Ultrasonic Pre-treatment and Optimization of Shaker Assisted Hexane Extraction kinetics and activation energy on Second Generation Biofuel Sources

Sri Aarthi Thangadurai M.*, Renganathan S., Shyamasundari M., Rajeswari G. and Priyanka M.
Department of Biotechnology, AC Tech Campus, Anna University, Chennai, INDIA
*sriarthi2007@gmail.com

Abstract
Aging of the world and depletion of all resources probes a path for discovering alternate energy sources. These Concerns on the search of bio fuels to replace the decaying fossil fuels. Identification of sustainable substitutes such as bio fuels (biodiesel, bio alcohol and biogas) from this study. The use of food crops may compete with the food chain, so non-edible crops are making an alternate for biodiesel production. As a result, scientists are now probing on the utilization of non-edible feedstock for biodiesel production.

In this study moisture content of certain seeds was analyzed by oven dry method. Moisture content of seeds less than 8% shows maximum oil yield as 23%, 32% and 34.54% for Caesalpinia sappan, Cleome gynandra and Cleome viscose respectively. Ultrasonic Pretreatment was carried out on seed sources such as Caesalpinia sappan, Cleome gynandra and Cleome viscose. Optimization of oil extraction were carried out on Cleome viscose by Shaker Assisted Hexane Extraction and the variables for optimization are Seed to solvent ratio, Rpm,Temperature,Time respectively effect the oil extraction. The optimized parameters are seed to solvent ratio as 1:12, 125 Rpm, 67°C temperature and time 66 mins. After optimization, the yield of oil extracted from Caesalpinia sappan, Cleome gynandra and Cleome viscose respectively were 25%, 32%, 34%.

The extraction kinetics determine the rate constant by applying Differential method. The reaction follows first order kinetics and the activation energy was interpreted as 7.1KJ and the Arrhenius constant was $3.77*10^{-3}$ $s^{-}1$*. The physiochemical properties of the seed oil were analyzed by AOCS methods. This biofuel second generation source was proved to be a suitable feedstock for biodiesel production.*

Keywords: Biofuel, Caesalpinia sappan, Cleome gynandra, Cleome viscose, Shaker, Utrasonication, Extraction kinetics, Activation energy.

Introduction

Biodiesel biorefinery facilitates and integrates biomass conversion process produces biofuel etc. Due to the drastic squandering of fossil fuels and to prevent green house gas emissions, foreign petroleum, air pollution and public health related risks biofuel production was probed over two decades [1]. Now, the third generation (algae) is encompassed hence forth the fourth generation (genetically modified crops) is on its development, even still broad area has to be explored on second generation (non edible seeds) was an alternate for first generation sources (edible seeds) [2]

Second generation sources offers fillip to convert wide range of ligninocellulosic feedstock to biofuel and other value added products compared to other generation sources. The truce we insist upon the barriers on the second generation sources yet another decade was required to make commercially viable [3]. The moisture content of the non edible seeds were determined by oven dry method because heat content calculated on a dry mass basis must be altered for the natural water content that can reduce by 20% to the net heat available [4].

Ultrasonic waves are generated by a transducer made out of a piezoelectric material [5]. In response to an alternating current piezoelectric material produces characteristic mechanical vibration of ultrasonic frequency was higher than 20 kHz. These ultrasonic waves produce pressure differences that enhances chemical and physical processes in the solution medium [6].The ultrasonic pretreatment was a powerful technique causes the ultrasonic waves through the vibrations that severely damage the biomass structure which will subsequently increase the yield of the oil content [7]. Ultrasound- pretreated was a low-cost technology that assists in the significant reduction of extraction time and temperature results in a higher yield [8].

The shaker extraction with hexane as a conventional solvent showed the highest yield when compared to other non conventional solvents (acetone, ethanol, isopropanol) [9].The Extraction kinetic of the solvent depends on the rate of formation of products and rate of dissociation of reactants [10, 11] studied the extraction kinetics that determining the rate of the reaction that demonstrates the reaction mechanism. Extraction kinetics also integrates the rate laws, and the reaction expressed in terms of Yield and Time [12.]

The objective of this paper were to determine the moisture content of certain seeds (*Caesalpinia sappan, Cleome gynandra and Cleome viscose*), ultrasonic pretreatment enhances the disruption of seed biomass and optimization of shaker assisted hexane extraction to recover the optimized parameters for extraction.The kinetics of extraction were determined by Differential method and rate equation, activation energy and Arrhenius constant also calculated. The physiochemical properties of the seeds were analyzed by AOCS methods. These study focuses on the non-edible seeds were the suitable Feedstock's for Biorefinery.

Material and Methods

Seed characterization: The Indigenous seeds (Caesalpinia sappan, Cleome gynandra and Cleome viscose) were collected from Anna University, Chennai, India. The damaged seeds and dirt were removed and good seeds were washed with distilled water. The seeds were sundried and dehulled to separate the kernels. The hulls and kernel weight were determined on weight basis. All chemicals used in this study were purchased from sigma Aldrich (India) is of analytical grade.

Moisture content determination: The moisture content of the seeds was removed by hot air oven at 105°C for 16 hrs by AOCS Ba 2b-82 method [13, 14.] The samples were dried and weighed. Formula 1 was used to calculate the moisture content. The loss in weight is found to be loss of moisture. The moisture content of seeds can be determined with respect to oil yield and time.

$$\%MC = \frac{\text{Original weight} - \text{Ovendry weight}}{\text{Oven dry weight}} * 100 \quad \rightarrow 1$$

Ultrasonic pre-treatment: Preliminary calibration of amplitude, frequency, pulse on and pulse off are made. Ultrasonic processor of model VC505 with power supply 500 watts used for pretreatment of seed biomass, 50 g of crushed and sieved seeds were probed separately at 24 kHz with constant temperature (50 ± 2 °C). Pretreatment for 5 min was processed on seed biomass along with distilled water (3:1 water to seed ratio). After destruction of cell wall of seeds, the biomass was dried in shade conditions and in oven at (60 ± 1 °C) as per method of [15]. The pretreated *Caesalpinia sappan, Cleome gynandra* and *Cleome viscose* seed biomass was loaded on shaker assisted hexane extraction.

Shaker Optimization: The shaker assisted hexane extraction was performed in Erlenmeyer flasks with rubber corks containing the ultrasonic pretreated *C.viscose* seeds and hexane was used as the solvent for extraction. The factors that has to be optimized are seed-to-solvent (w/v) ratios, rpm, temperature, time, respectively are the factors that affect the oil extraction. These assays were done separately on the *cleome viscose* seeds [9]. The oil yield was calculated from formula 2 and oil content was determined by AOCS Ba 3 –38 method[14.]

$$\text{Yield} = \frac{\text{Weight of oil extracted}}{\text{Weight of oil meal taken}} * 100 \quad \rightarrow 2$$

Extraction kinetics: Kinetics of extraction depends on the various chemical reactions that diffuses and control the chemistry of the extraction process. Extraction kinetics study was carried on the cleome viscose seeds.

The kinetics of the reaction rate depends on the time and the temperature factors that have to be optimized to calculate the formation of products (oil yield) [10]. The temperature ranges were conducted at 27, 37,47,57,67 degree celsius and the time ranges at 16, 26, 36,46,56,66 minutes was used to carry out the kinetic study on cleome viscose seeds.

Physiochemical properties: These seed oil were characterized for physiochemical analysis by AOAC methods. Seed oil was subjected to physical characterization. The colour and state of the oil at room temperature were not by visual inspection. While the refractive index was determined at room temperature using the Abbe refractometer (NAR-1T; Atago Co., Ltd., Tokyo, Japan).

The procedures for determination of chemical indices, such as the acid value (AOAC 969.17), peroxide value (AOAC 965.33), saponification number (AOAC 920.160), non saponification matter (AOAC 933.08), iodine value (AOAC 993.20), insoluble impurities (AOCS Ca 3a-46) and moist and volatile matter (AOAC 926.12), were carried out following the official method by [16].

Results and Discussion

Moisture content determination: Figure 1(a,b,c) of this study depicts that the moisture content of *caesalpinia sappan* at 26% yields 8% oil content when the moisture content decreases to 8.3% then the oil content was 23%, for Cleome gynandra the highest oil yield at 8.1% moisture content was 32% ,Cleome viscose seeds shows the oil yield at 8% of moisture content was 34% a gradual decrease in moisture content when time elapses, the oil yield also simultaneously increases, Adegmo et al. (2013) confirms this in his investigation on Moringa oleifera seeds.

According to [17], higher moisture content of seeds will disrupt the yield of oil so moisture content has to be reduced.Their report says maximum oil content obtained at 6% for sunflower seeds and decreased further increase in moisture content. [18], discuss the maximum oil recovery obtained at 8.1% for neem seed oil and minimum oil recovery at 16.1%.

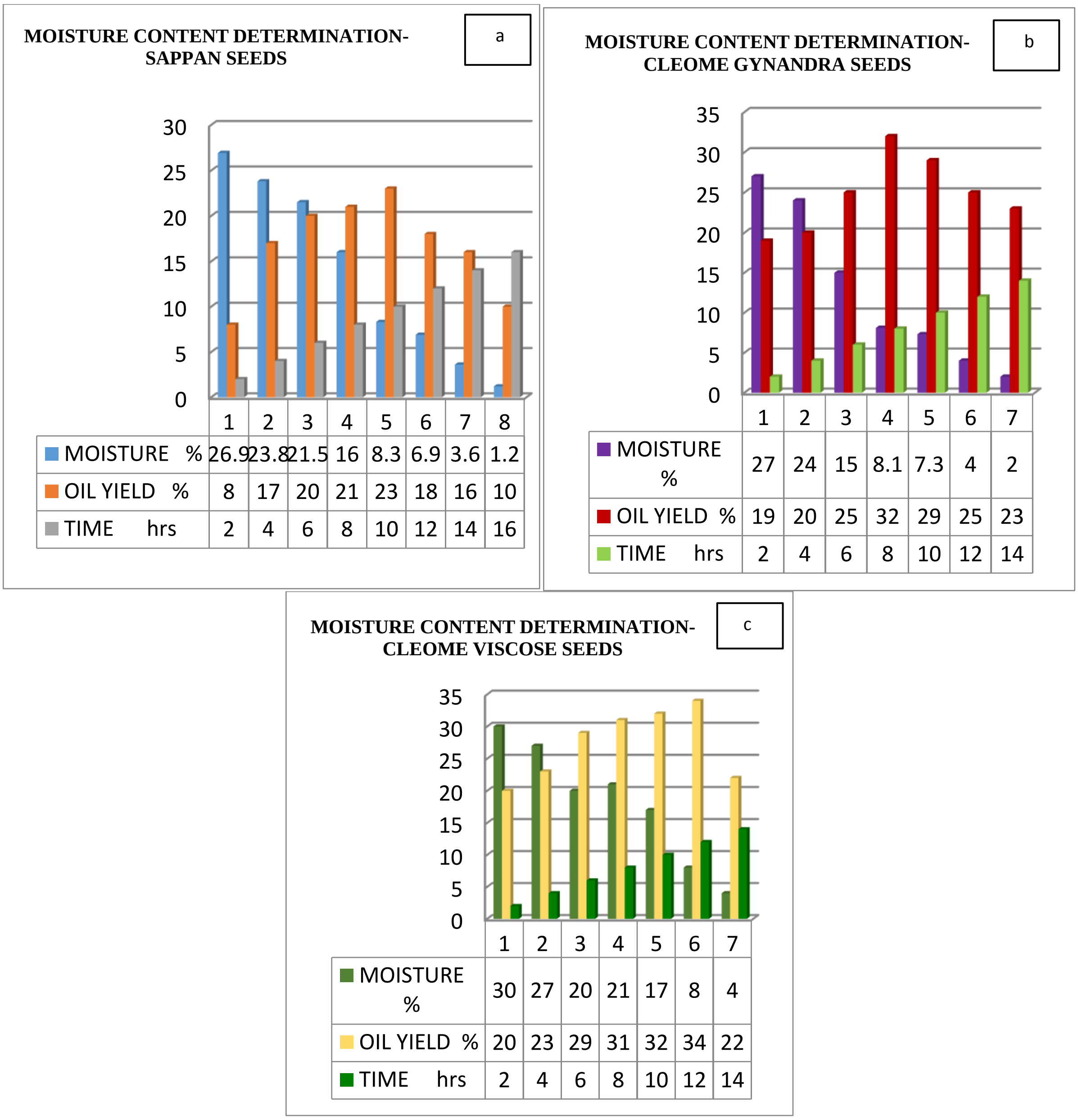

Figure 1: Moisture Content determination with respect to oil yield and time for (a) *Caesalpinia sappan*, (b) *Cleome gynandra*, (c) *Cleome viscose* seeds.

Shaker optimization:

Effect of polar and non polar solvents oil yield: The effects of the solvation nature determine the solubility of the desired products, [10].In this experiment various polar solvents and non polar solvents were used to extract on the cleome viscose seeds. The oil was extracted by ultrasonic pretreated shaker extraction to maximize the yield. The solvation nature of the solvents depends on the yield and the unsaponifiable matter, [19].

Table1: Effect of different non polar solvents on oil extraction from cleome viscose seeds

Solvent	Yield (wt %)	Unsaponifiable **matter** (wt %)
(non polar)		
n-Heptane	30	5
Benzene	31.54	9
Petroleum ether	32.59	4
Hexane	34.54	2
	33.89	8

Table 1 depicts the solvation nature of the polar and non polar solvents with respect to oil yield and unsaponifiable matter. In this study the order of solvation power of non polar solvents with respect to oil yield goes from high to low such as hexane, chloroform, petroleum ether, benzene, and n-heptane was compared with results of [20].The order of unsaponifiable matter in this study goes from low to high given as hexane, petroleum ether, heptane, chloroform, benzene. [19] expound in his study the lowest unsaponifiable matter yields higher oil yield. [21] decipher that unsaponifiable matter are the substances that should be less for determining the solvation nature of nonpolar solvents.

Effect of Seed to Solvent ratio: The optimization reaction was carried out on *cleome viscose* seeds. The ultrasonic pretreated sample for optimizing the seed to solvent ratio undergoes shaker extraction that ranges from 1:2, 1:4, 1:6, 1:8, 1:10,1:12,1:14 and 1:16.Figure 2 interpret the maximum oil yield of extraction was 34.54% at 1:12 seed to solvent ratio was the maximum yield manifested by [19].The further rise in the solvent ratio from 1:14 and 1:16 decreased the yield of oil extraction that was published by [19].

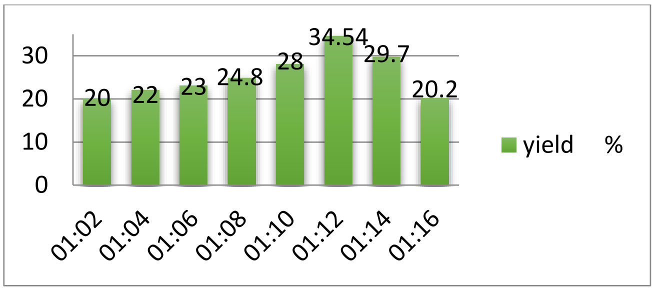

Figure 2: Effect of seed to solvent ratio on cleome viscose.

Effect of agitation speed (rpm): The agitation speed is also one of the parameter that affects the oil yield of cleome viscose seeds. Optimization of mixing intensity was carried out with different rate of mixing intensity ranging from 25 to 175 ppm. During optimization, other reaction parameters were kept constant at 37ºC temperature, seed to solvent ratio 1:12(g/ml), 66 min reaction time. From figure 3 the maximum oil yield was attained at 125 rpm further increase in rpm does not increase the yield of oil. Suganya et al. (2012) studied the maximum oil yield was obtained at 500 rpm from marine microalgae. [21] get across in his investigation that the maximum yield of oil from Linn seed was obtained at 100 rpm.

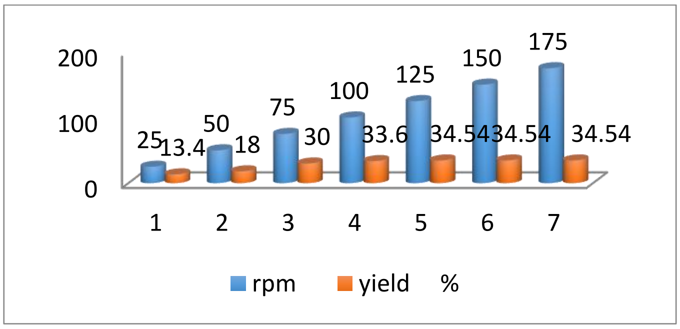

Figure 3: Effect of rpm on cleome viscose.

Effect of reaction temperature: The variation of temperature was considered as the parameter of study. The seed to solvent ratio 1:12, 125 rpm are kept constant on the ultrasonic pretreated shaker extraction. As the temperature increases from 27ºC to 67ºC, further increase in temperature abruptly does not increase the oil yield because the boiling point of hexane was 68ºC. Figure 4 bring out the maximum temperature attained was 67ºC and the yield obtained at 34.54 %. [22] in his findings the maximum temperature attained was 80 ºC optimizing the Linn seeds. Suganya et al. (2012) depicts the maximum temperature in her study on marine microalgae was 55 ºC. [23] in his paper reported the maximum temperature was 70 ºC.

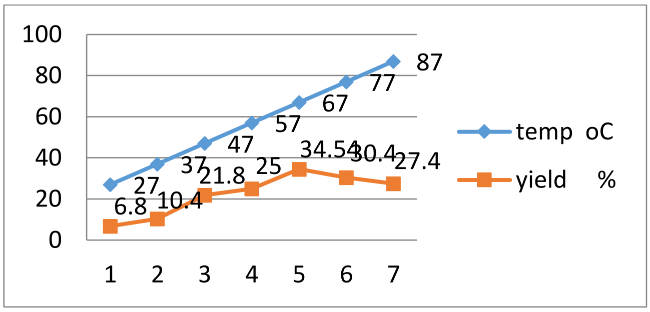

Figure4: Effect of reaction temperature on cleome viscose.

Effect of reaction time: The reaction time was also one of the important factors for extraction. Optimizing reaction time of the extraction process is much important as prolonged exposure of seed to the solvent can result in poor yield of the oil. In order to optimize the reaction time, the extraction process was allowed to execute from 16 to 76 min. The maximum time of extraction analyzed from this study was 66 min showed clearly in the figure 5.Further increase in the time will decrease in oil yield. [24] in his study justifies the maximum reaction time was 70 min extracts the maximum oil. Suganya et al. (2012) in her findings the maximum oil yield was obtained at 120 min.

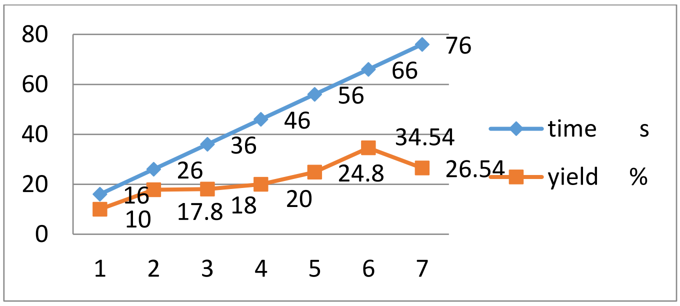

Figure 5: Effect of reaction time on cleome viscose.

Kinetics of Extraction: Table 2 depicts the oil yield of ultrasonic pretreated shaker extraction with respect to time and temperature was obtained from the cleome viscose seeds.

The reaction rate equation for extraction from cleome viscose seeds can be written as

$$dY/dt=kY^n \quad (3)$$

Y is the percent of oil yield, t is the time of extraction (min), k is the extraction constant, and n is the reaction order. The oil yield percentage increased with respect to time, the terms dY/dt have a positive sign. Applying differential method on Table 2, the graph was plotted between lndY/dt vs lnY and linear graph was obtained and the reaction follows First-order kinetics and the average of regression coefficient was 0.580. The slope and the values of n also reported from Figure 5b. Table 3 gives the values of reaction rate constant.[11, 21-23].

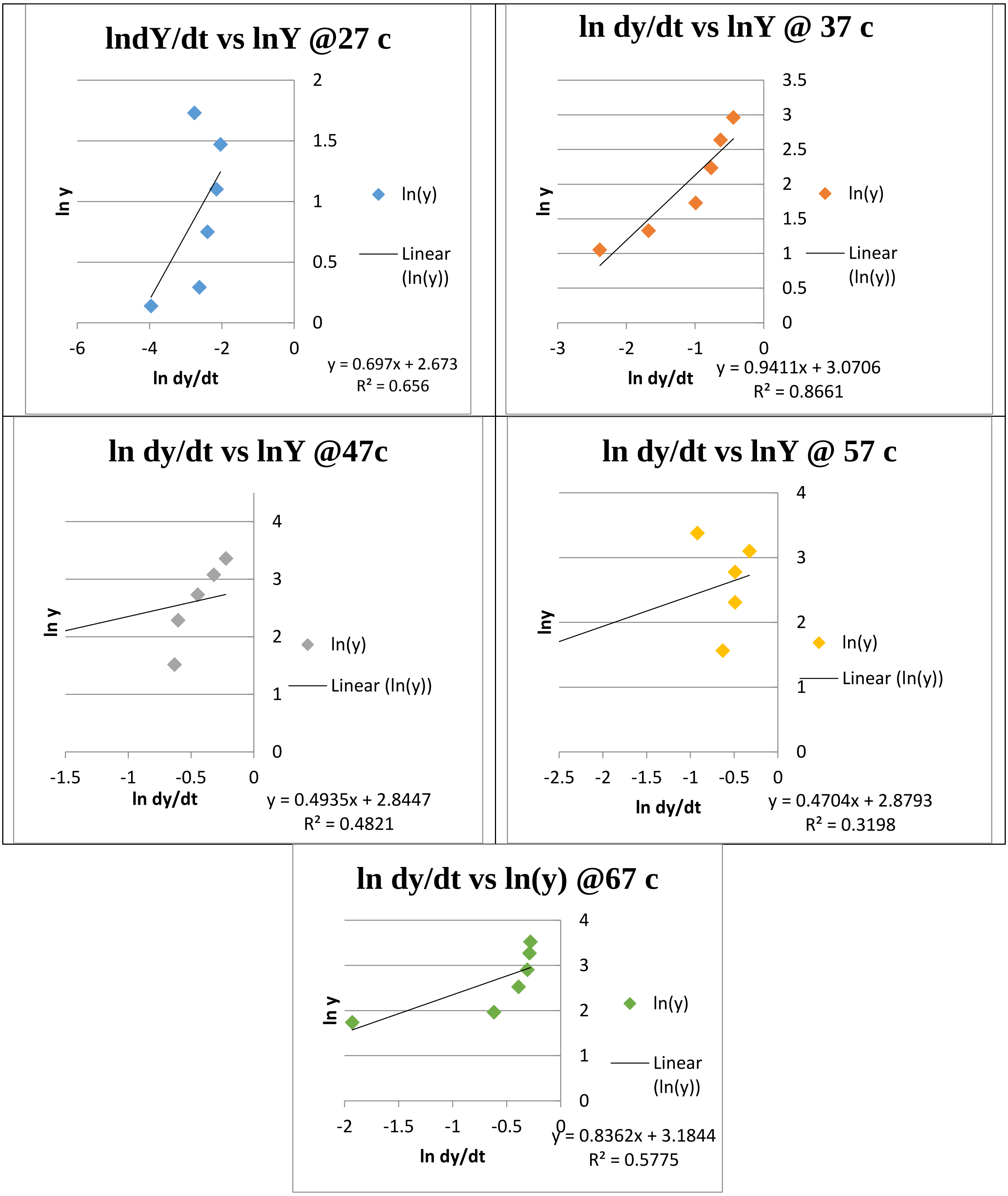

Figure 5b: A plot on ln dY/dt vs. ln Y at varying temperatures with respect to time has been plotted on cleome viscose seeds

Table 2
Oil yield percent of cleome viscose seeds with respect to temperature and time.

			Yield %			
Temp\Time	16	26	36	46	56	66
27	1.15	1.34	2.12	3.05	4.35	5.65
37	2.86	3.78	5.65	9.35	14	19.35
47	4.15	4.56	9.87	15.34	21.73	29
57	4.23	4.79	10.12	16.25	22.34	29.56
67	5.68	7.12	12.5	19.25	26.53	34.04

Activation energy: As the temperature of the reaction increases simultaneously, the rate of the reaction increases. The temperature changes affecting the reaction can be interpreted by the Arrhenius equation given below.

$$k=Ae^{-Ea/RT} \quad (4)$$

k is the reaction rate constant, A is the Arrhenius constant or frequency factor, Ea is the activation energy, R is the universal gas constant, and T is the absolute temperature. From table 3 the graph should be plotted between ln k vs 1/T gives the slope us –Ea/R and intercept as ln A (figure 6).Thus the Activation energy was interpreted as 7.1kJ and Arrhenius constant as $3.77*10^{-3}$ s^{-1}, confirmed by the studies of [11].

Table 3
Reaction rate constants from the slope at various temperatures

T	K min^{-1}
27	$6.98*10^{-2}$
37	$4.60*10^{-2}$
47	$5.8*10^{-2}$
57	$5.6*10^{-2}$
67	$4.1*10^{-2}$

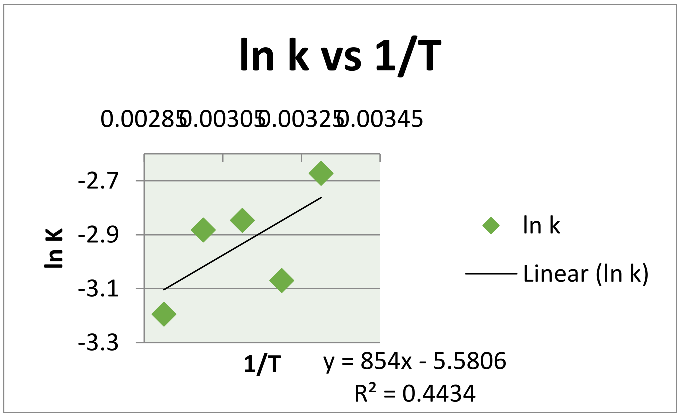

Figure 6: A plot on ln k vs. 1/T has been plotted on cleome viscose seeds.

Oil yield: Table 4 put in detail on the yield of oil by ultrasonic pretreated shaker assisted extraction on certain seeds such as *Caesalpinia sappan*, *Cleome gynandra* and *Cleome viscose*. [25] demonstrate in his studies on citrullus lanatus yields 35%.Suganya et al. (2012) on her research describes the maximum oil yield was 10.88%. Extraction on white pitaya seeds shows maximum yield was 7.78% by [26].

Table 4
Oil Yield of *Caesalpinia sappan, Cleome Gynandra, Cleome viscose* seeds

Source	**Yield %**
Cleome gynandra	34.54
Cleome viscose	32
Caesalpinia sappan	23

Physiochemical Analysis Table 5 depicts the Physiochemical analysis of certain seeds such as cleome viscose seeds oil analyzed by the AOCS standard methods. The colour of *c.sappan* was dark brown and for *cleome gynandra* and *cleome viscose* was dark green and yellowish green. The moisture content of the sappan, gynandra, viscose seeds was reduced to 3.48%, 7.70% and 5.40% respectively, this value was lower than Jatropha 5.54% and Parkia biglobbossa was 10.18% was reported by [27]. The yield of oil from sappan and gynandra seeds was 23%and 32% which is lower than the yield of oil reported by [28], but viscose seeds oil yield value was higher than reported. The Sappan oil Acid value is 5.04 mg KOH/g which was slightly higher, but for the cleome viscose and cleome gynandra was much higher than 3 mg KOH/g [29]. AOCS official Method Cd 3d-63 that makes the oil suitable for two step process for Biodiesel production i.e. esterification followed by transesterification.

The Saponification value describes the suitability of this oil for processing in soap manufacturing industries is 238 mg of KOH /g, 196 mg of KOH/g and 210 mg of KOH /g for sappan, gynandra and viscose seed oil compared with results of [30]. The Iodine value measures the degree of Unsaturation .The iodine value of these seeds from this study were compared with the results of [30,31]. The sappan fruit seed oil is placed in the semi-drying group of oils, gynandra and viscose seeds belong to non drying group of oils. From the above results, the sappan, gynandra, viscose seed oil by ultrasonic pretreated shaker extraction can be suitable second generation feed stocks for biodiesel production (Table 2).

Table 5
Physiochemical analysis of *Caesalpinia sappan, Cleome Gynandra, Cleome viscose* seeds

S.N.	Method	Units	AOCS number	Sappan seeds	Cleome gynandra	Cleme viscose
1	Moisture and volatile matter in the seed	g	Ba 2b-82	3.48%	7.70%	5.40%
2	Oil content	g	Ba 2 –38	23%	32%	34.54%
3	Average molecular weight of oil	MW		709.2	862	806.45
4	Oil colour			Dark brown	Dark green	Yellowish green
5	Odour		Cg 2-83	Mild pungent	Oily	Oily
6	Specific gravity @ 40ºc		Cc10a –25	0.933	0.8433	0.8949
7	Density @ 40 ºc	g/cm^3		0.926	0.867	0.92
8	Refractive index	nD	Cc 7 –25	1.479	1.475	1.47
9	Viscosity @ 40 ºc	Cst	Cc 13j-97	43.28	30	31
10	Physical state at room temperature	-		Liquid	Liquid	Liquid
11	Acid value	mg of KOH/g of oil	Cd 3d –63	5.04	44.31	35.34
12	Free fatty acid	%	Ca 5a –40	2.53	22.2	17.7
13	Saponification value	mg of KOH/g of oil	Cd 3a –94	238	196	210
14	Ester value	mg of KOH/g of oil		232.96	151.69	174.66
15	Iodine value	g I_2/0.1g of oil	Cd 1 –25	123.09	118.1	116.3
16	Peroxide value	mEq/Kg	Cd 8-53	2	1	1

Conclusion

This research throws light upon moisture content determination of non edible seeds to determine the amount of free moisture. Ultrasonic pretreatment was carried out to enhance the oil recovery on seeds. The pretreated samples were optimized by shaker extraction method, were the optimum conditions attained at 66 min, 67ºC, 125 rpm, 1:12 seed to solvent ratio with hexane as a suitable solvent compared with polar and nonpolar solvents. The extraction kinetic data reveals that extraction follows first order mechanism.

The rate constant, activation energy and Arrhenius constant were calculated. The Physiochemical analysis was determined by AOCS Methods. This proves the three seeds Caesalpinia sappan, Cleome gynandra, Cleome viscose second generation seeds are the suitable feedstock for biodiesel production.

References

1. Sheehan J., V. Cambreco, J. Dulfield, M. Garboski, and H. Shapouri. 1998. An Overview of Biodiesel and petroleum diesel life cycles, A report by us Department of Agriculture and Energy, Washington. DC: **1-35 (1998).**

2. Aro, E.M. 2016. From first generation biofuels to advanced solar biofuel. Ambio. 45 (Suppl 1): S24-S31.

3. Eisentraut, A. 2010. Sustainable Production of second generation biofuel. Renewable energy Division. International energy Agency: **1-16.**

4. Demirbas, A. 2008. Importance of biomass energy sources for Turkey. Energy Policy. **36: 834-842.**

5. Ushakov, V., 2005. Principles of the Development of Piezoelectric Transducers for Ultrasonic Testing of Articles with Flat and Curvilinear Surfaces, Russian Journal of Nondestructive Testing **41(6): 355-361.**

6. Mason, T. J., and J. P. Lorimer. 2003. Front Matter and Subject Index Applied Sonochemistry. Wiley-VCH Verlag GmbH & Co, KGaA: i-x.

7. Mason, T. J., L. Paniwnyk, and J. P. Lorimer. 1996. The uses of ultrasound in food Technology. Ultrasonics Sonochemistry **3(3): S253-S260.**

8. Hemwimol, S., P. Pavasant and A. Shotipru. 2006. Ultrasound-assisted extraction of Anthraquinones from roots of Morinda citrifolia. Ultrasonics Sonochemistry **13: 543–548 (2006).**

9. Ricardo Cardoso de Oliveira., Sueli Teresa Davantel de Barros, and Marcelino Luiz Gimenes. 2013. The extraction of passion fruit oil with green solvents, Journal of Food Engineering 117: 458–463.

10. Pier Roberto Danesi., 2004. Solvent extraction kinetics, Cheminform vol 35: issue 29, chapter 5.

11. Topallar, H., and U. Gecgel. 2000. Kinetics and thermodynamics of oil extraction from sunflowers seeds in the presence of aqueous acidic hexane solutions. Turk. J. Chem **24: 247−253.**

12. Peter Atkins., and Julio de Paula. 2009. Elements of physical chemistry, **219-230.**

13. Boone, R.S., and E.M. Wengert. 1998. Guide for Using the Oven-Dry Method for Determining the Moisture Content of Wood: 89.

14. Firestone, D. 1993. Champaign Official Methods and Recommended Practices of the American Oil Chemist's Society. AOCS Press: (4th ed).

15. Suganya, T., and S. Renganathan. 2014. Ultrasonic assisted acid base transesterification of algal oil from marine macroalgae Caulerpa peltata: Optimization and characterization studies, Fuel **128: 347–355.**

16. Liu, S., F. Yang., C. Zhang, P. Hong, and D. Deng. 2009. Optimization of process parameters for supercritical carbon dioxide extraction of Passiflora seed oil by response surface methodology. Journal of Supercritical Fluids. 48, 9–14.

17. Farsie, A., and M.S. Singh. 1985. Energy Models for Sunflower Oil Expression. Transactions of the American Society of Agricultural Engineers **28: 275 –279.**

18. Pandian Sivakumar, Karuppiah Subramanian Parthiban, Padmanaban Sivakumar, Mari Vinoba and Sahadevan Renganathan. 2012. Optimization of Extraction Process and Kinetics of Sterculia foetida Seed Oil and Its Process Augmentation for Biodiesel Production, Ind. Eng. Chem. Res **51: 8992−8998.**

19. Orhevba B. A., O. Chukwu, Z. D. Osunde, and V. Ogwuagw. 2013. Influence of moisture content on the yield of mechanically expressed neem seed kernel, Academic Research International **Vol. 4: No. 5.**

20. Muhammed Nasiru Abdulkadir, Isiaka Adekunle Amoo, Adeniyi Olufemi Adesina. 2013. Investigation of Solvents Efficiencies on Oil Extraction and Biodiesel Production from Hura Crepitans Seeds. International journal of research **vol 2: 3.**

21. Dari Linda. 2009. Effect of different solvents on the extraction of soya bean oil.

22. Balaji Panchal, Sanjay Deshmukh, Munish Sharma. 2014. Optimization of oil extraction and characterization from tamarindus indica Linn seed oil. International Journal of Oil Gas and Coal Engineering. **2(1): 1-6.**

23. Olakunle, M. S., M.J. Abdulhamid, and A. C. NEBO. 2014. Kinetics and thermodynamics of oil extraction from water-melon seeds. International Journal of Applied and Natural Sciences **Vol. 3: Issue 4, 93-102.**

24. Amin, S.H.K., Sh. Hawash, GEI. Diwani, and SEI. Rafei. 2010. Kinetics and Thermodynamics of Oil Extraction from Jatropha Curcas in Aqueous Acidic Hexane Solutions. Journal of American Science: **6(11).**

25. Sepidar Sayyar., Zurina Zainal Abidin, Robiah Yunus, and Azhari Muhammad. 2009. Extraction of Oil from Jatropha Seeds-Optimization and Kinetics, American Journal of Applied Sciences **6(7): 1390-1395.**

26. Ziyada, A.K., and S.A. Elhussien. 2008. Physical and Chemical Characteristics of Citrullus lanatus Var. Colocynthoide Seed Oil, Journal of Physical Science **Vol 19(2): 69–75.**

27. Hanming Rui, Liyan Zhang, Zuowei Li, Yanli Pan. 2009. Extraction and characteristics of seed kernel oil from white pitaya seeds. Journal of food science and engineering **93:482-486.**

28. Akintayo, E.K. 2004. Characteristics and composition of Parkia biglobbossa and Jatropha curcas oils and cakes. Bioresource Technology. **92: 307–310.**

29. Titil Datta Samanta and Subrata Laskar. 2013. Analysis of Oil and Fatty Acids from the Seeds of Erythrina variegata Linn, Biosciences biotechnology research Asia **10(1): 433-437.**

30. Zaku, S.G., S. A. Emmanual, A.H. Isa and A. Kabir. 2012. Comparative Studies on the Functional Properties of Neem, Jatropha, Castor, and Moringa Seeds Oil as Potential Feed Stocks for Biodiesel Production in Nigeria, Global Journal of Science Frontier Research Chemistry: **22-26.**

31. Mohammed, M. I., and Z. U. Hamza. 2008. Physicochemical Properties of Oil Extracts from Sesamum Indicum L. Seeds Grown in Jigawa State – Nigeria, J. Appl. Sci. Environ Vol. **12(2): 99 – 101**