Effect of Ultrasonication on Some Chemical Properties of Red Ginger
(Zingiber officinale var. rubrum) Oleoresin Nanoemulsion Bioactive
Compounds

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Received: 1 March 2023, Revised: 4 April 2023, Accepted: 26 April 2023, Published: 28 August 2023

Abstract

Nanotechnology has developed in various industrial fields. This encourages innovation in the use of additives such as antioxidants, dyes, flavors, and other active ingredients that can be modified in the form of nanoemulsions. Red ginger contains oleoresin, which has thick properties to form a paste. These properties make oleoresin difficult to mix with food, disperse, hydrophobic and susceptible to the effects of heat and light. One solution to overcome this problem is to convert oleoresin into a nanoemulsion so that it has high solubility, is stable, good bioavailability, and improves sensory properties. One technique for making nanoemulsions is ultrasonication. The high-intensity ultrasonication effect can cause the movement of the oil phase into the liquid phase in the form of droplets, thereby increasing the pressure in the liquid and resulting in the breakdown of the oil droplets into smaller sizes. The purpose of this research was to determine the effect of ultrasonication on some chemical properties of the red ginger oleoresin nanoemulsion bioactive compounds, which includes antioxidant activity, total phenol, gingerol content, and droplet microstructure. The study used a completely randomized design with four treatments based on sonication time. Data from the test results were analyzed using analysis of variance (ANOVA) and Tukey's advanced test. Based on the results of the analysis, it was found that ultrasonication had effect on the red ginger oleoresin nanoemulsion bioactive compounds. The best treatment is 90 min of sonication. The antioxidant activity (IC₅₀) was 11.45±3.48 ppm, the total phenolic content was 3342.74±283.00 mg GAE/g, and the gingerol content was 0.33 %. The nanoemulsion droplet microstructure complies with the standard; that is, the particles are in the form of small spheres, like balls, and there are no visible holes or cracks on the surface of the particles.

Keywords: Antioxidant, Droplet microstructure, Gingerol, Nanoemulsion, Oleoresin, Total phenolic content, Ultrasonication

Introduction

The development of nanotechnology in various sectors is very rapid, especially in the cosmetic, pharmaceutical, agricultural, and food industries [1]. The application of nanotechnology is focused on developing lipophilic substances such as fatty acids, flavors, dyes, and drugs [2]. In the industrial world, additives such as antioxidants, dyes, acids, flavors, benzoic acid preservatives, vitamins A and E, beta-carotene, omega-3 fats, and other bioactive compounds are often added to products to enhance taste, appearance, texture, fortification, and stability. This encourages innovation in the use of additives and active ingredients to be modified in the form of nanoemulsions [3].

Nanoemulsions are O/W (oil in water) or W/O (water in oil) emulsions that have droplet diameters ranging from 50 to 1000 nm [4]. Ashaolu [3] reported that the application of nanoemulsions has been shown to be very effective in the bioavailability, bioactivity, digestibility, stability, safety, quality, and sensory enhancement of food components. The use of nanoemulsions to stabilize essential oils in food also helps overcome their high volatility, hydrophobicity, and reactivity with other food components. In addition, essential oils in the form of nanoemulsions can enhance antioxidant and antimicrobial activity, compatibility, solubility, stability, and physicochemical balance [5].

One of the nanoemulsion manufacturing techniques is ultrasonication, which is a high-energy emulsification method [6]. Ultrasound uses ultrasonic waves with a frequency exceeding the limit of human
hearing, which is above 20 kHz. The energy for ultrasonication is generated from the sonicator probe. When the coarse emulsion (water and oil phases) touches the tip of the sonicator, it will produce mechanical vibrations and cavitation will occur so that nano-sized droplets are formed [7]. The longer the sonication time, the more the electric waves will turn into pressure waves, resulting in a reduction in particle size. This method can be applied to very thick types of oil with a high molecular weight [8].

Red ginger (Zingiber officinale var. rubrum) is commonly used in traditional medicine in Asia due to the presence of bioactive compounds such as gingerols and shogaols, which act as immunomodulators, anti-inflammatory agents, and antioxidants [9]. Red ginger contains more oleoresin than other types of ginger [10]. Oleoresin is a non-volatile component of oil obtained by extraction using organic solvents, after which the solvent is evaporated [11]. Oleoresin is a mixture of resin and essential oil, the resin consists of active components in the form of phenols such as gingerol, shogaol, and zingerone; which give it a spicy taste [12].

The physical properties of red ginger oleoresin are similar to thick oil that can be used to make a paste. These properties make oleoresin difficult to mix with food, disperse, hydrophobic, and susceptible to the effects of heat and light [13]. To assist mixing, permitted solvents such as propylene glycol or vegetable oil are often added [14]. One solution to overcome this problem is to convert oleoresin into a nanoemulsion, so that oleoresin in nanoemulsion form has high solubility, stability, absorption, improves sensory properties, and good bioavailability [15].

Information about the manufacture of red ginger oleoresin nanoemulsion using ultrasonication is still limited, its use will also affect the characteristics of the nanoemulsion [16]. The high-intensity ultrasonication effect can cause the movement of the oil phase into the liquid phase in the form of droplets, thereby increasing the pressure in the liquid, resulting in the breakdown of the oil droplets into smaller sizes and their complete dispersion in the liquid phase [17]. For this reason, it is very important to analyze bioactive nanoemulsion compounds such as antioxidant activity, total phenolic content, gingerol, and microstructure, so that they have the potential to be functional foods because they contain bioactive components that function as natural antioxidants.

Materials and methods

Materials

The materials used in this research are red ginger from East Lampung-Indonesia (the species is Zingiber officinale var. rubrum), aquadest, ethanol 96 % (Merck®), Tween 80 (Merck®), methanol (Merck®), DPPH (Himedia®) folin ciocalteu reagent (Merck®), sodium carbonate (Merck®), gallic acid (Sigma Aldrich®), standard 6-gingerol (Sigma Aldrich®), and filter paper (Whatman®). The instruments used were an analytical balance (Radwag-AS220.R2®), oven (Memmert®), hammer mill, incubator (Memmert®), hotplate magnetic stirrer (Chimarec+®), vacuum rotary evaporator (B-ONE®), ultrasonicator type bath (Branson 5800®), burette (Iwaki-pyrex®), erlenmeyer (Iwaki-pyrex®), beaker glass (Iwaki-pyrex®), measuring pipette (Iwaki-pyrex®), volumetric flask (Iwaki-pyrex®), measuring cup (Iwaki-pyrex®), test tube (Iwaki-pyrex®), micro pipette (Dragon Lab®), UV-Vis Single Beam Spectrophotometer (K-LAB®), TLC Scanner (Camac TLC Scanner 3®), and Scanning Electron Microscope (SEM) (type ZEISS EVO MA 10®).

Research design

The study used a one-factor, completely randomized design of 4 treatments based on sonication time. It is T0 (without sonication time), T1 (30 min), T2 (60 min), and T3 (90 min) with 4 replications. The research stages were: sample preparation, red ginger extraction, red ginger oleoresin nanoemulsion, and characterization of bioactive compounds.

Sample preparation

Red ginger is cleaned of soil and dirt, then washed, sliced to a thickness of ±1 - 3 mm and dried in an oven for 48 h at 50 °C until 12 % moisture is reached. Dried ginger is ground with a hammer mill and sieved with a 50 mesh sieve [18].

Red ginger extraction

Red ginger powder was extracted using the maceration method, the ratio of powder to solvent was 1:3. Maceration was carried out in stages for 72 h, and every 24 h it was replaced with a new solvent. The extraction results were separated from the solvent using a rotary evaporator at 50 °C for 60 min to obtain a thick extract called red ginger oleoresin [19].
Red ginger oleoresin nanoemulsion

Red ginger oleoresin is made into a nanoemulsion using the ultrasonication method, which has been modified [20,21]. 50 % red ginger oleoresin (oil phase) in 96 % ethanol (co-surfactant) (50 mL ginger oleoresin in 100 mL ethanol). Then, adding distilled water (the water phase) gently with a burette at a speed of 5 mL/min until a volume of 500 mL was obtained, accompanied by stirring at a speed of 750 rpm, I added Tween 80 (the surfactant) by 10 % of the oil phase added up to 5 mL and the water stages were then included. At a speed of 1500 rpm and a temperature of 30 °C, they stirred once more using a magnetic stirrer for 60 min. At the scheduled time, the mixture was added to a bath-style ultrasonicator operating at 40 kHz at 30 °C. The solvent was then separated from the solutes using a rotary evaporator at a temperature of 50 °C for 30 min after the results had been filtered through 0.45 micron filter paper.

Characterization of bioactive compounds

The bioactive compounds analyzed consisted of antioxidant activity, total phenolic content, gingerol content, and droplet microstructure.

Antioxidant activity

Antioxidant activity is measured using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method [21]. Antioxidant activity is expressed in the IC₅₀ value, or concentration capable of reducing 50 % of DPPH free radicals. The test was carried out by making a 50-ppm DPPH solution, a red ginger oleoresin nanoemulsion made in solution with 5 concentration levels. It is 200, 400, 600, 800 and 1000 ppm from each research sample. The percentage of antioxidant inhibition was determined by reacting the test solution with the DPPH solution in a ratio of 1:1 and then incubating for 30 min at 37 °C. Next, the absorbance was measured using a spectrophotometer at 517 nm. The percentage of antioxidant inhibition was calculated from the absorbance value obtained from the test was included in the percent inhibition formula. The IC₅₀ value is expressed as Ln x in the regression equation $y = ax + b$ from the curve of percent antioxidant inhibition as ordinate and Ln concentration as abscissa. “Y” is the value of 50; “a” and “b” are obtained from the regression equation [22].

Total phenolic content

The phenolic content was determined using the Folin-Ciocalteu method [23]. The test was carried out by making a standard solution of gallic acid in 5 concentration, namely 20, 40, 60, 80 and 100 ppm. From each of these concentrations, 400 µL was taken into a test tube, 400 µL Folin Ciocalteu reagent was added, vortexed, and incubated for 6 min, and then 4.2 mL of a 5 % sodium carbonate solution was added. The sample was vortexed and incubated for 90 min at room temperature and in dark place, then the absorbance was measured at 760 nm. The sample was weighed, and about 0.01 g was dissolved in distilled water up to 10 mL in a volumetric flask. Then treated in the same way as the gallic acid solution sample.

The phenolic content was determined using the gallic acid regression equation formula, $y = ax + b$. “y” denotes absorbance, “x” denotes gallic acid concentration, “a” denotes intercept, and “b” is a constant. Total phenol is expressed as mg gallic acid equivalent/g sample. Total phenol can be calculated using the following formula:

$$\text{phenolic content (mg GAE/g)} = \frac{x \text{ (mg/m)} \cdot \text{Sample solution volume (ml)}}{\text{sample (g)}} \cdot \text{xDF}$$

Information: X = Concentration from the linear regression equation of the gallic acid standard curve (mg/mL), and DF = Dilution factor

Gingerol content

The gingerol content was analyzed using thin layer chromatography [24]. A 0.5 g sample was placed in a 25-mL volumetric flask, and a third of the flask was filled with ethanol. Then it was shaken using a shaker for 2 h, measured, and allowed to stand for 24 h. The filtrate is filtered using filter paper. The 5 µL of the filtrate was placed on an aluminum silica gel plate measuring 20×20 cm², which had been heated in an oven at 105 °C for 30 min. In comparison, standard 6-gingerol at 500 ppm was found in as much as 5 µL of water. The plate is inserted into the chamber containing the hexane eluent; 30 mL of diethyl ether with a ratio of 3:7 was then eluted for 45 - 60 min until the elution limit was 15 cm. The plate was then air-dried before being measured with a TLC (Thin Layer Chromatography) scanner at = 282 nm. To calculate gingerol levels, the following formula was used:
Gingerol content (%) = \( \left( \frac{\text{area of sample}}{\text{area of standard}} \right) \times \frac{\text{ppm standard} \times \text{vol sample} \times \text{i} \times \text{DF}}{\text{mg sample}} \times 100 \% \)  

(2)

**Droplet microstructure**

SEM (Scanning Electron Microscopy) was used to examine the microstructure of red ginger nanoemulsion droplets, which had the best bioactive components. The sample is attached to the cell holder and coated with gold-palladium. Then, the SEM (ZEISS EVO MA 10 model) is operated in a state of WD 8.5 mm, using signal A SE1 and EHT 8 kV. Then the sample was enlarged using magnifications of 5000, 10,000, 15,000 and 20,000 times. The resulting image is in the form of a nanoemulsion droplet microstructure in the form of grains with all the protrusions, indentations, and holes on the surface [25].

**Statistical analysis method**

Data on antioxidant activity and total phenol content were processed using variance to determine the effect of treatment on the parameters tested using ANOVA at P ≤ 0.05. Furthermore, to find out which treatment was different, further tests were carried out using the Tukey test. As for the levels of gingerol and droplet microstructure, they were analyzed using descriptive statistics.

**Results and discussion**

**Antioxidant activity of red ginger oleoresin nanoemulsion**

Antioxidants are compounds that can slow down the oxidation process of free radicals, thereby protecting cells from damage caused by unstable molecules known as free radicals. These compounds work by donating their electrons (giving hydrogen atoms) to free radical molecules, thereby stopping the chain reaction and converting free radicals into a stable form. To ascertain the antioxidant potential in reducing free radicals as expressed by antioxidant activity (IC\(_{50}\)), i.e. the concentration capable of inhibiting free radicals by 50 %, The smaller the IC\(_{50}\), the higher the antioxidant activity of a compound or substance [26].

The principle of testing antioxidant activity using the DPPH method is the donation of hydrogen atoms from the tested samples to DPPH free radicals [21]. The active compound in the red ginger oleoresin nanoemulsion, which functions as an antioxidant, can reduce diphenylpicryl hydrazyl free radicals (DPPH) to diphenylpicryl hydrazine. The diphenyl picryl hydrazine compound is a derivative of the DPPH radical, which is a more stable antioxidant radical [27].

Antioxidant activity is expressed in IC\(_{50}\), namely the concentration capable of reducing free radicals by 50 %. The IC\(_{50}\) value is inversely proportional to the antioxidant activity. The smaller the IC\(_{50}\) value, the greater the antioxidant activity. The classification of antioxidants was divided into 5 classes namely very strong (< 50 ppm), strong (50 - 100 ppm), medium (100 - 150 ppm), weak (150 - 200 ppm) and very weak (> 200 ppm) [28]. In treatment T0, the IC\(_{50}\) value was greater than 50 ppm, indicating vigorous antioxidant activity. In contrast, in the T1, T2, and T3 treatments, the IC\(_{50}\) value was less than 50 ppm, which means that the antioxidant activity in this treatment was potent. The IC\(_{50}\) value of red ginger oleoresin nanoemulsion in the T1, T2, and T3 treatments was smaller than the red ginger extract in Sholikhati et al. [29], namely 41.27 ppm. That is, the antioxidant activity of red ginger in nanoemulsion form is stronger when compared to red ginger extract. Nanoemulsions are effective in increasing antioxidant activity, so they have the potential to improve bioactive components.

![Figure 1](image-url)  
**Figure 1** Antioxidant activity of red ginger oleoresin nanoemulsion based on sonication time.
Ultrasonication affects antioxidant activity, the longer the sonication time, the smaller the IC₅₀ value, which means stronger antioxidant activity. This is in accordance with the research of Lin et al. [30], who found that sonication time affects the IC₅₀ value. The use of ultrasonic waves can cause a cavitation effect on nanoemulsion which can break down the cell walls of the material so that the bioactive components that act as antioxidants can come out of the cell walls easily. So, the longer the sonication time, the stronger the antioxidant activity [31]. The ultrasonication process is able to reduce particle size down to the nanometer scale, thereby increasing the concentration of bioactive components and antioxidant activity [32].

The longer the sonication time, the greater the ultrasonic waves that propagate by vibration. This vibration results in friction between mass particles, which then generates heat [33]. Increasing the temperature of the ultrasonicator can increase the antioxidant activity of red ginger because temperature can increase the phenolic compounds that act as antioxidants [34]. The formation of phenolic compounds takes place at a temperature of about 60 °C [35]. This occurs due to the availability of precursor phenolic molecules through non-enzymatic interconversion between phenolic molecules. The process of enzyme inactivation is also associated with heating food. The higher the heating temperature, the higher the PPO (polyphenol oxidase) enzyme inactivation process, so that the degradation of phenol is lower. In addition, heating causes the release of antioxidant components as a result of damage to cell walls due to heat [34]. However, if the heating temperature is too high, it can damage the active substance of red ginger. The maximum temperature used to increase antioxidant activity is 60 °C. This is in line with the research of Shen et al. [36], which found that increased antioxidant activity is often associated with an increase in polyphenols. The maximum temperature limit for the ultrasonicator to increase antioxidant activity is 60 - 70 °C.

**Total phenolic content of red ginger oleoresin nanoemulsion**

Phenolic compounds are chemical compounds that have an aromatic ring that contains 1 or more hydroxy functions. Phenol compounds are an effective source of antioxidants, suppressing free radicals and chelating metal ions. The total phenol content is affected by the availability of phenolic compounds in a material. Total phenol is an important component that is antibacterial. Gingerol is a member of the phenol group, which is the most common disinfectant used in laboratories as a growth inhibitor and germ killer [37]. Ginger’s phenolic compounds are part of the oleoresin, which influences its spicy flavor. Phenol compounds consisting of shagaol, gingerol, sesquiterpene, zingiberen, zingiberol, kukumen, sesquiphellandran, zingeron, 6-dehydrogingerdion, ginger-glycolipid, and organic acids (lauric acid, oleic acid, linoleic acid, and stearate) have antioxidant, anti-inflammatory properties, and as an immunomodulator [38].

The total phenolic content of the red ginger oleoresin nanoemulsion can be seen in Figure 2. Ultrasonication affects the total phenolic content. Total phenolic content of red ginger oleoresin nanoemulsion at T0 was 2277.57±135.70 mg GAE/g, T1 was 2569.04±77.52 mg GAE/g, T2 was 2712.78±84.07 mg GAE/g, and T3 namely 3342.74±283.00 mg GAE/g. The T0, T1, and T2 treatments were not significantly different in the Tukey follow-up test. The highest total phenolic content was during sonication for 90 min (T3) was at T3. The total phenolic red ginger oleoresin nanoemulsion was much higher than the total phenolic red ginger extract compared to the study by Luhurningtyas et al. [38], namely 338.567 mg GAE/g. The ultrasonication treatment affected the total phenolic content. The longer sonication time causes the total phenolic content to increase [39]. The presence of heat generated by the ultrasonication process causes damage to the cell walls, thereby removing phenol from plant tissues, and as a result, the total phenol content increases [40].

![Figure 2](image-url) **Figure 2** Total phenolic content of red ginger oleoresin nanoemulsion based on sonication time.
Phenolic compounds function as antioxidants, so the total phenol content correlates with antioxidant activity. The higher the total phenol content, the stronger the antioxidant activity [31]. The higher the total phenol content (Figure 2), the smaller the IC$_{50}$ value (Figure 1), which means that the antioxidant activity is stronger. This is in line with research Ghafoor et al. [41] that total phenolic contents positively correlated with antioxidant activity.

**Gingerol content of red ginger oleoresin nanoemulsion**

The gingerol content in the red ginger oleoresin nanoemulsion tested was 6-gingerol. This is the main bioactive compound in red ginger. Gingerol content is part of the phenolic compounds that act as antioxidants. The content of 6-gingerol nanoemulsion oleoresin in red ginger was analyzed using a TLC scanner, which produced peaks as shown in Figure 3.

![Figure 3 Peak display of 6-gingerol red ginger oleoresin nanoemulsion.](image)

Based on the peak display, the area value of each sample is obtained, which is then calculated using the gingerol content formula, in order to obtain the gingerol content in percent as shown in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L. Area of sample</th>
<th>L. Area of standard</th>
<th>Ppm standard</th>
<th>Volume sample (L)</th>
<th>Dilution factor</th>
<th>mg sample</th>
<th>% gingerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>602.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>T1</td>
<td>689.15</td>
<td>6185.54</td>
<td>475.50</td>
<td>0.000005</td>
<td>5000</td>
<td>504.6</td>
<td>0.26</td>
</tr>
<tr>
<td>T2</td>
<td>708.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>505.0</td>
<td>0.27</td>
</tr>
<tr>
<td>T3</td>
<td>857.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>504.6</td>
<td>0.33</td>
</tr>
</tbody>
</table>

The 6-gingerol content was not analyzed statistically, but the test results were analyzed descriptively and compared with the oleoresin content of red ginger. The 6-gingerol content was 0.23 % without ultrasonication (T0), 0.26 % after 30 min of sonication (T1), 0.27 % after 60 min of sonication (T2), and 0.33 % after 90 min of sonication (T3).

The highest 6-gingerol content was at 90 min of sonication time (T3). Ultrasonication affects the bioactive content of red ginger; the longer the sonication time, the more bioactive content, especially gingerol, will increase [42]. The ultrasonication process causes damage to the cell and subcellular walls of the red ginger oleoresin nanoemulsion, thereby freeing the bioactive components contained therein [40].
The gingerol content is positively correlated with the total phenol content; the higher the total phenol content, the more gingerol content there is.

Information about the content of 6-gingerol in the form of nanoemulsions is not yet available. The 6-gingerol content in the red ginger oleoresin nanoemulsion is lower when compared to the 6-gingerol oleoresin content of 2.01% [43]. This is because in the process of making nanoemulsion, there is an added water phase, so the 6-gingerol content decreases. In addition, the 6-gingerol content is also affected by the heating process during drying, solvent evaporation, and ultrasonication so that the gingerol decomposes into shogaol [44].

**Droplet microstructure of the best bioactive compound**

The red ginger oleoresin nanoemulsion that had the best bioactive compound (T3) was analyzed for droplet microstructure using SEM with a magnification of 5000 - 20,000 times. The microstructure of the red ginger oleoresin nanoemulsion droplet is shown in Figure 4.

![Figure 4](image)

**Figure 4** Microstructure of red ginger oleoresin nanoemulsion droplet magnification: (a) 5000 times, (b) 10,000 times, (c) 15,000 times and (d) 20,000 times.

At a magnification of 5000 times, the surface structure of the nanoemulsion is shaped like a stretch of sand, meaning that the oil, water, surfactant, and co-surfactant phases are united and no sedimentation occurs. At a magnification of 10,000, the structure of the particle shape begins to be seen, and the grains become clearer at magnifications of 15,000 and 20,000 times. The use of ultrasonication results in the breakdown of the particles into smaller ones, even though the particle size is still not uniform (Figure 5). The ultrasonication process will affect the microstructure and surface of the nanoparticles to produce a more stable and easily dispersible nanoemulsion [45].

At 20,000 times (Figure 5), the size of the red ginger oleoresin nanoemulsion particles can be determined. Particle sizes are taken from the smallest size to the largest size. The first spot obtained a particle size of 205.1 nm, the second spot was 146.5 nm, the third spot was 263.6 nm, and the fourth spot was 380.8 nm. The particle size is not uniform because the difference in size is too large. But the particle size is included in the nanoemulsion size range because it is between 50 - 1000 nm in size [4].

The sonication time affects the size of the particles. The longer the sonication time, the smaller the particle size [45]. This is caused by the longer duration of the ultrasonic radiation power, which can separate the agglomerated particles and break them into smaller sizes [46]. The size of the particles is affected by temperature and sonication time [47]. The higher the temperature used in the ultrasonication process, the smaller the particle size will be. According to Koshani and Jafari [32], the optimum temperature for producing the smallest particle sizes (less than 120 nm) is 50 °C. If the temperature is above 50 °C, it will cause the particles to agglomerate so that the diameter is large. Meanwhile, the optimal time to produce
nanoparticle sizes less than 50 nm using the ultrasonic batch method is 3 h [48]. In addition to temperature and time factors, surfactant concentration also affects particle size. The lower the concentration, the less the surfactant is able to play a role in reducing the interfacial energy between the active compound and the liquid phase, so that the particle size is not optimal [49].

**Figure 5** Particle size of the best bioactive compound in 90 min of sonication.

The microstructure of the red ginger oleoresin nanoemulsion droplet is that of a solid colloid in the form of small dots with no visible holes or cracks on the surface of the particles. Ultrasonic waves cause precipitation to form spherical particles. Ultrasonication can break particles into smaller spheres (on the nanoscale), but it does not damage their atomic structure. The results of the nanoemulsion morphology are in accordance with the standards, namely spherical particles [50].

**Conclusions**

Ultrasonication has an effect on the red ginger oleoresin nanoemulsion bioactive compounds. The longer the sonication time, the greater the antioxidant activity, total phenolic content, and gingerol content. The best treatment is 90 min of sonication time. The antioxidant activity (IC\textsubscript{50}) was 11.45±3.48 ppm, the total phenolic content was 3342.74±283.00 mg GAE/g, and the gingerol content was 0.33 %. The best treatment for the nanoemulsion droplet microstructure on 90 min sonication time complies with the standard, namely the particles are in the form of small dots, like balls, with no visible holes or cracks on the surface of the particles.

**Acknowledgements**

The authors would like to thank Lampung State Polytechnic, Indonesia for providing facilities and equipment for this research.

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