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FABRICATION AND CHARACTERIZATION OF WATER-IN-PALM OIL NANOEMULSION AS A CARRIER FOR CATECHIN

(Fabrikasi dan Sifat Nanoemulsi Air dalam Minyak Kelapa Sawit sebagai Pembawa Katekin)

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Abstract

Catechins are known as potent antioxidants with numerous health benefits, including antimicrobial, antiviral, anti-inflammatory, anti-allergenic, and anti-cancer effects. However, there are some challenges to delivering catechins due to their low bioavailability, poor gastrointestinal absorption, and low stability. To overcome the limitations of catechin delivery, nanoemulsion technology with droplet sizes ranging from 10 to 1000 nm was used. In this study, water-in-oil (W/O) nanoemulsion was effectively prepared by employing palm oil (PO) as the oil phase, Span 80 as a lipophilic emulsifier, and Tween 80 as a hydrophilic emulsifier with high-energy approaches (high shear homogenizer). The optimum processing conditions for preparing water-in-palm oil nanoemulsion are as follows: the ratio of the oil phase to the water phase is 60:40, and the total concentrations of emulsifier mixtures are 11 w/w% with a hydrophilic-lipophilic balance (HLB) value of 7.1 using 15,500 rpm for 5 minutes of the high shear homogenizer. The best nanoemulsion showed an average of 185.6 nm particle size and a zeta potential of -38.4 mV. The optimized nanoemulsion containing catechin with a pH value of 6.82, showed a low conductivity value of this nanoemulsion (0.001mS/cm) which means that the continuous phase is oil. The nanoemulsion was stable at centrifugation of 4000rpm for 30 minutes and alongside 28 days of storage tests at 10˚C. Hence, this study successfully developed a stable water-in-palm oil nanoemulsion containing catechins (WPOC-NEs).

Keywords: catechin, nanoemulsion, emulsifiers, formulation, stability

Abstrak

Katekin dikenali sebagai antioksidan kuat yang menawarkan banyak manfaat kesihatan, termasuk kesan antimikrob, antivirus, anti-radang, anti-alergik, dan anti-barah. Walau bagaimanapun, terdapat beberapa cabaran dalam membuat penghantaran katekin disebabkan bioketersediaan mereka yang rendah, penyerapan gastrousus dan kestabilan yang lemah. Untuk mengatasi had penghantaran katekin, teknologi nanoemulsi dengan saiz zarah daripada 10 hingga 1000nm telah digunakan. Dalam kajian ini, nanoemulsi air-dalam-miyak telah disediakan dengan menggunakan minyak kelapa sawit sebagai fasa minyak, Span 80 sebagai pengemulsi lipofilik, dan Tween 80 sebagai pengemulsi hidrofilik dengan pendekatan tenaga tinggi (penghomogenatan ricih tinggi). Keadaan pemprosesan optimum bagi penyediaan nanoemulsi air-dalam-minyak mengandungi katekin adalah seperti berikut: nisbah fasa minyak kepada fasa air adalah 60:40, dan jumlah kepekatan campuran pengemulsi adalah 11% dengan nilai keseimbangan hidrofilik-lipofilik 7.1 menggunakan penghomogenatan ricih tinggi. Nanoemulsi mengandungi katekin yang terbaik menunjukkan saiz zarah 185.6 nm, dan potensi zeta -38.4mV. Tambahan lagi, nanoemulsi dengan nilai pH 6.82 ini, menunjukkan nilai konduktiviti yang rendah iaitu 0.001mS/cm, yang bermaksud fasa berterusannya adalah minyak. Nanoemulsi ini juga stabil selepas diemparkan pada 4000rpm selama 30 minit dan juga sepanjang ujian simpanan selama 28 hari di suhu 10°C. Oleh itu, kajian ini telah berjaya membangunkan nanoemulsi air-dalam-minyak dengan mengandungi katekin yang stabil.

Kata kunci: katekin, nanoemulsi, pengemulsi, formulasi, kestabilan

Introduction

Catechin is a polyphenol compound found in various fruits, vegetables, and plant-based beverages. Catechins have sparked much interest because of their antiviral, anticancer, antioxidant, antimicrobial, anti-inflammatory, and possible medicinal applications in various viral infections [1]. Catechins have been studied extensively in terms of their biological functions. However, the bioavailability of catechins in vivo has remained limited due to their volatility. As a result of the creation of microemulsions (MEs) or nanoemulsions (NEs) for encapsulation of bioactive chemicals like catechin, it is feasible to improve stability, hence increasing catechin bioavailability and biological activity [2]. Bhushani et al. [3] reported that nanoemulsified catechin can increase their bioaccessibility compared to non-emulsified catechin.

A nanoemulsion is a kinetically stable system composed of two immiscible liquids (oil and water) that are stabilized by surfactants with droplet sizes ranging from 10 to 1000 nm [4]. The advantages of nanoemulsions (NEs) are numerous. Due to a higher surface area, they are suitable for drug administration. This could boost drug absorption through a variety of pathways [5]. A wide surface area also has an impact on the drug's transport characteristics, which is a critical component in ensuring long-term and targeted drug delivery. Drugs are protected from environmental changes, such as pH shifts and enzymatic breakdown, by being encapsulated within the nanoemulsion core [6].

Nanoemulsions consist of two types known as oil-in-water (O/W), where the continuous phase is water, and water-in-oil (W/O), where oil is the continuous phase. W/O nanoemulsion has great potential for pharmaceutical, cosmetic, agricultural, and food applications. This type of nanoemulsion can encapsulate the hydrophilic drugs in the inner water phase by improving the effect of sustained release, maintaining the retention time of the drug in the body, and lowering the metabolic rate [7]. This research applied high-energy emulsification approaches such as high-shear homogenizers to obtain nanodroplet emulsions. The emulsion will be forced into the narrow space between the rotor and stator, causing the droplets to break down and form smaller particles [8].

Overall, this research study aimed to encapsulate the catechin within the water in palm oil nanoemulsion to improve their bioavailability after oral administration or for the fabrication of topical application. Therefore, to form a stable W/O nanoemulsion, the choice of oil and surfactants is essential. Palm oil was chosen in this study since it can help to overcome the issue of low bioavailability, high toxicity, and poor solubility of several drugs, as well as improved drug permeability and shelf life [9]. Non-ionic surfactants were used as they can form a stable nanoemulsion and have a low toxicity potential that helps to reduce irritation [10]. The optimal hydrophilic-lipophilic balance (HLB) surfactant mixtures were first determined to obtain stable water-in-palm oil nanoemulsions containing catechin (WPOC-NEs). Then, the stable nanoemulsions obtained were characterized, including their droplet size, polydispersity index (PDI), zeta potential, and conductivity. To our knowledge, no work has been done on water-in-palm oil nanoemulsion containing catechin.

Materials and Methods

Materials

Catechin Hydrate was purchased from Sigma-Aldrich Co. (St. Louis, MO). Tween 80 and Span 80 were purchased from Chemiz (M) Sdn.Bhd. Palm oil was purchased from a local market. Distilled water was used to prepare the water-in-palm oil nanoemulsion containing catechin.

Preparation of water-in-oil nanoemulsion

Water-in-palm oil (W/PO) nanoemulsion was prepared according to the method implied by Qonita et al. [11] and Baba Shekh et al. [12] with some modifications. The aqueous phase consisted of distilled water, Tween 80, Span 80, and catechin, which were mixed and stirred using a hotplate stirrer (Model HTS-1003, AB Labmart Malaysia) at 1200 rpm for 10 min. Then, the oil phase was added dropwise into the aqueous phase and homogenized at 15,500 rpm for 5 minutes using a high-shear homogenizer (T25 digital; IKA-Werk, GmbH & Co. KG, Staufen im Breisgau, Germany). All the procedures were standardized, that is (i) 36% water, 11% total surfactants mixture, 0.1% catechin, and 54% palm oil, and (ii) all procedures were performed at room temperature.

Selection of hydrophilic-lipophilic balance (HLB) of mixed surfactants

A range of 4.3-7.1 HLB values was used to prepare water in palm oil nanoemulsion for the HLB value determination. In all samples, the water-to-oil content ratio was maintained constant (40/60, w/w%). The surfactant consists of the mixtures Span 80 and Tween 80. The following calculation calculated calculation of HLB values of mixed surfactants:

$$HLB_{mix} = F_T\, HLB_T + F_S\, HLB_S, \qquad (1)$$

where $HLB_{mix,}$ HLB_T, and HLB_S are the HLB values of mixed surfactants, Tween 80 (T80, HLB =15) and Span 80 (S80, HLB=4.3), while F_T and F_S are the weight fractions of Tween 80 and Span 80. All standard conditions were kept constant to prepare the nanoemulsions which were then centrifuged to evaluate their stability. Based on the calculation using Equation (1), a suitable HLB value was investigated until a stable W/PO nanoemulsion containing catechin was obtained for this study.

Fourier transform infrared spectroscopy (FTIR) – based attenuated total reflection (ATR)

Functional groups and chemical structures in the composition of the pure palm oil, span 80, tween 80, catechin, and water in palm oil nanoemulsion containing catechin were evaluated using a Perkin Elmer Fourier Transform Infrared, FTIR-based ATR (Spectrum 400 Perkin Elmer, Waltham, MA, USA) spectrometer in the range of 400 to 4000 cm^{-1}. All the samples were analyzed in their original forms without any kind of preparation.

Droplet size, zeta potential, polydispersity index, conductivity determination, and transmission electron microscopy (TEM) image

The most significant parameter for this study is the measurement of droplet size. The smaller the particle size, the bigger the surface area of the absorption of the drug. The droplet size, zeta potential, conductivity, and polydispersity index of WPOC-NEs were measured using dynamic light scattering, which scattered at an angle of 173˚ and a temperature of 25˚C. This procedure used a droplet-size analyzer (Zetasizer Nano ZS90; Malvern Instruments, Malvern, UK). To certify that the system has reached equilibrium, the droplet size was measured a day after the formulations were made [13].

The TEM image of the WPOC-NEs was obtained using Transmission Electron Microscopy (TEM) (Tecnai G2 Spirit BioTwin). Samples were diluted with deionized water and homogenized. A Formvar-coated copper grid was placed on top of a drop of diluted sample and left at room temperature (25 ± 0.5˚C) for 3 min. The samples on the filled copper grid were then negatively stained using 2% w/w phosphotungstic acid for 2 min and were air-dried before analysis [14].

pH determination

An S20 SevenEasy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) was used to measure the pH of the optimized nanoemulsion containing catechin. The pH meter was calibrated with three pH standard buffer solutions (pH 4.00, 7.00, and 10.00) before taking pH measurements. The pH value was obtained by inserting the electrode directly into the sample.

Viscosity determination

A rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) was used to determine the viscosity of the optimized nanoemulsion containing catechin, with PP25 plate and plate geometries (gap of 1mm) at 25ºC. A controlled Peltier system (TEK150) with an accuracy of ± 0.1℃ was used. The experimental data were fitted to the Power Law Equation:

$$\eta = k\acute{y}^{\,n-1} \qquad (2)$$

Based on the above equation, η is the viscosity (Pa.s), ý is the shear rate (s^{-1}), and k and n are the consistency index and flow behavior index, respectively.

Accelerated stability tests

The stability of nanoemulsions is characterized by their capacity to maintain their physical appearance (in a certain system) without phase separation or physical changes during a specific time of storage and use [15]. The optimized nanoemulsion containing catechin was submitted to two stability tests, namely, the centrifugation test and storage stability at different temperatures (10, 25, 50℃) for 28 days. If the visible appearance of creaming or phase separation occurred, the nanoemulsion containing catechin was said to be unstable.

The centrifugation test was performed using a SIGMA 2-16P centrifuge. The nanoemulsion containing catechin was placed into a centrifugation tube and then centrifuged at 4000 rpm for 30 min. The optimized nanoemulsion was then observed for the presence or absence of phase separation.

The storage stability test was performed at different temperatures and the optimized nanoemulsion was carried out by observing the changes in physical appearance on days 1,7,14, and 28.

Results and Discussion

Formulation and screening of water-in-oil nanoemulsion

Table 1 tabulates the W/PO nanoemulsion containing catechin. This study aimed to encapsulate catechin into water in palm oil nanoemulsion. Water-in-oil nanoemulsions comprise a dispersed aqueous phase with tiny droplets and a continuous oil phase. Oil-in-water nanoemulsions, on the other hand, are the dispersed phase disseminated into the continuous water phase [16]. As reported by Qonita et al. [11], the percentage of oil phase used was more than 50%, to form W/O nanoemulsions which was used in this study.

Tween 80 (T80) and Span 80 (S80), both non-ionic surfactants, were chosen in this study due to their strong biological compatibility, insensitivity to changes in electrolyte concentration or pH, and their established safety and approval for inclusion in specific pharmaceuticals, cosmetics, and food products [12].

Selection of hydrophilic-lipophilic balance (HLB) value of mixed surfactants

An acceptable HLB value of the surfactant utilized in the formulation is one of the primary driving forces for stable nanoemulsions. As it reflects the ideal mixture of surfactants that can produce stable nanoemulsions, it serves as a scale for choosing the suitable surfactants or mixtures of surfactants that are necessary for the oil and water phase [17]. The HLB system predicts the ideal emulsifier when the HLB values of the emulsifier and oil are matched. Well-known non-ionic and ethoxylated sorbitan alkanoates, which are Spans and Tweens, had a wide range of HLB values that helped adjust the HLB [18].

Table 1 shows the result surfactant mixtures (Tween 80/Span 80) of 26.2/73.8 (HLB 7.1) were suitable for producing W/PO nanoemulsion containing catechin, while others show separation. This HLB value did not show any physical separation during centrifugation, supporting the conclusion that the HLB 7.1 of the surfactant mixtures effectively stabilized the W/O nanoemulsion. The selected HLB range (4-9) covers water in oil and wettability agents in the classical HLB scale and was experimentally proven as an effective stabilizer for W/O systems [19].

Table 1. Composition of the water in palm oil nanoemulsion containing catechin for HLB screening

Composition						Observation
Surfactants(w/w%)		HLB	Palm Oil (w/w%)	Distilled Water (w/w%)	Catechin (w/w%)	Centrifugation
Tween 80	Span 80					
0.00	11.00	4.30	60.00	40.00	0.10	X
0.50	10.50	4.70	60.00	40.00	0.10	X
1.00	10.00	5.20	60.00	40.00	0.10	X
1.60	9.40	5.70	60.00	40.00	0.10	X
2.10	8.90	6.10	60.00	40.00	0.10	X
2.70	8.60	6.80	60.00	40.00	0.10	X
2.90	8.10	7.10	60.00	40.00	0.10	/

/ = stable or no physical change X = not stable and physical changes occur

ATR analysis based on FTIR

Figure 1 and Table 2 present the FTIR spectrophotometer and wavenumbers of palm oil (PO), Tween 80, Span 80, catechin, and water in palm oil nanoemulsion containing catechin (WPOC-NEs). FTIR analysis was performed to determine whether catechin was present in the water in palm oil nanoemulsion containing catechin. Figure 1(a), displayed a carbonyl (C=O) stretching peak at 1743.7 cm^{-1} and C–H stretching of alkyl chains at 2854.6 and 2922.9 cm^{-1}, which corresponds to the molecular structure of palm oil [20]. FTIR transmittance spectrum of surfactant Tween 80 was depicted in Figure 1(b) where a broad peak at 3493.3cm^{-1} indicates the presence of the O-H group. The aliphatic CH_2 symmetric and asymmetric stretching vibration is responsible for the 2920.1-2862.9 cm^{-1} band. In the domain 1735.8 cm^{-1}, the presence of C=O stretching methods may be seen. The band from 1349.7 to 1247.4 cm^{-1} is due to C-H bending, whereas the peak at 1097.5 cm^{-1} is attributed to C-O-C in $(CH_2CH_2O)n$.

Figure 1(c) indicates the spectrum of pure Span 80. The OH group is allocated to the peaks at 3410.7 cm-1. It should be noted that the absorption peaks at 1738.8 cm^{-1}, and 1458.8 cm^{-1} are due to C=O and C-H bending in CH_3 and CH_2 [21]. Characteristic IR peaks of catechin in Figure 1(d) were observed at 3355.3 (phenolic hydroxyl group), 1640.3, 1461.2 (phenyl ring), 1155.4, and 1095.8 (stretching vibration of C-O-C) cm^{-1} [22]. From Figure 1(e), the WPOC-NEs spectrum shows all of the distinctive peaks of catechin were attained by WPOC-NEs. The catechin distinctive peak within the WPOC-NEs was observed at 3356.3 (phenolic hydroxyl group), 1640.0, 1460.5 (phenyl ring), 1159.5, and 1096.8 (stretching vibration of C-O-C) cm^{-1}. Based on these findings, it was determined that catechin was present within the core of water in palm oil nanoemulsion containing catechin.

Table 2. Wavenumbers of IR of palm oil, tween 80, span 80, catechin, and W/PO NEs containing catechin

Sample	Wavenumbers (cm^{-1})				
	O-H Stretching	C-H Stretching	C=O Stretching	Phenyl Ring	C-O-C Stretching
Palm Oil	-	2854.6	1743.7	-	1115.3
Tween 80	3493.3	2920.1	1735.8	-	1097.5
Span 80	3410.7	2923.3	1738.8	-	1085.5
Catechin	3355.3	2925.6	-	1640.3	1095.8
WPOC-NEs	3356.3	2925.2	1741.7	1640.0	1159.5

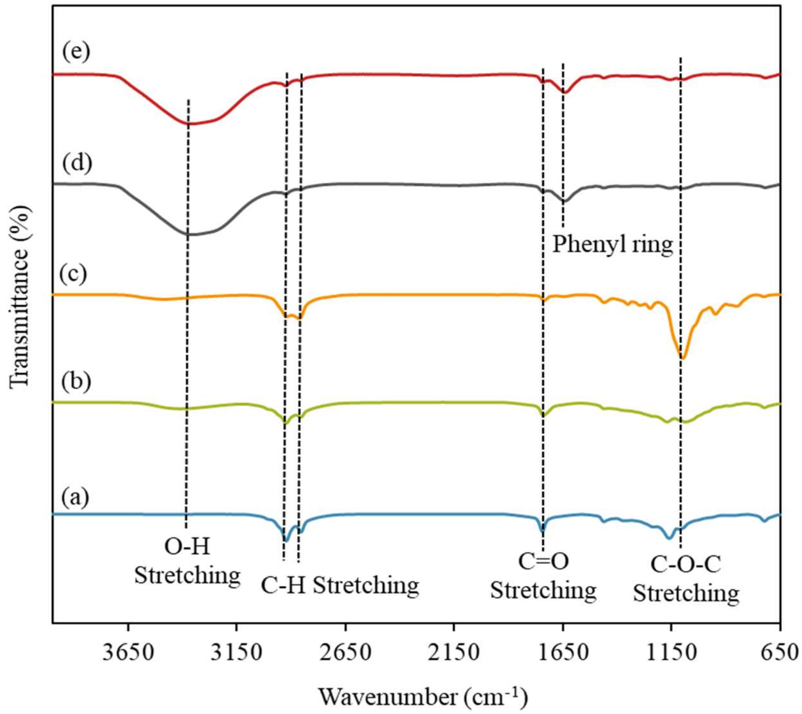

Figure 1. FTIR Spectra of (a)palm oil, (b)tween 80, (c)span 80, (d)catechin, and (e)WPOC-NEs

Droplet size, zeta potential, polydispersity index, conductivity determination, and transmission electron microscopy (TEM) image

The W/PO nanoemulsion containing catechin had mean droplet size, zeta potential, polydispersity index (PDI), and conductivity values of 185.60 nm, -38.4 mV, 0.251, and 0.001 mS/cm, respectively. The droplet size of a nanoemulsion ranging from 20 to 200 nm is substantially smaller than that of conventional colloidal systems. The small droplet size results in a larger colloidal surface area, which increases solubility and the rate of dissolution [23]. The zeta potential, which assesses a particle's electrokinetic potential, was utilized to determine the stability of the nanoemulsion. Readings higher than +30mV or lower than –30mV were said to be stable nanoemulsion [24]. The negatively charged zeta potential was caused by the polyoxyethylene chains in Tween 80, indicating sufficient Van der Waals electrostatic repulsions to stabilize and disperse the w/o system for an extended time [12].

The PDI value was used to examine the particle size distribution in the nanoemulsion. A PDI value close to zero implies a monodispersed system, whereas a PDI value close to one indicates a polydispersed system (a wide range of droplet sizes). Pongsumpun et al.[25] reported that a nanoemulsion system is termed monodispersed (with a restricted size distribution) if the PDI value is less than 0.3. Therefore, in this study, the optimized water in palm oil nanoemulsion containing catechin was found to be stable with a PDI value of 0.251, which conveys it is monodispersed (Figure 2) with a negative zeta potential value.

Conductivity is the measurement of the free amount of water and ions in a solution, as it will respond to any ions present. This parameter aids in determining the type of nanoemulsion produced [26]. The optimized nanoemulsion containing catechin had a low conductivity value of 0.001 mS/cm, indicating that the oil phase was a continuous phase and that the nanoemulsion created was a water-in-oil (W/O) nanoemulsion.

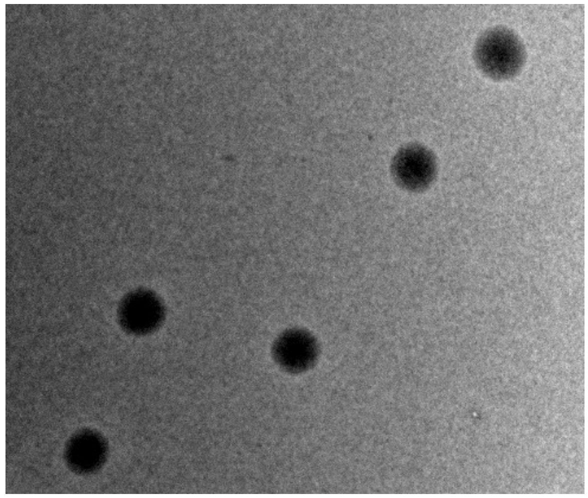

Figure 2. TEM image of WPOC-NEs

pH determination

The pH of the optimized W/PO nanoemulsion containing catechin was 6.82. These features are suitable for pharmaceutical applications, such as topical [27] and oral [28] applications.

Viscosity determination

A steady-state test was used to investigate the behavior of the W/PO nanoemulsion containing catechin. In the steady-state test, the W/PO nanoemulsion containing catechin displayed shear-thinning behavior (non-linear relationship), as shown in Figure 3. The flow behavior index (n) based on Equation (2) can be used to determine the rheological parameters of the W/PO nanoemulsion, as illustrated in Table 3. The value of n is 0.269 (n<1), indicating that the W/PO nanoemulsion was shear-thinning. The W/PO nanoemulsion's viscosity decreased with increasing shear rate, demonstrating that the W/PO nanoemulsion is a non-Newtonian pseudoplastic fluid (n<1). The non-Newtonian pseudo-plastic behavioral features of the W/PO nanoemulsion containing catechin are shown in Table 3. Notably, this type of behavior seen here matches the majority of over-the-counter pharmaceutical applications.

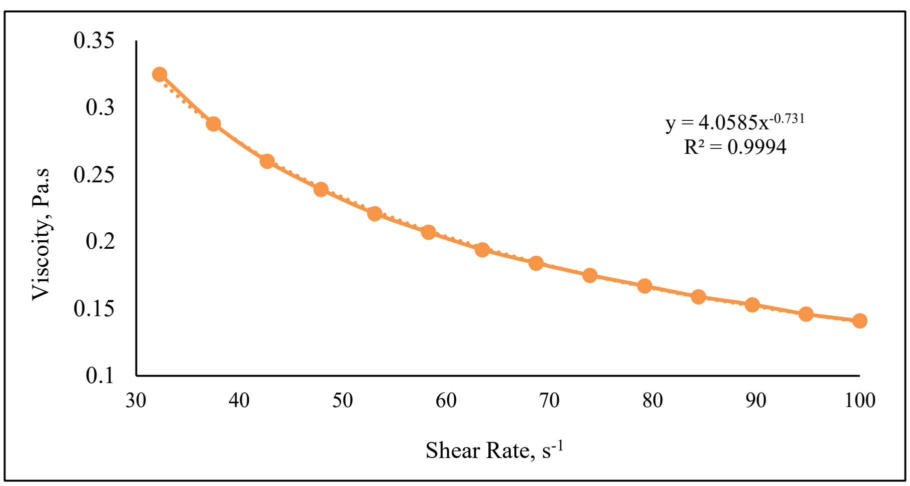

Figure 3. Graph of viscosity (Pa.s) versus shear rate (s^{-1})

Table 3. Flow behavior indices (n), consistency coefficients (k), and regression coefficients (R^2) of the water in palm oil nanoemulsion containing catechin

Sample	k	n	R^2
W/PO nanoemulsion containing catechin	4.0585	0.269	0.9994

Stability tests

For the centrifugation test, the W/PO nanoemulsion containing catechin showed no phase separation after centrifuging at 4000 rpm for 30 min. This indicates the formulation's remarkable stability against creaming or sedimentation and its expected long shelf life.

A storage stability study was conducted by exposing the W/PO nanoemulsion to three different temperatures (10, 25, 50 ℃) for 28 days. Table 4 denotes the physical stability of W/PO nanoemulsion containing catechin under three different storage temperatures. Based on the observations, the optimized nanoemulsion at 10 ℃ remained a homogeneous mixture and exhibited no physical or phase changes for 28 days. This shows that the W/PO nanoemulsion is stable at low temperatures.

However, at 25 and 50 ℃, the W/PO nanoemulsion was unstable after seven days and exhibited physical changes. This is due to the polyoxyethylene head groups of non-ionic surfactants of Tween 80 being known to be dehydrated when exposed to high storage temperatures. This alters the optimal curvature of the surfactant monolayer and reduces the range of steric repulsion, leading to instability [29].

Table 4. Physical stability of water in palm oil nanoemulsion containing catechin under different storage temperatures (10, 25, 50 ℃)

Storage Temperature (℃)	Storage Stability (days)				
	1	7	14	21	28
10	/	/	/	/	/
25	/	X	X	X	X
50	/	X	X	X	X

/ = stable or no physical change   X = unstable and physical changes occur

Conclusion

This study successfully developed a stable water in palm oil nanoemulsion containing catechin with 54% palm oil, a ratio of Tween 80: Span 80 (26.2:73.8) with HLB 7.1 and 0.10% of catechin. The physicochemical characterization indicated that the W/PO nanoemulsion containing catechin was in the nanosize range, had a zeta potential of -38.4mV, and a PDI of 0.251, showing that the nanoemulsion produced was stable and monodispersed. The TEM image obtained showed that the monodispersity of the WPOC-NEs was correlated with the PDI value. The pH of the nanoemulsion was 6.82, which was within the acceptable pH range for pharmaceutical products and its conductivity was low, indicating the developed nanoemulsion was a water-in-oil nanoemulsion type. The nanoemulsion produced remained stable and had no phase separation under centrifuge test and during storage at 10℃ over 28 days. These results show that it will be helpful for rationalizing design of water-in-oil nanoemulsion-based delivery systems to encapsulate catechin in food, beverage, cosmetic, and pharmaceutical products.

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References

1. Bae, J., Kim, N., Shin, Y., Kim, S. Y., and Kim, Y. J. (2020) Activity of catechins and their applications. *Biomedical Dermatology*, 4(1):1-10.
2. Tsai, Y. J., and Chen, B. H. (2016). Preparation of

catechin extracts and nanoemulsions from green tea leaf waste and their inhibition effect on prostate cancer cell PC-3. *International Journal of Nanomedicine*, 11: 1907-1926.

3. Bhushani, J. A., Karthik, P., and Anandharamakrishnan, C. (2016). Nanoemulsion based delivery system for improved bioaccessibility and Caco-2 cell monolayer permeability of green tea catechins. *Food Hydrocolloids*, 56: 372-382.
4. Wilson, R. J., Li, Y., Yang, G., and Zhao, C. X. (2022). Nanoemulsions for drug delivery. *Particuology*, 64: 85-97.
5. Li, P. H., and Chiang, B. H. (2012). Process optimization and stability of d-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. *Ultrasonics Sonochemistry*, 19(1): 192-197.
6. Tayeb, H. H., Felimban, R., Almaghrabi, S., and Hasaballah, N. (2021). Nanoemulsions: Formulation, characterization, biological fate, and potential role against COVID-19 and other viral outbreaks. *Colloids and Interface Science Communications,* 45: 1-19.
7. Liang, C. X., Qi, D. L., Zhang, L. N., Lu, P., and Liu, Z. D. (2021). Preparation and evaluation of a water-in-oil nanoemulsion drug delivery system loaded with salidroside. *Chinese Journal of Natural Medicines*, 19(3): 231-240.
8. Rai, V. K., Mishra, N., Yadav, K. S., and Yadav, N. P. (2018). Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: formulation development, stability issues, basic considerations and applications. *Journal of Controlled Release*, 270: 203-225.
9. Maluin, F. N., Hussein, M. Z., and Idris, A. S. (2020). An overview of the oil palm industry: Challenges and some emerging opportunities for nanotechnology development. *Agronomy*, 10(356): 1-20.
10. Goindi, S., Kaur, A., Kaur, R., Kalra, A., and Chauhan, P. (2016). Nanoemulsions: an Emerging technology in the food industry. *Emulsions*, 3: 651-688.
11. Qonita, H. A., Syafika, N., Valensie, V., Kamba, J., Maulana, A., and Permana, A. D. (2022). Development water in oil nanoemulsion of diethylcarbamazine for enhanced the characteristics for lymphatic targeting: A proof of concept study. *Journal of the Indian Chemical Society*, *99*(4): 1-5.
12. Baba Shekh, A. O., Abdul Wahab, R., and Yahya, N. A. (2022). Formulation of roselle extract water-in-oil nanoemulsion for controlled pulmonary delivery. *Journal of Dispersion Science and Technology*, 44(10): 1830-1841.
13. Ahmad, N., Alam, M. A., Ahmad, R., Umar, S., and Jalees Ahmad, F. (2018). Improvement of oral efficacy of irinotecan through biodegradable polymeric nanoparticles through in vitro and in vivo investigations. *Journal of Microencapsulation,* 35(4): 327-343.
14. Roselan M. A., Ashari S. E., Faujan N. H., Faudzi S. M. M., and Mohamad R. (2020). An improved nanoemulsion formulation containing kojic monooleate: optimization, characterization and in vitro studies. *Molecules*. 25(11): 1-23.
15. Shahidan, N. S., Salim, N., and Ashari, S. E. (2019). Preparation and optimization of ibuprofen-loaded nanoemulsion formulation. *Journal of Multidisciplinary Engineering Science and Technology,* 6(12): 89-96.
16. Liu, Q., Huang, H., Chen, H., Lin, J., and Wang, Q. (2019). Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds. *Molecules*, 24(23): 1-37.
17. Genot, C., Kabri, T. H., and Meynier, A. (2013). Stabilization of omega-3 oils and enriched foods using emulsifiers. *Food Enrichment with Omega-3 Fatty Acids*, 252: 150–193.
18. Wang, L., Dong, J., Chen, J., Eastoe, J., and Li, X. (2009). Design and optimization of a new self-nanoemulsifying drug delivery system. *Journal of Colloid and Interface Science*, 330(2): 443-448.
19. Colucci, G., Santamaria-Echart, A., Silva, S. C., Fernandes, I. P. M., Sipoli, C. C., and Barreiro, M. F. (2020). Development of water-in-oil emulsions as delivery vehicles and testing with a natural antimicrobial extract. *Molecules*, *25*(9): 1-15.
20. Sittinun, A., Pisitsak, P., Manuspiya, H., Thiangtham, S., Chang, Y. H., and Ummartyotin, S. (2020). Utilization of palm olein-based polyol for polyurethane foam sponge synthesis: potential as a

sorbent material. *Journal of Polymers and the Environment*, *28*(12): 3181-3191.

21. Branzoi, F., and Branzoi, V. (2017). Investigation of some nonionic surfactants as corrosion inhibitors for carbon steel in sulfuric acid medium. *International Journal of Electrochemical Science*, 12(8): 7638-7658.
22. Ahmad, N., Ahmad, R., Alrasheed, R. A., Almatar, H. M. A., Al-Ramadan, A. S., Amir, M., and Sarafroz, M. (2020). Quantification and evaluations of catechin hydrate polymeric nanoparticles used in brain targeting for the treatment of epilepsy. *Pharmaceutics*, 12(3): 1-34.
23. Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., and Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, 10(2): 1-17.
24. Marzuki, N. H. C., Wahab, R. A., and Hamid, M. A. (2019). An overview of nanoemulsion: concepts of development and cosmeceutical applications. *Biotechnology and Biotechnological Equipment*, 33(1): 779-797.
25. Pongsumpun, P., Iwamoto, S., and Siripatrawan, U. (2020). Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochemistry*, 60: 1-27.
26. Jiang, J., Mei, Z., Xu, J., and Sun, D. (2013). Effect of inorganic electrolytes on the formation and the stability of water-in-oil (w/o) emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 429: 82-90.
27. Yahya, N. A., Abdul Wahab, R., Attan, N., Abdul Hamid, M., Mohamed Noor, N., and Kobun, R. (2022). *Ananas comosus* peels extract as a new natural cosmetic ingredient: oil-in-water (o/w) topical nano cream stability and safety evaluation. *Evidence-Based Complementary and Alternative Medicine*, 2022: 1-9.
28. Attebäck, M., Hedin, B., and Mattsson, S. (2022). Formulation optimization of extemporaneous oral liquids containing naloxone and propranolol for pediatric use. *Scientia Pharmaceutica*, 90(1): 1-18.
29. Ryu, V., McClements, D. J., Corradini, M. G., and McLandsborough, L. (2018). Effect of ripening inhibitor type on formation, stability, and antimicrobial activity of thyme oil nanoemulsion. *Food Chemistry*, 245: 104-111.