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Research Article

Development and Characterization of Virgin Coconut Oil Nanoemulsions Stabilized with Soy Lecithin and Tween 20

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Abstract

Virgin coconut oil has attracted considerable attention due to its health-promoting properties and its potential applications in biodegradable coatings, natural preservatives, and nutraceutical delivery systems, particularly in nanoemulsion form. This study investigated the droplet size distribution as well as the physicochemical and functional properties of virgin coconut oil nanoemulsions produced by ultrasonication. Nanoemulsions stabilized with Tween 20 exhibited mean droplet sizes ranging from 327.6 ± 0.9 to 367.5 ± 0.4 nm, whereas those stabilized with soy lecithin ranged from 292.4 ± 1.6 to 576.7 ± 3.3 nm. All formulations showed polydispersity index values below 0.3, indicating relatively uniform droplet size distributions. Peroxide and acid values increased during storage, suggesting progressive lipid oxidation and hydrolysis. Fatty acid analysis using Gas Chromatography-Flame Ionization Detector (GC-FID) revealed that lauric acid is the predominant component (7.81 g/100 g fat) in the nanoemulsion system. Stability tests conducted at room temperature demonstrated that soy lecithin provided superior physical stability across most formulation ratios. These findings provide insights into the selection of natural emulsifiers for improving the stability of virgin coconut oil-based nanoemulsions intended for functional food and nutraceutical applications.

Keywords: Dynamic light scattering, Emulsion stability, Gas chromatography, Fatty acid profiles, Nanoparticle size, Ultrasonication

1 Introduction

Vietnam possesses abundant coconut resources, resulting in substantial production of virgin coconut oil (VCO) [1]. VCO is widely used in food preparation, including cooking, frying, baking, and as a substitute for margarine or animal fats [2], [3]. Nutritionally, VCO is considered a functional edible oil rich in phenolic compounds and antioxidants and characterized by a high content of medium-chain triglycerides (MCTs) [4]. In addition to myristic acid (C14:0), VCO contains beneficial MCTs such as caprylic acid (C8:0) and lauric acid (C12:0), with lauric acid accounting for approximately 50% of the total fatty acids and exhibiting antiviral, antifungal, and antibacterial properties [5]. MCTs have also been reported to benefit individuals with malabsorption, impaired glucose metabolism, and lipid disorders and are commonly incorporated into modified infant formulas [6]. In addition, VCO also contains a relatively small proportion of palmitic acid (C16 :0), accounting for approximately 9.5% of the saturated fatty acid fraction [7].

Although palmitic acid has long been associated with adverse health effects, its negative metabolic impacts are strongly influenced by factors such as excessive energy intake, high carbohydrate consumption, and sedentary lifestyle, which may contribute to metabolic disorders and inflammation [8].Furthermore, the antioxidants present in VCO are relatively heat stable, and thermal processing does not markedly compromise its nutritional quality due to the

high degree of saturation and the thermal stability of its phenolic constituents [9].

Despite these advantages, the incorporation of VCO into aqueous food systems remains challenging because of its hydrophobic nature and susceptibility to physicochemical instability. Nanoemulsion technology has emerged as an effective approach for encapsulating, protecting, and delivering lipophilic bioactive compounds, thereby improving their dispersibility, stability, and bioavailability [10-12]. Nanoemulsions generally consist of oil droplets dispersed in an aqueous phase with droplet sizes typically ranging from 20 to 500 nm [11-14]. The small droplet size provides a large interfacial surface area, which enhances physical stability and reduces instability phenomena such as coalescence, creaming, and sedimentation, making nanoemulsions attractive for food and nutraceutical applications [15-17]. In addition, nanoemulsions have been widely used to improve digestibility, encapsulation efficiency, and the delivery of bioactive compounds [18]. For example, incorporation of VCO nanoemulsions into surimi gel has been shown to improve sensory properties and increase the nutritional value by enriching the product with MCTs [19].

The selection of an appropriate emulsifier is a key factor affecting nanoemulsion formation and stability [20]. Food-grade emulsifiers such as lecithin and Tween 20 are widely used because of their high emulsifying efficiency and safety for food applications [21], [22]. Lecithin acts as a multifunctional ingredient in food systems, serving as an emulsifier, wetting agent, viscosity modifier, crystallization controller, and release agent. Soy lecithin is a natural phospholipid-based emulsifier that has demonstrated high stability and encapsulation efficiency in various emulsion systems [23], [24]. The major phospholipid component in soy lecithin, phosphatidylcholine, contains a polar phosphocholine-glycerol head group as well as a non-polar region consisting of two hydrocarbon fatty acid chains [25]. Due to hydrogen bonding between phosphate groups, lecithin is able to self-assemble at the oil/water interface and form a thick viscoelastic interfacial layer, resulting in strong stabilization of nanoemulsion systems [25]. The use of 1% soy lecithin effectively provided favorable stability in terms of viscosity, pH, and cream formation in VCO beverage emulsions, particularly in the formulation containing 20% VCO [26]. Tween 20, a synthetic nonionic surfactant, is also frequently employed due to its strong emulsifying capacity and cost effectiveness. Non-ionic surfactants contribute to the stability of the emulsion system by preventing droplets from interacting too intimately due to short-range repulsive forces such as steric overlap, hydration, and thermal fluctuation interactions [27]. It effectively stabilized oil-in-water nanoemulsions for encapsulating sensitive flavor oils and improved their physical stability for up to 600 days at 5 °C and 25 °C [28]. Previous studies have indicated that emulsifier type may influence lipid digestion kinetics; however, the relationship between emulsifier type and the overall physicochemical stability of VCO nanoemulsions remains insufficiently understood [29-31].

Among high-energy emulsification techniques, high-pressure homogenization and ultrasonication are commonly used to produce nanoemulsions with small droplet sizes and narrow size distributions. Ultrasonic homogenization generates intense cavitation forces through high-frequency sound waves, which disrupt oil droplets and promote the formation of kinetically stable emulsions [32], [33]. The resulting droplet size is influenced by formulation composition, sonication power, and processing conditions, with increased energy input generally producing smaller droplets [34], [35]. The formation of stable nanoemulsions depends on the type of surfactant and the ratio between the sample and surfactant, thereby requiring high energy inputs such as sonication for effective emulsification. In addition, the combination of sonication and high surfactant concentrations contributes to the reduction of nanoemulsion droplet size [36]. Nano-formulations treated with ultrasonication in the presence of Tween surfactants exhibited smaller and more uniform micelle sizes than non-sonicated systems, leading to improved nanoemulsion stability [37]. Soy lecithin combined with ultrasonication was successfully applied to maintain the physicochemical properties and antioxidant activity of a coconut oil-based lycopene-enriched nanoemulsion system and demonstrated potential for application in fat-rich probiotic food products [38].

Despite increasing interest in VCO-based nanoemulsions, limited studies have systematically compared the effects of natural and synthetic emulsifiers on the physicochemical stability and functional properties of VCO nanoemulsion systems prepared by ultrasonication. In particular, the influence of soy lecithin, a natural phospholipid emulsifier, in comparison with Tween 20 on droplet size distribution, oxidative stability, and antioxidant activity of VCO nanoemulsions remains insufficiently understood.

Interest in investigating natural emulsifiers as possible substitutes for synthetic surfactants in food nanoemulsion systems has also increased due to the growing demand for clean-label and naturally derived ingredients. Therefore, the objective of this study was to develop oil-in-water nanoemulsions of virgin coconut oil using ultrasonic homogenization and to investigate the effects of emulsifier types and concentration on droplet size distribution, physicochemical characteristics, oxidative stability, antioxidant activity, and storage stability. The findings of this study are expected to provide valuable insights into the formulation of stable VCO nanoemulsions and to support their potential application as delivery systems for lipophilic bioactive compounds in food and nutraceutical products. To the best of our knowledge, this study provides one of the few systematic comparisons between soy lecithin and Tween 20 in the ultrasonic preparation of VCO nanoemulsions.

2 Materials and Methods

2.1 *Material and chemicals*

Virgin coconut oil was purchased from Vietcoco Company (Vietnam). Soy lecithin (E322) and Tween 20 were used as surfactants. The reagents used in the analyses included 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol (absolute), ethanol (absolute), acetic acid, chloroform, sodium hydroxide (NaOH), phenolphthalein indicator, sodium thiosulfate (0.01 N $Na_2S_2O_3 \cdot 5H_2O$), potassium iodide (saturated solution), starch solution (1%), and deionized water. All chemicals and reagents were of analytical grade.

2.2 *Sample preparation*

2.2.1 Preparation of the aqueous and oil phases

The aqueous phase was prepared by dissolving surfactants, soy lecithin (E322) or Tween 20, in distilled water at concentrations of 0.5%, 1%, 1.5%, and 2% (w/v). The solution was maintained at 65 °C to ensure complete dissolution of the surfactants. The oil phase consisted of VCO, which was heated to 60 °C for 30 min using a hot plate with magnetic stirring (level 3, C-MAG HS7, IKA, Germany) to reduce interfacial tension and facilitate emulsification.

2.2.2 Preparation of VCO nanoemulsions

The oil phase was then gradually added to the aqueous phase at an oil-to-aqueous phase ratio of 20:80 (v/v) while continuously stirring with a magnetic stirrer (level 3, C-MAG HS7, IKA, Germany) to form a coarse emulsion. The resulting emulsion (100 mL) was subsequently subjected to ultrasonication using an ultrasonic processor (Sonics VCX 750, Newton, CT, USA) equipped with a 13 mm titanium alloy (Ti-6Al-4V) probe. Ultrasonication was performed at a frequency of 20 kHz and an amplitude of 60% for 5 min with a pulse cycle of 5 s on and 5 s off, and a maximum power of 750 W. The prepared oil-in-water (O/W) nanoemulsions were stored at 25 °C prior to further analyses. The effects of surfactant type (soy lecithin and Tween 20) and surfactant concentration (0.5-2%) on nanoemulsion formation and stability were subsequently evaluated.

2.3 *Functional properties of VCO nanoemulsion*

2.3.1 Determination of DPPH radical scavenging assay

Antioxidant capacity (AA, %) was determined using the method of Huynh & Nguyen [39] with slight modifications. Briefly, 1 mL of sample was mixed with 3 mL of 0.075 mM DPPH and incubated in the dark for 30 min (t = 30) at room temperature, after which absorbance was measured at 515 nm using a spectrophotometer (Jasco V730, Japan). A mixture of ethanol and DPPH with the same ratio served as the control and was measured immediately after mixing.

$$AA\ (\%) = (1 - (A/A_0)) \times 100 \quad (1)$$

Where A_o and A are the absorbance values of the control and nanoemulsion samples, respectively.

2.3.2 Determination of peroxide value

The peroxide value (PV) of the VCO nanoemulsions was determined using the iodometric titration method according to AOAC [40] with slight modifications. Briefly, 1 g of the sample was dissolved in 30 mL of an acetic acid-chloroform mixture (3:2, v/v) in a 250 mL Erlenmeyer flask. Subsequently, 3 mL of saturated potassium iodide (KI) solution and 30 mL of distilled water were added. The liberated iodine was titrated with 0.01 N sodium thiosulfate using 1% starch solution as an indicator until the dark blue color

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disappeared. A blank sample was analyzed under the same conditions. The peroxide value was calculated and expressed as milliequivalents of active oxygen per kilogram of sample (mEq/kg). Measurements were performed weekly during 30 days of storage.

$$PV = ((Vb - Vs) \times N \times 1000)/W \quad (2)$$

Where Vb and Vs are the volume of blank and sample titrant (mL), respectively; N is the normality of $Na_2S_2O_3.5H_2O$ solution (mEq/mL); 1000 is the conversion of units (g/kg); W is the sample mass (g).

2.3.3 Determination of acid value

The acid value (AV) of the nanoemulsion samples was determined according to AOAC [40] with slight modifications. Briefly, 10 g of the sample was weighed into a 250 mL Erlenmeyer flask and mixed with 50 mL of ethanol previously neutralized using 1 mL phenolphthalein indicator and 0.1 N NaOH. The mixture was heated at 60-65 °C for approximately 10 min and then titrated with 0.1 N NaOH until a stable light pink color appeared. The acid value was expressed as milligrams of potassium hydroxide per gram of sample (mg KOH/g) and calculated using the following equation :

$$AV = (V \times 56.1 \times N)/W \quad (3)$$

where V is the volume of used titrant (mL), W is the sample mass (g), N is the normality of KOH, and 56.1 is the molecular weight of KOH (g/mol).

2.3.4 Determination of fatty acid profile

The fatty acid composition of the VCO nanoemulsion was determined to evaluate potential changes in lipid profile following nanoemulsion formation. Fatty acids were converted to fatty acid methyl esters (FAMEs) prior to analysis and subsequently quantified using gas chromatography coupled with flame ionization detection (GC–FID). Identification of individual fatty acids was performed by comparing the retention times of sample peaks with those of standard FAME mixtures. The results were expressed as the relative composition of fatty acids (g/100 g oil).

Fatty acid profiles were determined following the method of Zeinalzadegan [41] with slight modifications. Approximately 1 mL of lyophilized nanoemulsion was used for analysis. The oil phase was methylated by adding 3 mL of 6% methanolic HCl and heating at 75 °C for 2 h. After extraction with n-hexane and 0.1 M KCl, the mixture was centrifuged at 1000 rpm for 3 min, and the resulting fatty acid methyl esters (FAMEs) were dried over an anhydrous Na_2SO_4 column [42]. FAMEs were analyzed using gas chromatography coupled with a flame ionization detector (GC-FID; HP 6890, Hewlett-Packard, USA) equipped with a Supelco™ SP-2380 capillary column (60 m × 0.25 mm × 0.20 μm; Sigma-Aldrich, USA). For analysis, approximately 15 mg of oil was dissolved in 1 mL of hexane, vortexed for 30 s, and reacted with 1 mL of sodium methoxide (0.4 M). After 15 min, the upper hexane layer containing FAMEs was collected for injection. The injector and detector temperatures were maintained at 250 °C. The oven temperature program was set from 140 °C (5 min) to 240 °C at 4 °C/min and held for 10 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Fatty acids were identified by comparing the retention times with those of standard FAME mixtures.

2.3.5 Determination of nano-droplet size and particle size distribution

The mean droplet size and polydispersity index (PDI) of the nanoemulsions were determined using the dynamic light scattering (DLS) technique [43]. Prior to analysis, the nanoemulsion samples were diluted with distilled water in the sample cell until 15–20% of the incident light was absorbed in order to minimize multiple scattering effects and ensure accurate measurements. Measurements were performed using a particle size analyzer (LB550, Horiba), which detects the intensity of scattered light at a 90° angle. The instrument was set with an equilibration time of 50 s, a refractive index of 1.09 (calculated based on the ratio of the refractive index of coconut oil - 1.45 and that of the medium - 1.33), viscosity setting of 0.8872 cP, and measurement temperature of 25 °C. The Z-average value obtained from the instrument was used to represent the mean droplet size of the nanoemulsions. The droplet size distribution and PDI of the nanoemulsion systems were further analyzed using a nanoparticle size analyzer (SZ-100, Horiba, Japan).

2.3.6 Determination of the stability of nanoemulsion

Following the procedure described by Pengon *et al.,* [44], approximately 20 mL of each nanoemulsion sample was transferred into Falcon tubes (29.6 mm in diameter and 114.7 mm in height) and tightly sealed (n = 3). The samples were stored in a chamber (VS-8111H-350, Vision, Korea) at room temperature (25 °C) and protected from direct sunlight for eight weeks, and visual observations were recorded weekly. During storage, phase separation occurred, resulting in the formation of an opaque upper cream layer and a turbid or transparent lower serum layer. The heights of the layers were measured using a millimeter-scale ruler. The total emulsion height (CT) was determined from the bottom of the sample to the upper surface of the emulsion system, whereas the cream layer height (CC) was measured from the separated cream layer formed at the top of the sample. Nanoemulsion stability was evaluated by monitoring cracking behavior and calculating the creaming index. The creaming index (CI, %) was calculated by the following formula :

$$CI\ (\%) = CC/CT \times 100 \quad (4)$$

2.4 ***Statistical analysis***

All experiments were conducted in triplicate, and results are expressed as mean values ± standard deviations (n = 3). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 20). One-way analysis of variance (ANOVA) was applied to evaluate significant differences among samples for Z-average, polydispersity index, and functional properties. When significant effects were detected, mean comparisons were performed using Fisher's test at a significant level of p-value ≤ 0.05.

3 Results and Discussion

3.1 *Effects of the surfactants on the formation of VCO nanoemulsion*

3.1.1 Effects of Tween 20 concentration on the formation of VCO nanoemulsion

According to Table 1, droplet size measured by dynamic light scattering (DLS) showed that the nanoemulsion containing 1.5% Tween 20 exhibited the smallest droplet size, whereas the formulation with 0.5% Tween 20 produced the largest droplets and differed significantly from the other samples. Surfactant concentrations in the range of 1-1.5% resulted in lower z-average values, suggesting improved emulsification efficiency within this concentration range. Instead of being adsorbed at the interfacial layer, the excess hydrophilic emulsifier (2%) would then disperse in the outer aqueous phase, encouraging the instability of the emulsion alter the crystallization of coconut oil [45]. In addition, since the surfactant at the interface has become saturated, excess surfactants may have surpassed the critical micelle concentration of nonionic surfactants like Tween 20 and tended to form self-micellar aggregates in the continuous phase [46]. This trend may be attributed to the surfactant concentration approaching the optimal hydrophilic-lipophilic balance (HLB) required to stabilize the oil-water interface in the VCO nanoemulsion system.

Table 1: Size characteristics of nanoemulsion made of VCO using Tween 20 as surfactant.

Surfactant ratio	Z-average (nm)	PDI
0.5T	367.500 ± 0.400^{a}	0.135 ± 0.025^{a}
1.0T	335.433 ± 2.177^{b}	0.132 ± 0.030^{a}
1.5T	327.600 ± 0.866^{c}	0.121 ± 0.02^{a}
2.0T	339.900 ± 2.800^{b}	0.122 ± 0.015^{a}

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C) using DLS analysis. 0.5T, 1.0T, 1.5T, and 2.0T represent 0.5%, 1.0%, 1.5%, and 2.0% Tween 20, respectively.

Considering the effect of concentration in more detail, Tween 20 produced a non-monotonic response in droplet size. The Z-average decreased significantly from 367.5 nm at 0.5% to a minimum of 327.6 nm at 1.5% (a reduction of approximately 11%) before increasing again to 339.9 nm at 2%. This behavior indicates that interfacial coverage improved up to 1.5%, beyond which the surfactant requirement for the available interfacial area was satisfied; the excess Tween 20 then remained in the continuous phase, where it can exceed the critical micelle concentration and promote droplet re-coalescence or depletion effects rather than further size reduction [46]. In contrast to droplet size, the PDI was largely concentration-independent, remaining low and statistically comparable across all four concentrations (0.121-0.135), which indicates that Tween 20 produced uniform droplet populations over the entire concentration range studied.

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All nanoemulsions produced exhibited droplet sizes within the typical nanoemulsion range (20-500 nm), which is consistent with nanoemulsions generated using ultrasonication techniques [13]. The polydispersity index values ranged below 0.5, indicating relatively narrow droplet size distributions and good dispersion uniformity [47], [48]. Furthermore, Figure 1 (a-d) showed that the particle size distributions of Tween 20-stabilized nanoemulsions were predominantly unimodal, suggesting homogeneous droplet populations with large interfacial surface areas. These characteristics indicate that 1.5% Tween 20 is an effective concentration for producing stable VCO nanoemulsions using ultrasonication.

The droplet sizes obtained in the present study are markedly smaller than those reported for VCO emulsions prepared by high-shear homogenization. Trivana *et al.,* [49], for example, produced VCO emulsions stabilized with Tween 80 and Span 80 using an Ultra-Turrax homogenizer and obtained droplets in the micrometer range (0.6-2.5 µm) with polydispersity index values above 0.5, indicative of non-uniform and physically less stable systems. The smaller and more uniform droplets achieved here can be attributed to the intense cavitation generated during ultrasonication, which has consistently been shown to produce finer coconut oil nanoemulsions than conventional rotor-stator devices [44]. The high emulsifying efficiency of Tween 20 under ultrasonic processing is also in agreement with previous reports, in which Tween 20 produced low Z-average values owing to its high hydrophilic-lipophilic balance and rapid adsorption at the oil-water interface [21].

3.1.2 Effects of soy lecithin concentration on the formation of VCO nanoemulsion

As shown in Table 2, increasing the concentration of soy lecithin (SL) from 0.5% to 2% resulted in a clear reduction in droplet size (z-average), with the 0.5% formulation exhibiting nearly twice the droplet size of the 2% sample. This trend can be attributed to the greater availability of surfactant molecules at higher concentrations, which rapidly adsorb onto newly formed oil–water interfaces during homogenization and provide sufficient interfacial coverage to stabilize smaller droplets [50]. In addition, higher surfactant concentrations reduce interfacial tension and facilitate droplet disruption during emulsification, leading to a larger interfacial area and smaller droplet sizes [32]. All lecithin-stabilized nanoemulsions exhibited polydispersity index (PDI) values below 0.5, indicating relatively narrow droplet size distributions and acceptable dispersion uniformity [47].

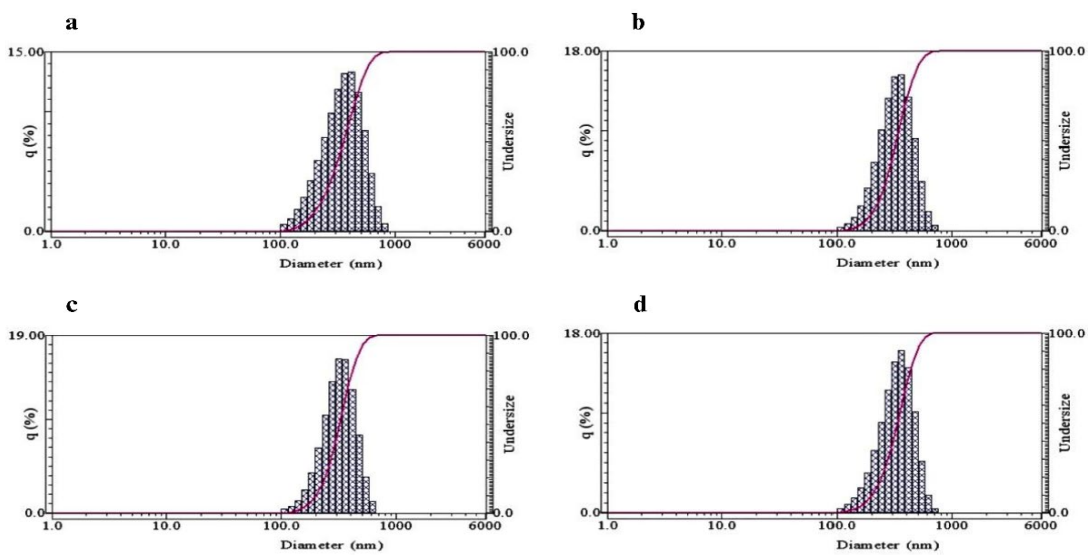

Figure 1: Size distribution of nanoemulsion with 0.5% (a), 1.0% (b), 1.5% (c), and 2.0% (d) of Tween 20 as surfactant.

Table 2: Size characteristics of VCO nanoemulsions using soy lecithin as surfactant.

Surfactant ratio	Z-average (nm)	PDI
0.5SL	576.666 ± 3.308^{a}	0.159 ± 0.016^{a}
1.0SL	359.066 ± 1.33^{ab}	0.098 ± 0.00^{ab}
1.5SL	303.633 ± 0.808^{c}	$0.117 \pm .0114^{ab}$
2.0SL	292.400 ± 1.571^{d}	0.122 ± 0.026^{ab}

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C) using DLS analysis. 0.5SL, 1.0SL, 1.5SL, and 2.0SL represent 0.5%, 1.0%, 1.5%, and 2.0% soy lecithin, respectively.

Unlike Tween 20, soy lecithin exhibited a clear monotonic concentration effect on droplet size. The Z-average decreased progressively and significantly with each increment in concentration, from 576.7 nm at 0.5% to 359.1 nm at 1.0%, 303.6 nm at 1.5%, and 292.4 nm at 2%, corresponding to an overall reduction of approximately 49% across the concentration range. The steepest decrease occurred between 0.5% and 1.0%, indicating that 0.5% lecithin provided insufficient phospholipid to fully cover the newly created interface, leaving droplets prone to recoalescence, whereas concentrations of 1.0% and above ensured rapid and complete interfacial coverage. The PDI of the lecithin systems was also low at all concentrations (0.098-0.159); the minimum value was obtained at 1.0% soy lecithin, and the slightly higher PDI at 0.5% is consistent with the less complete interfacial stabilization at this sub-optimal concentration. Taken together, these results show that 2% soy lecithin was the most effective concentration for minimizing droplet size, whereas droplet uniformity was acceptable across the whole range.

Consistently, the particle size distributions shown in Figure 2 (a-d) were predominantly unimodal, suggesting the formation of homogeneous droplet populations. Among the tested formulations, the nanoemulsion containing 2% SL produced the smallest and most uniform droplets, indicating improved colloidal stability compared with formulations containing lower SL concentrations. Droplet size and interfacial characteristics can also influence the chemical stability of nanoemulsions. Although smaller droplets provide a larger interfacial surface area that may increase lipid exposure to aqueous pro-oxidants, the phospholipid structure of soy lecithin can form a relatively thick interfacial layer that partially protects lipids from oxidative degradation. Consequently, the optimized formulation containing 2% SL not only achieved a favorable droplet size distribution but also provided a stable interfacial structure that supports the overall physicochemical stability of the VCO nanoemulsion system.

The reduction in droplet size with increasing soy lecithin concentration is consistent with the behavior reported for other food-grade emulsifier systems. Qian and McClements [50] and Silva *et al.,* [26] similarly demonstrated that raising the emulsifier-to-oil ratio lowers interfacial tension and provides sufficient surface coverage to stabilize smaller droplets during high-energy emulsification. The droplet size of the optimized lecithin formulation (292.4 nm at 2% soy lecithin) is comparable to, or smaller than, that of related coconut oil-soy lecithin nanoemulsions produced by ultrasonication, such as the lycopene-loaded system reported by Himanath *et al.,* [38], confirming that soy lecithin can serve as an effective natural emulsifier for VCO when combined with adequate ultrasonic energy. The performance of lecithin was nevertheless strongly concentration-dependent, as the sub-optimal level (0.5% soy lecithin) produced the largest droplets among all formulations, in line with reports that insufficient phospholipid coverage limits droplet disruption and promotes coalescence [26].

3.2 *Physicochemical properties of VCO nanoemulsion*

3.2.1 The stability of VCO nanoemulsion

Cracking behavior is an important indicator of nanoemulsion instability, as rapid increases in droplet size typically reflect droplet aggregation or coalescence within the system. As shown in Table 3, the stability of the nanoemulsions was monitored over eight weeks at room temperature (25 ± 2 °C). Based on the creaming index (CI, %), nanoemulsions stabilized with soy lecithin generally exhibited greater stability than those prepared with Tween 20. However, slight cracking was observed in the 0.5% soy lecithin formulation during the final week of storage, suggesting that insufficient surfactant concentration may reduce interfacial protection. In contrast, Tween 20 formulations showed improved stability at higher concentrations (1.5% and 2%), while lower concentrations (0.5% and 1%) exhibited

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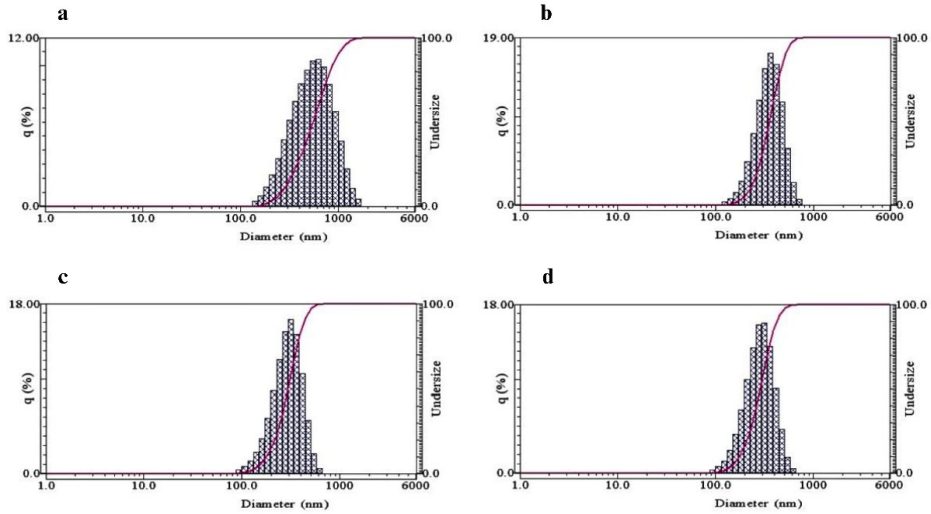

Figure 2: Size distribution of nanoemulsion with 0.5% (a), 1.0% (b), 1.5% (c), and 2.0% (d) of soy lecithin as surfactant.

reduced stability after approximately one month of storage, with CI values increasing over time.

The observed stability behavior can be related to interfacial properties and droplet characteristics within the nanoemulsion. Both surfactants were selected because of their widespread use as food-grade emulsifiers whose hydrophilic–lipophilic balance strongly influences nanoemulsion stability. Tween 20 is a nonionic surfactant known for its low toxicity and ability to produce small droplets using high-energy emulsification methods [51], [52]. In contrast, soy lecithin is a zwitterionic phospholipid, primarily composed of phosphatidylcholine, which can self-assemble at the oil–water interface to form a thicker and more viscoelastic interfacial film [53]. Previous studies have reported that lecithin forms a significantly thicker interfacial layer than Tween surfactants after adsorption at the interface, thereby improving resistance to droplet coalescence and phase separation [25]. This thicker interfacial film may help prevent droplet aggregation and contribute to the improved stability observed in lecithin-stabilized nanoemulsions. In addition, droplet size distribution plays a critical role in physical stability. Nanoemulsions with smaller and more uniform droplets tend to exhibit reduced gravitational separation and slower creaming rates due to lower buoyancy forces. Storage temperature can also

Table 3: VCO nanoemulsion stability during eight-week storage period.

Surfactants	Creaming index value (%)				
(n=3)	Week 1-4	Week 5	Week 6	Week 7	Week 8
0.5T	0	9.44 ± 0.55^{d}	11.33 ± 0.22^{c}	13.88 ± 0.55^{b}	16.66 ± 1.11^{a}
1.0T	0	13.33 ± 2.22^{d}	14.44 ± 1.11^{c}	16.66 ± 1.11^{b}	18.33 ± 0.55^{a}
1.5T	0	0	0	0	0
2.0T	0	0	0	0	0
0.5SL	0	0	0	0	1.92 ± 0.25^{a}
1.0SL	0	0	0	0	0
1.5SL	0	0	0	0	0
2.0SL	0	0	0	0	0

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C). 0.5T, 1.0T, 1.5T, and 2.0T represent 0.5%, 1.0%, 1.5%, and 2.0% Tween 20, respectively. 0.5SL, 1.0SL, 1.5SL, and 2.0SL represent 0.5%, 1.0%, 1.5%, and 2.0% soy lecithin, respectively.

influence stability, as higher temperatures decrease the viscosity of the continuous phase, facilitating droplet migration and accelerating creaming [54]. Creaming occurs when dispersed droplets move relative to the continuous phase, leading to partial phase separation and reduced emulsion stability over time.

A clear concentration threshold for physical stability was evident for both emulsifiers, mirroring the concentration-dependent reduction in droplet size described above. For Tween 20, the two lower concentrations (0.5% and 1.0%) began to cream from week 5 and reached creaming indices of 16.66% and 18.33%, respectively, by week 8, whereas the 1.5% and 2.0% formulations remained free of creaming throughout the eight-week period. A minimum of about 1.5% Tween 20 was therefore required to ensure full physical stability, consistent with the smallest droplets and most complete interfacial coverage being achieved at this concentration. Soy lecithin afforded stability at lower concentrations: the 1.0%, 1.5%, and 2.0% formulations showed no creaming over the entire period, and even the 0.5% formulation remained stable until a slight cream layer (1.92%) appeared only in the final week. This indicates that the threshold concentration for stable VCO nanoemulsions was lower for soy lecithin than for Tween 20, in agreement with the thicker, more cohesive interfacial film formed by the phospholipid and with the smaller droplets obtained at higher emulsifier loads.

These stability trends are in good agreement with previous studies on coconut oil and related nanoemulsions. Pengon *et al.,* [44] reported that the type of surfactant strongly governed the creaming index of coconut oil nanoemulsions, with poorly adsorbing surfactants producing higher creaming indices and faster phase separation. The superior physical stability of the lecithin-stabilized systems observed here supports the findings of Nash and Erk [25], who attributed the greater coalescence resistance of soy lecithin nanoemulsions to the formation of a thicker and more viscoelastic interfacial film than that produced by Tween 20. Similarly, the good long-term stability of the higher-concentration Tween 20 formulations is consistent with de Oliveira Felipe *et al.,* [28], who maintained Tween 20-stabilized oil-in-water nanoemulsions without appreciable phase separation for up to 600 days. The close relationship between physical stability and droplet characteristics also agrees with Trivana *et al.,* [49], who found that the physicochemical stability of VCO emulsions was significantly associated with droplet size, free fatty acid content, and the presence of antioxidants.

3.2.2 Antioxidant capacity of VCO nanoemulsion

The DPPH assay measures antioxidant activity based on the ability of antioxidants to donate hydrogen atoms to the DPPH radical, leading to a reduction in absorbance as the radical is converted from its purple form to a yellow-colored compound. As shown in Table 4, the DPPH radical scavenging activity of all nanoemulsion samples gradually decreased during the four-week storage period, indicating a progressive loss of antioxidant capacity over time. This reduction may be associated with oxidative degradation processes occurring within the nanoemulsion system during storage. Among the formulations, the nanoemulsion containing 0.5% soy lecithin exhibited

Table 4: DPPH scavenging capacity (%) of VCO nanoemulsion during four-week storage period.

Surfactants	DPPH radical scavenging (%)			
(n=3)	Week 1	Week 2	Week 3	Week 4
0.5T	60.263 ± 2.050^{a}	38.419 ± 0.110^{b}	26.161 ± 0.229^{c}	20.750 ± 0.083^{d}
1.0T	51.958 ± 1.890^{a}	36.040 ± 0.063^{b}	32.491 ± 0.110^{c}	21.889 ± 0.127^{d}
1.5T	63.337 ± 0.1900^{a}	34.138 ± 0.110^{b}	32.126 ± 0.063^{c}	24.444 ± 0.127^{d}
2.0T	70.325 ± 1.411^{a}	36.040 ± 0.167^{b}	36.000 ± 0.083^{b}	25.832 ± 0.063^{c}
0.5SL	78.521 ± 0.385^{a}	36.810 ± 0.063^{b}	32.530 ± 0.063^{c}	20.167 ± 0.167^{d}
1.0SL	48.810 ± 4.965^{a}	27.625 ± 0.063^{b}	23.271 ± 0.190^{bc}	21.444 ± 0.210^{d}
1.5SL	56.897 ± 1.100^{a}	32.382 ± 0.110^{b}	28.943 ± 0.541^{c}	24.083 ± 0.166^{d}
2.0SL	60.702 ± 0.720^{a}	37.724 ± 0.167^{b}	29.389 ± 0.210^{c}	23.417 ± 0.167^{d}

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C). 0.5T, 1.0T, 1.5T, and 2.0T represent 0.5%, 1.0%, 1.5%, and 2.0% Tween 20, respectively. 0.5SL, 1.0SL, 1.5SL, and 2.0SL represent 0.5%, 1.0%, 1.5%, and 2.0% soy lecithin, respectively.

the highest DPPH radical inhibition in the early storage period, whereas the formulation with 1% soy lecithin showed the lowest activity.

In general, formulations that maintained better physical stability tended to retain higher antioxidant capacity, suggesting that stable interfacial structures may help protect antioxidant compounds from degradation. By the end of the storage period, the nanoemulsion containing 2% Tween 20 exhibited the highest remaining antioxidant activity (25.83%). The decline in DPPH scavenging activity is consistent with the observed increases in peroxide and acid values during storage, which indicate ongoing lipid oxidation and hydrolysis reactions. As oxidation progresses, antioxidant compounds are gradually consumed in radical-scavenging reactions, leading to a decrease in measurable antioxidant capacity. These findings highlight the important role of nanoemulsion composition and interfacial properties in modulating oxidative stability and antioxidant retention during storage. The influence of emulsifier concentration on antioxidant capacity differed between the two surfactants. For Tween 20, both the initial and the retained DPPH activity rose with concentration. The week-1 activity increased from 60.26% at 0.5% to 70.33% at 2%, and the week-4 activity likewise increased from 20.75% at 0.5% to 25.83% at 2%, so that the 2% formulation retained the highest residual activity of all samples. This trend suggests that the more complete interfacial coverage obtained at higher Tween 20 concentrations slowed oxidative degradation and thereby preserved antioxidant compounds. For soy lecithin, the relationship was less monotonic; the 0.5% formulation showed the highest initial activity (78.52%) but also the steepest decline, falling to 20.17% by week 4, whereas the 1.5% and 2.0% formulations retained comparatively higher activity (24.08% and 23.42%, respectively). The high initial value at 0.5% soy lecithin is attributable to the antioxidant contribution of the phospholipid itself, while its rapid loss reflects the weaker interfacial protection at this sub-optimal concentration. Overall, increasing emulsifier concentration tended to improve the retention of antioxidant capacity during storage, in line with the concentration-dependent improvements observed for droplet size and physical stability.

The gradual loss of DPPH radical-scavenging activity during storage is consistent with the behavior of comparable coconut oil-based systems. Himanath *et al.,* [38] likewise observed that a coconut oil-soy lecithin nanoemulsion delivering lycopene retained but progressively lost antioxidant activity during storage, with the more physically stable formulations better preserving antioxidant capacity. The tendency of the more stable formulations in the present study to retain higher residual activity, therefore, supports the view that a well-organized interfacial layer helps shield antioxidant constituents from oxidative consumption [55].

3.2.3 Acid values and peroxide values of VCO nanoemulsion

Both acid value (AV) and peroxide value (PV) increased during the four-week storage period, indicating progressive hydrolytic and oxidative degradation in the nanoemulsion systems (Table 5 and Table 6). However, the extent of these changes depended strongly on the type and concentration of surfactant used. Nanoemulsions stabilized with Tween 20 generally exhibited lower AV values (<0.3 mg KOH/g) compared with those stabilized with soy lecithin, whereas lecithin-based formulations showed higher AV values (1.992-3.567 mg KOH/g). This difference may be attributed to the composition of soy lecithin, which contains hydrolysable phospholipids and triglycerides that can release free fatty acids during storage, thereby contributing to higher AV values. In addition, increasing AV of different Tween 20 concentrations after four weeks of storage revealed that the overabundance of polysorbate in the continuous phase might lead to the gradual chemical oxidation of its polyoxyethylene into free fatty acids [56].

In contrast, the oxidative stability trends showed a different pattern. Peroxide values increased in all formulations over time, reflecting the occurrence of lipid oxidation during storage. However, nanoemulsions stabilized with soy lecithin generally exhibited lower PV and a slower rate of increase compared with those stabilized with Tween 20. Notably, the formulation containing 2% soy lecithin showed the most gradual PV increase, while the nanoemulsion containing 0.5% Tween 20 exhibited the fastest oxidation rate. The higher oxidative susceptibility of Tween-stabilized systems may be associated with the polyether-based headgroups of Tween surfactants, which can facilitate hydroperoxide formation in the presence of water-soluble pro-oxidants and promote the oxidation of unsaturated fatty acids in VCO [55]. In addition, formulations containing lower emulsifier

concentrations (0.5% Tween 20, 1% Tween 20, and 0.5% soy lecithin) showed more pronounced increases in PV, likely due to insufficient interfacial coverage and greater exposure of lipid droplets to pro-oxidants. Nevertheless, all PV values remained within the acceptable limits for cold-pressed and virgin oils [57]. These results indicate that emulsifier type influences different degradation pathways in nanoemulsion systems. While soy lecithin may promote hydrolytic reactions leading to higher free fatty acid formation, it appears to enhance oxidative stability by forming a thicker interfacial layer that partially protects oil droplets from oxidation.

Emulsifier concentration exerted opposite effects on the two chemical-stability indices, and the direction of these effects was consistent for both surfactants. The acid value increased with emulsifier concentration. After four weeks, it rose from 0.166 to 0.293 mg KOH/g as Tween 20 increased from 0.5% to 2%, and from 1.992 to 3.567 mg KOH/g as soy lecithin increased over the same range. This concentration-dependent rise reflects the greater amount of hydrolysable material present at higher emulsifier loads, namely the polyoxyethylene chains of Tween 20, which can be oxidized to free fatty acids [56], and the hydrolysable phospholipids and associated acyl groups of soy lecithin. In contrast, the peroxide value decreased as emulsifier concentration increased: after four weeks, it declined from 5.481 to 2.639 meq/kg for Tween 20 and from 3.786 to 1.970 meq/kg for soy lecithin as the concentration was raised from 0.5% to 2%. The lowest-concentration formulations (0.5% Tween 20 and 0.5% soy lecithin) consistently exhibited the highest peroxide values, indicating that insufficient interfacial coverage left a larger fraction of the lipid exposed to aqueous pro-

Table 5: Acid values of VCO nanoemulsion during four-week storage period.

Surfactants	Acid Value mg KOH/g			
	Week 1	Week 2	Week 3	Week 4
0.5T	0.079 ± 0.004^{d}	0.113 ± 0.001^{c}	0.147 ± 0.006^{b}	0.166 ± 0.000^{a}
1.0T	0.083 ± 0.000^{d}	0.124 ± 0.004^{c}	0.167 ± 0.000^{b}	0.196 ± 0.000^{a}
1.5T	0.111 ± 0.000^{d}	0.139 ± 0.000^{c}	0.167 ± 0.000^{b}	0.208 ± 0.012^{a}
2.0T	0.122 ± 0.002^{d}	0.166 ± 0.001^{c}	0.266 ± 0.014^{b}	0.293 ± 0.013^{a}
0.5SL	0.238 ± 0.014^{d}	0.517 ± 0.013^{c}	1.483 ± 0.006^{b}	1.992 ± 0.122^{a}
1.0SL	0.245 ± 0.022^{d}	0.658 ± 0.013^{c}	1.534 ± 0.017^{b}	2.273 ± 0.055^{a}
1.5SL	0.573 ± 0.014^{d}	0.881 ± 0.013^{c}	1.679 ± 0.000^{b}	3.119 ± 0.046^{a}
2.0SL	0.616 ± 0.000^{d}	0.941 ± 0.011^{c}	1.755 ± 0.035^{b}	3.567 ± 0.098^{a}

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C). 0.5T, 1.0T, 1.5T, and 2.0T represent 0.5%, 1.0%, 1.5%, and 2.0% Tween 20, respectively. 0.5SL, 1.0SL, 1.5SL, and 2.0SL represent 0.5%, 1.0%, 1.5%, and 2.0% soy lecithin, respectively.

Table 6: Peroxide values of VCO nanoemulsion over four-week period.

Surfactants	Peroxide Value (meq peroxides/kg oil)			
	Week 1	Week 2	Week 3	Week 4
0.5T	0.937 ± 0.007^{d}	1.464 ± 0.035^{c}	2.409 ± 0.063^{b}	5.481 ± 0.027^{a}
1.0T	0.467 ± 0.007^{d}	0.945 ± 0.045^{c}	1.458 ± 0.026^{b}	3.062 ± 0.063^{a}
1.5T	0.475 ± 0.025^{d}	2.135 ± 0.135^{c}	2.392 ± 0.068^{b}	2.955 ± 0.014^{a}
2.0T	0.970 ± 0.010^{d}	1.441 ± 0.009^{c}	1.840 ± 0.140^{b}	2.639 ± 0.139^{a}
0.5SL	0.493 ± 0.003^{d}	0.990 ± 0.010^{c}	1.988 ± 0.203^{b}	3.786 ± 0.013^{a}
1.0SL	0.485 ± 0.005^{d}	0.503 ± 0.003^{c}	1.914 ± 0.009^{b}	2.690 ± 0.270^{a}
1.5SL	1.290 ± 0.014^{c}	1.774 ± 0.094^{b}	0.934 ± 0.009^{d}	2.392 ± 0.082^{a}
2.0SL	0.943 ± 0.001^{d}	0.979 ± 0.001^{c}	1.478 ± 0.008^{b}	1.970 ± 0.050^{a}

Each value represents means ± standard deviations (n = 3). Data with the same superscript letter within a column are not significantly different (p-value < 0.05) according to Fisher's test. Data were measured at room temperature (25°C). 0.5T, 1.0T, 1.5T, and 2.0T represent 0.5%, 1.0%, 1.5%, and 2.0% Tween 20, respectively. 0.5SL, 1.0SL, 1.5SL, and 2.0SL represent 0.5%, 1.0%, 1.5%, and 2.0% soy lecithin, respectively.

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oxidants, whereas higher concentrations formed a more complete and protective interfacial layer that limited oxidation [55]. Thus, raising the emulsifier concentration enhanced oxidative protection (lower PV) but simultaneously favored hydrolytic free-fatty-acid release (higher AV), revealing a concentration-dependent trade-off between the two degradation pathways.

Overall, the present study demonstrates that the physicochemical and oxidative stability of VCO nanoemulsions is strongly governed by surfactant type and concentration. Increasing emulsifier concentration generally improved physical stability by reducing droplet size and producing more uniform nanoemulsion systems. However, the two surfactants exhibited distinct influences on chemical stability. Tween 20 formulations showed lower acid values, indicating reduced hydrolytic degradation, whereas soy lecithin systems displayed relatively higher AV due to the presence of hydrolysable phospholipids.

Conversely, lecithin-stabilized nanoemulsions exhibited improved resistance to lipid oxidation, likely due to the formation of a thicker phospholipid interfacial layer that limited oxygen and pro-oxidant interactions at the oil-water interface. These findings highlight the critical role of interfacial composition in determining nanoemulsion stability and suggest that lecithin-based systems may offer advantages for developing stable VCO nanoemulsions for functional food and bioactive delivery applications.

3.3 *Fatty acids profile of VCO nanoemulsion*

Due to its superior stability and the smallest droplet size, the fatty acid (FA) composition (g/100 g) of the VCO nanoemulsion formulated with 2% soy lecithin was analyzed (Table 7). The major fatty acids identified in the nanoemulsion were lauric acid (7.81 g), caprylic acid (1.29 g), myristic acid (2.97 g), palmitic acid (1.58 g), and oleic acid (0.94 g). Determining the FA profile after nanoemulsion formation is important for quality evaluation and formulation optimization. The fatty acid composition of VCO plays a key role in influencing the physicochemical stability and functional properties of nanoemulsions, as interactions between fatty acids and other formulation components can affect emulsion stability and performance [58]. Furthermore, characterization of the FA profile provides essential information for nutritional labeling and supports the potential application of VCO nanoemulsions in food systems [59].

Table 7: Major fatty acids (g/100g) of VCO nanoemulsion produced by soy lecithin 2% as surfactant.

No.	Fatty acids	Results (g/100g)
1	C6:0 (Caproic acid)	0.08
2	C8:0 (Caprylic acid)	1.29
3	C10:0 (Capric acid)	1.03
4	C12:0 (Lauric acid)	7.81
5	C13:0 (Tridecanoic acid)	0.01
6	C14:0 (Myristic acid)	2.97
7	C16:0 (Palmitic acid)	1.58
8	C18:0 (Stearic acid)	0.56
9	C18:1n9C (Oleic acid)	0.94
10	C18:2n7C (Linoleic acid)	0.66
11	C18:3n3 (α-Linolenic acid)	0.05
12	C20:0 (Arachidic acid)	0.02
13	C20:1 (Eicosenoic acid)	0.01
14	C22:0 (Behenic acid)	0.01
15	C24:0 (Lignoceric acid)	0.01

The predominance of lauric acid, together with appreciable proportions of caprylic, capric, and myristic acids, confirms that the characteristic medium-chain fatty acid profile of VCO was retained after ultrasonic nanoemulsification. This finding agrees with previous reports indicating that emulsification and nanoemulsion formation do not substantially alter the fatty acid composition of the encapsulated oil [59] and that the medium-chain triglyceride fraction, particularly lauric acid, which typically accounts for approximately half of the total fatty acids in VCO, remains the dominant component [5]. Retention of this profile is important, as it underpins the antimicrobial and nutritional functionality for which VCO nanoemulsions are intended.

4 Conclusions

This study demonstrated the successful fabrication of virgin coconut oil (VCO) nanoemulsions using ultrasonic homogenization and food-grade emulsifiers. The optimized formulations (2% soy lecithin and 1.5% Tween 20) produced nanoemulsions with small droplet sizes and low polydispersity indices (PDI < 0.3), indicating uniform dispersion and good colloidal stability. Among the tested emulsifiers, soy lecithin provided superior

stability during storage while maintaining acceptable oxidative quality and antioxidant capacity. Fatty acid analysis confirmed retention of the characteristic medium-chain fatty acid profile of VCO following nanoemulsion formation. These findings highlight the potential of soy lecithin–stabilized VCO nanoemulsions as effective carriers for lipophilic bioactives in functional food, nutraceutical, and related delivery applications.

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Author Contributions

Amrutha D S: Conceptualization, data curation, Conceptualization : Ha Vu Hong Nguyen ; Performed the experiments : Nguyen Van Anh Doan and Ngoc Duy Nguyen ; Formal Analysis : Nguyen Van Anh Doan ; Writing-Original Draft Preparation : Nguyen Van Anh Doan and Quynh Thi Nhu Tran ; Supervision : Ha Vu Hong Nguyen ; Writing-Review & Editing : Ha Vu Hong Nguyen

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors have not utilized AI tools for preparing the article.

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