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SYNTHESIS AND CHARACTERISATION OF ANIONIC SURFACTANT FROM *AFZELIA AFRICANA* SEED OIL

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Abstract: **This research explores the possibility of synthesizing surfactant using vegetable oil from *Afzelia africana* seeds through a two-step transesterification and sulfonation processes. The oil was extracted by Soxhlet using n-hexane as the solvent. Characterization in terms of physicochemical parameters gave the following results: 24.72 % oil yield, a value that compares favourably with other fatty acids used for similar applications, specific gravity of 0.93, refractive index of 1.48, saponification value of 204.03 (mgKOH/g), an acid value of 5.6 (mgKOH/g), and an iodine value of 105.75 (Wij's) respectively. These values indicated that the semi-drying oil is suitable for the synthesis of surfactant. The oil was transesterified using ethanol with KOH catalyst. The ester product underwent sulfonation with $NaHSO_3$ as the sulfonation agent using Al_2O_3 catalyst. The sulfonated product was purified with ethanol and neutralised with NaOH. Both the fatty acid ethyl ester (FAEE) and the fatty acid ethyl ester sulfonate (FAEES) were analysed using Fourier Transform Infra-Red Spectroscopy which showed that both compounds contain their characteristic functional groups: the FAEE had a C=O stretch at 1736.9 cm^{-1} among other peaks, while the FAEES also showed a C=O stretch at 1640.0 cm^{-1}; 1230 cm^{-1} for the sulfonate group, 1140.6 cm^{-1} for the S=O group, and 924.4 cm^{-1} which indicated S-O group. These functional groups established the synthesized moiety to be a sulfonated ester (surfactant). Therefore, the results from this study show that *A. africana* seed oil can be a viable alternative feedstock for the production of surfactant for application across the chemical industry.**

***Keywords*: Surfactant, Two-step Transesterification, Sulfonation, FT-IR.**

1. INTRODUCTION

Surfactants are a special class of compounds that possess a distinct polar (hydrophilic) head and non-polar (hydrophobic) tail groups in the moiety [1,2]. They generally lower the surface tension of the liquid in which they are dissolved. As a result, they are used as detergents, wetting agents, emulsifiers, foaming agents, or dispersants across the chemical industry [3–7].

Surfactants are generally synthesised from petroleum and natural gas derivatives, although the process leaves a polluted ecosystem. Therefore, a viable alternative is the use of vegetable oil as feedstock for making surfactants [8]. Scientists have investigated the synthesis of methyl ester sulfonates using different edible and non-edible vegetable plants such as coconut oil, crude palm oil (CPO), palm kernel oil (PKO), soybeans oil, *Jatropha curcas* oil [9].

For large scale surfactant synthesis processes, SO_3 is usually employed as the sulfonation agent, with the advantages of being highly reactive and thus, enabling a more complete conversion of the reactants. Nevertheless, the sulfonation process is costly and resource intensive: it requires strict control due to the high reactivity of SO_3 and results in a black product

which needs bleaching for further application [8]. As a result, sodium bisulfite has been used for the synthesis of methyl ester surfactants [10]; its use, produces surfactant with a brighter appearance, suitable for use in both small and large scale to produce relevant products.

Surfactant synthesis by use of vegetable oil feedstock involved two-step transesterification and sulfonation processes [11,12]. A number of factors determine the characteristics of the surfactant viz; reaction temperature, neutralization time, type and concentration of catalyst (usually Al_2O_3 or CaO), pH, concentration of sulfonate groups and neutralization temperature [8].

Among surfactant types, two – anionic and non-ionic surfactants are most widely used [8]. This research discusses one of the vegetable oil surfactants – ethyl ester sulfonate (EES), synthesized from *Afzelia africana* oil. Alcoholic ethyl sulfonates are the most environmentally friendly of the anionic surfactants present in detergents; they have relatively excellent detergency properties – even in small quantities – to others such as linear alkylbenzenesulfonates (LAS) and Alkyl sulfates [8]. Anionic surfactants are used in **pharmaceutical** suspension to lower the surfactants surface tension between the suspended agent and the suspending medium. Anionic surfactants are classically used to enhance solubility or stability of a drug in a liquid preparation, stabilizing and modifying the texture of a semisolid preparation. Anionic Surfactants improve the efficacy bio performance of the product. Solubility of phenolic compounds such as cresol, chlorocresol and thymol with soap to form clear solutions for use in disinfection [9].

This study has several differences with other related studies in that: the fatty acid used is oil from *Afzelia africana* seed, ethanol is the choice of alcohol and the sulfonation catalyst is Al_2O_3. The vast availability of *Afzelia africana* trees in Nigeria and its relatively low edibility compared to other edible vegetable oils like palm oil and coconut oil, suggests its possible application in surfactant synthesis [13].

Therefore, the aim of this research is to determine the feasibility of synthesizing surfactant from the underutilized *Afzelia africana* oil using ethanol by analysing the physicochemical characteristics of the oil, analysing the fatty acid ester (the transesterification product) and the fatty acid ethyl ester sulfonate using Fourier Transform Infra-Red Spectroscopy.

2. MATERIALS AND METHODS

Materials

Afzelia africana seeds were obtained from a local market in Adikpo Local Government Area of Benue State. All the reagents used were of analytical grade: ethanol, diethyl ether, sodium hydroxide, potassium hydroxide, sodium bisulphite and aluminium oxide.

Collection and preparation of Afzelia africana seeds

Dried and matured *A. africana* seeds were bought from a local market in Adikpo, Kwande Local Government of Benue State, Nigeria. The seeds were sorted to discard some defective seeds, de-capped using a mortar and a pestle, winnowed, and washed with distilled water and sun-dried for one (1) week. The dried seeds were then reduced to granular sizes using a mortar and pestle [14]. Plate 1 shows *A. africana* pods and seeds:

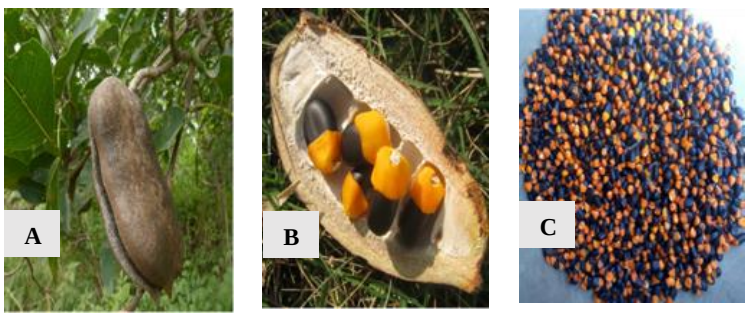

Plate 1: Afzelia africana (A): Plant with pod (B): Pod containing seeds (C): Oil-containing seeds

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Extraction of A. africana seed oil

Oil from the granular *A. africana* seeds was extracted using the Soxhlet extraction procedure as described by Redfern *et al* [15]. The ground product was neatly packed into a clean, white filter cloth and tied with white thread and placed in the thimble which was clamped onto a round-bottom flask. 250mL of the extracting solvent (n-hexane) was transferred into the round-bottom flask. The set-up was heated on a heating mantle to a temperature of 50 ºC. The solvent evaporated and moved up into the condenser where it was converted back to liquid which trickled into the extraction chamber through the sample and back into the boiling solvent. The cycle was allowed to continue for about 6h, after which the boiling flask content was removed and placed in a rotary evaporator to separate the *Afzelia* oil from the n-hexane. The extracted oil was collected and stored in a clean and dry transparent bottle [16].

Calculation of the percentage yield of the oil

The percentage yield of the extracted oil was determined from the following relationship:

$$\% \text{ oil yield} = \frac{\text{Wo}}{\text{Ws}} \text{x} \frac{100}{1}$$

Where;

Wo = weight of extracted oil

Ws = total weight of sample

Physicochemical Analyses of the extracted oil

The physical characteristics of the oil were carried out by methods provided by the AOAC [17-18], while the chemical parameters of the oil sample were determined by the official American Oil Chemist's Society (AOCS) methods reported by Ajiboye *et al* [16].

Determination of specific gravity

A clean empty 50mL density bottle was carefully weighed using an electronic weighing machine, filled with distilled water and reweighed. The water was discarded and the bottle dried. After drying, the bottle was filled with the oil and weighed. The specific gravity was calculated using the following equation:

$$\text{specific gravity} = \frac{W3 - W1}{W2 - W1}$$

Where;

W_1 = weight of empty density bottle

W_2 = weight of density bottle + water

W_3 = weight of density bottle + oil

Determination of refractive index

The refractive index of the oil was determined using an Abbe refractometer at 30 ºC after which the temperature correction was made to obtain the refractive index of the oil. The refractive index was calculated using the following equation:

$$R.I\ corr\ (R.corr) = Scale\ reading + [(T - 20)\ ℃ \times 0.0000078]$$

Where;

R. corr. = Refractive index after temperature correction

R.I = Refractive index obtained before temperature correction

T = Temperature at which the measurement was made

20 ºC = constant

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Determination of acid value

25mL of diethyl ether was measured with a 50 mL measuring cylinder and mixed with 25mL of ethanol in a clean beaker. 1 mL of 1 % phenolphthalein indicator solution was added to the mixture which was then neutralized with 3 drops of 0.1 M NaOH solution. The neutralized mixture was then used to dissolve 2 g of the oil which had been weighed in a conical flask. The solution was then titrated with 0.1 M NaOH solution, shaking continuously until the pink colour persisted for about 15 sec. The calculation was done using the following equation:

$$\text{Acid value} = \frac{titre\ value\ (mL) \times 56.1(mgKOH/g)}{weight\ of\ oil\ (g)}$$

Determination of iodine value

A dropping pipette was used to weigh 0.3g of the oil into a 250mL round-bottom flask. 10 mL of carbon tetrachloride was measured into the flask to dissolve the oil. Exactly 20mL of Wij's solution was added to the flask and the entire mixture was stoppered with a cork moistened with KI solution. The solution was properly mixed and allowed to stand in a dark place for about 30 min. 15mL of 10 % KI solution and 100mL of distilled water were added to the mixture and mixed. The mixture was then titrated against 0.1 M $Na_2S_2O_3.5H_2O$ solution using 1 % starch solution indicator. A blank test was also carried out. The iodine value was calculated using the following equation:

$$\text{Iodine value} = \frac{(b-a)mL\ \times\ 12.69\ (Wij's)}{weight\ of\ oil\ (g)}$$

Where;

a = titre value of titration with oil sample

b = titre value of blank titration

Determination of saponification value

A dropping pipetted was used to measure 2g of the oil into a flask. 25mL of the alcoholic KOH solution was added to it. Some anti-bumping chips were added. The mixture was then heated under reflux on a boiling water bath for 1 h with occasional shaking. After 1 h, 1 mL of phenolphthalein was added to the hot mixture and titrated with 0.5 M HCl solution. A blank test was also conducted. The saponification value was calculated using the following equation:

$$\text{saponification value} = \frac{(b-a)mL\ \times\ 28.05\ (mgKOH/g)}{weight\ of\ oil\ (g)}$$

Where;

a = titre value of titration with oil sample

b = titre value of blank titration

Esterification of *A. africana* seed oil

The esterification and transesterification processes were performed following a procedure by Kurniasih and Pardi [19]. The procedure was modified. 100mL of the oil was measured into a three-necked round-bottom flask. Using a hot plate, the oil was warmed to 60 ºC to prepare it for the reaction. At 60 ºC, a solution of the H_2SO_4 (2.5mL) and ethanol (42.0mL) was slowly added to the oil and a magnetic stirrer introduced for stirring. This point was noted as the starting time of the reaction which was carried out under reflux with temperature maintained at 60 ºC for 90min. After the reaction period, the product was allowed to cool and then transferred to a separatory funnel to enhance gravity separation between the organic layer

(ester) and the aqueous later (water and other by- and partially reacted – products). The ester part was collected into a conical flask after a settling period of about 24 h.

Transesterification of the ethyl ester

Exactly 80mL of the ester was measured into a three-necked round-bottom flask and warmed to 60 ºC, employing a water bath, hot plate stirrer and a magnetic stirrer. At 60 ºC, a solution of 0.8 g (1 % w/v of oil) of KOH and 33.9mL of ethanol was added to the reactor. The reaction was continued with stirring under a reflux apparatus for 1 h with the temperature maintained at 60 ºC. After the reaction, the product was transferred into a separatory funnel and allowed to separate for about 24 h. After draining out the lower layer of the products (glycerol and catalyst), the fatty acid ethyl ester was washed with a little quantity of warm distilled water (65 ºC) until no more bubbles appeared. The product was then heated at a temperature of 105 ºC for about 15 min using a hot water bath to evaporate the residual water [19].

Sulfonation of the fatty acid ethyl ester

The preparation of the ethyl ester sulfonate was performed through the following steps reported by Permadini *et al* [11] and Putra *et al* [12]:

Preparation of the ethyl ester sulfonate

The ethyl ester sulfonate was reacted in a ratio of 1:1.5. Exactly 21.6 g of $NaHSO_3$ was mixed with 0.616 g of Al_2O_3 catalyst (1 % w/w of the reactants) and transferred into a three-necked round-bottom and reacted under reflux. 40g of fatty acid ethyl ester was then added to the mixture which was preheated for 30 min at 90 ºC before raising the temperature to 100 ºC and allowing for another 4 h. After the reaction was completed, the product was centrifuged at 1500 rpm for 30 min to separate the residual $NaHSO_3$ from the ethyl ester sulfonate.

Purification of the ethyl ester sulfonate

The centrifuged product of the ethyl ester sulfonate was transferred into a three-necked round-bottom flask, about 30-40 % v/v ethanol was mixed to it and heated on a hot plate for 90min for purification. This was done using a reflux set up. The purified product was then heated (using a hot water bath) for another 30 min to 80-90 ºC to evaporate any residual ethanol and water.

Neutralization of the ethyl ester sulfonate

To the resulting product, 20 % NaOH was gradually added with gentle mixing to arrive at a neutral pH. The neutralized product was then heated for another 30min at 55 ºC with stirring. The product (surfactant) was ready for characterisation.

Characterisation of the Fatty acid ethyl ester and the fatty acid ethyl sulfonate using FTIR spectroscopy

The respective compounds were analysed for functional group identification using FTIR (Agilent – Cary 630) and the spectra were obtained in the frequency range of 4000 – 650 cm^{-1} with inbuilt KBr pellets. The analysis was conducted at the Ahmadu Bello University Multipurpose Laboratory, Zaria.

3. RESULTS AND DISCUSSION

Results

Table 1: Results from the Percentage Yield of the Extracted Oil and Physicochemical Characterisation of the Extracted A. africana Oil

Parameter	Value
Oil yield (%)	24.72
Specific gravity	0.93
Refractive index	1.48
Saponification value (mgKOH/g)	204.03
Acid value (mgKOH/g)	5.61
Iodine value (Wij's)	105.75

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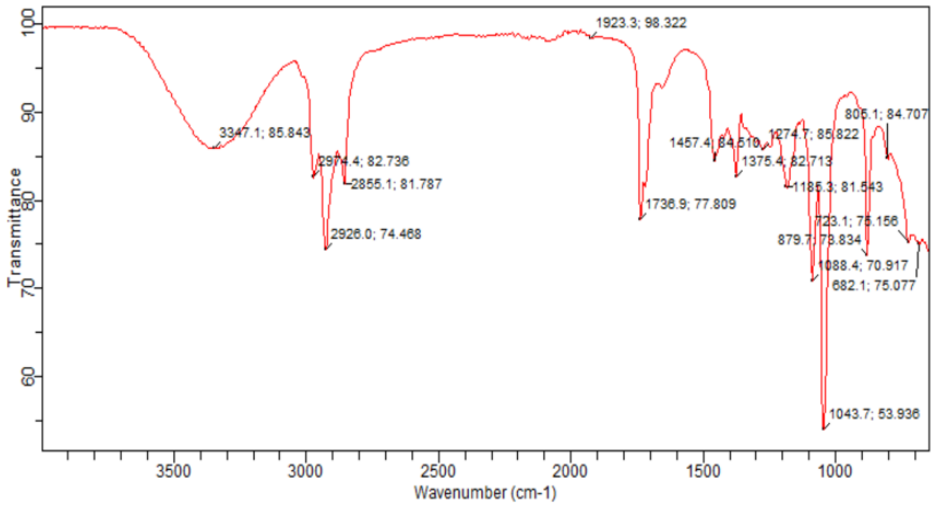

Figure 1: FTIR Spectrum of AASO Ethyl Ester

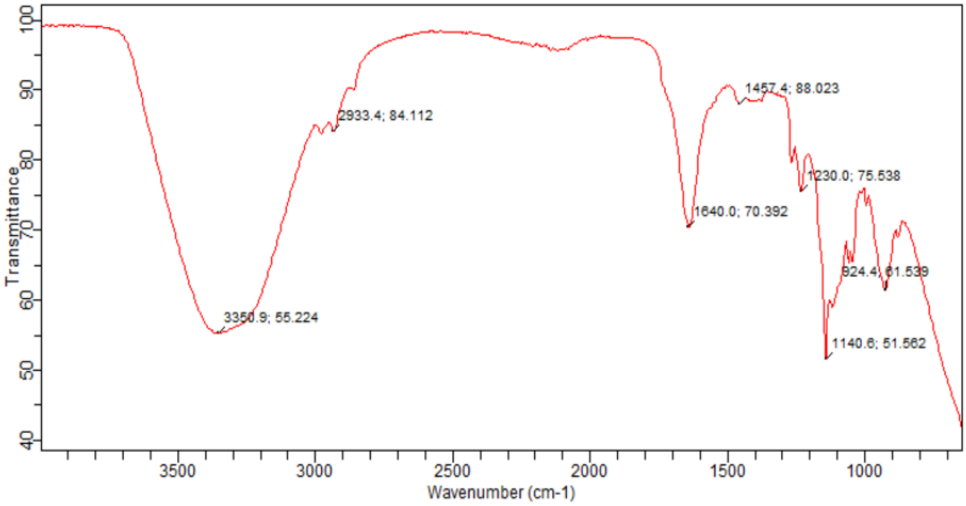

Figure 2: FTIR Spectrum of AASO Ethyl Ester Sulfonate

Discussion of results

Percentage yield of *A africana* seed oil

The oil yield from the extraction (24.72 %) compares well with the 25.8 % obtained for the same seeds by Ajiwe *et al* [20]. The value indicates that the seed has economical amounts of oil compared to other vegetable oils such as bitter kola seed (11.92 %) [21], and soybeans oil yield (19.74 %) [22]. The yield value of the oil falls in the same category as other vegetable oils as watermelon seed (28.68 %), although lower than oils from moringa seed, cashew, sesame seed, and melon seed with a yield range between 38-49 % [21]. The oil yield from the *A. africana* seed oil show that it is economical for industrial uses such as in the synthesis of surfactants.

Physicochemical characterisation of *A. africana* seed oil

Specific gravity

The specific gravity of the oil was 0.93, a value which falls in a similar category as most other vegetable oils such as coconut oil, corn oil, rapeseed oil (0.88-0.92) as reported in literature [23]–[25] etc. The value indicates that the oil is less dense than water and would be easily employed for the esterification and transesterification processes [26].

Refractive index

The refractive index of the oil was 1.48 at 31.6 °C. Usually, the refractive index of water is taken to be 1.33 and that of oil generally does not exceed 1.58. So, the value from this research falls within that range. The value also means that the oil has lower chances of spoilage due to oxidation since higher refractive index indicates the higher chances of rancidity development in oil [27]. Other vegetable oils such as peanut oil, sesame oil, mustard oil, soybean oil and palm oil have been reported by the AOAC to have refractive indices in the range of 1.46-1.48 [28].

Saponification value

Saponification value is a measure of the average molecular weight of all the fatty acids present in the sample as triglycerides. High saponification values implies lower average length of the fatty acids and the lighter the mean molecular weight of the triglycerides and the reverse case applies. The saponification value is effectively used to determine the average relative molecular mass of oils and fats. According to Aguebor-Ogie *et al*, and the value is relevant for efficiency of the transesterification reaction [29]. The saponification value of the oil was 204.03 mgKOH/g which yielded a high ester conversion in the transesterification reaction.

Acid value

For the efficiency of the transesterification process, the acid value of the oil is required to be less than 3mgKOH/g [30]. The acid value of the oil was 5.61 mgKOH/g, a relatively high value is probably due to some free fatty acids in the oil, resulting from some moisture in the oilseeds and long storage time. Acid-esterification of the oil using ethanol was employed to convert the free fatty acids to esters in order to facilitate the transesterification reaction.

Iodine value

The iodine value is a measure to determine the degree of unsaturation of fatty acids. Saturated oils, fats, and waxes take up no iodine; therefore, their iodine value is zero; unsaturated oils, fats, and waxes usually take up iodine. The more the iodine uptake, the higher the iodine value, and the more reactive, less stable, softer, and more susceptible to oxidation and rancidification is the fatty acid [31]. The iodine value of the oil was 105.75 (Wij's) and shows that the oil has a relatively high degree of unsaturation – the oil is non-drying in nature – and suitable for use for surfactant synthesis.

FTIR Result for ethyl ester of *A. africana* oil

Based on the FTIR results on the transesterified triglyceride, the spectrum of the fatty acid ethyl ester shows different peaks which identify the various functional groups to validate the actual conversion of the triglyceride to an ester.

The spectrum gives a broad peak at 3347 cm^{-1}, indicating the presence of the OH stretch. The sharp band at 2926.0 cm^{-1} indicates the C-H (Sp^3 hybridised carbon atoms). This means that the compound contains an alkyl group (from alkanes) which could be the ethyl part of the ester. The wavenumber also relates with that obtained by Slamet and Wulandari [32] (2921.79 cm^{-1} and 2852.64 cm^{-1}) for the C-H alkanes.

Probably the most important band is the C=O stretch with a medium transmittance at 1736.9 cm^{-1}. This indicates the presence of an ester group. Although the C=O group could also indicate the presence of other carbonyl compounds like aldehydes and ketones, the base values for ester compounds (1755-1650 cm^{-1}) further validates the result. The peak value is similar to the 1744 cm^{-1} obtained for C=O for crude palm oil methyl ester by Slamet and Wulandari [32].

The C–O group is transmitted at 1043.7 cm^{-1} with sharp intensity. This value is within the base value for the C–O (1300-1000 cm^{-1}), and also agrees with the 1159.9 cm^{-1} reported by Slamet and Wulandari [32]. These transmissions and functional groups identify the compound as an ethyl ester.

FTIR result of sulfonated ethyl ester

The FTIR spectrum from the analysis identifies the functional groups present and confirms the compound as the synthesised fatty acid ethyl sulfonate. The spectrum shows a medium transmittance at 3350.9 cm^{-1} which indicates the presence of the OH group. This further indicates that, at a mole ratio of 1:1.5, the reaction between ethyl ester and $NaHSO_3$ is most favourable, such that it forms an OH group that increases the surfactant solubility properties in water.

The transmittance at 2933.4 cm^{-1} shows the presence of the C–H alkanes (alkyl) in the compound. Based on the spectrum again, the sulfonate group is transmitted at 1230.0 cm^{-1}, and the value corresponds to the 1360.87 –1014.98 cm^{-1} obtained also by Slamet and Wulandari [32]. At the 1640.0 cm^{-1}, the C=O group has carbonyl stretch which indicates the presence of an ester.

Finally, in the fingerprint region of the spectrum, there is a strong transmittance at 1140.6 cm^{-1} which is characteristic of the S=O functional group, while at 924.4 cm^{-1}, we have the S–O functional group.

4. CONCLUSION

A. africana oil was extracted from its oil-bearing seeds and the percentage yield of the oil determined; the oil was characterised based on physicochemical parameters. The oil yield compared fairly with other vegetable oils and the physicochemical properties showed that the oil has economic importance and can be used for surfactant synthesis. FTIR analyses of the transesterified triglyceride and the sulfonated fatty acid ethyl ester showed that the oil has good potential for surfactant synthesis.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] D. Nikunj and J., Tejas. (2017). A Concise Review on Surfactants and its Significance. *Int. J. Appl. Chem.*,13, (3):663–672.

[2] T. Shachi, M. Chandrakanta, S. P. Prakash, M. Varanasi- Uttar (2018). Surfactant and its Applications: A Review. *Int. J. of Eng. Res. and App.* 8(9):61–66

[3] R. Azarmi and A. Ashjaran (2015). Type and Application of Some Common Surfactants. *J. Chm. & Pharm. Res.*, 7(2):632-640

[4] I. Anestopoulos, D.E. Kiousi, A. Klavaris, A. Galanis, K. Salek, S.R. Euston, A. Pappa and M.I. Panayiotidis (2020). Surface Active Afents and Their Health-Promoting Properties: Molecules of Multifunctional Significance. *J. Pharm.* 12(688):1-34

[5] R. Nagy, L. Bartha, and Á. Vágó (2014). Study on Characteristics of Micelles Formed by Surfactants and Polymer Mixtures for Enhanced Oil Recovery. *Chem. Eng. Trans.*, 36:217–222.

[6] F. Zanetti, A. Monti, and M. T. Berti (2013). Challenges and Opportunities for New Industrial Oilseed Crops in EU-27: A review, *Ind. Crop. Prod.*, 50:80–595.

[7] S. Sonawane, S. Pal, N. Bisht and S. Tayade (2015). Application of Surfactants in Various Fields. *Int. J. Sci. & Eng. Res.*, 6(12): 30-32

[8] E. Jaksen, Silviyati, I., Supraptiah, E., Daniar, and R., Margaretty, (2021). Effect of Sulfonation Temperature and Time on the Preparation of Methyl Ester Sulfonate Surfactant From Crude Palm Oil (CPO) Based Methyl Ester. *Proc. 4th Forum Res. Sci. Technol.*, 7:95–102.

[9] Q. Zhang, B. Cai, W. Xu, H. Gang, J. Liu, and S. Yang. (2015). The Rebirth of Waste Cooking Oil to Novel Bio-based Surfactants. *Nat. Publ. Gr.*, 1–7.

[10] L. Qadariyah and S. Sahila (2021). The Effect of Reaction Time and Temperature on the Synthesis of Methyl Ester Sulfonate Surfactant from Palm Oil as a Feedstock Using Microwave-Assisted Heating. *ASEAN J. Chem. Eng.*, 21(1):104.

[11] R. L. Permadani, M. Ibadurrohman, and Slamet. (2018). Utilization of Waste Cooking oil as Raw Material for Synthesis of Methyl Ester Sulfonates (MES) Surfactant. *IOP Conf. Ser. Earth Environ. Sci.*, 105 (1).

[12] A. D. Putra, R.A., Ismayanti, R., and Kalistaw (2018). Synthesis of Methyl Ester Sulfonate through Sulfonation of Methyl Ester Soybean Oil for Application in Chemical Flooding. *J. Mater. Sci. Indones.*

[13] D. O. Omokpariola, V. U. Okechukwu, and P. Leonard, (2018), Effects of Processing on the Nutritive and Anti-nutritive Properties of *Afzelia africana*. *Adv. J. of Chem.*, 3(2): 188-198

[14] B. Igbabul, O. Hiikyaa, and J. Amove. (2014). Effect of Fermentation on the Proximate Composition and Functional Properties of Mahogany Bean (*Afzelia africana*) Flour. *Curr. Res. Nutr. Food Sci.*, 2(1):1–7.

[15] J. Redfern, M. Kinninmonth, D. Burdass, and J. Verran ((2014). Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antibiotic Properties. *J. Microbio. & Biol. Edu.*, 15(1):45-46

[16] I. O. Ajiboye, O. B. Ajayi, and S. F. Akomolafe. (2018). Assessment of Physicochemical Properties , Fatty Acid Composition of *Afzelia africana* Seed Oil and the Effect of its Oil-Based Diet on Body Weight and Plasma Lipid Profile of Albino Rats, *Food Nutr. Sci.*, 9, 983–996.

[17] AOAC (2006). Official Method of Analysis of AOAC International 18th ed. American Oil Chemists' Society Press: Champaign IL.

[18] AOAC (2000). Official Methods of Analysis 17th ed. Association of Official Agric. Chem. Washington D.C. 1970.

[19] E. Kurniasih and P. Pardi (2018). Analysis of process variables on biodiesel transesterification reaction using Taguchi Method. *IOP Conf. Ser. Mater. Sci. Eng.*, 420(1):12–34.

[20] V.I.E. Ajiwe, C.A. Okeke, and H.U. Agbo. (1995). Extraction and Utilization of *Afzelia africana* seed oil. *Bioresour. Technol.*, 53(1): 89–90.

[21] E.A. Saeed, M.D., and Shola. (2015). Extraction and Physico Chemical Properties of Some Edible Seed Oils Sampled in Kano Metropolis, Kano State. *Bayero J. Pure App. Sci.*, 8(2):239–244.

[22] M.S. Sheikh and N. Dikshit (2015). Oil content and fatty acid composition of soybean (*Glycine max L.*) genotypes evaluated under rainfed conditions of Kashmir Himalayas in India Oil content and fatty acid composition of soybean (*Glysine max L.*) genotypes evaluated under rainfed condition, *J. Appl. Nat. Sci.*, 7(2): 910–915.

[23] S.T. Girisha, K. Ravikumar, and B. R. Mrunalini (2014). Comparative Study of Extraction Methods and Properties of Non-edible Oils for Biodiesel Production. *Asian J. Plant Sci. Res.*, 4(1):28–35.

[24] H. Noureddini, B.C., Teoh, and L.D. Clements (1992). Densities of Vegetable oils and Fatty Acids. *Chemical and Biomolecular Engineering Research and Publications*. University of Nebraska - Lincoln.

[25] A.K. Dubey, R.M. Sariya and A. Rehman. (2011). Characterization of Processed *Jatropha* Oil for Use as Engine Fuel. *Curr. World Environ.*, 6(1):101–107.

[26] ASTM D1429-13,. (2003). Standard Test Methods for Specific Gravity of Water and Brine, *ASTM International*, West Conshohicken, PA.

[27] A. Sarkar, J.P., Pandey, A., Singh, L., Tiwari, and A., Kumar. (2015). A Novel Method of Using Refractive Index as a Tool for Finding the Quality of Aqueous Enzymatic Extracted Algae Oil. *Advances in Applied Science Research*; 6(4):50-60.

[28] AOAC, (1993). Codex Alimentarius: Fats, Oils and Related Products. FAO, Rome.

[29] B.N. Aguebor-Ogier, P. Edokhume and F. Nwosu (2020). Evaluation of Saponification Value, Iodine Value, Peroxide Value and Free Fatty Acid Level of Essential Oil of Cayenne Pepper (*Capsicum annum*). *J. of Eng. Tech. & App. Sc.*, 5(2):14-16

[30] S. I. Baskar, G., Kalavathy, G., Aiswanrya, R., and Abarnaebenezer, (2019). Advances in Bio-oil Extraction from Non-edible Oil Seeds and Algal Biomass. *Adv. Eco-fuels a Sustain. Environ.*, 187–210.

[31] The Editors of Encyclopeadia Britannica (1998). Iodine Value, *Encyclopaedia Britannica*.

[32] M.I. Slamet, and P.P., Wulandari, (2017). Synthesis of Methyl Ester Sulfonate Surfactant from Crude Palm Oil as an Active Substance of Laundry Liquid Detergent. In S. Tursiloadi, & N, Rinaldi (Eds.), *Proceedings of the 3rd International Symposium on Applied Chemistry*, [020058] (AIP Conference proceedings; vol. 1904)