Development of Nanoemulsion Formulation Containing *Pandanus Amaryllifolius* Extract

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DECLARATION

I declare that this thesis entitled "Development of Nanoemulsion Formulation Containing *Pandanus Amaryllifolius* Extract" is the results of my own research except as cited in the references.

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Kajian mengenai formulasi yang stabil dan pencirian nanoemulsi yang mengandungi ekstrak *Pandanus Amaryllifolious*.

ABSTRAK

Penyelidikan ini bertujuan untuk mencipta nanoemulsi stabil yang mengandungi ekstrak *Pandanus Amaryllifolious* dan seterusnya menganalisis ciri-cirinya. Pencirian nanoemulsi melibatkan penilaian penampilannya, tahap pH, kestabilan, dan ujian kesan Tyndall. Langkah-langkah awal termasuk mendapatkan ekstrak *Pandanus Amaryllifolious* melalui proses ekstraksi Soxhlet dan penguapan rotari, dengan menggunakan air suling dan etanol mutlak sebagai pelarut. Pembentukan nanoemulsi berikutnya berdasarkan diafragma terna untuk kestabilan. Komponen seperti minyak soya, Tween 80, dan gliserol digunakan dalam nisbah 1:3:3, dengan pengenalan air semasa homogenisasi. Keputusan menunjukkan bahawa dua daripada empat formulasi tidak stabil, menunjukkan pemisahan. Pengukuran pH menunjukkan keasidan dalam semua formulasi. Kesan Tyndall mengesahkan klasifikasi semua formulasi sebagai nanoemulsi. Aplikasi potensial nanoemulsi dalam pertanian melibatkan formulasi dan penghantaran racun serangga dan baja, dengan memanfaatkan saiz titisan yang lebih kecil untuk liputan dan penyerapan yang lebih baik, mungkin mengurangkan impak alam sekitar agrokimia.

Kata kunci: Nanoemulsi, Pandanus Amaryllifolious, pencirian, tween 80, minyak soya

Study on the stable formulation and characterisation of nanoemulsion containing *Pandanus Amaryllifolious* extraction.

ABSTRACT

This research aims to create a stable nanoemulsion containing *Pandanus Amaryllifolious* extract and subsequently analyze its characteristics. The nanoemulsion characterization involved assessing its appearance, pH levels, stability, and Tyndall effect. Initial steps included obtaining *Pandanus Amaryllifolious* extract through Soxhlet extraction and rotary evaporator processes, utilizing distilled water and absolute ethanol as solvents. The nanoemulsion formulation followed, based on a ternary diaphragm for stability. Components such as soybean oil, Tween 80, and glycerol were used in a ratio of 1:3:3, with water introduced during homogenization. Results revealed that two out of four formulations were unstable, displaying separation. pH measurements indicated acidity in all formulations. The Tyndall effect confirmed the classification of all formulations as nanoemulsions. The potential application of nanoemulsions in agriculture involves formulating and delivering pesticides and fertilizers, leveraging the smaller droplet size for improved coverage and absorption, potentially minimizing the environmental impact of agrochemicals.

Keywords: Nanoemulsion, *Pandanus Amaryllifolious,* characterization, tween 80, soybean oil

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanotechnology was an outstanding technology that provided a wide range of potential in industries, including agriculture, medicines, electronics, and pesticides. It had a vast range of possible applications and advantages. Among these was the development of pesticides based on nanomaterials for the management of insect pests. Traditional approaches to pest management, such as integrated pest management, were insufficient, and the use of chemical pesticides had negative impacts on both people and animals, in addition to reducing soil fertility. Therefore, nanotechnology offered environmentally friendly and effective solutions for the management of insect pests without endangering the environment. This study focused on controlling insect pests and the promise of nanomaterials as innovative uses of nanotechnology.

Nanoemulsion is a colloidal dispersion made of two immiscible liquids, usually oil and water, that have been stabilised by an emulsifying substance or surfactant. It is distinguished by nanometer-sized droplets (usually between 20 and 200 nanometers in diameter), which give the substance a transparent or translucent appearance. In contrast to traditional emulsions, nanoemulsions have special qualities including higher stability, greater bioavailability, and increased solubilization capability. (Aswathanarayan JB and Vittal RR., 2019)

Pandan or also known as *Pandanus amaryllifolius* is a plant widely cultivated in Malaysia. *Pandan amaryllifolius* is an upright green plant with fan-shaped sprays of long, thin, bladelike leaves and woody aerial roots. *Pandan amaryllifolius* has fragrant leaves. The plant is sterile, only sometimes blooms, and is spread via cuttings. Although the plant is uncommon in the wild, it is frequently grown for use as a flavouring in food. In countries where the plant cannot be grown, the leaves are sold in Asian grocery shops in frozen form and may be used fresh or wilted.

Three different forms of alkaloid are among the chemical compounds present in *Pandan amaryllifolius*, and they start biological processes and have specific physiological effects that shield the plant from pest and disease infestation (Ismanto et al.,2020). Due to its cellular toxicity, saponin is regarded as an effective anti-insect agent fly (Ismanto et al.,2020).

1.2 Problem Statement

Nanoemulsion is an essential method for creating systems that are more efficient and have fewer negative effects is the creation of novel formulations based on nanotechnology for the encapsulation of repellents, both natural and synthetic. These sustained-release formulations offer regulated or gradual release of active substances into the environment, extending the duration of action and minimising human exposure to the substance (for instance, by skin penetration). The active ingredient is also shielded by encapsulation against early deterioration brought on by factors such as light, temperature, oxidation, humidity, and others (Campos et al., 2020)

The extraction and bioavailability of certain bioactive components from natural sources can be enhanced through the utilization of specific nanoemulsion formulations and techniques. However, creating a nanoemulsion formulation especially designed for pandan leaf extraction poses a number of difficulties. Making a stable nanoemulsion technology that can efficiently solubilize and distribute the active chemicals from *Pandanus amaryllifolius* while maintaining their bioactivity is a challenge. Therefore, more research is needed to develop optimized nanoemulsion systems and processes that can overcome the difficulties of extracting active compounds from pandan leaves while preserving their bioactivity.

1.3 Objectives

The objective of this study are:

1. To develop a stable formulation of nanoemulsion containing *pandanus amaryllifolius* extraction.
2. To characterize nanoemulsion containing *pandanus amaryllifolius* extraction.

1.4 Scope of Study

The scope of study are include improving the extraction procedure, creating a stable nanoemulsion formulation, characterising the formulation, ensuring its stability and safety, investigating its applicability, and performing comparative analyses.

Investigating the method suitable for extraction, such as solvent type, extraction period, temperature, and solid-to-liquid ratio, affect the number of active substances extracted from *Pandanus amaryllifolius* extraction. To maximise the extraction efficiency, the ideal circumstances must be found.

1.5 Significances of Study

The *Pandanus amaryllifolius* extraction's efficiency as a natural insect repellent can be increased by developing a nanoemulsion formulation containing *Pandanus amaryllifolius* extract as the active ingredient. Nanoemulsions can increase the active compounds' solubility, stability, and bioavailability, which will increase their ability to ward off insects and give more insect protection. The active ingredients of the *Pandanus amaryllifolius* extraction may be better preserved and delivered to the target insects by encapsulating it within a nanoemulsion, boosting the natural repellent's potency.

The use of natural repellents manufactured from *Pandanus amaryllifolius* reduces the need for repellents composed of synthetic chemicals, which have the potential to harm the environment. By developing a strong nanoemulsion formulation, the work advances the usage of environmentally friendly insect repellant alternatives. Those who prefer natural products or who are concerned about the potential harm that chemical repellents may do to their health and the environment may find this to be useful.

CHAPTER 2

LITERATURE REVIEW

2.1 *Pandanus amaryllifolius*

Pandanus amaryllifolius is a tropical plant in the screw pine (Pandanus) genus that is widely available in Malaysia. It is a tall, brilliant green plant with woody aerial roots and a fan-shaped spray of long, thin, spiky leaves. The plant must be grown using vegetative components because it is sterile and does not produce fruit. The *Pandanus amaryllifolius* may be extracted using a variety of techniques, such as carbon dioxide extraction, cold pressing, phytol extraction, and hydrodistillation (Mar A & Phyu P., 2019).

Figure 2.1: *Pandanus amaryllifolius* (Sources: iStock, 2023)

2.1.1 Benefit of *Pandanus amaryllifolius*.

Pandanus amaryllifolius has a distinctive flavour and scent that is frequently characterised as sweet, flowery, and nutty. They are frequently employed as a natural flavouring ingredient in Southeast Asian cuisines, giving foods, sweets, and beverages a distinctive and enticing scent. Due to their nutty and botanical fragrance provided by the key aroma compound (2-acetyl-1pyrroline), they are frequently used to enhance the flavour of dishes (Jimtaisong & Krisdaphong, 2016)

Bioactive substances including flavonoids and phenolic compounds, which have antioxidant characteristics, are found in *Pandanus amaryllifolius*. These antioxidants assist the body in scavenging dangerous free radicals and guard against oxidative stress, which is linked to a number of medical conditions.

According to research, *Pandanus amaryllifolius*'s extract is antibacterial and effective against a variety of bacteria and fungus. They may aid in preventing the development of specific infections and may be used as natural antibacterial agents and food preservers. Pandan leaves and roots contain phytochemicals as bioactive compounds and essential oil that inhibit the growth of some pathogenic bacteria (Lomthong et al, 2022).

2.1.2 *Pandanus amaryllifolius* as insect repellent

Terpenes, one of the natural components in *Pandanus amaryllifolius* leaves, can keep insects away. The scent of *Pandanus amaryllifolius* leaves is thought to deter some insects which include mosquitoes and cockroaches, making it a possible all-natural substitute for insect repellents made of chemicals.

Additionally, *Pandanus amaryllifolius* included polyphenols, which when mixed with saponin, impair an insect's ability to digest food and damage its respiratory system. *Pandanus* *amaryllifolius* also included tannin, which breaks down the bacterial cell wall and precipitates bacterial protein by deactivating the bacteria's generated enzyme. Other elements, such as flavonoids that bind protein and serve as antioxidants and essential oils with an unpleasant scent to flies, also prevent contamination of *Pandanus amaryllifolius* by flies (Ismanto et al.,2020)

Studies have established significant repellent activity of *Pandanus amaryllifolius* against American cockroaches (*Periplaneta americana L.*), but similar effects against other species of cockroaches have not yet been looked into. It is said that taxi drivers in Singapore and Malaysia keep bunches of *Pandanus amaryllifolius* in their taxis to ward off cockroaches. *Pandanus amaryllifolius* has the secondary benefit of adding visual and olfactory pleasure to humans (Biman Bhuyan., 2021).

2.2 Nanotechnology

According to Ragaei, M., & Sabry, A. H. (2014), the Greek term for "dwarf" is where the name "Nano" originated. The word "nano" is more specifically defined as 10^{-9}, or one billionth of anything. A virus, for instance, is about 100 nm in size. Naturally, the term "nanotechnology" developed as a result of the employment of particles with a size range of 1 to 100 nm. The uses and advantages of nanotechnology have great potential. Targeted nanoparticles frequently display out of the ordinary features, such as extraordinary strength, increased chemical reactivity, and high electrical conductivity, according to research by Ragaei, M., & Sabry, A. H. (2014). As a result, nanotechnology has emerged as one of the most promising emerging technologies in the last ten years. Nanoparticles have unique atomic strength-related physical, biological, and chemical capabilities.

2.3 Nanoemulsion

Nanoemulsions are emulsions with a size of the nanometer. These are thermodynamically stable isotropic systems in which an emulsifying agent, such as a surfactant and a co-surfactant, is used to combine two immiscible liquids into a single phase. Nanoemulsion generally has droplet sizes between 20 and 200 nm. The size and form of the particles scattered in the continuous phase is the primary distinction between an emulsion and a nanoemulsion.

	Nanoemulsion
Size	<200 nm
Thermodynamic Stability	Metastable
Kinetic stability	Stable
Optical property	Transparent/slightly translucent
Polydispersity	Low (<10–20%)
Preparation method	High and Low energy methods
Effect of temperature and pH	Stable to temperature and pH changes

Figure 2.2: Nanoemulsion size, thermodynamic stability, kinetic stability, optical stability, polydispersity, preparation method and effect of temperature and pH.

(Sources: Nanoemulsions and Their Potential Applications in Food Industry, 2019)

The droplet sizes of the nanoemulsions range from 200 to, in some cases, 100 nm (Jamuna Bai Aswathanarayan et al., 2019). As phase separation takes place over time, nanoemulsions are thermodynamically metastable. However, nanoemulsions are given kinetic stability since there is no gravitational separation and droplet aggregation due to the lower attractive attraction between the small sized droplets (Jamuna Bai Aswathanarayan et al., 2019). Temperature and pH fluctuations had little effect on the nanoemulsions' thermodynamically stable microemulsions. For their preparation, they require less surfactants. In addition to affecting a nanoemulsion's optical characteristics and stability, droplet size also affects its rheological and release characteristics. As a result, for a variety of applications, nanoemulsions are preferable to microemulsions.

2.3.1 High energy emulsification method

The mechanical apparatus used in high energy procedures includes ultrasonicators, microfluidizers, and high-pressure valve homogenizers. These devices are used to apply highly disruptive force for disrupting dispersed phase into tiny droplets of nanoemulsion (Jamuna Bai Aswathanarayan et al., 2019)

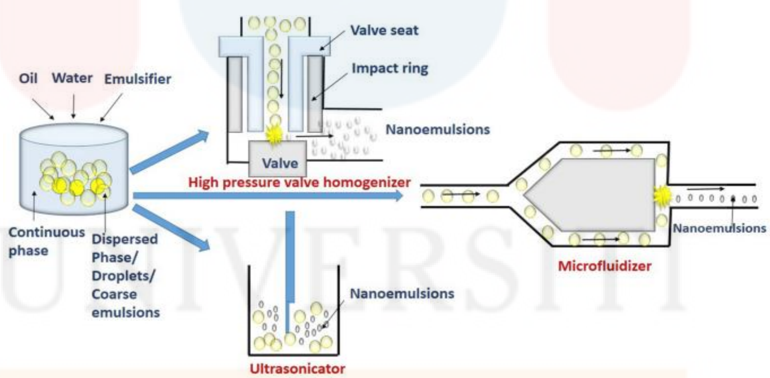

Figure 2.3: High energy method for nanoemulsion such as high-pressure valve, microfluidizer and ultrasonicater (Source: ResearchGate, 2019)

The high-pressure valve homogenization method comprises a pressure valve, a positive displacement pump, and homogenization and interaction chambers. The suction stroke of the pump draws the coarse emulsion into the homogenization chamber. A straightforward orifice plate, a colliding jet, or radial diffuser assembly can serve as the homogenization chamber (Ravishankar Rai Vittal., 2019).

The basic concept behind emulsification by microfluidization is comparable to that of a high-pressure valve homogenizer, with the exception that droplets are created using a unique microchannel with diameters between 50 and 300 μm (José Antonio.,2019).

In this ultrasonication method, coarse droplets are broken down into nanoemulsions by ultrasonic agitation caused by sound waves with a frequency greater than 20 kHz. Acoustic cavitation and mechanical vibration are created by the sonotrode's applied sound waves, and when the cavitations collapse, powerful shock waves are created that shatter the coarse droplets. The droplets collide due to high pressure and turbulence caused by the acoustic and shock waves. Nanoemulsions may also be created at high frequency (mega Hz) without the need of an emulsifier (Vittal et al., 2019).

2.3.2 Low energy emulsification method

The energy input for low-energy procedures comes from the chemical potential of the constituent parts in nanoemulsions. At the boundary between the phases of oil and water, nanoemulsions spontaneously develop after moderate component mixing. Two techniques can be used to regulate spontaneous emulsification. One technique is to adjust the temperature without changing the composition. The second technique is to maintain a steady temperature while varying the compositions and interfacial characteristics. As a result, physicochemical characteristics like temperature, composition, and solubility have a role in the creation of nanoemulsions using low energy techniques (Aswathanarayan & Vittal., 2019). Phase inversion temperature (PIT), phase inversion composition (PIC), and solvent diffusion method are low energy techniques used in the creation of nanoemulsions. These techniques generate less energy, which prevents the breakdown of heat-sensitive substances.

The phase inversion temperature approach alters the emulsifiers' hydrophilicity or lipophilicity in response to temperature at a given composition by taking use of the molecules' phase invasion characteristic. The oil-in-water emulsion forms at low temperatures. In contrast, as the solubility of the emulsifier in water diminishes with rising temperature, a water-in-oil emulsion forms at high temperatures. The temperature at which an emulsion changes from one of oil in water to one of water in oil is known as the phase inversion temperature (Aswathanarayan & Vittal., 2019).

In the phase inversion composition method, the hydrophilic-lipophilic behaviour of the emulsifier is altered by changing the element composition. Food-grade nanoemulsions with a mean particle diameter of 40 nm that are enhanced with vitamin E acetate have been created using the phase inversion composition technique. When using a high surfactant concentration, this approach proved superior to the microfluidization technique at creating nanoemulsions. However, the method was unfavourable for nanoemulsion formulations including label-friendly surfactants, such as Quillaja saponin, whey protein, casein, and sucrose monoesters (Aswathanarayan & Vittal., 2019).

2.4 Characterizations of nanoemulsion

The bulk physicochemical properties of nanoemulsions, such as droplet size, rheology, optical transparency, and stability against droplet aggregation and coalescence, determine whether they are suitable for a certain application (McClements & Jafari, 2018). Instrumental approaches that allow for the assessment of particle size, zeta potential, appearance, and pH measurement were necessary in order to characterise the properties of nanoemulsions.

2.4.1 Appearance

Nanoemulsion appearance can be seen in its transparency, color and the ability to stay unseparated.

2.4.2 Stability

Non-invasive For the stability of nanoemulsion, phase separation, which is the separation of oil and water phase, it leads to the formation of macroscopic droplets or layers should not occur over time in a stable nanoemulsion (Teo Chai Ting., 2020)

2.4.3 pH Measurement

The pH of a nanoemulsion refers to its level of acidity or alkalinity. It is determined by measuring the concentration of hydrogen ions (H+) in the system. The pH scale ranges from 0 to 14, where values below 7 indicate acidity, 7 is neutral, and values above 7 indicate alkalinity. The pH of nanoemulsion must be suitable for human skin contact which is 7 pH (Teo Chai Ting.,2020).

2.4.4 Tyndall effect test

The Tyndall Effect (TE), a phenomenon of light scattering named after the 19th-century physicist John Tyndall, occurs within a colloidal solution comprising particles with dimensions smaller than the wavelength of visible light, ranging from 400 to 760 nm (Wencheng Xiao et al, 2019). Nanoemulsions differ from traditional emulsions primarily in the size of their droplets, which fall within the nanometer range of 20 to 200 nm. Another distinguishing characteristic is their translucent, single-phase appearance, often exhibiting a bluish tint due to the Tyndall optical effect resulting from light dispersion.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The leaves of *Pandanus amaryllifolius* plants were harvested from workshop in University Malaysia Kelantan, Jeli. Samples were rinsed with tap water and dried for 24 hours. After 24 hours the samples were cut into small pieces using kitchen knife and chopping board. The leaves were kept in an aluminum bowl.

Chemicals such as ethanol absolute, soybean oils, tween 80, glycerol were used for this research. All chemicals listed above were obtained from laboratory of University Malaysia Kelantan, Campus Jeli.

3.2 Methods

3.2.1 Extraction of *Pandanus amaryllifolius*

The solvent ethanol absolute was heated to boiling temperature more than 78°C during the Soxhlet extraction procedure for 6 hours. The condenser unit keeps the evaporating ethanol inside the apparatus, but in case it escapes, the equipment needs to be put beneath a fume hood. It is not advised to leave the device alone overnight because it has a heat source and continuous running water. When handling the plant extract, personal protection equipment should be used, especially gloves because it may irritate the skin (Redfern et al., 2014). This process was repeated but the solvent was substituted with distilled water and the temperature was controlled between 78°C to 90°C to avoid the distilled water vaporized completely.

3.2.2 Rotary Evaporator

Rotary evaporator also known as “rotavap” is a machine used to remove any solvent from a sample through vaporization at low pressure. The solvent (in the round bottom flask) boils at a lower temperature than usual because of the apparatus's lowered pressure. The rate of evaporation is accelerated by rotating the flask with the spherical bottom. The solvent vapour enters the colder water condenser, condenses there, and drops into a different receiving flask. The solvent is eliminated using this procedure, leaving a concentrated substance in the original flask with a circular bottom. For absolute ethanol the boiling point were set to 58°C and for distilled water the boiling point were controlled between 60°C to 80°C. The special condition for the distilled water was to avoid the distilled water entering the receiving flask by accident.

3.2.3 Nanoemulsion formulation

Achieving a stable formulation of nanoemulsion involves employing soybean oil as the oil phase, tween 80 as the surfactant, glycerol as the co-surfactant, and distilled water as the aqueous phase. In this study, a 1:1 ratio of tween 80 to glycerol was utilized based on the ternary phase diagram by Ziheng Wang (2009) illustrated in Figure 3.1. Point A from the diagram, situated in the microemulsion phase (ME), was adopted for the nanoemulsion formulation in this investigation, as it exhibits a tendency to produce nanoemulsions. Each vertex of the triangular diagram corresponds to 100% water, oil, and surfactants. For the formulation at point A, 60% of surfactants, 30% distilled water, and 10% oil were employed. The surfactants consisted of a combination of tween 80 and glycerol, while soybean oil served as the oil phase, as determined from the study's findings.

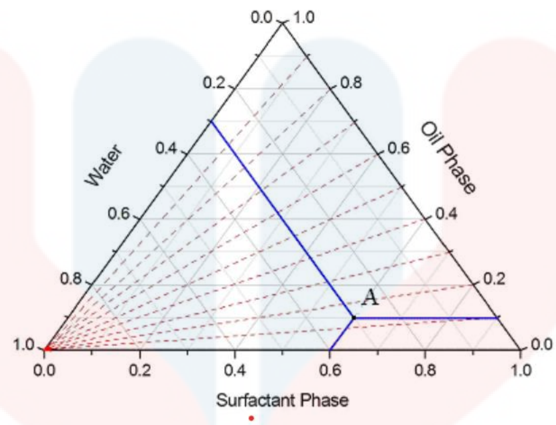

Figure 3.1:Ternary phase diagram.

3.2.4 Nanoemulsion formulation containing *Pandanus amaryllifolius* extraction.

In this research, the enhancement of four nanoemulsion samples was achieved by the incorporation of Pandanus amaryllifolius extraction into the oil phase and aqueous phase. There was different ratio for both extraction with different solvent (ethanol absolute and distilled water). The ratios were 8:2, 7:3 and 6:4. In the formulation of nanoemulsions with solvent extraction, where distilled water is employed in a 7:3 ratio, 0.3g of Pandanus amaryllifolius extraction was meticulously combined with 0.7g of soybean oil. Similarly, in the context of nanoemulsions with solvent extraction containing distilled water at a 6:4 ratio, 1.2g of Pandanus amaryllifolius extraction was harmoniously blended with 1.8g of distilled water. In the formulation involving ethanol as the solvent, two different tests were conducted using an 8:2 ratio. The first test utilized 0.8g of the extractive and 0.2g of soybean oil, while the second test incorporated a ratio of 2.4g of extraction and 0.6g of distilled water. The nanoemulsion formulation followed a methodical process, commencing with the meticulous blending of surfactants, specifically tween 80 and glycerol. Subsequently, soybean oil and Pandanus amaryllifolius extraction were introduced, followed by the addition of distilled water. The entirety of the mixture underwent stirring using a magnetic stirrer, conducted under ambient room temperature conditions.

3.2.5 Characteristics studies on nanoemulsion

3.2.5.1 Appearance

The colour and condition (contaminated) of the nanoemulsion were observed and recorded.

3.2.5.2 Stability

The stability of nanoemulsion was tested by looking for its time for it to become separated between oil and water (Teo Chai Ting.,2020).

3.2.5.3 pH measurements

The pH of nanoemulsion was determined by using a calibrated pH meter. The readings were obtained on average of three times.

3.2.5.4 Tyndall effect

A vial containing the nanoemulsion was utilized for the Tyndall effect test. The procedure involved directing a laser pointer beam through the sample, and the resulting observations were duly recorded.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterisation of nanoemulsion

The outcomes of the experiment have been documented in a series of images, providing a comprehensive record of observations conducted over a two-week period. The recorded results encompass various aspects, including the visual appearance of the nanoemulsion, an assessment of its stability, pH measurements, and the evaluation of the Tyndall effect. These multiple facets of analysis contribute to a thorough understanding of the nanoemulsion's characteristics and behavior over the duration of the study. Table 4.1 below will be used as guidance to better understand the findings.

Table 4.1: Table of nanoemulsion test

Sample	Surfactant (g)	Co-Surfactant (g)	Distilled water (g)	Soybean oil (g)	Extraction (g)
Control	3	3	3	1	0
A	3	3	3	0.7	0.3
F	3	3	1.8	1	1.2
EA	3	3	3	0.8	0.2
EM	3	3	0.6	1	2.4

4.1.1 Appearance

The visual assessment of color and condition, whether indicative of contamination or not, is readily discernible through direct observation with the unaided eye. The discernible coloration serves as a direct indicator of the nanoemulsion nature of the samples. The visual observations detailing the appearance of each sample's nanoemulsion have been systematically documented in Table 4.2.

Table 4.2: Colour and condition of nanoemulsion

Sample	**Colour**		**Contaminated**	
	Week 1	Week 2	Week 1	Week 2
Control	Light blue	Light blue	No	No
A	Cloudy	Cloudy	No	No
F	Cloudy	Cloudy	No	No
EA	Clear	Clear	No	No
EM	Greenish	Light Greenish	No	No

Based on Table 4.2, the color of the control sample (Figure 4.1) was light blue during the 2-week period with no signs of contamination observed. This stable light blue coloration aligns with established principles regarding the optical properties of nanoemulsions related to nanosized particle scattering effects. Specifically, the tiny droplets in the 20-200 nm range interact differently with light compared to larger micrometer-scale droplets, as described extensively in the literature (Maali & Mosavian, 2019; McClements & Xiao, 2020).In contrast, Sample A (Figure 4.2) and Sample F (Figure 4.3) exhibited a cloudy, opaque color over the 2-week period, also with no contamination. This milky or turbid appearance suggests the presence of larger, unstable emulsion droplets beyond the nanometer scale that likely formed through mechanisms like Ostwald ripening or coalescence (Bali et al., 2021; Maali & Mosavian, 2019).

Sample EA demonstrated a clear color during the testing, indicating successful formation of a stable nanoemulsion with sustained nanosized droplets. The visual clarity is desirable for commercial nanoemulsion products across various industries (Montalban et al., 2019). Sample EM (Figure 4.5) exhibited an initial greenish color during week 1, transitioning to a lighter and more transparent appearance by week 2. This shift suggests the sample evolved from a relatively unstable nanoemulsion with larger droplets to a more stable system as the droplets reduced in size over time back into the nanometer range. The kinetics of this apparent stabilization process align with established theories regarding the thermodynamic and kinetic factors influencing nanoemulsion stability (Hasan, 2023).

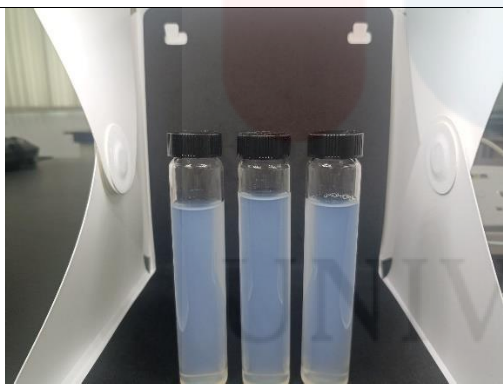	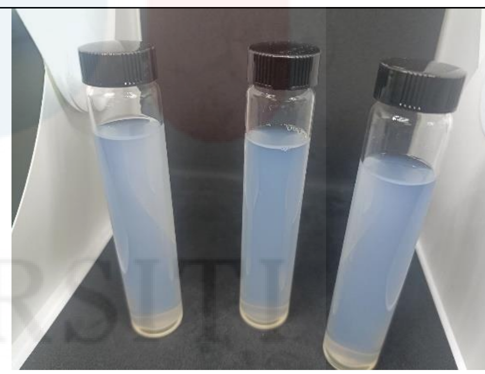
Week 1	Week 2

Figure 4.1: Appearance Sample Control

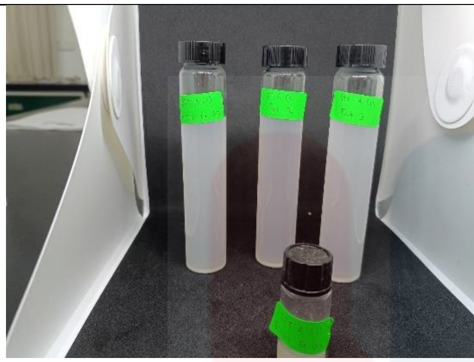	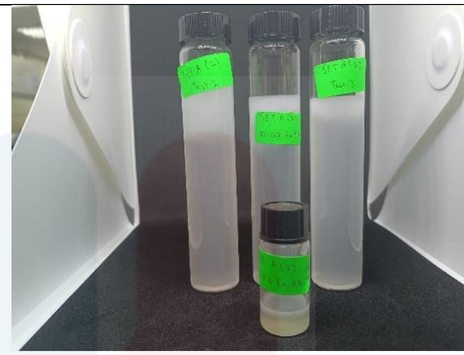
Week 1	Week 2

Figure 4.2: Appearance Sample A

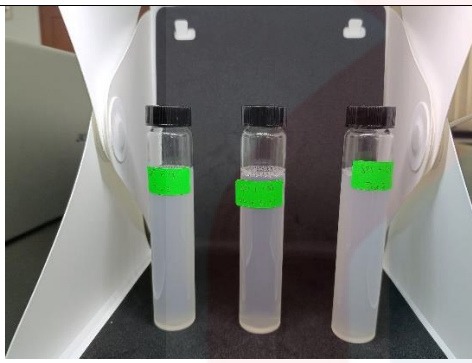	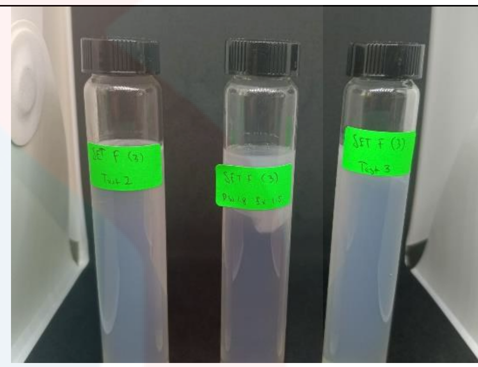
Week 1	Week 2

Figure 4.3 Appearance Sample F

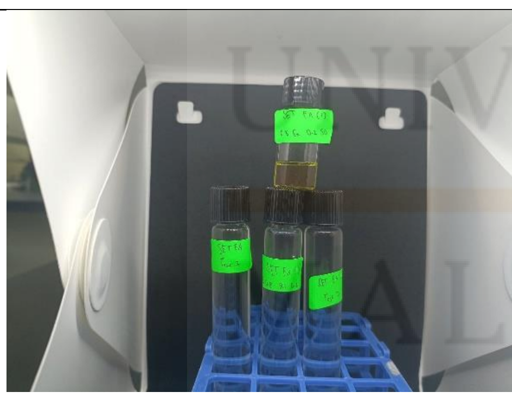	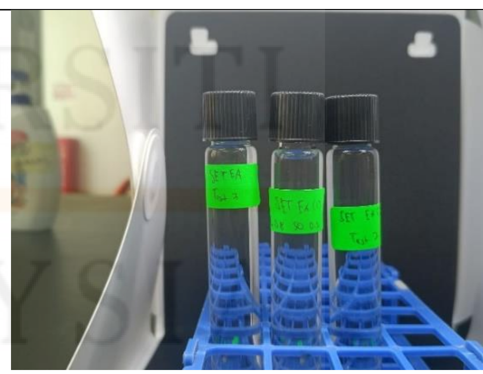
Week 1	Week 2

Figure 4.4: Appearance Sample EA

	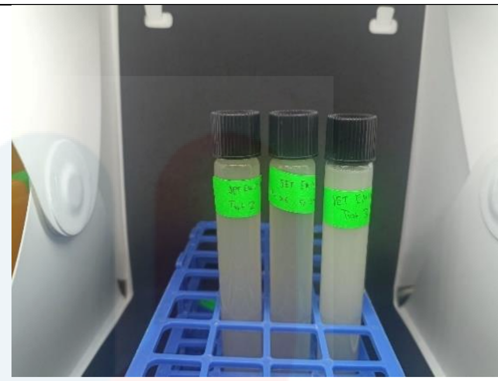
Week 1	Week 2

Figure 4.5: Appearance Sample EM

4.1.2 Stability

Stabilty of nanoemulsion refers to time for it to become separated between oil and water (Teo Chai Ting.,2020). Unstable formulation will lead to separation between the oils and the water in the formulation of nanoemulsion. Table 4.3 below were based on the observation from Figure 4.1 to 4.5.

Table 4.3: Stability of nanoemulsion

SAMPLE	SEPERATED	
	Week 1	Week 2
CONTROL	No	No
A	No	Yes
F	No	Yes
EA	No	No
EM	No	No

In Week 1, no observable separation is noted across all samples, indicating a consistent state in response to the initial experimental conditions. As the experiment progresses into Week 2, a notable shift occurs. Samples A and F exhibit visible separation, suggesting that the applied formulation induced changes in their physical properties, leading to discern. Thus far, the experiment has revealed that the principal instability observed in Oil-in-Water (O/W) nanoemulsions is attributed to the molecular exchange of oil molecules between individual droplets. This phenomenon is recognized as Ostwald ripening, a process identified as the predominant factor influencing the destabilization of the nanoemulsion system (Kaustav Bhattacharjee, 2019). Ostwald ripening influenced by various factors which included surface tension, diffusion coefficient, concentration of the dispersed phase and the nature of the dispersed phase.

4.1.3 pH measurements

All the sample of nanoemulsion formulation pH were recorded in Table 4.4. The average was also calculated to easily understand the characteristics of nanoemulsion.

Table 4.4: pH measurements for sample A, F, EA, and EM

Sample	*pH 1*	*pH 2*	*pH 3*	*Average*
Control	4	4.1	4	4.03
A	4.1	4	4.1	4.07
F	4.3	4.3	4.4	4.33
EA	4.5	4.4	4.4	4.43
EM	4.3	4.4	4.2	4.3

The analysis of various nanoemulsion formulations has consistently indicated an acidic nature, with average pH values falling within the range of 4.03 to 4.43. This acidity level may pose limitations on the suitability of these formulations for products intended for human contact, particularly in the fields of cosmetics and medicine. The importance of achieving a nearly neutral pH for such products is underscored by recommendations in the literature, as exemplified by Teo Chai Ting (2020), who suggests that an optimal pH range of 6.0 to 7.07 is desirable for enhanced compatibility with human skin and physiological conditions. Understanding the impact of pH on the stability, safety, and efficacy of cosmetic and medicinal products is paramount. An acidic pH can potentially lead to skin irritation and compromise the stability of certain active ingredients.

The observed acidity in all nanoemulsion samples can be attributed to multiple factors, primarily stemming from the inherently acidic nature of the chemicals employed in the formulation. Tween 80, soybean oil, and glycerol, constituting integral components of the nanoemulsion, inherently possess acidic properties (Were et al., 2019; Zhang et al., 2016). Furthermore, the extraction process itself introduces acidic compounds, exemplified by citric acid, present in the extracted material (Iglesias et al., 2022). The cumulative impact of these acidic components collectively influences the pH values within the nanoemulsion, establishing an acidic environment across all samples (McClements & Xiao, 2020).

4.1.4 Tyndall effect test

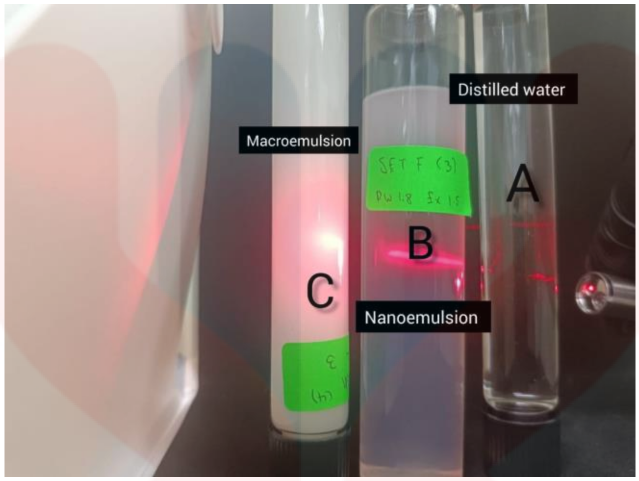

Figure 4.6: Laser beam pointed through three sample for Tyndall effect test.

When vials were illuminated with a laser beam, the light passed straight through the distilled water (A) while the nanoemulsion (B) scattered the beam visibly. The macroemulsion (C) fully obstructed the beam. This shows A is a true solution, B is a nanoemulsion colloid, and C is a hue solution with larger particles. Colloids contain particles from ~1nm to 1μm that scatter light through the Tyndall effect, observed for the nanoemulsion (Xiao et al., 2019). Hue solutions have larger micrometer-sized particles that block light transmission more strongly (Aguirre et al., 2022). Light scattering is a common optical phenomenon in nature, occurring when intense light is compelled to deviate from its straight path, influenced by localized irregularities created by colloidal particles within a gas or liquid medium through which the light traverses (Wencheng Xiao et al, 2019). Several researchers have employed the Tyndall effect as a qualitative tool to assess the nanoscale aggregation or formation of colloidal catalysts. Nevertheless, the complete analytical potential of the Tyndall effect remains largely untapped (Wencheng Xiao et al, 2019).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The visual assessment of nanoemulsion appearance, as depicted in Table 4.2, portrays distinctive features of each sample. The control sample, maintaining a light blue color throughout both observation weeks, signifies a stable formulation with no indications of contamination. Samples A and F, displaying a cloudy appearance, remain uncontaminated, showcasing the resilience of the nanoemulsion under certain conditions. Interestingly, sample EA attains a clear color, embodying the sought-after crystal-clear appearance desired in industries such as food, pharmaceuticals, and cosmetics. In contrast, sample EM undergoes a notable transformation from a greenish hue in Week 1 to a light greenish color in Week 2, hinting at underlying thermodynamic and kinetic phenomena.

Stability, a crucial aspect of nanoemulsions, is scrutinized in Table 4.3. In Week 1, all samples exhibit stability, but by Week 2, samples A and F manifest visible separation. This instability aligns with the Ostwald ripening phenomenon, where the molecular exchange of oil molecules between individual droplets emerges as the principal destabilizing factor in Oil-in-Water nanoemulsions. The observed instability underscores the importance of continuous monitoring and optimization strategies to enhance the stability profile of nanoemulsions.

pH measurements, as detailed in Table 4.4, reveal an overall acidic nature across all nanoemulsion samples. Average pH values ranging from 4.03 to 4.43 prompt considerations for potential limitations in applications involving human contact, particularly in cosmetics and medicine. Teo Chai Ting (2020) advocates for an optimal pH range of 6.0 to 7.07 in nanoemulsion formulations for enhanced compatibility with human skin and physiological conditions. The observed acidity, stemming from the inherently acidic nature of ingredients such as tween 80, soybean oil, glycerol, and the acidic compounds in the extraction process, necessitates a thoughtful approach to formulation adjustments and pH regulation.

The Tyndall effect test, illustrated in Figure 4.6, emerges as a pivotal qualitative tool for discerning the colloidal nature of nanoemulsions. As evidenced by the laser beam observations through the three samples, sample B, representing the nanoemulsion, exhibits visible light scattering, confirming its colloidal characteristics. The Tyndall effect, a result of light scattering by colloidal particles, proves to be an underutilized analytical advantage in nanoscale assessments. Its potential for providing qualitative insights into the aggregation or formation of colloidal systems warrants further exploration and optimization in nanoemulsion analyses. In summary, this thorough investigation into the characteristics of nanoemulsion reveals a dynamic interplay involving visual, chemical, and optical properties. The results not only enhance our comprehension of the present experiment but also set the stage for future research avenues. Areas demanding optimization, particularly the resolution of instability and pH challenges, emerge as focal points for refining nanoemulsion formulations further. The Tyndall effect's potential as a qualitative tool presents a promising avenue to elevate our analytical prowess in the domain of nanoscale colloidal systems. As the journey of nanoemulsions continues, these insights establish a solid groundwork for ongoing exploration, innovation, and practical application across diverse industries seeking formulations that are stable, visually appealing, and biocompatible.

Sample EA exhibited the most desirable characteristics among the tested formulations. It maintained a clear appearance and stability over the 2-week testing period, indicating successful formation and persistence of a nanoemulsion with sustained nanosized droplets. This optical clarity is advantageous for many commercial applications. Additionally, Sample EA had the highest average pH of 4.43 among the samples, making it the least acidic. While still slightly acidic, this higher pH improves its suitability for products intended for human use, compared to the other more acidic formulations. The stability, optical clarity, and higher pH of Sample EA collectively make it the most promising formulation for further development and optimization. Targeted adjustments to the formulation could potentially further enhance its pH to achieve biocompatibility, while retaining its superior visual and colloidal stability traits.

5.2 Recommendations

Given the acidic nature of the nanoemulsion formulations (pH ranging from 4.03 to 4.43), it is crucial to consider adjustments to achieve a more neutral pH range. Teo Chai Ting (2020) suggests an optimal pH range of 6.0 to 7.07 for nanoemulsion formulations to enhance compatibility with human skin and physiological conditions. Incorporating pH-regulating agents or exploring alternative components with neutralizing properties can be explored to align with these recommendations. The comprehensive findings from this study open doors to various research directions. Exploring the impact of different surfactants, co-surfactants, and oil phases on nanoemulsion characteristics, as well as investigating the influence of external factors like temperature and storage conditions, can provide a more comprehensive understanding. Additionally, studying the long-term stability of nanoemulsions and their performance in real-world applications will be valuable for practical implementation.

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APPENDIX A

Figure A: Soxhlet extraction process of the *Pandanus amaryllifolius*

APPENDIX B

Figure B: *Pandanus amaryllifolius* extract with solvent distilled water and ethanol.

APPENDIX C

Figure C: Process of removing solvent from extract using rotary evaporator.