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Pre-functionalized and lipid-dense post-hydrolysis rice bran as feedstock for FAME production via non-isothermal in-situ (trans) esterification with subcritical methanol

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ABSTRACT

In-situ (trans)esterification (ISTE) of lipids in post-hydrolyzed rice bran (PHRB) with methanol under subcritical conditions has proven to be a suitable feedstock for fatty acid methyl ester (FAME) production. The lipids from PHRB had a fatty acid profile which was primarily composed of oleic (39 wt%) and linoleic (36 wt%) acids, and could potentially result in biodiesel with favorable properties. The PHRBs which were lipid-dense (31.35 and 48.98 wt% on a dry basis) and pre-functionalized (0.55 and 1.21 mmol H^+/g dry and lipid-free PHRB), were successfully processed non-isothermally from 30 to 150 °C at high reactor loading of 85% and a solvent-to-solid ratio (SSR) of 4–6 mL/g dry PHRB, which resulted in yields of 26.48 and 35.11 g/100 g dry PHRB, equivalent to a conversion of ~90% of the fatty acids. Due to the acquired acid sites in the collected PHRB, no additional catalyst was required. Elemental analysis and FT-IR spectroscopy were carried out to test the presence of sulfur and sulfonic sites in the PHRB residues. Furthermore, the recovered solids still exhibited substantial acid sites which were tested for activity through the esterification of oleic acid in methanol and were reused up to 7 cycles.

1. Introduction

Rice bran (RB) is an inevitable by-product generated in rice mills. A recent review on utilizing RB lipids for biodiesel production indicated that countries in the South, Southeast, and East Asia generate significant amounts of RB annually that could be tapped as feedstock for the generation of renewable fuel in the form of FAME [1]. Rice bran typically contains lipids at 4 to 26 wt% [2,3] and varies with the paddy rice variety, its cultivation conditions, and the later milling process, but generally between 15 and 20 wt% [1,2]. With RB's lipid content generally do not go beyond 20 wt% and with its physical morphology being a powder-like substance, its practical extraction via mechanical means is often limited [4]. Although there are various ways to extract and convert lipids in oleaginous materials into biodiesel, *in-situ* (trans)esterification (ISTE) provides the advantage of reducing the required processing steps and solvent [5]. In addition, since the introduction of biodiesel synthesis under supercritical methanol conditions by Saka and Kusdiana in the early 2000s [6] and subcritical methanol conditions by Ju et al. [7], developments in ISTE also shifted towards the use of alcohols under these conditions to avoid or minimize the use of non-renewable catalyst while still having desired productivity and conversion [8–11].

The earliest attempt to carry out ISTE of lipids found in RB was by Kasim et al. [9] back in 2009. In their attempt, only ~51% of potential fatty acid methyl ester (FAME) was generated, with observed degradation and side reaction products from and/or the available lipids and the rice bran matrix, when the reaction was

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carried out at 300 °C. Although ISTE under supercritical methanol conditions was proven possible with Jatropha kernels as first reported by Lim et al. [12] in 2011, such an approach is not possible for RB. As an alternative, Go and Ju [13], proposed carrying out ISTE under milder conditions of 250 °C for an hour, using a mixture of acetic acid and methanol, which allowed higher FAME yields relative to the available lipids (67–85 wt%) translating to better reaction yields or conversion (~75–99%), depending on the gaseous co-solvent used. At about the same time, Zullaikah et al. [14,15] and Sutanto et al. [4,16,17] made separate attempts to maximize the use of RB components, not only to produce FAME but as well as to recover the carbohydrate fraction in the form of hydrolysates rich in sugar. The approach that Zullaikah et al. [15] took was to use a mixture of water and methanol to facilitate a one-pot process of carrying out ISTE and at the same time hydrolyzing the lignocellulosic components of RB. Even though such an approach is promising because of the milder reaction conditions of 200 °C, it required a reaction time of 3 h, and FAME yield relative to the available lipids was only ~67%, with hydrolysates containing sugar at a concentration of 1 g/L. In a series of attempts made by Suatanto et al. [4,16,17], a two-step approach was adopted to involve dilute acid hydrolysis (DAH) step for generating hydrolysates rich in sugar, followed by the ISTE of the lipid-dense post-hydrolysis RB (PHRB). Through such strategy, hydrolysates produced had a sugar content ranging from 40 to 50 g/L [4,16] and has been successfully used as part of the growth media lipid accumulation by oleaginous yeasts (*Yarrowia lipolytica* [4] and *Lipomyces starkeyi* [16]). In addition, the lipids were left intact in the PHRB at much higher concentrations and could be used to produce FAME via ISTE. The ISTE of lipids in PHRB resulted in a FAME yield as high as 87 wt% at temperatures less than 200 °C, which was said to be owing to the inherent sulfonic sites, that were grafted on the PHRB matrix upon curing of the PHRB obtained after hydrolysis with dilute acid [16,17].

Although lipids in PHRB could be converted into FAME, the advantage of utilizing such material as feedstock for FAME synthesis has not been fully explored. It only recently that lipids in PHRB have been studied for extraction and recovery where the lipid extraction is faster and easier as compared to native RB while requiring a lesser amount of solvent for a given quantity of lipid recovered [18]. An initial exploration of ISTE of lipids PHRB has been previously reported, however, owing to the limited quantity of the generated residue, tests were generally limited and favorable reaction temperature tended to vary from 165 [17] to 185 [16]. Although the difference may be attributed to the measured strong acid densities, this then gives rise to the concern of finding an appropriate process condition that is robust enough to accommodate such differences in the activity of the material to self-catalyze the reaction. Another concern in the generation and use of PHRB as feedstock for FAME production is the range of lipid content (29–48 wt%) in the resulting PHRB [18,19], as influenced by the inherent quality and composition of the starting RB material and subsequently the extent of hydrolysis that could be induced during the DAH step. The wide range of lipid content may entail the need for different solvent-to-solid ratios (SSR), reactor loading (RL), and space loading (SL). It is thus further needed to have a process that could accommodate such variability in feedstock quality. In addition, previous reports on the possibility of using PHRB were only explored for the ISTE process, with no further test performed to verify the catalytic activity of the residual solids obtained after ISTE.

Given the developments in the processing of lipid from RB for FAME production, the goal of the current study is to further maximize the use of PHRB as a raw material in producing FAME via non-isothermal ISTE with methanol under subcritical conditions and subsequent recovery of residues to serve as solid acid catalysts (SAC) in FAME synthesis via esterification of free fatty acids (FFA). Specifically, this study has the following objectives: (i) Investigate the influence of RL, SL, SSR, temperature, non-isothermal heating time, and isothermal holding time on the yield of FAME and conversion of available fatty acids during ISTE with subcritical methanol and without further addition of catalyst; (ii) Investigate the effect of PHRB quality (lipid content and acid density) on the convertibility of the lipids and activity of the material to produce the desired FAME; (iii) Determine the residual catalytic activity of post-ISTE PHRB as a catalyst for the esterification of FFA, using oleic acid and methanol as model substrates. It is hoped that the results of this work would contribute to the new possibility of maximized utilization of lipid-containing biomass for renewable energy production.

2. Materials and methods

Two separate batches of 10-kg RB were gathered from a rice mill in Kaoshiung, Taiwan. Upon receipt, the RB samples were contained in air-tight resealable bags and refrigerated at −4 °C until their use, to prevent hydrolysis of RB lipids. The collected RB were then transformed into PHRBs following the procedures laid out by Go et al. [18] with curing at 50 ± 5 °C for 5–7 days. The resulting dry PHRBs were labeled correspondingly as PHRB-A and PHRB-B. The dried PHRBs were stored in air-tight bottles. Other chemicals and reagents include concentrated (95 vol%) sulfuric acid from Scharlau, Spain, anhydrous (99.99 vol%) methanol from Aencore, Australia, concentrated (37 vol%) hydrochloric acid from Acros Organics, USA, analytical grade (95 wt%) sodium hydroxide from Fischer, USA, HPLC grade (99.9 vol%) ethyl acetate from Echo Chemical Co., Ltd, Taiwan, reagent grade (99.9 wt%) sodium chloride from Showa, Japan, reagent grade (85 wt%) potassium hydroxide from Acros organics, USA, technical grade (95 wt% n-hexane) hexane from Echo Chemical Co., Ltd, Taiwan, technical grade (88 wt%) oleic acid from Showa, Japan, and BF_3-methanol reagent (13–15% BF_3) & FAME 37-mix standard from Sigma-Aldrich, Germany. All chemicals and reagents were obtained through suppliers in Taiwan.

2.1. ISTE of PHRB with subcritical methanol

For the ISTE of PHRB-A, a stainless-steel (SS) batch reactor (292 mL capacity), with a glass container (192 mL capacity, but occupies a space of ~44 mL) was used to contain the reaction mixture. A pre-determined quantity of PHRB-A (m_{PHRB}), varying from about 12 g to 35 g, corresponding to the RL (55, 70, or 85%) and SSR (4, 6, 8 mL methanol/g moisture-free PHRB) investigated, was weighed into the glass reactor using a digital balance with an accuracy of up to 0.01 g. A measured amount of methanol (between 84.5 and 145 mL) was added to the weighed PHRB-A, and then manually stirred before placing it in the SS reactor. The reactor is tightly sealed with a Teflon lining to ensure no leaks and achieve the desired subcritical conditions upon heating from room temperature to the desired reaction temperature, before cooling down to 60 °C. The influence of RL and SSR was evaluated using a non-isothermal reaction approaching 150 °C, and then cooling down to 60 °C following the representative heating and cooling curves as presented in Fig. S1a in the supplementary file. To study the effect of temperature and time RL of 70% and SSR of 6 were adopted. The temperature range investigated are 100, 125, and 150 °C and isothermal holding times of 0, 15, and 30 min once the desired temperature was reached, before being cooled down to 60 °C. A typical heating curve for 30 min extension of the three temperatures is presented in Fig. S1b. After carrying out the reaction at the desired conditions, the glass container was taken out and the mixture was filtered under vacuum to separate the solids from the liquid crude products with the excess methanol. The solids,

including the insides of the reactor, were washed with 50 mL of hexane, succeeded by 2 frequencies of 30 mL hexane to ensure that all the product is collected in the filtrate. The solids were collected and then dried with the aid convective oven at 50 ± 5 °C for 1 day and stored in resealable bags until further use. After which, the filtrate was transferred into centrifuge bottles to which 60 mL of 5% w/w NaCl solution and 30 mL of hexane were added. The tubes were centrifuged for 5 min at 25 °C with a force of 10,000×g. The upper layer was decanted into a separatory funnel, which was washed with salt solution three times. The remaining liquid in the centrifuge bottle was reextracted with hexane amounting to 90 mL for the second time, and 30 mL for the third time, and transferred and washed with the salt solution as previously described. The upper layer from the separatory funnel was transferred to an empty evaporating flask (m_{flask}) and then concentrated in a low-pressure rotary evaporator. Concentration in the evaporator was done until the collected product did not have a change in weight, as all solvent was evaporated. The weight difference between the flask containing the products ($m_{flask+product}$) and the empty flask (m_{flask}) was taken to be the crude product weight ($m_{product}$), used to calculate the crude yield using Equation (1).

A sample of the crude product was injected in the GC with ethyl acetate at a concentration of 25 mg/mL to determine the FAME purity or content (Equation (2)). The FAME content of the crude sample was quantified using high-temperature gas chromatography. The gas chromatography system used was Shimadzu GC-2010 Plus, having a flame ionization detector, and installed with ZB-5HT Inferno column (15 m × 0.32 mm x 0.1 μm), while adopting an analysis program previously established and described elsewhere [21]. Finally, the FAME yield is calculated using Equation (3) for easier comparison of the desired product to the raw feedstock. The yield is compared to the maximum theoretical FAME that can be produced from PHRB lipids as a response to further evaluate the process conditions interns of the apparent conversion or reaction yield based on the collected products (Equation (4)). The maximum theoretical FAME was determined as the mass equivalent of the total fatty acid content of PHRB-A lipids following the approach described in a recent review [1]. A confirmatory run using optimum process conditions was carried out in duplicate using PHRB-B.

$$Crude\ Yield\ \left(\frac{g\ crude\ product}{100\ g\ PHRB}\right) = \frac{m_{flask+product} - m_{flask}}{m_{PHRB}} \times 100 \quad (1)$$

$$FAME\ Purity\ (\%) = \frac{m_{FAME}}{m_{product}} \times 100 \quad (2)$$

$$FAME\ Yield\ \left(\frac{g\ FAME}{100\ g\ PHRB}\right) = Crude\ yield\ \times\ FAME\ purity \quad (3)$$

$$Conversion\ (\%) = \frac{FAME\ yield}{Theo\ max\ FAME\ in\ PHRB\ lipid} = \frac{FAME\ yield}{\%\ TFA\ \times lipid\ content_{PHRB} \times 1.05} \quad (4)$$

2.2. Evaluating the catalytic activity of the collected solid residues

The activity of PHRB as an acid catalyst for (trans)esterification was primarily evaluated by determining the FAME yields and conversions from the ISTE of its lipids. From the ISTE yielding the optimum FAME, the residues of PHRB-A and PHRB-B, referred to as post-ISTE PHRB, were further evaluated for their catalytic activity. The primary response measured is the methyl oleate content in the reaction product from the esterification of oleic acid (OA) with methanol at a molar ratio of 20, whereby the catalyst was loaded as 10 wt% of the OA weight. The esterification reaction was carried out at 60 °C, and continuously stirred at 200 revolutions per minute, where an aliquot of 100 μL was taken at 5, 15, 30, 45, 60, 120, 240, 360, 480, 600, 720, and 1440 min. These aliquots were analyzed for their methyl oleate content using the GC. The solids were recovered and are recycled up to 6 times.

2.3. Characterization of PHRB and post-ISTE PHRB

In aid of evaluating the reaction yield and process performances, the residues were characterized accordingly. The resulting FAME yield was largely dependent on the total fatty acid available and the strength of the catalytic activity of the solid matrix where these lipids are contained. In the Characterization of the determination of the available lipids and their subsequent characterization, lipids were first extracted using Soxhlet extractor with hexane as solvent. Collected lipids were then saponified to determine total fatty acid (TFA) available and the fraction of unsaponifiable matter, following AOAC methods 993.08 and 972.28 with modifications by Loyao et al. [22]. The fatty acid profile was determined by comparing it with the chromatogram of an injected FAME-37 standard from Sigma-Aldrich. The fatty acid profile was then used to estimate the biodiesel properties using empirical correlations based on related literature [23,24] via an online tool (Biodiesel Analyzer©, "http://www.brteam.ir/biodieselanalyzer). In addition, DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay of selected lipid samples and the crude product was also determined following the procedures suggested by Sharma and Bhat [25], with ethanol used as a solvent to ensure all samples were completely soluble.

The components of the PHRB solid were determined by its proximate and elemental composition. Thermogravimetric analysis was done to determine the proximate composition of lipid-free solid fractions, following the program specified by Vounatsos et al. [26]. The residues were also analyzed of their elemental composition (CHONS) using an elemental analyzer (Elementar Vario EL cube, Germany). The acid sites (total and weak) in the PHRBs were determined by titration of the resulting solution from the reaction of 30 mL of a standardized solution of NaOH (0.1 M) and NaCl (2 M), respectively, as it was added to 0.5 g of dried lipid-free residue and stirred for 6 h following the procedures outlined by Macawile et al. [27] and Li et al. [28] with modification on the mass and concentrations of solutions and solids to ensure the determined acid densities were within detectable limits. In addition, samples were also analyzed using IR spectroscopy using Shimadzu Tracer-100 with the aid of LabSolutions IR software by scanning pellets of dried KBr as the carrier/background in aid of identifying possible functional groups that could have contributed to the activity of the solid matrices, as an acid catalyst for (trans)esterification. For all solid samples analyzed, these were first removed of their residual lipid using Soxhlet extraction to avoid interferences.

3. Results and discussion

The lipids of native RB samples were densified in the resulting PHRB-A and PHRB-B having lipid contents of 31.35 ± 2.20 and 48.98 ± 1.40 wt% on a dry basis, respectively. The PHRBs correspondingly have TFA contents of 89.29 ± 1.78 (PHRB-A) [19] and 80.78 ± 1.56 (PHRB-B) [18], as previously reported in previous related studies. The fatty acid profiles of PHRB lipids are summarized in Table S1. The fatty acid profile of PHRB-A is found to be similar to the previously reported PHRB fatty acid profile [18], with

oleic and linoleic acid as the 2 major unsaturated fatty acids present, followed by palmitic acid as the major unsaturated FA. Fatty acid profiles for both PHRB lipids are comparable to reported ranges of the fatty acid profile for rice bran lipids [1]. Although the fatty acid profile of PHRB-B was previously assessed to produce suitable for BD [18], some properties were left unassessed. To have a fair comparison, FA profiles from PHRB-A and PHRB-B [18] lipids were reprocessed using the same empirical correlations to have a fair comparison of their potential biodiesel properties (Table S1). The predicted cetane number for both PHRB biodiesel (>51) could pass both ASTM and EN standards. Cetane number, which is largely dependent on the length of the carbon chain and unsaturation with saturated long carbon chain fatty acids like palmitic acid preferred and resulting in good cetane number [30] for the biodiesel derivable from PHRB lipids. Due to the leading amounts of unsaturated fatty acids, the predicted cold flow properties of PHRB biodiesel are favorable. However, unsaturation in the fatty acids also presents a potential problem where it tends to form acid vapors upon autooxidation [31]. Lipids with having more bis-allylic unsaturation, such as linoleic acid, are more susceptible to autooxidation, and therefore will have poorer OSI [32], which is true for PHRB-A (5.86 h), when compared to PHRB-B (6.44). Nevertheless, the predicted OSI of potential PHRB FAME could pass the prescribed limits (≥3 h). The addition of antioxidants can address this problem, which is also coincidentally inherent in rice bran lipid [1]. The density of PHRB-B is within standard when compared to EN, however, PHRB-A was predicted to have a density of 835 kg/m^3 which is a few units off the minimum of 860 but is not an issue when based on ASTM standards. Less dense PHRB-A FAME tended to have a slightly lower viscosity (3.55 mm^2 s), compared to PHRB-B FAME (3.79 mm^2 s), but fall within the prescribed limits (1.9–6.0 mm^2 s). Overall, the lipids from PHRB have a favorable fatty acid profile and predicted properties when converted into FAME to serve as biodiesel (Table S1). The sections that follow detail the efficiency and extent of FAME production in the utilization of PHRB and its lipids as feedstock in an in-situ system under subcritical conditions of methanol via a non-isothermal reaction process.

3.1. Effect of reactor loading and SSR on FAME yield during ISTE with subcritical methanol

The influence of RL and SSR on the resulting FAME is driven by the proportion of reactants to facilitate the simultaneous reaction and/or extraction of the product or reactant into the bulk phase during the non-isothermal reaction approaching 150 °C. Space loading (SL) is another measure of reactor utilization that corresponds to the ratio of reactor volume to the solids loaded [5]. From Fig. 1a, it can be observed that increasing the RL and decreasing the SSR would decrease the SL, which would mean an increased solid loading for a fixed reactor volume. This was found to be ideal by Go et al. [10] in their utilization of *Jatropha curcas* L. seeds in ISTE, for optimum reactor productivity, and ensuring the solvent exposure to the lipid-containing solids by minimizing the headspace for vaporization. As seen in Fig. 1b, over a narrow range of SL (10.8–12.5 mL/g), there is an increase in FAME yield along with the increase in the RL from 55 to 70% and with an accompanying increase in the SSR from 4 to 6 mL/g, respectively. An increase in RL and SSR at a similar SL provided sufficient methanol to participate in the ISTE. Increasing further the RL to 85 and SSR to 8 did not further improve the FAME yield. Instead, a decrease in yield was observed, which may be attributed to dilution of the system by the excessive amounts of the solvent added and solvent present in the liquid phase, which resulted in a slower rate of reaction. The increase in amount of methanol in the liquid phase is indirectly indicated by the increase in the system pressure from 0.5 to 1.3 Mpa as the RL was increased from 55 to 85%, regardless of the SSR employed. Low system pressures achieved at lower RL need not mean that all methanol were vaporized, this is expected since components soluble in methanol specially lipids and the resulting FAME have exceptionally low vapor pressures, thus resulting in the lowering of the overall system pressure at the given temperature. The increase in pressure at higher RL of 70 and 85% is primarily attributed to the smaller volume available for expansion, which induces a higher system pressure. Considering that RL and SSR have mutual influence on the (trans)esterification of the lipids and extraction of the desired product (FAME), the effects of these parameters were further investigated in detail.

The influence of RL and SSR on extraction performance can be observed indirectly from the crude product yield (Fig. 1c). As could be observed, increasing the SSR tends to result in slight increases in crude yields, these increases are considered insignificant ($p = 0.0849$) at 95% confidence. While increasing the RL from 55 to 85% resulted in a significant decrease in the crude yield ($p = 0.0369$), particularly observed at SSR 4 and 6 ml/g. Analysis of variance also revealed that there is an interaction effect ($p = 0.0189$) which meant RL and SSR synergistically influence the amount of product extracted. Despite the complex relationship between RL and SSR to the corresponding crude yields under the conditions investigated, the lowest crude yield corresponds to at least 92.5% of the available lipids.

In view of product purity, at the studied range of RL ($p = 0.1092$) and SSR ($p = 0.2072$), these were found to not influence the resulting FAME purity or content in the crude product. However, it can be observed that low FAME contents were achieved at RL 55 and SSR 4, and RL 85 and SSR 8, which are around 72%. A low RL and SSR would result in increased amounts of the methanol being vaporized which reduces the effective volume of methanol interacting with a relatively large amount of PHRB in the system. Meanwhile, the increase in RL to 85% and SSR to 8 mL methanol/g, would dilute the lipids and the potential catalyst in the reaction system by simultaneously increasing the effective ratio of liquid methanol to the PHRB. Either case results in slower reaction rates, as indicated by the lesser amount of FAME in the products, with presences of higher unreacted acylglycerides (8.14 ± 0.06 wt%) as compared to RL of 70% (6.57 ± 0.09 wt% acylglyceride), owing to either mass transfer limitation or dilution, respectively.

Looking into the combined effect of extraction and reaction in terms of FAME yield, RL was found to predominantly ($p = 0.0180$) affect the FAME yield, while SSR had minimal influence ($p = 0.8534$). This is expected considering that the amount of methanol added is way above the stoichiometric requirement of 1 mol of methanol for every mole of available fatty acid, even at the lowest SSR of 4 mL/g (~100:1 mol FA). In view of RL, an RL of 70% provides a good balance that induces enough amount of methanol present to interact with the PHRB while avoiding dilution of the system. The highest FAME yield, 24.64 g/g PHRB, could be achieved at an RL of 70% and an SSR of 8 mL of methanol/g dried PHRB, corresponding to a conversion of 90.63%. However, a pairwise comparison with results from SSRs of 4 and 6 mL/g for the same RL, the FAME yields of 24.41 ± 1.44 ($p = 0.4203$) and 24.50 ± 0.32 g/g PHRB ($p = 0.2999$), respectively, were not significantly different. These results also coincide with the previous report by Sutanto et al. [17], where the reported optimum was found at an SSR of 5 at an RL of 61%, during the ISTE of PHRB lipids with methanol at 165 °C.

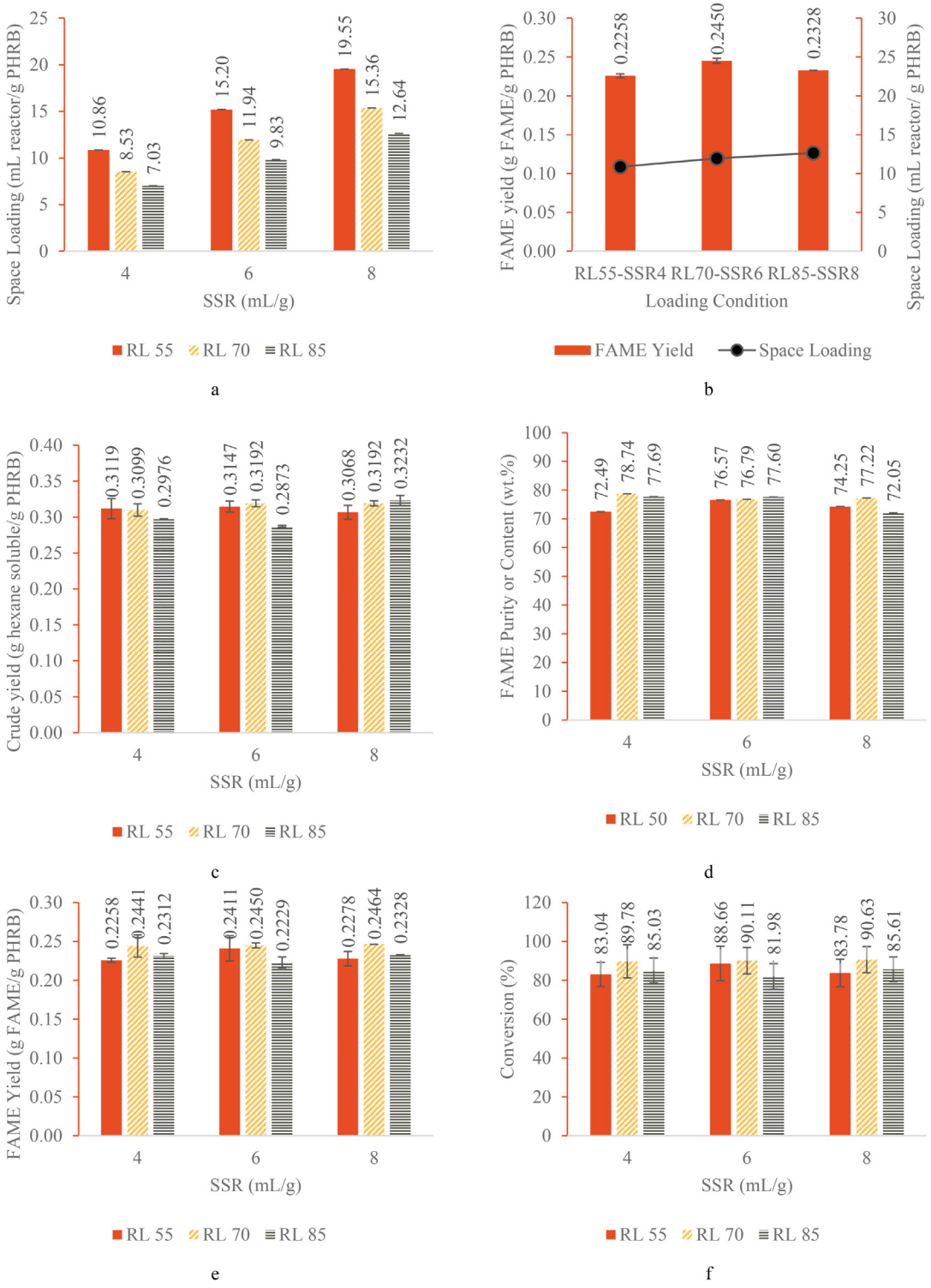

Fig. 1. Summary of ISTE at subcritical conditions (non-isothermal reaction from 25 to 150 °C, t = ~0.6 h for heating): Influence of reactor loading (55, 70, 85%) and SSR (4, 6, 8 mL/g PHRB) on the space loading* (a); Influence of SSR on FAME yield at a narrow SL (b), and Influence of RL and SSR on crude yield (c), FAME purity (d), FAME yields €, and Conversion (f). **Space loading is based on net void space of the reactor (247.89 mL) calculated from the difference of the subcritical reactor capacity (292 mL), and glass container volume (44.11 mL).*

3.2. Influence of temperature and time during ISTE with subcritical methanol

Since SSR of 4–8 mL/g was found to have no significant influence on the process yield, for practical considerations an SSR of 6 mL/g coupled with an RL of 70% was adopted in the investigation on the influence of temperature and time. From Fig. 2, it can be observed that even at the lowest temperature of 100 °C, where it took around 15.25 min to reach from room temperature, around 57% of the TFA were already converted to FAME. This implies that in the process of heating the contents inside the reactor, the (trans) esterification reaction has already commenced. This is indicative of

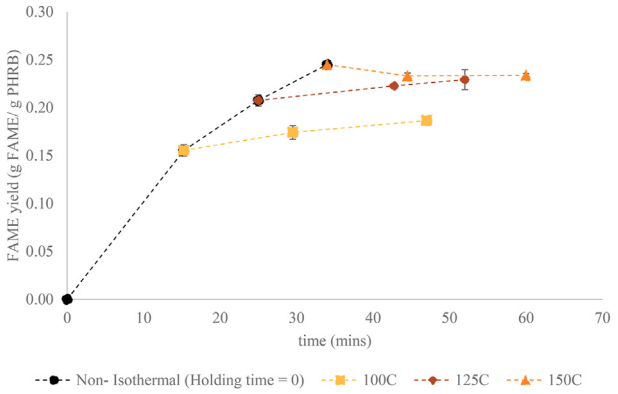

Fig. 2. FAME yield from for ISTE of PHRB lipids at RL = 70%, SSR = 6 mL methanol/g moisture-free PHRB, at different reaction temperatures and isothermal holding times.

the catalytic activity of the PHRB since it was previously reported that FAME yields of no more than 50% could be obtained from ISTE of RB lipids under subcritical conditions of methanol and water (T = 200 °C, P < 8 MPa), even after a prolonged period of 3 h [33]. Extending the time where the reaction was held to 15, and 30 min have increased the conversion to 64, and 69%, respectively, with significant amounts of the triacylglycerides (TAGs) and partial acylglycerides (PAG) still unreacted (~14, and ~6 wt% TAG, and ~1, and 6 wt% PAG). Increasing the temperature to 125 °C from room temperature, incurred an additional reaction time of 10 min from 100 °C and resulted in an increase in the conversion of TFAs to 76%. Holding the reaction temperature for an additional 15 and 30 min, has increased the conversion to 82 and 84%, respectively. Due to the incremental increase in conversion, by increasing temperature and time for both 100 and 125 °C, increasing the temperature to 150 °C was explored in hopes of improving the conversion at a relatively shorter time. The product yield (24.50 ± 0.32 g FAME/100 g of PHRB) was achieved during the non-isothermal reaction approaching 150 °C, which required about 34 min and has a corresponding apparent conversion of ~90%. However, holding the system at this temperature for an additional 15 and 30 min lowered the FAME yield by 5%. This can be attributed to possible degradation or side reaction in the FAME produced, which can be supported by the decrease in the unsaturated fatty acids based on the FFA ratios as presented in Table 1. Decrease in yield at elevated temperatures and prolonged reaction time were also previously observed by Kasim et al. [9] when carrying out ISTE of RB at 300 °C and 30 MPa where a low FAME yield of ~52 g/100 g RB was achieved. The low yield was attributed to the degradation of the fatty acids and the formation of unidentified hydrocarbon side products. In addition, comparing with the work by Sutanto et al. [16] where PHRB was also used as feedstock, the yield under the best conditions of 185 °C and SSR of 5 mL/g was also observed to decrease, by about 15%, when the reaction was prolonged for 30 min. The decrease was attributed to the thermo-chemical reactions which brought about degradation or side reaction of FAME with other components and hastened by the presence of acid sites at elevated temperatures.

In view of non-isothermal batch reaction, it must be noted that for batch reactor systems heated at a fixed power output, increasing the temperature setpoint, also meant increasing the heating and cooling time naturally. The recorded heating and cooling time along with the total reaction time is tabulated in Table 1. The maximum FAME yield with a conversion of ~90% was achieved after a total reaction time of 64 min by heating the system from room temperature to 150 °C followed by cooling to 60 °C.

3.3. Characteristics and activity of PHRB and post-ISTE PHRB as acid catalyst

Following the DAH pretreatment, ~55% of the initial RB was retrieved after separation via filtration, with other parts of the RB broken down forming part of the resulting hydrolysate. From previous works, the resulting hydrolysates which were rich in reducing sugars were successfully used in the cultivation of yeast for lipid accumulation [16,17]. In the preceding sections, the recovered PHRB containing significant amounts of lipids were found to be utilizable as feedstock for FAME production and hinted at a possible catalytic activity. Further characterizations were done with the collected solids to better understand the catalytic activity exhibited.

From the thermograms (Figs. 3c and 4d) of the lipid-free materials, it could be observed that PHRB-A has a lower stability than RB-A, which could be attributed to the breakdown of complex structures to simpler ones during the DAH pretreatment and the higher lipid content of PHRB-A (~31 wt%) as compared to RB (~16 wt%) and the higher FFA contents of up to 21 wt% in the lipids. These results coincide with observations by Go et al. [18] in a

Table 1
In-situ (trans)esterification of PHRB lipids at a reactor loading of 70%, SSR of 6 mL methanol/g PHRB at various temperatures and times.

T_r (°C)	t_{Heat}[a] (mins)	t_{HT}[b] (mins)	t_{Cool}[c] (mins)	t_{TOT}[d] (mins)	Hexane soluble fraction[e] (g/ 100 g PHRB)	FAME purity (g FAME/100 g hexane soluble yield)	FAME yield[f] (g FAME/ 100 g PHRB)	Conversion[g] (%)	FFA ratios (C18:2 + C18:10) to C16:0
100	15	0	31	46	28.17 ± 0.75	55.19 ± 0.64	15.54 ± 0.23	57.15 ± 4.33	4.73 ± 0.00
100	15	15	28	58	28.15 ± 0.03	61.89 ± 2.46	17.42 ± 0.71	64.07 ± 5.42	4.76 ± 0.07
100	17	30	21	68	29.48 ± 0.63	63.29 ± 2.09	18.65 ± 0.22	68.59 ± 5.16	4.87 ± 0.04
125	25	0	32	57	28.34 ± 0.34	73.26 ± 1.20	20.76 ± 0.59	76.35 ± 6.07	4.78 ± 0.02
125	27	15	26	68	30.08 ± 0.45	74.09 ± 1.81	22.28 ± 0.21	81.93 ± 6.13	4.84 ± 0.02
125	22	30	24	76	31.33 ± 0.62	73.18 ± 1.89	22.93 ± 1.05	84.32 ± 7.35	4.86 ± 0.03
150	34	0	30	64	31.92 ± 0.35	76.79 ± 2.16	24.50 ± 0.32	90.11 ± 6.80	4.79 ± 0.03
150	32	15	29	76	31.66 ± 0.59	73.81 ± 0.78	23.37 ± 0.19	85.94 ± 6.42	4.51 ± 0.00
150	33	30	26	89	31.78 ± 0.48	73.36 ± 0.16	23.31 ± 0.30	85.72 ± 6.46	4.49 ± 0.04

* Setpoint temperature (T).
[a] Duration of heating from room temperature (23–25 °C) to reaction temperature (Tr).
[b] Duration of time where reaction temperature (Tr) was held.
[c] Duration of time of cooling from reaction temperature (Tr) to 60 °C.
[d] Sum of heating, holding, and cooling times.
[e] Weight of hexane soluble yield from the filtrate of reaction mixture per 100 g of PHRB in wet basis (moisture content = 7.46 ± 0.11 wt%).
[f] Amount of FAME produced per 100 g of PHRB in wet basis (moisture content = 7.46 ± 0.11 wt%).
[g] Apparent conversion based on yield or the reaction yield: the amount of FAME in crude yield relative to the theoretical maximum amount of FAME in dry basis (29.39 ± 2.14 g FAME/100 g dried PHRB).

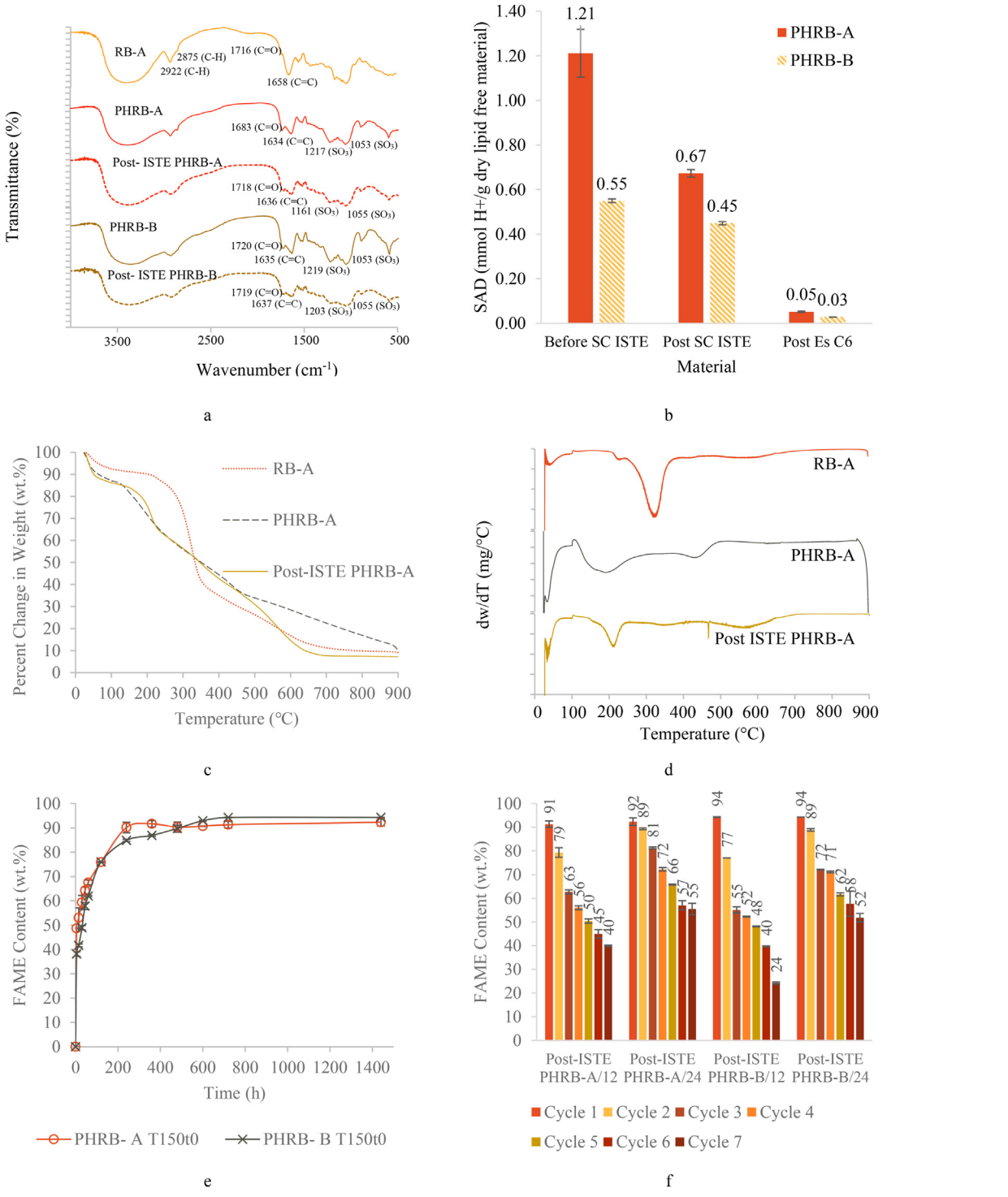

Fig. 3. Characterization of PHRBs and post-ISTE PHRBs: FTIR spectra (a), the acid density of delipidated PHRBs (Before SC ISTE), post-ISTE PHRB (Post SC ISTE), and residue after 6th cycle of esterification (Post Es C6) (b), their respective thermograms (c) and differential thermograms (d), FAME content during esterification at a methanol-to-oleic acid molar ratio of 20 over a period of 24 h at 60 °C and stirred at 200 rpm(e), reusability of post-ISTE PHRBs as a catalyst at 10 wt% catalyst loading (f).

related work where scanning electron micrograms revealed PHRB having oils/lipids exposed in the surface. The observed lower stability is also in agreement with related work on TGA of plant oils with different FFA content, with oils having higher FFA content starting to volatilize at temperatures of 200 [34]. Nevertheless, PHRB-A is still stable up to 200 °C, which still enabled its utilization under the investigated subcritical methanol conditions (100–150 °C). Compared to the native RB, the delipidated PHRB-A was found to have a 13-fold increase in its acid sites (1.21 ± 0.11 mmol H^+/g dry lipid-free material, Fig. 3b). Elemental analysis of both materials also confirms an increase in sulfur content, from 0.20 ± 0.03 to 5.56 ± 0.01 wt%, of RB-A and PHRB-A, respectively.

Observing a darkening in color, simultaneous carbonization and

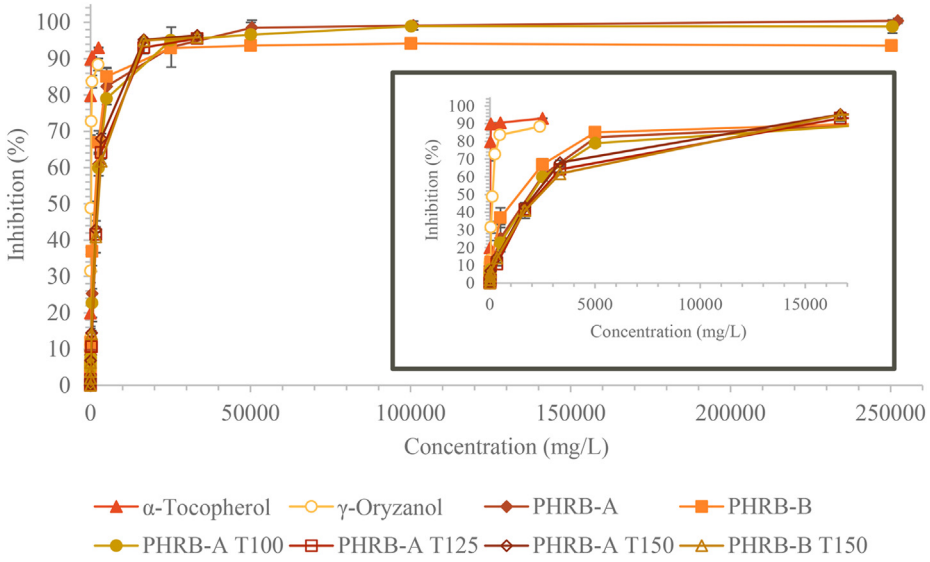

Fig. 4. Radical (DPPH) scavenging activity of PHRB lipids, crude FAME, γ-oryzanol, and α-tocopherol.

sulfonation might have occurred during the drying step, aided by the entrained residual dilute acid from the pretreatment. In agreement with the titrimetric and elemental analysis, spectral bands of sulfonic acid moieties were observable from FT-IR spectra of PHRB-A (1053, 1217 cm^{-1}), which are absent in the native RB-A (Fig. 3a). The observed peaks are in agreement with other works on the sulfonated catalyst with 1040–1100 cm^{-1} often attributed to $-SO_3H$ and 1150–1245 cm^{-1} being S=O of the $-SO_3H$ group [35,36]. A different batch of RB which had undergone the same DAH pretreatment also had the same peaks (1053, 1219 cm^{-1}) observable in the said regions. Characteristic peaks corresponding to –OH group (~3400 cm^{-1}) and C–H bonds (29–0 - 2870 cm^{-1}), representing cellulosic material [37] are present in the spectra of RB-A, PHRB-A, and PHRB-B. Carboxylic acid peaks were also found in all residues (C=O, Fig. 3a) at 1720–1680 cm^{-1} [35,38]. Strong peaks are observable from the 1670–1600 cm^{-1} region of the PHRB residues, corresponding C=C bonds, representing monosubstituted alkenes which are in agreement with previous studies which developed carbon-based acid catalysts from coffee [39,40] and RB [19,41].

To further confirm the catalytic activity of the solid matrix of PHRB, the residual solids from the subcritical ISTE experiments performed at optimum conditions were recovered, whose yield amounted to ~25% of the initial PHRB loaded. The recovered residues, referred to as post-ISTE PHRB-A, have a SAD ~45% lower than the PHRB before the subcritical ISTE experiment (Fig. 3b). From the elemental analysis of the post-ISTE residue, a significant amount of sulfur was still present (3.76 ± 0.03 wt%), which is ~67% of the initial sulfur content of the PHRB. The higher quantity of sulfur retained than the measured SAD equivalent could mean that the sulfur present in the material is not only in the sulfonic acid form. Despite having lower SAD compared to PHRB, post-ISTE PHRB A, and B still had appreciable strong acid sites 0.67 ± 0.02 and 0.45 ± 0.01 mmol H^+/g dry lipid-free material, respectively. Compared with other biomass-derived acid catalysts which have been used for esterification of fatty acids with acid densities ranging from 0.45 to 0.72 mmol SO_3H/g for coffee residue-derived catalyst [39], 0.59 mmol H^+/g for sugarcane bagasse-derived catalyst [42], and 0.459 mmol H^+/g dried and de-oiled microalgal biomass-derived catalyst [43], the residual acid sites of the post-ISTE residues are comparable.

The post-ISTE RBs having the said acid sites were verified of their activity to serve as SAC in the esterification of oleic acid and methanol (Fig. 3e). Having a higher SAD, PHRB-A achieved higher conversion (48.67 ± 0.12%) compared to PHRB-B (38.15 ± 0.95%) in the first 5 min. After 24 h, PHRB-A achieved 92.34 ± 1.58, while PHRB-B obtained a 94.33 ± 0.02% conversion, both being insignificantly different from each other ($p = 0.2183$). The stability of the acid sites was evaluated by reusing both PHRB-A and PHRB-B up to 7 cycles (Fig. 3f). With the SAD reduced to 0.05 and 0.03 mmol H^+/g post-ISTE PHRB-A and PHRB-B after the 6th cycle, the conversions achieved in the 7th cycle are 55.46 ± 2.43 and 51.84 ± 1.77%, respectively after a 24-h reaction time, it is evident that there are sites which are loosely attached. Thus, also corresponds to a decrease in the performance of both catalysts. The observed decrease is similar to those reported in the literature. For instance, Ngaosuwan et al. [39] also observed a decrease in the activity of coffee residue-derived catalysts by 67% after the 5th cycle was attributed to the poisoning of the catalytic sites by adsorption of reaction by-products such as water and leaching of acid sites estimating to cause ~40% of the deactivation. However, despite the significant decrease in the SAD, conversions of up to 50% could still be achieved and the process by which the catalyst was obtained did not generate unwanted acidic wastewater as compared to conventional approaches for carbon-based acid catalysts. Furthermore, the difference in the conversions achieved at the 6th and 7th cycles using post-ISTE PHRB-A ($p = 0.1423$) and post-ISTE PHRB-B ($p = 0.2599$) tend to approach a similar extent and are insignificantly different from each other. The same observation with regards to diminishing reduction in performance was observed by Flores et al. [42] in the use of SAC derived from sugarcane bagasse. Elemental analysis of the recovered catalyst after the 7th cycle still contained 0.52 ± 0.02 wt% sulfur, which is 14% of the initial sulfur content of post-ISTE PHRB but still enables conversions of up to 50%. Potential maximized utilization of PHRB through adopting the use of post-ISTE PHRB as a SAC in the esterification of high FFA feedstocks can contribute to the economic viability of the resulting biodiesel and further contributes to the potential of DAH as a pretreatment of lipid-containing residues in view of biofuel production.

3.4. Process comparison

The maximum FAME yield of 26.48 ± 0.51 g/100 g dried PHRB,

Table 2
Comparison of results from ISTE of lipids in PHRB with methanol under ambient pressure or subcritical conditions.

PHRB Characteristics[a]		Solvent Loading[b]		Catalyst Loading	Mixing/Irradiation/Heating	T (°C)/P (MPa)/t (h)[c]		Space Loading/ Reactor Loading[d]		Yields/ Conversion and FAME Content[e]		Material [Ref.]
MC:	7.46	SLR_n:	519	Strong acid Sites: 0.85 mmol/g solid[g]	No stirring	T	~65 to 70	SL:	-[f]	Y_S:	24	PHRB [19]
LC:	29.01	SLR:	54.2			P:	0.1	RL:	-[f]	Y_L:	83	
FFA:	21.13	SSR:	17	0.25 vol % H_2SO_4 – relative to the solvent[*]	Soxhlet Extractor	t:	8			Y_P:	88	
PS:	0.643			~4 wt% H_2SO_4 – relative to the solid[*]	Heating Mantle					C_{AE}:	84	
MC:	7.46	SLR_n:	611	Strong acid Sites: 0.85 mmol/g solid[g]	200 rpm	T	65	SL:	~30[k]	Y_S:	22	PHRB [19]
LC:	29.01	SLR:	63.8	*Added Acid: 5 wt% H_2SO_4*		P:	0.1	RL:	~65[k]	Y_L:	77	
FFA:	21.13	SSR:	20	0.25 vol % H_2SO_4 – relative to the solvent[*]	Hotplate Stirrer w/Water Bath	t:	12			Y_P:	82	
PS:	0.643			~9 wt% H_2SO_4 – relative to the solid[*]						C_{AE}:	85	
MC:	7.46	SLR_n:	458	Strong acid Sites: 0.85 mmol/g solid[g]	200 rpm	T	75	SL:	~22[k]	Y_S:	21	PHRB [19]
LC:	29.01	SLR:	48			P:	0.1	RL:	~66[k]	Y_L:	72	
FFA:	21.13	SSR:	15	0.14 vol % H_2SO_4 – relative to the solvent[*]	Hotplate Stirrer w/Water Bath	t:	8			Y_P:	76	
PS:	0.643			~4 wt% H_2SO_4 – relative to the solid[*]						C_{AE}:	91	
MC:	4	SLR_n:	91	Strong acid Sites:1.08 mmol/g solid[g]	No stirring	T:	185	SL:	9.7[k]	Y_S:	36	PHRB [16]
LC:	41	SLR:	12			P:	2.5	RL:	61[k]	Y_L:	88	
FFA:	33	SSR:	5	0.58 vol % H_2SO_4 – relative to the solvent[*]	Electric Heating Band at ~4 °C/min	t:	0 (~0.7)[h]			Y_P:	95	
PS:	n.s.			~5.29 wt% H_2SO_4 – relative to the solid[*]						C_{AE}:	-[f]	
MC:	9	SLR_n:	80	Strong acid Sites: 1.63 mmol/g solid[g]	No stirring	T:	165	SL:	9.7[k]	Y_S:	43	PHRB [17]
LC:	48	SLR:	10			P:	1.6	RL:	61[k]	Y_L:	89	
FFA:	n.s.	SSR:	5	0.87 vol % H_2SO_4 – relative to the solvent[*]	Electric Heating Band at ~5 °C/min	t:	0 (~0.5)[h]			Y_P:	97	
PS:	n.s.			~7.99 wt% H_2SO_4 – relative to the solid[*]						C_{AE}:	-[f]	
MC:	7.46	SLR_n:	93	Strong acid Sites: 0.77 mmol/g solid[g]	No stirring	T:	150	SL:	12[k]	Y_S:	24	PHRB A [This Study]
LC:	29.01	SLR:	13			P:	1.2–1.3	RL:	54[j]/70[k]	Y_L:	84	
FFA:	21.13	SSR:	4	0.51 vol % H_2SO_4 – relative to the solvent[*]	Electric Heating Band	t:	0 (0.56[h]/1.07[i])			Y_P:	90	
PS:	0.643			~3.77 wt% H_2SO_4 – relative to the solid[*]	at ~4 °C/min					C_{AE}:	79	
MC:	7.46	SLR_n:	135	Strong acid Sites: 0.77 mmol/g solid[g]	No stirring	T:	150	SL:	12[k]	Y_S:	25	PHRB A [This Study]
LC:	31.33	SLR:	19			P:	1.2–1.3	RL:	54[j]/70[k]	Y_L:	86	
FFA:	21.13	SSR:	6	0.34 vol % H_2SO_4 – relative to the solvent[a]	Electric Heating Band	t:	0 (0.56[h]/1.07[i])			Y_P:	90	
PS:	0.643			~3.77 wt% H_2SO_4 – relative to the solid[*]	at ~4 °C/min					C_{AE}:	77	
MC:	3.57	SLR_n:	86	Strong acid Sites: 0.28 mmol/g solid[g]	No stirring	T:	150	SL:	12[k]	Y_S:	34	PHRB B [This Study]
LC:	47.23	SLR:	12			P:	1.2–1.3	RL:	54[j]/70[k]	Y_L:	72	
FFA:	36.65	SSR:	6	0.13 vol % H_2SO_4 – relative to the solvent[*]	Electric Heating Band	t:	0 (0.56[h]/1.07[i])			Y_P:	89	
PS:	0.592			~1.37 wt% H_2SO_4 – relative to the solid[*]	at ~4 °C/min					C_{AE}:	74	

[*] Entries to the table are calculated based on available information to facilitate comparison.
[a] Rice bran quality in terms of moisture content (MC, wt.%), lipid content (LC, wt.%), free fatty acid content in the lipids (FFA, wt.%), and particle size (PS, mm).
[b] Solvent loading as expressed in terms of solvent-to-total fatty acid molar ratio (SLR_n), solvent-to-lipid volume to mass ratio (SLR, mL/g), and solvent-to-solid volume to dry biomass ratio (SSR, mL/g).
[c] Temperature (T), pressure (P), and time (t).
[d] Space loading (expressed as the reactor volume to the amount of biomass loaded, mL/g), and reactor loading (percentage of the reactor volume occupied by the reaction mixture).
[e] Yields expressed as the amount of FAAE with respect to the solid (Y_S), with respect to the total lipids (Y_L), and with respect to the theoretical maximum amount of alkyl ester (Y_P), with purity (C_{AE}) expressed as the alkyl ester content.
[f] Incomplete information to allow estimation.
[g] Acid sites relative to solid (including lipids).
[h] Time required to reach desired temperature before reaction was stopped, estimated based on the constant heating rate indicated.
[i] Total time required including heating and cooling.
[j] Relative to the glass chamber volume.
[k] Relative to the available reactor volume or space.

corresponding to a conversion of ~90%, from the subcritical ISTE of PHRB-A lipids was obtained at an RL of 70%, SSR of 6 mL/g dried PHRB, and under non-isothermal reaction approaching 150 °C. The FAME yield obtained from an SSR of 4 improving the reactor productivity, where 90% of lipid conversion was also achieved, is not significantly different ($p = 0.4694$). However, an SSR of 6 had a numerically higher result for the yield and conversion. For practical purposes, the optimum conditions (RL = 70%, SSR = 6 mL/g, non-isothermal reaction to T = 150 °C)) were applied to a different batch of post-hydrolyzed rice bran, PHRB-B, for verification. The FAME yield obtained when using PHRB-B was 35.11 ± 0.11 g/100 g dried PHRB, corresponding to a conversion of ~89%. The resulting FAME yield was higher than that from PHRB-A, due to the higher lipid content of PHRB-B (~47 wt%) as can be seen in Table 2. As for the extent of reaction of the two PHRB batches, the conversions were comparable despite PHRB-B's lower SAD. This can be attributed to the higher FFA content of PHRB-B at 37 wt% when compared to PHRB-A having only 21 wt%, making it more easily to convert lipids in PHRB-B into FAME. Likewise in Sutanto et al.'s previous work [16] where FFA content was 33 wt%, 95% conversion of available fatty acids was achieved through a non-isothermal reaction approaching 185 °C. The higher temperature might have been due to the higher lipid content of the hydrolyzed PHRB at 41%, which also means less solid present to serve as a catalyst in the ISTE system. Nevertheless, these results imply the versatility of PHRB when used as feedstock and processed via ISTE with methanol under subcritical conditions, allowing high FAME yields and conversions regardless of lipid content and differences in acid densities.

Compared to a system operated at ambient pressures from earlier work [19], employing subcritical conditions of methanol has substantially reduced the reaction time for the ISTE of PHRB, and with better yield. Batch systems at ambient conditions which obtained slightly lower yields as seen in Table 2, had longer reaction times of 8 and 12 h and higher SSR's of 15 and 20 mL/g, with the former requiring addition of acid catalyst. Compared with the use of a Soxhlet extractor for the ISTE, PHRB had to be subjected to extraction for 8 h to achieve a conversion of 88%. The higher conversion achieved using a Soxhlet extractor than a simple batch reactor under ambient pressures was attributed to the higher temperature (>100 °C) in the heating mantle of the Soxhlet system, where (trans)esterification could have occurred further. A subcritical methanol-water system (200 °C and 4 MPa) was also used in one study by Zullaikah et al. [44] to extract γ-oryzanol from RB. Likewise, the simultaneous conversion of lipids in RB to FAME required an extraction and reaction time of 7 h, which the authors suggest would enable an easier recovery of γ-oryzanol along with the purification of FAME produced due to the simplification of the bulk components. Despite the higher temperature and pressure, adopting subcritical conditions of methanol for ISTE of PHRB lipids have several advantages not limited to the reduced reaction time, but the higher temperature also shifts the equilibrium allowing higher yields and conversions of up to 90% to be achieved while requiring much lesser SSR. All these advantages translate to better overall process productivity.

Considering that natural antioxidants are inherently present in the lipids of PHRB, as previously reported to be part of the unsaponifiable matter [18,19], these may be taken advantage of to improve the oxidative stability of the obtained product from ISTE. Narayanasamy et al. [45] looked into the DPPH radical scavenging activity of the natural antioxidants added to Mahlua oil methyl ester (MOME), and then measured the oxidative stability index (OSI) through the Rancimat method. The authors then found the OSI's of MOME with the natural antioxidant to be significantly better and were positively correlated with the DDPH radical scavenging activity of the said antioxidants. In the study by Tokada et al. [46], the addition of methanol extracted oils from spent coffee grounds, which are known to contain tocopherol and tocotrienol, was explored and proven as an effective means of improving the oxidative stability of biodiesel derived from rapeseed oil, by up to 3 folds.

As a preliminary, and indirect means of gauging the ability of the crude products to resist oxidation, radical scavenging assay was carried out with DPPH and the half-maximal inhibitory concentration, IC_{50}, was determined for crude products from ISTE (PHRB-FAME), as well as for γ-oryzanol and α-tocopherol, as references (Fig. 4). As pure components, the IC_{50} values of α-tocopherol and γ-oryzanol, have lower values at 15.20 ± 1.15 and 50.85 ± 4.87 mg/L, respectively, which meant these require small amounts to effectively induce inhibition of free radicals. Lipids from PHRB-A and PHRB-B have IC_{50} values of 1887.53 ± 290.85 and 1361.58 ± 305.35, which are considerably higher (lower activity) given that phytosterols, triterpene alcohols, and their esters their ester (approximately 6.78 ± 1.34% and 7.74 ± 0.90% as oryzanol equivalent) are present but as components part of the entire lipid sample. Upon ISTE of PHRB-A at subcritical temperatures of 100, 125, and 150 °C, the radical scavenging activity decreased for crude PHRB-A FAME, with IC_{50} values at 1971.66 ± 52.06, 2292.53 ± 73.59, and 2139.46 ± 61.93 mg/L, respectively. Similarly, the IC_{50} of crude PHRB-B FAME is 2374.86 ± 221.87 mg/L. Nevertheless, radical scavenging activity was still observed in the crude PHRB-FAME samples and can be attributed to the residual bioactive components (approximately 3.33 ± 0.40% and 3.13 ± 0.10% as oryzanol equivalent) found in the crude products. These values may at first seem low, but natural antioxidants at a concentration of 2000 mg/L in MOME [45] and or 2000 mg/kg in rice bran oil [47] were previously found to improve the oxidative stability by at least twice. These may require further testing, but as a preliminary, these results are promising and open other possibilities for the crude FAME obtained from PHRBs through ISTE under subcritical conditions.

4. Conclusions

With the potentially favorable biodiesel properties from its fatty acid profile, pre-functionalized and lipid-dense (31.35 ± 2.20–48.98 ± 1.40 wt% in dry basis) PHRB can be used as feedstock in the production of FAME through ISTE of its lipids with methanol under subcritical conditions without additional catalyst added to the reaction mixture. Yields of 26.48 ± 0.51–35.11 ± 0.11 g FAME/100 g dried PHRB, corresponding to ~89–90% conversion of available total fatty acids, could be achieved with high RL (70–85%) and low SSR (4–6 mL methanol/g dry PHRB), regardless of lipid content. For the range of the SSR and RL investigated, high SSR and RL tend to dilute the system and reduce the process yields. The PHRB exhibited catalytic activity, serving as SAC, which enabled ISTE to be carried out non-isothermally under less severe conditions. The maximum FAME yield could be achieved by heating from 30 °C to 150 °C and then immediately cooling down to 60 °C, with a total time of less than 70 min. Extended reaction time at 150 °C using PHRB has tendencies to result in lower product yield as a result of possible product degradation or side reaction. The resulting crude FAME from PHRB lipids possesses radical scavenging activity which can either be recovered or may aid in improving the stability of the FAME during storage. The observed catalytic activity of PHRB is due to the acquired acid sites via partial carbonization and sulfonation during drying of the post-hydrolysis residue containing residual dilute acid solution. Recovered post-ISTE PHRB residues still demonstrated appreciable activity in catalyzing the esterification of oleic acid in methanol, while having the potential to be

recovered and subsequently reused at least 6 times and still retaining at least half of its initial catalytic performance.

CRediT authorship contribution statement

Alchris Woo Go: Writing – original draft, Writing – review & editing, Supervision, Project administration, Formal analysis, Funding acquisition, Conceptualization, Methodology, Visualization. **Kristelle L. Quijote:** Writing – original draft, Writing – review & editing, Formal analysis, Conceptualization, Methodology, Investigation, Data curation, Visualization. **Roxanne Kathlyn O. Alivio:** Writing – review & editing, Investigation. **Yi-Hsu Ju:** Writing – review & editing, Conceptualization, Resources. **Chintya Gunarto:** Writing – review & editing, Investigation. **Artik Elisa Angkawijaya:** Writing – review & editing, Resources, Supervision. **Shella Permatasari Santoso:** Writing – review & editing. **Maria Yuliana:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.renene.2022.02.089.

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