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# Development of standardized green coffee bean extract (*Coffea canephora*) into effervescent granules as an antioxidant supplement

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## ABSTRACT

This study aims to obtain the optimum formula for green coffee extract effervescent granules (EG-GCE). Dry extract is obtained by percolation using water as a solvent and a spray dryer drying system. Specific and non-specific standardization is carried out to ensure the quality of dry green coffee extract. The dose of the dried extract of green coffee used in the granule effervescent was 250 mg each sachet. EG-GCE was formulated using wet granulation method. The quality of effervescent granules was determined based on physical quality tests and the effectiveness of antioxidant power ($IC_{50}$ value) with DPPH reagent using a microplate reader. The optimum effervescent granule formula uses a factorial design method combining monohydrate citric acid and tartaric acid. As a response includes water content, flow rate, and effervescent granule dissolution time test. Furthermore, the data from the parametric experiments between bets and between formulas were analyzed using the One Way ANOVA (Yate's Treatment) statistical method. The test will continue using the Tukey post-hoc test method if there is a significant difference in the statistical analysis between formulas. The pH value of resulting EG-GCE products was within the range of 5.46-6.07, moisture content: 3.12-3.67%, flow rate: 25.78-28.53 g/s, angle of repose: 25.65-30.13°, Hausner ratio: 1.14-1.22, Carr's index: 12.50–17.83%, dissolving effervescent granule time test: 1.00-1.33 min. This study demonstrated that citric acid monohydrate, tartaric acid, and their interaction affected the moisture content, flow rate, and effervescent time of EG-GCE. The proportion of citric acid monohydrate (9.94%) and tartaric acid (17.46%) was found to be the optimum formula of EG-GCE, with the following responses: moisture content 3.26%, flow rate 25.72 g/s, and dissolving effervescent granule time test 1.19 min. The optimum formula show strong antioxidant activity with $IC_{50}$ free of radical scavenging 56.56 ± 0.97 µg/mL.

**Key words:** antioxidant**,** effervescent granules, green coffee, factorial design, citric acid monohydrate, tartaric acid

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**INTRODUCTION**

Oxidative stress is a condition with a decrease in endogenous antioxidant capacity and an increase in free radicals (reactive oxygen species - ROS) (Di Domenico et al., 2019). Free radicals are unstable reactive molecular species formed from natural metabolic processes that act as reducing agents or oxidants (Flieger et al., 2021). Free radicals have an impact on the pathogenesis of many diseases due to prolonged oxidative stress conditions triggered by increased free radicals causing cell damage (Flieger et al., 2021). Therefore, exogenous antioxidant supplementation is needed, such as bioactive polyphenolic compounds which can ward off free radicals to prevent oxidative stress conditions. One of the polyphenolic compounds that has antioxidant properties is chlorogenic acid (Boccellino & D'Angelo, 2020; Galanakis et al., 2020; Lammi & Arnoldi, 2021).

Pimple et al (2020) stated that chlorogenic acid is often found in robusta coffee beans at a concentration of around 7-14%. Green coffee has higher antioxidant activity because it does not undergo a roasting process, which risks degrading chlorogenic acid polyphenolic compounds (Asbaghi et al., 2020). The two types of coffee that dominate the global market include arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*) (Cui et al., 2020; Faria et al, 2020).

Green coffee contains polyphenol compounds (Ohishi et al., 2021) which show stability at temperatures of 160-200 °C, so during the drying process with an oven at a temperature of 45°C it does not affect the stability of polyphenol compounds (Abrahão et al., 2019 ; Piñón-Balderrama et al., 2020).

Robusta coffee contains the highest levels of chlorogenic acid compared to other coffee species (Pereira et al., 2021). Based on previous research, the chemical compounds found in green coffee include chlorogenic acid (6.7-9.2%), and caffeine (0.9-1.3%) (Pimpley et al., 2020). Green coffee was chosen because it has a higher polyphenol content than roasted coffee (Asbaghi et al., 2020).

Considering that green coffee robusta (*Coffea canephora*) contains high levels of chlorogenic acid, this research carried out the development of a health supplement formula containing dry extract of green coffee robusta (*Coffea canephora*) at a selected dose of 250 mg per sachet in the form of effervescent granules (Gorji et al., 2019 ; Sudeep & Shyam Prasad, 2021). Dry extract of green coffee may reduce the degradation of secondary metabolites caused by microorganisms in extract containing high water content (Lima et al., 2020) reported that the green coffee extract (GCE) formulated as effervescent granules may increase the release, absorption, and bioavailability of the bioactive compounds.

The development of green coffee into quality effervescent granules is largely determined by the composition of the acid source and base source. The combination of acid sources commonly used is citric acid monohydrate and tartaric acid (Bertuzi, 2021). Citric acid monohydrate can increase the stability and extend the storage period of effervescent granule preparations (Bertuzi, 2021). Meanwhile, sodium bicarbonate can cause a stronger effervescent reaction (Bertuzi, 2021). In effervescent formulations, the amount of tartaric acid used in the formula must be higher than citric acid, to achieve the correct stoichiometric equivalence, where tartaric acid is a diprotic acid, while citric acid is a triprotic acid (Bertuzi, 2021). The ideal combination of acid sources with a composition of citric acid monohydrate, tartaric acid, and sodium bicarbonate in a ratio of 1:2:3.4. An incorrect ratio will cause citric acid to form a sticky powder mixture making it difficult to granulate, while tartaric acid tends to produce brittle granules. In this study, the manufacture of effervescent granules used the wet granulation method because this method can provide better uniformity of active ingredients and improve the flow properties and porosity of the granules (Bertuzzi, 2021).

Based on the above, it is necessary to optimize the combination of acid sources to produce an optimal effervescent granule dosage formula using the factorial design method. The first factor is citric acid monohydrate and the second factor is tartaric acid with the selected responses including flow rate, water content, and dissolving time. The response of water content and dissolving granule effervescent time was chosen because both affect the granule flow rate, where the granule flow rate gets worse when the water content is high (Maysarah et al, 2020). The concentration of citric acid monohydrate used for formula optimization with a lower limit level (-1) of 8% an upper limit level (+1) of 12% and a concentration of tartaric acid for the lower limit level (-1) of 16% and an upper limit level (+1) of 24%. Determining the specification range in the optimization process refers to general requirements in the

literature and compendiums and is also based on test results on the innovator's product. This study aimed to obtain the optimum formula for effervescent granules containing green coffee extract.

## MATERIALS AND METHODS

In this study green coffee bean (*Coffea canephora*) dry extract was obtained from PT. Haldin Pacific Semesta (Indonesia). The dry extract was prepared by spray drying method without fillers and preservatives. Other materials used in this study were as follows: citric acid monohydrate (Weifang Ensign Industry Co., Ltd., China), tartaric acid (Badische Anilin- und SodaFabrik, Germany), sodium bicarbonate (Chruch and Dwilight Co., Inc., USA), PVP K-30 (Badische Anilin- und SodaFabrik, Germany), maltodextrin (Qin Huang Dao LiHua Starch Co., Ltd., China), aspartame (Shandong Tianjiao Biotech Co., Ltd., China), ethanol, and distilled water.

### Standardization of dry extracts

The main ingredient used in this research is a dry water extract of green coffee (*Coffea canephora*) obtained from PT. Haldin Pacific Semesta, Bekasi. The quality of the dry extract obtained needs to be guaranteed before use, using specific (i.e visual form, color, smell, flavor, pH of 1% w/v solution, water soluble extract level, and ethanol soluble extract level) and non-specific standardization (i.e total ash content, acid insoluble ash content, water content).

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### Factorial experimental design

The number of effervescent granules of green coffee extract (EG-GCE) formula was determined according to a complete $2^2$ factorial design. The factors studied in this study were citric acid monohydrate (8-12%) as $X_A$ and tartaric acid (16-24%) as $X_B$. Both factors studied were independent of each other. The following responses were evaluated: flow rate ($Y_1$), moisture content ($Y_2$), and effervescent time ($Y_3$).

### Formulation of EG-GCE

In this study, effervescent granules was formulated using wet granulation method. The proportion of citric acid monohydrate and tartaric acid as an acid source was 1:2, whereas sodium bicarbonate was used as a base source, as used by previous investigator. The optimum proportion of citric acid monohydrate and tartaric acid as an acid source was analysed by factorial design using Design Expert 12.0.3. The resulting EG-GCE was evaluated, including organoleptic, pH value after reconstitution, flow properties, moisture content, and dissolving effervescent time. According to the factorial experimental design, EG-GCE was formulated in four different formula (Table 1). Citric acid monohydrate, tartaric acid, and sodium bicarbonate were mixed and heated at 60 $^oC$ for approximately 3 hours. The purpose of heating is to remove hydrates, which reduces the risk of hygroscopicity and makes formulation easier. The mixture was subsequently crushed and mixed with other excipients. The mixture of excipients and GCE was mixed and binded with ethanolic solution of PVP K-30. The mixture was then sieved through mesh number 18 sieve. The wet granules obtained was then driedat 45 $^oC$ for approximately 3 hours or until the moisture content measured was lower than 5%.Finally, the EG-GCE was sieved with a mesh number 20 sieve.

**Table 1. Formula of EG-GCE**

| Ingredients | Weight (g) | | | |
|---|---|---|---|---|
| | Formula-1(F-1) | Formula a(Fa) | Formula b(Fb) | Formula ab(Fab) |
| Green coffee extract | 0.25 | 0.25 | 0.25 | 0.25 |
| **Citric acid monohydrate** | **0.32** | **0.48** | **0.32** | **0.48** |
| **Tartaric acid** | **0.64** | **0.64** | **0.96** | **0.96** |
| Sodium bicabonate | 1.24 | 1.24 | 1.24 | 1.24 |
| Aspartame | 0.12 | 0.12 | 0.12 | 0.12 |
| PVP K-30 | 0.08 | 0.08 | 0.08 | 0.08 |
| Maltodextrin | ad 4 | ad 4 | ad 4 | ad 4 |
| Water for reconstitution | 250 mL | 250 mL | 250 mL | 250 mL |

**Evaluation of the quality of EG-GCE before reconstitution**

***Organoleptic***

The organoleptic test was carried out visually, namely by observing the physical form of the green coffee dry extract effervescent granule (EG-GCE) preparation before it was dissolved, including observing the color, shape, and odor of the EG-GCE preparation. The organoleptic requirements for EG-GCE are that they are granular, yellowish-white, and have a distinctive coffee aroma.

***Flow rate test***

The purpose of the flow rate test is to determine the flow of granules directly (Maysarah et al., 2020). Flow speed tests were carried out using the funnel method referring to (Taylor & Aultons, 2022) with modifications. A total of 100 grams of effervescent granules were weighed and placed in a funnel with the end closed after that the funnel cover was opened, and the effervescent granules were allowed to flow until they ran out. The granule flow time is calculated using a stopwatch fromthe time the funnel cover is opened and stopped when the granules have finished flowing. The flow rate test was carried out three times in replication using equation 1. Effervescent granules have good flow properties if the granule flow rate is more than 10 grams/second (Tanjung et al, 2023). The flow rate specification for EG-GCE based on requirements is 27.50 ± 2.00 grams/second (U.S Pharmacopeia, 2020). The following Equation 1 is used to calculate the flow rate.

$$\text{Flow rate} = \text{Granule weight} / \text{Flow time} \quad \text{.....(1)}$$

**Angle of repose**

The purpose of the angle of repose test is to determine the flow of granules indirectly (Maysarah et al., 2020). The angle of repose test was carried out using the funnel method referring to (Taylor & Aulton, 2021) with modifications. A total of 100 grams of effervescent granules were weighed and placed in a funnel with the end closed after the funnel cover opened, and the effervescent granules were allowed to flow until they ran out, after which the angle formed was measured. The angle of repose test was carried out three times in replication using equation 2. Effervescent granules have good flow properties when forming an angle of repose between 25-40º (Taylor & Aulton, 2021; U.S Pharmacopeia, 2020). The Equation 2 used to calculate the angle of repose:

$$\tan \alpha = \text{cone height} / \text{radius of the base of the cone} \quad \text{.....(2)}$$

**Hausner ratio**

The Hausner ratio test aims to determine the flow properties of granules indirectly which are influenced by friction between particles (Taylor & Aulton, 2021). The Hausner ratio test is determined based on the compressible density and bulk density parameters. The compressible density and bulk density of the effervescent granules are determined first. A measuring cup with a volume of 100 mL is weighed (W1), then the effervescent granules are slowly added to the 100 mL mark (V1) and weighed again (W2). Next, the mouth of the measuring cup is plugged, the measuring cup is installed on the test tool, and the motor is run for the first 5 minutes, and the volume is measured (V5), and then the motor is run again until the twelve minute and the volume is measured (V12). Next, calculate the bulk density first and then the compressible density, and then calculate the Hausner ratio using Equation 3. The Hausner ratio specification for EG-GCE is 1.00-1.25 (U.S Pharmacopeia, 2020). The following Equation 3 is used to calculate the Hausner ratio.

Hausner ratio = *Tapped Density* (ρt) / *Bulk Density* (ρb) ……....(3)

**Carr's Index**

The purpose of the Carr's index test is to determine the flow properties of granules indirectly, and the compressibility of granule preparations based on the compressible density parameters and bulk density of granule preparations referring to (Taylor and Aultons, 2022) with modifications. The compressible density and bulk density of the effervescent granules are determined first. A measuring cup with a volume of 100 mL is weighed (W1), then the effervescent granules are slowlyadded to the 100 mL mark (V1) and weighed again (W2). Next, the mouth of the measuring cup is plugged, the measuring cup is installed on the test tool, and the motor is run for the first 5 minutes, and the volume is measured (V5), and then the motor is run again until the twelve minute and the volume is measured (V12). The change in volume that occurs (compressible volume) is recorded as (V2). After obtaining the results, they first calculate the bulk density and compressed density and then calculate Carr's index using equation 4. The Carr's index test was carried out three times in replication. The Carr's index specification for EG-GCE based on requirements is 1-20% (U.S Pharmacopeia, 2020). The Equation 4 is used to calculate the Carr's index:

$$\text{Carr's index} = \frac{\rho t - \rho b}{\rho t} \times 100 \quad \text{.....(4)}$$

**Water content test**

The purpose of the water content test is to determine the water content in the preparation. The water content test was carried out using a Moisture Analyzer tool referring to (Lima et al., 2020) with modifications. Approximately 3 grams of EG-GCE were put into the sample container, then the tool was run until the tool gave water content test results. The water content test was carried out three times in replication. The water content requirement for effervescent granules is ≤ 5% (Giyatmi & Lingga, 2019). The water content specification for EG-GCE based on requirements is 3.40 ± 0.30%.

**Evaluation of the quality of green coffee dry extract effervescent granules (EF-GCE) after reconstitution**

**Organoleptic**

The organoleptic test was carried out visually, namely by observing the physical form of the green coffee dry extract effervescent granule (EG-GCE) preparation after being dissolved, including observing the color, shape, and taste of the EG-GCE preparation after being dissolved, as well as observing the effervescent reaction through a microscope which was marked by the appearance of carbon dioxide gas bubbles. The organoleptic requirements for the EG-GCE solution are that it is a yellowish-white solution, has a sweet and sour taste, and forms carbon dioxide gas.

**pH Value**

The pH test used a pH meter refers to the research of (Jain & Patil, 2020) with modifications. The purpose of the pH test is to determine the acidity level of a preparation. Before taking pH measurements, the electrode is rinsed first with distilled water and dried. After that, calibration was done using a buffer solution of pH 4.0, 7.0, and 9.0. The calibrated electrode must be dried first so that can be used to measure the pH of the EG-GCE solution. The pH test was carried out by dissolving one sachet (4 grams) the EG-GCE in 250 mL of water at approximately 25 ℃ and continuing with pH testing. The pH test was carried out three times in replication. The pH specification for the EG-GCE solution based on the requirements is 6.00 ± 1.00.

**Dissolving effervescent granule test**

The dissolving effervescent granule time test aims to determine the time required for the preparation to dissolve completely in water. In this study, the late time test refers to the research of (Maysarah et al., 2020) with modifications. The dissolving effervescent granule was carried out by dissolving one sachet (4 grams) of EG-GCE granules in 250 mL of water at a temperature of approximately 25℃, the granules were considered to have dissolved when the release of carbon dioxide gas stopped. The solubility test was carried out three times in replication. The dissoving effervescent granule specification for EG-GCE based on requirements is 1.00 ± 0.50 minutes.

**Antioxidant activity test**

The antioxidant activity test in this research will be carried out on the optimum formula obtained based on the results of data analysis with the Design Expert program. Antioxidant activity test using the DPPH method refers to research by (Tasew et al., 2020) with modifications. The following are the stages of preparing the test solution.

**Preparation of ascorbic test solution**

In this study, ascorbic acid was used as a positive control. The stock solution was prepared by weighing 10 mg of ascorbic acid and dissolving it with 10 mL of mixed solvent (1,000 µg/mL). The ascorbic acid solution was then diluted using a volumetric flask to obtain four different concentrations, namely 75.00 µg/mL, 37.50 µg/mL, 18.75 µg/mL, and 9.38 µg/mL.

**Preparation of green coffee dry extract (GCE) test solution**

The main solution of dry green coffee extract is made by weighing 250 mg of GCE and dissolving it with 100 mL of mixed solvent (2,500 µg/mL). The first stock solution of GCE was then diluted using a volumetric flask to obtain four different concentrations, namely 75.00 µg/mL, 37.50 µg/mL, 18.75 µg/mL, and 9.38 µg/mL.

**Preparation of dry green coffee extract effervescent garnule (EG-GCE) test solution**

The test solution was made by dissolving one sachet of the preparation (equivalent to 250 mg of GCE) with 100 mL of mixed solvent (2,500 µg/mL). The test solution of EG-GCE was diluted using a volumetric flask to obtain four different concentrations, namely 75.00 µg/mL, 37.50 µg/mL, 18.75 µg/mL, and 9.38 µg/mL.

**Mixing Test Solution and DPPH solution**

DPPH stock solution is made by weighing 25 mg of DPPH dissolved in 25 mL of methanol, then vortexing until completely dissolved. After that, the DPPH stock solution was diluted by pipetting 1 mL of DPPH of the first stock solution and adding methanol to 25 mL (Tasew et al., 2020). Each diluted test solution reacted with a DPPH solution in a ratio of 1:4, while the control contained mixed solvent methanol: water (1:1% v/v) and DPPH solution. The mixture of the test solution and DPPH solution was left for thirty minutes in a dark place at room temperature until the reaction was complete. After that, the absorbance was observed using a UV-Vis spectrophotometer at a wavelength of 517 nm. The analysis was carried out three times in replication.

**Statistical analysis**

The experimental results were analyzed by one way ANOVA at a confidence level of 95% using IBM SPSS Statistics 25.0. The optimum formula of EG-GCE was determined by Yate's treatment using Design Expert version 12.0.3.

## RESULT AND DISCUSSION

### Results of standardization of green coffee dry extract (GCE)

The dry extract of green coffee (*Coffea canephora*) organoleptically has a brownish-green color and a distinctive coffee odor. The dry extract of green coffee is acidic with a pH value of 5.11 ± 0.03. The purpose of determining the levels of water-soluble extracts and ethanol-soluble extracts is to determine the compound content in certain solvents according to their level of polarity. The test results show that the percentage of water-soluble extract levels is higher than ethanol-soluble extract levels, which means that green coffee has a higher solubility in water than in ethanol solvent. The full results of the standardization of dried extracts can be seen in Table 2.

The active compound profile of GCE (*Coffea canephora*): chlorogenic acid was observed using thin layer chromatography under UV 254 and VIS 366 nm light. The active substance profile in the extract using the mobile phase Chloroform: Methanol: Formic Acid (44.1: 2.5: 2.15 %v/v) and the stationary phase silica gel 60 $GF_{254}$ showed that green coffee extract contained chlorogenic acid. The stain detected under 366 nm has a value of Rf 0.37 for a fluorescent blue color is chlorogenic acid. The theoretical Rf values for the active substances chlorogenic acid was 0.40 respectively (Bojić et al., 2013). The complete result of the TLC profile can be seen in Figure 1. The Rf value that is different from the theoretical Rf value is likely due to modifications to the analysis process and the type of solvent used. However, we can still say that green coffee bean extract still contains chlorogenic acid, which has anti-acne properties.

**Table 2. Results of standardization of green coffee** (*Coffea canephora*) **dry extract (GCE)**

| Standardization Parametre | Result |
|---|---|
| **Non Specific** | |
| Total Ash Content (%) | 8.89 ± 0.04 |
| Acid Insoluble Ash Content (%) | 0.26 ± 0.04 |
| Water Soluble Ash Content (%) | 8.19 ± 0.11 |
| Water content (%) | 3.45 ± 0.18 |
| **Specific** | |
| Organoleptic | |
| • Form | Powder Brownish |
| • Color | greenTypical |
| • Smell | coffee |
| • Flavor | Bitter |
| pH (1% w/v solution) | 5.11 ± 0.03 |
| Water Soluble Extract Level (%) | 92.03 ± 0.46 |
| Ethanol Soluble Extract Level (%) | 30.96 ± 0.68 |

### Evaluation results of green coffee dry extract effervescent granules (EG-GCE) before reconstitution

### Organoleptic

Organoleptic examination of the EG- GCE preparation was carried out visually as the preparation was yellowish-white in color, granular in shape, and hada distinctive coffee odor. The results of the organoleptic examination of the green coffee dry extract effervescent granule preparation can be viewed at Figure 2.

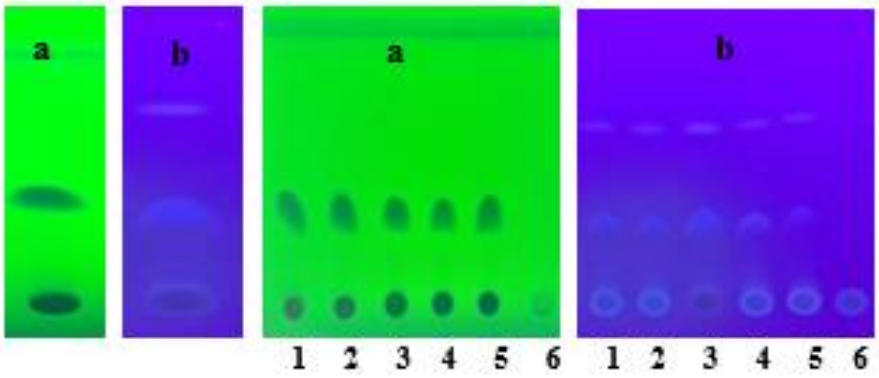


**Figure 1. Thin layer chromatography results of GCE and EF with mobile phase chloroform: methanol: formic acid (44.1: 2.5: 2.15 mL) under 254 nm UV light (a) and 366 nm UV light (b), 1 (formula -1); 2 (formula a); 3 (GCE); 4 (formula b); 5 (formula ab); 6 (formula blank)**

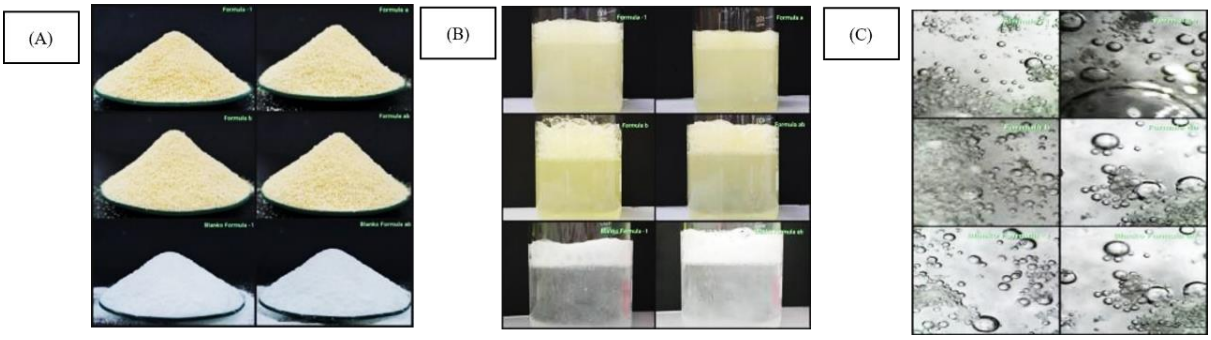


**Figure 2. EG-GCE before reconstitution (A) and after reconstitution (B) with carbon dioxide gas bubbles observed at 1000x magnification (C) using digital microscope TRM-DM1000**

## pH Measurement

The pH measurement was carried out to determine the acidity of EG-GCE after reconstitution. The four formulas gave significantly different pH values ($F_{value} > F_{0.05}$), which may be attributed to the different proportions of acid species. This findings was in agreement with Lowry-Brönsted theory stating that acids may act as a substance donating $H^+$, and hence the addition of acidic solute into water will result in an increased hydronium ion concentration (Aultons & Taylor, 2022). The pH values of all EG-GCE formula met the pHrequirement, i.e. within the range of 5-7 (Giyatmi & Lingga, 2019).

## Flow properties

The test results for all flow property parameters (flow rate, angle of repose, Hausner ratio, Carr index) show significant differences between the formulas (Table 3). Based on statistical analysis, it was found that $F_{value}$ of all parameters of flow properties was greater than the $F_{0.05\ (table)}$. In addition, all formula demonstrated a good flow properties of granules. Granules are considered to have good flow rates if they can flow at >10 g/s, angle of repose <350, Hausner ratio <1.25, and Carr's index <20% (Taylor & Aulton, 2021) According to (Qiu et al., 2017), the flow properties of granules are complex and affected by several factors including humidity, particle shape and size. The resulting water content value meets specifications: 3.40 ± 0.30%.

Based on the results of the quality control of flow properties that has been carried out in terms of the parameters of flow speed and angle of repose, all formulas are declared to have good flow properties when viewed from the Hausner ratio and Carr's index parameters, formula -1, formula a, and formula b stated to be quite good.

**Table 3. Flow properties parameters of EG-GCE**

| Parameter | F-1 | Fa | Fb | Fab | Fvalue | F0.05 |
|---|---|---|---|---|---|---|
| pH | 6.07 ± 0.05 | 5.94 ± 0.04 | 5.85 ± 0.05 | 5.46 ± 0.12 | 82.03 | |
| Flow rate (g/s) | 28.53 ± 1.05 | 26.48 ± 0.93 | 28.35 ± 0.53 | 25.78 ± 0.47 | 18.14 | |
| Angle of repose ($^0$) | 25.65 ± 0.89 | 27.47 ± 0.53 | 30.13 ± 0.56 | 29.56 ± 0.44 | 63.61 | 3.10 |
| Hausner ratio | 1.21 ± 0.01 | 1.22 ± 0.01 | 1.20 ± 0.02 | 1.14 ± 0.01 | 51.54 | |
| Carr's index (%) | 17.58 ± 0.49 | 17.83 ± 0.75 | 16.75 ± 1.25 | 12.50 ± 0.55 | 55.36 | |
| Water Content (%) | 3.16 ± 0.22 | 3.12 ± 0.06 | 3.28 ± 0.09 | 3.67 ± 0.14 | 18.66 | |

**Evaluation results of green coffee dry extract effervescent granules (EG-GCE) afterreconstitution**
**Organoleptic**

Organoleptic examination of the EF-GCE after reconstitution, provided specifications for the preparation being yellowish white in color, in solution form and with a sweet and sour taste, and producing carbon dioxide gas (Figure 2)**.**

***pH Measurement***

The purpose of pH measurements is to determine the acidity level of EG-GCE after reconstitution. The pH test results for each formula were -1 (6.07 ± 0.05), a (5.94 ± 0.04), b (5.85 ± 0.05), ab (5.46) respectively. ± 0.12), blank -1 (6.40 ± 0.03), blank ab (5.04 ± 0.07). The resulting pH value is in accordance with the specifications for the EG-GCE preparation, in the range of 6.00 ± 1.00. The four formulas give significantly different pH values ($F_{value} > F_{0.05}$), which may be attributed to the different proportions of acid species. This findings was in agreement with Lowry-Brönsted theory stating that acids may act as a substance donating $H^+$, and hence the addition of acidic solute into water will result in an increased hydronium ion concentration (Taylor & Aulton, 2021). The pH values of all EG-GCE formula met the pH requirement, i.e. within the range of 5-7 (Giyatmi & Lingga, 2019).

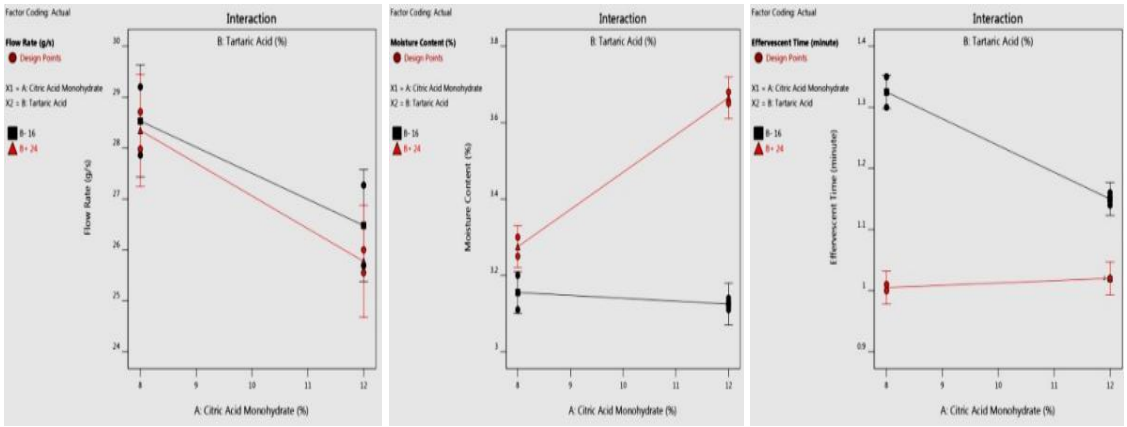


**Figure 3. Interaction of $X_A$ and $X_B$ on $Y_1$, $Y_2$, and $Y_3$**

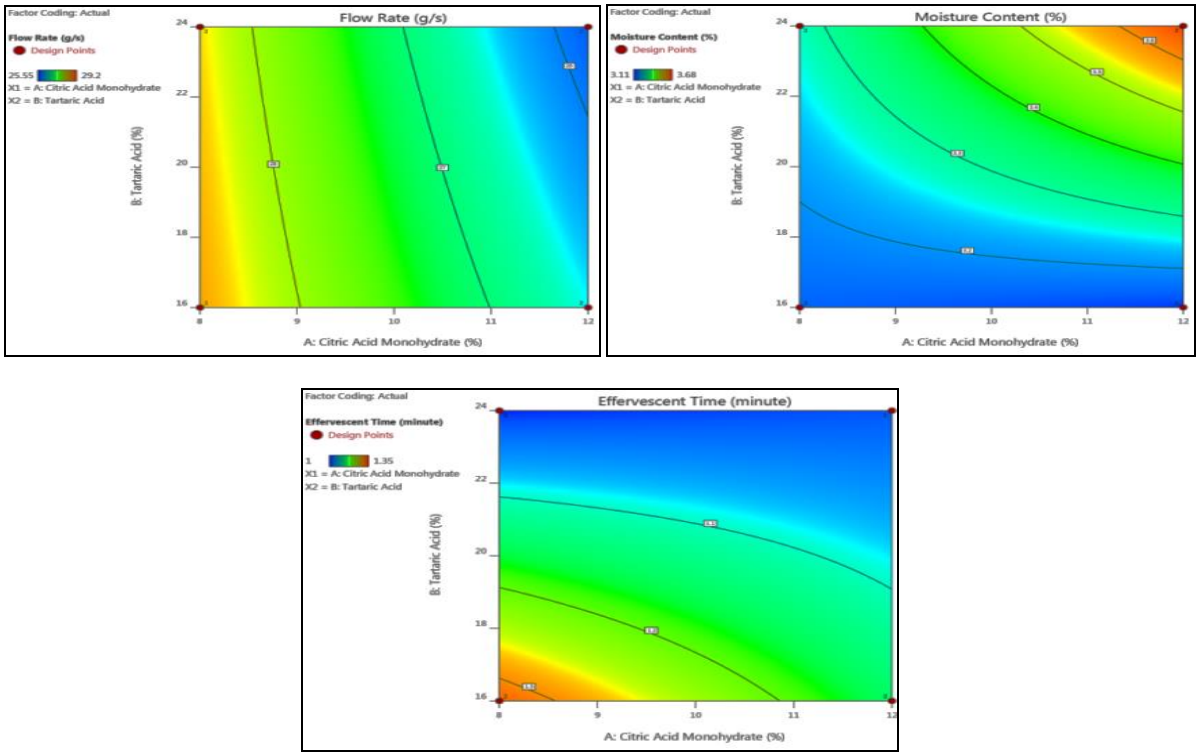


**Figure 4. Contour plot of $Y_1$, $Y_2$, and $Y_3$**

**Formula optimization flow rate response**

The flow rate test aims to ensure that granules may flow adequately through processing equipment, since poor flowability can lead to large weight variability among unit dose (sachet) in production scale (Qiu et al., 2017). In this study, all formula met the flow rate requirements, i.e. within the range of >10 g/s (Table 3). The polynomial equation for the flow rate was found to be $Y_1 = 27.28 - 1.16\ X_A - 0.22\ X_B - 0.13\ X_AX_B$. The equation suggested that citric acid monohydrate had more significant effect on the decreased flow rate than tartaric acid. According to (Rowe et al., 2020) citric acid monohydrate has orthorhombic crystal form, while tartaric acid is monoclinic. The difference in the crystal form of these two acidic substances may lead to a difference or change of flow rate (Qiu et al., 2017). Electrostatics forces, particle size, particle size distribution, and moisture content have also been shown to influence the flow rate (Gad, 2007; Qiu et al, 2017). In this study, it was also found that the interaction of citric acid monohydrate and tartaric acid did not affect the flow rate of the granules. (Figure 3 and 4).

**Moisture content response**

Effervescent granules should comply with the quality requirements related to moisture content, that is below 5% (Giyatmi & Lingga, 2019). The moisture content of all EG-GCE formula prepared in this study was within the range of 3.12-3.67% (Table 3). The polynomial equation for the moisture content was found to be $Y_2 = 3.31 + 0.09\ X_A + 0.17\ X_B + 0.11\ X_AX_B$. This equation suggested that tartaric acid exhibited more significant effect on the increased value of moisture content. This may be due to the hygroscopicity properties of the tartaric acid which is higher than citric acid monohydrate (Bertuzi, 2021). According to (Wilson & Koeberle, 2018; Kalman, 2021), hygroscopicity is the capacity of a chemical substance to adsorb and desorb water. Thus the increased concentration of tartaric acid which is highly hygroscopic excipient in the EG-GCE formula may enhance the water content in granules. This may be attributed to the increased capacity adsorption of water in the atmosphere. In this study, we found that the interaction of citric acid monohydrate and tartaric acid had no influence on the

moisture content of granules. In this study, all formulas had water content that met the requirement because the drying process of the wet granule mass was carried out at a relative humidity (RH) ≤ 25% with a temperature of around $25^0$C, thereby preventing the effervescent granules from absorbing water from the surrounding air.(Figure 4 and 5).

**Dissolving granule time test response**

Dissolving granule time evaluation aims to determine the dissolving time of EG-GCE in water. Effervescent granules should meet the quality requirements regarding to the effervescent time, that is less than 5 minutes (Taylor & Aulton, 2021). In this study, the effervescent time of EG-GCE wasfound to be within the range of 1.00-1.33 min (Table 3). Upon reconstitution of EG-GCE in water, citric acid monohydrate and tartaric acid will react with sodium bicarbonate, which in turn will produce its sodium salt, water, and $CO_{2(g)}$ The conversion of weak acid to its sodium salt leads to an increased ionic dissociation constant which is subsequently followed by the improved solubility of granules (Taylor & Aulton, 2021). The polynomial equation demonstrating the effervescent time of this EG-GCE was $Y_3 = 1.13 - 0.04\ X_A - 0.11\ X_B + 0.05\ X_AX_B$. The equation suggested that tartaric acid had more significant effect on the decreased effervescent time of granules. This may be due to the anhydrous form of tartaric acid as a substance used in this study. According to (Taylor & Aulton, 2021), the anhydrous form of a substance has a faster-dissolved rate than its respective hydrate form. This may explain our findings that tartaric acid had more significant effect on the effervescent time, compared with citric acid monohydrate. In thisstudy, the interaction of citric acid monohydrate and tartaric acid did not affect the effervescent time significantly (Figure 4 and 5).

**Optimum formula**

Based on the contour plot of the responses used in this study (Figure 5), it was found that the proportion of citric acid monohydrate and tartaric acid was responsible for the determination of optimum formula of EG-GCE. The contour plot was then overlaid (superimposed) to obtain the optimum formula (Figure 6). The yellow area is the prediction of the optimum formula of EG- GCE. The software recommended an optimum formula containing a combination of citric acid monohydrate (9.94%) and tartaric acid (17.46%), with a predicted flow rate between 25.62-29.29 g/s, moisture content between 3.11-3.29%, and effervescent time between 1.15-1.24 min. The suggested optimum formula was then prepared and the responses of this formula were verified. The optimum formula of EG-GCE showed a moisture content of 3.26%, flow rate of 25.72 g/s, and effervescent time of 1.19 min. All responses were within the predicted range and met the effervescent granules requirements.

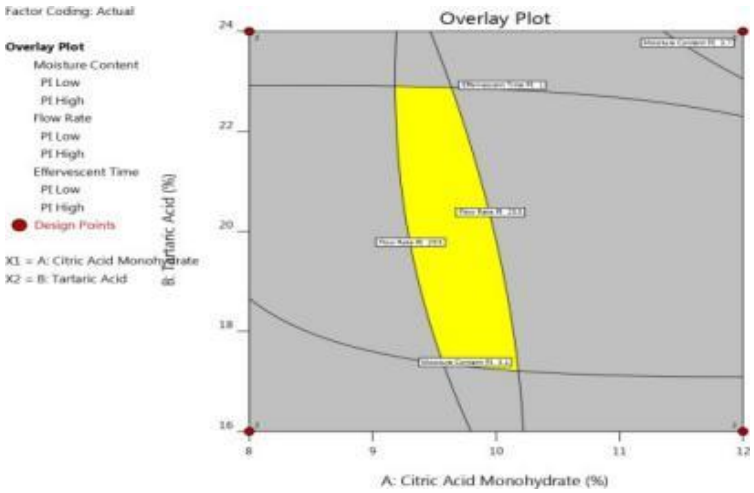


**Figure 5. Superimposed (overlay plot) of EG-GCE**

**DPPH scavening activity of EG-GCE**

Antioxidant activity tests were carried out on GCE and EG to determine the effect of formulation on GCE antioxidant activity. The results of the antioxidant activity test can be seen in Figure 6 with synthetic antioxidant compounds (ascorbic acid) as positive controls. The difference in $IC_{50}$ values for antioxidants occurs due to space obstructions caused by other additives or excipients that cover the hydroxyl, phenol, carbonyl, and aromatic amide groups, inhibiting their antioxidant activity (Faria et al., 2020). The results of this study are by the observations of the $IC_{50}$ of antioxidants using the DPPH method, which was reported by (Faria et al, 2020), where green coffee robusta thick extract has a stronger antioxidant $IC_{50}$ compared to green coffee robusta thick extract, which is encapsulated with maltodextrin: gum arabic (1:1). The Blois antioxidant activity classification system can be divided into four classification, very strong antioxidants ($IC_{50}$ < 50 µg/mL), strong antioxidants (50 µg/mL ≤ $IC_{50}$ ≤ 100 µg/mL), moderate antioxidants (101 µg/mL ≤ $IC_{50}$ ≤ 150 µg/mL), and weak antioxidants ( $IC_{50}$ > 150 µg/mL) (Faria et al., 2020). Based on this classification, ascorbic acid as a positive control has very strong antioxidant activity (37.36±0.29 µg/mL), while GCE and EG-GCE are classified as having strong antioxidant activity ($IC_{50}$ antioxidant 51.40±1.18 µg/mL and 56.56±0.97 µg/mL. Statistical analysis using the one-way ANOVA method for the $IC_{50}$ values of vitamin C, GCE, and EC-GCE gave a significant difference of $F_{count}$ (368.52) > $F_{table}$ (5.14).

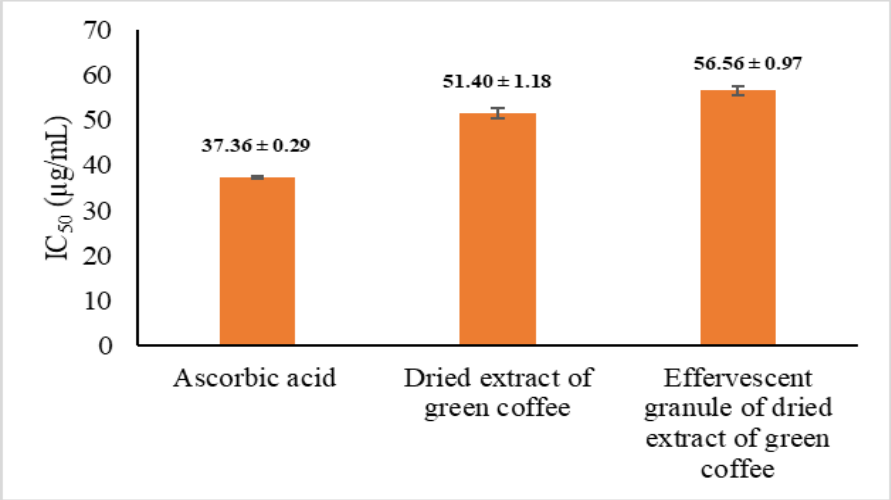


**Figure 6. $IC_{50}$ Antioxidant of ascorbic acid, GCE, and EG-GCE**

## CONCLUSION

EG-GCE has been formulated successfully as effervescent granules by wet granulation method and evaluated. The optimum formula, containing a combination of citric acid monohydrate (9.94%) and tartaric acid (17.46%), was obtained by factorial design method and has been verified, with the following parameters: moisture content 3.26%, flow rate 25.72 g/s, and effervescent time 1.19 min. The optimum formula show strong antioxidant activity with $IC_{50}$ free of radical scavenging 56.56 ± 0.97 µg/mL.

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