Effect of pH on the stability of W1/O/W2 double emulsion stabilized by combination of biosilica and Tween-20

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Abstract. Indonesia is amongst agricultural countries whose majority of the population consume rice as their staple food. Rice production tends to increase year by year followed by the increasing of their byproducts such as rice husks. The majority of rice husk waste has been generally thrown away by burning on site which cause pollution and may negatively impact on the environmental sustainability. In fact, rice husk waste contains of about 20% silica which potentially be used as emulsion stabilizers. Biosilica could stabilize the interface between water and oil due to their hydrophilicity and hydrophobicity characteristics in nature. However, their wettability was greatly influenced by pH. In this experiment, the effect of pH of the outer continuous phase (W₂) was investigated. The primary emulsion (W₁/O) was prepared by mixing a 40% water phase containing gelatin 3% w/w relative to the aqueous phase and Tween-20 of 1.1% w/w relative to the primary emulsion with the remaining oil phase for 5 minutes. The primary emulsions were then dispersed into the aqueous phase (W₂) of various pH (2; 3; and 5.7) by using a mixture of Tween-20 and biosilica as emulsifiers. The result showed that the most stable double emulsion was obtained upon the acidic pH of 2. There was no differences between the stability of double emulsion prepared at pH 2 and that of pH 3 after 7 days. However those prepared without pH adjustment (5.7) tended to be instable in the long-term. This implied that acidic pH would increase the packing density of biosilica in the interfaces thus enhancing the barrier properties of the droplets against coalescences. A stable food grade double emulsion would be very beneficial to develop low-fat emulsion products in various food applications. Furthermore, the inner aqueous phase could be used as a vehicle to encapsulate bio-active agents such as nutrients or antioxidants for the advancement of developments of functional food products.

1 Introduction

Indonesia is amongst one of the biggest agricultural countries with the majority of population consume rice as their staple foods. Paddy production in 2015 was 75.36 million tons which was increased of about 6.37% compared to the production in 2014 with the paddy field increase of 0.32 million ha according to Statistics Indonesia [1]. Paddy milling produce wastes. One of its wastes is rice husk which is usually burnt and still underutilized. In fact, rice husk is rich in silica which could be used as a substitute of synthetic emulsifier for food emulsions stabilization. Emulsion stabilized by particles was found by Pickering in 1907 so that the resulting emulsion has been known as pickering emulsion [2]. Silica has been used as food additives (E551) besides it has been considered as essential nutrition since it imparts health benefits such as strengthening bones and improving immune response, as well as neutronal and connective tissue health [3]. Pichot, *et. al.* (2010) studied on the effect of types and concentration of surfactants and colloidal particles on the emulsions stability. Tween-60 and Sodium

Caseinate were used as emulsifiers for O/W while lecithin and colloidal silica particles were used as emulsifiers for W/O emulsion. Emulsion stabilized by mixed surfactants and silica particles had smaller droplets compared to the emulsion stabilized by either surfactant or silica particle. Furthermore, the droplets were getting bigger with the increase of surfactant concentrations and silica particles were gradually detached from the interfaces. These effects were hardly seen in W/O emulsions [4]. Pichot, *et. al.* (2012) investigated the influence of pH on the stability of O/W emulsions stabilized with the combination of silica particles, Tween-60, and sodium caseinate and W/O emulsions stabilized with silica particles and lecithin. Surfactants were dispersed in aqueous phase followed by silica particles (0.2 – 2%) with the variation of pH of 2-10 of the aqueous phase. Vegetable oil was added to the aqueous phase and mixed with a high shear mixer. Emulsion formed at pH 2 showed the highest stability due to the smaller contact angle of silica particle in the O/W interfaces thus increasing its emulsifying properties [5]. There have been increasing applications of W/O or O/W emulsions in food

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industries. Nowadays, along with the increasing demands of healthy foods, W/O/W emulsions have been of interest to create food emulsions with lower fats. Cofrades, *et. al.* (2013) prepared W/O/W emulsions in meat systems. The primary W/O emulsion with 80% oil fraction was prepared using 6% PgPr as emulsifier. Olive oil was used for the oil phase. The emulsification was conducted at 3250 rpm for 5.5 minutes at 50°C. Furthermore, 6% whey protein, 0.5% sodium caseinate, and 0.6% NaCl was added into the outer aqueous phase. The 40% primary emulsion was then dispersed within the outer aqueous phase using a Thermomix blender at the mixing speed of 700 rpm for 5.5 minutes at a room temperature. The fat content was reduced from 11% to 7% [6]. L. Sapei, *et. al.* (2012) developed W/O/W emulsion for food applications. The inner aqueous phase was added with varying concentration of gelatin (0%, 3%, and 10% w/w) and NaCl (0 - 8% w/w). PgPr of 6% as emulsifier was added into the canola oil phase. The inner aqueous phase was then emulsified into the oil phase with the fraction of 40% using a rotor-stator homogenizer (Polytron PT 10/35, kinematik, CH-6010, Swiss) at 27,000 rpm for 3 minutes at 65°C. The resulting primary emulsion of 20% was then dispersed into the outer aqueous phase containing 1% w/w polysorbate 80 at 10,000 rpm for 2 minutes. The most stable W/O/W was obtained with gelatin concentration of 3%. The synergistic effect on the overall W/O/W stability due to the addition of gelatin and NaCl was observed [7]. The use of particles in stabilizing food-grade emulsions seems promising. Particles have to be combined with the polymeric emulsifiers in order to obtain emulsion with high level of stability [4, 8]. There have been few investigations of using biosilica as emulsifiers. The objective of this research was exploring the use of rice husk biosilica combined with Tween-20 in developing food-grade W/O/W double emulsions. These emulsions contained lower amount of fat with lower amount of polymeric emulsifier which were quite promising to be furthermore developed and incorporated into healthy and nutritious foods.

2 Materials and Methods

Materials

Rice husk wastes were obtained from Mojokerto, East Java, Indonesia; Tween-20 (Merck, Germany); Gelatin (SAP Chemicals, Indonesia); HCl 37% (Mallinckrodt, USA); palm oil consisting of about 40% of saturated oil and 60% of unsaturated oil (Sunco, PT. Milkie Olco Nabati Industri, Bekasi, Indonesia); demineralized water.

Biosilica preparation

Rice husk wastes were cleaned, washed with water, and dried at 105°C for 2 hours in an oven (Memmert, Germany) prior to leaching process. Process leaching was conducted in a vessel equipped with a condenser and an impeller. The solvent used was 5% citric acid. After leaching, the filtered rice husks were rinsed with

demineralized water for several times and then dried at 105°C for 2 hours in an oven (Memmert, Germany). The dried leached rice husks were subsequently thermally treated in a furnace (Ney VULCAN D-550, Denstply Ceramco, USA) at 750°C for 5 hours. The resulted white ashes were pure biosilica which were screened using 200 mesh screening prior to use.

Primary emulsion preparation

The primary emulsion was water in oil emulsion whereby 40% of aqueous phase (W_1) was dispersed in oil phase. Gelatin of 3% and Tween-20 of 2.8% was added to the aqueous phase in a 100 ml beaker glass. The mixture was mixed using a magnetic stirrer and slowly heated until 68°C for 30 minutes on a hotplate (Cimarec, Malaysia). The oil phase consisted of only oil was also preheated to 68°C for 30 minutes. The aqueous phase was dispersed into the oil phase by premixing using a magnetic bar at 68°C for 30 minutes followed by mixing using a rotor-stator homogenizer (IKA T25 digital ULTRA TURRAX, Germany) at 24,000 rpm for 5 minutes. The temperatures were well monitored using an alcohol thermometer. The beaker glasses were covered with aluminum foils in order to prevent the evaporation of liquids. The minor losses of liquid were compensated by adding several drops of liquid prior to emulsification.

Double emulsion preparation

Double emulsion ($W_1/O/W_2$) was prepared by dispersing the primary emulsion (W_1/O) into the outer aqueous phase (W_2). The outer aqueous phase was prepared by adding combination of biosilica of 0.5% and Tween-20 of 1% as mixed emulsifiers while the pH was brought into acidic environment at 2 and 3 by the addition of several drops of HCl 37% solution. The pH of outer aqueous phase prepared without pH adjustment was about 5.7. The outer aqueous phase was stirred using a magnetic bar while being slowly heated to 68°C. The primary emulsion (W_1/O) with a fraction of 20% w/w was dispersed into the W_2 phase using the rotor-stator homogenizer (IKA T25 digital ULTRA TURRAX, Germany) at 10,000 rpm for 2 minutes. The resulting double emulsions were then characterized macroscopically and microscopically for their stability. The stability was monitored for the first 2 hours and after 7 day storage.

Determination of double emulsion stability

The stability of double emulsion ($W_1/O/W_2$) was simply determined macroscopically by comparing the height of emulsion at final condition with that of initial condition. Double emulsion ($W_1/O/W_2$) was poured into a flat-bottomed 40 ml glass vial (ID = 25 mm and height = 95 mm) until $\frac{3}{4}$ part of the glass. The height of initial emulsion was measured as well as the height of emulsion after a certain time. Emulsion was distinguished from the cream or oil layer based on their milky appearance. The double emulsion stability was calculated according to the equation (1).

$$\%S = \frac{h_o - h_t}{h_o} \times 100\%$$

whereas h_o = initial height of emulsion, h_t = height of emulsion after a certain time, the emulsion height was monitored every 1 minute for the first ten minutes followed by every 5 minutes for the first one hour and every 10 minutes for the subsequent one hour. Afterwards, the emulsion height was monitored every 24 hours up to 7 days. Additionally, the stability of double emulsion ($W_1/O/W_2$) was investigated microscopically using an optical microscope (Olympus, Japan) equipped by a camera (Optilab, Indonesia). A drop of $W_1/O/W_2$ emulsion was put into an objective glass and covered by a cover glass. The droplet size of primary emulsion and inner aqueous droplet could be observed. The occurrence of double emulsion could also be further confirmed.

3 Results and Discussion

Food grade double emulsions stabilized by rice husk biosilica were developed. Biosilica were combined with Tween-20 as the emulsifier mixtures in the outer aqueous phase. The double emulsion was obtained from 2 stages emulsifications. The first stage was the emulsification in order to obtain W_1/O emulsion which was stabilized by Tween-20 and gelatin. The second stage was the emulsification of W_1/O into outer aqueous phase (W_2). The concentration of mixed emulsifier of Tween-20 and biosilica significantly influenced the overall stability of the resulting double emulsion as could be seen in Table 1. The double emulsions stabilized by Tween-20 or biosilica only were amongst the least stable emulsions with stabilities attainment of 60-70% after 7 days. The most stable double emulsion with the highest stability of ~93% after 7 days was obtained when 1% Tween-20 was combined with 0.5% biosilica. When biosilica concentration was decreased or increased, the long-term double emulsion stability after 7 day storage was getting lowered. There was a synergistic action between biosilica and Tween-20 when being combined together. Tween-20 was easily adsorbed into the interfaces followed by the attachment of biosilica particles on the interfaces protecting the oil droplets against flocculations or coalescences. When only polymeric emulsifier was used, it would be easily detached from the interfaces causing emulsion destabilisation in the long-term. On the other hand, when only particles were used, the attachment of the particles in the interfaces was quite difficult and leaving the emulsion unstabilised [4]. Furthermore, there was hardly creaming seen in the double emulsion stabilised by 1% Tween-20 and 0.5% biosilica as depicted in Figure 1 in contrast with those emulsions stabilised with Tween-20 or biosilica only which demonstrated creaming and even oil separation, respectively. The addition of Tween-20 as surfactant could decrease the interfacial tension thus facilitating the phase dispersion into another phase without the occurrence of flocculations [9]. Based on these results, the mixture of 1% Tween-20

and 0.5% biosilica was selected to formulate double emulsions with varying pH of the outer aqueous phase in order to investigate of the effects of pH on the overall double emulsion stability.

Table 1. Stability of double emulsion $W_1/O/W_2$ stabilized by mixture of biosilica and Tween-20 on the outer aqueous phase

Mixed emulsifiers	Stability (%)	
	after 2 hours	after 7 days
1% Tween-20 only	88	70
0.5% biosilica only	78	60
1% Tween-20 + 0.25% biosilica	100%	76.92%
1% Tween-20 + 0.5% biosilica	100%	92.98%
1% Tween-20 + 0.75% biosilica	73.84%	73.84%
1% Tween-20 + 1% biosilica	84.48%	77.58%
1% Tween-20 + 1.25% biosilica	84.48%	77.58%

Fig. 1. Observation of double emulsions $W_1/O/W_2$ stabilities after 7 day storage stabilized by different emulsifiers on the outer aqueous phase (W_2), (a) Tween-20 1% only; (b) Biosilica 0.5% only; (c) Tween-20 1% and biosilica 0.5%

The double emulsion $W_1/O/W_2$ developed as depicted in Table 1 were prepared without pH adjustment and the outer aqueous phase had the pH of ~5.7. The acidic pH of outer aqueous phase was adjusted by the addition of HCl solution into pH of 2 and 3. The stability profiles of the resulting double emulsions at several pH adjustments with storage time could be seen in Figure 2. It was obvious that the acidic pH seemed beneficial since there were increase of stability of double emulsions prepared at both pH 2 and 3 compared to that prepared without pH adjustment after 7 day storage. The appearances of double emulsions after 2 hours were shown in Figure 3. They all seemed similar and quite stable. There were no significant differences in

their appearance after 7 day storage. Furthermore, the double emulsions microstructures prepared at pH 2 and pH 5.7 after 2 hours and 7 days were demonstrated in Figure 4. In general, the double emulsion formations prepared at different pH of outer aqueous phase seemed similar up to 7 days.

Fig. 2. The effect of pH of the outer aqueous phase on the stability of double emulsions $W_1/O/W_2$

Fig. 3. Observation of double emulsions $W_1/O/W_2$ stabilities after 2 hours with adjusted outer aqueous pH, (a) 2; (b) 3; (c) 5.7

Emulsions stabilized by biosilica were more stable against coalescence when the pH of the system was shifted to acidic pH. As system pH approach acidic environment ($pH < 4$), biosilica particles tended to have no charge thus enabled the particles to form aggregates covering the oil-water interface. In turn, the aggregates could form a closely packed layer acting as a rigid barrier against droplets coalescences. At a higher pH ($pH > 4$), biosilica particles tended to dissociate to form SiO^- which had a negative charge. The negatively charged silica particle would be more hydrophilic [10] and tended to be withdrawn into the aqueous phase rather than staying on the oil-water interfaces. Additionally, the negatively charged silica particles did not form closely packed aggregates since they repelled each other thus lowering their properties as barriers against flocculations and coalescences in the long-term. The proposed mechanisms of biosilica particle stabilization at low ($pH < 4$) and higher pH ($pH > 4$) were depicted in Figure 5.

4 Conclusions and recommendations

Rice husk biosilica enhanced the stability of double emulsion $W_1/O/W_2$ with the combination of polymeric emulsifier such as Tween-20. Furthermore, the action of stabilizing silica particles on the oil/ water interfaces was favored by the acidic environment ($pH < 4$) of the outer aqueous phase. The silica particles tended to form a closely packed layer due to the biosilica particles aggregations thus increasing the barrier properties against flocculation and coalescence. Consequently, the resulting double emulsions had higher stabilities after 7 day storage. This implied the promising application of biosilica particles in stabilizing low fat food grade based emulsions for producing healthier and highly nutritious processed foods. However, the long-term stability of the double emulsion would still have to be investigated and improved either by the optimization of the formulation or by the modulation of operating conditions of the process.

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Fig. 4. The microstructures of double emulsions $W_1/O/W_2$ prepared at different pH after a certain time of storage, (a) pH 2, $t = 0$; (b) pH 2, $t = 7$ days; (c) pH 5.7, $t = 0$; (d) pH 5.7, $t = 7$ days

Fig. 5. Proposed models of pickering emulsion stabilized by biosilica under the influence of pH a) low pH ($pH < 4$) and b) higher pH ($pH > 4$).