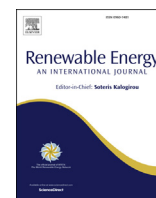


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## A non-catalytic *in situ* process to produce biodiesel from a rice milling by-product using a subcritical water-methanol mixture

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### ABSTRACT

A non-catalytic method to produce biodiesel *in situ* from a rice milling by-product, i.e. rice bran, using subcritical water-methanol mixture has been investigated. The method was found to be unaffected by initial moisture and free fatty acids (FFA) contents in rice bran so that no pretreatment was required. The yield and purity of biodiesel were higher under CO<sub>2</sub> atmosphere than those under N<sub>2</sub> atmosphere due to the ability of the gas to acidify water-methanol mixture. Oil extraction from the bran was identified as the limiting step and complete oil extraction could be achieved in 3 h at 200 °C, 4 MPa (under CO<sub>2</sub> atmosphere) and 43.8 wt% methanol concentration. Consequently, the highest biodiesel yield was also achieved at those operating conditions. The experimental data suggested that hydrolysis of rice bran oil into FFA followed by methyl-esterification of FFA into biodiesel could be the preferred reaction path to direct transesterification of oil. Subcritical water-methanol mixture was also able to break down complex carbohydrates in rice bran into simple sugars soluble in aqueous phase so that it could be separated easily from biodiesel.

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### 1. Introduction

Human activities, especially the burning of fossil fuels that emits greenhouse gasses into atmosphere, have been identified as the main cause of recent climate change [16]. The use of renewable fuels which can be considered carbon neutral can mitigate this issue. One kind of renewable fuel which is currently produced in industrial scale is biodiesel or fatty acid methyl esters (FAME). It can be blended with petrodiesel and requires practically no changes in the fuel distribution infrastructure [17]. Unfortunately, most biodiesel currently in use is produced from edible feedstocks, such as palm oil, soy oil and rapeseed oil [17,35], which can drive food price higher. Ideally, the feedstock should be available in large quantity, relatively cheap and inedible, such as rice bran.

Rice bran is produced during the milling of husked rice as a by-product and is traditionally used as cattle feed despite of its rich nutrient content. Husked rice contains about 8–12% rice bran while rice bran itself contains about 10–26% oil [5,19,24,35]. The potential of annual rice bran oil (RBO) production in China alone is about 6 million tonnes [23] while worldwide production could reach 8

million tonnes if all rice bran produced is harnessed for oil extraction [1,17]. RBO is rather unsuitable for human consumption due to higher free fatty acids (FFA), acetone-insoluble contents and darker appearance [6,23]. Complicated refining and stabilization process is required to transform RBO into edible oil suitable for human consumption, making it economically uncompetitive against other edible oils, such as palm, soy and rapeseed oils [17].

Since RBO has high FFA content, conventional biodiesel production method using base catalyst is unsuitable since the base reacts with FFA forming soap making FAME yield lower and purification process more complicated [17]. Various methods to produce biodiesel from crude and refined RBO have been proposed in the literature involve multiple steps. Zullaikah et al. [35] developed a two-step acid-catalyzed methanolysis method to produce FAME under atmospheric condition from dewaxed/degummed RBO with high FFA content (up to 76%). The first step was carried out at 60 °C mainly to convert FFA to FAME while the second step was carried out at 100 °C to convert the remaining triglycerides (TG) to FAME with a total reaction time up to 8 h. While [23] developed a three-step method to produce FAME from crude RBO. The first two pretreatment steps were carried out at 50 °C using acid catalyst to reduce FFA content to below 1 mg/g while the third step was carried out at 60 °C using base catalyst to convert TG into FAME. The total reaction time can be reduced to less than 3 h however, a

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**Abbreviation**

DRB	deffated rice bran
FAME	fatty acid methyl esters
FFA	free fatty acids
GC	gas chromatography
MS	mass spectrometry
RBO	rice bran oil
TG	triglycerides
V	volume

separation step was required between acid catalyzed steps and base catalyzed step. Although FFA contents in the feedstock and the methods were different, both of them reported that more than 98% of FFA and TG were converted into FAME under optimum conditions.

*In situ* methods to produce biodiesel from rice bran have also been proposed to reduce production cost since oil extraction and its conversion into FAME occur simultaneously. *In situ* method using methanol and sulfuric acid (1.5–5 vol%) at low temperature (60–65 °C) has been investigated by Özgül-Yücel and Türkay [27,28] and Gunawan et al. [9]. This method showed efficient esterification of FFA but transesterification of triglycerides (TG) was poor and therefore FAME yield increased with FFA content [17]. Shiu et al. [32] investigated a two-step *in situ* method where acid catalyst was used in the first step followed by basic catalyst in the second step. They found that lipid extraction from rice bran was a slow process that could take 4–5 h to extract most lipids in the bran using Soxhlet with n-hexane as solvent. *In situ* method under supercritical methanol at 300 °C and 30 MPa with CO<sub>2</sub> as the pressurizing gas was reported by Kasim et al. [20]. However, the result was rather disappointing with an overall conversion of 51.3%. Our preliminary experiment also showed that supercritical methanol featuring high temperature and pressure caused rice bran to char hindering oil extraction. Therefore, although *in situ* method could potentially reduce biodiesel production cost, there are still a lot of problems to be solved.

Another method to produce biodiesel *in situ* is by using subcritical water. It has been used widely for extraction of organic compounds [10,29] and has been employed to produce biodiesel *in situ* from algae [34], activated sludge [14] and *Jatropha curcas* seed kernels [8]. One advantage of this method is that it can be carried out without acid or base catalyst. Besides that, subcritical water is able to hydrolyze complex carbohydrates into soluble sugars which

can be utilized as a medium to grow yeast [8], feedstock for bioethanol production and other industrial applications [29].

In this work, subcritical water-methanol mixture was employed to produce biodiesel *in situ* from rice bran. The effects of reaction time, pressurizing gas type, temperature and methanol concentration were investigated. The composition of deffated rice bran (DRB) was also analyzed. Since this method requires no catalyst, it is expected that this work could provide an economical and environmentally friendly method to produce biodiesel from cheap, abundant and inedible feedstock to meet the requirement of renewable fuel in the future.

## 2. Experimental

### 2.1. Materials

Rice bran (from IR 64 rice variety) was obtained from local rice mill (Lamongan, East Java, Indonesia). The moisture and oil contents of the rice bran were  $12.9 \pm 0.1\%$  and  $14.9 \pm 0.2\%$ , respectively. The moisture content was determined by the drying method while the oil content was determined by the Soxhlet extraction method with n-hexane as solvent for 8 h. The rice bran oil content found in this work is in good agreement with those reported in the literature, i.e. between 10 and 26% depending on rice variety and degree of milling [5,9,10,19,23,35]. The FFA content in the RBO was found to be  $37.6 \pm 0.2\%$  determined by using the titration method according to [30]. The fatty acid composition in the RBO was determined by using a gas chromatography (GC) analyzer after methanolysis of RBO and the results are comparable to those reported in the literature as summarized in Table 1.

Pressurizing gas, either CO<sub>2</sub> or N<sub>2</sub>, was supplied by Genta Prima Gas (Surabaya, Indonesia). Standard of methyl linoleate and phenol was obtained from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade NaOH, sulfuric acid, phenolphthalein (PP) indicator and glucose were purchased from Merck (Kenilworth, NJ, USA). Methanol, n-hexane and 96% ethanol were purchased from Brataco (Surabaya, Indonesia). In all experiments, distilled water was used.

### 2.2. *In situ* biodiesel production

Rice bran (5 g) was mixed with a water-methanol mixture (40 ml) in a hydrothermal reactor (V = 86 ml) made of seamless 316 stainless steel tubing (SS-T12-S-083-6ME, Swagelok, Solon, OH, USA). The volume ratio of methanol to water was varied from 5/35 to 35/5 ml/ml, corresponding to methanol concentration from 10.0 to 84.5 wt%, respectively. Pressurizing gas (either CO<sub>2</sub> or N<sub>2</sub>) was

**Table 1**

Composition of fatty acids in rice bran oil (RBO) determined by using a gas chromatography (GC) analyzer.

Fatty acid	Composition (%)		
	Current study	Zullaikah et al. [35]	Bello and Oluboba [2]
Palmitic acid (C16:0)	22.8	17.7	15.0
Linoleic acid (C18:2)	29.5	35.6	38.7
Oleic acid (C18:1)	46.1	40.6	41.5
Arachidic acid (C20:0)	0.8	0.2	0.8
Myristic acid (C14:0)	0.8	0.8	0.1
Others	–	5.1	4.0
<b>Details of GC analysis</b>			
GC type	Agilent HP 6890	Shimadzu GC-17A	Agilent HP 6890
Column	HP 1 crosslinked methyl siloxane	DB-5HT (5%-Phenyl-) methylpolysiloxane	HP inno wax
Detector	Flame ionization detector	Flame ionization detector	Flame ionization detector
Initial temperature	125 °C	80 °C	60 °C
Temperature ramp	15 °C/min for 10 min	15 °C/min for 20 min	10 °C/min for 20 min + 15 °C/min for 4 min
Final temperature	275 °C	380 °C	320 °C

then introduced to increase the operating pressure to 40 bars and an electric heater (Schneider Electric, Rueil-Malmaison, France) was used to heat up the mixture to a pre-determined temperature (160–215 °C). The heating rate was about 10 °C/min and the temperature inside the reactor was monitored by using a thermocouple (Swagelok, Solon, OH, USA). After reaction (1–8 h), the mixture was cooled down to room temperature by quenching.

The oil phase was extracted by using n-hexane for five times (50 ml each) and it was then recovered by evaporating n-hexane in a rotary evaporator. The oil recovery is defined as the ratio of the amount of oil phase obtained using the subcritical water-methanol mixture to that obtained using the Soxhlet extraction method with n-hexane as described previously. The oil phase was then stored for further analyses of FAME content, FAME yield and FFA content. The aqueous phase was separated from the solid phase by filtration using filter paper (Whatman WHA 1201240, GE Healthcare, Little Chalfont, UK). The solid phase was washed with distilled water to ensure that all water soluble components were extracted completely. The aqueous phase was then analyzed for total sugar content while the solid phase was analyzed for cellulose, hemicellulose and lignin contents. All experiments were repeated at least twice and the standard deviations of most experimental data were found to be less than 5%.

### 2.3. Analysis of FAME

The concentration of biodiesel (FAME) in the oil phase was determined by using a gas chromatography (GC) analyzer (Agilent HP 6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. The separation was carried out on a HP 1 crosslinked methyl siloxane column (60 m × 0.25 mm i.d. × 1 µm film thickness, Agilent Technologies, Santa Clara, CA, USA). The sample (0.02 g) was diluted in n-hexane (1 ml) and 0.5 µL of this mixture was then injected into the GC column. The injector and detector temperatures were set initially at 125 °C and were increased at a rate of 15 °C/min. After reaching 275 °C, the temperatures were held constant for 10 min. The flow rate of carrier gas (helium) was set at 28 ml/min at 200 °C. External calibration standard curve was obtained using 0.2–20 mg methyl linoleate. The yield of FAME was determined as the weight ratio of FAME to that of oil in rice bran as follows:

$$\text{FAME yield(\%)} = \frac{\{\text{crude FAME(g)} \times \text{FAME content(\%)}\}}{\{\text{oil weight in rice bran(g)}\}} \times 100 \quad (1)$$

### 2.4. Analysis of total sugar and defatted rice bran composition

The total sugar content in the rice bran extract was determined by using the modified phenol–sulfuric acid method [4,10]. In general, 5 ml of sample (aqueous phase) or glucose solution as the standard was mixed with 1 ml of 5 wt% aqueous phenol solution and 5 ml of 98 wt% sulfuric acid and then mixed thoroughly with a vortex mixture for 30 s. The mixture was left for 10 min at ambient temperature and then cooled in a 25 °C water bath for 10 min. The total sugar content was evaluated using a spectrophotometer (UV-VIS Genesys 10S, Thermo Fischer Scientific, Waltham, MA, USA) at a wavelength of 490 nm.

The fractions of hemicellulose, cellulose and lignin in the defatted rice bran were determined by using the Chesson method as modified by Datta [3]. In general, dilute and concentrated sulfuric acids were used to convert hemicellulose and cellulose, respectively, into water soluble substances. While lignin with the most complex molecular structure practically does not react with

sulfuric acid.

## 3. Results and discussion

The exact reaction mechanism in the *in situ* biodiesel production using a subcritical water–methanol mixture is not yet fully understood. There could be two competing reaction mechanisms, i.e. direct transesterification of TG with methanol and hydrolysis of TG by water followed by methyl esterification of fatty acids [18]. However, the controlling step is still not clear.

The oil recovery, FAME yield and product purity (FAME and FFA contents) in the *in situ* biodiesel production using the subcritical water–methanol mixture were found to be affected by type of pressurizing gas, reaction time, temperature and methanol concentration as discussed below. Besides that, the effects of those parameters on the composition of the defatted rice bran (DRB) and sugar concentration are also discussed.

### 3.1. Effect of pressurizing gas type

The effects of pressurizing gas on oil extraction, FAME yield, FAME content and FFA content are shown in Fig. 1(a)–(d), respectively. In the first 3 h, oil recovery under CO<sub>2</sub> atmosphere was higher than that under N<sub>2</sub> atmosphere, however at longer reaction time, oil recovery under CO<sub>2</sub> atmosphere was lower (Fig. 1(a)). FAME yield and FAME content were higher under CO<sub>2</sub> atmosphere while FFA content was practically not affected by the type of pressurizing gas used.

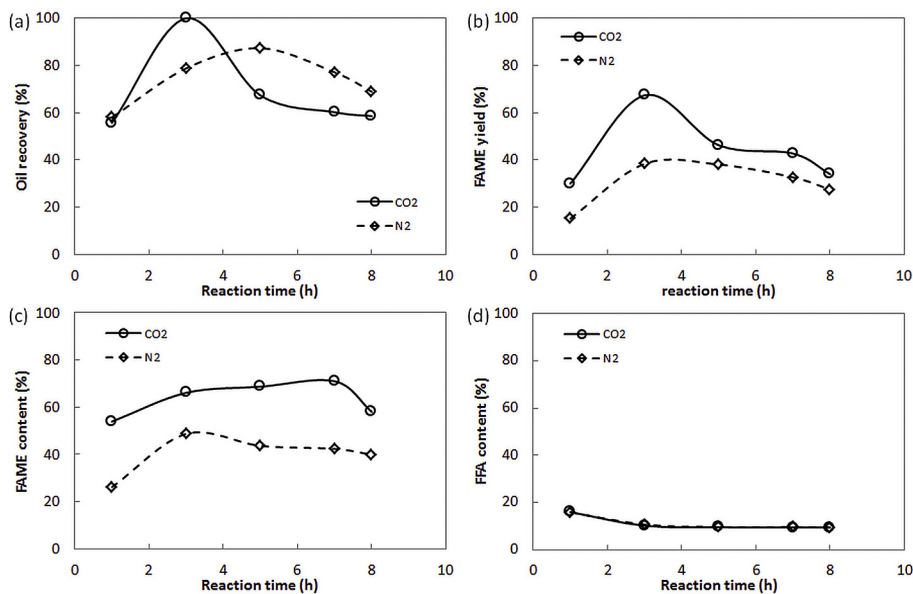
The use of CO<sub>2</sub> as pressurizing gas can increase the acidity of the water-methanol mixture because dissolved CO<sub>2</sub> reacts with water to produce carbonic acid (H<sub>2</sub>CO<sub>3</sub>) [25]. Since oil is more soluble in acidic medium, the oil extraction rate is faster under CO<sub>2</sub> atmosphere [8]. In the subcritical water-methanol mixture, transesterification of TG with methanol, hydrolysis of TG with water and esterification of FFA with methanol are all catalyzed by H<sup>+</sup> [17] and therefore they are faster under CO<sub>2</sub> atmosphere. On the other hand, N<sub>2</sub> does not affect the pH since it is an inert gas under these reaction conditions. However, acidic medium may also increase the polymerization and degradation rates of unsaturated oleic and linoleic fatty acids at a longer reaction time as discussed in the following section.

The oil extraction, FAME yield and FAME content at different reaction times, temperatures and methanol concentrations were also found to be higher under CO<sub>2</sub> atmosphere than those under N<sub>2</sub> atmosphere. Therefore, only results under CO<sub>2</sub> atmosphere are presented in this report to simplify the discussions.

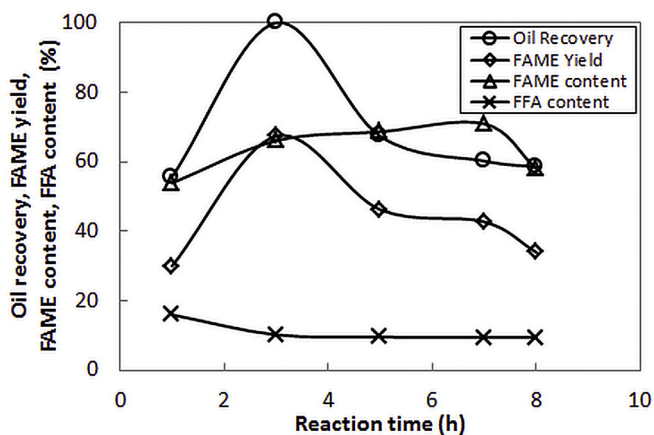
### 3.2. Effect of reaction time

The effects of reaction time on oil recovery (crude FAME), FAME yield, FAME content and FFA content under CO<sub>2</sub> atmosphere are shown in Fig. 2. The maximum oil recovery (100%) and FAME yield (67.4%) were achieved after 3 h of reaction time, while the maximum FAME content (71.0%) was achieved after 7 h. The FFA content tended to decrease with longer reaction time.

As the reaction time was extended from 1 to 3 h, the oil recovery and FAME yield increased by 44.3% (from 55.7% to 100%) and by 37.4% (from 30.0% to 67.4%), respectively, while FAME content only increased by 12.2% (from 53.9% to 66.1%). These results suggest that FAME yield depends more on the oil extraction rate from rice bran rather than the conversion rate of oil into FAME. Higher oil recovery will also shift the equilibrium towards the formation of FAME. Therefore, under reaction conditions investigated in this work, the oil extraction rate is the limiting factor. Shiu et al. [32] also reported that lipid extraction from rice bran was a slow process and could



**Fig. 1.** Effects of pressurizing gas on oil recovery (a), FAME yield (b), FAME content (c) and FFA content (d). T = 200 °C, P = 4 MPa, methanol concentration = 43.82 wt%. All data points are mean values of two replications.



**Fig. 2.** Effects of reaction time on oil recovery, FAME yield, FAME content and FFA content. T = 200 °C, P = 4 MPa (CO<sub>2</sub>), methanol concentration = 43.8 wt%. All data points are mean values of two replications.

take at least 4 h to complete using Soxhlet with n-hexane as solvent while [22] reported that oil extraction from rice bran using Soxhlet with methanol as solvent could take 5 h to finish.

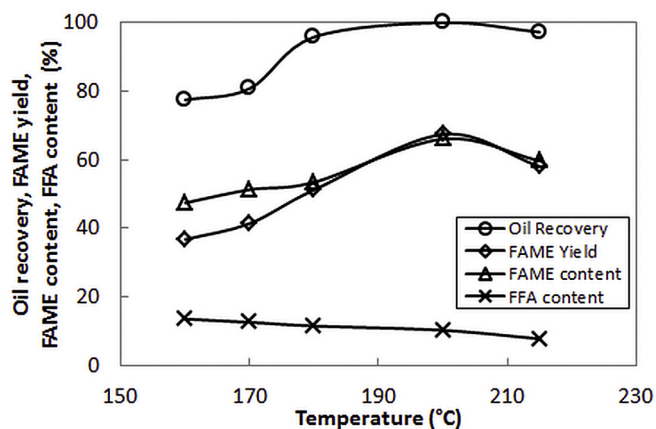
However, longer reaction time decreased both oil recovery and FAME yield probably due to polymerization and degradation of unsaturated oleic and linoleic fatty acids which constitute more than 75% of fatty acids in rice bran oil (Table 1). These unsaturated fatty acids are prone to polymerization and gum formation especially at high temperature [17]. The appearance of crude biodiesel obtained after 8 h of reaction was dark brown and was very different from that obtained after 3 h. This suggests that prolonged exposure to high temperature and pressure leads to the formation of by-products that were not detected by GC-MS used in this work.

Fig. 2 also shows that the FFA content after 1 h of reaction (16.0%) is much lower than that the initial FFA content in RBO (37.6%) suggesting that the subcritical water-methanol mixture can handle feedstock with high FFA content. However, the FFA content was still 9.4% even after 8 h of reaction due to the hydrolysis of FAME and TG in the presence of water.

### 3.3. Effect of temperature

The effects of temperature on oil recovery, FAME yield, FAME content and FFA content under CO<sub>2</sub> atmosphere are shown in Fig. 3. As the temperature was increased from 160 to 200 °C, oil recovery and FAME yield increased by 22.5% (from 77.5% to 100%) and by 30.6% (from 36.8% to 67.4%), respectively, while FAME content increased by only 18.6% (from 47.4% to 66.1%). The increases in both oil recovery and FAME content were similar, i.e. about 20%, since higher oil concentration pushed the equilibrium toward the formation of FAME. The increase in FAME yield was higher than those in oil recovery and FAME content since FAME yield depended on both oil recovery and FAME content. On the other hand, FFA content decreased by about 5% (from 13.5% to 7.8%) as the temperature was raised from 160 to 215 °C.

As the temperature is raised, the diffusion coefficient between oil and the water-methanol mixture increases leading to higher extraction rate of RBO, and therefore oil recovery. Increasing temperature also increases the solubility of oil in the water-methanol



**Fig. 3.** Effects of temperature on FAME yield, oil recovery, FAME content and FFA content. P = 4 MPa (CO<sub>2</sub>), methanol concentration = 43.8 wt%, reaction time = 3 h. All data points are mean values of two replications.



mixture since the dielectric constants of both methanol and water decrease and become more similar to that of oil. The dielectric constant of methanol decreases from 32 at room temperature to about 7 at critical point of 239.5 °C and 80.1 bar [36], while that of water decreases from 80 at room temperature to 27 at 250 °C [18]. The dielectric constant of vegetable oil is typically around 3–4 at room temperature.

Tang et al. [33] reported that the solubility of methanol in triolein in terms of mole fraction increased from about 0.7 to about 0.8 when the temperature was raised from 160 to 200 °C at 6 MPa while that of triolein in methanol was relatively constant (~1% in this temperature range). Completely miscibility between methanol and triolein can be achieved at 197–207 °C [7,12,33].

In this study, since 5 g of rice bran with 14.9% oil content was used in each experiment, the amount of RBO in the system was only about 0.7 g. If a mixture of 20 ml methanol and 20 ml water is used as solvent, the mole fraction of oil in methanol is only 0.2% (assuming that the oil molecular weight is 885). Therefore, at 160–215 °C, it is expected that all extracted oil is soluble in methanol. Since methanol is soluble in water, there could be only a single liquid phase in the reactor. The miscibility between methanol and oil can also be enhanced by the presence of FAME which is soluble in both methanol and oil. The formation of intermediate products (mono- and diglycerides) which have both polar and non-polar groups also increase the miscibility between oil and methanol.

Higher temperature generally also increases reaction rate due to increase in kinetic energy of reactant molecules and decrease in the strength of chemical bonds between molecules. Besides that, the dissociation constant of water increases from  $10^{-14}$  at room temperature to  $10^{-11}$  at 200 °C, making concentrations of  $H^+$  and  $OH^-$  higher at elevated temperature [21]. Although transesterification reaction can be catalyzed by acid and base, Ju et al. [18] believed that in the subcritical water-methanol mixture, it was catalyzed by acid. The hydrolysis and esterification reactions are also catalyzed by acid. Therefore, the rates of those reactions increase at higher temperature due to higher  $H^+$  concentration.

However, as the temperature was raised further to 215 °C, oil recovery, FAME yield and FAME content decreased. This is because the vapor pressure of methanol at 215 °C is about 5 MPa (calculated using Antoine equation [37], above the operating pressure in system (4 MPa). Therefore, most methanol existed as vapor at 215 °C and consequently both extraction and reactions rates were slower due to lower methanol concentration in the liquid phase.

### 3.4. Effect of methanol concentration

The effects of methanol concentration on oil recovery, FAME yield, FAME content and FFA content under  $CO_2$  atmosphere are shown in Fig. 4. The temperature, total pressure and reaction time were kept constant at 200 °C, 40 MPa under  $CO_2$  atmosphere and 3 h, respectively, while the methanol concentration was varied from 10.0 to 84.5 wt% (methanol/water volume ratio = 5/35–35/5 ml/ml, respectively). The maximum oil recovery (100%) and FAME yield (67.4%) were achieved at 43.8 wt% methanol, while the maximum FAME content occurred at 70.1 wt% methanol. On the other hand, FFA content tended to decrease with increasing methanol concentration. Even at the lowest methanol concentration investigated in this work, the FFA content (14.6 wt%) was significantly lower than that initially in RBO (37.6%).

Both transesterification of TG and esterification of FFA are reversible reactions. Excess methanol favors the formation of FAME and increases the rates of both transesterification and esterification reactions. Higher methanol concentration also leads to higher oil extraction rate since oil is more soluble in methanol than water. On

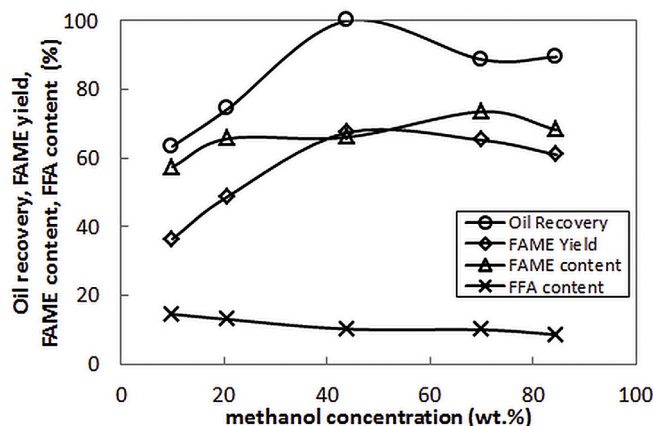


Fig. 4. Effects methanol concentration on FAME yield, oil recovery, FAME content and FFA content. T = 200 °C, P = 4 MPa ( $CO_2$ ), reaction time = 3 h. All data points are mean values of two replications.

the other hand, the water favors the reverse hydrolysis reaction of FAME into methanol and FFA and therefore complete conversion of FFA into FAME is not possible. As shown in Fig. 4, both oil recovery and FAME yield increased by 36.6% (from 63.4% to 100%) and by 30.9% (from 36.5% to 67.4%), respectively, as the methanol concentration was increased from 10.0 wt% to 43.8 wt%, while FAME content only increased by 8.6% (from 57.5% to 66.1%). Therefore, increasing methanol concentration up to 43.8% has a more profound effect on the oil extraction and subsequently FAME yield rather than the conversion of oil into FAME. This is because excess methanol has been used even at the lowest methanol concentration investigated in this work. At 10.0 wt% methanol, the molar ratio of methanol to oil is 144 which is much higher than 42 recommended by Saka and Minami [31] and consequently higher methanol concentration has limited effect on shifting the equilibrium toward FAME formation.

However, the experimental data showed that oil recovery and FAME yield decreased when methanol concentration was higher than 43.8 wt% (Fig. 4). Higher methanol concentration may lead to the extraction of more polar compounds such as carbohydrate and therefore lower the extraction rate of oil [14,32]. Besides that, Ju et al. [18] also reported that the formation rate of FAME from soybean oil in subcritical methanol increased in the presence a small amount of water than that in pure methanol suggesting that water may hydrolyze TG into FFA which subsequently was methyl esterified into FAME. This could be the preferred reaction mechanism in the subcritical water-methanol mixture since the rate constants of hydrolysis and esterification reactions are higher than that of transesterification [9,31].

### 3.5. Defatted rice bran (DRB) composition and total sugar content in aqueous phase

After the separation of oil phase, the solid phase (DRB) was dried, weighed and analyzed for its cellulose, hemicellulose and lignin contents while the aqueous phase was analyzed for total sugar content. The DRB weight is expressed as the relative weight of DRB to initial rice bran weight while the cellulose, hemicellulose and lignin contents in DRB are expressed as weight percentage. The total sugar content in the aqueous phase is expressed in g/l.

The effects of pressurizing gas type on DRB relative weight, DRB composition and total sugar concentration are shown in Table 2. There was no significant effect of using  $CO_2$  or  $N_2$  as pressurizing gas on DRB relative weight and DRB composition, however the total

**Table 2**

Effect of pressurizing gas type on DRB relative weight, DRB composition and sugar concentration.

Pressurizing gas type	DRB relative weight (%)	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Total sugar concentration (g/l)
CO <sub>2</sub>	30.6	7.6	16.7	58.4	0.7
N <sub>2</sub>	32.5	7.6	14.1	55.3	0.6

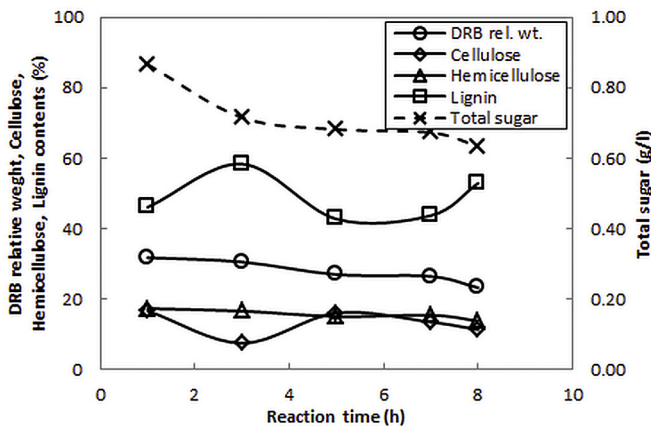
Operating condition: T = 200 °C, P = 4 MPa, methanol concentration = 43.82 wt%, reaction time = 3 h. All data are mean values of two replications.

sugar concentration under CO<sub>2</sub> atmosphere was about 31% higher than that under N<sub>2</sub> atmosphere. Since the majority of carbohydrate in rice bran is starch ([15,25]), the use of CO<sub>2</sub> as pressurizing gas may increase the hydrolysis rate of starch into sugar due to the increase in H<sup>+</sup> concentration. However, the increase in H<sup>+</sup> concentration due to CO<sub>2</sub> may not be strong enough to increase the hydrolysis rates of cellulose, hemicellulose and lignin and consequently their contents in DRB were similar under CO<sub>2</sub> and N<sub>2</sub> atmospheres. Therefore, only results under CO<sub>2</sub> atmosphere are presented in this report to simplify the discussion.

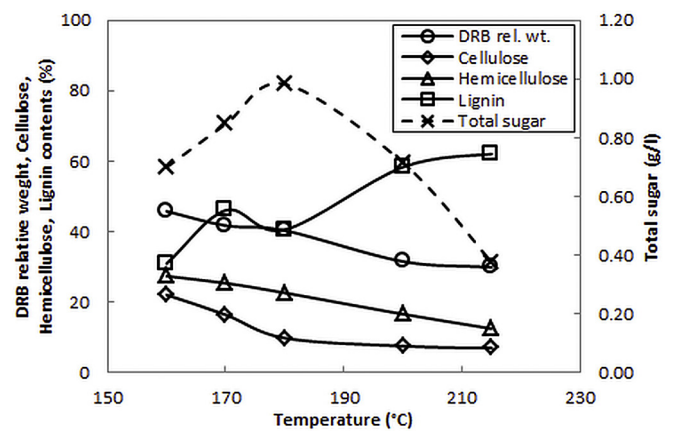
The effect of reaction time on DRB relative weight, DRB composition (in terms of cellulose, hemicellulose and lignin contents) and sugar concentration in aqueous phase is shown in Fig. 5. The relative weight of DRB decreased from 40.0% to 26.6% as reaction was extended from 1 h to 8 h due to the extraction of carbohydrates, proteins and oil in rice bran by subcritical water-methanol mixture. Most of these compounds (~60%) were extracted in the first hour of reaction and less than 14% in the remaining 7 h. It should be noted that oil extraction was completed after 3 h at this operating conditions (see Fig. 1).

Fig. 5 shows that cellulose and hemicellulose contents in DRB at 8 h were less than that at 1 h. Cellulose and hemicellulose decreased from 16.76% to 11.45% and 17.41%–13.91%, respectively, as reaction was extended from 1 h to 8 h. However, lignin content increased from 46.14% to 52.94% as reaction proceeded from 1 h to 8 h. These results show that the hydrolysis rates of cellulose and hemicellulose in subcritical water-methanol mixture are relatively high and the hydrolysis of lignin is very low. Its due to lignin is more difficult to degrade than cellulose and hemicellulose. On the other hand, sugar content in aqueous phase decreased with reaction time probably due to decomposition at high temperature.

The effect of temperature on DRB relative weight, DRB composition and total sugar concentration under CO<sub>2</sub> atmosphere is shown in Fig. 6. The relative weight of DRB, cellulose and hemicellulose contents in DRB decreased with increasing temperature due to higher hydrolysis rates. On the other hand, the lignin content



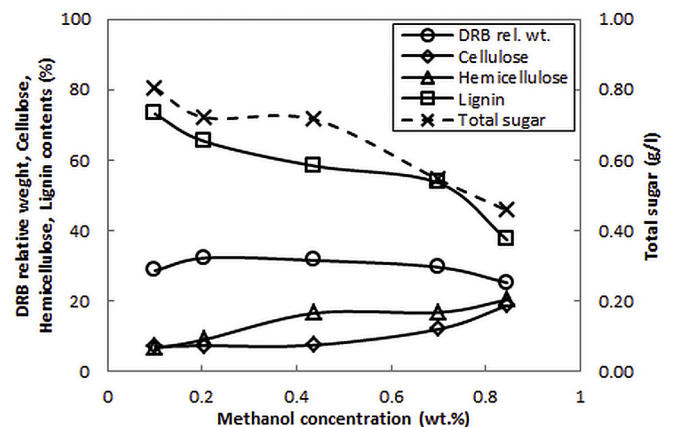
**Fig. 5.** Effects of reaction time on DRB relative weight, DRB composition and sugar concentration after treatment in subcritical water-methanol mixture. T = 200 °C, P = 4 MPa (CO<sub>2</sub>), methanol concentration = 43.8 wt%. All data points are mean values of two replications.



**Fig. 6.** Effects of temperature on DRB relative weight, DRB composition and sugar concentration after treatment in subcritical water-methanol mixture. P = 4 MPa (CO<sub>2</sub>), methanol concentration = 43.82 wt%, reaction time = 3 h. All data points are mean values of two replications.

in DRB increased with increasing temperature. Lignin is the most difficult to hydrolyze due to its complex three dimensional structure [26] and it is easier to decompose under basic rather than acidic condition [13]. Consequently, lignin content in DRB was higher than that initially in rice bran as cellulose and hemicellulose contents decreased during the reaction. The total sugar concentration in the aqueous phase increased with increasing temperature up to 180 °C due to faster hydrolysis reaction rate. However, at higher temperature, the total sugar concentration decreased due to decomposition of sugars into 5-HMF and furfural [34].

Fig. 7 shows the effect of methanol concentration on DRB relative weight, DRB composition and total sugar concentration under CO<sub>2</sub> atmosphere. The DRB relative weight was relatively unaffected by the methanol concentration while the cellulose and hemicellulose contents in DRB increased with the methanol concentration



**Fig. 7.** Effects of methanol concentration on DRB relative weight, DRB composition and sugar concentration after treatment in subcritical water-methanol mixture. T = 200 °C, P = 4 MPa (CO<sub>2</sub>), reaction time 3 h. All data points are mean values of two replications.

due to lower hydrolysis reaction rate at higher methanol concentration (lower water concentration) and consequently lignin content decreased. The total sugar concentration in the aqueous phase decreased with increasing methanol concentration since the rate of hydrolysis reaction decreases with decreasing water concentration.

#### 4. Conclusion

The effectiveness of a subcritical water-methanol mixture to produce FAME *in situ* from rice bran without a preliminary oil extraction step has been investigated. This process was found to be insensitive to initial moisture and FFA contents in the bran and therefore no pretreatment was required. Oil recovery, FAME yield and FAME content were higher under CO<sub>2</sub> atmosphere than those under N<sub>2</sub> atmosphere due to the ability of CO<sub>2</sub> to acidify the water-methanol mixture. The data suggested that the oil extraction from the bran was the limiting factor and that the hydrolysis of TG into FFA followed by methyl esterification of FFA into FAME may be the preferred reaction path rather than the direct transesterification of TG into FAME. Under optimum conditions (200 °C, 4 MPa under CO<sub>2</sub> atmosphere, 3 h reaction time and 43.8 wt% methanol), 100% oil in the bran can be recovered and 67.4% FAME yield can be achieved.

Complex carbohydrates in the bran were also hydrolyzed into soluble sugars. Sugar concentration in aqueous phase was higher under CO<sub>2</sub> atmosphere than that under N<sub>2</sub> atmosphere due higher hydrolysis rate of starch at lower pH, however the DRB relative weight, cellulose, hemicellulose and lignin contents in DRB were practically the same under both atmospheres. The highest sugar concentration of about 1 g/l was achieved at 180 °C. Higher temperature and longer reaction time caused sugar degradation while higher methanol concentration decreased the hydrolysis rate.

Therefore, subcritical water-methanol treatment of rice bran can produce biodiesel and sugar solution which can be fermented subsequently to produce ethanol. Besides that, the subcritical water-methanol mixture can extract active nutraceutical compounds in rice bran, such as  $\gamma$ -oryzanol. The purification and isolation methods of  $\gamma$ -oryzanol are currently under investigation. Consequently, by using biorefinery concept, the production cost of biodiesel can be lowered.

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