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BIO-TRANSFORMER OIL PRODUCTION BY ENZYMATIC TRANSESTERIFICATION OF PAPAYA SEED OIL

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ABSTRACT

Bio-transformer oil from vegetable oil has received quite attention as a substitute to mineral based transformer oil with a view of minimize mineral based oils and reduce greenhouse gases pollution. The seeds of papaya are potential feedstock for bio-transformer production owing to their high lipid contents and available. In this study, papaya seed oils was extracted via soxhlet apparatus using n-hexane and the oil yields were in between 34–35% which is commercially viable. The oil extracted was transesterified using immobilized Candida rugosa lipase as a catalyst at room temperature with various molar ratios of methanol to oil. The highest bio-transformer oil yield for papaya seed oil was found to be 96% at methanol-to-oil ratios of 6:1. The results obtained from the characterization of RPSO bio-transformer oil was analytically compared with that of the transformer mineral oil and ASTM standard requirement. Similarities and differences have been discussed. Owing to comparable results in the RPSO bio-transformer oil, mineral transformer oil studied and the ASTM standard requirement and its increasing breakdown voltage with increasing temperature application. The study revealed that papaya seed oils have the potential of being used as alternative feedstock for bio-transformer production.

Keywords: Bio-transformer oil, papaya seed oil, enzymatic transesterification, lipase, breakdown voltage.

Introduction

There is a worldwide pursuit for environmental conservation and sustainable development. This quest has increased the pace of researches on the use of biotechnology in search of alternative fuel (OECD, 2001). Consequently, there is a universal interest in searching for clean-burning, eco-friendly, cost effective and renewable alternative sources of energy such as energy from biomass, solar, wind, hydrogen, hydroelectricity and geothermal amongst others. Industrial production of esters is commonly carried out from high-quality oils (Issariyakul and Dalai, 2014) using transesterification process with methanol in the presence of acid or basic catalysts where esters and glycerol are produced (Yaakob *et al.,* 2013). This process has some drawback in the formation of soap which needs to be separated from the product mixtures resulting in increasing wastewater generation and energy consumption. Thus, considering the by-product recovery, processing costs and environmental complications call for the need to explore alternative processes (Stergiou *et al,* 2013). The use of enzymes for instance free lipases or immobilized created from diverse microorganisms (Yan *et al.,* 2014) for esters production has some benefits. Some of the benefits of enzymatic process is that is carried out under mild conditions without additional energy consumption and without soap generation (Lam *et al.,* 2010, Yaakob *et al.,* 2013). Nevertheless, enzymes also possess some drawback which makes the process unpopular from wide usage in the production of esters, for instance the high costs of enzymes.

Lipases as enzyme-catalysts for esters production have been categorized as environmentally friendly method. The use of enzyme appears to have overcome the drawback of using conventional catalysts-feedstock pretreatment, catalyst removal problems, waste water treatment and high energy requirements (Aransiola *et al.,* 2014). Gog *et al.,* (2012) and Antczak*et al.,* (2009) classified Lipases a biocatalysts into: intracellular and extracellular lipases produced by microorganisms, animals and plants. The core drawbacks of transesterification using lipase-catalyze process are the enzyme high cost of production, potentiality of explosion, higher reaction time relative to base-catalyzed reaction, regeneration and reuse of biocatalyst are limited. Conversely, to reduce the operation cost and increase catalyst stability and reusability, Oyelaran *et al.,* (2019) wrote that it can be immobilized on many materials. Koh *et al.,* (2011) and Dizge and Keskinler, (2008) wrote that the immobilized lipases have been characterized by high selectivity, efficiency, high yield of biodiesel and absence of side reactions.

Papaya fruit is an edible fruit and also used for the manufacture of cosmetics. Papaya fruit is available throughout the year and only a small portion is utilized as seed crop and the rest thrown away as waste. The seed of papaya is about 15% of the fruit and the oil content in the seeds about 30.7% (Daryono and Sinaga, 2017). When compared with sunflower seeds oil of 22.23% and 19.63% for soybeans oil, the oil content in the seeds of papaya have a great prospect if developed as feedstock for bio-transformer production. Utilization of Papaya seed oil as a cooking oil is not visible because of the benzyl isothiocyanate compound carcinogenic. Patel *et al.,* (2017) wrote that papaya fruits seeds thrown away, which result into bad experiences learnt from humans or animals that consumed it. The purpose of this research is to prepare an eco-friendly transformer oil from papaya seed oil by enzyme transesterification process using lipase as the catalyst. In this study, some of the physicochemical and chemical properties of the produced eco-friendly transformer oil were tested and compared with international standards and those of purchased mineral transformer oil.

Materials and Methods

Materials

Fresh papaya fruits were sourced from Eleru Farms in Ogbomoso-Nigeria. Other chemicals used such as methanol, sodium fluoride, Glutaraldehyde, magnetic particles (Fe3O4), [3-(2-aminoethylamino) propyl] trimethoxy-silane (APTS), and tetraethyl orthosilicate (TEOS) were purchased from a chemical store in Ibadan-Nigeria. All the reagents and chemicals used in this work were of analytical grade. While *Candida rugosa* lipase was purchased from a Biotechnology research Centre in Abuja -Nigeria.

Oil Extraction from Plant Seeds

The papaya fruits were cut into two halves and their seeds removed. To remove the gelatinous part, the seeds were washed 3 times with water and sundry for 3 days. Daryono and Sinaga, (2017) wrote that 1 kg of fresh feed required 3 days sun drying to completely dry. The dried seeds were milled into fine powder. The powdered seeds were then treated with n-hexane in a Soxhlet apparatus for 8 hours at 60°C. The solvent was evaporated using a rotary evaporator so as to recover the oil. The remaining solvent was detached by drying the oil in an oven for one hour at 60°C (Puangsri*et al.,* 2004). After filtration, the bio-transformer oil conversion experiments were performed using three-neck laboratory reactors (1 L) that was fitted with a thermocouple and reflux condenser and placed on a magnetic heater.

Preparation of Coated Magnetic Particles

Two grams of Fe_3O_4 (magnetite) was suspended in 100 ml distilled water. A mixture of 15 ml of methanol, 5 ml of sodium fluoride (NaF) solution and 5 ml [3-(2- aminoethylamino) propyl] trimethoxy-silane (APTS) solutions were mixed and stirred for 10 minutes. After which 20 ml of tetraethyl orthosilicate (TEOS) were added drop by drop slowly in the flask and vigorously stirred for 24 hours at room temperature. The precipitate produced was collected and washed three times with ethanol and water and dried in an oven for 10 hours at 40℃ (Dussan *et al.,* 2010).

Enzyme Immobilization of Candida rugosa Lipase

100 ml of glutaraldehyde was added to 4.4 g of coated particles at room temperature and stirred. 700 mg of *Candida rugosa* lipase was then dissolved in 100 ml solution of phosphate buffer (pH 7.0, 0.1 M) were added and stirred for 30 min at room temperature. The mixtures were then centrifuged for 20 minutes at 3000g, and the particles washed with phosphate buffer solution three times and then oven dry for 10 hours at 30ºC (Wong and. Othman, 2014).

Enzymatic Transesterification of Papaya Seed Oil

Bio-transformer was produced by enzymatic transesterification of papaya seed oils at room temperature for 24 hours with methanol-to-oil ratio of 6:1 .With oil basis of 5 g, 10% (w/w) of water was added. The reaction was carried out under a stirring speed of 200 rpm by adding 30% (w/w) of immobilized lipase. Methanol-to-oil ratio of 6:1 was used. Wong et al. (2015) stated that most of the vegetable oils behaved similarly and achieved highest biodiesel conversion at a 6:1 molar ratio. A three steps addition of methanol was executed to elude lipase denaturation. The amounts of oil and methanol used was 5 g and 0.32 g respectively as recommended by Wong and Othman, (2014). To separate the immobilized lipase a magnetic field was used and then washed with phosphate buffer after each reaction.

Biodiesel Yield and Analysis

The ester yield was calculated based on Equation 1.

$$B_y\ (\%) = \frac{W_b}{W_p} \times 100\% \qquad (1)$$

Where: B_y= Biodiesel yield
W_b = Weight of biodiesel produced
W_p = weight of plant oil used

In order to predict the performance of the oil produced, several properties of bio-transformer oil such as Odour, colour, acid value, Kinematic Viscosity iodine value, Specific gravity, flash point, cloud point, pour point and Electrical properties such as dielectric strength of the oil, and comparing the dielectric strength of produced oil with purchased mineral transformer oil at different temperatures were analyzed and compared

Determination of Physico-Chemical Properties of Purified papaya Seed oil (PPSO)

Physical state

Sensory evaluation was used for odour, and colour determination of the oil produced.

Density

The method used by Huseyin *et al.,* (2011) was used. The weight of a small beaker was determined using an LD model digital electronic balance and 2 ml of the sample was poured into it and the weight was noted. Density was determined using Equation 2.

$$\rho = \frac{M_O}{V_O} \text{ (g/cm}^3\text{)} \qquad (2)$$

Where: ρ = Density
M_o = Mass of oil
V_o = Volume of oil

Water Content

ASTM D1533 using 899 Karl Fischer coulometer (Metrohm) was used to measure the water content. This is determined using chemical analysis by adding an iodine solution of known concentration as a reagent in carefully measured amounts (Marta, 2015).

Specific gravity

The specific gravity of the oils was determined by weighing an empty 10 ml pycnometer bottle (W_1). The pycnometer bottle was then filled with water and weighed (W_2). The pycnometer bottle was then filled with the same volume of oil as that of the water and weighed (W_3). The specific gravity was then calculated using Equation 3.

$$\text{Specific gravity} = \frac{W_3 - W_1}{W_2 - W_1} \qquad (3)$$

Kinematic Viscosity

The ASTM D 445 standard procedure was used to determine the viscosity of the oils using suspended-level capillary viscometer. The U-shaped viscometer made of glassware was immersed into a hot water bath. It was then multiplied by a constant specific for S3 viscometer used, to give a measure of the sample viscosity at 27°C The initial temperature was set at 30°C (Abdelmalika*et al.,* 2010)

Acid value and free fatty acid (FFA)

In determining the FFA of the oil samples, the method used by Fares *et al.,* (2016) and Sidohounde *et al.,* (2014) was adopted for this work where 25ml diethyl ether was mixed with 25ml ethanol. 1ml of 1% phenolphthalein indicator solution was then added to the mixture and was then neutralize with 0.1M potassium hydroxide solution. 1 to 10g of the oil sample were then dissolved in the neutralized solvent mixture. Titration was the carried out with 0.1M potassium hydroxide solution with constant shaking until a pink colour is obtained persisting 115seconds. The acid value is calculated using Equation 4 and FFA using Equation 5:

$$\text{Acid value} = \frac{titration\ (ml) \times 5.61}{Weigh\ of\ sample\ used} \text{ (mgKOH/g)} \qquad (4)$$

And FFA% is approximately ½ acid value (Garba *et al.,* 2013). (5)

Iodine Value

The iodine value was determined by weighing 0.3g of the oil samples (with the acid of a dropping pipette) into a glass stopper flat bottom flask of 25ml, 10ml carbon tetrachloride was then dissolved in the oil sample. 20ml Wiji's solution was added and a stopper moistened with potassium iodide solution was inserted. It was then mixed and allow to stand for 30 minutes in a dark cupboard. 15ml of freshly prepared 10% potassium iodide solution and 100 ml water where added and mixed. The mixture was then titrated with 0.1M standard sodium thiosulphate solution with starch as an indicator just before the end point. A blank determination omitting the oil was carried out (Anastopoulos *et al.,* 2009). The iodine value was calculated using the Equation 6:

$$\text{Iodine value} = \frac{(b-a) \times 1.269}{W} \text{ (g100/g)} \qquad (6)$$

Where b = Blank titre value, a = Sample titre value, W = weight of oil and

12.69 = conversion factor from Meq. $Na_2S_2O_3$ to Iodine gram, molecular weight of iodine is 126.9g.

Boiling point

The temperature at which the vapour pressure of a liquid equals the pressure surrounding the liquid and the liquid changes into a vapour phase is termed boiling point. The sample oils were heated in a container with a thermometer inserted and the time the first bubble was observed and the temperature was noted, the experiment was repeated thrice and the average temperature calculated as the boiling point. (Garba*et al.,* 2013).

Determination of Flash Point of the Oils

The flash point was determined by pouring the sample oils (5ml) in a crucible and placed uncovered on a turned on hot-plate. A thermometer was then inserted into the sample oil and flame flashed on it intermittently using lighter. The temperature increase was carefully observed. The temperature when the fuel just starts to spark light was noted and recorded as the Flash Points. The observed flash points were recorded from the thermometer. Triplicate determinations were done and the mean value was calculated.

Determination of Cloud Point of Oils.

Cloud point of the sample oils were determined using the method of ASTM D2500. The sample of oil was poured into a small beaker placed inside a big beaker and the sample was cooled at a 2°C rate with ice block. It was then monitored until a cloud was seen on the tip of inserted thermometer. The temperature at which cloud first appeared was recorded. Triplicate determinations were done and the mean value used as cloud point.

Determination of Pour Point of Oils.

Pour point of the sample oils was determined using the method of ASTM D97. The sample of the oils were heated in a water bath maintained at 48°C to 45°C and poured into the capillary tube tied-up with thermometer and place in the beaker containing water. It was then cooled at decrements of 2°C and the container was tilted to check for any movement. The 2°C above temperature at which the sample oil stopped moving was recorded as the pour point. Triplicate determinations were done and the mean value as pour point

Determination of Breakdown voltage at Varying Temperatures

Dielectric breakdown voltage is a sign of the presence of contaminating agents such as air bubble, water, dirt, cellulosic fibers or conducting particles in the fluid. It is also a measure of an insulating fluid ability to resist electric stress without failure. In measuring the dielectric breakdown voltage, the IEC 60156 standard was used with the kit voltage capacity of 60KV. A spherical head electrode of standard diameter and a gap of 2.5 mm between them was used to carry out the experiment. 500ml of the sample oil was filled up to 40mm level above the electrodes. To allow air bubbles in the container to settle down about 5 – 15 minutes after filling of sample was allowed before increasing the test voltage at the rate of 2 kV/s. The voltage was applied at least 5 minutes after pouring the mineral oil sample into the test chamber (EC-156, 1995), and about 15 minutes for papaya oil (Rajab *et al.,* 2011). The delay time is necessary to guarantee gas bubbles formed during the pouring process have been expelled completely before the measurements. The measurements were performed six times with a time delay between two successive measurements at least 2 minutes for mineral oil (EC-156, 1995), and 6 minutes for papaya oil. The delay time after measurement was also carried out to allow breakdown of products to disperse and gas to expel, before succeeding measurement was conducted so that the later measurement was not influenced by the previous one (Cooper Power System, 2004). The longer delay time for papaya oil was due to the higher viscosity of the oil than that of mineral oil.

In order to understand the temperature effect on the breakdown voltage of oils, the measurement was performed at different temperatures. The temperature was carried out up to 120 ℃ with 10 ℃ increment. With the temperature kept relatively constant at each temperature using a temperature Sensor set in on the oil chamber's wall, heater, blower, controller and contactor as temperature control system. The temperature of the liquid was detects by the temperature sensor. Upon receiving information sent by the temperature sensor, the controller commands the contactor to connect or disconnect the electric heater from power supply. The blower helps to reduce the temperature when the temperature is higher than required.

Results and Discussion

Papaya Seed Oil Content

The papaya seeds yielded 34.5% quantity of oils on Soxhlet extraction. The result obtained was in agreement the result of Puangsri *et al.,* (2004) of 30.7%. Winayanuwattikun *et al.* (2008) also wrote that 25–48% is the range of oil yield from Carica papaya. From the result obtained papaya seeds are suitable resources of bio-transformer oil production owing to its high oil content is considerable for commercial or industrial applications.

Characterization of Bio-transformer oil from Refined Papaya Seed Oil.

After the transesterification of papaya seed oil, the two phases generated were the papaya oil methyl ester (POME) and glycerol. The POME was then separated. The produced bio-transformer oil was washed properly with distilled water and dried to purify the bio-transformer oil. The following parameters viscosity, specific gravity, flash point, cloud point, pour point, and breakdown voltage, among others characterized. The values were compared with that mineral transformer oil (MTO) and ASTM standard specification. The results of the characterization carried out are presented in Table 1.

Specific gravity and viscosity of oil are essential properties always considered in oil which will serve as feedstock for bio-transformer oil. The specific gravity of the refined papaya seed oil (RPSO) is comparable with specific gravity reported for cotton and corn seed oils that have been documented as good feedstock for bio-transformer (Shah and Tahir, 2011). The higher the value of specific gravity of the fuel, the denser the fuel will be which will affect some properties of the fuel, mainly the flow and the volatility. The specific gravity observed for RPSO was 0.840 and is comparable with MTO. Making it a suitable oil for bio-transformer synthesis trial. Additionally, the viscosity which is a measure of the flow characteristic of a fluid and defines ease of flow of vegetable oil when in operation, if was assessed when used directly. The kinematic viscosity was observed to be 8.78 cSt at 30℃ which is comparable with the viscosity also reported for castor and neem seed oils and with many other seed oils that have been applied for bio-transformer production (Okafor and Okafor, 2018).

Table 1. Physio-chemical properties of refined papaya seed oil (RPSO) with the ASTM standard specification for transformer oil and purchased mineral transformer oil (MTO).

PROPERTIES	MTO	RPSO	ASTM Specification
Specific gravity at 20°C	0.891	0.884	0.89–0.91
Density at 29.5°C (g/cm^3)	0.889	0.850	0.55-0.89
Viscosity at 30°C (cSt)	14.11	9.01	9.3–27
Cloud point (℃)	7	9	7–15
Pour point (℃)	-7	3	–8 to –6
Flash point (℃)	137	161	140–155
Acid value (mgKOH/g oil)	0.04	0.08	0.01–0.03
Boiling point (°C)	134	153	120–230°C
Iodine value (g100/g)	28.178	110.323	55–120
Appearance	Yellow tint	Yellow tint	White / yellow tint
Water content	0.013	0.042	

The viscosity of RPSO though lower than mineral transformer oil (MTO) but it met the minimum requirement of between 9.3 – 27 cSt required for bio transformer oil as result of the transesterification carried out on the seed oil that converted the triglyceride to methyl esters of the fatty acids which have shorter chains than the triglyceride (Agunbiade and Adewole, 2014). Hence, the reduction of the viscosity of the oil by transesterification is indispensable to its potential application as bio-transformer oil. It should also be noted that an increase in oil temperature results in decrease in viscosity, implying an inverse relationship between viscosity and temperature (Oyelaran *et al.,* 2020). Bashi et al. (2006) noted that for smooth oil operation in electrical equipment, the temperature is required to remain around the mild range. The 9.01 cst for RPSO is nice in this aspect. Rafiq et al. (2015) also noted that in electrical power transformers, the insulating fluid promotes heat dissipation, typically by natural convection. Hence, oils with lower viscosities can enhance heat dissipation efficiency, which can increase the transformer capacity and lifetime, in addition to more operational safety (Oyelaran *et al.,* 2020)

A higher density implies more mass of fuel per unit volume. In this case, the bio-transformer oil from RPSO has a relatively higher density when compared to mineral transformer oil. The RPSO has density of 0.85 g/cm^3, which is within 0.55 - 0.89 g/cm^3 ASTM specification. Raja et al. (2011) highlighted that high mass of oils will give better energy available for work output per unit volume. The measured value of the acidity of RPSO is 0.08 mgKOH/ g oil. The standard ASTM recommends a value of between 0.01 – 0.3 KOH/g oil. The obtained value is above the limit.

The iodine value of oil is that weight of iodine absorbed by 100 part by weight of the sample. It is a measure of the degree of unsaturation of the fatty acids in the oil (Okafor and Okafor, 2018). The higher the iodine value the greater the degree of unsaturation. The iodine value for RPSO was found to be 57.41 g 100/g oil as shown in Table 1. The value is lower than 100 and the oil can be classified as non-dry oil and it falls within the minimum standard ASTM requirement of between 55 - 120 g 100/g. The value also signifies the decrease in unsaturation of oil (Aliyu and Tijjani, 2017), which is a merit since lower unsaturation of oils and fats signifies greater oxidation stability.

Cloud point of a fluid is that temperature at which dissolved solids are no longer completely soluble and precipitating as a second phase making the fluid appearing cloudy (Foluso and. Adewole, 2014). Cloud point is the lowest temperature at which oil can flow. The cloud point for RPSO is 7°C and falls within the ASTM specifications as shown in Table 1, this is an indication that the oil can perform satisfactorily in cold climatic conditions. The pour point for RPSO is 3°C, even though it is very low, it is higher than the ASTM specification of between –8 - (–6)°C. A high pour point restricts the application of oils in cold climatic conditions. The pour point must be low so that the oil can remain flowing, even at low temperature (Achten*et al.,* 2010). When pour point is higher than the ambient temperature, wax precipitates in the oil and it loses its flow characteristics resulting in the blockage of the filters and fuel supply line due to wax formation. Storage and transporting fuels are challenging at temperatures below their pour points. It therefore shows that bio-transformer oil from RPSO will be difficult to store and transport particularly in temperate region. Chemical additives acknowledged as pour point depressants are often used to improve the flow properties of such fuel. Hence, the use of chemical additive is recommended for RPSO to improve the flow properties of the fuel.

Flash point is that temperature in which oil produces a certain vapour which mixes with air producing an ignitable mixture, causing a momentary flash. Flash point is an important specification for safety during transport, storage and handling (Oyelaran et al 2020). For the prevention of risk of fire that might happen in accidental ignition a minimum flash point is stated. The flash point of RPSO was found to be 161°C as seen in Table 1, it is slightly higher than 140-155°C specified by ASTM. The value obtained reveals that the oil can safely be used even where the temperature is very high.

The breakdown voltage measurements carried out at different temperatures between 25℃ and 120℃ are displaced in Figure 2. At each temperature, measurements were conducted six times, and the mean value of the six measurement are shown in Figure 2. Breakdown voltage of MTO and RPSO increased significantly with temperature up to 70℃. This as a result of the relative reduction of water content of the oils. This is as a result of the breakdown voltage of insulating fluid been inversely proportional to its relative water content (Suwarno and Darma, 2008). Increasing the temperature beyond 70℃ resulted in considerable decrease in the relative water content of the RPSO. Consequently, raising the breakdown voltage RPSO. However, a decrease in breakdown voltage was observed for MTO. A similar trend was reported by Rajab *et al.,* (2011), who carried out a research on breakdown voltage analysis on Palm Oil with Mineral and Synthetic types insulating liquid under temperature variations. From the result obtain from the breakdown voltage carried out at different temperatures and with the trend observed decreasing water content in PCSO and with higher breakdown voltage at higher temperature RPSO might be a good feedstock for bio-transformer fluid at higher temperature.

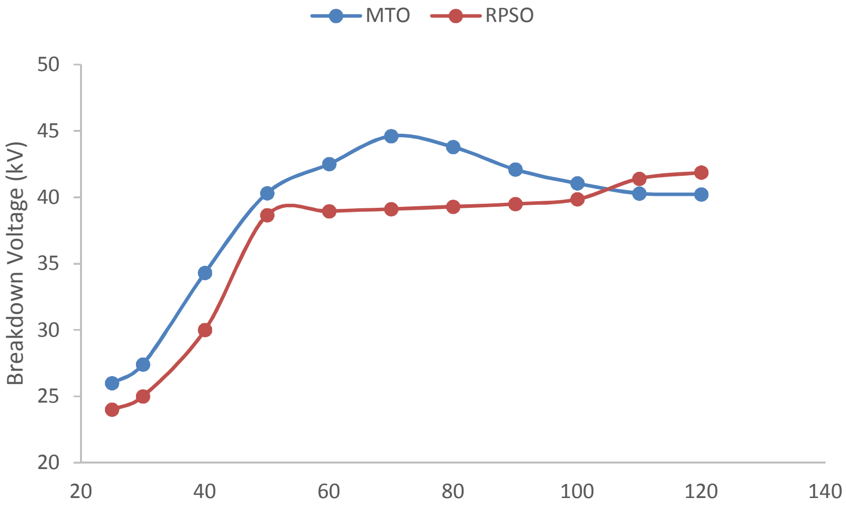

Figure1. The mean value of breakdown voltage of papaya oil and mineral oil as a function of temperature.

Conclusions

This study has proved that papaya seed oils have the potential of being used as feedstock for the production of bio-transformer oil using lipase via enzymatic transesterification. The highest yield of bio-transformer oil was obtained at methanol-to-oil molar ratio of 6:1. The usage of immobilized enzyme is essential for solving problems concerning the downstream separation of the acid or alkali catalyst from the bio-transformer oil via chemical transesterification process. This might possibly aid in the reduction of operating and energy costs in bio-transformer oil production. The properties of bio-transformer oil produced from papaya seed oil were also analyzed and found to have comparable results with mineral transformer oil and meeting most ASTM standard requirement and it also possesses increasing breakdown voltage with increasing temperature application.

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