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Comparison of Fabrication Technique and Carrier Oil to Curcumin Nanoemulsion Properties

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ABSTRACT

Turmeric (*Curcuma longa* L.) is included in the rhizome plant containing curcumin which has various therapeutic properties. However, curcumin has poor bioavailability. The manufacture of nanoemulsion is expected to increase curcumin bioavailability. This study aims to compare 3 methods (wet-ball milling, ultrasonication and microfluidic) and 3 carrier oils (soybean, virgin coconut, and olive oil) in the production of curcumin nanoemulsion. Wet-ball milling reduces particle size through grinding process using milling beads in liquid medium. Microfluidic decreases the particle size via collision of emulsion components in microchannel while ultrasound utilizes sound-wave energy to break down the particle size. Parameters compared were particle size, polydispersity index and entrapment efficiency. Particle size and polydispersity index were observed using Particle Size Analyzer with Dynamic Light Scattering technique while entrapment efficiency was measured based on the curcumin absorbance in UV-Visible spectrophotometer at 420 nm. Our study concludes that microfluidic is the most effective and efficient fabrication method which produces the smallest particle size and polydispersity index compared to ultrasonication and microfluidic. The resulting particle sizes using microfluidic are 154, 140 and 132 nm with polydispersity index of 0.224; 0.200 and 0.208 in soybean, virgin coconut, and olive oil respectively. However, entrapment efficiency is best achieved using wet-ball milling method with the average value of 49±10%. Soybean oil appears to be the most curcumin solubilizing oil compared to olive and virgin coconut oil. Thus, it can be concluded that fabrication methods and carrier oils determine curcumin nanoemulsion properties.

Keywords: curcumin nanoemulsion, ultrasonication, wet-ball milling, microfluidic, carrier oil

INTRODUCTION

Nano-size drugs have been developed extensively in medicinal world nowadays. Reducing the particle size to less than 500 nm has been shown to improve drug performance. This is due to the increased of surface area to volume ratio, specificity of the target site as well as its bioavailability [1,2]. In addition, nanoparticle technology provides room for modification of particle size, surface properties and materials used to facilitate more specific drugs. Another advantage of nanoparticles is the ease of drug-controlled release. Nanoparticle drug therapy has been proven to have a positive effect in the treatment of cancer and other diseases [2–4].

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The formation of stable nanoscale particle sizes is heavily influenced by the size reduction method used and the nanoemulsion composition applied [5–7]. Nanoemulsion preparation techniques are divided into two, low-energy and high-energy techniques. High energy techniques include microfluidization, sonication and high-pressure homogenization, while low energy techniques include phase inversion and self-assembly techniques. High energy techniques can produce small particle sizes with high uniformity, but require high energy and complicated instruments. Meanwhile, techniques with low energy are easier to apply with low energy requirements. However, low energy techniques usually produce larger particle sizes with low uniformity. On the other hand, the composition of the nanoemulsion also determines the result of nanoemulsion formation process. Determining the type and ratio of oil, surfactant, co-surfactant or active ingredient to be used greatly affects the character of the resulting nanoemulsion [8].

Currently, products from natural plants are often used as alternative medicine. Some literature mentions that the turmeric plant is one example of this type of plant. Turmeric (*Curcuma longa* L.) is classified as a rhizome plant which is widely used herbal medicine with versatile pharmacological activities. One of the compounds in turmeric is curcumin. Curcumin has anti-cancer [9], anti-oxidant [10], anti-bacterial [11] , and can treat various diseases such as liver disorders [12], respiratory diseases [13], diabetes mellitus [14], and inflammatory disorders [15]. However, curcumin has several weaknesses, namely its poor bioavailability and low solubility, thus, the levels of curcumin that spread in the body are detected very low [16,17].

One of the efforts attempted to overcome the problem is to make curcumin nanoemulsion by adding vegetable oil, surfactants, co-surfactants, and solvents to increase the stability and permeability of curcumin. Tween 80 is added to the nanoemulsion system because it has a hydrophobic-hydrophilic structure that serves to bridge the oil and water phases by reducing the interphase surface tension. HLB (Hydrophilic-Lipophilic Balance) of Tween 80 is 15 which makes Tween 80 is suitable for oil in water emulsion system [18]. In previous studies, various types of oils were used in the manufacture of nanoemulsion, such as soybean [19,20], coconut [21,21–23], olive [24], thyme, shyrazi thyme, rosemary [25], orange peel [26], celery [27] and many more oils.

Soybean oil contains flavonoids, isoflavonoids, phenolic acids, phytoalexins, phytosterols, proteins and peptides, saponins and various vitamin and mineral [28]. Meanwhile, coconut oil consists of medium-chain fatty acids which provide hypocholesterolemic, anticancer, antihepatosteatotic, antidiabetic, antioxidant, anti-inflammatory, antimicrobial and skin moisturizing properties [29]. Similarly, olive oil has phenolic compounds and shows anti-inflammatory actions, preventing cardiovascular diseases, diabetes mellitus, and breast cancer [30]. The usage of soybean oil together with lecithin increases curcumin nanoemulsion entrapment [20] while the combination of soybean and coconut oil improved curcumin nanoemulsion stability and entrapment [23,31]. Olive oil is also applied in curcumin nanoemulsion [24] and shows controlled release in human body [32]. In conclusion, these three carrier oils show a prominent role in determining curcumin nanoemulsion properties.

As previously mentioned, nanoemulsion preparation technique and carrier oils used influence differently to the properties of nanoemulsion produced. To compare the effect of preparation technique and carrier oil used in curcumin nanoemulsion making process, our study applies an identical formulation using three carrier oils by employing three different techniques in nanoemulsion preparation, which are ultrasonication (bath type), microfluidization and wet-ball milling. The techniques being compared are high-energy (microfluidization and ultrasonication) and low energy technique (wet-ball milling). Wet-ball milling reduces particle size through grinding process using milling beads in liquid medium. Microfluidic decreases the particle size via collision of emulsion components in microchannel while ultrasound utilizes sound-wave energy to break down the particle size. Nanoemulsion properties compared are particle size, polydispersity index and entrapment efficiency. Particle size and polydispersity index are measured using Particle Size Analyzer using Dynamic Light Scattering technique while entrapment efficiency is calculated based on the curcumin absorption using UV-Vis spectrophotometer at wavelength of 420 nm. Effectiveness will be measured based on the particle size, polydispersity index, entrapment efficiency. Efficiency will be determined based on the electrical power and time needed in the nanoemulsion manufacturing process. Thus, information regarding the difference effect of preparation technique and carrier oil applied to the properties of curcumin nanoemulsion then can be concluded.

METHOD

Chemical and Instrumentation

The ingredients used were curcumin powder (Health Ingredients, Pharmaceutical Grade, China), virgin coconut oil (food grade), olive oil (food grade), soybean oil (food grade), Tween-80 (Pharmaceutical Grade), ethanol 96%, methanol pa, acetone, nutrient agar (brand), yttrium-stabilized zirconium *beads* 0.5 mm (Hosokawa Alpine, Japan). The oils are edible and were obtained from local supermarket in Malang.

The tools/instruments used were Ultrasonic Bath (Skymen Cleaning Equipment Shenzen Co Ltd), a set of microfluidic made of acrylic with a channel with Y and Z design combination, water pump, potentiometer, analytical balance, Digital Imaging Microscope (Olympus BX51, Japan), Delsa ^{TM} Nano C Particle Analyzer (Beckman Coulter) instrument USA), magnetic stirrer, micropipette, UV-visible spectrophotometer (Shimadzu 1601), hot/stirrer plate and a set of laboratory glassware.

Preparation of curcumin nanoemulsion by microfluidization method

Microfluidization was initiated with pre-emulsion preparation by mixing 125 mg of curcumin powder with 300 µL of Tween 80 (1.2% w/w) and 1000 µL of soybean oil for 2 hours. Then, 25 ml of distilled water was added dropwise into the mixture while stirring was continued for the next 10 minutes. Pre-emulsion was then put in microfluidic set equipped with 5 pumps (water pump DC 12 Volt 100 Psi 2201) and potentiometer. The input tubing for each of the five pumps are placed in a beaker containing the preemulsion. The mixture was pushed into the microfluidic channel with 100 psi input pressure and continuously cycled in microfluidic set for 1 hour. Note that the volume of the pre-emulsion is 5 times larger than two other methods due to the required minimum volume needed in the microfluidization process, however, the composition of the pre-emulsion is identical. The steps then repeated for olive and coconut oil.

Preparation of curcumin nanoemulsion by ultrasonication method

The preparation of pre-emulsion was carried out in two steps. First, the water and oil phases were prepared separately. The oil phase was prepared by mixing 25 mg of curcumin powder with 200 µL of coconut oil (VCO) using magnetic stirrer on the stirrer plate for 2 hours. Meanwhile, 60 µL of Tween 80 (1.2% w/w) was mixed with 5 mL of water using magnetic stirrer on a stirrer plate for 2 hours to form water phase. Second, the water phase was then added to the oil phase dropwise and stirred for the next 15 minutes. Then the sample was sonicated by placing it in an ultrasonicator bath for 1 hour with a frequency of 40 kHz. The experiment was then repeated for olive and coconut oil.

Preparation of curcumin nanoemulsions using the wet-ball milling method

Curcumin (25 mg) was weighed in a 50 mL beaker, 60 µl of Tween 80 (1.2% w/w) was added together with 200 µL of soybean oil and 5 mL of distilled water. Then, the mixed solution is stirred until homogenous. The sample was then transferred to a vial containing 2.5 mL of milling beads (0.5 mm) and 1 magnetic stirrer. The sample was stirred for 24 hours with the help of a magnetic stirrer at 100 rpm. The procedure is then repeated for olive and coconut oil.

Measurement of particle size and polydispersity index

Particle size was determined using the Delsa™ Nano C Particle Analyzer Beckman Coulter instrument. Measurements were made using distilled water with a temperature of 25°C, index of refraction of 1.3328, viscosity of 0.8878, dielectric constant of 78.3 in a disposable cell. At each measurement, 50 µL of curcumin nanoparticle solution was dissolved in 2450 µL of distilled water.

Measurement of entrapment efficiency

Concentration of curcumin encapsulated is measured based on the curcumin absorbance using UV-Vis spectrophotometer at 420 nm wavelength. Sample is diluted followed with separation of precipitate and filtrate using centrifugation at 3500 rpm for 45 minutes. Free curcumin concentration was determined based on the absorbance of the filtrate. Meanwhile, total curcumin concentration was measured based on the absorbance of the remaining filtrate and precipitate which is further solubilized in methanol in a ratio of 1:1 and sonicated for 5 minutes to break all nanoemulsion droplets. Entrapment efficiency (EE) is then determined based on the difference between total and free curcumin concentration using the following formula:

$$\%EE = \frac{Total\ curcumin - Free\ curcumin}{Total\ curcumin} x\ 100\%$$

RESULTS AND DISCUSSION

Comparison of fabrication technique effectiveness in various carrier oils

The effectiveness of the techniques is determined by examining the particle size and polydispersity index resulting from each method which signifies the stability of the system. An effective nanoemulsion making method produces a stable system in which there is no further particle reduction or increase during storage. Thus, it is important to analyze which method can effectively produce stable system. Table 1 shows the particle size resulting from wet-ball milling, ultrasonication, and microfluidic methods. Wet-ball milling, which is a low energy method, seems to produce quite large particle sizes in all three carrier oils. The smallest size is achieved at 344 nm in the virgin coconut followed by 533 nm in soybean and 1196 nm in olive oil system. Meanwhile in our experiment, the high-energy method, ultrasonication, seems unable to produce smaller size than wet-ball milling method where the resulting particle sizes are 751, 774 and 1062 nm in the virgin coconut, soybean and olive oil systems respectively. Microfluidics appears to be the most effective method for producing small nanoemulsion particle size where the resulting particle sizes are 132, 140 and 154 nm in olive, coconut and soybean oil respectively.

Based on the average particle size from three carrier oils in each fabrication method, it appears that the type of oil affects the particle size produced. Olive oil produces the largest particle size (797 nm) while soybean and coconut oil show smaller nanoemulsion particle size (487 nm and 412 nm respectively). Viscosity of the oils is considered as the aspect influencing particle size produced. Particle size tends to have a linear correlation with viscosity. Higher viscosity will produce bigger particle size. Viscosity of the oils determine its diffusion, thus, influencing the extent of oil dispersion. Higher viscosity creates smaller extent of diffusion which limit the interaction of oil with other nanoemulsion curcumin. Thus, the particle size of viscous oil is larger than the less viscous oil. There is possibility of heat generated in the process which alter the oil viscosity; however, the change is negligible.

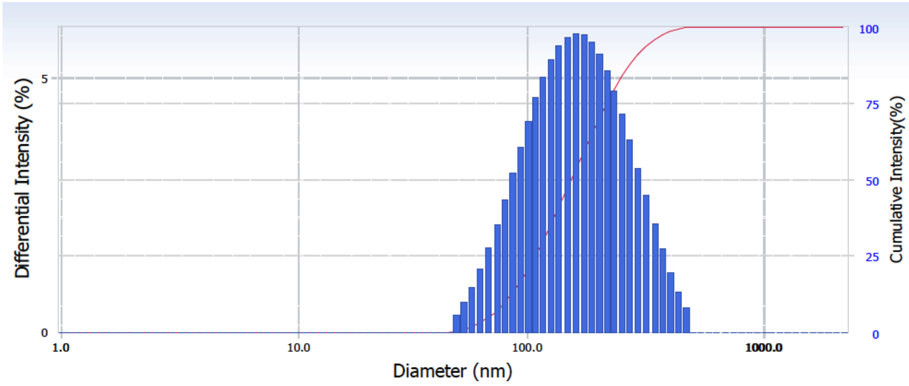
Our results show an agreement where olive oil, which has the highest viscosity (85 mPas at 20°C), produces the biggest particle size. Meanwhile, soybean and virgin coconut oil, with viscosity of 80 mPas at 20°C, exhibit smaller particle size (487 and 412 nm respectively). Interestingly, the results of nanoemulsion with microfluidic show a more uniform particle size in three oils where the particle size ranges from 132-154 nm. This signifies that microfluidics can produce smaller particle sizes regardless of the type of oil used in the system compared to wet-ball milling and ultrasonication.

Table 1. Particle size, polydispersity index and curcumin entrapment efficiency in various fabrication methods and carrier oils

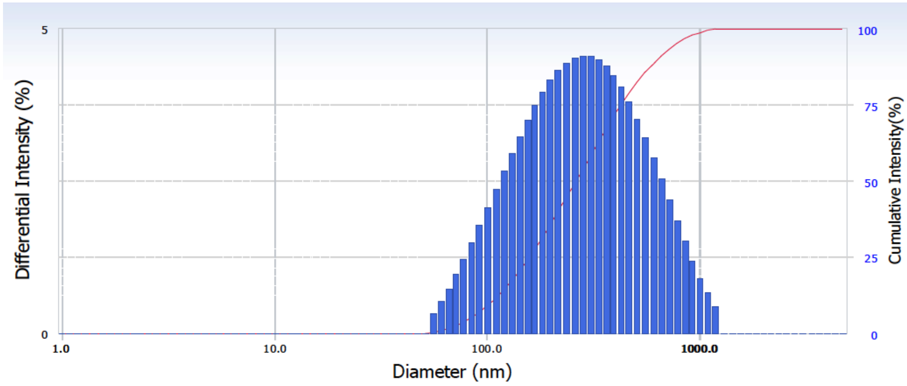
System	Particle Size (nm)	Polydispersity Index	Entrapment Efficiency (%)
Wet-ball milling			
Soybean oil	533	0.290	39
Virgin coconut oil	344	0.316	62
Olive oil	1,196	0.483	47
Ultrasonication			
Soybean oil	774	0.392	53
Virgin coconut oil	751	0.321	18
Olive oil	1,062	0.392	43
Microfluidic			
Soybean oil	154	0.224	49
Virgin coconut oil	140	0.200	26
Olive oil	132	0.208	32

The resulting polydispersity index, which shows the uniformity of the particle size in the system, is presented in Table 1. Similar with particle size, microfluidic method produced the lowest polydispersity index, namely 0.200; 0.208; and 0.224 in the coconut, olive, and soybean oil systems. The polydispersity index using ultrasonication and wet-ball milling methods produced a high polydispersity index in three oils. The polydispersity index produced by ultrasonication technique turned out to be higher even though this technique is included in high energy technique. This can be due to the type of ultrasonicator applied, which is bath type instead of probe-type ultrasonicator which may produce lower energy.

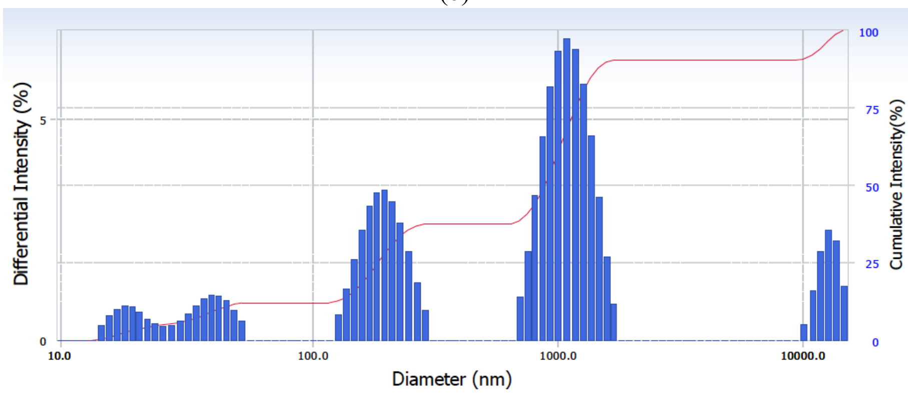
The graphs of differential intensity which illustrate the particle size distribution, which further determine polydispersity index, are presented in Figure 1(a-c). We present the graphs of virgin coconut oil system prepared using three methods. Single peak in microfluidic system (Figure 1.a) substantiates the uniformity of the particle size. Even though single-peak also observed in wet-ball milling system (Figure 1.b), the peak is broader than in microfluidic which shows that the system less uniform. In ultrasonication (Figure 1.c), there are many peaks which shows that this system is the most heterogeneous system.



(a)



(b)



(c)

Figure 1. Differential intensity graphs of nanoemulsion prepared using (a) microfluidic (b)wet-ball milling (c) ultrasonication.

From the two parameters previously mentioned, it can be concluded that microfluidic method is the most effective method in producing nanoemulsion with the lowest particle size and polydispersity index. Different types of oil do not determine the particle size and polydispersity index produced in this technique. Study by Fathordoobady *et.al* [5] confirms our results in which microfluidic produces smaller particle size and polydispersity index compared to ultrasonication in hempseed oil nanoemulsion production. In contrast, Paez-

Hernandez et.al [33] reported smaller particle size achieved using ultrasonication technique compared to microfluidic. However, the experiment setting and formula applied in this study is not identical to our system which hinder the meticulous comparison between methods.

In addition, previous studies have shown that one of the aspects that determine the size of the resulting particles is the type and amount of surfactant used [8]. In this experiment, the surfactant used is Tween 80 which has a high HLB (hydrophilic-lipophilic balance) value making it effective for forming oil-in-water nanoemulsions. The amount of Tween 80 used in this experiment is 1.2% (w/w) which is low compared to other nanoemulsion systems. Usually, surfactants applied is within the levels of 5-20%. Excessive use of surfactants might cause hypersensitivity reaction. This implies that with a low amount of surfactant, microfluidic method can produce particle sizes below 200 nm with a polydispersity index of around 0.2 in all types of oil. Thus, it can be concluded that microfluidic method is the most effective method compared to the ultrasonication and wet-ball milling in our system.

Table 2. Required energy in each nanoemulsion fabrication method.

Methods	Process	Required energy (watts/hour)	Duration (hour)	Total energy
Wet-ball milling	Stirring	25	24	600
			Total	600
Ultrasonication	Stirring	25	4.25	106.25
	Ultrasonication	60	1	60
			Total	166.25
Microfluidic	Mixing	25	2.17	54.25
	Microfluidization	125	1	125
			Total	179.16

Comparison of fabrication technique efficiency

Process efficiency is calculated based on the total energy required during the nanoemulsion formation process. The total energy required is calculated from the power used in equipment involved in each techniques. In wet-ball milling, the required power is the power for the stirring process using a hot plate and a magnetic stirrer of 25 watts/hour for 24 hours. Hence, the total power in this process is 600 watts. In microfluidic technique, the power used consists of power for pre-emulsion mixing of 25 watts/hour for 2.17 hours (total 54.25 watts) and the microfluidization process is 25 watts/hour/pump for 1 hour using 5 pumps (total 125 watts). Thus, the total power required is 179.18 watts. For the ultrasonication method, the power involved consists of a pre-emulsion stirring process of 25 watts/hour for 4.25 hours both for water and oil phases (total 106.25 watts) and ultrasonication process of 60 watts/hour for 1 hour so that the total power used in this process 166.25 watts. Table 2 summarizes the required energy in each method.

Ultrasonication and microfluidics are considered as high energy methods compared to wet-ball milling method. Nonetheless, to achieve the same particle size and polydispersity index as the microfluidic technique, wet-ball milling technique takes 24 hours or 600 watts which is 3.3 times higher than microfluidic method. Ultrasonication used a lower energy at 166.25 watts, however, particle size and polydispersity index achieved are bigger than the other two methods. From the above results it can be concluded that microfluidic method is an efficient method for producing curcumin nanoemulsions with the smallest particle size and polydispersity index since it uses lower energy in shorter duration of time.

Comparison of curcumin entrapment efficiency

Comparison of curcumin entrapment efficiency in the 3 oil systems is presented also in Table 1. Wet-ball milling method appears to produce the highest entrapment efficiency among the three methods. On average, curcumin entrapment efficiency produced by wet-ball milling method in three carrier oils is 49±10%. This value is the highest compared to the entrapment efficiency in ultrasonication and microfluidic which are 38±15% and 36±10% respectively. Meanwhile, the average entrapment efficiency in soybean oil is the highest regardless of the fabrication method applied, which is 47±6%. Standard deviation calculated is low, showing that soybean oil solubilize curcumin better regardless of the fabrication method employed. Olive oil, shows lesser entrapment efficiency to soybean oil, 41±6%. Entrapment efficiency of curcumin in virgin coconut oil is the lowest among three methods with high standard deviation value (35±19%) which signifies that fabrication method determines the entrapment efficiency produced. Using wet-ball milling, 62% curcumin added in the system is incorporated in virgin coconut oil nanoemulsion system. In contrast, only 18% and 26% of curcumin is trapped in oil droplet if ultrasonication and microfluidic is applied respectively.

Our results confirm the previous study which showed that curcumin solubilize slightly higher in soybean rather than in olive oil [34]. Previous study reported that curcumin solubility in soybean oil is 0.47 mg/g which is higher than in olive oil which is 0.45 mg/g. Other study stated that curcumin solubility was higher in soybean oil (0.08%) followed with virgin coconut oil (0.06%) and olive oil (0.04%) [35]. Franklyne [32] also showed that coconut oil solubilize curcumin as much as 58.62 mg/ml which is higher than in olive oil (54.36 mg/ml). From these findings it can be concluded that soybean oil produces the highest curcumin entrapment efficiency compared to virgin coconut and olive oil. Our experiment shows that olive oil entrapped curcumin better than virgin coconut oil which is in contrast with other studies. However, high standard deviation value calculated in our experiment open possibilities that virgin coconut oil can solubilize curcumin better depend on the fabrication method applied. Polarity plays a significant role in determining the degree of curcumin solubilization in carrier oil as well as formulation and fabrication method applied.

Further analysis reveals that particle size does not linearly correlate to the entrapment efficiency. Linear plot of entrapment efficiency as a function of particle size shows a very low correlation coefficient which signifies that entrapment efficiency could not be estimated based on the particle size. It implies that bigger particle size does not contain higher concentration of curcumin and vice versa. This could be due to the possibility of water-contain liposome formation in the system. Tween 80 might form void liposome which entrapped water instead of curcumin loaded oil. Our previous simulation study shows that the encapsulated curcumin molecules tend to position their molecule closer to the hydrophilic groups rather than at the innermost part of the micelle [36]. This phenomenon might lead to the formation of big particle size with low curcumin content, thus, making particle size does not correlate linearly with entrapment efficiency.

CONCLUSION

Our study concludes that microfluidic is the most effective and efficient fabrication method which produces the smallest particle size and polydispersity index compared to ultrasonication and microfluidic. The resulting particle sizes using microfluidic are 132, 140 and 154 nm with polydispersity index of 0.224; 0.200 and 0.208 in olive, coconut, and soybean oil respectively. However, entrapment efficiency is best achieved using wet-ball milling method with average value of 49±10%. Soybean oil appears to be the most curcumin solubilizing oil compared to olive and virgin coconut oil. Thus, it can be concluded that fabrication methods and carrier oils determine curcumin nanoemulsion properties.

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