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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 nanoparticle (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 produced 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).

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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 monooleate (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)

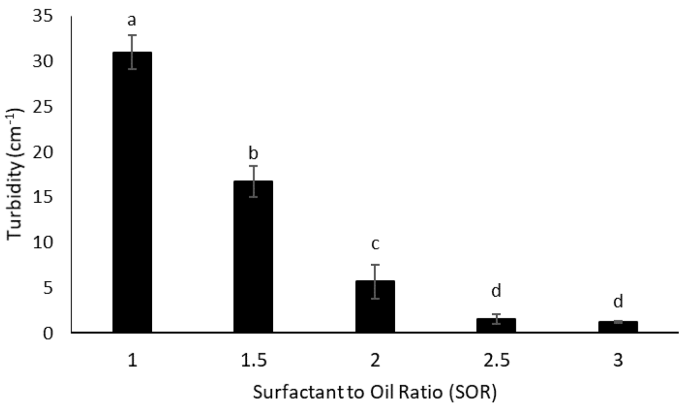

Figure 1. Effect of surfactant to oil ratio (SOR) on turbidity of nanoemulsions produced by emulsion phase inversion method. Nanoemulsions were prepared using virgin coconut oil (VCO), surfactant phase (Tween 80) and 80% (w/w) deionized water at a stirring speed 800-1100 rpm at ambient temperature.

Initially, we examined the effect of surfactant to oil ratio (SOR) on the turbidity of nanoemulsion. Virgin coconut oil (VCO) and Tween 80 were selected as oil phase and surfactant, respectively. The SORs were varied by 1-3 and turbidity was the main parameter in this section. From Figure 1, we know that the turbidity of nanoemulsion was a decline as increasing in SOR. The 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

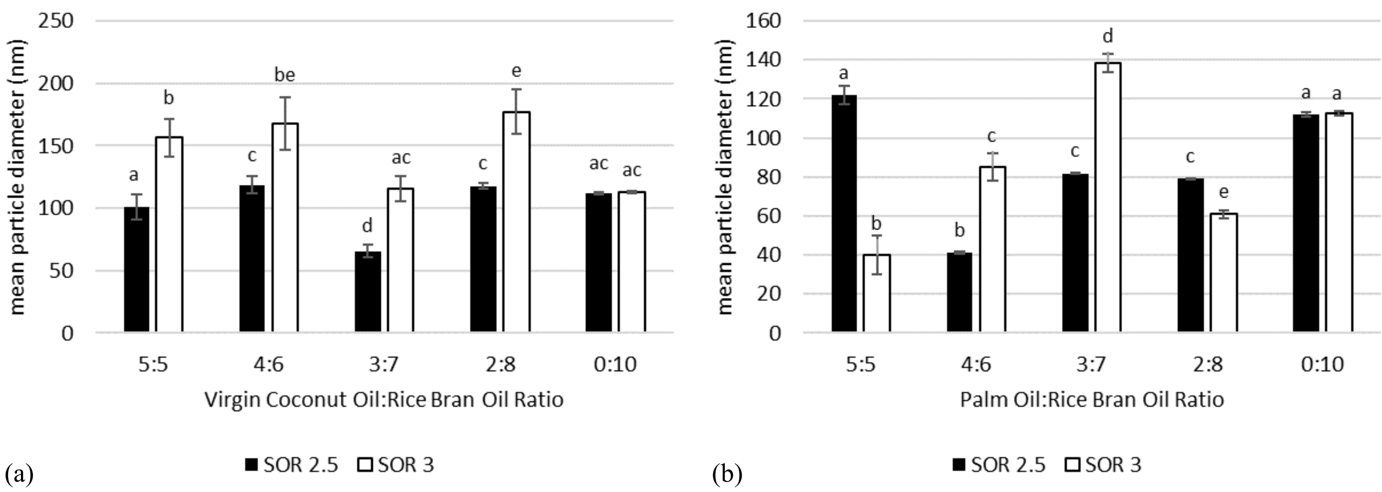

Figure 2. Effect of oil ratio, virgin coconut oil: rice bran oil (a) and palm oil: rice bran oil (b) on mean particle diameter of nanoemulsions prepared by emulsion phase inversion at room temperature. The surfactant phase was Tween 80 and deionized water was used as aqueous phase.

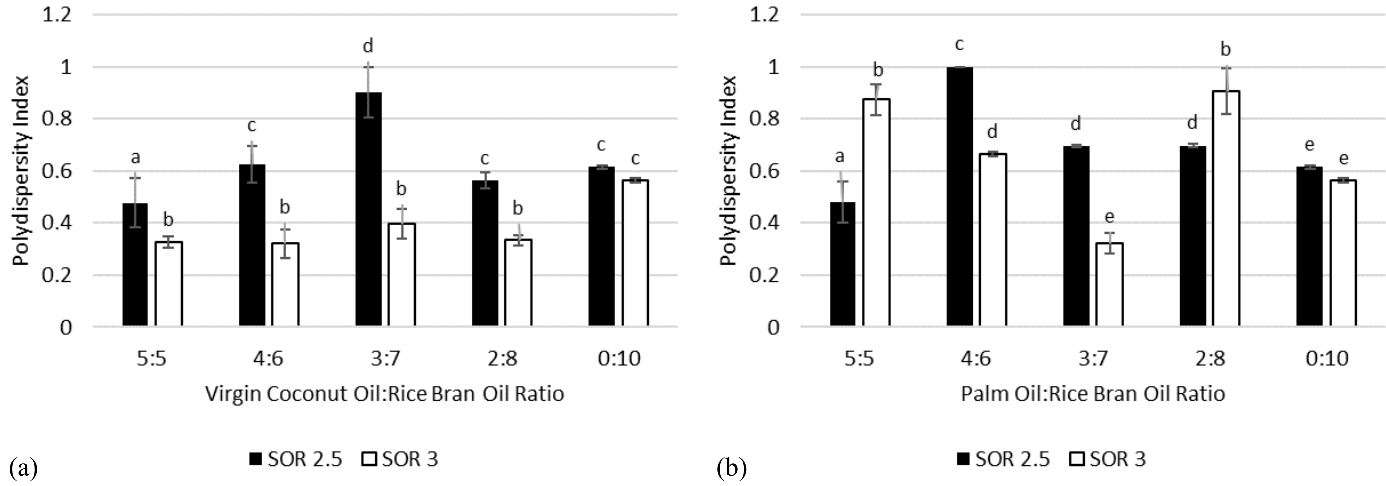

Figure 3. Effect of oil ratio, virgin coconut oil: rice bran oil (a) and palm oil: rice bran oil (b) on polydispersity index of nanoemulsions prepared by emulsion phase inversion at room temperature. The surfactant phase was Tween 80 and deionized water was used as aqueous phase.

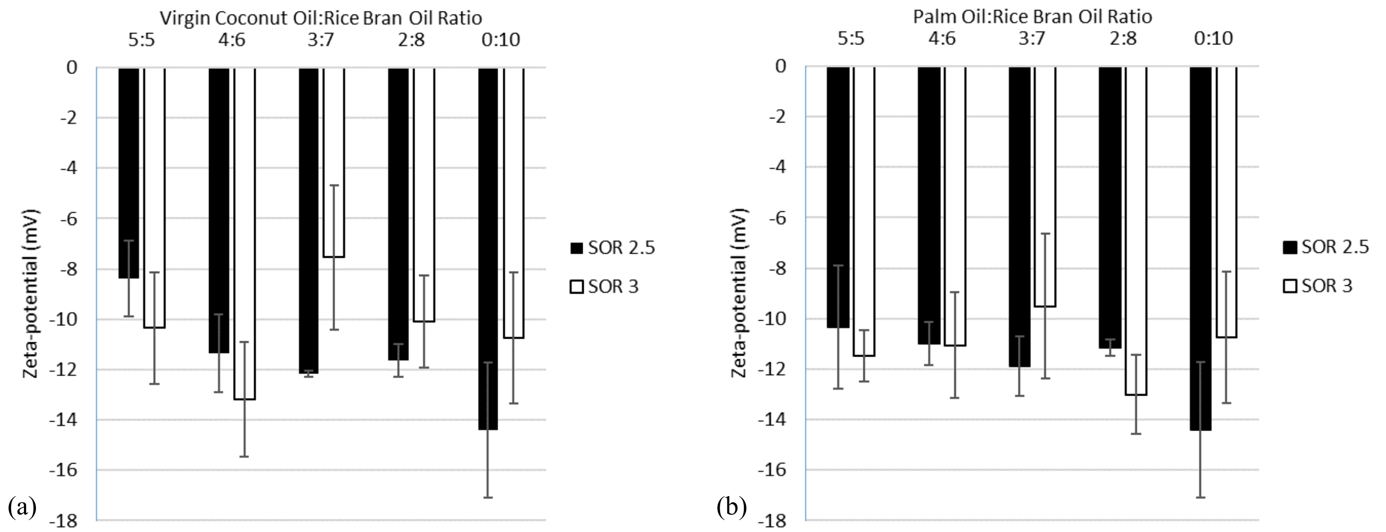

Figure 4. Zeta-potential values of all samples. Either nanoemulsion were prepared by combination of VCO: RBO (a) or PO: RBO (b), SOR at 2.5 and 3. Tween 80 and deionized water (80% w/w) were used as surfactant and aqueous phase, respectively.

Table 1. The changes of turbidity nanoemulsion before and after stability test by accelerated test

Nanoemulsion formula	Before	After centrifugation	After heating
PO: RBO 4:6, SOR 2.5	9.208±1.084aA	7.854±0.352a	1.423±0.099B*
PO: RBO 4:6, SOR 3	2.436±0.296aA	2.429±0.117a	4.662±0.708A*
VCO: RBO 3:7, SOR 2.5	4.09±0.381aA	3.633±0.565a	3.633±0.565A*
VCO: RBO 3:7, SOR 3	3.768±0.053aA	3.56±0.642a	4.746+ 0.416B*

Data are expressed as mean±SD. PO: Palm Oil; VCO: Virgin Coconut Oil; RBO: Rice Bran Oil
*Phase separation in nanoemulsion.
Different small letters indicate significant differences after centrifugation ($p<0.05$). Different capital letters indicate significant differences after heating ($p<0.05$).

of nanoemulsions with combination either of virgin coconut oil or palm oil with rice bran oil as oil phase.

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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References

Alfaro, L., Hayes, D., Boeneke, C., Xu, Z., Bankston, D., Bechtel, P.J. and Sathivel, S. (2015). Physical properties of a frozen yogurt fortified with a nano-emulsion containing purple rice bran oil. *LWT - Food Science and Technology*, 62(2), 1184–1191. https://doi.org/10.1016/j.lwt.2015.01.055

Ariviani, S., Raharjo, S., Anggrahini, S. and Naruki, S. (2015). Formulasi dan Stabilitas Mikroemulsi O/W dengan Metode Emulsifikasi Spontan Menggunakan VCO dan Minyak Sawit sebagai Fase Minyak: Pengaruh Rasio Surfaktan-Minyak. *Agritech*, 35(1), 27–34. https://doi.org/10.22146/agritech.9416. [In Bahasa Indonesia].

Basiron, Y. (2005). Palm Oil. In Shahidi, F. (Ed). Bailey's Industrial Oil and Fat Products. Vol. 2. 6th ed., p. 333-429. New Jersey, Canada: John Wiley and Sons, Inc. https://doi.org/10.1002/047167849X.bio071

Chusut, T., Pathompak, P., Charoenchai, L., Saingam, W., Monton, C., Kraisintu, K. and Amnuaikit, T. (2015). Development of gamma Oryzanol-Loaded Nanostructured Lipid Carriers by Using Glyceryl Stearate Blended PEG-100 Stearate as Solid Lipid and Cold Pressed Rice Bran Oil as Liquid Lipid. *Bulletin of Health, Science and Technology*, 13(2), 47–55.

Cuevas, M.S., de Souza, P.T., Rodrigues, C.E.C. and Meirelles, A.J.A. (2017). Quantification and Determination of Composition of Steryl Ferulates in Refined Rice Bran Oils Using an UPLC-MS Method. *Journal of the American Oil Chemists' Society*, 94(3), 375–385. https://doi.org/10.1007/s11746-017-2955-5

Davidov-Pardo, G., and McClements, D.J. (2015). Nutraceutical delivery systems: Resveratrol encapsulation in grape seed oil nanoemulsions formed by spontaneous emulsification. *Food Chemistry*, 167, 205–212. https://doi.org/10.1016/j.foodchem.2014.06.082

Dhavamani, S., Rao, Y.P.C. and Lokesh, B.R. (2014). Total antioxidant activity of selected vegetable oils and their influence on total antioxidant values in vivo : A photochemiluminescence based analysis. *Food Chemistry*, 164, 551–555. https://doi.org/10.1016/j.foodchem.2014.05.064

Guttoff, M., Saberi, A.H. and McClements, D.J. (2015). Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry*, 171, 117–122. https://doi.org/10.1016/j.foodchem.2014.08.087

Hategekimana, J., Chamba, M.V.M., Shoemaker, C.F., Majeed, H. and Zhong, F. (2015). Vitamin E nanoemulsions by emulsion phase inversion: Effect of environmental stress and long-term storage on stability and degradation in different carrier oil types. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 483, 70–80. https://doi.org/10.1016/j.colsurfa.2015.03.020

Hentschel, A., Gramdorf, S., Müller, R.H. and Kurz, T. (2008). β-Carotene-Loaded Nanostructured Lipid Carriers. *Journal of Food Science*, 73(2), N1–N6. https://doi.org/10.1111/j.1750-3841.2007.00641.x

Ishaka, A., Imam, M.U., Ismail, M., Mahmud, R. and Bakar, Z.Z.A. (2016). Nanoemulsified gamma-oryzanol rich fraction blend regulates hepatic cholesterol metabolism and cardiovascular disease risk in hypercholesterolaemic rats. *Journal of Functional Foods*, 26, 338–349. https://doi.org/10.1016/j.jff.2016.08.015

Jo, Y.-J. and Kwon, Y.-J. (2014). Characterization of β-Carotene Nanoemulsions Prepared by Microfluidization Technique. *Food Science and Biotechnology*, 23(1), 107–113. https://doi.org/10.1007/s10068-014-0014-7

Komaiko, J. and McClements, D.J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering*, 146, 122–128. https://doi.org/10.1016/j.jfoodeng.2014.09.003

Komaiko, J.S. and McClements, D.J. (2016). Formation of Food-Grade Nanoemulsions Using Low-Energy Preparation Methods: A Review of Available Methods. *Comprehensive Reviews in Food Science*

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and Food Safety, 15(2), 331–352. https://doi.org/10.1111/1541-4337.12189

Lou, Z., Chen, J., Yu, F., Wang, H., Kou, X., Ma, C. and Zhu, S. (2017). The antioxidant, antibacterial, antibiofilm activity of essential oil from *Citrus medica L. var. sarcodactylis* and its nanoemulsion. *LWT - Food Science and Technology*, 80, 371–377. https://doi.org/10.1016/j.lwt.2017.02.037

Marina, A.M., Che Man, Y.B., Nazimah, S.A.H. and Amin, I. (2009). Chemical Properties of Virgin Coconut Oil. *Journal of the American Oil Chemists' Society*, 86(4), 301–307. https://doi.org/10.1007/s11746-009-1351-1

Mayer, S., Weiss, J. and McClements, D.J. (2013). Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: Factors influencing droplet size and stability. *Journal of Colloid and Interface Science*, 402, 122–130. https://doi.org/10.1016/j.jcis.2013.04.016

Patel, M. and Naik, S.N. (2004). Gamma-oryzanol from rice bran oil – A review. *Journal of Scientific and Industrial Research*, 63(7), 569–578.

Pestana, V.R., Zambiazi, R.C., Mendonça, C.R B., Bruscatto, M.H., Lerma-García, M.J. and Ramis-Ramos, G. (2008). Quality Changes and Tocopherols and γ-Orizanol Concentrations in Rice Bran Oil During the Refining Process. *Journal of the American Oil Chemists' Society*, 85(11), 1013–1019. https://doi.org/10.1007/s11746-008-1300-4

Rohmah, M., Raharjo, S., Chusnul, H. and Martien, R. (2019). Application of Response Surface Methodology for the Optimization of β-Carotene-Loaded Nanostructured Lipid Carrier from Mixtures of Palm Stearin and Palm Olein. *Journal of the American Oil Chemists' Society*, 97(2), 213-223. https://doi.org/10.1002/aocs.12310

Saberi, A.H., Fang, Y. and McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science*, 391, 95–102. https://doi.org/10.1016/j.jcis.2012.08.069

Seetapan, N., Bejrapha, P., Srinuanchai, W. and Ruktanonchai, U.R. (2010). Rheological and morphological characterizations on physical stability of gamma-oryzanol-loaded solid lipid nanoparticles (SLNs). *Micron,* 41(1), 51–58. https://doi.org/10.1016/j.micron.2009.08.003

Suhendra, L., Raharjo, S., Hastuti, P. and Chusnul, H. (2012). Formulasi dan Stabilitas Mikroemulsi O/W sebagai Pembawa Fucoxanthin. *Agritech*, 32(3), 230–239 [In Bahasa Indonesia].

Yuwanti, S., Raharjo, S., Hastuti, P. and Supriyadi. (2012). Mikroemulsi Minyak dalam Air (O/W) sebagai Pembawa α-Tokoferol untuk Menghambat *Sunlight Flavor* Pada Susu *Full Cream* Akibat Fotooksidasi. *Agritech*, 32(2), 179–185 [In Bahasa Indonesia].

Zhong, J., Liu, X., Wang, Y., Qin, X. and Li, Z. (2017). γ-Oryzanol Nanoemulsions Produced by a Low-Energy Emulsification Method: an Evaluation of Process Parameters and Physicochemical Stability. *The Royal Society of Chemistry*, 8(6), 2202-2211. https://doi.org/10.1039/C7FO00023E

