Physical Characterization of Red Palm Oil Emulsion

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Abstract: The oil/water emulsion is widely studied as a carrier system of oil-soluble bioactive components. This study aimed to investigate the formation of a whey protein-based red palm oil (RPO) emulsion system and its stability during long-term storage. The RPO emulsion utilizing three different emulsifiers (WPCa, WPCb, and WPI) with three different emulsifier concentrations (*i.e.*, 2.5, 5, 10, and 15% w/v) in the aqueous phase was prepared. A mixture of whey protein solution containing 30% (v/v) of RPO was prepared and emulsification was performed with two-steps homogenization processes. Emulsions produced were stored at ambient temperature and evaluated for their physical stability by determining the qualitative rating of destabilization and oil droplet viability and measuring its separation index (SI, %). The results showed that the type and concentration of emulsifiers affected the emulsions' stability during long-term storage at ambient temperature. The system emulsified with WPCa and WPCb was found to be more stable with a lower SI value than that of WPI. Furthermore, emulsions with higher emulsifier concentrations were found to be more stable, with lower SI values. The RPO emulsion with 15% of WPCb was the most stable system, having SI of 0% after 105 days of storage.

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1 INTRODUCTION

The oil-in-water (O/W) emulsion is one of the carrier systems widely reported increasing the stability of the fat-soluble food bioactive components (Aswathanarayan & Vittal, 2019; Banasaz, Morozova, Ferrentino, & Scampicchio, 2020; Gasa-Falcon, Odriozola-Serrano, Oms-Oliu, & Martín-Belloso, 2020; D. J. McClements, Decker, & Weiss, 2007; David Julian McClements, 2010; Özbek & Günç Ergönül, 2017; Wang, Neves, Isoda, & Nakajima, 2015). The O/W system is known to provide a protection mechanism for the entrapped components by forming a "wall" composed of the emulsifier (D. J. McClements et al., 2007; Wang et al., 2015). The ionic interaction and repulsive forces exerted by the emulsifier and other constituent components of the emulsion (i.e., co-emulsifier, co-solvent, or other stabilizers) can also enhance the protection mechanisms provided by the system (Wilde, Mackie, Husband, Gunning, & Morris, 2004). The "wall" defines the entrapped oil portion from interactions with environmental factors that can reduce its stability (*i.e.*, oxygen, light, or metal cations).

Red palm oil (RPO) is a type of edible oil rich in natural fat-soluble bioactive micronutrients (Chong et al., 2018; Dauqan, Sani, Abdullah, Muhamad, & Md. Top, 2011; Loganathan, Subramaniam, Radhakrishnan, Choo, & Teng, 2017; Nagendran B., Unnithan U., Choo, Sundram, 2000; Scrimshaw, 2000). RPO contains high levels of carotenoids with potential natural antioxidants and pro-vitamin A activity. RPO also contains high levels of vitamin E in the form of tocotrienols and tocopherols, and other food bioactive compounds in a considerable amount

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(phytosterols, ubiquinone, and squalene). RPO is potential to be used as a source of natural fat-soluble bioactive micronutrients based on its rich nutritional value. However, the RPO tends to be less stable to oxidation during storage (Ayu, Andarwulan, Hariyadi, & Purnomo, 2016; de ALMEIDA, Viana, Costa, Silva, & Feitosa, 2019; Sohail, Ahmed, Akhtar, Durrani, & Section, 2010). This has led RPO has not been intensively used as a source of fat-soluble bioactive micronutrients. Therefore, there is a need to enhance the stability of RPO during storage to improve its utilization as a source of fat-soluble food bioactive compounds.

The formation of the O/W system has the potential to be used to increase the stability of fat-soluble bioactive micronutrients in RPO. In this study, we studied the production of RPO-in-water emulsion system. Emulsions stabilized with natural emulsifiers could provide additional functional value to the system (i.e., nutritional value) (Adjonu, Doran, Torley, & Agboola, 2014). Concerning the current trend in using natural emulsifiers (*i.e.*, protein, pectin, gum, or other biopolymer compounds) in emulsions (Charoen et al., 2011; Matalanis, Jones, & McClements, 2011; David Julian McClements, 2009; Qian & McClements, 2011; Wilde et al., 2004), we considered that whey protein has good potential as a single emulsifier in the RPO emulsion system. To the best of our knowledge, emulsion systems with whey protein as single emulsifiers have never been reported to be an emulsifier in RPO emulsion systems. A stable whey protein-based RPO emulsion system is needed to increase the stability of RPO emulsified as well as to maintain the emulsion stability.

This study then aimed to investigate the formation of a whey protein-based RPO emulsion system and its physical stability during long-term storage. Three types of commercially available whey protein were used as the single emulsifier in our RPO emulsion system.

2 MATERIALS AND METHODS

2.1 Materials

RPO was obtained from the Indonesian Oil Palm Research Institute (IOPRI). Whey Protein Concentrate (WPCa) (Avonlac™ 180) had a total protein of >78 %, water ≤5.0 %, fat <12.0 %, mineral <5.0 %, and sugar 5.0. Whey Protein Concentrate (WPCb) (OptiSol® 1007) had a total protein >76 %, water <6.0 %, fat <13.5 %, mineral <6.0 %, and sugar 5.0. Whey Protein Isolate (WPI) 90 (Provon 190) had a total protein >90 %, water <5.0 %, fat <0.7 %, mineral <3.5 %, and sugar 0.7. These whey protein products were obtained from Glanbia Nutritionals Singapore Pte Ltd., Singapore. The deionized water was obtained from Hach Lange GmbH, Germany. Sodium azide (NaN_3) (pro-analysis grade) was purchased from Merck KGaA, Darmstadt, Germany.

2.2 Emulsification of Whey Protein-based RPO Emulsions

The WPI, WPCa, or WPCb (2.5, 5, 10, 15 %; w/v) were dissolved in deionized water containing 0.02 % (w/v) NaN_3 and stirred (IKA C-MAG HS7, Staufen, Germany) for one hour at ambient temperature. The whey protein solutions were then stored overnight (±18-20 h) at 4±1 °C to allow protein hydration. Whey protein-based RPO emulsions were prepared by combining phase inversion technique and rotor-stator homogenization. Whey protein solution was added gently to RPO to produce a mixture containing 30 % (v/v) of RPO. Emulsification was carried out with two-steps homogenization. Initially, the rotor-stator homogenizer (Silverson L4r, Silverson Machines, Ltd, Bucks, UK) was set to 9000 rpm for two minutes and followed by an increased speed at 18000 rpm for five minutes. The emulsions was produced in duplicate

2.3 Physical Stability of Emulsion

A 40 mL of RPO/W emulsion was transferred immediately to a 50-mL measuring cylindrical glass (Iwaki Pyrex) after emulsification and stored at ambient temperature. The stability of emulsions was analysed by visual observation and was expressed in as three categories. For the first category, the emulsions destabilisation was rated into discontinuing values (0 to 6) defined in Table 1 and noted as rating of destabilisation (RoD). Second category was focused on the oil droplet viability (ODV) on the surface of the emulsion system, expressed in zero to six scale (*i.e.*, 0 = oil separation was not identified, and 6 = high amount of oil creamed at the surface. In the third category, the emulsion stability was evaluated based on the separation index (SI, %). SIs of the stored emulsions were evaluated on the 1st, 7th, 14th, and 105th days of storage. SI was calculated by measuring the ratio of separated portion volume (H_S) to the volume of total system (H_E) (Equation 1).

$$SI\ (\%) = \frac{H_s}{H_E} \times 100 \quad (1)$$

Table 1: Description of emulsion destabilisation score.

RoD	Description
1	Emulsion was visually stable; no change or separation was observed
2	Emulsion started to separate, identified by a visually thin color gradation
3	Emulsion experienced one type of separation; such as creaming development (at the top layer of emulsion) or oil droplet separation (at the top surface of emulsion) or serum separation (at the bottom layer of emulsion) or formation of whey powder sediments (at the bottom of emulsion).
4	Emulsion experienced two types of separation.
5	Emulsion experienced three types of separation.
6	Emulsion experienced four types of separation.

3 RESULTS AND DISCUSSIONS

The RPO emulsion emulsified with whey protein formed by two-step homogenization employing rotor-stator homogenizer. Previously, we performed a preliminary study to estimate the required amount of whey protein used. Based on preliminary research results, whey protein started at 2.5% concentration in the continuous phase showed good performances on the initial physical appearances of the emulsions. Therefore, the RPO emulsion was fabricated utilizing a whey protein at 2.5-15% in the continuous phase. The emulsions with emulsifier concentrations of 2.5% and 5% had relatively small amounts of cream droplets on the top surface as soon as the emulsification process finished. There were 12 variants of RPO emulsions produced and showed a yellowish visual appearance and were either dense or not translucent. The RPO emulsions emulsified with WPI had a more dilute consistency than those with WPCa and WPCb.

3.1 SI (%) of the Whey Protein-based RPO Emulsions

The whey protein-based RPO emulsions showed different destabilization phenomena influenced by the type of emulsifier. RPO emulsion emulsified with WPI was observed to experience a different destabilization phenomenon compared to RPO emulsion emulsified with WPCa and WPCb. The visually observed destabilization phenomenon is presented in Figure 1. The RPO emulsion emulsified with WPI experienced destabilization in the form of splitting the system into three noticeable parts, whereas the RPO emulsion emulsified with WPC experienced separation into only two noticeable parts. Several previous studies also reported differences in the appearance of phase separation due to destabilization into two parts or three separate parts with different emulsifiers and system composition (Anvari & Joyner (Melito), 2017; Zhang et al., 2019). The separation of the emulsion prepared with WPI could be identified in the form of cream, emulsion, and serum; whereas the separation of RPO emulsions emulsified with WPCa and WPCb was indicated by the formation serum (see Figure 1). The destabilization phenomena were differed presumably due to the inherent emulsifier's physicochemical characteristics (*i.e.*, protein concentration, charge, structures). WPI with higher protein concentration is considered to have more intense protein intermolecular interactions in the resulting emulsion system. Herein, the creaming process is noticeable.

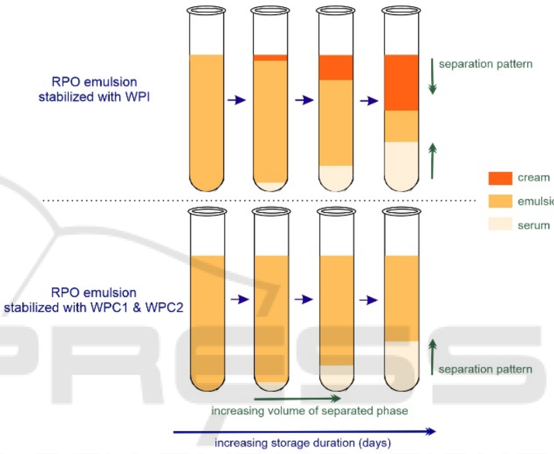

Figure 1: Destabilisation pattern observed on the whey protein-based RPO emulsions during storage.

The stability of the whey protein-based RPO emulsion was evaluated based on the separation index (SI, %). Based on the destabilization phenomena described previously, we propose the use of SI to describe the stability characteristics of a protein-based emulsion system based on visual observation. Generally, a creaming index (CI, %) is used to describe emulsion stability in evaluating visual observations over long-term storage (David Julian McClements, 2007). However, the CI value could not indicate the system's differences in destabilization when the separation of the cream and serum phases (the emulsion separated into three parts) was noticeable, or the separation of the serum layer was unnoticeable even though the separation of the cream layer was observed (the emulsion separated into two parts). SI value defined in this study was calculated based on the ratio of the separated parts' volume to the total system's volume (see Equation 1). SI values and the documentation of the whey-protein based RPO emulsion are shown in Figure 2.

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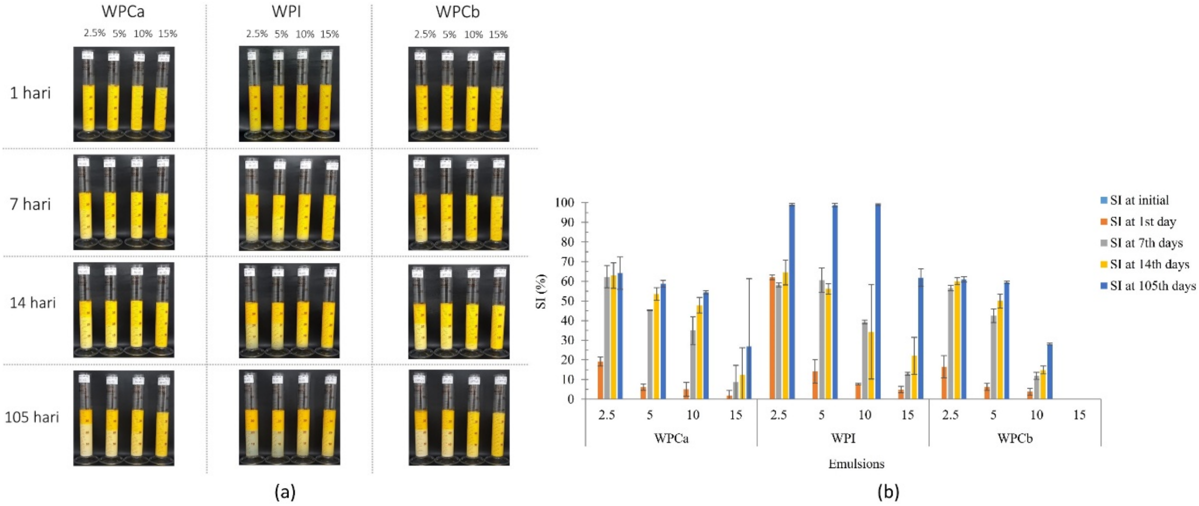

Figure 2: The documentation of physical appearance (a) and SI (%) (b) of the whey protein-based RPO emulsions after 1, 7, 14, and 105 days of storage at ambient temperature.

The results showed that the systems emulsified with WPCa and WPCb had lower SI values than the ones emulsified with WPI. The systems prepared with WPCa and WPCb had relatively similar SI values. The systems emulsified with WPI experienced increased SI values respective to the storage time. On the other hand, SI values for the systems emulsified with WPCa and WPCb did not change significantly after experiencing separation. The systems emulsified with WPCa and WPCb experienced separation after 14 days of storage. At longer storage time (*i.e.*, more than 14 days), these systems did not show significant changes.

The concentration of whey protein used also influenced the stability of the emulsion. Emulsions with higher emulsifier concentrations were found to be more stable, as indicated by lower SI values. The system with an emulsifier concentration of 15% was observed to have the best stability among the concentrations tested for each type of emulsifier tested. The results showed that a system stabilized with WPCb at 15% showed the best stability with a separation index value of 0% after 105 days of storage. At extended storage, the RPO emulsion system emulsified with 15% WPCb emulsifier was stable for up to 5.5 months. RPO emulsion prepared with WPCb was more stable than that of WPCa at 15% concentration. This was presumably due to differences in fat and mineral content in WPCb and WPCa. The differences in whey protein' physicochemical characteristics can result in differences in repulsive forces and ionic interactions, shown through the emulsion's physical stability (Abd El-Salam, El-Shibiny, & Salem, 2009; Adjonu et al., 2014; Dybowska, 2011; Ravindran, Williams, Ward, & Gillies, 2018).

3.2 Destabilization and Oil Droplet Viability of the Whey Protein-based RPO Emulsions

The destabilization of whey protein-based RPO emulsions was also evaluated based on the rating of destabilization (RoD) and the rating of oil droplet viability (ODV) at the top of emulsions. The system was evaluated for the appearance of destabilization at the beginning of storage and also after 1st, 7th, 14th, and 105th days of storage. RoD and ODV scores of the whey protein-based RPO emulsion are presented in Figure 3. The results confirm the findings described in section 3.1 that the twelve whey protein-based RPO emulsions had varying stability.

A whey protein-based RPO emulsion system with a higher emulsifier concentration was observed to be more stable on storage. There is a trend that shows the type of emulsifier affects the stability of the emulsion system. The difference in stability was perceived from the destabilization that first occurred in the system with WPI, namely the separation in the form of cream formation (shown in Figure 1). In fresh emulsions, systems with higher emulsifying concentrations, such as 10% and 15%, were observed to be more stable with no appearance of cream droplets on the top surface except in the emulsion emulsified with WPI. Emulsion emulsified with WPI was observed to experience complex destabilization earlier than the system with WPC; the higher rating of destabilization system indicates this since the beginning of storage.

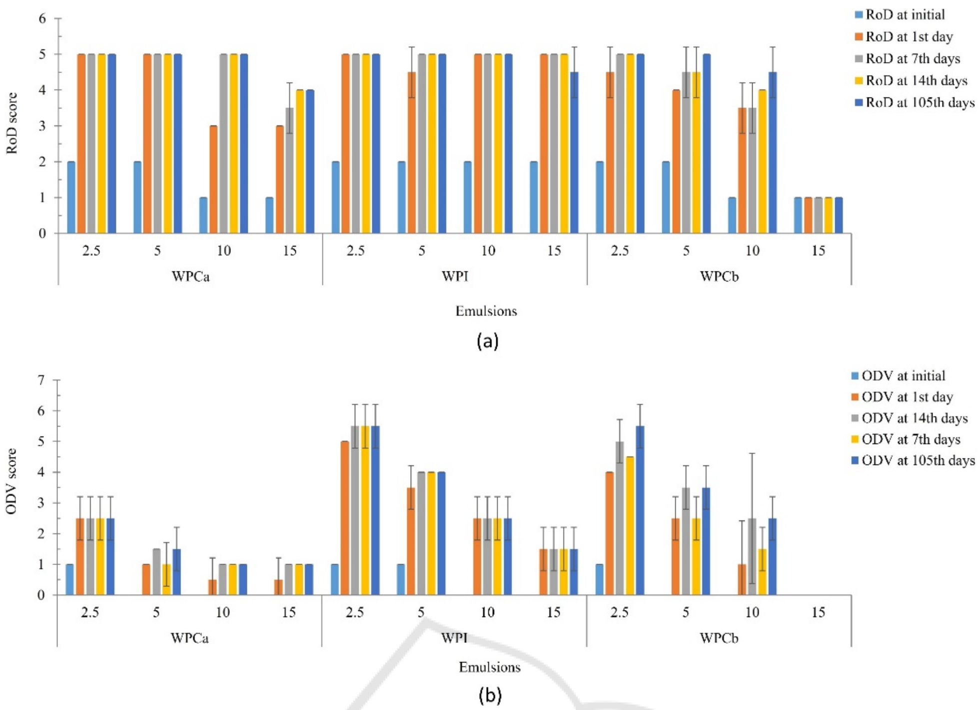

Figure 3: The rating of destabilisation (a) and oil droplet viability (b) of the whey protein-based RPO emulsions produced during storage.

The whey protein-based RPO emulsion system was not observed to undergo a large amount of oil phase separation. Oil separation was observed in the form of oil droplets that did not form an oil layer. This was due to the characteristic of protein that can absorb oil in the emulsion system. The WPI-stabilized emulsion was observed to have more separated oil droplets than that of WPCa and WPCb. This results are corresponding to the findings we described earlier that the systems emulsified with WPCa and WPCb were more stable than the ones emulsified with WPI. The systems with lower emulsifying concentration were observed to have more oil droplets separated. Therefore, the whey protein-based RPO system had better stability to oil droplet separation when the concentration used was high (*i.e.*, 15%).

4 CONCLUSIONS

Whey protein-based RPO emulsion was produced with a rotor-stator in this study. The emulsion's stability was investigated based on visual observation. Whey protein emulsifier type and its concentration were found to influence the stability of RPO emulsion. A stable emulsion system was obtained by having WPCb as the emulsifier at 15% concentration. Conclusively, WPCb is proposed as a potent emulsifier for producing RPO emulsion with better stability.

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