Preparation of Double Emulsion of Vitamin C with Two Different Emulsifiers in the Outer Aqueous Phase

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Abstract: Vitamin C is one of common bioactive compound widely known and used by many people, especially in Indonesia. The weakness of vitamin C stablity are sensitive with extreme pH, temperature, oxygen and direct light. Because of that, the encapsulation of itamin C with $W_1/O/W_2$ emulsion can be the solution for maintaining the stability of vitamin C. This study is aimed to emulsify Vitamin C in the inner aqueous phase of water-in-oil-water ($W_1/O/W_2$) emulsions with soybean oil as the oil phase. Two type of emulsifier Tween 80 and WPC-Pectin in the outer aqueous phase used to compare their stability during storage. The result of this research showed that emulsion with WPC-Pectin had bigger droplet size 1.34 μm than emulsion with tween 80 0.91 μm. Tween 80 had a better stability with 14.41% of creming index compared to WPC-Pectin with 23.06% of creaming index. Morphology of $W_1/O/W_2$ emulsion with Tween 80 can be described as $W_1/O/W_2$ when emulsion with WPC Pectin cannot be described as $W_1/O/W_2$ Emulsion. Tween 80 is a better emulsifier than WPC-Pectin to stabilized the $W_1/O/W_2$ emulsion in the outer aqueous phase.

1 INTRODUCTION

Vitamin C is a water-soluble vitamin which is widely known and popularly used in Indonesia. This is proven by the number of food products that contain vitamin C in it. Vitamin C has the main function as a compound that can maintain body endurance and can increase skin moisture (Gregory, 2017). Vitamin C consists of ascorbic acid compounds that have a low pH and sour taste that is obtained from citrus fruits such as oranges, lemons, lime and berries (strawberries, blueberries and others). However the stability of Vitamin C is very prone and it would affect it functionality. Vitamin C is unstable to extreme temperatures, extreme pH, high oxygen, and light (Levine et al., 2004). Prolonged contact can cause damage to vitamin C and ultimately can be bad for health. Therefore, further efforts are needed so that the vitamin C compound remains stable during processing and storage.

Emulsification is one method of maintaining vitamin C compounds in food products. Emulsion is a dispersion system which consists of two solutions which do not dissolve each other. There are two basic types of emulsions, namely oil-in-water (O/W) emulsion and water-in-oil (W/O) emulsion. This emulsion is called as single emulsion. Another emulsion system namely water-in-oil-in-water ($W_1/O/W_2$) is a method for emulsifying hydrophilic bioactive components. $W_1/O/W_2$ consists of an internal water phase (W_1) which can be filled with bioactive components such as vitamin c, intermediate oil phase (O) and external water phase (W_2). The use of water as an outer phase of the emulsion system aims to keep vitamin C soluble in water so that it is easily applied in various processed food products.

The emulsion system $W_1/O/W_2$ uses two emulsifiers where the first emulsifier has high hydrophobic properties while the second emulsifier has high hydrophilic properties. The use of these two types of emulsifiers play an important role in the stability of the emulsions formed (Anton, Vandamme, Ding, Yu, & Serra, 2018). The use of emulsifiers to be studied are high molecular weight emulsifiers such as bio complex proteins (whey protein concentrate, and pectin) and low molecular weights such as tween 80 in the outer phase of the system. The effect of both type emulsifiers on the

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stability of the double emulsion during storage was studied.

2 MATERIAL AND METHODS

2.1 Material

Vitamin C (Brataco, Indonesia), Aqua Deminerals (Brataco, Indonesia), Commercial soybean oil (Salim Ivomas, PT., Indonesia), Natrium Chloride (Brataco, Indonesia), Propyl Paraben (Brataco, Indonesia), Citric Acid (Weifang Ensign Industry Co. Ltd., China), Magnesium Sulfate (Brataco, Indonesia), Tween 80 (Croda International, UK), Span 65 (Futura Ingredients Pte, Ltd., Singapore), Pectin (CP Kelco, U.S.) and Whey Protein Concentrate 80% (Avonlac, Glanbia USA).

2.2 Methods

2.2.1 Preparation of the W_1 / O (Water in Oil) Emulsion System

Aqueous phase in the form of a 10% vitamin C solution was prepared by mixing 10 g (10% w / v) into 100 ml aqua demineralized and adding 2 grams of $MgSO_4$. The oil phase is made by adding 3.5 grams (5% w / v) Span 65 to 70 ml of soybean oil at 70°C until it dissolves at a speed of 500 rpm. W_1/O emulsions were prepared by adding 30 ml of the water phase to 70 ml of the oil phase at room temperature (around 25 °C) at 800 rpm, followed by the first stage emulsification at 4000 rpm for 2 minute using homogenizer.

2.2.2 Preparation of W_2 Biopolymer Solution and Tween 80

Biopolymer solution was used as a second emulsifier made from a mixture of 80% WPC (Whey Protein Concentrate) and pectin. Biopolymer solution was prepared by dissolving 3.1 g (3.5% w/v) WPC into 100 ml of aqua demineralized at 50˚C for 30 minutes then adding 0.1 g of pectin, 0.7 g of citric acid, 3.9 g of NaCl and 0.5 g of propyl parabens. After 30 minutes, the solution is lowered to room temperature and stored for 24 hours at 4°C. Preparation of tween 80 solution refers to Gharehbeglou *et al.*, (2019) with a slight modification. Tween 80 of 3.6% (w/v) was dissolved into aqua demineralized at room temperature, also added 3.3% NaCl (w/v) to maintain the osmotic pressure of the emulsion system that will be formed. The solution is then stored at a chiller temperature before use.

2.2.3 Preparation of $W_1/O/W_2$ (Water in Oil in Water) Emulsion Systems

Preparation of the $W_1/O/W_2$ emulsion system was firstly done by mixing 20 ml of W_1/O emulsion into 80 ml of W_2 solution, then the mixture was homogenized with Ultrasonic Homogenizer (Qsonica Q500, US.) for 10 minutes with a pulse of 3 seconds on 3 seconds off 70% amplitude. The homogeneity process aims to homogenize the solution and reduce the size of the droplet emulsion. The $W_1/O/W_2$ emulsion formed is stored in a bottle at 25℃ for stability testing for 14 days. The process of double emulsion can be seen in Figure 1.

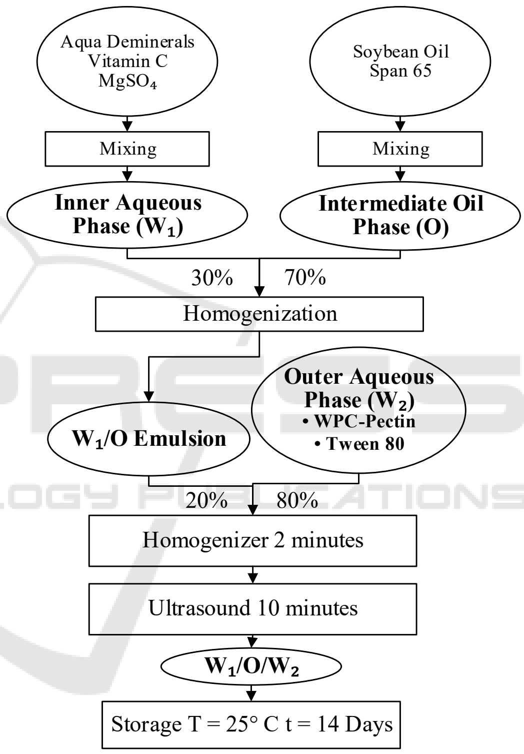

Figure 1: Preparation of Double Emulsion.

2.2.4 Particle Size Measurement

The particle size of droplet was analyzed with particle size analyzer (Beckman Coulter LS 13 320, Beckman Coulter, Inc. US). Particle size was measured for the fresh emulsion immediately after preparation (H0), Days 7 (H7) and after 14 Days (H14) storage at 25℃.

2.2.5 Creaming Index Measurment

Measurement of the creaming index by calculating the height of the cream formed divided by the height of the sample. The creaming index measurement was carried out every 4 days for 14 days at 25℃ then the creaming index percentage was calculated using the formula:

$$CI = (\frac{h_0}{h_1}) \times 100\%$$

CI = Creaming Index (%)
h_0 = Cream Height
h_1 = Sample Height

2.2.6 Morphology Particle of $W_1/O/W_2$

Morphology particle of W/O/W emulsions were made by Transmission Electronic Microscope (Jeol, Japan) after 14 days of storage.

3 RESULT AND DISCUSSION

Particle size of the emulsion with tween 80 increased after storage for 7 and 14 days. Emulsion with biopolymer solution has a bigger droplet compare to tween 80. Emulsion with biopolymer solution start from 1.34 µm and tween 80 start from 0.91 µm. According to Gharehbeglou et al., (2019) WPC and Pectin have larger droplet sizes due to interactions between the WPC and pectin forming a massive complex and trapped the oil phased inside it.

Particle size was increased significantly in the emulsion with WPC-Pectin than tween 80. Figure 2 shows droplet size of both emulsions increased on the 7th day, on the 14th day, only the droplet of emulsion with tween 80 increased by 1.15 µm while the emulsion with WPC Pectin decreased from 1.32 µm to 0.87 µm. The decrease in droplet emulsion in Figure 2 can occur due to the broken of the W_1/O emulsion in the $W_1/O/W_2$ emulsion so it changes into an O/W.

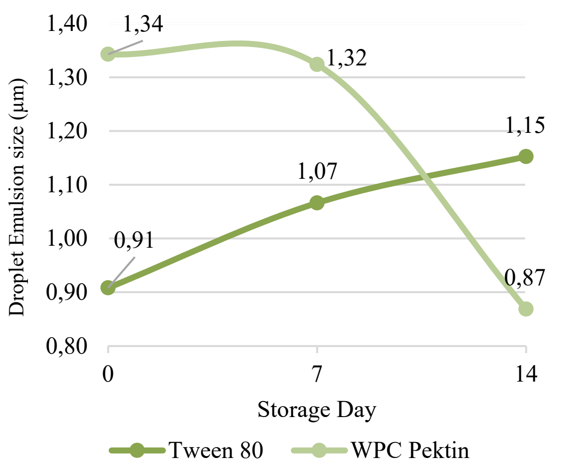

Figure 2: Particle Size of $W_1/O/W_2$ Emulsion Vitamin C.

Increasing the size of the droplet emulsion causes the emulsion system to become unstable. Research from Mohammadi *et al.*, (2016) states that the large size of the emulsion droplet with the WPC Pectin emulsifier because of the addition of WPC and Pectin together causing the thickening of the biopolymer compound around the W/O emulsion, thereby increasing the size of the WPC and Pectin droplet emulsion and reduce the stability of the emulsifier proving by cream formed.

Another thing that enlarging the droplet size of the emulsion and causes the W/OW emulsion system to be unstable is the coalescence process of the W/O emulsion during storage. According to Chung and Mcclements (2018), coalescence causes the emulsion droplet fuse into a larger droplet resulting the increase of droplet size during storage. This is also supported by Schuch *et al.*, (2014) where the emulsifier concentration also affects the stability of the emulsion system formed. If the concentration of the emulsifier are less, the emulsifier is not able to bind the water completely, causing the water molecules to fuse and coalescence occurs so the droplet enlarges. Other studies that support coalescence as a cause of increasing droplet size and as a sign of instability of the emulsion system were carried out by (Vicente et al., 2018). Coalescence occurs because the lack of hydrocolloid compounds as a stabilizer for emulsifiers can initiate the Brownian motion on the droplet emulsion.

Brownian motion is random motion that occurs in droplets. When the emulsion system is stable, brownian motion will maintain the distribution of dissolved phase droplets evenly throughout the solvent phase (Dickinson, 2010). If the emulsion is not stable, there will be a tendency for droplets to separate from each other because they have different densities so the brownian motion can no longer move evenly. If the density of the droplet is small, the brownian motion will make the droplet emulsion move towards the solution whereas if the density of the droplet is large, then the brownian motion will make the droplet move downward (Zhang & Mcclements, 2018).

Based on the results in figure 3, $W_1/O/W_2$ Emulsion with Tween 80 as the outer phase emulsifier are better than with WPC Pectin as the outer phase emulsifier. Figure 3 (a) can be described as $W_1/O/W_2$ emulsion when the dark phase are oil and bubble phases inside the dark one are the water phase (W_1) and the white phase around the dark are the W_2. This is supported by Mainz (2006) that the dark particle are made from mass thickness contrast method depends on mass weight of the particle that used. Figure 3 (b) cannot be described as $W_1/O/W_2$ emulsion because there is no dark phase between the light phase like figure 3 (a). it means that WPC-Pectin cannot be outer phase emulsifier. This phenomena happens because the amplitude from sonication process doesn't match with WPC-Pectin as a emulsifier, if the amplitude is high, then proteins that contained in WPC-Pectin are denaturized (Hubinger, 2018).

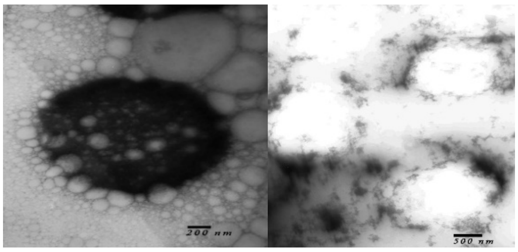

Figure 3: Morphology of Double Emulsio with Tween 80 (Left) and WPC-Pectin (Right).

Based on the experimental results, the WPC Pectin emulsifier cannot be used as an emulsifier for the $W_1/O/W_2$ vitamin C emulsion system. The increase in the creaming index value is more significant in the WPC Pectin emulsifier compared to the Tween 80 emulsifier based on the curves in Figure 4. The higher creaming index value indicates that the emulsion system formed is increasingly unstable. Tween 80 emulsifier has better stability than the Pectin WPC emulsifier with the same concentration.

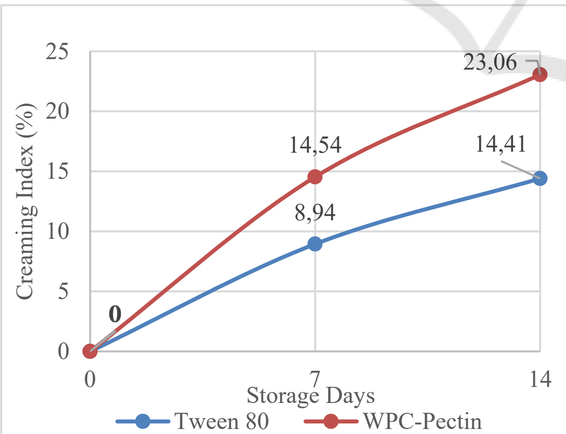

Figure 4: Creaming Index of W/O/W Vitamin C.

The high value of the creaming index on the WPC Pectin emulsifier can be caused by flocculation of the formed emulsion system components. This is supported by Khalid, Kobayashi, Neves, Uemura, & Nakajima, (2013) who stated that the speed of creaming is influenced by the flocculation that occurs in the W_1/O emulsion system components. The occurrence of flocculation can be due to the droplet aggregation process, thereby accelerating the process of creaming (Schmidts, Dobler, Nissing, & Runkel, 2009). According to Dickinson (2010), the flocculation process is the process that most influences the creaming of an emulsion system. Flocculation has a big role because the droplets that have undergone flocculation move faster than individual droplets in the emulsion solution. This occurs because the emulsion droplets that have undergone flocculation are much larger than the intact emulsion droplets.

Research from Schuch, Helfenritter, Funck, & Schuchmann, (2014) stated that the instability of the $W_1/O/W_2$ emulsion can be caused by differences in the surface tension of the emulsifier used. In addition, the active components that become surfactants in the Pectin WPC emulsifier can also cause instability of the $W_1/O/W_2$ emulsion system that has been formed. According to Schuch et al., (2014), the instability of an emulsifier using a protein which is a hydrophilic emulsifier can occur due to the lack of a stabilizer concentration in the form of a hydrocolloid compound. Hydrocolloid compounds that can be used as stabilizers are gum arabic, CMC and pectin. Pectin has been added to the WPC as a stabilizer but the small pectin concentration (0.1%) is not able to maintain the stability of the $W_1/O/W_2$ emulsion system.

Based on the results of the experiments conducted, the tween 80 emulsifier is more stable than the WPC Pectin emulsifier for the $W_1/O/W_2$ emulsion system so that the tween 80 emulsifier is more suitable for use as a second emulsifier for vitamin C emulsions.

4 CONCLUSIONS

Emulsion $W_1/O/W_2$ Vitamin C with WPC-Pectin as emulsifier in the outer aqueous phase bigger particle size 1.34 µm compared with Tween 80 with 0.91 µm after preparation. Tween 80 as emulsifier was more stable during storage with little cream formed in the surface. When the droplet size become bigger, the stability of the emulsion have been decreased. TEM Result also showed that the morphology of emulsion with Tween 80 remained stable as a double emulsion after storage for 14 days. tween 80 is the best emulsion to maintain the stability of $W_1/O/W_2$ emulsion containing vitamin C.

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