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Enhancement of the Stability of W/O/W Double Emulsion by Chitosan Modified Rice Husk Silica

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Abstract. The awareness of the need for healthy food continues to increase, hence, the demand for healthy food is also increasing. Water-in-oil-in-water (W/O/W) double emulsion can provide low-fat emulsion-based products due to a lower fraction of oil compared to the corresponding O/W emulsion. However, the stability of double emulsion has been quite tricky to be established due to the presence of two interfacial layers between oil and water. This research aims to study the use of biosilica in the form of rice husk ash (RHA) combined with both chitosan particles and chitosan solution to stabilize the interfacial layer between oil droplets and the outer aqueous phase. The concentrations of chitosan and biosilica, as well as pH of the outer aqueous phase in the acidic ranges, were varied to obtain W/O/W emulsion with higher stability. The primary emulsion was prepared by using the emulsifier mixtures of Tween 20 and Span 80. The resulting primary emulsion was subsequently dispersed into the outer aqueous phase, loaded with the combination of biosilica and chitosan particles/ solution. Emulsification processes were conducted using a rotor-stator homogenizer. The resulting W/O/W emulsions with high stability of ~80-100% were obtained by using the combination of 0.5% biosilica and 1.25% chitosan particles/ 5.25% chitosan solution at the pH of 4. The use of biosilica and chitosan seemed promising to be applied in the manufacturing of healthier food emulsion-based products.

Keywords: Chitosan; Emulsion; pH; Rice husk silica; W/O/W

1. Introduction

The emulsion has been widely found in many diverse processed foods both in the forms of oil-in-water (O/W) and water-in-oil (W/O) emulsions. One of those examples is mayonnaise which is an oil in water emulsion and contains a high concentration of oil of about 60 to 70% (Morley, 2016). However, nowadays the demand for healthier food products with much lower oil content has been increasing. The oil content was reduced in W/O/W emulsion, leading to the development of lower-calorie foods with acceptable taste (Yildirim, 2016).

Pickering emulsion has recently gained an attraction to reduce the use of polymeric emulsifiers since it had demonstrated higher stability against shear stress and changes in

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pH or temperatures (Yang et al., 2017). The particles could form multilayers which act as a physical barrier against coalescence. Moreover, the particles were much more strongly deposited on the interfacial layers in contrast to the polymeric emulsifiers which tend to easily detach from the interfaces with time (Aveyard et al., 2003). Pickering emulsion has increasingly been investigated to be used in different fields such as food, pharmacy, and cosmetics (Muharam et al., 2015; Shah et al., 2016; Wu et al., 2020). Several particles which have been used for Pickering emulsions were silica, clays, polymer latex, magnetic particles, graphene, and polymethylpolymethylmethacrylate particles (Lee & Choi, 2018; Bao et al., 2019). The use of silica for the stabilization of O/W emulsion has been widely investigated (Sapei et al., 2017a; Sapei et al., 2017b; Sapei et al., 2018; Sapei, 2020). However, the use of bare silica was not effective and had to be combined with the use of polymeric emulsifiers to help its adsorption onto the interfacial layers (Pichot et al., 2012; Sapei et al., 2017a; Sapei et al., 2017b).

Chitosan is a biopolymer derived from chitin which is biodegradable, biocompatible, not toxic, and has mucoadhesive properties (Muharam et al., 2015; Krisanti et al., 2020). Chitosan as a polycationic biopolymer may be acted as an emulsifier and emulsion stabilizer through adsorption of the protective layer at oil-water interfaces, viscosity enhancement, and interaction with surface-active agents (Klinkesorn, 2013). Chitosan behaved as a weak cationic polyelectrolyte at $\text{pH} < 6.5$ when most of its amino groups were protonated (Tiraferri et al., 2014; Bhutto et al., 2021). At higher pH of about 7-8, dissolved chitosan was able to strengthen the interfacial layer O/W emulsion due to the polymer chains aggregation (Mwangi et al., 2016). Silica accumulated in living tissues generally referred to as biosilica was amorphous and tended to be hydrophilic due to the presence of hydroxyl groups on their surface (Sapei et al., 2008; Dhaneswara et al., 2019; Sapei, 2020). It is well known that natural silica particles are negatively charged in pure water. As the pH was increased above pH 2, the silica surface developed a net negative charge primarily due to the deprotonation of the silanol group (Dyab, 2012). The high positive charge on $-NH_3$ groups of chitosan when dissolved in aqueous acidic media at $\text{pH} < 6.5$ would likely adhere to negatively charged surfaces such as anionic polysaccharides and synthetic polyanions to give polyelectrolyte complexes and multilayer surfaces (Klinkesorn, 2013; Tiraferri et al., 2014). Furthermore, O/W emulsion was stabilized by silica particles of which hydrophobicity was modified by chitosan particles addition (Alison et al., 2016; Alison et al., 2018). The hydroxyl groups of silica were suggested to play an important role in the interaction with the polyelectrolyte of chitosan in the formation of a stable adsorption layer (Tiraferri et al., 2014). Furthermore, the stability of W/O/W stabilized by silica-chitosan was dependent on pH since pH may have affected particle charge and rheological properties of the emulsion (Klinkesorn, 2013; Alison et al., 2016). At acidic pH, rice husk silica tended to be chargeless (Sapei et al., 2018) which may have limited its interaction with chitosan. On the other hand, the positive charges of chitosan tended to be decreased as pH was progressing towards neutral pH (Tiraferri et al., 2014; Mwangi et al., 2016; Alison et al., 2018). The chitosan concentration may have affected its adsorption behavior on the silica surface (Li & Xia, 2011; Tiraferri et al., 2014). A recent study demonstrated the use of chitosan solution to coat the oil globules which entrapped insulin-containing internal aqueous phase due to polyelectrolyte complex formation with alginate present in the outer aqueous phase (Faghmous et al., 2020). This chitosan-coated W/O/W multiple emulsion seemed to be promising to be developed as a drug delivery system.

Chitosan can be applied for bodyweight reduction, maintenance of LDL-cholesterol, amelioration of inflammation, and reduction of intestinal transit time according to the European Food Safety Authority (Manigandan et al., 2018). Biosilica could also be used as a food additive and used for biomedical applications because of its health benefits (Alshatwi et al., 2015). The electrostatic interaction between oppositely charged chitosan and biosilica particles has not been extensively explored yet. Let alone its role in strengthening the interfacial layer between oil and water, which became the main concern in this article. This principle has been exploited in wastewater treatment using the coagulation-flocculation process described in the recent studies (Bahrodin et al., 2021; Fard et al., 2021; Iloamaeke et al., 2021). The pollutant removal efficiency was highly dictated by the wastewater pH depending on the coagulant agent and its mechanism (Bahrodin et al., 2021; Sibiyaet al., 2021). Alum as the coagulant became soluble and positively charged in the acidic environment was easily adsorbed onto the negatively charged surface of the colloids leading to charge neutralization and pollutant removal (Bahrodin et al., 2021). Furthermore, the color removal efficiency increased as pH decreased from 10 to 2 when *Mercenaria mercenaria* shell with a zero-point charge value (pH_{zpc}) of 7.8 used as the coagulant became positively charged thus, enhancing the coagulation of negatively charged colloid particles making them to floc due to the increase in the force of attraction which led to charge neutralization (Iloamaeke et al., 2021). In this research, rice husk silica in combination with chitosan was used to stabilize food-based W/O/W double emulsion. Chitosan both as particles and solution were combined with biosilica and their different mechanisms dependent on pH were studied. Chitosan, both as particles and as a solution, was combined with biosilica, and the various mechanisms dependent on pH were investigated. It is expected that the outcome of this research will be useful for the development of healthier and low-calorie emulsion-based novel food products.

2. Materials and Methods

2.1. Materials

Rice husk was obtained from paddy milling in Mojokerto, East Java, Indonesia; chitosan powder with a deacetylation degree of 88% (Merck, Germany), palm cooking oil (Sunco, PT. Milkie Oleo Nabati Industri, Bekasi, Indonesia); citric acid powder (Merck, Germany); sodium dihydrogen citrate/ monosodium citrate powder (Merck, Germany); disodium hydrogen citrate/ disodium citrate (Merck, Germany); acetic acid glacial (Merck, Germany); sorbitan monooleate/ Span-80 (Sigma Aldrich, Germany); polyethylene glycol sorbitan monolaurate/ Tween-20 (Merck, Germany); demineralized water.

2.2. Preparation of rice husk ash

Rice husks were separated from the debris and dust before being washed with water and rinsed with demineralized water. Clean rice husks were subjected to drying at 105°C for 2 hours in an oven (Memmert, Germany) followed by calcination at 750°C for 5 hours in the furnace (Ney VULCAN D-550, DentsplyCeramco, USA) to remove the organic compounds. The remaining rice husk ash (RHA) was grayish and consisted of silicon (Si) of ~85% (Sapei et al., 2015). The rice husk ash was milled and screened with a 200 mesh screen for further use as the emulsion stabilizer.

2.3. Preparation of w/o emulsion

Tween-20 of 2.1% relative to the aqueous phase was added to water and stirred with a magnetic bar at 300 rpm for 7 minutes to obtain the aqueous phase. Furthermore, a Span-80 of 5.3% relative to the oil phase was added into the oil and mixed with a magnetic bar at 800 rpm for 7 minutes. Afterward, the aqueous phase with a fraction of 40% was dispersed in the oil phase using a rotor-stator (IKA T25 digital ULTRA TURRAX, Germany) at a mixing speed of 20,000 rpm for 6 minutes. This primary emulsion was highly stable for several weeks. The experimental setup for the preparation of the primary W/O emulsion can be seen in Figure 1A.

2.4. *Preparation of buffer solution*

The buffer solution of different pH (2, 3, and 4) was prepared for the outer aqueous phase of the W/O/W emulsion. The buffer solution of pH 2 was prepared by mixing 47 mL of 0.065 M citric acid with 3 mL of 4.628 × 10^{-3} M monosodium citrate into the 100 mL volumetric flask. Water was added up to 100 mL and the solution was well shaken. The buffer solution of pH 3 was prepared by mixing 35 mL of 0.011 M citric acid with 15 mL of 7.46 × 10^{-3} M monosodium citrate; while the buffer solution of pH 4 was prepared by mixing 45 mL of 5.08 × 10^{-3}M disodium citrate with 15 mL of 7.46 × 10^{-3} M monosodium citrate in the 100 mL volumetric flask. The flask was filled with water until reaching the mark and was shaken several times till it was homogeneous. The outer aqueous phase without pH adjustment with a pH value of about 5.7 was used as the control.

2.5. *Preparation of W/O/W double emulsion*

The outer aqueous phase was prepared before the emulsification of the primary emulsion to obtain the double emulsion. The mixture of rice husk silica particles or chitosan particles and rice husk silica particles or soluble chitosan was added into the outer aqueous phase with adjusted pH (2, 3, and 4) to stabilize the interfacial layer between oil droplets with the outer aqueous phase. Rice husk silica of 0.5% was mixed with various concentrations of chitosan powder (1.25%; 3.75%; and 5.25% relative to the outer aqueous phase) in the outer aqueous phase. Chitosan solution was prepared by solubilizing the amount of chitosan powder at the corresponding concentrations in 1% acetic acid solution. The amount of acetic acid solution was 37.5% relative to the outer aqueous phase. Rice husk silica of 0.5% was added into the outer aqueous phase containing chitosan solution followed by mixing using a magnetic stirrer at 800 rpm for 10 minutes. The addition of 0.5% rice husk silica only without chitosan was used as the control.

W/O/W double emulsion was prepared by dispersing the primary emulsion W/O emulsion with the fraction of 0.2 into the outer aqueous phase using a rotor-stator at 8000 rpm for 3 minutes. The emulsion was poured into a transparent 40 mL glass vial (ID= 25 mm, height= 95 mm) and stored at a room temperature of ~ 28°C. The percentages used belonged to weight %. The experimental setup for the preparation of W/O/W double emulsion stabilized using chitosan modified rice husk silica can be seen in Figures 1B and 1C.

2.6. *Determination of W/O/W emulsion stability*

The emulsion stability was determined using equation 1. The height of emulsion was measured right after the double emulsion preparations and for several consecutive days up to 7 days. The stable emulsion layer was indicated by a milky appearance excluding the oil, cream, and sediment layers.

$$\%S = \frac{h_t}{h_0} x\,100\% \tag{1}$$

Whereas h_t is emulsion height at a certain time and h_0 is initial emulsion height.

2.7. *Determination of W/O/W emulsion viscosity*

The viscosity of W/O/W emulsion was determined using DV-III Ultra Rheometer (Brookfield, USA) using the spindle type SC4-21. A double emulsion of about 8 mL was put into the small sample holder and the spindle speed was adjusted at 0.5 rpm until constant and the viscosity value was measured.

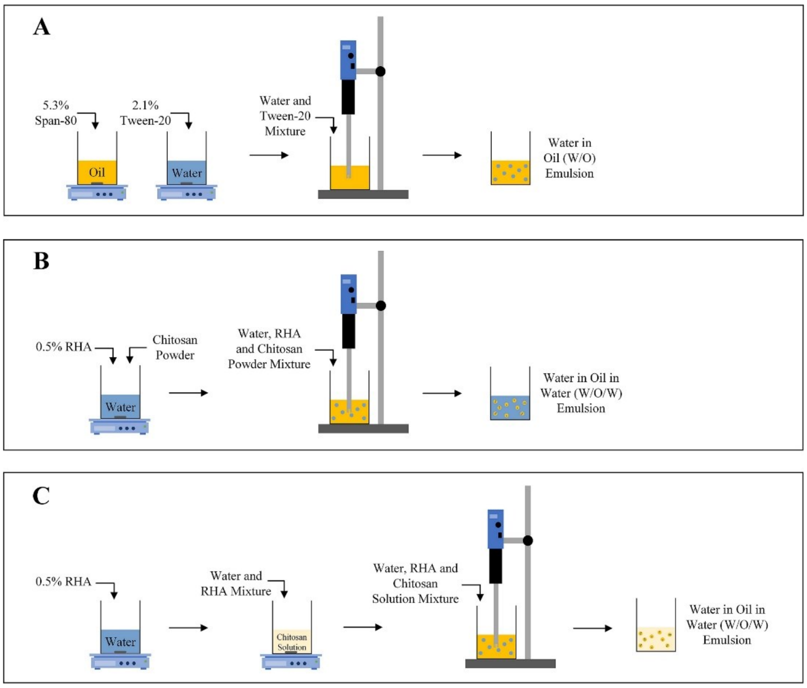


Figure 1 Schematic diagram of the experimental setup. A) Preparation of primary W/O emulsion; B) Preparation of W/O/W double emulsion stabilised with rice husk silica/ chitosan particle; C) Preparation of W/O/W double emulsion stabilised with rice husk silica/ soluble chitosan

3. Results and Discussion

3.1. The effect of chitosan particle concentration and outer aqueous pH on the stability of W/O/W emulsion

The combination of rice husk silica of 0.5% with various concentrations of chitosan powder (0%; 1.25%; 3.75%; and 5.25%) dispersed at the outer aqueous phase of W/O/W emulsion demonstrated different double emulsion stability patterns at different continuous phase pH (2; 3; 4; and ~ 5.7) as could be seen in Figure 2. It turned out that the addition of chitosan powder to rice husk silica enhanced the stability of the double emulsion at all pH ranges except pH 2. It seemed possible that electrostatic attraction occurred between positively charged chitosan particles and negatively charged rice husk silica at the mild pH condition. Chitosan particles attached to silica particles would in turn help increase the adsorption of silica particles at the oil-water interfaces by increasing the wettability of silica particles on the interfacial layer (Tiraferri et al., 2014; Alison et al., 2016; Alison et al., 2018) hence improving the overall stability of the double emulsion. This interaction was similar to the electrostatic interaction between chitosan with negatively charged small-molecule surfactants, protein, and polysaccharides which formed multilayer surfaces rendering the electrosteric stabilization of the emulsion (Klinkesorn, 2013). It was observed that the emulsion stability was increasing with the decrease in chitosan particle concentration (Figure 2B, 2C, and 2D). The chitosan powder concentration of 1.25% seemed to be the optimum concentration which showed the effective adsorption of chitosan particles onto the rice husk silica particles and deposited on the interfacial layer. Moreover, the adsorption process of chitosan into silica surface was found to be irreversible and always stable in the solution (Tiraferri et al., 2014). The number of chitosan particles adsorbed on the silica surface was dependent on the saturation condition which was dictated by pH (Tiraferri et al., 2014). The more concentrated the chitosan powder was, the thicker the sediment layer of chitosan particles observed with time resulting in the decrease of W/O/W emulsion stability. This could be explained by the previous investigation finding out that the increase in chitosan concentration reduced the interaction between chitosan and oil due to a stronger molecular interaction among chitosan molecules because of high electrostatic forces (Li & Xia, 2011).

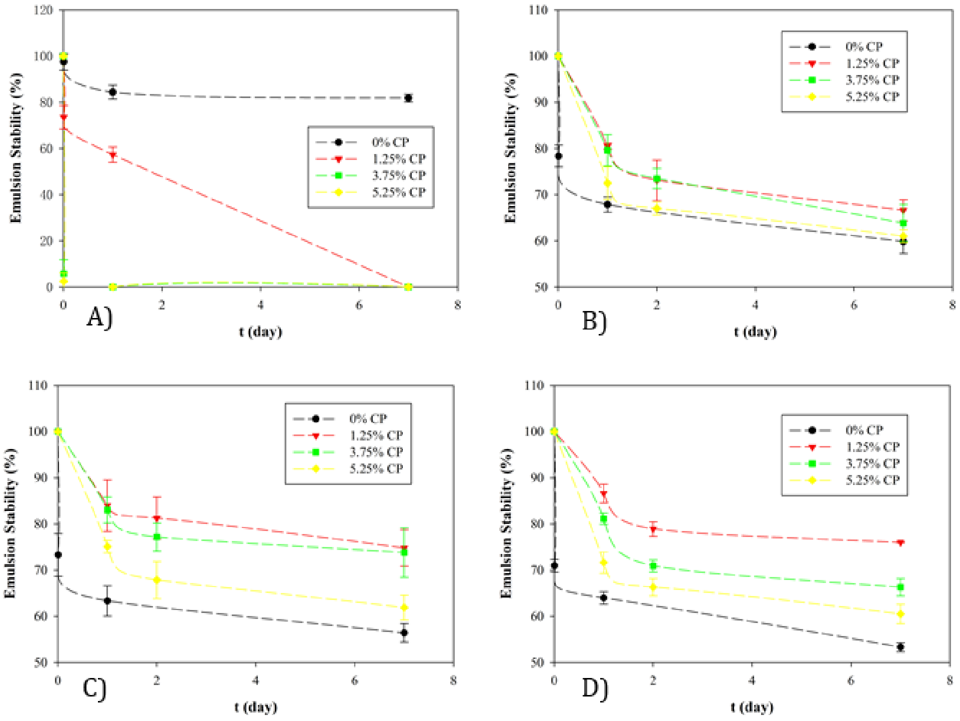
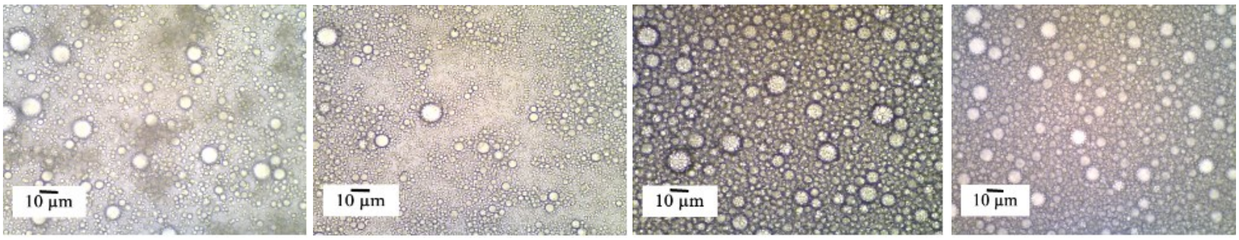


Figure 2 The effect of various chitosan particle concentrations combined with 0.5% rice husk silica in the outer aqueous phase on the stability of W/O/W emulsion. Primary emulsion W/O stabilized with Tween 20/ Span 80 was prepared by dispersing 40% aqueous phase in palm oil. W/O/W double emulsion was obtained by emulsification of 20% primary emulsion into the outer aqueous phase at a certain pH value. a) pH = 2; b) pH = 3; c) pH = 4; d) pH = ~ 5.7. CP was denoted as a chitosan particle

The pH of the outer aqueous phase played a significant role in the W/O/W emulsion stability. The highest stability of W/O/W emulsion was attained at pH 4 and the lowest stability was seen at pH 2 at all ranges of chitosan particle concentrations (see the representative figure in the supplementary file). There were only slight differences in double emulsion stability between those prepared at pH 3 and 5.7 with that prepared at pH 4. The visual macroscopic observation showed that the emulsions prepared at pH 4 demonstrated creaming but without the appearance of an oil layer on the top as observed on the other emulsions prepared at other pHs after 7-day storage (data not shown). Furthermore, the emulsions prepared at pH 4 also showed the least particles sediments at the bottom implying the adherence of both silica and chitosan particles on the interfacial layers. The adsorption process of chitosan particles onto the silica surface at pH 4 was transport-controlled, meaning that all chitosan molecules approaching the silica surface were successfully attached due to electrostatic attraction between chitosan molecules and substrates at acidic pH (Tiraferri et al., 2014). Furthermore, silica particles were partially hydrophobized upon the addition of chitosan rendering their adsorption at the oil-water interface and the formation of Pickering emulsion (Alison et al., 2018). Within the pH ranges of 3 to 5.7, rice husk silica underwent a dissociation process forming SiO^- groups with negative charges on the surface which tended to be more hydrophilic, whereas chitosan particles became positively charged due to the formation of protonated amine group NH_3^+. Consequently, electrostatic interaction between negatively charged silica particles and positively charge chitosan particles was facilitated. The interacted particles improved their role as rigid emulsifiers on the interfacial layers mainly acting as barriers against flocculation and coalescences of oil globules. Such synergistic interaction between silica and chitosan particles did not occur when pH was reduced to 2. The double emulsion stability was abruptly reduced to zero upon the addition of chitosan particles after 7-day storage as seen in Figure 2A. It was already known that silica was negatively charged above the isoelectric point (IEP) and point of zero charges, i.e. around pH 2 (Dyab, 2012; Pichot et al., 2012; Sapei et al., 2018; Alison et al., 2018). Therefore, the electrostatic interaction between silica and chitosan did not occur since silica was chargeless although chitosan was positively charged below its pKa value of about 6 (Tiraferri et al., 2014; Alison et al., 2018). Furthermore, the use of rice husk silica only without chitosan particles at pH 2 demonstrated the highest W/O/W emulsion stability over time (Figure 2A) since silica particles tended to be adsorbed and aggregated at the interfaces forming a multilayer barrier against oil droplet coalescences.

The microstructure of W/O/W double emulsion stabilized by 0.5% rice husk silica/ 1.25% chitosan particles prepared at various pHs of the outer aqueous phase could be seen in Figure 3. It was seen that the formation of the double emulsion was most pronounced on the W/O/W emulsion prepared at pH 4. Moreover, the highest double emulsion stability of about 78% was still retained after 7 days. This again confirmed the possibility of the formation of multilayer surfaces at pH 4 consisting of silica and chitosan layer surrounding the oil globules, which provided the electrostatic stabilization against coalescences. Moreover, these rigid layers could also prevent the diffusion of the inner aqueous phase towards the outer aqueous phase therefore, the presence of inner aqueous droplets inside the oil globules seemed to be more preserved compared to others prepared at other pH values.



(a) (b) (c) (d)

Figure 3 The microstructures of W/O/W emulsion stabilized with 0.5% rice husk silica/ 1.25% chitosan particles at various pH of outer aqueous phase in day 1. a) pH = 2; b) pH = 3; c) pH = 4; d) pH = ~ 5.7

The interaction between rice husk silica and chitosan particles at the interfacial layer of the secondary emulsion could be explained pertained to the pH value of the outer continuous phase. It was figured out that the strongest interaction between silica and chitosan particles occurred at pH 4 as negatively charged silica particles and positively charged chitosan particles are conductively formed. Silica particles were strongly adsorbed on the interfaces between oil droplets and the outer aqueous phase. The rigid barrier of silica-chitosan particles on the interfaces provided high stability against coalescence and flocculation of the resulting W/O/W emulsion indicated by stability of about 80% after 7 days. As pH was getting lower than 4, the dissociation of silica particles into SiO^- were decreased while chitosan particles were increasingly positively charged. The interaction between silica and chitosan particles was diminishing. At pH 2 silica particles were presumed to be chargeless and the interaction between silica and chitosan particles was hardly seen even though silica particles could aggregate and formed a multilayer surrounding the oil globules against flocculation and coalescences. However, the silica particles would likely be detached from the interfaces with time. As the pH was getting higher at pH ~5.7, silica particles retained the negative charges as their hydroxyl groups were continued to be dissociated while the positively charged chitosan particles were decreased since the protonation of the amine group were decreased as pH was increasing, thus lowering the interaction between silica and chitosan particles.

3.2. The effect of chitosan solution concentration and outer aqueous pH on the stability of W/O/W emulsion

The stability of W/O/W double emulsion stabilized with rice husk silica combined with the addition of soluble chitosan of various concentrations on the outer aqueous phase was investigated and compared with those added with chitosan particles. It was obvious that the stability of W/O/W emulsion increased with the increasing concentration of soluble chitosan on the outer aqueous phase as depicted in Figure 4. Moreover, at the soluble chitosan concentration of 3.75% and 5.25%, the stability of W/O/W emulsion was extraordinarily stable for up to 7 days without the occurrence of any creaming or sedimentation regardless of the pH values of the outer continuous phase.

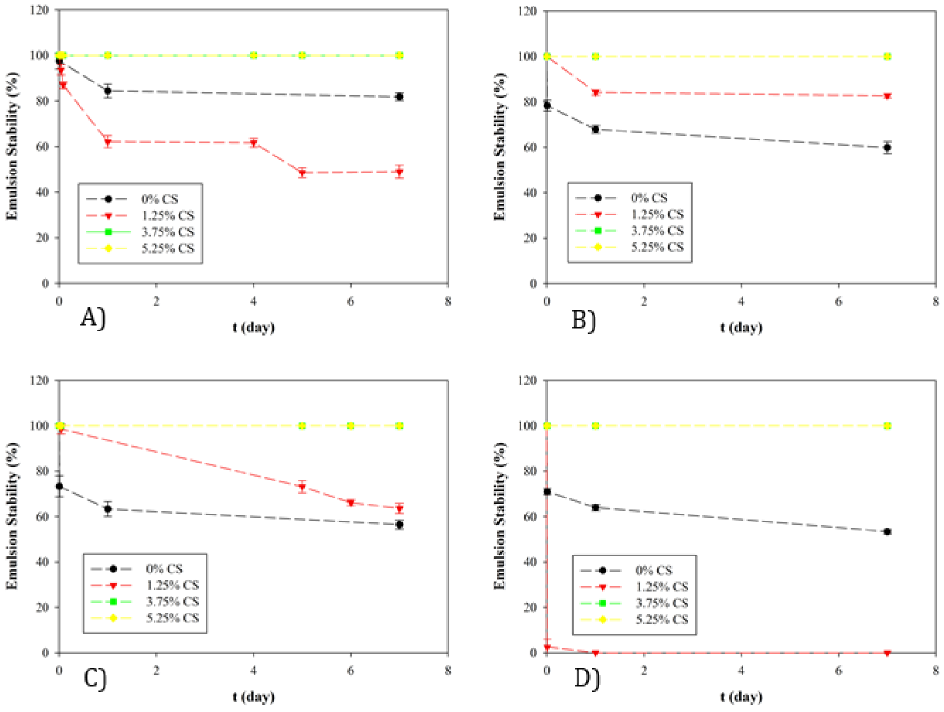
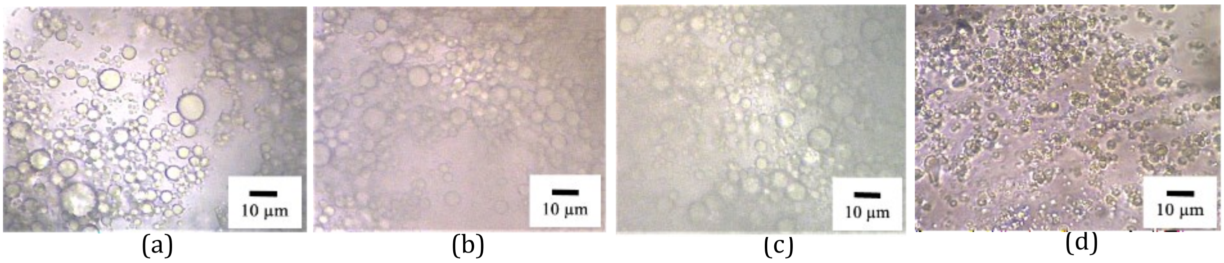


Figure 4 Effect of various chitosan solution concentrations combined with 0.5% rice husk silica in the outer aqueous phase on the stability of W/O/W emulsion. Primary emulsion W/O stabilized with Tween 20/ Span 80 was prepared by dispersing 40% aqueous phase in palm oil. W/O/W double emulsion was obtained by emulsification of 20% primary emulsion into the outer aqueous phase at a certain pH value. A) pH = 2; B) pH = 3; C) pH = 4; D) pH = ~ 5.7. CS denoted as chitosan solution

The remarkable stability of W/O/W emulsions added with soluble chitosan was due to the significantly increased viscosity of the outer aqueous phase which slowed down the rate of flocculation or coalescence of oil globules. Once chitosan dissolved in a 1% acetic acid solution, it readily formed a viscous solution which gave rise to the viscosity of the outer continuous phase. The increase in chitosan concentration increased the viscosity of the outer continuous phase hence increasing the overall stability of W/O/W double emulsion. The viscosity was increased by more than 100 folds to about 186,000 cP when soluble chitosan of 5.25% was added into rice husk silica stabilized W/O/W emulsion as compared with that added with 1.25% chitosan particle at the outer aqueous phase pH of 4 as could be seen in Table 1. The increased viscosity of the continuous phase decreased the creaming rate due to the decrease in oil droplets and flocs diffusion (Klinkesorn, 2013). This was confirmed by the demonstration of tiny oil globules dispersion in the quite thick outer aqueous phase in all W/O/W double emulsions stabilized by 0.5% rice husk silica and 5.75% soluble chitosan prepared at various outer aqueous pH as could be seen in Figure 5.

Tabel 1 Viscosity of W/O/W emulsion stabilized with 0.5% rice husk silica/ chitosan at various pH of the outer aqueous phase

Chitosan	pH	Viscosity (cP)
1.25% Chitosan particles	4	1,600
5.25% Soluble chitosan	2	90,200
5.25% Soluble chitosan	3	137,000
5.25% Soluble chitosan	4	186,000
5.25% Soluble chitosan	±5.7	2,400



(a) (b) (c) (d)

Figure 5 The microstructures of W/O/W emulsion stabilized with 0.5% rice husk silica/ 5.25% soluble chitosan at various pH of outer aqueous phase in day 1. A) pH = 2; B) pH = 3; C) pH = 4; D) pH = ~ 5.7

The effect of pH on the W/O/W emulsion stability was seen in the double emulsion with the addition of 1.25% soluble chitosan. The double emulsion stability dropped to zero at pH 5.7. Moreover, the viscosities of W/O/W emulsions stabilized by 0.5% rice husk silica/ 5.25% soluble silica tremendously increased until pH 4 and then abruptly dropped when pH was 5.7 (Table 1) even though it still looked completely stable until day 7 (Figure 4D). With increasing pH, chitosan becomes progressively neutral thus decreasing the repulsion between adsorbed molecules (Tiraferri et al., 2014). The loss of electrostatic repulsion between the charged groups leads to the formation of insoluble chitosan aggregates at the O/W interfaces and the formation of a chitosan network in the continuous phase (Klinkesorn, 2013; Mwangi et al., 2016). It seemed plausible that the increased insolubility of chitosan molecules at pH 5.7 resulted in the decrease of the viscosity of W/O/W emulsion.

The mechanism of how soluble chitosan improved the stability of rice husk silica stabilized W/O/W emulsion was different from that of chitosan particles. The soluble chitosan behaved as a hydrocolloid biopolymer, which enabled the retardation of creaming phenomena by modifying the rheology properties, particularly the viscosity of the outer aqueous phase. The soluble chitosan significantly increased the viscosity of the outer aqueous phase which improved the stability of the W/O/W double emulsion by preventing the flocculation and coalescences of oil globules. Rice husk silica which was deposited on the interfacial layer would even not be easily detached due to the increasing resistance of the silica particles' motion in the outer continuous phase. This mechanism was different than the stabilization mechanism of chitosan particles which were deposited at the interfacial layer due to electrostatic attraction between silica and chitosan particles. The increased viscosity due to the dissolved chitosan increased the W/O/W stability by hindering oil globules' flocculation or coalescences (see the supplementary file).

4. Conclusions

The stability of W/O/W double emulsion stabilized by rice husk silica was much improved from approximately 60% to about 80-100% when chitosan in the form of particles and solution was added into the outer aqueous phase, respectively. The stabilization mechanisms seemed different between those added with chitosan particles and those added with soluble chitosan. Chitosan particles electrostatically adsorbed on the silica particles at the oil-water interfaces forming a rigid barrier thus preventing flocculation and coalescences of globules. On the other hand, soluble chitosan drastically enhanced the viscosity of the outer aqueous phase thus retarding the flocculation and coalescence rates of oil globules. The pH of the outer aqueous phase played an important role in dictating the positive charge of chitosan particles and the negative charge of silica particles thus influencing the extent of their electrostatic attraction. The pH 4 seemed optimal for achieving W/O/W double emulsion with high stability. The presence of the inner aqueous phase within the microstructure of W/O/W double emulsion was more pronounced at pH 4 compared to those prepared at another pH. The chitosan concentration also affected the overall stability of the W/O/W double emulsion. Fewer chitosan particles seemed preferable to have effectively adhered to the silica particles while increased soluble chitosan was advantageous for improving the viscosity of the outer aqueous phase. The combination of rice husk silica and chitosan as emulsifiers of the secondary emulsions seemed potential to be used for the development of low-calorie and healthy food emulsion-based products.

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