



## Physico-Chemical Properties and Antioxidant Stability of Liquid and Powdered Red Ginger Aquaresin: Modification of Plating Method with Silicon Dioxide and $\gamma$ -Cyclodextrin

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### Abstract

Red ginger extract (RGE) boasts high antioxidant activity due to its bioactive compounds but suffers from poor water solubility and dispersibility. This study aimed to improve these properties by converting RGE into liquid and powdered aquaresins. Diacetyl tartaric acid ester of mono- and diglycerides (DATEM) were used as emulsifiers due to their balanced hydrophile-lipophile balance (HLB). Aquaresins were prepared using plating methods due to their practicability and vacuum methods for the powdered form. The optimal RGE:DATEM ratio and characteristics of the aquaresins were investigated. A 45% RGE and 5% DATEM formulation yielded the highest levels of phenolic compounds, flavonoids, and 6-gingerol, while maintaining potent antioxidant activity over 60 days. The plating method significantly enhanced bioactive compound concentration compared to the vacuum method. Adding silicon dioxide and  $\gamma$ -cyclodextrin improved physical properties and antioxidant stability. The 45:5 RGE:DATEM ratio offered superior chemical properties and antioxidant activity in liquid aquaresin, while the plating method contributed to better chemical characteristics and antioxidant activity in powdered form. This study paves the way for incorporating RGE into various food and pharmaceutical applications.



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DATEM;  
Plating;  
Red Ginger Extract.

### Introduction


Red ginger (*Zingiber officinale* var. *rubrum*), a rhizome of an Indonesian spice plant, is commonly used as a cooking spice and traditional herbal medicine. Its extensive use in Indonesian traditional medicine can be attributed to its perceived effectiveness in treating various ailments like the flu, and indigestion, and

providing a warming effect on the body.<sup>1,2</sup> Numerous studies have highlighted the physiological and pharmacological effects of red ginger consumption, primarily linked to its notable antioxidant activity.<sup>2-5</sup> The antioxidant activity is closely associated with the presence of bioactive compounds like gingerol and shogaol.<sup>6,7</sup>

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Among red ginger's derivative products, red ginger extract (RGE) stands out due to its high concentration of bioactive compounds and potent antioxidant activity. Typically prepared through extraction techniques like maceration, RGE forms a thick oil paste with a dark brown resinous appearance.<sup>7</sup> However, its poor solubility and dispersibility in water limit its application in the water-based food industry. Therefore, developing a modification to enhance its solubility and dispersibility is crucial for expanding its potential industrial use.

Red ginger liquid aquaresin is a water-dispersible emulsion composed of RGE, emulsifier, and diluent. The emulsifier facilitates the dispersion of RGE by binding its non-polar tail to the extract and interacting with water molecules through its polar surface.<sup>8</sup> Diacetyl Tartaric Acid Ester of Mono- and Diglycerides (DATEM), an emulsifier with a hydrophilic-lipophilic balance (HLB) value of<sup>8-12</sup> is often employed in the formulation of bread. DATEM possesses a negative charge, which prevents particle aggregation by electrostatic repulsion, further highlighting its suitability for liquid red ginger production.<sup>9</sup> Red ginger liquid aquaresin can also be converted into a powder form for improved water solubility.

Several methods exist for converting red ginger liquid aquaresin into powder, one of which is the plating method. For materials amenable to the plating method, its ease and speed offer an attractive option for industrial powdering. Plating involves mixing the liquid with a crystalline carrier material, such as granulated sugar, to achieve powder formation. However, the stability of powdered liquid obtained through this method is compromised during storage.<sup>10</sup> To address this issue, partial substitution of granulated sugar with compounds like silicon dioxide and  $\gamma$ -cyclodextrin has been proposed to enhance stability. These alternatives hold promise for improved stability and preservation of red ginger aquaresin's valuable properties.

Despite the increasing interest in red ginger aquaresin, the comprehensive characterization of its physical and chemical properties, as well as the stability of its antioxidant activity over storage, remains limited in the existing scientific literature. Hence, this study aims to investigate the properties of liquid red ginger aquaresin using

different formulations, including its physical and chemical composition, as well as the stability of its antioxidant activity. Additionally, this study aims to compare the stability of antioxidant activity between the plating method and modern powdering methods like vacuum drying, while exploring the influence of various carriers and carrier substitutes.

Overall, this research aims to contribute to a better understanding of red ginger aquaresin and its potential applications in the food industry, particularly by elucidating its stability and properties in both liquid and powdered forms.

## Materials and Methods

### Materials

Dried red ginger (*Zingiber officinale* var. *Rubrum*) rhizomes were obtained from local collectors in Bandung, Indonesia. Liquid DATEM was obtained from BASF group, Germany. Vegetable glycerine pharmaceutical grade and propylene glycol DOW USP grade were obtained from Buana Chemical, Bandung, Indonesia.  $\gamma$ -cyclodextrin was obtained from Xi'an Bpanda Biological Technology Co., Ltd. Silicon dioxide was obtained from Solvay Silica Korea Co., Ltd. Ethanol 96% was obtained from PT. Sumber Kita Indah. Gallic acid standard, methanol 99.9%, potassium acetate, and aluminum chloride were obtained from Merck, USA. Folin-Ciocalteu reagent, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay kit, sodium carbonate, quercetin standard, and 6-gingerol standard were obtained from Sigma-Aldrich, USA.

### Extraction of Red Ginger

Dried red ginger rhizomes were dried first in a cabinet dryer at 50°C for 5 hours to homogenize the moisture content. Dried red ginger rhizomes were then grounded using GETRA Spice Herb Grinder IC-10B and sieved using a 60 mesh sieve. Then, powdered red ginger rhizomes were macerated using ethanol 96% in a 3L glass jar with a ratio of 1:10 for 24 hours without agitation, and then filtered using a vacuum filter. The solvent was then evaporated using BÜCHI rotary vacuum evaporator R-300 at 40°C. Each extract was stored in an amber bottle and placed in the refrigerator below 4°C.

### Production of Liquid Red Ginger Aquaresin

The formulation of RGE and DATEM compared to the diluent (propylene glycol and vegetable glycerine)

was kept fixed in each treatment at 50:25:25. Five different ratios of RGE and DATEM were used for the production of liquid aquaresin; 45:5 (D5), 40:10 (D10), 35:15 (D15), 30:20 (D20), and 25:25 (D25) respectively. The emulsification process was carried out using a homogenizer at 12000 rpm. Liquid aquaresin was then stored using an amber bottle and placed in the freezer at below -20°C to be analyzed for its physical and chemical characteristics, while some were stored in a desiccator without silica gels for 60 days to be analyzed for their antioxidant activity every 30 days.

### Production of Powdered Red Ginger Aquaresin

One of the five treatments of liquid red ginger aquaresin with the best quality was chosen as the basic ingredient for making powdered red ginger aquaresin (D5). 10% of the liquid red ginger aquaresin is grounded for two minutes with 90% carrier to ensure even distribution, called plating method. From this plating method, Four treatments have been investigated, each with a different carrier formulation; 90% granulated sugar (P1), 89% granulated sugar + 1% silicon dioxide (P2), 80% granulated sugar + 10%  $\gamma$ -cyclodextrin (P3), 70% granulated sugar + 20%  $\gamma$ - cyclodextrin (P4).

Vacuum drying was used to produce one treatment of powdered red ginger aquaresin using 10% liquid red ginger aquaresin and 90% granulated sugar. Granulated sugar was first dissolved in distilled water in a 1:1 ratio using a hotplate stirrer at 60°C for 30 minutes, then cooled down until it reached room temperature. Then liquid red ginger aquaresin was added and stirred for 30 minutes. The solution is then poured into a silica cup and placed in a vacuum oven at 50°C for  $\pm$ 5 hours. Dried aquaresin then grounded for 2 minutes. Every powdered red ginger aquaresin was then placed in a clear zipper-lock plastic and covered with aluminum zipper-lock, then placed in the freezer at below -20°C for immediate analysis. Some powdered red ginger aquaresin was placed in a clear zipper lock and stored in a desiccator without silica gels for 60 days to be analyzed for its antioxidant activity every 30 days.

### Total Phenolic Content (TPC) Analysis

Total phenolic content was analyzed using Siddhuraju & Becker<sup>11</sup> method. 1 mL of diluted sample each was mixed with 0.5 mL of 1:1 Folin-Ciocalteu reagent with distilled water and 2,5 mL of Na<sub>2</sub>CO<sub>3</sub> 20%. The

solution was then adjusted with distilled water until it reached 25 mL final mixture. The final mixture was then incubated in the dark for 40 minutes and then measured at 725 nm using a spectrophotometer UV-9200 from Beijing Rayleigh Analytical Instrument Co., Ltd., China. The data was then presented in gallic acid equivalent.

### Total Flavonoid Content Analysis

Total flavonoid content was examined by using a modified method from Chang *et al.*<sup>12</sup> 1 mL of diluted sample each was mixed with 3 mL methanol, 0.2 mL of AlCl<sub>3</sub> 20%, 0.2 mL of CH<sub>3</sub>COOK 1 M, and adjusted using distilled water until it reached 10 mL of the final mixture. The final mixture was then incubated in a dark room for 30 minutes, then measured using a spectrophotometer UV-9200 at 415 nm. The data was then expressed in quercetin equivalent.

### 6-Gingerol Content Analysis

6-gingerol content was determined using HPLC method based on Simon-Brown *et al.*<sup>13</sup> 200 mg samples each were mixed with 10 mL methanol in a volumetric flask, ultrasonic for 10 minutes, then adjusted with methanol until it reached 50 mL. The diluted sample was then filtered using a 0.2  $\mu$ m filter membrane. The resulting supernatant then utilized for analysis in the HPLC system consisted of HPLC Waters Alliance e2696 PDA/UV Detector with reverse phase C18 inertsil ODS-3.5  $\mu$ m x 150 mm at 280 nm wavelength and quantified based on the retention time of the 6-gingerol standard.

### Color Analysis

Color analysis was performed using Konica Minolta Head CR-400 based on Iijima & Joh 14 method. Each sample was put in a clear glass petri dish and measured spectrometrically. The data was then recorded in CIE-L\*, a\*, and b\* values, with added values of hue angle (°) and chroma using formula;

$$\text{Hue angle } (^{\circ}) = \tan^{-1} b^*/a^*$$

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$$

### Water Solubility Index Analysis

Water solubility index (WSI) was used to quantify the percentage of liquid & powdered aquaresin that can be dissolved in water. 0.5 g of sample were added to 50 mL of aquades each and stirred using a hotplate stirrer at 40°C for 5 minutes. The mixture was then

filtered with Whatman filter paper No. 1. 10 mL of each supernatant was then placed inside constant petri-dish and then dried in the oven at 105°C until constant. WSI was then determined using the formula from Jafari *et al.*<sup>15</sup>, which is;

$$\text{WSI (\%)} = \frac{\text{Dried supernatant weight (g)}}{\text{Initial sample weight (g)}} \times 100$$

### Angle of Repose Analysis

Angle of repose was used to determine the flowability of a powdered sample, measured based on Zhao *et al.*<sup>16</sup> methods. 10 grams of sample each were passed through a fixed funnel vertically above graph paper and allowed to fall completely until a cone was formed. Then the diameter (y) and the height (x) of the cone were measured to calculate the angle of repose ( $\theta$ ) using formula;

$$\theta = \tan^{-1} y/x$$

### Higroscopy Analysis

Powdered aquaresin hygroscopicity was measured using supersaturated NaCl with  $a_w$  0.762. 0.5 g sample of each powdered aquaresin was put inside the desiccator above the supersaturated NaCl and its weight was measured every 15 minutes in the first 60 minutes of storage. Then the weight was measured every 30 minutes until it reached 300 minutes of storage. The data were shown in the changes of moisture content (%db) per time of storage.

### Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy (SEM) was applied to assess the morphology of powdered aquaresin. Each sample was put above the specimen stub and put inside the specimen stage to be analyzed using JEOL JSM-6360 Scanning Electron Microscope, Japan. The analysis was done using 100x magnification.

### ABTS Assay Antioxidant Activity Analysis

2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay kit was used to determine the antioxidant activity of liquid and powdered aquaresin during 60 days of storage using the method from Mareček *et al.*<sup>17</sup> with modification. 0.2 M of acetate buffer was prepared using sodium acetate trihydrate, and adjusted to pH 4.5 using acetic acid glacial. Acetate buffer was then used to dilute 33.18 mg  $K_2S_2O_8$  in a 50 mL volumetric flask to make 2.45 mM  $K_2S_2O_8$  reagent, and 38.4 mg ABTS powder in

a 10 mL volumetric flask to make ABTS stock. Both  $K_2S_2O_8$  reagent and ABTS stock were mixed with a ratio of 1:1 to make ABTS+ reagent and were then incubated for 12-16 hours in a dark place. After the incubation period, ABTS+ reagent was then diluted with acetate buffer until its absorbance was within the optimal value of  $0.700 \pm 0.02$  at 734 nm.

For sample analysis, each sample was divided into 5 dilution solutions (ex: 100, 50, 25, 12.5, 6.25 ppm) and was prepared using a mixture of sample, diluent (ex: methanol), and diluted ABTS+ reagent. Each solution absorbance was then measured using spectrophotometer UV-9200 at 734 nm. The % inhibition can be calculated using the formula;

$$\% \text{ inhibition} = \frac{A_{\text{(control negative)}} - A_{\text{sample}}}{A_{\text{(control negative)}}} \times 100$$

The  $IC_{50}$  value then can be determined using the sample linear equation obtained from % inhibition and absorbance value using the formula;

$$C_{50} = (50 - \text{intercept}) / \text{slope}$$

### Data Analysis

The experimental analysis, conducted in triplicate, yielded mean values for phenolic content, flavonoid content, 6-gingerol content,  $IC_{50}$ , color, WSI, angle of repose, and hygroscoy. These values were calculated from 3 replicates, with standard deviations expressed in error bars.

## Results and Discussion

### Chemical Characteristics of Liquid Red Ginger Aquaresin

Analysis of 6-gingerol, total phenolic content, and total flavonoid content in liquid red ginger aquaresin revealed that higher concentrations of RGE and lower amounts of DATEM resulted in greater levels of bioactive compounds. RGE, being a derivative product rich in bioactive compounds, influenced the content of these compounds in the aquaresin.<sup>7,18</sup> Among the formulations, formulation D5 exhibited the highest content of 6-gingerol ( $4.66 \pm 0.41\%$ ), total phenolic content ( $17.79 \pm 0.49\%$  gallic acid equivalent), and total flavonoid content ( $3.66 \pm 0.02\%$  quercetin equivalent). Notably, approximately 30% of the total phenolic content in each treatment was attributed to 6-gingerol. However, the content of

these valuable compounds can be affected by processing factors like heat treatment.

The concentration of 6-gingerol in the liquid red ginger aquaresin is influenced by the treatment applied to the red ginger rhizomes during the extraction process. It is important to note that heat treatment during handling could potentially lead to a decrease in the percentage of 6-gingerol retained in the aquaresin. This is because gingerols are thermolabile compounds, sensitive to temperature.<sup>19</sup> Upon heating, the β-hydroxy keto group in gingerol can lose the -OH group and transform into shogaol.<sup>21</sup> While shogaol offers higher antioxidant activity, its formation can affect the desired flavour profile of the liquid red ginger aquaresin.<sup>22</sup>

**Physical Characteristics of Liquid Red Ginger Aquaresin**

The analysis of color intensity in the five liquid red ginger aquaresin treatments did not show a consistent trend based on the L\* and a\* parameters. However, there was an increase in the b\* value with a decrease in the amount of RGE used, and the three treatments with the lowest RGE content exhibited higher hue angle values. Overall, the liquid red ginger aquaresin samples had a dark color with a yellowish-red hue.

The color of the liquid red ginger aquaresin is likely influenced by the presence of pigments in the RGE. Previous research by Iijima & Joh<sup>14</sup> identified curcumin, demethoxycurcumin, and 6-dehydrogingerdione as the pigments responsible for the color of RGE.

The higher usage of DATEM in the liquid red ginger aquaresin likely contributed to the increase in the b\* value and hue angle. DATEM itself has a yellowish-white color in its liquid form. While no specific studies have been conducted on the effect of DATEM on the color of aquaresin, it has been demonstrated that the use of DATEM in bread-making can alter the color of the final product.<sup>23</sup>

In addition to color, the analysis also revealed the solubility of liquid red ginger aquaresin. Analysis revealed that the liquid red ginger aquaresin samples exhibited poor water solubility index (WSI). This is attributed to the inherent nature of aquaresin, which is dispersed rather than dissolved in water. Interestingly, the lower the content of RGE in the aquaresin, the higher the WSI level observed. This can be explained by the low polarity of the constituents of RGE, such as gingerol and shogaol, which hinder their dissolution in water.<sup>24</sup>

**Table 1: Total Phenolic Content, Total Flavonoid Content, 6-Gingerol Content, Color Analysis, and Water Solubility Index of Liquid Red Ginger Aquaresin Sample**

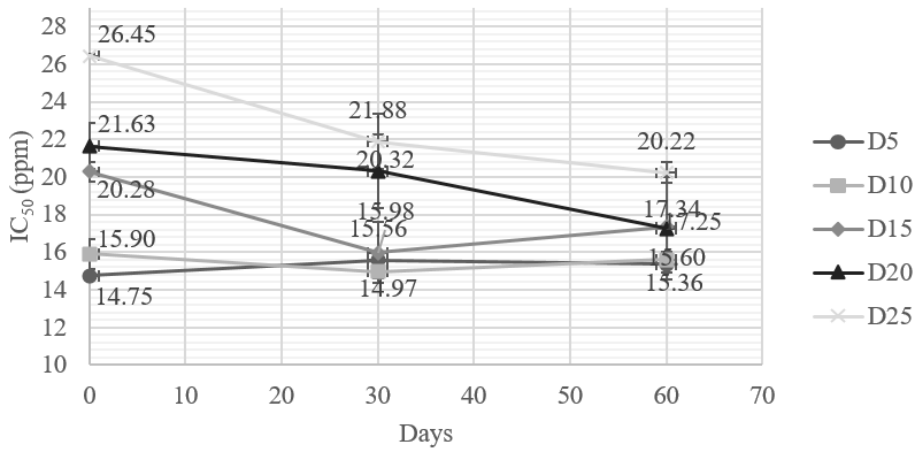
Sam-ple	6-Gingerol Content (%)	Phenolic Content (% Gallic Acid Equivalent)	Flavonoid Content (% Quercetin Equivalent)	L*	a*	b*	Chroma	Hue Angle (°)	Solubility (%)
D5	4.66±0.41	17.79±0.49	3.66±0.02	5.21±0.04	4.06±0.26	3.66±0.00	5.46±0.19	42.10±1.84	2.10±0.00
D10	4.03±0.04	14.92±0.18	1.98±0.03	6.89±0.02	4.88±0.01	4.30±0.08	6.50±0.05	41.38±0.56	2.60±0.28
D15	3.61±0.01	10.54±1.05	1.65±0.02	5.32±0.08	3.79±0.20	4.58±0.13	5.95±0.03	50.40±2.25	3.15±0.07
D20	2.92±0.03	8.66±0.20	1.29±0.03	5.28±0.04	4.05±0.14	4.61±0.07	6.14±0.04	48.70±1.43	3.20±0.42
D25	2.48±0.04	6.91±0.03	1.17±0.07	5.56±0.03	4.56±0.21	5.19±0.01	6.90±0.14	48.71±1.24	3.78±0.28

\*Values are means ± SD of duplicate determination. D5 = 5% DATEM & 45% RGE; D10 = 10% DATEM & 40% RGE; D15 = 15% DATEM & 35% RGE; D20 = 20% DATEM & 30% RGE; D25 = 25% DATEM & 25% RGE.

**Liquid Red Ginger Aquaresin Antioxidant Activity Stability**

The ABTS assay is a method of testing antioxidant activity based on the reaction of antioxidants with organic cation radicals known as ABTS<sup>+</sup>. This

method was chosen to assess the stability of the antioxidant activity of liquid red ginger aquaresin as several studies found that the ABTS assay was suitable when reacted with ginger bioactive compounds.<sup>25-27</sup>



**Fig. 1: Antioxidant Activity of Liquid Red Ginger Aqaresin over 60 Days of Storage. D5 = 5% DATEM & 45% RGE; D10 = 10% DATEM & 40% RGE; D15 = 15% DATEM & 35% RGE; D20 = 20% DATEM & 30% RGE; D25 = 25% DATEM & 25% RGE**

The results of the analysis of the antioxidant activity stability for 60 days in the five liquid red ginger aquaresin samples can be interpreted as follows: the lower the RGE content in the liquid aquaresin formulation, the sharper the increase in antioxidant activity of liquid red ginger aquaresin. It was also noted that the two treatments with the highest levels of RGE (D5 & D10) had the strongest and most stable antioxidant activity during the storage period of 60 days.

The observed increase in antioxidant activity of red ginger aquaresin is likely attributed to structural changes in 6-gingerol during storage. Over time, 6-gingerol can convert into its dehydrated form, known as 6-shogaol. Since 6-shogaol possesses greater antioxidant potential than 6-gingerol, its presence may explain the increased antioxidant activity during storage.<sup>28</sup> As the ratio of RGE decreases, the dispersion between particles of 6-gingerol becomes more delicate, making it more susceptible to degradation during storage, which could contribute to the reduced stability of antioxidant activity.

The analysis indicates that liquid red ginger aquaresin treatment D5 exhibited superior antioxidant activity, stability, and chemical properties compared to the other samples. Furthermore, there were no significant differences in physical properties observed between liquid red ginger aquaresin D5 and the other samples. Thus, treatment D5 will be

used as the primary ingredient in the production of powdered red ginger aquaresin in the subsequent stage of these experiments, considering these three evaluation criteria.

### Chemical Characteristics of Powdered Red Ginger Aqaresin

The analysis of powdered red ginger aquaresin revealed that the sample produced using the plating method exhibited a higher total phenolic content compared to the sample obtained through vacuum drying. The analysis also revealed that there was no significant difference in total phenolic content between the four samples made using plating method, with values ranging between  $2.13 \pm 0.14$  and  $2.39 \pm 0.36$  (% gallic acid equivalent).

The lower total phenolic content in the vacuum-dried red ginger aquaresin powder can be attributed to the fact that not all of the liquid aquaresin used during the drying process was effectively bound to the granulated sugar carrier. As a result, the concentration of bioactive compounds in the powdered form is affected. Meanwhile, the concentration of bioactive compounds in red ginger aquaresin in the plating method is higher due to the plating principle, which simply disperses the liquid on the carrier's surface.<sup>10</sup>

The analysis also indicated that the total flavonoid content in all five treatments was very low, measuring less than 0.05%. It is believed that the heat

generated during the powdering process has an adverse effect on the flavonoid content. When grinding is performed in the plating method, the friction between particles and the blades generates heat, leading to an increase in material temperature. Zainol *et al.*<sup>29</sup> demonstrated that drying *Centella asiatica* samples in a vacuum oven can cause a reduction in flavonoid content by 63-87%.

**Physical Characteristics of Powdered Red Ginger Aqauesin**

The analysis revealed that the vacuum drying treatment (V) of red ginger aqauesin powder resulted in a brighter and paler color compared to the plating method. This was evidenced by higher L\* value and lower a\*, b\*, and chroma values, which can be attributed to the lower concentration of bioactive compounds in vacuum-dried red ginger aqauesin powder.

To further enhance the color of the final product, silicon dioxide and  $\gamma$ -cyclodextrin were used as carrier substitutes in the plating method. Among the samples, P2 (1% silicon dioxide substitute) exhibited the brightest color with a mean L\* value of 73.86±0.45. Previous studies have indicated that the addition of silicon dioxide to the powdering process can improve the L\* value and reduce the material's yellowness (b\* value).<sup>30</sup> This could be due to the nature of silicon dioxide nanoparticles, which are light, white in color, and easily adsorbed on the surface of larger particles.<sup>31</sup> Moreover, the hydrophobic inner wall of  $\gamma$ -cyclodextrin contributes to color brightening by capturing some of the red ginger bioactive compounds within its structure, unlike the granulated sugar carrier that merely disperses the bioactive compounds on the surface.<sup>32</sup>

**Table 2: Total Phenolic Content, Total Flavonoid Content, Color Analysis, Water Solubility Index, and Flowability of Powdered Red Ginger Sample**

Sam-ple	Phenolic Content (% Gallic Acid Equivalent)	Flavonoid Content (% Quercetin Equivalent)	L*	a*	b*	Chroma	Hue Angle (°)	Solubility (%)	Angle of Repose (°)
P1	2.39±0.36	0.019±0.002	66.90±0.04	8.00±0.27	40.40±0.70	41.18±0.74	78.80±0.18	79.94±0.21	47.96±1.39
P2	2.38±0.26	0.0355±0.005	73.86±0.45	5.13±0.02	38.40±0.43	38.74±0.42	82.39±0.12	77.40±0.35	33.89±1.39
P3	2.13±0.14	0.024±0.004	68.94±0.22	7.57±0.09	39.22±0.22	39.94±0.20	79.08±0.19	77.71±0.63	45.04±3.06
P4	2.23±0.15	0.029±0.002	70.04±0.20	7.33±0.16	39.02±0.41	39.70±0.44	79.37±0.12	78.56±0.00	35.51±0.73
V	1.45±0.18	0.021±0.002	76.36±0.07	3.92±0.11	29.12±0.35	29.39±0.36	82.33±0.13	80.76±0.42	58.62±0.88

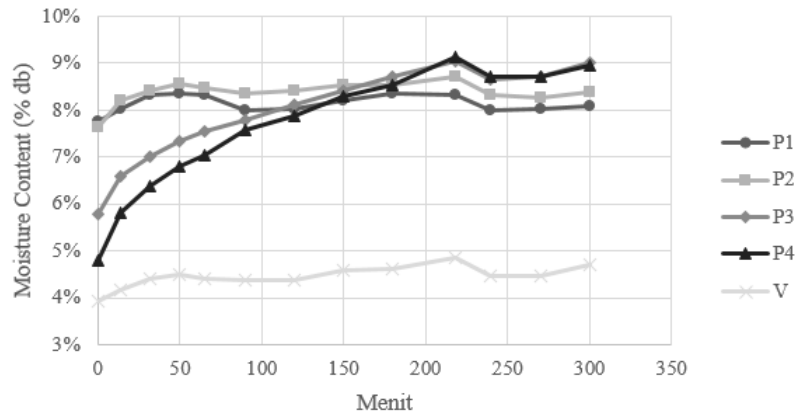
\*Values are means ± SD of duplicate determination. P1 = plated 90% granulated sugar; P2 = plated 89% granulated sugar + 1% silicon dioxide; P3 = plated 80% granulated sugar + 10%  $\gamma$ -cyclodextrin; P4 = plated 70% granulated sugar + 20%  $\gamma$ -cyclodextrin; V = vacuum-dried 90% granulated sugar

The WSI index analysis demonstrated the significant impact of using granulated sugar as a carrier on the solubility of red ginger aqauesin powder. Additionally, the red ginger aqauesin powder produced by the vacuum drying method exhibited slightly better solubility compared to the plating method.

The addition of silicon dioxide and  $\gamma$ -cyclodextrin reduced the solubility of powdered red ginger aqauesin in water. This can be attributed to the role of silicon dioxide as a particle barrier, impeding the absorption of water particles into the material.<sup>33</sup> The

decrease in solubility associated with  $\gamma$ -cyclodextrin substitution can be attributed to its lower solubility in water compared to granulated sugar, which was measured as 23.2% (w/v at 25°C).

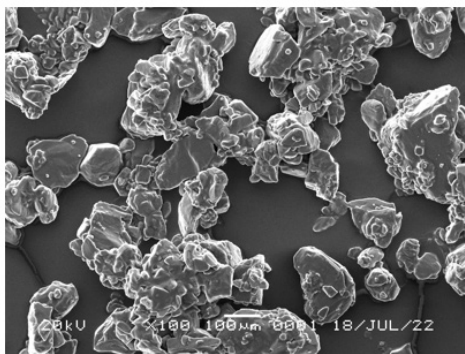
The data also demonstrates the notable impact of silicon dioxide and  $\gamma$ -cyclodextrin on the flowability of red ginger aqauesin powder. The addition of silicon dioxide reduces cohesiveness, thereby improving the flowability of the powder.<sup>34</sup> Similarly, several studies in the pharmaceutical industry have reported the flowability-enhancing properties of cyclodextrins for various powdered drugs.<sup>35,36</sup>



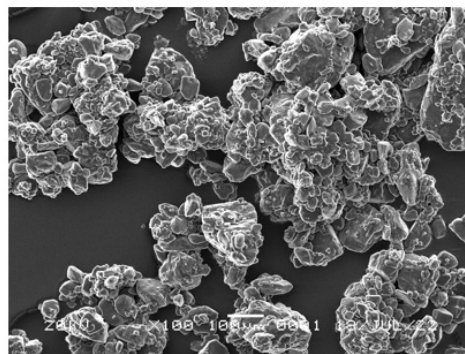
**Fig. 2: Change of Moisture Content (%db) of Each Powdered Red Ginger Aquaresin over 300 minutes of Storage. P1 = plated 90% granulated sugar; P2 = plated 89% granulated sugar + 1% silicon dioxide; P3 = plated 80% granulated sugar + 10%  $\gamma$ -cyclodextrin; P4 = plated 70% granulated sugar + 20%  $\gamma$ -cyclodextrin; V = vacuum-dried 90% granulated sugar**

In terms of hygroscopicity, the carrier material used during the production of red ginger aquaresin powder exerts a more substantial influence than the powdering method. The hygroscopicity rate analysis further reveals a direct relationship between the hygroscopicity rate and the amount of

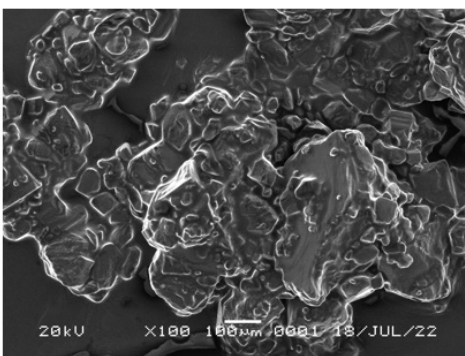
$\gamma$ -cyclodextrin utilized in the production of powdered red ginger aquaresin. This can be attributed to the hydrophilic outer wall structure of  $\gamma$ -cyclodextrin, which enables it to capture water particles present in the environment.<sup>37</sup>



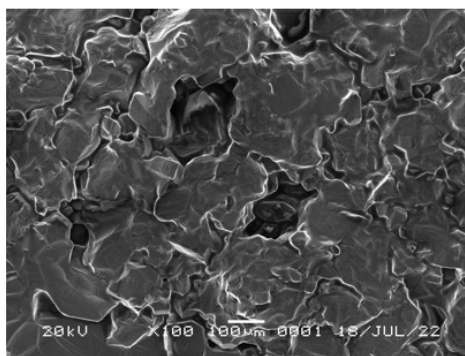
(a)



(b)

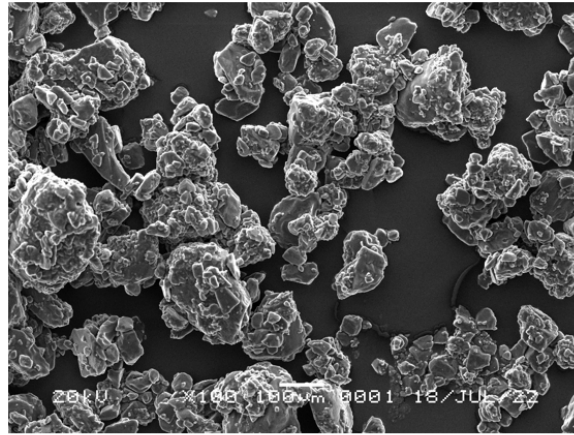


(c)



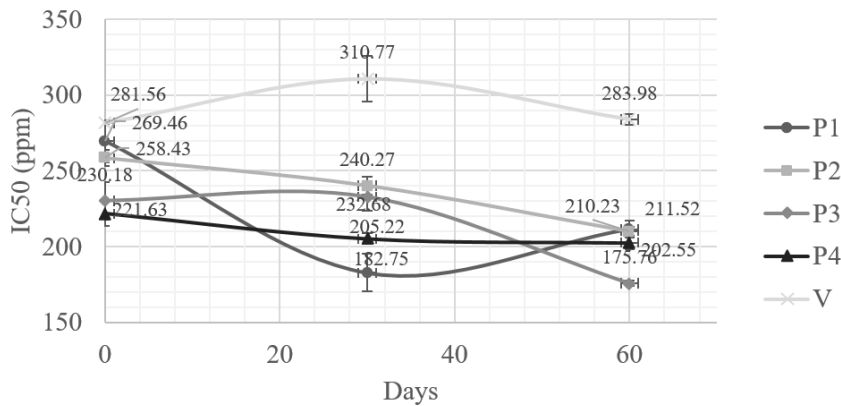
(d)





(e)

**Fig. 3:** Scanning Electron Micrographs (x100) of the Powdered Red Ginger Aquaresin: (a) P1; (b) P2; (c) P3; (d) P4; (e) V. P1 = plated 90% granulated sugar; P2 = plated 89% granulated sugar + 1% silicon dioxide; P3 = plated 80% granulated sugar + 10%  $\gamma$ -cyclodextrin; P4 = plated 70% granulated sugar + 20%  $\gamma$ -cyclodextrin; V = vacuum-dried 90% granulated sugar



**Fig. 4:** Antioxidant Activity of Each Powdered Red Ginger Aquaresin over 60 Days of Storage. P1 = plated 90% granulated sugar; P2 = plated 89% granulated sugar + 1% silicon dioxide; P3 = plated 80% granulated sugar + 10%  $\gamma$ -cyclodextrin; P4 = plated 70% granulated sugar + 20%  $\gamma$ -cyclodextrin; V = vacuum-dried 90% granulated sugar

Scanning Electron Microscopy (SEM) analysis provides insights into the distribution of liquid aquaresin within the powdered red ginger aquaresin samples. Specifically, in samples that solely employed granulated sugar as a carrier, the liquid aquaresin was found to be dispersed on the surface of the granulated sugar. Conversely, samples incorporating silicon dioxide as a carrier substitute exhibited increased adherence of smaller particles to the sugar walls, indicating the role of silicon dioxide as an outer wall component within the powder.

Furthermore, SEM analysis revealed the formation of a complex between  $\gamma$ -cyclodextrin substitution and liquid aquaresin, with a greater amount of  $\gamma$ -cyclodextrin leading to the formation of a more substantial complex.

**Liquid Red Ginger Aquaresin Antioxidant Activity Stability**

The antioxidant stability analysis conducted revealed that all five red ginger aquaresin treatments exhibited

low antioxidant activity, as indicated by an  $IC_{50}$  value of less than 150 ppm.<sup>38</sup> Notably, the vacuum drying method yielded red ginger aquaresin powder with the lowest antioxidant activity among the four samples tested. Sample P1 exhibited a significant increase in antioxidant activity on day 30, while sample P3 demonstrated a similar trend on day 60. Sample P2 also experienced an increase, albeit less pronounced than in sample P3, whereas sample P4 displayed the highest antioxidant activity stability compared to the other samples.

In addressing the limitations of the plating method using conventional carriers like granulated sugar, the incorporation of  $\gamma$ -cyclodextrin appears to offer potential improvements. This can be attributed to the propensity of  $\gamma$ -cyclodextrins to form complexes with bioactive compounds. It is believed that the formation of these complexes effectively preserves the chemical structure of bioactive compounds, thereby minimizing potential damage caused by external factors.<sup>25</sup>

### Conclusion

The utilization of a RGE and DATEM ratio of 45:5 in the production of liquid red ginger aquaresin has demonstrated superior chemical properties and antioxidant activity. Notably, this specific ratio has exhibited the highest stability among all the liquid aquaresin formulations tested. Moreover, when employing the plating method, red ginger aquaresin powder has displayed improved chemical characteristics and antioxidant activity compared to the vacuum drying method. The incorporation of silicon dioxide and  $\gamma$ -cyclodextrin as carrier substitutes has yielded powdered red ginger aquaresin with more favorable physical properties. Particularly,  $\gamma$ -cyclodextrin has proven to be effective in producing powdered red ginger aquaresin with the highest antioxidant activity and satisfactory stability over a 60-day storage period.

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### Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Authors Contribution

Bambang Nurhadi: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); supervision (equal); writing – review and editing (equal). Bayu Rezaharsamto: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). Edy Subroto: Conceptualization (equal); data curation (equal); supervision (equal); writing – review and editing (equal). Siti Nurhasanah: Conceptualization (equal); data curation (equal); supervision (equal); writing – review and editing (equal). Rudy Adi Saputra: Data curation (equal); formal analysis (equal); methodology (equal); supervision (equal).

### Data Availability

All data is provided in full in the tables and figures section of this paper.

### Ethics Approval Statement

This study did not involve any experiments on humans and/or animals.

### References

1. Mulia K, Risqi UY, Pane IF, Krisanti EA. Formulation, Characterization, and Release Property of Antioxidant Supplement Capsule with Red Ginger Oleoresin Extract-loaded Chitosan Microparticles. *J Phys Conf Ser.* 2019;1198(6):062008. doi:10.1088/1742-6596/1198/6/062008
2. Zhang S, Kou X, Zhao H, Mak K-K, Balijepalli MK, Pichika MR. *Zingiber officinale* var. *rubrum*: *Red Ginger's Medicinal Uses. Molecules.* 2022;27(3):775. doi:10.3390/molecules27030775

3. Jayanudin, Fahrurrozi M, Wirawan SK, Rochmadi. Antioxidant activity and controlled release analysis of red ginger oleoresin (*Zingiber officinale* var. rubrum) encapsulated in chitosan cross-linked by glutaraldehyde saturated toluene. *Sustain Chem Pharm.* 2019;12:100132. doi:10.1016/j.scp.2019.100132
4. Syafitri DM, Levita J, Mutakin M, Diantini A. A Review: Is Ginger (*Zingiber officinale* var. Roscoe) Potential for Future Phytomedicine? *Indones J Appl Sci.* 2018;8(1):8-13. doi:10.24198/ijas.v8i1.16466
5. Azizah N, Nihayati E, Khotimah H, *et al.* Impact of potassium fertilization on yield, nutrient use and response efficiency, and antioxidant content of red ginger (*Zingiber officinale* var. rubrum Theilade). *Chil J Agric Res.* 2022;82(3):380-389. doi:10.4067/S0718-58392022000300380
6. Levita J, Syafitri D, Supu R, *et al.* Pharmacokinetics of 10-gingerol and 6-shogaol in the plasma of healthy subjects treated with red ginger (*Zingiber officinale* var. Rubrum) suspension. *Biomed Reports.* 2018;9(6):474-482. doi:10.3892/br.2018.1163
7. Nurhadi B, Suriati, Tensiska, Saputra RA, Sukri N. The role of encapsulant materials on the stability of bioactive compounds of red ginger (*Zingiber officinale* Roscoe. var. Rubrum) extract powder during storage. *Food Chem.* 2020;333:127490. doi:10.1016/j.foodchem.2020.127490
8. McClements DJ, Jafari SM. Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Adv Colloid Interface Sci.* 2018;251:55-79. doi:10.1016/j.cis.2017.12.001
9. Miller R. Emulsifiers: Types and Uses. In: Caballero B, Finglas PM, Toldrá FBT-E of F and H, eds. *Encyclopedia of Food and Health.* Oxford: Elsevier; 2016:498-502. doi:10.1016/B978-0-12-384947-2.00249-X
10. Baines D, Knights J. Applications I: Flavors. In: *Chemistry and Technology of Flavors and Fragrances.* Oxford, UK: Wiley; 2004:274-304. doi:10.1002/9781444305517.ch12
11. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem.* 2007;101(1):10-19. doi:10.1016/j.foodchem.2006.01.004
12. Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2020;10(3):178-182. doi:10.38212/2224-6614.2748
13. Simon-Brown K, Solval KM, Chotiko A, *et al.* Microencapsulation of ginger (*Zingiber officinale*) extract by spray drying technology. *LWT.* 2016;70:119-125. doi:10.1016/j.lwt.2016.02.030
14. Iijima Y, Joh A. Pigment Composition Responsible for the Pale Yellow Color of Ginger (*Zingiber officinale*) Rhizomes. *Food Sci Technol Res.* 2014;20(5):971-978. doi:10.3136/fstr.20.971
15. Jafari SM, Ghalegi Ghalenoei M, Dehnad D. Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technol.* 2017;311:59-65. doi:10.1016/j.powtec.2017.01.070
16. Zhao X, Yang Z, Gai G, Yang Y. Effect of superfine grinding on properties of ginger powder. *J Food Eng.* 2009;91(2):217-222. doi:10.1016/j.jfoodeng.2008.08.024
17. Mareček V, Mikyška A, Hampel D, *et al.* ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *J Cereal Sci.* 2017;73:40-45. doi:10.1016/j.jcs.2016.11.004
18. Ghafoor K, Al Juhaimi F, Özcan MM, Uslu N, Babiker EE, Mohamed Ahmed IA. Total phenolics, total carotenoids, individual phenolics and antioxidant activity of ginger (*Zingiber officinale*) rhizome as affected by drying methods. *LWT.* 2020;126:109354. doi:10.1016/j.lwt.2020.109354
19. Ko M-J, Nam H-H, Chung M-S. Conversion of 6-gingerol to 6-shogaol in ginger (*Zingiber officinale*) pulp and peel during subcritical water extraction. *Food Chem.* 2019;270:149-155. doi:10.1016/j.foodchem.2018.07.078
20. Dalsasso RR, Valencia GA, Monteiro AR. Impact of drying and extractions processes on the recovery of gingerols and shogaols, the main bioactive compounds of ginger. *Food Res Int.* 2022;154:111043. doi:10.1016/j.foodres.2022.111043
21. Jacob JN. Comparative Studies in Relation to the Structure and Biochemical Properties

- of the Active Compounds in the Volatile and Nonvolatile Fractions of Turmeric (*C. longa*) and Ginger (*Z. officinale*). In: Atta-ur-Rahman BT-S in NPC, ed. Vol 48. Elsevier; 2016:101-135. doi:10.1016/B978-0-444-63602-7.00004-7
22. Teng H, Seuseu KT, Lee W-Y, Chen L. Comparing the effects of microwave radiation on 6-gingerol and 6-shogaol from ginger rhizomes (*Zingiber officinale* Rosc.). *Restani P*, ed. PLoS One. 2019;14(6):e0214893. doi:10.1371/journal.pone.0214893
  23. Pourfarzad A, Haddad Khodaparast MH, Karimi M, Mortazavi SA. Optimization of a novel improver gel formulation for Barbari flat bread using response surface methodology. *J Food Sci Technol*. 2014;51(10):2344-2356. doi:10.1007/s13197-012-0778-9
  24. Md Sarip MS, Morad NA, Mohamad Ali NA, Mohd Yusof YA, Che Yunus MA. The kinetics of extraction of the medicinal ginger bioactive compounds using hot compressed water. *Sep Purif Technol*. 2014;124:141-147. doi:10.1016/j.seppur.2014.01.008
  25. Pais JM, Pereira B, Paz FAA, Cardoso SM, Braga SS. Solid  $\gamma$ -Cyclodextrin Inclusion Compound with Gingerols, a Multi-Component Guest: Preparation, Properties and Application in Yogurt. *Biomolecules*. 2020;10(2):344. doi:10.3390/biom10020344
  26. AliAMA, El-NourMEM, Yagi SM. Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors. *J Genet Eng Biotechnol*. 2018;16(2):677-682. doi:10.1016/j.jgeb.2018.03.003
  27. Roli OI, Adetunji CO, Mishra RR, Adetunji JB, Mishra P, Fatoki TH. Rediscovering Medicinal Activity and Food Significance of Shogaol (4, 6, 8, 10, and 12): Comprehensive Review. In: Mishra P, Mishra RR, Adetunji CO, eds. *Innovations in Food Technology*. Singapore: Springer Singapore; 2020:125-145. doi:10.1007/978-981-15-6121-4\_9
  28. Kou X, Li X, Rahman MRT, *et al.* Efficient dehydration of 6-gingerol to 6-shogaol catalyzed by an acidic ionic liquid under ultrasound irradiation. *Food Chem*. 2017;215:193-199. doi:10.1016/j.foodchem.2016.07.106
  29. Zainol K, Abdul Hamid A, Bakar F, Dek M. Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*. 2009;16:531-537.
  30. Pui LP, Karim R, Yusof YA, Wong CW, Ghazali HM. Anti-caking Agent Effects on the Properties of Spray-dried 'Cempedak' Fruit Powder. *Pertanika J Trop Agric Sci*. 2020;43(4). doi:10.47836/pjtas.43.4.15
  31. Elemike E, Uzoh I, Onwudiwe D, Babalola O. The Role of Nanotechnology in the Fortification of Plant Nutrients and Improvement of Crop Production. *Appl Sci*. 2019;9(3):499. doi:10.3390/app9030499
  32. Mangelings D, Eeltink S, Vander Heyden Y. Recent developments in liquid and supercritical fluid chromatographic enantioseparations. In: Valkó KI, BT-H of AS, ed. *Separation Methods in Drug Synthesis and Purification*. Vol 8. Elsevier Science B.V.; 2020:453-521. doi:10.1016/B978-0-444-64070-3.00009-6
  33. Madany MMY, Saleh AM, Habeeb TH, Hozzein WN, AbdElgawad H. Silicon dioxide nanoparticles alleviate the threats of broomrape infection in tomato by inducing cell wall fortification and modulating ROS homeostasis. *Environ Sci Nano*. 2020;7(5):1415-1430. doi:10.1039/C9EN01255A
  34. Blanco D, Antikainen O, Rääkkönen H, Yliruusi J, Juppo AM. Effect of colloidal silicon dioxide and moisture on powder flow properties: Predicting in-process performance using image-based analysis. *Int J Pharm*. 2021;597:120344. doi:10.1016/j.ijpharm.2021.120344
  35. Hosny KM, Alahdal AM, Hosny KM. Utilization of Solid Dispersion Technique to Improve Solubility and Flowability of Acyclovir. *Life Sci J*. 2013;10(4):40-44.
  36. Zhou Y, Zhao Y, Niu B, *et al.* Cyclodextrin-based metal-organic frameworks for pulmonary delivery of curcumin with improved solubility and fine aerodynamic performance. *Int J Pharm*. 2020;588:119777. doi:10.1016/j.ijpharm.2020.119777
  37. Del Valle EMM. Cyclodextrins and their uses: a review. *Process Biochem*. 2004;39(9):1033-1046. doi:10.1016/S0032-9592(03)00258-9
  38. Sukandar D, Nurbayti S, Rudiana T, Husna TW. Isolation and Structure Determination of

Antioxidants Active Compounds from Ethyl  
Acetate Extract of Heartwood Namnam  
(*Cynometra cauliflora* L.). *J Kim Terap*

*Indones.* 2017;19(1):11-17. doi:10.14203/jkti.  
v19i1.325