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ORIGINAL PAPER

Taguchi Method and Grey Relational Analysis to Improve *in Situ* Production of FAME from Sunflower and *Jatropha curcas* Kernels with Subcritical Solvent Mixture

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Abstract This study investigates the possibility of employing *in situ* (*trans*)esterification (ISTE) under the subcritical condition (200–250 °C) of the solvent mixture (methanol + acetic acid) with a high solid loading and a low solvent to solid ratio (SSR). The Taguchi method together with grey relational analysis was used to improve both FAME yield and productivity. It was found that temperature reaction time and SSR were factors which contributed the most in obtaining high FAME yields. In addition to the above-mentioned factors the addition of acetic acid also significantly improved the productivity. Employing the following conditions: 250 °C; 8.8 MPa; 3–7 mL/g SSR; 10 % acetic acid was found to provide an improved FAME yield and productivity. A confirmatory test resulted in a FAME yield of 87.5–92.7 % for sunflower kernels and 88.2–97.22 % for *Jatropha curcas* L. kernels and productivity up to 37.5 kg/m^3/h can be obtained with good repeatability. Furthermore, the process developed in this study can tolerate moisture and a free fatty acid content of up to 25 %. The direct application of the method using whole kernels was also investigated.

Keywords Biodiesel · *Jatropha curcas* · Subcritical methanol · Subcritical acetic acid · Sunflower oil · (*trans*) Esterification

Abbreviations

FAME	Fatty acid methyl ester(s)
FFA	Free fatty acid(s)
AA	Acetic acid
JCL	*Jatropha curcas* L
SF	Sunflower
ISTE	*In-situ* (*trans*)esterification
SSR	Solvent to solid ratio

Introduction

In-situ (*trans*)esterification (ISTE) was first employed using sunflower kernels in acidified methanol by Harrington and D'Arcy-Evans in the 1980s [1, 2]. Acid as a catalyst was preferred as it is more tolerant to moisture and high free fatty acid (FFA) content. The main advantage of ISTE is the elimination of the extraction step and higher yield of fatty acid methyl esters (FAME) compared to conventional methods [1–3]. Depending on the amounts of alcohol and catalyst used, the ISTE reaction usually requires 10–24 h [1–4]. Considering that both extraction and reaction occurred simultaneously, the time required is still shorter than that of the conventional process where oil

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S. Sutanto and A. W. Go have made equal contributions to this work.

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is firstly extracted before it can be reacted with methanol to produce FAME. Its main disadvantage is the need to use high amounts of solvents, typically with a solvent to solid ratio (SSR) of at least 10 mL/g. Haas *et al.* first employed a base-catalyzed ISTE using soybean flakes, distillers dried grains and meat and bone meal as feedstock [4]. In their process, the SSR can be lowered down to 2.4 mL/g but feedstock drying to a moisture content of ~2.6 % and long reaction time (~10 h) were required [4, 5]. Although a conventional base catalyst acts as a stronger catalyst, which leads to lower SSR, it should be noted that the amount of solvent required greatly depends on the amount of oil in the solid feedstock. Georgogianni *et al.* [6] employed a base catalyzed ISTE on sunflower kernels with an SSR of 10 mL/g and this resulted in a high FAME yield (~95 %) in just 20 min. The use of high SSR is undesirable as this leads to low productivity, hence a high cost and high energy consumption in recovering the unreacted methanol [4, 5]. Moreover the use of a catalyst also implies additional cost in its removal and treatment.

For ISTE the use of a recoverable (heterogeneous/solid) and environmentally benign (enzyme) catalyst would require specially designed reactors. These types of catalyst are usually more expensive but can be reused. Unlike conventional biodiesel production ISTE involves solid feedstock, which poses recovery or separation issues when using a solid catalyst.

The other possibility is employing supercritical ISTE in which low SSR is required without the need of a catalyst. One of the early attempts to produce biodiesel via ISTE in supercritical methanol and carbon dioxide utilized rice bran as the feedstock [7]. Despite the short reaction time (10 min) it resulted in a low FAME yield, which was attributed to thermal degradation. Factors affecting ISTE of *Jatropha curcas* L. (JCL) in supercritical solvents were extensively studied including the effects of pretreating (drying, grinding and particle size) JCL seed kernels [8, 9], choice of co-solvent [8, 10] and other key factors [11, 12]. An optimized process was developed resulting in a high FAME yield (>92 %) by using an SSR of 5.9 mL/g at 300 °C and an initial pressure of 2.0 MPa using CO_2 for a reaction time of 12.3 min (a heating up time of 80 to 90 min was required) [12]. In most of these studies a high reaction temperature (300 °C) and drying of the kernels (12 h) were required. Moreover high space loadings (30–54 mL/g) were employed [11, 12], implying low utilization of the reactor's effective volume. In our previous works, acetic acid was employed as a co-solvent and as a catalyst [13, 14]. Acetic acid as a catalyst under subcritical condition enhanced extraction rate and allowed the use of feedstock with moisture up to ~10 % which resulted in faster FAME conversion rate [13]. It was also observed that filling the reactor to its maximum allowable capacity resulted in a decrease in FAME yield [13]. Although a lower operating temperature (250 °C) may be employed for a similar reaction duration as those carried out under supercritical methanol conditions, it has disadvantages including: The need to use high SSR of 7 mL/g; the use of high amount of acetic acid (25 % of the solvent mixture); and requiring the addition of carbon dioxide as a co-solvent. In a separate study utilizing refined oils, it was observed that upon maximizing the utilization of the reactors effective volume, the amount of methanol and acetic acid could be decreased while still achieving high conversion [15]. In this study, the use of high space loading and low SSR at a fixed total reaction volume was investigated. The possibility of using a low acetic acid concentration was also explored. Previous researches only considered FAME yield as the criteria for the process assessment, in this study both FAME yield and productivity were taken as response variables. The Taguchi method coupled with grey relational analysis was carried out for improving the process and identifying the main factors contributing to the process response. Sunflower (SF) kernels were first used as a model feedstock for the ISTE process. The improved conditions were then applied and confirmed for the said feedstock. Furthermore, the robustness of the said process was further investigated by applying it to a different feedstock (JCL kernels), moisture containing kernels and kernels with lipid containing high FFA.

Materials and Methods

Materials

SF seeds were purchased from a local supplier in Taiwan while JCL seeds were obtained from Muhammadiyah University (Malang, Indonesia). Seeds obtained were sun dried and de-hulled manually. Kernels obtained were ground to particle sizes between 0.71 to 1.0 mm and kept at −20 °C prior to use. Standards of fatty acid (FA), acylglycerides (AG) such as monoolein, diolein and triolein and fatty acid methyl esters (FAME) were supplied by Supelco (Bellafonte, PA). All solvents and reagents used were either high performance liquid chromatography (HPLC) or analytical reagent grade, obtained from commercial sources.

Sample Preparation and Characterization

Ground kernels (~5 g) were lyophilized to determine their moisture content [13, 14]. To estimate the amount of extractable lipid, a sample (5 g) was extracted using *n*-hexane in a Soxhlet extractor. Unsaponifiable matter and FFA contents in the extracted lipid was analyzed using AOCS official methods (Method 6b-53 and Method Ca 5a-40, respectively). Saponified lipids obtained after the determination of unsaponifiable matter were collected for an FA profile and theoretical maximum FAME yield determinations. Detailed analysis procedures can be found elsewhere [14, 15]. Dewaxing and degumming of the extracted crude lipid was carried out before its AG composition was analyzed using high temperature gas chromatography (HTGC) described elsewhere [16, 17].

***In-situ* Transesterification**

Ground seed kernels (21–42 g) containing ~5 % moisture were loaded into a glass chamber (190 mL) and placed in a high-pressure reactor (290 mL). The ground kernels were mixed and suspended in a mixture of methanol (120–140 mL) and acetic acid (7–35 mL). A detailed reactor description and can be found elsewhere [17]. After the sample was put into the reaction chamber, the reactor was sealed and the chamber was purged with N_2.

The reactor was equipped with external electric heating and the temperature inside the reactor was controlled to within ±2 °C. The reaction was carried out at 200–250 °C for a predetermined time (0–90 min). The heating rate of the reactor was ~5–6 °C/min with a heating period of 40–45 min. The moment the desired reaction temperature was reached in the reactor was taken as the reaction time zero. After the reaction, the reactor was rapidly cooled, pressure inside the reactor was released and the product was collected at room temperature.

The reaction product was vacuum filtered using a Buchner funnel with Advantec No.2 filter paper (8 µm pore size) to separate the solid residue from the reaction product. The retained solid was washed three times (each time with 30 mL methanol) to recover the FAME produced. Methanol and acetic acid in the filtrate were removed and recovered using a rotary evaporator operated at 60 °C and 13.3 kPa for 30 min. The concentrated organic extract was then redissolved in 100 mL *n*-hexane and transferred to a separation funnel. Salt solution (5 % NaCl) was added to aid phase separation. To recover the FAME produced the top FAME-rich hexane layer was transferred to another separation funnel, the organic extract in the first funnel was then re-extracted four times (each time using 25 mL *n*-hexane, with approx. 15 min between extractions). The recovered FAME-rich layers were pooled and washed four times (each using 25 mL, 5 % NaCl solution, approx. 15 min between washings) in the separation funnel to remove non-lipid products co-extracted by methanol. The solution was allowed to clarify in between washings. The upper hexane phase, which contained FAME, was withdrawn. Hexane from the combined extract was evaporated. The recovered product was weighed and analyzed for its FAME, FA and AG contents.

Results of the reaction were evaluated in terms of FAME yield, conversion and productivity. The FAME yield is defined as the mass of FAME (M_{FAME}) produced per mass of extractable oil (M_{Oil}) loaded into the reactor. This was calculated using Eq. 1, where A_{FAME} is the area calculated from the GC analysis and f_c is the external calibration factor (slope of the calibration curve) while C_s and V_s are the concentration and volume of the samples prepared and injected to the GC for analysis, respectively.

$$\text{FAME yield }(\%) = \frac{M_{\mathrm{FAME}}}{M_{\mathrm{Oil}}} \times 100\% = \frac{M_{\mathrm{product}}}{M_{\mathrm{Oil}}} \times \frac{\sum A_{\mathrm{FAME}} \cdot f_c}{C_s V_s} \times 100\,\% \tag{1}$$

Conversion of FAME was calculated using Eq. 2. The theoretical FAME yield was obtained based on the total convertible lipids to FAME from the extracted crude lipid.

$$\text{Conversion }(\%) = \frac{\text{FAME yield}}{\text{Theoretical FAME yield}} \times 100\,\% \tag{2}$$

while Eq. 3 was utilized to calculate productivity, which is defined as the amount of FAME (M_{FAME}) produced per reactor volume (V_r = 240 mL) per reaction time (t in h), including the time to heat up the reactor.

$$\text{Pr oductivity}\left(\frac{\mathrm{kg}}{L \cdot \mathrm{h}}\right) = \frac{M_{\mathrm{FAME}}}{V_r t} \tag{3}$$

All values reported are the averages of two experimental trials.

Gas Chromatography Analysis

A 20-mg aliquot of lipid sample was dissolved in ethyl acetate and filtered through a PTFE hydrophobic filter to remove moisture. External calibration curves were generated using 0.2–20 mg of pure standards dissolved in *n*-heptane or ethyl acetate. Each calibration curve was generated by fitting a straight line with the *y*-intercept passing through zero ($R^2 > 0.99$).

Qualitative and quantitative analyses of FAME and unreacted FA in each sample were performed using a Shimadzu GC2010 instrument (Kyoto, Japan) equipped with a split-injector and a flame ionization detector. Separation was carried out on a ZB-5HT (5 % phenyl)-methylpolysiloxane nonpolar column (15 m × 0.32 mm i.d., 0.1 mm film thickness) (Zebron, Phenomenex, Torrence, CA). Both injector and detector temperatures were set at 370 °C. The temperature program was set at 80 °C, increased to 365 °C at 15 °C/min and held for 8 min. The total analysis time was 29 min. Nitrogen was used as the carrier gas with a linear velocity of 30 cm/s at 80 °C. Data analyses were carried out by the software "GC Solution version 2.3", Shimadzu.

The method described above was adopted from a previously established protocol [16, 17].

Analysis of recovered solvents was carried out using a Finnigan TSQ-700 GC–MS, equipped with a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) and mass spectra detector (MSD). The sample was injected in split mode with a ratio of 20:1 under helium flow at 1.0 cm^3/min. The initial oven temperature was set at 353 K and ramped to 553 K at 8 K/min and held for 2 min. Temperatures of the ion source and interface of the detector were set at 473 and 513 K, respectively. Identification of components in bio-oil was conducted by comparing the mass chromatogram of the sample with standard database from the National Institute of Standards and Technology.

Experimental Design

In order to develop a good design of the experiment, some preliminary trials were carried out to avoid unnecessary experiments. Previous studies on ISTE of JCL in subcritical solvent (methanol + acetic acid) required to use 25 % acetic acid (AA) in the solvent mixture and reacted at 250 °C [13, 14]. In a separate study utilizing refined soybean oil an AA of 6.25 % was sufficient to achieve a high FAME yield (>90 %) and at a lower solvent requirement once a larger reactor space was utilized [15]. Thus in this work, preliminary studies with SF kernels were done by first utilizing acidified methanol solution containing 0, 6.25 and 25 % AA, where reactions were carried out at 250 °C for 0 and 1 h. All experiments were carried out by filling the glass chamber (190 mL) in the reactor to ~87 % of its capacity, which is about ~70 % of the reactor's effective volume (240 mL).

Experiments based on the Taguchi method were carried out and analyzed statistically using analysis of variance (ANOVA). In the Taguchi method it utilizes the signal-to-noise ratio (S/N) to evaluate the response of experimental trials [18, 19]. In this study, high FAME yield and productivity were preferred. The S/N ratio with the higher the better function is defined as [14, 18, 19]:

$$\frac{S}{N} = -10 \times \log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (4)$$

where y_i is the ith quality parameter (yield) and n is the number of trials.

A factorial design of 3^4 was carried out using the Taguchi L_9 orthogonal array and the levels of variables are based on the preliminary experiment. Table S1 in the electronic supplementary is a summary of the operating conditions and parameters studied together with its corresponding responses. The individual experiments were carried out at random.

In the Taguchi method the average S/N ratios for each parameter and level are plotted in a response graph. From the response graph, the level resulting in the highest response for each factor investigated is taken as the factor level providing an improvement to the response investigated. With the level for each factor identified, it then assumes that the contribution of each factor to the overall response is additive.

$$y_{\text{pred}} = y_{\text{m}} + \sum_{i=1}^{q}\bar{y}_i - y_{\text{m}} \quad (5)$$

where y_{pred} is the predicted response of the combination of the identified factors to provide an improved response of the process, q is the number of significant processing parameters, y_{m} is the overall mean of all responses and $\bar{y}_i$ is the mean response value of the ith level of the significant processing parameter.

The predicted responses (yield and productivity of FAME) are confirmed by running a confirmatory test employing the optimum factor levels as analyzed. A confidence interval can be calculated using the equation:

$$\text{CI} = \sqrt{F_{\alpha;1,\nu 2} \times V_{\text{e}} \times \left(\frac{1}{n_{\text{eff}}} + \frac{1}{r}\right)} \quad (6)$$

where $F_{\alpha:1,\nu 2}$ is the F ratio of significant level α, α is the significant level, $1-\alpha$ is the confidence level, V_2 is the degree-of-freedom of pooled error variance, V_{e} is the pooled error variance and r is the number of repeated trials. On the other hand n_{eff} is the number of effective measured results defined as:

$$n_{\text{eff}} = \frac{\text{total number of experiments}}{\text{sum of degrees of freedom used in estimating the response} + 1} \quad (7)$$

The Taguchi method was first successfully applied to improve *in situ* FAME production from JCL under subcritical solvent conditions [14] through improving a single quantity characteristic (FAME yield). In most processes more than one process characteristics (FAME yield and productivity) are involved. Generally a pool of data from various processing and operating conditions are collected and analyzed to understand the relationship between quality characteristics, processing parameters and target values, requiring complicated statistical analysis and sophisticated modeling tools for regression.

Grey relational analysis has been found to efficiently transform multiple quality responses into a single grade by measuring the correlation degree between factors based on similarity and/or differences between them [19]. A detailed discussion of the method can be found elsewhere [19]. In brief, similar to the Taguchi method, it converts the quantity characteristics (FAME yield productivity) measured into grey relational generation (GRG). Since both high yield and productivity are the preferred responses the higher-the-better principle is again adopted and the quality characteristics are first normalized through:

$$x_i^*(k) = \frac{x_i(k) - \min x_i(k)}{\max x_i(k) - \min x_i(k)} \tag{8}$$

where x_i can either be the actual characteristic response or the S/N ratio from the Taguchi method. The normalized characteristics are further converted into its difference sequence ($\Delta_{0,i}$ (k)) to calculate the grey relational coefficient:

$$\Delta_{0,i}(k) = |x_0(k) - x_i(k)| \tag{9}$$

Grey relational coefficient (γ) was determined by:

$$\gamma_{0,i}(k) = \gamma\left(x_i(k), x_j(k)\right) = \frac{\Delta_{\min} + \zeta\,\Delta_{\max}}{\Delta_{0,i}(k) + \zeta\,\Delta_{\max}} \tag{10}$$

where the identification coefficient (ζ), ranging from zero to one, in general is set to a value of 0.5 and the average value of the grey relational coefficient is the GRG. The GRG is then sorted in a descending order as the evaluation criterion. A summary of the calculated results is provided in Table S2.

Results and Discussion

Table 1 summarizes the basic characteristics of sunflower and JCL seed kernels used in this study. Kernels utilized in the following experiments were not dried after grinding and both kernels have a moisture content of ~5 %. The average extractable lipids from both kernels are within the range reported in the literature [1–7]. Although JCL kernels contain higher amounts of extractable lipid than SF kernels, their theoretical maximum yields are very similar, due to the higher wax/gum and lower AG contents in JCL kernels.

Preliminary Evaluation of Process Parameters

Product yields at various solvent to solid ratios (SSR) and AA concentrations are presented in Fig. 1. It is evident that with increasing SSR and AA concentrations, extraction yield increases in all cases. This clearly shows that AA acted as a co-solvent and an acid catalyst.

Without AA, FAME decreased with increasing SSR, which is probably due to dilution by methanol. In ISTE extraction and transesterification occur simultaneously. During extraction, a large amount of solvent or higher SSR results in faster extraction due to an increased solvation capacity and the increased lipid concentration gradient between solid matrix and solvent. In transesterification, the reaction rate depends on the concentration of lipids and methanol. Since excess methanol was used in this study, methanol concentration may be assumed relatively unchanged, leaving lipid concentration as the main driving force for the reaction. The presence of more methanol would result in dilution of the reaction system, specifically in decreasing lipid concentration. Thus the addition of more methanol improved the extraction of other lipid components but at the same time resulted in a more dilute system and thus a slower reaction rate.

In a previous study with JCL kernels [13], the dilution effect was observed at an SSR above 10 mL/g, which is significantly higher than that used in this study. The main difference lies in the reactor volume utilized. Only ~15 % of the effective reactor volume was initially occupied in the previous study [13], providing a relatively large void for expansion. In this study, ~87 % of the reactor volume was utilized. Although nitrogen gas was previously used to pressurize the system, a significant portion of methanol was still in the vapor phase. By reducing the reactors void space, the space for gaseous methanol was reduced, which in turn resulted in a pressurized system without the need of

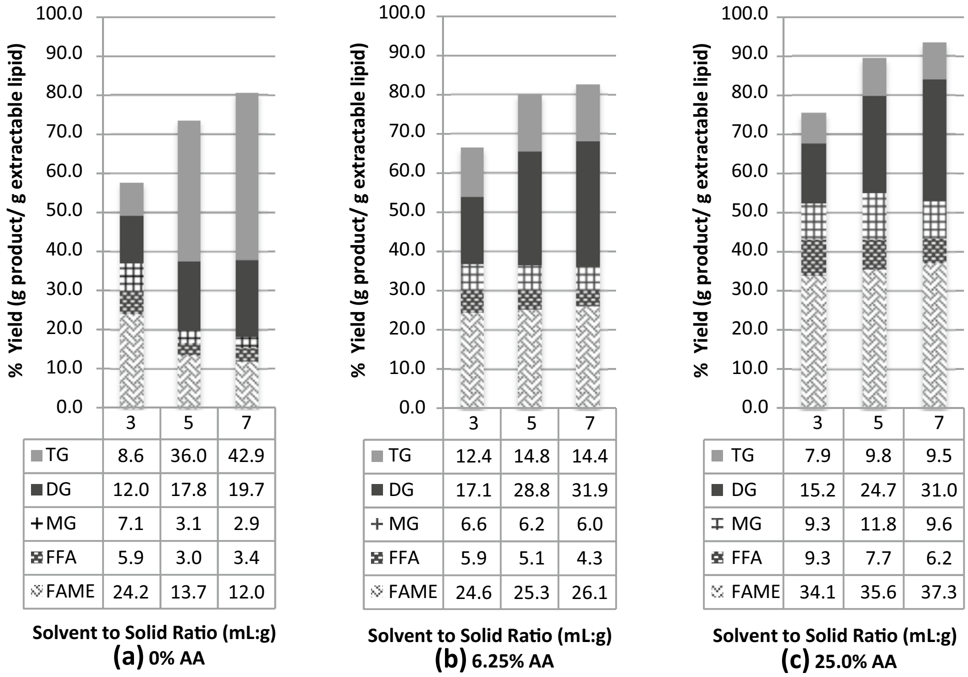
Table 1 Characteristics of seed kernels used in this study

Components	Sunflower		*Jatropha*[a]	
	Lipid	Kernel[b]	Lipid	Kernel[b]
Moisture (%)[c]	–	5.38 ± 0.03	–	4.53 ± 0.08
Lipid content	–	45.22 ± .0.44	–	54.42 ± .0.86
FFA (%)	2.98 ± 0.11	1.35	2.16 ± 0.21	1.16
Wax and gum (%)	9.86 ± 0.52	4.45	13.73 ± 1.07	7.43
Unsaponifiables (%)	1.49 ± 0.15	0.67	1.18 ± 0.06	0.64
AG (%)	~85.67	~38.74	~82.93	~44.84
Hydrolyzable (%)	87.68 ± 0.42	49.28	87.38 ± 1.61	47.25
Theoretical FAME yield (%)	~92.06	~41.63	~91.74	~49.61

[a] Ref [14]

[b] Based on dry weight of the kernel

[c] Wet basis



(a) 0% AA	3	5	7
TG	8.6	36.0	42.9
DG	12.0	17.8	19.7
MG	7.1	3.1	2.9
FFA	5.9	3.0	3.4
FAME	24.2	13.7	12.0

(b) 6.25% AA	3	5	7
TG	12.4	14.8	14.4
DG	17.1	28.8	31.9
MG	6.6	6.2	6.0
FFA	5.9	5.1	4.3
FAME	24.6	25.3	26.1

(c) 25.0% AA	3	5	7
TG	7.9	9.8	9.5
DG	15.2	24.7	31.0
MG	9.3	11.8	9.6
FFA	9.3	7.7	6.2
FAME	34.1	35.6	37.3

Fig. 1 Product yields and extraction efficiencies for *in situ* transesterification of sunflower kernels at different SSR and AA concentrations. Reaction mixtures were heated to 250 °C (0 h) and rapidly cooled down once this temperature was reached

adding pressurizing gas and this also kept most methanol initially fed into the reactor in the liquid phase. The same phenomenon was observed in the (*trans*)esterification of refined soybean oil, which was carried out in a subcritical solvent mixture of methanol and acetic acid at a high reactor loading [15].

Since acetic acid also acted as an acid catalyst, its presence improved FAME yield at a fixed SSR. Without adding AA, FAME yield decreased with increasing SSR due to dilution effect of methanol as describe previously. In the presence of AA, FAME yield remained almost the same with increasing SSR. The dilution effect of methanol at higher SSR still occurred, but, when AA was added, FAME yield did not change with increasing SSR possibly due to the catalytic effect of AA, which kept the rate of FAME formation almost constant.

With initial experiments carried out by heating the reactor to 250 °C (0 h reaction time), it is clear that the addition of AA and more methanol (higher SSR) could substantially improve the extraction yield but the yields of FAME are unsatisfactory even after an approximately fourfold increase in the AA added.

Upon reaching 250 °C, the reaction was allowed to continue for 1 h and the results of product yield are summarized in Table S3. At the end of 1 h, lipids in the SF kernels were completely extracted. The addition of 6.25 % AA in the solvent mixture can achieve comparable FAME yield to that of using pure methanol but at a lower SSR (3 *vs* 7 mL/g). Reactions carried out in the presence of AA resulted in higher FFA in the final product, which suggests that TG was converted first to FFA and subsequently esterified, in agreement with the previous findings of Go *et al.* [15]. At the same acetic acid concentration (6.25 %) but at a higher SSR of 7 mL/g, FFA was reduced significantly which resulted in a FAME yield of 92.75 %.

From the preliminary experimental runs it was found that both SSR and addition of AA in the solvent improved FAME yield. Addition of AA at 25 % v/v of the solvent used resulted in better extraction efficiency, however at 250 °C prolonged reaction for an hour using 6.25 % AA not only resulted in high extraction efficiency but also high FAME yield. In a practical perspective addition of less amount of AA is preferable. Acetic acid is more expensive and would require more energy to recover after the reaction when compared to methanol. Temperature upper limit was set at 250 °C in order to keep the reaction system within subcritical condition (details are discussed in "Process Evaluation"). With information from the preliminary experiments it was decided that improvements to the reaction response would be carried out by varying SSR, % AA, time and temperature.

The most common measure or criteria of the performance of an ISTE process is the FAME yield and/or conversion. Another important parameter in chemical processes is process productivity, which is often overlooked. Since ISTE reactions are carried out in the same reactor with fixed volume, a decrease in SSR also implies that more oil bearing seed kernels are being processed, leading to more FAME being produced. In the following experimental design study both yield and productivity were taken into consideration in improving the ISTE process for producing biodiesel.

Yield and Productivity of FAME

FAME yield and productivity obtained by ISTE of SF kernels are summarized in Table S1. Based on S/N ratios obtained from experiments, the effects of each factor can be calculated and its average response graph is presented in Fig. 2. In the Taguchi design of experiments, the main effect is determined by calculating the difference between the maximum and minimum of the average S/N ratio of each factor.

In the case of FAME yield, the main contributing factor was found to be the reaction time (C), while results for FAME productivity showed that temperature (D) was the main contributing factor. The factor response graph (Fig. 2) reveals that improved responses were achieved at factor levels A3, B3, C3 and D3 for yield and A3, B1, C3 and D3 for productivity.

To provide a more objective judgment on the effects of the factors investigated, ANOVA was employed to the raw responses (yield and productivity). From the experiments carried out in this study an F ratio greater than 4.3 implies a significant contribution of the factor to the response. From Table 2, we can see that for FAME yield all factors were found to have significant contribution except for acetic acid (A), while time (C) has the highest contribution. For productivity, all factors also contributed significantly with temperature (D) having the greatest contribution.

Fig. 2 Response graph of higher-the-better signal-to-noise ratios of FAME yield (**a**) and productivity (**b**) from ISTE of SF kernels

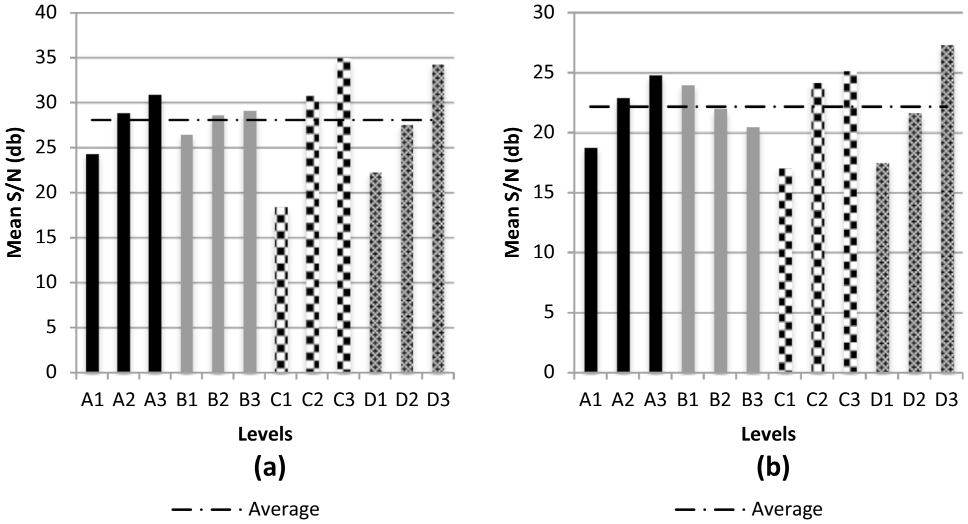


Table 2 Summary of ANOVA analysis on FAME yield and productivity for *in situ* (*trans*) esterification of SF kernels

Factor	DOF	SS	SSE	F_0	*p* value	Contribution (%)	Rank
FAME yield							
A (acetic acid)	2	35.10	31.19	3.76	0.06501987	0.58	4
B (SSR)	2	168.29	164.39	19.81	0.00050515	3.04	3
C (time)	2	3283.80	3279.90	395.33	0.00000000	60.74	1
D (temperature)	2	1895.29	1891.39	227.97	0.00000002	35.03	2
Error	9	17.569	37.335			0.69	
Total	17	5400.05				100.08	
Pooled error	11	185.86					
FAME productivity							
A (acetic acid)	2	269.21	264.32	28.6	0.00012596	12.23	4
B (SSR)	2	555.06	550.17	59.59	0.00000644	25.46	2
C (time)	2	473.84	468.95	50.79	0.00001252	21.70	3
D (temperature)	2	840.52	835.63	90.50	0.00000110	38.68	1
Error	9	22.00	41.55	1.00		1.92	
Total	17	2160.62				100.00	
Pooled error	9	22.00					

F_0: F ratio, p: p value ($F_{crit} = 4.3$ at $p = 0.05$)

DOF degrees of freedom, *SS* sum of squares, *SSE* sum of square error

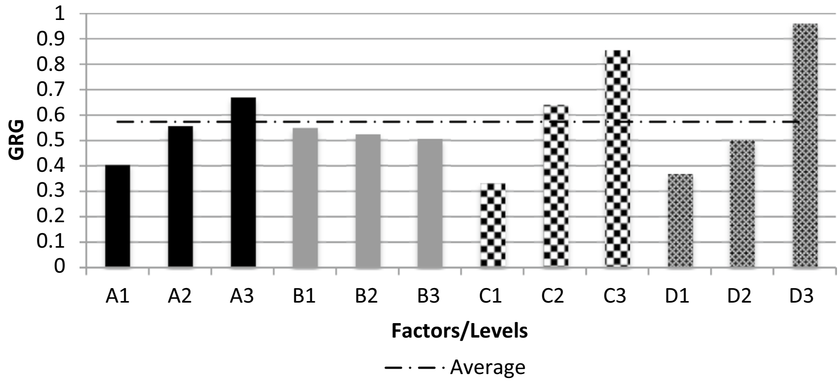


Fig. 3 Response graph of higher-the-better GRG of FAME yield and productivity from ISTE of SF kernels

As can be seen from the preliminary investigation AA acted both as a co-solvent and a catalyst. By adding AA, SSR and time required for extraction were reduced through improved solubility [13, 15]; and the reaction rate was increased by the catalytic effect of AA. Thus AA played a more significant role in FAME productivity than in FAME yield. On the other hand, other factors also contributed significantly to both yield and productivity with time and temperature as the main contributing factors.

Taguchi analysis on yield and productivity resulted in two different sets of improved process conditions, with yield having a preference in higher SSR while productivity in lower SSR. To resolve this, grey relational analysis was employed to obtain a single grade response from two different responses simultaneously. From the response graph in Fig. 3, the favored response levels for each factor considering both yield and productivity are A3, B1, C3 and D3.

By employing the improved conditions (250 °C, 8.8 MPa, 3 mL/g SSR, 10 % v/v acetic acid), high FAME yield and productivity can be obtained. The required reaction time was about an hour at 250 °C. It took 45 min to heat the reaction mixture from room temperature to 250 °C, thus an overall reaction time of 1.75 h was required.

Using Eqs. (5) and (6) the predicted yield and productivity based on the identified factor levels (A3, B1, C3, D3) was found to be 88.69 ± 2.20 % for yield (96.34 % conversion) and 40.59 ± 3.24 kg/m^3/h for productivity. Conformational runs carried out in triplicate resulted in an average FAME yield of 87.09 ± 0.70 % (S/N = 38.79) and a productivity of 37.46 ± 0.42 kg/m^3/h (S/N = 31.47). These tests show that improved conditions can be well predicted by a simple addition model and the results falls within 95 % CI, showing good reliability of the model while measured responses by the conformational runs ($n = 3$) show repeatability of the method.

Effects of Water

After sun drying, seed kernels retain some moisture. Haas [4, 5, 20] reported that the removal of moisture to <1 % substantially improved the efficiency of alkaline catalyzed ISTE biodiesel production. ISTE of SF oil using kernels with 5.5–6.2 % moisture content [1, 2, 21] for acid catalyzed reactions and 4.6–5.6 % for base catalyzed reactions [6, 22] required 1 h drying at 85 °C prior to use. A process capable of tolerating high moisture and/or water content in the reaction mixture is important for two main reasons. Firstly, energy required for drying can be saved. Secondly methanol and AA are very hygroscopic, if the presence of water cannot be tolerated it would require to have a specially designed process for recovery and storage of solvents.

Figure 4 shows the effect of moisture on the ISTE process carried out under the previously improved conditions. It can be seen that insignificant decrease in yield and productivity was observed even up to a moisture content of ~25 %. Kernel from oil seed typically contains moisture less than 6 %, but other potential feedstock for biodiesel like rice bran and biomass of microbial origins mostly contain moisture over 10 %. With this process the drying step can be eliminated or the extent of drying feedstock can be greatly reduced.

Applicability to JCL Kernels

One of the most studied oil seeds is the JCL kernel. A detailed summary of ISTE reactions carried out with JCL kernels is mentioned elsewhere [13]. The use of JCL kernels as feedstock is favored due to their high lipid content. The JCL kernels used in this study contained ~54 % extractable lipid and had a potential of producing ~0.5 g FAME per gram of dry kernels. Table S4 summarizes the results of ISTE reactions of JCL kernels using improved reaction conditions adopted from the ISTE of SF kernels.

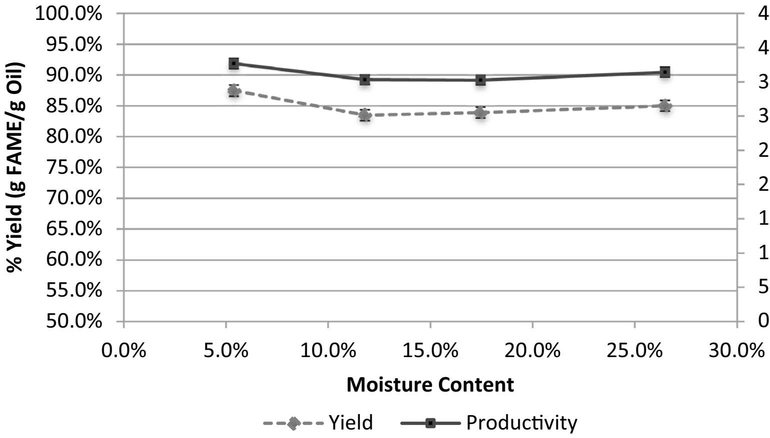


Fig. 4 Effect of moisture content on yield and productivity of FAME (results are average values from duplicate runs)

Extraction efficiency (99–109 %) and FAME yield (88–97 %) based on extractable lipids obtained in this study are comparable to those reported in literature, while a lower space loading of 3 mL/g and a higher fraction of reactor volume (~70 %) were utilized leading to higher overall productivity in this study. Compared to SF kernels, JCL kernels gave higher yield despite more oils being required to be extracted and the same amounts of solvents were used. Considering the stoichiometry of reactions involved the amount of methanol used was in great excess which resulted in a high yield. In ISTE the presence of solid will affect the reaction. Since SF and JCL kernels were ground to the same particle size, the composition and properties of the solid matrix involved will have an effect on FAME yield. A difference between the two kinds of kernel is that the lignin content of SF kernels is ~6–12 % (lipid free basis) [23] and JCL kernels contain only ~0.3–0.5 % lignin [24]. Kernels with a lower lignin content require a shorter time to breakdown their solid matrix, which leads to easier penetration of solvent and faster extraction.

Maximal utilization of reactor volume allowed a subsequent decrease in acetic acid required for the reaction. The need to use carbon dioxide as a co-solvent was also eliminated thus lowering the reaction pressure. The severity of this process being lower than that of the supercritical ISTE [11, 12] may lead to lower costs in terms of energy consumption and process design. Moreover, ISTE was also conducted with JCL kernels which were left at room temperature for 9 months (FFA content ~25 %); high FAME yield (89.72 %) can still be achieved. In addition to being flexible to feedstock source, this process also can tolerant feedstock with a high FFA content.

The ISTE process developed in this study does not require stirring. From previous studies it was found that stirring significantly improved FAME yield [13], shortened reaction time [13, 14] and further lowered down SSR required [13, 15]. The effect of stirring was not investigated in this study. Because low space loading and low SSR were used which resulted in high solid loading in the reactor, stirring with a magnetic stir bar was not possible.

Process Intensification

As discussed in the previous section, the interaction between solid material and reacting solvent plays a role in the ISTE process. If whole kernels can be used in ISTE reaction, the size reduction step could be eliminated. It was reported that subcritical water-pretreated whole JCL kernels could be utilized directly without further size reduction [14]. Since the ISTE process developed in this study is relatively water tolerant, the possibility of directly utilizing whole SF kernels was also investigated and results are summarized in Table S5.

Runs 1 and 2 (R1, R2) show that at a fixed SSR of 3 mL/g, an increase in AA concentration from 10 to 25 % did not result in higher FAME yield due to a decrease in methanol content in the reaction system. Compared to R3 and R6, an increase in AA concentration resulted in an increase in FAME yield, this is due to the difference in the SSR (7 mL/g) employed.

Even at 7 mL/g SSR and 25 % AA, FAME yields (R6 to R9) were still lower than the maximum FAME yield (~88–92 %) achieved with ground kernels. Addition of CO_2 (R4), and prolonged reaction time (1.5 h, R5, R9) had very little effect on FAME yield despite an additional 0.5 h of reaction time. Without extra water addition (R8) resulted in the lowest FAME yield at 7 mL/g SSR and 25 % AA. Adding 1.0 mL/g kernel water (R7) also resulted in a lower yield than R6. At a water to kernel ratio of 0.3 mL/g kernel (R6), a reasonably high FAME yield (77.68 %) can be achieved in 1 h when whole kernels were utilized.

Apart from lower FAME yield, extraction efficiencies were also lower. The dried solid residue after the reaction was re-extracted with hexane in a Soxhlet extractor for 4 h. Results of ISTE and Soxhlet extraction of the residual solids are summarized in Table S6. Compared to ground kernels a significant amount of lipid components was left in the solid residue. Surprisingly, extract from the solid residue contained a significant amount of FAME, resulting in an overall FAME yield of over 85 %. This results suggest that the reaction not only take place with the extracted oil but also with oil trapped in solid matrix. This phenomenon was also observed in alkaline catalyzed ISTE of rapeseed by Zakaria and Harvey [25], which they supported with stained microscopic images of the solids.

Although lower FAME yield was obtained by using whole SF kernels, the solid residues were easier to separate after the reaction. The use of higher SSR resulted in a similar overall FAME yield to that of ground kernels. Despite the lower productivity due to higher SSR required, the direct use of whole kernel eliminated the size reduction step. Although further optimization on the washing step to fully recover all products from spent kernels and detailed economic analysis on the process are required, this approach offers certain advantages which might be compensated for the lower productivity in the reaction step.

Process Evaluation

From the Constantinou and Gani method of group contributions [26] and the pseudo-triglyceride model proposed by Espinosa *et al.* [27], critical temperature (T_c) and critical pressure (P_c) of SF were found to be 698.3 °C and 0.34 Mpa, respectively; and for JCL T_c and P_c were found to be 704.1 °C and 0.36 MPa, respectively. For simplicity it is assumed that the system is mainly composed of oil, methanol ($T_c = 240$ °C, $P_c = 8.1$ MPa) and AA ($T_c = 319$ °C, $P_c = 5.8$ MPa). Figure 5 shows the pseudo T_c and P_c of methanol–oil mixture obtained by Lorentz–Berthelot type mixing rules [10, 28]. It indicates that with increasing solvent content, T_c decreases while P_c increases. The addition of AA on the other hand resulted in an increase in T_c and a decrease in P_c. The temperature employed in this study (250 °C) is below the predicted T_c (265–272 °C). The operating pressure of 8.5 to 11.0 MPa are much higher than the predicted P_c. If water ($T_c = 373.9$ °C $P_c = 22.1$ MPa) and solid are taken into consideration, the mixtures pseudo T_c should be much higher and thus it is safe to assume that the system is under subcritical conditions.

The use of AA as a catalyst and a co-solvent in this process offers certain advantages. Less severe operating temperature and pressure are required to achieve high FAME yield than that required for reactions carried out under supercritical conditions. Unlike conventional acid and base catalyst (Table S7), AA maybe recovered easily by distillation or evaporation under vacuum since its boiling point (~118 °C) is lower than that of mineral acids such as sulfuric acid.

Compared to other processes, the subcritical ISTE developed in this study requires less solvent but still achieves comparable conversion without using strong alkali or acid. Although it requires much higher temperature and longer reaction time than those of alkaline catalyzed reactions, the process can tolerate high FFA and water contents, which are common characteristics of potential non-edible feedstock for biodiesel production. For conventional acid catalyzed process, high yield can also be achieved with kernels containing ~5 % moisture in 1 h reaction time and at a relatively low SSR; however it requires the use of large amount of strong acid (100 % H_2SO_4 based on oil) [21]. The use of co-solvent allows the reduction in SSR [22], but requires higher amount of catalyst and the cost of co-solvent like DEM may offset the advantage of reducing the amount of methanol. While stirring was not required in this study, most other studies on ISTE required stirring or sonication [6] to promote contact between reacting materials.

GC–MS analysis of the recovered methanol revealed the presence of methyl acetate, which was the product of esterification of AA and methanol. This leads to the possibility of simultaneously producing methyl acetate, which is easier to recover than AA due to its lower boiling point (56.9 °C).

The use of refined SF oil to verify the catalytic effect of AA was carried out by adding 6.25 % AA or methyl acetate to the solvent mixture and at a solvent to oil ratio of 1 mL/g based on previous study [15]. Reactions were then carried out at 250 °C for 1 h. The use of AA resulted in a FAME yield of 88.5 % while using methyl acetate only resulted in 44.4 %. Methyl acetate may not have the same catalytic effect as AA, but it

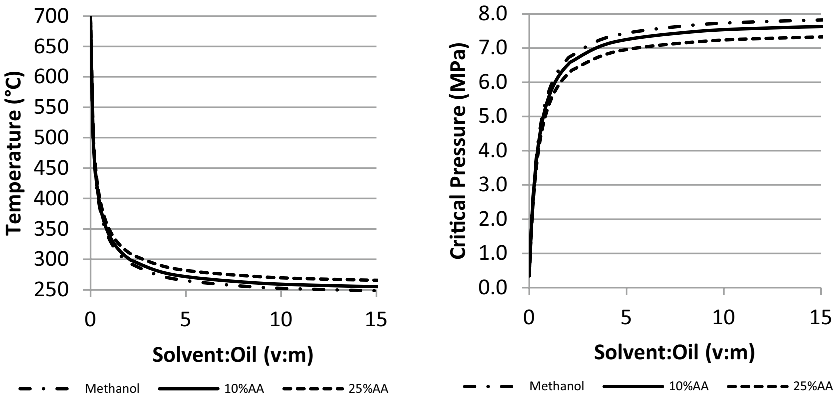


Fig. 5 Pseudo-critical temperature and pressure of methanol–oil mixture estimated by Lorentz–Berthelot type mixing rules [10, 28]

may have acted as a co-solvent since reactions between refined SF oil and methanol only resulted in yield less than 34 %.

Conclusion

In this study, an improvement in FAME productivity (37.46 kg/m^3/h) and FAME yield (87.47 %) in the ISTE of SF oil in subcritical solvent was successfully carried out by using the Taguchi method and grey relational analysis. The improved process allowed the use of low SSR (3–7 mL/g) and space loading as low as 6 mL reactor space per gram of solid. CO_2 as a co-solvent was not required. Maximizing utilization of reactor volume allowed the use of a lower amount of AA. The developed method can tolerate the presence of water (~25 %) and FFA (~25 %). Its applicability to other oil-bearing seeds like *J. curcas* L. was tested and resulted in a high FAME yield (88–97 %). Direct utilization of whole seed kernels was also looked into and preliminary results seem encouraging.

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