

POTENTIAL OF PUMPKIN SEEDS FOR BIODIESEL PRODUCTION AND COMPARISON OF VARIOUS PARAMETERS FROM VARIOUS SEED

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Abstract:

*As in current scenario, the cost of all commercial fuels are of higher cost and the rates are increasing dramatically day by day and even their usage leads to pollution, that in turn increases the global warming. In the present study, the different seeds were taken for the study pumpkin (*Cucurbita pepo*), Peanut (*Arachis hypogaea*) and Soybean (*Glycine max*) for the biodiesel production. The oil was extracted from those seeds after drying and powered. The extracted oil was estimated for the total percentage of yield and it was determined as pumpkin 83.00 % yield of weight. The percentage yield for peanut and soybean was determined as 85.00%, 79.00 % respectively. The high yield for oil was identified in peanut. Various parameters of oil were predicted. The parameters are pH, Color, appearance at 20°C, percentage yield, moisture content, protein content, fiber content, fat content, gravity, Solubility in water were predicted. Further the production of biodiesel was carried out from Pumpkin seed oil and it was characterized through flame test and GS-MS report. The main fatty acids present were palmitic acid (54.64%), oleic acid (28.38%) and EPA (4.72%). It will help to produce maximum amount of biodiesel in economic way.*

Keywords: Biodiesel, Pumpkin seed, Peanut, Soybean, Oil

1. Introduction

This biodiesel was developed by inventor Rudolph diesel in the year 1890s which has become a choice of power, reliability and high fuel economy over the world wide. Modern biodiesel involves the conversion of vegetable oil into a compound called fatty acid methyl esters, research

conducted in Belgium 1930's, but today's biodiesel industry was not established in Europe until the late 1980's. The diesel was developed for a particular reason to improve efficiency, cumbersome and dangerous steam engines of the late 1800s. Diesel became known to worldwide for his innovative engine which could use variety of fuels [1].

The continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide to the environment. Global warming is thought to be due to increasing levels of carbon dioxide and water vapor in the atmosphere caused by large scale burning of fossil fuels. Every day hazardous emissions are dumped in to the atmosphere the results in the increase of green house gases [2]. Biodiesel is an alternative fuel produced from renewable vegetable oils, animal fats or recycled cooking oils by transesterification reaction. Finding alternative energy resources is a pressing mission for countries, especially for those countries lacking conventional fuel resources in. In 1930s and 1940s, vegetable oils have been used as biodiesel fuels in the emergency situation. With rapid development the modern industry, the demand for energy has been greatly increased in recent years.

Biodiesel appears to be an attractive energy resource for following reasons. It is a renewable source of energy that could be sustainably supplied. It has several favorable environmental properties resulting in no net increased release of carbon dioxide and very low sulfur content [3]. It is better than diesel fuel in terms of flash point and. Its emission profile is clear than diesel fuel. It can be used in diesel engines without modification and can be blended with petro-diesel fuel effectively. Biodiesel obtained from oil crops, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels [4]. The production cost is high for biodiesel. The price of biodiesel is a feasible substitute for conventional energy.

In the present study the effect of salinity and pH on the growth of *Pumpkin seeds* is carried out. The growth was assessed by measuring the absorbance at 750 nm and pigment concentration, the total lipid was extracted and estimated for biodiesel production. The main advantages of biodiesel given in the literature include its domestic origin, which would help reduce a country's dependency on imported petroleum, its biodegradability, high flash point, and inherent lubricate in the neat form reported by Moser [4]. The advantages of biodiesel as

conventional fuel are its portability, renewability, higher combustion efficiency and lower sulphur and aromatic content reported by (Arjun et al., 2008) higher cetane number and higher biodegradability reported by (Chingjuan et al., 2011). Biodiesel has significant potential as an alternative fuel in compression ignition engines reported by (Farizul et al., 2011). It is estimated that the proportionate consumption of petroleum products in India is as follows and it is also represented (India biofuels annuals 2012). Transport (petrol, diesel, CNG, and aviation fuel), 51% industry (petrol, diesel, fuel oil, naphtha, natural gas), 14 % commercial and others, 13 % domestic (LPG and kerosene), 18 % agriculture (diesel) 4 %.

2. Materials and Methods

The pumpkin (*Cucurbita pepo*), peanut (*Arachis hypogaea*) and soybean (*Glycine max*) were obtained from local garden and market respectively at Thiruvallur (Latitude: 13.144443, DMS Lat: 13° 8' 39.9948" N, Longitude: 79.894005, DMS Long: 79° 53' 38.4180" E). The species was verified with the Botanist, Madras University, Chennai. The pumpkin fruits were broken and the seeds removed for further processing. The seeds were cleaned by washing with distilled water and then with 1 % normal saline to clean and remove the microbial toxin. The seeds were dehulled with the knife and it was dried at 40°C for approximately 72 hours. The low temperature was maintained to prevent the evaporation of volatile constituents in the seeds. The seeds were broken into smaller bits, ground into coarse particles and ready for oil extraction (Ossai Emmanuel C and Njoku Obi U, 2011). The dried peanut and soybean was directly used further for making into coarse particles.

2.1. Extraction of Oil from Various Powdered Seeds: The extraction was carried out by soxhlet extraction method. The ground seeds (100g) were properly packed into the thimble of the soxhlet extractor, and n-hexane (300ml) was poured into the round bottom flask of the soxhlet extractor. The complete soxhlet extractor (i.e. with its condenser) was then mounted on a heating mantle which had its temperature gauge set at 80°C. At this temperature, the extractor set up was left to stand for 6 hours. The percentage (%) yield of the seed oil was determined.

2.2. Physico-Chemical analysis of oil extracted from various seeds: The parameters are pH, Color, appearance at 20°C, percentage yield, moisture content, protein content, fiber content, fat content, gravity, Solubility in water, solubility in ethanol was determined. Using with pH meter

the samples were tested for their acid content level. Color was predicted using with standard color chart (IEEE Guide Std 637-1985). Appearance of the oil was estimated through visible prediction.

2.3. Production of Biodiesel: The production was carried out using Freedman method, 1984. Biodiesel (fatty acid methyl esters or simply FAME) was produced from the pumpkin seed oil using the classical method. The extracted biodiesel was compared and the maximum producer was finely characterized further.

2.4. Characterization of Biodiesel: The first and basic confirmatory test for the biodiesel was the flame test which determines the presence of FAME (fatty acid methyl ester) components involved in the emission process in engines and machineries. TLC can also provide a chromatographic measurement known as an Rf value. 9:1 (Hexane : Diethyl ether) or in the ratio 85:15:1 (Petroleum ether : Diethyl ether : Acetic acid) was added for solvent phase. The GC-MS was an analytical method that unites the features of both Gas-chromatography and Mass-spectrometry.

3. Result and Discussion

The collected seeds were identified at species level Pumpkin (*Cucurbita pepo*), Peanut (*Arachis hypogaea*) and Soybean (*Glycine max*). The extracted oil was estimated for the total percentage of yield and it was determined as pumpkin 83.00 % yield of weight. The percentage yield for peanut and soybean was determined as 85.00%, 79.00 % respectively. The high yield for oil was identified in peanut.

The physico chemical parameters were interpreted and results were tabulated. The pH was found low for pumpkin and represented as little acidic, the color for peanut was identified as strong yellow. The appearance at 20°C was resulting that transparent for soybean, thickness is high in pumpkin and peanut. The moisture content was identified as 1.16 % in pumpkin (Table 1). The high protein content was estimated as peanut, high fiber content in soybean. The gravity is high in soybean. All seed oil is partially soluble in water. Among the different environmental conditions, the salinity and pH plays a major role in the growth of the organism. A slight change

in these conditions can provoke important changes in the growth and biochemical composition of microbial/plant samples [7]

Table 1: Analysis of Physico-chemical parameters

Parameter	Pumpkin	Peanut	Soybean
pH	5.6 (20 ^o C)	6.7	6.5
Color	Yellow	strong yellow	Yellow
appearance at 20°C	Oily, thickness	Oily, thickness	Oily, Transparent
percentage yield	83.00	85.00	79.00
moisture content	1.16 %	1.31 %	2.39 %
protein content	0.83	0.97	0.9
fiber content	0.025	2.099	2.133
fat content	12 gms	93 gms	95 gms
gravity	0.8	0.9	0.92
Solubility in water	Partially Soluble	Partially Soluble	Partially Soluble
solubility in ethanol	Soluble	Partially soluble	Partially soluble

Biodiesel Production Estimation: Further the pumpkin seed oil was considered for biodiesel production and determined the physico-chemical properties. The FAME obtained was sent for GC-MS analysis for characterization of the fatty acid present in it. The fatty acid profile was obtained and the maximum content of fatty acid was found to be palmitic acid which is of 54.64% of the total FAME, which was followed by oleic acid of 28.38% of the FAME. The third highest fatty acid obtained was eicosapentaenoic acid of 4.72% (Table 2). From the Physico-chemical characterization, the TLC analysis preliminarily confirms the presence of methyl ester groups present in the biodiesel by visualization of TLC plates. The GC-MS analysis of the

biodiesel identified the various fatty acid groups present within the biodiesel called the FAME along with their chemical compositions.

Table 2. Fatty acid profile of the test sample

S. No.	Fatty acid profile	Percentage
1	Capric acid	0.06
2	Lauric acid	0.84
3	Myristic acid	7.0
4	Pentadecanoic acid	1.0
5	Palmitic acid	54.64
6	Palmitioleic acid	0.51
7	Margaric acid	0.57
8	Stearic acid	0.59
9	Oleic acid	28.38
10	Linoleic acid	0.38
11	Arachidonic acid	1.30
12	Eicosapentaenoic acid	4.72

The first and basic confirmatory test for the biodiesel was the flame test which confirms the presence of methyl ester groups in the biodiesel. Thus the presence of methyl esters group in the biodiesel was confirmed by this flame test which was depicted in the figure. This confirms the FAME (fatty acid methyl esters) was the important components involved in the emission process of the engines and machineries during the combustion of biodiesel.

The Thin layer chromatography was another important confirmatory test of the biodiesel which confirms the presence of methyl esters in the biodiesel by the separation of the compounds in the TLC plates.

The GC – MS report determines the the Hydrocarbon grouping that are differentiated based on their Retention Time (RT) of Standard hydrocarbons. The analysis revealed the

presence of 15 major Fatty acid components that are present as Hydrocarbon chains within the Biodiesel sample (Table 3 and Fig 1-2).

Table 3. Determination of Fatty acid groups under GC-MS

No	Fatty acid name	Retention time (R _T)	Ions	Molecular weight (g/mol)	Molecular formula
1	9-Amino-1,8-Dimethyl-3,6-diazahomoadamantane	25.65	132	181.278	C ₁₀ H ₁₉
2	Heptadecanoic acid	28.33	53	270.457	C ₁₇ H ₃₄ O ₂
3	Octadecyl ester	30.03	111	536.9557	C ₃₆ H ₇₂ O ₂
4	Decane	7.55	60	142.282	C ₁₀ H ₂₂
5	Nonanoic acid, methyl ester	12.92	82	172.2646	C ₁₀ H ₂₀ O ₂
6	9-Hexadecenoic acid	17.09	227	254.4082	C ₁₆ H ₃₀ O ₂
7	Dodecanethioamide,N,N-diethyl	18.22	177	271.50492	C ₁₆ H ₃₃ NS
8	Dihydroapohemanthamine	18.48	188	271.31108	C ₁₆ H ₁₇ NO ₃
9	Ethanol, 2-[6-chloro-4-(4-methyl-1-piperidyl)-1,3,5triazin-2ylamino]	18.92	221	46.069	C ₂ H ₆ O
10	14, 17-Octadecadienoic acid, methyl ester	20.3	134	294.4721	C ₁₉ H ₃₄ O ₂
11	16-Octadecenoic acid, methyl ester	21.3	248	296.495	C ₁₉ H ₃₆ O ₂
12	N-Cyclooct-4-enylacetamide	22.63	132	87.122	-
13	1,8-Dimethyl-3,6-diazahomoadamantan-9-ol	24.02	151	224.348	C ₁₃ H ₂₄ N ₂ O
14	N-[3-Diethylaminopropyl]-4-oxo-1,2,3,4,5,6,7,8-octohydroquinoline	24.57	173	-	-
15	2-Octadecenoic acid, methyl ester	24.97	254	294.4721	C ₁₉ H ₃₆ O ₂

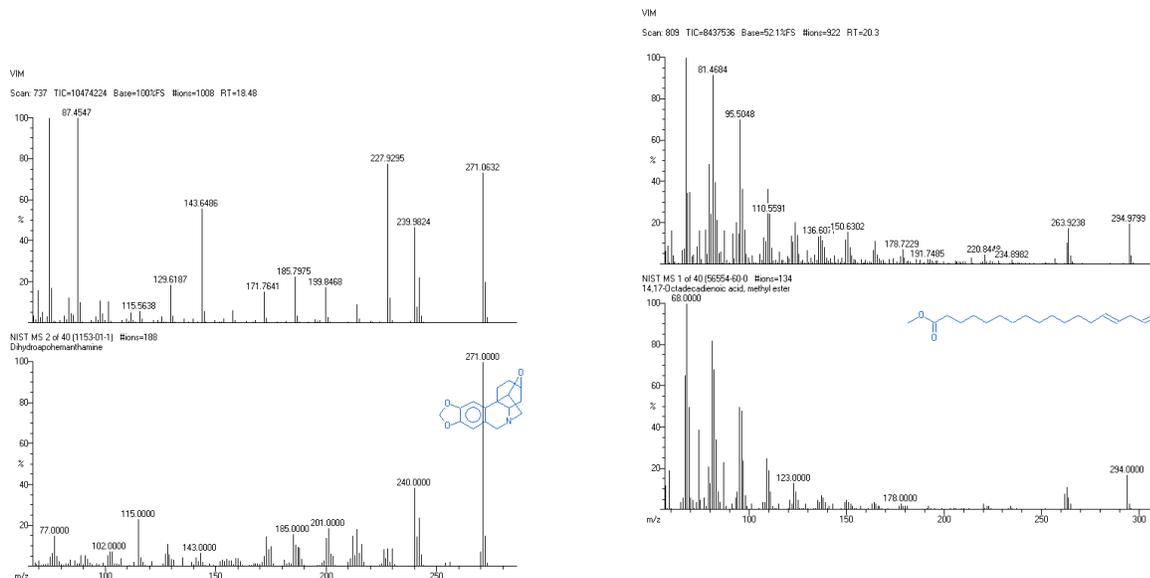


Fig 1. GC-MS Spectrum of fatty acid- Dihydroapohemanthamine & 14, 17-Octadecadienoic acid, methyl ester

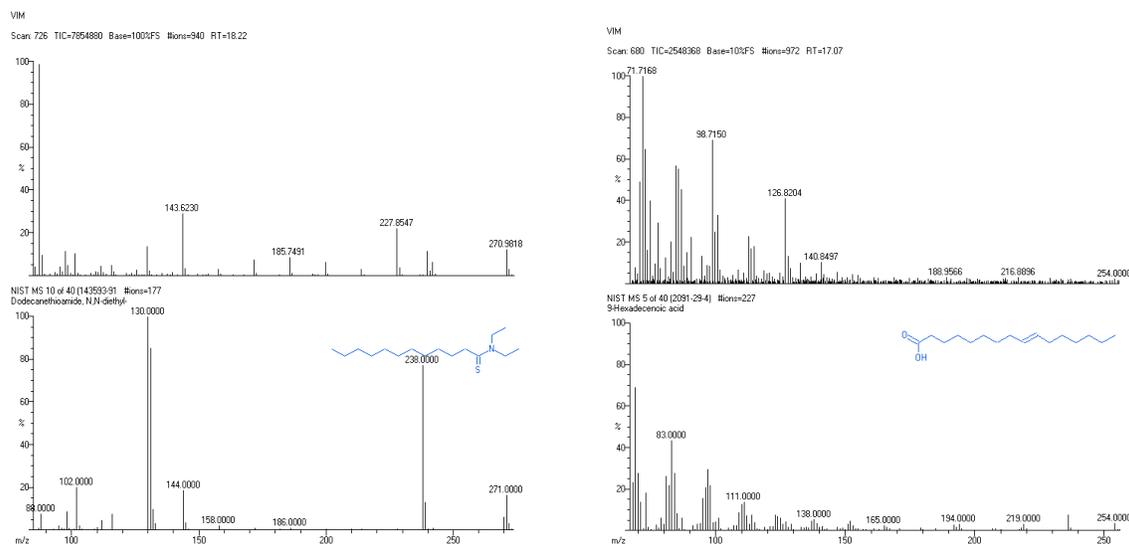


Fig 2. GC-MS Spectrum of fatty acid 9-Hexadecenoic acid & Dodecanethioamide,N,N-diethyl

The main fatty acids present were palmitic acid (54.64%), oleic acid (28.38%) and EPA (4.72%). The future use of biodiesel not only limits the use of fossil fuel but also helps in the

reduction of harmful air pollutants that are released during the combustion of conventional fuels, which therefore mentioned to be Green-fuel.

The characterized the biodiesel from the pumpkin seed was effectively producing the biodiesel and it was confirmed through the GC-MS. According to the interpretation it was identified as it can produce less amount pollution in the environment when compared with fossil fuels.

5. Conclusion

This study revealed that opportunities exist to enhance lipid production by manipulating various medium components and environmental factors. Moreover, an investigation of detailed physiological behavior of the nutrient consumption patterns, and fatty acid compositions, etc., The future use of biodiesel not only limits the use of fossil fuel but also helps in the reduction of harmful air pollutants that are released during the combustion of conventional fuels, which therefore mentioned to be Green-fuel.

6. References:

- [1] Ossai Emmanuel C and Njoku Obi U. *Asian J. Research Chem.* 4(10): (2011); Pp 1582-1586.
- [2] Freedman B.; Pryde E. H. and Mounts T. L. Variables affecting the yields of fatty esters from transesterified vegetable oils. *Journal of American Oil Chemists' Society.* **61**: (1984). 1638–1643.
- [3] Gayathri AK, GeethaPriya L, Haripriya U, M. Thenmozhi, P. Bama. Isolation and optimization of textile dye degrading bacteria from effluent dye containing soil sample. *Bulletin of Trends in Biological Sciences* (2017) 1 (1): 1-5.
- [4] Moser, B.R. “Biodiesel production, properties and feedstocks. In vitro cellular and Developmental Biology”. *Plant.* **45**: (2009). 229-266.
- [5] Ramos, M.J.; Fernandez, C.M.; Cacas, A.; Rodriguez, L. and Perez, A. Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresour. Technol.*, **100**: (2009). 261 – 268.

- [6] Oluba, O.M.; Ogunlowo, Y.R.; Ojieh, G.C.; Adebisi, K.E.; Eidangbe, G.O. and Isiosio, F.O “Physicochemical properties and fatty acid composition of *Citrullus lanatus* (Egusi melon) seed oil”. *Journal of Biological science*. **8**(4): (2008).814-817.
- [7] Pratibha Sanjenbam, Mohankumar Thenmozhi, Krishnan Kannabiran. Screening of glycolytic enzyme inhibitory activity of *Streptomyces* isolates from brine spring and marine sediments of India. *International journal of pharma research and review* (2013) 2(2): 5-11.