Statistical Optimisation using Taguchi Method for Transesterification of Reutealis Trisperma Oil to Biodiesel on CaO-ZnO Catalysts

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Abstract
Optimisation of biodiesel production from non-edible Reutealis Trisperma oil (RTO) was investigated using Taguchi method. Biodiesel was produced via consecutive esterification and transesterification reactions. Esterification of RTO was carried out using acid catalyst to decrease the amount of free fatty acid from 2.24% to 0.09%. Subsequent transesterification of the treated oil with methanol over a series of CaO-ZnO catalysts was optimized based on the L₉ Taguchi orthogonal approach. The optimization parameters are Ca/Zn ratio (0.25, 0.5, and 1), methanol/oil ratio (10, 20, and 30) and reaction time (0.5, 1, and 2 h). The CaO-ZnO catalysts at variation of Ca/Zn ratios were prepared using co-precipitation method and characterized using XRD, SEM, TEM, and FTIR analysis. The amount of methyl ester yield was used as the response parameter in the S/N ratio analysis and Analysis of Variance (ANOVA). The optimum parameter for RTO transesterification to biodiesel was determined at Ca/Zn ratio of 1, methanol oil ratio of 30 and reaction time for 2 h. Transesterification under these optimized parameter generated 98% of biodiesel yield, inferring the validity of the statistical approach. Furthermore, ANOVA analysis also confirmed that all the parameters were significantly contributed at approximately equal percentage towards the amount of biodiesel.

Keywords: Biodiesel; Reutealis trisperma oil; Taguchi; optimization; Transesterification

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1. Introduction
Environmental pollution caused by the excessive use of fossil fuel as energy sources has urged the development of green alternative fuels. Biodiesel has gained enormous interest as green fuel due to its high cetane number, renewable and biodegradable properties, low toxicity, low emission of sulphur oxides (SOₓ), carbon monoxide (CO) and particulate matter during
treating the data [22]. The reaction parameters studied number of experiments without compromising experimental design following Taguchi method is possible to study simultaneously. Experimental result based on the theoretical calculation designed by Taguchi Design of Experiment [13,23,24].

2. Materials and Methods

2.1 Materials

Reutealis trisperma oil was supplied from PT Agrindo, Gresik, Indonesia. Zinc acetate dihydrate (≥99.8%) and calcium acetate hydrate (≥99%) were obtained from Merck, Germany. Ethylene glycol (99.8%) and dichloromethane (≥99.8) were purchased from Sigma Aldrich, Germany. All materials used in this work were analytical grade.

2.2 Catalyst Preparation

CaO-ZnO catalysts were prepared via co-precipitation of a mixed zinc acetate and calcium acetate solution with oxalic acid was used as precipitant agent. The precursors were dissolved in ethylene glycol separately to obtain 0.1 M solution. Calcium acetate solution was mixed with zinc acetate solution at different volume to obtain Ca/Zn molar ratios at 0.25, 0.5 and 1. An aqueous solution of oxalic acid 0.15 M was added gradually to the solution and the mixture was stirred for 12 h at room temperature. The resulting precipitates were centrifuged and washed with acetone for three times. The catalyst was dried at 120 °C for 12 h and calcined at 800 °C for 6 h. The final catalyst was labelled as CaO-ZnO X, where X means the Ca/Zn molar ratio.

2.3 Catalyst Characterization

Crystallinity and phase identification were determined using powder X-ray diffraction (XRD) using X-ray Diffractometer (Phillips X-pert). The diffractograms were collected at 40 kV and 40 mA using filtered CuKα radiation (λ = 1.5405 Å), and in steps of 0.02 over the range of 2θ=20<θ=70. Morphological study of the catalyst was performed by a Zeiss Evo MA10.
Scanning Electron Microscope (SEM) equipped with EDX. The functional group of catalysts were characterized using FTIR (a Shimadzu Instrument Spectrum One 8400S), recorded in the wavelength range of 400–3600 cm⁻¹ with KBr disk.

2.4 Transesterification reaction

Transesterification reaction was carried out using a three neck round bottom flask with condenser that was submerged into oil bath to control the temperature at 60 °C. *Reutealis trisperma* used for transesterification was characterised to obtain the acid value via titration using NaOH. The free fatty acids (FFA) content of *Reutealis trisperma* oil was determined at 2.44%. Ideally for biodiesel production, the feedstock should have less than 0.5% of Free Fatty Acid (FFA) [13,25] in order to reduce saponification reaction [1]. Esterification of bio-oil using acid catalyst was employed to reduce the acid value by conversion of FFA to Fatty acid methyl ester. 100 mL of *Reutealis trisperma* oil was heated at 65 °C and a mixture of 50 ml methanol and 0.5 ml H₂SO₄ was added to the preheated oil under stirring at 900 rpm for 1 hour. The mixture was transferred into separating funnel to separate the polar layer comprising methanol, catalyst, and impurities from the fatty acid methyl ester.

The transesterification reactions were performed under different condition following the matrix of parameter developed by the Taguchi design of experiment (Table 2). Transesterification reaction was carried out using 3 %wt of catalysts suspended in methanol and heated up to 60 °C. The esterified *Reutealis trisperma* oil was added into the flask under constant stirring of 1200 rpm. Hydrochloric acid was added into the reaction mixtures to stop the reaction. Dichloromethane was added to extract methyl ester and the mixture was settled in the separating funnel to form two separate layers. After excess methanol and other impurities were removed, the nonpolar layer was then loaded to a rotary evaporator to remove dichloromethane. Product of transesterification reactions were analysed using a GC Techcomp 7900 Gas Chromatography equipped with FID detector and capillary column (ECTM-5, 30 m x 0.25 mm x 0.25 µm).Injector and detector temperature were 250 and 275 °C, respectively. The oven temperature was increased from 50 to 250 °C at 4 °C/min and held for 1 min.

The yield of biodiesel was determined based on the methyl ester content determined from GC analysis. The yield was calculated by following equation:

\[
Yield = \frac{W_{ME}}{W_o} \times 100\%
\]

where \(W_{ME}\) is the mass of methyl ester produced, and \(W_o\) is mass of oil.

2.5 Taguchi Design of Experiment

Taguchi method applied orthogonal array to design the experiment parameters in which the matrix comprises of parameter and its level. This orthogonal array applied a fraction of the combination of variables considered, allowing the reduction of the experimental number. In this study, three reaction parameters were evaluated: Ca/Zn ratio of CaO-ZnO catalyst, the reaction time, and the methanol/oil ratio. Each parameter has three levels as shown in Table 1, which were then employed to the orthogonal L₉ (Table 2). The matrix in Table 2

| Parameter             | Level 1 | Level 2 | Level 3 |
|-----------------------|---------|---------|---------|
| Ca/Zn ratio           | 0.25    | 0.5     | 1       |
| Methanol/oil ratio    | 10      | 20      | 30      |
| Reaction time (h)     | 0.5     | 1       | 2       |

| Experiment | Ca/Zn Ratio | Methanol/oil | Reaction time (h) | Yield (%) |
|------------|-------------|--------------|-------------------|-----------|
| 1          | 0.25        | 10           | 0.5               | 48.2      |
| 2          | 0.25        | 20           | 1                 | 57.6      |
| 3          | 0.25        | 30           | 2                 | 92.9      |
| 4          | 0.5         | 10           | 1                 | 75.6      |
| 5          | 0.5         | 20           | 2                 | 59.3      |
| 6          | 0.5         | 30           | 0.5               | 67.5      |
| 7          | 1           | 10           | 2                 | 92.3      |
| 8          | 1           | 20           | 0.5               | 69.6      |
| 9          | 1           | 30           | 1                 | 94.7      |
was adapted as reaction condition for trans-esterification of RTO and methyl ester conversion was considered as the response.

Taguchi design of experiment has been used for process optimisation of manufactured goods, especially engineering and biotechnology. Taguchi suggested calculating the deviation of experimental result to the desired value. This deviation was then converted into a signal to noise ratio (S/N ratio). The S/N ratio is the log function of the expected outcome that is used for the objective of the optimisation of the desired response. There are three types of S/N ratio in Taguchi method, nominal is the best, smaller the better and larger the better [26]. In this study, the larger the better the S/N ratio was applied which was calculated by the following formula:

\[ S/N = -10 \log \left( \frac{1}{10} \sum_{i=1}^{n} \frac{1}{y_i^2} \right) \]  

(2)

where \( i \) is response variable and \( y_i \) is number of experiments.

The value of S/N ratio represents the significance of each level of parameter on the biodiesel yield which optimal level is the level with highest S/N ratio. Subsequently, a new set of optimised reaction condition can be obtained from each parameter that can be applied in a validation reaction to produce the best yield. However, S/N ratio is unable to calculate contribution of each parameter and to determine the most influencing parameter. This limitation can be addressed through the application of statistical analysis of variance (ANOVA) [13,23,24]. The percentage of contribution was calculated by the following equation:

\[ \text{Percentage of contribution} = \frac{SS_p}{SS_T} \times 100\% \]  

(3)

where \( SS_p \) is the sum of squares of each parameter and \( SS_T \) is sum square of parameters as determined according to the equation:

\[ SS_p = \sum_{j=1}^{R} \left( \frac{S/N}{y_j^2} \right)^2 \]  

(4)

\[ SS_T = \sum_{i=1}^{M} \left( \frac{S/N}{y_i} \right)^2 \]  

(5)

where \( R \) is the number of repetition of each level and \( M \) is the number of experiment. In this study, the analysis of S/N ratio and ANOVA were performed in Minitab software.

3. Results and Discussion

3.1 Characterization of Catalyst

FTIR spectra of CaO-ZnO with various Ca/Zn ratios in Figure 1 showed the adsorption band at 490 cm\(^{-1}\) indicated the vibration of CaO and ZnO. The carbonate was confirmed by the formation of intense absorption bands at 875 cm\(^{-1}\) and 1400 cm\(^{-1}\), which were corresponded to bending and asymmetric stretching vibrational of O–C–O bonds, respectively [21], [27]. The band intensity were significantly increased at high CaO/ZnO ratios. The vibration band of hydroxyl group was observed at 3600 cm\(^{-1}\) indicating the presence of Ca(OH)\(_2\) on the catalyst surface [19,20,28]. The intensity of Ca(OH)\(_2\) band was increased with increasing the CaO/ZnO ratios from 0.25 to 1. The presence of physically adsorbed water on the catalyst was confirmed by a wide absorption band at 3400 cm\(^{-1}\) and the OH bending at 1600 cm\(^{-1}\) [29,30]. The absence of CaCO\(_3\) peaks in the catalysts at Ca/Zn ratios of 0.24 implied that the calcination process successfully eliminated the

![Figure 1. FTIR spectra of CaO-ZnO catalysts.](image1)

![Figure 2. XRD patterns of CaO-ZnO catalysts.](image2)
carbonate to form CaO. The presence of ZnO was suggested to reduce the calcination temperature for decomposition of CaCO$_3$ to CaO. The removal of carbonate resulting in the formation metal oxides with void or pore within the particles that allowed the heat transfer and gas diffusion during calcination [2].

The XRD pattern of CaO-ZnO catalysts at different Ca/Zn atomic ratios in Figure 2 showed the presence of peaks at 31.80°, 34.45°, 36.28°, 47.56°, 56.6° and 67.5°, assigned to the ZnO with cubic structure [2,21,22,31]. The presence of CaO was confirmed by the peaks at 32.2°, 37.4°, 53.9°, 64.1° and 67.4° which attributed to the formation of wurtzite structure [32–34]. No additional peaks were observed suggesting both metal oxides existed as separate clusters. The intensity of CaO peaks were only visible when the Ca/Zn ratio was increased to 0.5 and 1. At Ca/Zn ratios of 0.25, the low concentration of CaO produced a well dispersed particles that cannot be detected by XRD. Increasing the CaO loading also significantly improved the intensity of ZnO peaks. The FWHM of ZnO peaks were also increased with further addition of CaO suggesting the formation of large crystallites with high crystallinity.

Rietveld refinement program using Rietica software was adapted to obtain the particle size and the percentage of ZnO and CaO in the catalysts using X-ray diffractogram. The analysis determined the catalyst phase by comparison with the crystallographic database (CaO = ICSD 75785 and ZnO = ICSD 67849). Table 3 summarizes the crystalline phases and the par-

![Figure 3](image.png)

**Figure 3.** The SEM image of CaO-ZnO 0.25 (a), CaO-ZnO 0.5 (b), and CaO-ZnO 1 (c) and TEM image of CaO-ZnO 0.5 catalysts.

| Sample         | Composition % | Particle size (nm) | Elemental content (% wt) | EDX | Rietica |
|----------------|---------------|--------------------|--------------------------|-----|---------|
|                | CaO | ZnO | CaO | ZnO | Ca     | Zn    | O     | Ca     | Zn    | O     |
| CaO-ZnO 0.25   | 0.5  | 99.5 | 233.8 | 31.1 | 3.97   | 76.52 | 19.51 | 0.37   | 79.92 | 19.70 |
| CaO-ZnO 0.5    | 27.1 | 72.9 | 219.6 | 36.1 | 12.30  | 60.99 | 26.14 | 19.35  | 58.58 | 22.07 |
| CaO-ZnO 1      | 48.7 | 52.3 | 175.5 | 37.6 | 25.71  | 45.72 | 28.57 | 30.55  | 42.04 | 22.51 |

Table 3. Composition and particle size estimation using Rietveld and Elemental analysis from EDX.
particle size of CaO and ZnO determined using Rietveld analysis. The concentration of Ca and Zn were also compared with EDX elemental analysis (Table 3). The CaO particle size was significantly reduced from 233 nm to 175 nm when the concentration of CaO was increased. The size of ZnO crystallites was significantly smaller than CaO at 31–37 nm, and the size was less affected by increasing the Ca/Zn ratios.

CaO-ZnO morphology was analysed using SEM (Figure 3) that showed the CaO-ZnO were formed as non-uniformed agglomerates with random structures. Increasing the Ca/Zn molar ratios from 0.25 to 1 significantly increased the size of agglomerates. TEM analysis was also conducted on CaO/ZnO with Ca/Zn ratios of 0.5 to provide detail morphology within nanoscale region. The TEM analysis (Figure 3d) showed the formation of two clusters with different sizes of crystallites. Large crystallites observed at ~300 nm size were corresponded to CaO. Aggregates with significantly smaller size were assigned to ZnO. ZnO cluster was in a close proximity with CaO, however a clear phase separation was observed indicating a non-homogeneous dispersion between CaO and ZnO.

The presence of CaO and ZnO was important to ensure the availability of the active sites for transesterification reaction. All the characterization techniques confirmed the presence of both CaO and ZnO clusters in every samples. CaO and ZnO possessed basic sites for transesterification reaction with different level of basicity. The characterization results also confirmed the effect of Ca/Zn ratio on the CaO-ZnO properties such as crystallinity and morphology. The performance of the catalyst CaO-ZnO was determined on transesterification of RTO to confirm the effect of different Ca/Zn ratio and the structural properties on the catalytic activity.

3.2 Transesterification of Reutealis Trisperma Oil and Optimisation Result

The yield of FAME from RTO by transesterification reaction conducted based on Taguchi method in Table 2 revealed that the yield was significantly affected following the variation of Ca/Zn molar ratios, temperature, and methanol/oil ratios. Low yield of FAME was obtained at 48.2% from experiment no. 1 in which the parameters were at the lowest level than the other sets of experiments. High yield of FAME was obtained at 94.7% from experiment no. 9. It is interesting to note that, high yield of FAME was not generated from the highest level of each parameter.

Figure 4. Effect of parameter and level on the methyl ester conversion.
3.2.1 Effect of methanol/oil ratio

The effect of methanol/oil ratio ratios towards transesterification of RTO oil was analysed using statistical analysis ANOVA. The biodiesel conversion determined from the experimental orthogonal array was converted to S/N ratios. In this study, methanol/oil ratio is varied to 10, 20, and 30 and the effect of variation based on the value of S/N ratio was shown in Figure 4a. Increasing the methanol to oil ratio from 10 to 20 showed no significant changes on the S/N ratios. The S/N ratio was significantly increased when the methanol/oil ratio was enhanced to 30. In transesterification reaction, three moles of methanol are required to convert one mole of triglycerides to methyl ester. However, the excess amount of methanol is important in order to shift the equilibrium towards biodiesel production [13,26].

3.2.2 Ca/Zn ratio

The S/N ratio was improved with increasing the Ca/Zn ratios (Figure 4b). Transesterification reaction occurred on the basic site of catalyst. As evidence by XRD and SEM analysis, increasing the Ca/Zn ratios significantly reduced the crystallites size of the CaO and also increased the number of basic sites for high biodiesel. CaO is more basic than ZnO and important to provide basicity on the catalysts [2]. However, the presence of ZnO was crucial to increase the dispersion of CaO and to reduce its agglomeration during reaction. Transesterification of soybean oil showed a constant methyl ester yield in the range of 93-94% when the Ca/Zn ratios were varied from 0.25 to 1. However, further increasing the Ca/Zn ratios to 4 reduced the yield presumably due to the reduction of basic sites and the formation of larger crystallites [2].

3.2.3 Effect of reaction time

Increasing the reaction time has significantly enhanced the S/N value from 36.4 at 0.5 h to 38.0 when the reaction extended to 2 h (Figure 4c). An adequate reaction time is required to accommodate the interaction between reactants and catalysts. Prolonging the reaction time from 0.5 to 2 h resulted in the increase of S/N ratio due the enhanced mass transfer between methanol and RTO. Biodiesel production from crude corn oil suggested the reaction time as the most influencing parameter surpassing the effect of methanol/oil, reaction temperature, and catalyst concentration [10]. The S/N ratio was reported to decrease over prolonged reaction time due to the formation of glycerol as by-product [11]. The excessive formation of glycerol affected the reaction equilibrium due to the solubility of glycerol in methanol.

3.3 Optimum condition and method validation

The Analysis of Variance (ANOVA) results of biodiesel production from RTO are depicted in Table 4. The result includes the important test such as Fischer’s statistical test (F value) and sum squares to check the level of significance for each parameter. The larger F value represents the higher level of influence. The sum square values were used to estimate the percentage of contribution for individual parameter as calculated using equation (3). Table 4 indicated a narrow range of contribution from 26.4–35.3% deducing an approximately similar significance of each parameter. Reaction time was found to be the least influencing parameter with the percentage contribution was determined at 26.6%. On the other hand, the percentage of contribution for methanol/oil ratios and the Ca/Zn ratios were determined at approximately similar values ~35%. The parameters mainly affected the biodiesel production varied depending on the type of bio-oil. The use of crude tall oil as feedstock for biodiesel showed that reaction time was the most contributing parameter to enhance biodiesel formation [26]. In contrast to biodiesel production from Manilkara zapota oil, reaction time only contributed to 1.6% percentage, meanwhile catalyst concentration was mainly affect-

| Source                  | Degree of Freedom | Sum of Square | Mean square | F-value | Percentage of contribution | Optimum value |
|-------------------------|-------------------|---------------|-------------|---------|----------------------------|---------------|
| Ca/Zn ratio             | 2                 | 11.22         | 5.61        | 10.9    | 34.9                       | 1             |
| Methanol/oil molar ratio| 2                 | 8.55          | 4.27        | 8.31    | 26.6                       | 30            |
| Reaction time (h)       | 2                 | 11.34         | 5.67        | 11.02   | 35.3                       | 2             |
| Residual error          | 2                 | 1.03          | 0.51        |         | 3.2                        |               |
| Total                   | 8                 | 32.15         |             |         |                            |               |

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ed the reaction at 67% of contribution followed by methanol/oil ratio at 25.85% [24]. Transesterification of biodiesel from castor oil reported that methanol/oil ratios as the main parameters at 59.6% contribution. Based on the S/N ratios, the optimised reaction condition was suggested as Ca/Zn ratio of 1, reaction time at 2 h and methanol/oil ratio of 30. Transesterification of RTO under this condition produced 98% of methyl ester conversion which was the highest conversion in comparison to the parameters derived from the orthogonal array. Therefore, it is validated that the statistical analysis was capable of predicting the optimised condition accurately.

As confirmed in this study, Taguchi method promises the efficiency of biodiesel optimization based on relatively a small number of experiments compared to conventional experimental design. A large number of experiments in the conventional optimisation method also contributed to a high cost of production. Therefore, employing the Taguchi method will be beneficial for large scale biodiesel production that can consequently reduce the biodiesel price from the edible RTO.

4. Conclusion

Biodiesel was produced via transesterification of RTO with CaO-ZnO catalysts. The parameter for biodiesel production such as methanol/oil ratio, reaction time and Ca/Zn composition were optimised using Taguchi method. The set of experiments at variation of reaction parameters were obtained using orthogonal array L9. The statistical analysis indicated similar percentages of contribution for reaction time and Ca/Zn composition parameters which were at ~35%, meanwhile the effect of methanol to oil ratio was determined at 26.6% of contribution. The optimum condition predicted by the S/N ratios were Ca/Zn composition of 1, reaction time at 2 h and methanol/oil ratio of 30. The confirmation reaction under the following optimised parameters produced 98% of biodiesel yield. This result verified the applicability of Taguchi design experiment for the optimisation of biodiesel production from RTO.

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