Eco. Env. & Cons. 28 (1) : 2022; pp. (535-539)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i01.081

Effect of Ultrasonication in Lipid Extraction and Production of Biodiesel from Groundnut Shells

Hariprasath Ragupathy* and Erumalla Venkatanagaraju

Department of Life sciences, CHRIST (Deemed to be University), Hosur Rd, Bhavani Nagar, S.G. Palya, Bengaluru 560 029, Karnataka, India

(Received 18 June, 2021; Accepted 27 August, 2021)

ABSTRACT

Agro-industrial wastes are at no cost and contain phenolic content; utilizing them in biodiesel production is a beneficial idea. Biodiesel is a choice of fossil fuels. It can be used in diesel engines alone or blended with diesel. In this study, we have chosen groundnut shells as Agro-industrial waste. Groundnut shells were chopped, dried, and ground into powder, and taken into acid pretreatment. After acid pretreatment, one sample was subjected to Ultrasonication, and then lipid contents were isolated using the modified Bligh and Dyer method. In the transesterification process, the lipids were turned into a product such as esters of fatty acids and glycerol in the presence of catalyst KOH and solvent methanol. The characterization and confirmation of esters of fatty acids were done by Gas chromatography-Mass spectroscopy (GC-MS). This research mainly focuses on studying the effect of Ultrasonication in lipid extraction and encourages biodiesel production from groundnut shells.

Key words: *Groundnut shells, Biodiesel, Transesterification, Lipids, Ultrasonication.*

Introduction

Groundnut (*Arachis hypogaea*), also known as peanut, is a nutritious leguminous seed. It is primarily grown for seed and oil all over the world. Groundnut shells are the final product of the extraction of the groundnut seed from its pod. Groundnut shell is a significant agro-industrial waste that degrades very slowly in environmental conditions. Most abandoned groundnut shells are burned or buried, dumping into the soil and polluting the atmosphere (Duc *et al.,* 2019). In comparison to several agricultural wastes, groundnut shells seem to have the highest lignocellulosic biomass production potential. Thus, it is thought to be impressive lignocellulosic biomass to produce biofuels such as biodiesel, bioethanol, biogas, etc. (Deeba *et al.,* 2017). Petroleum-based fuel is a big polluter of the atmosphere. Carbon dioxide, a contributor to global climate change, is one of the emissions emitted due to hydrocarbon combustion. The pollutants released from the diesel cause several diseases in humans, lead to cancer and cardiopulmonary health issues, and causes air, water, and soil pollution (Lloyd and Cackette, 2001). The need for fuels is increasing day by day, and on the other hand, prices are also increasing. One of the best solutions for these problems is biofuel (Krishnaprabu, 2019). Biodiesel has proven to be a safe and promising alternative to petroleum diesel. Biodiesel is less volatile than conventional diesel, in addition to being biodegradable and made from renewable resources. For using biodiesel, no major engine modifications are required. Biodiesel is a mixture of fatty acid alkyl esters. It is produced by transesterification of oils such as vegetable oil or animal fat in the presence of a

short chain of alcohols. Methanol and ethanol are the most widely used (Yusoff, 2014). The creation of economically and environmentally safer fuel substitutes from renewable resources is vital to solving these problems. It has a higher-octane number than Petro-diesel, leading to substantial decreases in particulate matter, carbon monoxide, sulphur, polyaromatics, hydrocarbons, smoke, and noise pollution when used in diesel engines (Krishnaprabu, 2019). There are four significant steps in biodiesel production from agro-industrial wastes: Collection of samples, pre-treatment, lipid extraction, and transesterification (Biodiesel synthesis) (Deeba *et al.,* 2017). The first fatty acid esters to be used as biodiesel were fatty acid methyl esters (FAME). However, the use of fatty acid ethyl esters (FAEE) in biodiesel is gaining popularity. Because of its suitable physicochemical parameters, cheap, low toxicity, and ease of phase separation, methanol has been the most commonly used alcohol for biodiesel processing (Yusoff *et al.,* 2014) (Donoso *et al.,* 2020). Mechanical and non-mechanical disturbance are the two categorized pre-treatment procedures. Non-mechanical methods include desiccation and lysis, while mechanical methods include solid and liquid shear methods. Ultrasonication is one of the best cell disruptions while comparing with the non-mechanical techniques. By using Ultrasonication before solvent extraction enhances the yield of lipid. It consumes the least number of solvents and less time, thus enhancing lipid release and producing excellent results (Mubarak *et al.,* 2016) (Zhang *et al.,* 2014). In this study, lipids were isolated from the groundnut shells by being subjected to ultrasonication techniques, and comparison was taken between Ultrasonicated samples and non-sonicated samples.

Methodology

Collection and processing of Groundnut shells

Industrially waste groundnut shells were collected from the oil industries from around Erode district, Tamil Nadu. The sample was collected in a sterile plastic bag and taken into a laboratory for further processing. The sample was kept in a hot air oven at 70 °C for 62hr to remove moisture and microbial content. Then it was transferred into a clean mixer and grounded into fine powder. The obtained fine powder was stored in the sterile plastic bag for further process.

Extraction of Lipids by subjecting Ultrasonication

The 20g of powdered samples were weighed and pre-treated with 2-fold of 2M HCl in two conical flasks, set A and set B, and kept in an orbital shaker for 24hr. After 24 hours, sample set A was named an Ultrasonicated sample and subjected to Ultrasonication (GTSonic-GT-1730QTS) at 33KHz for 20 min at 35±2 ºC. The Ultrasonicated sample A and Non sonicated sample B both were centrifuged at 5000 rpm for 10mins. After centrifugation, the supernatant from the sonicated sample and pellet from non-sonicated was taken and mixed with chloroform and methanol in the ratio of 2:1. The mixed sample was centrifuged, and the chloroform layer was transferred into the round bottom flask. The chloroform layer thus contains the purified lipids. The lipid extraction was done separately by using the Rotary evaporator at 70 °C. After this process, the solid residue, which contains lipids were taken into the transesterification process (George *et al.,* 2020).

Determination of Lipid

After the round bottom flask was taken from the Rotary evaporator, the flask was covered with aluminium foil and then taken into a weighing machine. The total lipid content was calculated by subtracting the weight of the round bottom flask with the lipid content with the initial weight of the round bottom flask. The formula was noted below (George *et al.,* 2020)

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

Transesterification process

In the transesterification process, 40mL of methanol and 1g of potassium hydroxide were mixed in a separate 100 mL beaker and kept in a magnetic stirrer for 10 min at 100rpm. Then the mixture was added into a round bottom flask containing the lipid sample. It was kept under a heating mantle, and the condenser was attached to the top of the round bottom flask. To prevent the evaporation of methanol, the cooling system was attached to the condenser. The temperature was adjusted to 60 °C for 3hr. After this process, the solvent was removed by using a rotary evaporator. After this process, 20 mL ethyl acetate was added and stirred, and an equal volume of H_2O was added. It resulted in two layers which

will be separated by using a separating funnel. The upper layer was collected, and few grams of Sodium sulfate were added in order to remove the water contents. The sample solvents were removed in the rotary evaporator, and then it was stored in chloroform and sent for characterization. The same transesterification process was performed with the supernatant collected from the non-sonicated sample (George *et al.,* 2020).

GC-MS Analysis

The NIST/EPA/NIH mass spectral library system, version 1.0, was used to perform the GC-MS analysis. GC-MS did the confirmation and characterization of the obtained result. Gas chromatography interfaced with a Mass Spectrometer (GC-MS) to determine the methyl esters and other esters present in the obtained biodiesel products (Elkady *et al.,* 2015). The constituents were identified by comparing them to those in the computer library (NIST) attached to the GC-MS instrument, and the results obtained were tabulated (Starlin *et al.,* 2019) (George *et al.,* 2020).

Results and Discussion

In this research, Ultrasonication was employed to break down the cell wall and separate the lignin and cellulosic contents. The cell disruption leads to the release of closely intact lipid particles present inside the groundnut shell. Lipids are insoluble in a polar solvent.

The initial weight of the round bottom flask was found to be 132.057g and the weight of the round bottom flask with the lipid content was found to be 135.815g. Finally, the modified Bligh and Dyer method showed that the sonicated sample's total lipid content was 3.758g. When comparing with the non-sonicated sample, the Ultrasonicated sample contains double the times of lipid contents. It thus, confirm that an ultrasonicated employed a major role in lipid extraction.

The obtained lipids were taken into the transesterification process. After 3 hours, the lipids were converted into esters of fatty acid and glycerol. The final biodiesel product was separated from the glycerol by adding co-solvent ethyl acetate and water.

Fig.1 represents fatty acids retention times, chromatography and peak areas. The ester compounds obtained from the Ultrasonicated groundnut shells sample was found to be Peak 1 2,5-cyclohexadiene-1,4-dione,2-(1,1-dimethylethyl)-5-(2-methyl-2-propen-1-yl)-, Peak 2 2,4-Di-tert-butylphenol, Peak 3 8-Heptadecene, Peak 4 Tetradecanoic acid, Peak 5 n-Hexadecanoic acid (Palmitic acid), Peak 6 n-Heptadecanol-1, Peak 7 cis-Vaccenic acid, Peak 8 Octadecanoic acid (Stearic acid), Peak 9 2-Hexyldecyl isobutyrate, Peak 10 9-Octadecenoic acid (Z)-,2-hydroxyethyl ester. These are all the major compounds that confirm the presence of Biodiesel compounds in the Groundnut shell. The detailed profile of these compounds is mentioned in Table 1.

Some of the other major compounds detected at other hits were Pentadecanoic acid (Pentadecylic acid), Oleic Acid(Elaidoic acid), Dodecanoic acid (Lauric acid), 6-Octadecenoic acid, (Z)- (Petroselinic *acid*), Acetic acid n-octadecyl ester, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, Oleic acid, 3-hydroxypropyl ester, etc., which were all suggests that the sonicated sample have ester compounds and also confirmed that it has the characteristics of biodiesel.

Fig. 2 represents the GCMS profile of the non-

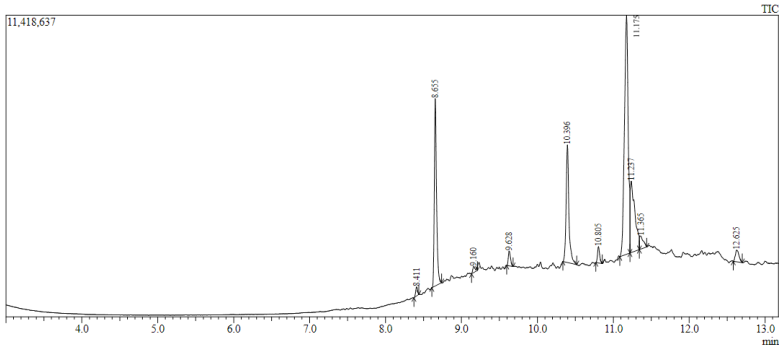

Fig. 1. The above graph represents the GC-MS profile of fatty acids retention times and peak areas – sonicated sample

Table 1. Ultrasonicated sample - groundnut shell biodiesel ester profile

S.No	Compounds	Molecular Formula	Peak	Retention Time	Area%	Height%
1	2,5-cyclohexadiene-1,4-dione, 2-(1,1-dimethylethyl)-5-(2-methyl-2-propen-1-yl)-	$C_{14}H_{18}O_2$	1	8.411	0.80	1.33
2	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	2	8.655	19.11	27.30
3	8-Heptadecene	$C_{17}H_{34}$	3	9.160	0.80	0.87
4	Tetradecanoic acid	$C_{14}H_{28}O_2$	4	9.628	1.51	2.17
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5	10.396	15.05	17.13
6	n-Heptadecanol-1	$C_{17}H_{36}O$	6	10.805	1.57	2.40
7	cis-Vaccenic acid	$C_{18}H_{34}O_2$	7	11.175	41.95	34.80
8	Octadecanoic acid	$C_{18}H_{36}O_2$	8	11.237	15.18	10.41
9	2-Hexyldecyl isobutyrate	$C_{20}H_{40}O_2$	9	11.365	2.07	1.87
10	9-Octadecenoic acid (Z)-,2-hydroxyethyl ester	$C_{20}H_{38}O_3$	10	12.625	1.96	1.72

Table 2. Non-sonicated sample groundnut shell biodiesel ester profile

S.No	Compounds	Molecular Formula	Peak	Retention Time	Area%	Height%
1	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	1	8.655	17.98	23.23
2	n-Hexadecanoic acid	$C_{16}H32O_2$	2	10.380	8.38	10.49
3	cis-Vaccenic acid	$C_{18}H_{34}O_2$	3	11.148	53.39	44.41
4	n-Decyl alpha.-d-2-deoxyglucoside	$C_{16}H_{32}O_5$	4	11.215	8.55	12.04
5	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	5	12.625	11.70	9.82

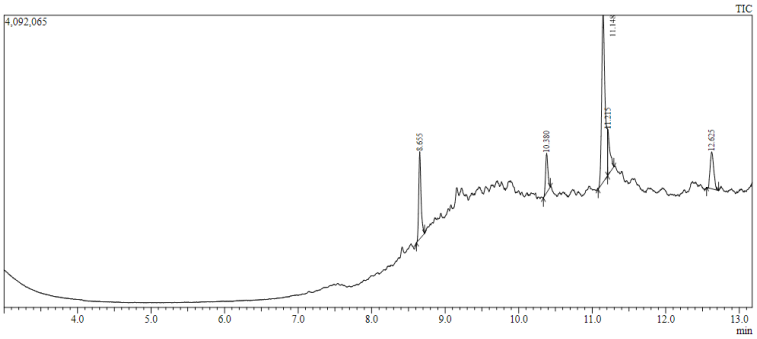

Fig. 2. Fatty acid profile of fatty acid esters of groundnut shell – non-sonicated sample

sonicated groundnut shells sample. The ester compounds obtained from the Non sonicated groundnut shell sample was found to be Peak 1 2,4-Di-tert-butylphenol, Peak 2 n-Hexadecanoic acid, Peak 3 n-Decyl. alpha. -d-2-deoxyglucoside, Peak 4 Oleic acid, Peak 5 3-hydroxypropyl ester. Non-sonicated sample groundnut shell biodiesel ester profile was mentioned in Table 3.

The number of the ester and other compounds obtained from the Ultrasonicated sample of groundnut shell was higher than the Non sonicated sample. The amount of biodiesel obtained from the Ultrasonicated sample was found to be 1.2mL. The amount of biodiesel obtained from the non-Sonicated sample was found to be 0.5mL. It showed that Ultrasonication has a high potential for lipid extraction and helps produce a high amount of biodiesel and lipids compared with the Non sonicated sample.

Conclusion

Agro-industrial waste, Groundnut shells showed that it has a rich source of lipid content and it was successfully converted into biodiesel product. This research highlighted that in comparison with Non

sonicated sample, the biodiesel obtained from the Ultrasonicated sample showed more ester compounds and a higher yield of lipid and biodiesel. It indicates that Ultrasonication played a major role in this production process. We conclude that biodiesel from the groundnut shells blended with diesel fuel could be used as an alternative fuel in conventional diesel engines without significant modifications. It indicates that Ultrasonication played a promising role in separation and extraction of lipids, leading to an increased production. Further studies such as Flashpoint, specific gravity, pour point, moisture content, kinematic viscosity would be taken to check the properties and efficiency of biodiesel.

Acknowledgements

This project was selected under the 44th series of student project programmes and funded by KSCST & IISc. The authors would like to thank the VC, Pro VC, and CHRIST (Deemed to be University) management for supporting this work. The authors would also like to thank Mr.Jyothis Devasia from common insturmentaion facility for his help in sample preparation and GCMS instrumentation.

References

Deeba, F., Pruthi, V. and Negi, Y. S. 2017. Fostering triacylglycerol accumulation in novel oleaginous yeast Cryptococcus psychrotolerans IITRFD utilizing groundnut shell for improved biodiesel production. *Biores. Technol.* 242 : 113–120.

Donoso, D., Bolonio, D., Lapuerta, M. and Canoira, L. 2020. Oxidation Stability: The Bottleneck for the Development of a Fully Renewable Biofuel from Wine Industry Waste. *ACS Omega.* 5(27) : 16645–16653.

Duc, P., Dharanipriya, P., Velmurugan, B. and Shanmugavadivu, M. 2019. Groundnut shell -a beneficial bio-waste. *Biocatal. Agric. Biotechnol.* 20: 101206.

Elkady, M. F., Zaatout, A. and Balbaa, O. Production of Biodiesel from Waste Vegetable Oil via KM Micromixer. *J. Chem. Eng* : 1-9.

George, A.B., Joy, M., Xavier, F.J. and Venkatanagaraju, E. 2020. Production of biodiesel from agro-industrial waste. *Int. J. Pharm. Sci. Res.* 11(2) : 978-86.

Krishnaprabu, S., Department of Agronomy, and Annamalai University. 2019. A Review on Biodiesel Production as Alternative Fuel. *Ind. J. Pure. App. Biosci.* 7(2) : 258–266.

Lloyd, A. C. and Cackette, T. A. 2001. Diesel engines: environmental impact and control. *J. Air. Waste. Manage.* 51(6) : 809–847.

Mubarak, M., Shaija, A. and Suchithra, T.-2016. Ultrasonication: An effective pre-treatment method for extracting lipid from *Salvinia molesta* for biodiesel production. *Res. Efficient. Technol.* 2(3):126-132.

StarlinT., Prabha, P. S., Thayakumar, B. K. A. and Gopalakrishnan, V. K. 2019. Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. *Bioinfo.* 15(6) : 425–429.

Yusoff, M. F. M., Xu, X. and Guo, Z. 2014. Comparison of Fatty Acid Methyl and Ethyl Esters as Biodiesel Base Stock: a Review on Processing and Production Requirements. *J Am Oil Chem' Soc.* 91(4) : 525–531.

Zhang, X., Yan, S., Tyagi, R. D., Drogui, P. and Surampalli, R. Y. 2014. Ultrasonication assisted lipid extraction from oleaginous microorganisms. *Biores. Technol.* 158 : 253–261.