# Green tea and pomegranate hull extracts as additives for biodiesel

Vegetable oil/animal fat is made up of one mole of glycerol and three moles of fatty acids, referred to as triglycerides. They differ in the nature of their carbon chain and the amount of unsaturation. They are highly viscous, water-insoluble/hydrophobic and contain larger fractions of free fatty acids apart from phospholipids, sterols, water, odourants and other impurities[1]. These qualities impede their direct use in engines and require modifications.

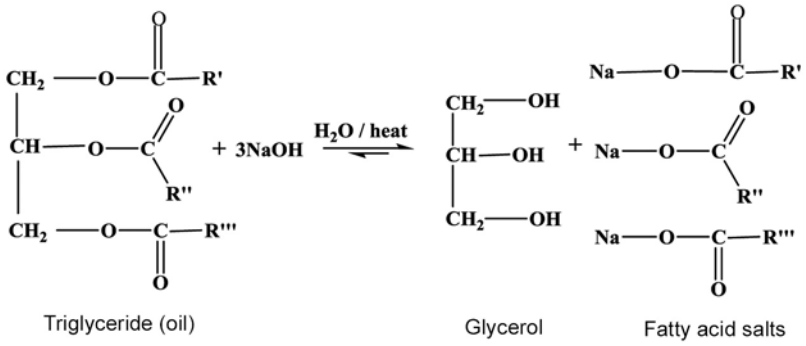
Biodiesel as its name implies is obtained from biological sources like vegetable oils and animal fats. They are the simple alkyl esters of long-chain fatty acids which are obtained from glyceride sources by employing appropriate methods for production. Presence of even a small quantity of water in the source or methanol will support saponification reaction (Scheme 1) resulting in emulsion formation[2], making the separation process of biodiesel difficult. Moreover, free fatty acid (FFA) content should be normally within a limit of 0.5% to get good conversion efficiency for base-catalysed transesterification[3], above which saponification reaction will dominate reducing the yield of desired product since soap formation results in emulsification. Also, phospholipids must be degummed using an acid before production of biodiesel; otherwise it will lead to difficulty in separation of the product[4].

Biodiesel from high acid value (FFA content) rubber seed oil (46.41 mg KOH/g) was obtained by two-step method[5] – esterification using an acid catalyst (Scheme 2), followed by base-catalysed transesterification (Scheme 3). Crude rubber seed oil along with methanol in the molar ratio 1 : 15 with 1.7% v/v concentrated hydrochloric acid at 95°C (optimum condition) was mixed well and refluxed in a water bath using a water condenser. The progress of the reaction was monitored frequently, by titrating 1 ml of the solution against standard potassium hydroxide, till the FFA content was reduced below 0.5%. On completion of this reaction, excess methanol and hydrochloric acid moved to the top surface and the clear bottom layer was separated easily, discarding the lipophilic solid substance collected as the middle layer. The clear bottom layer was transesterified with methanol/oil/KOH in the molar ratio 6 : 1 : 0.18 at 65°C for 15 min (optimum condition) using ultrasonic waves (33 ± 3 kHz) in order to strengthen the mass transfer between liquid–liquid heterogeneous systems, since fats and alcohols are not totally miscible. The denser glycerol layer moved to the bottom and the methyl ester layer was separated by allowing it to settle for 12 h. Then it was washed by spraying hot water (10% v/v) gently until the layer was clear. Vigorous shaking with water was avoided as it would lead to emulsification of methyl esters. Anhydrous sodium sulphate was used to dry the product.

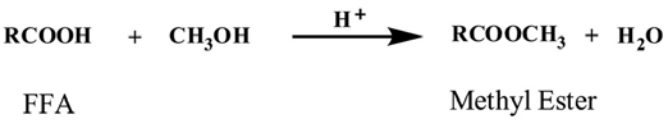
The biodiesel produced and characterized by GC-MS and $^1$H-NMR chromatographic and spectroscopic techniques revealed normal fatty acid profile comprising 18 : 2, 18 : 1, 18 : 0, 16 : 0 and 18 : 3 fatty acid methyl esters (FAMEs). Further, it was found to have fuel properties (nearly the same as produced by magnetic stirring technique) as specified by ASTM D6751 (ref. 6).

Oxidative stability in terms of induction period was determined according to IP 306 at regular intervals for the biodiesel samples stored at 30°C loaded with GT-M or PH-M at varied concentrations. The FAMEs sample was heated to the required temperature (ASTM standard, 110°C) and air was allowed to pass over the sample by establishing a closed system. The air was then passed into water when the conductivity was measured. The natural antioxidants already available with the FAMEs would suppress the oxidation of the biofuel, until such a time when oxidation of FAMEs begins. With consumption of these antioxidants, the conductivity of water increased suddenly due to the evolution of volatile organic acids[7]. The time at which a sudden rise in the conductivity of water was observed was recorded as the induction period of the sample.

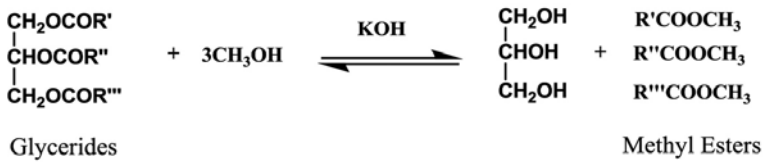
FAMEs obtained from rubber seed oil exhibit 60 days of storage stability against autoxidation when protected against light and air[6]. Since oxidative




**Scheme 1.** Saponification of triglycerides.




**Scheme 2.** Acid esterification of free fatty acids (FFAs).




**Scheme 3.** Base-catalysed transesterification of triglycerides.

**Table 1.** Polyphenols and flavonoids in green tea and pomegranate hull extracts

| Source | Polyphenols (mg/g extract) | Flavonoids (mg/g extract) |
|---|---|---|
| Green tea – methanol | 475.74 ± 0.043 | 11.65 ± 0.031 |
| Pomegranate hull – methanol | 363.64 ± 0.022 | 35.8 ± 0.026 |

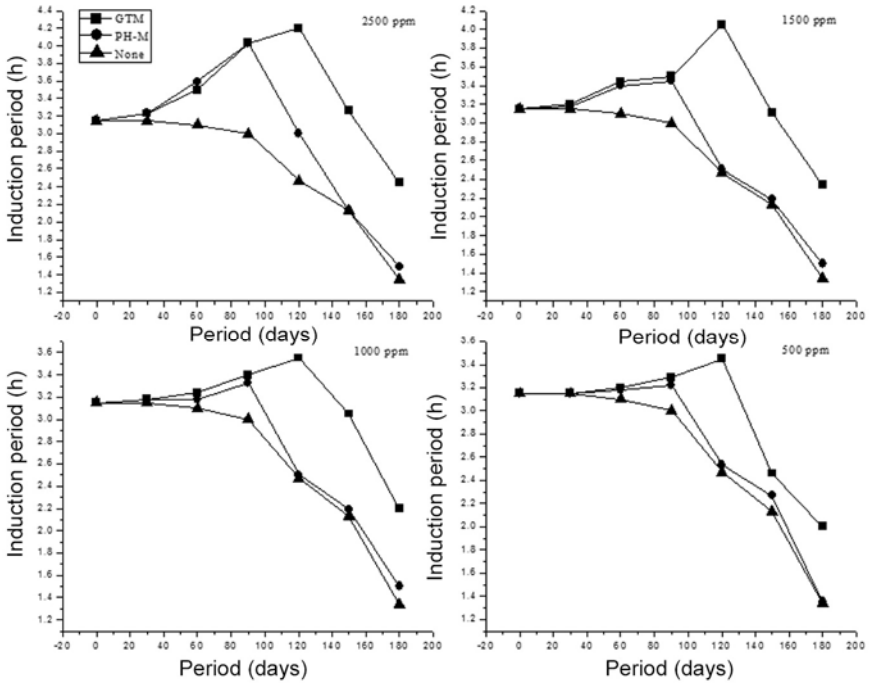



**Figure 1.** Induction period of biodiesel samples with loaded antioxidants.

stability of biodiesel is a key factor for its future use, a trial attempt to improve its oxidative stability was made using some natural antioxidant sources.

Methanol extracts of green tea (GT-M) and pomegranate hull (PH-M) rich in antioxidants[8,9] were prepared from the dried samples and the excess solvent was drained out using a vacuum evaporator. The extracts were allowed to dry at room temperature and stored below 15°C.

The dried extracts collected were characterized by UV–visible spectrophotometer Systronics 2203 double beam spectrophotometer. The methanolic extract of green tea showed absorption at 272 nm representing epigallocatechin gallate[9] (polyphenol) and peaks at 412, 603 and 657 nm indicate the presence of chlorophyll *a* pigment[10]. For the methanolic extract of pomegranate hull, absorption peaks were observed at 250 nm and 364 nm. The peaks at 250 and 364 nm depict the presence of quercetin, a flavonoid in pomegranate hull[11].

Polyphenols were estimated following the procedure of McDonald *et al.*[12]. Exactly 0.5 ml of 1 : 10 g ml$^{-1}$ extract was added to 4 ml sodium carbonate (1 M) and 5 ml Folin–Ciocalteu reagent (1 : 10 diution with distilled water). The solution was allowed to stand for 15 min and absorbance was measured at 765 nm. A calibration plot was drawn using the values obtained in the following concentrations of gallic acid: 83, 114, 125, 250, 500 and 750 µg/ml.

Total flavonoids were estimated by aluminium chloride colorimetric method following the procedure of Chang *et al.*[13]. Exactly 0.5 ml of 1 : 10 g ml$^{-1}$ extract was added to 4.3 ml methanol. To the above solution 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1 M potassium acetate were added. Absorbance at 415 nm was measured after 30 min. A calibration plot was drawn using the values obtained in various concentrations of quercetin 5–30 µg/ml.

Polyphenols and flavonoids estimated in green tea and pomegranate hull methanolic extracts indicate the presence of higher polyphenol content in green tea compared to pomegranate hull extract (Table 1). However, flavonoid content is greater in pomegranate hull extract.

ASTM D6751 has specified a minimum requirement of 3 h stability against oxidation. The green tea extract was found to improve the induction period to another 150 days, whereas for those samples loaded with the pomegranate hull extract induction period retains ASTM standard for another 90 days with 1500 ppm (Figure 1). With higher concentration of 2500 ppm, further increase in induction period was noted (120 days for PH-M and 160 days for GT-M). Since the polyphenol content recorded was greater in green tea extracts, green tea shows a remarkable increase in the induction period compared to the pomegranate hull extract.

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