

Research Article

Spectroscopic Analysis of Structural Transformation in Biodiesel Degradation

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Abstract: The vegetable oil, fats and their biodiesel suffer with the drawback of deterioration of its quality during long term storage unlike petroleum diesel. The oxidation and thermal stability of two biodiesels of different origins, viz. palm oil derived biodiesel and used cooking oil based biodiesel were analyzed. The structural transformation of Fatty Acid Methyl Ester (FAME) of the biodiesels was analyzed by an infrared spectrometer and an ultraviolet absorption spectrometer. The infrared spectra of the samples were recorded by FTIR spectroscopy. The absorbance values of the spectrum bands were observed and it was determined that some of the chemical groups of oxidized oils caused changes in absorbance. The spectroscopic data of degraded biodiesel suggested oxidative polymerization. The results demonstrated that the oxidation behavior of biodiesels of different origins was closely related to the composition and distribution of FAMES. Higher concentration of unsaturated FAME with multi-double bonds exhibited poorer oxidation resistance. In this study, in order to increase the stability of biodiesel, against oxidation process during the storage and distribution, different percentages (0.02, 0.05, 0.1 and 0.2% (w/v), respectively) of caffeic acid, were added as natural antioxidants. The antioxidant effect increased with concentration up to an optimal level. Above the optimal level, the increase in antioxidant effect with its concentration was relatively small.

Keywords: Antioxidants, FTIR, microwave irradiation, oxidative and thermal degradation, structural transformation, used frying oil

INTRODUCTION

Biodiesel, also commonly referred to as Fatty Acid Methyl Esters (FAME), is a renewable fuel produced from new and used animal and vegetable oil via a process of transesterification (Kalam and Masjuki, 2002; Demirbas, 2002, 2007). Biodiesel is continuously gaining attention and significance as an alternative diesel fuel. It is virtually, similar to conventional petrodiesel in terms of the fuel properties and is competitive with petro-diesel and offers a number of advantages over petro-diesel such as enhanced biodegradability, reduced toxicity, lower emissions and increased lubricity (Knothe, 2005). It could be used to replace at least a portion of the diesel fuel consumed worldwide. The finished biodiesel can only be used in ignition engine when the quality of the product is guaranteed. However, biodiesel has been reported to have some performance disadvantages (McCormick *et al.*, 2007; Geller *et al.*, 2008; Haseeb *et al.*, 2010; Fazal *et al.*, 2010, 2011). Most vegetable oils and animal fats are triacylglycerols with long-chain fatty acid groups attached by ester linkages to a glycerol backbone. Biodiesel derived from rapeseed, soybean or palm oil is made up of six main fatty acid alkyl esters: palmitic acid, C16:0, palmitoleic acid, C16:1, stearic acid,

C18:0, oleic acid, C18:1, linoleic acid, C18:2 and linolenic acid, C18:3. Biodiesel derived from microalgae also contains a variety of other FAME types with generally longer chain lengths and higher levels of unsaturation. Fuels with higher proportions of polyunsaturated organic compounds are significantly more reactive to oxidation than monounsaturated which in turn are more unstable than saturated compounds. For instance, in linoleic acids, polyunsaturated fatty acid chains of linoleic acid contain two (C18:2) double bonds, while linolenic acids contain three (C18:3) double bonds. As a result, undesirable products like gums, organic acids and aldehydes, formed as a result of degradation, may cause injector and filter blockages resulting in engine problems (Chen and Luo, 2011). Acid value and kinematic viscosity, however, are two facile methods for rapid assessment of biodiesel fuel quality as they continuously increase with deteriorating fuel quality. The oxidative stability of biodiesel is also worse than the oxidative stability of diesel. Currently, the biodiesel used in automobiles is employed in mixtures from B5 to B20 (diesel with 5 and 20% vol. of biodiesel, respectively) depending on the location. Due to problems associated with the compatibility of automotive parts, higher percentages of biodiesel have not been used. The corrosive effects of biodiesel are

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mainly caused by the presence of water and free fatty acids (Haseeb *et al.*, 2010). In vehicles, the fuel is in direct contact with different parts of the engine, such as the fuel pump, fuel injector, pistons and piston rings. The incompatibility of copper, aluminum, zinc, brass and bronze with biodiesel has been proven (Kaul *et al.*, 2007; Haseeb *et al.*, 2010; Fazal *et al.*, 2011). In fact, biodiesel has been shown to cause swelling of nitrile rubber (Frame and McCormick, 2005; Trakarnpruk and Porntangjitlikit, 2008; Haseeb *et al.*, 2011).

A number of mechanisms have been proposed detailing how biodiesel can undergo oxidation. The primary mechanism, the auto-oxidation, proceeds with the formation of a radical hydrocarbon species on the bis-allylic carbon. The double bonds will isomerize into a conjugated structure. This radical can then react with oxygen to form a peroxide species propagating the reaction. The peroxides are thought to break down into oxygenated intermediates which further degrade into small chain acids, ketones, alkenes and aldehydes (Knothe, 2007; Bannister *et al.*, 2011). The peroxide species' can also form dimers and oligomers via peroxy, ether or carbon-carbon bonds (Monyem *et al.*, 2000). Recently, further evidence has suggested that the propagating species is not a mono substituted hydro peroxide but an oligomeric species with more than one peroxide linker which propagates the reaction by rapidly breaking down into aldehydes, acids and a radical alcohol (Morita and Tokita, 2006). This is not the only reaction path discussed however and it is thought that a saturated and unsaturated aldehyde can be formed on the decomposition of a biodiesel chain with more than one peroxide group (Schneider *et al.*, 2008).

There are various methods for measuring the quality of finished biodiesel, the commonest one is the use of Gas Chromatography (GC) based on ASTM and EN standards, but the main shortcoming of Gas chromatographic method is that it consumes time in sample preparation which involve the derivatization of fuel sample prior to GC analysis (Zagonel and Peralta, 2004).

Spectroscopy is another common technique used to evaluate biodiesel properties as well as to monitor the transesterification reaction. Among the techniques used are ^1H and ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transformed Infrared (FTIR) and Ultraviolet (UV) spectroscopy (Lewis *et al.*, 2004; Knothe, 2006). FTIR spectroscopy may be used to verify the biodiesel degradation products resulting from accelerated oxidation, evaluating the size of the carbonyl bands. Biodiesel oxidation may present several degradation mechanisms, like reverse transesterification, secondary products and carbonyl compounds formation (Knothe, 2007).

Most vegetable oils and animal fats are triacylglycerols with long-chain fatty acid groups

attached by ester linkages to a glycerol backbone. Depending on the origin of the vegetable oil, the biodiesel derived from such feed stocks will contain a low (10-15%) to relatively high (70-80%) concentration of unsaturated long-chain fatty acid alkyl esters. Unsaturated organic compounds are significantly more reactive to oxidation than saturated compounds. Therefore biodiesel can be easily oxidized during storage, negatively affecting fuel quality and thus engine performance. Maintaining fuel quality of biodiesel for widespread use will depend on the development of technologies to increase its resistance to oxidation during long-term storage. Used frying oil, which is much less expensive than pure vegetable oil, is a promising alternative to vegetable oil for biodiesel production. The quantity of waste cooking oil generated per year by any country is huge. Deep-fat frying is a widely used procedure for the preparation of food. During deep-fat frying, fats and oils are continuously or repeatedly heated at high temperatures (up to 190°C) for prolonged periods of time in the presence of air. Under these conditions both thermal and oxidative reactions of the oils may occur.

Maintaining fuel quality of biodiesel for widespread use will depend on the development of technologies to increase its resistance to oxidation during long-term storage. As a result, oxidation prevention of biodiesel has in recent years attracted considerable attention, mainly focusing on the oxidation evaluation methods (Jain and Sharma, 2011; Bannister *et al.*, 2012) and the oxidation inhibiting technologies (Caramit *et al.*, 2013; Kivevele and Mbarawa, 2011; Moser, 2012). Investigations on oxidation mechanism of biodiesel derived from used frying oil, especially the structural transformation during biodiesel oxidation, are scarcely reported. It is indeed important to understand the behavior of structural transformation of biodiesel in order to better improve the fuel quality and thus promote practical applications of biodiesel. Natural antioxidant extracted from plant is renewable, safe and is widely used in food industry. So far, few works about the natural additives stabilizing biodiesel were reported. In this study, the structural transformation during oxidation and thermal degradation of biodiesel derived from Malaysian palm oil and used frying cooking oil (predominantly palm oil) were analyzed by IR spectroscopy and peroxide value changes. In this study, natural antioxidant caffeic acid, an antioxidant abundantly found in plants was added into both, the palm oil and the used frying oil derived biodiesel to estimate how effective these additives are to protect the biodiesel from oxidation. Degradation of both used and fresh palm oil derived biodiesel with or without antioxidants was investigated by IR spectroscopy and the changes using peroxide values. Primary products of oxidation are detectable on the basis of determination of peroxide value or

conjugated fatty acids, polyunsaturated fatty acids exhibiting pairs of unsaturated carbons not separated by at least one saturated carbon. The present investigation on the thermal and oxidative stability of biodiesel synthesized from used frying oil will give a better understanding on the quality of biodiesel and to promote wider applications of biodiesel derived from used frying oil.

METHODOLOGY

Materials: Waste Frying Oil (WFO) in Malaysia is routinely collected mostly from the major food outlets and manufacturers. In this study waste cooking oil was collected from a chip cracker potatoes factory in the state of Selangor in Malaysia. All the WFO samples collected were of similar quality having the acid value of between 3.2-3.6 mgKOH/g oil. Fresh Malaysian palm cooking oil was purchased from a local supermarket having the same brand as the one used by the chip cracker factory. The physio-chemical properties were determined as shown in Table 1. The values were determined by established standard methods.

All chemicals were of Analytical grade (AR) and purchased from Sigma Aldrich, Malaysia. The natural antioxidant used was Caffeic acid (3, 4-Dihydroxycinnamic acid) (98%); a natural dietary phenolic compound found in plants that is an antioxidant, was purchased from Sigma-Aldrich (M) Sdn. Bhd.

Synthesis of biodiesel: The biodiesel synthesis was carried out using the previously mentioned method with slight modification (Saifuddin and Chua, 2004). Typically the biodiesel samples were prepared by dissolving by first dissolving 1.25 g of sodium hydroxide (as catalyst) in 72 g ethanol. The solid catalyst was dried using microwave oven for 1 min at high power (output power, 750 Watts), then quickly added to the ethanol and was vigorously stirred with slight heating until completely dissolved. This mixture was added in the 250 mL of fresh palm cooking oil or waste palm frying oil and the resultant volume was vigorously mixed using a magnetic stirrer for 10 min. The reaction mixture was microwave irradiated for 3 min at a power of 400 W. The reaction mixture was agitated and after 1 min at room temperature the reaction mixture was again irradiated with microwave

for 3 min at 400 W. This step was repeated again for total of 4 cycles of irradiation. After the fourth cycle, the mixture was poured into a separation funnel and due to difference of absolute density; the biodiesel was separated of the glycerin (major by-product). The top ester layer (biodiesel) was poured into another cylinder. Water was sprayed into the top of the cylinder at a low velocity. The excess alcohol and catalyst were removed by the water as it percolated through the cylinder. Microwave heating at maximum power (750 Watt) for 2-3 min was again used to speed up the separation process. After microwave irradiation the cylinder was left to stand at room temperature for 10 min. After 10 min the water phase containing the un-reacted alcohol and catalyst would settle, leaving a clear ester (biodiesel) phase on top. Both sample of biodiesel (from used frying oil and fresh Malaysia palm oil) were analyzed by FTIR spectroscopy.

Sample oxidation test and peroxide value: Determination of the oxidative states (peroxide value) was performed by the oven storage test.

The biodiesel samples were placed in a 100 mL vessel of glass, kept at a constant temperature 70°C, in the presence of air atmosphere (21% oxygen) at flow rate of 200 mL/min.

For stability investigation of samples treated with various percentages of caffeic acid, a rapid and reliable method, such as oven test in which samples are kept at 25-80°C (preferably 70°C) in an oven, repeatedly controlling the peroxide value. The intermediate compounds formed during the oxidation of lipids may react further to form compounds that can cause rancidity. Unsaturated fatty acids in oils are able to react with oxygen and produce peroxide on their double bonds, which is quantified with the iodimetry method. Determination of peroxide value was done according to AOCS Cd 8b-90. The biodiesel samples after oxidation were also analyzed by FTIR spectroscopy.

Structural analysis of biodiesel: The structures of biodiesel (fresh palm oil biodiesel and waste palm frying oil biodiesel) before and after oxidation were characterized by a Fourier Transform Infrared (FTIR) spectrometer. The structural transformation of used frying oil biodiesel and fresh palm oil biodiesel before and after oxidation was evaluated from the increase of the area of the carbonyl band in a FTIR spectrum.

The FTIR spectra measurements were performed using the Shimadzu FTIR spectrophotometer-model

Table 1: Physiochemical properties of waste and fresh Malaysian palm frying oil

Properties	Waste frying oil	Fresh palm frying oil
Acid value (mg KOH/g oil)	2.830±0.03	0.870±0.02
Saponification value (mg KOH/g oil)	113.550±0.36	83.700±0.42
Iodine value (g iodine/100 g oil)	33.680±0.53	48.250±0.91
Density at 25°C (g/mL)	0.927±0.07	0.899±0.03
Kinematic viscosity at 40°C (mm ² /sec)	54.130±0.13	41.210±0.13
Moisture content (wt%)	1.200	0.110
Flash point (°C)	161-164	222-224

Values are expressed as mean of three replicates ±S.D.

IRPrestige-21 (Shimadzu Corporation Japan) equipped with temperature controlled DLATGS (deuterated, L-alanine doped triglycine sulfate) detector. The scan settings were set as follows; resolution: 4 cm^{-1} , accumulation: 20 scans, measurement mode: Transmittance (%T), wave number 4000 to 650 cm^{-1} . In a typical analysis, a drop of oil sample (using a Pasteur pipette) from various samples was deposited on the surface of the horizontal Attenuated Total Reflectance (ATR) crystal disc (Diamond Type II crystal) at controlled ambient temperature (23°C). A background measurement of air spectrum was performed. Spectra were processed using IRsolution-window based software version 1.4 (Shimadzu). After every scan, a new reference air background spectrum was taken. After each sample, the oil was removed with dry tissue and the surface of crystal disc was washed with acetone and finally it was dried and cleaned with tissue.

Thermal stressing experiments: In the thermal stressing experiments, the biodiesel fuels were heated in a thermal bath. In our experiments a convection oven with temperature range from 60-200°C was used the heat treatment. During the high temperature experiments, the biodiesel fuel was immersed in the sand bath that provides excellent heat transfer and temperature uniformity. The sand bath was heated to required temperatures. Thermal degradation experiments of biodiesel samples were performed at 60 to 200°C for a residence time from 6 to 72 h.

Formulations antioxidant/biodiesel: The biodiesel from Malaysian palm oil and the used frying obtained as mentioned earlier, were added with additives caffeic acid, an antioxidants at the concentration of 0.02, 0.05, 0.1 and 0.2%, respectively by simple mixing.

RESULTS AND DISCUSSION

The basic constituent of vegetable oils is triglyceride. Figure 1 shows a typical triglyceride molecule. Vegetable oils comprise 90 to 98% triglycerides and small amounts of mono- and diglycerides. Triglycerides are esters of three fatty acids and one glycerol. Fatty acids vary in their carbon chain length and in the number of double bonds. During frying process, oils are subjected to three agents that contributing to modify their structure: the water released by the food (hydrolytic effect); the oxygen of the atmosphere (oxidative effect); and finally the high temperature (thermal effect) (Encinar *et al.*, 2005). The Table 1 shows the physiochemical properties of fresh palm cooking oil and waste palm cooking oil as determined by the already established methods.

Vegetable oils undergo changes in terms of chemical and physical properties when they interact with the food or atmosphere. From Table 1, it can be

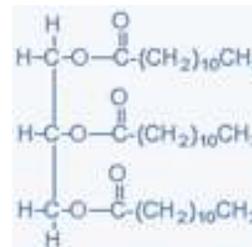


Fig. 1: Structure of a typical triglyceride

seen that waste frying oil exhibited slightly poorer properties relative to fresh frying oil in terms of FFA, PV, saponification value and iodine value due to oil deterioration during excessive frying. The heat, air and moisture to which the oil is exposed lead to oxidation and hydrolysis in the oil. Vegetable oils contain natural antioxidants such as tocopherols, phenolic compounds and carotenoids which provide protection against the oxidation reaction. The oxidative stability of the oils is mostly based on these compounds (Bashir *et al.*, 2004; Boskou, 2006). One of the unique characteristics of palm oil is its high content of carotenoids and tocopherols, both of which contribute to the stability of palm oil. It is oxidatively stable due to a fatty acid composition which is low in polyunsaturation and high antioxidant content (Ming *et al.*, 2009).

The FTIR spectroscopy provides information about the functional groups in molecules and the structure of the molecules based on energies associated with molecular vibration. The previously reported spectral evaluation of palm oil and extra virgin olive oil is shown Table 2 (Rohman and Che Man, 2010). From the present study, in a first view, the FTIR spectra (Fig. 2) of Waste Frying Oil (WFO) and fresh frying oil shows similar spectra features and this can be related to the same principle component (triglyceride) in both samples. Most of the peaks and shoulders seen in the spectrum of WFO and the fresh frying oil are attributed to the specific functional group vibrations as reported in Table 2, which are similar to the other previous reports (Rohman and Che Man, 2010; Kumar and Singh, 2012). Although both the sample show similar spectra features, there are some important features to note. Interesting point to note are the existence of slight differences in terms of band intensities around 3007 cm^{-1} , assigned to the (= C-H) stretching vibration of the *cis*-carbon-carbon double bond. The height of the 3007 cm^{-1} band for the waste cooking oil is noticeably smaller than it is for the fresh frying oil. This corresponds to the *cis* vinyl = C-H stretching. It is well known that with heating process (during the cooking) the *cis* configuration is converted into *trans*. This effect is indicated by the decrease in the peak at 3007 cm^{-1} . The 2922 and 2851 cm^{-1} band is attributed to the symmetric stretching of the aliphatic CH₂ group. The heights of both these peaks were also slightly smaller (although not as distinct as the 3007 cm^{-1}) in the waste

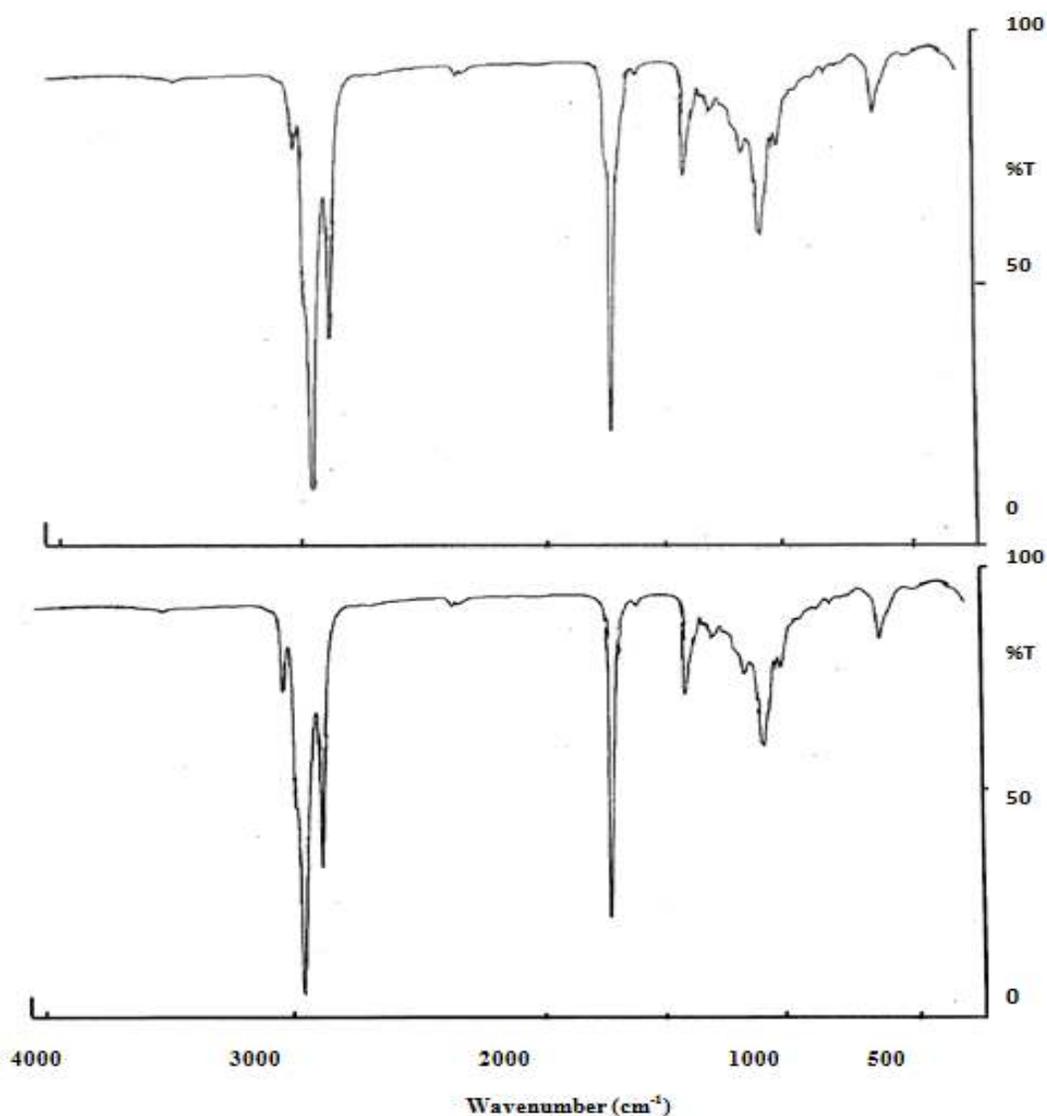


Fig. 2: FTIR spectra of (top): waste cooking palm oil and (bottom): fresh palm cooking oil

Table 2: Functional groups and modes of vibration in the spectrum of extra virgin olive oil and palm oil

Frequency (cm ⁻¹)	Functional group assignment
3007	cis double-bond stretching
2924 and 2852	Asymmetrical and symmetrical stretching vibration of methylene (CH ₂) group
1744	Ester carbonyl functional group of the triglycerides
1465	Bending vibrations of the CH ₂ and CH ₃ aliphatic groups
1417	Rocking vibrations of CH bonds of cis-disubstitutedolefins
1402	= C-H bending vibration
1377	Bending vibrations of CH ₂ groups
1236 and 1160	C-O stretching
1117 and 1098	Stretching vibration of the C-O ester group
1030	C-O stretching
962	Bending vibration of CH functional groups of isolated transolefin
850	= CH ₂ wagging
721	Overlapping of the methylene (-CH ₂) rocking vibration and to the out of plane vibration of cis-disubstitutedolefins

Rohman and Che Man (2010)

frying oil than those in fresh frying palm oil. It can be concluded that these minor changes are brought about by oxidation process due to heating. Another interesting point to note is the slight change in the spectral in the C-

O region (~1744 cm⁻¹). Here, the study shows a slight widening of the band for the waste frying oil sample. This observation can be attributed to production of saturated aldehyde functional groups or other secondary

oxidation products that will also have an absorbance at 1728 cm^{-1} , which overlaps with the stretching vibration at 1744 cm^{-1} of the ester carbonyl functional group of the triglycerides. When new carbonyls are formed, their maximum peaks are in the region between 1700 and 1726 cm^{-1} , hence resulting in a widening of the 1743 cm^{-1} band. Similar observation have been reported previously by Kumar and Singh (2012) which reported that there was increase in carbonyl compound after sample of olive oil were heated to 200°C for 30 min, which resulted in slight widening of the 1743 cm^{-1} . Besides that it can be said that the FTIR spectra of both used palm cooking oil and fresh palm cooking oil have high degree of similarity showing almost unappreciable variations either in the frequency or band intensities. Consequently, all absorption bands observed in the IR spectrum are in agreement with the structure of a triacylglycerol bearing unsaturated fatty acids in its structure. These findings suggest that no significant structural changes have been produced in the palm cooking oil after using it for frying except for the minor few changes as indicated earlier.

Differences related to the oxidative stability of oil from various origins have been reported (Guillén and Ruiz, 2005). The differences in the stability could be attributed to quantity of unsaturated fatty acids with multi-double bonds in the oil. Bis-allylic hydrogens in linoleic and linolenic fatty acids are susceptible to free radical attacks, peroxide formation and production of polar oxidation products (Erhan *et al.*, 2006). Concerns about possible problems caused by biodiesel on engines were often assigned to high iodine values although studies have shown that biodiesel stability depends, besides the degree of unsaturation, on the content of antioxidants and the production technology used. The

presence of antioxidant components, play an important role in the oxidation rate of the oils. Several published work has shown that oxidative stability of many oils was affected by their fatty acid profiles and tocopherol (γ - and δ -) and tocotrienol contents of the oil (Miraliakbari and Shahidi, 2008; Azadmard-Damirchi and Dutta, 2008; Madawala *et al.*, 2012). Study by Knothe (2007) have shown that esters of polyunsaturated fatty acids like linolenate and linoleate methyl esters have higher oxidative rate than that of monounsaturated fatty acid like oleate methyl esters. In a comparison study of oxidative stability of some biodiesel, Zuleta *et al.* (2012) reported that the biodiesel made from palm oil has the highest induction time (25 h) as compared with biodiesel from sunflower (0.8 h), soybean (1.3 h), corn (1.2 h), rapeseed (9.1 h) and olive oil (3.3 h). Accordingly, the Malaysian palm oil is very stable edible oil because of its low polyunsaturated fatty acid composition (49% saturated, 39% monounsaturated and 10% polyunsaturated) and high content of natural antioxidant, the tocopherol and tocotrienol (vitamin E).

Analysis of structural changes of biodiesel after oxidation by FTIR and peroxide value:

The biodiesel susceptibility to oxidation is mainly due to the double bonds existing in the polyunsaturated fatty acid chains (content of linoleic and linolenic acids). Palm oil biodiesel contains only 9% of Linoleic and Linolenic acids (polyunsaturated). This is similar to the polyunsaturated acid content in olive oil. Among the vegetable oils, both of these oils are among the lowest in terms of polyunsaturated content. Fatty acid methyl esters of animal origin are less stable for oxidation than fatty acid methyl esters of vegetable origin. This

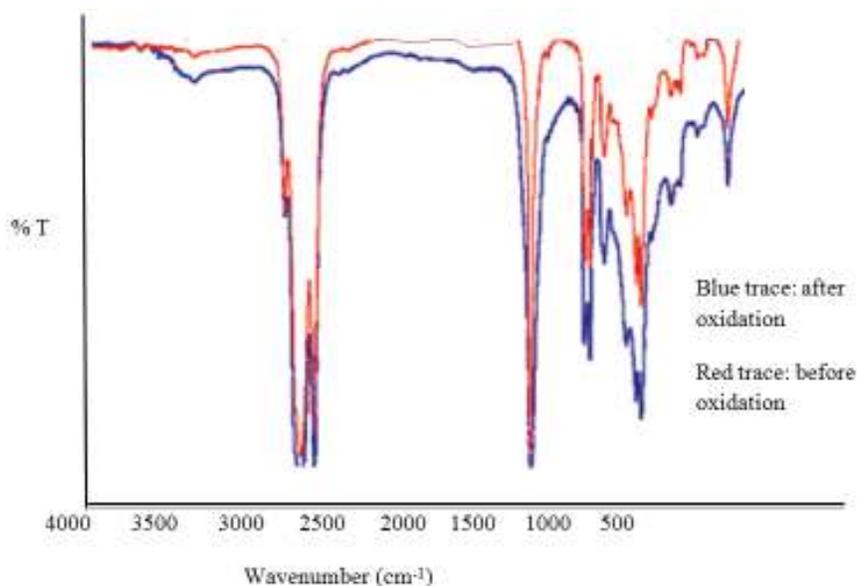


Fig. 3: FTIR spectrum of biodiesel from fresh palm frying oil before and after oxidation

difference may be explained by the absence of natural antioxidants in fatty acid methyl esters of animal fat (Sendzikiene *et al.*, 2005). Figure 3 shows the FTIR spectra of Palm oil methyl ester (biodiesel). In a typical FTIR spectrum of biodiesel from vegetable oils (spectrum in Fig. 3, before oxidation), the characteristic peaks found include the strongest peaks at 1750 cm^{-1} (the C = O vibration), the C-O ester vibrations of approximately $1170\text{--}1200\text{ cm}^{-1}$ and a signal at 1435 cm^{-1} , which is the methylester group (-O-CH₃) with its deformation vibration (Conceição *et al.*, 2007; Ivanoiu *et al.*, 2011). In addition, peaks that indicate the double bond (3000 and 725 cm^{-1}), will be prominent in the spectra. Figure 3 also shows the FTIR spectra of Palm oil methyl ester (biodiesel) after oxidation. Upon degradation, the bands at approximately 3000 cm^{-1} (HC = CH stretching) and 725 cm^{-1} (out-of-plane bending) will show a slight decrease in the intensity, indicating decrease in unsaturation. As discussed before, this band is corresponding to the *cis* double-bond (= CH) stretching vibration therefore its intensity is affected by the number of *cis* double bonds present. Thus, the changes observed indicate a decrease in the number of *cis* double-bond, therefore a decrease in degree of unsaturation as a consequence of oxidation process (Conceição *et al.*, 2007).

As oxidation proceeds, the concentration of hydroperoxides increases. The increase in concentration of hydroperoxides can be reflected by increase band intensity at 3470 cm^{-1} (Guillén and Ruiz, 2005). A slight increase of the peak area was found at $1700\text{--}1750\text{ cm}^{-1}$, which might be resulted from the oxidation decomposition products of -OOH radicals, such as

ketone and aldehyde. At $980\text{--}960\text{ cm}^{-1}$, the increase in the peak intensity of C = C *trans*-double bond structure could also be observed. The results indicated that *cis-trans* isomerization transformation occurred in unsaturated FAME molecules during oxidation, which could be verified through variations of *cis-trans* transmittance values of biodiesel before and after oxidation. As can be seen from the FTIR spectrum (Fig. 4) *cis-trans* variation is not large and this can be used as an indirect indicator to judge the degree of oxidation, which is a reflection of deterioration of the biodiesel. Similarly as compared with the FTIR spectrum of biodiesel from used frying oil (Fig. 4), the *cis-trans* variation is not large and shows similar pattern of FTIR spectrum as compared with the biodiesel from fresh frying oil. It can be noted that there is only a slightly higher deterioration compared with biodiesel from fresh frying oil as depicted in the FTIR spectrum in Fig. 4. High percentage of saturated fatty acids in palm oil caused this oil to have higher stability as compared with other vegetable oils such as olive oil (Mc Donald and Eskin, 2006). Tocopherols and tocotrienols are fat-soluble vitamin E isomers and the major antioxidants of vegetable oils. Tocotrienols are rarely seen in vegetable oils with the exception of palm and rice bran oils. Crude palm oil contains about 230 and 817 mg/kg oil of total tocopherol and tocotrienols, respectively (Sundram *et al.*, 2003). Refining reduces the level down to 155 mg/kg oil (total tocopherols) and 595 mg/kg oil (total tocotrienols). Hence, the refined palm oil still retains 67% tocopherols and 73% of the tocotrienols (Sundram *et al.*, 2003). Carotenes, which gives the crude palm oil its distinctive red color is

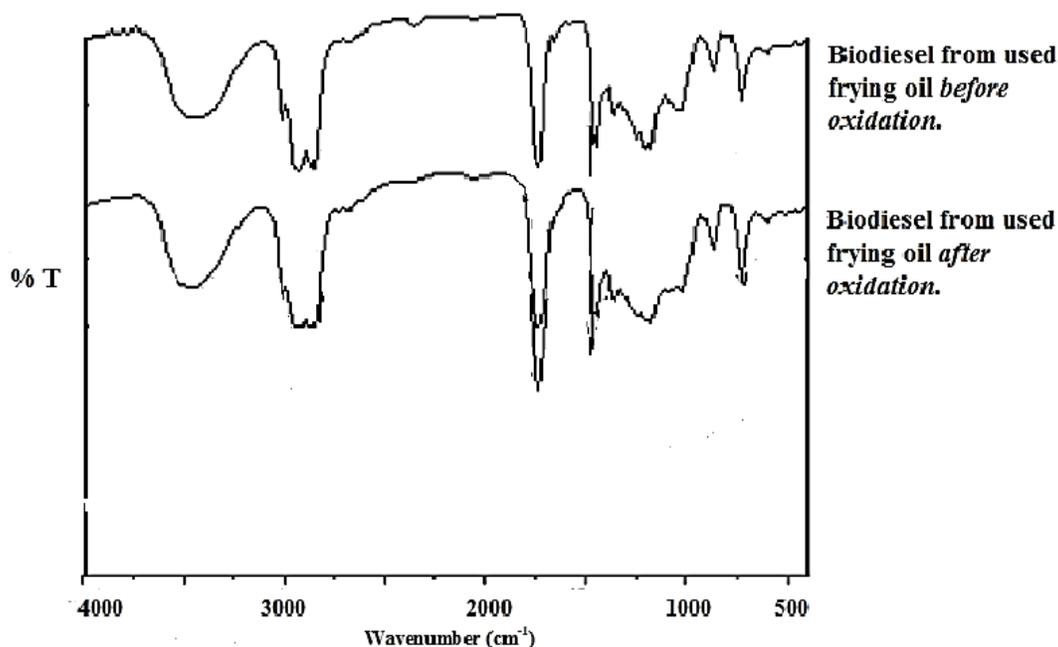


Fig. 4: FTIR spectrum of biodiesel from used palm frying oil before and after oxidation

Table 3: The variation of peroxide value of biodiesel from waste frying oil and fresh cooking oil (palm oil) and biodiesel containing different percentages of antioxidant (w/v %)

No.	Sample	Peroxide value (meq/kg) after incubation time in oven at 70°C			
		48 h	96 h	144 h	192 h
1	Biodiesel from used frying oil	0.90	2.10	3.20	5.10
2	Biodiesel (used oil) +0.02% (w/v) caffeic acid	0.90	1.45	3.19	4.85
3	Biodiesel (used oil) +0.05% (w/v) caffeic acid	0.90	1.43	3.14	4.65
4	Biodiesel (used oil) +0.1% (w/v) caffeic acid	0.47	0.85	2.25	3.65
5	Biodiesel (used oil) +0.2% (w/v) caffeic acid	0.46	0.80	2.15	3.45
6	Biodiesel from fresh cooking oil	0.80	1.90	3.00	4.74
7	Biodiesel (fresh oil) +0.02% (w/v) caffeic acid	0.80	1.30	2.90	4.53
8	Biodiesel (fresh oil) +0.05% (w/v) caffeic acid	0.80	1.30	2.70	4.50
9	Biodiesel (fresh oil) +0.1% (w/v) caffeic acid	0.45	0.78	2.15	3.60
10	Biodiesel (fresh oil) +0.2% (w/v) caffeic acid	0.44	0.81	2.20	3.35

almost all removed during refinery. After being converted to biodiesel, its ester is expected to have even less oxidation stability. Attempts must be made therefore to ensure that these tocopherols and tocotrienols remain near their original levels in the methyl esters when the oils are esterified. Waste frying oil contains much lesser amounts of these natural antioxidants. The antioxidants are destroyed during frying and only 40-50% of the original amount remains. However, it is interesting to note that microwave assisted transesterification reaction can prevent the degradation of the products molecule since the reaction is carried out at moderate temperature as oppose to conventional transesterification method which requires higher temperature (80-90°C). About 6-8 min was sufficient for microwave heating while 105 min were required for conventional heating to achieve comparable biodiesel yields. Shorter reaction time and lower temperature will minimize unwanted side reactions and gives less by-products.

The oxidation test data in Table 3, revealed that there were differences between the treatments after 48 h. Samples 1, 2, 3 and 4, 5, respectively had the similar peroxide value, indicating their identical susceptibility to oxidative rancidity. Similar result was observed for sample 6, 7, 8 and 9, 10, respectively. Sample 3 had lower peroxide values and therefore higher stability, but it was not significantly different from samples 1 and 2. Similar observation was seen for test within the group of sample 6, 7 and 8, respectively in which sample 8 had lower peroxide values and therefore higher stability, but it was not significantly different from samples 6 and 7. Still greater significant differences were found in all treatments after 144 h. Samples 1, 2, 4 and 5, respectively had higher values (each by approximately 3 meq/kg of oil) and sample 5, with 0.2% caffeic acid, had a lower value indicating a positive effect of the antioxidant presence in delaying oil oxidation.

The differences between the samples were significant after 192 h. Sample 1 had the highest peroxide value (5.1 meq/kg of oil), sample 5 the lowest (3.0 meq/kg of oil). The latter was not significantly different from samples 4. The progressive increase of

peroxide value in the controls revealed the deleterious effect of heat on oil stability. The treatments containing 0.02 and 0.05% of caffeic acid had decreased in peroxide values; however, the decreases were not significant compared to the controls. The overall study suggests that the addition of caffeic acid has a positive effect on the shelf life of biodiesel. The higher the concentrations of caffeic acid, the greater were the stability of biodiesel towards oxidation degradation. It was also revealed that 0.1 and 0.2% of caffeic acid had the same effect on biodiesel stability. Therefore, 0.1% caffeic acid is more economic and probably a threshold dose for stabilizing the biodiesel.

Thermal decomposition of biodiesel: It is well known in the diesel industry that diesel fuel generally darkens in colors as it degrades. This phenomenon was also observed in our thermal stressing experiments. The undegraded biodiesel sample is colorless or in very light yellow color while the post degradation samples showed darkened color (dark yellow) at high temperatures. Thermal degradation experiments of biodiesel samples were performed at 80 to 200°C for a residence time from 6 to 72 h. Thermal degradation study was carried out with the aim to find out how biodiesel evolve with various residence times at different temperatures. In the process of collecting heat treated biodiesel samples, the observed color changed responsible for thermal degradation of biodiesel fuel was noted.

The visual observation of thermal treated biodiesel samples (both used frying oil biodiesel and fresh palm oil biodiesel) shows that higher temperature and longer residence resulted in greater degradation. It is clear that there was no pronounced variation of color at temperatures up to 200°C for residence time of 24 h for both the biodiesel samples (used frying oil biodiesel and fresh palm oil biodiesel) as shown in Table 4. The color retained colorless as untreated biodiesel. With a further increase in thermal stressing residence time, the color change of biodiesel was more significant. Both samples become slight yellow at 200°C after 48 h. After 72 h at 200°C, both samples became dark yellow.

Table 4: Observations of selected thermal stressed biodiesel samples

	Temp (°C)	Heating time (h)	Color
1.	80, 100, 120, 140, 160, 180, 200	24	Colorless
2.	80, 100, 120, 140, 160	60	Colorless
3.	80, 100, 120, 140	72	Colorless
4.	180, 200	36	Mild tint of yellow
4.	180	60	Slight yellow
5.	160, 180	66	Slight yellow
6.	200	48	Slight yellow
7.	180, 200	72	Dark yellow

For temperatures 80, 100, 120, 140 and 160°C, respectively both samples did not show signs of color change after 60 h. At temperature 160 and 180°C, both samples showed slight yellow color after 66 h (Table 4). It is interesting to note that both the biodiesel samples have quite similar thermal stability and both are stable for 24 h at 200°C. Thermal decomposition caused by excessive temperature may denature the fuel. The denaturalization of fatty acid chain of the methyl esters has a direct impact on fuel quality. *cis-trans* isomerization reaction, Diels-Alder reaction and pyrolysis reaction are predominantly involved in the thermal decomposition of biodiesel. As temperature rises, biodiesel will initially go through reversible isomerization and Diels-Alder reaction to form isomers and dimers, respectively. When the temperature is increased further, produced isomers, dimers and remaining fatty acid methyl ester will decompose to form lower molecular weight methyl esters, hydrocarbons and gaseous products (Bondioli *et al.*, 1995; Jain and Sharma, 2011).

Application of microwave energy during the production of biodiesel offers a fast, easy route to the production of this valuable biodiesel with advantages of enhancing the reaction rate and improving the separation process (Saifuddin and Chua, 2004; Leadbeater and Stencel, 2006; Barnard *et al.*, 2007). It can be said that around the temperature of 100°-180°C the specific microwave effect becomes more prominent as the rate of reaction increases significantly. In this study, both biodiesel were produced using the same method of microwave assisted transesterification to take the advantage of not only fast rate of reaction but faster separation. Dipolar polarisation depends on polar groups within the material having the necessary freedom of movement to align with the waves of the microwave field (Kappe, 2004). We can therefore assume that before the microwave exposure, the polar groups in the biodiesel molecule are more restrained by structural constraints and cannot rotate. After sufficient microwave exposure there will be interaction between the microwave energy and biodiesel and this allows increase in rotation of some ionic groups within the molecule. Microwave energy has been reported to lower the activation energies in organic synthesis. This, along with the increased mobility allows for increased motion of hydroxyls in the molecule and as result increased crystallinity of the molecule (Perreux and

Loupy, 2001; Di Blasi *et al.*, 2007). Crystalline biodiesel contains a more ordered hydrogen bonded network which can increase the overall structural rigidity of the biodiesel molecule. It can be postulated that microwave irradiation has brought about structural conformational changes to the methyl ester molecules and this morphological change in the molecular structure, stabilizes the molecule by strengthening the intra molecular hydrogen bonding. This results in the thermal stability of the biodiesel prepared using microwave assisted reaction.

CONCLUSION

The increasing emphasis on the use of quality biodiesel in automobiles and electricity generating plants required a rapid and reliable analysis, so as to reveal some properties that are so insensitive to manual analysis. The usage of modern instrumentation such as the FTIR spectrometry as an emerging technique for analysis of biodiesel, for evaluating functional groups to indicate biodiesel reactivity and stability is now more readily available. Frying oils constitute an interesting source for obtaining biodiesel after prior purification to remove water which interferes with both production and the presence of salts which cause corrosion in engines. The analysis of peroxides and unsaturated fatty acids are important to estimate the stability of the product. Higher concentration of unsaturated Fatty Acid Methyl Ester (FAME) with multi-double bonds exhibited poorer oxidation resistance. It is interesting to note that microwave irradiation during the biodiesel production can bring about structural conformational changes to the methyl ester molecules and this morphological change in the molecular structure, stabilizes the molecule by strengthening the intra molecular hydrogen bonding. Adding antioxidant is an easy way to delay the rate of oxidation. However, the optimum antioxidant concentration depends on type of biodiesel and biodiesel production process as well. The use of natural antioxidants can replace artificial antioxidants in good yield.

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