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Gingerol and Shogaol Nanoemulsion of Ginger Extract-based Subcritical Water Extraction

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ABSTRACT: The aim of the present study was determine optimum processing conditions and nanoemulsion formulation for the preparation of gingerol and shogaol nanoemulsion from ginger extract-based subcritical water. For this purpose, nanoemulsions were prepared with rotor-stator homogenizer and varied surfactant and cosurfactant concentrations (24%, 26% and 28% v/v), and stirring speed (22,000 rpm, 24,000 rpm). The stirring time is adjusted to 30 minutes. The density, viscosity, pH, % transmittance, nanoemulsion type test, centrifugation test, particle size, zeta potential, and polydispersion index was observed. Nanoemulsion with 28% emulsifier has a % transmittance value closest to 100% indicating that the particle size is estimated at nanometers. The results of test showed that the formed ginger extract nanoemulsion was unstable. Therefore, further studies are needed to find the optimum process conditions and nanoemulsion formulations to form stable

KEYWORDS: Emulsifier; Ginger extract; Nanoemulsion; Stability; Subcritical Water Extraction.

1. INTRODUCTION

Cancer is a non-communicable disease (NCDs) with huge negative effects on the world population. This is caused by increased abnormal body cells which lead to the formation of tumors with the possibility of metastases (migrating to other body tissues) [1]. Several treatments have been developed such as chemotherapy, radiotherapy, synthetic medicine, etc., but they have a toxic effect on non-target tissues that impact patients' quality of life [1,3] Therefore, it is necessary for alternative treatment such as using the medical plant.

Ginger has been used as a traditional drug because it has nutraceutical effects from the bioactive compound in ginger i.e. gingerol, zingiberene dan shogaol [4,5]. In the delivery of a traditional drug, the drug is distributed into the body, and the targeted cells only absorb part of the drug, depending on the location cells. However, the important part of the drug is excreted from the body without being used, leading to drug wastage [6]. According to Indonesian Ministry of Industry data, imported pharmaceutical raw materials are still more than 90 %. In addition, drug production also requires production costs such as raw material costs, operating costs, marketing, and advertising costs, distribution costs, and others (administration) which leads to expensive drug costs [7]. So it is necessary to develop traditional medicine from ginger.

Ginger is a medicinal plant with excellent prophylactic and curative anti-cancer properties. 6-shogaol, 8-shogaol, 10-shogaols, 6-gingerol, 6-paradol, dan zingerone in ginger shown activity anticancer in the lung, colon, colorectal, ovary, prostate [1]. However, 6-gingerol (6G), 8-gingerol (8G), 10-gingerol, and 6-shogaol (6S), have low water solubility and low oral bioavailability, resulting in limited nutraceutical effects [8]. In addition, these compounds are also sensitive to light, heat, and oxygen, causing the degradation of bioactive compounds [9]. This can be overcome with nanoemulsions because nanotechnology can be an alternative to increase the stability of bioactive compounds and increase bioavailability, water solubility, and absorption of bioactive compounds [9,10].

Nanoemulsion oil in water (O/W) has been used for a pharmaceutical compound with hydrophobic properties, encapsulation as well as delivery of active compounds, especially in the biomedical industry [11]. They consist core-shell structure, where the core (oil) internal phase contains nonpolar molecules, and this internal phase is stabilized by the shell external phase which is a surfactant [12,13]. There have been several studies to prepare ginger extract nanoemulsions using the high-energy approach method. [14] used an Ultra Turrax T8 homogenizer at 22,000 rpm for 10 minutes to make nano gingerol. [9] also used Ultra Turrax T8 homogenizer at 7,200 rpm for 3 minutes to make ginger extract nanoemulsion-based maceration extraction. [15] made a ginger extract nanoemulsion based on enzymatic extraction using ng Ultra Turrax homogenizer with a stirring speed of 22000 rpm and a time of 15-25 minutes. The previous study is limited to selecting the optimal conditions of operation in prepared nanoemulsions (stirring speed) and nanoemulsion formulations. However, the main challenge in developing nanoemulsion is the selection of optimal processing conditions and formulation of nanoemulsion that can produce nanoemulsion with great stabilization and afford bioactive compound delivery into cell target, have good solubility and absorption.

Preliminary research has been carried out in the preparation of gingerol and shogaol nanoemulsions without using co-surfactants. However, the result was that the ginger extract in the nanoemulsion precipitated after 3 days of storage. Therefore the use of cosurfactants is important to stabilize the film on nanoemulsion globules [16]. The combination of surfactants and cosurfactants used in the preparation of nanoemulsions is necessary to produce the very low interfacial tension required to reduce droplet size down to the nanometer range. HLB calculation also has a necessary position in the preparation of nanoemulsions. The HLB value takes parts of the hydrophilic and lipophilic of the surfactant molecule, so it will promote the formation of a physically stable NE [17].

This study aims to determine optimum processing conditions and nanoemulsion formulation for the preparation of gingerol and shogaol nanoemulsion from ginger extract-based subcritical water extraction with the smallest particle size and zeta potential value.

2. RESEARCH METHOD

2.1. Ginger Extraction

Ginger extract was obtained through subcritical water extraction. 300 gr ginger dreg powder was added into 7-liter aquadest. The mixture was put in stainless tube cells with a lid. Nitrogen gas was entered into the cell for 2 minutes to remove oxygen. The temperature was set to 130ºC and the pressure was 2 bar. The heating process takes 3 - 5 minutes to reach the desired temperature. Extraction was carried out for 20 minutes. The extract was cooled in a cooling cell at 25°C and 1 MPa for 1 min [18].

2.2. Preparation Gingerol and shogaol Nanoemulsion

The preparation of gingerol and shogaol nanoemulsion was referred to research by [19] with modification. 1% v/v ginger extract, tween 80, and PEG 400 were varied into 24% v/v, 26% v/v, and 18% v/v, and 2% VCO were mixed as the oil phase and stirred continuously. Aquadest was added to obtain 100% of the mixture. The nanoemulsion was formed using homogenizer D-500 with the speed 20.000 rpm and 24.000 rpm, with stirring time adjusted to 30 minutes.

2.3. Viscosity

Viscometer Ostwald was used to determine the viscosity value [20]. Low viscosity indicates that the formed nanoemulsion is oil in water and high viscosity indicates that the formed nanoemulsion is water in oil [21].

2.4. Density

Density analysis on the nanoemulsion is carried out using a pycnometer to measure the density of the nanoemulsion formed [22].

2.5. pH

pH value was determined using a pH meter at room temperature. pH value was measured Repeat three times to get accurate results [23].

2.6. Transmittant Test

The test was carried out by spectrophotometry UV-VIS with wavelength 650 nm and aquadest as a blank solution. This test aims to measure the clarity of the nanoemulsion. A good nanoemulsion has a transmittance value close to that of water (100%) [24].

2.7. Nanoemulsion type test

This test was used to determine the type of nanoemulsion. The test of nanoemulsion type was performed by placing nanoemulsion on object glass and adding a tiny bit of methylene blue. If nanoemulsion is in type o/w, the methylene blue will be dissolved and dispersed in it. If the nanoemulsion is type w/o, the methylene blue will form a blue spot in it [25].

2.8. Centrifugation Test

Nanoemulsion was centrifuged at 3000 rpm for 30 minutes [26]. The result of the test is said to be good and stable if there is no separation after centrifugation [27]

2.9. Particle size, zeta potential, and Polydispersion Index Test

The measurement particle size, zeta potential, and Polydispersion Index of nanoemulsion were determined using a particle size analyzer with triplicate replication [28].

3. RESULTS AND ANALYSIS

3.1 Effect of Stirring Speed and Emulsifier Concentration of Ginger Extract Nanoemulsion

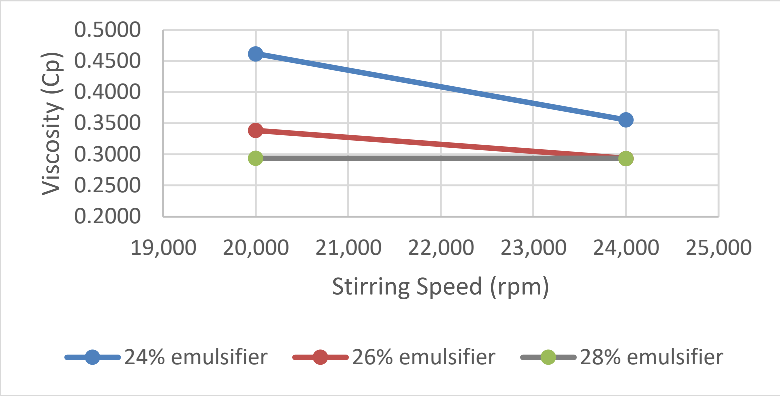

Figure 1. Effect of Stirring Speed and Emulsifier Concentration on Viscosity of Ginger Extract Nanoemulsion

Viscosity affects the release of the active substance, if the viscosity value is high it can make it difficult to release the active substance, and vice versa [29]. Figure 1 presents the effect of stirring speed and emulsifier concentration on the viscosity of ginger extract nanoemulsion and figure 2 presents the effect of stirring speed and emulsifier concentration on the density of ginger extract nanoemulsion. A faster stirring speed for nanoemulsion produces a smaller viscosity and density of the nanoemulsion. It is in line with [22], the faster stirring speed can reduce the viscosity of the nanoemulsion but also increase the separation time of the oil emulsion in water. The density value is directly proportional to the viscosity. So, when the viscosity decreases, the density will also decrease.

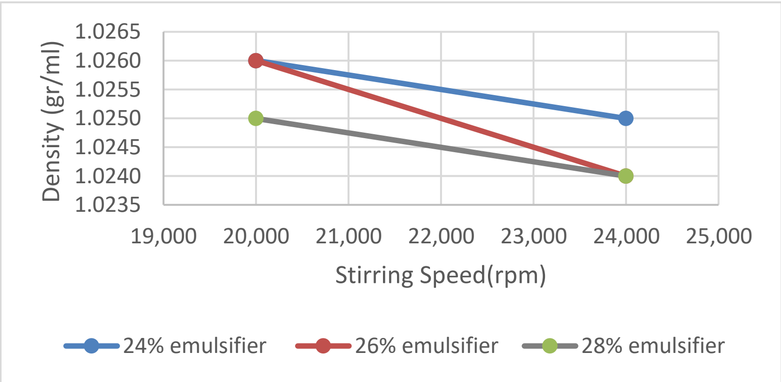

Figure 2. . Effect of Stirring Speed and Emulsifier Concentration on Densityof Ginger Extract Nanoemulsion

Emulsion separation is influenced by viscosity. Viscosity affects the emulsion separation rate. The higher the viscosity, the smaller the separation rate. The viscosity of the continuous phase will prevent the flocculation and/or coalescence of the dispersion phase. With the intention that, the emulsion separation speed is reduced [22]. [30] stated that decreasing the number of surfactants and cosurfactants will increase the interfacial tension between water and oil so that the viscosity increases[30]. In other words, the higher the concentration of surfactants and cosurfactants used, the lower the viscosity value

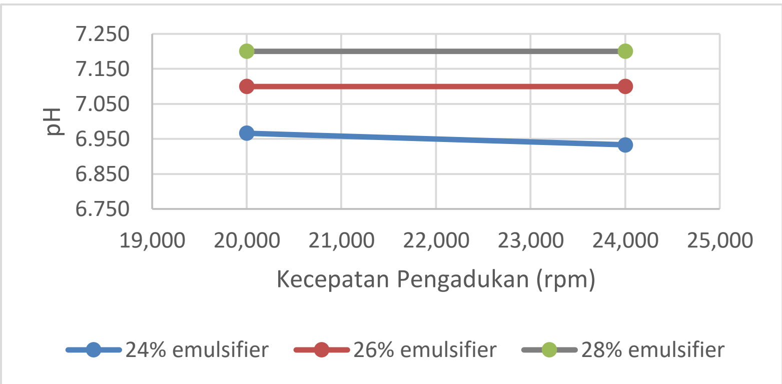

Figure 3. Effect of Stirring Speed and Emulsifier Concentration on pH of Ginger Extract Nanoemulsion

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Figure 3. shows the effect of stirring speed and emulsifier Concentration on the pH of ginger extract nanoemulsion. It can be seen that the speed of stirring does not affect the pH of the nanoemulsion. [31] reported that with various variations of stirring speed, there has been a change in the pH of the emulsion, but the change is not very significant. However, the higher the surfactant/emulsifier used the pH of the nanoemulsion decreased. When adding 24%, 26%, and 28% emulsifier, the pH of the nanoemulsion was 7.2; 7.1; and 6,9. This is in accordance with the research of [32], the higher the surfactant concentration, the pH of the emulsion also decreases. According to [33], pH 6-8 O/W nanoemulsion using surfactants will produce a large negative charge, so it can prevent adjacent droplets and form particle aggregation.

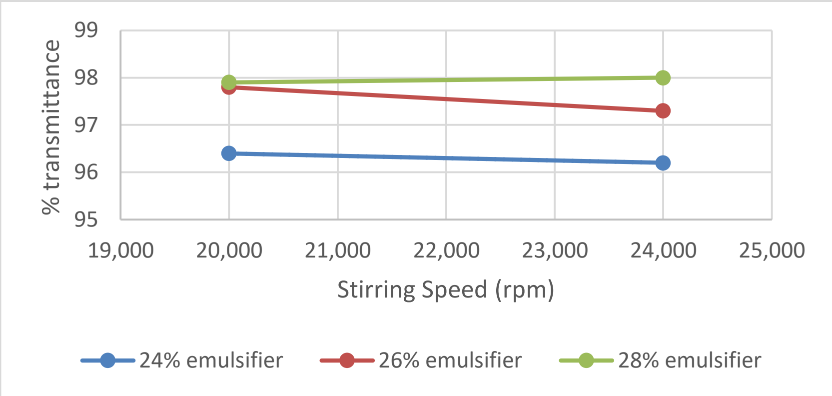

Figure 4. Effect of Stirring Speed and Emulsifier Concentration on % transmittance of Ginger Extract Nanoemulsion

Figure 4 are shown that increasing the concentration of the emulsifier will increase the percent transmittance. Higher concentrations of tween 80 lead to a large drop in surface tension leading to the spontaneous generation of interfacial turbulence droplets. Increasing the concentration of tween 80 results in a stable interface between oil and water and particle size is smaller. PEG as a cosurfactant reduces the interfacial tension. In this study, the cosurfactant concentration was lower than the surfactant used. Cosurfactants with high levels of concentration produce nanoemulsions with lower transmittance percentages [29].

The transmittance percentage indicates the level of clarity of the formed nanoemulsion. The transmittance percentage close to 100% indicates that the nanoemulsion formula produces a clear and transparent dispersion with a droplet size estimated at nanometers. Nanoemulsion with 28% concentration emulsifier had a transmittance value closest to 100% with a value of 98%. Therefore, the sample was further tested with a Particle Size Analyzer to determine particle size, PDI, and zeta potential, and its stability was tested using centrifugation to determine the occurrence of phase separation.

3.2 Analysis of Particle Size, Zeta Potential, and PDI Nanoemulsion of Ginger Extract

The particle size value of ginger extract nanoemulsion is 617 nm. According to [34], nanoemulsion is an emulsion with droplet sizes ranging from 20 to 500 nm. In other words, the nanoemulsion ginger extracts with a 28% concentration emulsifier do not include the nanoemulsion size range, because the particle size of the nanoemulsion in this study is more than 500 nm. The droplet size distribution of the emulsion is affected by the composition of the nanoemulsion system and/or the modification of the homogenization conditions [34]. There is a homogenization condition called "over-processing", which means that the energy input is too high to the emulsion system, such as too long homogenization time. The additional input of kinetic energy into the maximum dispersed emulsion will cause the particle movement faster, which enables overcoming steric or electrostatic barriers. According to the DLVO theory, over-processing promotes coalescence and the growth of droplet size [35]. This study used a stirring time of 30 minutes. A stirring process time of 10 minutes is included in the over-processing category [35]. Therefore, this study has experienced the phenomenon of over-processing. Based on the DLVO theory, this phenomenon will encourage coalescence and droplet size growth and make the function of the emulsifier to get poorer, an increase in Brownian motion which lead to a higher probability of collisions and mergers when input energy is high in the nanoemulsion system [35,36].

Table 1. Results of Analysis of Particle Size, Zeta Potential, and PDI Nanoemulsion of Ginger Extract

Particle Size (nm)	Zeta Potential (mV)	Polydispersity index
617	0,4	0.383

The zeta potential value generated from ginger extract nanoemulsion with 28% emulsifier concentration is 0.4 mV. The higher the surface charge material, the more stable the resulting nanoemulsion will be. In the case of low particle charge, the droplets tend to be attracted to each other and leading to increasing droplet size, which causes a decrease in the nanoemulsion stability [34]. [23] stated that a good zeta potential value to produce a stable preparation is less than -30 mV or more than +30 mV. However, the results of the analysis, the zeta potential value of the nanoemulsion in this study is 0.4 mV, so the nanoemulsion was less stable. Nanoemulsion may experience very large particle aggregation and flocculation due to van der Waals forces which result in physical instability in the nanoemulsion preparation. This is caused by the surfactants and the amount of surfactants added to the nanoemulsion formulation [23]. In further research, it is necessary to determine the type and amount of surfactants to produce stable ginger extract nanoemulsions.

Polydispersity is defined as the ratio of standard deviation to the average droplet size which implies the uniformity of droplet size in a nanoemulsion formulation. For a higher uniform droplet size in a nanoemulsion, the polydispersity in the formulation must be lower. The stable nanoemulsion has a PDI value between 0.2 and 0.5. A higher PDI value indicates a more dispersed particle size distribution which can make the nanoemulsion susceptible to delamination or precipitation [34]. The polydispersity index value of the resulting ginger extract nanoemulsion was 0.383, in other words, the nanoemulsion in this study had a uniform size distribution.

3.3 Nanoemulsion Type Test

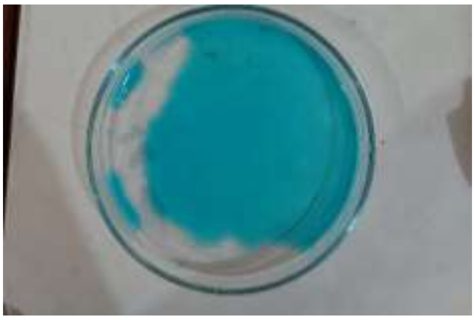

Figure 5. The result of nanoemulsion type test

This test shows that the type of nanoemulsion is an oil in water, by dripping methylene blue, the methylene blue dissolves and diffuses into the nanoemulsion sample. [17] stated that oil-in-water (o/w) nanoemulsions are more likely to form in the HLB range of 8-18. Tween 80 has an HLB value of 15 and PEG 400 has a value of 13.1, so the nanoemulsion is an oil-in-water type.

3.4 Centrifuge Test

The results of the centrifugation test at 3000 rpm for 30 minutes, the nanoemulsion with 28% emulsifier precipitation occurred. The particle size of the nanoemulsion is 617 nm which is larger than the nanoemulsion criteria, which is below 500 nm [34]. This is in accordance with research [37] which states that the smaller the particle size, the smaller the sedimentation rate, therefore at the same centrifugation rate and the same centrifugation time, a suspension with a much smaller particle size will have a much lower sedimentation rate than a suspension with a larger particle size.

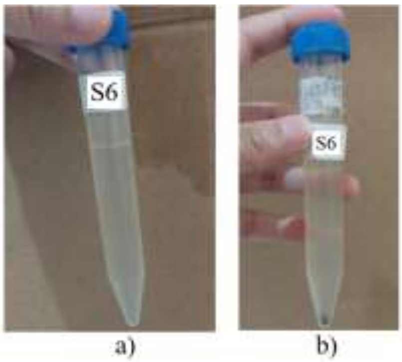

Figure 6 The resullt of the centrifugation test, (a) before centrifugation (b) after centrifugation

4. CONCLUSION

The formed nanoemulsion is O/W type. The stirring speed of ginger extract nanoemulsion has affect to viscosity and density. Emulsifier concentration of ginger extract nanoemulsion has affect to viscosity, density, transmittan, and pH of the nanoemulsion. The nanoemulsion with 28% emulsifier has % transmitan value closest to 100% whis is indicate that the particle size estimated at nanometers. The result of particle size, zeta potential, except PDI test shown that the formed nanoemulsion is unstable, and sedimentation after centrifugation occurred. It is necessary to find optimum processing conditions and nanoemulsion formulation for the nanoemulsion from the ginger extract in future research to form a stable nanoemulsion.

REFERENCES

1) Balogun FO, Tayo AdeyeOluwa E, Omotayo Tom Ashafa A. Pharmacological Potentials of Ginger. Ginger Cultiv Its Antimicrob Pharmacol Potentials 2020. https://doi.org/10.5772/intechopen.88848.
2) Mutiah R. Peran fitofarmaka dalam pencegahab dan terapi kanker. Anal Fitokimia Daun Pepaya (Carica Papaya L) Di Balai Penelit Tanam Aneka Kacang dan Umbi, Kendalpayak, Malang 2021:134–7.
3) Ma RH, Ni ZJ, Zhu YY, Thakur K, Zhang F, Zhang YY, dkk. A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food Funct 2021;12:519–42. https://doi.org/10.1039/d0fo02834g.
4) Kiyama R. Nutritional implications of ginger : chemistry , biological activities and signaling pathways. J Nutr Biochem 2020;86:108486. https://doi.org/10.1016/j.jnutbio.2020.108486.
5) Semwal RB, Semwal DK, Combrinck S, Viljoen AM. Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 2015;117:554–68. https://doi.org/10.1016/j.phytochem.2015.07.012.
6) Tayarani S sadat, Khayatzadeh J, Es-haghi A, Behnaz Shadan. Anti-cancer effects of nanoemulsion prepared using Zingiber Officinale L . tincture against PC3 prostate cancer cells 2022;9:241–51.
7) Perindustrian K. Membangun Kemandirian Industri Farmasi Nasional. Buku Anal Pembang Ind 2021:1–33.
8) Ogino M, Nakazawa A, ichi Shiokawa K, Kikuchi H, Sato H, Onoue S. Krill oil-based self-emulsifying drug delivery system to improve oral absorption and renoprotective function of ginger extract. PharmaNutrition 2022;19. https://doi.org/10.1016/j.phanu.2021.100285.
9) Mulia K, Zaenal MF, Krisanti EA. Encapsulation of oleoresin from ginger (Zingiber officinale var . Roscoe) in vegetable oils based nanoemulsions : Preparation and characterization for oral delivery formulation Encapsulation of Oleoresin from Ginger (Zingiber officinale var . Roscoe) 2019;030011. https://doi.org/10.1063/1.5096715.
10) Limthin D, Phromyothin D. Improving Stability of Nanoemulsion Containing Centella asiatica, Lycopersicon Esculentum Mil. And Moringa oleifera Lam. Extract. Mater Today Proc 2019;17:1852–63. https://doi.org/10.1016/j.matpr.2019.06.223.
11) Chen F, Kowaleguet MGGM, Shi W, Zhang S, Dai J, Ban Z, dkk. Associating chitosan and nanoemulsion as a delivery system of essential oil; the potential on quality maintenance of minimally processed produce. Lwt 2022;155:112925. https://doi.org/10.1016/j.lwt.2021.112925.
12) Aswathanarayan JB, Vittal RR. Nanoemulsions and Their Potential Applications in Food Industry. Front Sustain Food Syst 2019;3:1–21. https://doi.org/10.3389/fsufs.2019.00095.
13) Rosso A, Lollo G, Chevalier Y, Troung N, Bordes C, Bourgeois S, dkk. Development and structural characterization of a novel nanoemulsion for oral drug delivery. Colloids Surfaces A Physicochem Eng Asp 2020;593. https://doi.org/10.1016/j.colsurfa.2020.124614.
14) Noor E, Harmi L, Maddu A, Yusron M. Fabrication of Nanogingerol by Combining Phase Inversion Composition and Temperature. Res J Pharm 2015;6:38–47.
15) Yulianto ME, Siswanto AP, Handayani D, Nugraheni FS, Hartati I, Yudanto YA. Advanced nanoemulsion production on ginger (Zingiber officinale) for product quality improvement. AIP Conf Proc 2019;2114. https://doi.org/10.1063/1.5112415.
16) Hanifah N, Achmad YF, Humaira A, Salasia SIO. Red ginger-extract nanoemulsion modulates high blood pressure in rats by regulating angiotensin-converting enzyme production. Vet World 2021;14:176–81. https://doi.org/10.14202/VETWORLD.2021.176-181.
17) Smail SS, Ghareeb MM, Omer HK, Al-Kinani AA, Alany RG. Studies on surfactants, cosurfactants, and oils for prospective use in formulation of ketorolac tromethamine ophthalmic nanoemulsions. Pharmaceutics 2021;13. https://doi.org/10.3390/pharmaceutics13040467.
18) Endy Yulianto M, Paramita V, Amalia R, Wahyuningsih N, Dwi Nyamiati R. Production of bioactive compounds from ginger (Zingiber officianale) dregs through subcritical water extraction. Mater Today Proc 2022;63:S188–94. https://doi.org/10.1016/j.matpr.2022.02.216.
19) Wuttikul K, Sainakham M. In vitro bioactivities and preparation of nanoemulsion from coconut oil loaded Curcuma aromatica extracts for cosmeceutical delivery systems. Saudi J Biol Sci 2022;29:103435. https://doi.org/10.1016/j.sjbs.2022.103435.
20) Namira ZR, Paramita V, Kusumayanti H. The Effect of Rotational Speed of Homogenization on Emulsion Results Obtained

Using Soy Lecithin Emulsifier 2021;3:14–7.
21) Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci 2018;80:781–9.
22) Hariyatno SP, Paramita V, Amalia R. The Effect of surfactant, time, and speed of Stirring in the emulsification process of soybeaan oil in water. J Vocat Stud Appl Res 2021;3:21–5.
23) Mardhiani YD, A DP, Fadilah L. Astaxanthin Nanoemulsion Formulation and Evaluation 2022;3:139–49.
24) Wiwiek A, Martodihardjo S, Soenardi, Jumina, Budiana IGMN, Mustofa. Preparation and In-Vitro characterization of Self-Nano emulsifying system of C- Phenylcalix-[4]-Resorcinaryl Octacinnamate and C-Methylcalix-[4]-Resorcinaryl Octabenzoate as ultraviolet absorbers 2017. https://doi.org/10.15562/bmj.v6i3.699.
25) Maha HL, Sinaga KR, Masfria. Formulation and evaluation of miconazole nitrate nanoemulsion and cream. Asian J Pharm Clin Res 2018;11:319–21. https://doi.org/10.22159/ajpcr.2018.v11i3.22056.
26) Amit C, Rajeshwar A, Kant K, Raj PG, Bhawna T. Formulation and Evaluation of Ginger Extract Loaded Nanoemulgel for the Treatment of Rheumatoid Arthritis 2019;9:559–70.
27) Nurhidayati LG. Formulasi Dan Uji Sifat Fisik Sediaan Nanoemulsi Natrium Diklofenak Dengan Kombinasi Tween 80 Dan Transkutol. Sainteks 2020;17:33. https://doi.org/10.30595/sainteks.v17i1.6896.
28) Uthumpa C, Indranupakorn R, Asasutjarit R. Development of nanoemulsion formulations of ginger extract. Adv Mater Res 2013;684:12–5. https://doi.org/10.4028/www.scientific.net/AMR.684.12.
29) Ningsih IY, Faradisa H, Cahyani MD, Rosyidi VA, Hidayat MA. The formulation of ginger oil nanoemulsions of three varieties of ginger (Zingiber officinale rosc.) as natural antioxidant. J Res Pharm 2020;24:914–24. https://doi.org/10.35333/jrp.2020.251.
30) Chime SA, Kenechukwu FC, Attama AA. Nanoemulsions — Advances in Formulation, Characterization and Applications in Drug Delivery. Appl Nanotechnol Drug Deliv 2014. https://doi.org/10.5772/58673.
31) Haslinah A, Yani S, Aladin A. Optimization Of The Rotational Speed Of Homogenizers In The Production Of VCO Emulsion Using Soy Lecithin As The Emulsifier 2019;165:16–9. https://doi.org/10.2991/icmeme-18.2019.4.
32) Adak T, Barik N, Patil NB, Govindharaj GPP, Gadratagi BG, Annamalai M, dkk. Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (Sitophilus oryzae (L.) and Tribolium castaneum (Herbst)) of rice. Ind Crops Prod 2020;143:111849. https://doi.org/10.1016/j.indcrop.2019.111849.
33) McClements DJ. Food Emultions: Principles, Practices and Techniques. CRC Press. New York; 2005.
34) Sneha K, Kumar A. Nanoemulsions : Techniques for the preparation and the recent advances in their food applications. Innov Food Sci Emerg Technol 2022;76:102914. https://doi.org/10.1016/j.ifset.2021.102914.
35) Scholz P, Keck CM. Nanoemulsions produced by rotor-stator high speed stirring. Int J Pharm 2015;482:110–7. https://doi.org/10.1016/j.ijpharm.2014.12.040.
36) Mahdi Jafari S, He Y, Bhandari B. Nano-emulsion production by sonication and microfluidization - A comparison. Int J Food Prop 2006;9:475–85. https://doi.org/10.1080/10942910600596464.
37) Bangun H, Tandiono S, Arianto A. Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent. J Appl Pharm Sci 2018;8:147–56. https://doi.org/10.7324/JAPS.2018.81217.
38) Balogun FO, Tayo AdeyeOluwa E, Omotayo Tom Ashafa A. Pharmacological Potentials of Ginger. Ginger Cultiv Its Antimicrob Pharmacol Potentials 2020. https://doi.org/10.5772/intechopen.88848.
39) Mutiah R. Peran fitofarmaka dalam pencegahab dan terapi kanker. Anal Fitokimia Daun Pepaya (Carica Papaya L) Di Balai Penelit Tanam Aneka Kacang dan Umbi, Kendalpayak, Malang 2021:134–7.
40) [Ma RH, Ni ZJ, Zhu YY, Thakur K, Zhang F, Zhang YY, dkk. A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food Funct 2021;12:519–42. https://doi.org/10.1039/d0fo02834g.
41) Kiyama R. Nutritional implications of ginger : chemistry , biological activities and signaling pathways. J Nutr Biochem 2020;86:108486. https://doi.org/10.1016/j.jnutbio.2020.108486.
42) Semwal RB, Semwal DK, Combrinck S, Viljoen AM. Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 2015;117:554–68. https://doi.org/10.1016/j.phytochem.2015.07.012.
43) Tayarani S sadat, Khayatzadeh J, Es-haghi A, Behnaz Shadan. Anti-cancer effects of nanoemulsion prepared using Zingiber Officinale L . tincture against PC3 prostate cancer cells 2022;9:241–51.
44) Perindustrian K. Membangun Kemandirian Industri Farmasi Nasional. Buku Anal Pembang Ind 2021:1–33.
45) Ogino M, Nakazawa A, ichi Shiokawa K, Kikuchi H, Sato H, Onoue S. Krill oil-based self-emulsifying drug delivery system to improve oral absorption and renoprotective function of ginger extract. PharmaNutrition 2022;19. https://doi.org/10.1016/j.phanu.2021.100285.

46) Mulia K, Zaenal MF, Krisanti EA. Encapsulation of oleoresin from ginger (Zingiber officinale var . Roscoe) in vegetable oils based nanoemulsions : Preparation and characterization for oral delivery formulation Encapsulation of Oleoresin from Ginger (Zingiber officinale var . Roscoe) 2019;030011. https://doi.org/10.1063/1.5096715.
47) Limthin D, Phromyothin D. Improving Stability of Nanoemulsion Containing Centella asiatica, Lycopersicon Esculentum Mil. And Moringa oleifera Lam. Extract. Mater Today Proc 2019;17:1852–63. https://doi.org/10.1016/j.matpr.2019.06.223.
48) Chen F, Kowaleguet MGGM, Shi W, Zhang S, Dai J, Ban Z, dkk. Associating chitosan and nanoemulsion as a delivery system of essential oil; the potential on quality maintenance of minimally processed produce. Lwt 2022;155:112925. https://doi.org/10.1016/j.lwt.2021.112925.
49) Aswathanarayan JB, Vittal RR. Nanoemulsions and Their Potential Applications in Food Industry. Front Sustain Food Syst 2019;3:1–21. https://doi.org/10.3389/fsufs.2019.00095.
50) Rosso A, Lollo G, Chevalier Y, Troung N, Bordes C, Bourgeois S, dkk. Development and structural characterization of a novel nanoemulsion for oral drug delivery. Colloids Surfaces A Physicochem Eng Asp 2020;593. https://doi.org/10.1016/j.colsurfa.2020.124614.
51) [Noor E, Harmi L, Maddu A, Yusron M. Fabrication of Nanogingerol by Combining Phase Inversion Composition and Temperature. Res J Pharm 2015;6:38–47.
52) Yulianto ME, Siswanto AP, Handayani D, Nugraheni FS, Hartati I, Yudanto YA. Advanced nanoemulsion production on ginger (Zingiber officinale) for product quality improvement. AIP Conf Proc 2019;2114. https://doi.org/10.1063/1.5112415.
53) Hanifah N, Achmad YF, Humaira A, Salasia SIO. Red ginger-extract nanoemulsion modulates high blood pressure in rats by regulating angiotensin-converting enzyme production. Vet World 2021;14:176–81. https://doi.org/10.14202/VETWORLD.2021.176-181.
54) Smail SS, Ghareeb MM, Omer HK, Al-Kinani AA, Alany RG. Studies on surfactants, cosurfactants, and oils for prospective use in formulation of ketorolac tromethamine ophthalmic nanoemulsions. Pharmaceutics 2021;13. https://doi.org/10.3390/pharmaceutics13040467.
55) Endy Yulianto M, Paramita V, Amalia R, Wahyuningsih N, Dwi Nyamiati R. Production of bioactive compounds from ginger (Zingiber officianale) dregs through subcritical water extraction. Mater Today Proc 2022;63:S188–94. https://doi.org/10.1016/j.matpr.2022.02.216.
56) Wuttikul K, Sainakham M. In vitro bioactivities and preparation of nanoemulsion from coconut oil loaded Curcuma aromatica extracts for cosmeceutical delivery systems. Saudi J Biol Sci 2022;29:103435. https://doi.org/10.1016/j.sjbs.2022.103435.
57) Namira ZR, Paramita V, Kusumayanti H. The Effect of Rotational Speed of Homogenization on Emulsion Results Obtained Using Soy Lecithin Emulsifier 2021;3:14–7.
58) Gurpreet K, Singh SK. Review of nanoemulsion formulation and characterization techniques. Indian J Pharm Sci 2018;80:781–9.
59) Hariyatno SP, Paramita V, Amalia R. The Effect of surfactant, time, and speed of Stirring in the emulsification process of soybeaan oil in water. J Vocat Stud Appl Res 2021;3:21–5.
60) Mardhiani YD, A DP, Fadilah L. Astaxanthin Nanoemulsion Formulation and Evaluation 2022;3:139–49.
61) Wiwiek A, Martodihardjo S, Soenardi, Jumina, Budiana IGMN, Mustofa. Preparation and In-Vitro characterization of Self-Nano emulsifying system of C- Phenylcalix-[4]-Resorcinaryl Octacinnamate and C-Methylcalix-[4]-Resorcinaryl Octabenzoate as ultraviolet absorbers 2017. https://doi.org/10.15562/bmj.v6i3.699.
62) Maha HL, Sinaga KR, Masfria. Formulation and evaluation of miconazole nitrate nanoemulsion and cream. Asian J Pharm Clin Res 2018;11:319–21. https://doi.org/10.22159/ajpcr.2018.v11i3.22056.
63) Amit C, Rajeshwar A, Kant K, Raj PG, Bhawna T. Formulation and Evaluation of Ginger Extract Loaded Nanoemulgel for the Treatment of Rheumatoid Arthritis 2019;9:559–70.
64) Nurhidayati LG. Formulasi Dan Uji Sifat Fisik Sediaan Nanoemulsi Natrium Diklofenak Dengan Kombinasi Tween 80 Dan Transkutol. Sainteks 2020;17:33. https://doi.org/10.30595/sainteks.v17i1.6896.
65) Uthumpa C, Indranupakorn R, Asasutjarit R. Development of nanoemulsion formulations of ginger extract. Adv Mater Res 2013;684:12–5. https://doi.org/10.4028/www.scientific.net/AMR.684.12.
66) Ningsih IY, Faradisa H, Cahyani MD, Rosyidi VA, Hidayat MA. The formulation of ginger oil nanoemulsions of three varieties of ginger (Zingiber officinale rosc.) as natural antioxidant. J Res Pharm 2020;24:914–24. https://doi.org/10.35333/jrp.2020.251.

67) Chime SA, Kenechukwu FC, Attama AA. Nanoemulsions — Advances in Formulation, Characterization and Applications in Drug Delivery. Appl Nanotechnol Drug Deliv 2014. https://doi.org/10.5772/58673.
68) Haslinah A, Yani S, Aladin A. Optimization Of The Rotational Speed Of Homogenizers In The Production Of VCO Emulsion Using Soy Lecithin As The Emulsifier 2019;165:16–9. https://doi.org/10.2991/icmeme-18.2019.4.
69) Adak T, Barik N, Patil NB, Govindharaj GPP, Gadratagi BG, Annamalai M, dkk. Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (Sitophilus oryzae (L.) and Tribolium castaneum (Herbst)) of rice. Ind Crops Prod 2020;143:111849. https://doi.org/10.1016/j.indcrop.2019.111849.
70) McClements DJ. Food Emultions: Principles, Practices and Techniques. CRC Press. New York; 2005.
71) Sneha K, Kumar A. Nanoemulsions : Techniques for the preparation and the recent advances in their food applications. Innov Food Sci Emerg Technol 2022;76:102914. https://doi.org/10.1016/j.ifset.2021.102914.
72) Scholz P, Keck CM. Nanoemulsions produced by rotor-stator high speed stirring. Int J Pharm 2015;482:110–7. https://doi.org/10.1016/j.ijpharm.2014.12.040.
73) Mahdi Jafari S, He Y, Bhandari B. Nano-emulsion production by sonication and microfluidization - A comparison. Int J Food Prop 2006;9:475–85. https://doi.org/10.1080/10942910600596464.
74) Bangun H, Tandiono S, Arianto A. Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent. J Appl Pharm Sci 2018;8:147–56. https://doi.org/10.7324/JAPS.2018.81217.