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PRODUCTION OF BIODIESEL FROM AGRO-INDUSTRIAL WASTE

Aishwarya B. George, Maria Joy, Fr. Jobi Xavier and Erumalla Venkatanagaraju *

Department of Life Sciences, CHRIST (Deemed to be University), Hosur Road, Bengaluru - 560029, Karnataka, India.

Keywords:

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Correspondence to Author:
Dr. Erumalla Venkatanagaraju

Assistant Professor,
Department of Life Sciences,
CHRIST (Deemed to be University),
Hosur Road, Bengaluru - 560029,
Karnataka, India.

E-mail:venkatanagarajue@gmail.com

ABSTRACT: Fossil fuels are significantly depleted nowadays due to excess utilization. Therefore, it is always better to find alternatives to produce eco-friendly fuels. This paper deals with the production of biodiesel from agro-industrial waste such as young coconut husk, custard apple seeds and broiler chicken skin. Lipids were isolated from the substrates using the modified Bligh and Dyer method and transesterification was conducted to the isolated lipids in an inert condition to produce biodiesel. Obtained biodiesel was characterized by Gas Chromatography-Mass Spectroscopy (GC-MS) and confirmation of esters was done using Fourier Transform Infrared (FTIR). This study encourages the production of biodiesel from waste plant and animal sources.

INTRODUCTION: The most fundamental requirement for human beings is energy. It is one of the major factors for socio-economic development. Fossil fuel usage increasing day by day to meet the demands of an increasing population. Excess consumption of these fuels drastically changes the ecosystem balance by increasing greenhouse gases. To balance all these issues researchers focusing on the production of eco-friendly biofuels such as biodiesel, from agro-industrial residues. Biodiesels produced from different sources like vegetable oils and animal fats [1-5]. The current research was carried out to produce biodiesel from agro-industrial waste materials such as young coconut husk, custard apple seeds and broiler chicken skin. The botanical name of coconut in *Cocus nucifera*. It belongs to the family Aceraceae [6].

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Coconut is an unbranched and monoecious tree. The coconut fruit is botanically known to be a drupe. Coconut has 3 layers called the exocarp (outer layer), mesocarp (middle layer) and the endocarp (inner layer). The exocarp and mesocarp together forms the 'husk' of the coconut [7]. The botanical name of Custard apple is *Annona squamosa* and it belongs to the family Annonaceae. It is also known as sugar apple. The seeds of *Annona squamosa* contains a good source of oil of almost 26.8% [8].

The botanical name of broiler chicken is *Gallusgallus domesticus*. Chickens that are breed and that are specifically raised for meat production are called as a broiler [9]. Broilers generally have yellow skin and white feathers [10, 11]. The products or waste produced from food processing methods and other agricultural products are generally considered to be a problem as a pollutant. After processing, a large amount of plant material and animal waste often remains without any application. When such materials are converted into valuable resources, it contributes to reduction of residues [12].

Lipids of biological materials, plant waste *etc*. can be extracted and purified in a single-step operation. This requires a mixture of chloroform and methanol that had wide use as lipid extractants. Common methods employed to reduce the viscosity of vegetable oils and other oils include blending with diesel, micro-emulsions, thermal cracking, and transesterification [13]. The transesterification process is generally a sequence of reversible reactions, where there is a conversion of triglycerides to diglycerides and then to monoglycerides. The glycerides formed were then converted into glycerol and one ester molecule at every step [14] **Fig. 1**.

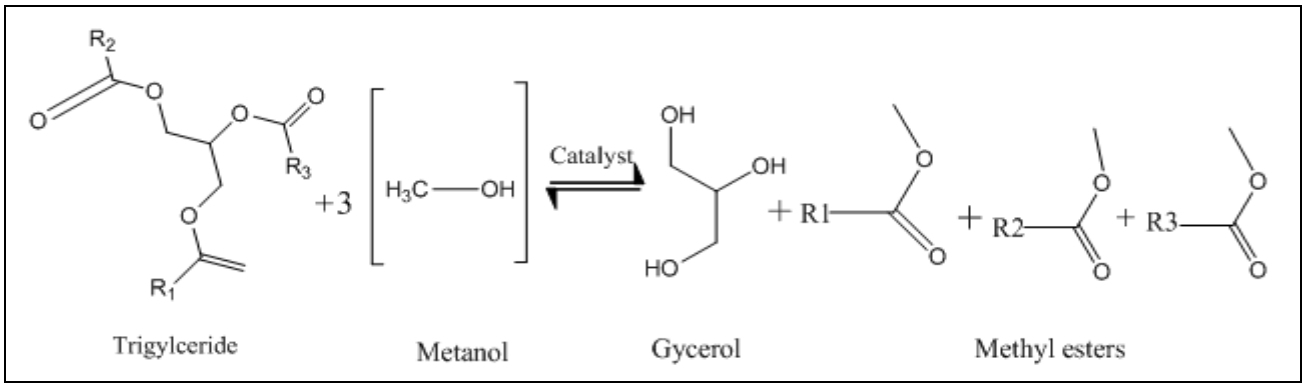


FIG. 1: TRANSESTERIFICATION REACTION

R1, R2, R3 are long-chain hydrocarbons and are generally called fatty acid chains. It involves the conversion of triglyceride to diglyceride and then to monoglyceride, and finally to glycerol. Therefore, 1 mol of a fatty ester is liberated at each step. Methanol is usually used as the alcohol because it has a low cost of [15]. The transesterification usually requires about 1 h with the temperature of 60-65 °C (for methanol) [13].[16].

MATERIALS AND METHODS:

Collection of Raw Materials: Commercially available tender coconut husk, custard apple seeds, and broiler chicken skin were collected from vendors in and around the area of Krishna Raja Puram, Bengaluru, India. The sample was collected in sterile plastic bags and it was brought to the laboratory for additional processing [17]. Coconut husk was separated from the shell and was kept in a sterile container for further usage [18]. Custard apples collected from fruit sellers were stored in airtight containers for further studies.

Processing of Samples: The young coconut husk was removed from the shell 250 g was weighed on a weighing balance. The samples that are large were cut into small pieces [19]. This was then transferred to a mixer and ground until a fine paste was obtained. After collection, the custard apple seeds were sun-dried for a week to remove moisture content. A total of 250 g of dried custard apple seeds were obtained and ground in mortar and pestle [20]. 250 g of skin were excised from broiler chickens [21]. This was then transferred to a mixer and ground until a fine paste was obtained.

Extraction of Lipids: The Bligh and Dyer method was used to isolate lipids from the substrates. 2:1 ratio of chloroform and methanol was added. This was placed in a water bath for 15 min at 55 °C. The homogenate was filtered and the cell debris was removed by using Whatman filter paper no. 1 [22]. For washing the crude extract, to the filtrate, 20ml of 50 mM NaCl was added instead of water [23]. After the addition of NaCl, it is allowed to separate further. This was transferred to a 500 ml measuring cylinder for complete and accurate separation. The methanolic layer is removed along with a small volume of chloroform layer to make sure complete removal of the alcoholic layer. The chloroform layer thus contains the purified lipids [22]. The oils were then recovered from the chloroform layer completely using the rotary evaporator at 40 °C [25].

Determination of Lipid Content: The chloroform layer is evaporated to dryness by heating in a water bath at 50-60 °C. After complete evaporation, the residue was transferred into a tare round bottom flask by adding a small amount of chloroform. Non-lipid particles are insoluble in chloroform while lipids are soluble [22]. This is evaporated completely by placing on a heating mantle at 55 °C.

The initial weight of the round bottom flask is taken and the weight of round bottom flask with lipids is noted.

Total lipids(g) = Weight of round bottom flask + lipids – the initial weight of the round bottom flask

Transesterification: On a heating mantle, a two neck round bottom flask equipped with a reflex water-cooled condenser to reduce the evaporative loss of methanol was placed. The second neck is used for the addition of chemicals. For each transesterification run, lipids were added into the reactor and were heated at 60 ℃ before adding the catalyst and methanol [24]. 100 ml of methanol and 10 g of sodium hydroxide was added to the oil. The reaction was maintained in an inert condition in the presence of nitrogen gas. This was carried out for 90 min [8]. The mixture was transferred to a separating funnel to settle. Two layers will be formed where the upper layer is the biodiesel and the lower layer is glycerine. Biodiesel is thus separated from the glycerine layer [8]. The upper layer is collected and is transferred to a funnel containing sodium sulphate to completely remove the presence of water. The filtrate was subjected to complete evaporation of the solvents by placing it in a rotary evaporator at 40 °C [25]. The purified FAME was analyzed by Fourier Transform Infrared and Gas Chromatography-Mass Spectroscopy.

GC-MS Analysis: GC-MS analysis was performed using a NIST/EPA/NIH mass spectral library system, version 1.0, comprising of a Gas Chromatograph interfaced with a Mass Spectrometer (GC-MS) to determine the methyl esters present in the obtained biodiesel products. For GC-MS detection, an electron ionization system (EI) was operated in electron impact mode with ionization energy of 70 eV. As a charge carrier, Helium gas (99.999%) was used at a constant flow rate of 1.2 ml/min, and an injection volume of 2 µl was employed with a split ratio of 2:0. The injector temperature was maintained at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 60 °C (isothermal for 2 min), with an increase of 10 °C/min to 200 °C. The constituents were recognized after comparison with those constituents available in the computer library (NIST) which is attached to the GC-MS instrument and the results obtained have been tabulated [26].

FTIR Analysis: The purified FAME were analyzed by Fourier Transform Infrared (FTIR). FTIR relies on the fact that most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000-600 cm^{-1}. The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly associated with the sample's absorption spectrum [27].

Determination of Moisture Content: SHIMADZU UniBloc Moisture Analyzer MOC63u was used to analyze the moisture content of the fuel [28].

RESULTS AND DISCUSSION: Modified Bligh and Dyer method showed that broiler chicken skin had the maximum amount of lipids corresponding to 5.40 ml. The least amount of lipids was found in young coconut husk corresponding to 2.72 ml and custard apple seeds had 3.31g of lipids. It thus confirms that chicken skin was the best source of lipids. The methyl esters obtained from young coconut husk were Peak3-Hexadecanoic acid ($C_{17}H_34O_2$), Peak4-n-hexadecanoic acid ($C_{16}H_{32}O_2$), Peak 5-9-Octadecenoic acid ($C_{19}H_{36}O_2$) and Peak 6-9,12-Octadecadienoic acid ($C_{18}H_{32}O_2$). From **Table 1**, the main methyl esters present in the biodiesel are hexadecanoic acid ($C_{17}H_{34}O_2$) and 9-Octadecenoic acid ($C_{19}H_{36}O_2$). Their respective percentages by composition were 6.47 and 15.36 %. Other esters found in the biodiesel were n-hexadecanoic acid ($C_{16}H_{32}O_2$); 12-Octadecadienoic acid ($C_{18}H_{32}O_2$); having respective percentages of 6.47 and 6.06 %. The profile revealed, n-hexadecanoic acid ($C_{16}H_{32}O_2$) as the predominant compound in the mixture having the highest percentage of 18.40%. The fatty acid methyl ester profile is one of the factors that decide the suitability of substrates for biodiesel production.

From **Fig. 2**, the quantification was done with the use of the infrared spectra. The region 1742 confirms the presence of an ester. The compound that confirms an ester is an n-hexadecanoic acid with a retention time of 19.306. From Table 4, the main methyl esters present in the biodiesel are hexadecanoic acid ($C_{17}H_{34}O_2$); 9,12-octa-

decadienoic acid ($C_{19}H_{34}O_2$); 9-Octadecenoic acid ($C_{19}H_{36}O_2$) and methyl stearate ($C_{19}H_{38}O_2$). Their respective percentages by composition were 12.67, 12.73, 31.40, 12.73%. Other esters found in the biodiesel were oleic acid ($C_{18}H_{34}O_2$); octadecanoic acid ($C_{18}H_{36}O_2$) and methyl 18-methyl-nona-decanoate ($C_{21}H_{42}O_2$); having respective percentages of 11.56, 3.61, 1.19%. The profile revealed, 9-octadecenoic acid ($C_{19}H_{36}O_2$) as the predominant compound in the mixture having the highest percentage of 31.40%.

From **Fig. 4**, the quantification of fatty acid methyl ester content in the produced biodiesel. The region 1719 confirms the presence of an ester. The compound that confirms an ester is 9-Octadecenoic acid with a retention time of 21.725. From **Table 7**, the main methyl esters present in the biodiesel are dodecanoic acid ($C_{13}H_{26}O_2$); pentadecanoic acid ($C_{16}H_{32}O_2$); 9-hexadecenoic acid ($C_{17}H_{32}O_2$); hexadecanoic acid ($C_{17}H_{34}O_2$); 10-heptadecenoic acid ($C_{18}H_{34}O_2$); cis-10-heptadecenoic acid ($C_{18}H_{34}O_2$); heptadecanoic acid ($C_{18}H_{36}O_2$); 9,12-octadecadienoic acid ($C_{19}H_{34}O_2$); 9-octadecenoic acid ($C_{19}H_{36}O_2$) and 5,8,11,14-eicosatetraenoic acid ($C_{21}H_{34}O_2$). Their respective percentages by composition were 0.05, 0.12, 5.82, 0.06, 0.15, 11.12, 29.38, 0.47, 24.93%. Other esters found in the biodiesel were methyl myristoleate ($C_{15}H_{28}O_2$); methyl tetradecanoate ($C_{15}H_{30}O_2$); methyl stearate ($C_{19}H_{38}O_2$) and phenol 4, 4-methylethylidene ($C_{15}H_{16}O_2$), having respective percentages of 0.19, 0.82, 7.58, 1.52%. The profile revealed, 9-Octadecenoic acid ($C_{19}H_{36}O_2$) as the predominant compound in the mixture having the highest percentage of 29.38%. The fatty acid methyl ester profile is one of the key factors that decide the suitability of stock in biodiesel production. From **Fig. 6**, the quantification of fatty acid methyl ester content in the produced biodiesel. The region 1743.25 confirms the presence of an ester. The compound that confirms an ester is hexadecanoic acid with a retention time of 18.940.

TABLE 1: YOUNG COCONUT HUSK BIODIESELMETHYL ESTERS PROFILE

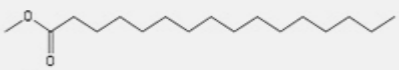
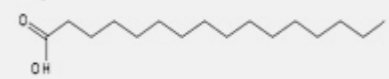
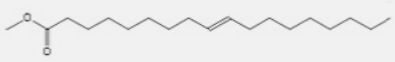
S. no.	Peak no.	Compound	Retention time	Peak area percentage	Structure
1	3	Hexadecanoic acid	18.785	6.47	
2	4	n-Hexadecanoic acid	19.306	18.40	
3	5	9-Octadecenoic acid	21.520	15.36	
4	6	9,12-Octadecadienoic acid	21.990	6.06	

TABLE 2: YOUNG COCONUT HUSK BIODIESELFATTY ACIDS PROFILE

Name of the ester	Name of fatty acid
Hexadecanoic acid	Galic acid
n-Hexadecanoic acid	Palmitic acid
9-Octadecenoic acid	Oleic acid
9,12-Octadecadienoic acid	Linoleic acid

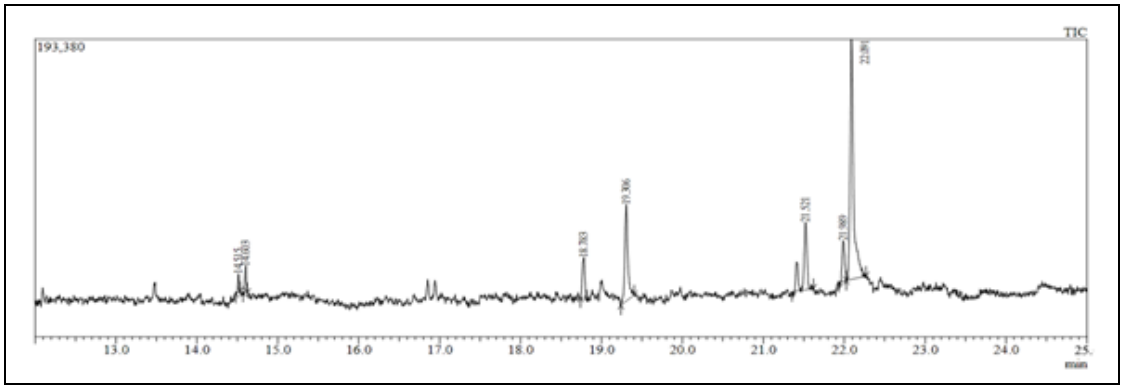


FIG. 2: GC-MS PROFILE OF FATTY ACID METHYL ESTERS OF YOUNG COCONUT HUSK BIODIESEL

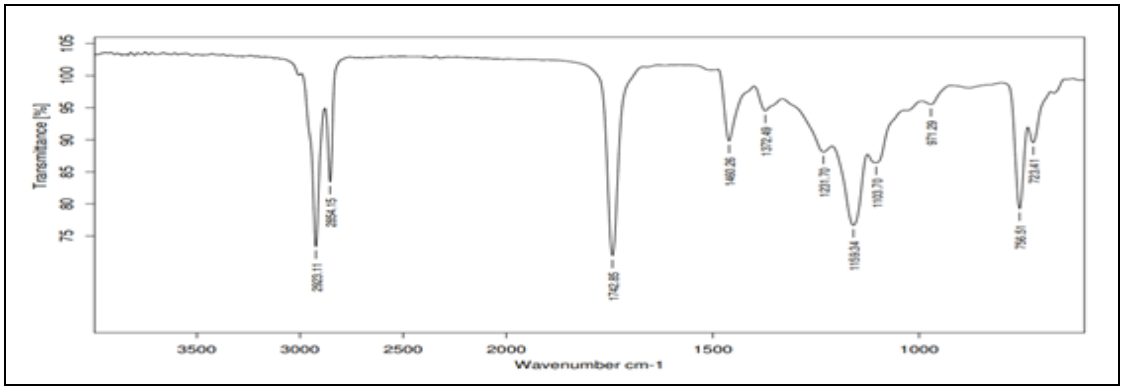


FIG. 3: FTIR PROFILE OF FATTY ACID METHYL ESTERS OF YOUNG COCONUT HUSK

The region 1742 confirms the presence of an ester. The compound that confirms an ester is an n-hexadecanoic acid with a retention time of 19.306.

TABLE 3: MOISTURE CONTENT OF YOUNG COCONUT HUSK BIODIESEL

Weight of oil (g)	Moisture content (%)
1.12	0.18

The moisture content showed that 1.12g of oil has a moisture content of 0.18%. The methyl esters obtained from custard apple seeds were Peak 1-hexadecanoic acid ($C_{17}H_{34}O_2$); Peak 2-9, 12-Octadecadienoic acid ($C_{19}H_{34}O_2$); Peak 4-9-Octadecenoic acid ($C_{19}H_{36}O_2$); Peak 5- Methyl stearate ($C_{19}H_{38}O_2$); Peak 6- Oleic Acid ($C_{18}H_{34}O_2$); Peak 7-Octadecanoic acid ($C_{18}H_{36}O_2$) and Peak 8-Methyl 18-methylnonadecanoate ($C_{21}H_{42}O_2$).

TABLE 4: CUSTARD APPLE SEEDS BIODIESEL METHYL ESTERS PROFILE

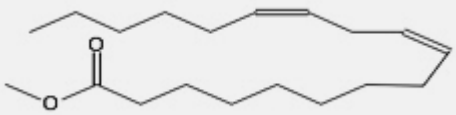
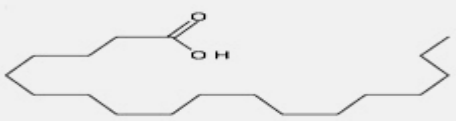
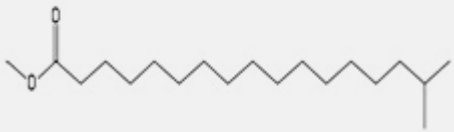
S. no.	Peak no.	Compound	Retention time	Peak area percentage	Structure
1	2	Hexadecanoic acid	18.855	12.67	
2	3	9,12-Octadecadienoic acid	21.530	18.28	
3	4	9-Octadecenoic acid	21.725	31.40	
4	5	Methyl stearate	22.045	12.73	
5	6	Oleic Acid	22.370	11.56	
6	7	Octadecanoic acid	22.640	3.61	
7	8	Methyl 18-methylnonadecanoate	24.935	1.19	

TABLE 5: CUSTARD APPLE SEEDS BIODIESELFATTY ACIDS PROFILE

Name of Ester	Name of Fatty Acid
Hexadecanoic acid	Gaidic acid
9,12-Octadecadienoic acid	Octadeca-9,12-dienoic acid
9-Octadecenoic acid	Elaidic acid
Methyl stearate	Stearic acid
Oleic Acid	Elaidoic acid
Octadecanoic acid	Stearic acid
Methyl 18-methylnonadecanoate	Nonadecanoic acid

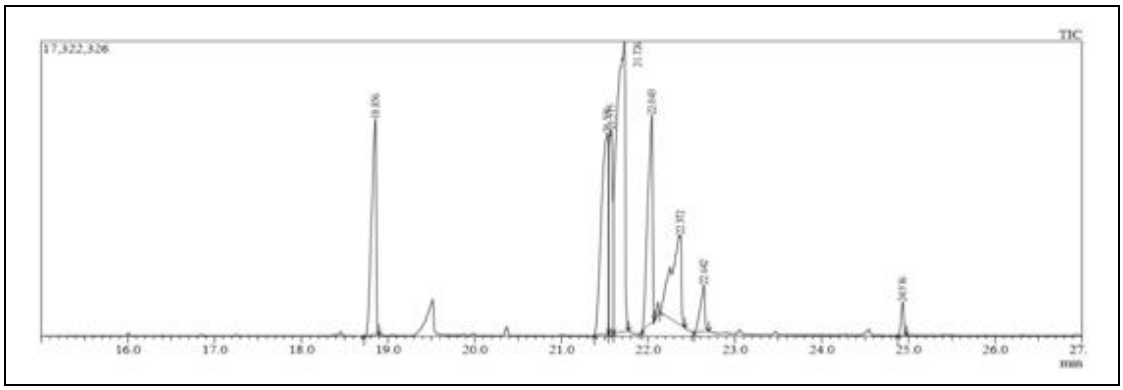


FIG. 4: GC-MS PROFILE OF FATTY ACID METHYL ESTERS OF CUSTARD APPLE SEEDS BIODIESEL

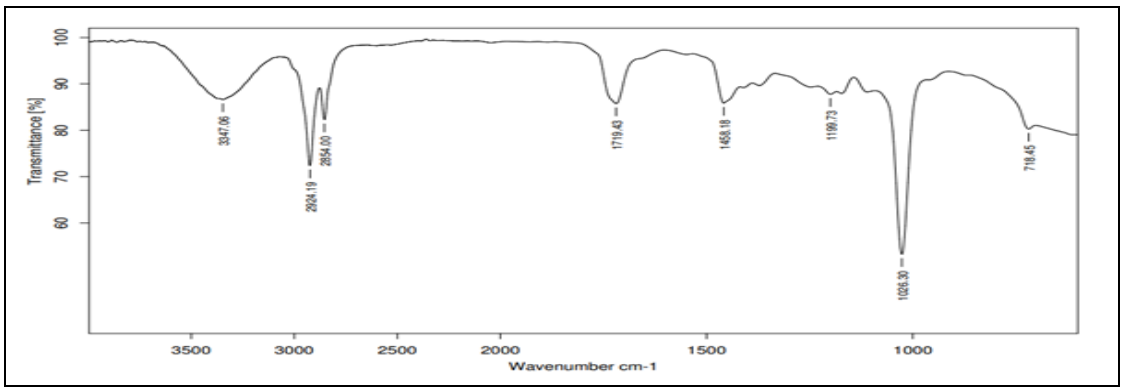


FIG. 5: FTIR PROFILE OF FATTY ACID METHYL ESTERS OF CUSTARD APPLE SEEDS BIODIESEL

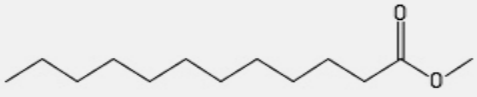
The region 1719 confirms the presence of an ester. The compound that confirms an ester is 9-Octadecenoic acid with a retention time 21.725.

TABLE 6: MOISTURE CONTENT OF CUSTARD APPLE SEEDS BIODIESEL

Weight of oil (g)	Moisture content (%)
0.355	0.21

The methyl esters obtained from broiler chicken skin were found to be Peak 1-dodecanoic acid, methyl ester ($C_{13}H_{26}O2$); Peak 2-methyl myristoleate ($C_{15}H_{28}O_2$); Peak 3-methyl tetra-decanoate ($C_{15}H_{30}O_2$); Peak 4-pentadecanoic acid ($C_{16}H_{32}O_2$); Peak 7- 9-hexadecenoic acid ($C_{17}H_{32}O_2$); Peak 8-hexadecanoic acid ($C_{17}H_{34}O_2$); Peak 9-cis-10-heptadecenoic acid ($C_{18}H_{34}O_2$); Peak 10-heptadecanoic acid ($C_{18}H_{36}O_2$); Peak 12-9,12-octadecadienoic acid ($C_{19}H_{34}O_2$); Peak 14-9-octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$); Peak 16-methyl stearate ($C_{19}H_{38}O_2$); Peak 18-phenol, 4,4'-(1-methylethylidene)bis-($c_{15}h_{16}o_2$) and peak 19- 5, 8, 11, 14-Eicosatetraenoic acid ($C_{21}H_{34}O_2$).

TABLE 7: BROILER CHICKEN SKIN BIODIESEL METHYL ESTERS PROFILE

S. no.	Peak no.	Compound	Retention time	Area percentage	Structure
1	1	Dodecanoic acid	13.665	0.05	

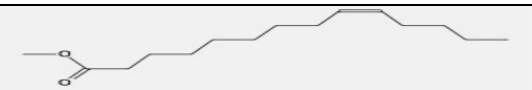
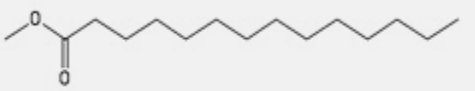
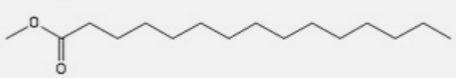
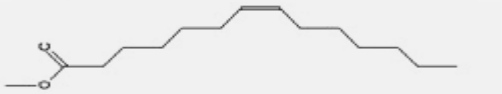
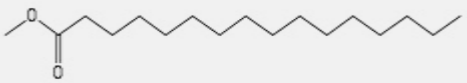
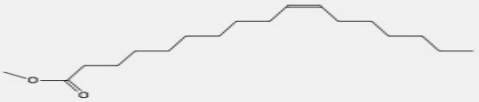
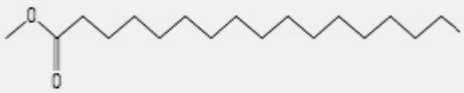
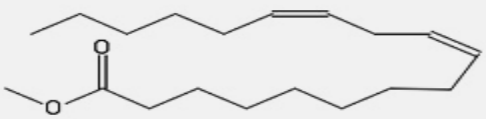
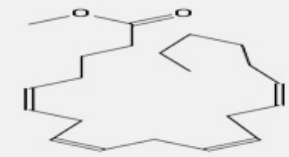
2	2	Methyl myristoleate	15.880	0.19	
3	3	Methyl tetradecanoate	16.020	0.82	
4	4	Pentadecanoic acid	17.260	0.12	
5	7	9-Hexadecenoic acid	18.515	5.82	
6	8	Hexadecanoic acid	18.940	24.93	
7	9	cis-10-Heptadecenoic acid	19.995	0.06	
8	10	Heptadecanoic acid	20.375	0.15	
9	12	9,12-Octadecadienoic acid	21.520	11.12	
10	14	9-Octadecenoic acid	21.770	29.38	
11	16	Methyl stearate	22.050	7.58	
12	18	Phenol, 4,4'-(1-methylethylidene)	22.755	1.52	
13	19	5,8,11,14-Eicosatetraenoic acid	23.900	0.47	

TABLE 8: BROILER CHICKEN SKIN BIODIESELFATTY ACID PROFILE

Name of Ester	Name of Fatty Acid
Dodecanoic acid	Lauric acid
Methyl myristoleate	Myristoleic acid
Methyl tetradecanoate	Myristic acid
Pentadecanoic acid	Pentadecylic acid
9-Hexadecenoic acid	Palmitoleic acid
Hexadecanoic acid	Palmitic acid
cis-10-Heptadecenoic acid	Enoic acid
Heptadecanoic acid	Heptadecanoic acid
9,12-Octadecadienoic acid	Linoleic acid
9-Octadecenoic acid	Elaidic acid
Methyl stearate	Stearic acid
Phenol, 4,4'-(1-methylethylidene)	Propeonoic acid
5,8,11,14-Eicosatetraenoic acid	Arachidonic acid

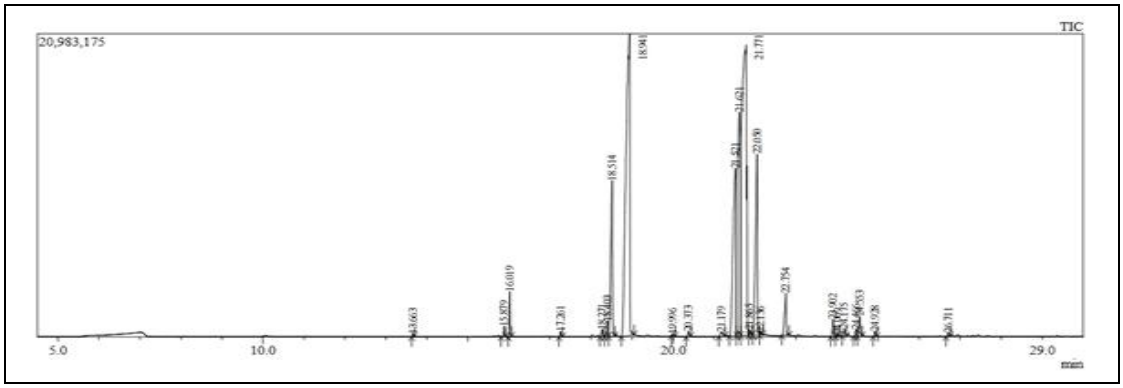


FIG. 6: GC-MS PROFILE OF FATTY ACID METHYL ESTERS OF BROILER CHICKEN SKIN BIODIESEL

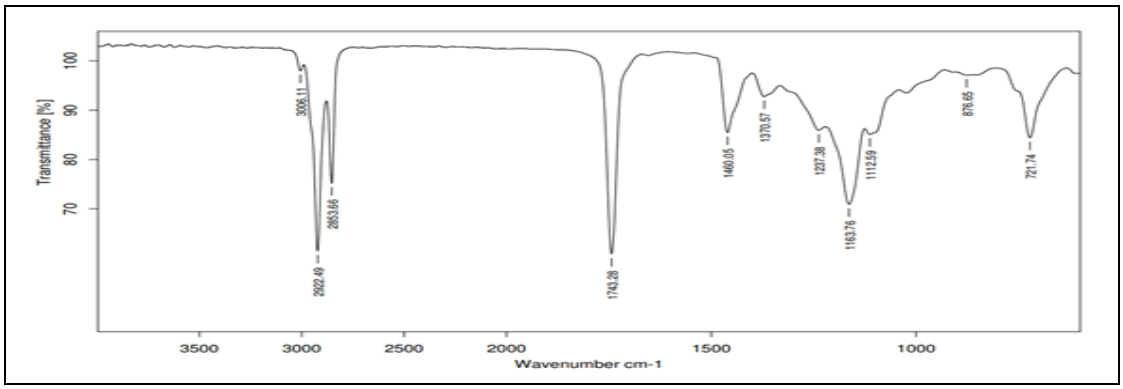


FIG. 7: FTIR PROFILE OF FATTY ACID METHYL ESTERS OF BROILER CHICKEN BIODIESEL

The region 1743.25 confirms the presence of an ester and thus biodiesel. The compound that confirms an ester is hexadecanoic acid with a retention time of 18.940.

TABLE 9: MOISTURE CONTENT OF BROILER CHICKEN SKIN BIODIESEL

Weight of oil (g)	Moisture content (%)
1.926	0.16

CONCLUSION: One can understand that though the production of bioethanol is highly researched and even various evidence of its being commercialized is known, still many challenges lie ahead in making its sustainable source. It is understood that it's always better to choose substrates that have a higher lipid content. This favors the transesterification reaction. In this work, the three substrates selected were young coconut husk, custard apple seeds, and chicken skin. Among the 3 substrates, it was clear that broiler chicken skin had more percentage yield when compared to young coconut husk and custard apple seeds. The waste produced from food processing methods and other agricultural products is generally considered to be a problem as a pollutant. After processing, a large amount of plant material often remains without any application. When such materials are converted into valuable resources, it contributes to the reduction of residues. Utilization of agro-industrial waste, its low operating cost in biodiesel production makes this study a promising one for possible green technological applications. The rendered oils with high FFA could be converted to good quality biodiesel by two-step process *viz.* acid-catalyzed esterification of FFA followed by alkali catalyzed transesterification of triglycerides. The oil methyl ester from the substrates blended with diesel fuel could be used as an alternative fuel in conventional diesel engines without any major modifications.

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