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Effects of Oil Content, Mixing Time, and Temperature on the Performance Stability of Water-in-Crude Oil Emulsion using *Hylocereus undatus* Foliage as Biosurfactants

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Poor control of the emulsification mechanism may result in increased operational costs, particularly due to complications within the pipeline system in the oil and gas industry. The development of stable water-in-crude oil emulsion is highly undesirable yet unavoidable in petroleum processing. To mitigate this, the chemical surfactant is commonly used to break the emulsion. However, excessive use of chemical surfactants poses serious environmental concerns, especially to aquatic ecosystems. The discovery of *Hylocereus undatus (H. undatus)* foliage as a biosurfactant offers a promising alternative, owing to its lower toxicity, biodegradability, and eco-friendly attributes. Emulsion stability is influenced by several factors, including surface tension, viscosity, oil content, mixing time, and temperature. This study investigates the effects of oil content (at varying oil-to-water ratios), mixing time, and temperature on the performance of *H. undatus* foliage as a biosurfactant. The stability of the water-in-oil emulsion was evaluated using a tensiometer, rheometer, FTIR spectroscopy, and creaming index measurements. Results revealed that emulsions with a 5:5 oil-to-water ratio demonstrated the highest stability, with the greatest average viscosity (4.217 mPa.s), the lowest creaming index (0%), and the greatest phase separation behaviour at 88.24%. Furthermore, an optimal mixing time of 10 mins produced the most stable emulsion. FTIR analysis further confirmed this, indicating that the emulsion's high stability was associated with a lower O-H stretching wavenumber as well as having the lowest creaming index value (0%). Similarly, samples processed at an optimal temperature of 50 °C displayed the lowest surface tension (27.6 mN/m) and a reduced creaming index (less than 5%), indicating a highly stable emulsion system. In conclusion, these findings support the adoption of sustainable industrial practices by replacing synthetic surfactants with natural, biodegradable alternatives derived from *H. undatus* foliage, aligning with SDG 12: Responsible Consumption and Production.

Keywords: *Hylocereus undatus* foliage; oil content; mixing time; mixing temperature

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Generally, an emulsion is a mixture of two immiscible liquids (water and oil), where the water molecules are dispersed within oil molecules as tiny droplets, with a mean particle size of around 1 µm [1]. The three main forms of emulsions are water-in-oil (W/O), oil-in-water (O/W), and more complex emulsions like water-in-oil-in-water (W/O/W) emulsions. In water-in-oil emulsions, water droplets are dispersed in a continuous oil phase, whereas in oil-in-water emulsions, oil droplets are dispersed in a continuous water phase. The water content in emulsions is no more than 74% [2]. These emulsions can be found across various sectors, either in beneficial or undesirable forms. The formation of O/W and W/O emulsions is common in the oil and gas sector, food system, and the pharmaceutical industry [3]. Notably, water-in-oil emulsions are prevalent in the petroleum industry. Emulsions of crude oil and water can occur during drilling, production, transportation, and processing stages. These can occur at various points, including refineries, surface facilities, water and oil networks, hydrocarbon reservoirs, and wellbores [4]. Emulsion in the petroleum industry is primarily caused by turbulent flows, pressure variations in choke valves, and other valves used in crude oil extraction [5].

The formation of stable water-in-oil emulsion is heavily undesirable in the petroleum industry; however, it is often unavoidable. Understanding the emulsification mechanism is necessary in the oil and gas industry, as it affects the production and transportation processes. Emulsions can cause challenges during the refining and transportation of crude oil. Poor control of the emulsification mechanism

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may result in additional costs, particularly due to issues in the pipeline system. Additionally, a previous study has discovered that asphaltenes, resins, and the content of the oil are the most important elements in the emulsion formation [3]. However, emulsions also have beneficial applications in certain industries. Water-in-oil emulsions are widely used in the pharmaceutical industry, particularly in skincare products [6]. For example, cold creams help prevent moisture loss from the skin's surface. So, ensuring the stability of the emulsion is essential for such applications.

Surfactants, also known as surface-active molecules, typically contain significant amounts of nonpolar molecules [7]. They are widely utilised to lower the interfacial tension through adsorption at the oil-water interface, thereby lowering the energy required for the emulsion formation [8]. Incorporating surfactants into a mixture can enhance the stability of emulsions [9], and their effectiveness is determined by their ability to lower the surface tension of the medium [10]. Emulsion stability can be evaluated based on the time required for the emulsion to break, which is correlated with surfactant concentration. The most common technique for breaking emulsions involves the use of a chemical surfactant [11]. However, excessive use of chemical surfactants in the environment, particularly in aquatic systems, can have disastrous impacts on the living organisms [7]. These surfactants contain toxic compounds, which may negatively affect both the environment and the equipment utilised [12]. Consequently, biosurfactants have been introduced as an alternative due to their lower toxicity, biodegradability, and environmentally friendly properties [7].

Biosurfactants have been extensively applied across various industries, including the petroleum industry. They are more adaptable and serve as alternatives to conventional surfactants [13]. Emulsion stability is a key consideration in the environmental application of biosurfactants [14]. Variations in oil phase concentrations at a specific surfactant level result in differences in emulsion droplet size [15].

Hylocereus undatus (*H. undatus*), known as white dragon fruit, belongs to the cactus species *Cactaceae* [16]. Its foliage has been the subject of extensive research due to its high potential for improvement in various applications [17]. Previous research has reported the application of *H. undatus* foliage as a natural coagulant in the treatment of latex concentrate effluent [18], palm oil mill effluent (POME) [19], and paint wastewater [20, 21]. Som et al. [4] previously evaluated the performance stability of water-in-oil emulsions using the *H. undatus* foliage as a biosurfactant, comparing it with a chemical surfactant, methyl-α-D-glucopyranoside (A-MeG). The findings observed comparable stability performance between both surfactants, suggesting that *H. undatus* foliage has strong potential as a superior biosurfactant, particularly for water-in-oil emulsions. Despite prior research demonstrating the potential of *H. undatus* foliage as a biosurfactant, there remains a limited understanding of how key formulation variables affect emulsion stability in systems where it is applied. Therefore, this study addresses this gap by investigating the effects of oil content, mixing time, and mixing temperature on water-in-oil emulsion stability. A combination of analytical techniques was employed to provide a comprehensive evaluation of the emulsion's physicochemical properties, including tensiometry, rheometry, FTIR spectroscopy, creaming index analysis, centrifugation, and freeze-thaw cycle testing.

MATERIALS AND METHODS

In this section, the sample preparation procedures for each experiment, examining the effects of oil content, mixing time, and temperature of the water-in-oil emulsions, are described in the following subsections. The preparations of *H. undatus* foliage and the methods used for stability measurement were the same across all experiments.

Crude oil samples, sourced from identified oilfields and provided by the School of Chemical Engineering, along with deionised water obtained from the laboratory of the School of Chemistry and Environment, Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, were used in the experimental procedures.

H. undatus Foliage Sample Preparation

Thirty-five kilograms of *H. undatus* foliage were taken from a dragon fruit farm in Sepang, Selangor. The foliage was cleaned by removing its thorns and washing with tap water. The foliage was then oven-dried at a temperature of 70 °C for 24 hours [18]. The dried foliage was subsequently cut, milled, and sieved to a particle size of 0.25 mm in diameter using a cutting mill (SM 2000, Retsch, Germany). Finally, the powdered foliage was kept in a sealed plastic bag. A fixed amount of 0.3 g powder was used as the biosurfactant in each water-in-oil emulsion sample. Stability performance at each oil-to-water ratio was evaluated through rheological analysis, creaming index measurement, functional group identification, phase separation behaviour assessment, and dewatering ratio determination. All experiments were performed in triplicate, and the results are reported as average values.

Sample Preparation for the Effect of Oil Content

In this experiment, varying crude oil contents were used (5, 6, 7, and 8 mL). A total of 10 mL of a crude oil-water mixture was prepared with the ratios of crude oil to water of 8:2, 7:3, 6:4, and 5:5, labelled as samples A, B, C, and D, respectively. The emulsions were then mixed with 0.3 g of *H. undatus* foliage powder as the biosurfactant and mixed using a vortex mixture for 10 seconds.

Sample Preparation for the Effect of Mixing Time

Five samples were prepared with a fixed biosurfactant concentration of 0.3 g and the water-to-oil ratio of 1: 9 mL, but mixed for 2, 4, 6, 8, and 10 mins, respectively, and labelled as samples A, B, C, D, and E. Stability performance at each mixing time was evaluated through surface tension measurement, rheological analysis, functional group identification, and creaming index measurement. All experiments were conducted in triplicate, and the results are reported as average values.

Sample Preparation for the Effect of Temperature

Four samples were prepared with a fixed biosurfactant concentration of 0.3 g and a water-to-oil ratio of 1: 9 mL, but at varying temperatures of 30 °C, 40 °C, 50 °C and 60 °C. These were labelled as samples A, B, C and D, respectively. Stability performance at each temperature was analysed in terms of surface tension, rheology, functional groups, and creaming index. All experiments were performed in triplicate, and the results are reported as average values.

Surface Tension Measurement

Tensiometer (K20, KRUSS, Germany) is a device used to measure the surface tension of a liquid. It consists of a tube with a porous bulb at one end and a scale or digital display at the other. When the porous bulb is immersed in a liquid, surface tension generates a pressure difference across the pores of the bulb, which is proportional to the surface tension of the liquid. This pressure difference is measured by the tensiometer and used to determine the surface tension [22]. For measurement, the procedure is as follows: the platinum plate was immersed in acetone and distilled water before washing it with acetone. Then, the platinum plate was heated using a Bunsen burner, allowed to cool before attaching it to the sensitive force-measuring device. Ten mL of the emulsion sample was poured into the vessel, which was then placed on the tensiometer stage. Finally, the stage position was adjusted until the platinum plate made contact with the emulsion surface, and the instrument's software recorded all readings. The surface tension of liquids was then calculated by applying a force to the plate, using the formula in Equation 1.

$$F = 2\,(I \times L)\,\gamma \cos\theta \qquad (1)$$

where: F = Force
I = Thickness
L = Length
γ = Surface tension
θ = Contact angle

Rheological Measurement

The rheological properties of the emulsion system were measured using a rheometer (Physica MCR 300, Anton Paar, Austria). Firstly, the system temperature was adjusted and maintained by the Paar Physica circulating bath and Peltier system, following the method by [23]. Then, the emulsion sample was placed on the stage and its viscosity was measured at different spindle rotational speeds of 50 rpm, 150 rpm, and 250 rpm. The viscosity readings were taken 20 times over a period of 100 seconds. The average values were then calculated and recorded. The shear rate of the emulsion was measured using Equations 2 and 3, as follows:

$$Velocity = \frac{Flowrate}{Area} \qquad (2)$$

$$Sheer\ Rate = \frac{Velocity}{Radius} \qquad (3)$$

Creaming Index Measurement

The emulsion stability was measured using the creaming index measurement. The emulsion sample was mixed with the biosurfactant and then filled into a test tube containing 10 mL of emulsion. The samples were kept at room temperature, and the creaming index readings were recorded on the first day and subsequently every 4 days for a total of 32 days.

The creaming index was calculated to predict the creaming behaviour of the emulsions [24]. The creaming index was evaluated using the method proposed by Firebaugh and Daubert [25], with some modifications. The formula for calculating the creaming index (CI) is shown in Equation 4:

$$CI\% = \frac{Height\ of\ serum\ layer}{Total\ height\ of\ the\ emulsion\ mixture} \times 100\% \qquad (4)$$

Functional Group Identification

Fourier-transform infrared spectroscopy, FTIR (Spectrum One FTIR Spectrometer, PerkinElmer, USA), is a procedure used to identify a sample's chemical composition by analysing the sample's infrared spectrum. This method is based on the principle that different chemical bonds absorb specific wavelengths of infrared radiation [26]. By measuring the absorption of infrared radiation, it is possible to determine the types of chemical bonds present in the sample and identify the functional groups responsible for the absorption [27]. This study conducted Fourier Transform Infrared (FTIR) analysis using a PerkinElmer Spectrum One FTIR spectrometer. A few drops of the emulsion sample were placed at the centre of the aperture plate and covered with a second plate to form a thin film. The assembly was securely fastened before spectral acquisition. The infrared spectra were recorded and subsequently analysed using the Spectrum One software.

Centrifugation Test

A centrifugation test was carried out by placing the water-in-oil emulsion systems in the centrifuge tubes, followed by centrifugation at 3500 rpm for 20 minutes using a Centrifuge 5810 R (Eppendorf, Germany). After centrifugation, the samples were examined for any phase separation. The emulsion stability was then calculated using the formula as presented in Equation 5:

$$Emulsion\ stability\ (\%) = \frac{Hs}{Ht} \times 100\% \qquad (5)$$

where: Hs = Height of the emulsion separation
Ht = Total height of the emulsion

Freeze-thaw Cycle

A freeze-thaw cycle experiment was carried out by subjecting a sample to repeated cycles of freezing and thawing. Each cycle involved storing the emulsion systems at 5 °C for 24 hours in a freezer, followed by keeping them at room temperature for 15 hours. This process was repeated for 3 cycles. This experiment aimed to study the effects of freeze-thaw cycles on the stability and properties of water-in-oil emulsions. The observation was made after each cycle to monitor any changes to the emulsion systems. The dewatering ratio, D_w, of the emulsion system was calculated to determine its stability. The dewatering ratio of an emulsion refers to the efficiency of separating water from the emulsion. The formula for calculating the dewatering ratio is presented in Equation 6 as follows:

$$Dewatering\ ratio, Dw = \frac{v_{inital\ water} - v_{final\ water}}{v_{inital\ water}} \qquad (6)$$

RESULT AND DISCUSSION

Effect of Oil Contents on the Stability of Water-in-Oil Emulsion

Rheological Properties

The rheological properties of an emulsion, particularly its viscosity, play a crucial role in determining its stability. Viscosity affects the ability of droplets to coalesce and the resistance of the emulsion to shear forces. Table 1 shows the average viscosity of the emulsion system for each oil-to-water ratio.

In water-in-crude oil emulsions, viscosity is not only influenced by the volume fraction of the continuous phase (crude oil) but also by the microstructure, droplet interactions, and phase inversion. Within a certain range of oil-to-water ratios, emulsions with lower oil content (i.e., higher dispersed water phase) may exhibit higher viscosity (Table 1) due to the following reasons: a) When the water content is relatively high but still within the W/O regime, a large number of water droplets are dispersed in a smaller volume of oil. This can lead to higher droplet packing density and increased interfacial area between water and oil phases. As these droplets become more closely packed, hydrodynamic interactions and collisions among them increase, resulting in higher resistance to flow and thus, higher viscosity [28, 29]. b) At specific oil-to-water ratios near the emulsion stability limit (just before phase inversion), emulsions may form transient structured networks or pseudo-gels due to the crowding of dispersed droplets. These networks restrict the movement of the droplets and cause a sharp increase in viscosity, even if the continuous oil phase is relatively low in volume [30, 31].

From the results collected, shown in Table 1, Sample C had the highest average viscosity of 4.7635 mPa.s. A high value of viscosity generally contributes to higher emulsion stability. In contrast, Sample A had the lowest viscosity with 0.5305 mPa.s. The emulsion was the least stable due to the weak interaction between particles, which resulted in faster coalescence. The observed emulsion instability was primarily attributed to the weak interactions between stabilising agents at the droplet interface, facilitating droplet coalescence. In emulsion systems, stability is largely determined by the ability of surfactants or stabilising agents to form a robust interfacial film around dispersed droplets. When these interfacial films are weak due to low surface activity, inadequate adsorption, or insufficient electrostatic or steric repulsion, droplets are prone to close contact during Brownian motion or external disturbances, leading to thinning and eventual rupture of the interfacial film [31, 29]. Such weak interfacial interactions fail to provide the necessary mechanical strength to resist droplet deformation or coalescence upon collision. As a result, droplet coalescence occurs more rapidly, increasing average droplet size and accelerating phase separation. This behaviour is in agreement with the previous findings, emphasising that insufficient interfacial stabilisation significantly reduces the energy barrier against coalescence, thereby reducing emulsion longevity [30]. Therefore, the lack of strong particle-particle or surfactant interface interactions in this system directly contributed to the rapid coalescence and reduced stability of the emulsion.

Table 1. Average viscosity of the emulsion system containing *H. undatus* foliage.

Sample Crude Oil: Water	Average Viscosity (mPa.s)
Crude Oil	0.08
A (8:2)	0.5305
B (7:3)	4.7210
C (6:4)	4.7635
D (5:5)	4.2170

Table 2. Percentage of the creaming index of emulsion mixtures containing *H. undatus* foliage over 32 days of observation.

	Creaming Index (%)									
Sample	**Day 1**	**Day 4**	**Day 8**	**Day 12**	**Day 16**	**Day 20**	**Day 24**	**Day 28**	**Day 32**	**Mean ± SD**
A (8:2)	39.4	39.4	39.4	33.3	30.3	27.3	27.3	27.3	27.3	31.83±5.66
B (7:3)	18.2	18.2	18.2	18.2	18.2	15.2	12.1	12.1	12.1	15.46 ±2.96
C (6:4)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.00 ±0
D (5:5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00 ±0

*SD: Standard deviation

Creaming Index

The creaming index measurement plays a crucial role in assessing the tendency of the emulsion to separate over time. Emulsions with a lower creaming index and slower creaming rate generally exhibit better creaming behaviour and high emulsion stability [31]. A lower creaming index indicates that the droplets are settling slowly, resulting in a more stable emulsion. Table 2 shows the creaming index results over 32 days of observation.

From the results presented in Table 2, Sample D exhibited the lowest creaming index percentage (0%). No serum layer was observed in Sample D, which had the oil-to-water ratio of 5:5. Therefore, based on Equation 4, its creaming index percentage was 0.0%, indicating the emulsions are completely stable with no droplet settling or phase separation occurring in the emulsion. In contrast, Sample A recorded the highest creaming index (27.3%), followed by samples B and C with 12.1% and 3%, respectively, after 32 days of observation. Stable W/O emulsions are defined as those that persist without phase separation for five days or more [32].

Functional Group Identification

Fourier-transform infrared (FTIR) spectroscopy is a technique used to identify the chemical bonds present in a molecule by producing an infrared absorption spectrum. Molecules absorb light in the infrared region of the electromagnetic spectrum. Table 3 summarises the FTIR results for each water-in-oil emulsion sample. The water-in-oil emulsion sample containing *H. undatus* foliage powder showed the presence of an amine N-H / O-H functional group, detected at 3384.55 cm^{-1}. On the other hand, the aromatic ring C-H functional group was observed in the range between 780 and 950 cm^{-1}, while the alkenyl C-H bend functional groups were detected in the range from 900 to 945 cm^{-1}. Two other functional groups, aromatic C-H bend and C-O-C stretch, were detected in the frequency ranges from 690 to 850 cm^{-1} and 1000 to 1300 cm^{-1}, respectively.

Centrifugation Test

A centrifugation test was conducted to analyse and characterise the emulsion system based on its stability and phase separation behaviour. This test aims to separate the emulsion into its constituent phases (typically oil and water) and to evaluate the emulsion's stability.

Table 4 presents the emulsion stability percentages of different water-in-oil (W/O) emulsions, evaluated through centrifugation, a widely accepted method to assess physical stability. Centrifugation accelerates the separation of immiscible phases by simulating gravity-driven separation under stress, making it useful for identifying emulsion's resistance to phase breakdown [33, 34].

Table 3. Functional group detected in the emulsion samples containing *H. undatus* foliage.

Functional Group	Frequency Range, cm^{-1}
Amine N-H / OH	3384.55
Alkyl C-H	2923.67
H-bonded carboxylic acid, O-H	2871.91
C=C	1641.66
Aromatic ring C=C	1456.57
Aromatic ring C-H	780-950
Alkenyl C-H bend	900-945
Aromatic C-H bend	690-850
C-O-C stretch	1000-1300

Table 4. Emulsion stability percentage (%).

Sample Crude Oil: Water	Emulsion Stability (%)
A (8:2)	65.88
B (7:3)	82.35
C (6:4)	84.71
D (5:5)	88.24

In this study, Sample D, with an oil-to-water ratio of 5:5, exhibited the highest stability at 88.24%, indicating strong resistance to phase separation. This observation suggests that a balanced ratio between oil and water allows better dispersion of water droplets within the continuous oil phase, enhancing the emulsion's structural integrity. Adequate droplet interaction and compact packing reduced the tendency of droplet coalescence during centrifugation, resulting in better stability [35, 36].

In contrast, Sample A, which had the highest oil content, showed the lowest stability at 65.88%. Excess oil results in fewer dispersed water droplets and lower droplet collision frequency, reducing the effectiveness of the surfactant film and allowing faster separation under centrifugal force [37]. The weaker interfacial film may not sufficiently withstand the shear forces during centrifugation, promoting phase separation.

Samples B and C, with intermediate oil-to-water ratios, showed moderate stabilities of 82.35% and 84.71%, respectively. These findings suggest that emulsion stability is optimised at a balanced oil-to-water ratio, where the interfacial area is sufficient for the biosurfactant to stabilise the droplets effectively [38].

Freeze-Thaw Cycle

A freeze-thaw cycle was conducted by subjecting a sample to repeated freezing and thawing. The results of the cycle were focused on the stability and properties of emulsions, with any changes observed after each cycle. The dewatering ratio, $D_{w,}$ of the water-in-oil emulsion was calculated to determine stability and efficiency of the emulsion to separate water, where a lower dewatering ratio indicates better stability.

As shown in Table 5, Sample D exhibited the lowest dewatering ratio of 0%, indicating no water was separated from the emulsion after undergoing freeze-thaw cycles. This observation suggests that the water droplets remained effectively entrapped within the continuous oil phase, reflecting a high level of emulsion stability.

Freeze-thaw cycles expose emulsions to extreme temperature fluctuations, which can destabilise the system through ice crystal formation, droplet coalescence, and phase separation [39, 40]. When water freezes, it expands and forms ice crystals that may disrupt the droplet interfaces, weakening the emulsion. Upon thawing, if the interfacial film is not strong enough, coalescence occurs, leading to water separation.

However, in the case of Sample D (1:1 oil-to-water ratio), the balanced composition likely resulted in optimal droplet packing and a stronger interfacial film formed by the biosurfactant, preventing droplet rupture and coalescence during freezing and thawing [41, 36]. This structural integrity effectively prevented water migration.

Conversely, emulsions with higher oil content (such as Sample A) tend to have lower dispersed phase volume, resulting in fewer droplet interactions and weaker interfacial strength. These factors make them more vulnerable to freeze-thaw-induced breakdown and water separation [42, 38].

Thus, a balanced oil-to-water ratio, as in Sample D, enhances the freeze-thaw resistance by promoting a more stable microstructure that effectively resists phase separation.

Table 5. Average dewatering ratio of water-in-oil emulsion containing *H. undatus* foliage.

Sample Crude Oil: Water	Average Dewatering Ratio, D_w
A (8:2)	3.90
B (7:3)	3.03
C (6:4)	4.80
D (5:5)	0.00

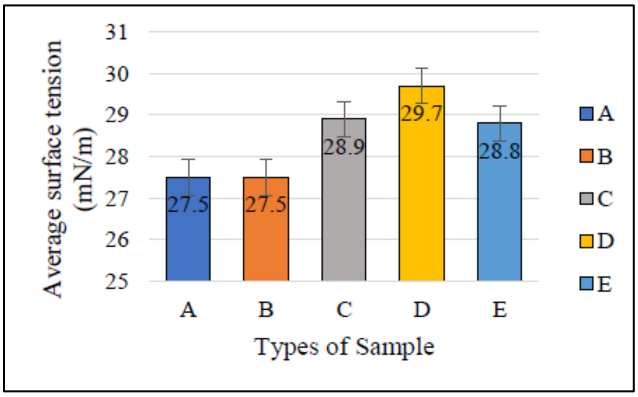

Figure 1. Average surface tension versus mixing time with five different durations.

Effect of Mixing Time on the Stability of Water-in-Oil Emulsion

Surface Tension

The surface tension of an emulsification system is independent of mixing time. Longer mixing times are not recommended, as they can lead to unnecessary energy consumption and unintended consequences, such as emulsion foaming, which in turn diminishes the surfactant's efficiency. It is also important to note that increasing the surfactant concentration can reduce the surface tension. However, the optimal mixing time to generate the most stable emulsion in the system must be investigated. Five samples were prepared using both fixed amounts of biosurfactant extracted from *H. undatus* foliage (0.3 g) and water to an oil ratio of 1:9 mL and then mixed for varying durations (from 2 mins to 10 mins). The measurements were taken five times for each sample to obtain the average surface tension. As previously noted, the most stable emulsion corresponds to the lowest surface tension.

Figure 1 shows that the surface tension measurements for samples A and B were identical at 27.5 mN/m, indicating that 2 and 4 minutes were the best durations for mixing. The surface tension values for samples C, D, and E were higher at 28.9 mN/m, 29.5 mN/m, and 28.8 mN/m, respectively. This does not rule out the possibility that emulsion stability might be improved by a longer mixing period. While sufficient mixing is essential to facilitate droplet breakup and surfactant adsorption at the oil–water interface, excessively long mixing times do not always lead to improved emulsion stability. Once a critical mixing threshold is reached, where surfactant molecules have adequately coated the dispersed phase, further mixing can result in diminishing returns or even negative effects. Prolonged shear may lead to coalescence or flocculation due to mechanical stress disrupting the interfacial film, especially when natural or weak surfactants are used [43]. Additionally, extended mixing can promote foaming, introducing air into the system, destabilising droplet structures, and impairing surfactant performance [44].

Moreover, continued agitation beyond the optimal emulsification point may generate smaller droplets, increasing the total interfacial area, thereby requiring more surfactant than is available. This imbalance leaves some droplet surfaces unprotected, promoting droplet coalescence and phase separation over time [45, 46]. Therefore, optimising mixing time is critical, as over-processing may unintentionally reduce emulsion stability despite initial improvements in dispersion.

A smaller range of droplet sizes and a lower probability of phase separation or droplet coalescence are both outcomes of increased mixing time. Longer mixing also enables more complete and prolonged interaction between the surfactant, water, and oil. Better emulsification can be achieved when smaller water droplets are dispersed throughout the oil phase. Coalescence of emulsion droplets may be avoided if the surfactant forms a high-coverage interfacial coating on the surface of the dispersed droplets [47]. In conclusion, while the surface tension of a W/O emulsion is not directly affected by the mixing time, longer mixing durations enhance the emulsion's stability and uniformity.

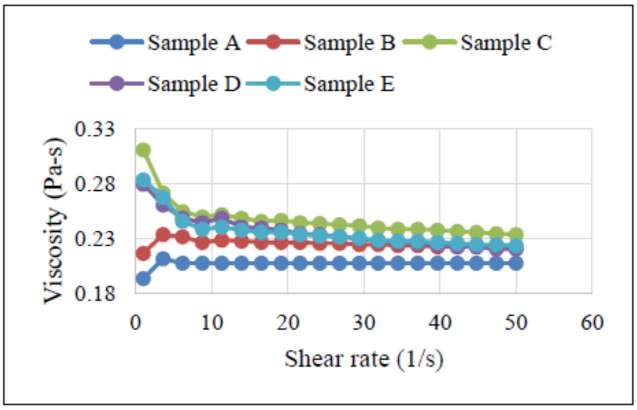

Figure 2. Effect of shear rate on apparent viscosity.

Measurement of Viscosity

The viscosity of an emulsion is one of its most crucial characteristics. Viscosity refers to a fluid's resistance to shear and extensional deformation [48]. Experimental data have shown that viscosity increases as the average droplet size decreases, as smaller and more uniformly distributed droplets increase interfacial area and enhance internal friction, thereby increasing viscosity. During mixing, physical changes occur within the emulsion system, including droplet size reduction, phase rearrangement, and redistribution or reorientation of surfactant molecules at the interface. These structural modifications significantly influence the flow behaviour and viscosity of the emulsion [49, 50]. Although enhanced shear during mixing leads to finer droplet sizes and more stable emulsions, excessive shear may destabilise the system or alter the surfactant film properties, thereby impacting viscosity [41]. Hence, viscosity was measured to examine the relationship between mixing time and viscosity. The influence of apparent viscosity on shear rate is shown in Figure 2. All samples exhibit the same tendencies. As the shear rate increases, the viscosity of the samples decreases, indicating shear-thinning behaviour characteristic of pseudoplastic fluids [51, 52].

Average viscosity tends to rise with mixing time, as shown in Figure 3. Generally, the viscosity of an emulsion tends to decrease with prolonged mixing time. This behaviour is attributed to the progressive breakdown of larger droplets into smaller ones under sustained shear. While smaller droplets increase the total interfacial area, enhancing droplet–droplet interactions, they also promote more efficient packing and reduced internal friction, which can lower the bulk viscosity of the emulsion [53, 54]. Prolonged mixing may also cause surfactant redistribution at the interface, stabilising the smaller droplets but potentially reducing viscosity due to improved fluidity [55]. By 6 minutes (Sample C), the trend reached its peak, yielding the maximum viscosity (Figure 3). Nonetheless, the average viscosity readings for 6, 8, and 10 minutes (samples C, D and E) were 0.2477 Pa.s, 0.2364 Pa.s, and 0.2365 Pa.s, respectively. Therefore, further testing is recommended to determine the optimal mixing duration.

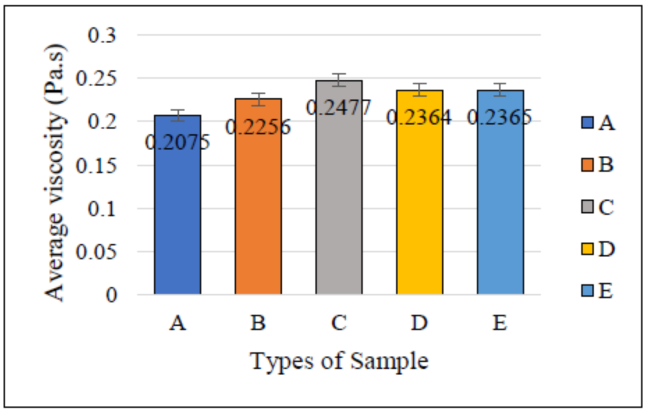

Figure 3. Average viscosity versus mixing time with five different mixing times.

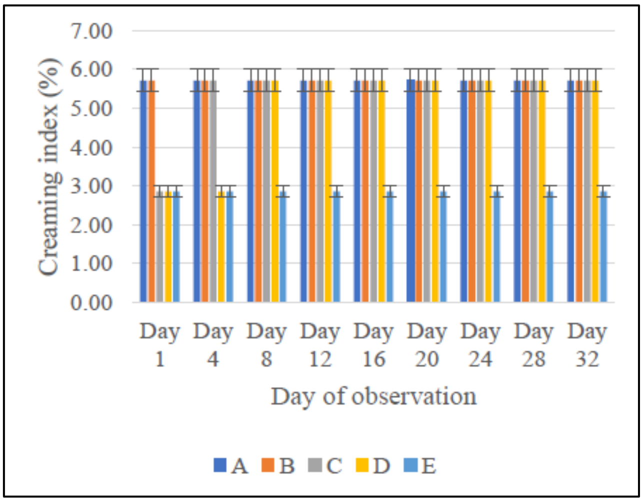

Figure 4. Creaming index (%) of five samples over 32 days.

Measurement of Creaming Index

Emulsions are thermodynamically unstable systems that change gradually over time [56]. They tend to separate into two distinct layers, with oil forming the top layer and water the bottom. Therefore, the creaming index measurement is important to assess the stability and quality of an emulsion. This study prepared five samples with different mixing times and observed them every four days within 32 days to compare the mixing time and emulsion stability. A low creaming index indicates a stable emulsion, with oil droplets remaining dispersed in the water phase without separation. Conversely, a high creaming index reflects poor stability, suggesting that the emulsion is susceptible to creaming, which may eventually undergo irreversible phase separation [57].

Figure 4 presents the creaming index values of five samples for 32 days. Among the samples, sample E, mixed for 10 minutes, exhibited the highest emulsion stability, as indicated by its low creaming index. Sample C likely demonstrated an increase in its creaming index after four days, while Sample D's rose in the subsequent days and weeks. From the observation, the insoluble *H. undatus* foliage powder apparently settled beneath the emulsion layer, and only the top layer was used as part of the study. Compared to the other samples, Sample A exhibited greater layer height. In addition, the overall layer height decreases as mixing time increases, indicating that longer mixing times result in more stable emulsions. Sample E demonstrated outstanding creaming behaviour and greater emulsion stability compared to the others.

Identification of Functional Group

Emulsion stability can be evaluated using FTIR, as most molecules absorb light within the electromagnetic spectrum's infrared region, making FTIR the most useful for identifying chemical bonds. FTIR analysis is applicable to samples in various forms, including liquids, solutions, pastes, powders, and films. When infrared light interacts with a sample, part of it is absorbed while the remainder passes through and is transmitted [58]. The resulting spectrum represents the absorption of molecules. As compared to traditional infrared (IR) spectrometers, FTIR offers a variety of advantages, including higher accuracy and sensitivity, the ability to simultaneously measure frequencies for all functional groups, and faster operation [59]. Table 6 represents the functional groups detected in the emulsion samples, including C-H (alkane/aromatic/methyl and alcohol), C=O (ketones or ether), C=C (alkene/aromatic), C-O (aromatic amines/secondary alcohol/ether), C-N (amine), and C-Cl (halo compound). Previous studies have shown that surfactants interact with water molecules in the interfacial layer of W/O emulsions, weakening the hydrogen bonding network and preventing the coalescence of water droplets. Accordingly, the –OH stretching vibration band serves as a sensor for the molecular interactions that stabilise W/O emulsion [60].

Table 6. Functional groups of emulsion samples with *H. undatus*.

Functional Group	Frequency Range, cm^{-1}
Amine N-H / OH	3374
C-H (alkane)	2925
C-H (methyl group)/ O-H (alcohol)	1375
C-H (aromatic compound)	783
C=O (ketones/esther)	1748
C=C (Alkene)	1646
C=C (aromatic compound)	1458
C-O (aromatic amine)/C-N (aromatic esther)	1295
C-O (secondary alcohol)	1098
C-O (ether)	1026
C-N (amine)	1247
C-Cl (halo compound)	841

Table 7. Comparison of O-H stretching wavenumber.

Sample	Mixing Time (Minutes)	O-H Stretching Wavenumber (cm^{-1})
A	2	3372.88
B	4	3385.68
C	6	3372.27
D	8	3370.85
E	10	3370.62

The O-H stretching wavenumber (in cm^{-1}) for five samples with varied mixing periods is shown in Table 7. The results revealed that the range of O-H stretching wavenumbers was from 3370 to 3385 cm^{-1}, with each sample exhibiting a unique peak intensity. Maximum O-H stretching band wavenumbers for samples mixed for 2, 4 and 6 minutes were 3372. 88, 3385.68, and 3372.27 cm^{-1}, respectively. Higher wavenumber components imply that water is not entirely coordinated, resulting in stronger covalent bonds vibrating at higher frequencies [61]. In such cases, the absence of sufficient surfactant leads to reduced water coordination, making the sample less stable. However, samples mixed for 8 and 10 minutes revealed the lowest O-H stretching wavenumbers, 3370.85 and 3370.62 cm^{-1}, respectively. This observation indicates that the most stable emulsion has the lowest O-H stretching band wavenumber (i.e., sample E). Lower O–H stretching frequencies are generally associated with stronger hydrogen bonding, reflecting enhanced water coordination within the emulsion matrix. These interactions may arise from surfactant-assisted structuring at the oil–water interface, where hydrogen bonding occurs between surfactant headgroups and water molecules [62, 53]. In contrast, higher wavenumbers indicate weaker hydrogen bonding or a greater proportion of "free" water, often associated with insufficient surfactant coverage or interface instability.

Effect of Mixing Temperature on the Stability of Water-in-oil Emulsion

Measurement of Surface Tension

Temperature plays a significant role in modulating the surface tension and stability of emulsification systems. Generally, increasing the temperature reduces the viscosity of both the dispersed and continuous phases, promoting better diffusion and mobility of surfactant molecules toward the oil–water interface, thereby enhancing interfacial coverage and emulsion stability [63]. Elevated temperatures can also reduce coalescence by promoting the rapid formation of stable interfacial films around droplets [64]. However, lowering the temperature can increase interfacial tension, which may negatively affect droplet formation and stability during emulsification [65]. Thus, optimising temperature is crucial for achieving efficient emulsification and long-term stability of the system.

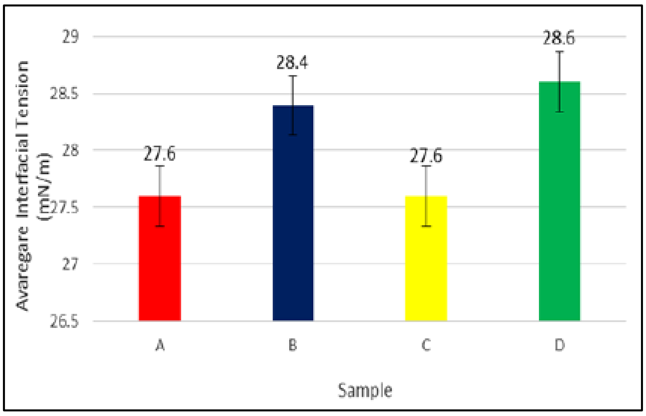

Figure 5. Average surface tension of water-in-oil emulsion that varies with temperature.

The average surface tension of the samples is presented in Figure 5. The average surface tension of samples A, B, C, and D containing *H. undatus* foliage powder was in the range of 27.6 to 28.6 mN/m. Sample D, freshly prepared at a temperature of 60 °C, exhibited the highest surface tension of 28.6 mN/m, indicating the least stable emulsion. Samples A and C both recorded a surface tension of 27.6 mN/m. Although these values are identical, increased temperature significantly increases the thermal energy that can hinder the coalescence and flocculation of emulsion droplets by increasing the repulsive forces between them. Therefore, Sample C is more stable than Sample A.

Measurement of Viscosity

In the context of emulsions, high viscosity refers to the thickness or resistance to the flow of a substance. A highly viscous substance is thick and flows slowly. In emulsions, high viscosity can enhance stability by preventing phase separation or coalescence of the dispersed phase (usually oil droplets) from the continuous phase (usually water). In general, the viscosity of most fluids decreases with increasing temperature, a trend observed in both liquids and gases. As the temperature rises, the kinetic energy of the molecules increases, resulting in enhanced molecular motion and a corresponding reduction in intermolecular attractive forces. This decrease in molecular cohesion facilitates easier molecular displacement, thereby reducing viscosity and allowing the fluid to flow more readily [49, 66]. In emulsions, elevated temperatures can also reduce interfacial tension and modify the structural arrangement of dispersed droplets, further contributing to decreased viscosity [67]. Hence, viscosity was measured to examine the relationship between temperature and viscosity.

As illustrated in Figure 6, average viscosity decreases with rising temperature. This occurs because higher temperatures reduce viscosity. In certain cases, increasing temperature further reduces viscosity, causing shear-thinning fluids to exhibit lower viscosities at higher shear rates. Such high shear rates can cause emulsion droplets to break into smaller sizes. Among the samples, Sample A recorded the highest average viscosity, while Sample D recorded the lowest average viscosity, both were 0.24835 Pa.s and 0.20615 Pa.s, respectively.

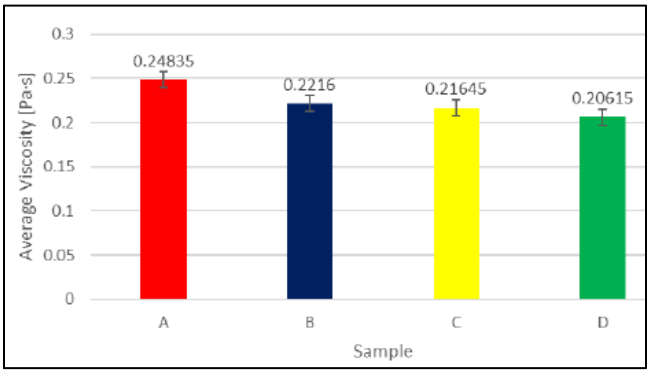

Figure 6. Average viscosity of water-in-oil emulsion that varies with temperature.

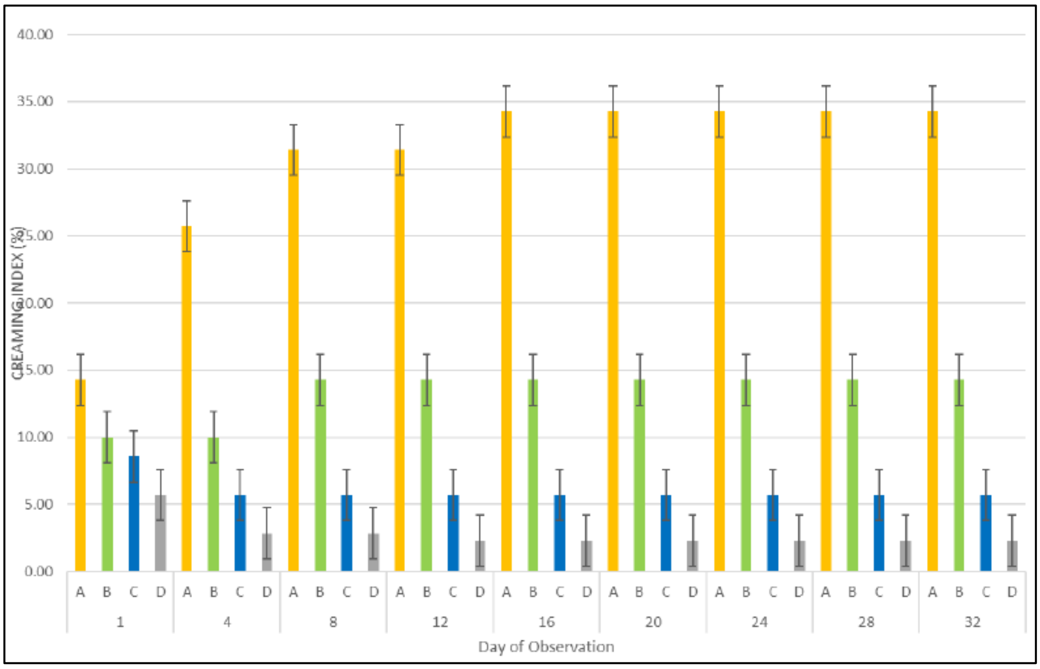

Figure 7. Creaming index (%) of four samples in 32 days.

Measurement of Creaming Index

The creaming index and emulsion stability have an inverse relationship. A lower creaming index indicates increased emulsion stability, whereas a higher creaming index indicates decreased stability. In other words, emulsions with lower creaming indices are considered more stable, since they show less creaming or phase separation over time. The creaming index is typically determined by measuring the height of the creamed layer (the separated layer) in the emulsion after a specified time period. The creamed layer height is then compared to the total height of the emulsion to calculate the creaming index as a percentage.

Four temperature-varying samples were prepared and examined every four days for 32 days to investigate the relationship between temperature and emulsion stability. Figure 7 depicts the creaming index of four samples during this period. The highest emulsion stability was observed in Sample D, maintained at 60 °C, due to its low creaming index. In contrast, Sample A's creaming index apparently rose after four days, followed by a similar rise in Sample B during the subsequent week. Based on the observations, the insoluble *H. undatus* foliage powder settled at the bottom layer, with the top layer used for creaming index measurement. Furthermore, an increase in temperature corresponded with a reduction in overall layer height, indicating enhanced emulsion stability at higher temperatures. Among all samples, Sample D exhibited the most favourable creaming behaviour and emulsion stability compared to the other samples.

FTIR Observation

FTIR spectroscopy can be used to analyse the composition and behaviour of surfactants in emulsions. By examining the FTIR spectra of emulsifier molecules, it is possible to gain insights into their chemical structure, interactions with both the dispersed and continuous phases, and their adsorption behaviour at the oil-water interface. This information can provide a deeper understanding of how surfactants contribute to emulsion stability. Table 8 shows the functional groups detected in the emulsion samples. Based on the table, amine N-H/O-H group can be observed within the frequency range of 3608.85 cm^{-1}, alkyl C-H group (2905.5 cm^{-1}), H-bonded carboxylic acid group (2670.69 cm^{-1}), C=C group (1661.77 cm^{-1}), aromatic ring (675–995 cm^{-1}, 1607.6 cm^{-1}), alkenyl C-H bond (900–940 cm^{-1}), aromatic C-H bond (680–860 cm^{-1}) and C-O-C group (1000–1300 cm^{-1}).

Table 8. Functional groups present in water-in-oil emulsion samples.

Functional Group	Frequency Range, cm^{-1}
Amine N-H / OH	3608.85
Alkyl C-H	2905.5
H-bonded carboxylic acid, O-H	2670.69
C=C	1661.77
Aromatic ring C=C	1607.6
Aromatic ring C-H	675-995
Alkenyl C-H bend	900-940
Aromatic C-H bend	680-860
C-O-C stretch	1000-1300

CONCLUSION

The effects of oil content, mixing time, and temperature of the water-in-oil emulsion system using *H. undatus* foliage as biosurfactants were successfully determined in this study. For the effect of oil content, it can be concluded that Sample D (oil-to-water ratio of 5:5) was the most stable emulsion system due to its highest average viscosity (4.217 mPa.s), the lowest creaming index (0%), the lowest dewatering ratio (0), and high phase separation behaviour (88.24%). For the effect of mixing time, the most stable emulsion was sample E (10 mins), which had the lowest creaming index and a low O-H stretching wavenumber analysed based on FTIR analysis. However, Sample C (6 mins) recorded the highest average viscosity (0.2477 Pa.s), which also indicated good stability. Samples A and B had the lowest surface tension (27.5 mN/m), suggesting high stability as well. It was found that the surface tension was not directly affected by the mixing time, although longer mixing times might increase the emulsion's stability and uniformity. For the effect of mixing temperature, Sample C (50 °C) showed the lowest surface tension at 27.6 mN/m, and was chosen as the most stable emulsion. In contrast, Sample A (30 °C) recorded the highest average viscosity (0.24835 Pa.s). However, Sample D (60°C) had the lowest creaming index and demonstrated superior emulsion stability when compared to other samples. In conclusion, this study highlights the potential of using *H. undatus* foliage as a biosurfactant in the water-in-oil emulsion system.

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