these 3 different types of extractions (HO, LO and Soxhlet). However, in glycolipid classes there was a significant difference in Soxhlet RBO compared with oil extracted using SFE. As shown in Table 1 the estimated amount of NL in HO and LO were about 0.97±0.01 g and 0.95±0.01g which was almost 90%. As expected, NL is a major lipid in RBO as showed by Shin and Godber (1996) where they found that amount of NL in RBO was 89.2%. Meanwhile, the estimated amount of neutral lipid in Soxhlet RBO was lower from RBO that extracted using SFE.

The estimated amount of neutral lipid in Soxhlet RBO was about 0.72 ±0.43g. Yet, there was no significant difference in amount of NL in each different type of oil extractions. Instead, the estimated amount of GL in Soxhlet RBO was lower than that of HO and LO which was 0.08±0.03 g ($p<0.05$). Meanwhile, the estimated amounts of GL in HO and LO were about 0.03±0.08 g and 0.03±0.000 g. The different extraction of RBO slightly gives different amounts of GL extraction. It seems that SFE method increased the amount of GL classes in rice bran oil. In addition, there were no significant differences in amount of PL in the different type of oil extraction. Amount of PL in HO and LO extracted using SFE were slightly similar which was estimated about

0.01±0.01 g and 0.01±0.01 g, respectively. However, the amount of phospholipids (0.06±0.03 g) in a Soxhlet extracted oil was significantly ($p<0.05$) higher.

3.1.1. Analysis of unsaponifiable matters of rice bran oils

Table 2. Compounds in Unsaponifiable Matters in Different Extract of Rice Bran Oil

Sample (mg/g)	a-Tocopherol	Tocotrienol	^{x}Oryzanol	^{y}Oryzanol	Ferulic acid	Phytosterol
HO	11.61±0.8^{a}	2.62±0.2^{a}	7.84±0.04^{a}	ND	ND	ND
LO	6.47±0.1^{a}	3.24±0.6^{a}	6.17±0.02^{b}	ND	ND	ND
Soxhlet	23.12±3.0^{b}	2.45±0.1^{a}	4.81±0.11^{c}	ND	ND	ND

a-c: The different letters within the same column indicate significant difference ($p < 0.05$) Concentration of gamma-oryzanol in RBO samples with (x) and without saponification (y). *ND=not detected. HO- high oryzanol, LO- low oryzanol

Table 2 shows the composition of unsaponifiable matters in RBOs. Tocopherols, tocotrienols, oryzanols, phytosterols and ferulic acid are typical compounds that can be found in unsaponfiable portion. In this study, α-tocopherol had been detected by fluorescence detector. Alpha -tocopherol is abundant in oil and it is the main type of tocopherol or vitamin E in oil. The three different types of oil extractions contained different concentrations of α-tocopherol. In this study Soxhlet RBOs contained significantly ($p<0.05$) higher amount of α-tocopherol compared to HO and LO which was 23.12±3.0 mg/g oil. In contrast, the study by Sarmento et al. (2006) found that the concentration of tocols in hexane extraction was lower compared to SFE due to higher temperature during solvent extraction and longer time of oil exposed to air. There was no significant difference between *α*- tocopherol in HO and LO. The concentration of *α*-tocopherol in HO and LO was 11.61±0.8 mg/g oil while LO 6.47±0.01 mg/g oil. The possibilities of higher amount of tocopherol in RBO might be due to the solubility of this compound in hexane. The concentration of tocotrienol in HO, LO and Soxhlet RBOs was 2.62±0.2 mg/g oil, 3.24±0.6 mg/g oil and 2.45±0.05 mg/g oil, respectively. Sarmento et al. (2006) found that SFE yielded a high amount of tocopherol and tocotrienol particularly at 200bars and 40 ºC.

In the three types of oils using different extraction method, the compound of oryzanol or gamma-oryzanol was detected using a diode array detector (DAD). In this study, there was a significant difference ($p<0.05$) of the oryzanol content of the different type of oil extractions. Table 2 shows HO extracted using SFE was significantly containing high amount of oryzanol as compared to LO and Soxhlet extraction. Concentration of oryzanol in HO was 7.84±0.04 mg/g oil while in LO and Soxhlet RBO was 6.17±0.02 mg/g oil and 4.81±0.11 mg/g oil. Xu and Godber (2000) found that SFE extraction yields four times more gamma- oryzanol in rice bran oil compared to solvent extraction. This is due to the effect of higher temperature in SFE system that alters the physical properties of rice bran matrix. The alteration of rice bran matrix makes it more penetrable by the extraction fluid of SFE (Xu and Godber (2000). Quantification of gamma-oryzanol in the oil with and without the saponification was done to compare the concentration of gamma-oryzanol in rice bran oil. Saponification process was an important process in most lipid extracted samples because it can remove interfering triglycerides and other hydrolysable materials. Besides, it also aids the release of lipids from a sample matrix. However, gamma- oryzanol that undergo the saponification process was not detected. This is

because bonds between ferulic acid and triterpene alcohol could be hydrolyzed under alkali condition (Xu and Godber 2000). In this study, 4 major isomers were detected by HPLC in RBOs samples (Fig. 2). These isomers were cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and beta- sitosteryl ferulate. In this study, ferulic acid could not be detected by a detector in the HPLC assay. Ferulic acid is a part of oryzanol, appeared as a mixture of ferulic acid esters of phytosterols. To obtain the ferulic acid component oils needs to be hydrolyzed to break down the bond between ferulic ester of phytosterol.

3.1.2 Analysis of neutral lipid of rice bran oils

In this study, neutral lipid was separated by HPLC using a C18 Supelco column into monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG) and free fatty acids (FFA) classes. Separation of components in NL using the HPLC was depending on the polarity of components. In this study MAG was eluted at an earlier retention time as this component more polar than TAG, while TAG as the least component that was eluted.

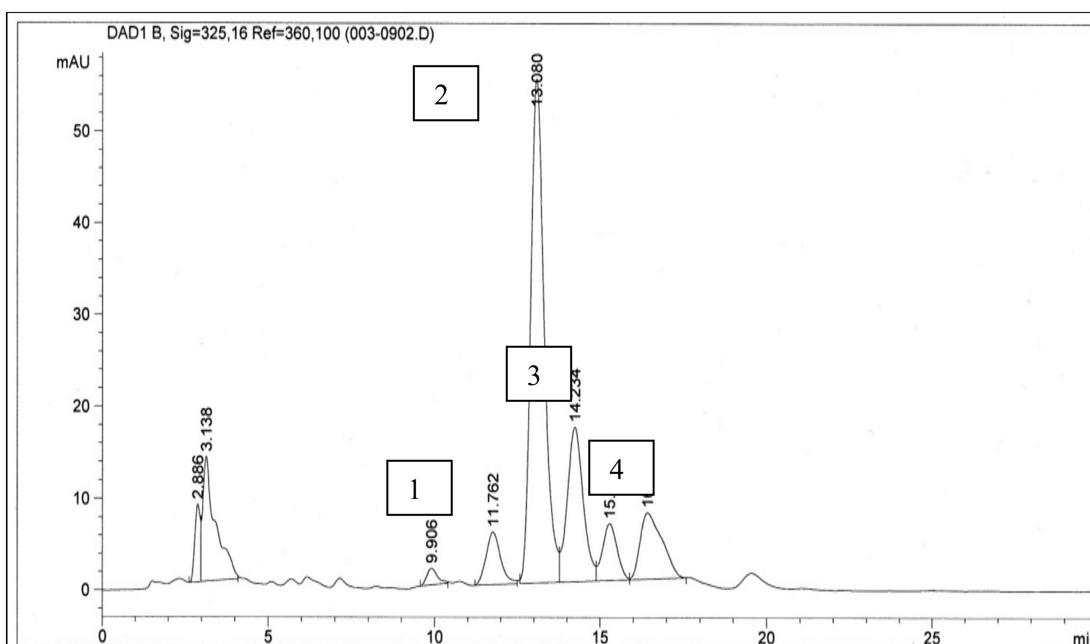

Figure 2. Ultraviolet detection of gamma-oryzanol in HO extracted by SFE. 1= cycloartenyl ferulate; 2= 24-methylene cycloartanyl ferulate; 3= campesteryl ferulate; 4=–β-sitosteryl ferulate and cycloartanyl ferulate.

Table 3. Components of Neutral Lipid in Different Extracted of Rice Bran Oil

Sample	**MAG**	**DAG**	**TAG**	**FFA**
High oryzanol SFE(mg/g)	32.05±3.0^{a}	14.05±0.3^{a}	137.52±0.6^{a}	39.22±4.8^{a}
Low oryzanol SFE(mg/g)	56.09±1.3^{b}	15.51±0.15^{b}	135.28±0.4^{ab}	46.52±4.4^{a}
Soxhlet(mg/g)	29.94±5.03^{a}	15.01±0.74^{b}	138.09±1.6^{b}	37.63±3.0^{a}

a-b: The different letters within the same column indicate a significant difference ($p < 0.05$). Abb: MAG- monoacylglycerol, DAG- diacylglycerol, TAG- triacylglycerol, FFA- free fatty acid

According to Table 3, MAG in LO (56.09±1.3 mg/g oil) was significantly ($p < 0.05$) higher than HO (32.05±3.0) and Soxhlet oil (29.94±5.03). Meanwhile, the amounts of MAG in both HO and Soxhlet extractions were not significantly different. DAG compound in the three different types of oil extractions contributed about 1-2%. This was slightly different with data reported by Orthoefer, (2005) who reported that DAG was about 2-4% (Orthoefer, 2005). In this study, the amount of DAG in HO and LO that have been extracted using SFE was 14.05±0.3 mg/g oil and 15.51±0.15 mg/g oil. Meanwhile, the amount of DAG in Soxhlet oil was slightly similar with LO which was 15.01±0.74 mg/g oil. Study by Marimuthu et al. (2010), found rice bran oil contain high amount of DAG which was 2.46%. DAG in vegetable oil is formed by oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids in a ratio of 7:3:1. Thus, at high temperature and pressure of the SFE extraction the compounds of DAG might be maximized. There was a significant difference ($p<0.05$) of TAG in Soxhlet and HO. TAG of Soxhlet RBO contributed 138.085±1.6 mg/g oil (13.81%) compared to HO and LO which were 137.52±0.6 mg/g (13.75%) and 135.28±0.4 mg/g (13.53%) of oil, respectively. However, the finding was in contrast with previous study where the amount of TAG of oil approximately more than 80% (Krishna et al. 2006). This is due to the solubility problems presented by solid glycerides in each mono- , di-, and tri-. It is difficult to find a solvent as mobile phase in which this component soluble and can be separated in reverse phase HPLC (Marimuthu et al. 2010). There was a high amount of FFA mainly oleic acid in LO compared to HO and Soxhlet RBO ($p>0.05$). Amount of FFA in LO was 46.52±4.4 mg/g oils compared to HO and Soxhlet oil, which was 39.22±4.8 mg/g oil and 37.63±3.0 mg/g oil, respectively.

3.1.3 Fatty acid composition in rice bran oil

The fatty acid methyl ester (FAME) analysis was quantified using Thermo Fisher Scientific Triple Quad GCMS (St. Waltham, MA, USA). The experiment was carried out in duplicate due to the limited sample and time constraint. The result shows that RBOs contain myristic acid (C14), palmitic acid (C16), heptadecanoic acid (C17), stearic acid (C18), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C:20) and eicosenoic acid (C20:1). The amount of fatty acid in RBOs samples per one gram of oil is shown in Table 4.

Table 4. Fatty Acid Composition in Rice Ban Oil Samples

	Sample (g/g)		
Fatty acids composition	**High oryzanol SFE**	**Low oryzanol SFE**	**Soxhlet**
C14:0	0.028±0.01	0.027± 0.01	0.029± 0.02
C16:0	0.163±0.01	0.058± 0.06	0.030±0.01
C17:0	0.013±0.02	0.00± 0.06	0.025±0.01
C18:0	0.029±0.00	0.008±0.01	0.010±0.02
C18:1	0.316±0.01	0.059± 0.08	0.053± 0.01
C18:2	0.484±0.02	0.120± 0.04	0.095±0.01
C20:0	0.020± 0.00	-	-
C20:1	0.072± 0.02	-	-
Saturated fatty acid	0.252 ± 0.2	0.093 ± 0.1	0.094± 0.03
Monounsaturated fatty acid	0.388±0.01	0.059±0.08	0.053±0.01
Polyunsaturated fatty acid	0.484±0.02	0.120±0.04	0.095±0.01

These results were not consistent, particularly for LO and Soxhlet RBOs and might be due to an error during FAME preparation. During the FAME preparation, transmethylation reaction showed a precipitate in solution. This might be due to the high content of wax in RBO samples. Transmethylation is much better if dewaxed oil is used for fatty acid analysis. According to Table 4, eight fatty acids were detected in HO compare to LO and Soxhlet RBO. The highest amount of fatty acids in HO was oleic acid and linoleic acid, which was 0.316±0.01 g/g and 0.484±0.02 g/g oils. Kim et al. (2005), reported that SFE of RBO yielded more essential fatty acids (EFA) compared to solvent extraction. In addition, the amount of MUFA and PUFA in HO oils was higher compared to LO and Soxhlet RBO. Amount of MUFA and PUFA in HO was 0.388±0.011 g/g oil and 0.48±0.02 g/g oil.

4. Conclusions

Comparing supercritical fluid and Soxhlet extraction systems (SFE and SE) for concentrating bioactive components of rice bran oil. A distinctive concentrations of tocopherols, tocotrienols, and oryzanols were separated by the two methods. Oryzanol in RBO demonstrated a significant difference ($p < 0.05$) between diverse fractions of RBOs. Consequently, extraction by SFE impacted the sum, composition, and antioxidant of lipid in RBOs

5.References

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