Formulation and physical stability analysis of red beetroots (Beta vulgaris l.) effervescent granules

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ABSTRACT

Introduction: Red beetroots (*Beta vulgaris L.)* are a natural ingredient rich in nutrients and provides various notable health benefits such as mediating cardiology effect, antioxidant, anti-inflammation, anti-tumor and antibacterial. In dentistry, red beetroots have the potential as a plaque-disclosing agent. Several studies showed significant drawbacks of red beetroots preparation in gels or chewable forms. Therefore, this study aimed to analyze the ideal formula and evaluate the physical stability of effervescent granules made from red beetroots extract (*Beta vulgaris L.)* in different doses. **Methods:** This study was experimental, descriptive research conducted from August 2022 to January 2023. Instruments used were a digital stopwatch, digital balance sheet, blender, stirring rod, erlenmeyer, vial, measuring cup, rotary rotavapor, vortex, oven, knife, pH paper, calipers, plastic bottle, glass bottle, aluminum foil, a container for granulation process, mortar, stamper, granulate tool tester, sieve mesh. The extract obtained was made into effervescent granules using the granulation method. The formulation of effervescent granules contains 8.8% of citric acid, 17.6% of tartaric acid, 30.2% of sodium bicarbonate, 1% aspartame, 1% PVP, 1% aerosil. The amount of lactose is based on the concentration of red beetroots extract formulation in each sample. After the formulation of granules, physical stability tests were carried out, including a flow rate test, density test, dissolution time, and pH test.

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Result: At 1% and 3% concentrations, red beetroots effervescent granules passed all the physical stability tests and revealed a relatively good result. Meanwhile, the 4% red beetroots extract formula showed poorer results than the other two concentrations and failed two physical tests. **Conclusion:** F1 (1%) and F2 (3%) have the best formulation and physical stability of all three formulas. F2 (3%) and F3 (4%) are potential for alternating plaque disclosing agents.

***Keywords**: formulation, physical stability, red beetroots, Beta vulgaris L., effervescent granules*

INTRODUCTION

Red beetroots *(Beta vulgaris L*) are one of the natural ingredients in root vegetables that can quickly be grown in Indonesia. It contains high antioxidants and carbohydrates (Hidayat et al., 2019). Various nutritional content in red beetroots is helpful as an antibacterial, anticancer and protection of the digestive and cardiovascular systems (Khosasi et al., 2021; Babarykin et al., 2019; Tan & Hamid, 2021; Milton-Laskibar, 2021). Besides its health benefits, red beetroots can be used as natural dyes (Lembong & Utama, 2021). The beetroots have a rounded shape and purplish-red color characteristics (Alizar, 2021). The purplish-red pigment in beets is betalain pigment. Betalain combines the purple pigment betacyanin and the yellow pigment betaxanthin (Saula et al., 2021). Betacyanin is a dye categorized in polar betalain pigments which have functions as antioxidants, anti-inflammatories, antiviral, anticarcinogen, antibacterial, and antiprotozoal (Purbaningtyas, 2020). Betacyanin, a natural pigment, can also be utilized as an alternative to dental plaque-disclosing agents (Mangiri et al., 2018).

Apart from red beetroots, betacyanin pigment can be found in red dragon fruit (*Hylocereus polyrhiyus*), cactus fruit (*Opuntia elatior Mill.*), and *Inflorescence celosia* (Halimfanezi et al., 2020). Research showed that super red dragon fruit juice was effective as disclosing agent, and its ability to color plaque was similar to chemical disclosing solutions (Mangiri et al., 2018). Other studies have proven that purple sweet potato extract (Fione & Adam, 2020) and turmeric extract (*Curcuma domestica Val.*) (Oktaviani et al., 2019) were also influential in identifying the presence of dental plaque.

Disclosing solutions sold in the market contain carcinogenic chemicals, such as potassium iodide, iodine crystals, and glycerin (Jung et al., 2020). It is also pricey and rare in many regions (Erwin et al., 2021). The development of disclosing solutions made from natural ingredients arises for these reasons (Mega et al., 2019). Disclosing solutions can be prepared as solutions, tablets, and lozenges (Fione & Adam, 2020). All these preparations can be easily cleaned with water, but they are less effective than gel form. However, the gel tastes unpleasant (Ratnaningsih et al., 2018).

The sweet characteristics of beetroots are suitable to be made into the form of effervescent granules. Granulation formulation helps reduce and mask the bitter medicinal taste (Lestari et al., 2021). Effervescent granules are simple to use, easily dissolved into water, well mixed, and distributed more evenly than tablets and powders (Salim et al., 2018; Grajang et al., 2018). The liquid form is easier to diffuse and spread into inaccessible areas in the oral cavity (Wati et al., 2019). Effervescent

granules were made from the addition of active ingredients: carbonic acid, tartaric acid, citric acid, and sodium bicarbonate, to name a few. These compounds encourage faster disintegration and dissolving of tablets when added to water or watery drinks (Rusita et al., 2015; Lynatra *et al*., 2018). It is perfectly compatible with the stomach (İpçi et al., 2016), while the gas bubbles byproduct from the reaction (carbon dioxide) boosts the penetration of active ingredients into the paracellular space (Aprilia et al., 2021).

Effervescent granules can be processed either dry or melting, or wet. The dry granulation method maintains active ingredients with relatively heat-sensitive, poor flow, and compressibility properties (Murtini and Elisa, 2018). The wet granulation method is used when the active substance resists moisture and heat. The advantages of the wet granulation method include increasing the cohesiveness and compressibility of the powder, good distribution and uniformity of content for small doses of active substances, and preventing the separation of mixed components during the production process (Pratiwi et al., 2017).

The effervescent agent's type and ratio are the principal aspects affecting granules' physical characteristics, stability, and acceptability (Rani et al., 2021). Several tests were required to evaluate the physical stability of the effervescent granules. For the application and acceptability of the formulation, these physical test results must meet the ideal values. Nonetheless, a little study was found to analyze the stability of effervescent granule formulation from red beetroots extract with dose variants. This research aims to analyze the best formulation and its physical stability for red beetroots effervescent granules by measuring the flow rate test, bulk density test, dissolution time, and pH value.

METHODS

This research design was an experimental, descriptive study approved by the Faculty of Medicine, Dentistry and Health Sciences Universitas Prima Indonesia. The plant was determined at MEDA Herbarium Laboratory. This research was conducted between October 2022 to January 2023.

Extraction of Red Beetroots

Twenty kilograms of freshly picked red beet fruits (*Beta vulgaris L.*) were thoroughly cleaned and peeled. Approximately 16.9 kg of red beet fruit flesh was obtained, thinly grated, and air-dried. Around 965 grams of dried beets simplisia were acquired, blended, and soaked in 96% ethanol solvent for 4x24 hours with periodic stirring, then filtered using a medical cotton roll (maceration). The liquid was strained, and the marc was pressed. The marc was macerated twice for the next four days. The filtrates from both macerations were combined and stored. Subsequently, the result was concentrated using a rotary evaporator at ± 50°C, and a water bath was continued to obtain a 100% concentration of red beetroots (Nugraha et al., 2020).

Groups and Treatment

The study of effervescent granules was grouped into three groups of formulation: group 1 (F1) was formulated using 1% red beetroots extract, group 2 (F2) was formulated from 2% red beetroots extract, and group 3 (F3) was formulated using 4% red beetroots extract respectively. All groups were analyzed for organoleptic characteristics, flow rate and angle of repose, bulk and tapped density, and dissolving time.

Preparation of Red Beetroots Effervescent Granules

Initially, acidic granules and alkaline granules were prepared separately. Acidic granules were made by grinding and mixing beetroots extract, aerosil, citric acid, tartaric acid, and lactose. Alkaline granules were made by mixing sodium bicarbonate with the rest of the PVP. Both acidic and alkaline granules were then meshed by sieve no 16 and dried using the drying cabinet separately. The acid granules and base granules were then mixed and meshed together using sieve no 20 and continued to be dried in the drying cabinet (Wati et al., 2019)

Table 1. Effervescent formulations of various concentrations (1%, 2%, and 4%)

Material	Formula 1 (1%)	Formula 2 (3%)	Formula 3 (4%)
Extract	1	3	4
Citric acid	8.8	8.8	8.8
Tartaric acid	17.6	17.6	17.6
Sodium bicarbonate	30.2	30.2	30.2
Aspartame	1	1	1
Lactose	39.4	37.4	36.4
PVP	1	1	1
Aerosil	1	1	1
Total	100	100	100

Evaluation of Red Beetroots Effervescent Granules

a. Organoleptic Test

   Test performed by observing and tasting the effervescent granules. This examination includes the granules' color, aroma, flavor, and condition.

b. Flow rate test and angle of repose

   Twenty-five grams of granules were placed into the *flowability tester funnel,* then the base cover of the funnel was opened, and flow time was measured (USP, 2007). The result of the flow rate would be expressed in grams/second. The angle of repose measurements includes the vertical height and radius of the conical pile of flowed-down granules. The angle of repose can be calculated from the following:

$$\theta = \tan^{-1}(h/r),$$

   with h=height, r=radius and θ=angle of repose in degree

c. Bulk Density and Tapped Density

   Twenty-five grams of granules were placed into a 100 mL graduated cylinder, and the initial volume (V0) was recorded (Sholikhati, 2022). The cylinder was attached to the bulk density apparatus, and the machine was turned on. The tapping process was carried out 20 times. Afterward, the constant volume (Vt) was measured. The compressibility of the granules was

calculated using the *formulas of Carr's index* and *Hausner's ratio*, which both require the results of *bulk* density and *tapped density*.

d. Dissolving time test

Five grams of granules were dissolved in 200 mL of aqueous. Dissolving time is determined from when the granules are put into the glass until all the granules dissolve in the distilled water (Grajang et al., 2018).

e. pH test

Five grams of granules were dissolved in 200 mL of aqueous. Instructions on the pH paper box were followed, and the color shown was observed to determine the pH level (Grajang et al., 2018).

RESULTS

The Red Beet Fruit Extract (*Beta Vulgaris L.*) effervescent granules had been tested for their physical properties and stability as follows:

Organoleptic test

Table 2. Result of organoleptic test

Formulation	Organoleptic		
	Color	Consistency	Taste
F1 (1%)	Light brown	Dry	Less bitter taste
F2 (3%)	Brown	Dry	Less bitter taste
F3 (4%)	Dark brown	Slightly moist	Less bitter taste

Based on examination, F1(1%) had light brown color, but F2(3%) was brown and F3(4%) was dark brown. In terms of consistency, F1(1%) and F2(3%) were dry, while F3(4%) was slightly moist. All three formulas had the same smell with a less bitter taste.

Angle of repose

The results of the evaluation angle of repose of effervescent granules can be seen in the table below.

Table 3. Angle of repose (°)

Formulation	Angle of repose (°)
F1 (1%)	24
F2 (3%)	23
F3 (4%)	27

Based on the results above, F2 has the lowest angle value of 23°, and F3 has the highest value of repose angle of 27°. All the results above pass the condition where the angle of repose must be ≤ 40° (Santosa et al., 2017).

Bulk density

The results of the bulk density evaluation of the effervescent granules are shown in the table below.

Table 4. Bulk density (g/ml)

Formulation	Bulk Density (g/ml)
F1 (1%)	0.7
F2 (3%)	0.46
F3 (4%)	0.48

Based on the results above, F1 has the highest bulk density value of 0.7, and F3 has the lowest bulk density value of 0.48.

Tapped density

The results of the tapped density evaluation of effervescent granules can be seen in the table below.

Table 5. Tapped density (g/ml)

Formulation	Tapped Density (g/ml)
F1 (1%)	0.8
F2 (3%)	0.51
F3 (4%)	0.56

Based on the results above, F1 has the highest tapped density value of 0.8, and F2 has the lowest tapped density value of 0.51.

Hausner ratio

The results of evaluating the Hausner ratio of effervescent granules can be seen in the table below.

Table 6. Hausner's Ratio

Formulation	Hausner Ratio
F1 (1%)	1.14
F2 (3%)	1.11
F3 (4%)	1.16

Based on the table above, F3 has the highest Hausner ratio value of 1.16, and F2 has the lowest Hausner ratio value of 1.11. All the results above have fulfilled the conditions, which must be close to 1 or more than 1 (Octavia, 2012)

Carr's index

The results of the evaluation of carr's index of effervescent granules can be seen in the table below.

Table 7. Carr's index (%)

Formulation	Carr's index (%)
F1 (1%)	12.5
F2 (3%)	9.8
F3 (4%)	14.3

Based on the table above results, F3 has the highest carr's index value of 14.3, and F2 has the lowest Carr's index value of 9.8. All the results above have met the conditions where a good Carr's index is <15% (Reddy et al., 2014).

Flow rate

The results of the flow rate evaluation of effervescent granules can be seen in the table below.

Table 8. Flow Rate (g/s)

Formulation	Flow Rate (g/s)
F1 (1%)	12.3
F2 (3%)	10.5
F3 (4%)	9.2

Based on the table above, F1 has the fastest flow rate, 12.3 g/s, and F3 has the slowest flow rate, 9.2 g/s. F1 and F2 have met the conditions, which must be greater than ten g/s (Widyantari et al., 2021)

Dissolution time

The results of evaluating the dissolution time of effervescent granules can be seen in the table below.

Table 9. Dissolution Time

Formulation	Dissolution time
F1 (1%)	2 Minutes 5 seconds
F2 (3%)	2 minutes 19 seconds
F3 (4%)	2 minutes 39 seconds

Based on the evaluation results above, F1 has the fastest dissolution time of 2 minutes 5 seconds, and F3 has the slowest dissolution time of 2 minutes 39 seconds. F2 has a dissolution time of 2 minutes and 19 seconds. All the above results pass the provisions where a good dissolution test has a dissolution time of < 5 minutes (Forestryana et al., 2020; Santosa et al., 2017).

pH

The results of the pH test of effervescent granules can be seen in the table below.

Table 10. pH Test

Formulation	pH
F1 (1%)	6
F2 (3%)	6
F3 (4%)	6

Based on the results above, all formulas have the same pH value of 6 and have passed the conditions where the pH of an excellent effervescent granule is between 6-7 (Grajang et al., 2018).

DISCUSSION

Effervescent granules preparations typically contain an agent able to release CO_2 (sodium bicarbonate and sodium carbonate) and agents that trigger CO_2 releases (malic acid, adipic acid, ascorbic acid, fumaric acid, maleic acid, succinic acid, or in the research they are tartaric acid and citric acid)

(Advankar et al., 2019). It is compulsory to evaluate effervescent granules to ensure that each and any formulation when put into use or further study meets the requirements of the *European Pharmacopoeia 8th ed*. and indicates formulation with good features, which perhaps will be a potential alternative to present dental disclosing agents (Szumilo et al., 2017). Evaluation of *effervescent* granules includes an organoleptic test, flowability study, angle of repose test, density test, dissolution time test, and pH test (Arisanty et al., 2021).

Organoleptic testing assesses the product's flavor, odor, appearance, and mouthfeel, which is critical to guarantee that it satisfies all organizational and customer demands (Aslani et al., 2013). The results of the organoleptic test of all three formulas bring pleasant experiences (taste, aroma, mouthfeel) to testers. The brown color of the granules slightly hints at the presence of the natural pigment *betacyanins*, the primary source of developing herbal disclosing solutions. *Formula 1 (1%)* and *formula 2 (3%)* are dehydrated and meet the requirement of *effervescent* granules. Meanwhile, *formula 3 (4%)* is still slightly moist. With high moisture content, *effervescent* granules become unstable, acids and bases in the product will react much faster, and the doses are undoubtedly unmaintained (Kuswardhani et al., 2020). Therefore, *F3(4%)* formulation fails the test, primarily due to its higher extract concentration and relatively low lactose.

The flow rate test or hopper discharge rate is a straightforward method to determine the flowability of the granules; the flow rate can also be measured simultaneously during the angle of repose test (Persson et al., 2013). The granule flow rate indicates the number of granules flowing every second; if it takes less than 10 seconds to flow 100 grams or more than 10 grams/second, the granule flow rate is satisfactory (Widyantari et al., 2021). The more granules flow, the better. Based on the result of the research, *F2(3%)* and *F3(4%)* complies with the requirement (12.3 g/s and 10.5 g/s respectively). As for *F3(4%)*, the results do not meet the criteria of a good flow (9.2 g/s). Flow rate is influenced by particle size, particle shape, and humidity (Widyantari et al., 2021). A product with inconsistent granule sizes and shapes results in undesired flow rates; in granules, particles should be small, round, and uniform (İpçi et al., 2016). *Polyvinylpyrrolidone* (*PVP)* is a binder in the wet granulation method and a stabilizer for amorphous drugs in a solid-dispersion system. Its typical concentration is 0.5%–5% w/w (Hiremath et al., 2019). Adding *PVP* as a lactose binder will increase the granule particle size and the high affinity of *PVP* for *lactose* compared with other binder-diluent affinities, such as *PVP/mannitol* or *HPMC/lactose,* will produce larger sizes (Morkhade et al., 2017). The increase in particle granule size decreases the cohesive force that improves the flow rate (Syahrina D & Noval et al.,2021). Granular flowability is also affected by the variations in concentration between *citric acid* and *tartaric acid* (1:2). As an effervescent base, *tartaric acid* and *citric acid* are combined rather than either acid alone since when used separately, *tartaric acid* alone produces chalky friable granules. *Citric acid* alone creates sticky mixtures that are hard to granulate (Diyya et al., 2017). *Tartaric acid* has a higher density than *citric acid*; hence granules containing more *tartaric acid* will have higher density indicating large molecular weight and, as a result, will flow more readily due to the higher gravity (Grajang et al., 2018).

Effervescent preparation made by the wet method should be done in a room with maximum relative humidity (RH) of 25% with a temperature of 20-25^{o}C (Wati et al., 2019). However, all three formulas

above were done in a room with high relative humidity, causing the *effervescent* agents to react rapidly. Because of such restrictions, granules sucked out moisture from their surroundings and increased their moisture content. However, granules were dried in the oven, and the result was still unable to attain ideal moisture levels of 0.4%–0.7%. The flowability and the dissolution rate will be impacted if the *effervescent* granule moisture content is unmet (Grajang et al., 2018).

The maximum angle between a pile of powder's surface and a horizontal plane is identified as the angle of repose; angle of repose measurements can be used to determine the frictional force present in granules and serves as a good indicator of the powder's flow characteristics (Patel et al., 2018). Generally, the higher the angle of repose, the poorer the flow (Firodiya et al., 2017). All three formulas' results pass the test for being ≤ 40° (Santosa et al., 2017). However, their flowability is classified according to table Prajapati (2021):

Table 11. Specifications for an angle of repose (Prajapati et al., 2021)

Flowability	The angle of repose (°)
Excellent	< 25
Good	25-30
Moderate flow	30-40
Poor	>40

As a consequence, the evaluation of flowability by the angle of repose is as follows:

Table 12. Flowability of F1 (1%), F2 (3%), and F3 (4%) based on the angle of repose.

Formula	The angle of repose (°)	Flowability
F1 (1%)	24	Excellent
F2 (3%)	23	Excellent
F3 (4%)	27	Good

Hausner ratio and Carrs' index are indicators of the flowability of bulk solids (Yusof et al., 2013). According to Reddy (2014), the flowability is evaluated in *Table 13*.

Table 13. Specifications for Carr’s index and Hausner ratio (Reddy et al., 2014).

Flowability	Carr’s Compressibility index (%)	Hausner ratio
Excellent	0-10	1.00-1.11
Good	10-15	1.12-1.18
Fair	16-20	1.19-1.25
Possible	21-25	1.26-1.34
Poor	26-31	1.35-1.45
Very poor	32-37	1.46-1.59
Very, very poor	>38	>1.60

Evaluation of flowability of effervescent granules based on Carr's index and Hausner ratio is as follows:

Table 14. Flowability of F1 (1%), F2 (3%), and F3 (4%) based on Carr's index and Hausner ratio.

Formula	Bulk density (g/ml)	Tapped density (g/ml)	Carr’s index (%)	Hausner ratio	Flowability
F1 (1%)	0.7	0.8	12.5	1.14	Good
F2 (3%)	0.46	0.51	9.8	1.11	Excellent
F3 (4%)	0.48	0.56	14.3	1.16	Good

The flow rate test results, angle of repose, Carr's index, and Hausner ratio confirmed that *F1 (1%)* and *F2 (3%)* have the best flowability. Overall, the flowability of the granules decreased with increased moisture and cohesivity; Carr's index, Hausner ratio, and repose angle strongly correlate with moisture content (Chinwan et al., 2019).

The dissolution test results were assessed according to Forestryana (2020) and Santosa (2017). All recorded results pass the demand on an excellent dissolution rate of effervescent granules, which is less than 5 minutes (Forestryana et al., 2020; Santosa et al., 2017). *F1,* with the most lactose, has the fastest dissolution rate, *F2* is the second highest, and *F3* is the slowest, with the most negligible lactose content. An increased lactose concentration in the formula will improve the drug's dissolution rate since the drastic dissolution of lactose has helped open up the matrix structure and deagglomerate the particles (Hiremath et al., 2019).

The pH test is essential because if the effervescent solution is too acidic, it can irritate the stomach. At the same time, if it is too alkaline, it will have a bitter and unpleasant taste. The pH of the effervescent solution is suitable if it is between 6-7 (Grajang et al., 2018). All three formulas acquired good pH values.

Limitations: The physical evaluation tests were done in a room with relative humidity higher than 25%, possibly affecting the final results somewhat.

Suggestion: In further research, it is necessary to formulate a higher concentrated preparation and examine the application of its plaque-disclosing capability. Physical stability tests should be strictly carried out in a room with a relative humidity of 25% with a temperature of 20-25^{o}C (Wati et al., 2019).

CONCLUSIONS

Based on the results of the physical property tests, F1 and F2 have the best physical stability by passing all the tests. Meanwhile, F2 has not had as good results as the other two and failed two of the tests (organoleptic test and flow rate test). F1 and F2 obtain the most stable formulation of all three.

As for the potential of replacing the disclosing agent, only F2 and F3 promise thanks to the brown and dark brown color of granules and effervescent solution observed. Unfortunately, F1 granules and effervescent solution show light brown due to its low beetroots extract concentration, indicating that it is not qualified for the staining of dental plaque.

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