

Enhancing the Hydrolysis of Africa Pear Seed Oil

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Abstract:- Recent trend in terms of waste seed oil application has shown vast industrial relevance. Therefore, the high fatty acid concentration of African Pear (*Dacryodes edulis*) Seed-oil (APSO) is the reason for its utilization, thereby according it the possible potential as feedstock for industrial applications. However, pretreatment of the oil extract is key to its functionality as feedstock in industrial processes. Therefore the process of hydrolysis as a pretreatment route based on the types of catalysts, and a variety of reaction circumstances, including concentration, temperature as well as reaction time was adopted and characterization was affirmed with FTIR and GC-MS analytical methods. The results showed that the highest percentage Free Fatty Acid (% FFA) hydrolysis was obtained at optimum concentration (2 M), temperature (60 °C) and reaction time (2 hrs). Rapid hydrolysis was observed at 1.5 M for both ethanolic KOH and NaOH, thereby reporting a percentage yield of 70.58 % and 63.37 % of the % FFA for KOH and NaOH respectively. Further characterization findings by GC-MS confirmed a positive identification of % FFA composition. Likewise the FTIR analysis also exhibited high carboxylic acid peak absorption for NaOH and KOH catalyst at 1781 cm^{-1} and 1179 cm^{-1} respectively. Therefore, the result is a pointer that catalyst and the choice of a suitable reaction condition has the potential to influence the rate of hydrolysis in oil.

Keywords:- Hydrolysis, Fatty Acids, Pretreatment, Catalyst, Physicochemical.

I. INTRODUCTION

The continuous search for an alternative industrial raw material for sustainable green practices, has unveiled several quest towards the use of waste sources [1]. The rapid global growth in the area of industrialization and modernization has triggered the high dependence on synthetic products that has created more cause of concern in the ecosystem. Evidence in this practices is a reflection in the dwindling petroleum reserves and numerous environmental pollution challenges occasioned by emission of greenhouse gases. Adopting Africa Pear Seed-oil (APSO) as a potential feedstock for industrial application is based on the high oil yield as well as important FFA content [2]. It also contains bioactive chemicals with a variety of functional qualities, including antioxidant and anti-cancer effects, antiinflammatory, etc. [3, 4, 5]. These functional properties have revealed new potential application in pharmaceuticals and foods [5, 6].

The extraction of FAs and glycerol from oils is critical, particularly in the oleo-chemical industry [7]. This reaction can be catalyzed via acid, base, or enzymatic route [8, 9]. These techniques is employed in food, cosmetics, and pharmaceutical industries [8, 10], as well as soap, synthetic

detergents, greases, cosmetics, and a variety of other things [11].

One of the most important steps in the manufacturing of seed oil is extraction as reported [12]. Therefore pretreatment of the oil extract is key to its functionality as feedstock in industrial processes. Therefore, these pretreatment practices; enzyme digestion, ultrasonication, microvating, pulse electric fields etc., have been reported and grouped based on chemical, physical, physicochemical and biological as captured in figure 1.

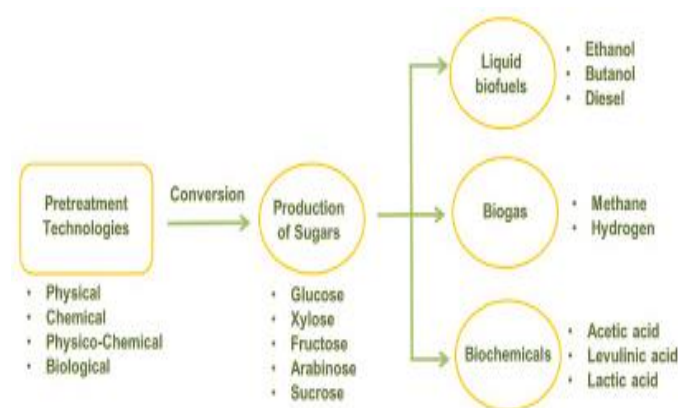


Fig 1 Pretreatment techniques of oil [5, 13]

In seed oil processing, physicochemical properties are essential quality criteria that has the potential to enhance oxidative stability and functional components of seed oil thereby improving its commercial value and shelf life [5, 14, 15]. Although pretreatment of seeds has been shown to have a considerable impact on factors such as oil output and colour [5, 16, 17 18].

The alkaline hydrolysis of triacylglycerol is known as saponification as described in Figure 2. Most oleo-chemical productions begin with FFA, which are produced by these processes. To maximize the economics of large-scale production, reaction pathways and conditions with effective glycerol recovery are necessary [8, 19]. Nowadays, researchers employ a one-way rather than reversible alkaline saponification as the catalytic route to the hydrolysis of esters based on the ease via which the product is separated. [11]. furthermore, it has been reported that FFA hydrolysis is affected by the type of catalysts, as well as the oil type [11, 20, 21]. It is also affected by variables such as temperature and time to accomplish 100 percent hydrolysis. Findings from research using NaOH and KOH revealed that when methanol and NaOH were used, the experiment was carried out with the best molar ratio (6:1). However, when KOH was used, the experiment was carried out with the best molar ratio (8:1) while maintaining the catalyst concentration (1 percent

KOH), 70°C reaction temperature as well as a reaction duration of 3¹/₂ h.

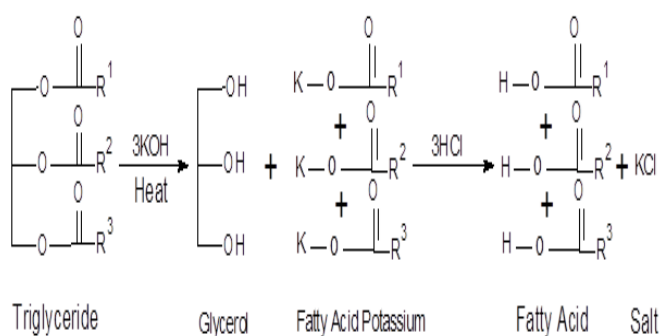


Fig 2 Alkaline hydrolysis of triglycerides [8]

To improve the nutritional quality of seed oil, maximum fatty acid recovery during oil extraction is required [22]. Pretreatment of seeds has a little influence on fatty acid content, according to scientists, which could be favourable from a nutritional standpoint. This assertion was based on study conducted to examine the effect of microwave pretreatment (2450 MHz, 100, 250, 600 W, 2, 6 min) on fatty acids composition [5, 23, 24]. According to their report, microwave pretreatment did not affect the FFA content [17]. However, in another finding by Mohseni et al. [25], reported that pretreatment procedures strongly affected the FFA content. They discovered that the amount of oleic and linoleic acids had reduced considerably. Thermal deterioration, according to the authors, was the cause of the drop in oleic acid and linoleic acid. Therefore as a means of sorting an effective catalytic route for the production of the required FFA for industrial application, this study will focus on a comparative study involving the use of KOH and NaOH in enhancing an effective hydrolysis of APSO.

II. EXPERIMENTAL METHODS

➤ Materials and Apparatus

African Pear Seeds (*Dacryodes Edulis*) oil, Wij's solution, n-hexane as well as other chemicals are products of Sigma-Aldrich, USA and Merck Co Ltd. Laboratory oven (DHG 9030) magnetic stirrer with hotplate (UNICON). A three-necked round-bottom flask, a measuring cylinder, a beaker, a separating funnel, a burette, a density bottle, funnels, thermometers, and a measuring flask were all utilized.

➤ Sample Collection and Treatment

Samples were purchased from Otovwodo market in Ughelli North Local Government Area of Delta State, Nigeria. To separate the seed from the pulp, the seeds were dehulled using a sharp stainless knife. The produced pulp sample was then dried for 48 hours in a Gallenkamp hot air oven at 70 °C. The powdered dried materials were pulverized into a fine powder.

➤ Hydrolysis

APSO hydrolysis for the production of FFA was obtained as described by A.O.A.C. [26] with some modification. In a typical experiment, APSO (25 g) was mixed in the reactor with 150 mL of saponifying solution comprising of ethanolic KOH and NaOH concentration (0.5-2.00 M), and ethanol (150 mL: 90% v/v). The hydrolysis was carried out in a 250 mL temperature-controlled reactor at different reaction temperature 40-70°C and different reaction time 1-2.5 h. Following hydrolysis, 20 mL of water was added to the mixture. Thereafter, separation by extraction with 50 mL of hexane was done to remove unsaponifiables components. The soap-containing aqueous alcohol phase was acidified to pH 1 with HCl 6N, and the FFA was extracted with hexane. To achieve a neutral pH, the extract was rinsed with distilled water. A separating funnel was used to remove the lowest layer, which was then discarded. Anhydrous magnesium sulfate was used to dry the FFA-containing top layer, and the solvent was evaporated at 35°C in a vacuum rotary evaporator. Following that, the FFA percentage was calculated.

➤ Determination of % Free Fatty Acid (FFA)

Method according to Mahesar et al., [27] was adopted for the determination of the percent FFA in the hydrolyzed seed oil. In a flask, 50 mL isopropanol was added, followed by 0.5 mL phenolphthalein, which was then neutralized with sodium hydroxide (NaOH, 0.5 N) until a permanent pink colour was obtained. The neutralized isopropanol was added to the 5 g of FFA, which will be placed into an Erlenmeyer flask, and about 0.5 mL of phenolphthalein was added. After shaking the mixture gently, the mixture is neutralized by the addition of NaOH, 0.5 N until the first permanent pink colour is obtained. The % FFA was calculated by using the equation.

$$\% \text{ FFA as oleic} = \frac{28.2 \times N \times V}{W}$$

Where; V = Volume in ml of 0.5 N NaOH required for titration in ml.

W = Weight in g of sample taken.

➤ FTIR spectroscopic analysis

For this investigation, we used a SHIMADZU FTIR-8400S equipped with deuterated triglycine sulphate (DTGS) as the detector and potassium bromide (KBr) as the beam splitter, as described by Liang et al., [28]. The measurements were at room temperature in the IR range of 4000–450 cm⁻¹ by aggregating 40 scans with a resolution of 4 cm⁻¹.

➤ GC-MS Analysis

On a GCMS-3800 equipment, gas chromatography-mass spectrometry analysis was done. This technique was adopted from Adams [29]. Hexane (99 percent, Sigma-Aldrich, Germany) was used to dilute 20 microliters of the extracted oil sample to 1 mL. The flow rate of helium carrier gas (99.999 percent, AGA Lithuania) was set at 1.23 mL/min. After injection, the oven temperature was maintained at 40 °C for 2 minutes before being programmed at 3 °C/min to 210 °C, where the column was kept for 10 minutes. The split ratio was set at 1:10 with a mass detector electron ionization of 70

eV. A search of a mass spectra database was used to identify volatile chemicals (NIST 14).

III. RESULTS AND DISCUSSION

➤ Percentage of free fatty acid (% FFA)

Experiments on percent FFA for KOH and NaOH under reaction conditions of concentration, temperature and reaction time as captured in Figure 3, 4 and 5 respectively yielded the following findings. The findings demonstrate the hydrolysis performance of these catalysts as well as their impact under various experimental settings. The results showed that increasing the KOH concentration as shown in figure 3 indicates a corresponding increase in the % FFA. Results from this study is in agreement with findings from observation involving *C. rugosa* lipase hydrolysis of various vegetable oil [30-31]. However, increasing the concentration of NaOH did not result in any significant changes in the percentage of FFA. Furthermore, the impact of temperature as captured in figure 4, showed that when the temperature was raised from 50 to 70°C, the percent FFA increased, with a maximum yield obtained at 60°C. This is also an important indicator that the hydrolysis of APSO is influenced by temperature. The result also in figure 5, describe the effect of reaction time (1-2.5 hrs) on the % FFA, which showed an increased in % FFA with an increase in the reaction time for both catalyst. However, the highest yield of % FFA was recorded after 2 hrs for both catalyst.

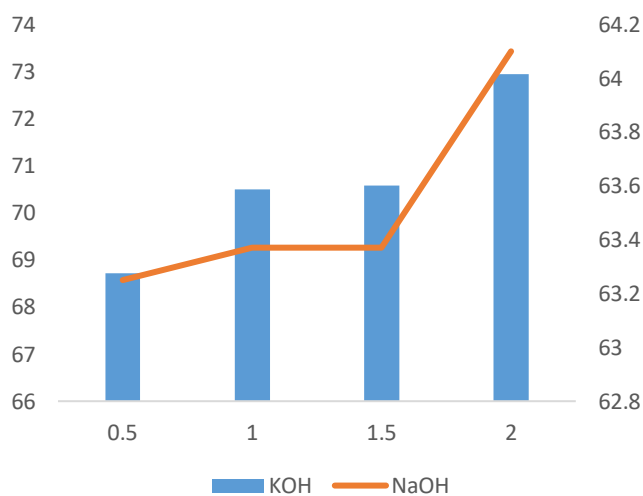


Fig. 3 Effect of Concentration (M) on the % FFA

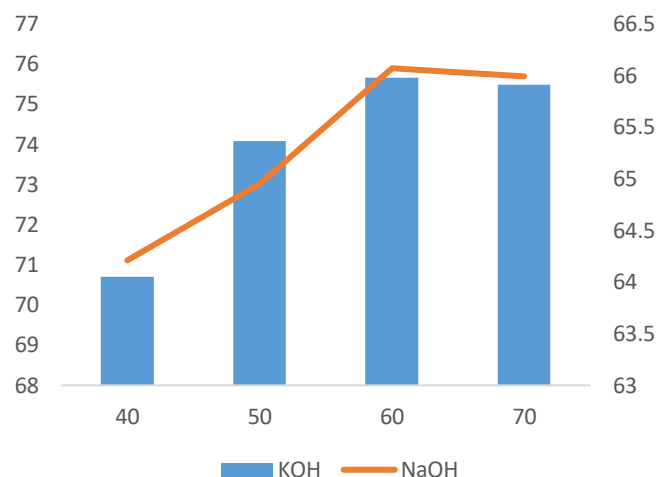


Fig. 4 Effect of Temperature (°C) on % FFA

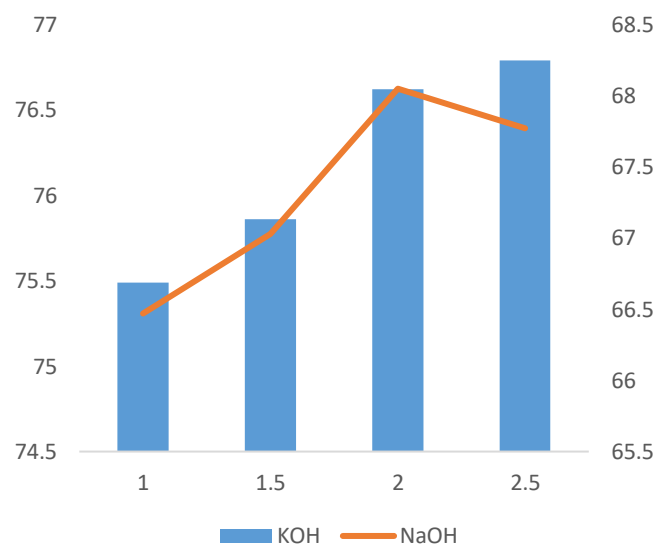


Fig. 5 Effect of Reaction time (h) on % FFA

➤ FTIR Analysis of Fatty Acids

The hydrolysis of APSO sample revealed by FTIR spectroscopy as summarized in Table 1, displayed the main peaks as well as their functional groups. For the FTIR study, the reaction conditions that yielded the highest yield (2 M, 60 °C, and 2 hours) were used.

Table 1 FTIR wavelengths of functional groups of APSO before and after hydrolysis

S/N	Wavelengths			Functional group
	Fresh oil sample	Hydrolyzed oil sample with KOH	Hydrolyzed oil sample with NaOH	
1	3468 cm ⁻¹	-	3468 cm ⁻¹	The vibration of O-H stretching
2	2917 cm ⁻¹	2912 cm ⁻¹	2917 cm ⁻¹	Unsaturated C-H stretching of carbon chain
3	2851 cm ⁻¹	2849 cm ⁻¹	2851 cm ⁻¹	Saturated carbon chain C-H stretching vibration
3	1736 cm ⁻¹	1738 cm ⁻¹	1734 cm ⁻¹	C=O stretching
4	1472 cm ⁻¹	1472 cm ⁻¹	1472 cm ⁻¹	Bending symmetric vibration of CH ₂ (methylene) groups' C-H bonds
5	1179 cm ⁻¹	1179 cm ⁻¹	1181 cm ⁻¹	The stretching vibration peak of C-O in triglyceride
6	723 cm ⁻¹	-	723 cm ⁻¹	Carbon skeleton vibration peak

7	718 cm ⁻¹	718 cm ⁻¹	716 cm ⁻¹	The out-of-plane vibration of the cis-HC=CH group of disubstituted olefins overlaps with the rocking vibration of CH ₂
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The comparison between APSO hydrolyzed by KOH and NaOH as captured in table 1 reveal the spectra and key peaks, as well as the functional groupings to which they belong. Peaks for carboxylic acid carbonyl functional groups (C = O) were found at 1733 cm⁻¹ for KOH while there was increased intensity with NaOH at 1734 cm⁻¹. Absorption bands of hydrolysis at 1472 cm⁻¹ for stretching vibration was same for both catalyst. A higher intensity at 1181 cm⁻¹ for

stretching asymmetric was observed for NaOH with no change observed for KOH. The result also indicated the absence of ester functional groups at 1746 cm⁻¹ for both catalyst. Other peaks at 2912 cm⁻¹ and 2917 cm⁻¹ denote the presence of CH₂ and CH₃ scissoring for both catalyst. The absorption bands for (C-H) group vibration in the NaOH catalyst were also seen in the FTIR spectrum at 7233 cm⁻¹.

Table 2 GC-MS characterization of APSO after hydrolysis

S/N	Fatty acids	Systematic names	Composition (%)	
			KOH	NaOH
1	Palmitic acid; C16:0	Hexadecanoic (C ₁₆ H ₃₂ O ₂)	9.37	2.87
2	Palmitoleic acid; C16:1	cis-9-hexadecenoic C ₁₆ H ₃₀ O ₂	3.62	2.02
3	Stearic acid; C18:0	Octadecanoic (C ₁₈ H ₃₆ O ₂)	14.35	12.21
4	Oleic acid; C18:1	cis-9-octadecenoic (C ₁₈ H ₃₄ O ₂)	51.32	43.19
5	Linoleic acid; C18:2	cis-9-cis-12-octadecadienoic (C ₁₈ H ₃₀ O ₂)	9.65	16.09
6	Linolenic acid; C18:3	cis,cis,cis-9,12,15-octadactrienoic C ₁₈ H ₃₀ O ₂	2.76	3.15
	SAFA		23.72	15.08
	UFA		67.35	64.45
	others		8.93	35.55

Further characterization study by GC-MS as captured in table 2, confirmed a positive identification of FFA composition. Fatty acids identified from this study for KOH catalyst include the following: palmitic acid (9.37 %) Palmitoleic acid (3.62 %), Stearic acid (14.35 %), Oleic acid (51.32 %), Linoleic acid (9.65 %) and Linolenic acid (2.76 %). While for NaOH, the follow percentage of fatty acids were identified; palmitic acid (2.87 %) Palmitoleic acid (2.02 %), Stearic acid (12.21 %), Oleic acid (43.19 %), Linoleic acid (16.09 %) and Linolenic acid (3.15 %). The results from this study showed variations in the percentage composition as reported in other findings [32, 33]. This variations could be traced to environmental conditions, species of the seed plant, extraction technique etc.

IV. CONCLUSION

Investigation of APSO hydrolysis with GCMS and FTIR monitored under optimum conditions, revealed that hydrolysis was achieved using KOH and NaOH catalyst respectively. The results showed that the highest % FFA hydrolysis was obtained at optimum concentration (2 M), temperature (60 °C) and reaction time (2 hrs). Rapid hydrolysis was observed at 1.5 M for both catalyst, thereby reporting a percentage yield of 70.58 % and 63.37 % of the % FFA for KOH and NaOH respectively. Further characterization study by GC-MS confirmed a positive identification of FFA composition. FTIR studies of the KOH and NaOH catalysts revealed substantial absorption of carboxylic acid peaks at 1738 cm⁻¹ and 1134 cm⁻¹, respectively. Therefore the result is an indication that KOH showed a better reactivity over NaOH under the studied condition of hydrolysis. Nevertheless, caution must be taken to ensure that above certain limits, productivity is not

hampered as seen in the case of increase in reaction time and temperature.

Conflict of Interest. The author declare that he has no conflict of interest.

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