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ORIGINAL PAPER

Crab and Cockle Shells as Catalysts for the Preparation of Methyl Esters from Low Free Fatty Acid Chicken Fat

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Abstract The use of waste oils/fats, as well as catalysts from waste materials, for producing biodiesel via transesterification have been of recent interest. As such, in the present work, the transesterification of chicken fat using crab and cockle shells as a catalyst was attempted. X-ray diffraction (XRD) and electron dispersive X-ray (EDX) results show that, upon thermal activation, both shells transformed into CaO, the active ingredient that catalyzes the reaction. In addition, the catalytic performance of individual shells as well as the combination of both shells in various ratios was similar. Under the optimal reaction conditions of 4.9 wt% catalyst amount and 0.55:1 methanol to oil mass ratio, methyl esters conversion above 98% was achieved in 3 h. The chicken fat methyl esters that were produced were found to meet several key specifications of biodiesel based on EN 14214 and ASTM D6751 test procedures.

Keywords Biodiesel · Transesterification · Chicken fat · Crab shell · Cockle shell · Methyl ester

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Introduction

In recent years, biodiesel (methyl esters) has emerged as a sustainable alternate or extender for fossil fuel, mainly due to its renewable and green properties. Biodiesel is produced via the transesterification of vegetable oils or animal fats with methanol or ethanol. It has been found to be the best substitute for petro-diesel fuel because of its comparable calorific value, low toxic emission, as well as being carbon-neutral and environmentally friendly [1]. However, despite its advantages, its full commercialization is hindered by the production cost. Currently, costly edible oil sources are being utilized, which not only raises the cost but also contributes to the 'food-for-fuel' crisis. Hence, the exploitation of raw materials and catalysts from waste sources has been of recent interest. Researchers have effectively utilized lard, beef tallow and chicken fat [2], waste oil adsorbed onto clay [3], as well as waste crab [4, 5], oyster [6], and egg [7] shells as catalysts in the transesterification reaction.

Furthermore, in Peninsular Malaysia, the production of 550 million chickens was projected for the year 2009 (491 million chickens in 2008) [8]. Based on an estimation of 2 kg per chicken and 11% of the total body fat, this translates to 121,000 tonnes of fat being produced in 2009 [9]. Chicken fat can be collected from the respective local slaughterhouses and with more countries opting to centralize their abattoirs, the collection of the low-cost animal fats will be much convenient. This huge number of chickens produces abundant fat that, at present, is discarded as waste. In addition, the current health consciousness has resulted in chicken fat being avoided for human consumption. This scenario means that chicken fat is a potential source or co-source for the preparation of methyl esters. Transesterification is usually carried out

under the conventional homogeneous catalysts (sodium or potassium hydroxide); however, they posses a few drawbacks, such as the catalyst being non-reusable, impossible to recover the catalyst, and the generation of abundant waste water in neutralizing and separating the catalyst [10]. In such a situation, the use of heterogeneous catalysts is a better solution. Moreover, the increase in crab and cockle aquaculture activities (aquaculture retail values of crab and cockle were over RM 1 million and over RM 101 million, respectively, in 2008; RM 1 = USD 0.31) [11] has indirectly generated abundant waste shells that have no practical use. The activated shells, whose main composition is CaO, have a potential use as a heterogeneous catalyst [12]. To the best of our knowledge, no one has reported the use of waste shells in the preparation of methyl esters using chicken fat.

Consequently, in this study, the low free fatty acid (FFA) chicken fat and the shells (crab and cockle) were utilized to produce methyl esters. In order to optimize the reaction variables and to assess their influence in the reaction, statistical analysis was performed using a central composite design (CCD). Individual performances of the activated crab and cockle shells, as well as their combination performances, have also been appraised. To evaluate its potential as a renewable fuel, the produced methyl esters were subjected to a few recommended key established biodiesel tests.

Experimental

Materials

Methyl esters standards, internal standards, and Hammett indicators of chromatographic grade were obtained from Sigma-Aldrich (Switzerland). Methanol and anhydrous sodium sulphate of analytical reagent grade were purchased from Merck (Darmstadt, Germany).

Catalyst Preparation and Characterization

Chicken fat, crab (*Scylla serrata*), and cockle (*Anadara granosa*) shells were obtained from the local market (Penang, Malaysia). The fat was cleaned with warm water to remove any visible non-fat solid matters, air-dried at room temperature, and slow-melted in an oven from room temperature to 80 °C, followed by filtration to obtain the oil. The oil was then transferred to an amber glass bottle and kept in a refrigerator. The acid value of the oil was determined following the standard EN 14104 and the moisture content was analyzed using the Karl Fischer titration method (784 KFP Titrino, Metrohm). The shells were cleaned to remove protein and other unwanted substances and washed thoroughly with warm water several times. They were then dried overnight in an oven at 105 °C. Crushed and powdered shell was then sieved (<1 mm) before being subjected to heat treatment in a furnace (900 °C for 2 h). The basic strength of the activated catalysts was tested using Hammett indicators. The following Hammett indicators were used: phenolphthalein ($H_- = 8.2$), 2,4-dinitroaniline ($H_- = 15$), and 4-nitroaniline ($H_- = 18.4$). The surface analysis of the catalysts was examined using Micromeritics ASAP 2000. The sample was degassed at 105 °C prior to analysis and the adsorption of N_2 was measured at −196 °C; the particle size of the catalysts was measured using transmission electron microscopy (TEM) with a Philips CM12 equipped with an image analysis system; X-ray diffraction (XRD) on a Siemens Diffraktometer D5000 using Cu Kα radiation, 2θ range from 25° to 125°, with step sizes of 0.1°, at a scanning speed of 1° min^{-1}; scanning electron microscopy coupled with electron dispersive X-ray (SEM-EDX) was obtained using the Leo Supra 50VP Field Emission SEM system with a 5-kV accelerating voltage.

Reaction

Transesterification reactions were performed in a 25-mL, two-neck glass reactor with a condenser, immersed in a water bath. In a typical reaction, 10.0 g of oil was added onto the mixture of catalyst and methanol. The contents were refluxed under magnetic stirring for 3 h. Reaction parameters (methanol/oil mass ratio and catalyst amount) were studied to identify the optimum reaction conditions (MeOH/oil mass ratio 0.5:1 is equivalent to 13.8:1 in terms of molar ratio; molecular weight of chicken oil is assumed to be 885 g/mol). The molecular weight of chicken fat was calculated by using acid and saponification values of 4.1 and 194.3 mgKOH/g, respectively [13]. The reaction mixture was then allowed to cool, resulting in the glycerol to separate by gravity. A centrifuge (Hettich, Universal) (2,500 rpm, 15 min) was used to further separate the layers (methyl esters, glycerol, and catalyst), and the uppermost layer of methyl esters was decanted and washed with warm distilled water to neutralize, and dried over anhydrous sodium sulphate. Residual methanol in the methyl esters was evaporated out using a rotary evaporator (Rotavapor R II, Buchi, Switzerland) at 80 °C to obtain a pure methyl ester. Finally, the methyl esters were filtered through a 0.45-μm syringe filter.

Methyl Esters Analysis

The produced methyl esters specifications were determined by following the EN 14214:2003 and ASTM D6751 test procedures as listed in Table 5. The fatty acid profile was

determined using a gas chromatographer (Perkin-Elmer, Clarus 500) fitted with a polar capillary column (Supelco Wax, 30 m × 0.32 mm × 0.25 μm) following the EN 14103 procedure.

Statistical Analysis

The CCD under response surface methodology was used to analyze the collected data statistically using Design-Expert® 8.0 (Stat-Ease, Inc., Minneapolis, MN, USA). As depicted by Table 1, CCD utilized four factorial points (entries 1–4), four star (axial) points with an alpha value of 1.414 (entries 5–8), and five center points (entries 9–13). The alpha value (1.414) was recommended by the design and was defined as the distance that the star points are from the center of the design space. Negative and positive signs of the alpha values indicate the extreme lowest and highest points from the center point, respectively.

Results and Discussion

Chicken Fat and Catalyst Characterization

The acid value of the chicken oil was found to be 4.1 mgKOH/g (equivalent to 2.1% FFA as oleic acid) and 0.14% moisture content. As tabulated in Table 2, oleic acid was the predominant fatty acid in chicken fat and the results were in agreement with a previous report [14]. With about 30% of the acids being of the saturated type, the remaining 70% of the content accounted for unsaturated fatty acids. The basic strength of the activated catalyst was tested using Hammett indicators. If the indicator exhibited a color change, then the catalyst was labeled as stronger than the indicator, and if not, then the catalyst was said to be weaker than the indicator [15]. The catalysts could change the color of both phenolphthalein ($H_- = 8.2$) from colorless to pink and of 2,4-dinitroaniline ($H_- = 15$) from yellow to mauve, but failed to change the color of 4-nitroaniline ($H_- = 18.4$). Therefore, the catalyst's basic strength was assigned as $15 < H_- < 18.4$, and was regarded as a strong base for the transesterification reaction. The surface area and particle size of the shells' CaO were compared against laboratory CaO (Table 3) and were found to be in good agreement with each other. As depicted by Fig. 1, CaO was the only component in all of the activated catalysts, regardless of their origin. The XRD profile of the activated shells also closely matched the profile of the laboratory CaO. In addition, the EDX results also reveal that the activated shells mainly consist of CaO. The SEM micrographs of calcined catalyst (not shown here) demonstrated many pores on its surface and the presence of similar morphologies explain its higher catalytic activity. On the contrary, the micrographs of uncalcined catalyst showed a bulky substance without any clear pores on its surface.

Statistical Analysis and Reaction Optimization

Table 1 shows the methyl esters purity under different reaction conditions. The results indicate that any changes in any one of the factors has a serious consequence on the purity of the product. Analysis of variance (ANOVA) suggested that the quadratic model is a better fit than the

Table 1 Factorial design (entries 1–4), star points (entries 5–8), and center points (entries 9–13) with their corresponding methyl esters purity

Entry	*C* (wt%)	MR (g:g)	X_C	X_{MR}	Purity (wt%)[a]
1	3	0.25	−1	−1	60.7
2	7	0.25	1	−1	68.4
3	3	0.75	−1	1	58.8
4	7	0.75	1	1	97.2
5	2.2	0.50	−1.41	0	60.8
6	7.8	0.50	1.41	0	90.7
7	5	0.15	0	−1.41	65.8
8	5	0.85	0	1.41	95.2
9	5	0.50	0	0	98.1
10	5	0.50	0	0	99.0
11	5	0.50	0	0	97.8
12	5	0.50	0	0	98.3
13	5	0.50	0	0	98.1

C catalyst amount (crab:cockle = 1:1); *MR* MeOH/oil mass ratio; *X* coded value of the respective factor

[a] Results of duplicate determinations

Table 2 Fatty acid profile of chicken fat

Fatty acid	Composition (%)	
	Present work	Previous work [14]
Saturated		
Myristic ($C_{14:0}$)	0.6	0.7
Palmitic ($C_{16:0}$)	24.7	25.2
Stearic ($C_{18:0}$)	4.5	5.9
Subtotal	29.8	31.8
Unsaturated		
Myristoleic ($C_{14:1}$)	0.2	0.3
Palmitoleic ($C_{16:1}$)	6.3	7.8
Oleic ($C_{18:1}$)	44.1	40.5
Linoleic ($C_{18:2}$)	18.4	18.4
Linolenic ($C_{18:3}$)	0.2	0.7
Gadoleic ($C_{20:1}$)	1.0	0.5
Subtotal	70.2	68.2
Total	100.0	100.0

Table 3 Physical properties of catalysts from different origins

Physical properties	Catalyst (CaO)		
	Laboratory	Crab	Cockle
BET surface area (uncalcined) (m^2 g^{-1})	4.5	3.9	4.3
BET surface area (calcined)[a] (m^2 g^{-1})	12.6	14.2	11.4
TEM particle size (calcined)[a] (nm)	22.2 ± 9.6[b]	25.6 ± 10.5[b]	24.6 ± 9.9[b]

[a] Calcined at 900 °C for 2 h

[b] Standard deviation of the mean value

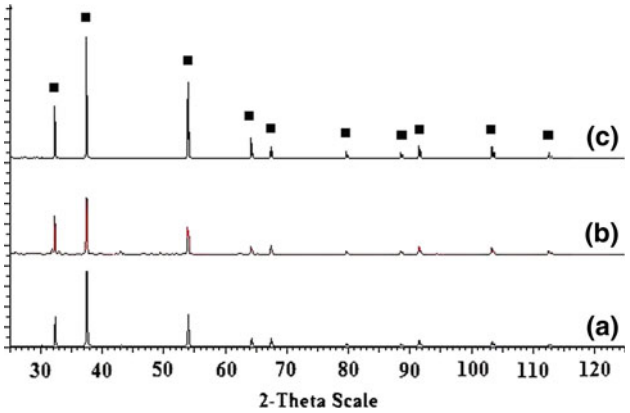

Fig. 1a–c Powder X-ray diffraction (XRD) patterns of the calcined (900 °C for 2 h) catalysts. **a** Laboratory CaO. **b** Crab shell. **c** Cockle shell. The *squares* indicate CaO

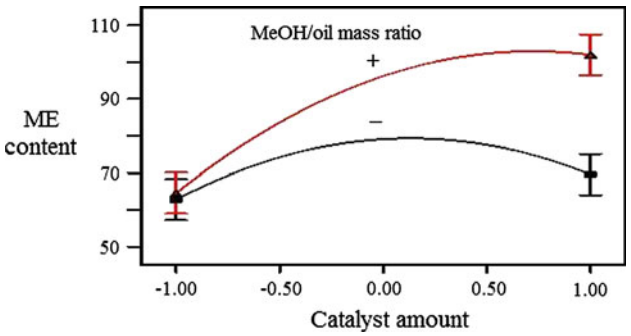

Fig. 2 Influence of the interaction effect of reaction variables on the methyl ester content. The *plus* denotes the factor's highest level and the *minus* denotes the factor's lowest level

linear model. As per suggestion, the following model was obtained:

$$\begin{aligned}\text{Methyl esters content} &= 98.26 \\ &+ (11.05 \times \text{catalyst amount}) \\ &+ (8.56 \times \text{MeOH/oil mass ratio}) \\ &+ (7.68 \times \text{catalyst amount} \times \text{MeOH/oil mass ratio}) \\ &- \left(12.97 \times \text{catalyst amount}^2\right) \\ &- \left(10.59 \times \text{MeOH/oil mass ratio}^2\right)\end{aligned}$$

The simultaneous effects of the variables under study can be modeled with the quadratic model rather than a linear regression [16]. The model's *P*-value was recorded to be less than 0.0001; any value below 0.05 indicates that the model is a strongly significant model. Adequate precision, which measures the signal-to-noise ratio, should be greater than a value of 4. Our adequate precision value was more than 15, implying that the selected model can navigate the design space. In addition, the model was also well validated with a higher regression coefficient, $R^2 = 0.97$, and a lower coefficient of variation (error percentage), CV = 5%. While both factors exhibit a positive response to the generation of methyl esters (indicated by the positive sign), the catalyst amount had a greater impact in transesterifying the chicken fat into methyl esters, as seen by the higher magnitude term of 11.05. The methanol/oil mass ratio was the second most influential factor of the reaction.

As depicted by Fig. 2, at the lowest catalyst level (3 wt%, coded as −1.00), both extreme levels of methanol recorded a very similar content of methyl esters (around 63 wt%). In contrast, at the highest catalyst level (7 wt%, coded as 1.00), both low and high levels of methanol yield a very different amount of the product (63 vs. 100 wt%). It can be deduced that the methanol effect is only observed when coupled with a sufficient amount of catalyst that is needed to drive the reaction. Too much solid catalyst can affect the stirring effectiveness and a large amount of methanol could dilute the oil and reduce the reaction rate [17]; as a result, lower conversions were recorded with higher amounts of catalyst and methanol.

Using the Design-Expert® 8.0 software, numerical optimization of the reaction was performed with the two reaction variables, which were set 'in range' and the response (methyl esters purity) was set as 99%, the highest experimental value. With this criteria, the software selected 4.9 wt% catalyst amount (corresponding to −0.04 in coded value) and 0.55:1 methanol to oil mass ratio (corresponding to 0.20 in coded value), as one of the best solutions, in which 99 wt% of methyl esters was predicted, as shown by a contour plot in Fig. 3. To validate this prediction, a set of three experiments were carried out based on the suggested optimal conditions and, experimentally, an average of 98.6 wt% of methyl esters purity was obtained in comparison with the predicted value of 99 wt%. With the average difference of 0.4 wt% between the predicted and experimental values and a standard deviation of 0.36, it can be concluded that the experimental design used in this work is valid and reproducible.

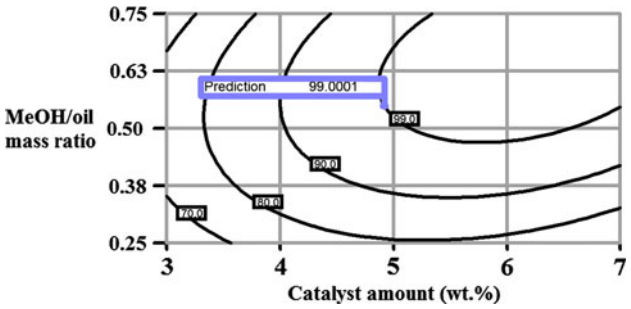

Fig. 3 Contour plot of methyl esters content as a function of catalyst amount and MeOH/oil mass ratio under the optimal reaction conditions

Table 4 Produced methyl esters using individual and combined catalysts at various ratios

Catalyst composition (crab:cockle, %)	Purity (wt%)[a]
0:100	98.3 ± 0.4[b]
25:75	99.0 ± 0.3[b]
50:50	98.7 ± 0.4[b]
75:25	98.6 ± 0.5[b]
100:0	98.2 ± 0.4[b]

[a] Reaction conditions: MeOH/oil mass ratio, 0.55:1; catalyst amount, 4.9 wt% (based on oil weight); reaction temperature, at methanol refluxing temperature (65 °C); reaction period, 3 h

[b] Standard deviation of the mean value ($n = 3$)

Performance of the Combined Catalysts

In order to study the effect of the combined catalysts from two different sources, experiments with crab and cockle shells in different combinations were conducted under the suggested optimal conditions. From Table 4, it can be seen that the catalyst source as well as the combination ratios have no appreciable effect on the methyl esters produced. The combined catalysts performed equally well when compared to their individual performances. As such, these results prove that the CaO from the crab and cockle shells have a very similar catalytical activity in the reaction, and the different origin of the catalysts has no observable effect on the produced methyl esters. The results have opened up the possibility of combining all of the possible waste sources of CaO as solid catalysts in transesterifying triglycerides into methyl esters.

Properties of the Prepared Methyl Esters

The prepared methyl esters from low FFA chicken fat was found to comply with all of the important key physical and chemical properties of biodiesel, as tabulated in Table 5. Nonetheless, work with more representative samples (higher FFA content than 2.1%) need to be carried out before the method can be considered for biodiesel production from chicken fat. In order to use the fuel in cold climate countries, the cold flow properties are of critical consideration. The problems associated with cold flow properties can be overcome by either blending the produced methyl esters with methyl esters derived from oil with a higher content of unsaturated fatty acids [18] or by using cold flow additives [19].

Conclusion

Activated waste crab and cockle shells have been successfully utilized as heterogeneous catalysts in the transesterification of chicken fat. It was found that different

Table 5 Properties of the produced methyl esters

Property	Unit	Specification	Results	Test method
Ester content	% (m/m)	96.5 min	98.8	EN 14103
Density (at 15 °C)	kg/m^3	860–900	880	EN ISO 3675
Viscosity (at 40 °C)	mm^2/s	3.5–5.0	4.0	EN ISO 3104
Cetane number	–	47 min.	63	ASTM D6890
Cloud point	°C	Report	10	ASTM D2500
Pour point	°C	Report	8	ASTM D97
Cold filter plugging point	°C	Report	5	EN 116
Monoglyceride content	% (m/m)	0.80 max.	0.17	EN 14105
Diglyceride content	% (m/m)	0.20 max.	0.01	EN 14105
Triglyceride content	% (m/m)	0.20 max.	Not detected	EN 14105
Free glycerine	% (m/m)	0.02 max.	0.01	EN 14105
Total glycerine	% (m/m)	0.25 max.	0.05	EN 14105
Methanol content	% (m/m)	0.20 max.	0.08	EN 14110
Acid value	mgKOH/g	0.50 max.	0.14	EN 14104
Water content	wt%	0.05 max.	0.02	KF method

Reaction conditions: MeOH/oil mass ratio, 0.55:1; catalyst amount, 4.9 wt% (based on oil weight, crab:cockle = 1:1); reaction temperature, at methanol refluxing temperature (65 °C); reaction period, 3 h

combinations of the shells perform equally in the reaction, regardless of their origin. This enhances the chances of combining all of the possible waste CaO to produce methyl esters. Using statistical software, the transesterification of chicken fat has been successfully optimized (4.9 wt% catalyst amount and 0.55:1 methanol to oil mass ratio) and has been validated experimentally. Since the final product meets several key biodiesel specifications, the low free fatty acid (FFA) chicken fat can be a potential lipid feedstock in the preparation of renewable fuel. In order to make it a sustainable process, the exploration of materials from waste sources should be given priority.

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