Impact of different homogenization methods on properties of tea polyphenol loaded on solid in oil - water emulsion

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ABSTRACT:

Polyphenols of tea origin are prone to oxidation which limits their bioavailability. The unstable nature of polyphenols limits their application in the prevention of major human diseases. Therefore, in the current study, we aimed to encapsulate tea polyphenol powder in (S/O/W) emulsions stabilized by Whey Protein Isolate (WPI) by using three different of homogenization processes, the emulsions were characterized for their physicochemical propertiesincluding particle size distribution, microstructure, mean emulsion size, zeta potential, and physical stability under environmental stress. The finding showed that the encapsulation efficiency of S/O/W emulsion homogenized by high-intensity ultrasound (HIU) had more encapsulation efficiency (95.95 ± 0.14) of tea polyphenol. The HPH and HIU emulsions had minimum droplet size (d3.2) between 0.45 and 0.48 μm respectively and were relatively more stable. We suggest that S/O/W emulsion could be used as an efficient delivery system of tea polyphenol in food for improved stability and bioavailability during storage.

Keywords: S/O/W emulsion, Tea polyphenol, Encapsulation, Ultrasound emulsification, Homogenization.

INTRODUCTION.

In the past decades, emulsions have been widely used as delivery systems for incorporating bioactive compounds into foods. Numerous delivery systems have been studied for lipophilic bioactive ingredients, e.g., oil-soluble vitamins, flavors, preservatives, colors pharmaceuticals, and nutraceuticals.(Joye & McClements, 2014). However, delivery systems for hydrophilic bioactive food ingredients have not been studied in detail. The multilayer emulsions could provide chemical stability under different conditions of temperature, storage, acidity, various digestion processes, and improved chemical stability to bioactive components. (Benjamin et al., 2012). Proteins are able to form an interfacial layer through the repulsive electric forces generated between oil droplets leading to increased stability of emulsions during storage. Whey protein isolate is a type of protein that is widely used as emulsifiers in the food industry to increase the stability of emulsions (Livney, 2010; McClements, 2004). Lecithin is a natural emulsifier with a zwitterionic surface characteristic used in the formulation of emulsifiers to reduce surface tension between emulsion droplets (Mottola et al., 2015). Lecithin function could be attributed to its amphiphilic structure, which contains groups of fatty acids as a lipophilic part and phosphoric esters group as a hydrophilic group. High-intensity Ultrasound (HIU) has been classified into low and high-power ultrasound based on their frequency range (Awad et al., 2012). An increase in the power or duration of ultrasound results in a reduction of oil droplets size in the emulsions which contain a mixture of whey protein isolate and xanthan (Kaltsa et al., 2014). High-pressure homogenization (HPH) technology is widely used in the food industry to modify the physicochemical properties of food and increase the stability of emulsions. Also, the HPH affects the secondary structure of most the globular proteins (Zhao et al., 2018). In High-pressure homogenization treatment, the fluid was exposed to the integration of high shear stress, cavitation, and high turbulence flow leading to decrease in the size of oil droplets and provide droplet size distribution (PSD) with the monomodal curve to increase the stability of emulsions (Gadkari et al., 2017). For many years, the various range of bioactive compounds and food with different functional and nutritional properties created using emulsion as a colloidal delivery system. In recent years, the role of green tea has improved from traditional beverage and spread around the world as an important source of bioactive components with many health benefits. The (-)-epigallocatechin gallate (EGCG) and (- - epigallocatechin (EGC) (−)-epicatechin (EC) and (−) epigallocatechin-gallate (EGCG), are the main groups present in tea polyphenol (Liang et al., 2008). Many of beneficial health effect including protection of cardiovascular, (Kuriyama et al., 2006) cancer (Xiang et al., 2016), Anti-diabetes (Fu et al., 2017), antioxidative and anti-inflammatory were associated with consummation of green

tea and tea polyphenol (Oz et al., 2013). The bioavailability of green tea polyphenol is very low due to their poor absorption, limited permeability, and less stability in the small intestine. For example, (Lin et al., 2007) reported that less than 5 % of the oral dose of catechins amount to the systemic blood circulation taken by rats. (Green et al., 2007) reported that more than 80 % of total catechins which occur in green tea deteriorated in simulated digestive models. Interest in the bioavailability of tea polyphenols has increased from tea-based foods and beverages. Food grade is generally abundant in terms of biocompatibility, biodegradability, low cost, and functions, which have significant potential for the delivery of biologically active compounds through the human digestive tract to the target tissues. (Nesterenko et al., 2013). Nevertheless, increasing pieces of evidence suggest that tea polyphenols are highly unstable, limiting their application. Several factors such as temperature, light, pH, oxygen, polyphenols concentration, and the level of oxidants affect the stability of polyphenols (Li et al., 2012). The advantage of (S/O/W) emulsion is the delivery efficiency of bioactive compounds without dissolution in any phases to maintain the efficiency of the active ingredients. In contrast, water emulsions in water (W/O/W) are used to deliver hydrophilic bioactive compounds by dissolving them in the internal phase (Garti & Bisperink, 1998). However, the loading of (W/O/W) emulsions is low, and the inner drops are unstable in the emulsion, and the bioactive compounds have low activity during storage and different food treatments (Vasiljevic et al., 2006). Therefore, in this study, we aime to investigate the influence of different methods of homogenization on physicochemical properties of tea polyphenol-loaded in S/O/W emulsions stabilized by Whey Protein Isolate WPI and fabricated by high shear, high-pressure homogenization, and high-intensity ultrasounds, as well as, evaluation of the critical measurements of the emulsion's stability through different conditions e.g. various temperature, pH and ionic strength. The findings of our study could provide knowledge about the ability of effectiveness of S/O/W emulsions to encapsulated tea polyphenol as hydrophilic bioactive compounds and prevent them from degradation during storage in food or gastrointestinal conditions. Furthermore, our findings could also facilitate the design of a colloidal delivery system that may control the gradual release of bioactive compounds and lipid digestion in the application of food industries and pharmaceuticals.

MATERIAL AND METHODS.

Material.

60 (G) Polyphenol from green tea powder contain (carbon 54.0 - 56.5 %, nitrogen < 3.5 %; total catechin content > 60 %) were obtained from Sigma-Aldrich. Soybeans oil were purchased from the local market in Wuhan, China. Lecithin, span 80®, sodium azide, Calcium chloride ($CaCl_2$), hydrochloric acid (HCl), sodium chloride (NaCl), and sodium hydroxide (NaOH) were purchased from Shanghai Yuanye Technology Co., Ltd., China). Whey protein isolate (WPI), pepsin, mucin from porcine Bile salt (porcine) were purchased from Aladdin Co. Shanghai, China). Pancreatic lipase, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and Folin-Ciocalteu reagent were procured from Sigma Aldrich (St. Louis, MO, USA). All reagents and chemical used were of analytical grade, and ultrapure water was obtained from milli-Q-water system (Millipore, MA, USA).

Methods

Emulsions Preparation.

Whey Protein Isolate (WPI) solution (2%, w/v) were prepared with 1.0 wt.% lecithin and sodium azide (0.02 wt.% of emulsion) as an antimicrobial agent which were stirred using magnetic stirrer for 2 hr. after that the WPI solution was stored overnight at 4 ^{0}C for complete hydration of the protein. Solid oil suspension (S/O) were prepared with 0.05% (w/v) tea polyphenols with 90 % (w/v) soybean oil and 10.0 % (w/w) sorbitan monooleate. Initially, a coarse emulsion was prepared with conventional homogenization by a high shear homogenizer (HSH) (Cyclone I.Q. microprocessor homogenizer) at 12.000 rpm for 2.0 min (Zhang & Zhong, 2015). The pre-emulsion was further processed through a high-pressure homogenizer (Model JN-10HC, Guangzhou Juneng Nano & Bio-Technology Co., Ltd) driven by a piston pump. The High-Pressure Homogenization (HPH) process was performed at 50 MPa with five cycles. The pressure and homogenization cycles were chosen based on previous study (Gadkari et al., 2017). The high-intensity sonication emulsion (HIU) prepared by sonicating using a 20 kHz, an ultrasonic processor with 10 mm diameter probe (Ning Bo Scientz, Biotechnology Co. Ltd., Ningbo, China) at an amplitude of 40 % for 8 min, on-time 5s and off-time 10s. To avoid the denaturation of

whey protein isolate, an ice-water bath surrounded with coarse emulsion, the time and energy densities were chosen based on the High-intensity sonication used in relevant previous studies (Sui et al., 2017).

Stability evaluation of S/O/W emulsion under different conditions of environmental stress.

The physicochemical stability of S/O/W emulsions was estimated under different environmental stress conditions as a change in temperatures, pH value of the emulsion, and Sodium chloride concentration. The fresh emulsions were stored at different temperature; (4 ± 1 °C) refrigerator, (25 ± 2 °C) ambient temperature and (37 ± 1 °C) oven up to 14 days. The emulsions were adjusted to different pH value; 2.0, 5.0, 7.0, 9.0 using 0.1 M HCl and 0. 1 M NaOH. The emulsions were diluted to (1:1 (v/v) NaCl solution: Emulsions) using different concentrations of sodium chloride solution 0.1 M (10, 50, 100 and 150 mM) (Wei & Gao, 2016). During stability studies, the particle sizes (D3.2), particle size distribution creaming index, and visual images of emulsions were obtained over 14 days of storage period.

Particle size measurement.

The mean particle size and particle size distribution were analyzed using laser diffraction equipment (M2000 MasterSizer, Malvern Instrument. Ltd., Worcestershire, UK). The emulsions were dispersed using a phosphate buffer at the same pH conditions. The refractive index of soybean oil and dispersion medium were 1.472 and 1.330, respectively. The mean particle size diameter was recorded triplicate and presented as average surface weighted Eq. (1) (Chen et al., 2017).

$$D_{3,2} = \frac{\Sigma\, n_i\, d_i^3}{\Sigma\, n_i d^2} \quad (1)$$

$$D_{4,3} = \frac{\Sigma\, n_i d_i^3}{\Sigma n_i d_i^2} \quad (2)$$

$$\text{Span} = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \quad (3)$$

Zeta potential (ζ) evaluation.

The ζ- zeta potential of the emulsions was evaluated by Malvern- Zetasizer (Nano-ZEN3600; Malvern Instruments, Worcestershire, U.K.). The samples were diluted before testing as volume ration 1:1000 with buffer at the same pH as an original emulsion to avoid effects of multiple scattering. The temperature of the cuvette was maintained at 25°C. Each sample was evaluated at tree time average, and the standard deviation was reported.

Creaming stability.

The creaming stability of S/O/W emulsion was measured by calculating the creaming index described recently by (Akhtar et al., 2014). With some modification, the freshly prepared emulsions were stored in glass vials with stopper and kept it at (25 ± 2 ºC). For emulsion prepared with different pH and different sodium chloride concentration. For studying the effect of storage temperature, the samples were stored at (4 ± 1 °C, 25 ± 2 °C, and 37 ± 1 °C) the emulsions were separated at two layers, as the top (cream) and bottom layers (serum). The creaming index (CI) % was calculated by measure the total height of emulsion (H_t) and height of cream layer (H_c) (mm, millimeter) by ruler after specific different time intervals (1, 3, 5, 10 and 14 days). The percentage of creaming index was calculated using the following formula:

$$\%\ \text{Creaming index (CI)} = \frac{H_c}{H_t} \times 100 \quad (4)$$

Optical microscopy.

The microstructure of the emulsion droplets was evaluated under optical microscopy (Olympus, model CX 40, USA) with 100X magnification. A droplet of emulsion (10μl) was placed on a microscope slide which was covered by a coverslip. The images were captured by (Digital camera, Leica model DFC 320, UK).

Encapsulation efficiency.

Encapsulation of tea polyphenol is determined by measuring total polyphenol Folin-Ciocalteu in the aqueous phase (free total polyphenol) according to (Anesini et al., 2008) With some modification, 4 ml of tea polyphenol emulsion was placed in a Falcon™ tube centrifuged at 16000 g for 20 min using (MiniSpin Plus, Eppendorf, Inc. Hauppauge, NY, USA). The serum will be collected using a (0.22-lm syringe filter), then 100 μL of sample emulsion was transferred in duplicate test tubes containing 500 μL of Folin-Ciocalteu reagent 10% (v/v) in water within to 8 min of incubation. Then, 400 μl of 7.5% (w/v) sodium carbonate was added. The mixtures were left for 45 min at room temperature after the incubation of the samples were measured at an absorbance of 765 nm. The total polyphenol content in the extract was calculated, expressed as gallic acid equivalent (GAE) mg ml-1 using gallic acid (0-100 mg/mL) standard

curve. The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency (EE)} = \frac{M_1 - M_2}{M\_1} \times 100 \quad (5)$$

Where M_1 is the total tea polyphenol add to the emulsion and M_2 is the amount of free tea polyphenol in an aqueous phase.

Statistical analysis.

All experiments were prepared in triplicates. One-Way ANOVA analysis and Duncan's test at a P level of 0.05. Using SPSS software (IBM SPSS statistics 24.0. Inc., Chicago, IL, USA).

Results and discussion.

Particle size and microstructure of emulsion.

Determine the particle size of the droplet is the most critical factor in evaluating food emulsions for their shelf life, texture, appearance, stability, and release properties. The emulsions with small size droplets are usually stable having creamy mouth feeling (McClements, 2015). The particle size variation ($D_{3.2}$), particle size distribution (PSD), and microscope images of S/O/W emulsion are shown in Fig.1, 2, and Table 1. The tea polyphenols were added to Solid/Oil suspensions and all the emulsions treated with HPH and HIU had significantly reduced particle size ($D_{3.2}$) than emulsions treated with HSH. The particle size diameter of emulsion droplets was fairly similar and relatively small about (0.45 and 0.48 μm) for HPH and HIU, respectively (Table 1). These results are in accordance with the previous findings with (Kaltsa et al., 2016), (Paximada et al., 2017). The diameters of droplets were much smaller than the droplet size used in S/O/W for glutamine (Y. Zhang & Zhong, 2015) and lactose (Y. Zhang & Zhong, 2017). It was apparent that the particle size distribution for HPH and HIU emulsions had unimodal distribution, and the peaks were disappeared in HSH (Fig. 1). Besides, the large particle size was reduced to less than 1 μm to given around 0.45 μm as the average of ($D_{3.2}$). This increase was due to the high shear forces generated by high-pressure treatment and high-intensity ultrasound. The high forces resulting from high-pressure treatments caused disruption in the hydrophobic electrostatic interaction and the induce shear, turbulence, and cavitation in the simultaneously (Dissanayake & Vasiljevic, 2009). The forces generated through High-intensity ultrasound led to an increase in high shear stress which deactivated non- covalent interaction in protein aggregates including electrostatic, hydrophobic interaction and hydrogen bonding which led to a reduction of particle size and tight particle size distribution (Ma et al., 2019). The microstructure images of S/O/W emulsion stabilized by WPI during storage showed significant flocculation in HSH emulsion. On the other hand, HPH and HIU emulsion were un-flocculated showing individual droplets (Fig. 2). These images were consistent with our results of droplets diameter D3.2 (Table. 1) and Particle size distribution (Fig. 2). Therefore, it is difficult to distinguish among the individual emulsion droplets because of maximum resolution limit of the microscope.

Impact of storage environmental conditions on the stability of S/O/W emulsions.

It is important to study the physical stability of emulsions under environmental stresses. Thus, the impact of storage temperature, pH, and various salt concentration was investigated in this study.

Effect of temperature storage on emulsion stability.

It's known that food products are usually exposed to temperature fluctuations during storage. Thus, it was necessary to investigate the impact of storage temperatures on the stability of emulsions. The effect of storage temperature (4, 25 and 37° C) was assessed on emulsions HSH, HPH, and HIU containing tea polyphenol by storing for 14 days. The particles flocculation and discoloration of emulsion stored at 37 °C were observed after 14 days of storage. Where (Jafari et al., 2008) indicated that the storage for a long time at high temperature for whey protein isolate might cause interaction between the interactive site of thiol- group (SH) to produce the disulfide bonds, which led to degradation and aggregation of proteins. Thus, particle diameter was studied to evaluate the stability of emulsions after storage at different temperatures. The particle means the diameter of HSH emulsions stored at 37 °C increased from 3.93 ±0.01 (before storage) to 7.98 ± 0.48 after 14-day storage. A stark contrast to this, the HIU and HPH emulsions exhibited a negligible increase in the diameter size. In addition, the particle size distribution of HSH showed a multimodal peak with flocculating distribution (Fig. 1A).

On the other hand, The HIU and HPH emulsions stored at 4 °C and 25 °C exhibited unimodal distribution with peak value around 0.5 μm. while all HSH emulsion showed the

flocculated distribution with droplet aggregation (Fig. 1A). The mean particle diameter of fresh (HSH) emulsion was (3.93±0.01) μm and increased to (7.98±0.48) μm after stored to 14 days at 37°C (Table. 1). The storage time and temperature of storage significantly affected the diameter of droplets in all high (HSH) emulsions. Moreover, small changes in droplet size (d3.2) for (HIU) and (HPH) emulsion except for the samples at 37 ° C were observed. The increase in the particle size of globular-protein emulsions during storage was due to flocculation (Qian et al., 2012). This suggested that the emulsions stored at 4, 25 C° had better stability. These results indicated that HSP and HPH emulsions, when stored for a short period under different conditions of homogenization, were more stable S/O/W emulsion systems. Furthermore, lipids particles did not have flocculation and coalescence after a storage period.

Effect of pH on emulsion stability and Zeta potential.

Food products and beverage emulsions are usually affected by the acidity of the water phase. Therefore, the physical stability of emulsions has significant roles in their industrial and commercial application in food and beverage products, which depend on their emulsifiers. Thus, the average of particle size and the particle size distribution were measured at various pH values (2, 5, 7, and 9) then stored at room temperature (±2, 25°C) for 14 days (Fig. 1B). Furthermore, the emulsions stored at pH 2.0 and 9.0 were highly unstable, and the particle size diameter showed a large increase of droplets coalescence at pH 5.0 and this value is close to the isoelectric point of whey proteins.

Consequently, the charge of droplets has a net or little charge. Furthermore, the electrostatic repulsion forces were not strong enough to overcome the hydrophobic attraction, and van der walls force hence leading to flocculation droplets. The results indicated that the tea polyphenol-enriched in S/O/W emulsion have more stability in neutral medium (pH 7.0) as evident from particle distribution (Fig 1B) which showed the monomodal distribution with $D_{3.2}$ value 0.46 and 0.68 μm for HPH and HIU emulsions respectively.

The change in the electrical properties of oil particles in S/O/W emulsion at different pH gradients (2, 5, 7 and 9) provide information about the properties of the interfacial emulsion. Evidence has shown the effect of high intensity ultrasonic on the particles size of the protein. The ζ-potential value illustrated in Fig. (3) indicates a negative surface charge in all samples except those at lowest pH 2.0 below isoelectric point around about pH 4.9 for WPI (Teo et al., 2016). The magnitude of ζ-zeta potential for droplets was positively charged. This may be attributed to the fact that the surface of protein molecules has double charge, amino groups ($–NH^2$) and carboxyl groups (–COOH) whereas, at low pH the amino groups become positive (–NH3+) while the carboxyl group is neutrally charged ($–COO^-$). Conversely, when the pH is higher than the PI the amino groups become neutral (-NH2) and Carboxylic groups become negatively charged (-COO-) (Z. Zhang et al., 2015).

Interestingly, it was found that the emulsions had higher ζ-potential values at pH 9.0, where the negative charge on the droplets was increased at magnitudes. It could be due to many possibilities that demonstrate the changes in the physicochemical properties observed in the electric charger of droplets. The increase of negative surface charge in alkaline condition at pH 9.0 was due to anionic groups generated during hydrolysis of oil to produce free fatty acids or presence of a hydroxyl group (-OH) in NaOH which used adjusting pH of emulsion (Jo & Kwon, 2014). The consumption of free amine groups of the adsorbed protein particles by Schiff base reaction (Feng Jiao et al., 2011). A significant increase ($p < 0.05$) of negative charge was observed in all emulsions. At pH 7.0, the samples had a negative electric charge and similar magnitudes.

Effect of ionic strength on emulsion stability

In this study, the physical stability of S/O/W emulsions prepared by different homogenization was investigated under various ionic strengths (10-150mM) (Fig. 1C). Our results indicated a significant increase in the mean particle diameter of emulsion with an increase in sodium chloride concentration. The diameter size of HSH emulsions increased from 3.70±0.01 μm at 10 mM NaCl to 4.95 ±0.54 μm under 150 mM of NaCl concentration. In contrast, there was a slight change in the particle size of HPH and HIU emulsions under the same conditions of molarities. However, HPH emulsion showed a unimodal distribution during the storage period at 10 and 50 mM of NaCl (Fig. 2C). Furthermore, the mean of particle diameter was 4.0, 0.4 and 0.6 μm for HSH, HPH, and HIU respectively with a slight increase in the diameter with the

prolonged duration of storage time (Table. 1). A significant increase in diameter was observed for HSH emulsion stored for 14 days, which indicated that the HPH emulsions were more stable under different ionic strength conditions than other emulsion systems. In contrast, the HIU emulsions were more susceptible to salt, especially at 100 and 150mM NaCl. The HSH emulsion attained a particle size of 3.70 μm with bimodal flocculation at 10mM NaCl. However, the HSH emulsion showed multimodal peaks at all salt concentrations (Fig. 1C) and had a higher droplet size around 7.02 mM after 14 days. The higher ionic strength of NaCl led to reducing repulsion forces between emulsion droplets which caused instability of physicochemical properties of emulsions (Qian et al., 2012)

Cream index and visual observation of emulsions.

Cream index or gravitational separation is one of the most important reasons that affect the instability and quality in food emulsions (McClements & Rao, 2011). The emulsion is more stable if the CI is lower. Higher temperature and prolonged storage period result in a higher accumulation of oil droplets due to a slight increase in the size of oil droplets (Dickinson, 2010) (Fig 4 A-C). This could be attributed to one of the reasons that (HIS) emulsion is more stable because the mean size of droplets is very small (Hu et al., 2013), i.e., less than 0.5 μm. It also indicated that HIS is an effective method of forming polysaccharide and protein and that protein solubility increased significantly after use (HIS) (Mu et al., 2010). With increased storage at different degrees, there was an increase in the mean size of oil droplets and an increase in CI. The emulsion prepared by HIS and HPH demonstrated a low rate of CI compared to high shear homogenization. The emulsion made by HIS and HPH showed a low rate of CI compared to High shear homogenization which is an indication of increased stability of the emulsion. A similar behavior for creaming index has been noticed (Winuprasith & Suphantharika, 2015). The instability of the lipid emulsion ultimately affects the separation of the visual stage into separate visible areas of the serum. In this study, the phase separation was observed beginning in HSH emulsion when stored at 37°C under increased NaCl concentration. Instability of physical properties of HSH emulsion was marked in all the treatment (Fig. 4A-C). Visual observation of samples revealed that High shear emulsion in all treatment had significantly higher creaming index than HPH and HIS emulsions**.** For HPH and HIS, there were no significant differences in creaming index, possibly due to similarities in droplet size diameter and partial size distribution. Creaming index and visual observation of emulsion indicated that the emulsion became creamy and more turbid in pH 5 due to this point near to isoelectric point (PI 5.1) for whey protein isolate (You et al., 2018). At this pH, the electrostatic repulsion between protein molecules is weak, which does not suffice to overcome the hydrophobic and the van der Waals forces. Therefore, the proteins accumulate and precipitate. In alkaline conditions, the deprotonation of the amino group ($-NH^2$) and Schiff -base reaction were the main reasons for the change of emulsion color to yellow.

On the other hand, in acidic conditions, the color of emulsions became darker. This is attributed to the repression process of the protonated amino group ($-NH^{3+}$) (Mercadé-Prieto & Chen, 2006). As well as with increasing the temperature and time the yellow color could come from poly-sulfides groups formed after removal of β-cystines (Fan et al., 2019).

Encapsulation Efficiency.

Encapsulation efficiency for emulsion was determined by indirectly measuring the total polyphenols by *Folin- Ciocalteu* in the aqueous phase (total polyphenol equivalent GAE mg/ml) (Fig. 5). The tea polyphenols are highly susceptible to light, oxidation, and degradation due to change in pH, NaCl concentration, and temperature during different storage periods. Therefore, it is very important to study the effect of storage times for advanced emulsion formula. In this study, the encapsulation efficiency of tea polyphenols was investigated using various homogenization during storage at 4 °C. Our results indicated that the encapsulation Efficiency of emulsions showed variation between (HSH), (HPH), and (HIU) emulsification methods. The lowest percentage of EE values in HSH emulsion is (82 - 71%) while the highest EE value (93 - 90%), (95 - 87%) for HPH and HIU respectively, was observed.

These results are almost consistent with (Paximada et al., 2017) and (Y. Zhang & Zhong, 2017) where the encapsulation efficiency of glutamine, lactose and L. salivarius using S/O/W emulsion system was found to be 95%, 67%, and 87% respectively. This may be directly related to the particles size diameter and stability of the droplets

during the homogenization process and storage. Indeed, HSH emulsions have particles with larger diameters as indicated in section (**3.1**) therefore, generally unstable compared to HPH and HIU emulsion which has smaller particles size and a more declared particle size distribution.

CONCLUSION.

In summary, the S/O/W emulsion is an effective way to encapsulate tea polyphenols as solid particles in S/O/W emulsions were obtained. The HIU and HPH emulsions had improved encapsulation efficiency and enhanced retention of tea polyphenol during storage compared with different homogenization methods. HSH emulsion exhibited the highest flocculation rate among all emulsions after mixing the emulsions with different concentration of NaCl solutions. The encapsulation efficiency of up to 95.65% in HIU and 93.72 in HPH emulsion was achieved. The diameter of the emulsion's droplets decreased from 3.93 μm at HSH to 0.45, 0.47 μm for HPH and HIU respectively.

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Table 1: Sauter mean diameter (d3,2), volume mean diameter (μm) and Span of S/O/W emulsions prepared by different homogenization (High shear homogenizer, High-pressure Homogenizer, and High-intensity Ultrasonication).

Storage days	Fresh			14 Days		
Treatment	D3.2	D3.4	Span	D3.2	D3.4	Span
	Droplet diameter (μm)			Droplet diameter (μm)		
HSH	3.93±0.02^{Ab}	10.75±0.89^{Ab}	5.37±0.59^{Aa}	5.26±0.45 ^{Aa}	16.22±0.4 ^{Aa}	3.64±1.17^{Ab}
HPH	0.45±0.0^{Ba}	0.54±0.0 ^{Ba}	1.26±0.0 ^{Bb}	0.46±0.02 ^{Ba}	0.60±0.0 ^{Ba}	1.54±0.01 ^{Ba}
HIU	0.48±0.02^{Bb}	0.66±0.0 ^{Bb}	1.75±0.0 ^{Ba}	0.68±0.02 ^{Ba}	0.83±0.07 ^{Ba}	1.51±0.13 ^{Bb}

The different uppercase (A-B) along the columns are significantly at (P< 0.05.) among the different homogenization methods, the different lowercase (a-b) in each storage condition along rows are significantly at (P< 0.05) among the different.

figures

تأثير طرق التجانس المختلفة على خواص فينولات الشاي المحتل على المادة الصلبة في الزيت في مستحلب الماء.

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الملخص العربي

فينولات الشاي عرضة للأكسدة مما يحد من الاستفادة بها حيويا. حيث الطبيعة غير المستقرة للفينولات من تطبيقها في الوقاية من الأمراض الرئيسية. لذلك ، الغرض من الدراسة الحالية هو تغليف مسحوق فينولات الشاي في مستحلبات (S / O / W) مثبتة بواسطة WPI باستخدام ثلاث عمليات تجانس مختلفة ، وتم تقييم المستحلبات لخصائصها الفيزيائية والكيميائية. بما في ذلك توزيع حجم الجسيمات ، والبنية المجهرية ، ومتوسط حجم المستحلب ، زيتا المحتملة ، والاستقرار المادي في ظل الإجهاد البيئي. أظهرت النتائج أن كفاءة تغليف مستحلب S / O / W المتجانس بواسطة الموجات فوق الصوتية عالية الكثافة لها كفاءة تغليف أكبر (٩٥,٩٥ ± ٠,١٤) من فينولات الشاي. كان لمستحلبات HPH و HIU حجم قطيرات أدنى (d3.2) بين ٠,٤٥ و ٠,٤٨ ميكرومتر على التوالي وكانت أكثر استقرارًا نسبيًا. نقترح أنه يمكن استخدام مستحلب S / O / W كنظام توصيل فعال لبوليفينول الشاي في الطعام لتحسين الاستقرار والتوافر البيولوجي أثناء التخزين.

الكلمات الاسترشادية: مستحلب S / O / W ، بوليفينول الشاي ، التغليف ، الاستحلاب بالموجات فوق الصوتية.