



Scaling up stirring-assisted extraction and transformation of roselle anthocyanins into dried powder using spray-drying and oven-drying

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is a source of anthocyanins with double functions as natural colorants and pharmacologically active ingredients in the food and pharmaceutical industries. Optimizing its extraction by upscaling and engineering the derived extract into its concentrated form or dry powder are thus needed. This study optimized the conditions of stirring-assisted extraction (SAE) of roselle calyces at an upscaled volume, determined the physicochemical and antioxidant properties of extract concentrated with rotary evaporation, and evaluated the effects of spray-drying and oven-drying on the powdered extract characterized from both properties. Results show that anthocyanin extraction from roselle was successfully upscaled using SAE with these optimized conditions: 50 % ethanol acidified with 2 % citric acid as the solvent, an S/L ratio of 1:15 g/mL, and three extraction cycles with a duration of 60 min each. Concentrated extracts (moisture content = 1.38–2.35 %) can be used as an alternative form of storage. Spray-drying with the matrix maltodextrin proved effective in producing dry powder with superior properties than oven-drying. Spray-drying and a 1:1 extract-to-maltodextrin ratio created more favorable dry powder: smooth surface, light red color, amorphous structure, spherical morphology, homogenous particle size (avg. 1.951 μm), total anthocyanin of 2.15 mg/g, antioxidant activity at IC_{50} = 203.56 $\mu\text{g/mL}$, and color stability maintained at pH = 2.6–4.0. These conditions enable extraction scale-ups for industrial production. Further, storage in thick concentrations and oven-drying can alternatives in specific cases when spray-drying is not doable.

1. Introduction

Colorants, dyes, or pigments are substances added to various foods or medicinal products to give or enhance color. These additives are crucial in the food manufacturing chain for covering undesirable characteristics or improving the inherent qualities of food products (Luzardo-Ocampo et al., 2021). Additionally, colorants are used in pharmaceutical products for numerous purposes, including preserving the active ingredients' stability, increasing acceptance, and serving as product identification throughout production and distribution (Biswal et al., 2015).

The industrial sectors have been relying on synthetic colorants for their stability, appealing colors, and relatively low costs. However, as people become more aware of the adverse health consequences and the rising environmental problems due to artificial dyes, consumer habits are changing towards environmentally-friendly choices that increase the

demand for natural dyes. The United States and the European Union have accordingly approved the use of various natural dyes, including anthocyanins (Luzardo-Ocampo et al., 2021).

Anthocyanins are blue, red, or purple pigments found in plants, especially flowers, fruits, and tubers (Khoo et al., 2017). Roselle (*Hibiscus sabdariffa* L.) is one of the sources of red to purplish-red anthocyanins, e.g., the compounds delphinidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-sambubioside, and cyanidin-3-sambubioside (Borrás-Linares et al., 2015). They possess a wide range of biological properties, including antioxidant, anti-inflammatory, antibacterial, antitumor, antidiabetic, antihypertensive, and hepatoprotective (Borrás-Linares et al., 2015; Hapsari & Setyaningsih, 2021; Jabeur et al., 2017). Consequently, roselle's anthocyanins have multiple functions: natural dyes and pharmacologically active ingredients. The food and pharmaceutical industries can draw upon this multifunctionality to explore

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anthocyanins not only as coloring agents but also for enhancing and enriching products' characteristics and functions (e.g., functional food and pharmaceutical preparations with specific activities) (Jabeur et al., 2017).

Several extraction methods have been developed to separate anthocyanins from roselle calyces: heat-assisted extraction (HAE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), maceration, infusion, supercritical fluid extraction (SFE), and stirring-assisted extraction (SAE) (Aryanti et al., 2019; Chumsri et al., 2008; Hapsari & Setyaningsih, 2021; Jabeur et al., 2017; Pham et al., 2019; Pinela et al., 2019; Setyawan & Kartini, 2023; Wu et al., 2018). Setyawan and Kartini (2023) optimized the SAE of anthocyanins from roselle at the laboratory scale, resulting in these optimal conditions: 50 % ethanol acidified with 2 % citric acid as the solvent, 15-min extraction, and an S/L ratio of 1:15 g/mL (Setyawan & Kartini, 2023). However, optimal SAE conditions at this scale cannot be directly replicated for larger manufacturing. To efficiently remove bioactive compounds at a broader scale where large amounts of raw materials are used, they should be adjusted or upscaled from the laboratory to the industrial level (Belwal et al., 2019).

In addition, for ease of use, transportation, storage, and increased shelf life, roselle extract should be separated from the solvent by concentration to create a thick preparation or drying to make powder (Gonzalez-Palomares et al., 2009). Rotary evaporation is a process of removing solvents from extracts using moderate heat and low pressure. While efficient for removing organic solvents, it cannot completely dry aqueous extract (Bennour et al., 2020). Spray-drying is the most common drying method to produce roselle powder as a natural coloring agent (Cid-Ortega et al., 2022; Fitriani et al., 2021; Gonzalez-Palomares et al., 2009; Minh, 2020; Nguyen et al., 2022a, b). However, the equipment and overall cost of the procedure can be quite expensive, which remains an obstacle to its utilization and development in some countries and industries. Moreover, with many process variables and different matrix types and concentrations, their combinations must be optimized to create powders with the desired physicochemical characteristics (Gonzalez-Palomares et al., 2009). A more affordable option is oven-drying, which can be implemented using readily available equipment at a low cost, including a conventional oven. Oven-drying has been shown to improve the phytochemical profiles of medicinal and food crops depending on the selected temperature; for instance, in previous work, samples dried at 40 °C presented higher total polyphenol content and antioxidant activity than fresh samples. Oven-drying prove as effective as lyophilization in preserving metabolites in the 50 % ethanol extract of *Arthrocnemum macrostachyum* shoots (ElNaker et al., 2021; Stepień et al., 2019). Even though the oven-drying of post-harvest (pre-extraction) roselle calyces has been broadly studied in terms of process variables and products (Ashaye, 2013; Juhari et al., 2021; Lema et al., 2022; Tham et al., 2018), its effects on roselle extract (post-extraction) are still under researched.

The objectives of this study were: (i) to optimize the SAE conditions (number of cycles and duration of extraction) of roselle calyces for production scale-up, (ii) to determine the physicochemical and antioxidant properties of concentrated roselle extract obtained through rotary evaporation, and (iii) to evaluate the effects of spray and oven-drying on powdered roselle extract, as seen from both properties. This study contributes to providing initial data for further optimization and scale-up studies of SAE conditions and enriching the methodical options for transforming liquid roselle extract into its concentrated form or dry powder with their respective advantages and disadvantages.

2. Material and methods

2.1. Plant materials and chemicals

Purple roselle calyces were handpicked in April 2021 in Magetan (East Java, Indonesia) and then verified by the Center for Traditional

Medicine Information and Development, University of Surabaya. Using a Philips HR 222 blender (Amsterdam, the Netherlands), air-dried calyces were crushed into powder and sifted using a 20-mesh strainer. The chemicals used were ethanol (analytical reagent), potassium chloride (KCl), hydrochloric acid (HCl), disodium phosphate (Na_2HPO_4), sodium acetate, tartaric acid, and citric acid monohydrate from Merck KGaA (Darmstadt, Germany); food-grade DE10–12 maltodextrin from Qingdao Shengda Commercial & Trade Co., Ltd. (Qingdao, China); ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from HiMedia Laboratories, LLC (Pennsylvania, USA); and demineralized water (Aqua DM) from the Chemistry Laboratory, Faculty of Pharmacy, University of Surabaya (Indonesia).

2.2. Optimization of stirring-assisted extraction (SAE) scale-up

A preliminary work found optimal SAE conditions for roselle calyces on a laboratory scale: 50 % ethanol acidified with 2 % citric acid as the solvent, 15-min extraction, and a solid-to-liquid ratio of 1:15 g/mL (Setyawan & Kartini, 2023). In this study, the extraction scale was increased to 100 times the lab-scale volume, and two extraction parameters were tested for optimization: number of repetitions (1, 2, 3, 4, and 5 cycles) and duration (30, 60, and 90 min).

To extract anthocyanins, 100 g of the crude drug was added with 1500 mL of 50 % ethanol acidified with 2 % citric acid and then stirred with an overhead stirrer (IKA RW 20; IKA-WERKE, Staufen, Germany) for 30 min at 500 rpm. The extract was removed and set aside, while the residue was extracted again using the procedure above. This process was repeated five times to determine the optimal number of extraction cycles (1, 2, 3, 4, or 5). Afterward, the filtrate derived from each cycle was observed to characterize its color, pH, total anthocyanins (TA), and antioxidant activity. The optimal cycle was later applied with different extraction durations (30, 60, or 90 min) for SAE optimization.

2.3. Extract concentration using rotary evaporation

Extracts made with optimal SAE conditions (number of cycles and duration) were combined, concentrated with a rotary evaporator (Buchi R-300; BÜCHI Labortechnik AG, Flawil, Switzerland) at 50 °C and 11.7 cmHg pressure to reach a specific °Bx, and then atmospherically evaporated in a water bath at 50 °C (WBB 22; Memmert GmbH, Schwabach, Germany) until a thick extract was obtained. Afterward, its organoleptic characteristics, yield, TA, and antioxidant activity were determined.

2.4. Extract drying

2.4.1. Spray-drying

Spray-drying (SD) was applied with three different ratios of total solids in roselle extract to the matrix maltodextrin (RE:MD), namely 1:1 (referred to as SD1), 1:0.43 (SD2), and 1:0.25 (SD3). The concentrated extract (8.8°Bx) was poured into a beaker glass, added with maltodextrin little by little, and then mixed using a stirring rod until homogeneous. The mixture was then dried using a Buchi Mini spray dryer (BÜCHI Labortechnik AG) with the inlet and outlet temperatures of 150 °C and 85–90 °C, respectively, and at a feed flow rate of 8 mL/min (Archaina et al., 2019; Nguyen et al., 2022a, b). Afterward, the dried extract powder was stored in a dark glass bottle at 4 °C for further analysis (organoleptic properties, moisture content, total anthocyanin, and color stability at various pH levels). Powder with the most favorable characteristics was then tested for its antioxidant activity, particle size and its distribution, shape and morphology, and crystallinity.

2.4.2. Oven-drying

Oven-drying (OD) on roselle extract was adapted from Juhari et al. (Juhari et al., 2021). OD also used maltodextrin as the matrix and three variations of extract-to-matrix ratios, namely 1:20 (referred to as OD1), 1:10 (OD2), and 1:5 (OD3). Concentrated rosella extract (11.9°Bx) was

placed in a porcelain pestle, added with maltodextrin little by little, and mixed using a mortar until homogenous. This mixture was then flattened on a glass tin to form a thin layer and heated in a drying oven (Memmert UN 110) at 60 °C for 24 h. The dry extract was scraped off the tin, placed in a porcelain pestle, and ground into powder using a mortar. The dried powder was stored in a dark glass bottle at 4 °C and tested with the same analysis as the spray-dried extract powder.

2.5. Characterization of liquid, concentrated, and dried extracts

2.5.1. pH measurement

A 50 mL beaker glass was filled with 10 mL of the liquid extract. pH was measured at room temperature by dipping the calibrated pH electrode of the pH meter (Mettler Toledo FP-20; Ohio, USA) in the extract.

2.5.2. Moisture content analysis

Concentrated extracts and spray-dried and oven-dried powders were heated at 105 °C for 1.5 min. Then, the moisture content was measured using a moisture analyzer (Ohaus MB 120; New Jersey, US). Moisture content (%) was calculated as the ratio of the difference between the initial weight of the sample (W_0) and the weight after heating (W_1) to W_1 multiplied by 100, as written below:

$$\text{Moisture Content (\%)} = \frac{W_0 - W_1}{W_1} \times 100\%$$

2.5.3. Determination of color stability at various pH levels

To determine the color stability, 50 mg of spray-dried powder and 400 mg of oven-dried powder were dissolved in 5.0 mL phosphate-citrate buffer with different pH levels: 2.6, 3.0, 4.0, 5.0, 6.0, and 7.0. The solution's color intensity (lightness, redness, and yellowness) was read using a color reader (Konika Minolta CR-10 Plus; Konika Minolta, Inc., Tokyo, Japan).

2.5.4. Determination of total anthocyanin (TA) content

The modified version of the pH differential method proposed by Lee et al. was used to determine the total anthocyanins (TA) of the liquid, concentrated, and dried extracts (Lee et al., 2005). To start, 250 μ L of the sample (liquid extract, concentrated extract, or the dried powder re-dissolved in water) was mixed with 5 mL of a KCl buffer solution (pH 1.0). Then, 250 μ L of the same sample was given 5 mL of sodium acetate buffer solution (pH 4.5). Using a UV-Vis spectrophotometer (Shimadzu UV 1900; Shimadzu, Kyoto, Japan), their absorbance values were gauged at 520 nm (A_{520}) and 700 nm (A_{700}).

TA (mg/L) was calculated by multiplying final absorbance (A) by molecular weight (MW), dilution factor (DF), and the weight conversion factor ($1 \text{ g} = 10^3 \text{ mg}$) and then dividing the result by molar extinction coefficient (ϵ) and cuvette width ($l = 1 \text{ cm}$). Because TA was defined as cyanidin-3-glucoside equivalent, the molecular weight and molar extinction coefficient of cyanidin-3-glucoside were used, namely MW = 449.2 g/mol and $\epsilon = 26,900 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$. The equation is written below:

$$\text{Total anthocyanin content (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

where, final absorbance (A):

$$A = [(A_{520} - A_{700})pH_{1.0}] - [(A_{520} - A_{700})pH_{4.5}]$$

The TA concentrations in the liquid, concentrated, and dried extracts were converted from mg/L into mg/g according to the weight of the crude drug, concentrated extract, or dried powder.

2.5.5. Determination of antioxidant activity

The antioxidant activity was determined using the free-radical (DPPH) scavenging method (Kartini et al., 2019). A series of five-fold dilutions were made from the roselle extract and the reference

compound, ascorbic acid, separately. One hundred μ L of the sample (extract or reference compound) was moved into a 96-well clear polystyrene microplate using a pipette and given 50 μ L of DPPH solution in ethanol (0.026 %). After homogenization, the mixture was incubated for 10 min in a dark room, and then using a microplate reader (UVM 340 Biochrom; Biochrom Ltd., Cambridge, UK), the absorbance was detected at 517 nm. In addition, a blank sample was created for each diluted concentration by mixing 100 μ L of the sample and 50 μ L of ethanol. The control absorbance was obtained from the mixture of 100 μ L of DPPH solution and 50 μ L of ethanol. The DPPH radical scavenging was calculated as a percentage of inhibition based on the sample's absorbance (A_s), the blank sample's absorbance (A_{bl}), and the control absorbance (A_c), as expressed below:

$$\% \text{Inhibition} = \frac{(A_c - (A_s - A_{bl}))}{A_c} \times 100\%$$

The percentage of inhibition (y) and the series of sample concentrations (x) were analyzed statistically to produce a regression linear equation ($y = bx + a$) for half-maximal inhibitory concentration (IC_{50}). IC_{50} of the extract against DPPH measures the sample's effectiveness in inhibiting free radicals' activities.

2.5.6. Characterization of morphology, crystallinity, and particle size distribution of dried extracts

Powdered extract from the best formulation of each dry method (SD and OD) was observed to determine its morphological properties, crystallinity, and particle size and its distribution. For analysis of morphological properties, the samples were placed in the sample container and coated with gold aluminum with a thickness of 10 nm. Samples were observed at various magnifications using scanning electron microscope or SEM (FEI Inspect S 50, USA) which was regulated with a voltage of 20 kV and 12 mA.

Crystal lattice formed was analyzed using Philips X-ray diffractometer or PXRD (Philips X'Pert, Netherlands) at room temperature. Target conditions required in the analysis were Cu, $K\alpha$ filter, with a voltage of 40 kV and 15–30 mA, carried out at $2\theta = 5\text{--}40^\circ$ ($0.2\text{--}0.5^\circ/\text{minute}$). The samples were placed on the sample holder and flatted to prevent particle orientation during preparation.

Particle size and size distribution were measured using a particle size analyzer (Nanotrac Wave II, Microtrac, Germany) using Dynamic Light Scattering (DLS) method. 400 mg sample was dispersed in 10 mL of distilled water and homogenized using a vortex. The homogenized sample was then inserted into the instrument and analyzed at temperature of 25 °C and an angle of 90° .

2.6. Statistical analysis

The data were statistically analyzed in SPSS 26 program (SPSS Inc.; Illinois, US) using the feature one-way ANOVA, Kruskal-Wallis, or student t-test ($P < 0.05$) depending on their distribution and variance and the number of data groups being compared. Post hoc test, i.e., least significant difference (LSD) or Mann Whitney-U, was applied to determine if the mean values were significantly different at a 5 % significance level. Unless stated otherwise, all experiments were performed in triplicates.

3. Results and discussion

3.1. Optimal number of cycles for the upscaled stirring-assisted extraction (SAE)

Stirring-assisted extraction (SAE) is an equilibrium technique, meaning some compounds are left in crude drug residues after the extraction ends. Therefore, repeating extraction has been proposed to increase the yield. On the one hand, with more cycles, SAE produces more yields and fewer (closer to 0) compound is left in the residue. On

Table 1
Characteristics of roselle extracts made with different cycles of extraction for optimization.

Cycle i th	Color	pH	Volume (mL)	TA (mg/g)	IC ₅₀ (μg/mL)*
1	Purple	3.13	1370	1.925±0.02 ^a	800.95±24.78 ^a
2	Purplish-red	3.10	1500	0.450±0.02 ^b	5425.59±449.13 ^b
3	Red	3.04	1500	0.071±0.00 ^c	22,872.32±233.52 ^c
4	Light red	2.81	1500	0.017±0.00 ^d	59,744.67±2823.37 ^d
5	Pale red	2.77	1500	0.017±0.00 ^d	65,180.83±828.37 ^e

Notes:

TA (total anthocyanins) and IC₅₀ are expressed as mean±SD (n = 3).

TA contents are based on the weight of the crude drug.

Means followed by different notations in the same column show significant differences from one another (P<0.05).

* IC₅₀ of the positive control (ascorbic acid) = 10.59±0.50 μg/mL.

the other hand, this technique spends more solvent, time, and energy.

Five cycles of extraction, each with 30 min of stirring, were conducted to study the effect of the number of cycles on the SAE's ability to extract anthocyanins from roselle. In the first cycle, the crude drug absorbed some solvents, producing an extract with the lowest volume (1370 mL) (Table 1). The extract's color became lighter from purple in the first cycle to purplish-red and then red in the third one, suggesting a significantly decreasing anthocyanin content from one cycle to the next (Fig. 1). On the contrary, no significant change in color was observed after the third, fourth, and fifth cycles (red to light red then pale red). The pH varied between 2.77–3.33 (acidic) or within the pH range where anthocyanins are stable (Khoo et al., 2017). However, results also show that pH decreased with the number of extraction repetitions.

TA content from each cycle showed a different pattern from its cumulative sum (Fig. 1). After the third cycle, TA dramatically decreased and remained stable at a very low amount. In contrast, cumulative TA rose substantially and then relatively unchanged after the third cycle,

meaning it would no longer increase after the fourth and fifth cycles. This corresponds to the patterns of phenolics extracted from *Ficus virens* leaves and polysaccharides from the fruit of *Capparis spinosa* (Chen et al., 2013; Ji et al., 2012).

IC₅₀ multiplied significantly after each cycle (P<0.05), indicating weakening antioxidant activity (Table 1). This result corresponds to TA, with its significant decrease (P<0.05), especially after the second cycle. In addition to organic and phenolic acids, anthocyanins are a group of compounds contributing to the antioxidant activity of roselle (Hapsari & Setyaningsih, 2021). Considering the extracts' characteristics and production aspects (cost, time, energy), it was concluded that the optimal number of cycles for SAE scale-up would be three.

3.2. Optimal duration for the upscaled SAE

Compounds can be removed from plant cells after an ample time of contact between solvents and crude drugs. For instance, maceration

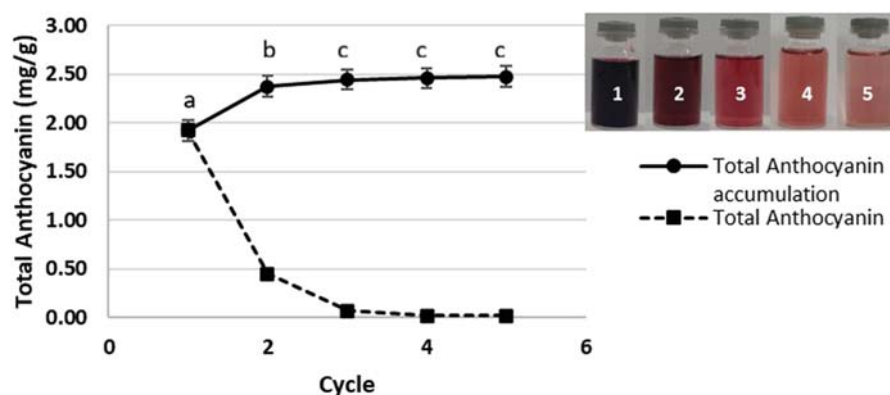


Fig. 1. Total anthocyanin content and the cumulative sum obtained from five extraction cycles. Notes: Means followed by different notations show significant differences from one another (P<0.05). Number 1–5 in the inset image shows the extraction cycle in order, from the first to the fifth.

Table 2
Characteristics of roselle extracts made with different duration of extraction for optimization.

Duration (min)	Cycle i th	Color	pH	Volume (mL)	TA (mg/g)	IC ₅₀ (μg/mL)*
30	1	Purple	3.13	1370	1.925±0.02 ^a	800.95±24.78 ^a
	2	Purplish red	3.10	1500	0.450±0.02 ^b	5425.59±449.14 ^c
	3	Red	3.04	1500	0.071±0.00 ^c	22,872.32±233.52 ^d
60	1	Purple	3.01	1380	2.326±0.04 ^d	633.79±53.58 ^b
	2	Purplish red	2.95	1500	0.343±0.02 ^e	5911.81±582.31 ^c
	3	Red	2.79	1500	0.029±0.00 ^f	28,533.81±3091.69 ^e
90	1	Purple	3.00	1396	2.489±0.02 ^e	591.08±14.99 ^b
	2	Purplish red	2.98	1500	0.318±0.02 ^e	6203.39±309.26 ^c
	3	Red	2.88	1500	0.035±0.00 ^f	26,083.57±677.23 ^e

Notes:

TA (total anthocyanins) and IC₅₀ are expressed as mean±SD (n = 3).

TA contents are based on the weight of the crude drug.

Means followed by different notations in the same column show significant differences from one another (P<0.05).

* IC₅₀ of the positive control (ascorbic acid) = 10.59±0.50 μg/mL.

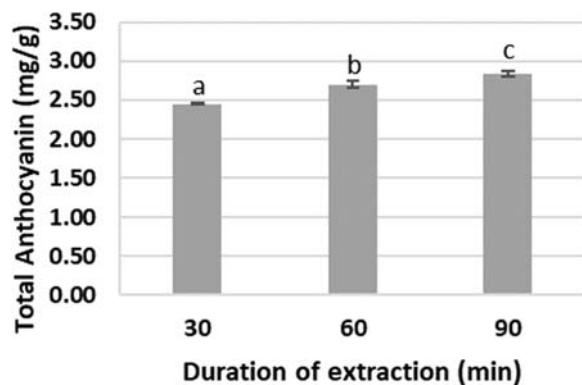


Fig. 2. Cumulative total anthocyanins of roselle extracts from three cycles of extractions with different durations. Notes: Values with different notations show significant differences from one another ($P < 0.05$).

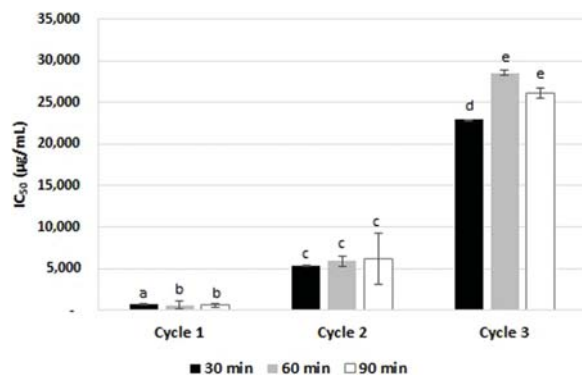


Fig. 3. Antioxidant activities of roselle extracts obtained with different duration. Notes: Values with different notations show significant differences from one another ($P < 0.05$).

typically takes anything between an hour to five days to finish. However, if lasting too long, extraction might unfavorably break down some compounds in the resulting extract (Cacique et al., 2020). Stirring during maceration is proposed to shorten the extraction time (Setyawan & Kartini, 2023).

While optimizing the duration (Table 2), the colors of extracts from the same cycle did not differ significantly from one another, but sharp variations were observed between durations. The extracts became more acidic as the duration and the number of cycles increased. The second and third cycles produced high TA when performed for 30 min instead of 60 or 90 min, which might be caused by larger amounts of anthocyanins that had already been extracted in the first cycle.

Cumulative TA from the three durations differed significantly ($P < 0.05$) (Fig. 2). Durations producing the highest to the lowest TA ($2.84 > 2.70 > 2.45$ mg/g) were in the following order: 90 min > 60 min > 30 min. A longer extraction allows more time for solvents and crude

drugs to interact and, consequently, increases the presence of anthocyanins in the extract, as confirmed by Ji et al. (Ji et al., 2012). However, based on IC_{50} , the first extractions performed in 60 and 90 min showed the most potent antioxidant activities equivalent to IC_{50} of 633.79 and 591.08 µg/mL, calculated based on the crude drug (Fig. 3). No significant difference was identified between the two ($P > 0.05$). Compared to the first cycle, the antioxidant properties were lower after the second and third cycles, as marked by higher IC_{50} values. From the cumulative TA and antioxidant activity in the first cycle, it can be inferred that the optimal duration for the upscaled SAE is 60 min.

3.3. Characteristics of concentrated extracts

Previous research has shown that vacuum evaporation (44 cmHg, 70 °C) produces concentrated dry roselle extract with higher total phenolic content and antioxidant capacity than atmospheric evaporation (90 °C) (Chumsri et al., 2008). In this study, the liquid extracts (first to third cycle) from each duration were combined and concentrated using a rotary evaporator, followed by heating with a water bath (Table 3). Extraction yields varied from 46.90 to 49.89 %, which meets the quality standard of no less than 19.1 %, according to the second edition of the Indonesian Herbal Pharmacopeia (IHP II) (Health, 2017). The high yields might be attributed to the different solvent from the one recommended by IHP II, a mixture of ethanol and hydrochloric acid (85:15). These results are in agreement with a previous work that obtained an extraction yield of 34.5 to 62.8 % (Pinela et al., 2019).

Concentrated extracts from the three durations had the same color, purplish-red. However, their TA and antioxidant activity were different, with the highest attained after 60 and 90 min of extraction. These results confirm the previous finding (Section 3.3), i.e., the optimal duration for upscaled SAE of roselle calyces is 60 min. With low moisture content, concentrated extracts guarantee good stability during storage.

3.4. Physical characteristics, TA, and antioxidant activities of powdered extracts from spray-drying and oven-drying

Water is the most common solvent used for anthocyanin extraction, although several food processing industries prefer alcoholic solutions (Khoo et al., 2017). However, storing roselle extracts in liquid forms is not recommended because this creates favorable conditions for microbial growth. While concentrated forms help improve the compounds' stability in the extract, applying them for food and medicine coloring can be difficult due to their consistency. Drying is thereby recommended to solve this problem.

Spray-drying and oven-drying made powders with different organoleptic characteristics. SD produced fine red powder (Fig. 4), with color intensity increasing as the amount of maltodextrin in the mixture decreased. On the contrary, with OD, coarser-textured powders were obtained.

SD yields were reduced with fewer concentrations of maltodextrin (matrix). The RE:MD ratios of 1:1, 1:0.43, and 1:0.25 presented 67.6, 53.2, and 23.6 % yields, respectively. Unlike SD, OD did not show a clear

Table 3
Characteristics of concentrated roselle extracts.

Duration (min)	Color	MC (%)	Yield (%)	TA (mg/g)	IC_{50} (µg/mL)*
30	Purplish-red	1.48	46.90	2.14 ± 0.04^a	468.40 ± 2.53^a
60	Purplish-red	1.38	48.91	3.17 ± 0.02^b	384.98 ± 19.61^b
90	Purplish-red	2.35	49.89	3.25 ± 0.10^b	368.95 ± 11.90^b

Notes:

TA (total anthocyanins) and IC_{50} are expressed as mean \pm SD ($n = 3$).

TA contents are based on the weight of the concentrated extract.

Means followed by different notations in the same column show significant differences from one another ($P < 0.05$).

* IC_{50} of the positive control (ascorbic acid) = 10.59 ± 0.50 µg/mL.

MC: moisture content.



Fig. 4. Visual characteristics of roselle extract powders after spray-drying and oven-drying with various extract-to-maltodextrin ratios. Notes: SD1, SD2, and SD3 show spray-drying results with the roselle extract-to-maltodextrin (RE:MD) ratios of 1:1, 1:0.43, and 1:0.25. OD1, OD2, and OD3 show oven-drying results with RE:MD ratios of 1:20, 1:10, and 1:5.

correlation between the yields and the RE:MD ratios. The 1:10 ratio produced the highest yield, followed by 1:20 and 1:5. In general, OD gave higher yields than SD. In SD, the extract and matrix mixture was put into a spray dryer and atomized through a nozzle. The heated drying gas evaporated water upon contact with the atomized material. Dried powders falling to the bottom of the dryer were collected in a collection jar (Fang & Bhandari, 2010). In practice, a lot of extract powder might be deposited onto the walls of the drying chamber and cyclone, making collecting the entire dried powder difficult. As a result, SD likely produces a lower yield than OD. SD1 and SD2 generated comparable yields to a previous study, 56.48–72.07 % (Cid-Ortega & Guerrero-Beltrán, 2020).

In instant powder goods, the moisture content is a good indicator of quality. Products of high quality are often associated with low moisture content (Vardin & Yasar, 2012). In SD, the derived moisture content can be undesirably high due to the low evaporation rate of the solvent and

ineffective heat exchange, which happen with a high feed flow rate that allows a very short contact time between the drying gas and the atomized particles (Tonon et al., 2008). Based on preliminary research, the extract and matrix mixture was sprayed at a flow rate of 8 mL/min.

With SD, the product's moisture content (1.31–4.58 %) increased with more proportion of maltodextrin in the ratio (Table 4). This result contradicts Nguyen et al., which found that a decrease in moisture content was associated with a higher concentration of maltodextrin (Nguyen et al., 2022b). Moisture contents in this research are similar to previous studies: 1.79–2.48 % (Cid-Ortega & Guerrero-Beltrán, 2020), 3.0–5.0 % (Gonzalez-Palomares et al., 2009), 6.5–12.1 % (Nguyen et al., 2022a), and 7.66–10.19 % (Nguyen et al., 2022b). Besides feed rate, this variable is also shaped by filler type, extract-to-filler ratio, and inlet temperature. As for OD, the powders obtained had a moisture content of 3.07–4.47 %. Therefore, it can be concluded that SD and OD create powders with appropriate moisture content, except for SD3 with the RE:

Table 4
Characteristics of roselle extract powders from spray-drying and oven-drying.

Sample	Yield (%)	MC (%)	Organoleptic properties	TA (mg/g)		IC ₅₀ (μg/mL)*	
				Bulk	Native	Bulk	Native
SD1	67.6	4.58	Light red, fine, slightly hygroscopic, slightly sticky, clump-free powder	1.08 ±0.02 ^a	2.15±0.04 ^a	407.13±54.51 ^a	203.56 ±27.25 ^a
SD2	53.2	3.92	Light red, fine, slightly hygroscopic, slightly sticky, easily clumped powder	1.47 ±0.03 ^b	2.10±0.05 ^a	ND	ND
SD3	23.6	1.31	Dark red, slightly coarse, hygroscopic, very sticky, easily clumped powder	1.85 ±0.01 ^c	2.32±0.01 ^b	ND	ND
OD1	90.8	4.47	Light red, fine, not hygroscopic, not sticky, clump-free powder	0.06 ±0.00 ^d	1.24±0.01 ^c	ND	ND
OD2	92.0	3.20	Light red, coarse-textured, not hygroscopic, not sticky, clump-free powder	0.12 ±0.00 ^e	1.29±0.02 ^d	ND	ND
OD3	88.0	3.07	Light red, coarse-textured, slightly hygroscopic, slightly sticky, clump-free powder	0.21 ±0.01 ^f	1.27±0.03 ^{c,d}	1835.50 ±40.01 ^b	305.92±6.67 ^b

Notes:

TA (total anthocyanins) and IC₅₀ are expressed as mean±SD (n = 3).

TA and IC₅₀ are based on the weight of the dried extract: bulk and native.

Means followed by different notations in the same column show significant differences from one another (P<0.05).

* IC₅₀ of the positive control (ascorbic acid) = 10.59±0.50 μg/mL.

ND: not determined.

MD ratio of 1:0.25. SD3 powder had a very low moisture content (1.31 %) and was stickier than other samples.

Produced with several RE:MD ratios, spray and oven-dried powders showed a significant reduction in TA ($P < 0.05$) when the proportion of maltodextrin in the mixture increased (Table 4) because maltodextrin dilutes anthocyanins. TA of the bulk extract obtained from SD varied between 1.08 and 1.85 mg/g, while TA from OD was 0.06–0.21 mg/g. TA contents of oven-dried powder were 8.8 to 18x lower than those of spray-dried powder because OD used higher RE:MD ratios. However, when calculated based on the native extract, the TA contents were 2.10–2.32 mg/g for SD and 1.24–1.29 mg/g for OD, meaning that the former produced two times higher anthocyanins. Compared to OD, the drying process in SD is run at a higher temperature, but the material is exposed to heat in a shorter time, creating products with better compound stability. Prolonged contact with heat can decompose anthocyanins from aglycones to chalcones (Nasrullah et al., 2020). In conclusion, SD1 and OD3 formulas produce the best extracts for each drying method.

In addition to anthocyanins as the main component, roselle calyces also contain various other phenolics in high concentrations. Therefore, it is necessary to evaluate the effects of drying methods and conditions on the dried extract's antioxidant activity to maintain its pharmacological properties. Results show that SD1 had significantly higher antioxidant activity than OD3 ($P < 0.05$), as evident in IC_{50} of 203.56 and 305.92 $\mu\text{g/mL}$, respectively (calculated based on the weight of the native extract).

Overall, the IC_{50} value of liquid, thick and dry roselle extract obtained in this study was higher than the IC_{50} value of ascorbic acid (Table 1, Table 2, Table 3, Table 4). This makes sense because ascorbic acid is a pure compound commonly used as a standard in antioxidant assay. On the other hand, roselle extract is composed of various compounds that either have antioxidant activity or not. In this study, ascorbic acid was used to validate the testing method carried out, so that the IC_{50} value was not compared with rosella extract. The IC_{50} value of roselle extract obtained in this study is in accordance with the previous research, which found that the IC_{50} value of liquid and thick roselle extract is 44,770–50,480 $\mu\text{g/mL}$ and 47,530–52,940 $\mu\text{g/mL}$, respectively (Chumsri et al., 2008). Other researchers concluded that the IC_{50} value of roselle extract was 4060 $\mu\text{g/mL}$ (Wu et al., 2018).

3.5. Color stability of powdered roselle extracts from spray-drying and oven-drying at various pH levels

Color is an important parameter in food products as it influences not only the overall quality but also the buyer's decision (Gerard & Roberts, 2004). The pH of the products' surrounding determines if roselle extracts' distinctive red to purple color can be maintained during storage. Therefore, testing the effect of pH on the appearance of anthocyanins is needed to determine the extract's suitability for coloring food and

medicinal products with different acidity levels. SD1 powder (1:1 ratio) showed that the intensity of L^* (lightness) increased at pH of 2.6–4.0, declined at pH of 5.0, and rose again at pH of 6.0–7.0 (Fig. 5). A similar pattern was observed in OD3 powder (1:5 ratio) (Fig. 6). The intensity of redness (a^*) and yellowness (b^*) of the two samples seemed to decrease with an increase in pH.

Visual observations found color changes from dark red at pH = 2.6 to light red at pH = 4.0 and to brownish-red at pH = 5.0–7.0 (see inset pictures in Fig. 5 and Fig. 6). In acidic environments, anthocyanins appear as red flavylium cations at pH < 4.0 and are hydrolyzed at glycosidic bonds at pH = 4.0, opening aglycone rings to form various labile aglycones and colorless carbinol and chalcone groups (Khoo et al., 2017). In conclusion, spray-dried and oven-dried powders maintain their redness intensity in acidic environments and can thus be used as a natural dye for food and medicinal products with pH = 2.6–4.0.

3.6. Morphology, crystallinity, and particle size distribution of powdered roselle extracts

In addition to measuring antioxidant activity and stability at various pH levels, the best formulas in SD (i.e., SD1) and OD (OD3) were also used to determine the powdered extract's morphology, crystallinity, and particle size and its distribution. SD1 powder observed with scanning electron microscopy (SEM) showed spherical particles with a smooth surface and visible bridges and voids between particles, indicating agglomeration (Fig. 7a). Meanwhile, OD3 powder had equant or cube-shaped particles, nearly homogenous thickness and width, and no agglomeration (Fig. 7b). However, the particles showed an uneven surface with some visible cracks. Intergranular voids in SD1 powder are caused by air incorporated into liquid droplets during spraying or liquid atomization. Atomization in SD is responsible for smaller particle sizes than the oven-dried powder. Moreover, the very fine size of the particles is distributed uniformly, which accelerates the drying process (Fang & Bhandari, 2010). The equant shape and cracks on the surface of the OD3 particles can be attributed to the manual grinding of the oven-dried powder using a mortar and a pestle.

The XRD analysis provides information on the amorphous and crystalline structures of extracts, matrices, physical mixtures of extracts and matrices, and their drying results. The diffractogram shows that the pure roselle extract (RE) had a wide peak at 2θ 27.6947°, indicating amorphous extract (Fig. 8 and Table 5). Meanwhile, maltodextrin (MD) had two broad peaks at 2θ 10.9533° and 27.4653°, suggesting that this matrix tended to have an amorphous structure. In the physical mixture (RE-MD), two peaks similar to both components were observed at 2θ 11.2591° and 26.1658°, illustrating no interaction in the physical mixture of roselle extract (8°Bx) and maltodextrin before drying. After SD, the peaks of both materials did not appear on the diffraction pattern of the physical mixture RE-MD_(SD). Instead, there was a new wide peak at

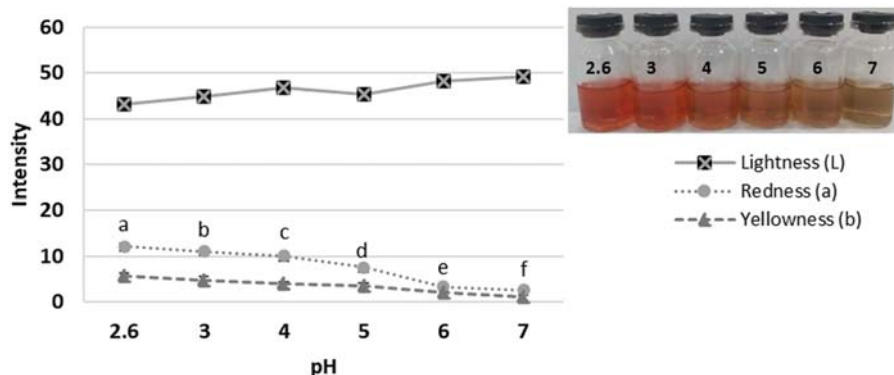


Fig. 5. Color stability of powdered roselle extracts from SD1 at various pH levels. Notes: Degrees of intensity with different notations on the redness line (a^*) show significant differences from one another ($P < 0.05$). SD1: spray-drying with the 1:1 RE:MD ratio.

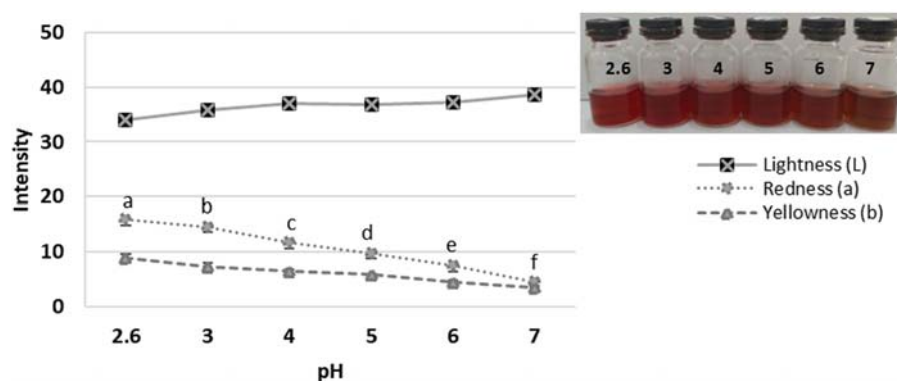


Fig. 6. Color stability of powdered roselle extracts from OD3 at various pH levels. Notes: Degrees of intensity with different notations on the redness line (a*) show significant differences from one another ($P < 0.05$). OD3: oven-drying with 1:5 RE:MD ratio.

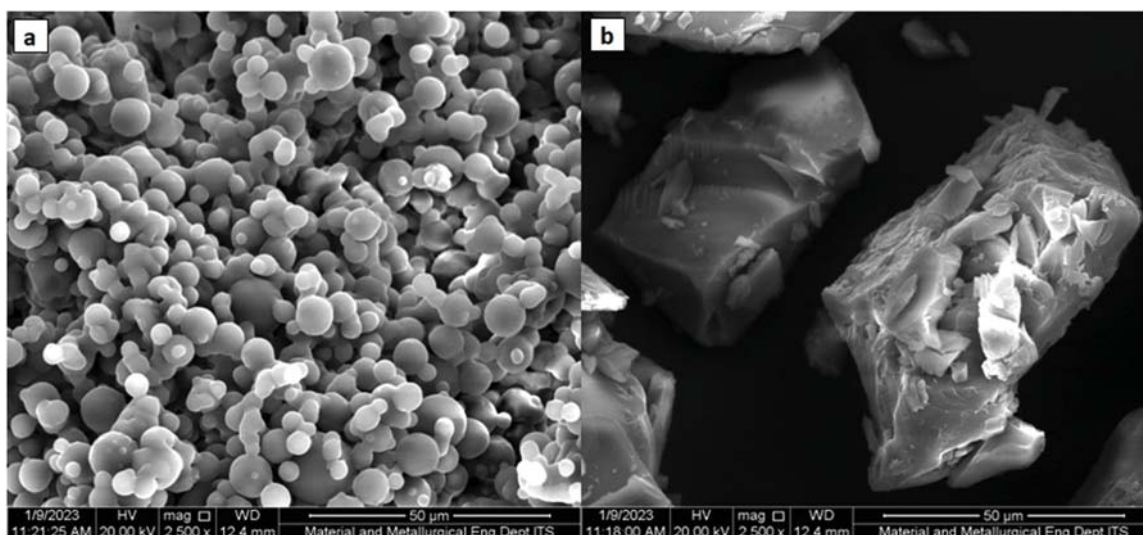


Fig. 7. Micrographs of powdered roselle extract from spray-drying with the SD1 formula (a) and oven-drying with the OD3 formula (b) at 2500x magnification.

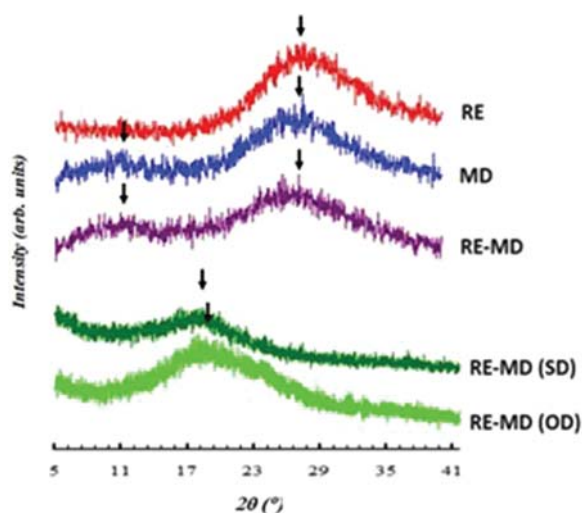


Fig. 8. Diffractograms of pure roselle extract (RE), maltodextrin (MD), physical mixture of roselle and maltodextrin (RE-MD), spray-dried roselle powder (RE-MD_(SD)), and oven-dried roselle powder (RE-MD_(OD)).

2θ 19.8800°, suggesting an extract-maltodextrin interaction and amorphous powder. The same case was true for the oven-dried powder RE-MD_(OD), with one broad peak at 2θ 18.5619°. Similar XRD results of

Table 5

X-ray diffraction profiles of roselle extract.

Material	Peak th	Peak position (° 2θ)	Peak height (cts)	Relative intensity (%)
Roselle extract (RE)	1	27.6947	41.48	100
Maltodextrin (MD)	1	10.9533	101.43	100
	2	27.4653	54.60	53.83
Roselle extract- maltodextrin physical mixture (RE-MD)	1	11.2591	100.32	56.66
	2	26.1658	56.84	100
Spray-dried roselle powder (RE-MD _(SD))	1	19.8800	47.52	100
Oven-dried roselle powder (RE-MD _(OD))	1	18.5619	76.94	100

powdered roselle extract obtained under various conditions of SD have been reported. Amorphous particles are also observed in mango powder, produced by dehydrating mango juice using SD with different matrices (Cano-Chauca et al., 2005; Navidad-Murrieta et al., 2020). Amorphous forms are the highest energy forms of solid materials that do not have long-range molecular order. Due to their high internal energy, amorphous materials generally have greater molecular movement and better thermodynamic properties compared to the crystalline state, resulting in higher solubility (Hancock & Parks, 2000; Laitinen et al., 2013).

Besides moisture content, shape, and particle surface morphology, the two other factors influencing material flow rate are particle size and

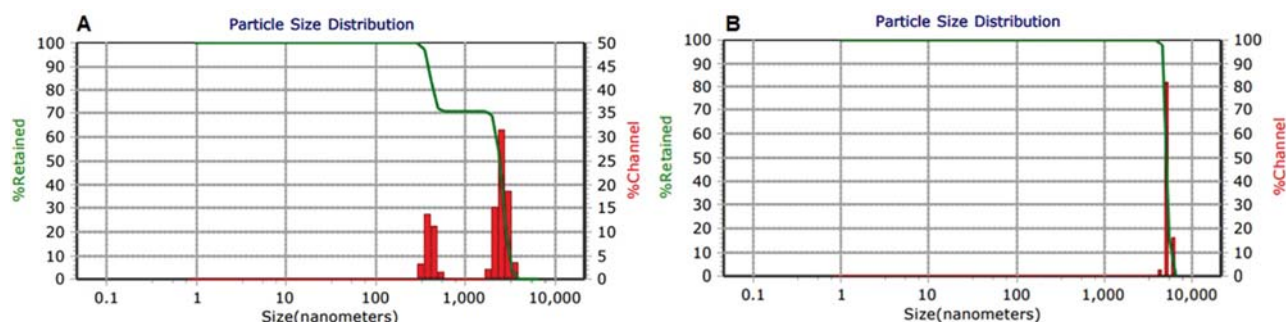


Fig. 9. Particle size profiles of spray-dried roselle powder (A) and oven-dried roselle powder (B) determined with a particle size analyzer (PSA).

Table 6

Particle size and polydispersity index of spray-dried and oven-dried roselle powders.

Sample	Particle size (μm, avg.)	Polydispersity index
SD1	1.951	0.398
OD3	5.160	0.00469

its distribution (Liu et al., 2008). A particle size analyzer (PSA) was used at 25 °C and an angle of 90°, with an overall sizing range of 0.0001–10 μm. SD1 and OD3 powders showed an average size of 1.951 and 5.160 μm with polydispersity index (PDI) of 0.398 and 0.00469 (Fig. 9 and Table 6). PDI is a measure of homogeneity (or heterogeneity) based on particle size. The size distribution of the powder is homogenous if PDI < 0.7 and heterogenous if PDI > 7. Results show SD1 and OD3 powders had homogeneously distributed microparticles (1–1000 μm), with SD1 producing smaller particles and OD3 powder showing more homogeneity (Table 6)—the latter results from manually grinding oven-dried powder using a mortar and a pestle.

4. Conclusions

Roselle petals are a promising source of natural dyes for food and medicinal products. The red-purple pigment, anthocyanin, can be extracted on a large (industrial) scale by stirring-assisted extraction (SAE) with the following optimal conditions: 50 % ethanol acidified with 2 % citric acid as the solvent, an S/L ratio of 1:15 g/mL, three cycles of extraction, and a duration of 60 min for each cycle. Roselle extracts concentrated by rotary evaporation contain 1.38–2.35 % moisture, which can thus be used as an alternative form of storage. However, the extracts should be converted into dry powder for quick application as a natural dye. Spray-drying using maltodextrin as matrix has proven effective in producing dry powder with more favorable characteristics than oven-drying. With the 1:1 extract-to-maltodextrin ratio, the resulting dry powder has good features: light red color, amorphous structure, spherical morphology, homogeneous particle size with an average of 1.951 μm, total anthocyanin content of 2.15 mg/g, IC₅₀ of 203.56 μg/mL (antioxidant activity), and color stability at pH 2.6–4.0.

Author contribution

K.K., R.N. conceptualized the research, designed the methodology, acquired funding, provided resources, and supervised the work; M.B.H., Z.M.H. and N.S. conducted the data curation, analyzed the data, verified and visualized the results, did the research administration, and discussed the data; K.K. drafted the manuscript; K.K., R.N. carried out review, editing, and critical revision of manuscript.

Ethical statement

This article does not contain any studies with human participants or

animals performed by any of the authors.

Declaration of Competing Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

No data was used for the research described in the article.

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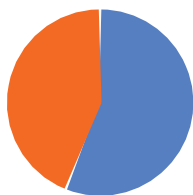
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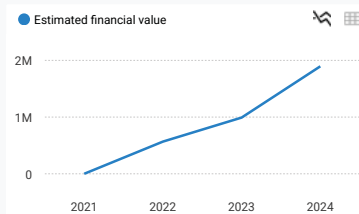
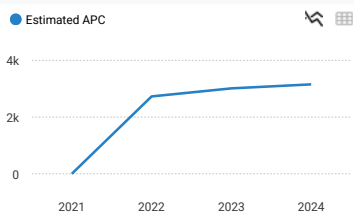
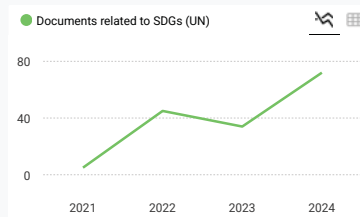
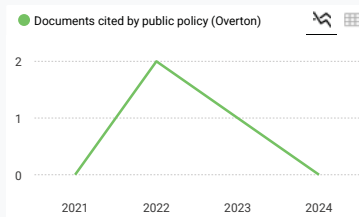
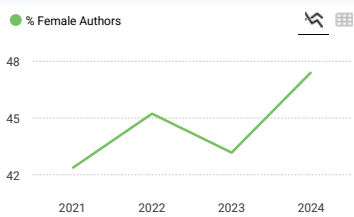
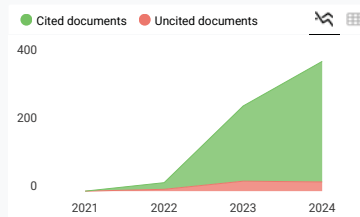
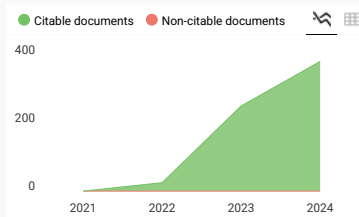
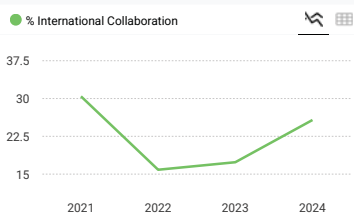
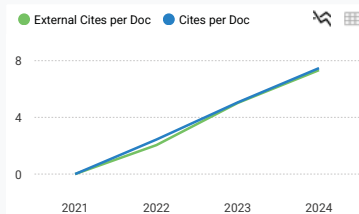
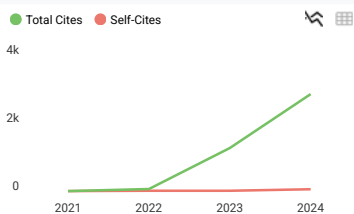
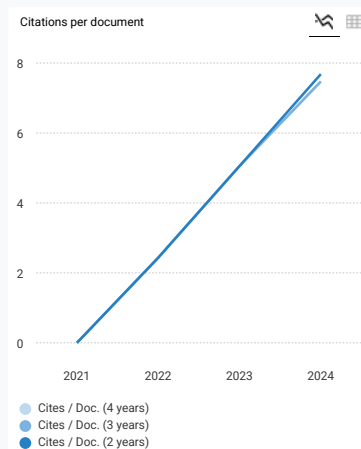
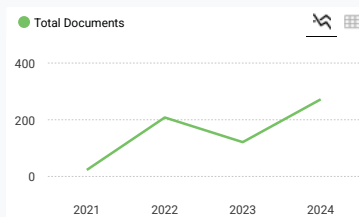
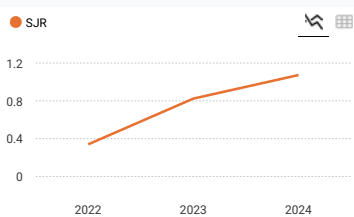
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