

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Antioxidant Properties and Storage Stability of Spray-dried *Melastoma malabathricum* **L. Fruit Extract for Natural Colorant**

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ABSTRACT

Melastoma malabathricum L. is a plant that is rich in anthocyanin, though its anthocyanin is low in stability. Therefore, this study aims to characterize the physicochemical properties of the encapsulated fruit extracts of *M. malabathricum,* to determine the antioxidant activities before and after encapsulation using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino-bis-3 ethylbenzothiazoline-6-sulphonic acid (ABTS), and Ferric Reducing Antioxidant Power (FRAP) assays, expressed as IC50 values, and to assess the storage stability of the encapsulated fruit extract in different conditions based on the Total Anthocyanin Content (TAC) and Degradation Index (DI). The encapsulation was performed using a spray dryer and maltodextrin as the wall material. Crude extract with maltodextrin ratios of 1:1, 1:2 and 1:3 were tested, and TAC of the samples were determined using pH differential methods. The encapsulated extract was characterized according to their physical

ARTICLE INFO

Article history: Received: 25 April 2024 Accepted: 4 July 2024 Published: 28 January 2025

DOI: https://doi.org/10.47836/pjtas.48.1.17

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and chemical properties. Stability was evaluated under different conditions, including 20°C, 4°C and 25°C in the dark, and 25°C with light exposure for 90 days. Ratio 1:3 exhibited the lowest moisture content (5.77%) and solubility time (3s), while ratio 1:1 demonstrated the lowest bulk and tapped densities (0.366 g/ml and 0.5 g/ml). All ratios were acidic and displayed spherical particles with irregular surfaces. Ratio 1:1 showed the highest antioxidant activity with IC50 values of 28.50 μg/ml (DPPH), 49.03 μg/ ml (ABTS), and 39.97 μg/ml (FRAP). The most

ISSN: 1511-3701

stable condition was at 4° C in the dark, while the ratio 1:2 was found to be the best ratio. Both factors can be considered to achieve stability for future applications.

Keywords: Antioxidant, anthocyanin, natural color, spray-dried, storage stability

INTRODUCTION

The rising demand for all-natural products has sparked the exploration of novel natural colorants (Novais et al., 2022). Natural colorants pose no health risks to humans and biodegrade quickly, making them ideal for use (Usman et al., 2017). Besides, synthetic colorants have produced potentially harmful consequences compared to natural ones. It can be seen in the case of various studies on the harmful consequences of using synthetic colorants (Oplatowska et al., 2017). Thus, this issue has created an alarm for people to be concerned about the effects of shifting to a natural colorant.

Natural colorants are widely used in many industries but are most commonly used in food and beverage products. According to the market research report, the market for natural food colors was estimated to be worth USD 1.54 billion in 2021. By 2030, it is anticipated to expand at a compound annual growth rate (CAGR) of 7.4%. Due to the increasing demand for natural colorants, exploring new and alternative sources is crucial to focusing on the underutilized plant species (Adnan et al., 2011).

Numerous underutilized natural plant species have not been researched for a few reasons. As Malaysia has a tropical climate with the highest levels of biodiversity in the world, an underutilized plant known as senduduk, *Melastoma malabathricum* L., shown in Figure 1, is a promising candidate for becoming a source of natural colorant. Many

Figure 1. A mature plant of *Melastoma malabathricum* L. in its wild habitat

studies have demonstrated this plant's medicinal properties, which can benefit from all parts, including fruits, leaves, flowers, and roots.

The fruit of *M. malabathricum*, as shown in Figure 2, has been emerging as a promising source of natural colorants due to the presence of anthocyanin pigments, which are able to offer a color ranging from red to purple, marked as a significant advancement for different sectors. Anthocyanin from *M. malabathricum* has attracted attention lately because it possesses potential health benefits, especially as an antioxidant. Significantly, this plant's development of natural colorants aligns with the growing interest in food natural colorants with higher antioxidant activities. However, the food natural colorant from plant pigment faces challenges because they are vulnerable to degradation and low stability (Echegaray et al., 2020). They usually often lose their color, and their biological activity is particularly affected by the light, temperature of the storage, and pH (Roobha et al., 2011). Following storage stability testing by World Health Organization (WHO), the storage conditions were simulated to include room temperature, refrigerator, and freezer conditions. This finding broadly influences the selection of storage stability testing for anthocyanins due to their potential stability in stable conditions, as they are easily degraded at higher temperatures. Besides that, the pH will remain constant throughout the study to accurately depict how anthocyanins act in their native state.

Figure 2. *Melastoma malabathricum* L. fruits

Existing studies on encapsulation of *M. malabathricum* fruit extracts have primarily focused on evaluating wall materials instead of stability. Therefore, this study has successfully established the connection by assessing the storage stability of the encapsulated fruit extract of *M. malabathricum* in different conditions based on the Total Anthocyanin Content (TAC) and Degradation Index (DI). These studies concern the physicochemical properties of the encapsulated fruit extract in producing high-quality powder and highlight its potential to provide health benefits, such as antioxidant activity.

MATERIALS AND METHODS

Sample Collection

Fresh samples of *M. malabathricum* fruits were collected from UTHM Campus Pagoh, Johor, Malaysia, from January to March during the northeast monsoon season. According to Kasunmala et al. (2020), the fruits were collected at two different maturation stages: maturity stage 2, which represents mature fruits, and maturity stage 3, which represents ripened fruits. *Melastoma malabathricum* L. species were identified and deposited at the National Herbarium in Forest Research Institute Malaysia (FRIM) Kepong. Subsequently, five kilograms of *M. malabathricum* fruits were washed, cleaned, and air-dried at room temperature. The dried samples were then ground using a blender and stored at 4°C until further use (Kasunmala et al., 2020).

Sample Extraction

Ultrasound-Assisted Extraction (UAE) was conducted with some modifications. Fruits of *M. malabathricum* were extracted using a wt/vol ratio of 1:10 (mass of sample in g: volume of solvent in ml) with absolute ethanol (Thermo Fisher, America) in a beaker. The ultrasonic frequency and power set were fixed at 280 W 37 kHz, 70°C temperature, and 19 min extraction time. After that, the extract was filtered using Whatman No. 1 filter paper, and the solvent was removed using a rotary evaporator (Buchi Switzerland R-100, Switzerland) under 200 mbar at a temperature of 70°C to obtain the crude extract.

Encapsulation Using Spray Dryer of *Melastoma malabathricum* **L.**

The spray-drying process was conducted using maltodextrin, following the methods outlined by Narayanan et al. (2018), Mokhtar et al. (2013), and Krishnainah et al. (2012) with certain modifications. Three different ratios of the encapsulated fruit extracts to maltodextrin were (1:1), (1:2), and (1:3). The maltodextrin (Sigma Aldrich, Malaysia) was mixed with the crude extract together with 1000 ml distilled water, as shown in Table 1. The mixture was then stirred using a magnetic stirrer for 1 hr (Nayak & Rastogi, 2010a). The mixture was spray dried using a spray dryer with an inlet of 160°C and an outlet temperature

maintained at 80°C. Then, the powders were stored over silica gel in desiccators at room temperature (25° C \pm 2°C) for further experiments.

Table 1 *Total sample and maltodextrin in different ratio*

Ratio	Sample (g)	Maltodextrin (g)
1:1	50	50
1:2	33	66
1:3		75

Encapsulation Efficiency (EE)

The encapsulation efficiency was the amount of anthocyanin encapsulated relative to the extract's content. It was calculated using the formula [1] (Kar et al., 2019).

$$
EE (%) = \frac{Total Anthocyanin Content in the feed}{Total Anthocyanin Content in the powder} X 100
$$
 [1]

Physicochemical Properties of Encapsulated Extracts

Moisture Content

According to Kathiman et al. (2020), the encapsulated fruit extracts' moisture content was determined using a moisture analyzer (MX-50, A&D Weighing, Malaysia). One gram of the sample powder was placed into the moisture analyzer. The data was recorded, and the evaluation was done in triplicate.

Bulk and Tapped Density

The bulk density and tapped density of the encapsulated fruit extract were determined based on the weight-to-volume ratio. Two grams of powder were transferred to a 10 ml graduated cylinder, and the reading was recorded. For tapped density, the encapsulated fruit extracts were transferred and battered 20 times on a hard surface until the volume was fixed. All samples were run in triplicate (George et al., 2021). The density was calculated by using formula 2:

Bulk and Tapped density
$$
\left(\frac{g}{ml}\right) = \frac{Weight \ of \ sample}{Volume \ of \ sample}
$$
 [2]

pH Value

Ten grams of encapsulated fruit extracts were added to 90 ml of distilled water, and the pH value was determined using a pH meter (pH 700, Eutech Instruments, Singapore) (Caglar et al., 2020).

Dissolution Test

Fifty milligrams of encapsulated fruit extracts were added to 1 ml of distilled water at 25℃. The time required to reconstitute the powder was recorded using an electronic timer (s) (Quelal et al., 2023).

Color Determination

The color changes in the anthocyanin content of the encapsulated fruit extract and crude extracts were determined using a colorimeter (MSE-4500L, HunterLab, USA) based on CIELab color space. The data $L^*a^*b^*$ from the colorimeter was then simulated into a color palette using the software easyrgb.com (EasyRGB 1.0, USA), according to Lam et al. (2023).

Surface Morphology by Scanning Electron Microscopy (SEM)

Five milligrams microcapsules were dried at 110℃ for 24 hours before starting. SEM evaluated encapsulated fruit extracts and crude extracts at a voltage of 20 kV, and SEM images were recorded at different magnifications (George et al., 2021).

Antioxidant Activities

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The free radical scavenging activity was analyzed using the DPPH method with minor modifications (Chandra et al., 2014). Each prepared sample solution of the encapsulated fruit extracts and crude extract was mixed with 1 mM DPPH solution. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer (U-3900H, Hitachi, Japan). The assay was done in triplicate. The free radical scavenging activity was calculated based on the following formula 3:

Radical Scavening activity (
$$
\%
$$
) = $\frac{\Delta C - \Delta S}{\Delta C}$ x 100 [3]

Where ΔC is the absorbance reading of the negative control, and ΔS is the absorbance reading of the sample.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic Acid (ABTS) Assay

The ABTS assay was performed by preparing 7 mM ABTS and mixing it with 2.45 mM Potassium persulfate (Sigma Aldrich, Malaysia). To each 1 ml of prepared solution, 10 µl of each encapsulated fruit extract and crude extract was added. The mixtures were kept for 15 min at room temperature, and the absorbance was read at 734 nm. The assay was

done in triplicate. The free radical scavenging activity was calculated using the radical scavenging activity as formula [3] above.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric-reducing activity (FRAP) of the encapsulated fruit extracts and crude extract was performed. Firstly, the FRAP reagent was prepared by mixing 25 ml of acetate (Merck, Germany) buffer, 2.5 ml of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) solution, and 2.5 ml of Iron (III) chloride (Thermo Fisher, America). Fifty (50) μl aliquots of each extract or standard were mixed with 1.5 ml of the freshly prepared FRAP reagent. The absorbance was measured 30 minutes after the addition of the FRAP reagent. The percentage of ferric-reducing antioxidant activity was calculated using formula 4 (Bejeli et al., 2012). All determinations were carried out in triplicate.

FERIC reducing antioxidant activity (%) =
$$
\frac{\Delta C - \Delta S}{\Delta C} \times 100
$$
 [4]

Where ΔC is the absorbance reading of the negative control, and ΔS is the absorbance reading of the sample.

Total Anthocyanin Content (TAC)

The total anthocyanin was measured using the pH-differential method by Giusti and Wrolstad (2001), using two buffer systems: (1) 0.025M potassium chloride (Merck, Germany) buffer at pH 1.0 and (2) 0.4 M sodium acetate (Merck, Germany), buffer at pH 4.5 as referenced in Association of Official Analytical Chemists (AOAC) (2005). One (1) ml of the encapsulated fruit extracts and the crude extract were added into a buffer solution of pH 1 and pH 4.5. Then, the absorbance of each sample was determined using a UV-Vis Spectrophotometer and was read at 520 nm and 700 nm. Absorbance (A) of dilutions was calculated using the following formula 5:

$$
TAC\ \left(\frac{mg}{ml}\right) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \tag{5}
$$

Where, $A = (A520nm - A700nm)$ pH $1.0 - (A520nm - A700nm)$ pH 4.5. *MW* (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu). $DF =$ dilution factor established in D. $l =$ pathlength in cm.

 ε = 26 900 molar extinction coefficients, in L \times mol⁻¹ \times cm⁻¹, for cyd-3-glu 1000 = factor for conversion from g to mg

Stability Test

The stability test of the encapsulated fruit extracts and crude extract was referred to the studies by Yusoff et al. (2014), and Janna et al. (2006), focusing on the anthocyanin stability of *M. malabathricum* under different light conditions and different storage temperatures. Encapsulated fruit extract from each ratio $(1:1, 1:2, 1:3)$ and crude extract were located and evaluated at 4°C, -20°C, and 25°C with the absence of light wrapped with aluminum foil. In comparison, another sample for 25°C was located in the presence of light. The total anthocyanin content of each sample was recorded on days 0, 14, 30, 60, and 90 using a UV-Vis spectrophotometer in triplicate.

Degradation Index (DI)

The degradation constant (K) for the anthocyanin content in the encapsulated fruit extracts and the crude extract was determined considering first-order degradation kinetics, as described by formula [6], where *Co* represents the initial anthocyanin content, and Ct represents the anthocyanin content at a specified time.

$$
ln \frac{Co}{Ct} = -Kt
$$
 [6]

Statistical Analysis

All data in triplicate readings were recorded and reported as mean \pm standard deviation for every analysis. Experimental data were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistic 27. Tukey's test determined Significant differences between means (Kim, 2017). Minitab software 19 was also utilized to determine the best ratio and storage condition using a main effect plot graph (Qaziyani et al., 2019).

RESULTS AND DISCUSSION

Encapsulation Efficiency (EE%)

Encapsulation efficiency (EE) was calculated by considering the Total Anthocyanin Content (TAC) present in the solution before drying and in the powder after drying. Table 2 shows the TAC of the encapsulated fruit extracts and encapsulation efficiency with different ratios.

From Table 2, different ratios of maltodextrin showed a significant effect on TAC after encapsulation and EE, ranging from 69.76% to 96.82%. It indicates that the encapsulation process successfully preserved the anthocyanins in the fruit extract**.** EE increased significantly $(p < 0.05)$ with higher sample ratios or maltodextrin concentrations. Ratio 1:1 exhibited the highest EE at 89.10%, followed by ratio 1:2 (78.54%), and ratio 1:3 showed the lowest EE at 69.76%. What stands out in these results is the correlation between the

amount of TAC and EE, with a ratio of 1:1 showing the highest TAC after encapsulation and the most effective encapsulation percentage among the other ratios.

Table 2 *Total anthocyanin content of the encapsulated fruit extracts and encapsulation efficiency with different ratios*

Sample	Total Anthocyanin Content (TAC) after encapsulated (mg/ml)	Encapsulation efficiency % (EE)
1:1	246.66 ± 0.34 ^a	$96.82 \pm 0.13^{\circ}$
1:2	184.54 ± 1.77^b	$78.54 \pm 0.81^{\circ}$
1:3	$155.99 \pm 1.91^{\circ}$	69.76 ± 0.79 °

Note. Data are expressed as means \pm SD ($n = 3$), means were compared by the Tukey test ($p < 0.05$). The mean is significantly different *p* < 0.05

Generally, TAC may decrease as maltodextrin concentration increases because a thicker wall surrounding the anthocyanin droplets due to higher maltodextrin concentrations may reduce the diffusion of water and other solutes into the droplets (Deng et al., 2023; Nafiunisa et al., 2017; Padzil et al., 2018). As the amount of maltodextrin in the encapsulated fruit extracts increased, the TAC decreased, as depicted in Figure 3. It is likely because the bulky nature of maltodextrin as wall materials may dilute the anthocyanin sample. Interestingly, the samples before encapsulation had a substantially higher TAC than the encapsulated samples, even with the highest maltodextrin content. It suggests that anthocyanins can be effectively encapsulated and preserved even with a small amount of maltodextrin.

Figure 3. Total Anthocyanin Content (TAC) of *Melastoma malabathricum* L. fruit extract and encapsulated fruit extract 1:1, 1:2, and 1:3

Contradicting the findings, Akdeniz et al. (2017) subsequently stated that the EE decreased as the ratio of core to coating increased. The study revealed that a ratio of 1:10 has higher encapsulation efficiency than a ratio of 1:20. In addition, Akdeniz also acknowledged the use of maltodextrin as wall materials, giving higher encapsulation efficiency than gum Arabic.

Physicochemical Properties of Encapsulated Fruit Extract

Physical and chemical properties were included in characterizing encapsulated fruit extracts from *M. malabathricum*. Table 3 summarizes the results of the physicochemical properties of the encapsulated samples, while Table 4 presents the surface morphology of the encapsulated fruit extracts and crude extract.

Moisture Content

The moisture content of the encapsulated fruit extracts at different ratios is shown in Table 3, ranging from 5.77%–6.66 %. Ratio 1:3 exhibited the lowest moisture content (5.77%) compared to ratios 1:2 and 1:1, which were 6.22% and 6.66%, respectively. The differences in moisture content were attributed to the concentration of the encapsulating agent, where moisture content decreased as maltodextrin concentration increased. The lower moisture content observed in ratio 1:3 can be attributed to the higher amount of maltodextrin present in the sample compared to ratios 1:1 and 1:2. Generally, higher amounts of maltodextrin slow down the dispersion of water molecules, thus aiding in maintaining the moisture content of the encapsulated fruit extract.

Previous studies from Quelal et al. (2023) have also observed a reduction in moisture content with increasing maltodextrin concentration. Quelal et al. (2023) used different maltodextrin concentrations ranging from 3% to 7%, resulting in moisture content ranging from 5.44% to 1.96%. Similarly, findings from Narayanan et al. (2018) indicate that the moisture content decreases with increasing maltodextrin concentration. The water content of the feed significantly affects the final moisture content of the powder produced in a spray drying system.

Lower moisture content benefits the long-term storage and stability of the samples. According to Bell (2020), reduced moisture content improves chemical stability by lowering oxidation rates and hydrolytic degradation, which can degrade the active compounds and eventually reduce the sample's efficacy. At the same time, the safety of the sample and the extension of its shelf life depend on this microbial stability. Lower moisture content also prevents microbial growth (Rezaei & VanderGheynst, 2010), which means that contamination and deterioration are prevented by maintaining conditions in which bacteria, molds, and yeasts are unable to thrive. Physically, less moisture keeps the extract powder from caking and clumping, which happens when there is too much moisture, and the

Table 3
Physicochemical properties of encapsulated fruit extracts *Physicochemical properties of encapsulated fruit extracts*

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particles attach (Carter, 2020). It ensures the powder will flow freely, making handling, processing, and dosing uniformly during manufacture easier. According to Gaikwad et al. (2019), lower moisture content extracts are less likely to absorb more moisture from the surroundings, which may reduce cost by reducing the need for expensive, specialized, moisture-proof packing materials. It also might improve storage efficiency while simultaneously reducing the cost of packaging. Maintaining batch-to-batch consistency and product reliability also depends on the sample's uniform physical and chemical qualities, which a stable moisture content ensures.

Bulk and Tapped Density

The encapsulated fruit extracts at difference ratios $(1:1,1:2,1:3)$ exhibited a bulk density range of 0.362–0.501 g/ml, and tapped density ranged from 0.5–0.769 g/ml, as shown in Table 3. Significant differences ($p < 0.05$) in bulk density were observed among all ratios, indicating that the concentration of the wall material affected both bulk and tapped density. Ratio 1:1 showed the lowest values for both bulk dan tapped density (0.362 g/ml) and 0.5 g/ml, respectively), followed by ratio 1:2 (0.429 g/ml and 0.601 g/ml), and ratio 1:3 had the highest values (0.501 g/ml and 0.769 g/ml). Many studies, including those by Al-Maqtari et al. (2021), Rashid et al. (2022), and Shadordizadeh et al. (2023), have found that tapped density is correlated with bulk density. To better understand this relationship, Amidon et al. (2017) explained that the tapped density is always higher than bulk density because tapping and vibration during the procedure compact the powder, reducing the spaces between particles.

The highest bulk density observed for ratio 1:3 can be attributed to the increase in maltodextrin concentration, which increases solid content in the feed solutions, making the particles heavier and enhancing bulk density (Rodríguez-Díaz et al., 2014). Similarly, moisture content affects the density of the powder, with higher maltodextrin concentrations typically resulting in lower moisture content. Bulk density and moisture content generally have an inverse relationship. As moisture content increases, bulk density decreases because water molecules take up space between sample particles, reducing the total volume filled by solid material. Previous studies from Šavikin et al. (2021) have also observed that the concentration of the wall material can affect the bulk density of encapsulated fruit extract. For instance, powders with 120% whey protein (WP) concentrations had higher bulk density than others.

The stability and long-term storage of extracts are significantly influenced by bulk and tapped densities, which affect a few important variables. First, space is used efficiently, and fewer materials are needed for packaging when bulk density-based optimization is used for packaging (Ding et al., 2020). Tapped density reduces empty areas inside the container by indicating how much a powder can be compacted. Reducing access to air and moisture is crucial since these factors can trigger degradation processes such as oxidation and hydrolysis. The extract's shelf life can be increased by preserving its chemical stability by limiting exposure to these substances (Sornsomboonsuk et al., 2019). Moreover, higher densities enhance the extract powder's flow properties, which is helpful for consistent handling and dosing throughout the production and packaging stages (Akseli et al., 2019). This avoids problems like clumping or caking, which can reduce the effectiveness and efficacy of the extract. Optimized densities have the potential to decrease transportation and warehousing costs by enabling the transportation and storage of a greater quantity of products inside a given container. Furthermore, uniform bulk and tapped densities provide batch-to-batch consistency, essential for preserving the extract's purity and functionality in various applications (Stranzinger et al., 2019).

pH Value

The results from Table 3 show a significant difference $(p < 0.05)$ in the pH value of the encapsulated fruit extracts of *M. malabathricum* across different ratios (1:1, 1:2, 1:3). The pH of the powder ranges from pH 5.35 to 2.87, with the highest pH value (5.35 \pm 0.02) recorded for ratio 1:1, followed by ratio 1:2 with a pH value of 3.60 ± 0.02 , and the lowest pH for ratio 1:3 (2.87 \pm 0.02). Kobo et al. (2022) noted that these pH values indicate acidic conditions. The low and acidic pH values observed in this study suggest that all the powder samples may be stable on the shelf, indirectly contributing to the stability of the product.

It is important to consider that the pH of each ratio may have been affected by the amount of crude extract present in the ratio. For instance, in the sample ratio 1:1, which had the highest crude extract content compared to other ratios, the pH value was $5.35 \pm$ 0.02. A study by Aishah et al. (2013) reported that the crude extract of *M. malabathricum* fruit collected from Muar, Johor, had a pH value of 5.56 ± 0.30 , which is similar to the pH of ratio 1:1. In contrast, the pH values of ratios 1:2 and 1:3 was lower. It could be attributed to the decrease in crude extract content and increased maltodextrin concentration in these ratios, leading to a decrease in pH value. Additionally, Chatpun et al. (2016) found that as the concentration of maltodextrin increased from 1% to 10%, the pH of tapioca maltodextrin solutions containing DE1 slightly dropped from 5.4 to 5.1. It suggests that higher concentrations of maltodextrin can contribute to decreased pH.

Solubility

The encapsulated fruit extracts of *M. malabathricum* at different ratios (1:1, 1:2, 1:3) exhibited solubility times ranging from 4.637 to 3.027 s. The results in Table 3 indicate significant differences ($p < 0.05$) in solubility time among all ratios, suggesting that the different concentrations of maltodextrin affect the powder's solubility. Specifically, ratio 1:3 had the lowest solubility time $(3.027 \pm 0.06 \text{ s})$ compared to ratio 1:1 $(4.637 \pm 0.36 \text{ s})$ and ratio 1:2 (3.943 \pm 0.92 s).

As Nawi et al. (2015) described, maltodextrin is highly soluble compared to other wall materials such as gum Arabic. Maltodextrin is produced exclusively through hydrolysis catalyzed by acids, resulting in linear chains that are easily retrograded. Thus, the highwater solubility of maltodextrins is achieved by combining acid catalysis with amylasecatalyzed hydrolysis (Tiefenbacher et al., 2017). The different ratios, representing varying maltodextrin concentrations, appear to be linked to the solubility of the powder. Ratio 1:3, which contained more maltodextrin compared to the other ratios, exhibited the highest solubility. The findings are consistent with those of George et al. (2021), who attributed the high solubility index of maltodextrin samples to its inherent solubility properties.

Color Determination

Three variables typically represent color properties: *L*, a*,* and *b*.* The color properties of encapsulated fruit extracts with different ratios were analyzed (Table 3), and the powder color was presented using the Red, Green, Blue System (RGB) color palette. Ratio 1:1 showed the lowest L^* value (56.86 \pm 3.13), followed by ratio 1:2 (70.505 \pm 0.62), and the highest was ratio 1:3 (76.75 \pm 1.83). Similarly, ratio 1:1 showed the lowest a^* value (39.72 ± 4.43) , followed by 1:2 and 1:3 with 42.48 ± 0.26 and 45.90 ± 0.77 , respectively. However, the b^* value was highest for ratio 1:1 (2.43 \pm 0.61), followed by ratio 1:2 (1.27) \pm 0.17), and the lowest value was ratio 1:3 (1.75 \pm 0.39).

It is noteworthy that increasing maltodextrin concentrations in the powder tends to increase lightness (L*) due to maltodextrin's white color. Similarly, an increase in the crude extract content in the sample reflects the dark purple nature of the original *M. malabathricum* fruit color. A study by Laqui-Vilca et al. (2018) supports the correlation between maltodextrin concentration and the *L** value of microencapsulated quinoa betalain. They found that *L** values increased as maltodextrin concentration increased. The betalain microencapsulated from beetroot juice has the highest *L** value with a 12.5% maltodextrin content. Similarly, Santiago et al. (2016) observed that anthocyanin pomegranate juice powder exhibited *a** value within the red range, indicating the presence of anthocyanins. Therefore, the analysis of the powder color shows the presence of anthocyanins and how wall materials influence the color values with a 12.5% maltodextrin content.

Surface Morphology by the Scanning Electron Microscopy (SEM)

SEM micrographs of the encapsulated fruit extracts of *M. malabathricum*, with different ratios and the crude extract, obtained through spray drying at magnifications of 2000 and 5000 (Table 4). The encapsulated fruit extracts (1:1, 1:2, 1:3) displayed spherical particles and particles with irregular, concave, and wrinkled surfaces, all equal size with no significant differences observed ranging from 21.13 ± 0.78 µm, 21.20 ± 0.05 µm, and 21.35 ± 0.19 µm, respectively. Meanwhile, the crude extract exhibited an agglomerated and disordered structure.

Quelal et al. (2023) attribute this phenomenon to spray drying, explaining that wrinkled particles are more susceptible to oxidation. However, their concavity and wrinkles facilitate quick moisture evaporation during spray drying. However, these features may also lead to agglomerate formation, impeding the dust's ability to disperse when reconstituted in a solvent (Kurniawan et al., 2019). In addition, mechanisms causing shrinkage and deformation are generally more obvious during drying, especially at low temperatures where slower water diffusion allows particles more time to shrink, collapse, and undergo other deformations. Previous studies by George et al. (2021) noted slight differences in the morphological shape of *Moringa oleifera* leaf powder due to the different encapsulation agents used.

Antioxidant Activity

In this study, the encapsulated fruit extracts (1:1, 1:2, 1:3) and crude extract of *M. malabathricum* underwent screening for their potential antioxidant activities using DPPH, ABTS, and FRAP assay methods. The IC50 values for DPPH and ABTS radicals were determined as the concentration required to achieve 50% inhibition, indicating the amount of antioxidants needed to reduce the initial concentration of the assay solution by half.

DPPH and ABTS Radical Scavenging Activity

IC50 values of the encapsulated fruit extracts and crude extract with positive controls (ascorbic acid) ranged from 25.17 ± 0.06 μg/ml to 178.5 ± 7.53 μg/ml (Table 5). From the table, ratio 1:1, crude extract and ascorbic acid exhibited no significant difference (*p* > 0.05) and demonstrated excellent antiradical activity by inhibiting DPPH and ABTS assay with lower IC50 values of (1:1, 28.5 ± 2.78 μg/ml), (crude, 27.37 ± 7.91 μg/ml), and (ascorbic acid, 25.17 ± 0.06 μ g/ml) for DPPH assay. Similarly, for the ABTS assay, the IC50 values were (1:1, 49.03 \pm 5.94 μg/ml) (crude, 35.33 \pm 3.4 μg/ml), and (ascorbic

IC_{50} (μ g/ml)		
	2,2-Diphenyl-1-picryhydrazyl (DPPH)	2,2'-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid (ABTS)
Ascorbic Acid $25.17 \pm 0.06^{\circ}$		26.67 ± 0.12 ^{de}
Crude	27.37 ± 7.91 °	35.33 ± 3.40 ^{cd}
1:1	28.50 ± 2.78 °	$49.03 \pm 5.94^{\circ}$
1:2	73.93 ± 4.06^b	$67.43 \pm 7.98^{\rm b}$
1:3	86.27 ± 2.61 ^a	$92.83 \pm 0.76^{\circ}$

Table 5 *The IC50 value for both DPPH and ABTS assay of samples*

Note. Data are expressed as mean \pm SD ($n = 3$), means were compared by the Tukey test ($p < 0.05$). The mean is significantly different *p* < 0.05

acid 26.67 ± 0.12 μg/ml). However, ratios 1:2 and 1:3 significantly differed from other DPPH and ABTS assay samples.

There is a significant difference ($p < 0.05$) among the samples, ratio1:1 showed the highest antioxidant activity followed by ratio 1:2 and ratio 1:3 for DPPH (28.5 \pm 2.78 µg/ ml, 73.93 ± 4.06 μg/ml, 86.27 ± 2.61 μg/ml) and ABTS assays $(49.03 \pm 5.94, 67.43 \pm 7.98,$ 92.83 ± 0.76), respectively. With that, all the encapsulated fruit extracts were considered to have greater antioxidant activity. In addition, a ratio of 1:1 successfully preserved the antioxidant activity of the extract, as the results show no significant difference $(p >$ 0.05) compared to the crude extract. It can be proven by the previous result, where the encapsulation efficiency of ratio 1:1 was the highest among other ratios.

To date, little evidence has been found associating the different ratios of plant extract and/or wall material concentrations with the antioxidant activity of encapsulated fruit extract of *M. malabathricum*. However, the study by Sayuti et al. (2015) showed that the increasing amount of *M. malabathricum* fruit extract in jackfruit jam correlated with a lower IC50 value, indicating higher antioxidant activity. It was related to the fruit's flavonoid content in the form of anthocyanin and phenolic compounds. Meanwhile, Wariyah and Riyanto (2016) showed that increasing the concentration of maltodextrin will decrease antioxidant activity. Adding maltodextrin caused the total phenol of the encapsulated fruit extract to become lower.

By contrast, the DPPH assay conducted by Purwaningsih et al. (2023) showed a higher IC50 value for the methanolic extract of *M. malabathricum* fruit (99.79 μg/ml) than the ethanolic extract reported by Isnaini et al., (2019) (16.82 \pm 0.24 μg/ml). This difference can be explained by the presence of –OH groups bound to the carbon of the aromatic ring, which enables flavonoids and phenols to act as antioxidants. The ability of phenolic compounds to scavenge free radicals depends on the quantity and position of hydroxyl groups within the molecule. Greater antioxidant activity is generated in proportion to the quantity of hydroxyl groups present. Antioxidant activity may be influenced by how the compound interacts with DPPH free radicals (Shahidi & Ambigaipalan, 2015).

These results suggest that the antioxidant activity in both DPPH and ABTS assays may vary due to different concentrations and variations in localities and solvent extraction methods used to obtain the crude sample. This finding suggests that the fruit of *M. malabathricum* plants may serve as a promising source of natural colorants with higher antioxidant activity, holding potential for commercialization in the future.

FRAP Ferric Ion Reducing Activity

Meanwhile, the FRAP assay determines antioxidant activity by measuring electron transport. The result of IC50 values of the FRAP assay of the encapsulated fruit extracts ratios 1:1, 1:2, 1:3, and the crude extract sample is presented in Figure 4. The IC50 values ranged from 24.67 to 186.80 μg/ml. A lower IC50 value indicates a higher antioxidant activity in the sample. The graph depicted that the highest IC50 value was observed for ratio 1:3, indicating a reducing activity of 50% at 84.93 μg/ml, followed by ratio 1:2 $(66.43 \,\mu\text{g/ml})$. Additionally, there was no significant difference between ratios 1:1 and the crude extract ($p > 0.05$), with IC50 values of 39.97 μ g/ml and 28.03 μ g/ml, respectively. Moreover, the crude extract showed no significant difference compared to the standard.

Figure 4. Comparison of IC50 value for Ferric reducing antioxidant power (FRAP) assay of samples

Among the encapsulated fruit extracts, ratio 1:1 exhibited the lowest IC50 value, indicating the highest antioxidant activity among the other ratios. The results showed a significant difference between the encapsulated fruit extracts, with a ratio of 1:1 at 39.97 μg/ml, ratio 1:2 at 66.43 μg/ml, and ratio 1:3 at 84.93 μg/ml. Previous results indicate this outcome is due to the highest anthocyanin compound content. Anthocyanin compounds in the sample contribute to the antioxidant capacity by functioning as free radical scavengers against harmful oxidants, such as reactive oxygen and nitrogen species (Mattioli et al., 2020).

According to Karageçili et al. (2023), compounds capable of breaking the chain of free radicals by donating a hydrogen atom are associated with the reducing properties of any sample. Phenolic compounds, such as anthocyanins, act as hydrogen donors, singlet oxygen quenchers, and reducing agents due to their redox characteristics, exhibiting high

reducing power on Fe3+–TPTZ. The higher antioxidant activity in the FRAP assay for *M. malabathricum* is supported by previous studies by Nayak & Basak (2015) and Kasunmala et al. (2020), which found that *M. malabathricum* exhibits the highest FRAP.

Stability Test

This study evaluated different storage conditions within 90 days for the encapsulated fruit extract ratios (1:1, 1:2, and 1:3) and crude extract based on Total Anthocyanin Content (TAC). Table 6 summarizes the TAC for each ratio and the crude extract from day 0 to day 90. Meanwhile, Figure 5 demonstrates the degradation of total anthocyanin content from day 0 under dark conditions at temperatures of -20°C, 4°C, and 25°C, as well as under conditions exposed to light at 25°C.

Figure 5. Degradation of Total Anthocyanin Content (TAC) at different storage conditions in 90 days for (a) Ratio 1:1, (b) Ratio 1:2, (c) Ratio 1:3, and (d) Crude

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The overall trend shown in Figure 5 indicates that the anthocyanin contents were at their lowest at the highest temperature (25°C) and when exposed to light conditions. This result aligns with a previous study by Enaru et al. (2021), which stated that temperature affects the degradation of anthocyanin content. The hypothesis proposed by Hocine et al. (2018), suggesting that increasing temperature decreases the stability of encapsulated fruit extract, may closely correlate with their chemical structure. Indeed, the chemical structure of the encapsulated fruit extract plays a crucial role in determining its stability. This structure can accelerate chemical reactions, alter its configuration, facilitate the release of encapsulated material more easily, potentially induce degradation reactions, or render the powder more susceptible to environmental factors such as oxidation and moisture.

Research has indicated that anthocyanins undergo degradation during storage conditions (Enaru et al., 2021). Elevations in polymeric color values correspond with a reduction in total anthocyanin content. It is anticipated that during storage, anthocyanins undergo significant polymerization (Ochoa et al., 1999). There could be several reasons for the significant rise in polymeric color values and the consequent decrease in anthocyanins, such as residual enzyme activity or condensation interactions with other phenolics (Cheynier et al., 2012). Unfortunately, establishing the stability of anthocyanins is challenging due to their complex mechanisms, but their chemical structure strongly influences their stability.

These findings were also reported by Muche et al. (2018), indicating that temperature affects the rate of anthocyanin degradation at 25°C during storage, with higher temperatures resulting in a noticeably larger loss of anthocyanins. By the end of storage at 25°C, anthocyanin loss was significantly higher (95%–99.9%) than at 5°C, where the loss was 50%–60%. Additionally, light exposure contributes to reducing the anthocyanin content in the sample due to the absorption of light photons by organic molecules in the sample, causing chemical disruption to the conjugated double bonds, such as in aromatic rings, double rings, and compounds, including disulfide bonds, thereby reducing the anthocyanin stability.

The findings reveal that although encapsulation has some protective effect, it is not enough to stabilize anthocyanin considerably since Total Anthocyanin Content (TAC) decreases slowly over 90 days under various storage conditions. Compared to crude extracts, the encapsulation technique probably slows down the degradation process, which protects anthocyanins from direct exposure to conditions that can cause them to degrade, such as light, oxygen, and temperature changes. This result also can be seen clearly from the previous study in which Baeza et al. (2021) reported that the degradation of anthocyanins during storage indicates a half-life of 63 days at room temperature. Meanwhile, Tonon et al. (2010) evaluated the storage stability of spray-dried acai juice within six months and reported the degradation of anthocyanins during storage, indicating a decrease in anthocyanin content. The encapsulation methods or materials could not achieve

optimal protection, which could allow the slow deterioration to continue. It implies that while encapsulation slows down the rate at which anthocyanins break down, it does not completely resolve the anthocyanins' underlying instability under the studied conditions. Therefore, more encapsulating materials and technique optimization are required for better stabilization and storage conditions.

Degradation Index (DI)

According to Nayak and Rastogi (2010b), the stability and quality of the anthocyanin DI were calculated for further analysis of deterioration. This study measured the first-order kinetics for anthocyanin degradation for different ratios from day 0 until the end of the 90-day storage period under different storage conditions.

Figure 6 shows the anthocyanin's DI of encapsulated fruit extract of *M. malabathricum* produced with different ratios and crude extract, following first-order kinetics throughout a 90-day storage period. From the figure, the results are consistent for all three ratios, and the crude extract showed an increase in DI from lower to higher temperatures, except for -20°C, with the encapsulated fruit extract at the highest temperature exposed to light

showing the highest DI value. The most distinctive pattern found for all different ratios and crude extract was that the DI of anthocyanin contents increased progressively when stored at 25°C in light conditions over 90 days. However, the DI of all three ratios and the crude extract was relatively low when the temperature was maintained at 4°C. The most likely causes of anthocyanin degradation are the compound's susceptibility to higher temperatures and light exposure. In addition, the results also showed that the sample is likely unsuitable at extremely low temperatures, -20°C.

Azarpazhooh et al. (2018) stated that, generally, the anthocyanin content of the encapsulated fruit extract decreased as the storage period increased. The degradation index measures the extent of degradation or damage to a material over time. A lower degradation index is considered more stable than a higher degradation index because it indicates that the anthocyanin content has experienced less degradation or deterioration, as demonstrated in the figure. The experimental results align with previous studies on the stability of *Melastoma malabathricum* L., as reported by Yusoff et al. (2014) and Janna et al. (2006). These studies found that anthocyanin pigments degraded more at higher temperatures, such as 25°C, compared to lower temperatures, like 4°C. Furthermore, Jiang et al. (2019) stated that the degradation and polymerization of anthocyanins at high temperatures ultimately cause a greater DI. Kirca et al. (2006) also demonstrated this scientific phenomenon and observed that black carrot anthocyanins degraded more quickly when stored at 37°C compared to refrigerated conditions at 4°C. Furthermore, Kumar et al. (2022) explained that the increase in DI is due to the reaction between two different compounds, tannins and anthocyanin. Reduced values of DI suggest increased anthocyanin stability and a decreased degradation rate. The formation of polymeric colors occurs when monomeric anthocyanins react with condensed tannins, such as epicatechin or catechin. The interaction between anthocyanins and other hydroxy residues from phenols in condensed tannins leads to the formation of a chemical complex, contributing to degradation.

Storage Stability Determination

The analysis proceeded using ANOVA to determine the significant effects between two factors, the ratio and the condition, on the main effect (DI value). Table 7 indicates a significant effect $(p < 0.05)$ for the condition, while for the ratio, there is no significant effect ($p > 0.05$). Similarly, the interaction for both factors showed no significance ($p > 0.05$). Thus, these results suggest that the main factor affecting DI is the condition, contributing to lower DI values.

Figure 7 presents the main effect plot for each factor, condition, and ratio to assist in interpreting the results for stability determination. Since there is no significant interaction between the ratio and condition, both factors act as independent main effects. The condition, rather than the ratio, is the significant factor determining what most affects the lowest degradation value.

Source	Degrees of Freedom (DF)	Adjusted Sum of Squares (Adj SS)	Adjusted Mean of Squares (Adj MS)	F-Value	p-Value
RATIO		0.000002	0.000001	1.13	0.340
CONDITON	3	0.000149	0.000050	46.98	0.000
RATIO [*] CONDITON	6	0.000009	0.000001	1.38	0.264
Error	24	0.000025	0.000001		
Total	35	0.000186			

Table 7 *Analysis of variance (ANOVA) of the main effect between two factors*

Figure 7. Main Effect Plot (a) Condition main effects plot for Degradation Index (DI), and (b) Ratio main effects plot for DI

As previously mentioned, a lower degradation index signifies greater stability, indicating less anthocyanin content degradation or deterioration. From the graph in Figure 7, the condition factor showed the lowest mean DI at 4°C (0.00314) in the dark, followed by -20°C (0.00589) in the dark, 25° C (0.00693) in the dark, and the highest DI was at 25° C (0.00877) in the light. As for the ratio, the lowest mean DI was ratio 1:2 (0.00585), followed by ratio 1:3 (0.006228), and the highest mean DI was 1:1 (0.00648). Limited research supports the stability of ratio 1:2 compared to other ratios. However, the composition of wall materials may contribute to why the ratio 1:2 was more stable. This finding is in line with a previous study by Dobroslavić et al. (2023), which suggested that a ratio of 1:2 may be more stable due to the optimal balance between the sample and maltodextrin, creating a suitable protective layer that protects against external factors and prevents anthocyanin degradation. In comparison, a ratio of 1:3 contains a higher amount of maltodextrin, which may delay structural formation and potentially lead to instability.

The study revealed notable differences in anthocyanin stability when stored under -20°C, 4°C, and 25°C. The DI at 4°C was 1.282×10^{-3} , slightly higher at -20°C with 1.286 \times 10⁻³, while at 25^oC, the anthocyanin pigments degraded faster with a DI of 6.261 \times 10⁻³. A study by Yusof et al. (2014) observed a similar pattern for *Hibiscus rosa-sinensis* but not for *Codiaeum variegatum*, suggesting that anthocyanin pigments are more stable at -20°C. Thus, this suggests that the anthocyanin at -20° C provides good stability for three months, but the optimal temperature for storage stability was still 4°C. The analysis of the variance table and the main effect plot graph leads to the conclusion that the most stable condition was at 4°C in the dark, while the best ratio was 1:2. These factors can be considered essential in achieving a lower DI and indirectly ensuring stability for future applications.

CONCLUSION

This research highlights the potential of encapsulated fruit extracts from *Melastoma malabathricum* L. as a valuable source of natural color. This study has demonstrated an overall characterization, including moisture content, bulk and tapped density, pH value, solubility, color, and surface morphology for potential future applications. Among the ratios tested, ratio 1:3 exhibited the lowest moisture content and solubility time, while ratio 1:1 showed the lowest values for bulk and tapped density. The pH of the encapsulated fruit extracts demonstrated an acidic condition. Regarding color properties, ratio 1:1 exhibited the lowest L^* and a^* values but the highest b^* value. All encapsulated fruit extracts showed the same shape, and no significant differences were observed in particle size among the ratios. This study also focused on assessing antioxidant activity before and after encapsulation. Ratio 1:1 exhibited no significant difference $(p > 0.05)$ compared to the crude extract *from M. malabathricum*, indicating successful preservation of antioxidant activity. For storage stability, the most stable condition was at 4° C in dark conditions, while a ratio of 1:2 was the best condition. These factors contribute to achieving lower DI values and indirectly enhance stability for future applications. To further enhance anthocyanin stability, optimizing methods like nanoencapsulation and using superior encapsulation materials could be applied and considered, especially during storage stability, by taking into account other parameters, including humidity levels and the type of containers used for storing the samples.

ACKNOWLEDGEMENTS

This research was supported by Universiti Tun Hussein Onn Malaysia (UTHM) through RE-GG (vot Q209) and Tier 1 (vot Q148), and communication of this research is made possible through monetary assistance by the UTHM Publisher's Office via Publication Fund E15216.

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