7. An alternative fatty acid alkyl esters production method: In situ transesterification

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Abstract. Fatty acid alkyl esters are valuable reagents which have many applications in detergent, cosmetic, pharmaceutical and food industry. Fatty acid esters, especially methyl esters which are produced with methanol and oils are generally used as an alternative fuel called biodiesel. Alcoholysis of oils and fats is the traditional method that has been extensively employed to synthesize fatty acid esters over the past decades. In this method raw material is extracted oil mostly from edible or inedible oil sources. In situ esterification is another biodiesel production method which provides the direct use of an oil bearing raw material because extraction and transesterification proceed in one step. Till 1985, the researchers have been focused on in situ transesterification to improve the use of different raw materials and economic feasibility of biodiesel.

The aim of this chapter is to review the articles published about in situ transesterification. In this chapter several aspects of in situ
fatty acid esters production will be discussed. The factors affecting the process (catalyst, raw material, stirring, time, temperature etc.), advantages and disadvantages of in situ fatty acid ester production by making comparison with other methods will be analyzed.

**Introduction**

Fatty acid esters are the product of the esterification reaction of fatty acids with monohydroxy alcohols, which have many industrial applications ranged from cosmetics, pharmaceuticals to food due to their softening and lubricating properties [1]. Fatty acid monoalkyl esters are also referred to as biodiesel which is a clean energy source [2]. Development of alternative, renewable, economical energy sources is the main framework of country’s sustainable energy agendas. Biodiesel seems to be the most suitable alternative to petroleum-based diesel fuel with unique properties such as being biodegradable, renewable, and environmentally safe fuel. It has also lower sulphur and aromatic content, a high flash point (>130 °C) and similar energy density to diesel fuel [3].

The economic feasibility of biodiesel is the significant aspect to make it capable of competing with petroleum-based diesel fuel. The cost of production method and feedstock are features that have to be overcome. Alcoholysis (also called transesterification) of lipids (mainly triglycerides) is the traditional biodiesel production method which is widely used for industrial biodiesel production. In this traditional method, triglycerides are transformed into fatty acid alkyl esters in the presence of an either primary or secondary short-chain alcohol (methanol, ethanol, isopropanol etc.), and a catalyst by either an acid, enzyme or a base [4]. In recent years, researches have been directed to produce biodiesel by use of microwave assisted, ultrasound assisted and supercritical production methods. In all of these methods raw material is pre-extracted oil derived from vegetable sources. In situ esterification, also called as reactive extraction, is an alternative biodiesel production method in which raw material is an oil bearing source because extraction and transesterification proceed in one step [5]. In last two decades, the researchers have been focused on in situ esterification to improve the use of different raw materials (e.g. unrefined high-acidity oils) and economic feasibility of biodiesel. By using in situ transesterification the production cost of biodiesel may be further reduced since the direct use of vegetable oils will eliminate the oil extraction and purification step [1].

This chapter briefly reviews the articles published about in situ transesterification. In this chapter several variables affecting the in situ transesterification process including the type of alcohol, catalyst and raw
In situ esterification method

material, ratio of alcohol to oil, time, and temperature will be discussed. The microwave, supercritical and ultrasound assisted in situ fatty acid esters production will also be explained. The advantages and disadvantages of in situ transesterification will be compared with other transesterification methods.

**In situ transesterification**

In situ transesterification differs from the conventional transesterification in that processing has fewer steps as can be seen from Figure 1. Predominantly, pre-extracted and partially purified oils are transesterified in the presence of a simple monohydroxy alcohol in conventional method (a). Contrarily, the extraction and transesterification steps are combined in the synthesis of fatty acid esters by in situ transesterification (b).

**Figure 1.** The scheme of biodiesel production by use of a) conventional and b) in situ transesterification method.

The in situ transesterification of oils was firstly reported in 1985 by Harrington and D’arcy Evans [6]. During the past two decade, many researchers have experimented on the use of several feedstocks, catalyst and reaction conditions for in situ transesterification of oils to obtain a feasible process.

**Variables affecting the in situ transesterification process**

The molar ratio of alcohol to vegetable oil, reaction temperature, reaction time, catalyst, acidity and moisture content of feedstock are the variables affecting the ester yield during the in situ transesterification reaction [1, 7, 8].
It is important to ascertain the reaction conditions necessary for achieving the maximum yield with optimum conditions [9].

**Effect of raw material on in situ transesterification**

Selection of feedstock is a very important issue since the cost of raw materials accounts about 60–80% of the total cost of biodiesel production [10]. The presence of high free fatty acid concentrations in the feedstock is a limiting factor for conventional transesterification. Free fatty acid content of feedstock must be lower than 0.5 wt. % to prevent undesirable soap formation [11]. However, low cost feedstocks mainly non–edible oils such as *Jatropha curcas*, *Pongamina pinnata*, palanga has high free fatty acid content. Rice bran oil is also has high acidity oil which can not be converted to fatty acid ester in one step. Rice bran oil methyl esters were obtained with 86.4-97.4 %yield and ethyl esters were obtained with 97.6% conversion from high acidity rice bran oil by in situ esterification method [1,7,12]. In situ transesterification is a suitable method for these kinds of feedstock and almost any oil bearing feedstock.

In situ production of fatty acid esters from different species including canola [13], *Capparis deciduas* seed [14], castor seed [4,15], *Chlorella vulgaris* microalgae [16, 17, 18], *Chlorella pyrenoidosa* [19], coconut waste [20],*cottonseed* [8, 21, 22],*crude palm oil* [23, 24], *cyanobacteria* [25], *desert date* (*Balanites aegyptiaca*) [26], *grape seed* [27], Iberian pig subcutaneous adipose tissue [28], *Jatropha curcas* [9, 29-37], *marine algae* (*Fucus Spiralis* and *Pelvetia Canaliculata*)[38], *milk weed seeds* [39], *municipal and activated sludge* [3, 40], *Pistacia chinens is* Bunge seed [41], *rapeseed* [42, 43], *residual oil recovered from spent bleaching earth* [44], *palm seed waste pulp* [45], *rice bran* [1, 7, 46-50], *soybean* [51-54], *sunflower seed* [5, 6,55, 56], *undi seed* (*Calophyllum innophyllum* L.) [57] were investigated for their suitability as biodiesel. These studies are also summarized in Table 1.

**Effect of moisture content of raw material on in situ transesterification**

Moisture content of raw material has a great importance on the fatty acid ester yield. In alkali catalyzed transesterification reactions excess water causes saponification of free fatty acids which consumes the catalyst, reduces the catalytic efficiency and hardens the glycerol separation step by formation of gels [63]. Also, the solubility of triglycerides in methanol might reduce due to polar structure of water and increase of the mass transfer resistance [36, 42].
In situ esterification method

### Table 1. Data on in situ transesterification studies

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Solvent</th>
<th>Reaction Mixture</th>
<th>Temperature (°C) / Time(min)</th>
<th>Catalyst</th>
<th>Stirring Speed (rpm)</th>
<th>Maximum Performance (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>Methanol</td>
<td>25 mL solvent:1 g freeze dried activated sludge</td>
<td>55/1440</td>
<td>H₂SO₄</td>
<td>-</td>
<td>4.88 (yield)</td>
<td>[3]</td>
</tr>
<tr>
<td>Canola (Brassica napus)</td>
<td>Methanol</td>
<td>275:1 Molar ratio of solvent: triacylglycerol</td>
<td>60/360</td>
<td>KOH (Molar ratio of catalyst: triacylglycerol 1:0.65:1)</td>
<td>200</td>
<td>80 (conversion)</td>
<td>[13]</td>
</tr>
<tr>
<td>Capparis decidua</td>
<td>Ethanol</td>
<td>6:1 Molar ratio of solvent:oil</td>
<td>80/60</td>
<td>KOH (0.08 N)</td>
<td>300</td>
<td>63.75 (conversion)</td>
<td>[14]</td>
</tr>
<tr>
<td>Castor seed (red)</td>
<td>Ethanol-Hexane (hexane 20% v/v based on the oil volume)</td>
<td>60:1 Molar ratio of solvent:oil</td>
<td>60/120</td>
<td>H₂SO₄ (1%), KOH (1%)</td>
<td>300</td>
<td>65.6 (conversion)</td>
<td>[4]</td>
</tr>
<tr>
<td>Castor seed</td>
<td>Methanol</td>
<td>225:1 Molar ratio of solvent:oil</td>
<td>55/180</td>
<td>KOH</td>
<td>350</td>
<td>88.3 (yield)</td>
<td>[15]</td>
</tr>
<tr>
<td>Chaetoceros gracilis microalgae</td>
<td>Methanol</td>
<td>2 mL solvent:100 mg algal biomass</td>
<td>60/100</td>
<td>H₂SO₄ (1.8%)</td>
<td>-</td>
<td>35.6 (yield)</td>
<td>[25]</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa microalgae</td>
<td>Methanol-Hexane</td>
<td>0.6 mL n-hexane:4mL methanol:1 g algal biomass</td>
<td>90/120</td>
<td>H₂SO₄</td>
<td>-</td>
<td>95 (yield)</td>
<td>[19]</td>
</tr>
<tr>
<td>Chlorella sp. microalgae</td>
<td>Methanol</td>
<td>315:1 Molar ratio of solvent:oil</td>
<td>60/480</td>
<td>H₂SO₄</td>
<td>500</td>
<td>88.5 (conversion)</td>
<td>[58]</td>
</tr>
<tr>
<td>Chlorella vulgaris microalgae</td>
<td>Methanol</td>
<td>600:1 Molar ratio of solvent:oil</td>
<td>60/75</td>
<td>NaOH (molar ratio of catalyst: lipid 0.15:1)</td>
<td>380</td>
<td>77.6 ± 2.3 (yield)</td>
<td>[16]</td>
</tr>
<tr>
<td>Chlorella vulgaris microalgae</td>
<td>Methanol</td>
<td>20 mL solvent:5 g algal biomass</td>
<td>175/240</td>
<td>-</td>
<td>-</td>
<td>89.71 (yield)</td>
<td>[18]</td>
</tr>
<tr>
<td>Coconut waste</td>
<td>Methanol</td>
<td>100 mL solvent:10 g waste</td>
<td>62/420</td>
<td>KOH</td>
<td>700</td>
<td>88.5 (yield)</td>
<td>[20]</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>Methanol</td>
<td>130:1 molar</td>
<td>40/180</td>
<td>NaOH</td>
<td>-</td>
<td>98</td>
<td>[21]</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Table Entry</th>
<th>Methanol</th>
<th>673.1 Molar ratio of solvent : oil</th>
<th>60/20</th>
<th>NaOH (2.0%)</th>
<th>600</th>
<th>95 (yield) [8]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottonseed</td>
<td>Methanol</td>
<td>133:1 Molar ratio of solvent : oil</td>
<td>40/180</td>
<td>NaOH (0.1 mol/L)</td>
<td>-</td>
<td>98 [22]</td>
</tr>
<tr>
<td>Desert date</td>
<td>Ethanol</td>
<td>200 mL solvent : 1 g desert date</td>
<td>25/420</td>
<td>KOH (2.0 wt. %)</td>
<td>-</td>
<td>91.87 (yield) [26]</td>
</tr>
<tr>
<td>(Balanitesa</td>
<td>Supercrit</td>
<td>300/5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51.28 (yield) [59]</td>
</tr>
<tr>
<td>gypsicca)</td>
<td>al Methanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distillers</td>
<td>Methanol</td>
<td>655:1 Molar ratio of solvent : oil</td>
<td>35/72</td>
<td>NaOH</td>
<td>-</td>
<td>91.1 [51]</td>
</tr>
<tr>
<td>dried</td>
<td></td>
<td>180 mL solvent : 50 g seed</td>
<td>50/600</td>
<td>KOH (0.4 N)</td>
<td>-</td>
<td>95.5 (methyl ester wt.) [27]</td>
</tr>
<tr>
<td>grains with</td>
<td></td>
<td>140 mL solvent : 20 g kernel</td>
<td>30/120</td>
<td>CH3ONA (2.0 wt. %)</td>
<td>600</td>
<td>99.98 (yield) [29]</td>
</tr>
<tr>
<td>solubles</td>
<td></td>
<td>7.5 mL solvent : 1 g seed</td>
<td>30/150</td>
<td>NaOH (Mass ratio of catalyst to seed: 0.675:1)</td>
<td>300</td>
<td>99.3 (yield) [30]</td>
</tr>
<tr>
<td>Jatropha</td>
<td>Dimethyl</td>
<td>10:1 Molar ratio of solvent : oil</td>
<td>50/1440</td>
<td>Novozym 435</td>
<td>180</td>
<td>95.9 (yield) [60]</td>
</tr>
<tr>
<td>curcas L.</td>
<td>carbonate</td>
<td>7.5:1 Molar ratio of solvent : oil</td>
<td>50/1440</td>
<td>Novozym 435</td>
<td>180</td>
<td>87.2 (yield) [41]</td>
</tr>
<tr>
<td>Jatropha</td>
<td>Ethyl</td>
<td>400:1 Molar ratio of solvent : oil</td>
<td>30/30</td>
<td>NaOH</td>
<td>300</td>
<td>81.9 (yield) [32]</td>
</tr>
<tr>
<td>curcas L.</td>
<td>acetate</td>
<td>6:1 Molar ratio of solvent : oil</td>
<td>45/720</td>
<td>Lipozyme TLIM</td>
<td>180</td>
<td>90.6 (yield) [33]</td>
</tr>
<tr>
<td>Jatropha</td>
<td>Methanol</td>
<td>6.5:1 Mass ratio of solvent : oil</td>
<td>30/210</td>
<td>NaOH, C10H10NO (Benzyltrimethyl ammonium hydroxide)</td>
<td>~400</td>
<td>99.2 (yield) [31]</td>
</tr>
<tr>
<td>curcas L.</td>
<td>Ethanol</td>
<td>7.8:1 Mass ratio of solvent : seed</td>
<td>65/60</td>
<td>NaOH (0.1 M)</td>
<td>-</td>
<td>98 (conversion) [9]</td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Solvent</th>
<th>Molar ratio of solvent : oil</th>
<th>Molar ratio of catalyst : oil</th>
<th>Reaction Temperature</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Jatropha curcas</em> L.</td>
<td>Methanol</td>
<td>7.5 mL solvent: 1 g seed</td>
<td>60/1440</td>
<td>-</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td><em>Jatropha curcas</em> L.</td>
<td>Ethanol</td>
<td>10 mL solvent: 1 g seed</td>
<td>300/80</td>
<td>-</td>
<td>103.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Marine macroalgae</td>
<td>Methanol</td>
<td>300/1 Molar ratio of solvent : oil</td>
<td>60/660</td>
<td>NaOH</td>
<td>-</td>
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<td></td>
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<td></td>
<td></td>
<td>17.1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Municipal primary sludges</td>
<td>Methanol</td>
<td>12.1 Molar ratio of solvent : sludge</td>
<td>75/480</td>
<td>H₂SO₄ (5% [v/v])</td>
<td>-</td>
</tr>
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<td></td>
<td>14.5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Palm seed pulp</td>
<td>Ethanol</td>
<td>35:1:1 Molar ratio of solvent : oil</td>
<td>78/720</td>
<td>H₂SO₄</td>
<td>-</td>
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<td></td>
<td></td>
<td></td>
<td>99.35</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Palm oil (crude)</td>
<td>Methanol</td>
<td>150:1 Molar ratio of solvent : oil</td>
<td>60/120</td>
<td>KOH</td>
<td>-</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>75.2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(conversion)</td>
</tr>
<tr>
<td>Palm oil (crude)</td>
<td>Methanol</td>
<td>225:1 Molar ratio of solvent : oil</td>
<td>60/540</td>
<td>KOH</td>
<td>300</td>
</tr>
<tr>
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<td></td>
<td>97.25</td>
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<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td><em>Pistacia chinensis</em></td>
<td>Ethanol</td>
<td>10:1 Molar ratio of solvent : oil</td>
<td>50/1440</td>
<td>Novozym 435</td>
<td>180</td>
</tr>
<tr>
<td>Burge</td>
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<td></td>
<td></td>
<td>90.7</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td><em>Pistacia chinensis</em></td>
<td>Ethanol</td>
<td>7.5:1 Molar ratio of solvent : oil</td>
<td>50/1440</td>
<td>Novozym 435</td>
<td>180</td>
</tr>
<tr>
<td>Burge</td>
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<td></td>
<td></td>
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<td>92.8</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>Methanol</td>
<td>475.1 Molar ratio of solvent : oil</td>
<td>60/60</td>
<td>NaOH (0.1 molal)</td>
<td>200</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>88.8</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>Methanol</td>
<td>720:1 Molar ratio of solvent : oil</td>
<td>65/60</td>
<td>KOH</td>
<td>-</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>(conversion)</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Methanol</td>
<td>200 mL solvent: 50 g rice bran</td>
<td>65/300</td>
<td>H₂SO₄</td>
<td>-</td>
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<tr>
<td></td>
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<td></td>
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<td>86.4</td>
</tr>
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<td></td>
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<td>(yield)</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Ethanol</td>
<td>200 mL solvent: 50 g rice bran</td>
<td>78/60</td>
<td>H₂SO₄</td>
<td>-</td>
</tr>
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<td>78.3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Ethanol</td>
<td>200 mL solvent: 50 g rice bran</td>
<td>25/60</td>
<td>H₂SO₄ (7.5 mL)</td>
<td>-</td>
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<tr>
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<td>97.6</td>
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<td>(conversion)</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Methanol</td>
<td>15 mL solvent: 1 g rice bran</td>
<td>60/15</td>
<td>H₂SO₄ (mass ratio of catalyst to rice bran 0.18)</td>
<td>600</td>
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<td></td>
<td></td>
<td></td>
<td>(yield)</td>
</tr>
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</table>
Qian and Yun [21], compared the effect of different water removal pretreatment methods of milled cottonseed to see the influence of moisture on cottonseed oil conversion into fatty acid methyl ester. The highest conversion (98%) of oil into fatty acid methyl ester was obtained by use of methanol washed seeds. The conversions of crude cottonseed meal and vacuum dried (70 °C in an oven for 3 h) cottonseed meal into fatty acid methyl ester were 21 and 35 %, respectively.
The in situ biodiesel production from microalgae was studied by Ehimen et al. [58] who investigated ten moisture content levels of 72.5%, 56.0%, 40.9%, 31.7%, 19.5%, 8.7%, 3.2%, 1.4%, 0.7% and 0.0%. The equilibrium conversion of oil to biodiesel by the acid catalyzed in situ transesterification of algae was shown to decrease significantly with the increase in the biomass moisture content. The results indicated that when the moisture content was greater than 31.7%, the conversion was almost inhibited [58].

Hass and Scott [51], reported that when the soybean flakes dried to 0% water, the methanol requirement decreased 60% compared with transesterification of full-moisture flakes. In contrast to this study, Zakaria and Harvey [42], found that drying the rapeseeds from 6.7% to 0% water content does not change the ester yield and required methanol amount.

Especially, in enzymatic catalyzed transesterification reactions the presence of an oil–water interface required because it provides a suitable environment for enzyme activation [64, 65]. Enzymatic activity cannot be possible in a water free media. However, excess water cause reverse reaction of hydrolysis [2]. Lipase catalyzed reactive extraction of *Pistacia chinensis* Bunge seeds and *Jatropha curcas* L. seed in the presence of different acyl acceptors was investigated by Su et al. [60]. The optimum water content was found to be 3.14% in the presence of dimethyl carbonate and 2.34% in the presence of diethyl carbonate to achieve the highest *Pistacia chinensis* Bunge seeds methyl ester yield. It was determined that after these optimum water contents the ester yields decreased. The *Jatropha curcas* L. Seed transesterification results showed that the highest ester yields were obtained at 3.02% of water content for both acyl acceptors.

Su et al. [41], also investigated the in situ transesterification of *Pistacia chinensis* Bunge seeds and *Jatropha curcas* L. seeds (5 g) with the use of methyl acetate and ethyl acetate as extraction solvents in the presence of 30% (w/w) Novozym 435 at 50°C and 180 rpm. The highest yields were obtained at 4.26% and 4.62% of water content for *Pistacia chinensis* Bunge seeds and *Jatropha curcas* L. seed, respectively.

Consequently, the effect of moisture content on yield is especially related to the type of raw material.

**Effect of particle size of raw material on in situ transesterification**

The seed particle size is an important factor to obtain higher reaction rate and fatty acid methyl ester yield.

In situ transesterification of *Jatropha curcas* L. seeds with methanol was investigated by Kasim and Harvey [32]. Influence of different seed particle sizes in the ranges of <0.5–4 mm were tried besides several process variables.
The best results were obtained with <0.5 mm particle size as 86.1 % yield at a 1:400 alcohol /oil ratio and 0.1 N NaOH concentration. However, increasing the particle size to 0.71–1 mm, and further to 1–2 mm decreased the yield from approximately 50% to 40%. When the transesterification proceed with 2-4 mm seed particle size, the lowest yield (35.5 %) was obtained.

Kıldıran et al. [54] reported the effect of two particle size (<0.5 and <1 mm) on in situ alcoholysis of soybean oil at 1h,3h,5h with ethanol. According to Kıldıran et al. [54], extraction rate and purity of the products increased as the particle size of soybeans decreased and at 5h approximately 95% and 78% of oil was dissolved in alcohol with <0.5 and <1 mm particle size, respectively.

Kaul et al. [9] studied the reactive extraction of \textit{Jatropha} seeds with different sizes ranging from < 0.85 mm to >2.46 mm and noted that the biggest seed size (>2.46 mm) showed better results than <0.85 and >0.85 to <2.46 seed sizes at a 1:5.2 seed/methanol ratio (w/w) and a catalyst (NaOH) concentration of 0.1 N.

**Effect of alcohol type on in situ transesterification**

The in situ transesterification of oils was proposed for different sorts of alcohols, ranging from methanol, ethanol, \textit{n}-propanol, isopropanol to \textit{n}-butanol. Mostly, a short chain alcohol, methanol has been used in reactive extraction reactions due to its low price and availability.

The in situ transesterification of soybean oil with monohydroxy alcohols (ethanol, methanol, \textit{n}-propanol and \textit{n}-butanol) was investigated by Kıldıran et al. [54]. It was observed that the highest yield (84.6%) was obtained by use of \textit{n}-propanol. The in situ alcoholysis of soybean oil with ethanol and \textit{n}-butanol also resulted in high yields 80.8% and 78%, respectively. Methanol was determined as an inefficient solvent for transesterification of soybean oil which gave 41.5% yield.

Özgül and Türkay [12], investigated the in situ esterification of rice bran oil with two kind of monohydroxy alcohol (methanol and ethanol) with acid catalyst. It was demonstrated that the use of ethanol was superior to methanol as the ester conversions were 98.7 and 85.9, respectively.

Wahlen et al. [25], studied the in situ transesterification of the microalgae \textit{Chaetoceros gracilis} by the use of primary alcohols of longer chains (methanol, ethanol, \textit{1}-butanol, 2-methyl-\textit{1}-propanol, and 3-methyl-\textit{1}-butanol) to determine the appropriate alcohol. The transesterification of microalgae by the use of methanol resulted in the lowest conversion. However, fatty acid alkyl esters production yield approximately same when the reaction proceeded by use of an acid catalyst (H\textsubscript{2}SO\textsubscript{4}). Thus, methanol was chosen for the optimization studies due to its low price.
Effect of alcohol volume on in situ transesterification

The amount of alcohol in the reaction media played a major role in affecting the product yield. In traditional transesterification system, product yield always increased due to the molar excess of alcohol over fatty acids in triglycerides [66]. The transesterification reaction is reversible, and thus an increase in the amount of one of the reactants will shift the reaction equilibrium toward the products side and result in higher ester yield [21]. At three molar equivalents of methanol to oil are required minimally for the complete conversion [67]. Alcohol has two roles during in situ transesterification. Firstly, it plays as a solvent to extract lipids from oil bearing seed and then transforms the lipids to fatty acid esters as the transesterification reagent (acyl acceptor) [25].

Hailegiorgis et al [30], investigated the in situ transesterification of *Jatropha curcas* L. with alkaline ethanol at 30°C, 300 rpm after 2.5 h by use of cetyltrimethylammonium bromide and NaOH to seed weight ratio of 0.675. The volume of ethanol to weight of seed of 7.5 gave the highest yield of fatty acid ethyl ester was 99.3 wt. %.

The influence of methanol amount on fatty acid methyl ester content in the in situ transesterification of wet *Chlorella vulgaris* biomass at 175 °C and after 4 h under subcritical condition was studied by Tsigie et al. [18]. It was observed that a wet microalgae biomass to methanol ratio of 1:4 (w/w) was the optimum rate and further increase in methanol amount decreased the conversion of crude microalgal oil to biodiesel.

Özgül-Yücel and Türkay [7], studied the in esterification of rice bran oil using sulfuric acid catalyst and found that increasing the amount of methanol from 200 to 250 mL had an insignificant effect.

Ehimen et al. [58], examined the in situ methanolysis of *Chlorella* biomass with a reacting molar alcohol to oil ratio range of 105:1–524:1. These authors showed that as previous studies after an optimum methanol volume (i.e. a molar ratio of methanol to microalgae oil greater than 315:1) no significant changes occured in the yield.

The in situ lipase catalyzed transesterification of *Pistacia chinensis* Bunge seeds and *Jatropha curcas* L. seed with different solvent/seed ratios were investigated by Su et al. 2009 [60]. The highest yields of Pistacia chinensis Bunge methyl ester, *Pistacia chinensis* Bunge ethyl ester, *Jatropha curcas* L. methyl ester and *Jatropha curcas* L. ethyl ester was attained at a solvent/seed ratio of 10 mL/g.

Kasim and Harvey [32], focused on investigating the reactive extraction of *Jatropha curcas* L. seeds in the ranges of 100–600 molar ratio of methanol to oil. The transesterification did not proceed at a molar ratio of 100. The
yield of methyl ester was obtained at 200, 300, 400, 500, 600 were 52%, 74.7%, 81.9%, 85.7% and 86.9%, respectively. When the methanol to oil ratio reached 600, the yield did not change greatly and higher methanol addition might increase the load on downstream separation step. Thus the ratio of 400 was selected as the best condition.

The alkali catalyzed in situ transesterification of rapeseed was investigated by Abo El-Enin et al. [43], to detect the effect of variations in molar ratio of methanol to oil in seeds on the yield. The experiments were conducted with the variation from 360 to 1440 molar ratio of methanol to oil. The yield was 90% when the molar ratio of methanol to oil was very close to 720:1. Despite the yield was higher (96%) at 1440:1 ratio, the ratio of 720:1 was chosen as an optimum because of economic reasons.

Siler-Marinkovic and Tomasevic [55], investigated the production of biodiesel from sunflower seed by reaction with the oil in situ. A good conversion was obtained at a 200:1 molar ratio and after 4 h in the presence of 100% acid catalyst (on oil basis).

As reported by numerous researchers, better yields were obtained when the ratio of methanol to oil was higher than calculated according to stoichiometry of the transesterification reaction [56,68].

Even though the required alcohol volume is higher in reactive extraction process, the possibility of recycling and reusing solvent in the process in a closed loop make it an alternative method for commercial biodiesel production by reducing the process costs [69].

**Effect of temperature on in situ transesterification**

Temperature is one of the key factor for in situ transesterification that has a strong effect on the rate of diffusion and reaction [42]. Various researches have been carried out to find out the effect of temperature on in situ biodiesel production. The studies about the effect of temperature for in situ transesterification are shown in Table 2. As can be seen from the table optimum temperature is specific for each production and the in situ biodiesel production can be achieved at both high and low temperatures [43,62].

**Effect of catalyst on in situ transesterification**

Type and amount of catalyst present in the reaction media are critical parameters. The in situ transesterification reaction does not proceed in the absence of catalyst. Both homogeneous (alkalies and acids) and heterogeneous catalysts can be used for in situ transesterification reaction [11]. Acid catalysts are preferred during the in situ transesterification of high
In situ esterification method

Table 2. Data on optimum temperature for in situ transesterification

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Solvent</th>
<th>Catalyst</th>
<th>Performed Temperatures In The Range(°C)</th>
<th>Optimum Temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castor seed</td>
<td>Methanol</td>
<td>KOH</td>
<td>45-65</td>
<td>55</td>
<td>[15]</td>
</tr>
<tr>
<td>Chlorella pyrenoidosa</td>
<td>Methanol</td>
<td>H₂SO₄</td>
<td>20-110</td>
<td>90</td>
<td>[19]</td>
</tr>
<tr>
<td>Coconut waste</td>
<td>Methanol</td>
<td>KOH</td>
<td>55-65</td>
<td>62</td>
<td>[20]</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>Methanol</td>
<td>NaOH</td>
<td>35-60</td>
<td>40</td>
<td>[21]</td>
</tr>
<tr>
<td>Jatropha curcas L.</td>
<td>Ethanol</td>
<td>CH₂ONa</td>
<td>30-70</td>
<td>30</td>
<td>[29]</td>
</tr>
<tr>
<td>Jatropha curcas L.</td>
<td>Ethanol</td>
<td>NaOH</td>
<td>30-70</td>
<td>30</td>
<td>[30]</td>
</tr>
<tr>
<td>Jatropha curcas L.</td>
<td>Methanol</td>
<td>NaOH</td>
<td>30-60</td>
<td>30</td>
<td>[32]</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>Methanol</td>
<td>NaOH</td>
<td>30-60</td>
<td>30</td>
<td>[42]</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>Methanol</td>
<td>KOH</td>
<td>30-70</td>
<td>65</td>
<td>[43]</td>
</tr>
<tr>
<td>Rice bran</td>
<td>Methanol</td>
<td>H₂SO₄</td>
<td>20-65</td>
<td>65</td>
<td>[7]</td>
</tr>
<tr>
<td>Soy flour</td>
<td>Methanol</td>
<td>CH₂Cl₂</td>
<td>90-120</td>
<td>110</td>
<td>[70]</td>
</tr>
</tbody>
</table>

Acidity raw materials such as rice bran oil and microalgae lipids. The free fatty acid content of reaction medium increased through dissolution and esterification [1]. Acid catalysts provide the rapid esterification of the excess free fatty acids situated in the reaction medium, and also the acid catalysts break down the cell membrane of oil bearing material which can enhance the oil extraction step. Especially, sulfuric acid has been widely used which could convert both triacylglycerols and free fatty acids into fatty acid esters [69].

The effect of catalyst concentration on in situ transesterification of Jatropha curcas L. seeds with ethanol at 70 °C, 600 rpm with five different catalyst concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 wt.% for 2 h was explored by Ginting and Yusup [29]. Sodium methoxide, sodium hydroxide and potassium hydroxide were used as alkaline catalyst to catalyze the transesterification. Even when the sodium methoxide concentration was raised from 2 to 2.5 wt.%, the transesterification rate kept apparently constant. Thus, the required amount of sodium methoxide was 2.0 wt.% and less than the other catalyst (NaOH and KOH).

Kasim and Harvey [32], studied the in situ transesterification of Jatropha curcas L. seeds with and without alkali catalyst. It was demonstrated that the fatty acid methyl ester formation did not proceed without catalyst. The use of 0.15 N NaOH gave higher yield than the 0.1 N (76.2%). After further
increment in NaOH concentration to 0.2 N, the yield decreased from 87.8% to 80.8%. It was reported that the reduction in yield was related to the soap formation which increases the viscosity of the reaction products that agree with Abo El-Enin et al., Pradhan et al. and Zakaria and Harvey, [15,42,43].

Özgül-Yücel and Türkay [7], explored the effect of sulfuric acid catalyst dosage on in situ methanolic esterification of rice bran oil and observed that increasing the amount of sulfuric acid from 5 mL to 7.5 mL and 10 mL did not enhance fatty acid methyl ester yield.

The in situ production of fatty acid methyl ester from rice bran oil with both acid (H2SO4) and alkali (NaOH) catalyst was investigated by Lei et al. [48]. It was reported that the acid catalyzed transesterification rate (47.3%) was lower than alkaline catalyzed (80.4%). As a matter of fact, the use of alkali catalyst is more common in commercial biodiesel production because higher conversion rate can be obtained with no intermediate steps under a low temperature and pressure environment [71]. Also, it is less corrosive and faster than acid catalyzed transesterification [52]. But if the oil contains free fatty acid more than 50-60% which called as high acidity oil, higher yields will be achieved by selection of an acid catalyst.

As a result, the presence of catalyst is essential for biodiesel production. The amount and type of catalyst will influence the ester yields inevitably. The determination of optimum catalyst dosage is very important to achieve high product yields. It is known that at low catalyst dosages sufficient reactive sides may not exist to speedily produce fatty acid methyl esters [70]. On the other hand, lower conversion of triacylglycerol to fatty acid methyl esters occurred due to reverse reactions caused by high catalyst dosage [70].

**Effect of reaction time on in situ transesterification**

The reaction time is an important parameter in the designing of in situ transesterification experiments. Initially, the transesterification reaction is slow. After a time alcohol mixes and disperses into the oil [71], and thus the conversion rate of triacylglycerol to fatty acid methyl esters increases with increment of reaction time. The yield of biodiesel rests relatively constant upon a further increase in reaction time [72]. Furthermore, after an optimum reaction time the yield of biodiesel decreases due to the reverse mechanism of transesterification reactions which results in soap formation.

Qian and Yun [21], studied the effect of reaction time on cottonseed oil conversion by in situ alkaline methanolysis under the condition of methanol to oil ratio of 130:1, and at 40°C. A conversion of 80% was observed after 1 h. The optimum reaction time was determined as 3 h which resulted in 98% conversion.
Tsige et al. [18], observed a common trend on the influence of reaction time (0, 2, 4, 6 or 8 h) for in situ transesterification of *Chlorella vulgaris* microalgae under subcritical conditions. The fatty acid methyl esters yield increased with the increment of the time. The largest amount of biodiesel is obtained in 4 h with about 88.65% yield.

Abo El-Enin et al. [43], presented the influence of reaction time on the alkali in situ transesterification of rapeseed. The results showed that when the reaction time was 1 h, the amount of yield reached 90%. It was determined that the further increment of reaction time from 1 to 3 h did not have any effect on biodiesel yield.

Özgül-Yücel and Türkay [7], studied the in situ esterification of rice bran oil with reaction times of 0.5 h, 1 h, 3 h, 5 h and found that the conversion rate of fatty acid esters increases from 80.5% to 86.9% when the reaction proceeded at 0.5 and 5 h, respectively.

Hailegiorgis et al. [30], transesterified *Jatropha curcas* L. with ethanol in the presence of cetyltrimethylammonium bromide and in the absence of cetyltrimethylammonium bromide at different times ranging from 30 to 240 minute. A cetyltrimethylammonium bromide to NaOH molar ratio of 1, NaOH to seed weight ratio of 0.675, ratio of volume of ethanol to weight of seed of 7.5, reaction temperature of 30°C and 400 rpm stirring were the other reaction conditions. The highest yield of fatty acid ethyl ester (89.2%) obtained at 3 h in the absence of cetyltrimethylammonium bromide and 99.5% yield obtained at 2.5 h in the presence of cetyltrimethylammonium bromide.

Kasim and Harvey [32], reported a comprehensive study on effect of various parameters on reactive extraction of Jatropha seeds for biodiesel production. Six different time (10, 20, 30, 40, 50, 60 minute) were tried to study the effect of time towards in situ transesterification. It was observed that a longer time than 10 minute has a positive effect on the formation of fatty acid methyl esters. The results showed that 30 minute was enough to reach a good methyl ester yield. Reactive extraction of Jatropha seeds with alkali based catalyst takes place in shorter times than acid based as reported by Shuit et al. [35].

It is clear that during the past two decade numerous investigations have been made to determine the optimal time for in situ transesterification. As a result, the optimum time to achieve a good conversion rate is changing due to the reagents and reaction environment. However, it is necessary to determine the appropriate time especially for the industrialization of biodiesel production.

### Effect of stirring on in situ transesterification

In transesterification reactions, stirring avoids clumping and poor mixing, provides adequate contact of biomass-solvent-catalyst, and thus improves the
reaction progress. Stirring can be carried out by use of a mechanical or magnetic stirrer. A number of studies were also reported on the effect of stirring on in situ biodiesel production [15, 18, 20, 30, 32].

As pointed out by Tsigie et al. [18], stirring had a significant effect on transesterification of *Chlorella vulgaris*. It was observed that approximately the same fatty acid methyl ester yield was obtained at 4 h with stirring and at 8 h without stirring. On the other hand, there was an insignificant effect of stirring on fatty acid methyl ester yield at 6 h and 8 h.

Kasim and Harvey [32], investigated the effect of stirring speed on efficiency of *Jatropha curcas*’s reactive extraction. At the lower stirring speeds the methyl ester yield was 37.2 and 85.7 at 100 and 200 rpm, respectively. 300 rpm was found to be the optimum speed, produced the highest yield, at 94.8%. However, further increase of agitation speed did not show a substantial impact on the yield.

Ehimen et al. [58], studied the influence of stirring on the acid catalyst in situ transesterification process as a potentially important economic reaction factor. It was determined that the equilibrium conversion of the *Chlorella* microalgae oil content to biodiesel was influenced by stirring. The biodiesel yield of 87% was obtained with 1 h stirring at 500 rpm.

The reactive extraction of castor seed at different rotations ranging from 100 to 600 rpm was investigated by Pradhan et al. [15]. The optimum mixing intensity was found to be 350 rpm and the results showed that there was not any significant increase in the yield above 350 rpm mixing speed.

**Effect of co-solvent on in situ transesterification**

The co-solvent has a crucial role on in situ transesterification process. The combined use of alcohol and co-solvent has been evaluated to overcome the alcohol’s inability to extract lipid from raw material. By the incorporation of an appropriate co-solvent mass transfer can be improved, the alcohol requirement and extraction time can be reduced [69]. Also, co-solvent assisted in situ transesterification process can be carried out at low temperatures without stirring that demands less energy [61].

Bollin and Viamajala [70], used dichloromethane, ethyl acetate, and chloroform as potential co-solvents for in situ methanolysis of fatty acid methyl esters directly from soy flour with a Lewis acidic 1-ethyl-3-methylimidazolium chloroaluminate ionic liquid. The fatty acid methyl ester yield was lower in the presence of ethyl acetate among the other co-solvents. Dichloromethane was found to be the most suitable co-solvent to reduce the viscosity of the reaction media due to its low toxicity and boiling point which provides easy separation.
Xu and Mi [61], reported the performance of toluene, dichloromethane and diethyl ether co-solvents on alkali catalyzed direct transesterification of *Spirulina* oil. The results showed that use of toluene gave the highest yield, 42.6 %. When the toluene/methanol system, was used the biodiesel yield increased from 42.6 % to 76%.

The effect of different co-solvents (chloroform, hexane and petroleum ether) on the acid catalyzed in-situ methanolysis of *Schizochytrium limacinum* heterotrophic microalgae was investigated by Johnson and Wen [73]. In situ transesterification of microalgae with hexane and petroleum ether gave very low conversion rates 10.5% and 11.1 %, respectively. The highest conversion of oil to fatty acid methyl ester, 72.8% was achieved by use of chloroform.

The conversion of *Chlorella* oil to methyl esters in the presence of n-pentane and diethyl ether was investigated by Ehimen et al. [58]. It was observed that the alcohol requirement reduced from 60 mL to 15 mL to achieve 99% conversion of *Chlorella* oil to fatty acid methyl ester when either diethyl ether or n-pentane was integrated as co-solvent in the reaction medium with ultrasound agitation after a reaction time of 2 h.

As a result, alcohol requirement and mass transfer limitations can be mitigated in the presence of a suitable co-solvent.

**Combined methods with in situ transesterification process**

Researches have investigated alternative biodiesel production methods to overcome the drawbacks of conventional in situ transesterification process. Microwave, supercritical and ultrasound assisted biodiesel production methods are promising alternative processes from an environmental point of view. The details are described below.

**Microwave heating assisted in situ transesterification**

The microwave heating aided transesterification is one of the alternative biodiesel production method. The microwave irradiation produces an efficient internal heating by direct coupling of microwave energy with the molecules (solvents, reagents, catalysts) that are presented in the reaction mixture, thus significantly reduces reaction times and enhances the reaction rate [74].

Many authors [75,76] have been investigated the use of microwave irradiation on in situ fatty acid ester production. The literature results showed that microwave assisted in situ transesterification is an energy efficient and economical route for transesterification of dry algal biomass [75].

The simultaneous extraction and methanolysis of *Nannochloropsis* algae by microwave irradiation (2.45 GHz) in the presence of SrO as a catalyst at
60°C was investigated by Koberg et al. [76] and 99.9% conversion of triglyceride to biodiesel was achieved. The algal biodiesel production from Nannochloropsis sp. by direct methanolysis under the microwave irradiation (800W) with KOH as a homogeneous alkaline catalyst around 60-64°C was studied by Patil et al. and 80.13 fatty acid methyl ester yield was observed [75]. The results showed that microwave-assisted in situ transesterification of dry algal biomass is a fast and simply method.

However, further research studies would be performed to achieve the industrial implementation of this laboratory small-scale application.

**Supercritical fluid assisted in situ transesterification**

Supercritical fluid technology is an alternative processing method that has been applied to biodiesel production to handle long reaction times for high conversion and use of catalyst. This technology also provides reducing the purification operations in which only the evaporation of alcohol and the settling of glycerol is needed [77].

In Kasim’s study [32], in situ transesterification of rice bran and dewaxed-degummed rice bran oil with supercritical methanol and CO₂ as the co-solvent was carried out at 30 MPa and 300°C for 5 min. The purity (52.52%) and yield (51.28%) of fatty acid methyl esters did not high when rice bran oil was reacted. Thus, this method was not suitable for transesterification of rice bran oil. However, the transesterification of dewaxed-degummed rice bran resulted in high purity and yield as 89.25% and 94.84%, respectively.

The effect of reaction temperature (275 or 325 °C), time (60 or 120 min) and ethanol amount (approximately 2-8 w/w ethanol/biomass) on in situ transesterification of lipid rich wet algal biomass (*Chlorella vulgaris*) with supercritical ethanol was investigated by Levine et al. [17]. The results showed that 100% biodiesel yield was obtained at 325 °C and after 120 minute with 6.6% ethanol loading.

Tsigie et al. [18], reported the in situ preparation of fatty acid methyl esters from *Chlorella vulgaris* at 175°C and after 4 h using subcritical water as catalyst with reacting 5 gram biomass and 20 mL methanol. The setup can produce biodiesel with a high yield (0.29 g FAME/g dry biomass) which is better than the theoretical yield (0.20 g FAME/g dry biomass).

The in situ supercritical methanol transesterification of *Jatropha curcas* seeds was investigated by Ani et al. [78]. The yield of fatty acid methyl esters was 97.9% after 30 minute at 280°C and 12.7 MPa, with reacting 1 gram seeds and 40 mL methanol. In supercritical technology, the degradation of
unreacted triglycerides and polyunsaturated methyl esters occurred after postcritical state as can be seen from this study that the yield slightly decreased to 90.9 % at 300°C.

An experimental study had been conducted by Lim and Lee [36] to investigate the effect of solvent/seed ratio (2.5–15.0 mL/g), co-solvent (n-hexane)/seed ratio (0.0–6.0 mL/g), space loading (18.0–90.0 mL/g), temperature (240–320°C), reaction time (0-35 min) and mixing intensity (0-500 rpm) on supercritical reactive extraction of Jatropha curcas with methanol. The highest fatty acid methyl ester yield (99.67%) was obtained at 54.0 mL/g space loading, 5.0 mL/g methanol to seed ratio, 300°C, 9.5 MPa, and without n-hexane after 30 min.

As a consequence, supercritical in situ transesterification process which is an environmentally friendly method may bring many benefits such as shortening the reaction times, simplifying the downstream processes, providing a catalyst free reaction possibility, supplying the reuse and recycling of byproducts. However, this process is energy intensive due to high temperature and pressure requirement. This drawback would be eliminated with an adequate integrated heat recovery system as pointed out in the literature [36, 79, 80]. Thus, supercritical fluid assisted in situ transesterification would be an effective biodiesel production technology.

**Ultrasound assisted in situ transesterification**

Recently, sonochemistry has become more popular due to the applicability of ultrasonic waves in wide range of areas as well as ultrasound assisted transesterification. The use of low frequency ultrasonic irradiation could be advantageous for transesterification which maintains the mechanical energy for mixing and activation energy required to initiate the reaction [81]. Ultrasound assisted transesterification is an efficient and economic method which also provides shorter reaction times, less energy consumption and less alcohol requirement when compared with the conventional transesterification [82]. The ultrasound aided in situ production of fatty acid esters from different species including cotton seed [8], crude palm seed [23], sunflower seed [5], rice bran [50] and Chlorella microalgae lipids [58] has been increasingly investigated.

The methyl ester production from crude palm oil adsorbed on spent bleaching clay by ultrasound (47 kHz) assisted in situ transesterification was achieved at 60 ± 2°C with a methanol to oil molar ratio of 150:1, with the aid of KOH (20%) and 3 mL co-solvent after 2 hour [23]. When petroleum ether and ethyl methyl ketone was used as co-solvent, the methyl ester yield was 75.2 ± 1.7% and 60.0 ± 0.8%, respectively.
Georgogianni et al. [8], compared the effect of ultrasonic irradiation (24 KHz) and mechanical stirring (600 rpm) on alkaline conventional and in situ transesterification of cotton seed oil in the presence of ethanol and methanol. The obtained yields of conventional and in situ transesterification were similar. The yield of methyl esters (95%) were equal by the use of ultrasonication and mechanical stirring. The use of ultrasonication was found superior to mechanical stirring when ethanol was used. The use of ultrasonication resulted in the highest yield (98%) after 40 minute in the presence of ethanol. Only 78% ethyl ester yield was achieved after 4 hour when mechanical stirring was used. Also, in situ transesterification of sunflower seed with using low frequency ultrasonic irradiation (24 kHz) and mechanical stirring (600 rpm) in the presence of ethanol gave similar results. The obtained ethyl ester yields were 98% and 88% by use of ultrasonication after 40 minute and mechanical stirring after 4 h, respectively [5]. Thus, it can be proposed that the use of ultrasonication method for in situ biodiesel production will provide higher yields in a short reaction time.

**Advantages and disadvantages of in situ transesterification**

There are a lot of advantages of using in situ transesterification instead of conventional transesterification as; there is no need to extract oil from the oil bearing seed which will provide to use directly seeds as a raw material. The high acidity oils can be used directly as a raw material. Hence, the refining step that is used to remove excess free fatty acids is eliminated by use of in situ transesterification. Thus, the numbers of unit operations are lesser than conventional production method which shows the simplicity of the method. Also it can be said that direct transesterification process is a time effective method and the required amount of solvent is less due to the reduced unit operations compared to conventional transesterification [40]. These advantages will also provide opportunities for cost reduction.

Noteworthy byproducts were generated from the in situ transesterification process. Cottonseed meal is a protein source for monogastric animals which contains a toxic compound called gossypol. It is necessary to reduce the gossypol content of cottonseed meal to maximum 0.045% according to FAO. Therefore, a refining step is needful for cottonseed to use as an animal meal. The gossypol content of cottonseed can be reduced far below the value of FAO standard by in situ transesterification which will enhance the economic value of cottonseed meal due to improved quality of extracted seeds [21, 22]. Additionally, feedstocks such as soybean and distillers dried grains with soluble can be used as an animal feed product after direct transesterification reaction.
soybeans preserve almost all of its protein content [51]. Thus, the byproduct that can be called as ‘lipid free soy meal’ is an attractive animal feed component which has similar protein content as commercial ones [52].

Hence, these advantages will provide industrial implementation of in situ transesterification method for biodiesel production.

Besides the several advantages of using in situ transesterification method for biodiesel production, the impossibility of using low cost raw materials such as waste cooking oil sand animal fats is a drawback [11].

Conclusion

Currently, the requirement of using renewable alternative energy sources increasing due to the growing energy necessity and environmental pollution problem. Biodiesel is a sustainable, nontoxic and biodegradable biofuel which can be produced using different methods. Among the different methods, the in situ transesterification method may be taken into consideration as a promissory alternative which could facilitate the fatty acid esters production process and provides use of various feedstocks.

Biodiesel have been shown to be effectively produced by in situ transesterification and also, numerous researches have been performed to optimize process conditions for biodiesel production which can be influenced by several factors including alcohol type, molar ratio of alcohol to biomass, catalyst, presence of water, temperature, time and stirring speed. The optimization of process and the coupling of microwave, supercritical and ultrasound processes with in situ transesterification would allow the improvement of a scale-up cost effective biodiesel production.

References

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In situ esterification method