Impact of Surfactants on Nanoemulsions based on Fractionated Coconut Oil: Emulsification Stability and in vitro Digestion

Wei Gao¹, Zefang Jiang¹, Xiaojing Du¹, Fangfang Zhang¹, Yawen Liu¹, Xinpeng Bai¹,²* and Guangyou Sun³

¹ College of Food Science and Engineering, Hainan University, Haikou, 570203, CHINA
² Tropical Polysaccharide Resources Utilization Engineering Research Center of the Ministry of Education, Hainan University, Haikou, 570203, CHINA
³ Hainan Dabai Kangjian Pharmaceutical Co., Ltd., Haikou, 570203, CHINA

Abstract: Functional oils have broad application prospects in functional foods and beverages because of their rich beneficial ingredients and healthier intake. The small droplets of the nanoemulsion enhance the effective delivery, solubility and bioavailability of the various hydrophobic food components. This study used a mixed oil phase of green tea seed oil and fractionated coconut oil, compared the emulsifying properties of natural surfactants: Whey protein isolate, soy lecithin, tea saponin and synthetic surfactant: Tween 80 in the preparation of nanoemulsions by ultrasonic method. In particular, the impact of emulsifier type and concentration, pH, ionic strength, and heat treatment on the mean particle size and ζ-potential were investigated. The long-term storage stability of the fabricated nanoemulsions was also monitored during storage at different temperatures. In addition, the effects of emulsifier type on the bioavailability of nanoemulsions were evaluated. For all nanoemulsions studied, the mean particle size decreasing with increasing emulsifier concentration. Tea saponin and soy lecithin can produce smaller droplets of nanoemulsion than Whey protein isolate. Tea saponin has the same emulsifying ability as Tween 80. Presumably tea saponin-stabilized droplets may be maintained by electrostatic repulsion and steric repulsion. All of the nanoemulsions significantly improved the bioavailability of the mixed oil phase compared to the unemulsified oil phase. This study highlights the potential of natural surfactants in the ultrasonic preparation of nanoemulsions containing functional oils, and provides a basis for the application of natural surfactants and new functional oils in food industry.

Key words: nanoemulsions, natural surfactants, ultrasonic treatment, tea saponin, stability, bioavailability

1 Introduction

In recent years, food and beverage products consisting of functional ingredients have had more health benefits and have attracted more and more attention in the food industry⁹. *Camellia sinensis* L. belonging to the Camellia L. (Theaceae), is a small evergreen shrub or tree and the tea it produces is one of the most widely consumed drinks in the world. *Camellia sinensis* L. originated in the Yunnan-Guizhou Plateau of China and are mainly distributed in East Asia and Southeast Asia. Tea seeds of *Camellia sinensis* L. contain a lot of valuable nutrients, rich in tea polyphenols, VE, linoleic acid and other nutrients needed by the human body. Green tea seed oil is extracted from the seeds of *Camellia sinensis* L. which is the fruit of tea trees and currently a by-product of tea production. According to relevant data, the fatty acid composition ratio of green tea seed oil is most similar to that of olive oil and oleifera oil, but green tea seed oil is rich in natural tea polyphenols with characteristic indexes which is different from oleifera oil. Coconut oil is a mixture of various triglycerides, the content of lauric acid is more than 50%, and the content of C6, C8 and C10 fatty acids is above 15%. Therefore, it is considered to be the richest source of medium-chain fatty acids. In addition, virgin coconut oil (VCO) retains a large amount of natural active substances such as vitamins, polyphenols and phytosterols because it
is obtained from fresh, mature coconut pulp using cold pressing without the need for heating and refining\(^3\)\(^-\)\(^4\), which can have antioxidant, antithrombotic and anti-inflammatory effects\(^5\)\(^-\)\(^8\). However, the melting point of virgin coconut oil is 25\(^\circ\)C to 27\(^\circ\)C, which causes it to be easily solidified, this feature limits its application range in food industry. Constant temperature gradient field fractionation is a method which allows the oil to crystallize continuously at an environment of constant temperature difference. Glyceride molecules in oils can keep a multi-degree-of-freedom movement in non-isothermal environments. Molecules with higher melting points have enough time to form crystal nuclei in the temperature gradient field. By this method, a solid extract having a high melting point and a high purity can be formed and the VCO can be physically fractionated to obtain a fractionated coconut oil\(^9\). In this study, we used green tea seed oil and fractionated coconut oil to obtain a mixed functional oil phase, and explored the application of new oil phase in nano-transport systems.

The development of nanoparticle-based colloidal delivery systems has been one of the most important applications of nanotechnology within the food industry\(^10\). The small droplets of the nanoemulsion enhance the effective delivery, solubility and bioavailability of the various hydrophobic food components\(^11\). Due to their extremely small size, the nanocarrier has the advantages of improving water solubility, increasing residence time of the gastrointestinal tract, improving physicochemical stability of the gastrointestinal tract, increasing intestinal permeation, and controlling release\(^12\). Among various strategies, nanocarrier systems have been widely developed worldwide to effectively provide lipophilic nutraceuticals. When nanocarriers are used in food systems or oral delivery systems, they should be considered stable, non-toxic, biodegradable in food formulations, and suitable for use in a variety of food processing systems\(^13\)\(^-\)\(^16\). Recently, consumers have begun to focus on more natural and healthier products, which have led to a trend to replace synthetic surfactants with natural surfactants and natural plant substitutes, such as the use of phospholipids, biosurfactants, polysaccharides, proteins and biological products. Therefore, it is necessary to develop safe and efficient natural surfactants for nano-transport systems.

Nanoemulsions are made from a variety of food grade ingredients as surfactants, including synthetic surfactants, natural surfactants (e.g. phospholipids, proteins, polysaccharides), and sometimes these components are used in combination to form composite nanoparticles\(^13\). Currently, surfactants used in food processing are classified into synthetic surfactants and natural surfactants. Synthetic surfactants have the ability to promote nanodroplet formation with high efficiency, but their toxicity restricts their use in food grade nanoemulsions. Consequently, research on the application of natural surfactants in food processing has been widely concerned. For example, it has been reported that saponins, phospholipids, polysaccharides, and proteins can be used as good emulsifiers for the preparation of nanoemulsions\(^11\)\(^-\)\(^16\), and the nanoemulsions formed have significant differences in stability. The type and amount of emulsifier have a significant effect on droplet size distribution, optical properties, stability, bioavailability, and controlled release characteristics\(^17\). Therefore, it is necessary to compare the functional properties of different types of emulsifiers in order to prepare nanoemulsions in the food industry by selecting the most suitable emulsifiers according to different production needs. Ultrasonic can be used to prepare nanoemulsions with droplet sizes between 30 and 600 nm\(^18\). At present, there are some studies on the formation of nanoemulsions with different emulsifiers. However, little research has been done on the effects of emulsifier type (saponin, protein, phospholipid, synthetic surfactant) and content on the stability and bioavailability of nanoemulsions under ultrasonic methods.

The aim of this study to use green tea seed oil and fractionated coconut oil as the mixed oil phase to study the application of new functional oils in nanoemulsions. The stability and bioavailability of the nanoemulsion prepared by ultrasonication of whey protein isolate, soy lecithin, tea saponin and Tween 80 were compared. The results obtained from this study are very important for the development and production of “tag-friendly” nanoemulsions which is suitable for food industry applications using natural emulsifiers and functional oils.

2 Materials and Methods

2.1 Materials

Virgin coconut oil (VCO) and green tea seed oil were kindly supplied by Hainan Dabai Kangjian Pharmaceutical Co., Ltd. Fractionated coconut oil is prepared from VCO by constant temperature gradient field extraction. Soy lecithin, Whey protein isolate, Tea saponin and Tween 80 was purchased from XiLong Chemical Co., Ltd. Distilled and deionized water was used in all microemulsions.

2.2 Preparation of mixed oil phase

A temperature gradient field device used (Fig. 1) is a device for crystallization of oils and fats. It is mainly composed of a temperature control system, ultrasonic generator, double layer surface crystallizer and spiral tube heater\(^5\). Two liter VCO was poured into the crystallization vessel. The temperature of the spiral tube heater was maintained at 35\(^\circ\)C, and a constant temperature water of 10\(^\circ\)C was injected into the two-layer surface crystallizer through the circulating water temperature control system. Keep the ultrasonic generator power at 60 W. The crystallization lasted for 2 h and separated uncrystallized parts to
Impact of Surfactants on Nanoemulsions based on Fractionated Coconut Oil

J. Oleo Sci.

Fig. 1 Schematic diagram of the experimental device used for coconut oil extraction with gradient temperatures. Representing: 1. Temperature control system, 2. Mezzanine of the container, 3. Temperature control system, 4. Heating equipment, 5. Ultrasonic generator, 6. Condensation rod, 7. Cooling equipment.

get fractionated coconut oil.

Tea seed oil and fractionated coconut oil were kept in a constant temperature water bath at 60°C for 30 min to eliminate their crystal structure. Then mixed tea seed oil and fractionated coconut oil in a 3:2 ratio on a magnetic stirrer for 30 min. After that, the mixed oil was subjected to sonication for 15 min to obtain the mixed oil phase.

2.3 Nanoemulsion preparation

Preparation of nanoemulsions by ultrasonic method and study of the effects of emulsifiers and their concentrations on the formation of nanoemulsions. A water-in-oil nanoemulsion was prepared by homogenizing 10 wt% of the oil phase (mixed oil phase) with the aqueous phase. The aqueous phase consists of an emulsifier (0.5 wt%-10 wt% Whey protein isolate, soy lecithin, tea saponin or Tween 80) and a phosphate buffer solution (10 mM sodium phosphate buffer, pH 7.0). All the concentration of emulsifier used is reported on an active ingredient basis. All the emulsifiers were fully dissolved after being added to the aqueous phase by stirring with a magnetic stirrer for 12 h to fully hydrate. High-intensity ultrasonic homogenization were used to prepare nanoemulsions. To prepare the nanoemulsions, the oil phase and aqueous phase were pre-homogenized with UltraTurrax at 20,000 rpm for 2 min to get coarse emulsions. Later on, nanoemulsions were prepared by inducing coarse emulsions into ultrasonic homogenization at an output power of 320 W and 15 min. In order to avoid excessive heating caused by ultrasonic treatment during the experiments, all nanoemulsions were cooled by an ice bath during ultrasonic homogenization to keep the reaction stable at room temperature (25°C). After the formation, sodium azide (0.02 wt%) was added to the nanoemulsions as an antimicrobial agent.

2.4 Determination of mean particle size

The mean particle size distribution of the nanoemulsions was measured using static light scattering (Mastersizer 2000; Malvern Instruments, Malvern, UK). Before analysis, samples were diluted with phosphate buffer (10 mM, pH 7.0) to avoid multiple scattering effects. The refractive index was taken as 1.46 for nanoemulsion and 1.33 for phosphate buffer. The results were reported using mean particle size that is an average particle diameter calculated from the particle size distribution.

2.5 Determination of ζ-potential

ζ-potential were determined using an electrophoresis instrument (Mastersizer 2000). To avoid multiple scattering effects, samples were diluted with 10 mM pH 7.0 phosphate buffer prior to analysis.

2.6 Nanoemulsion stability testing

Nanoemulsion containing 10% (w/w) oil phase and 90% (w/w) aqueous phase (2.5% Whey protein isolate, soy lecithin, tea saponin or Tween 80 in buffer solution) were prepared as described in Section 2.2. The stability of different types of emulsifier-stabilized nanoemulsions under environmental stresses that may occur during food industry production and processing was tested.

2.6.1 pH stability

Newly produced nanoemulsions were placed in a series of different glass beakers and the samples were adjusted to different pH values (3-8) with 1 M HCl or 1 M NaOH solution. Then the samples were transferred to test tubes for storage and analysis. The sample was stored at room temperature for 24 h and then the mean particle size and ζ-potential were measured.

2.6.2 Ionic strength stability

Newly produced nanoemulsions (2 mL) were placed in a series of different glass test tubes and the final salt concentrations were adjusted to 100-500 mM by the addition of 2 mL of saline solution (NaCl solution). The sample was stored at room temperature for 24 h and then the mean particle size and ζ-potential were measured.

2.6.3 Thermal processing stability

Newly produced nanoemulsions (5 mL) were transferred into a series of different sealed glass test tubes that were placed into water baths set at different temperatures (40-80°C) for 30 min and then placed the glass test tubes in an iced-water bath and cooled to room temperature. After that, the sample was stored at room temperature for

J. Oleo Sci.
24 h and then the mean particle size and ζ-potential were measured.

2.6.4 Storage stability

The newly prepared nanoemulsions (5 mL) were transferred to different sealed tubes. They were then stored at different ambient temperatures (5°C, 27°C, 50°C), and the mean particle size of the nanoemulsions stored at different storage temperatures were measured at different storage times (7 day, 15 day, 30 day, 45 day).

2.7 In vitro digestion

Simulation of gastrointestinal conditions was performed by an international consensus approach\(^1\), including simulated gastric fluid and simulated intestinal fluid in order to investigate the in vitro simulated digestion process of nanoemulsions. The mean particle size of the nanoemulsion after simulated gastric phase digestion were determined, and the in vitro digestibility of the nanoemulsions was evaluated by measuring the release rate of free fatty acids (FFAs) in the simulated intestinal digestion stage.

To simulate the gastric phase, placed 5 mL aliquot of nanoemulsion in several Brown glass tubes, then 5 mL Milli-Q water was mixed, and added 7.5 mL of Simulated Gastric Fluid (SGF), 5 μL of CaCl\(_2\)(0.3 M) and HCl (2 M) to reach pH 3. After that, added 1.6 mL of pepsin solution dissolved in SGF to achieve 2000 U/mL in the final chyme. The total volume of the was 20 mL. For the small intestinal phase, added 11 mL Simulated Intestinal Fluid (SIF) to the gastric phase. The rest of the solutions were dissolved in SIF. Subsequently, added 2.5 mL bile solution (160 mM) and 40 μL CaCl\(_2\)(0.3 M), used Milli-Q water and NaOH (1 M) to bring the pH to 7. After that, added 5 mL pancreatic solution consisting of pancreatic lipase and pancreatin. The final lipase activity was 1600 U/mL of pancreatic solution, thus the final lipase activity in the digest was 200 U/mL. And the particle size of the nanoemulsions were measured.

3 Results and Discussion

3.1 Effect of emulsifier type and concentration on nanoemulsion characteristics

In industrial production, it is often necessary to select the appropriate type and concentration of emulsifier to meet the specific requirements for the droplet properties of the nanoemulsion. To evaluate the effect of emulsifier type and concentration on the formation and stability of nanoemulsions under the ultrasonic treatments, a series of 10% oil-containing oil-in-water nanoemulsions were prepared using different emulsifiers (whey protein isolate, soy lecithin, tea saponin, Tween 80), and each emulsifier was used at different concentrations (0.5, 1, 1.5, 2, 2.5, 5, 10%). After storage at room temperature for 24 h, the mean particle size and ζ-potential of these nanoemulsions were measured.

Initially, the effects of the types and concentration of several natural or synthetic emulsifiers (whey protein isolate, soy lecithin, tea saponin, Tween 80) on the particle size of nanoemulsions were investigated. Overall, the mean particle size of the nanoemulsions showed a significant decrease with the increase in the concentration of emulsifiers for all emulsifier types \((p < 0.05)\) (Fig. 2a). The mean particle size exhibited two trends that are common in emulsion formation: Significantly decreases when the surfactant concentration is at a low level, and remains stable at a high surfactant concentration\(^2\). The increased absolute ζ-potential values of emulsions provided a high energy barrier between emulsion droplets, thereby providing good electrostatic repulsion (Fig. 2b). For these emulsifiers, when the emulsifier concentration was at low levels from 0.5 to 2.5 wt%, the mean particle size of the nanoemulsions decreased with the increase of the emulsifier concentration. The nanoemulsion prepared by the synthetic emulsifier Tween 80 exhibited the smallest mean particle size, decreased from 297 nm to 130 nm. Similarly, the other three natural emulsifiers also significantly decreased the particle size of the nanoemulsion, in which tea saponin exhibited the best effect of decreasing the particle size and reduced the particle size of the nanoemulsion from 235 nm to 130 nm. And the particle size of nanoemulsion decreased from 568 nm to 204 nm for soy lecithin, and from 528 nm to 270 nm for whey protein isolate. The decrease in the mean particle size of the nanoemulsion is due to the increase of the interfacial area caused by the accumulation of surfactant molecules at the water-oil interface, which reduces the interfacial tension and prevents the droplets from flocculating\(^3\). There are two main reasons for this trend: (i) When the concentration of emulsifier is at a higher level, during the process of ultrasonic formation of the nanoemulsion, more emulsifier molecules can cover the surface of the oil droplet to stabilize the droplet. (ii) The higher the level of emulsifier concentration, the faster the emulsifier molecules adsorb to the surface of the oil droplets, thereby pre-
Impact of Surfactants on Nanoemulsions based on Fractionated Coconut Oil

Fig. 2 Influence of emulsifier type and concentration on the (a) mean particle size (b) \( \zeta \)-potential of 10 wt% mixed oil-in-water nanoemulsions produced by ultrasonic treatment (320 W, 15 min).

Inventing the re-agglomeration of the nanoemulsion more effectively\( ^{22} \). When the emulsifier concentration continued to increase to a high level at 5% and 10%, the mean particle size of the nanoemulsion prepared by Tween 80 was stable at 126 nm and 203 nm for soy lecithin, 267 nm for whey protein isolate, 133 nm for tea saponin. Overall, nanoemulsification maintained a small particle size, but there was no significant change, indicating that the droplet size was limited by the cavitation energy generated by sonication under operating conditions, rather than by emulsifier type and concentration.

Among these emulsifiers, tea saponin exhibited the best emulsifying properties of these natural surfactants when preparing nanoemulsions using ultrasonic treatments. The mean particle size of the nanoemulsion can be reduced to 200 nm by only 1 wt% of tea saponin. However, under the same sonication conditions, 2.5 wt% soy lecithin or 2 wt% Tween 80 was required to achieve the same effect. Besides, even in the high emulsifier concentration at 5 wt% and 10 wt%, whey protein isolate can only make the mean particle size of the nanoemulsions reach 267 nm. This behavior may be due to the relatively small emulsifier molecules being able to adsorb to the surface of the oil droplets faster, thereby promoting droplet formation and inhibiting droplet condensation during ultrasonication\( ^{23-25} \). Tea saponins are small molecules emulsifier belong to saponins, which can promote the formation of droplets at the water-oil interface faster. In addition, soy lecithin also exhibited better emulsifying properties than whey protein isolates and finally maintained the mean particle size of the nanoemulsions at 203 nm, this is mainly because that saponins and lecithins have smaller molecules than proteins, which can attach to the oil-water interface more quickly\( ^{26} \), thus exhibiting better emulsifying properties. Studies have shown that the non-ionic surfactant Tween80 can significantly reduce the surface tension of nanoemulsion systems. And because of the neutral Zeta-potential value of non-ionic surfactants, it can provide a more effective surface area, which is conducive to the formation and stability of oil-in-water nanoemulsions\( ^{27} \).

3.2 Nanoemulsion stability

Foods or beverages based on emulsifying systems may undergo a range of environmental stresses (e.g., pH, ionic strength, or heat treatment) during the production, storage, transportation, and processing of the food industry. Changes in these factors may adversely affect the stability of the nanoemulsion. Therefore, determining the impact of these factors on the nanoemulsion is very important. In this section, we investigated the effects of pH, ionic strength, and heat treatment on the mean particle size and \( \zeta \)-potential of nanoemulsions prepared with different emulsifiers. A series of nanoemulsions prepared with an oil content of 10% and an emulsifier content of 2.5% were selected for research.

3.2.1 Effect of pH

The effect of \( \mathrm{pH}(3 \sim 8) \) on the mean particle size and \( \zeta \)-potential of different emulsifier nanoemulsions was investigated (Fig. 3). Whey protein isolate-nanoemulsions were stable at \( \mathrm{pH} 6 \sim 8 \), but the mean particle size increased at \( \mathrm{pH} 3 \sim 5 \), and overall, the mean particle size of it had been at a high level. Soy lecithin-nanoemulsion maintained a stable mean particle size in the environment of \( \mathrm{pH} 6 \sim 8 \), but the mean particle size increased in the range of \( \mathrm{pH} 3 \sim 5 \). Tea saponin-nanoemulsion had always maintained a small and stable mean particle size in the environment of \( \mathrm{pH} 3 \sim 8 \) (around 134 nm) which was similar to Tween 80-nanoemulsion (Fig. 3a).

To clarify the effect of \( \mathrm{pH} \) on the stability of nanoemulsions stabilized by different emulsifiers, we measured the \( \zeta \)-potential of the nanoemulsions (Fig. 3b). Studies have shown that the nanoemulsion system has good stability when the \( \zeta \)-potential of the nanoemulsion is above 30 mV (absolute value)\( ^{28} \). For the Whey protein isolate-nanoemulsions, the \( \zeta \)-potential on the droplets went from moderately positive at \( \mathrm{pH} 3( \pm 25.6 \text{ mV}) \) to strongly negative at \( \mathrm{pH} 7( -52.8 \text{ mV}) \). At \( \mathrm{pH} 3 \sim 5 \), the absolute value of the
potential of the nanoemulsion was detected to be lower than 30 mV, resulting in the nanoemulsion exhibiting instability, which corresponds to an increase in the particle size of the nanoemulsion in this range. The absolute value of the potential is high at pH 6~8, and correspondingly, the nanoemulsion in this range exhibits better stability and smaller particle size. This is because the isoelectric point of the adsorbed protein is around pH 4, and the droplets had the lowest charge magnitude near the isoelectric point, thereby weakening the electrostatic repulsion\(^2\), which results in a large nanoemulsion droplet diameter. Whey protein isolates act as emulsifiers to stabilize nanoemulsions, the stability of nanoemulsions is primarily maintained by strong electrostatic repulsion between the droplets. When the pH is close to the isoelectric point, the electrostatic repulsion is not sufficient to overcome the attraction interaction between the particles (e.g., van der Waals and hydrophobic). For soy lecithin-nanoemulsion, the absolute value of the \(\zeta\)-potential in the range of pH 5~8 is kept above 30 mV, from -32 mV at pH 5 to -62.9 mV at pH 8. The good stability of the nanoemulsions at high pH range may therefore be partly due to a strong electrostatic repulsion between the highly charged droplets. In the range of pH 3~4, the absolute value of the \(\zeta\)-potential of soybean lecithin-nanoemulsion is above 15 mV and it is much lower than pH 6~8 and the mean particle size is increased. This is because soy lecithin contains phospholipids such as phosphatidylcholine, phosphatidylchinositol and phosphatidic acid, and the tail of these phospholipids is combined with a hydrophilic group. The phospholipid group contains an ionic anionic group having a pKa value in the acidic pH range. Therefore, when it is at lower pH values may lose its charge, resulting in a decrease in the stability of the nanoemulsion. For tea saponin-nanoemulsion, the mean droplet diameter remained relatively small and stable across the range of pH 5~8, the \(\zeta\)-potential of the tea saponin-nanoemulsion changes significantly with the change of pH, and the \(\zeta\)-potential changes from a strong negative at high pH 5~8 (Maximum -60.8 mV) to a weak negative at low pH 3~4 (Minimum -23.4 mV). The result indicates that the tea saponin-nanoemulsion maintains the stability mainly by electrostatic repulsion between the droplets. Therefore, the stability of the nanoemulsion at low pH values may be deteriorated due to the weakening of the electrostatic repulsion. The negative \(\zeta\)-potential exhibited by the droplets is caused by the carboxylic acid groups on the adsorbed saponin molecule, and the \(\zeta\)-potential becomes strongly negative when the gradual ionization of the carboxyl group on the saponin molecule\(^3\sim^\ref{footnote Add 8}.\) The results of our study are consistent with previous studies on the \(\zeta\)-potential of saponin-stabilized droplets\(^3\). However, tea saponin is able to maintain a small mean particle size, because the droplets of tea saponin-nanoemulsion appeared to be stabilized by electrostatic and steric repulsion in the same time. The Tween 80-nanoemulsions remained stable throughout the pH range, although they had weak negative \(\zeta\)-potential (around -15 mV). This is because their large polymeric head groups, so that they mainly maintain the stability of the droplets by steric repulsion\(^3\). 

3.2.2 Effect of ionic strength

In order to study the effect of ionic strength on the stability of nanoemulsions, different levels of salt (100~500 mM NaCl) were added to different emulsifier-stabilized nanoemulsions, and then the mean particle size and \(\zeta\)-potential were measured (Fig. 4). The soybean lecithin-nanoemulsion did not observe a significant change in the mean particle size at an ionic strength of 100 mM, however, when the ionic strength was increased from 100 mM to 500 mM, the mean particle size was significantly increased. At each ionic strength studied, the whey protein-stabilized nanoemulsion was not observed to have a significant change in the mean particle size. On the other hand, we observed only a slight change in the mean particle size of tea saponin-nanoemulsion and Tween 80-nanoemulsion in the range of ionic strengths studied (Fig. 4a).

The determination of the \(\zeta\)-potential showed that the
potential of the soybean lecithin-nanoemulsion changed from a strong negative value (−61.6 mV) to a weak negative value (-15 mV) as the ionic strength increased (Fig. 4b). We attribute the decrease in the absolute value of the potential to the electrostatic screening effect. It is indicated that the addition of the NaCl solution reduces the amount of charge around the droplets, and the electrostatic repulsion that maintains the stability of the droplets is weakened, resulting in an increase in the mean particle size. When no salt solution is added or the salt concentration is low, the hydrophobic tail region of the surfactant was isolated, and the polar groups are in contact with the surrounding emulsion, so that the droplets of the oil-in-water nanoemulsion can remain stable. As the salt concentration increases, the addition of salt ions adsorbed a part of water molecules, which reduced the number of water molecules that interacted with the charged part of the surfactant, the repulsive force between the polar groups decreases, and the hydrophilic head begins to gather in the center, causing the oil-in-water droplets to be destroyed and the average droplet diameter to increase. For the whey protein isolate, there was no significant change in the absolute value of the \( \zeta \)-potential in the range of ionic strength from 100 mM to 500 mM, which corresponds to the stability of the mean particle size. This effect was probably due to the whey protein isolate formed partial steric interaction between the droplets, which is sufficiently large enough to prevent the droplet from aggregation. On the other hand, it may be the electrostatic screening effect caused by the addition of the salt solution is not strong enough to lead the electrostatic repulsion to weaken. The potential of the tea saponin-nanoemulsion changed from a high negative value at 100 mM to a low negative value at 500 mM throughout the range of ionic strengths studied, which is inconsistent with the fact that it was found to have a slight change in the mean particle size. This result is consistent with previously reported results, based on the analysis, it is flocculation rather than coalescence causes the aggregation observed at high salt levels. When the ionic strength is at a low level, the long-range electrostatic repulsion between the droplets is strong enough to prevent aggregation and keep the nanoemulsions stable. However, at high ionic strength, van der Waals force between the droplets is stronger than the electrostatic repulsion, causing the nanoemulsions to flocculate. However, at high ionic strength, the short-range steric repulsion is still strong enough to prevent the droplets from coalescing.

As the ionic strength increased, the Tween 80-nanoemulsion maintained a low negative potential, and the mean particle size increased slightly in the range of ionic strength from 200 mM to 500 mM. Presumably because the dehydration of the hydrophilic polymer head group of the non-ionic surfactant after the ionic strength is increased. However, Tween 80-nanoemulsions exhibited good stability in the ionic strengths studied. The results of the study proved that Tween 80-nanoemulsions are mainly resistant to aggregation by steric repulsion, rather than electrostatic repulsion.

3.2.3 Effect of thermal treatment

In order to evaluate the thermal stability of different emulsifier nanoemulsions, the mean particle size and \( \zeta \)-potential of nanoemulsions after heat treatment at several temperatures (30°C ~ 80°C) for 45 min were studied (Fig. 5). Soy lecithin-nanoemulsion exhibits good thermal stability, maintains high negative \( \zeta \)-potential, and has no significant instability over the entire temperature range (Fig. 5b). The result indicates that a strong electrostatic repulsion can still be maintained between the droplets of the soybean lecithin-nanoemulsion during the heat treatment. Similarly, the tea saponin-nanoemulsion was also detected to have a small mean particle size after heat treatment (Fig. 5a), which is related to the potential of the nanoemulsion to maintain a stable and high negative value, leading to a strong electrostatic repulsion. The whey protein isolate-nanoemulsion was measured to have a mean

![Fig. 4](image_url) Influence of Ionic Strength on (a) the mean particle size (b) the \( \zeta \)-potential of 10 wt% mixed oil-in-water nanoemulsions containing 2.5 wt% emulsifiers produced by ultrasonic treatment (320 W, 15 min).
trostatic repulsion is relatively weak, which is related to a decrease in the absolute value of the ζ-potential. Presumably, heat treatment caused a certain degree of denaturation of the protein, thereby reducing the amount of negative charges on the surface of the droplets, caused the electrostatic repulsion provided was not enough to maintain the stability of the droplet. It has been reported that when spheroidal proteins are used to stabilize oil droplets, the temperature of the heat treatment exceeds the thermal denaturation temperature of the protein, which leads to the accumulation of droplets, particularly when the electrostatic repulsion is relatively weak. This is because as the adsorbed globular protein unfolded, the non-polar group of proteins was exposed to the surrounding water phase, causing an increase in hydrophobic attraction between the droplets. The Tween80-nanoemulsion remained stable throughout the heat treatment range except for a significant increase in the mean particle size after treatment at 80°C. This phenomenon can be explained by the fact that when the heat treatment temperature is close to the phase inversion temperature of the nonionic surfactant, the droplets tend to have a tendency to coalescence, resulting in instability of the nanoemulsions.

3.2.4 Storage stability

The storage stability of soybean lecithin-, whey protein isolate-, tea saponin-, Tween 80-nanoemulsion was studied at three storage temperatures (5°C, 27°C, 50°C). The mean particle size of the nanoemulsions were measured at 7 days, 15 days, 30 days, and 45 days, respectively (Fig. 6). Soy lecithin-nanoemulsion were not measured to have significant increase in the mean particle size during storage at 5°C, and the mean particle size appeared slightly increase after storage for 45 days at 27°C storage temperature, thus we consider them to be generally stable. In contrast, these nanoemulsions have better storage stability, and this result can be considered as the rate of gravity separation decreases with the reduction in droplet size. Additionally, the Brownian motion between small droplets can overcome the gravity separation forces that often lead to the instability of nanodispersions. In addition, the negative charge around the droplets provides sufficient electrostatic repulsion to inhibit the accumulation of droplets during storage. However, during storage at 50°C, soy lecithin-nanoemulsions observed significant phase separation and an increase in the mean particle size after 30 days of storage compared to the newly prepared nanoemulsions (Fig. 7), it may be that long-term heat preservation enhances the frequency of droplet-droplet collisions or changes in the interfacial properties to make them more susceptible to breakage and then causes phase separation. The mean particle size of the nanoemulsion increased with the increase of storage time because the emulsion droplets coalesced during storage. The size of the nanoemulsion droplets and their Brownian motion determines the stability of the nanoemulsion system during storage. Therefore, the smaller the average particle size of the nanoemulsion, the better the storage stability. Whey protein isolate also maintains good storage stability after storage for 45 days at 5°C and 27°C, this is because the whey protein isolate formed the strong electrostatic surface potential which combined with the steric repulsion between the droplets can prevent the droplets from aggregation. But, the whey protein isolate-nanoemulsion was measured for a significant mean particle size increase after storage for 15 days at 50°C. The reason for this phenomenon is that the continuous heating during storage denatures the whey protein isolate, resulting in a decrease in the amount of negative charge around the droplets, thus promoting droplets aggregation. When adsorbed globular proteins are maintained under high temperature conditions, they tend to undergo a conformational transition ("surface denaturation"), causing some non-polar groups that are originally located in the hydrophobic surface to be exposed. Therefore, the hydrophobic at-
traction between the lipid droplets is increased, thereby promoting the flocculation of the droplets. On the other hand, tea saponin-nanoemulsions maintain good stability at all storage temperatures studied and have little change in mean particle sizes. The result indicates that the tea saponin can effectively prevent the droplets from agglom-

Fig. 6 Influence of storage time at (a) 5°C (b) 27°C (c) 50°C on the stability of 10 wt% mixed oil-in-water nanoemulsions containing 2.5 wt% emulsifiers produced by ultrasonic treatment (320 W, 15 min).

Fig. 7 10 wt% mixed oil-in-water nanoemulsions prepared with 2.5 wt% different emulsifiers by ultrasonic treatment (320 W, 15 min): (a) New preparation, and after storage at (b) 5°C (c) 27°C (d) 50°C for 45 days.
Generating, which is related to the fact that tea saponin can provide strong electrostatic repulsion for droplets. This study did not observe significant changes in the phase separation or the mean particle size of the Tween 80-nanoemulsions after stored at 5°C and 27°C for 45 days. Conversely, we observed a significant increase in the mean particle size after 45 days storage at 50°C. Presumably due to changes in the optimal curvature or hydrolysis of the surfactant head group under high temperature storage conditions. Similar results with published reports, demonstrating that Tween 80-nanoemulsions may be unstable during long-term storage at high temperatures.

3.5 In vitro digestion

The mean particle size and stability of the nanoemulsion affect its bioavailability in gastrointestinal digestion. In order to evaluate the release properties of nanoemulsions stabilized by different emulsifiers, the mean particle size of nanoemulsions after simulated gastric phase digestion for 1 hour and the release rates of free fatty acids (FFAs) at different stages (7.5, 15, 30, 45, 60, 90, 120 min) during simulated intestinal digestion were studied.

3.5.1 Effect of simulated gastric phase digestion on the mean particle size

The type of emulsifier used to stabilize the nanoemulsions had a significant impact on the rate and extent of lipid in vitro digestion (Fig. 8). After simulated gastric phase digestion, the mean particle size of whey protein isolate-nanoemulsion increased significantly. This phenomenon may be due to partial hydrolysis of part of the whey protein isolated by the emulsion interface by pepsin, causing partial emulsion droplet aggregation. This is similar to the reported enzymatic hydrolysis results using soy protein to prepare a stable oil-in-water emulsion. The mean particle size of soy lecithin-nanoemulsions increased slightly, presumably related to its instability at low pH levels in the previous study. On the other hand, tea saponin and Tween 80 stabilized nanoemulsions showed good stability after simulated gastric phase digestion which are well resistant to digestion of gastric phase, which is related to their ability to maintain a small particle size at high pH.

3.5.2 Effect of emulsifier types on bioavailability of gastrointestinal digestion

The bioavailability of nanoemulsions stabilized by different emulsifiers in the simulated intestinal digestion stage was determined by measuring the percentage of FFA release. After the nanoemulsion enters the small intestine, the triglyceride is rapidly decomposed into a diglyceride or a monoglyceride and a free fatty acid by the action of a lipase. In the small intestine simulated digestion, the volume of NaOH consumed to maintain the neutral pH of the system can be converted to the release rate of FFAs. The FFAs release rate is an important indicator for measuring the bioavailability of bioactive components. On the whole, the FFAs release of several different emulsifier-stabilized nanoemulsions increased rapidly and then slowly increased (Fig. 9). The FFAs release rate after simulated digestion of whey protein isolate-nanoemulsion was 68%, 88% for soy lecithin-nanoemulsion, 98.6% for tea saponin-nanoemulsion and 92.8% for Tween 80-nanoemulsion. In contrast, the uncoated oil phase FFAs release rate was only 41.8%. Overall, the nanoemulsion system we studied significantly improved the digestibility of the oil phase.

The release of free fatty acids of the whey protein iso-

---

Fig. 8 Effect of simulated gastric phase digestion on the mean particle size of 10 wt% mixed oil-in-water nanoemulsions prepared with 2.5 wt% different emulsifiers by ultrasonic treatment (320 W, 15 min).

Fig. 9 The change of FFAs release rate of the 10 wt% mixed oil-in-water nanoemulsions prepared with 2.5 wt% different emulsifiers by ultrasonic treatment (320 W, 15 min) at different times (7.5, 15, 30, 45, 60, 90, 120 min) during the simulated intestinal phase digestion.
Impact of Surfactants on Nanoemulsions based on Fractionated Coconut Oil

J. Oleo Sci.

4 Conclusions

This study compared the effectiveness of different natural emulsifiers in fabricating nanoemulsions using an ultrasonic method. The current work evidences that these natural emulsifiers we studied can be used to effectively form and stabilize nanoemulsions containing tea seed oil and fractionated coconut oil. The mean particle size of all types of nanoemulsions decreased with increasing emulsifier concentration. Tea saponin and soy lecithin were capable of producing smaller droplet diameters than whey protein isolates, wherein the ability of tea saponin to form small emulsion droplets was close to that of the synthetic surfactant Tween 80. In addition, the bioavailability of the oil phase was significantly improved after the formation of nanoemulsion droplets using these natural surfactants.

The stability of nanoemulsions under environmental stresses was highly dependent on the type of emulsifier. The stability of Whey protein isolate-nanoemulsion was less affected by ionic strength but decreased in the range of pH 3 ~ 5 or heat treatment temperature is higher than 60°C. Soy lecithin-nanoemulsion exhibited instability at low pH value and high ionic strength, but was less affected by heat treatment. The Tea saponin-nanoemulsion was well tolerated to ambient pressure and was capable of maintaining good stability over the range studied so exhibited good emulsifying ability. All the nanoemulsions studied stabilized by natural surfactants exhibited good stability when stored at 5°C. However, only Tea saponin-nanoemulsion maintained stability during long-term storage at 27°C and 50°C. Compared to the synthetic surfactant Tween 80, tea saponin has the potential to be an effective natural surfactant for the formation and stabilization of nanoemulsions, suitable for use in food and other industrial applications, and it has an advantage in emulsifying ability compared with other natural emulsifiers such as protein or phospholipid. However, more research is needed on the toxicity and suitability of Tea saponin compared to Whey protein isolate and soy lecithin. In summary, our research shows that a stable nanoemulsion system containing green tea seed oil and fractionated coconut oil can be prepared by ultrasonic method using different natural emulsifiers. Therefore, the results of this study are useful for applying natural emulsifiers and functional oils to foods and beverages.

Acknowledgements

Funding for this work was provided by the Key Research and Development Program Project of Hainan Province (ZDYF2019021) and the Comprehensive Processing and Industrialization of Woody Oils and Fats (HD-KYH2017205). We also thank Hainan Daai Kangjian Pharmaceutical Co., Ltd. For donating Coconut oil and Green tea seed oil.

References

1) Piorkowski, D.T.; McClements, D.J. Beverage emulsions: Recent developments in formulation, production, and applications. Food Hydrocoll. 42, 5-41 (2014).
2) Marina, A.M.; Man, Y.B.C.; Amin, I. Virgin coconut oil: emerging functional food oil. Trends Food Sci. Tech. 20, 481-487 (2009).
3) Seneviratne, K.N.; Dissanayake, D.M.S. Variation of phenolic content in coconut oil extracted by two conventional methods. Int. J. Food Sci. Tech. 43, 597-602 (2008).
4) Seneviratne, K.N.; Hapuarachchi, C.D.; Ekanayake, S.
Comparison of the phenolic-dependent antioxidant properties of coconut oil extracted under cold and hot conditions. *Food Chem.* **114**, 1444-1449 (2008).

5) Cardoso, D.A.; Moreira, A.S.; de Oliveira, G.M.; Raggio, L.R.; Rosa, G. A coconut extra virgin oil-rich diet increases HDL cholesterol and decreases waist circumference and body mass in coronary artery disease patients. *Nutr. Hosp.* **32**, 2144-2152 (2015).

6) Dosumu, O.O.; Duru, F.I.O.; Osmibiu, A.A.; Oremosu, A.A.; Noronha, C.C. Influence of virgin coconut oil (VCNO) on oxidative stress, serum testosterone and gonadotropic hormones (FSH, LH) in chronic ethanol ingestion. *Agriculture & Biology Journal of North America* **1**, 1126-1132 (2010).

7) Vysakh, A.; Ratheesh, M.; Rajmohan, T.P.; Pramod, C.; Premal, S.; Kumar, B.G.; Sibi, P.I. Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. *Int. Immunopharmac.* **20**, 124-130 (2014).

8) Zakaria, Z.A.; Ahmad, Z.; Somchit, M.N.; Arifah, A.K.; Khairi, H.M.; Sulaiman, M.R.; Long, K. Anti hypercholesterolemia property and fatty acid composition of mardi-produced virgin coconut oils. *African Journal of Pharmacy & Pharmacology* **4**, 636-644 (2010).

9) Wu, L.; Cao, J.; Bai, X.; Chen, H.; Zhang, Y.; Wu, Q. Effects of ultrasonic parameters on the crystallization behavior of virgin coconut oil. *J. Oleo Sci.* **65**, 967-976 (2016).

10) McClements, D.J.; Decker, E.A.; Park, Y.; Weiss, J. Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Crit. Rev. Food Sci. Nutr.* **49**, 577-606 (2009).

11) Cheng, Q.; McClements, D.J. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocoll.* **25**, 1009-1018 (2011).

12) Oehlke, K.; Adamiku, M.; Behsnilian, D.; Gräf, V.; Mayer-Miebach, E.; Walz, E.; Greiner, R. Potential bioavailability enhancement of bioactive compounds using food-grade engineered nanomaterials: a review of the existing evidence. *Food Funct.* **5**, 1341-1359 (2014).

13) McClements, D.J. The future of food colloids: Next-generation nanoparticle delivery systems. *Curr. Opin. Colloid Interface Sci.* **28**, 7-14 (2016).

14) Long, B.; Huan, S.; Gu, J.; McClements, D.J. Fabrication of oil-in-water nanoemulsions by dual-channel microfluidization using natural emulsifiers: Saponins, phospholipids, proteins, and polysaccharides. *Food Hydrocoll.* **61**, 703-716 (2016).

15) Xu, X.; Sun, Q.; McClements, D.J. Enhancing the formation and stability of emulsions using mixed natural emulsifiers: Hydrolyzed rice glutelin and quillaja saponin. *Food Hydrocoll.* **89**, 396-405 (2019).

16) Zhu, Z.; Wen, Y.; Yi, J.; Cao, Y.; Liu, F.; McClements, D.J. Comparison of natural and synthetic surfactants at forming and stabilizing nanoemulsions: Tea saponin, Quillaja saponin, and Tween 80. *J. Colloid Interface Sci.* **536**, 80-87 (2019).

17) Chang, Y.; McClements, D.J. Influence of emulsifier type on the *in vitro* digestion of fish oil-in-water emulsions in the presence of an anionic marine polysaccharide (fucoidan): Caseinate, whey protein, lecithin, or Tween 80. *Food Hydrocoll.* **61**, 92-101 (2016).

18) McClements, D.J. Edible nanoemulsions: Fabrication, properties, and functional performance. *Soft Matter* **7**, 2297-2316 (2011).

19) Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Brodkorb, A. A standardised static in-vitro digestion method suitable for food – an international consensus. *Food Funct.* **5**, 1113-1124 (2014).

20) McClements, D.E. Food Emulsions: Principles, Practices, and Techniques. 2nd ed. CRC Press, Boca Raton, FL (2004).

21) Bera, A.; Mandal, A.; Kumar, T. Physicochemical characterization of anionic and cationic microemulsions: Water solubilization, particle size distribution, surface tension, and structural parameters. *J. Chem. Eng. Data* **59**, 2490-2498 (2014).

22) Yang, Y.; Leser, M.; Sher, A.A.; McClements, D.J. Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale(R)). *Food Hydrocoll.* **30**, 589-596 (2013).

23) McClements, D.J.; Gumus, C.E. Natural emulsifiers—Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv. Colloid Interface Sci.* **234**, 3-26 (2016).

24) Qian, C.; Decker, E.A.; Xiao, H.; McClements, D.J. Comparison of biopolymer emulsifier performance in formation and stabilization of orange oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **88**, 47-55 (2011).

25) Qian, C.; Decker, E.A.; Xiao, H.; McClements, D.J. Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chem.* **132**, 1221-1229 (2012).

26) Bouyer, E.; Mekhloufi, G.; Rosilio, V.; Grossiord, J.L.; Agnely, F. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field? *Int. J. Pharm.* **436**, 359-378 (2012).

27) Kumar, N.; Mandal, A. Thermodynamic and physicochemical properties evaluation for formation and characterization of oil-in-water nanoemulsion. *J. Mol. Liq.* **266**, 147-159 (2018).

28) Jacobs, C.; Kayser, O.; Müller, R.H. Nanosuspensions as a new approach for the formulation for the poorly
soluble drug tarazepide. *Int. J. Pharm.* 196, 161-164 (2000).

29) Xu, X.; Luo, L.; Liu, C.; Zhang, Z.; McClements, D. J. Influence of electrostatic interactions on behavior of mixed rice glutelin and alginate systems: pH and ionic strength effects. *Food Hydrocoll.* 63, 301-308 (2017).

30) Böttcher, S.; Drusch, S. Saponins — Self-assembly and behavior at aqueous interfaces. *Adv. Colloid Interface Sci.* 243, S0001868616302962 (2017).

31) Ozturk, B.; Argin, S.; Ozilgen, M.; McClements, D.J. Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin. *J. Food Eng.* 142, 57-63 (2014).

32) Shu, G.; Khalid, N.; Chen, Z.; Neves, M.A.; Barrow, C.J.; Nakajima, M. Formulation and characterization of astaxanthin-enriched nanoemulsions stabilized using ginseng saponins as natural emulsifiers. *Food Chem.* 255, 67-74 (2018).

33) Kenar, J. Food emulsions: Principles, practices, and techniques. *International News on Fats, Oils.* (December) (2005).

34) Bera, A.; Kumar, T.; Ojha, K.; Mandal, A. Screening of microemulsion properties for application in enhanced oil recovery. *Fuel* 121 (4), 198-207 (2013).

35) Pal, N.; Saxena, N.; Mandal, A. Phase behavior, solubilization, and phase transition of a microemulsion system stabilized by a novel surfactant synthesized from castor oil. *J. Chem. Eng. Data* 62, 1278-1291 (2017).

36) Amir Hossein, S.; Yuan, F.; David Julian, M. Stabilization of vitamin E-enriched nanoemulsions: Influence of post-homogenization cosurfactant addition. *J. Agric. Food Chem.* 62, 1625-1633 (2014).

37) McClements, D.J. Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter* 8, 1719-1729 (2012).

38) Kim, H.J.; Decker, E.A.; McClements, D.J. Impact of protein surface denaturation on droplet flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin. *J. Agric. Food Chem.* 50, 7131-7137 (2002).

39) Kishore, R.S.K.; Pappenberger, A.; Dauphin, I.B.; Ross, A.; Buergi, B.; Staempfli, A.; Mahler, H.C. Degradation of polysorbates 20 and 80: Studies on thermal autoxidation and hydrolysis. *J. Pharm. Sci.* 100, 721-731 (2011).

40) Nik, A.M.; Wright, A.J.; Corredig, M. Micellization of beta-carotene from soy-protein stabilized oil-in-water emulsions under *in vitro* conditions of lipolysis. *J. Am. Oil Chem. Soc.* 88, 1397-1407 (2011).

41) McClements, D.J.; Li, Y. Review of *in vitro* digestion models for rapid screening of emulsion-based systems. *Food Funct.* 1, 32-59 (2010).

42) Howles, P.N.; Carter, C.P.; Hui, D.Y. Dietary free and esterified cholesterol absorption in cholesterol esterase (bile salt-stimulated lipase) gene-targeted mice. *J. Biol. Chem.* 271, 7196-7202 (1996).