

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Prospects and Potential of Green Fuel from some Non Traditional Seed Oils Used as Biodiesel

Mushtaq Ahmad, Lee Keat Teong,
Muhammad Zafar, Shazia Sultana,
Haleema Sadia and Mir Ajab Khan

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/52031>

1. Introduction

Today's diesel engines require a clean-burning, stable fuel that performs well under a variety of operating conditions. Biodiesel is the only alternative fuel that can be used directly in any existing, unmodified diesel engine. Because it has similar properties to petroleum diesel fuel, biodiesel can be blended in any ratio with petroleum diesel fuel. Many federal and state fleet vehicles in USA are already using biodiesel blends in their existing diesel engines (Harwood, 1981). The low emissions of biodiesel make it an ideal fuel for use in marine areas, national parks and forests, and heavily polluted cities. Biodiesel has many advantages as a transport fuel. For example, biodiesel can be produced from domestically grown oilseed plants. Producing biodiesel from domestic crops reduces the dependence on foreign petroleum, increases agricultural revenue, and creates jobs.

Presently world's energy needs are met through non-renewable resources such as petrochemicals, natural gas and coal. Since the demand and cost of petroleum based fuel is growing rapidly, and if present pattern of consumption continues, these resources will be depleted in near future. It is the need of time to explore alternative sources of fuel energy. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable and easily available. Fatty acid methyl esters derived from renewable sources such as vegetable oils has gained importance as an alternative fuel for diesel engines. The edible oils such as soybean oil in USA, rapeseed oil in Europe and palm oil in countries with tropical climate such as Malaysia are being used for the production of biodiesel (Knothe, 2002).

1.1. Historical background

Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oils and animal fats. It is biodegradable and nontoxic, has low emission profiles and so far is environmentally beneficial (Krawczyk, 1996). Bio-diesel production is not something new, because the concept of using vegetable oil as fuel dates back to 1895. Rudolf Diesel developed the first diesel engine which was run with vegetable oil in 1900. The first engine was run using groundnut oil as fuel (Bijalwan *et al.*, 2006). In 1911, Rudolf Diesel stated that the diesel engine can be fed with vegetable oil and would help considerably in the agricultural development of the countries which use it. In 1912, Rudolf Diesel said, the use of vegetable oils for engine fuels may seem insignificant today. But such oils may become in course of time as important as petroleum and the coal tar products of the present time (Babu and Devarad-jany, 2003). After eight decades, the awareness about environment rose among the people to search for an alternative fuel that could burn with less pollution. Rudolf Diesel's prediction is becoming true today with more and more bio-diesel being used all over the world. With the advent of cheap petroleum, appropriate crude oil fractions were refined to serve as fuel and diesel fuels and diesel engines evolved together. In the 1930s and 1940s vegetable oils were used as diesel fuels from time to time, but usually only in emergency situations. Recently, because of increases in crude oil prices, limited resources of fossil oil and environmental concerns there has been a renewed focus on vegetable oils and animal fats to make biodiesel fuels. Continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by CO₂ (Shay, 1993). In a particular case, such as the emission of pollutants in the closed environments of underground mines, biodiesel fuel has the potential to reduce the level of pollutants and the level of potential or probable carcinogens (Krawczyk, 1996).

Considerable research has been done on vegetable oils as diesel fuel. That research included palm oil, soybean oil, sunflower oil, coconut oil, rapeseed oil and tung oil. Animal fats, although mentioned frequently, have not been studied to the same extent as vegetable oils. Some methods applicable to vegetable oils are not applicable to animal fats because of natural property differences. Oil from algae, bacteria and fungi also has been investigated (Shay, 1993). Terpenes and latexes also were studied as diesel fuels. Microalgae have been examined as a source of methyl ester diesel fuel (Nagel and Lemke, 1990).

1.2. Sources of biodiesel

Alternative diesel fuels are made from natural, renewable sources such as vegetable oil and fats (Ratledge *et al.*, 1985; Lee *et al.*, 1995). There are more than 350 oil-bearing crops identified, among which only soybean, palm, sunflower, safflower, cottonseed, rapeseed and peanut oils are considered as potential alternative fuels for diesel engines (Pryor *et al.*, 1982).

Vegetable oils are promising feedstocks for biodiesel production since they are renewable in nature, and can be produced on a large scale and environmentally friendly (Patil & Deng, 2009). Vegetable oils include edible and non-edible oils. More than 95% of biodiesel production feed stocks come from edible oils since they are mainly produced in many regions and the properties of biodiesel produced from these oils are much suitable to be used as diesel fuel

substitute (Gui *et al.*, 2008). However, it may cause some problems such as the competition with the edible oil market, which increases both the cost of edible oils and biodiesel (Kansedo *et al.*, 2009).

In order to overcome these disadvantages, many researchers are interested in non-edible oils which are not suitable for human consumption because of the presence of some toxic components in the oil. Non edible oil crops can be grown in waste lands that are not suitable for food crops and the cost of cultivation is much lower because these crops can still sustain reasonably high yield without intensive care (Kumar *et al.*, 2007; Gui *et al.*, 2008)

Animal fats contain higher level of saturated fatty acids therefore they are solid at room temperature that may cause problems in the production process. Its cost is also higher than vegetable oils (Singh, 2009). The source of Biodiesel usually depends on the crops amenable to the regional climate. In the United States, soybean oil is the most commonly Biodiesel feedstock, whereas the rapeseed (canola) oil and palm oil are the most common source for Biodiesel, in Europe, and in tropical countries respectively (Knothe, 2002). A suitable source to produce Biodiesel should not compete with other applications that rise prices, for example pharmaceutical raw materials. But the demand for pharmaceutical raw material is lower than for fuel sources. As much as possible the Biodiesel source should fulfill two requirements: low production costs and large production scale. Refined oils have high production costs, but low production scale; on the other side, non-edible seeds, algae and sewerage have low production costs and are more available than refined or recycled oils. The oil percentage and the yield per hectare are important parameters to consider as Biodiesel source.

Algae can grow practically in every place where there is enough sunshine. Some algae can grow in saline water. The most significant difference of algal oil is in the yield and hence its biodiesel yield. According to some estimates, the yield (per acre) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils (Sheehan *et al.*, 1998b).

1.3. Biodiesel production

The seed oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities. Because of these the oil cannot be used as fuel directly. To overcome these problem the oil requires slight chemical modification mainly pyrolysis, microemulsion, dilution and transesterification. Pyrolysis is a method of conversion of one substance into another by mean of heat or by heat with the aid of the catalyst in the absence of air or oxygen (Sonntag, 1979). The process is simple, waste less, pollution free and effective compared with other cracking processes.

The vegetable oil is diluted with petroleum diesel to run the engine. Caterpillar Brazil, in 1980, used pre-combustion chamber engines with the mixture of 10% vegetable oil to maintain total power without any alteration or adjustment to the engine. At that point it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% diesel fuel was successful. Some short-term experiments used up to a 50/50 ratio.

A micro emulsion define as a colloidal equilibrium dispersion of optically isotropic fluid microstructure with dimensions generally into 1–150 range formed spontaneously from two

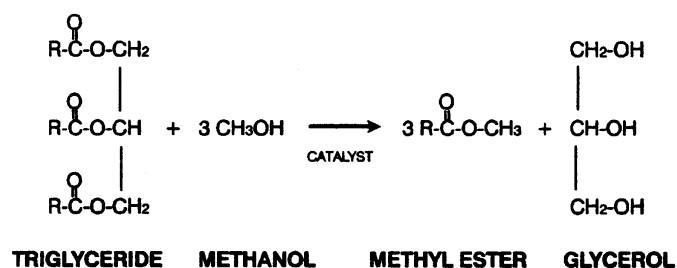


Figure 1. Transestrification Reaction

normally immiscible liquids and one and more ionic or more ionic amphiphiles (Schwab *et al.*, 1988). They can improve spray characteristics by explosive vaporization of the low boiling constituents in micelles (Pryde, 1984). The engine performances were the same for a microemulsion of 53% sunflower oil and the 25% blend of sunflower oil in diesel (Ziejewski *et al.*, 1983). A microemulsion prepared by blending soyabean oil, methanol, and 2-octanol and cetane improver in ratio of 52.7:13.3:33.3:1.0 also passed the 200 h EMA test (Goering, 1984).

Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water. This process has been widely used to reduce the high viscosity of triglycerides. A catalyst is usually used to improve the reaction rate and yield. Excess alcohol is used to shift the equilibrium toward the product because of reversible nature of reaction. For this purpose primary and secondary monohybrid aliphatic alcohols having 1-8 carbon atoms are used (Sprules and Price, 1950).

The main factors affecting transesterification are the alcohol to oil molar ratio, catalyst concentration, reaction temperature and reaction time. The methanol to oil ratio needs to be higher than stoichiometric ratio to drive the equilibrium to a maximum ester yields. The molar ratio is associated with the type of vegetable oil used. Ikwuagwu *et al.*, 2000 stated that molar ratio was 6: 1 for rubber seed oil. It was also undertaken sunflower oil was used (Vicente *et al.*, 2005). Catalysts are classified as alkali, acid and alkali-alcoholic. Transesterification of jojoba oil catalysed with sodium metoxide (Canoira *et al.*, 2006). Sodium hydroxide was also chosen to catalyse the transesterification of rubber seed oil because it is cheaper. Different homogeneous catalysts were used to transesterify sunflower oil to obtain 100% biodiesel yield by using sodium methoxide catalyst (Vicente *et al.*, 2005).

Transesterification consist of a number of consecutive, reversible reactions. It is usually reaction of vegetable or waste oil respectively with a low molecular weight alcohol, such as ethanol and methanol. During this process, the triglyceride molecule from vegetable oil is removed in the form of glycerin. The triglycerides are broken step wise into diglycerides, monoglyceride and finally converted into methyl esters and glycerol (Fig: 1).

There are various types of transesterification that includes based, acid and lipase catalyzed. The petroleum and other fossil fuels contain sulfur, ring molecules & aromatics while the biodiesel molecules are very simple hydrocarbon chains, containing no sulfur, ring molecules or aromatics. Biodiesel is thus essentially free of sulfur and aromatics. Biodiesel is made up of almost 10% oxygen, making it a naturally "oxygenated" fuel (Nouredini & Zhu).



Figure 2. Electric Oil Expeller



Figure 3. Mechanical Oil Expeller



Figure 4. Preparation of Methoxide catalyst



Figure 5. Mixing of catalyst



Figure 6. Separation of glycerin from Biodiesel



Figure 7. Biodiesel Filtration



Figure 8. Milk Thistle Flower



Figure 9. Milk Thistle Seeds

1.4. Biodiesel Scenario at Global Level

Use of bio-diesel is catching up all over the world especially in developed countries.

- In **Malaysia**, the tropical climate encourages production of bio-diesel from palm oil (Meher *et al.*, 2006; Lam and Lee, 2011).
- The **United States** is contributing 25% of the world green house gases: i.e., oil and coal. We also need to reorganize its 70% of oil consumption is in transportation. The cost of bio-diesel is \$3.00 a gallon. With the tax subsidy available in the law now, it could be sold for about \$1.80. It is clearly known that the future depends on bio-fuels as replacement for fossil fuels. At present, USA uses 50 million gallons and **European countries** use 350 million gallons of bio-diesel annually. It is mixed with 20% of bio-diesel in fossil diesel.
- **France** is the country which uses 50% of bio-diesel mixed with diesel fuel.
- In **Zimbabwe**, 4 million jatropha has been planted in 2000 ha by the end of 1997.
- In **Nicaragua**, one million *Jatropha curcas* has been planted in 1000 ha. The harvest of pods reached 333000 tonnes in the 5th year with a seed of 5000 tonnes and the oil extracted was approximately 1600 tonnes per annum.
- In **Nepal**, 22.5 ha of area are planted with 40,000 rooted cuttings of *Jatropha curcas*. The rural women co-operative have been trained to extract oil, produce soap and use 30:70 mix (oil/kerosene) of oil and kerosene in stove without smoke (Bijalwan *et al.*, 2006; Paramathama *et al.*, 2007).

1.5. Non traditional seed oils

Various plant resources either edible or non-edible are used for biodiesel production. While in this study three oil seed plants i.e. wild safflower, safflower and milk thistle belonging to family Asteraceae were selected as non edible oil feed stocks for biodiesel production. These oil seeds are considered as nontraditional energy crops as these are un commonly cultivated and mainly found as weed. The raw material (crude oil) from these oil seeds can be used as a feed stock for biodiesel production.

***Carthamus oxyacantha* M. Bieb.**

Carthamus oxyacantha M. B. (Figure 9) is a spiny-leaved annual herb up to 1.5 m tall commonly known as Wild Safflower. It is a hardy and xerophytic noxious weed of winter crops. Like other spiny plants in the genus *Carthamus*, this species is not eaten by livestock, enabling it to spread on grazing lands. It also competes with and reduces the yield of cereal crops. It is a valuable source of non edible and drying oil (28-29% oil content) from waste lands (Deshpande, 1952). However, it was almost eradicated through regular campaigns due to noxious weedy nature. Fruit an achene, obovate or elliptic, 3–5.5 mm long, 2–3.5 mm wide, 1.5–2 mm thick, truncate at apex, marginal notch at base, cross sectional outline broadly elliptic to slightly 4-sided. Glabrous, smooth and glossy, bone-white to ivory, less frequently beige, with densely distributed blotches and speckles in shades of brown. Scar subbasal, an outlined, diamond-shaped cavity containing a rough, vertical ridge. Pappus early deciduous, absent. Apex a

round, rough, flat to uneven area, surrounded by irregularly edged black ring; style base deciduous. Embryo spatulate, cotyledons broad; endosperm absent. It is widely distributed in Afghanistan, Azerbaijan, India, Iran, Iraq, Kyrgyzstan, Pakistan, Tajikistan, Turkmenistan. *Carthamus oxycantha* seeds yields two types of oils: oleic oil and linoleic oil. Fatty acid oil composition of oleic oil is, palmitic acid 5-6%, stearic acid 1.5 -2%, oleic acid 74-80%, linoleic acid 13-18% and traces of linoleic acid and longer chain fatty acids. The fatty acid composition of linoleic oil, palmitic acid 5-8%, stearic acid 2-3%, oleic acid 8-30%, linoleic acid 67-89% and also traces of linoleic acid and longer chain fatty acids. *Carthamus oxycantha* fruit also contains proteins 20-25%, hull 60%, residual fat 2-15%. Flowers of *Carthamus oxycantha* contain two major pigments, the water soluble, yellow carthamidin and the formally important dye carthamin, flavonone which is orange red (Fernandez-Martinez *et al.*, 1993; Anjani, 2005). Flowers also contain 0.3-0.6% carthamin. Flavonoids, glycosides, sterols and serotonin derivatives have been identified from flowers and seeds (Figure 10) (Firestone, 1999). Two new glycosides, 2-O-methylglucopyranosyl-carthamoside and beta-D-fructofuranosyl carthamoside, along with the known compound 3', 4', 5, 7-tetrahydroxyflavanone have been isolated from *Carthamus oxycantha* using recycling preparative HPLC. The structures of these compounds were established by mass spectrometric and extensive spectroscopic analysis (Hassan *et al.*, 2010). This oil seed plant is commonly found as noxious weed after harvesting of cash crop wheat. Throughout the world due to its weedy nature it is generally burnt after wheat harvesting, while in this study it is targeted as energy crop for biodiesel production.



Figure 10. Wild Safflower



Figure 11. Wild Safflower seeds

***Carthamus tinctorious* L. (Safflower)**

Safflower is cultivated nontraditional seed oil crop which contains a higher percentage of essential unsaturated fatty acids and a lower percentage of saturated fatty acids than other vegetable seed oils. The oil, light colored and easily clarified, is used in liqueurs, candles, and as a drying oil in paints, linoleum, varnishes, and wax cloths. The flowers (Figure 11) have been the source of yellow and red dyes, largely replaced by synthetics, but still used in rouge. Annual thistle-like herb, branching above with a strong central stem to 1-2 m tall; leaves spiny, oblong or lanceolate, the upper ones clasping, minutely spinose-toothed; flowers in 1-6 heads per plant, 3-4.5 cm across, each head developing 20-60 seeds; corollas yellow, orange, white or red, surrounded by a cluster of leafy spiny bracts, which pass over gradually into the bracts of the involucre; achenes (fruits or seeds) (Figure 12) white, 7-9 mm long, shining. Many cultivars have been developed differing in flower color, degree of spininess, head size, oil content, resistance to disease and ease of harvest. Most common varieties have yellow or orange flowers, but red and white flowered varieties are known. Reported from the Central Asian and Near Eastern Center of Diversity, safflower thereof is reported to tolerate bacteria, disease, drought, frost, fungus, high pH, phage, salt, sand, rust, virus, wind, and wild. Wu and Jain (1977) discuss germplasm diversity in the World Collections of Safflower. ($2n = 24, 32$). Believed to have originated in southern Asia and is known to have been cultivated in China, India, Pakistan, Persia and Egypt almost from prehistoric times. During Middle Ages it was cultivated in Italy, France, and Spain, and soon after discovery of America, the Spanish took it to Mexico and then to Venezuela and Colombia. It was introduced into United States in 1925

from the Mediterranean region and is now grown in all parts west of 100th meridian. Safflower grows in the temperate zone in areas where wheat and barley do well, and grows slowly during periods of cool short days in early part of season. Seedlings can withstand temperatures lower than many species; however, varieties differ greatly in their tolerance to frost; in general, frost damages budding and flowering thus reducing yields and quality. It thrives in heavy clays with good waterholding capacity, but will grow satisfactorily in deep sandy or clay loams with good drainage, and needs soil moisture from planting through flowering. Soils approaching neutral pH are best (Duke, 1978, 1979). Propagation is by seed, which are usually pretreated with insecticides and fungicides. Same machinery used for small grains may be used for planting, cultivation and harvesting. Seed should be planted in a soil prepared and completely free of weeds, when the soil temperature is about 4.4°C and the upper 10 cm of soil is moist. Seed germinates quickly at 15.5°C. Safflower matures in from 110-160 days from planting to harvest as a spring crop, as most of it is grown, and from 200 or more days as fall crop. It should be harvested when the plant is thoroughly dried. Since the seeds do not shatter easily, it may be harvested by direct combining. The crop is allowed to dry in the fields before threshing. Its average yields are 1,900 kg/ha, but yields above 4,500 kg/ha are not uncommon; in the Great Plains yields run about 850 kg/ha (C.S.I.R., 1948-1976). The world low production yield was 244 kg/ha in Israel, the international production yield was 789 kg/ha, and the world high production yield was 1,900 kg/ha in U.S.A. Yields higher than 4,000 kg/ha have been attained. Oil yields approach 50%, leaving a meal with ca 21% protein, 35% fiber, and 1-3% fat. Safflower is self-pollinated with some cross-pollination. Pollen and nectaries are abundant with insect working the flowers. Safflower is attacked by many fungi: *Alternaria carthami* (leaf spot and bud rot), *A. zinniae*, *Bremia lactucae*, *Cercospora carthami*, *Cercospora carthami*, *Carthamus tinctorius* is only cultivated species of genus *Carthamus* commonly known as Safflower. It is cultivated since ancient times for not only the dye obtained from its flowers and medicinal uses but also for its seed oil and ornamental purposes.

***Silybum marianum* (L.) Gaertner**

Milk thistle (*Silybum marianum* Gaertn.) (Figure 7) is a winter annual or a biennial. Its current distribution includes most temperate areas of the world. It is a broad-leaved species belonging to Asteraceae that reaches a height of 200–250 cm. Milk thistle is grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina but it has been reported as a noxious weed in many other countries. Stems glabrous or slightly tomentose. Leaves: basal wing-petioled, blades 15–60 cm, margins coarsely lobed; cauline leaves clasping, progressively smaller and less divided, bases spiny, coiled, auriculate. Phyllary appendages spreading, ovate, 1–4 cm including long-tapered spine tips. Corollas 26–35 mm; tubes 13–25 mm, throats campanulate, 2–3 mm, lobes 5–9 mm. Cypselae brown and black spotted, 6–8 mm; pappus scales 15–20 mm. $2n = 34$. *Silybum marianum* is sometimes cultivated as an ornamental, a minor vegetable, or as a medicinal herb. Young shoots can be boiled and eaten like cabbage and young leaves can be added to salads. The seeds (Figure 8) can be used as a coffee substitute. Extracts of *S. marianum* are used as an herbal treatment for liver ailments. Milk thistle is toxic to livestock when consumed in large quantities, and it forms dense stands in pastures and rangelands. California reports up to 4 tons per acre in heavily infested areas. The leaves are very distinctive,



Figure 12. Safflower



Figure 13. Safflower seeds

with white marbling on the shiny green leaves. An annual or biennial, found in rough pasture, on grassy banks, in hedgerows and on waste ground. It is locally well-established and persistent, especially in coastal habitats in S. England, but is also a widespread casual. Lowland. Native of the Mediterranean region; naturalised or casual throughout much of

Europe and in N. America and Australia. The plant grows wild in Egypt on canal banks and in wet ground regions in the Nile Valley. The soil supporting this plant is fine-textured and moist. It occurs in two types, the most abundant has purple flowers while the least abundant has white flowers (Ahmad et al., 2008). Milk thistle is commonly found as a noxious weed in waste land and in along with cultivated field of traditional crops. In this project this energy crop was first time reported as a feed stock for biodiesel production at global perspectives.

In this project these species were selected for biodiesel potential at global interest as renewable energy because of their oil which is non edible and species found as weeds on waste and marginal lands. The study conducted with aims to extract the seed oils from these resources for production of biodiesel through base catalyzed transesterification. Study may also confined to quality standards of biodiesel obtained from these species according to ASTM standards.

2. Methodology

The oil from these three resources was extracted by two methods;

1. Chemical method (Soxhlet Apparatus)
2. Mechanical method (Electric oil expeller) (Figure 1-2)

The oil seeds were oven-dried at 40°C over night and then ground with blender. 250ml of petroleum ether was poured into round bottom flask. Five gram of the sample was placed in the thimble and inserted in the centre of the extractor. The Soxhlet was heated at 60°C. When the solvent was boiling, the vapour rises through the vertical tube into the condenser at the top. The condensed liquid drips into the filter paper thimble in the centre, which contains the solid sample to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This process was allowed to continue for 3-4 hrs. Distinct layers of oil and petroleum ether appeared in round bottom flask. In this process of oil extraction, the solvent was recovered and reused. The resulting mixture containing oil was heated to evaporate solvent and weighed again to determine the amount of oil extracted. (AOAC, 1990). While in electric oil expelling method oil was extracted mechanically from seeds by using electric oil expeller (KEK P0015, 10127) and crude oil was collected in beakers for further processing. After an average of 5-6 turns, the oil is fully extracted from seeds.

2.1. Determination of free fatty acid number of seed oils

Free fatty acid content of oil seeds was determined by aqueous acid-base titration (Trajkovic *et al.*, 1983). Two types of titration were performed i.e. blank titration and sample titration. In case of blank titration 0.14 g KOH was dissolved in 100 ml of distilled water to prepare 0.025M KOH solution and this solution was poured in burette. 10 ml of isopropyl alcohol and 2-3 drops of phenolphthalein were mixed in a conical flask and titrate it against 0.025 M KOH from burette until the color of solution became pink. Note the volume of KOH used. This was repeated three times to calculate mean volume of KOH used for blank titration. While in sample titration 9 ml

isopropyl alcohol, 1 ml of wild safflower oil and 2-3 drops of phenolphthalein were taken in conical flask and titrate against 0.025M KOH from burette until end point i.e pink color appeared. Note the volume of KOH used and three readings were taken by repeating the same experiment to calculate the mean volume of KOH used to titrate the sample.

$$\text{Acid number} = (A-B) \times C/D$$

A = Volume used in Sample/Actual titration, B = Volume used in Blank titration

C = Mass of Catalyst in g/l, D = Volume of oil used

2.2. Biodiesel synthesis

The method used for synthesis of fatty acid methyl esters (Biodiesel) from crude oil was alkali catalyzed transesterification (Ahmad *et al.*, 2010) (Figure 3,4,5 &6). There are numerous transesterification citations in the scientific and patent literature (Bradshaw and Meuly, 1944; Freedman *et al.*, 1984; Freedman *et al.*, 1986; Schwab *et al.*, 1987; Allen *et al.*, 1945; Trent, 1945; Tanaka *et al.*, 1981; Wimmer, 1992b; Ma *et al.*, 1998a; Ma *et al.* 1998b; and Ma *et al.* 1999). Crude oil contains impurities which could affect the quality, yield and process of transesterification. The filtration of crude oil was done by using whattmann paper NO: 42 (See Plate 1). The filtered oil was heated up to 125 °C on hot plate (VELP Scientifica F20520166) in order to decompose triglycerides into monoglycerides and diglycerides. Transesterification of one liter oil (Plate 2) was carried out for the production of methyl esters by using different alkali catalysts (Ahmad *et al.*, 2011). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) were used as catalyst. A specific amount of each alkali hydroxide (6.3 g for one liter oil) was added to methanol (200 ml) to make alkali methoxide which was used as a catalyst in reaction. The prepared methoxide was added to oil at 65°C and stirred for 35-40 min at 600 rpm.

After stirring the reaction mixture was kept overnight at room temperature to settle down distinct layers i.e. upper thin layer of soap, middle layer of FAME (fatty acid methyl ester) and the bottom dense layer of glycerin. These layers were then separated through separating glass funnel. Biodiesel washing is done with ordinary tap water in order to remove impurities and suspended particles. 3-4 washings were performed for complete clearance of biodiesel. Few drops of acetic acid were also added. The residual water was eliminated by treatment with anhydrous sodium sulphate (Na₂SO₄) followed by filtration.

3. Results and discussion

Catalyst	Wild Safflower	Safflower	Milk Thistle
NaOH	2.74	1.75	2.32
KOH	2.81	1.82	2.46

Table 1. Determination of FFA (%) contents through aqueous acid base titration

Catalyst	Catalyst concentration (g)	Wild Safflower (%)			Safflower (%)			Milk Thistle (%)		
		Biodiesel	Glycerin	Soap	Biodiesel	Glycerin	Soap	Biodiesel	Glycerin	Soap
NaOH	6.3	88	12	0	82	17	1	84	15	1
KOH	6.3	80	20	0	78	20	2	82	18	0

Table 2. yield of biodiesel and by-products by using various catalysts

Fuel Properties	Method	Wild Safflower	Safflower	Milk Thistle	HSD
Color	ASTM D-1500	2	2	2	2.0
Density @40°C Kg/L	ASTM D-1298	0.8980	0.8623	0.8990	0.8343
Kinematic Viscosity @ 40°C c St	ASTM D-445	6.45	6.13	6.23	4.223
Sulphur % wt	ASTM D-4294	0.1103	0.00041	0.0123	0.05
Total Acid No. mg KOH/gm	ASTM D-664	0.14	0.63	0.92	0.8
Flash Point °C (PMCC)	ASTM D-93	110	80	92	60-80
Pour Point °C	ASTM D-97	-12	-9	-6	-35 to -15
Distillation @ 90% recovery °C	ASTM D-86	358	352	354	360.4
Cloud Point °C	ASTM-2500	+15	+9	+7	-15 to 5
Calorific Value BTU/LB	ASTM-240	16977	16566	16472	20,400
Cetane Index	ASTM-976	50	52	51	46
Phosphorus % wt.	ASTM D-6728	-	-	-	-

Table 3. Fuel Properties of Biodiesel (B100) in comparison with HSD

The major share of all energy consumed worldwide comes from fossil sources (petroleum, coal and natural gas). However these sources are limited, and will be exhausted by the near future. Thus looking for alternative sources of new and renewable energy such as biomass is of vital importance. Alternative and renewable fuels have the potential to solve many of the current social problems and concerns, from air pollution to global warming to other environmental improvements and sustainability issues (MacLeana and Laveb, 2003).

During the decade of 1930s and 1940s, neat vegetable oils were used in diesel engines under an emergency situation (Ma and Hanna, 1999). Currently, most of the biodiesel is produced from the edible or vegetable oils using methanol and an alkaline catalyst such as sunflower

(Vicente *et al.*, 2004), canola (Singh *et al.*, 2006), palm (Darnoko and Cheryman, 2000; Cheng *et al.*, 2004), soybean oil (Encinar *et al.*, 2005) and waste vegetable oils (Felizardo *et al.*, 2006; Dorado *et al.*, 2002; Cetinkaya and Karaosmanolu, 2004). However, large amount of non-edible oils and fats are available such as safflower (Meka *et al.*, 2007), Pongame (Ahmad *et al.*, 2009), Sesame (Ahmad *et al.*, 2011), and tigernut oil (Ugheoke *et al.*, 2007) have been intensively investigated as potential low priced biodiesel sources. This study supports the production of biodiesel from non edible seed oils i.e. (wild safflower, safflower and milk thistle oil biodiesels as a viable sources of alternative to the diesel fuel.

3.1. FAMES production

The percentage conversion of oil to biodiesel with NaOH and KOH at 65°C is given in table 2. The results illustrated the greater oil to FAMES conversion with NaOH as compared to KOH. The most common way to produce biodiesel is transesterification reaction in which triglycerides react with an alcohol to produce fatty acid mono-alkyl esters (Biodiesel) and glycerol. Methanol is the most common alcohol because of its low price compared to other alcohols. This reaction is referred as methanolysis. Generally transesterification is catalysed by a basic or an acid catalyst. However, the basic catalysts are the most commonly used in industry, because the process proves faster and the reaction conditions are moderated (Freedman *et al.*, 1984; Reid, 1911). In this project biodiesel was synthesized from wild safflower, safflower and milk thistle oil by base (NaOH and KOH) catalyzed transesterification with methanol. Most studies of the basic-catalysed transesterification of vegetable oils involve the calculations of the triglyceride conversion rate and the changes in product composition during reaction (Feuge and Gros, 1949; Freedman *et al.*, 1984, 1986; Schwab *et al.*, 1987; Peterson *et al.*, 1991; Mittelbach and Trathnigg, 1990; Chang *et al.*, 1996; Mittelbach, 1996; Coteron *et al.*, 1997; Boocock *et al.*, 1998; Nouredini *et al.*, 1998; Vicente *et al.*, 1998; Darnoko and Cheryan, 2000).

3.2. Fuel properties

Biodiesel is characterized by their viscosity, density, cetane number, cloud and pour points, calorific value, distillation range, flash point, ash content, sulfur content, acid value, and phosphorus contents. These parameters are specified through the ASTM (American Standard Testing Methods) standards. This standard identifies the parameters the pure biodiesel (B100) must meet before being used as a pure fuel or being blended with petroleum-based diesel fuel. The properties of these oils methyl esters (B100) are given in table 3. These values are in the close range and comparable with high speed diesel (HSD).

The viscosity difference forms the basis of an analytical method, i.e. viscometry, applied to determine the conversion of vegetable oil to methyl ester. The viscosity difference between the componential triacylglycerols of vegetable oils and their corresponding methyl esters resulting from transesterification is approximately one digit (Knothe, 2001). Kinematic viscosity has been included in biodiesel standards (1.9-6.0 mm²/s in ASTM D6751 and 3.5-5.0 mm²/s in EN 14214) (Knothe, 2005). The viscosity of these oil biodiesel were near to ASTM standards. Biodiesels have a viscosity close to that of diesel fuels. As the oil temperature increases its viscosity decreases (Sarin & Sharma, 2007). The lower the

viscosity of the biodiesel, the easier it is to pump and atomize and achieve finer droplets (Goodrum, 2007). The calorific value of edible and non-edible methyl ester was lower than that of diesel because of their oxygen content. The presence of oxygen in the biodiesel helps for a complete combustion of the fuels in the engine (Pramanik, 2003). Calorific value of these oil biodiesel were comparable to ASTM standard. The cetane number is one of the most commonly cited indicators of diesel fuel quality. It measures the readiness of the fuel to auto-ignite when injected into the engine. It is generally dependent on the composition of the fuel and can impact the engine's startability, noise level, and exhaust emissions. Cetane index of these three species were also in accordance with ASTM standards. The higher the cetane number, the more efficient the ignition is. Because of the higher oxygen content, biodiesel has a higher cetane number as compared to petroleum diesel. (Arjun *et al.*, 2008). Flash point is the important temperature specified for safety during transport, storage, and handling (Krisnangkura, 1992). Flash point of these oil methyl esters were found to be higher as compared to HSD. The flash point of bio-diesel is higher than the petro-diesel, which is safe for transport purpose. The ASTM standard for total acid number for pure biodiesel is 0.8 mg KOH/g. The TAN or acid value is the total amount of potassium hydroxide necessary to neutralize the free acids in biodiesel sample (Arjun *et al.*, 2008). Higher acid number could also cause degradation of rubber parts in older engines resulting in filter clogging. The test result for the total acid number of these oil biodiesel were found to be ideal (Guo & Leung, 2003). Two important parameters for low-temperature applications of a fuel are cloud point (CP) and pour point (PP). The CP is the temperature at which wax first becomes visible when the fuel is cooled. The cloud point of methyl ester produced from these oils were found to be in accordance with ASTM standards. The PP is the temperature at which the amount of wax from solution is sufficient to gel the fuel; thus it is the lowest temperature at which the fuel can flow. The pour point of methyl ester produced from these oil were near to HSD. Biodiesel has a higher CP and PP compared to conventional diesel. The cloud points were affected by the presence of monoglycerides, however, the pour points were not affected. Moreover, the *cis* double bond present in the erucic acid of rapeseed oil hampered the lowering of the pour point of esters. The type of fatty acid branched chain available in the original oil has an impact on the pour point (Lee *et al.*, 1995). Biodiesel contains virtually trace amount of sulfur, so SO₂ emissions are reduced in direct proportion to the petrodiesel replacement (Demirbas, 2007). Sulphur contents in these three oil yielding plants were very low as compared to HSD.

4. Conclusion and recommendations

Based on above findings these three species of family Asteraceae have higher potential as a raw material source for biodiesel production at global interest and application. Following are some key recommendations which might be useful for production of raw material availability, production and consumption of biodiesel at global perspective;

1. In all developed countries, research and development has always played a vital role in profitable development of industry. In developed and some developing countries more and more R & D activities are being sponsored by the private sector and their Governments are assisting them and taking part in these activities by way of tax incentives and award schemes.
2. It is recommended that policies should be designed and incentives be offered by government to develop biodiesel companies and industries in the country.
3. Serious consideration should be given to establish a mega tree plantation for production of oil seeds in biodiesel application.
4. It is recommended that production of biodiesel to final use by consumer, quality should be given priority. Number of strategies should be given importance such as collection of seeds, extractions, processing, handling, storage and marketing. Therefore positive inspection system for all these sectors including agriculture, private sector and farming system.
5. In view of the present study as presented in this issue about the economic importance of national plants resources used for biodiesel production, research, development and cultivation efforts should be focused on these plants and identified other resources.
6. These three species are fast growing but cultivated on a small scale by rural farmers, could be produced on large scale for consumption and to be used as fuel. These species are more economical and need minimal quantity of water, fertilizer and pesticides. Such type of study on plant resources will make their data readily available for identifying promising species for future consideration for cultivation of biodiesel yielding crops.
7. It is proposed to further extend the project of bio-diesel. There is need to establish pilot projects to commercialize bio-diesel and set up its supply chain. The project may be extended step wise like conversion of vehicle fleets of designated departments on bio-diesel.

List of abbreviations

ASTM = American Society for Testing and Materials

FA = Fatty Acid

FAME = Fatty Acid Methyl Esters

FFA = Free Fatty Acid

EM = Engine Manufacturing Association

Author details

Mushtaq Ahmad^{1*}, Lee Keat Teong², Muhammad Zafar¹, Shazia Sultana¹, Haleema Sadia¹ and Mir Ajab Khan¹

*Address all correspondence to: mushtaqflora@hotmail.com

1 Biofuel Lab., Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan

2 School of Chemical Engineering, University of Sains Malaysia, Malaysia

References

- [1] Ahmad, M., A. Khan, M. Zafar and S. Sultana. 2010. Environment friendly renewable energy from Sesame biodiesel. *Energy Sources, Part A*. 32(2): 189-196.
- [2] Ahmad, M., K. Ullah, M. A. Khan, S. Ali, M. Zafar and S. Sultana. 2011. Quantitative and qualitative analysis of sesame oil biodiesel. *Energy Sources, Part A*. 33: 1239-1249.
- [3] Ahmad, M., M. A. Khan, A. Hasan, M. Zafar, and S. Sultana. 2008. Chemotaxonomic standardization of herbal drugs Milk thistle and Globe thistle. *Asian J. of Chem.* 6(20): 4443-4459.
- [4] Ahmad, M., M. Zafar, M. A. Khan and S. Sultana. 2009. *Pongamia pinnata* as a biodiesel resource in Pakistan. *Energy Sources, Part A*. 31: 1436- 1442.
- [5] Allen, H. D., G. Rock and W. A. Kline. 1945. Process for treating fats and fatty oils. US Patent, 2: 383-579.
- [6] Anjani, (2005). Genetic variability and character association in wild safflower (*Carthamus oxycantha*).
- [7] AOAC, 1990. Official methods of analysis, (13th edition). Association of Official Analytical Chemists. Washington, DC.
- [8] Arjun, B., L. K. Chhetri, C. Watts & M. R. Islam. 2008. Waste Cooking Oil as an Alternate Feedstock for Biodiesel Production. *Energies*. 1: 3-18.
- [9] Bijalwan, A., C. M. Sharma and V. K. Kediya. 2006. Bio-diesel revolution. *Science Reporter*, January 2006. pp: 14-17.
- [10] Boocock, D.G.B., S. K. Konar, V. Mao, C. Lee and S. Buligan. 1998. Fast formation of high-purity methyl esters from vegetable oils. *J. Am. Oil Chem. Soc.*, 75 (9): 1167-1172.
- [11] Bradshaw, G. B. and W. C. Meuly. 1994. Preparation of detergents. US Patent, 2: 360-844.
- [12] C.S.I.R. (Council of Scientific and Industrial Research). 1948-1976. The wealth of India. 11 vols. New Delhi.

- [13] Canoira, L., R. Alcantara, M. J. Garcia-Martinez and J. Carrasco. 2006. Biodiesel from Jojoba oil-wax: transesterification with methanol and properties as a fuel. *Biomass and Bioenergy*. 30: 76-81.
- [14] carthamosides from *Carthamus oxycantha*. *Natural Product Communication*. 5(3), 419-422.
- [15] Cetinkaya, M. and F. Karaosmanolu. 2004. Optimization of base-catalyzed transesterification reaction of used cooking oil. *Energ. Fuel*. 18 (6): 1888-1895.
- [16] Chang, D.Y. Z., J. H. Van Gerpen, I. Lee, L. A. Johnson, E. J. Hammond and S. J. Marley. 1996. Fuel properties and emissions of soybean oil esters as diesel fuel. *J. Am. Oil Chem. Soc.*, 73 (11): 1549–1555.
- [17] Cheng, S. F., Y. M. Choo, A. N. Ma and C.H. Chuah. 2004. Kinetics study on transesterification of Palm oil. *J. Oil Palm Res.*, 16 (2): 19-29.
- [18] Coteron, A., G. Vicente, M. Martinez and J. Aracil. 1997. Biodiesel production from vegetable oils. Influence of catalysts and operating conditions. In: Pandalai, S.G. (Ed.), Recent Res. Developments in Oil Chemistry, vol. 1. Transworld Research Network, India, pp. 109–114.
- [19] Darnoko, D. and M. Cheryan, 2000. Continuous production of palm methyl esters. *J. Am. Oil Chem. Soc.*, 77 (12): 1269–1272.
- [20] Deshpande, R. B. 1952. Wild safflower (*Carthamus oxyacantha*)- A possible oilseed crop for the desert and arid regions. *Indian J. Genet.*, 12: 10-14.
- [21] Dorado, M. P., E. Ballesteros, J. A. de Almeida, C. Schellert, H. P. Löhrlein and R. Krause. 2002. Transesterification of karanja (*Pongamia pinnata*) oil by solid basic catalysts. *Am. Soc. Agr. Biol. Eng.*, 45 (3): 525-529.
- [22] Duke, J.A. 1978. The quest for tolerant germplasm. p. 1-61. In: ASA Special Symposium 32, Crop tolerance to suboptimal land conditions. Am. Soc. Agron. Madison, WI.
- [23] Encinar, J. M., F. Juan, J. F. Gonzalez, and A. Rodriguez-Reinares. 2005. Biodiesel from used frying oil: Variables affecting the yields and characteristics of the biodiesel. *Ind. Eng. Chem. Res.*, 44 (15): 5491-5499.
- [24] Felizardo, P., M. J. Correia, I. Raposo, J. F. Mendes, R. Berkemeier and J. M. Bordado. 2006. Production of biodiesel from waste frying oils. *Waste Manage.*, 26 (5): 487-494.
- [25] Fernandez-Martinez, M., Del-Rio, M. and de Haro, A. (1993). Survey of safflower (*Carthamus oxycantha* L.) germplasm for variants in fatty acid composition and other seed characters. *Euphytica*. 69,115-122.
- [26] Feuge, R.O. and A. T. Gros. 1949. Modification of vegetables oils. VII. Alkali-catalyzed interestification of peanut oil with ethanol. *J. Am. Oil Chem. Soc.* 26 (3): 97–102.
- [27] Firestone, D. (1999). Physical and chemical characteristics of oils, fats, and waxes. AOCS Press, Champaign, United States. pp 152-153.

- [28] Freedman, B., E. H. Pryde and T. L. Mounts. 1984. Variables affecting the yields of fatty esters from transesterified vegetable oils. *J. Am. Oil Chem. Soc.*, 61(10): 1638–1643.
- [29] Freedman, B., Kwolek, W. F., and E. H. Pryde. 1986. Quantitation in the analysis of transesterified soybean oil by capillary gas chromatography. *J. Am. Oil Chem. Soc.*, 63: 1370–1375.
- [30] Goodrum, J.W. 2002. Volatility and boiling points of biodiesel from vegetable oils and tallow. *Biomass Bioenergy*. 22: 205–211.
- [31] Gui, M. M., K. T. Lee and S. Bhatia. 2008. Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. *Energy*. 33: 1646–1653.
- [32] Guo, Y. and Y. C. Leung. 2003. Analysis on the biodiesel production using grease trap oils from restaurants, Macro Review. Japan, *Jpn. Macro Eng. Soc.*, 16: 421-426.
- [33] Hassan, Z., Ahmed, V.U., Hussain Z.J., Zahoor A, Siddiqui, I.N., Rasool Z.M.. (2010). Two new
- [34] Ikwuagwu, O. E., I. C. Ononogbu and O. U. Njoku. 2000. Production of biodiesel using rubber, *Hevea Brasiliensis* (kunth. Muell.) seed oil. *Ind. Crops and Product*. 12: 57-62.
- [35] *Indian Journal of Agricultural Science*. 75(8), 516-518.
- [36] Kansedo, J., K. T. Lee and S. Bhatia. 2009. *Cerbera odollam* (sea mango) oil as a promising non-edible feedstock for biodiesel production. *Fuel*. 88: 1148–1150.
- [37] Knothe, G. 2000. Monitoring a progressing transesterification reaction by fiber optic NIR with correlation to ¹H NMR spectroscopy. *J. Am. Oil Chem. Soc.*, 77: 489–493.
- [38] Knothe, G. 2001. Analytical methods used in the production and fuel quality assessment of biodiesel. *Am. Soc. Agr. Eng.*, 44 (2): 193-200.
- [39] Knothe, G. 2002. Current perspectives on biodiesel. *INFORM.*, 13: 900–903.
- [40] Knothe, G. 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process. Tech.*, 86: 1059-1070.
- [41] Krawczyk, T. 1996. Biodiesel as alternative fuel makes in roads but hurdles remain. *INFORM.*, 7: 801-829.
- [42] Kumar, A., A. Tiwari and H. Raheman. 2007. Biodiesel production from jatropha oil (*Jatropha curcas*) with high free fatty acids: an optimized process. *Biomass and Bioenergy*. 31: 569–575.
- [43] Lam, M.K. and Lee, K. T., “Renewable and sustainable bioenergies production from palm oil mill effluent (POME): Win–win strategies toward better environmental protection”, *Biotechnology Advances*, Elsevier, Vol. 29 (1) 2011, pp 124-141.
- [44] Lee, I., L. A. Johnson and E. G. Hammond. 1995. Use of branched-chain esters to reduce the crystallization temperature of Biodiesel. *JAOCS.*, 72: 1155–60.

- [45] Ma, F. and M. A. Hanna. 1999. Biodiesel production: a review. *Bioresource Technology*. 70: 1-15.
- [46] Ma, F., L. D. Clements and M. A. Hanna. 1998a. The effects of catalyst free fatty acids and water on transesterification of beef tallow. *Trans. ASAE.*, 41: 1261-1264.
- [47] Ma, F., L. D. Clements and M. A. Hanna. 1998b. Biodiesel fuel from animal fat. Ancillary studies on transesterification of beef tallow. *Ind. Eng. Chem Res.*, 37: 3768-3771.
- [48] Ma, F., L. D. Clements and M. A. Hanna. 1999. The effects of mixing on transesterification of beef tallow. *Bioresource Technology*. 69: 289-293.
- [49] MacLeana, H. and L. B. Laveb. 2003. Evaluating automobile fuel propulsion system technologies. *Prog. Energy Combust Sci.* 29: 1-69.
- [50] Meher, L. C., S. Vidya, S. Dharmagadda and S. N. Naik. 2006. Optimization of alkali catalyzed transesterification of *Pongamia pinnata* oil for production of biodiesel. *Bioresour Technol.*, 97: 1392-1397.
- [51] Meka, P. K., V. Tripathi and R. P. Singh. 2007. Synthesis of biodiesel fuel from safflower oil using various reaction parameters. *J. Oleo Sci.*, 56(1): 9-12.
- [52] Mittelbach, M. and B. Trathnigg. 1990. Kinetics of alkaline catalyzed methanolysis of sunflower oil. *Fat Sci. Technol.*, 92 (4): 145-148.
- [53] Nagel, N. and P. Lemke. 1990. Production of methyl fuel from microalgae. *Appl. Biochem. Biotechnol.* 24: 355-361. Calvin, M. 1985. Fuel oils from higher plants. *Ann. Proc. Phytochem. Soc. Eur.* 26: 147-160.
- [54] Nouredini, H. and Zhu, D. 1997. Kinetics of transesterification of soybean oil. *J. Am. Oil Chem. Soc.* 74: 1457-1463.
- [55] Paramathma, M., P. Venkatachalam and A. S. Rajan. 2007. Need for jatropha cultivation and bio-diesel production, We need to move to a biofuel future. *BioSpectr J.*, pp: 30-32.
- [56] Patil, P. D. and S. Deng. 2009. Optimization of biodiesel production from edible and non edible vegetable oils. *Fuel*. 88: 1302-1306.
- [57] Peterson, C.L., M. Feldman, R. Korus and D. L. Auld. 1991. Batch type transesterification process for winter rape oil. *Appl. Eng. Agric.*, 7 (6): 711-716.
- [58] Pramanik, K. 2003. Properties and use of *Jatropha curcas* oil and diesel fuel blends in compression ignition engine. *Renewable Energy*. 28: 239- 248.
- [59] Pryde, E. H. 1984. Vegetable oils as fuel alternatives—symposium overview. *JAOCS*. 61: 1609-1610.
- [60] Pryor, R.W., M. A. Hanna, J. L. Schinstock and L. L. Bashford. 1982. Soybean oil fuel in a small diesel engine. *Transactions of the ASAE*. 26: 333-338.
- [61] Ratledge, S. and C. A. Boulton. 1985. Fats and oils. In: *Comparative Biotechnology in industry, agriculture and medicine*, New York: Pergamon Press, vol. 3. pp: 983-1003.

- [62] Sarin, R. and M. Sharma. 2007. Jatropha Palm biodiesel blends: An optimum mix for Asia. *FUEL*. Vol. 86: 1365-1371.
- [63] Schwab, A. W., G. J. Dykstra, E. Selke, S. C. Sorenson and E. H. Pryde. 1988. Diesel fuel from thermal decomposition of soybean oil. *JAOCs*. 65: 1781-1786.
- [64] Shay, E. G. 1993. Diesel fuel from vegetable oils: status and opportunities. *Biomass and Bioenergy*. 4: 227-242.
- [65] Sheehan, J., T. Dunahay, J. Benemann and P. Roessler. 1998b. A look back at the US Department of Energy's aquatic species program—biodiesel from Algae. National Renewable Energy Laboratory (NREL) Report: NREL/TP-580-24190, Golden, CO.
- [66] Singh, A. B. H., J. Thompson and J. V. Gerpen. 2006. Process optimization of biodiesel production using different alkaline catalysts. *Appl. Eng. Agric.*, 22 (4): 597-600.
- [67] Sonntag, N.O.V. 1979a. Structure and composition of fats and oils. Bailey's industrial oil and fat products, vol. 1, 4th edition, Swern, D. John Wiley and Sons, New York, p. 1-14.
- [68] Tanaka, Y., A. Okabe and S. Ando. 1981. Method for the preparation of a lower alkyl ester of fatty acids. US Patent, 4: 305-590.
- [69] Trent, W. R. 1945. Process of treating fatty glycerides. US Patent, 2: 383-632.
- [70] Ugheoke, B. I., D. O. Patrick, H. M. Kefas and E.O. Onche. 2007. Determination of optimal catalyst concentration for maximum biodiesel yield from tigernut (*Cyperus Esculentus*) oil. *Leonardo J. Sci.*, 10: 131-136.
- [71] Vicente, G., A. Coteron, M. Marti'nez and J. Aracil. 1998. Application of the factorial design of experiments and response surface methodology to optimize biodiesel production. *Ind. Crops Pro.*, 8 (1): 29-35.
- [72] Wimmer, T. 1992b. Preparation of ester of fatty acids with short-chain alcohols. [In Austrian], pp: 349-571.
- [73] Wu, K.K. and Jain, S.K. 1977. A note on germplasm diversity in the world collections of safflower. *Econ. Bot.* 31:72-75.
- [74] Ziejewski, M. Z., K. R. Kaufman and G. L. Pratt. 1983. Vegetable oil as diesel fuel, USDA. *Agric. Rev. Man.*, ARM-NC-28. pp: 106-111.