Formulation, characterization and stability of o/w nanoemulsion containing rice bran oil prepared by emulsion phase inversion

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Abstract

Rice bran oil (RBO) contains naturally occurring antioxidants such as carotenoids, tocopherol, and γ-oryzanol. The aim of this research was to formulate and evaluate the characteristics of nanoemulsion which was prepared using RBO containing naturally occurring antioxidants. The RBO-in-water nanoemulsion was prepared by the emulsion phase inversion method. The oil phase of the nanoemulsion was prepared by either virgin coconut oil (VCO) or palm oil (PO) combined with RBO with the ratio of 5:5, 4:6, 3:7, 2:8 and 0:10. Tween 80 was used as a surfactant. The surfactant to oil ratios was predetermined at 2:5:1.0 and 3.0:1.0. The aqueous phase (80% w/w) was titrated into an organic phase that consisted of Tween 80 and oil phase (approximately 20% w/w). Droplet size, zeta-potential and polydispersity index of the nanoemulsion were used as the main parameters. The results showed that the smallest droplet (<100 nm) of the nanoemulsion was obtained when the ratio of VCO: RBO at 3:7 and the ratio of PO: RBO at 4:6 with the surfactant to oil ratio (SOR) was 2.5. Nanoemulsion with a relatively small polydispersity index of 0.3 was achieved when the ratio of PO: RBO was 3:7 and SOR at 3. All of the freshly prepared RBO containing nanoemulsion have good stability with zeta-potential values of < -30 mV. Nanoemulsions were stable against centrifugation at 2300 rpm for 15 mins, but they were not stable against heating at 105°C for 5 hrs. The RBO-in-water nanoemulsion could be successfully prepared by phase inversion method, by combining RBO with either VCO or PO at different ratios.

1. Introduction

Rice bran oil (RBO) contains naturally occurring antioxidants such as carotenoids, tocopherol, and γ-oryzanol as the main antioxidant. The amounts of γ-oryzanol in rice bran oil was <0,1-1.4% (Patel and Naik, 2004; Pestana et al., 2008; Dhavamani et al., 2014; Cuevas et al., 2017). To optimize the utilization of it into an aqueous product, γ-oryzanol should be carried in delivery systems due to its characteristic as lipophilic. Lipophilic delivery systems in nano-sizes are solid lipid nanoparticles (SLN), nano lipid carrier (NLC) and nanoemulsion (NE). The main difference of them is in the lipid phase. SLN has a solid lipid phase while the lipid phase of NLC is a mixture of solid and liquid lipids. Nanoemulsion has liquid oil as the oil phase (Hentschel et al., 2008; Seetapan et al., 2010; Rohmah et al., 2019). Many researchers have been producing delivery systems for γ-oryzanol in SLN, NLC and NE for application in pharmaceutical and food products (Seetapan et al., 2010; Alfaro et al., 2015; Chusut et al., 2015; Ishaka et al., 2016).

Nanoemulsions are defined as emulsions with the mean droplet diameter about 20-200 nm, so it is transparent or slightly turbid. Although nanoemulsion is more unstable and requires less surfactant than microemulsion (Suhendra et al., 2012; Yuwanti et al., 2012; Komaiko and McClements, 2016). The high-energy methods like using microfluidizer, high-shear homogenizer or ultrasonication able to produce nanoemulsion. But it is costly and more samples were needed. Many researchers produced nanoemulsion using low energy method by emulsion phase inversion or spontaneous emulsification, successfully (Mayer et al., 2013; Hategekimana et al., 2015; Komaiko and McClements, 2015). Either emulsion phase inversion or spontaneous emulsification is simply a titrating method to produce nanoemulsion. In the spontaneous emulsification method, an organic phase containing oil and surfactant mixed were titrated into the water with continuous stirring. The EPI method is described as titration of organic phase using an aqueous solution with continuous stirring (Komaiko and McClements, 2015).
Therefore, spontaneous emulsification and emulsion phase inversion were categorized as low energy isothermal method.

We consider that the EPI method is easier to implement than spontaneous emulsification. But, the disadvantage of the low energy method is that it needs more surfactant to produce nanoemulsion than the high-energy methods. Therefore, it is important to obtain the right formula of the nanoemulsion and followed by its characterization.

In this research, either virgin coconut oil or palm oil was selected to combine with rice bran oil as the oil phase. Virgin coconut oil is rich in medium fatty acids (46.64-48.03% as lauric acid) (Marina et al., 2009), and its characteristic is important to produce nanoemulsion by low energy method. Palm oil that rich in palmitic acid (44%) was also selected to compare with VCO (Basiron, 2005). Tween 80 or polyoxyethylene-20-sorbitan monooate (hydrophilic-lipophilic balance = 15) was used as a surfactant. The aqueous phase is deionized water.

In this study, we aimed to formulate and evaluate the characteristics of nanoemulsion which was prepared using RBO containing naturally occurring antioxidants. Turbidity, particle size, polydispersity index, and zeta-potential were used to evaluate the nanoemulsion formula.

2. Materials and methods

2.1 Materials

Rice bran oil (RBO), virgin coconut oil (VCO) and palm oil were bought from the local market. Tween 80 (Merck) and deionized water were used as a surfactant and aqueous phases, respectively.

2.2 Nanoemulsion preparation

The RBO-in-water nanoemulsion was prepared by the emulsion phase inversion method. The oil phase of the nanoemulsion was prepared by either virgin coconut oil (VCO) or palm oil (PO) combined with RBO with the ratio of 5:5; 4:6; 3:7; 2:8 and 0:10 (w/w) by magnetically stirring (AREC digital ceramic hot plate stirrer, VELP Scientifica, Italy) at 800 rpm for 20 mins. Tween 80 was added into the oil phase then it called an organic phase. The surfactant-to-oil ratio (SOR) was varied by 1.0; 1.5; 2.0; 2.5 and 3 (w/w). For SOR determination, only VCO was selected as an oil phase. The organic phase was mixed at 800 rpm for 20 mins. Deionized water (80% w/w) was titrated into the organic phase at 800 rpm for 10 min. Added mixing was done at 1100 rpm for 20 mins.

2.3 Nanoemulsion stability

Stability test for nanoemulsion was done by accelerated tests (Ariviani et al., 2015). Either nanoemulsion were centrifuged at 2300 x g for 15 mins at 27°C or heated at 105°C for 5 hrs. The turbidity of nanoemulsion was analyzed in triplicate.

2.4 Nanoemulsion characterization

Mean particle diameter (z-average), zeta-potential, and polydispersity index of the nanoemulsion were analyzed by dynamic light scattering using Zetasizer Nano ZS (Malvern Instruments, UK). Samples were diluted in deionized water prior to analysis to avoid multiple scattering effects. Turbidities of selected nanoemulsions were analyzed at 600 nm using UV-Vis Spectrophotometer (Zhong et al., 2017).

2.5 Experimental design

All measurements were analyzed at least in triplicate. Results are presented as mean±standard deviation. One way ANOVA (SPSS version 24) followed the Duncan Multiple Range Test (DMRT) was done to analyze the data.

3. Results and discussion

3.1 Determination of surfactant to oil ratio (SOR)

Initially, we examined the effect of surfactant to oil ratio (SOR) on the turbidity of nanoemulsions produced by emulsion phase inversion method. Nanoemulsions were prepared using virgin coconut oil (VCO), surfactant phase (Tween 80) and 80% (w/w) deionized water at a stirring speed 800-1100 rpm at ambient temperature.

Figure 1. Effect of surfactant to oil ratio (SOR) on turbidity of nanoemulsions produced by emulsion phase inversion method. Nanoemulsions were prepared using virgin coconut oil (VCO), surfactant phase (Tween 80) and 80% (w/w) deionized water at a stirring speed 800-1100 rpm at ambient temperature.
greater amount of surfactant up to a certain level will decrease in the interfacial tension so small droplets could be formed (Saberi et al., 2013). The turbidity of nanoemulsion that produced by SOR at 2.5 and 3 was not statistically significant (p<0.05). Therefore, the optimum of SOR in this system was 2.5. In the next experiment, SOR at 2.5 and 3 were selected to formulate nanoemulsion with oil combinations. SOR 3 was used to confirm the effect of higher SOR on nanoemulsions characterization.

3.2 Characterization of nanoemulsions

The oil phase of the nanoemulsion was prepared by either virgin coconut oil (VCO) or palm oil (PO) combined with RBO with the ratio of 5:5; 4:6; 3:7; 2:8 and 0:10. Tween 80 was used as a surfactant at SOR 2.5 and 3. The aqueous phase was standardized at 80% (w/w). In this research, we aimed to maximize the utilization of rice bran oil to deliver γ-oryzanol. For all the different oil compositions and SOR, nanoemulsion with a diameter below 200 nm could be formed using the emulsion phase inversion method. The results showed that the smallest droplet (<100 nm) of the nanoemulsion was obtained when the ratio of VCO: RBO at 3:7 and the ratio of PO: RBO at 4:6 with the surfactant to oil ratio (SOR) was 2.5 (Figure 2). Our experiment indicated that these formulae were optimum oil phase compositions required for the formation of nanoemulsion. The viscosity of VCO is lower than the RBO and PO. In the lower oil viscosity, the surfactant molecules will move faster, and therefore the smaller the droplets will be produced (Saberi et al., 2013). When the ratio of VCO: RBO at 5:5 and 4:6, the mean particle diameter of this formula were higher than at 3:7. Besides, Saberi and co-workers in 2013 also reported that the composition and physicochemical properties of surfactant-oil-water mixtures are more important than the characteristics of pure oil. One might expect that a combination of PO and RBO could produce nano size emulsion due to the relatively high SOR in this system (SOR 2.5 and 3). Therefore, nanoemulsion with tiny droplets successfully achieved by this method.

For most of the samples, the mean particle diameter of nanoemulsions produced by SOR at 3 was higher than nanoemulsions produced by SOR at 2.5. It was assumed that the spontaneous breakup of the oil-water interface depends on the formation of associated liquid crystal. Thus, at a certain surfactant level, the high order of the crystalline, the droplet size will increase (Saberi et al., 2013). Most of the freshly prepared RBO containing nanoemulsion were polydisperse (Figure 3). The nanoemulsion which produced by a combination of VCO and RBO has a more homogeneous particle size distribution than the PO and RBO combination. Zeta-potential values of all of the nanoemulsions formula were < -30 mV (Figure 4). This parameter measures charges on droplet nanoemulsion and its contribution to the stabilization of nanoemulsion. Although Tween 80 was non-ionic surfactant, the negative values of zeta-potential maybe are due to the adsorption of OH- species from the aqueous phase onto the interface (Jo and Kwon, 2014).

There are some reports on the combination of medium and long-chain triglyceride oil could produce nanoemulsions by low energy methods. Zhong et al. (2017) had combined medium-chain triglyceride and fish oil at ratio 7:3, SOR at 1, Tween 80 and Span 20 (3:1) were used as a surfactant, could produce nanoemulsion with 150 nm diameter. The tiny particle diameter of nanoemulsions also could be obtained by the combination of grape seed oil with orange oil (1:1) and medium-chain triglyceride with citrus essential oil (1:1) (Davidov-Pardo and McClements, 2015; Lou et al., 2017). To the best of our knowledge, none of the reports in the scientific literature producing and characterization of nanoemulsions produced by SOR at 3 were described.
3.3 Stability test of nanoemulsion

Nanoemulsion with the smallest particle diameter from each oil combination group was selected to stability test against centrifugation at 2300 rpm for 15 mins and heating at 105°C for 5 hrs (accelerated test). All nanoemulsion formula were stable after centrifugation but not stable after the heating test (Table 1). Turbidity of nanoemulsion is not significantly affected between before and after centrifugation. But, after heating, nanoemulsion tends to unstable due to dehydration of Tween 80’s head groups leading to changes in their solubility and optimum curvature (Guttoff et al., 2015).

4. Conclusion

The smallest particle (<100 nm) of the nanoemulsion was obtained when the ratio of VCO: RBO at 3:7 and the
ratio of PO: RBO at 4: 6 with the surfactant to oil ratio (SOR) was 2.5. All of the freshly prepared RBO containing nanoemulsion have good stability with zeta-potential values of < -30 mV. The RBO-in-water nanoemulsion could be successfully prepared by phase inversion method, by combining RBO with either VCO or PO at different ratios.

**Conflict of Interest**
The authors declare no conflict of interest.

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