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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 provide various notable health benefits. In dentistry, red beetroots have the potential to act as a plaque-disclosing agent. Previous studies showed that red beetroot preparation in gelform is more effective compared to chewable tablets, but has significant drawbacks in taste. Effervescent granules have the potential to conceal the unpleasant taste of beetroot substances. This study aims to determine the ideal formula and evaluate the physical properties and stability of effervescent granules made from red beetroot extract. **Methods:** This was an experimental design study with descriptive analysis. The maceration method was used to extract the red beetroot constituents. Fresh fruits were collected, air-dried, and macerated with 96% ethanol twice. The filtrates were then concentrated. The wet granulation method was used to formulate effervescent granules. The samples were grouped into formula 1 (F1), formula 2 (F2), and formula 3 (F3) using 1%, 3%, and 4% red beetroot extract, respectively. All sample groups were analyzed for physical stability, namely: organoleptic, flowability, density, dissolving time, and pH tests. **Results**: All samples surpassed the sensory evaluation for physical properties. Formula 1 (1%) and formula 2 (3%) models passed all the physical stability tests. Meanwhile, the 4% formula (F3) failed two physical tests. F1 (1%) samples also showed lighter brown color for its lower beetroot extract concentration, indicating under-qualification as dental plaque staining materials. Therefore, 3% concentration (F2) has the best formulation regarding physical properties and stability. **Conclusion:** Formulas F1 and F2 have the best physical properties and stability by passing all the tests, while Formula 3 failed the organoleptic and flow rate tests.

KEYWORDS
formulation, physical stability, *Beta vulgaris* L, effervescent granules

INTRODUCTION

Red beetroot *(Beta vulgaris* L) is one of the natural ingredients in root vegetables that grows very well in Indonesia. It contains high antioxidants and carbohydrates.[1] Various nutritional content in red beetroots is helpful as an antibacterial, anticancer and protection of the digestive and cardiovascular systems.[2–5] Besides their health benefits, red beetroots can be used as natural dyes.[6] The beetroots have a rounded shape and purplish-red color characteristics.[7] The purplish-red pigment in beets is betalain pigment. Betalain combines the purple pigment betacyanin and the yellow pigment betaxanthin.[8] Betacyanin is a dye categorized in polar betalain pigments, which have functions as antioxidants, anti-inflammatories, antivirals, anticarcinogens, antibacterials, and antiprotozoals.[9] Betacyanin, a natural pigment, can also be utilized as an alternative to dental plaque-disclosing agents.[10]

Apart from red beetroots, betacyanin pigment can be found in red dragon fruit (*Hylocereus polyrhiyus*), cactus fruit (*Opuntia elatior* Mill*.),* and Inflorescence celosia.[11] 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.[10] Other studies have proven that purple sweet potato extract and turmeric extract (*Curcuma domestica* Val.*)* were also influential in identifying the presence of dental plaque.[12,13] Nonetheless, beetroot is easier to find, available all year long in Indonesia, and relatively cheap.

Disclosing solutions sold in the market could possibly contain carcinogenic chemicals, such as potassium iodide, iodine crystals, and glycerin.[14,15] It is also expensive and rarely used for these reasons.[16] Disclosing solutions can be prepared as solutions, tablets, or lozenges.[12] All these preparations can be easily cleaned with water, but they are less effective than gel forms. However, the gel tastes unpleasant.[17]

The sweet characteristics of beetroots make them suitable to be made into effervescent granules. The granulation formulation helps reduce and mask the bitter and unpleasant medicinal taste.[18] Effervescent granules are simple to use, easily dissolved into water, well mixed, and distributed more evenly than tablets and powders.[19] The liquid form is easier to diffuse and spread into inaccessible areas in the oral cavity.[20] 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 dissolution of tablets when added to water or watery drinks.[21,22] It is perfectly compatible with the stomach, while the gas bubble byproduct from the reaction (carbon dioxide) boosts the penetration of active ingredients into the paracellular space.[23,24]

Effervescent granules can be processed either dry melting, or wet. The dry granulation method maintains active ingredients with relatively moisture-sensitive, poor flow, and compressibility properties.[25] 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, ensuring good distribution, and uniformity of content for small doses of active substances and preventing the separation of mixed components during the production process.[26]

The type and ratio of the effervescent agent are the principal aspects affecting granules' physical characteristics, stability, and acceptability.[27] 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, very few studies were found to analyze the stability of effervescent granule formulation from red beetroot extract with dose variants. This research aims to determine the best formulation and its physical properties and stability for red beetroots effervescent granules. Analysis was made by measuring the organoleptic characteristics, flow properties, dissolution time, and pH value tests.

METHODS

This research design was an experimental study with descriptive analysis approved by the Faculty of Medicine, Dentistry and Health Sciences Universitas Prima Indonesia. The plant was determined at MEDA Herbarium Laboratory. The extraction of red beetroots was done by collecting, thoroughly cleaning, and peeling 20 kilograms of freshly picked red beet fruits (*Beta vulgaris* L.). Approximately, 16.9 kg of red beets fruit's flesh was obtained, thinly grated, and air-dried. Around 965 grams of dried *beets simplicia* 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 processes were combined, stored and subsequently concentrated using a rotary evaporator at ± 50°C, and a water bath, to obtain a 100% concentration of red beetroots.[28]

Initially, red beetroot effervescent granules were formulated by preparing acidic and alkaline granules separately. Acidic granules were made by grinding and mixing beetroot 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 strained using sieve #16 and dried separately in the cabinet. The acid granules and base granules were then mixed and strained using sieve #20, and the drying cabinet was used to complete the drying process.[20]

The effervescent granule samples were grouped into three groups of formulation: group 1 (F1) was formulated using 1% red beetroots extract, group 2 (F2) was formulated using 3% red beetroots extract, and group 3 (F3) was formulated using 4% red beetroot extract. [The samples of effervescent granules were divided into three formulations: group 1 (F1), group 2 (F2), and group 3 (F3), using 1%, 3%, and 4% of the red beetroot extract respectively.] The complete formula used in preparing effervescent granules in this study are shown in 1.

Table 1. Effervescent formulations of various concentrations

Materials	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**

All groups were analyzed for organoleptic characteristics, flow properties, dissolving time, and pH. The organoleptic test was performed in a double-blinded condition, by observing and tasting the effervescent granules. The selected panelists assessors analyze the granules' color, consistency, smell, and taste. Afterward, flow properties were measured related to angle of repose, bulk density, tapped density, compressibility, as well as flow rate of the granules. The flowability related to angle of repose was carried out in which 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.[29] The result of the flow rate would be expressed in grams per 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 as $\theta = \tan^{-1}(h/r)$, with h=height, r=radius and θ=angle of repose in degree. Next, bulk density and tapped density value were evaluated by placing twenty-five grams of granules into a 100 mL graduated cylinder, and the initial volume (V0) was recorded.[30] The cylinder was attached to the bulk density apparatus, and the machine was activated .

The tapping process was carried out 20 times before measuring the constant volume (Vt). The compressibility of the granules was calculated using the *formulas of Carr's index* and *Hausner's ratio*, which both required the results of *bulk density* and *tapped density*. The specifications for Carr's compressibility index (%) and Hausner ratio with regards to flowability were explained in Table 2. The dissolving time of granules were measured by taking five grams of granules and dissolved it 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.[19] Lastly, pH test was completed by dissolving five grams of granules in 200 mL of aqueous. Instructions on the pH paper box were followed, and the color shown was observed to determine the pH level.[19]

Table 2. Specifications for Carr's index and Hausner ratio[32]

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

RESULTS

The physical properties and stability of red beets fruit extract (*Beta Vulgaris* L.) effervescent granules had been sensory and physically evaluated. Based on organoleptic examination (Fig. 1), F1(1%) had lighter brown color, while F2(3%) color was brown and F3(4%) color was dark brown. In terms of consistency, F1(1%) and F2(3%) were dry, while F3(4%) was slightly moist. Table 3 reveals that all three formulas had the same smell with a less bitter taste. The granules were spherical in shape.

Table 3. Result of organoleptic test

Formulation	Organoleptic test			
	Color	Consistency	Taste	Shape
F1	Light brown	Dry	Less bitter taste	Spherical
F2	Brown	Dry	Less bitter taste	Spherical
F3	Dark brown	Slightly moist	Less bitter taste	Spherical

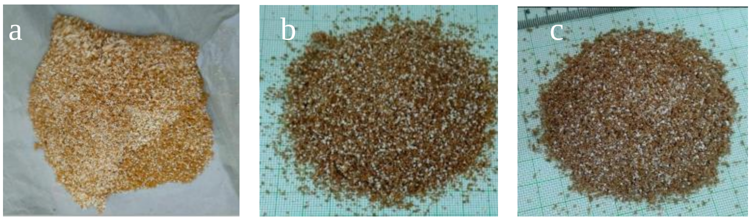

Figure 1. Red beetroots effervescent granules of the formula F1 is lighter Brown: (a) formula F2 is brown; (b) formula F3 is darker brown; (c) in color.

The flow properties of beetroot's effervescent granules were assessed in relation to angle of repose, bulk density, tapped density, Carr's index, Hausner ratio, and flow rate. Table 4 below demonstrates the flowability condition of beetroot's effervescent granules based on angle of repose's evaluation. Based on the results collected, 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°.[31]

Table 4. Flowability of F1, F2, and F3 based on the angle of repose

Formula	The angle of repose (°)	Flowability
F1	24	Excellent (<25°)
F2	23	Excellent (<25°)
F3	27	Good (within 25-30°)

The results of the flowability characteristics of red beetroot effervescent granules corresponding to the analysis of bulk density, tapped density, Carr's index, and Hausner ratio are shown in the table 5. It can be seen that F1 has the highest bulk density value of 0.7, while F2 and F3 have the lowest bulk density value of less than 0.5. Meanwhile, the tapped density evaluation of effervescent granules (table 5) revealed that F1 has the highest tapped density value of 0.8, and F2 has the lowest tapped density value of 0.51. On Carr's index examination of effervescent granules, 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 data studied have met the conditions where a good Carr's index is <15%.[32] In this study, F3 was shown with 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.[32]

Table 5. Flowability of 3 Formulas based on Carr's index and Hausner ratio.

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

The results on table 6 below showed that 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 10 g/s.[33]

Table 6. Flow Rate of Formulation (g/s)

Formulation	Flow Rate (g/s)
F1	12.3
F2	10.5
F3	9.2

Based on the evaluation results on table 7, 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.[31]

Table 7. Dissolution Time of Formulation

Formulation	Dissolution time
F1	2 Minutes 5 seconds
F2	2 minutes 19 seconds
F3	2 minutes 39 seconds

Table 8 revealed the pH test analysis. 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.[19]

Table 8. pH Test of Formulation

Formulation	pH
F1	6
F2	6
F3	6

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).[1] 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.[34] Dental disclosing solution available in the market possibly contain carcinogenic chemicals, such as potassium iodide, iodine crystals, and glycerin, which had strong cytotoxicity on gingival epithelial cells.[14] However, aspartame, an artificial sweetener, used in this study possibly cause liver cancer in human although the evidence was limited.[35]

Evaluation of effervescent granules includes an organoleptic, flow properties, angle of repose, density test, dissolution time, and pH tests.[5] Organoleptic testing assesses the product's flavor, odor, appearance, and mouthfeel, which is critical to guarantee that it satisfies all organizational and customer demands.[36] 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. F1(1%) and F2(3%) are dehydrated and meet the requirement of effervescent granules. Meanwhile, F3(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.[37] 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.[38] 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.[33] The more granules flow, the better. Based on the result of the research, F1(1%) and F2(3%) 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.[33] A product with inconsistent granule sizes and shapes results in undesired flow rates; in granules, particles should be small, round, and uniform.[23] 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.[39] 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.[40]

The increase in particle granule size decreases the cohesive force that improves the flow rate.[41] 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.[42] 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.[19]

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°C.[20] 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 degree is not achieved.[19]

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.[34] Generally, the higher the angle of repose, the weaker the flow.[43] Two formulas (F1 and F2) attained results of ≤25°, which showed an excellent flowability. On the other hand, F3 revealed a slightly weaker flowability (≤30°) but still within the range of good features.[31] The specifications for an angle of repose and flowability characteristics was classified based on Prajapati's study.[44]

Hausner ratio and Carrs' index are indicators of the flowability of bulk solids. According to Reddy et al,[32] the flowability evaluation concluded that only formula 2 (3%) had an excellent flowability with Carr's index ≤10, and Hausner ratio ≤1.11.

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, at a rate of less than 10g/s. 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.[45] Factors affected flowability rate of effervescent granules are the particle shape and the crystalline citric acid monohydrate used in this study.[46,47]

The dissolution test results were assessed according to Santosa.[31] All recorded results pass the demand on an excellent dissolution rate of effervescent granules, which is less than 5 minutes.[31] F1, with the most lactose, has the fastest dissolution rate, F2(3%) is the second highest, and F3(4%) 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 deagglomeration the particles.[39]

The pH test is essential to evaluate the acidity of the effervescent solution. 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 effervescent solution is suitable between pH 6-7.[19] All three formulas acquired good pH values.

CONCLUSION

Formula F1 and F2 have the best physical properties and stability by passing all the tests, while F3 failed the organoleptic and flow rate tests. However, formula 1 is not qualified for staining dental plaque because of its very light brown color due to its minimum concentration of red beets extract, making it not suitable as plaque disclosing agents. Overall, F2(3% beetroots effervescent granules) have the best formula, physical properties and stability compared to the other formulas, and have the greatest potential to be developed further.

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