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Fabrication of Nanogingerol by Combining Phase Inversion Composition and Temperature.

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ABSTRACT

Nanogingerol is an active compound derived from an extract ginger. As a lipophylic active component nanogingerol could improve the effectiveness when use in a delivery system, such as drug, flavor, antioxidant and antimicrobial agent. The nano form would overcome the gingerol extract form that has a highly hydrophobic compound with low water solubility and poor bioavailability. This study was examined the impact of system composition and temperature conditions on the formations of nanogingerol by using a low energy homogenizer. The processes were used Ultra Turrax with a speed of 22,000 rpm in 10 minutes. The compositions of organic phase were 10-50% and temperature set at 30-50 ^{o}C, with the addition of Tween 80 as emulsifier. The nanogingerol gained with the droplet size less than 100 nm , by using a variation of the composition organic phase 30 % at the temperature 30-50 ^{o}C and organic phase 50 % at the temperature 30 ^{o}C. The nanogingerol showed increased solubility, stability and bioavailability (up to 13.22 %) compared to the ginger extract emulsion. Change the droplet size to adding composition organic phase the factors viscosity, but in temperature increase droplet size droplet changes effected by force between advance.

Keywords: phase inversion composition, phase inversion temperature, homogenization, nanogingerol

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INTRODUCTION

Gingerols as pungent compounds of ginger rhizomes extract has been used widely for pharmaceutical (Nakatani 1995; Kikuzaki 2000; Babu 2004) and in food as a flavor, antioxidant and antimicrobial agent. Many bioactive agents used for oral ingestion are highly hydrophobic, which having low water solubility and poor bioavailability (Kleberg et al. 2010; Li et al. 2012). One of the natural active agent that has been used widely is gingerol, however as many other bioactive compound it behaves as hydrophobic. Therefore it has low solubility and less absorb in the ingestion system. To improve the solubility, bioavailability and the emulsion transform to nanoemulsion.

One of the processes that can be developed is nanoemulsion since it has many benefits such as to increase component solubility, enhance bioavailability, improve absorption and reduce the dose usage (Huang and Chang 2009). Nanoemulsion base delivery system are particularly convenient means of delivering poorly water soluble nutraceutical and drug via the oral route for both functional food and pharmaceutical applications (McClements and Li, 2010).The particle size of active substances in herbs is associated with the absorption level by the body. Micron size can be absorbed only 50% while the nano herbs can be absorbed by the body almost 100% (Suptijah 2009).

Many of preparation methods have been developed to prepare nanoemulsions, and these can conveniently be classified as either high energy or low energy approaches (Qian and McClements, 2011). High energy approaches utilize mechanical devices capable of generating intense disruptive forces that breakup the oil and water phases and lead to the formation of tiny oil droplets, such as high-pressure homogenizers, microfluidizers and sonication methods (Qian and McClements, 2011). On the other hand, low energy approaches rely on the spontaneous formation of tiny oil droplets within mixed oil/water-emulsifier systems when the solution or environmental conditions are altered (Qian and McClements, 2011). In low-energy methods, the particle size mainly depends on system composition (surfactant -oil– water ratio, surfactant type, ionic strength), preparation method (such as order of addition, initial location of ingredients, stirring speed), and environmental conditions (such as temperature history) (Anton and Vandamme 2009; McClement 2013). The organic solvent is selected based on its water miscibility, boiling point, safety, and legal status (Lee and McClements 2010) and environmental temperature conditions selected based on its stability of active component from emulsion preparations.

This study was examining the influence of organic phase and temperature conditions on the formation of oil-in water nanogingerols using with low energy homogenizer. The solubility and bioavailability of nanogingerol product were tested and compared to ginger extract.

METHODS

Materials and Equipments

The ginger (*Zingiber officinale Rosc*) was 10 months. The chemicals used were distilled water, ethanol (98%), surfactant (Tween 80), phosphate buffer, NaOH, HCl, hexane, ethyl acetate, acetone, and methanol.

The equipments are homogenizer (Ultra Turrax T8), screen (40 mesh), rotary vacuum evaporator, viscometer, digital scale, pH meter, magnetic stirrer and Particle Size Analyzer (Vasco).

Preparation of Ginger Extract

Ginger powder was extracted by ethanol in a ratio of 1 : 5 (w / v) at 40 ^{o}C for 3 hours (Anam 2010). The extract was separated from its solution by Rotary Vacuum Evaporator set at 40 ^{o}C and 5 bars. The properties of the extract were characterized (such as density, viscosity, pH, conductivity and active compounds).

Preparation of Nanogingerols

Emulsion were prepared from aqueous and organic phase. The organic phase produce by dissolving 10% ginger extract into 96% ethanol in the composition of 10%, 30%, and 50% (v/v) and were stirred for 30 min at room temperature until completely dissolved. An aqueous phase contain Tween 80 as an emulsifier and 5mM phosphate buffer solution (pH 7.0) containing 0.02% sodium azide was prepared by stirring for at least 10 min. The premulsion of solution prepared by mixing the organic phase and an aqueous phase using homogenizer Ultra Turrax T8 at a speed of 22,000 rpm for 10 minutes at room temperature.

Furthermore, the process of manufacture nanoemulsion with a method that combined fase inversion composition and phase inversion temperature where the temperature at the time of nanoemulsion making process was conditioned at 30 ^{o}C, 40 ^{o}C, and 50 ^{o}C. The available nanoemulsion was stirred using homogenizer Ultra Turax T8 with a speed of 22,000 rpm for 10 minutes. The available nanoemulsion used in this stage is the ready nanoemulsion that has been done in the process of pre-emulsion stage process.

Characterization of nanogingerols properties

Particle size measurements

The influence of organic phase and temperature from preparation of nanoemulsion on mean particle size and polydispersity index (PDI) was determained using a Particle Size Analyzer (PSA) instrument.

Solubility

Nanoemulsion mixed with solvent at room temperature in the ratio 1:1, the solvents were hexane, ethyl acetate, acetone, ethanol, methanol and water. The solution well mixed and allowed to stand for 6 hours before observed

In-vitro drug releasing of Nanoemulsion

The bioavailibilty tested by using Franz diffusion cells, it measured the amount of gingers that transfer via a membrane during a specific time interval. The membrane used in the testing is a goat intestines. Phosphat buffer solution (pH 7.4) was used in the compartment receptor side and stirred using magnetic stirrer at 300 rpm to homogenized the active compound that dissolved in compartment receptor. Temperature of solution in the compartment receptor is maintained at 37 ± 0.5 ^{o}C (appropriate body temperature). Membrane was placed between the receptor and donor compartment. Samplings were analyzed after 480 minutes. The absorbance of samples were measured by using spectrofotometer at 280 nm wavelength . The number of

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active substances gingerol diffused in the cell fluid were tested by HPLC method.

RESULTS AND DISCUSSION

Nanogingerol size

Nanogingerol particles were measured using PSA (Particle size analyzer). The result of this study was an average value of the readings of polydispersity index (PDI) less than 0.3, where the value of PDI on nanoemulsion between 0.1-0.3 indicated that the particle size distribution was narrow (Li et al. 2012).

The result show the droplet size smaller than 100 nm are generated at concentrations organic phase above 30%, but the droplet size regrow (> 100 nm) when the temperature is rised to 50 ^{o}C at concentrasions above 50% (Fig 1). According to Mason et al (2006) one of the mechanisms that can destabilize the emulsion and cause the droplet size distribution to change is the coalescence, caused by the rupturing of film of the continuous phase and the fusion of two or more droplets into a single larger droplet. Study on formulation of nanogingerol using these surfactants where Tween 80 is very unstable in the presence of heat. Tween 80 is hydrophilic at low temperature but becomes lipophilic with increasing temperature because of dehydration polyoxyethylene chain (Herrera 2012). Nanogingerol at concentrations of 50% at temperature of 40-50 ^{o}C the molecule has a large activation energy and concentration of droplets the more droplets so that more often interact so some droplets merge into larger caused by the rupture of a layer of Tween 80 due to dehydration the polyoxyethylen chains caused increase in operating temperature to 50 ^{o}C. While the concentration of 30% with a temperature of 40-50 ^{o}C emulsion system can still maintain the surfactant layer on the surface of the droplet due to electrostatic force can negate the van der walls so as to produce a strong repulsive force that can prevent droplet approaching and joining. Anggraeni (2014) also stated that if the van der walls and electrostatic forces cancel each other and the more its value is close to zero, the resultant force generally produce a greater repulsive force.

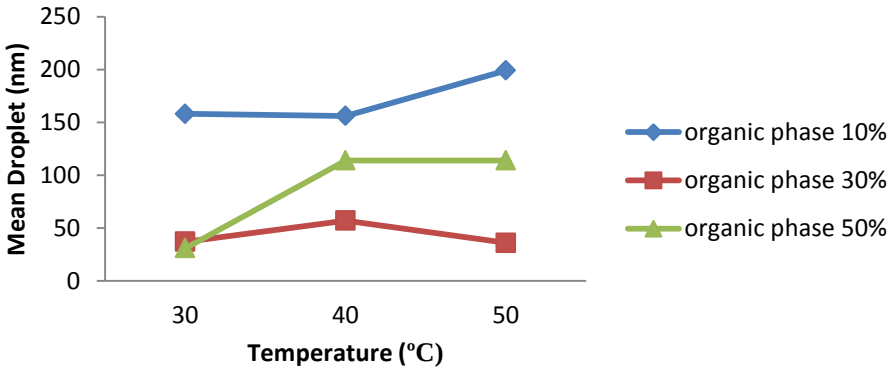

Figure 1: Mean droplets nanogingerols the combination method of phase inversion composition and temperature

Influence temperature, Shinoda and Saitu (1969) has also conducted research influence type emulsion with method inverse temperature stating that emulsion containing quaternary polioksietilena nonylphenylether 3 %, size droplet produced very small but less stable against smelting because the outbreak layer quaternary influenced by the increase of temperature, type emulsify oils in water this relative stable at temperature 20-65 ^{o}C but the increase of temperature above 65 ^{o}C will make coalescence droplet in an emulsion. This is in line with the research conducted by Troncoso et al. (2012) concerning the nanoemulsion manufacture from corn oil in hexane and its characteristics that the resultant particle size of nanoemulsion

obtained decreased around 44% after homogenization with the increased composition of the organic phase from 0% until 95%.

The size of droplet in research is effected by the concentration of organic phase and temperature in the process of making nanogingerol. Temperature and concentration of organic phase was influenced by change in the viscosity of resultant nanogingerol. The higher the temperature in the process make the lower the viscosity would be. The results obtained at temperatute 30-50^{o}C, concentration of organic phase 10-50% would have a decreased viscosity of respectively (Fig 2). The average value of the viscosity of each treatment had increased with the higher concentration of the organic phase which was added to the available nanogingerol. Trancoso et al. (2012) in his research regarding the ratio of corn oil in hexane (oil phase) in which the homogenization condition, the amount and type of emulsion and constant water phase explained that the amount of oil added to organic phase (oil phase) in the emulsion making process affected the value of nanoemulsion viscosity, both oil in water and water in oil. However, at a concentration rised of 50% organic phase an average droplet size of nanoemulsion consecutively smaller than 100 nm. But an average droplet size nanoemulsion respectively larger than 100 nm if the temperature is rised from 40-50 ^{o}C. In general, the viscosity decreased as the temperature increased so that the droplet size also tended to increase. The decrease in viscosity is consistent with the opinion of Astuti (2008), according to Marpaung (2014), heating a liquid caused the molecules to gain energy, so that the molecules moved and the interaction force between molecules weakened; thereby, the viscosity of the fluid would go down as the temperature rises.

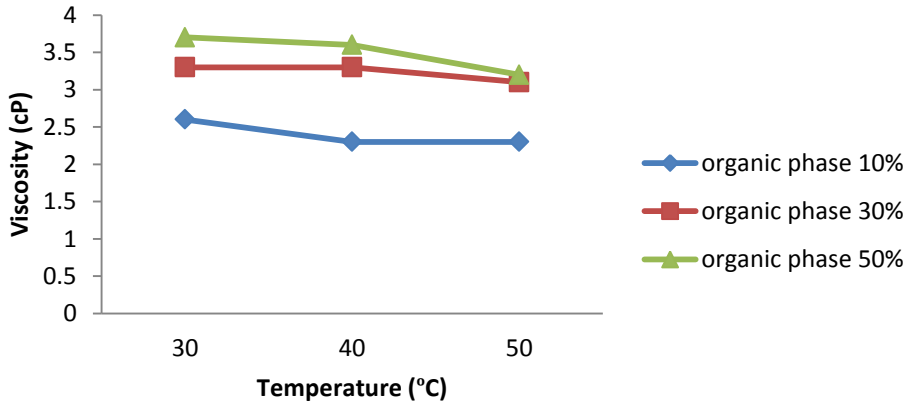

Figure 2: Viscosity values of nanogingerol on the combination method of phase inversion composition and temperature

In liquids, viscosity is caused by the cohesive forces between molecules (Giancoli 1996). Cohesion is the attractive forces between similar particles. This led to the increasing number of droplets contained in the emulsion system so that the greater the force of cohesion that occured in nanoemulsion generated, the larger the viscosity would become. *In this case an* increased concentration of above 50% followed by increase in temperature of 50 ^{o}C on process of making nanogingerol cause nanogingerol having high energy activation on the surface and the molecules interact so that energy in environmental it can prevent the recombining droplet.

Stability

The nanogingerol generated by a combination method of phase inversion composition and temperature tended to be homogeneous and stable when it was assessed qualitatively where the emulsion

stability test results were observed at room temperature (± 30 °C) for 3 days to 30 days.

The nanogingerol which was made at 30- 50 °C with 10% concentration of the organic phase was only stable in storage at room temperature for 3 days but not stable after 30 days, while the oil phase addition of 30% and 50% was relatively stable after being stored at room temperature for 30 days (Table 1). McClement (2005) also explained that the emulsion containing Tween 80 (nonionic surfactant) was resistant to aggregation and formation of sediment but could be ionized on the active surface. Nanogingerol was made at 30-50 °C with 10% organic phase stored for 30 days was found that the sediment was present due to the larger droplet size, larger than 150 nm compared with 30% and 50% organic phase, that is about 150 nm. The decreasing size of droplet would make nanogingerol kinetically stable so that it could prevent the occurrence of sedimentation during storage (Solans et al. 2005).

Table 1: Stability of nanogingerols

Organic Phase (%)	Temperature (°C)	Mean droplet (nm)	Storage 3 day	Storage 30 day
10	30	158	-	+
10	40	156	-	+
10	50	199	-	+
30	30	37	-	-
30	40	57	-	-
30	50	36	-	-
50	30	31	-	-
50	40	114	-	-
50	50	114	-	-

Note: -: stable, +: have agregations

Solubility

The nanogingerol used in solubility test in this experiment was the nanogingerol availibility with the smallest droplet size, that is the combination process of organic phase 50% with a temperature of 30 °C with an average droplet size of 31 nm, pH 6.82. The solubility of nanogingerol would be compared with the extracts of ginger. Test on the solubility properties of nanogingerol was done by mixing in a measuring cup (10 ml nanoemulsion) with organic solvent (1:1) of varying degrees of polarity such as hexane, ethyl acetate, acetone, ethanol, methanol and water with successive polarity values of: 0 , 38 , 47, 68, 73 and 90. Each phase of the organic solvent was then measured to see the addition of its volume before and after being mixed. The increase in the volume of the organic phase was the solubility value.

The solubility of a substance, which is largely due to the polarity of its solvent, is a very important factor in a dosage formulation process of nanogingerol for further utilization. Nanogingerol generated after solubility test at various levels of polar solvent would be insoluble in n-hexane and dissolved completely in the solvents such as ethyl acetate, acetone, ethanol, methanol and water (Table 2.). When compared with the ginger extract that could be perfectly dissolved in n-hexane solvent, ethyl acetate, acetone and ethanol could not dissolve completely in methanol and water. This nanoemulsion could perfectly dissolve in polar solvents. This suggested that changes in the nature of the ginger extract which increased solubility because it could dissolve completely at different polar solvent levels. This is supported by Pujaatmaka (1986) which stated that the solubility of a substance in a solvent was determined by the nature of the match in properties between the solvent (like dissolve like) and among others caused by their polarity.

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Table 2: Solubility value of nanogingerol

Solvent	Polarity	Solubility of nanoemulsion (%)	Solubility of Ginger extracts (%)
Heksan	0	0	100
Etil asetat	38	100	100
Aseton	47	100	100
Etanol	68	100	100
Metanol	73	100	50
Air	90	100	10

pH value also affected the nanogingerol solubility and its further processing. Experiment on manufacture nanogingerol with a combination method of phases inversion composition and temperature, the resultant nanogingerol pH value ranged from 6.82 to 7.04, close to the pH value of human biological fluid where Utami (2012) stated that the pH condition of human biological fluid was ±7.4, making it more easily absorbed by the body. Furthermore, this nanogingerol used surfactant Tween 80, and if further exploited the condition of solusion pH should get a speacial attention because according to the American Pharmaceutical Association (1994) the emulsion using Tween 80 might be saponification reaction in strong acid or strong base environments.

Bioavailability

For the experiment, the penetration of nanogingerol availability selected was the nanogingerol with the smallest droplet size, that is with the phase inversion composition process of organic phase 50 % with a temperature of 30°C with an average droplet size of 31 nm to be compared with the penetration of pure ginger extract. The absorption of the active substance gingerol on cell fluid was taken every interval of 60 minutes during 8 hour (Fig 3). Testing the amount of gingerol was chosen at the highest absorbance, that is 120 minutes after penetration. During the 120 minutes penetration, the nanogingerol penetration was 13.22 %. Meanwhile, the availability of pure ginger extract penetration was through a very little intestine viewed from absorbance at a wavelength of 280 nm which was low so that the levels of penetrated active substances that could not be counted with HPLC method.

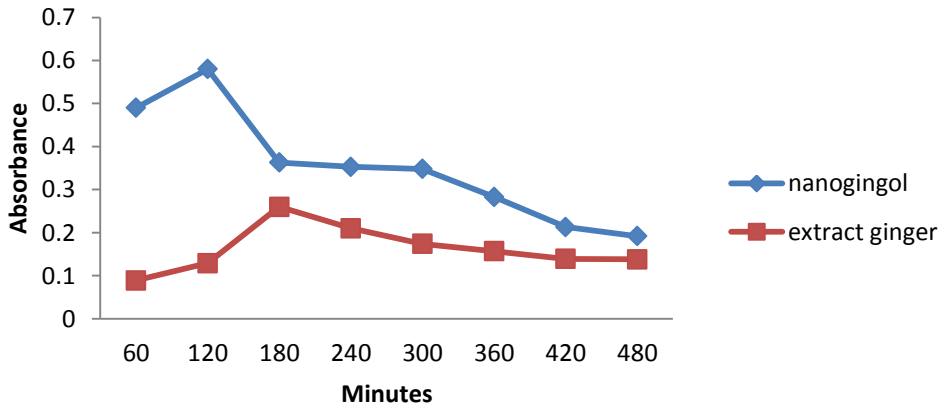

Figure 3: Nanogingerol penetration from absorbance at a 280 nm wavelength.

Penetration capability of nanogingerol availibility was faster and bigger than the availibility of ginger

extract because the smaller droplet size, that is of nanoscale compared to ginger extract droplet of micro-sized. This was also stated by Huang *et al.* (2010) that nanoemulsion had many benefits such as to increase solubility of components, enhance bioavailability, improve absorption and reduce the dose usage.

In addition to size, the electrical conductivity also affected the penetration ability of nanogingerol. The nanoemulsion obtained from the combination method of composition inversion and temperature inversion produced nanoemulsion with a negative charge. The available nanoemulsion tested had a charge of -12 mV while the pure ginger extract was charged with +76 mV. The nanoemulsion with a negative charge would affect the distribution and its bioavalability into cells or body because the cells with negative charge had electrostative affinity for a positive charge, making the cationic or neutral surface of nanoemulsion able to be modified into positively charged to improve the efficacy (Couureurr et al. 2002; Winarti 2013).

CONCLUSION

The best of nanogingerol with droplet size < 100 nm are obtained on a combination of the organic phase of 30% and the relative size of temperature stable at 30-50 ^{o}C. Nanogingerol has an advantage compared to extract ginger with the stability for 30 days, the solubility that can dissolve perfectly on polar solvent, and bioavailability higher (13,22 %). Change the droplet size to adding composition organic phase the factors viscosity, but in temperature increase droplet size droplet changes effected by force between advance.

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