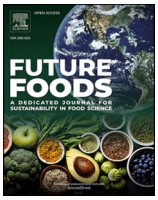
Future Foods 9 (2024) 100300

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



Microencapsulation of roselle (*Hibiscus sabdariffa* L.) anthocyanins: Effects of maltodextrin and trehalose matrix on selected physicochemical properties and antioxidant activities of spray-dried powder

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ARTICLE INFO

Keywords:
Maltodextrin matrix
Microencapsulation
Natural colorant
Roselle anthocyanins
Spray-drying
Trehalose matrix

ABSTRACT

This research was conducted to improve the stability of roselle anthocyanins by spray-drying using a single matrix (maltodextrin) and a binary matrix (maltodextrin-trehalose = 1:1). Each matrix was added to the core material at three different ratios: 30:70, 40:60, and 50:50. The physicochemical characteristics and antioxidant activity of microencapsulated powders were compared. The process using a single matrix with a 50:50 maltodextrin-to-extract ratio (MD-3) was identified as optimal. Spray-drying yield of MD-3 was 73.6 %, significantly higher ($P < 0.05$) than the other formulas. Encapsulation efficiency of MD-3 was 50.28 % and the powders was light red with a non-sticky texture, 2.5 % moisture content, and its red color was stable at pH 2.6–5. Average particle size of MD-3 was 4.48 μm with homogeneous distribution (PDI = 0.41400), spherical and amorphous particles. In the DPPH scavenging method, MD-3 powders exhibited antioxidant activity with an IC_{50} of 3397.55 μg/mL. Although the addition of trehalose to maltodextrin in the binary matrix did not produce better physicochemical characteristics, it significantly ($P < 0.05$) improved encapsulation efficiency (66.65–76.53 %). Therefore, further exploration is necessary to determine the appropriate maltodextrin-to-trehalose ratio in microencapsulating roselle extract. These findings enable further industrial application of different matrices to encapsulate anthocyanin as well as other bioactive compounds.

Introduction

Food and pharmaceutical additives are added to edible products or drug preparations to improve their appearance, taste, and texture. Based on function, additives can be categorized into colorants, preservatives, sweeteners, or thickeners, among others. Colorants, one of the most universal additives used in food and pharmaceutical products, can be sourced naturally or synthetically. Synthetic dyes are used more extensively, even though many problems have been associated with increasing utilization, including misuse, excessive application, and toxicity (Wu et al., 2022). It is, meanwhile, recommended to use natural dyes extracted from certain plant parts, which prove empirically safe for human health (Luzardo-Ocampo et al., 2021). Roselle (*Hibiscus sabdariffa* L.) is a plant that gives red color because the calyces contain anthocyanins (red pigment). Anthocyanins have double purposes; other than providing a source of natural dye, they also exhibit certain biological activities, e.g., antioxidant, antibacterial, antihypertensive, and antidiabetic (Borrás-Linares et al., 2015; Hapsari and Setyaningsih, 2021; Jabeur et al., 2017).

Like most other natural compounds, anthocyanins have limitations. The stability of these pigments vary across types and is susceptible to slight changes in environmental pH, light exposure, temperature, metal ions, oxygen levels, and enzymes (Khoo et al., 2017). Microencapsulation has been proposed as an appropriate strategy to increase stability. This technique "entraps" a core material (active compound) in a matrix (carrier) made of another material to create particles sizing a few micrometers to a few nanometers. The particles or droplets are surrounded by walls (i.e., a coating) or embedded in a homogeneous or heterogeneous matrix, forming tiny capsules (Nedovic et al., 2011; Nguyen et al., 2022). Some microencapsulation methods are spray-drying, freeze--drying, and coacervation (Özkan and Bilek, 2014; Piñón-Balderrama et al., 2020). In spray-drying, the core material is dissolved or suspended in a polymer solution, which is pumped to a nozzle and atomized into fine droplets. The droplets accumulating in the drying chamber are

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https://doi.org/10.1016/j.fufo.2024.100300
Received 28 October 2023; Received in revised form 3 January 2024; Accepted 10 January 2024
Available online 12 January 2024
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vaporized upon contact with hot gas, leaving the core material trapped in the matrix and forming dry particles (Khandbahale, 2020). The method can be used for non-heat-resistant compounds because it applies short contact with heat and a relatively low drying temperature (the inlet temperature is generally not greater than 200 °C) (Idham et al., 2012; Lu et al., 2021).

Most of the properties of microencapsulated powders are related to the structural properties of the matrix. Therefore, matrix selection is an important factor influencing the properties of microcapsules. Ideally, materials used as a matrix or coating must have low viscosity, be able to disperse or emulsify and have a high capacity to trap the encapsulated active ingredient, be non-reactive to it during drying and storage, and be able to provide maximum protection to the active ingredient against environmental exposure (oxygen, heat, light, and humidity) (Piñón-Balderrama et al., 2020). In addition to a single matrix, a mixture of two matrices (binary matrix) has also been widely applied in the microencapsulation process and has been proven to be able to improve the characteristics of microencapsulated powders (Archaina et al., 2019; Cid-Ortega and Guerrero-Beltrán, 2020; Idham et al., 2012; Nguyen et al., 2022; Sarabandi et al., 2017; Younesi et al., 2023). Anthocyanins are hydrophilic compounds and, therefore, compatible with matrices derived from carbohydrates, proteins, and polymers. Maltodextrins of the carbohydrate group (oligosaccharides) are commonly used as a microencapsulation matrix because they are heat-resistant and with a large molecular weight (900–9000)—prerequisites for forming a good coating (Rowe et al., 2009). Despite to high availability, ease of application and safety, low cost, better solubility, neutral flavor, and low viscosity, they lack surface activity and provide weak film-forming ability (Mahdavi et al., 2014). Thus, supplementary matrix such as trehalose opens up new possibility to overcome these disadvantages. Trehalose is a disaccharide formed by a 1,1-glycosidic bond between two α-glucose units, which can interact intensively with the surface of macromolecules due to its great associativity and flexibility. Compared with other disaccharides, trehalose has a higher glass transition temperature (Tg, up to 120 °C) and is less hygroscopic, and these properties make trehalose an ideal protective agent for spray dried products (Zhang et al., 2021). Moreover, previous studies found that trehalose played an important role in preventing the encapsulated components from agglomeration and degradation, and improving the redispersibility of powders (Lim et al., 2016; Lim and Roos, 2017).

There has been no research combining maltodextrin and trehalose as a matrix in the microencapsulation of roselle calyx extract. Therefore, the objective of this study was to improve the stability of roselle anthocyanins by spray-drying using a single and a binary matrix. In this study, we compared the physicochemical characteristics and antioxidant activities of roselle extract-microencapsulated powder with a single matrix, i.e., maltodextrin (MD), and a maltodextrin-trehalose binary matrix (MD/TH at a ratio of 1:1). Both single and binary matrices were added to the core material at three matrix-to-extract ratios: 30:70, 40:60, and 50:50. Adding trehalose to maltodextrin when microencapsulating roselle extract by spray-drying is expected to increase the encapsulation efficiency and improve the characteristics of the resulting powder. This research lays the stepping stone to accelerating the application of natural dyes in the food and pharmaceutical industries.

Materials and methods

Plant materials and chemicals

In April 2021, samples of purple roselle calyces were manually collected from a plantation in Magetan Regency (Indonesia). After the authentication by the Center for Traditional Medicine Information and Development (PIPOT, University of Surabaya), the samples were air-dried, ground into fine powder using a blender (Philips HR 222, Amsterdam), and passed through a 20-mesh screen. The laboratory chemicals used were citric acid monohydrate, disodium phosphate (Na_2HPO_4), ethanol, hydrochloric acid (HCl), potassium chloride (KCl), sodium acetate (NaOAc), and tartaric acid ($C_4H_6O_6$) from Merck KGaA, (Germany); 2,2-diphenyl-1-picryhydrazyl (DPPH) and ascorbic acid from HiMedia Laboratories (Pennsylvania, USA); food-grade trehalose from tapioca (Lot No. 2A161, Niaga Kimia Raya) (Indonesia); food-grade maltodextrin (DE10–12) from Qingdao Shengda Commercial & Trade Co., Ltd. (China); and Aqua DM or demineralized water from the Laboratory of Chemistry, University of Surabaya (Indonesia).

Preparation of roselle extract

Roselle calyx powder was extracted by stirring-assisted extraction (SAE) using the procedure from (Kartini et al., 2023). This process used the crude drug-to-solvent ratio of 1:15 g/mL. The solvent used was 50 % ethanol acidified with 2 % citric acid. Three cycles of extraction were performed, each with 30 min of stirring using an overhead stirrer (IKA RW 20; IKA-WERKE, Staufen, Germany) at 500 rpm. The extract obtained was then evaporated at 50 °C under reduced pressure (11.7 cmHg) using a rotary evaporator (Buchi R-300; BÜCHI Labortechnik AG, Flawil, Switzerland). Evaporation was carried out until a concentrated extract was produced with a total solid of 8°Bx (pocket digital refractometer PAL-alpha, Atago Co., Ltd., Japan).

Preparation of microencapsulation system

Anthocyanins from the roselle extract were microencapsulated using a single matrix (maltodextrin) and a maltodextrin-trehalose binary matrix (1:1) with three matrix-to-extract ratios (30:70, 40:60, and 50:50). The microencapsulation system was made referring to (Idham et al., 2012). The processes for each matrix were as follows: one gram of the matrix was gradually added to the roselle extract (8°Bx) according to the appropriate ratio every 2 min while stirred using a magnetic stirrer (Cimarec from Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 40 °C until all ingredients were dissolved. The mixture was then homogenized using a T25 digital ULTRA-TURRAX (IKA-Werke, Staufen, Germany) by stirring at a speed of 14,000 rpm for 1 hour at room temperature. Afterward, it was dried using a Buchi Mini spray dryer (BÜCHI Labortechnik AG) with inlet and outlet temperatures of 120 °C and 85 °C, respectively, and a flow rate of 10 mL/min.

The dried microencapsulated powder was then stored in a dark glass bottle, closed tightly with a lid, and added with silica gel. Then, its total anthocyanin content, encapsulation efficiency, and physicochemical characteristics (including yield, organoleptic properties, moisture content, color stability at various pH levels, particle size, and particle size distribution) were evaluated. The best microencapsulated powder from each single and binary matrix was further observed to determine the physicochemical characteristics (morphology and crystallinity) and tested for antioxidant activity.

Characterization of roselle extract-microencapsulated powders

Yield calculation and organoleptic evaluation

Yield (%) or recovery was calculated as the ratio between the weight of the microencapsulated powder obtained from spray-drying and the total weight of solids in the extract plus the weight of the matrix. Organoleptic properties observed were the color and texture of the microencapsulated powders.

Quantification of moisture content

The microencapsulated powder was weighed before heating (initial weight, W_0) and after heating at 105 °C for 1.5 min (W_1). The moisture content (MC) was read using an Ohaus MB 120 moisture analyzer (New Jersey) and manually calculated as the percentage of the difference between W_0 and W_1 divided by W_1 or expressed as $\%MC = (W_0 - W_1)/W_1 \times 100\ \%$.

Determination of total anthocyanin (TA) and encapsulation efficiency (EE)

Encapsulation efficiency (EE) was calculated as a function of total anthocyanin (TA) and surface anthocyanin (SA), as written below:

$$EE\ (\%) = \frac{TA - SA}{TA}\ x\ 100\%$$

TA and SA were determined spectrophotometrically using the pH differential method (Lee et al., 2005). The sample for TA measurement was prepared using these steps: 750 mg of the microencapsulated powder was added with 2 mL of distilled water and then crushed using a mortar and pestle to rupture the microencapsulation membrane. The mixture was then transferred into a test tube, added with 13 mL of ethanol, homogenized using a Maxi Mix II vortex mixer (Thermo Fisher Scientific, Waltham, Massachusetts) for 5 min, and then filtered. TA was then determined from the collected filtrate. Meanwhile, to quantify SA, the sample was prepared as follows: 750 mg of microencapsulated powder was added with 15 mL of ethanol and homogenized using a vortex for 30 s. The mixture was then centrifuged (Hettich Zentrifugen Rotofix 32A, Massachusetts, USA) at 3000 rpm for 10 min. The supernatant was filtered with a 0.22 μm PTFE membrane filter (ANPEL Laboratory Technologies Inc., Shanghai, China). Finally, SA was quantified from the collected filtrate.

For the next step, 5 mL of the sample was combined with 5 mL of KCl buffer (pH 1.0) to create solution I, and 5 mL of the same sample was added with 5 mL of NaOAc buffer (pH 4.5) to make solution II. The absorbance of each solution was measured at 520 and 700 nm using a Shimadzu UV-1900 UV–Vis spectrophotometer (Kyoto, Japan). To calculate the final absorbance (A), the absorbance values of the two solutions read at different wavelengths were inputted into this formula $A = [(A_{520} - A_{700})\ pH_{1.0} - (A_{520} - A_{700})\ pH_{4.5}]$. TA or SA (cyanidin-3-glucoside equivalents, mg/g) is a multiplication of final absorbance (A), molecular weight of cyanidin-3-glucoside (MW = 449.2 g/mol), dilution factor (DF = 2), sample volume after preparation ($V = 0.015$ L), and the g-to-mg conversion factor (10^3) divided by the coefficient molar extinction of cyanidin-3-glucoside (ε = 26,900 L.mol^{-1}.cm^{-1}), cuvette width (l = 1 cm), and the sample's weight (W = 0.75 g), as written below:

$$TA\ or\ SA\ (mg/g) = \frac{A \times MW \times DF \times V \times 10^3}{\varepsilon \times l \times W}$$

Color stability testing at various pH levels

This analysis dissolved 100 mg of the microencapsulated powder in 5.0 mL of citrate-phosphate buffer solution at six pH levels (2.6, 3.0, 4.0, 5.0, 6.0, and 7.0). The color intensity of this mixture was measured using a CR-10 Plus color reader (Konika Minolta, Inc., Tokyo, Japan) and described in three parameters: lightness, redness, and yellowness.

Granulometry and measurements of particle morphology and crystallinity

The microencapsulated powder was physically characterized to determine particle size and size distribution with a nanoparticle size analyzer (NANOTRAC WAVE II, Microtrac, Germany), morphology with a scanning electron microscope (FEI Inspect S50), and crystallinity with X-ray diffraction (Kartini et al., 2023). Encapsulates were analyzed in a diffractometer (X'pert Philips, Netherlands). Target conditions in the analysis were Cu, Kα filter, with a voltage of 40 kV and 15–30 mA, carried out at 2θ = 5–40° (0.2–0.5°/minute) (Navidad-Murrieta et al., 2020).

DPPH assay for antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method was employed to measure the antioxidant activity of the roselle extract-microencapsulated powder (Kartini et al., 2019). Solutions of the microencapsulated powder and the reference compound (trolox) were prepared separately into a series of five dilutions. First, 100 μL of each solution was pipetted into a 96-well microplate (clear polystyrene) and homogenized with 50 μL of 0.026 % DPPH solution in ethanol. Then, after 10 min of incubation in a dark room, the absorbance of the mixture was measured at 517 nm with a UVM 340 microplate reader (Biochrom, Cambridge). Besides the above solutions, a blank sample was also made by combining 100 μL of the sample of each diluted concentration with 50 μL of ethanol. Also, a control solution was prepared by mixing 50 μL of DPPH solution with 100 μL of ethanol (control absorbance). The absorbance values of the sample (A_s), blank sample (A_{bl}), and control (A_c) were inputted into the equation of the percent inhibition below to determine the powder's DPPH scavenging activity:

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

Afterward, a regression linear ($y = bx + a$) or logarithmic equation ($y = b\ \ln(x) + a$) linking percent inhibition (y) to diluted concentration (x) was generated to determine the sample's IC_{50}, i.e., the amount of a particular active substance required to hamper a biological process by 50 %. The sample's IC_{50} indicates its potency in inhibiting the activity of DPPH or free radicals.

Statistical analysis

Depending on data distribution, variance, and the number of compared groups, the data were analyzed using Kruskal-Wallis, one-way ANOVA, or student *t*-test (α= 0.05) in SPSS 26 (SPSS Inc., Illinois). This analysis was followed by the post-hoc Mann Whitney U or Tukey's test to identify whether or not the mean values showed a statistically significant difference ($P < 0.05$).

Results and discussion

Formation of microencapsulation systems

The XRD analysis ensures that the microencapsulation system for the roselle calyx extract has been formed. It was conducted on the microencapsulated powder made with the 40:60 matrix-to-extract ratio. As a control, XRD analysis was also performed on the 8°Bx roselle extract (RE), maltodextrin (MD), trehalose (TH), and the physical mixtures of the extract and the matrix (RE-MD and RE-MD/TH). XRD was set at a shooting angle ~2θ and a scanning distance of 5–40° The diffraction profiles reported in Fig. 1 and Table 1 show that the roselle extract (RE) had one wide peak at 2θ 27.6947°, suggesting an amorphous solid. Maltodextrin (MD) had two broad peaks at 2θ 10.9533° and 54.60° (amorphous), while trehalose (TH) had many sharp peaks, with the highest at 2θ 23.9272° (crystalline). The diffraction pattern of the RE-MD mixture shows two specific angles at 2θ 11.2591° (RE) and 26.1658° (MD), meaning the roselle extract and the matrix in the mixture are still separate. Similarly, the RE-MD/TH mixture appeared as two peaks with close positions to those of the extract and pure maltodextrin, namely at 2θ 10.0360° and 26.7773°

The powder microencapsulated with a single matrix, maltodextrin (ME-MD), showed no overlapping peaks with RE and MD. However, a new peak was formed at 2θ 18.2920°, identified as the MD matrix that is bound to RE. Therefore, it can be concluded that the roselle extract is successfully microencapsulated in maltodextrin. This was also found in the diffraction pattern of the powder microencapsulated with a maltodextrin-trehalose binary matrix (ME-MD/TH), where peaks of extract, maltodextrin, and trehalose were no longer detected. Instead, a new broad peak appeared at 2θ 18.2929° Also, the sharp peak of trehalose did not appear in the microencapsulated powder, suggesting a transformation from crystalline to amorphous structure after spray-drying.

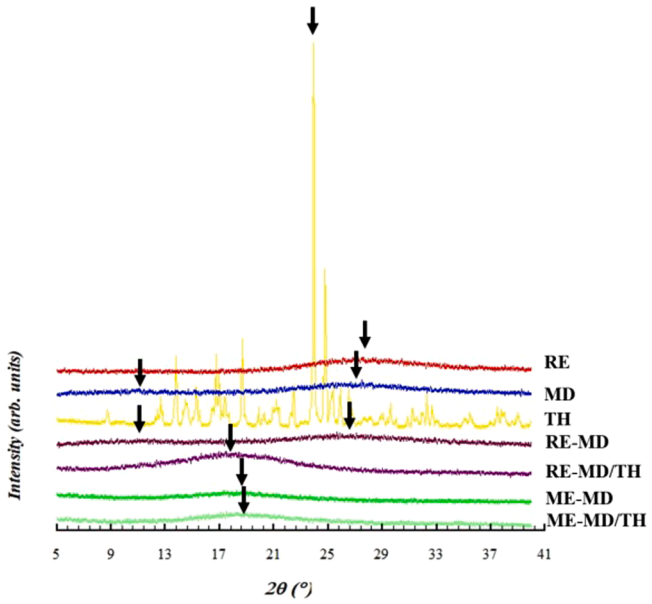

Fig. 1. Diffractograms of the roselle extract (RE), maltodextrin (MD), trehalose (TH), the physical mixture of RE and MD (RE-MD), the physical mixture of RE and MD/TH (RE-MD/TH), and the roselle extract-microencapsulated powders with a single matrix (ME-MD) and a binary matrix (ME-MD/TH).

Table 1
X-ray diffraction profiles of the roselle extract-microencapsulated powders and their compositions.

Material	Peak, *i* th	Position (°2θ)	Height (Cts)	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
Trehalose (TH)	1	23.9272	7600.92	100
Physical mixture of RE and MD (RE-MD)	1	11.2591	54.60	53.83
	2	26.1658	100.32	100
Physical mixture of RE and MD/TH (RE-MD/TH)	1	10.0360	104.82	100
	2	26.7773	55.21	52.67
Microencapsulated powder with the MD matrix (ME-MD)	1	18.2920	58.83	100
Microencapsulated powder with the MD/TH combination matrix (ME-MD/TH)	1	18.2929	56.21	100

Physical characteristics, yields, and moisture contents of microencapsulated powder

Fig. 2 and Table 2 show the visual characteristics of roselle extract powders microencapsulated with MD and MD/TH matrices. With a higher matrix-to-extract ratio, the microencapsulation produced lighter red and less sticky powder. Maltodextrin and trehalose are white fillers, which can reduce the powder's redness. MD/TH-1 powder was dark red, quite different from the other powders. This can be attributed to a higher concentration of the core material (roselle extract) and caramelization of the trehalose matrix during spray drying (Lu et al., 2021).

Yield and encapsulation efficiency quantitatively measure whether a matrix is suitable for microencapsulation, which can reflect the degree of surface attachment of the core material and the quality of the resulting powder (Lu et al., 2021). Table 2 illustrates a positive correlation between the matrix concentration and the yield. The yields were in the range of 63.2–73.6 % for the use of an MD matrix and were lower, 44.4–68.0 %, for an MD/TH binary matrix. From these data, it can be inferred that adding trehalose to maltodextrin to create a binary matrix does not increase yield. The MD/TH-1 formula produced the lowest yield because a lot of the highly hygroscopic powder adhered to the walls of the drying tube and the cyclone during spray-drying and was difficult to remove.

Moisture content describes the quantity of water contained in a material and it is related to the process of production of the microcapsules. Moisture analysis is commonly used during the formulation, processing, and testing of food and pharmaceutical products, especially instant powders. Good-quality products tend to have low moisture content (Sulieman, 2014; Vardin and Yasar, 2012). The moisture contents of MD-1, MD-2, and MD-3 were 2.3 %, 3.3 %, and 2.5 %, while those of MD/TH-1, MD/TH-2, and MD/TH-3 were 1.2 %, 2.3 %, and 3.1 %. These numbers are consistent with the moisture contents of the roselle extract-microencapsulated powders in (Cid-Ortega and Guerrero-Beltrán, 2020), 1.79–2.48 %, and (Gonzalez-Palomares et al., 2009), 3.0–5.0 %. Also, they suggested that moisture content increases with the matrix concentration. Feed rate, matrix type, extract-to-matrix ratio, and inlet temperature influence the powder's moisture content.

An unusual result was found in MD/TH-1 powder, which had the lowest moisture content (1.2 %) but was very sticky. The glass transition of trehalose is predicted to have caused this anomaly. Trehalose can form an amorphous gel (or glass) when water is evaporated during spray drying. The glass becomes sticky if the material's transition temperature (Tg) is exceeded or with increasing water content (Nabors, 2012). However, these results contradict the high Tg (up to 120 °C) and low hygroscopicity of trehalose described by (Zhang et al., 2021). Therefore, further evaluation using, for instance, thermal analysis by differential scanning calorimetry (DSC) is needed to determine crystallization stability, glass formation, and activation energy in the glass crystallization process.

Encapsulation efficiency

Table 3 summarizes the total anthocyanin (TA) and surface anthocyanin (SA) contents of the microencapsulated powders and encapsulation efficiency after spray-drying. Overall, TA decreases with increasing matrix concentration, which can be attributed to the dilution of anthocyanin by the matrix. Also, for microencapsulated powders with a maltodextrin matrix, there is a positive correlation between matrix concentration and encapsulation efficiency (EE), which corresponds to (Hasrini et al., 2017). EE reflects the potential of a matrix to entrap or retain a core material in microcapsules and is also related to the shelf life of anthocyanins in powder. In general, it appears that the maltodextrin-trehalose binary matrix created higher EE than maltodextrin, with the highest efficiency produced by MD/TH-1 powder (76.53 %). However, MD/TH-1 powder had poor physical characteristics: very low yield, sticky texture, and low moisture content (see Fig. 2 and Table 2).

Other than the type of matrix and its ratio to the extract, EE is also determined by the percentage of each matrix in the binary product. (Zhang et al., 2007) discovered that mixing 60 % maltodextrin and 40 % gum Arabic for the matrix gave the optimal conditions for encapsulating procyanidins. In the current research, the matrix combined maltodextrin and trehalose at a 1:1 ratio. Therefore, further studies are required to optimize the combination ratio between the two matrices for the microencapsulation of roselle extract.

Color stability of roselle extract-microencapsulated powder at different pH levels

Color significantly dictates the overall quality of a food product and a consumer's first impression that influences their purchasing interest (Gerard and Roberts, 2004). In pharmaceutical preparations for pediatrics, color also determines drug acceptability and patient compliance (Alessandrini et al., 2023). The red-to-purple color of anthocyanins is highly sensitive to changes in environmental pH. Therefore, to ensure suitability as a colorant in foods and medicines that have different

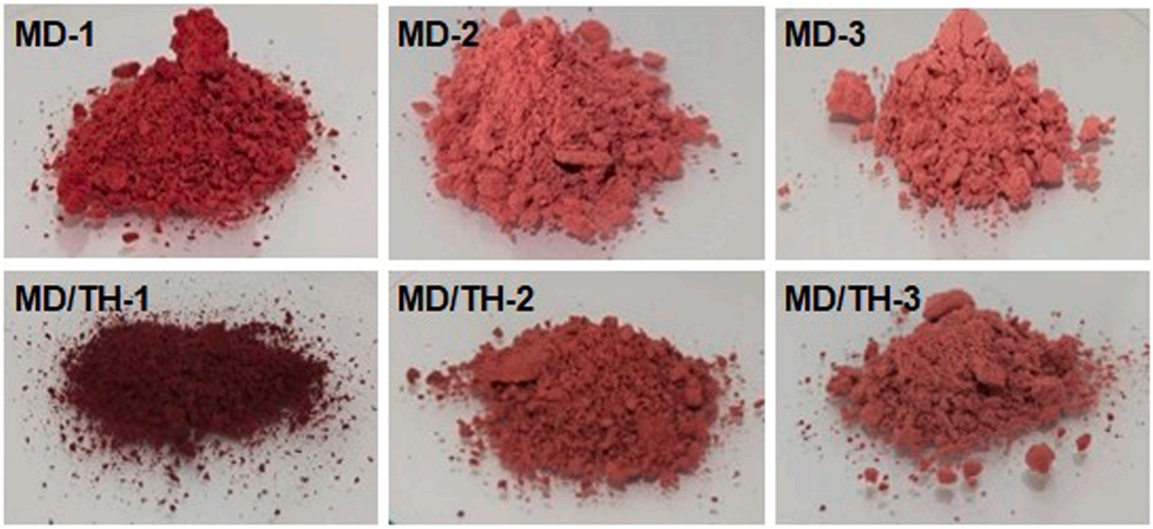

Fig. 2. Visual characteristics of the roselle extract-microencapsulated powders formulated with different matrix compositions.
MD-1, MD-2, MD-3: microencapsulated powders with a maltodextrin matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios. MD/TH-1, MD/TH-2, MD/TH-3: microencapsulated powders with a maltodextrin-trehalose binary matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios.

Table 2
Yields, visual descriptions, and moisture contents of the roselle extract-microencapsulated powders.

Microencapsulated powder	Yield (%)	Color and texture	Moisture content (%)
MD-1	63.2 ± 6.8^{a,c}	Dark red and sticky powder	2.3 ± 0.4^{a}
MD-2	66.4 ± 2.3^{a,c}	Red and slightly sticky powder	3.3 ± 0.7^{b}
MD-3	73.6 ± 5.1^{a,d}	Light red and not-sticky powder	2.5 ± 0.1^{a,b}
MD/TH-1	44.4 ± 1.6^{b}	Dark red and very sticky powder	1.2 ± 0.1^{c}
MD/TH-2	61.2 ± 0.6^{c}	Red and slightly sticky powder	2.3 ± 0.1^{a}
MD/TH-3	68.0 ± 1.1^{c,d}	Light red and slightly sticky powder	3.1 ± 0.1^{a,b}

Notes:.
MD-1, MD-2, MD-3: microencapsulated powders with a maltodextrin matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios.
MD/TH-1, MD/TH-2, MD/TH-3: microencapsulated powders with a maltodextrin-trehalose binary matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios.
Yield data are expressed as mean±SD ($n = 2$).
Moisture content data are expressed as mean±SD ($n = 3$).
Values followed by different letters in the same column indicate a significant difference from one another ($P < 0.05$).

degrees of acidity, it is necessary to analyze the effect of environmental pH on the powder's color stability. Table 4 shows the three color parameters measured using a color reader: L* (lightness), a* (redness), and b* (yellowness). This research, however, focused on a* to describe any change or instability in the red color of anthocyanins.

Fig. 3 illustrates that the redness intensity (a*) decreases with increasing pH and that the color change from red to dark red occurs at a pH of 6–7. These results can be explained by the structural transformation of anthocyanins as a function of pH. In aqueous media, anthocyanins can be in equilibrium when present in one of these four structures depending on pH: red flavylium cation, blue quinoidal base, colorless carbinol pseudobase, and colorless chalcone. Results indicate that the flavylium anthocyanin cation could maintain its redness intensity in an environment with a pH of 2.6–5 and change to a darker color in the pH range of 6–7, suggesting structural transformation to a

Table 3
Encapsulation efficiencies of roselle extract with a maltodextrin matrix and a maltodextrin-trehalose binary matrix.

Microencapsulated powder	TA (mg/g)	SA (mg/g)	EE (%)
MD-1	0.74±0.007^{a}	0.44±0.005^{a}	40.65±0.009^{a}
MD-2	0.60±0.005^{b}	0.32±0.009^{b}	46.93±0.014^{b}
MD-3	0.53±0.004^{c}	0.26±0.001^{c}	50.28±0.003^{c}
MD/TH-1	0.80±0.013^{d}	0.19±0.001^{d}	76.53±0.410^{d}
MD/TH-2	0.65±0.008^{e}	0.22±0.007^{e}	66.65±1.130^{e}
MD/TH-3	0.65±0.004^{e}	0.20±0.002^{d}	68.92±0.500^{e}

Notes:.
MD-1, MD-2, MD-3: microencapsulated powders with a maltodextrin matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios.
MD/TH-1, MD/TH-2, MD/TH-3: microencapsulated powders with a maltodextrin-trehalose binary matrix and 30:70, 40:60, 50:50 matrix-to-extract ratios.
TA, SA, and EE are expressed as mean±SD ($n = 3$).
Values followed by the different letter in the same column indicate a significant difference from one another ($P < 0.05$).

quinoidal base (Sigurdson et al., 2017). In conclusion, roselle extract-microencapsulated powder can be applied as a coloring agent in products with a pH of 2.6–5. These results are, however, slightly different from (Selim et al., 2008), which found that the powder's red color was stable at pH≤3.0, substantially faded at pH 4–5, and almost disappeared (colorless) at pH 6.0. In addition, increasing the pH continuously to 7 and then 8–9 changed the color to purple and blue, respectively.

Particle size of roselle extract-microencapsulated powder

Particle size and size distribution are physical characteristics of powder that determine flow rate and are necessary for product packaging (Liu et al., 2008). Particles with a small size and homogeneous size distribution have good flow properties, resulting in products with a uniform weight. A particle size analyzer (PSA) determines the size distribution by calculating the polydispersity index (PDI). Particles are deemed homogeneous if the PDI is less than 0.7. In contrast, a PDI value closer to 1 indicates heterogeneity. Homogeneous size distribution creates physical stability, preventing particles from agglomerating, whereas heterogeneous distribution can be linked to an uneven drying process that induces agglomeration (Septevani et al., 2018).

Table 4
Lightness (L*), redness (a*), and yellowness (b*) parameters of the microencapsulated powders in different pH buffer solutions.

Micro-encapsulated powder	Parameter	pH 2.6	3	4	5	6	7
MD-1	L*	20.60 ± 1.30	22.70 ± 0.44	20.20 ± 2.47	21.10 ± 2.69	20.20 ± 1.19	15.10 ± 1.30
	a*	5.67 ± 0.29	5.67 ± 0.06	4.63 ± 0.15	4.27 ± 0.31	3.53 ± 0.32	1.50 ± 0.26
	b*	1.10 ± 0.25	0.87 ± 0.23	0.77 ± 0.06	0.53 ± 0.15	0.53 ± 0.45	0.33 ± 0.23
MD-2	L*	18.20 ± 1.15	21.30 ± 0.30	21.90 ± 0.40	21.50 ± 0.57	17.40 ± 2.46	20.60 ± 1.86
	a*	4.70 ± 0.26	4.20 ± 0.10	3.60 ± 0.29	3.10 ± 0.15	2.80 ± 0.32	1.90 ± 0.06
	b*	0.80 ± 0.26	0.30 ± 0.12	0.50 ± 0.20	0.40 ± 0.35	1.00 ± 0.91	0.60 ± 0.35
MD-3	L*	19.60 ± 2.08	19.5 ± 2.95	20.30 ± 1.33	21.40 ± 1.30	19.50 ± 2.79	19.40 ± 1.00
	a*	3.90 ± 0.20	3.10 ± 0.06	2.50 ± 0.15	2.30 ± 0.23	1.90 ± 0.06	0.90 ± 0.17
	b*	0.90 ± 0.46	0.20 ± 0.06	0.40 ± 0.31	0.10 ± 0.06	0.00 ± 0.38	−0.80 ± 0.12
MD/TH-1	L*	19.07 ± 1.10	18.8 ± 0.30	18.37 ± 0.55	20.17 ± 0.76	21.13 ± 1.29	20.83 ± 0.47
	a*	7.17 ± 0.12	6.33 ± 0.30	5.57 ± 0.15	5.53 ± 0.21	4.17 ± 0.12	1.67 ± 0.25
	b*	1.73 ± 0.06	1.73 ± 0.40	1.20 ± 0.10	1.40 ± 0.40	0.90 ± 0.20	0.10 ± 0.00
MD/TH-2	L*	19.36 ± 1.21	19.40 ± 0.26	20.40 ± 1.06	20.57 ± 0.58	19.97 ± 1.21	19.90 ± 0.96
	a*	4.37 ± 0.21	3.63 ± 0.12	3.53 ± 0.06	3.23 ± 0.06	2.63 ± 0.21	1.60 ± 0.17
	b*	1.07 ± 0.06	0.87 ± 0.25	0.73 ± 0.15	0.57 ± 0.05	0.53 ± 0.15	0.13 ± 0.15
MD/TH-3	L*	19.90 ± 0.72	20.50 ± 0.17	21.20 ± 0.10	19.77 ± 1.77	19.63 ± 0.55	19.43 ± 1.89
	a*	4.63 ± 0.29	3.10 ± 0.12	2.77 ± 0.12	2.60 ± 0.30	1.80 ± 0.10	0.87 ± 0.06
	b*	0.63 ± 0.15	0.50 ± 0.20	0.17 ± 0.15	0.17 ± 0.06	0.07 ± 0.06	−0.13 ± 0.12

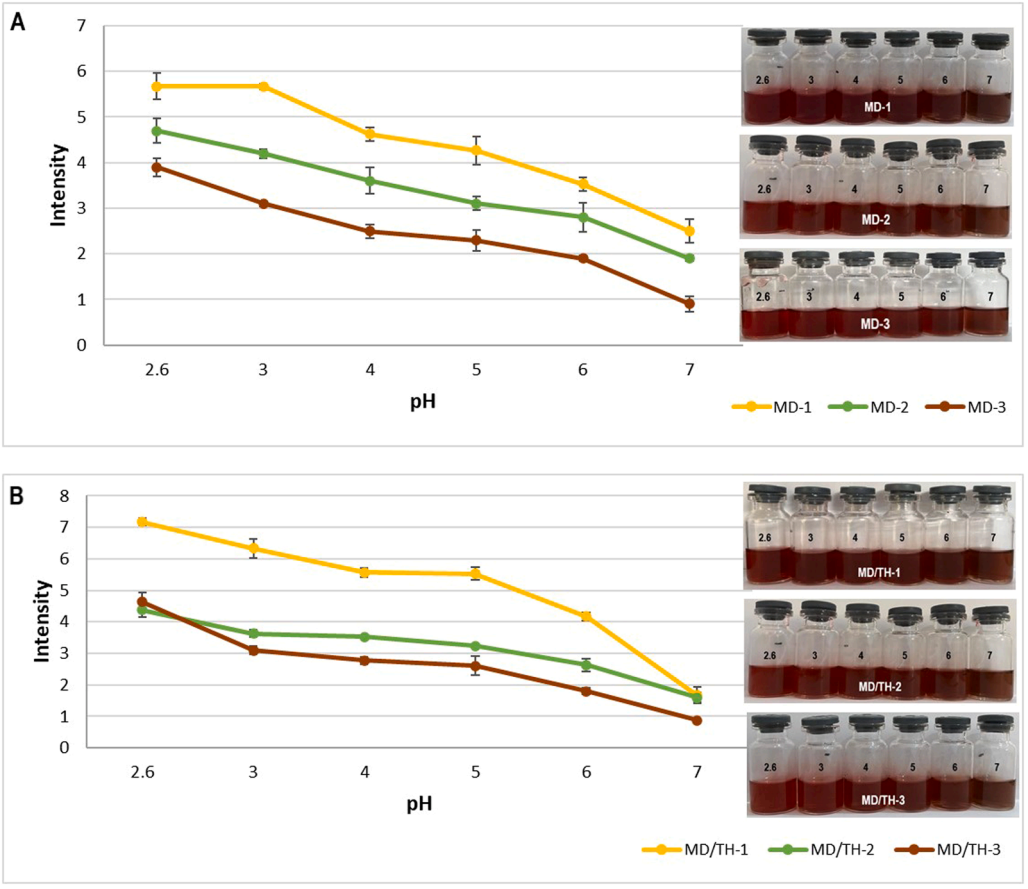

Fig. 3. Redness intensity of roselle extract-microencapsulated powders with maltodextrin matrix (A) and maltodextrin-trehalose binary matrix (B) at various pH levels.

Table 5 summarizes the particle size and particle size distribution of the roselle extract-microencapsulated powders. All the formulas met the particle size requirements for microencapsulated powders, namely 1–1000 μm. MD-2 had the largest mean particle size with the lowest PDI. This is assumed to result from agglomeration, as confirmed by the moisture content of MD-2, which was the highest among the formulas (3.3 %). As for the binary matrix, MD/TH-2 and MD/TH-3 powders showed a homogeneous particle size distribution, while MD/TH-1 powder was heterogeneous (PDI>0.7).

Surface morphology and crystallinity of roselle extract-microencapsulated powder

Learning from the microencapsulation yields, organoleptic properties, moisture contents, total anthocyanins and encapsulation efficiencies, particle size and size distributions, and pH-dependent redness stability, it can be concluded that 50:50 is the optimal ratio for a maltodextrin matrix and a maltodextrin-trehalose binary matrix, as observed in MD-3 and MD/TH-3 powders. Therefore, the surface

Table 5
Particle size and polydispersity index of roselle extract-microencapsulated powders.

Microencapsulated powder	Mean particle size (μm)	Polydispersity index
MD-1	4.66	0.06280
MD-2	5.62	0.00586
MD-3	4.48	0.41400
MD/TH-1	1.99	1.65100
MD/TH-2	5.49	0.01055
MD/TH-3	4.20	0.34400

morphology, crystallinity, and antioxidant activity of the two powders were further analyzed.

MD-3 powder had spherical particles, a curved surface (dented), and visible bridges and cavities between adjoining particles (Fig. 4A). The curved shape can be associated with particle shrinkage during drying and cooling. Similar dents have been observed in several previous studies (Idham et al., 2012; Obón et al., 2009; Tonon et al., 2009). Different matrices create different coating wall densities that protect anthocyanins in microcapsules from exposure to or interaction with the surrounding environment. MD/TH-3 powder was spherical with a smooth surface and very low cohesion between the particles (Fig. 4B). These characteristics are congruent with (Domian et al., 2017), which concluded that using small sugars such as trehalose as a microencapsulation matrix can produce spherical particles. Spherical particles have better flow properties than those with irregular shapes.

The XRD analysis of MD-3 and MD/TH-3 powders provided information on the amorphous or crystalline structure of the microencapsulated powders. The diffraction profiles in Fig. 5 and Table 6 show that ME-MD-3 and ME-MD/TH-3 powders had two broad, instead of sharp, peaks at 2θ 18.7506° and 2θ 18.9035°, indicating low crystallinity. This means the formed powders tend to have amorphous particles, which possess higher solubility but lower stability than the crystalline form (Hancock and Parks, 2000; Laitinen et al., 2013).

Antioxidant activity of roselle extract-microencapsulated powder

Apart from anthocyanins as the main component, roselle calyces also contain various other phenolic compounds in high concentrations. Therefore, its coloring capacity can be better complemented with comprehensive information about the effect of microencapsulation by spray-drying using MD and MD/TH matrices on the antioxidant activity of the resulting powder. Fig. 6 and Table 7 show the antioxidant

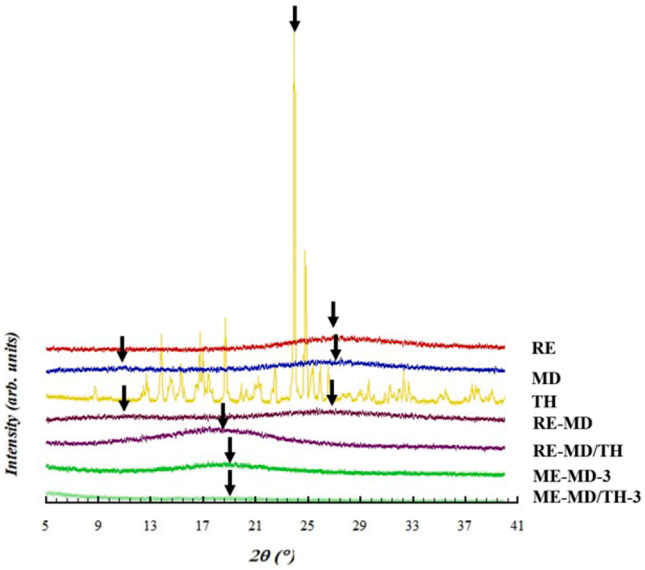

Fig. 5. Diffractograms of the roselle extract (RE), maltodextrin (MD), trehalose (TH), the physical mixture of RE and MD (RE-MD), the physical mixture of RE and MD/TH (RE-MD/TH), and the microencapsulated powders with a maltodextrin matrix at 50:50 matrix-to-extract ratio (ME-MD-3) and a maltodextrin-trehalose binary matrix at 50:50 matrix-to-extract ratio (ME-MD/TH-3).

activities of MD-3 and MD/TH-3 powders using the DPPH-scavenging method. The IC_{50} value of the bulk powder of MD/TH-3 was 5943.97 μg/mL, and this figure was lower than those of MD-3, 6795.10 μg/mL. If calculated as native powder, the IC_{50} of MD/TH-3 and MD-3 were 2971.48 and 3397.55 μg/mL, respectively. The weight of the bulk powder is the total weight of the extract and the matrix in the microencapsulated powder, while the weight of the native powder refers to that of the extract contained in the powder.

Conclusions

Spray-drying is a popular and effective method for microencapsulating and protecting the activity of phenolic compounds, especially anthocyanins. Choosing the right type of matrix determines the success of the microencapsulation. This research microencapsulates roselle calyx extract using a single matrix, maltodextrin (MD), and a

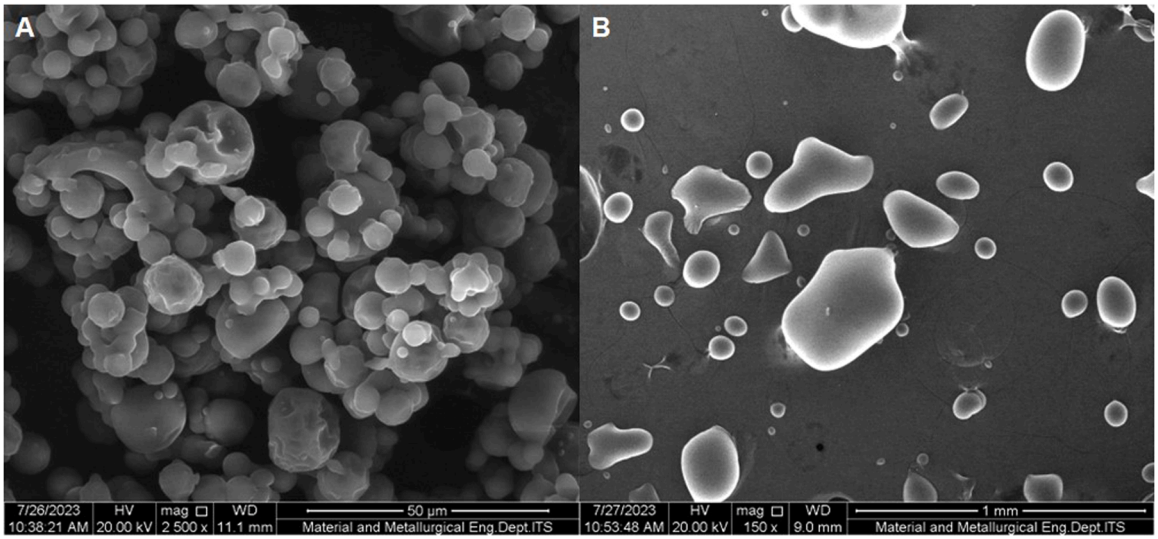

Fig. 4. Micrographs of roselle-extract microencapsulated powders made with the MD-3 formula at 2500x magnification (A) and MD/TH-3 at 150x magnification (B).

Table 6
X-ray diffraction profiles of the roselle extract-microencapsulated powders.

Microencapsulated powders	Peak, *i* th	Position (°2θ)	Height (Cts)	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
Trehalose (TH)	1	23.9272	7600.92	100
Physical mixture of RE and MD (RE-MD)	1	11.2591	54.60	53.83
	2	26.1658	100.32	100
Physical mixture of RE and MD/TH (RE-MD/TH)	1	10.0360	104.82	100
	2	26.7773	55.21	52.67
Microencapsulated powder with the MD matrix (ME-MD) at a 50:50 ratio (ME-MD-3)	1	18.7506	46.15	100
Microencapsulated powder with the MD/TH combination matrix (ME-MD/TH) at a 50:50 ratio (ME-MD/TH-3)	1	18.9035	51.07	100

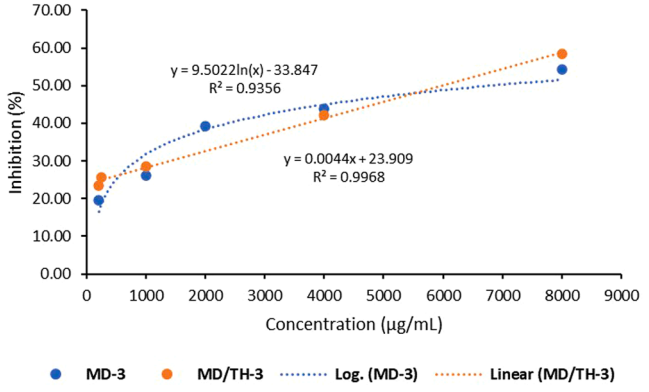

Fig. 6. Correlation between the concentration of the roselle extract-microencapsulated powder and the percentage of DPPH inhibition.

Table 7
Inhibitory activity of roselle extract-microencapsulated powders on DPPH.

Material	IC_{50} of bulk powder (µg/mL)	IC_{50} of native powder (µg/mL)
MD-3	6795.10 ± 375.16	3397.55 ± 187.58
MD/TH-3	5943.97 ± 241.82	2971.48 ± 120.91
Trolox (reference compound)	NA	31.95 ± 0.19

maltodextrin-trehalose (1:1) combination (binary matrix), with three matrix-to-extract ratios: 30:70, 40:60, and 50:50. It has been highlighted that 50:50 (MD-3 formula) is the optimal matrix-to-extract ratio for producing powders with good physicochemical characteristics and activity. These characteristics include 73.6 % yield, light red powder with a non-sticky texture, 2.5 % moisture content, 50.28 % encapsulation efficiency, average particle size of 4.48 µm with homogeneous distribution (PDI = 0.41400), red color stability at pH 2.6–5, and spherical and amorphous particles. In the DPPH (free radical) scavenging method, the microencapsulated powders exhibit antioxidant activity with an IC_{50} of 3397.55 µg/mL (calculated with the native extract). Even though adding trehalose to maltodextrin to create a binary matrix does not produce powders with better physicochemical characteristics, it can improve encapsulation efficiency. Therefore, there is an opportunity to investigate further the appropriate maltodextrin-to-trehalose ratio for microencapsulating roselle extract.

CRediT authorship contribution statement

Bertha Ladesta Millinia: Writing – original draft, Visualization, Project administration, Formal analysis, Data curation. **Dewi Mashithah:** Writing – original draft, Project administration, Formal analysis, Data curation. **Roisah Nawatila:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Conceptualization. **Kartini Kartini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Ethical Statement

This study does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgments

This research was supported by the Indonesian Ministry of Education, Culture, Research and Technology (Grant Number 011/SP-Lit/LPPM-01/KemendikbudRistek/FF/V/2023) and University of Surabaya (Grant Number 016/SP-Lit/LPPM-01/FF/Int/FF/IV/2023).

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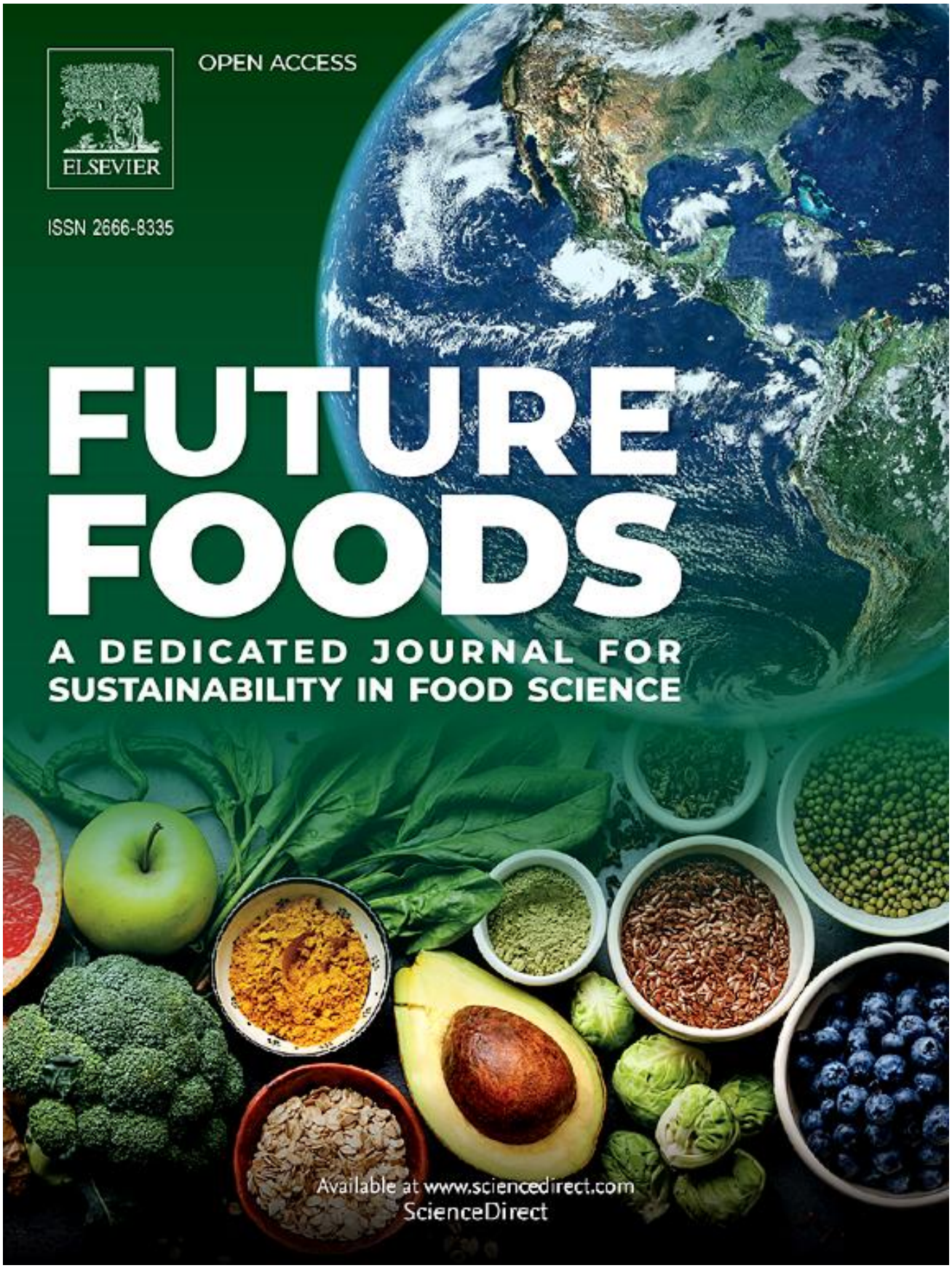
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