The stabilization of kenaf leaves extract co-loaded W 1 /O/W 2 double emulsion by emulsifier mixtures of sodium caseinate-Tween20-β-cyclodextrin

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Research Article

Keywords: double nanoemulsion, physical stability, ultrasonication, sodium caseinate, β-cyclodextrin, kenaf leaves

Posted Date: November 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2272510/v1

Abstract

Water-in-oil-in-water ($W_1/O/W_2$) double emulsion (DE) is often used for hydrophilic bioactives encapsulation. However, the stabilization of food-grade DE is difficult due to its complex structure and limited emulsifier choices. Thus, this paper studies the stabilization of DE containing ethanolic kenaf leaves extract by emulsifier mixture (EM) containing sodium caseinate, Tween-20, and β-cyclodextrin. The particle size, polydispersity index, creaming index, encapsulation efficiency, and droplet morphology were observed for 5 weeks storage. Overall, all DE samples did not undergo phase separation, with a noticeable increase in viscosity throughout storage. The morphology and functional groups also showed spherical droplet shape and interaction changes after emulsification. DE stabilized by 2.5% and 4.0% (w/w) EM had consistent droplet size and distribution, encapsulation efficiency > 98%, and highest viscosity value up to 35-day storage at 25℃. Specifically, DE with 4.0% (w/w) EM showed good resistance under pH changes. In conclusion, double emulsion stabilized by sodium caseinate, T20, and β-cyclodextrin are stable up to 5 weeks at 25°C, potentially to be applied in food and beverage applications.

Introduction

Double emulsion (DE) is a three-phase emulsion system that can be classified into water-in-oil-in-water ($W_1/O/W_2$) and oil-in-water-in-oil ($O_1/W/O_2$) emulsion. The former is more commonly studied because it has numerous advantages in terms of functionality, cost-effectiveness, and feasible application. The reduced-fat can be achieved in $W_1/O/W_2$ because of the inner aqueous phase incorporated within the middle oil phase (Paximada et al., 2021; Saffarionpour and Diosady, 2021). (Jolayemi et al., 2021)Besides, DE can encapsulate both hydrophilic and lipophilic carriers simultaneously within a single carrier. Research done by Harimurti et al. (2021) showed PGPR and Tween 20-stabilized DE had encapsulated both hydrophobic temulawak extract and hydrophilic red dragon fruit extract which impart higher antioxidant properties observed by the one-tenth lower IC_{50} than unencapsulated extract.

The stabilization of double emulsion is essentially important as it has three phases with two interfacial layers. DE is normally stabilized by both hydrophobic and hydrophilic emulsifiers (Herzi and Essafı, 2021). PGPR is the classic hydrophobic emulsifier to stabilize primary emulsion (W_1/O) as there are not many variations for food-grade hydrophobic emulsifiers. In most cases, synthetic emulsifiers like spans and Tweens could produce smaller droplet sizes with their fast adsorption during emulsification (Saffarionpour and Diosady, 2021). However, there is concern about the dosage use of synthetic emulsifiers for oral consumption on a long-term basis.

Biopolymers like protein and polysaccharides are commonly used as hydrophilic emulsifiers in DE due to their lower toxicity as compared to synthetic surfactants. Some biopolymers have their charge and surface active properties which affect rheological behaviours of $W_1/O/W_2$ (Kabakci et al., 2021). Milk proteins such as caseins and whey are common protein biopolymers due to its excellent emulsifying properties (Silva et al., 2020). While some have been discovering the uses of plant-based protein emulsifier from pea and soy, it was still not as stable with reported stability of 9-days at 4°C (Hua et al.,

2022). Meanwhile, Teixé-Roig et al. (2022) reported the synergistic effect of NaCas with PGPR in producing a viscoelastic adsorbed layer which minimize diffusion from between aqueous phase of DE. Studies reported the formation of DE gel by combining both PGPR and caseinate to stabilize mangiferin phenolic compound (Kabakci et al., 2021; Xing et al., 2022).

Meanwhile, β-cyclodextrin (β-CD) is the polysaccharide-based pickering emulsifier that stabilizes the emulsion by forming crystal particles. Besides, β-CD can form a strong solid network structure in the aqueous phase. However, recent studies of β-cyclodextrin-stabilized DE by Eslami et al. (2017) and Tian et al. (2022) have only obtained 7-day and 14-day storage stability of probiotic, respectively. This means DE stabilized by β-CD alone has shorter storage stability. Considering the structural complexity of the DE system using biopolymers, current DE is still more unstable than conventional O/W nanoemulsion. The food application of double emulsion remains more challenging as there are limited options of suitable food-grade emulsifiers. Research shows that combined emulsifiers could have better stability than a single biopolymer only in stabilizing DE. However, the former often face the problem of competitive adsorption that leads to instant phase separation after emulsification.

In most studies, a stable W/O/W is often demanded to encapsulate hydrophilic bioactives for potential functional food application (Jolayemi et al., 2021; Heidari et al., 2022). Kenaf (*Hibiscus cannabinus* L.) leaf contains lots of bioactive compounds such as kaempferol, phenolic acids, and quercetin, which have antioxidant, anti-inflammatory, and anti-proliferative properties (Sim et al., 2019). However, plant extract often has unpleasant sensorial taste and degrades easily when subjected to long-term exposure to oxygen, light, and heat (Herzi and Essafi, 2021). Thus, this paper aims to study the effect of biopolymer mixture containing sodium caseinate, Tween20, and β-cyclodextrin as hydrophilic emulsifiers on the physical stability of DE co-loaded with kenaf leaves extract.

Materials And Methods

2.1. Materials

Fresh *Hibiscus cannabinus* L. KR9 (kenaf) leaves were obtained from Lembaga Kenaf and Tembakau Negara (LKTN). Refined corn oil was purchased at local store (Maizola, US), sodium caseinate and β-cyclodextrin were purchased from VIS-Ingredient, Malaysia. PGPR 1750 was generously provided by Paalsgard, Malaysia. The ultra-pure water (Sartorius, Germany) was used throughout the analysis. Tween-20, PBS tablet, PTSA (1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt hydrate), Trichloroacetic acid, nile red were purchased from Sigma-Aldrich (Germany), 99% ethanol was purchased from Chemiz, (UK), sodium hydroxide and HCl were purchased from Merck (Germany)

2.2. Kenaf Leaves Extract Preparation

According to Sim et al. (2019), the kenaf leaves were extracted with 95% ethanol with a sample to solvent ratio of 1:10 (w/v). The ultrasonication (Sartorius, Germany) parameters were set at 50% amplitude, frequency of 20 kHz for three cycles of 1 min on time and 1 min off time under controlled temperature

(18–22°C). The mixture was centrifuged at 5000 rpm for 10 minutes, followed by filtration with Whatman filter paper no.1. The solvent evaporated under reduced pressure at 45°C. The sample was transferred into a storage tube and purged with nitrogen gas. The tube was then wrapped with aluminum foil and stored at -18°C for future use.

2.3. Biopolymer Solution Preparation

According to Cheong et al. (2016), the optimized ratio of sodium caseinate:Tween-20:β-cyclodextrin were 57.9%:27.6%:14.5% (w/w/w). In this study, phosphate buffer saline (PBS) solution (pH 7.4) was the aqueous medium to dissolve biopolymers. PBS contains salts to maintain the osmotic balance between the inner and external aqueous phases. Sodium caseinate and Tween-20 were fully dissolved by magnetic stirring at 45°C whereas β-cyclodextrin was fully dissolved at 75°C. These two solutions were mixed at 45°C for 1 hour and shaken overnight at room temperature for complete hydration. For preservation effect, 0.02% (w/v) of sodium azide was added to the solution.

2.4. Double Emulsion Two-Step Emulsification

The kenaf leaves extract (KLE) co-loaded water-in-oil-in-water ($W_1/O/W_2$) double emulsion (DE) will be prepared by the two-step emulsification method. The primary emulsion (W_1/O) will be prepared and incorporated into the secondary aqueous phase to form $W_1/O/W_2$. The inner aqueous phase (W_1) contained 400 µg/mL ethanolic KLE which was dissolved in a biopolymer solution. For the oil phase (O), 5% (w/w) PGPR as the hydrophobic emulsifier was dissolved in corn oil under continuous stirring for 10 minutes. The coarse primary emulsion (W_1/O) was formed by adding W_1 into the O phase at the ratio of 20:80 (w/w) at a stirring condition of 700 rpm for 10 minutes. The coarse emulsion was formed with a rotor-stator homogenizer (IKA T25, Germany) at 8800 rpm for 3 minutes. It was then ultrasonicated at 20 kHz, 50% amplitude for 3 minutes with a 0.5 cycle pulse setting (Sartorius, Germany). The temperature was maintained at 50 °C throughout the emulsification process.

External aqueous phase consists of biopolymer solution with different concentrations (2%, 2.5%, 3%, 3.5%, 4% (w/v) in PBS (pH 7.4)). The double emulsion was formed through secondary emulsification by encapsulating the primary emulsion (W_1/O) into the external aqueous phase (W_2) by rotor-stator homogenization at 6600 rpm for 3 minutes. Lastly, the coarse double emulsion was subjected to ultrasonication with similar parameter settings as the primary emulsification mentioned above.

2.5. Characterization of Double Emulsion

All double emulsion samples were characterized weekly throughout 35-day storage.

2.5.1. Creaming Index

The emulsion samples were put into a 15 mL conical tube, sealed, and stored at room temperature. During storage, the sample vials were photographed for interval assessment. When separation was observed in the sample tube, the creaming index was calculated through Eq:

Creaming index (%) = $\frac{height\,of\,separation\,layer\,on\,top(cm)}{height\,of\,the\,emulsion\,in\,vials(cm)}$

2.5.2. Particle Size and Distribution

The particle size and distribution were determined by dynamic light scattering (DLS) using Zetasier Nano ZS (Malvern, UK) according to Barbosa & Garcia-Rojas (2022). The undiluted sample was directly analyzed in triplicate, at an angle of 173° with an equilibration time of 120 s at 25°C.

2.5.3. Encapsulation Efficiency

Encapsulation efficiency was determined by observing the leakage of the PTSA dye marker from W_1 to W_2 of DE (Jolayemi et al., 2021). The 0.2% (w/w) PTSA was pre-dissolved in W_1 prior to double emulsion formation. The W_2 from the $W_1/O/W_2$ sample was extracted by centrifuging at 8000 g for 15 min. Then, the bottom aqueous phase was collected and re-centrifuged at the same condition as before. The collected aqueous phase was added with 20% (v/v) trichloroacetic acid solution to precipitate out the NaCas. The mixture was re-centrifuged and filtered with a 0.45 µm filter. The absorbance was measured at a wavelength of 374 nm. The PTSA standard curve (0.1–0.000625 % (v/w), R^2 = 0.996 to determine the PTSA concentration leaked into W_2.

2.5.4. Viscosity

The Brookfield viscometer attached to LV-64 spindle was immersed into double emulsion samples under a rotation speed of 100 rpm to measure the viscosity of the sample (Sim and Nyam, 2021).

2.5.5. FTIR Spectra

The FTIR spectra of biopolymer mixture containing NaCas:T20:β-cyclodextrin, biopolymer solution only, primary emulsion and double emulsion samples were studied using FTIR with a scan range of 500–4000 cm^{-1} (Perkin Elmer, USA).

2.5.6. Morphology Observation

The double emulsion was observed with a fluorescence microscope Axiovert (Carl Zeiss Axiocom, Germany) at a magnification lens of ×40 lens. Double emulsion was pre-stained with Nile red (0.01% w/w) in the oil phase, it was placed on glass slides and covered with a cover slip. Fluorescence from the sample was collected using filter set 43 for Nile red which had a wavelength of excitation to emission at a range between 559 to 637 nm (Aditya *et al.* 2015; Velderrain-Rodríguez *et al.* 2019).

2.6. The pH stability

All freshly prepared double emulsion samples were subjected to different pH conditions (2.5–8.5). Creaming index, particle size, and polydispersity index were then assessed to determine which formulation is stable to pH changes.

2.7. Statistical Analysis

All results were presented in mean ± standard deviation (SD) (n = 3) which were analyzed with One-way Analysis of Variance (ANOVA) using Minitab 16.2.1 (Minitab Inc., Pennsylvania, USA), with Tukey's test to determine the significant difference ($p < 0.05$).

Results And Discussion

3.1. Creaming Index (CI) and Morphology of Double Emulsion

Figure 1 (a) shows the visual observation of double emulsion stabilized with emulsifier mixture (EM) of sodium caseinate:Tween20:β-cyclodextrin (NaCas:T20:β-CD). All double emulsion (DE) samples did not undergo sedimentation, creaming, or phase separation throughout 35-day storage at 25°C. Thus, all samples showed a constant CI of 100% throughout the storage study, indicating homogeneity at the macroscopic scale and physically stable (Yang et al., 2021). Synergistic stabilization by NaCas and T20 which impart electrosteric repulsion and steric interaction while β-CD stabilized the interface through pickering interaction at the interface. Overall, the 2–4 % (w/w) concentation of EM in external aqueous phase (W_2) of DE are optimal to stabilize DE against creaming destabilization.

The droplet morphology of primary and double emulsion were observed by dyeing the oil with nile red fluorescence dye dye. The less spherical shape and higher droplet size corresponded to lower stability. Overall, Fig. 1(b – d) shows spherical droplets of primary emulsion, double emulsion with 2% and 4% EM, accordingly. The morphology of DE with 4% EM showed slightly smaller spherical droplets than DE with 2% EM.

1.1. Particle Size and Distribution

Overall, DE stabilized with 2–4% (w/w) of EM in W_2 showed nano-sized droplets throughout the 35-day storage. Reducing droplet size to the nanoscale could contribute to better kinetic stability of DE (Harimurti et al., 2021). It can be affected by emulsifier concentration and types of emulsification methods. In comparing to other authors, some reported DE in micron-sized droplets (Silva et al., 2020; Yang et al., 2021; Teixé-Roig et al., 2022) while some obtained nano-sized droplets (Harimurti et al., 2021; Barbosa and Garcia-Rojas, 2022).

As observed in Fig. 2 (a), DE with 2% EM had the highest droplet size whereas DE with 4% EM had the lowest droplet size (210 nm) on day 1. Likewise, the former still has the highest droplet size than the latter (214.17 nm) on day 35. Theoretically, the higher the emulsifier concentration, the lower the particle size produced. However, this seems not applicable throughout the storage duration. Starting from Day 7 to 28, the DE samples had undergone both increase and decrease value (Fig. 2a). Among all samples, only DE with 2.5, 3.5, and 4% (w/w) EM show slight changes of droplet size throughout storage days, indicating better stability than DE with 2 and 3% (w/w) EM. Meanwhile, the latter had drastic changes of droplet size throughout the storage study.

Both reduction and increment of droplet size within double emulsion samples are common as the double emulsion is a very thermodynamically unstable. DE with a high initial larger droplet size attracts neighbouring droplets due to kinetic instability, leading to aggregation forming even larger droplets (Silva et al., 2020). Meanwhile, if droplet size decreased over time, it indicates the possible shrinkage of the inner aqueous phase (Harimurti et al., 2021). This is commonly happened in DE due to the diffusion of dissolved molecules between aqueous phases through the middle oil phase (Teixé-Roig et al., 2022).

Nevertheless, all DE samples in this study showed relatively consistent droplet distribution. The polydispersity index of double emulsion samples ranges from 0.445 to 0.516 (Supplementary material 1). This range is reasonable for double emulsion as it encapsulates another emulsion system. Furthermore, biopolymer-stabilized DE tends to have a higher PDI value as reported by Jo et al. (2021) PDI value ranges around 0.608–0.634. However, larger droplets with high PDI in DE are less desired because it indicates inconsistencies in droplet sizes, different densities which could lead to phase separation (Heidari et al., 2022).

In this study, set asiding from EM used in W_2, ultrasonic emulsification might also be the reason for the formation of nano-sized droplets in all DE samples (Kabakci et al., 2021). Ultrasonication can induce structural changes of casein in this study which lower the interfacial tension. This was induced by pH, electrical charge, and hydrophobic interaction of the emulsion droplets (Huck-Iriart et al., 2016). The self-assembling properties of NaCas affected the mean average droplet size after formation significantly, which explained the slight changes in droplet size throughout storage days (Fig. 2a). It might impart flocculation behaviour that could either attach to the interface or form micelles in the continuous phase.

1.2. Encapsulation Efficiency

Encapsulation efficiency of kenaf leaves extract incorporated in W_1 was assessed by measuring the concentration of PTSA dye released into the external aqueous phase (Jolayemi et al., 2021). Figure 2(b) shows that DE stabilized with 2–4 % EM of NaCas : T20: β-CD showed > 98% encapsulation efficiency after 35 days storing at 25℃. When emulsion samples were freshly prepared (Day 1), all samples except 2% EM showed > 99% encapsulation efficiency. DE with 4% EM shows a slightly decreasing trend throughout the storage study, as observed there were slight differences in the EE%. Meanwhile, DE with 3.5% EM had consistent encapsulation efficiency throughout 35-day storage. However, as compared to other concentrations of DE, it did not have the highest encapsulation efficiency, except on Day 7.

1.3. Viscosity

The viscosity of the double emulsion (DE) could affect the rate of phase separation and physical stability (Yang et al., 2021). Based on the viscosity reading at different shear rates, DE with different concentrations (2–4% (w/v)) of emulsifier mixtures exhibited a nearly Newtonian-like behavior (preliminary data was not shown). As observed in Table 1, all DE samples have viscosity values ranging from 257 to 1189 $Pa.s^{-1}$ at a shear rate of 100 rpm. This is considered quite viscous, which could be due to NaCas and the fixed ratio of primary emulsion stands for 30% (w/w) in the whole DE system (Goibier et

al., 2020). The higher viscosity favors emulsion stability, resulting in better protection of KLE for this formulation. The high viscosity of the continuous phase decreases the movement velocity of inner aqueous phase, contributes to better stability and retains the encapsulation efficiency (Xing et al., 2022).

Table 1
Viscosity of Double Emulsion at Different Emulsifier Concentrations Throughout Storage Study

Storage days	Viscosity (Pa. s^{-1})				
	2	2.5	3	3.5	4
1	564.00 ± 3.61^{Af}	403.00 ± 3.00^{Cf}	380.23 ± 2.36^{De}	257.03 ± 2.05^{Ef}	472.83 ± 2.75^{Bf}
7	668.47 ± 3.09^{Be}	502.73 ± 2.75^{De}	450.90 ± 3.44^{Ed}	595.57 ± 4.81^{Ce}	782.20 ± 2.31^{Ae}
14	769.60 ± 4.41^{Bd}	512.43 ± 3.78^{Cd}	462.03 ± 3.00^{Dc}	790.10 ± 1.01^{Ad}	796.80 ± 3.56^{Ad}
21	804.63 ± 1.48^{Cc}	550.00 ± 3.00^{Dc}	505.43 ± 3.23^{Eb}	865.87 ± 2.42^{Ac}	817.93 ± 3.69^{Bc}
28	840.13 ± 3.83^{Db}	758.13 ± 2.52^{Eb}	1058.00 ± 3.46^{Ba}	919.83 ± 3.44^{Cb}	1108.60 ± 2.42^{Ab}
35	855.47 ± 3.02^{Ea}	1040.00 ± 4.04^{Da}	1060.00 ± 3.46^{Ca}	1078.0 ± 3.46^{Ba}	1189.00 ± 4.58^{Aa}

A-E indicates significant difference between the emulsifier concentration within same day; a-e indicates significant difference at different storage days on the same emulsifier concentration

However, the viscosity of double emulsion did not increase linearly with biopolymer concentration (Table 1). In most cases, viscosity value would increase with higher biopolymer concentration due to the compact structure of the biopolymer (Paximada et al., 2021; Xing et al., 2022). However, DE with 4% (w/w) EM showed the highest viscosity whereas DE with 3.5% (w/w) EM showed the lowest viscosity value among all samples after formation. But with an increase in storage days, DE with 3.5% (w/w) EM has increased significantly to reach the second-highest viscosity value at the 35th storage day. All DE samples showed a linear increase in viscosity with different rates throughout storage duration. Some stated that different rates of viscosity increase could be due to un-emulsified droplets and unadsorbed emulsifiers in the continuous phase. However, some stated that the self-reassembling properties of sodium caseinate may contribute to a viscosity increase during storage (Goibier et al., 2020). Considering that no phase separation occurred even on the last day of storage (Day 35), the bulk stabilization by sodium caseinate has stabilized the system which can be observed by viscosity increase throughout the storage study.

1.4. Functional Group Assessment of Double Emulsion

Each phase of double emulsion were investigated in terms of its FTIR spectra. As NaCas constituted about 57.9% (w/w) of biopolymer mixture concentration, thus, the FTIR spectra of double emulsion (Fig. 3b), biopolymer solution and W_1 (Supplementary material 2) had the sharp peak representing NaCas at the range between 3500 to 3000 cm^{-1}. The spectrum of NaCas at 3284 cm^{-1} represented O-H stretching absorption bands Bai et al. (2019). A band ranging from 3291.5–3293 cm^{-1} could also correspond to O-H bending vibrations in β-CD. In some circumstances, the successful host-guest completion of β-cyclodextrin could diminish some vibration peaks which can be observed that DE (b) had lesser peaks than primary emulsion (a).

Primary emulsion mainly consists of corn oil, with 20% of W_1, thus shorter peak of NaCas still can be observed at area of 3000–3500 cm^{-1}, followed by lots of smaller peaks at 1463.97, 1161.15, and 1118.71 cm^{-1}, accordingly (Fig. 3a). As primary emulsion contains mainly lipid phase, noticeable peaks might represent composition (PGPR, corn oil, W_1) in the oil phase. The strong peak at 1744 cm^{-1} represented C = O stretching absorption of the free fatty acids (oleic and linoleic acids) in corn oil. Furthermore, it also had strong band absorptions in the region of 3000–2800 cm^{-1} caused by C–H stretching vibrations Hady et al. (2022). Successful encapsulation can be observed with the shift in wavenumbers representing changes in terms of interaction between the emulsion components.

1.5. The Effect of Environmental Condition pH on stability of DE

DE stabilized by NaCas:T20:β-CD showed no phase separation when the pH of DE was adjusted from 4.5 to 8.5 (Supplementary Material 3). This indicates good stability to be incorporated into food matrices that have different acidic conditions (Teixeira et al., 2022). The pH value of double emulsion (DE) samples was 6.5 which is similar with the pH of the biopolymer solution before emulsification. The physical stability can be affected by pH in terms of the solubility and charge intensity of biopolymers. Thus it greatly affected the particle size of DE (Table 2).

Table 2
The particle size of double emulsion samples under different pH conditions

Emulsifier concentration (%)	pH			
	2.5	4.5	6.5*	8.5
2	21.04 ± 0.77^{a}	1.79 ± 0.12^{b}	0.76 ± 0.00^{c}	0.63 ± 0.01^{c}
2.5	19.98 ± 0.60^{a}	1.01 ± 0.04^{b}	0.53 ± 0.00^{b}	0.57 ± 0.01^{b}
3	9.87 ± 0.31^{a}	0.66 ± 0.03^{b}	0.46 ± 0.00^{b}	0.53 ± 0.01^{b}
3.5	2.26 ± 0.95^{a}	0.97 ± 0.02^{b}	0.35 ± 0.01^{c}	0.50 ± 0.02^{d}
4	0.61 ± 0.01^{c}	0.97 ± 0.02^{a}	0.21 ± 0.00^{d}	0.80 ± 0.05^{b}

*Control sample (no pH adjustment); a-e indicates significant difference at different pH on the same emulsifier concentration

DE with 4% EM (w/w) EM remained relatively small (< 1 μm) when subjected to pH changes whereas the other DE samples had micron-sized droplets. The former had smaller size could be due to its initial lower droplet size after formation. With the decreasing pH towards acidic condition, those particle sizes become increasingly large due to the aggregation of sodium caseinate. On the other hand, DE with 2.5% and 3.0% EC had a relatively more consistent droplet size when subjected to pH ranges from 4.5 to 8.5 ($p > 0.05$) (Table 2). This could justify that DE with 2.5% EM also showed relatively good resistance at pH > pI of NaCas with insignificant changes in droplet size.

Conclusion

The emulsifier mixture (EM) containing NaCas, T20, β-cyclodextrin has successfully stabilized double emulsion (DE) for 35-day storage at 25°C. In this study, DE stabilized by 2.5% and 4.0% (w/w) EM are the optimized formulation based on its consistent droplet size distribution, > 98% encapsulation efficiency, higher viscosity throughout 35-day storage. Specifically, the latter has good pH resistance with insignificant droplet size changes when pH ranges from 4.5 to 8.5. DE with 4% (w/w) EM shows spherical droplet shape and the interaction changes of functional groups before and after emulsification indicates a successful encapsulation. Currently, a complete synthetic surfactant-free double emulsion is still quite difficult to achieve, thus still requires the complementation of synthetic surfactant to achieve the physical stability of the double emulsion. This study shows the potential hydrophilic emulsifier of food-grade biopolymer mixture in stabilizing the double emulsion, which could be applied for functional food, pharmaceutical or nutraceutical applications.

Declarations

Acknowledgment

Funding This work was supported by UCSI University Kuala Lumpur through Research Excellence & Innovation Grant, Project number REIG-FAS-2020/029.

Authors' contributions

Elaine: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - Drafting. **Chin Ping Tan:** Writing – Review & Editing. **Md Jahurul Haque Akanda:** Writing – Review & Editing. **Kar Lin Nyam:** Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

Data availability statements

Data available on request from the authors.

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Figures

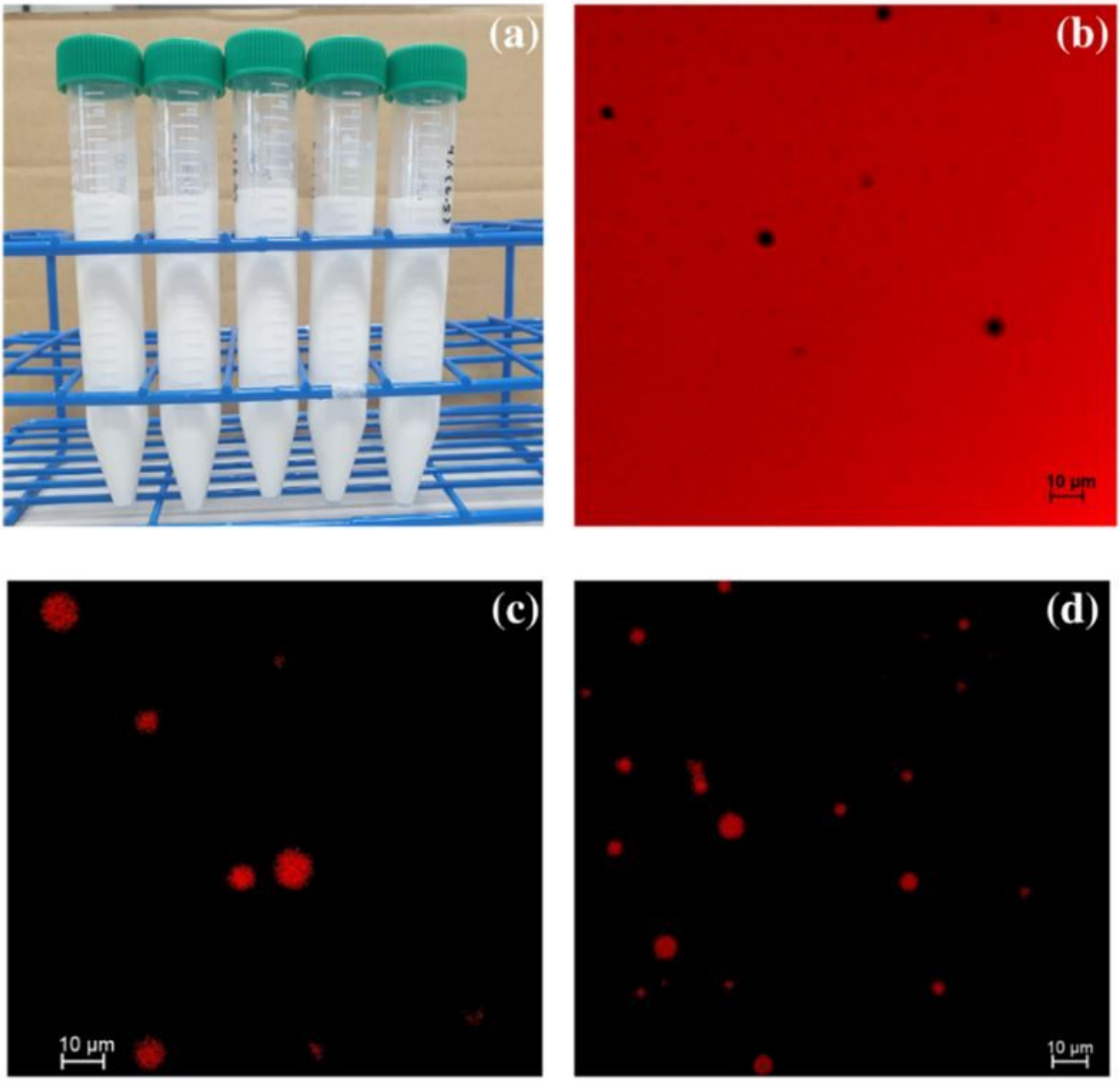
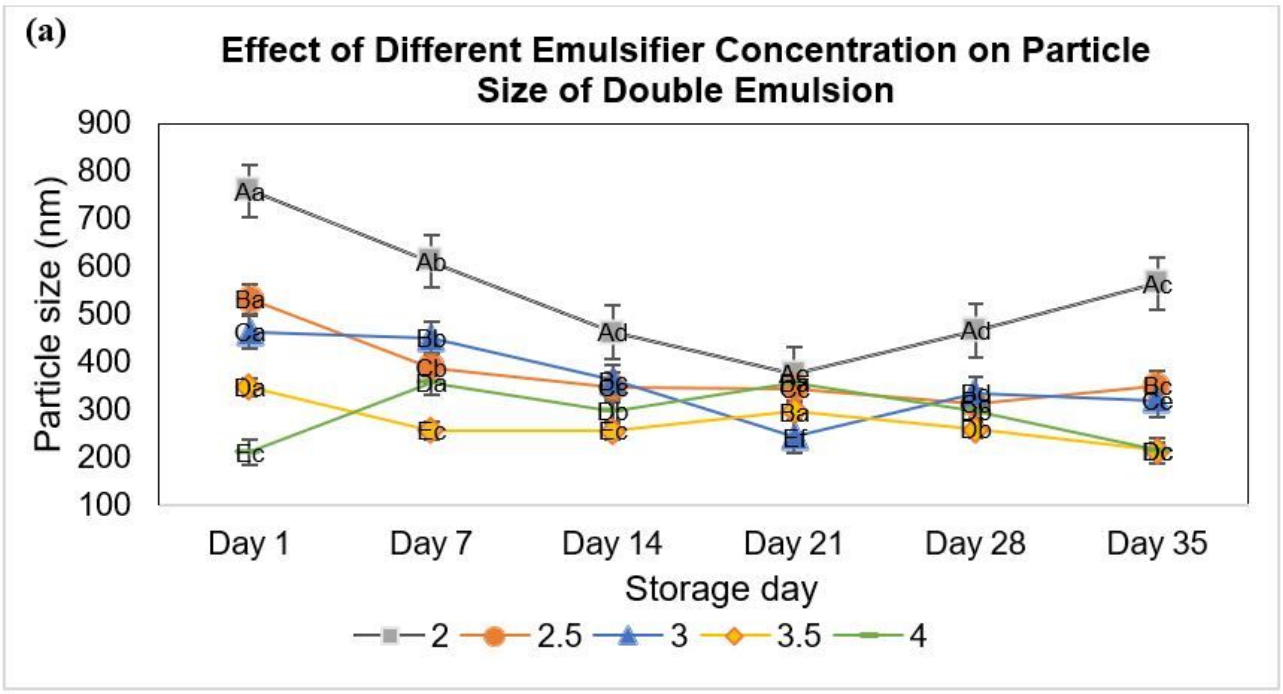


Figure 1

Visual Observation of DE stabilized by NaCas:T20:β-cyclodextrin from the left to right were 2, 2.5,3, 3.5, 4% emulsifier concentration (a); the morphology of DE droplets under CLSM observation at magnification of X40 lens of primary emulsion (W_1/O) (b), DE ($W_1/O/W_2$) with 2% EM (c) and 4% EM (d)



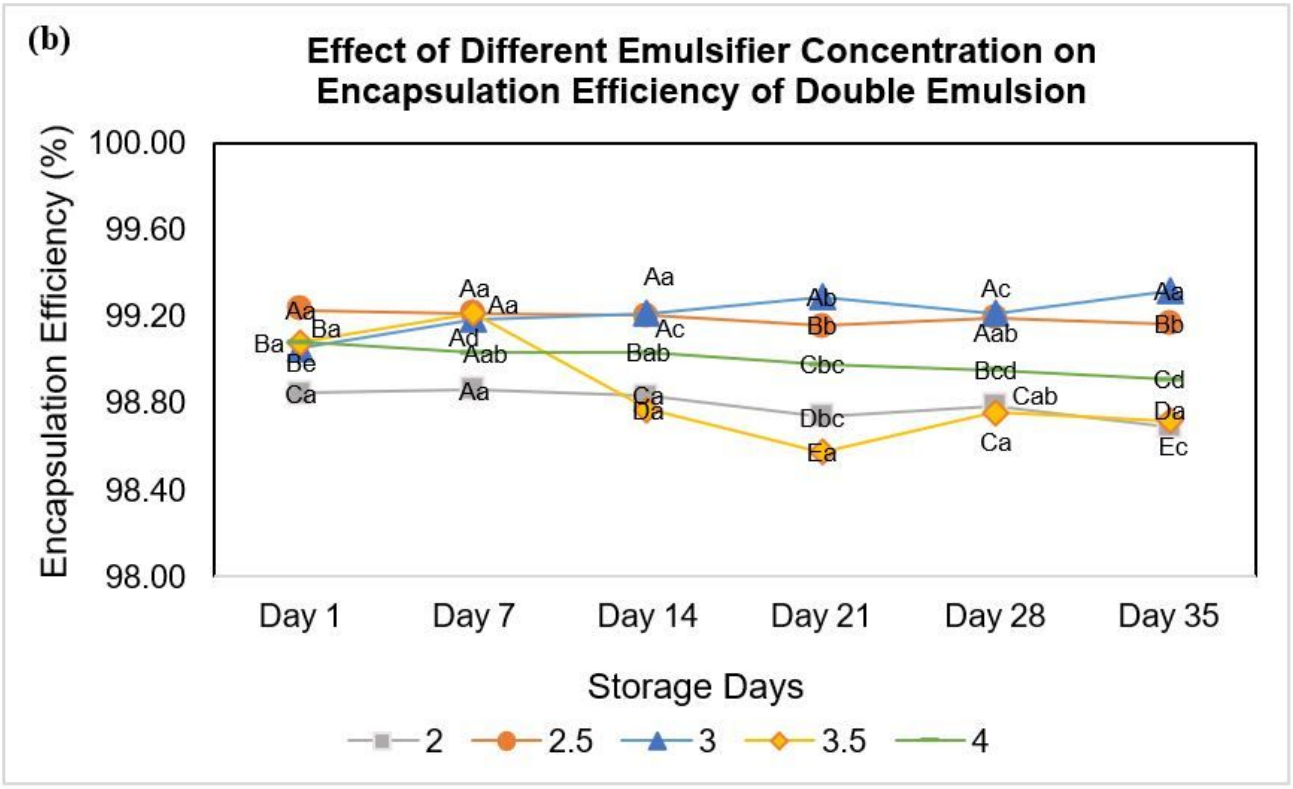


Figure 2

The storage stability of DE samples in terms of particle size (a) and encapsulation efficiency (b) (A-E indicates significant difference between the emulsifier concentration within same day; a-e indicates significant difference at different storage days on the same emulsifier concentration)

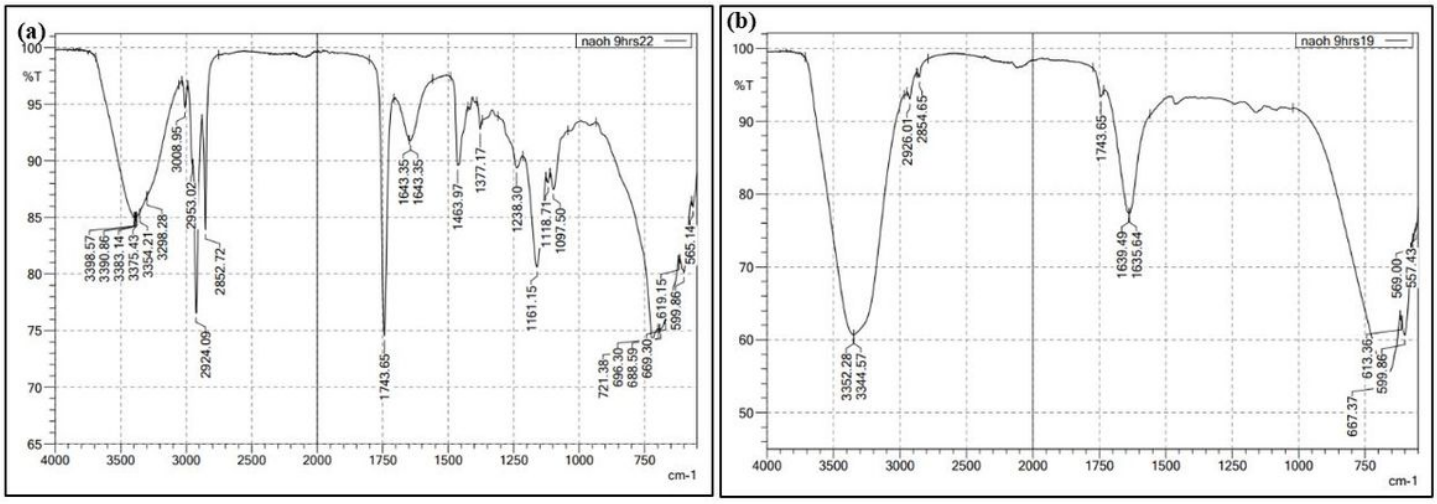


Figure 3

FTIR Spectrum of primary Emulsion (W_1/O) (a); optimized DE + 3.5% EM (b)

Supplementary Files

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- 281022SupplementaryMaterials.docx