Surface Activity and Thermodynamic Assessment of Surfactants Derived from Oreochromis Niloticus Oil (Nile Tilapia Fish)

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ABSTRACT

Surfactants were synthesized from *Oreochromis niloticus* (Nile Tilapia) oil via sulphonation and evaluated for surface-active and thermodynamic properties. The extracted oil had an acid value of 9.56 mg KOH/g, refractive index of 1.46, density of 1341 kg/m³, viscosity of 19.52 mPa.s, and lipid content of 18.20%. Fourier Transform Infrared Spectroscopy (FTIR) identified carboxylic acids and esters as major functional groups, while Gas Chromatography–Mass Spectrometry (GC-MS) detected oleic, palmitoleic, and decanoic acids among others. Three surfactants (NT-25, NT-30, NT-45) were obtained at sulphonation temperatures of 25°C, 30°C, and 45°C, respectively. They effectively reduced surface tension to 31.0, 36.0, and 44.0 mN/m, with critical micelle concentrations (CMC) of 2.86, 3.16, and 3.55 mmol/L, respectively. Wetting times ranged from 7.21 to 19.00 seconds, and foam stability reached 96% (NT-25), 95% (NT-30), and 96% (NT-45). NT-25 and NT-30 exhibited higher surface pressure at CMC (∏cmc), greater surface excess concentration (Γmax), and lower minimum surface area per molecule (A), enhancing their surface tension reduction compared to NT-45. These results suggest NT-25 and NT-30 possess excellent wetting and foam stability, making them promising for applications in soaps, detergents, and cosmetics.

Keywords: Surfactant; Sulphonation; *Oreochromis niloticus*; critical micelle concentration; surface tension

INTRODUCTION

Surfactants are surface-active compounds made up of molecules that have both water-loving and water-repelling parts. This special structure allows them to form clusters called micelles [1]. Surfactants are indispensable in everyday life, finding widespread application in food products, pharmaceuticals, agrochemicals, paints, plastics, textiles, petroleum, emulsion polymerization, and corrosion prevention [1]. Surfactants can be produced from various sources, including plant oils, animal fats, and petroleum. Due to the environmental impact of surfactant discharge into water, there is a growing need for biodegradable surfactants [2]. Traditional surfactants made from petroleum are not fully biodegradable and pose ecological concerns [3, 4]. Most conventional surfactants reduce dissolved oxygen in water due to microbial breakdown, leading to environmental issues. Consequently, there is a shift towards developing eco-friendly surfactants from natural resources that are fully biodegradable. Lipid-derived surfactants are a promising alternative, being both biologically compatible and environmentally friendly [5].

The global availability of fish oil and the utilization of by-products from the fish processing industry make it an appealing alternative to petroleum-based surfactants. However, challenges such as temperature stability,

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potential solidification at low temperatures depending on composition, purification requirements, and optimization of cost-effective synthesis methods remain areas for improvement [6].

The development of eco-friendly surfactants from fish oil offers a sustainable alternative to conventional synthetic surfactants. Utilizing fish-derived lipids as precursors, combined with the use of heterogeneous catalysts and a simple work-up process, can help overcome challenges associated with traditional surfactant production. This approach aligns with current efforts to create biodegradable and environmentally benign surfactants from renewable biological sources. To achieve this, several surfactants based on the specific structure of fish oil and fish lipids need to be synthesized and fully characterized to ensure their surfactant properties and behaviour. Additionally, these surfactants require specific modifications using fish oil and fish lipids as precursors to enhance their surfactant properties [6]. Despite not being commonly used, these natural detergents are a viable option.

Traditional soaps are typically anionic surfactants composed of free fatty acids and alkyl chains, known for their excellent cleaning abilities due to their negative charge and oil-dissolving properties [7]. Instead of converting fats into soap traditionally, it is possible to use reactive ester derivatives of fatty acids and peptides derived from natural proteins directly [8]. This method simplifies the production of amphiphiles similar to anionic surfactants [9]. Modern dermatological practices favour detergents and surfactants that do not affect skin cell physiology, barrier function, or wound healing cells [10, 11]. This shift is driven by the widespread availability of anionic and nonionic synthetic surfactants [12]. Recent studies have shown that various natural sources, such as *Clarias anguillaris* (Mudfish) [13], marine waste [14], *Sapindus mukorossi* and *Sapindus trifoliatus (Soapnuts)* [15], *Sapindus laurifolius* [16], and fish waste [17], have been used to produce biosurfactants for different applications. These studies are possible because research is geared towards the sustainability and biodegradability of surfactants. Moreover, fish oil-based surfactants have garnered attention due to the high content of long-chain fatty acids, which enhance surfactant performance by improving properties such as emulsification, foaming, and detergency. Studies by Mukhin [17] demonstrated the potential of fish oil-derived surfactants in various applications, including cosmetics and bioremediation, where their biodegradability and non-toxicity were particularly valued. Research on surfactants derived from *Oreochromis niloticus* (ON) fish oil is relatively scarce. The high yield of oil from ON fish and its favorable fatty acid profile make it an attractive candidate for surfactant synthesis. Studies by Suseno et al., [18] and Li et al. [19] highlighted the extraction of ON fish oil and its characterization. Nevertheless, despite these promising findings, no study has investigated the potential of ON fish oil in the production of surfactant, surface properties analysis and thermodynamics study of this surfactant which are critical for understanding their stability, self-assembly, and performance in various industrial applications. This study aims to address this gap by providing a detailed evaluation of a surfactant derived from *Oreochromis niloticus* (Nile Tilapia) fish oil through a sulphonation-based approach. The research focuses on a comprehensive functional analysis, including surface activity, thermodynamic behavior, and adsorption characteristics. By investigating these properties, the study contributes to the broader understanding of bio-based surfactants and highlights their potential application in sustainable industrial practices.

Oreochromis niloticus, or Nile tilapia is an edible fish with high lipid content, originally from Africa, and widely cultivated due to its adaptability and fast growth rate [20, 21]. It is known for its laterally flattened bodies, long dorsal fins, and grey or silver colour, varying based on environment and diet [20, 22]. The species thrives in various freshwater habitats and performs best at temperatures between 25°C to 30°C [23, 24]. Given its availability and low cost, Nile tilapia is an attractive substitute for synthetic surfactant precursors and other fish oil-derived surfactants. This study explores the extraction and characterization of oil from *Oreochromis niloticus* and the development of surfactants from the extracted oil through a sulphonation-based process. The performance of the resulting surfactants was evaluated through foaming tests, wetting power assessments, and surface tension measurements, complemented by thermodynamic and adsorption studies related to micellization.

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MATERIALS AND METHODS

Sample Preparation

The fish samples were procured from fishermen residing near the Niger River, Onitsha, Anambra State. The fish was thoroughly washed and cut into smaller sections to facilitate faster oven drying, with the gills and intestines discarded. The fish's moisture content was lowered through oven drying at 125°C because water is immiscible with oil. Once dried, the sample was further reduced in size and finely blended using an electronic blender.

Chemicals and Reagents

The following chemicals and solvents were used in this study: n-Hexane (99.5%, analytical grade, Sigma-Aldrich, USA): used as extraction solvent. Methanol (99.5%, analytical grade, Sigma-Aldrich, USA): used for transesterification. Potassium hydroxide (KOH) pellets (85%, qualikems, India): used as catalyst for transesterification and for acid value determination. Sulphuric acid (H_2SO_4), 25 wt% (97.99% purity, JHD, India): used as sulphonating agent in surfactant preparation. Sodium hydroxide (NaOH) pellets (98%, Unicorn, India): prepared into 0.05 M solution for titration and used for neutralization in surfactant preparation. Carbon tetrachloride (CCl_4) (99.5%, analytical grade, JHD, India): used as solvent in acid value test. Phenolphthalein indicator solution (1% in ethanol, analytical grade, Sigma-Aldrich, USA): used as titration indicator. Ethylenediaminetetraacetic acid (EDTA) (≥99%, analytical grade, Sigma-Aldrich, USA): used as a chelating agent in sulphonation to sequester trace metal ions. Distilled water (laboratory grade): used for cleaning, dilution, and solution preparation. Fish oils extracted from *Oreochromis niloticus* (Nile tilapia) using Soxhlet extraction with n-hexane.

Oil Extraction and Trans-esterification

According to the method described by Effiong and Fakunle [25], fish oil extraction was performed using a Soxhlet extractor and n-hexane solvent. The extraction involved placing 100 g of dried, blended fish sample into a round-bottom flask with 600 mL of n-hexane at around 343K. The oil was extracted by evaporating the solvent under reduced pressure with a rotary evaporator at 323K. The extracted oil was then collected, measured, stored in sample tubes, and labelled for further use. The oil extracted from the fish oil was trans-esterified using an acid and base as a catalyst. The oil obtained was mixed with methanol and KOH was added to catalyze the reaction (Figure. 1) [26].

Fish Oil Characterization

The fish oil was characterized instrumentally using a refractometer (Abbey model number BK-R2S), a viscometer, GC-MS (Agilent Technologies 7890A GC coupled with a 5975C Mass Selective Detector), and FTIR spectrometers (Bruker USA Model ALPHA II 2012 with an ATR Smart iTR unit). Additionally, the acid value and total lipid content were tested using standard methods [25].

Determination of Refractive Index

To measure the refractive index of the oil, an Abbe refractometer (model BK-R2S) was used. The measuring prism was cleaned with a solvent & distilled water and then dried with a clean towel. The mode selector was set to the appropriate mode. A drop of oil was placed on the prism surface with a glass dropper and covered. The illumination arm was adjusted so the upper prism's exposed face was fully illuminated. Viewing through the eyepiece, the dark position was aligned with the crosshair. With no parallax error, the scale's pointer showed the refractive index, which was then recorded. This value represents the refractive index of the oil sample [27].

Determination of acid value

A 1 g fat sample was dissolved in carbon tetrachloride and titrated with 0.05 M NaOH using phenolphthalein as an indicator. The solution was constantly shaken until a dark color was seen, and the value was noted [25].

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

$$\text{FFA (\%)} = \frac{mL\ of\ alkali\ \times N \times 28.2}{W} \quad 1$$

Where N is the normality of the NaOH solution (mol/L), and W is the weight of the oil in grams (g).

Determination of Viscosity

To determine the viscosity, the capillary tube viscometer method was used, the most common technique for measuring kinematic viscosity in a lab is setting. This method involves placing the oil sample in a glass capillary U-tube and drawing it up to a start mark using suction. Upon releasing the suction, the sample flows back through the tube by gravity. The narrow capillary section controls the flow rate, with higher viscosity oils taking longer to flow compared to lower-viscosity oils [28, 29].

Determination of Lipid Content

For lipid extraction, 5 g of the sample was loaded into a Soxhlet apparatus, with a 500 mL round bottom flask attached to the base and secured on a retort stand. A thimble containing 300 mL of n-hexane solvent was inserted, and the unit was placed on an electrothermal heater, connected to a reflux condenser. Heat and water were applied to enable the solvent in the flask to boil and extract lipids from the sample for three hours. After extraction, the thimble was removed, and the solvent was distilled off. The flask with the extracted lipid was then heated briefly at 70°C to remove any remaining solvent residues and cooled in a desiccator [30].

The percentage of lipids was calculated using the equation below:

Weight of lipid = Weight of flask and content after extraction – Weight of flask before extraction

Determination of Relative Density

The density bottle was weighed first. Subsequently, 10 milliliters of oil were introduced into the bottle, which was then reweighed together with its contents [31]. This process was replicated using an equal volume of water. Finally, the relative density was calculated employing the specified method.

$$Density = \frac{Mass}{Volume} \quad 2$$

$$Relative\ density = \frac{Density\ of\ the\ sample}{Density\ of\ water} \quad 3$$

Preparation of surfactant

The fatty acid methyl ester (Compound 1), obtained via transesterification, was used in the preparation of the surfactants through a sulphonation process (Figure 2) [26]. The experiments employed 25 wt % sulphuric acid and methyl ester (compound 1). This preparation involves a two-step sulphonation process. In the first step, sulphuric acid was added to fish oil at temperatures of 25°C, 30°C, and 45°C throughout the reaction period, which lasted between 60 and 180 minutes. EDTA (ethylenediaminetetraacetic acid) was introduced subsequently, not as a conventional acid catalyst, but as a chelating and reaction-modifying agent. This is the fatty acid methyl esters used were derived from fish oil, a biological feedstock known to contain trace amounts of metal ions such as iron (Fe), copper (Cu), and manganese (Mn), which may originate from the marine environment or processing equipment. These metal ions are known to catalyze undesirable side reactions, including oxidative degradation of unsaturated fatty acids and decomposition of sulphonated intermediates, particularly under acidic and elevated temperature conditions typical of sulphonation processes. The presence of EDTA helps to sequester these trace metal impurities by forming stable, inactive complexes, thereby minimizing their catalytic activity. This improves the selectivity, consistency, and stability of the sulphonation reaction. The use of EDTA in this context aligns with its established application in surfactant chemistry and industrial formulations, where it is routinely employed to prevent metal-induced degradation and enhance product integrity [32, 33]. The second step involved neutralizing the acids in the mixture with sodium

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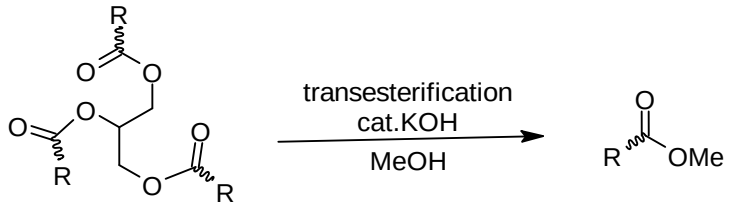
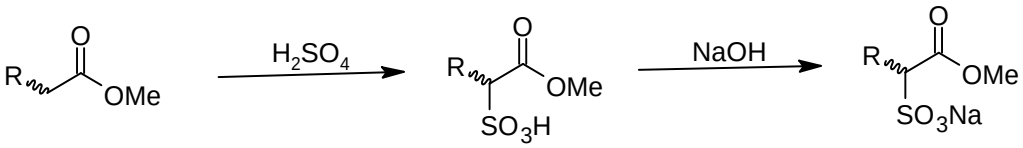
hydroxide (NaOH) [17]. The reaction is shown in Figure 1 and 2. The reaction in Figure 2 resulted in three technical surfactant samples with varying active component contents (from 25°C to 45°C). These were labelled as follows: NT-25 (Nile tilapia surfactant produced at 25°C), NT-30 (Nile tilapia surfactant produced at 30°C), and NT-45 (Nile tilapia surfactant produced at 45°C).



Triglyceride (oil) compound 1 (fatty acid methyl ester)

Figure 1: Reaction route for the generation of the fatty methyl ester



fatty acid ester sulfonated methyl ester

Figure 2: Reaction scheme illustrating the formation of sulphonated methyl ester (Surfactant)

Characterization of the surfactants

Determination of Surface tension

The surface tension measurements were performed with a Krüss tensiometer (Krüss GmbH USA) using a platinum-iridium ring at a constant temperature of 25 ± 1ºC. For each surfactant concentration, measurements were taken until at least three consecutive readings showed nearly identical values. The average of these consistent readings was adjusted for the tensiometer's configuration to obtain a corrected surface tension value. A correction factor, F, was multiplied by the average dial reading to get the corrected surface tension (ST). The surface tension value was recorded once it stabilized. The ring and wire had dimensions of 9.545 mm and 0.185 mm, respectively [34].

CMC measurement

Critical Micelle concentration, CMC values were derived from a standard plot of surface tension against surfactant concentration [35]. The CMC concentration is identified at the point where the surfactant first displays the lowest surface tension. Following this point, the surface tension remains fairly constant.

Draves wetting test

According to ASTM D2281-68 [36], the Draves wetting test was conducted by pouring 500 mL of surfactant solution into a 500 mL graduated cylinder (38 cm in height) and dropping a 5.0 g standard skein attached to a lead anchor into the solution. The skein floats due to trapped air and sinks when fully wetted, with the sinking time recorded as the wetting time. This experiment was carried out at constant concentrations of 0.05 wt% and 0.15 wt%, temperatures of 25 ºC, 30 ºC and 45 ºC, water hardness (80 mg/L), and ageing period (24 hrs).

Ross-miles foaming test

A 50 mL surfactant solution was carefully poured into the receiver tube of a 1-meter glass column, ensuring no foam was generated during transfer. A 200 mL foam pipette filled with the surfactant solution was placed 90

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cm above the receiver, and the solution was allowed to drain into the receiver. The foam height was measured initially at 0 minutes and then after 5 minutes [36]. The stability of the foam was determined using the following equation [37]:

$$\text{Foam stability (\%)} = \frac{Foam\ volume\ after\ 5\ minutes}{Foam\ volume\ after\ 0\ minutes} \times 100 \qquad 4$$

RESULTS AND DISCUSSION

Characterization of the ON oil extracted

Table 1 illustrates the properties of ON fish oil. The acid value of fish oil is an important measure of its quality and stability, indicating the extent of lipid oxidation and hydrolysis, as described by Romanova et al. [38]. High acid values generally indicate oil degradation, poor storage conditions, or extended storage times, resulting in increased free fatty acids [39]. The acceptable acid value range is 7–8 mg/KOH g [40]. In this research (Table 1), the acid value is found to be 9.56 mg/KOH g ± 0.86 with the free fatty acid content of ON fish oil calculated to be ($19.01\% \pm 1.57$) from the acid value. The standard for free fatty acids is ≤1.5% [41]. Several factors, including composition, extraction process, sample preparation, and raw material freshness, affect the acid value and free fatty acid content, as reported by Wrolstad et al., [39]. The high values observed in ON fish oil could be attributed to these factors. Suseno et al. [18] obtained free fatty acid levels of 1.12–4.30% from Nile tilapia using the Dry Rendering method at various temperatures and times. The acid values of tilapia oil obtained through diluted alkaline hydrolysis, enzymatic hydrolysis, and microwave-assisted methods are 0.77, 1.12, and 1.41 mg KOH g, respectively [19]. The refractive index of ON fish oil is 1.46 ± 0.39, which falls within the standard range of 1.4–1.473 [42], consistent with findings by Mota et al. [43] and Menegazzo et al. [44], who reported refractive indices of 1.468 and 1.46, respectively, for Nile tilapia and its viscera oils. The viscosity of ON fish oil is 19.54 mPa.s ± 2.34. This did not compare well with the 37.07 mm²/s reported by Mota et al. [43] may be due to the method used in the synthesis. The results also show that the density and total lipid content of ON fish oil are 0.93 g/cm³ ± 0.23 and 18.20% ± 3.26, aligning with the density and total lipid values for Nile tilapia reported by others [45, 18].

Parameters	Values
Acid value (mg/KOH g)	9.56 ± 0.86
FFA (%)	19.01 ± 1.57
Refractive index	1.46 ± 0.39
Density (g/cm^3)	0.93 ± 0.23
Viscosity (mPa.s)	19.54 ± 2.34
Total lipids (%)	18.20 ± 3.26

Table 1: Properties of ON fish oil

FTIR Analysis of ON oil

The result of Fourier Transform Infrared Spectroscopy, FTIR is presented in Figure 3. The peak observed at 3008.06 cm^{-1} denotes the O-H stretching vibration from the carboxylic group. Absorbance at 2853.13 cm^{-1}, and 2922.42 cm^{-1}, signifies C-H stretching vibration which indicates C-H bonds from alkane group. At 1743.94 cm^{-1}, peaks is C=O stretching vibration suggesting the presence of ester groups within the oil. Saturated fatty acids are characterized by single bonds between carbon atoms, while double bonds indicate unsaturated fatty acids. Peak at 1656.19 cm^{-1} is C=C stretching vibration which signifies the alkene functional group, confirming the presence of unsaturated fatty acids in ON fish oils. Specific to fish oil composition, the

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aliphatic methyl functional group is identified by peaks at 1432.72 cm^{-1} and 1418.16 cm^{-1}. The band at 1377.23 cm^{-1} indicates O-H bending of the phenol group, while spectral bands at 1159.66 cm^{-1}, 1117.19 cm^{-1}, and 1097.46 cm^{-1} correspond to C-O stretching of primary, secondary, and tertiary alcohols, respectively. The bending/rocking vibrations of methylene ($-CH_2$) groups, observed at 1235.25 cm^{-1}, 757.73 cm^{-1}, and 721.80 cm^{-1}, confirm the presence of long, linear aliphatic hydrocarbons in the fish oils. This analysis underscores that ON fish oil primarily consists of saturated, unsaturated, and polyunsaturated fatty acids, corroborating findings from previous studies [30, 46, 47].

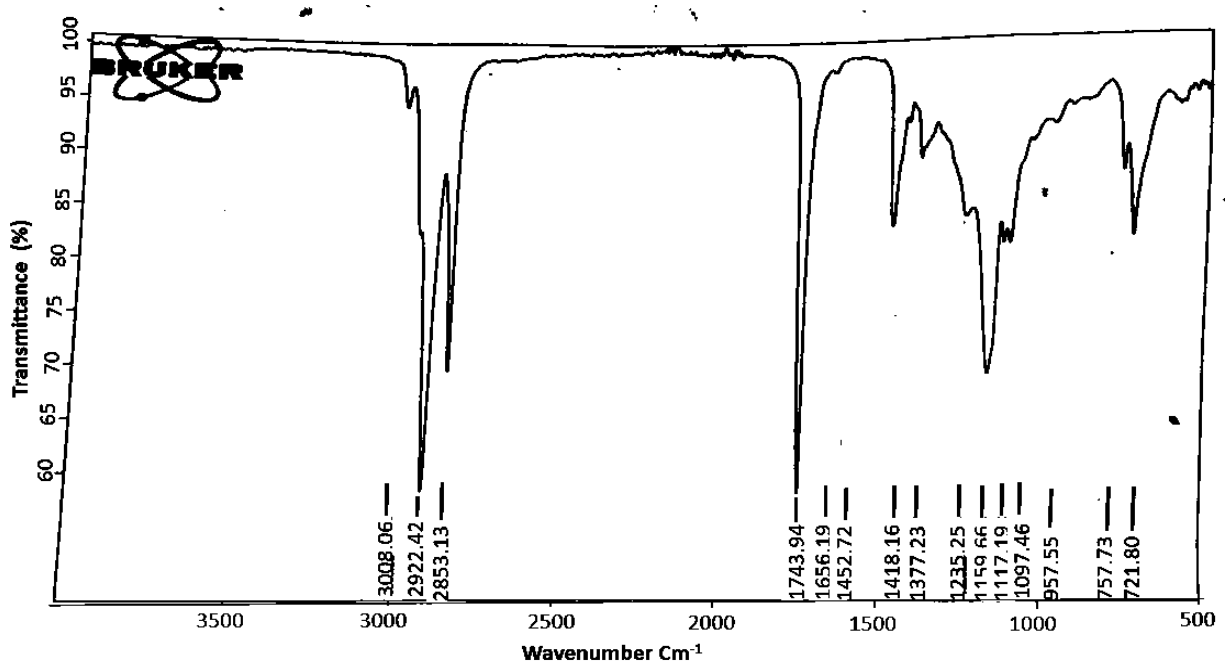

Figure 3: FTIR Spectrum of the ON Oil

GC-MS result of ON oil

A total of 22 different compounds were identified through Gas Chromatography–Mass Spectrometry, GC-MS analysis in Table 2 and Figure 4, with fatty acids predominating. These compounds were characterized by their retention time, percentage area, molecular weight, and formulas. The major fatty acids which have alkyl carbon chain structures detected were categorized as follows: (1) Saturated fatty acids including tetradecanoic acid (C14:0), which constitutes 2.79%, pentadecanoic acid, 14-methyl-, methyl ester (C15:0) at 0.94%, n-hexadecanoic acid (palmitic acid, C16:0) at 17.28%, and octadecanoic acid (stearic acid, C18:0) at 3.72%. These fatty acids have a limited ability to reduce surface tension due to the absence of double bonds, making them poor surfactants. Additionally, they contribute to rigid structures in self-assembled surfactant systems, such as micelles. (2) Monounsaturated fatty acids contain one double bond, which enhances their surface activity [47]. The monounsaturated fatty acids identified in this ON oil sample include palmitoleic acid (C16:1) at 8.55%, 9-octadecenoic acid (Z)-, methyl ester (C18:1) at 1.79%, cis-11-eicosenoic acid (C20:1) at 0.86%, and 9-octadecenoic acid (Z)-, 2-hydroxyethyl ester (C18:1 derivative) at 4.24%. Lastly, the presence of a double bond introduces a kink in the molecular structure, reducing packing efficiency and increasing surface activity compared to saturated fatty acids. These monounsaturated fatty acids also improve solubility and wetting properties, making them better emulsifiers. The Z-configuration of oleic acid further enhances fluidity, making it beneficial for surfactant formulations [48].

Polyunsaturated fatty acids have multiple bonds making them highly reactive and significantly influential in surfactant properties. The polyunsaturated fatty acids present in this fish oil sample include 9,12-octadecadienoic acid, methyl ester (a linoleic acid derivative, C18:2) at 2.04%, 9,12-octadecadienoic acid (Z,Z) (linolelaidic acid, C18:2) at 42.28%, which is the most abundant component, and 9,12,15-octadecatrien-1-ol (a C18:3 derivative) at 0.85%. These compounds exhibit very high surface activity due to the presence of multiple double bonds, which introduce greater structural flexibility [49]. As a result, they enhance spreadability, emulsification, and detergency, making them highly effective in surfactant applications.

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Other listed compounds in the same Table 2, although present in smaller amounts, may be due to moisture or impurity during processing of the oil. FTIR (Figure 3) analysis of the ON oil further confirmed the functional groups of these findings. These results are consistent with the research conducted by Cheng et al. [50] and Abo-Raya et al. [51].

Table 2: GC-MS analysis of ON oil

S/N	Compounds	Relative amount (% wt)
1	Hexane, 2-bromo-	1.18
2	2,4-Decadienal, (E,E)-	0.79
3	Tetradecanoic acid	2.79
4	Pentadecanoic acid, 14-methyl-, me thyl ester	0.94
5	Palmitoleic acid	8.55
6	n-Hexadecanoic acid	17.28
7	9,12-Octadecadienoic acid, methyl ester	2.04
8	9-Octadecenoic acid (Z)-, methyl ester	1.79
9	Methyl stearate	0.52
10	9,12-Octadecadienoic acid (Z,Z/ Linoelaidic	42.28
11	Octadecanoic acid	3.72
12	2-Decanone	1.12
13	9,12,15-Octadecatrien-1-ol,/Arachidonic	0.85
14	cis-11-Eicosenoic	0.86
15	Carbonic acid, eicosyl prop-1-en-2-yl ester	1.32
16	Bis(2-(Dimethylamino)ethyl) ether	1.24
17	S-[2-[N,N-Dimethylamino]ethyl]morp	1.29
18	Glycerol 1-palmitate	4.39
19	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	4.24
20	Ethanol, 2-(tetradecyloxy)	0.53
21	gamma.-Sitosterol	1.26
22	Squalene	1.06

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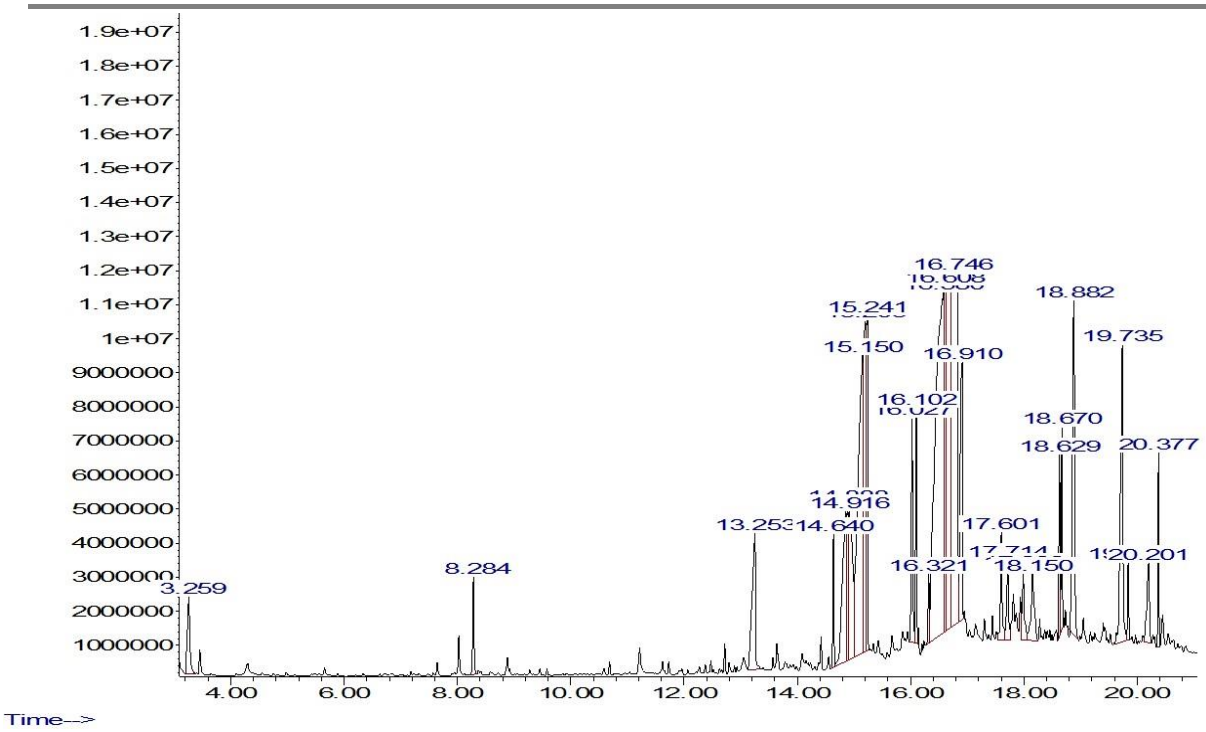

Figure 4: GC-MS result of ON oil

Characteristic of the Surfactant from ON oil

RESULT OF SURFACE TENSION

Surface tension is a characteristic of liquids that causes their surfaces to mimic the behaviour of a tightly stretched elastic membrane [52]. This results from the cohesive forces acting between the molecules in the liquid, particularly at the liquid-air interface, where the intermolecular attraction is uneven. Surfactants, also called surface-active agents, work by lowering the surface tension of a liquid and the interfacial tension between liquids or between a liquid and a solid, achieved through adsorption at liquid-air or liquid-liquid interfaces [53]. Surface tension reduction by surfactants enhances wetting, spreading, emulsification, and foam stability, playing a crucial role in a wide range of industrial, agricultural, pharmaceutical, and household applications [54].

Table 3 presents the surface tension (γ) and critical micelle concentration (CMC) of the NT surfactants prepared at different temperatures. The results highlight the impact of temperature on surfactant performance, particularly in terms of surface tension reduction and micelle formation. In NT-45, decreased packing efficiency results in higher surface tension values, making it less effective at lowering surface tension compared to NT-25 and NT-30. This demonstrates how temperature influences the molecular structure and packing behavior of surfactants. Higher temperatures can reduce a surfactant's ability to pack tightly at the interface, leading to increased surface tension [55]. Elevated preparation temperatures may lead to changes in the molecular packing or orientation of surfactants, which in turn affect their surface activity and overall performance. Heat can induce structural modifications, such as changes in the alkyl chain length, branching, or headgroup composition [56, 57]. These changes disrupt the balance between the hydrophilic (water-loving) and hydrophobic (water-repelling) regions of the molecule, ultimately affecting its interfacial behavior. A key consequence of these temperature-induced structural changes is a decrease in packing efficiency. Surfactant molecules typically align closely at the air-water interface, but structural alterations can hinder this arrangement. As a result, molecules are less densely packed, leading to higher surface tension values, as observed in Table 3**.** Conversely, the lower preparation temperatures of NT-25 and NT-30 promote better molecular alignment and packing at the interface, resulting in more effective surface tension reduction**.**

Figure 5 depicts the relationship between surface tension (γ) and the logarithm of surfactant concentrations (log C). It shows that as surfactant concentration increases, the surface tension decreases until it reaches a

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plateau, indicating the critical micelle concentration (CMC). The CMC is determined at the point where the linear portions of the plots intersect [58]. The surface tension curves in Figure 5 show a noticeable decline as surfactant concentration increases, which means surfactant molecules are adsorbing at the water interface. Once a certain concentration is reached, the surface tension becomes constant, depicting the CMC. It was observed from the Figure 5 that produced surfactants (NT-25, NT-30 and NT-45) exhibit lower CMC values than commercial SLS (8 mmol/dm³). Performance property studies indicated that the developed surfactants NT-25 have the highest surface activity followed by NT-30 and the least is NT-45. Surface tension values measured at 25°C are comparable to those of commercially available Sodium Lauryl Sulphate (32 mN/m) and Methyl ester surfactant (MES) (32.26 mN/m). This result aligns with the findings of Jin et al. [59]**,** who reported similar surface tension values for MES**,** and Fujiwara et al. [61]**,** who observed a **low** critical micelle concentration (CMC) for surfactants produced from methyl esters with varying carbon chain lengths at different temperatures. A comparison of these values is presented also in Table 3**.**

Prabhakara and Rati [62] also reported low CMC values for their synthesized surfactants, highlighting the utility of CMC in selecting surfactants for specific applications. The same goes with Obuebite et al. [63]. Low CMC values generally indicate that surfactant molecules have a stronger tendency to aggregate, leading to micelle formation at lower concentrations. This often results in more stable micelles in solution, which can contribute to reduced irritancy and improved performance. Additionally, surfactants with lower CMC values may exhibit better foam stability over time, as their molecular arrangement enhances foam formation and persistence. The developed surfactants in Table 3, with lower CMC values compared to SLS, are expected to be more stable. NT-25 surfactant which was prepared at lower temperature is well-suited for applications needing high surface activity, such as dishwashing liquids, laundry detergents, and general household cleaners [64]. Moderate temperature developed surfactant NT-30 finds application in personal care products, such as shampoo, body and facial cleaner, pharmaceuticals etc. NT-45, with its higher preparation temperature and comparatively higher surface tension, is less effective in surface tension reduction but exhibits improved stability at elevated temperatures, making it ideal for high-temperature or industrial settings such as oil recovery and textile processing.

Table 3: Surface tension (γ) and Critical Micelle Concentration (CMC) of the surfactants produced at different temperatures

Surfactant code	$\boldsymbol{\gamma_{cmc}(mNm^{-1})}$	**CMC (mmol/L)**	**References**
NT-25	31.0	2.86	Current
NT-30	36.0	3.16	Current
NT-45	44.0	3.55	Current
Sodium Lauryl sulphate (SLS)	32.5	8.10	[60]
R-MES (25°C)	32.6	4.96	[59]
W-MES (25°C)	32.3	5.38	[59]
S-MES (25°C)	32.5	5.49	[59]
C_{14}MES-Na (13°C)	-	2.80	[61]
C_{16}MES-Na (23°C)	-	0.730	[61]
C_{18}MES-Na(33°C)	-	0.18	[61]

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C_{18}MES-Na(30ºC)	-	0.660	[61]
C_{16}MES-Ca (45ºC)	-	0.190	[61]
C_{18}MES-Ca (50ºC)	-	0.0420	[61]

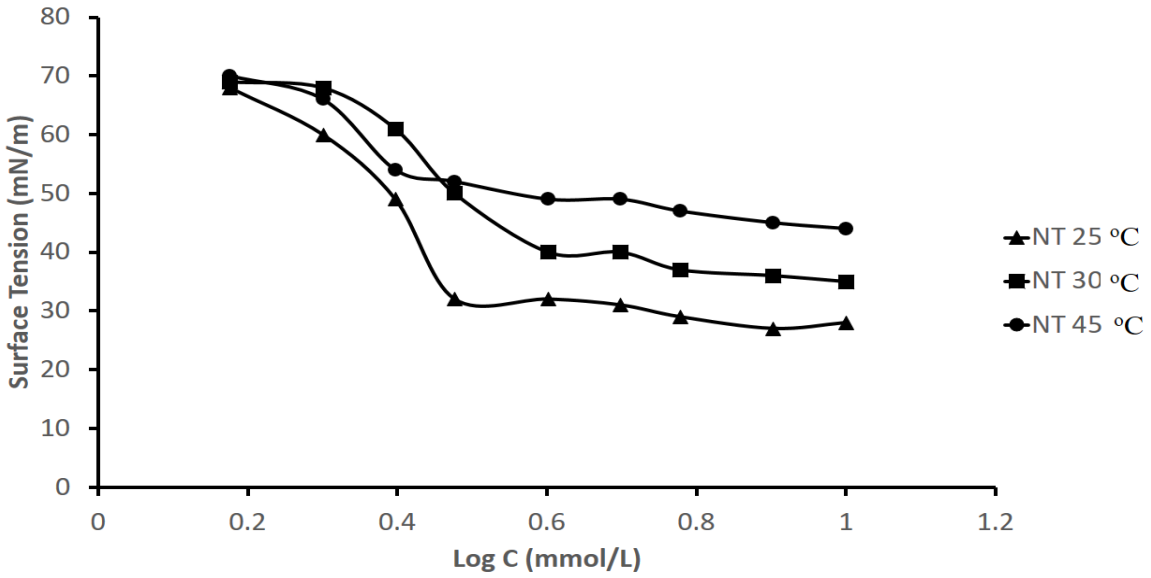

Figure. 5: Surface tension plot of ON oil surfactants produced at temperatures of 25ºC, 30ºC and 45ºC

Result of Ross- miles foaming test

Foamability is essential in cleaning products such as shampoos, dishwashing detergents, laundry detergents etc. The foam produced assists in lifting and suspending dirt and oils, facilitating their removal during rinsing. Additionally, the appearance of foam provides users with a visual indication that the product is functioning efficiently [65]. Table 4 details the initial and 5-minute foam heights for the prepared surfactants and their percentages of foam stability. A foam height of ≤ 40 mm is considered low [66]. The developed surfactants demonstrated very high foam heights and stabilities compared to the standard, sodium lauryl sulphate (SLS), with foam stabilities ranging from of 95-96.0%. at the temperature 25ºC, 30ºC, 45ºC, respectively. This compared well with the standard, Sodium Lauryl sulphate with foam stability of 96%. Surfactants with modest foam heights and stabilities were synthesized by El-Ghaffar et al. [67], making them appropriate for treating oilfield water. Prabhakara and Rati [62], on the other hand, produced surfactants that have limited foaming capabilities but great emulsion stability, which makes them superior to traditional emulsifiers in a range of industrial applications. Conversely, surfactants that were synthesized from renewable castor oil by Pandari et al. [68] demonstrated subpar foam height and stability, falling short of the typical ranges for foaminess and stability. These were recommended for use in sectors where low-foam surfactants are necessary. Thus, the surfactants synthesized in this study exhibit excellent foam heights and stabilities. Based on this characteristic surfactant

from ON oil is suitable for applications where high foam is desired, such as manual dishwashing detergents, hair shampoos, or manual textile washing detergents, cosmetics, oil field etc.

Table 4: Display of initial foam heights, 5-minute foam heights, and foam stability percentages

Surfactant	**Initial foam height (mm)**	**Foam height (mm) after 5 mins**	**Foam stability (%)**

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NT-25	124	119	96
NT-30	129	122	95
NT-45	128	123	96
Sodium Lauryl sulphate (SLS)	135	130	96

Wetting result

The Draves wetting test [68] is a standard laboratory procedure used to assess the wetting efficiency of surfactants in aqueous solutions. This test measures the time it takes for a cotton skein to be wetted by dilute surfactant solutions, with shorter times indicating better wetting efficiency. The process involves the displacement of air from the skein by the surfactant solution, causing the skein to sink once sufficient air has been replaced. Table 5 reveals the Draves wetting times for the surfactants at concentrations of 0.05 wt% and 0.15 wt%. NT-25 demonstrated the shortest wetting time among the surfactants, with times as low as 7.21 seconds at higher concentrations. This result reflects strong wetting efficiency, as NT-25 can quickly displace air from the cotton surface, allowing it to sink rapidly. This efficiency is due to the surfactant's ability to reduce surface tension significantly, a characteristic of surfactants produced at lower temperature. NT-30 displayed slightly longer wetting times than NT-25 but still achieved efficient wetting, with times around 7.22 seconds at higher concentrations. This suggests that NT-30 can effectively lower surface tension and achieve good spreading and penetration, albeit with slightly reduced efficiency compared to NT-25. NT-45 exhibited the longest wetting time, around 19 seconds at higher concentrations. The longer wetting time is likely due to the higher preparation temperature, which results in reduced surface activity and a higher minimum surface area per molecule. This limits NT-45's ability to rapidly displace air from surfaces, making it less efficient as a wetting agent than NT-25 and NT-30. According to Stepan Company [66], rapid wetting is defined as ≤40 seconds. It is evident from Table 5 that these surfactants are excellent wetting agents because the more efficient a wetting agent is, the lower the concentration of surfactant required for sinking in a given time. This result is consistent with the findings of [58, 62, 67].

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

Surfactant	**Draves wetting power time (sec) at 0.05 wt %**	**Drave wetting power (sec) at 0.15 wt%**
NT-25	29.49	7.210
NT-30	29.49	7.220
NT-45	30.07	19.00
Sodium Lauryl sulphate (SLS)	60.00	30.00

Adsorption and Thermodynamics studies of Micellization

Surfactant molecules are made up of a hydrophilic head and a hydrophobic tail. On dissolution in water at concentrations below the critical micelle concentration (CMC), surfactant molecules are found as individual monomers. But as the concentration increases and reaches the CMC, the hydrophobic tails tend to avoid water and start aggregating, thus forming micelles. Within the micelle, the hydrophobic tails are confined to the interior, shielded from water. At the same time, the hydrophilic heads remain on the surface, interacting with the aqueous surroundings, thus reducing the system's free energy [71, 72]. The efficacy of a surfactant is

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measured by its ability to greatly lower the surface tension in comparison to a pure solvent [73]. This can be calculated;

$$\prod_{cmc} = \gamma_o - \gamma_{cmc} \quad 5$$

Where $\prod_{cmc}$ represents the surface pressure at the CMC, which is related to the reduction in surface tension achieved by the surfactant at the CMC. It is also the effectiveness of the adsorption of NT surfactant, γ_o is the surface tension of pure water at a particular temperature and γ_{cmc} is the surface tension of the surfactant at CMC. The reduction in surface tension by a surfactant can be quantified using the maximum surface concentration isotherm in the following expression [74]:

$$\Gamma_{max} = -\frac{1}{RT}\left(\frac{\delta\gamma}{\delta \ln C}\right)_T \quad 6$$

Γ_{max} denotes the maximum surface excess concentration, R is the gas constant, T is the temperature, γ is the surface tension and C is the concentration of the surfactant. It indicates how effectively molecules accumulate at interfaces. This accumulation can stabilize emulsions, foams, and colloidal suspensions by reducing surface tension. A plot of γ against Ln C is made and the slope equal to $\frac{\delta\gamma}{\delta \ln C}$.

The surface area occupied by each surfactant molecule at the interface can be estimated using the following equation [75]:

$$A = \frac{10^{18}}{\Gamma_{max} N_A} \quad 7$$

Where A is the minimum surface area per molecule, Γ_{max} is the maximum surface excess concentration and N_A is Avogadro's number.

This parameter is important because the extent to which a surfactant can reduce surface or interfacial tension depends on how effectively it covers the interface. A lower surface area per molecule indicates that fewer molecules are needed to significantly reduce tension and improve stability [76].

The formation of micelles is measured in terms of Gibbs free energy, which quantifies both their thermodynamic stability and the spontaneity of the process [77]. It is estimated using the critical micelle concentration (CMC) and the standard Gibbs free energy equation.

$$\Delta G_{mic} = RT\ In\ CMC \quad 8$$

R, remains the gas constant =8.314 $JK^{-1}mol^{-1}$, T is the temperature in kelvin

The Gibbs free energy of adsorption assesses the thermodynamic possibility and spontaneity of the adsorption process, and it can be determined using the following equation 2.0 [75].

$$\Delta G_{ads} = \Delta G_{mic} - \frac{\prod_{cmc}}{\Gamma_{max}} \quad 9$$

Table 6 presents the adsorption and thermodynamics parameters calculated. A higher $\prod_{cmc}$ demonstrates a surfactant's effectiveness in lowering surface tension, which is essential for processes such as emulsification, cleaning, and wetting [76]. As shown in Table 6, the surfactants NT-25 (41 mN/m) and NT-30 (36 mN/m) demonstrated better ability to reduce surface tension than NT-45 (28 mN/m), indicating superior performance at lower preparation temperatures. Surfactants with a high Γ_{max} are generally more efficient at reducing surface tension because they can cover the surface more densely [76]. The efficiency is due to their ability to form a dense layer at the surface, significantly decreasing surface tension even when present in small amounts. This makes them effective even at lower concentrations. Also the efficiency of this NT surfactant is compromised at elevated temperatures, whereas lower temperatures significantly improve its surface tension reduction capabilities. Table 6 further reveals that the highest Γmax was obtained for NT-25, followed by NT-30, with

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the lowest value recorded for NT-45. A smaller minimum surface area (A) means higher surface coverage, more efficient packing of molecules at the surface, higher surface pressure (Π_{cmc}) and greater reduction in surface tension [78, 79]. Therefore, Table 6 also portrays that as the minimum surface area per molecule increases, the surface pressure decreases, leading to a higher surface tension. Conversely, smaller minimum surface area, A values correspond to higher surface pressure and thus lower surface tension. Which means that surfactant coded with NT-25 is the best surfactant out of the three because of it lowest minimum surface coverage, A. ΔG_{mic} and ΔG_{ads} are negative, indicating that the processes of micellization and adsorption is feasible and spontaneous.

Table 6: Adsorption and Thermodynamics parameters

Parameters	$\prod_{cmc}$ **(mN/m)**	Γ_{max} **(mol/m^2)**	A **(nm^2)**	ΔG_{mic} **(J/mol**	ΔG_{ads} **(J/mol)**
NT-25^{o}C	41	8.597×10^{-6}	0.194	-14510.97	-19280.08
NT-30 ^{o}C	36.	8.182×10^{-6}	0.203	-14503.16	−18903.06
NT-45 ^{o}C	28	4.964×10^{-6}	0.330	−14913.46	−20554.07

CONCLUSION

This study successfully produced surfactants from *Oreochromis niloticus* fish oil using a sulphonation process. The fish oil, with an acid value of 9.56 mg/KOH g $\pm$0.86 a refractive index of 1.46 $\pm$0.39, a density of 1.34 g/cm^3 $\pm$0.23, a viscosity of 19.52 mPa.s $\pm$2.34, and a total lipid content of 18.20% $\pm$3.26, was confirmed as a suitable raw material for surfactant production. FTIR analysis revealed the presence of carboxylic acids, esters, and alkyl groups, while GC-MS analysis identified significant fatty acids including oleic acid, palmitoleic acid, and decanoic acid. These temperature-specific surfactants designated NT-25, NT-30, and NT-45 exhibited distinct physicochemical properties that impact their suitability for various industrial applications. NT-25, showed the highest surface activity, achieving significant surface tension reduction, rapid wetting, and stable foaming, making it ideal for applications such as household detergents, firefighting foams, and agricultural adjuvants. NT-30, produced at 30°C, demonstrated moderate surface activity with balanced foamability and wetting efficiency, suitable for use in personal care products and textile processing. Meanwhile, NT-45, prepared at 45°C, exhibited higher thermal stability but lower surface activity and foamability. Its reduced foamability may be advantageous for industrial processes that require controlled wetting and minimal foam formation. The ability of a surfactant to lower surface tension is quantified by the surface pressure at CMC (Π_{cmc}) and the maximum surface excess concentration (Γ_{max}) measures how effectively surfactant molecules accumulate at interfaces. The minimum surface area per molecule (A) affects the surface coverage and efficiency of surface tension reduction. The NT-25 and NT-30 surfactants have higher Π_{cmc} ,leading to greater surface tension reduction compared to NT-45. Moreover, free energy of micellization and adsorption, ΔG_{mic} and ΔG_{ads} demonstrate that the process is feasible and spontaneous. The results show that *Oreochromis niloticus* oil is a potential and sustainable source for surfactant production. It offers eco-friendly, biodegradable, and application-specific alternatives to traditional synthetic surfactants. The NT-25, NT-30, and NT-45 surfactants demonstrated notable surface-active properties. However, a limitation of the present study is the lack of detailed molecular characterization and classification of the developed products (NT-25, NT-30 and NT-45). Therefore, further research is recommended to elucidate their molecular structures, determine critical physicochemical parameters such as Krafft point and disodium salt content and evaluate their performance in real-world formulations for industrial and environmental applications.

Declaration of Competing Interest

The authors declare no financial ties or personal relationships that might have influenced the research presented in this paper.

CRediT authorship contribution statement

Ifeoma M. Iloamaeke**:** Conceptualization, Supervision, Draft Review, Peter Ekemezie**:** Methodology, Writing-Original Draft, Data Curation, Chidimma V. Ezeuduji**:** Investigation, Formal Analysis, Writing-Review & Editing, Phina C. Ezeagwu: Writing-Review & Editing, Visualization, Juliana O. Ndubuisi**:** Validation, Resources, Draft Review, Onyeka F. Obumselu**:** Software, Data Curation, Writing- Review & Editing. All the authors gave their final approval and are accountable for the work.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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