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Surfactant from the Oil of *Clarias anguillaris* (Mudfish): Synthesis and Characterization

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Abstract
This study deals with extraction, synthesis and characterization of surfactants from oil of Clarias anguillaris (mud fish). The oil was extracted using Soxhlet extractor and n-hexane as solvent while the synthesis of the surfactant was done by sulphonation method. To determine the quality of the oil produced, physicochemical analysis such as acid value test, free fatty acid, refractive index, density, total lipid and viscosity were conducted on the fish oil. The result showed acid value (7.18 mg/KOH g), refractive index (1.38), density (1160.70 Kg/cm^3), viscosity (20.46 mPa.s) and total lipids (17.83 %) implying that mud fish oil is a precursor to surfactant. Instrumental characterization such as FTIR and GC-MS indicated that mud fish oil is rich in fatty acids. Surface properties analysis also depicted that the surfactants had good critical micelle concentration (CMC) of 3.0 mmol/L for MUD-25, 4.0 mmol/L for MUD-30 and 3.0 mmol/L for MUD-45 and surface tension (γCMC) values 31.0 mNm^{-1} 39.0 mNm^{-1}, 51.0 mNm^{-1} for MUD- 25-45 respectively and they compared well with standard, sodium lauryl sulphate (SLS). The surfactant produced also exhibited high formability of 86.21 % (MUD-25), 87.50 % (MUD 30) and 88.80 % (MUD 45) and excellent wetting time. These results suggest that mud fish surfactant may find application in the industries especially in soap and detergent industries where it is desirous to have a surfactant with high foam and good wetting properties.

Keywords: Synthesis, Characterization, Surfactant, Sulphonation, Clarias anguillaris

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I. Introduction

Surfactants are one of the most versatile and powerful classes of materials used in chemical industry throughout the world[1]. They are surface-active agents, which are made up of a non-polar hydrophobic tail attached to polar head group and these two parts provide a compound with interfacial activity and give rise to a wide range of surface chemistry functions: wetting, emulsifying, softening, solubilizing, foaming/defoaming, rheology-modifying, detergency and surface conditioning[2]. The chemical classification of surfactants is thus based on the nature of the hydrophilic group with subgroup being defined by the nature of the hydrophobic group[3]. Surfactants can be derived from both petrochemical feedstock and renewable resources (plant, animal and microorganisms). A surfactant with one of the main building blocks, the polar head group or the hydrophobic tail, obtained from a natural source is often referred to as a natural surfactant[4]. Surfactants derived from renewable raw materials (natural surfactant) are characterized by their positive impact on the environment such as biodegradability, low or non-toxicity and safe to human health[5, 6].

In addition, the use of natural surfactant can contribute to save fossil resources, such as crude oil and natural gas, reduction of fossil carbon dioxide emissions (CO_2) and hence could be part of a strategy to mitigate the greenhouse effect[7]. There is a desire to find new surfactants in the industry with improved properties in comparison to conventional surfactants[8]. Moreover, there is a trend to move towards a more sustainable production, using renewable natural products instead of petrochemical ones.

Fishes or fish by-products are important sources of energy, food, and industrial feedstock[9]. Fish oils are a valuable source for fatty acids, primarily oleic ($C_{17}H_{33}COOH$) and linoleic acids, as well as saturated and poly (unsaturated) compounds. These compounds and their derivatives can be used as a part of compositions with different technical products for use as surfactants, film-forming and antifriction materials[9]. Fish oils containing free fatty acids, mono-, di- and triglycerides can be the raw material for production of biodegradable surfactants which can be used in the development of environmentally benign products.

In the past, many researchers have carried out studies on the fatty acid composition of freshwater fishes, but little has been done on the utilization of these resources in the production of surfactants. The last few years showed an increased interest in work involving the preparation of surfactants based on natural products. Examples are surfactants based on sugars and fatty acids[10], Castor oil[11], Rosin Gemini from soft wood[12].

Cashew nut oil[13], technical fish oil[14], *Litsea glutinosa*[6], coconut oil and corn starch[15] and Schiff Bases Vanillin[16].

Clarias anguillaris is a species of African air breathing catfish known as mudfish. It grows to a length of 100 cm TL (39.4 inches). They are common in flooded areas and mostly bury themselves in the mud when the pools are drying up. They feed mainly on fish, molluscs; crustaceans, diatoms, detritus and bottom organisms[17].

This study is aimed at extraction of oil from the *clarias anguillaris*, synthesizing the extracted oil to surfactant and characterizing the surfactants oil produced.

II. Materials and Methods

Sample Collection and Preparation

Fresh captured *Clarias anguillaris* (mud fish) was bought from fishermen living close to the Niger River, Onitsha, Anambra state, Nigeria and stored in freezer. The fish was identified by Mr. Monday Akakpo, a senior technologist in the Department of Marine Science, Faculty of Science, University of Lagos. The frozen fish were washed thoroughly in order to remove dirt that might get stuck to the body after undergoing a de-freezing process. They were cut into sizes to enhance speedy oven drying as unwanted such as gills and intestines were removed. The moisture content of the fishes was reduced by oven drying (at a temperature of 125^{o}C). The samples were further reduced in size by pulverizing using an electronic blender after undergoing moisture content elimination in the oven.

Fish Oil Extraction Process

The fish oil was extracted using soxhlet extractor and n-Hexane as the solvent. The extraction procedure is as follows: 100g of crushed dried fish sample was placed in a round bottom flask with 600 mL of n-hexane solvent, under the approximate temperature of 343k. The oil was extracted by removing the solvent under reduced pressure by a rotary evaporator at a temperature of 323k[18]. The oil extracted was collected, measured, labelled and stored in sample bottles.

Determination of refractive index

Refractive index is the ratio of the speed of light at a definite wave length in a vacuum to its speed in the medium and this varies with the wave length of light and temperature.

Abbey refractometer model number BK-R2S was used in determining the refractive index of the oil. The measuring prism surface was cleaned with solvent and distilled water, and then wiped with a clean towel after which the mode selector was regulated to the desired mode position. A drop of oil was made on the prism surface using a glass dropper and covered. The illumination arm was positioned so that the exposed face of the upper prism will be fully illuminated. The refractometer was used through the eyepiece, the dark position viewed was adjusted to be in line with the cross line. At no parallax error, the pointer to the scale pointed in the refractive index, the reading was then taken[19].

Determination of acid value

1g of oil sample was taken and dissolved in carbon tetrachloride and the solution was titrated with 0.04 M NaOH; using phenolphthalein as indicator with constant shaking until a dark colour was observed and the value recorded[20].

The free fatty acid content (% FFA) was calculated using the equation:

$$\text{FFA (\%)} = \frac{mL\ of\ NaOH \times N \times 28.2}{W} \qquad (1.1)$$

Where N = Normality of NaOH solution

W = weight of oil (g)

Determination of viscosity

The viscosity was tested using the Capillary tube viscometer test method. The most common method of determining kinematic viscosity in the lab utilizes the capillary tube viscometer. In this method, the oil sample was placed into a glass capillary U-tube and the sample is drawn through the tube using suction until it reaches the start position indicated on the tube's side.

The suction is then released, allowing the sample to flow back through the tube under gravity. The narrow capillary section of the tube controls the oil's flow rate; more viscous grades of oil take longer to flow than thinner grades of oil[21, 22].

Determination of lipid content

500 mL round bottom flask was attached to the base of the extractor and clamped to a retort stand. 5g of sample was placed into a soxhlet apparatus. 300 mL volume of n-hexane solvent was poured into the thimble

and the assemble unit placed on an electro-thermal heater with the top of the extractor connected to the reflux condenser. The source of heat was turned on as well as water source supplied to enable the solvent in the flask to boil and extract the lipid in the sample for about three hours. On completion, the thimble was removed and the solvent reclaimed by distillation. The flask and extracted lipid was placed in an oven at700^{0}C for a few minute to completely remove all the solvent residues and kept in a dessicator to cool[23].
The percentage of lipid was calculated using the equation below:
Weight of lipid = Weight of flask and content after extraction – Weight of flask before extraction 1.2

Determination of relative density

A density bottle was weighed and 10 mL of oil was poured into the bottle. The density bottle was weighed with its content[24]. This was repeated for equal volume of water. The relative density was then calculated using:
Density = mass/volume 1.3
Relative density = density of sample/density of water 1.4

GC-MS procedure

The gas chromatographic model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with mass selective detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9 % purity) was used as carrier gas, while capillary pole type HP-MS (with dimensions 30 mm x 0.25 mm x 0.320 µm) was used as the stationary phase. The separation process was conducted in accordance with the thermal program GC on 50^{o}C for a minute and then raised to 150^{o}C for two minutes at a rate of 5^{o}C per minute and then raised 250^{o}C at a rate of 5^{o}C per minute. The temperature was stabilized at 250^{o}C for a minute. The injection process was carried out using the automatic injector type[25].

FTIR procedure

The IR Fourier spectrometer, Bruker USA Model ALPHA II 2012, supplied with the unit of a single Attenuated total reflection (ATR) *Smart iTR*. Spectral range of the instrument is 7800–350 cm^{-1}; spectral range of the ATR crystal (*ZnSe*) is 20000–650 cm^{-1}. Three Drops of oil samples were placed in contact with the ATR. The spectra were recorded in the region between 4000 and 650 cm^{-1} with a spectral resolution of 6 cm^{-1} and an aperture of 5.0mm. The wave numbers accuracy is 0.01 cm^{-1}. For each spectrum, 32 scans were averaged. The internal software Advanced ATR Correction Algorithm was used for correction of collected spectrums. The calculation takes into account the reflective index of a sample, the reflective index of the ATR, the angle of incident, and the number of reflections[23].

Synthesis of surfactants

This synthesis was carried out using sulphonation process which involved a two-step procedure. In the first step, Sulphuric acid was added to the fish oil at 25^{0}C, 30^{0}C and 45 ^{0}C all through the reaction time. The time of the synthesis was 60–180 min. EDTA was added to the reacting mixture as catalyst on a magnetic stirrer. The second step involved the neutralization of acids in the obtained mixture by introducing sodium hydroxide NaOH.

Fatty acids + $SO_3 \xrightarrow{neutralization}$ $RCHCOOCH_3SO_3Na$ (α- sulpho methyl esters)

Three samples of technical surfactants (from 25^{0}C to 45^{0}C) were obtained and were labelled thus: MD-25 (mud fish oil surfactant at 25^{0}C), MD-30 (mud fish oil surfactant at 30^{0}C), MD-45 (mud fish oil surfactant at 45^{0}C).

Surface tension measurement

The surface tension measurements were carried out with Krüss tensiometer (Krüss Gmb USA, using a platinum-iridium ring at constant temperature (25±1ºC). Krüss tensiometer operates on the Du Nouy principle, in which a platinum-iridium ring is suspended from a torsion balance, and the force (mN/m) necessary to pull the ring free from the surface film is measured. Reading was obtained for a given surfactant concentration (1.5 - 10 mmol/L), as indicated by at least three consecutive measurements having nearly the same value. The average of a series of consistent readings for each sample was then corrected to account for the tensiometer configuration, yielding a corrected surface tension value. A correction factor, F is multiplied by the average dial reading in order to obtain the corrected value for surface tension. Surface tension value was taken when stable[26].

Draves wetting test

500 mL of surfactant solution was poured into a 500 mL graduated cylinder (38 cm in height), and 5.0 g of a standard skein hooked with a lead anchor was dropped in the solution. The Skein floats in the solution because of the trapped air and sinks when wetted, and the time taken to sink after initially being added to the solution was recorded as the time of wetting[27]. This was carried out with fixed concentration, temperature, hardness of water and period of aging.

Ross- miles foaming test

50 mL of surfactant solution also known as the receiver was carefully poured into the 1.0 m glass column, without creating any foam. A 20 mL pipette with the surfactant solution was placed 90 cm above the receiver and the solution was allowed to drain into the foam receiver. The height of the foam produced was measured at 0 minute and after 5 minutes[28].

The stability of foam was calculated by using the following equation (Saito *et.al*, 1990):

$$\text{Foam stability (\%)} = \frac{Foam\ volume\ after\ 5\ minutes}{Foam\ volume\ after\ 0\ minutes} \times 100 \qquad (1.2)$$

III. Results and Discussion

Table 1 shows the acid value, % free fatty acid, refractive index, density, viscosity and % total lipids present in the extracted oils. These parameters provide the quality index of the fish oils. Parameters in Table 1 also indicted that mud fish can serve as a precursor to surfactant production. Those values are acid value of 7.18 mg/KOHg, free fatty acid (FFA) 14.27% refractive index 1.38, density 1160.70 Kg/cm^3, viscosity of 20.46 mPa.s, and lipid 17.83%.

Table 1: Result of the characteristics of Mud fish .

	Acid value (mg/KOH g)	% FFA	Refractive index	Density Kg/cm^3)	Viscosity (mPa.s)	Total lipids (%)
Mud fish	7.18	14.27	1.38	1160.70	20.46	17.83

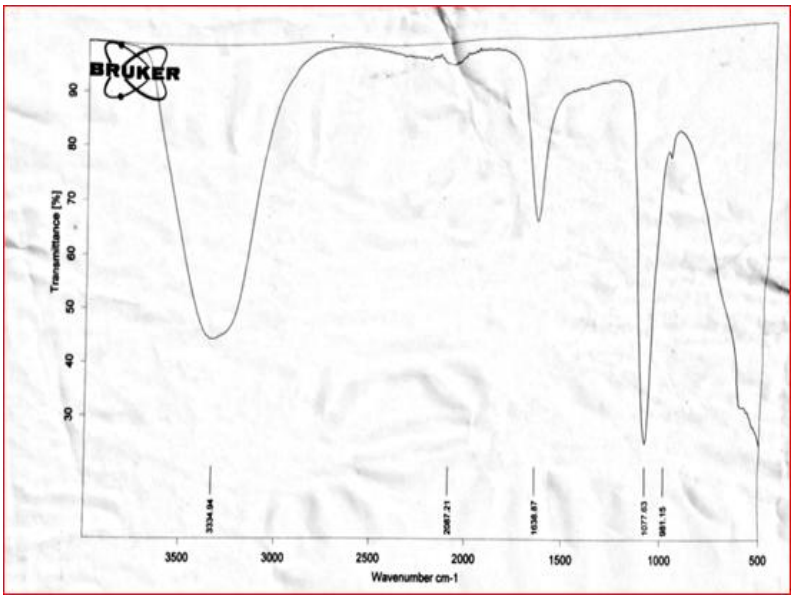

Figure 1: FTIR spectrum

Table 2: FTIR spectrum interpretation of Mud fish oils

Frequency range (cM^{-1}) of MF	Assignment	Class of compound
3335.00	Strong broad O-H stretch	Carboxylic acid R-COOH
1638.87	Medium C= C stretching	Alkene
1077.63	C-O stretching	Aliphatic ether
981.15	Strong $C = C$ bending	Monosubstituted Alkene

Fatty acid chains in the triglycerides are made up of long chains of hydrocarbon. Mostly, they consist of even number of carbon atoms from 4 to 36 carbon atoms. The presence of double bonds indicates unsaturated fatty acids. Based on the information in Table 2 (Figure 1), absorption band of 3335.00 cm^{-1} is assigned to O-H stretch of carboxylic acid, the peak at 1638.87cm^{-1} is assigned to alkene group, indicating unsaturated fatty acid in the mud fish oil. 1077.63 cm^{-1} is C-O stretch of aliphatic ether and peak at 981.15 cm^{-1} is C=C bend of monosubstituted alkene. According to this analysis, mud fish consists of saturated, unsaturated and poly unsaturated fatty acids. This result correlate with that of Ugoala *et al.,* (2008)[29] who indicated the presence of saturated fatty acids, unsaturated fatty acids and poly unsaturated acids in the oil extracted from marine fish.

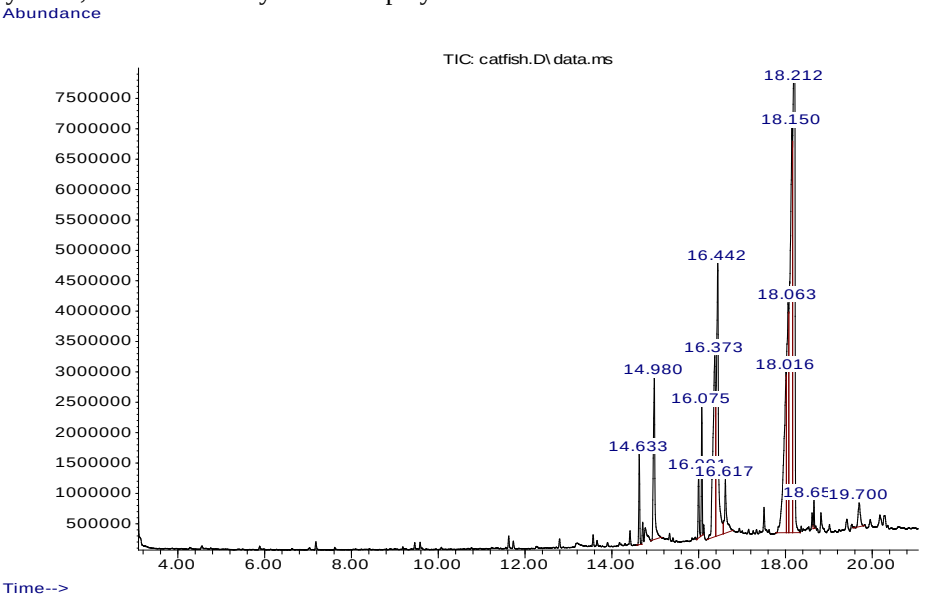

Figure 2: GC-MS spectrum of mud fish oil

Table 3: GC-MS interpretation of mud fish oil

S/N	Compounds	Relative amount (% wt)
1	n-hexadecanoic acid	8.53
2	9-Octadecenoic acid (Z)-methyl ester	2.36
3	9,12-Octadecadienoic acid (Z,Z) Linoelaidic	9.67
4	Octadecanoic acid	2.28
5	Hexadecanoic acid, methyl ester	1.71
6	9, 12-octadecanoic acid, methyl ester, (E,E)-	1.37
7	9-octadecanoic acid, (E)-Oleic Acid	12.25
8	17(-1,5- Dimethyl hexyl)- 10, 13 – dimethyl	8.70
9	Cholesterol	45-32
10	9-oxabicyclo (6,1,0) nonane	0.47
11	2, E-2, 13-octadecadien-1- ol	1.15

A total of 11 different compounds were identified by the GC-MS as shown in Table 3 (Figure 2) with fatty acids dominating. The compounds were confirmed by their retention time, percentage area, molecular weight and formulae respectively. The major fatty acids revealed were n-hexadecanoic acid (8.53%), linoleic acid (9.67%),, hexadecanoic acid methyl esters (1.71%), 9, 12-octadecanoic acid, and oleic acid (12.25%), The common neutral lipids are cholesterol and cholesterol esters. The results of the GCMS analysis in Table 3 agrees with the work of Wanten and Calder, (2007)[30] and Achionye-Nzeh and Omoridion (2002)[31]..

Surface tension

The relationship between the concentration of surfactants in aqueous solution and surface tension of MD-25^{o}C, MD-30^{o}C and MD-45^{o}C are shown in the Figures 3. As the surfactant concentration increases, surface tension decreases. Once surfactant concentration reaches a certain value, the value of surface tension becomes invariable. The surface tension curve shows an obvious decrease as the concentration of surfactant solution increases. In this work it is very clear from surface tension data that an increase in surfactant concentration lowers the surface tension of the solution. Decrease in surface tension values of surfactant in their aqueous solutions is attributed to the increase in surfactant molecules adsorption at the solution interface. Adsorption of surfactant molecules at air–aqueous solution interface occurred due to the interaction between surfactant and water molecules[32]**.**

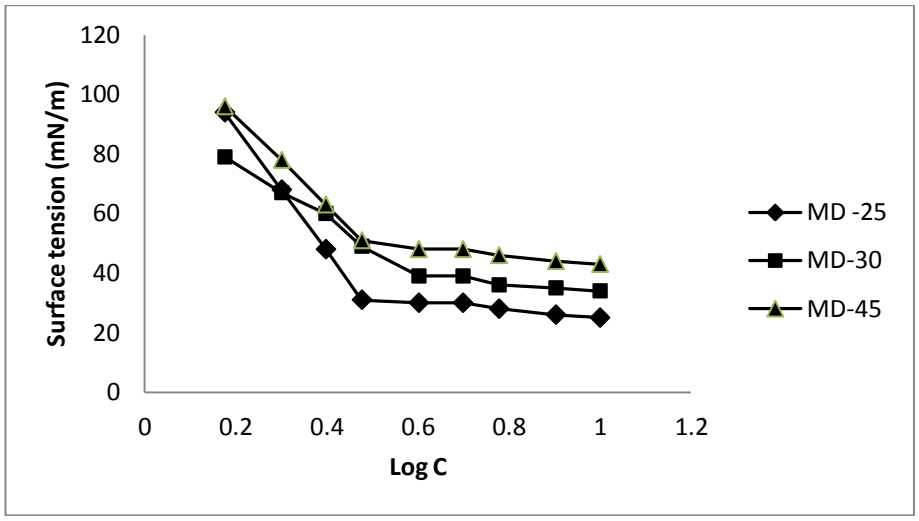

Fig 3: Surface tension curves of Mud fish oil surfactants synthesized at temperatures of 25^0C, 30^0 C and 45^0C

The concentration at which surfactants start to form micelles is called critical micelle concentration (CMC.). The CMC, of surfactant is an important value that can be obtained from surface tension concentration profile of the surfactants in aqueous solutions. The CMC was taken as the intersection of the linear portions of the plots in Figure 3, beyond which no considerable changes were noticed. When these two regions are intercepted, this gives the concentration at which the surfactant micelles are formed (CMC) [33]. The lower the CMC value the more stable it will be. The synthesized surfactants in Table 4 depicted lower CMC values compared to the commercially available surfactant SLS – (8.0 mmol/L) signifying they will be more stable compared to standard SLS.

The observed surface tension values (Table 4) at the CMC (γCMC) were in the range of 31.0 – 51.0 mN/m, which was not highly surface active. The above result of surface tension and CMS aligned with the findings of the research [34, 35].

Table 4: The initial foam heights, foam heights after 5 minutes and % foam stability, CMC and surface tension values

Samples	Initial foam height (mm)	Foam height after 5 minutes (mm)	Foam stability (%)	CMC (mmol/L)	$\gamma = (mNm^{-1})$
MD-25	116	100	86.21	3.0	31
MD-30	120	105	87.50	4.0	39
MD-45	125	111	88.80	3.0	51
SLS	135	130	96	8.0	32.5

Foaming Test

The foaminess of surfactants is generally defined by the foam volume obtained from a unit volume of the surfactant. Table 4 demonstrates the initial and 5-minutes foam heights for the prepared surfactant and their various % foam stability. Low foam is defined to be $\leq 40\,mm$ initial foam[36]. Surfactants synthesized from mud fish oil displayed very high foam heights and foam stabilities when compared with their synthetic counterpart, SLS (sodium lauryl sulphate). The foam stabilities were in the range of 86.0 – 96.0 %. The above result also agrees with the result of Jin *et al*., (2016) who prepared a surfactant with an excellent stable foamability[34] which proved surfactant from mud fish oil a potential detergent material.

Wetting

Table 5 above presents the Draves wetting times for the surfactants. Rapid wetting is defined to be ≤ 40 seconds [36]. it is clear from the Table 5 that these surfactants are excellent wetting agents because of their low wetting values when compared to their synthetic counterpart. This result has been obtained elsewhere[37]. This therefore affirms that surfactant from synthesized mud fish oil is a good wetting agent and may be potential candidate for manual dish-washing, detergents, hair shampoos and detergents for manual textile washing.

Table 5: Draves wetting times for the prepared surfactants at concentrations of 0.05 wt % and 0.15 wt % of surfactants solutions for the mud fish oil.

Surfactant	Draves wetting time (sec) at 0.05 wt%	Draves wetting time (sec) at 0.15 wt%
MD-25	26.36	4.10
MD-30	27.30	5.00
MD-45	28.11	6.50
SLS	8.10	8.70

IV. Conclusion

Oil extracted from mud fish were characterized by instrumental (FTIR, GC/MS) and physicochemical analysis. The result of this characterization showed that this oil is rich in fatty acids and oleochemicals. Surfactant was successfully synthesized from this oil extracted from mud fish through the sulfonation method and surface properties were investigated. Surface tension measurements obtained indicated that the prepared surfactants MD-25, MD-30, MD45 exhibits good CMC and γCMC values when compared to their synthetic counterpart (SLS). The prepared surfactants MD-25, MD-30, MD45 also showed high and stable foamability and excellent wetting properties. These results indicate that these surfactants may find applications in some industries, especially the soap/detergent industries where it is important to have a surfactant with high foam and good wetting properties.

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