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Caffeine-Containing Emulsion: Influence of the HLB and Mixing Proportions, the Oil's Chemical Composition, and the Existence of Caffeine on Emulsion Properties

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Cite This: *ACS Omega* 2024, 9, 2113−2122

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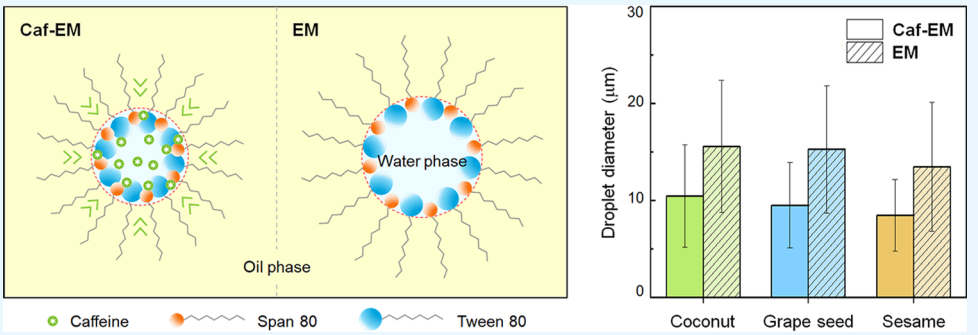


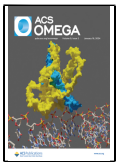
ABSTRACT: This study employs a low-energy emulsification method to prepare caffeine-containing emulsions, denoted as Caf-EM. Three different oils, including coconut, sesame, and grape seed oils, are utilized along with the surfactants Span 80 and Tween 80. We investigate the influence of various factors, including (i) the hydrophilic−lipophilic balance (HLB) and surfactant ratio, (ii) the chemical composition of the oils, and (iii) the presence of caffeine, on the stability and size of emulsions. The results indicate that the HLB value and surfactant ratio are the most crucial factors affecting the emulsions' stability. The most stable Caf-EM formulation is achieved by combining mixed surfactants of Span 80 and Tween 80 with an optimal HLB value of 6.4 at a concentration of 15% (S15 to 6.4) across all oil types. This specific ratio also leads to significantly smaller emulsion droplet sizes than other ratios and is the only ratio that produces stable emulsions even without caffeine (denoted as EM). Notably, formulation S15−6.4 additionally causes a phase inversion from oil-in-water (O/W) to water-in-oil (W/O). Furthermore, the presence of caffeine in the water phase contributes to the formation of smaller and more stable emulsions. The particle size of Caf-EM is approximately 1.5 times smaller than that of EM. Regarding the oil's chemical composition, while there is a discernible trend in emulsion droplet size (coconut oil > grape seed oil > sesame oil), the differences within this sequence are insignificant, suggesting that the oil's chemical composition does not have a pronounced effect.

1. INTRODUCTION

Caffeine, which is a naturally occurring alkaloid, is known for stimulating the central nervous system. It is consumed globally in various forms, such as coffee, tea, and energy drinks, due to its potential to enhance alertness, concentration, and overall cognitive performance. The absorption of caffeine from the gastrointestinal tract into the circulatory system is rapid and can reach maximum concentration in plasma after ingestion in only 30−60 min.[1] Moreover, there has been a notable endeavor to broaden the utilization of caffeine in personal care items and cosmetics owing to its significant biological potency and capacity to permeate the skin barrier. Caffeine has potent antioxidant properties. It helps protect cells from free radical damage from, for instance, UV radiation, thus slowing down the skin's aging.[2] The microcirculation of blood in the skin could also be enhanced and stimulate hair growth by inhibiting the 5-α-reductase activity.[3] Consequently, the popularity of caffeine as a functional ingredient has led to a growing demand.

Nonetheless, caffeine falls short of possessing the characteristics of an optimal skin penetrant due to its hydrophilic nature. Various approaches were employed to overcome this limitation, including using physical enhancement techniques such as mechanical pressure, ultrasound, iontophoresis, and microneedles as well as formulation strategies like penetration enhancer combining and nanoparticle encapsulation. Another

Received: May 25, 2023
Revised: November 22, 2023
Accepted: December 21, 2023
Published: January 4, 2024



potential approach is to encapsulate caffeine within an oil-based formulation.[4] Caffeine finds application in various oil-based products across different domains. In skin care, caffeine-infused serums, creams, and lotions are utilized to rejuvenate the skin, harnessing its antioxidant properties, and enhancing blood circulation. This can lead to diminished puffiness, dark circles, and fine lines. In hair care, caffeine-containing oils are incorporated into scalp treatments to promote hair growth, reduce hair loss, and enhance overall scalp health. The oil-based formulation ensures effective penetration into the scalp and hair follicles, facilitating the delivery of caffeine's benefits. Caffeine-infused massage oils combine caffeine's stimulating effects with massage therapy's soothing properties, offering a revitalizing experience, potentially boosting circulation, and alleviating muscle fatigue. In this regard, the concept of "emulsion" becomes relevant when mixing substances with different properties, such as caffeine (hydrophilic) and oil (hydrophobic).

Emulsions can be described as a system comprising an immiscible liquid in the droplet form dispersed in another immiscible liquid aided by surfactants. Depending on the continuous phase, they can fall into two main categories: oil-in-water (O/W) and water-in-oil (W/O) emulsions.[5] More complex emulsions called double emulsions or multiple emulsions like water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions have also been developed.[6] All systems are well-known encapsulation technologies, offering considerable opportunities for targeted drug delivery to and via the skin. Emulsions are extensively utilized systems for delivering cosmetic and pharmaceutically active ingredients through topical applications. Within skincare, they can take the form of lotions or creams. These formulations can effectively transport both hydrophilic and lipophilic active compounds, safeguarding them against degradation while regulating their absorption into or through the skin. Additionally, emulsions exhibit moisturizing properties and typically offer appealing sensory qualities, enhancing their acceptance and usability when applied.[7]

Many findings showed that the bioavailability of active ingredients increased while decreasing emulsion droplet sizes to nanoscales, enhancing the desired responses.[8,9] Briefly, emulsions can be classified based on droplet size and stability: coarse, micro-, and nanoemulsions. Coarse emulsions (or conventional/macroemulsions) have larger particles (>200 nm) and are optically turbid and unstable over time. Microemulsions have smaller droplets (<100 nm) and are optically transparent and thermodynamically stable but sensitive to environmental changes. Nanoemulsions are similar in size to microemulsions (<200 nm, sometimes <100 nm). Like conventional emulsions, nanoemulsions are thermodynamically metastable, undergoing phase separation over time. However, they maintain kinetic stability due to reduced attractive forces between tiny droplets, preventing gravitational separation and clumping. Unlike stable microemulsions, nanoemulsions stay resilient against changes in temperature, pH, and other factors. Besides, they need fewer surfactants for preparation.[10] Creating these emulsions relies on crucial physicochemical factors such as solubility, composition, and temperature. Their ease of preparation, excellent performance, and outstanding stability make them highly appealing for large-scale production.[11] Although some studies on caffeine emulsion in various systems, including emulsifier-free emulsions,[12] multiple emulsions (W/O/W),[13] O/W emulsions,[14] and W/O emulsions,[15,16] have been reported, the impact of oil's chemical composition on emulsions' properties has not been explored for all formulations.

In this study, caffeine-containing emulsions, so-called Caf-EM, were prepared using a low-energy emulsification method, i.e., mechanical stirring. Three natural oils, including coconut, grape seed, and sesame oils, were chosen as base oils. Their structural differences are what we are interested in inspecting. Specifically, while coconut oil is saturated fat having a saturated medium chain length (lauric acid, C12:0) as the primary fatty acid, grape seed and sesame oils are unsaturated fats. Both grape seed and sesame oils have long-chain (C18) unsaturated fatty acids as primary fatty acids with a total of >80%. However, while most fatty acids in grape seed oil are polyunsaturated fatty acids (~75%), approximately equal amounts of mono- and poly-unsaturated fatty acids exist in sesame oil. Two nonionic surfactants, Span 80 and Tween 80, were used. They were selected based on their nontoxic nature and hydrophilic–lipophilic balance (HLB) value. The HLB values of Span 80 and Tween 80 are 4.3 and 15.0, respectively. Generally, surfactants with HLB values in the 3.5–6.0 ranges are more suitable for W/O emulsions, while those of O/W are in the range of 8–18.[17] Combining two surfactants is therefore helpful for achieving a stable emulsion. The stability of the emulsions was investigated under varied HLB and volume ratios between a surfactant and oil. The influence of the oil's chemical composition on the droplet size and stability of the emulsion was investigated. Eventually, the effect of caffeine existing in the system was explored.

2. MATERIALS AND METHODS

2.1. Materials. Coconut oil (Product No. B001CM), grape seed oil (Product No. B003GS), and sesame oil (Product No. B006SM) were purchased from Krungthepchemi Co., Ltd., Bangkok, Thailand. Palm oil (Morakot; Morakot Public Co., Ltd., Samutprakan, Thailand; Product number 8850154000041), soybean oil (Cook; Thanakorn Vegetable Oil Products Co., Ltd., Samutprakan, Thailand; Product number 8850180010045), corn oil (Mazola; ACH Food Companies, Inc., Illinois; Product number 7640129897865), and sunflower oil (Cook; Thanakorn Vegetable Oil Products Co., Ltd., Samutprakan, Thailand; Product number 8850180010090) were used as supplementary oils. No additional purification was conducted on any of the oils before use.

Caffeine anhydrous powder (Loba Chemie, AR grade, 99% purity) was purchased from Apexchemical Co., Ltd., Bangkok, Thailand. Span 80 (sorbitan oleate, 100% purity) and Tween 80 (polysorbate 80, 100% purity) were purchased from Phitsanuchemicals Co., Ltd., Phitsanulok, Thailand. Deionized water was used in the dispersed phase. All reagents were of analytical grade and used without further purification.

2.2. Preparation of Caf-EM. Emulsions loaded with caffeine were prepared by mixing the water and oil phases and stirring them on a magnetic stirrer at 400 rpm for 5 min at room temperature (~25 °C). The water phase was 2.5%w/v caffeine solution, abbreviated as W. The solution volume was fixed at 40% of the total volume of the emulsion. The remaining 60% contained surfactants (S) and oil (O). Based on the ratios of surfactants, samples were divided into five groups, having surfactant volume ratios of 1, 3, 5, 10, and 15%. Within these percentages, the ratios between Span 80 and Tween 80 were adjusted to have HLB values of 4.3 (pure Span 80), 6.4, 8.5, and 15.0 (pure Tween 80). The HLB of the mixed surfactant was calculated from the following equation.[18]

https://doi.org/10.1021/acsomega.3c03674
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$$\mathrm{HLB} = \frac{[(H_{\mathrm{Span80}}W_{\mathrm{Span80}}) + (H_{\mathrm{Tween80}}W_{\mathrm{Tween80}})]}{W_{\mathrm{Span80}} + W_{\mathrm{Tween80}}}$$

where "H" is the HLB value of the pure surfactant and "W" is the weight of the surfactant in the mixture. Proportions of the constituents in each sample and the designated names of samples are shown in Table 1. Note that the "HLB" and "ratio of surfactants" are separate variables. Specifically, the surfactants with desired "HLB" values are derived from mixing two surfactants. Meanwhile, the "ratio of surfactant" in this research means the volume fraction of the surfactant relative to the water and oil phases.

Table 1. Proportions of Constituents in the Caf-EM Sample and the Designated Names of Samples

group	S (%)	O (%)	W (%)	HLB	sample name
1	1	59	40	4.3	S1−4.3
				6.4	S1−6.4
				8.5	S1−8.5
				15.0	S1−15.0
2	3	57	40	4.3	S3−4.3
				6.4	S3−6.4
				8.5	S3−8.5
				15.0	S3−15.0
3	5	55	40	4.3	S5−4.3
				6.4	S5−6.4
				8.5	S5−8.5
				15.0	S5−15.0
4	10	50	40	4.3	S10−4.3
				6.4	S10−6.4
				8.5	S10−8.5
				15.0	S10−15.0
5	15	45	40	4.3	S15−4.3
				6.4	S15−6.4
				8.5	S15−8.5
				15.0	S15−15.0

Since lower HLB values indicate high oil affinity and, on the other hand, high HLB values indicate high water solubility, Span 80 was added in the oil phase; in contrast, Tween 80 was added in the water phase before mixing to form emulsions. The total volume of the emulsion for each preparation was 40 mL. Besides, to explore the influence of caffeine in the system on emulsion properties, emulsions without caffeine, denoted as "EM" (the water phase contains water only), were additionally prepared and characterized.

2.3. Emulsion Stability Test. The stability of the emulsions to phase separation was evaluated by visual observation to determine the emulsion shelf life. The test was based on two contiguous circumstances: (i) the real-time storage condition and (ii) the accelerated storage condition. The methods of each test are described as follows.

2.3.1. Real-Time Storage Stability. The emulsion's real-time storage stability was assessed by storing the as-prepared emulsion at room temperature for one month and observing their phase separation by visual observation. The conditions in which no phase separation occurs in this experiment were selected for further testing, i.e., accelerated storage stability.

2.3.2. Accelerated Storage Stability. This test was conducted to determine the change in physical properties of the emulsion after being stored under high-stress conditions. Two tests were conducted.

2.3.2.1. Heating−Cooling Test. The heating−cooling test was reported as a practical approach for assessing emulsion stability within a brief time frame. It involves analyzing structural alterations and replicating temperature fluctuations encountered during various stages, such as processing, production, storage, and transportation.[19] The emulsion was stored at an extreme temperature in a temperature control chamber at 4 °C for 24 h. Then, it was moved to an incubator, where the temperature was kept at 50 °C for the next 24 h (counted as one round). This procedure was repeated for two rounds. Then, emulsion stratification was observed.

2.3.2.2. Centrifugation. Centrifugation is a fundamental tool to accelerate the sedimentation of undissolved particles from a liquid or separate liquids with different specific gravities. The emulsion was centrifuged at 5000 rpm for 30 min. Then, emulsion stratification was observed.

2.4. Emulsion Size Analysis. *2.4.1. Particle Size Analyzer (Mastersizer).* The emulsion's droplet size was measured by a Mastersizer 2000 (Malvern, Hydro mode). A 0.5 mL emulsion was introduced into 500 mL of deionized water at a pump speed of 2000 rpm. After complete dispersion, droplet sizes were

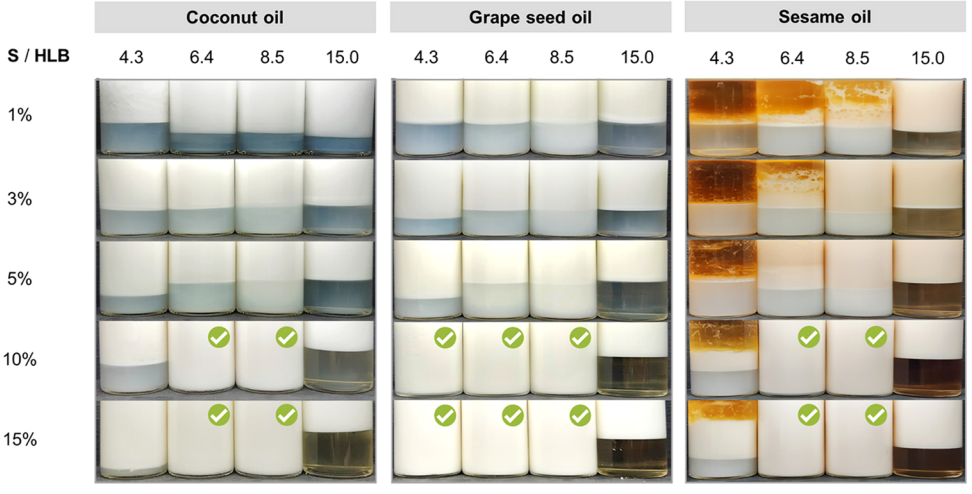

Figure 1. Appearance of Caf-EM prepared using different oil types, percent by volume of surfactant, and HLB values after one month of storage at room temperature. Unstratified samples are highlighted with a tick mark symbol.

https://doi.org/10.1021/acsomega.3c03674
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Figure 2. Appearance of Caf-EM prepared using different oil types, percent by volumes of surfactant, and HLB values after heating−cooling (4 °C, 24 h and 50 °C, 24 h) and centrifugation (5000 rpm, 30 min) tests. Unstratified samples are highlighted with a tick mark symbol. Note that the compositions of S15−6.4 for all types of oil were stable.

measured using the hydro mode, providing precise insights into emulsion characteristics. The provided result for each condition represents the average of nine values acquired from three distinct samples with three measurements taken for each sample. The statistical significance of the data was assessed using a one-way ANOVA post hoc test following Fisher's least significant difference (LSD).

2.4.2. Optical Microscopy. The microstructure of the emulsions was determined with an optical microscope (Olympus-BX53M). A drop of the as-prepared emulsion was placed on a microscope slide, covered with a coverslip, and then observed at 50× magnification. High-quality images were acquired. Then, the average particle size was identified using image-based analysis from approximately 200−350 droplets for each sample.

3. RESULTS

3.1. Emulsion Stability under Real-Time Storage Conditions. Figure 1 shows photographs of Caf-EM prepared using different oil types, percent by volumes of surfactant, and HLB values after one month of storage at room temperature. Emulsions prepared using high concentrations of surfactant, i.e., 10 and 15%, and the mixed surfactant of HLB values of 6.4 and 8.5 for all oil types were homogeneous with time. For grape seed oil, an HLB of 4.3 was additionally found to give rise to a stable emulsion.

The results also revealed that the system containing all types of oil resulted in creaming and two-phase separation. Additionally, three-phase separation was found for sesame oil (S10−4.3 and S15−4.3). This finding could be related to the oil's chemical composition and physical properties. Regarding the oils' density, creaming occurring in emulsions of all oil types resulted from the lower density of the oil than water.[20] The results also demonstrated that the obtained emulsions are O/W. Therefore, the oil-containing particles can coalesce, then float upward, and separate from the water phase after storage. For sesame oil, "bicontinuous microemulsion" could form in the middle phase, and thus, three-phase separation occurred.[21] The pattern of this separation based on the Winsor system followed the Winsor-III pattern, which was reported to be found in a system with a low surfactant or a similar ratio of water and oil phases.[22]

3.2. Emulsion Stability under Accelerated Conditions. From the real-time storage stability test results, stable samples from all oil types, including S10−6.4, S10−8.5, S15−6.4, and S15−8.5 of coconut oil; S10−4.3, S10−6.4, S10−8.5, S15−4.3, S15−6.4, and S15−8.5 of grape seed oil; and S10−6.4, S10−8.5, S15−6.4, and S15−8.5 of sesame oil, were selected for two acceleration tests, i.e., heating−cooling and centrifugation. The results are shown in Figure 2. For the heating−cooling test, most samples were stable after being accelerated at extreme temperatures. However, emulsion stratification after centrifugation occurred more than in the first case, i.e., half of the samples stratified. Remarkably, emulsions of all oil types with the composition of S15−6.4 were stable.

3.3. Emulsion Droplet Size. One crucial factor influencing emulsion stability is droplet size. Using a particle size analyzer and optical microscope images, emulsions with high stability under real-time storage conditions were measured for their sizes. The particle sizes measured by the particle size analyzer are listed in Figure 3a. Results indicated that the composition of S15−6.4 for all oil types exhibited the smallest droplet size with narrower size distributions. The dimensions were smaller than all other formulations at a significance level of 0.1 ($p < 0.1$). This result was consistent with those seen under an optical microscope (Figure 3b) in which the S15−6.4 composition displayed the smallest droplet size and narrower size distributions across all oil types. The charts comparing particle sizes measured by the particle size analyzer with the droplet diameter obtained through the optical microscope are additionally offered in the Supporting Information (Figure S1). Size distribution graphs of the S15−6.4 sample for all oil types (from the optical microscope) are further shown in Figure 3c. This finding agrees with the accelerated emulsion stability test results (Figure 2) that S15 to 6.4 for all types of oil was the most stable condition. Comparing three types of oil, the particle sizes of emulsions of formulation S15−6.4 were in the following order:

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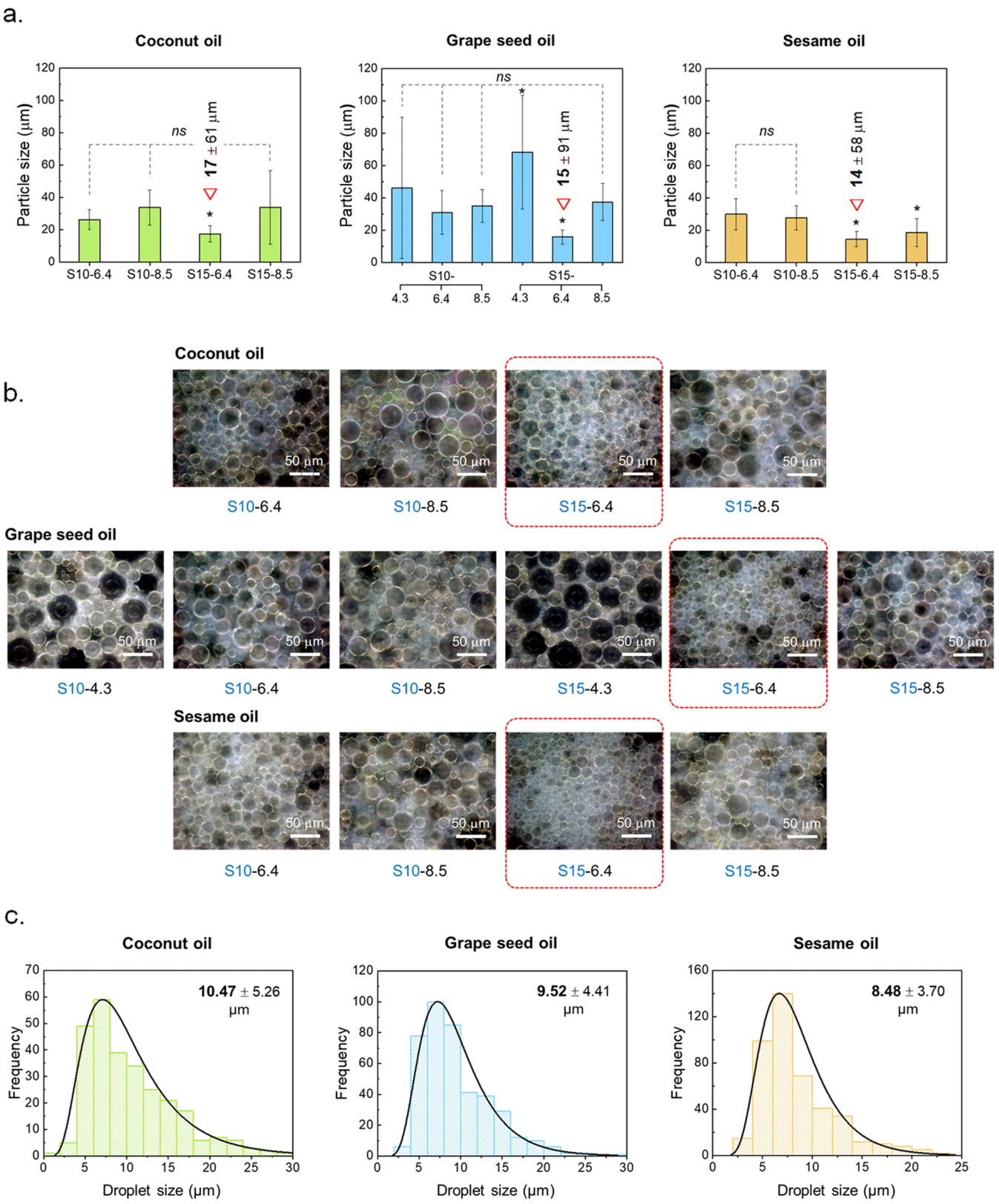

Figure 3. (a) Emulsion particle sizes measured by a particle size analyzer. The abbreviation "ns" denotes statistical insignificance at the 0.1 level, while the asterisk (*) signifies statistical significance at the 0.1 level across all conditions. (b) Optical microscopy images of Caf-EM prepared using different oil types, percent by volume of surfactant, and HLB values. The composition of S15−6.4 for all oil types exhibited the smallest size, having size distribution graphs as shown in (c).

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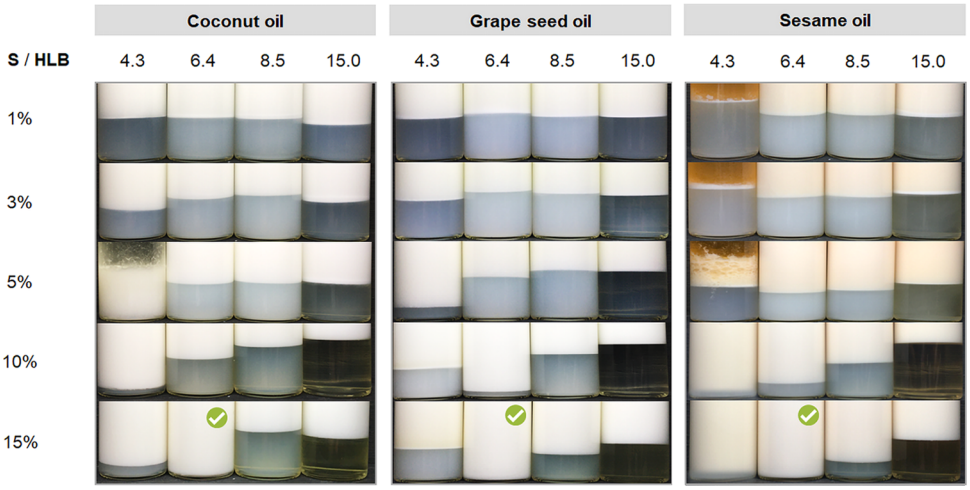

Figure 4. Appearance of EM prepared using different oil types, percent by volume of surfactant, and HLB values after one month of storage at room temperature. Unstratified samples are highlighted with a tick mark symbol.

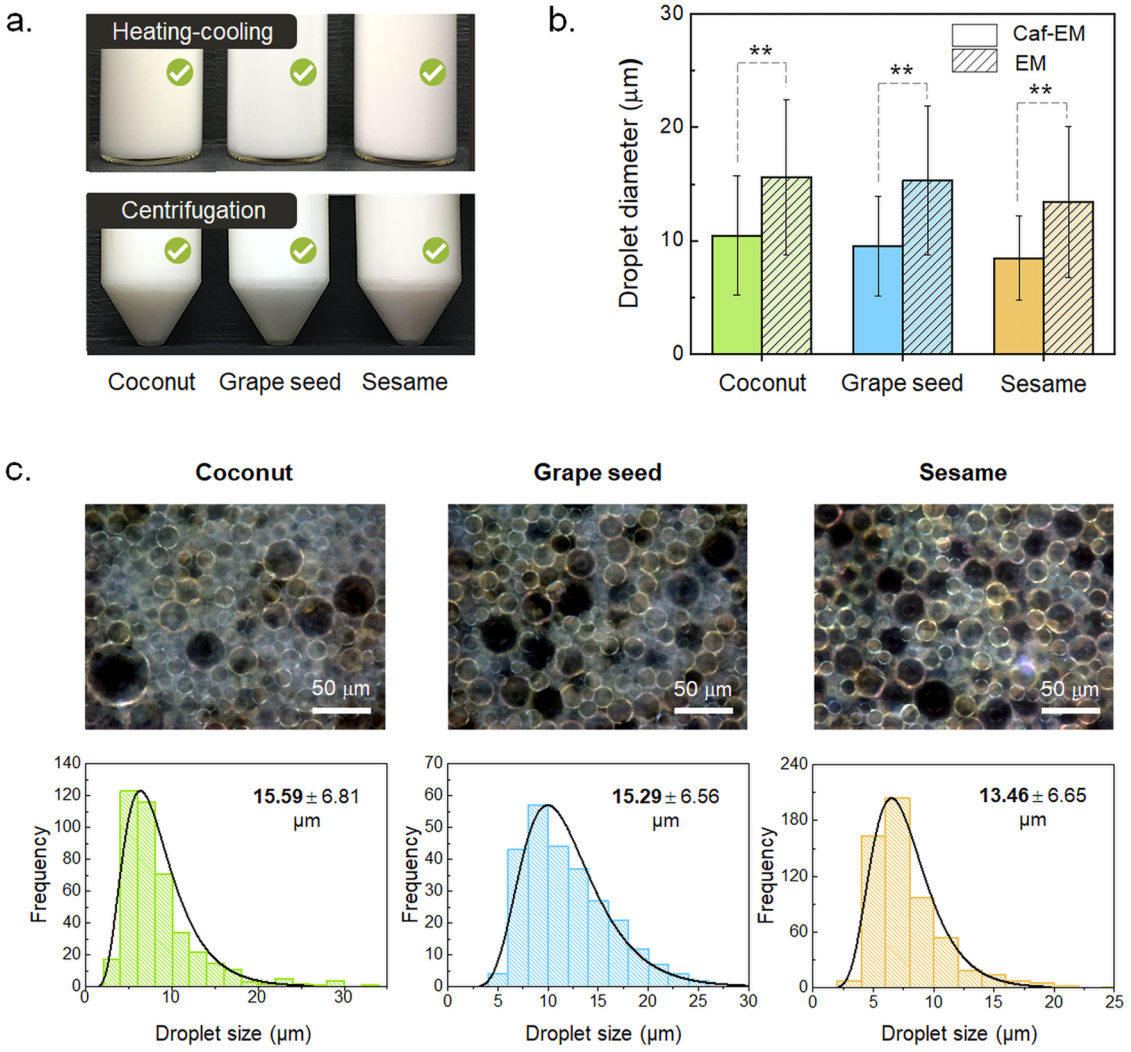

Figure 5. (a) Appearance of EM prepared using different oil types at a composition of S15−6.4 after heating−cooling and centrifugation tests. (b) Corresponding samples' droplet sizes, measured from OM images, compared with the Caf-EM ones. Double asterisks (**) indicate statistical significance at the 0.05 level. (c) Optical microscopy images of the related samples and size distribution graphs obtained from the images.

https://doi.org/10.1021/acsomega.3c03674
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coconut oil > grape seed oil > sesame oil. Nevertheless, statistical analysis indicated that no noteworthy distinctions were observed.

3.4. Effect of Caffeine on Emulsion Properties. The effect of caffeine on the emulsion properties was also studied. Emulsions without caffeine (EM) were additionally prepared for comparison. Figure 4 demonstrates their appearance after real-time storage stability testing. We found that the number of conditions that remained stable after leaving for one month was less than that found in the caffeine-containing emulsions. The most stable formulation for all oil types was also S15−6.4. Moreover, the observed instability of emulsions was two-phase and three-phase separation, similar to that found in the former test. These results suggested that the most critical factor affecting the stability of emulsions is the optimal HLB and ratio of surfactants. Modulating these parameters is therefore a key to preparing stable emulsions. Furthermore, the oil's chemical composition affects the types of emulsion's instability.

Afterward, stable samples from all oil types were tested for accelerated storage stability and droplet size. As shown in Figure 5a, all samples remained stable. Figure 5b,c indicates that the emulsion's droplet sizes of different oil types were in the following order: coconut oil > grape seed oil > sesame oil. However, the distinctions within this sequence do not exhibit any significant variance ($p > 0.05$). Above all, it was noticeable that the droplet sizes of EM were significantly larger than the Caf-EM in all cases (approximately 1.5 times, $p < 0.05$) and with narrower size distributions (Figure 5b). These results pointed out that caffeine helps create smaller emulsion sizes and thus more stable emulsions.

4. DISCUSSION

Here, stable Caf-EMs with a relatively high amount of caffeine (i.e., 40%v/v) are prepared using a low-energy emulsification method. We are interested in inspecting the influence of the following factors: (i) the optimal HLB and ratio of surfactants in the system, (ii) the oil's chemical composition, and (iii) the existence of caffeine.

Regarding the optimal HLB and ratio of surfactants, the most stable emulsion for Caf-EM formulations obtained in this study is the formulation using a mixed surfactant with an optimum HLB value of 6.4 at a concentration of 15% (referred to as S15−6.4). This condition additionally has an appreciable effect on the particle size and size distribution.

Table 2 summarizes typical literature values of the fatty acid composition of coconut oil, grapeseed oil, and sesame oil along with the chemical structures of the primary fatty acid shown in Figure 6. The influence of the oil's chemical composition is described as follows.

Table 2. Typical Literature Values of the Fatty Acid Composition (%) of Coconut, Grapeseed, and Sesame Oils

types of fatty acid	coconut oil[a]	grape seed oil[a]	sesame oil[b]
saturated fatty acids	92.1	10.4	16.3
monounsaturated fatty acids	6.2	14.8	41.9
polyunsaturated fatty acids	1.6	74.9	42.3

[a]23. [b]24.

Generally, the fatty acid composition of vegetable oils varies with the species of plants producing oils and geographical region. However, the primary fatty acid in each type of oil is noticeable. Specifically, "lauric acid" is the primary fatty acid in coconut oil, consisting of approximately 45−53%. It is a saturated fatty acid with a 12-carbon atom chain without double bonds (C12:0). The remaining acids are caprylic acid, C8:0 (∼8%); capric acid, C10:0 (∼7%); myristic acid, C14:0 (∼8%); palmitic acid, C16:0 (∼8%); stearic acid, C18:0 (∼2%); oleic acid, C18:1 (∼6%); and linoleic acid, C18:2 (∼2%). Overall, most fatty acids in coconut oil are saturated fatty acids (∼92%). Note that fatty acids with aliphatic tails of 6−12 carbons and 13−21 carbons are considered medium-chain and long-chain fatty acids, respectively.[25] Therefore, coconut oil is a mixture of triglycerides consisting of glycerol groups and medium-chain fatty acids.[26,27] Its benefits include reducing inflammation, keeping the skin moisturized, and helping heal wounds.[28]

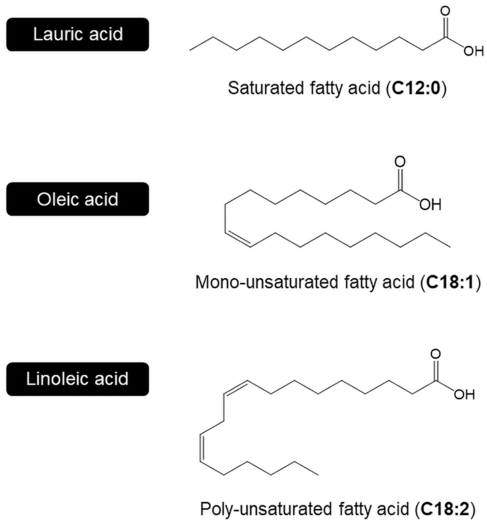

Figure 6. Chemical structures of lauric acid, oleic acid, and linoleic acid; primary fatty acids in the oils used in this study.

Grape seed oil's attractiveness is its high levels of hydrophilic constituents, such as phenolic compounds, and lipophilic constituents, such as vitamin E, phytosterols, and unsaturated fatty acids.[29] Most fatty acids in grapeseed oil are polyunsaturated fatty acids (∼75%), and the primary polyunsaturated fatty acid is linoleic acid (C18:2), comprising 58−78%. Oleic acid (C18:1), a monounsaturated fatty acid, is also found.[23]

Sesame oil is rich in vitamins B and E, helping soothe skin rashes and fade scars. Skin inflammation could also be alleviated. Sesame oil consists of two primary fatty acids: "oleic acid (C18:1)" and "linoleic acid (C18:2)", mono- and polyunsaturated fatty acids, respectively. Each comprised more than 40% (total > 80% of all fatty acids).[30,31] It should be postulated that approximately equal amounts of mono- and polyunsaturated fatty acids exist in sesame oil.

We hypothesize that the chemical composition of oils may also impact the emulsion's droplet dimensions. The observed ranking of emulsion sizes of a formulation S15−6.4 in both Caf-EM and EM in the following order: coconut oil > grape seed oil > sesame oil suggests that oils with unsaturated fatty acids may result in smaller emulsions than saturated fatty acids. Besides, approximately equal amounts of mono- and polyunsaturated fatty acids in oils may result in smaller emulsions than in oils having only a high amount of polyunsaturated fatty acids. To provide more comprehensive data, additional oils likely to be classified in these three categories were prepared as Caf-EM using the ideal formulation S15−6.4. Typical literature values of their fatty acid composition (%) are shown in Table S1. Results

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ACS Omega 2024, 9, 2113−2122

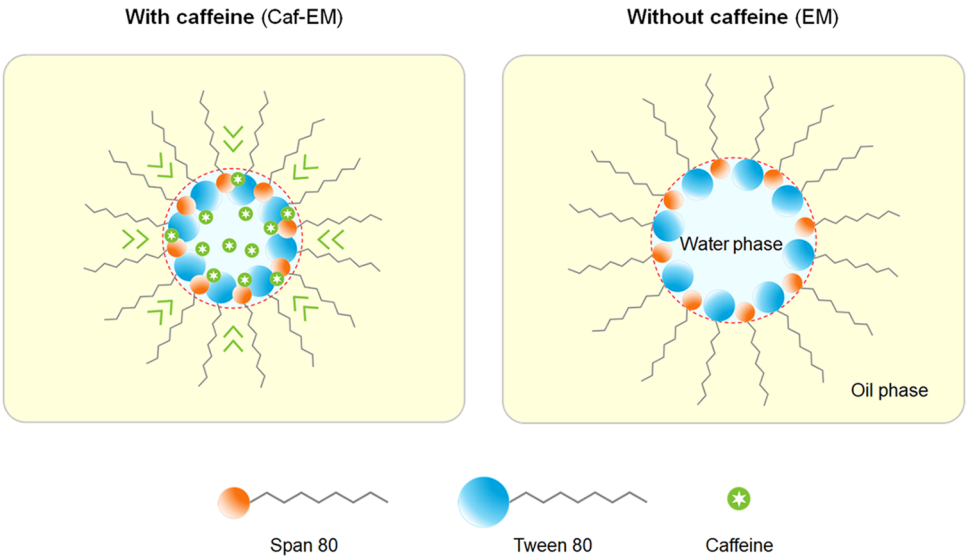

Figure 7. Schematic drawing illustrating the effect of caffeine on the emulsion droplet size. Briefly, the formulation S15−6.4 induces a phase inversion from O/W to W/O for both Caf-EM and EM. For the Caf-EM, caffeine increases the hydrophobicity of the water phase, allowing greater integration of oil molecules into the core of water-phase droplets, fostering stronger attraction among polar molecules in the W/O droplets due to the reduced molecular distance facilitated by electrostatic attraction.

depicted in Figure S2 indicated trends consistent with those hypothesized. However, when considering other interconnected factors, such as the amount of carbon in fatty acid molecules and the proportions of saturated, monounsaturated, and polyunsaturated fatty acids, unlike the oils examined in the primary investigation, these variables might not exhibit a well-defined balance of their composition. As a result, they may not effectively illustrate the influence of the oil type. The obtained outcome, when considering the impact of different oils (the primary oils) on alternate formulations (i.e., S10, 6.4; S10, 8.5; and S15, 8.5), is additionally depicted in Figure S3. The results affirm no discernible influence of the oil's chemical composition.

Lastly, we examine the influence of caffeine's presence in the system by considering the formulation S15−6.4, which provides stable emulsions in both Caf-EM and EM. It was found that the existence of caffeine molecules contributes to the creation of significantly smaller emulsion droplets. We hypothesize that formulation S15−6.4 induces a phase inversion from O/W to W/O. This hypothesis was substantiated through an experiment involving applying a water-soluble dye (methylene blue) solution onto both Caf-EM and EM of the S15−6.4 formulation. The results, illustrated in Figure S4, reveal that the dye did not disperse in both emulsions, providing empirical support for our hypothesis. This transition is likely to occur in this system for the following reasons: (i) the HLB value of 6.4 is notably compatible with W/O emulsions, and (ii) as indicated in Table 1, the proportions of the constituents have been set to ensure that the oil phase ratio exceeds that of the water phase in all formulations.

Despite being polar, caffeine exhibits relative hydrophobicity due to multiple functional groups with varying hydration patterns and flat hydrophobic surfaces.[32] Therefore, caffeine increases the interfacial area between the water and oil phases by enhancing the hydrophobicity of the water phase. This phenomenon could foster stronger attraction among polar molecules in the W/O droplets due to the reduced molecular distance facilitated by electrostatic attraction. Consequently, the sizes of Caf-EM were remarkably smaller than those of EM (approximately 1.5 times) in all formulations. Figure 7 illustrates a schematic representation of the effect of caffeine on the emulsion droplet size.

All of the descriptions mentioned above collectively indicate that the optimal HLB value and surfactant ratio exert the most significant influence among the factors under investigation, followed by the existence of caffeine. Specifically, the highest stability for both Caf-EM and EM formulations is achieved by employing a combination of nonionic surfactants (Span 80 and Tween 80) at a 15% concentration with an HLB value of 6.4, referred to as S15−6.4. This formulation demonstrates robust stability during real-time storage and accelerated conditions involving heating and cooling cycles and centrifugation. The notably smaller droplet size compared to other conditions is the primary contributing factor to this stability. Finally, while some trends have been observed concerning the impact of the oil's chemical composition, this factor does not appear to exert significant influence.

5. CONCLUSIONS

This study has provided insights into the factors influencing the formation and stability of emulsions containing caffeine prepared by mechanical stirring, including the HLB value and surfactant ratio, the oil's chemical composition, and the caffeine's presence. Among these factors, the optimal HLB value and surfactant ratio have the most significant impact. Specifically, the highest stability for both the Caf-EM and the EM formulations is attained by employing a combination of nonionic surfactants (Span 80 and Tween 80) at a concentration of 15% with an HLB value of 6.4 (referred to as S15−6.4). This formulation demonstrates stability under real-time storage conditions and accelerated conditions involving heating−cooling cycles and centrifugation. The main reason for this stability is the significantly smaller droplet size than the other conditions. Additionally, the formulation S15−6.4 induces a phase inversion from O/W to W/O in both Caf-EM and EM. Caffeine in Caf-EM enhances the water phase hydrophobicity, promoting greater integration of oil molecules into water-phase droplets, fostering stronger attraction among polar molecules in

https://doi.org/10.1021/acsomega.3c03674
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the W/O droplets due to the reduced molecular distance facilitated by electrostatic attraction, and resulting in smaller emulsion sizes. Notably, the particle size of Caf-EM is approximately 1.5 times smaller than that of EM. Regarding the influence of the oil's chemical composition, although there is an observable trend in the emulsion droplet size with coconut oil > grape seed oil > sesame oil, these distinctions within the sequence lack notable variability, leading to the presumption that the oil's chemical composition does not have a discernible effect.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03674.

Typical literature values of the fatty acid composition (%) of additional oils; compared droplet sizes of Caf-EM measured by a particle size analyzer (Mastersizer) and an optical microscope; particle sizes, measured by a particle size analyzer (Mastersizer), of Caf-EM prepared from additional oils using the ideal formulation, S15−6.4; compared particle sizes, measured by a particle size analyzer (Mastersizer), of Caf-EM prepared at the same formulations but different oil types; and visual examination of water-soluble dye (methylene blue) droplets on Caf-EM and EM of the S15−6.4 formulation indicates a lack of dispersion substantiating that the specified formulation exhibits a water-in-oil (W/O) configuration (PDF)

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J.M.: Conceptualization, methodology, formal analysis, investigation. S.T.: Methodology, formal analysis, investigation. R.R.: Conceptualization, resources, validation. S.T.: Validation. A.W.: Conceptualization, supervision, data curation, investigation, visualization, writing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the Thailand Science Research and Innovation (TSRI) Fund (CU_FRB640001_01_63_1). The authors would also like to express their appreciation to Mahidol University Frontier Research Facility (MU-FRF) for supporting equipment for characterizations. Special thanks to Chawalit Takoon from MU-FRF and Miss Thammika Srisaman, a master's degree student of the Faculty of Science, Mahidol University, for their kind assistance in the operation of the particle size analyzer.

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