

# Comparison of Gidyea gum, gum Arabic, and maltodextrin in the microencapsulation and colour stabilisation of anthocyanin-rich powders using freeze-drying and spray-drying techniques

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## ABSTRACT

Traditional foods in Australia have been under-explored as both food colourants and microencapsulates. Many of these native foods are high in anthocyanins. Anthocyanins, known for their vibrant colours and health benefits, face stability challenges in food applications due to sensitivity to environmental factors. Microencapsulation is a common technique used to preserve the stability of anthocyanins. This study investigates the microencapsulation of anthocyanins extracted from *Antidesma erostrae*. The native Gidyea gum, as compared to gum Arabic and maltodextrin, is used as wall material, employing both freeze-drying and spray-drying techniques. The encapsulated powders were evaluated for colour stability, encapsulation efficiency, and morphological characteristics over a 30-day storage period. Results demonstrated that spray-drying was more effective than freeze-drying in preserving colour stability, with minimal changes observed in CIELAB metrics. Among the wall materials, maltodextrin combined with either gum Arabic or Gidyea gum exhibited superior encapsulation efficiency and anthocyanin retention. Gidyea gum was a reliable microencapsulation material with comparable performance to gum Arabic. The study provides valuable insights for food manufacturers seeking to enhance anthocyanin-rich products' nutritional and aesthetic qualities while highlighting the importance of selecting appropriate wall materials and drying techniques. The findings support the development of natural, sustainable, and consumer-friendly solutions for colour stabilisation in food products.

## 1. Introduction

Anthocyanins are a class of flavonoids within the polyphenol family that are responsible for the red, purple, and blue colours in many fruits and vegetables (Ali, Cottrell, & Dunshea, 2022; Cömert, Mogol, & Gökmen, 2020; Cosme et al., 2022; Nastasi, Fitzgerald, & Kontogiorgos, 2023). Within the field of food science, compounds have been extensively studied for their health-promoting properties, including antioxidant, anti-inflammatory, and anti-carcinogenic activity (Khoo, Azlan, Tang, & Lim, 2017). It has also been reported that regular consumption of anthocyanin-rich foods can reduce the risk of cardiovascular diseases, improve cognitive function, and protect against cancer (Khoo et al., 2017; Singh, Bhatt, Kumar, & Singh, 2024). These associated health benefits are attributed to the ability of anthocyanins to efficiently scavenge free radicals by donating a hydrogen atom from the many hydroxyl groups present along their cyclic ring structure (de Souza Farias, da Costa, & Martins, 2021).

Beyond their health benefits, anthocyanins are increasingly used as natural colourants in the food industry (Baeza & Chirife, 2021; Gençdağ, Özdemir, Demirci, Görgüç, & Yılmaz, 2022; Rocha et al., 2023; Suriano, Balconi, Valoti, & Redaelli, 2021). Synthetic colourants have faced scrutiny due to potential health risks, leading to growing consumer demand for natural alternatives (Mabuza, Sonnenberg, & Marx-Pienaar, 2023). Anthocyanins offer a vibrant and diverse colour palette, making them ideal for use in beverages, confectioneries, dairy products, and more (Caldeira, Lopes, Delgado, Canas, & Anjos, 2018; Mahdavi, Jafari, Assadpour, & Ghorbani, 2016a; Oliveira et al., 2021). However, their stability in processed foods is challenged due to sensitivity to oxidation, moisture, temperature changes, and enzymatic degradation (Liu et al., 2018; Patras, Brunton, O'Donnell, & Tiwari, 2010). Therefore, various techniques are employed to improve the stability of anthocyanins within a food matrix (Darniadi, Ho, & Murray, 2018; Li, Zhu, & Zeng, 2021; Weber, Boch, & Schieber, 2017).

Microencapsulation is a dynamic technology used in food and

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pharmaceutical formulation, particularly for enhancing the stability and bioavailability of sensitive compounds such as anthocyanins (Huang, Yuan, & Baojun, 2023; Sousa, Parente, Marques, Forte, & Tavares, 2022; Yan, Kim, Ruiz, & Farmer, 2022). Specifically, techniques such as spray-drying and freeze-drying have proven to be effective in the microencapsulation of naturally pigmented compounds (da Rosa et al., 2019; Kanha, Surawang, Pitchakarn, & Laokuldilok, 2020; Mahdavee Khazaei, Jafari, Ghorbani, & Hemmati Kakhki, 2014; Yan et al., 2022). Common molecules used for the encapsulation of bioactive compounds include polysaccharides, proteins, lipids and synthetic polymers (Yan et al., 2022). For the encapsulation of anthocyanins, maltodextrin has shown a higher efficacy in preserving colour and antioxidant activity during storage compared to other biopolymers (da Rosa et al., 2019). Maltodextrin is often used in conjunction with other food gums such as pectin or gum Arabic to modify powder dynamics and improve anthocyanin stability (Estupiñan-Amaya, Fuenmayor, & López-Córdoba, 2022; Mahdavee Khazaei et al., 2014; Mahdavi, Jafari, Assadpoor, & Dehnad, 2016b; Sarabandi, Jafari, Mahoonak, & Mohammadi, 2019). Microencapsulation effectively protects pure anthocyanins from environmental changes while offering physical stability, controlled release, improved bioavailability, and ease of handling and storage (Mohammadalinejad & Kurek, 2021). However, it has become commonplace to use fruit extracts for their anthocyanin contents, with a high research output for blueberry (da Rosa et al., 2019; Estupiñan-Amaya et al., 2022), raspberry (Mansour, Salah, & Xu, 2020), and various other berries (Estupiñan-Amaya et al., 2022; Klisurova et al., 2019; Mahdavi, Jafari, Assadpoor, & Dehnad, 2016a). As the variety of microencapsulated fruit extracts is growing, there is still a vast range of fruit to be investigated.

Our recent works, which characterised a new food hydrocolloid (Hay, Kontogiorgos, Thompson, Nastasi, & Fitzgerald, 2024), as well as reviewing a range of Traditional foods of potential colourant sources (Hay, Prakash, Daygon, & Fitzgerald, 2022), demonstrates the potential for underutilised food sources to be used as natural food colourants and microencapsulation materials. Continuing research on the microencapsulation of novel anthocyanin sources from Traditional foods showcases new alternatives for food ingredients, which can supplement the current range in the food industry and promote industry development within Indigenous communities. Research of this nature supports the development of natural, sustainable, and consumer-friendly solutions across various industries which can contribute to a circular economy (Booth

et al., 2023; Ohlin, Trueb, Raven, & Robinson, 2024).

This study focuses on comparing the efficacy of different wall materials; Gidyea gum, gum Arabic, and maltodextrin in the microencapsulation of anthocyanins extracted from *Antidesma erosre*. The microencapsulation processes (Fig. 1) examined include freeze-drying and spray-drying, both widely used in the food industry for preserving bioactive compounds. By evaluating the colour stability, encapsulation efficiency, and morphological characteristics of the resultant powders, this research aims to identify the most effective combination of wall material and drying method for maintaining anthocyanin stability. These findings will provide valuable insights for food manufacturers seeking to enhance their product's nutritional and aesthetic qualities and potential new business opportunities for Indigenous communities.

## 2. Materials and methods

### 2.1. Materials

*Antidesma erosre* was sourced from Widjutgrub Bush Food Nursery (GPS Coordinates  $-26.64148, 152.81429$ ). Plant samples were collected directly from plants and kept on ice in transit to be stored at  $-80^{\circ}\text{C}$  until use. The following materials were used to produce microparticles: Gidyea gum was collected from a private location in Traditional Iningai country with help from the Yumbangu Aboriginal Cultural Heritage and Tourism Development Aboriginal Corporation (YACHATDAC). With the help of YACHATDAC the authors of this study acquired informed consent for the use of all Gidyea gum-related Traditional Knowledge, including an active benefit-sharing initiative. Gum Arabic standards (CAS# 9000-01-5) were acquired from ThermoFischer, (Darmstadt, Germany). Maltodextrin DE20 (CAS# 9050-36-6) was from Sigma Aldrich (St. Louis, MO, USA). All chemicals, organic solvents, and materials were supplied by Sigma Aldrich (St. Louis, MO, USA) and reagents were prepared freshly on the day of analysis.

### 2.2. Extraction protocol

*Antidesma erosre* samples were frozen to  $-80^{\circ}\text{C}$  and dried for 72 h in a Christ alpha 1–2 LD freeze drier (Martin Christ, Osterode, Germany) at 1.0 mPa. The freeze-dried sample matter was immersed in liquid nitrogen before grinding in a Tissuelyser II ball and socket mill (Qiagen, Tokyo, Japan) at 30x1/s Hz for 30 s and then sieved through a 50  $\mu\text{m}$

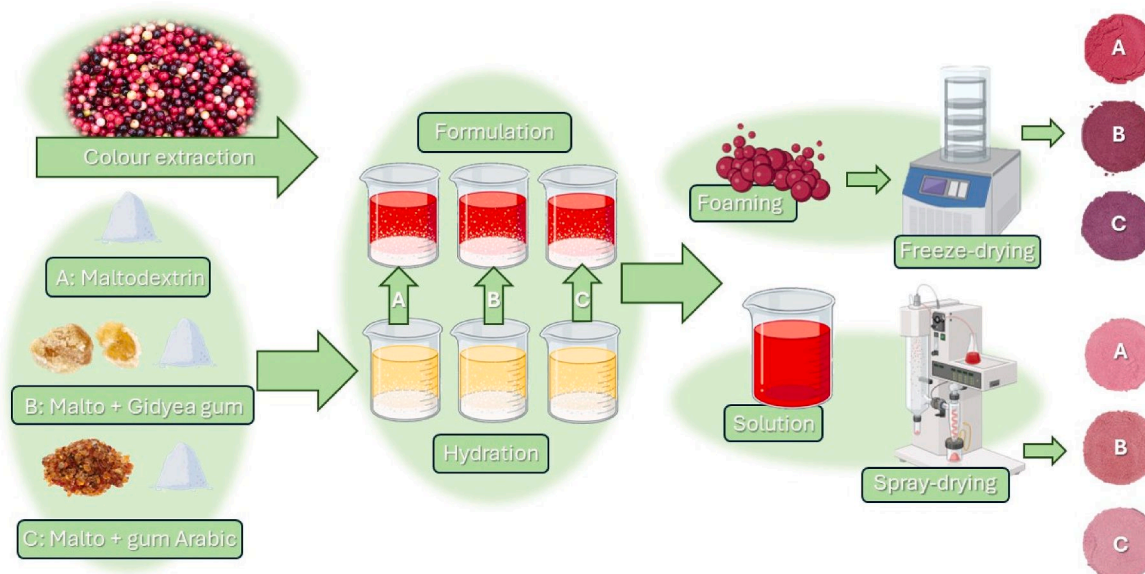


Fig. 1. Diagram of processing conditions for microencapsulated native currant powders.

mesh. The sieved sample (1g) was twice extracted in 10 mL of 80 % ethanol acidified to 2 pH with 6N HCl). Each 10 mL extract was sonicated (Soniclean 160TD, Mektronics, Australia) for 15 min at 100 Hz, vortexed for 30 min in the dark, and incubated at 4 °C for 2 h. Next, the supernatants were combined and centrifuged (Thermo Fischer Scientific, Waltham, MA, USA) at 4500 g for 15 min, and the pooled supernatant was filtered using Wattman filter paper strip (item number: 1001 110). Extracts were made in triplicate and then stored at –80 °C for no longer than 48 h before testing.

### 2.3. Anthocyanin profiling

Anthocyanin identification and quantification were performed using an Ultra Performance Liquid Chromatography-Quadrupole Time of Flight Tandem Mass Spectrometry (UPLC-Q-ToF-MS/MS) The instrument information is as follows: A Shimadzu Nexera UHPLC system (Kyoto, Japan; LC-30AD pump, SIL-30AC autosampler and CTO-30A column oven) was equipped with a Shimadzu Q-TOFMS-9030 detector. The separation of the sample analytes was conducted on a Restek Biphenyl, 2.7 µm (100 × 2.1 mm Column, Product Code: 9309A12, Restek, Saunderton, England). The mobile phase consisted of A (0.05 % [v/v] formic acid, 40 µM ammonium nitrate in water) and B (0.05 % [v/v] formic acid). The sample injection volume was 1 µL with a consistent flow rate of 0.4 mL/min. The gradient program was as follows: 0 % B for 0–1.5 min, 5 % B for 1.5–2.5 min, 10 % B for 2.5–4.5 min, 30 % B for 4.5–8 min, 40 % B for 8.0–12.0 min, 60 % B for 12.0–13.0 min, 80 % B for 13.0–14.0 min, 100 % B for 14.0–15.0 min, 100 % B for 15.5–16.0 min, 0 % B for 16.0–17.0 min. The column temperature and auto-sampler were set at 40 °C. Ionisation for positive and negative modes was assessed. The mass spectrometer was operated using an electrospray ionisation (ESI) source with collision energy set at 70 eV, the fragmentor voltage was 100 V. The settings were: Data independent acquisition, nebulizing gas flow of 3.0 L min<sup>-1</sup>, Drying and heating gas flow was 10.0 L min<sup>-1</sup>. The nebulizer pressure was 230 kPa. The source temperature was 120 °C, and the desolvation temperature was 200 °C. Anthocyanin quantification was determined via a 5-point standard curve expressed as cyanidin-3-glucoside equivalence.

### 2.4. Processing of LC-MS/MS data

Data files were extracted from the Shimadzu Lab Solutions Software (Shimadzu, Kyoto, Japan) in the MzML format. Next, the MzML files were imported to MS-DIAL v5.1 to process the positive and negative ionisation mode datasets. All samples were aligned using a mixed QC file, and the alignment file was normalised by the total ion chromatogram method. Features were reference matched with MS Dial using positive and negative mode libraries with a match threshold of >85%. The reference-matched compounds were each annotated using their MS2 spectral matching and cross-referenced with *in silico* modelling using MS Finder score. Compounds fragmented in positive and negative ionisation modes were resolved based on their highest abundance across the sample groups. The reference-matched peak list was exported to Microsoft Excel for import into statistical software.

### 2.5. Microencapsulation stock solution preparations

Fig. 1 presents a diagram of the workflow for microencapsulation. Three treatments were prepared for each of the freeze-drying and spray-drying microencapsulation methods. Firstly, three wall material stocks were prepared: maltodextrin (100 %), maltodextrin/gum Arabic (3:1) and maltodextrin/Gidyea gum (3:1). Each wall material stock was hydrated in deionised water (mass ratio 0.25) for 3 h at 20 °C under constant stirring at 120 rpm. Native currant extractions (50 mg mL<sup>-1</sup>) made as per section 2.2 were added to wall stocks at a 1:3 ratio. Stock solutions were pH adjusted to 3.5 using 2N HCl. All stock solutions were prepared and microencapsulated in triplicate. The final total soluble

solids (TSS) were 18–21° Brix, and the pH was 3.5–3.6. TSS was measured using a PAL-1 BLT/A-W digital hand-held pocket refractometer (Atago, Japan).

### 2.6. Microencapsulation produced by freeze-drying

Stock solutions were foamed using a T25 Ultra-Turrax at 15000 rpm for 5 min before freezing at –80 °C and subsequently freeze-dried for 48 h using a Christ alpha 1–2 LD freeze drier (Martin Christ, Osterode, Germany) at 1.0 mPa. Microencapsulated pellets were ground in mortar and pestle and sieved through a 250 µm mesh sieve. Powders were stored in 20 mL water-tight glass vials at –80 °C until use.

### 2.7. Microencapsulation produced by spray-drying

Stock solutions were fed into a Büchi B-290 mini spray dryer with the following operating conditions: aspirator rate 100 % (35 m<sup>3</sup>/h); atomisation air rotameter 30 mm (439 L/h) with a co-current flow; drying air inlet temperature 150 °C, while pump rate was adjusted between 8 and 10 % to maintain an outlet temperature of 90 °C. After the completion of the experiment and when the air inlet temperature fell below 50 °C, the samples were collected from the product collection vessel. Powders were stored in 20 mL water-tight glass vials at –80 °C until use.

### 2.8. Moisture content and water activity ( $A_w$ )

The moisture content was determined by oven drying at 105 °C until constant weight was attained, according to the AOAC method 925.10. (Horwitz, Chichilo, & Reynolds, 1970). The water activity ( $A_w$ ) was performed using an Aqualab Pawkit (Decagon, Malaysia) operated at 25 °C. Each analysis was performed in triplicate.

### 2.9. Stability of microencapsulated compounds under storage

The stability analysis was adapted from da Rosa et al. (2019) with modifications. The microcapsules were packed in 2 mL Eppendorf vials and kept in the dark at 45 °C. The samples were analysed every five days for 30 days to determine the total monomeric anthocyanin compounds. The degradation constant ( $k$ ) was determined using a first-order kinetic model according to Equation (1) and the half-life of the capsules was determined according to Equation (2).

$$\ln(C) = \ln(C_0 - k(t)) \quad (1)$$

Where:  $C$  = concentration of anthocyanins at time  $t$  (mg mL<sup>-1</sup>);  $C_0$  = initial concentration of anthocyanins (mg mL<sup>-1</sup>);  $t$  = storage time (days).

$$t_{1/2} = \ln(2) / kt \quad (2)$$

Where:  $t_{1/2}$  = half-life time in days;  $k$  = kinetics degradation constant;  $t$  = time in days.

### 2.10. Encapsulation efficiency

The surface anthocyanin compounds (SAC) and encapsulation efficiency (EE) were calculated as described by Robert et al. (2010) according to the following equations (3) and (4):

$$SAC (\%) = (SAC / TTAC) \times 100 \quad (3)$$

Where: SAC = surface anthocyanin compounds; TTAC = theoretical total anthocyanin compounds.

$$EE (\%) = 100 - SAC (\%) \quad (4)$$

### 2.11. Colour of microencapsulated powders

The method used for colour analysis was adapted from Nastasi et al. (2023) with changes. The colour coordinates  $L^*$ ,  $a^*$ , and  $b^*$  were measured using an FRU Precise Colour Reader (Shenzhen Wave Optoelectronics Technology Co., Ltd.). The colourimeter was calibrated using a white reference plate, and an 8 mm adapter was attached to the colourimeter for sample measurement. For analysis, microencapsulated powders were poured to fill a  $35 \times 10$  mm cell culture dish (Corning Incorporated, Corning, NY, USA) and measured at day 0 and day 30 of storage ( $n = 3$ ). Illumination was provided from two 63 LED light bars (12,000–13000 LM) and samples were photographed using a smartphone camera in a  $45 \text{ cm}^3$  photo box. The Hue angle ( $^\circ$ ) and Chroma (%) were calculated by eqs. (5) and (6) respectively:

$$\text{Hue} = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad (5)$$

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad (6)$$

### 2.12. Total monomeric Anthocyanin content (TMAC)

The total monomeric anthocyanin content (TMAC) was determined according to the UV–Vis spectrophotometric pH differential method (Lee, Durst, Wrolstad, Kupina, & JD, 2005). In brief, each extract was diluted with 0.025 M potassium chloride buffer (pH 1) and measured on a spectrophotometer (Shimadzu UV1800, Shimadzu, Kyoto, Japan) at 520 nm and 700 nm. This process was repeated with a 0.4 M sodium acetate buffer (pH 4.5). The blank consisted of an 80 % ethanol and 20 % distilled water solution, and anthocyanin concentration was expressed as cyanidin-3-glucoside equivalents.

### 2.13. Microparticle morphology

Before microscopy, samples were mounted on a  $\varnothing$  9.6 mm pin stub and placed in a vacuum oven overnight at  $45 \text{ }^\circ\text{C}$ , plasma cleaned (Evactron Plasma Cleaner) and carbon-coated (Quorum Q150T carbon coater) from above and at  $2 \text{ } 45^\circ$  angles for 3 pulses. Field Emission Scanning Electron Microscopy (FE-SEM) was performed on a JEOL JSM – 7100F using a secondary electron detector (SE). Accelerating voltage ranged between 2 and 4 keV for electron imaging, with a working distance of 10 mm. Samples were measured at day 0 and day 30 to test the stability of the samples.

### 2.14. Pigmentation analysis

Differences in the pigmentation of the powders were analysed by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy to evaluate if the functional groups of the anthocyanins were forming unique configurations with the wall materials. The instrument information is as follows: An ALPHA II FTIR spectrometer (Bruker Fig. 1. Optics GmbH, Ettlingen, Germany) equipped with a diamond crystal ATR cell that was used to measure the spectra between  $4000$  and  $400 \text{ cm}^{-1}$ . Samples were measured from individual microencapsulations ( $n = 3$ ). Samples were measured at room temperature, and the spectrum was assembled from 24 co-added spectra at a resolution of  $4 \text{ cm}^{-1}$ . Spectra were imported into SIMCA 18 (Umetrics, Sweden) for pre-processing.

### 2.15. Statistical analysis

Univariate analysis was performed using GraphPad Prism version 9.4.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). One-way ANOVA and Tukey post-hoc analysis was conducted to a confidence interval of  $p < 0.05$ . IR spectra were analysed with SIMCA 18 (Umetrics, Sweden).

## 3. Results & discussion

### 3.1. Anthocyanin quantification in native currant extract

A range of anthocyanins were detected in the native currant. In this study, we follow recommended guidelines for MS/MS data reporting to ensure transparency in the emerging field of Australian Traditional food research (Alseekh, Aharoni, Brotman, Contrepolis, D'Auria, Ewald et al., 2021). Using positive ionisation, the protonated molecule ( $[M+H]^+$ ), and negative ionisation ( $[M+H]^-$ ), were identified through MS-DIAL, providing exact mass data and fragmentation patterns for potential molecular formula prediction. Sugar moiety positions were determined by analysing MS/MS fragmentation patterns, focusing on neutral loss analysis (e.g., loss of a glucose moiety) and characteristic fragmentation of sugar linkages induced by collision-induced dissociation. For features with common sugar masses, such as galactose and glucose, we utilised *in silico* identification through MS Finder. This process provided exact mass and compound identification aligned with MS-DIAL feature annotations. While we acknowledge the limitations of *in silico* matching, where compound identification is less definitive compared to reference standards, it represents the most accurate theoretical interpretation for compounds with similar sugar moieties. The compounds are reported in Table 1 as cyanidin-3-glycoside micrograms per gram dry weight ( $\mu\text{g g}^{-1}$  dw). One anthocyanidin and three anthocyanins were detected with relatively high abundance. delphinidin was the most abundant, measured at  $2486.2 \mu\text{g/g}$ , showcasing its significant presence within the sample. Other delphinidin anthocyanins had a moderate content including delphinidin-3-*O*-beta-glucopyranoside, delphinidin-3-galactoside, and delphinidin-3-*O*-sambubioside. Following closely was cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside albeit with a higher standard deviation that suggests variability in its concentration across different sample extractions. petunidin-3-*O*-beta-glucoside was identified at  $407.0 \mu\text{g/g}$ . Trace content of peonidin derivatives was also present including peonidin-3-*O*-galactoside and peonidin-3-*O*-arabioside. Base delphinidin and glycosides typically exhibit a blue to violet colour. The exact shade can vary depending on the pH of the environment and the presence of other factors such as co-pigments and metal ions. At acidic pH levels, delphinidins may appear redder, while in neutral to basic conditions, they tend to be blue or violet (Husain et al., 2022). Cyanidins and petunidins range from red to purple, being redder at low pH and moving to bluer in basic conditions (Rakić & Poklar Ulrih, 2021; Tang & Giusti, 2020). Peonidin ranges from pink to red depending on the pH environment (Narbona, del Valle, Arista, Buide, & Ortiz, 2021). Several taxonomies of *Antidesma* subspecies grow throughout southeast Asia and Australia (Nguyen-Ngoc, Le-Thi-Phuong, Vu-Van, Pham-Ha-Thanh, & Nguyen-Huu, 2024). Krongyut and Sutthanut (2019) reported the major phytochemical content in *Antidesma bunius*, finding  $13.4 \text{ mg g}^{-1}$  cyanidin-3-glucoside via HPLC analysis. Jorjong, Butkhup, and Samappito (2015) also investigated *A. bunius*, finding a significant content of phenolic and bioactivity. The *Antidesma* ssp. Presented here grow throughout Northern Queensland. The present findings agree with

**Table 1**  
Anthocyanin content of Native currant.

Anthocyanins	Content ( $\mu\text{g/g DW}$ )
Cyanidin-3- <i>O</i> -glucoside	$891.2 \pm 120.0$
Delphinidin	$2486.2 \pm 86.8$
Pelargonidin-3- <i>O</i> -glucoside	$0.9 \pm 0.3$
Cyanidin-3- <i>O</i> -sophoroside	$19.7 \pm 3.3$
Cyanidine-3- <i>O</i> -sambubioside	$1991.5 \pm 82.2$
Delphinidin-3- <i>O</i> -beta-glucopyranoside	$23.7 \pm 5.4$
Delphinidin-3-galactoside	$151 \pm 2.6$
Delphinidin-3- <i>O</i> -sambubioside	$30.1 \pm 1.6$
Petunidin-3- <i>O</i> -beta-glucoside	$407.0 \pm 178.2$
Peonidin-3- <i>O</i> -alpha-arabioside	$16.9 \pm 1.5$
Peonidin-3- <i>O</i> -beta-galactoside	$33.8 \pm 5.1$

Anthocyanin content reported as cyanidin-3-glucoside equivalent. Data shown are the mean  $\pm$  SD ( $n = 3$ ).

the emerging literature, which indicates that *Antidesma* ssp. fruit are a valuable source of colouring and bioactive compounds. As a point of reference, blueberries can range in anthocyanin composition to include a wide variety of delphinidin, cyanidin, petunidin, peonidin and malvidin glycosides (Wang et al., 2022), with anthocyanin content typically ranging from 300 to 500 mg 100 g<sup>-1</sup> fresh weight in cultivated varieties (Cesa et al., 2017; Faria et al., 2005; Wang et al., 2022; Yan, Pico, Gerbrandt, Dossett, & Castellarin, 2023). The total anthocyanin content of the native currant was ~591 mg 100 g<sup>-1</sup>. As such, the native currant has a higher anthocyanin content than blueberry, as reported in previous literature. This is not unexpected, as Traditional foods have a high content of bioactive compounds given their retention of protective physiological adaptations to their environment compared to cultivated food species (Dissanayake et al., 2023; Hay et al., 2022).

### 3.2. Colour stability of powders

The colour stability of the freeze-dried and spray-dried microencapsulated powders shown in Table 2 was analysed by accelerated shelf-life assessment. The colour of the samples was measured using the CIELAB system commonly employed to monitor anthocyanin colours in microencapsulated powders (Baeza & Chirife, 2021; Sarabandi et al., 2019). Samples measured via CIELAB colourimetry revealed significant change over time in all CIELAB metrics, aside from a\* in maltodextrin Spray-Dried (MSD) and L\* and a\* in Gidyea gum/maltodextrin spray-dried (GGSD). Within the CIELAB colour system, chroma is a valuable metric for the representing saturation of a colour. It indicates the degree of departure of a colour from a grey of the same lightness or, in simpler terms, the intensity or purity of the colour. Chroma is measured from the centre of the colour space (neutral grey) outward, with higher values representing more vivid colours. The maltodextrin freeze-dried (MFD) exhibited the highest chroma at 25.86 %, followed by gum Arabic/maltodextrin spray-dried (GASD), Gidyea gum/maltodextrin spray-dried (GGSD), gum Arabic/maltodextrin freeze-dried (GAFD), maltodextrin Spray-Dried (MSD), and Gidyea gum/maltodextrin freeze-dried (GGFD). By day 30, the MSD and GGSD chroma % had slight increases, whereas GASD and MFD maintained the same colour intensity indicating stability or minimal change in the colour purity over the 30 days. The Gum Arabic/maltodextrin freeze-dried (GAFD) and Gidyea gum/maltodextrin freeze-dried (GGFD) powders decreased in chroma by 2.26 % and 0.36 % respectively. The  $\Delta E$  reported measures the colour difference between days 0 and 30. The most substantial changes were observed in GAFD (23.43) and GGFD (21.90), indicating substantial colour shift. In contrast, GGSD (0.41), GASD (1.67) and MSD (4.30) showed minimal change suggesting higher colour stability over the storage period.

**Table 2**  
Colour stability of microencapsulated powders.

	Day 0				Day 30				
	L	a	b	Chroma %	L	a	b	Chroma %	$\Delta E_{lab}$
MSD	80.09 ± 0.11	12.85 ± 0.02	-2.81 ± 0.02	13.15 ± 0.02 <sup>A</sup>	82.46 ± 0.15*	12.99 ± 0.01	-4.19 ± 0.01*	13.65 ± 0.00 <sup>A</sup>	4.30
	80.59 ± 0.15	10.79 ± 0.09	-4.65 ± 0.07	12.25 ± 0.76 <sup>B</sup>	80.49 ± 0.09	10.92 ± 0.01	-5.19 ± 0.00*	12.61 ± 0.98 <sup>B</sup>	0.41
GGSD	77.84 ± 0.12	15.96 ± 0.04	-4.16 ± 0.02	14.87 ± 2.79 <sup>C</sup>	79.11 ± 0.04*	15.64 ± 0.01*	-4.70 ± 0.01*	14.92 ± 2.44 <sup>C</sup>	1.67
GASD	62.13 ± 0.18	24.19 ± 0.08	-1.20 ± 0.01	25.86 ± 0.10 <sup>D</sup>	67.90 ± 0.08*	25.82 ± 0.09*	-1.52 ± 0.02*	25.86 ± 0.12 <sup>D</sup>	8.51
MFD	59.18 ± 0.03	15.83 ± 0.04	-3.07 ± 0.02	16.13 ± 0.03 <sup>E</sup>	63.20 ± 0.07*	14.15 ± 0.11*	-6.95 ± 0.07*	15.77 ± 0.13 <sup>E</sup>	21.90
GGFD	62.71 ± 0.11	13.29 ± 0.04	-6.06 ± 0.02	14.61 ± 0.04 <sup>F</sup>	68.08 ± 0.05*	9.47 ± 0.03*	-7.93 ± 0.05*	12.35 ± 0.06 <sup>F</sup>	23.43
GAFD									

The data are shown in means ± SD (n = 3). CIELAB metrics with \* significantly different over 30 days, p < 0.05 by Tukey's test p < 0.05. Different letters in the Chroma % values within the same column are significantly different by Tukey's test p < 0.05.  $\Delta E_{lab}$  = measured distance between day 0 and day 30. MSD = maltodextrin spray-dried, GGSD = Gidyea gum/maltodextrin spray-dried, GASD = gum Arabic/maltodextrin spray-dried, MFD = maltodextrin freeze-dried, GGFD = Gidyea gum/maltodextrin freeze-dried, GAFD = gum Arabic/maltodextrin freeze-dried.

The CIELAB analysis (Table 2) of the freeze-dried samples shows a significant increase in lightness compared to spray-dried samples, indicating a higher degree of colour lightning over time. This suggests that freeze drying may be less effective at preserving the original lightness of the samples compared to spray drying. Likewise in both a\* and b\* values, the freeze-dried samples a\* value decreased significantly, indicating a substantial reduction in red intensity, while also having notable shifts towards more negative b\* values (bluer shades). Spray-drying had a lower variation in the a\* and b\* values over 30 days, again indicating better preservation of the initial colour. Mahdavee Khazaei et al. (2014) reported a similar shift in colour within saffron extract microencapsulation using maltodextrin/gum Arabic composites, where spray-dried powders maintained chroma over time, reflecting higher preservation of colour intensity than freeze-drying. Previous comparisons of spray-drying and freeze-drying showed the same disparity between drying methods for colour retention for black glutinous rice (Laokuldilok & Kanha, 2017). Analysis of spray-drying anthocyanins from maltodextrin and gum Arabic have also shown similar retention of colour over time to the present analysis, with no significant decrease in saffron and black carrot anthocyanin contents (Ersus & Yurdagel, 2007; Mahdavee Khazaei et al., 2014). Among previous freeze-drying studies, saffron anthocyanins encapsulation using maltodextrin at 100 %, and 50:50 maltodextrin to gum Arabic also showed similar colour change during accelerated shelf-life storage at 35 °C (Jafari, Mahdavi-Khazaei, & Hemmati-Kakhki, 2016). In the present study, we reported the same retention of chroma for maltodextrin microencapsulation, although a decrease in chroma for the gum-containing microencapsulation. Likewise, the freeze-dried samples became significantly (P < 0.05) 'bluer' which is also seen in the literature (Jafari et al., 2016; Mahdavee Khazaei et al., 2014). Overall, spray-dried samples demonstrate better colour preservation over the 30 days compared to freeze-dried samples.

### 3.3. Powder moisture content and encapsulation efficiency

The stability of anthocyanins in microencapsulated powders, the structural integrity of the powder, and the overall quality of the microencapsulated product rely on optimizing formulation and processing techniques to maintain the beneficial properties of anthocyanins over time. To predict degradation due to thermal activity and oxidation, metrics such as moisture content, water activity, encapsulation efficiency and the anthocyanin half-life can be employed. Higher moisture content and water activity can accelerate hydrolytic reactions and promote microbial growth, leading to anthocyanin degradation (Oxley, 2012). Anthocyanin content (TMAC) and encapsulation efficiency (EE %) indicate the amount of active compound retained and protected within the encapsulate, respectively, reflecting the success of the

encapsulation process (da Rosa et al., 2019). Lastly, monitoring the powder half-life determined by TMAC over time provides a measure of the stability and shelf-life of the encapsulated anthocyanins, predicting potential degradation due to thermal activity and oxidation (da Rosa et al., 2019). The key parameters displayed in Table 3 include moisture content, water activity ( $A_w$ ), anthocyanin content (TMAC), encapsulation efficiency (EE %), and the powder half-life determined by TMAC over time. The TMAC over time is shown in Fig. 2. GAFD had the lowest moisture content at 4.2 %, followed by the GGFD and MFD, where the GGFD was not significantly higher in moisture ( $p < 0.05$ ). Spray-drying was less efficient in water removal from the powders, with all spray-dried samples showing higher moisture content than freeze-dried samples. The  $A_w$  reflected the same trend, where freeze-drying was more efficient in removing unbound water. The moisture content showed the same trend as seen previously where the addition of gum to maltodextrin improved water removal due to the capacity of the gum to bind and retain moisture within its structure, reducing the free water, and increasing the film-forming effects of the wall material (da Rosa et al., 2019; Kanakdande, Bhosale, & Singhal, 2007; Mahdavi, Jafari, Assadpour, & Dehnad, 2016a). Adding gum Arabic and Gidyea gum lowered the water content of the spray-dried powder, with no significant difference between the two gums ( $p < 0.05$ ). Previously, Mahdavi, Jafari, Assadpour, and Ghorbani (2016b) reported the same effect of lower moisture content when adding gums to wall materials of powders. The freeze-dried powders showed a similar moisture removal effect, where adding gum Arabic or Gidyea gum lowered the moisture content compared to the maltodextrin alone. Typically, spray-drying is more efficient in moisture removal compared to freeze-drying (Dadi, Emire, Hagos, & Eun, 2020; Darniadi et al., 2018). The lower moisture in the freeze-dried powders may be explained by the comparison of a relatively lower inlet air temperature of 120 °C in spray-drying to a foaming method in the freeze-dried samples. The lower inlet temperature is less efficient in water removal, while foam-mat freeze-drying has been shown as effective in moisture removal (da Rosa et al., 2019; Dadi et al., 2020).

All wall materials successfully encapsulated between 81.8 and 88.0 % of the anthocyanins. The GGSD and GASD had significantly higher encapsulation efficiency than the MSD. The MSD powder has an encapsulation efficiency of 81.8 %, which is the lowest among all powders. Both GGFD and GAFD demonstrate high encapsulation efficiencies at 86.7 % and 87.6 %, respectively. There was no significant difference in encapsulation efficiency between the freeze-dried samples, though the MFD encapsulation efficiency was lowest at 85.1 %. The encapsulation efficiency reported by da Rosa et al. (2019) ranged between 74.4 and 85.2 % for spray-dried powders, where efficiency increased due to inlet temperature and wall material solubility. Likewise in the freeze-dried powders, encapsulation efficiency was consistent with the previous literature (Azarpazhooh, Sharayei, Zomorodi, & Ramaswamy, 2019; Tran et al., 2022). Azarpazhooh et al. (2019) suggest that higher encapsulation efficiencies for freeze-dried powders are related to wall materials having a lower moisture content. In the present study, there is a lower moisture content correlated to a higher encapsulation efficiency. Herein, we support the improved efficiency being a

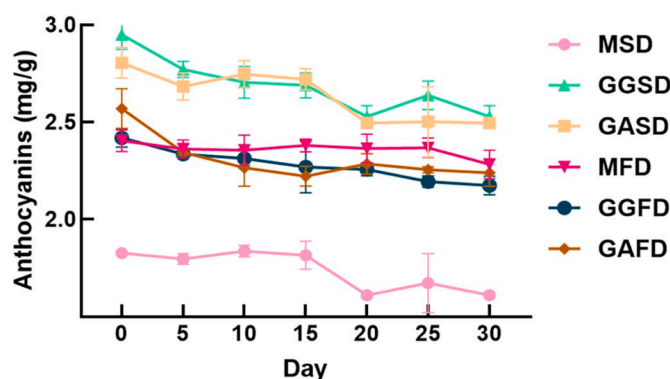


Fig. 2. Total monomeric anthocyanin content over 30 days in spray-dried and freeze-dried microencapsulated powders. Data are presented as means  $\pm$  SD ( $n = 3$ ). Significance was determined by one-way ANOVA with Tukey post hoc analysis ( $p < 0.05$ ). MSD = maltodextrin spray-dried, GASD = gum Arabic spray-dried, GGSD = Gidyea gum spray-dried, MFD = maltodextrin freeze-dried, GAFD = gum Arabic freeze-dried, GGFD = Gidyea gum freeze-dried.

function of moisture content. In effect, within this study, each processing method and wall material have a minor impact on the encapsulation efficiency and moisture content. In terms of the change in colour over time, there are no apparent connections that could be drawn from the encapsulation efficiency and moisture content, given the freeze-dried powders had a slightly lower moisture content and higher efficiency, their colour retention was not significantly better than in spray-drying.

In both the spray-dried and freeze-dried samples, the addition of gum to the wall materials improved the encapsulation efficiency (Table 3). In the spray-dried powders, Gidyea gum had no significant difference in encapsulation efficiency to gum Arabic, though both gum walls were significantly increased compared to maltodextrin alone. Mahdavi, Jafari, Assadpour, and Dehnad (2016b) also reported the inclusion of gum Arabic to maltodextrin improved the encapsulation efficiency. The gum Arabic and Gidyea gum improvement of encapsulation efficiency is dependent on their structure. Each of the gums is a heteropolymer with a branching polysaccharide chain covalently linked to globular proteins (Hay et al., 2024; Prasad, Thombare, Sharma, & Kumar, 2022). Each gum acts as an excellent film-forming agent which better entraps the flavylium cation of anthocyanins due to the increased degree of hydrogen and  $\pi$ - $\pi$  bonding (Mahdavi, Jafari, Assadpour, & Dehnad, 2016a; Sarabandi et al., 2019; Sethuraman & Rajendran, 2019).

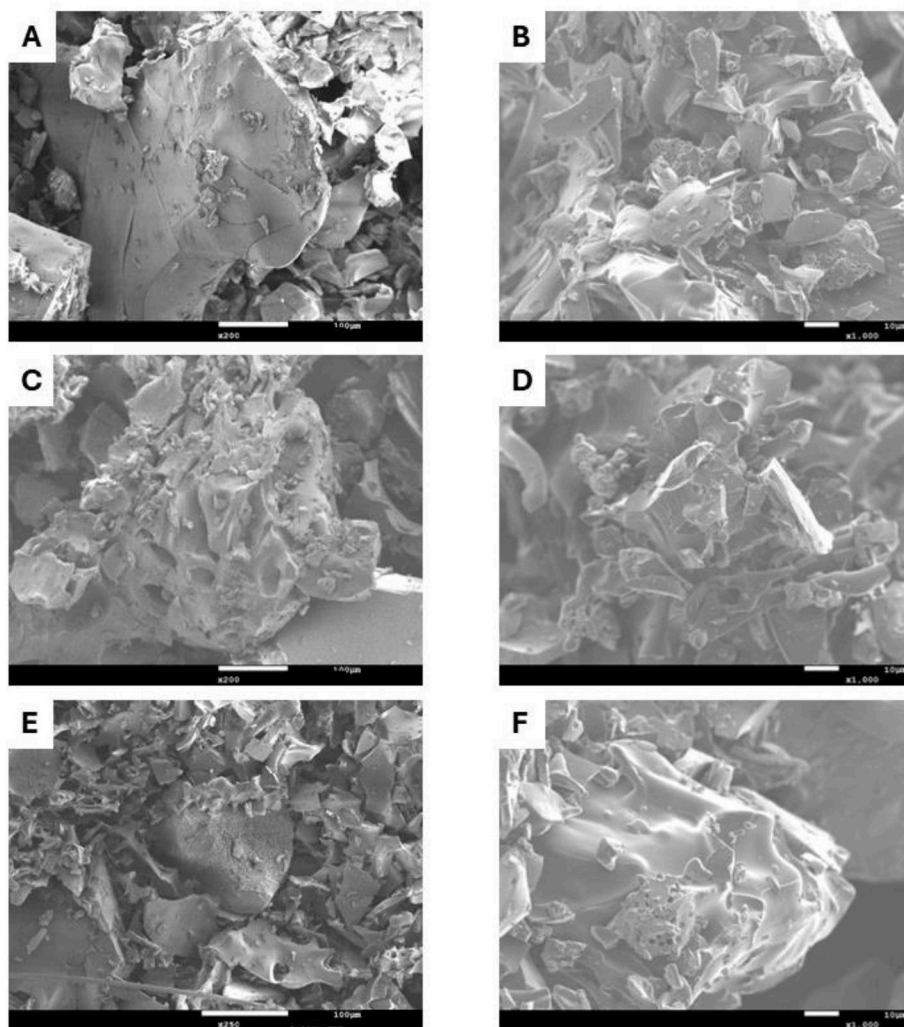
### 3.4. Morphology of powders

To evaluate the impact of structural changes on colour retention, field emission scanning electron microscopy was employed to assess the morphological differences between each wall treatment, as well as each treatment over the 30-day storage trial. The freeze-dried powders are shown in Fig. 3, where all freeze-dried samples had characteristically irregular, flake-like substances. The MFD day 0 exhibited a relatively smooth and more homogeneous surface structure (Fig. 3A) and showed

Table 3  
Stability of microencapsulated powders.

Sample	Moisture content (%)	$A_w$	Day 0 TMAC (mg/g DW)	Day 30 TMAC (mg/g DW)	EE (%)	Half-life (days)
MSD	6.2 $\pm$ 0.2 <sup>A</sup>	0.28 $\pm$ 0.01 <sup>A</sup>	1.83 $\pm$ 0.02 <sup>A</sup>	1.61 $\pm$ 0.02 <sup>A</sup>	81.8 $\pm$ 1.4 <sup>A</sup>	162 $\pm$ 7 <sup>A</sup>
GGSD	5.00 $\pm$ 0.7 <sup>B</sup>	0.26 $\pm$ 0.01 <sup>AB</sup>	2.94 $\pm$ 0.07 <sup>B</sup>	2.52 $\pm$ 0.06 <sup>B</sup>	88.0 $\pm$ 1.1 <sup>B</sup>	134 $\pm$ 7 <sup>A</sup>
GASD	5.63 $\pm$ 0.5 <sup>B</sup>	0.26 $\pm$ 0.00 <sup>B</sup>	2.80 $\pm$ 0.08 <sup>C</sup>	2.49 $\pm$ 0.02 <sup>B</sup>	87.2 $\pm$ 0.2 <sup>B</sup>	167 $\pm$ 14 <sup>A</sup>
MFD	4.7 $\pm$ 0.2 <sup>BC</sup>	0.22 $\pm$ 0.01 <sup>C</sup>	2.46 $\pm$ 0.05 <sup>D</sup>	2.20 $\pm$ 0.07 <sup>CD</sup>	85.1 $\pm$ 1.7 <sup>C</sup>	178 $\pm$ 11 <sup>A</sup>
GGFD	4.3 $\pm$ 0.5 <sup>BC</sup>	0.21 $\pm$ 0.01 <sup>D</sup>	2.42 $\pm$ 0.05 <sup>D</sup>	2.14 $\pm$ 0.05 <sup>C</sup>	86.7 $\pm$ 1.2 <sup>BC</sup>	153 $\pm$ 10 <sup>A</sup>
GAFD	4.2 $\pm$ 0.1 <sup>C</sup>	0.20 $\pm$ 0.00 <sup>D</sup>	2.57 $\pm$ 0.10 <sup>E</sup>	2.24 $\pm$ 0.07 <sup>D</sup>	87.6 $\pm$ 1.9 <sup>BC</sup>	138 $\pm$ 1 <sup>A</sup>

The data are shown in means  $\pm$  SD ( $n = 3$ ). When followed by the same letter in the column they do not differ significantly by Tukey's test,  $p < 0.05$ . Efficiency of encapsulation (EE%). Water activity ( $A_w$ ). MSD = maltodextrin spray-dried, GGSD = Gidyea gum/maltodextrin spray-dried, GASD = gum Arabic/maltodextrin spray-dried, MFD = maltodextrin freeze-dried, GGFD = Gidyea gum/maltodextrin freeze-dried, GAFD = gum Arabic/maltodextrin freeze-dried.



**Fig. 3.** FE-SEM images of freeze-dried powders. A. maltodextrin day 0. B. maltodextrin day 30. Figure C. Maltodextrin and Gidyea gum day 0. D. Maltodextrin and Gidyea gum day 30. E. Maltodextrin and gum Arabic day 0. F. Maltodextrin and gum Arabic day 30.

minimal structural change over the 30 days (Fig. 3B). The GGFD displayed a more complex and textured surface compared to maltodextrin alone, including evidence of higher pore formation (Fig. 3C). Again, the 30-day imaging of GGFD indicated minimal morphological changes (Fig. 3D). The GAFD sample had a consistent structure to the GGFD though exhibited some evidence of micropore formation (Fig. 3E). The effect of elevated temperature was slightly more pronounced in the GAFD morphology, where shard structures appear more amorphous than in the MFD and GGFD. The irregular, flake-like morphology of freeze-dried powders often provides a larger surface area but can be associated with lower encapsulation efficiency compared to the smooth, spherical shapes typically observed in spray-dried powders (Stabrauskiene, Pudziulyte, & Bernatoniene, 2024). In the present study, there were no significant differences in the encapsulation efficiency between processing methods for the gum-containing formulations. The maltodextrin formulation had a significantly higher encapsulation efficiency for the freeze-dried processing. A higher encapsulation efficiency in the MFD versus the MSD is unexpected given the characteristic pore formation in the MFD samples. Due to the foaming in the freeze-drying process, the created micropores may have enhanced the film-forming properties around the active compounds. These films can still retain anthocyanins effectively, contributing to higher encapsulation efficiency despite the visible pores (Bleiel, Kent, & Brodtkorb, 2017).

The spray-dried microencapsulation powders are shown in Fig. 4. The spray-dried particles had various sizes and contours within each sample. The MSD (Fig. 4a) had a high degree of smooth spheres across large spheres and moderate shrinkage in smaller spheres. MSD had a low incidence of cracking on the surface. Post-storage MSD (Fig. 4b) exhibited no apparent morphological change. The GGSD showed a higher degree of shrinkage compared to the MSD (Fig. 4c). Post-storage, samples showed no difference in morphology over time, except for minor surface cracking in the GGSD that was not seen in the day 0 imaging. Gidyea gum may exhibit differential drying behaviour due to its unique protein-polysaccharide composition, making it more prone to cracking. GASD particle size appeared consistent with the other spray-dried samples, though had a much higher shrinkage (Fig. 4e). As with the GGSD, post-storage images showed surface cracking. Surface cracking observed in spray-dried powders can influence moisture ingress and subsequent degradation of anthocyanins (Fitzpatrick, 2021, pp. 73–89). The minimal morphological changes in maltodextrin-only formulations versus gum-based formulations did not translate to an improvement in encapsulation efficiency. During spray drying, the atomisation step exposes the feed solution to high temperatures for a short duration. This rapid exposure can cause partial volatilisation or degradation of sensitive compounds like anthocyanins, leading to lower encapsulation efficiency for the spray-dried samples. However, the significantly lower efficiency of the MSD compared to the

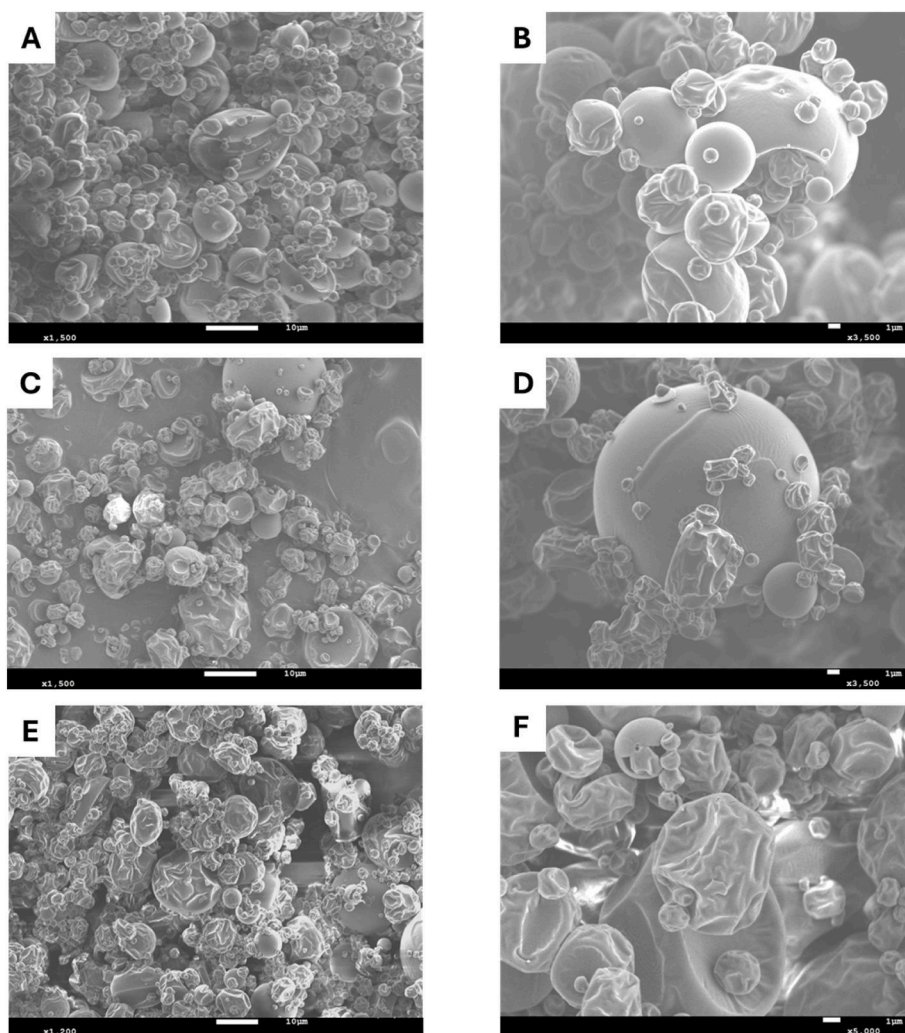


Fig. 4. FE-SEM images of spray-dried powders. A. maltodextrin day 0. B. maltodextrin day 30. C. Maltodextrin and Gidyea gum day 0. D. Maltodextrin and Gidyea gum day 30. E. Maltodextrin and gum Arabic day 0. F. Maltodextrin and gum Arabic day 30.

gum-containing powders demonstrated the film formation typical of gums (Liu et al., 2020). Their ability to create a cohesive matrix during drying, and trapped anthocyanins effectively. While the morphology of the particles showed crackling, there was no significant reduction in powder shelf life.

### 3.5. Anthocyanin stability

Over the 30 days of the stability trial, all samples showed a decrease in anthocyanin contents (Fig. 2), suggesting degradation of the micro-encapsulated anthocyanins. There were significant differences ( $p < 0.05$ ) in the initial powder content, with anthocyanins ranging between 2.94 and 1.83 mg g<sup>-1</sup>. The GGSD had the highest initial anthocyanin content yet had poorer retention of 42 mg g<sup>-1</sup> over 30 days. GASD had a lower initial anthocyanin content at day 0 but had a higher retention rate, losing 31 mg g<sup>-1</sup> over 30 days. The MSD had the lowest initial anthocyanin content and had a minimal loss of 22 mg g<sup>-1</sup>. Within the spray-dried powders, the gums improved the initial anthocyanin content but did not improve stability compared with maltodextrin alone. Among the freeze-dried powders, the GAFD sample had the highest initial anthocyanin content, followed by MFD and GGFD. The MFD had the highest retention in anthocyanins, dropping by 26 mg g<sup>-1</sup>. GGFD dropped by 28 mg g<sup>-1</sup> during storage, while the GAFD dropped by 33 mg g<sup>-1</sup>. As within the spray-dried powders, the freeze-dried powders showed that maltodextrin alone had the highest impact on powder

stability over time.

The addition of gum Arabic significantly improved the initial anthocyanin concentration in the freeze-dried processing, while the addition of Gidyea gum significantly improved the anthocyanin content in the spray-dried powder. It is 151 reported in the literature that changes in the yield and stability of encapsulated anthocyanins are highly subjective to the wall material used (Azarpazhooh et al., 2019; Estupiñan-Amaya et al., 2022; Mahdavee Khazaei et al., 2014). The varying anthocyanin contents between the Gidyea gum and gum Arabic, and the different drying methods (freeze-drying and spray-drying) can be attributed to the complex differences in their macrostructure and protein content (Hay et al., 2024), where Gidyea gum has a lower branching in the polysaccharide yet a larger protein structure. Anthocyanins being glycosylated anthocyanidins, vary in hydroxylation, bounds sugar moieties and degree of methylation (Patras et al., 2010). The stability of anthocyanins is influenced by structural change, mostly due to oxidation during heating (da Rosa et al., 2019). The spray-drying process exposes the product to a high temperature for a short interval (Bhandari, Patel, & Chen, 2008), and the degradation by heating has also been suggested to be minimal (Yu & Lv, 2019). The higher anthocyanin content in the two spray-dried gum samples compared to the maltodextrin alone indicates that the gums were protected against thermal degradation during processing. When comparing the use of gums between processing methods, the spray-dried samples had a higher anthocyanin content than the freeze-dried samples, whereas typically

comparisons of the two processing methods have indicated that freeze-drying has a greater yield between 17 and 20 % anthocyanins (Baeza et al., 2021; Yu et al., 2019). However, other studies have indicated that the difference in anthocyanin yield between processing methods can be minimal (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Fredes, Becerra, Parada, & Robert, 2018).

The stability of microencapsulated anthocyanins is often assessed using kinetic analysis (Baeza & Chirife, 2021). In the case of the present study, the modelling of the half-life of anthocyanin content was based on the accelerated 30-day storage at 35 °C. While extrapolating the powder half-life introduces a margin of error, a basis for relative comparison can be made between samples stored in consistent conditions. There was no significant difference in half-life between any of the powders ( $p < 0.05$ ). The shelf-life modelling found that MFD exhibits the longest half-life of 178 days. GGFD and GAFD have a half-life of 153 days and 138 days respectively. The spray-dried powders varied more between the inclusion of gum with maltodextrin, where GGSD had a half-life of 134 days, while GASD and MSD showed similar half-lives of 167 days and 162 days. Half-life projections range in literature from conservative estimations of ~30 days (da Rosa et al., 2019), to greater than 400 days (Baeza & Chirife, 2021). In the present study, there was minimal half-life variation between the gum samples for their respective processing methods.

Cumulatively, the change in wall material demonstrated that the inclusion of gums improved the anthocyanin yield in spray-drying, while the gum Arabic showed only minor improvement in freeze-drying. There was no significant improvement in anthocyanin retention over time compared to maltodextrin alone. The colour stability varied between wall materials used, with the gums having comparable retention in both spray-drying and freeze-drying methods. Overall, the spray-dried samples had higher colour stability, which is explained by the higher surface area in the freeze-dried sample due to micropore formation when drying. Interestingly, the variance in colour saturation between processing methods was noteworthy, as shown in Fig. 5 and requires further investigation by FTIR and UV-Vis.

### 3.6. Co-pigmentation of anthocyanins and their chemometric evaluation

Distinct colour differences were evident between the six encapsulated powders (Fig. 5). The colour change was affected by both the drying method and the types of wall materials used. Specifically, the chroma values observed in freeze-dried powders significantly increased compared to the spray-dried powders, as shown in Table 2. Some degree of colour difference may have been due to the addition of different gums. Sarabandi et al. (2019) explained colour variation in microencapsulation of eggplant extract with maltodextrin and gum Arabic because of gum Arabic's yellow/reddish colour. However, when comparing the colour of the freeze-dried to spray-dried powder of the same wall material, there is an obvious change in both saturation and hue. Given that the feed solutions had a standardised pH of 3.5, the colour change was determined to be caused by a separate mechanism. Herein, we see that there is either coacervation of pigment compounds, and/or co-pigmentation of anthocyanins occurring *via* colloidal encapsulation within the maltodextrin, and gum/maltodextrin wall materials. Coacervation of pigments typically occurs under conditions where the electrostatic interactions between the oppositely charged polymers are strong enough to overcome the thermal motion and solvation forces. Factors influencing coacervation include pH, ionic strength, temperature, and polymer concentration (Rocha et al., 2023). The co-pigmentation of anthocyanins in macromolecules acts to protect the anthocyanin flavylium cation from nucleophilic attack (Gençdağ et al., 2022), thereby stabilising the anthocyanins while influencing the colour expression. Fourier transform infrared (FT-IR) spectroscopy has previously been shown to effectively determine the structural differences influenced by the co-pigmentation of anthocyanins within polymers (Kanha et al., 2020). Moreover, using predictive modelling of IR spectra

coupled with chemometric data aids in statistically evaluating how small analytes affect complex matrices (Alagappan et al., 2024; Ali Redha et al., 2023). Therefore, we may compare the impact of colour change on the IR spectra of like materials. As such, FT-IR spectroscopy was performed (Fig. 5) to identify any differences in molecular interaction between processing methods which may be attributed to the co-pigmentation of the anthocyanins.

The paired comparisons of FT-IR spectra of the maltodextrin (MFD, MSD), Gidyea gum/maltodextrin (GGFD, GGSD), and gum Arabic/maltodextrin (GAFD, GASD) are shown in Fig. 5a, b, and 5c respectively. The spectral waveforms match those previously shown for maltodextrin (Capron, Robert, Colonna, Brogly, & Planchot, 2007), and gum Arabic/Gidyea gum (Hay et al., 2024). Within each of the three comparisons, the freeze-dried and spray-dried powders revealed noticeable differences within 1200  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$  regions, with secondary spectra variations occurring in each of the individual comparisons. Within the Gidyea gum powders (Fig. 5b), GGFD had an increased absorption peak intensity of 3600  $\text{cm}^{-1}$  to 3000  $\text{cm}^{-1}$  associated with water content and hydrogen bonding (Kanha et al., 2020). Likewise, the freeze-dried gum Arabic (GAFD) exhibited increased absorption within 3600  $\text{cm}^{-1}$  to 3000  $\text{cm}^{-1}$  regions (Fig. 5c).

Discriminate analysis (PLS-DA) was conducted using the infrared spectra and the CIELAB colour coordinates to interpret changes in the colour of the encapsulated powders. A model was developed for each of the maltodextrin (MFD, MSD), Gidyea gum/maltodextrin (GGFD, GGSD), and gum Arabic/maltodextrin (GAFD, GASD) comparisons and the scores plot for each are shown in Fig. 6a, c and 6e, respectively. The model statistics for Fig. 6 are provided in Table S1. The small separation of powder groupings along PC1 and PC2 in each case showed the high similarity between each processing condition, as expected given their common composition. However, there was a small separation along PC1 in each model. The groupings were determined as a change in the spectra based on the processing method and moisture content. Spectra shift is attributed to differences in moisture content and non-covalent bonding related to anthocyanin-colloid complexation (Kanha et al., 2020). The discriminate analysis was performed using CIELAB metrics as latent variables in the spectral data that best discriminate between powder samples. Therefore, the PLS-DA model can be interpreted to understand which spectral regions are most important for distinguishing between different colours (as defined by  $L^*$ ,  $a^*$ ,  $b^*$  values), are shown in the loadings plots (Fig. 6b, d and 6e). Each model used the first component for loadings comparison with a high regression in the gum/maltodextrin and the maltodextrin powders (Table S1). The regions indicated in red show the most significant difference ( $p < 0.05$ ) between powder comparisons, as determined by the variable importance of projection ( $\text{VIP} > 1.5$ ). Concisely, the PLS-DA models determine the waveform region in each paired comparison that is most influenced by colour difference. In each case, the colour difference was explained by the region between 1200  $\text{cm}^{-1}$  to 800  $\text{cm}^{-1}$ . The gum-containing powder comparisons showed a peak shift from 980  $\text{cm}^{-1}$  to 760  $\text{cm}^{-1}$ , previously determined as aromatic ring shifting associated with anthocyanin co-pigment microencapsulation (Kanha et al., 2020). In the maltodextrin, and gum Arabic/maltodextrin comparisons, the two peak bands at around 1146 and 1076  $\text{cm}^{-1}$  that have been determined by Kanha et al. (2020) as the coupling of C=O, C-C and O-H bond stretching, bending and asymmetric stretching of the C-O-C glycosidic bonds. Interestingly, the Gidyea gum powders lacked the same differentiations, which may explain the difference in hue between the two gum powders. Both gum powder comparisons showed an increase of peaks from 1758  $\text{cm}^{-1}$  to 1560  $\text{cm}^{-1}$  and a peak shift at wavenumber 1636  $\text{cm}^{-1}$  which are previously associated with C=C scissoring vibration in pyran rings typical of flavonoid compounds (Molaeafard, Jamei, & Marjani, 2021).

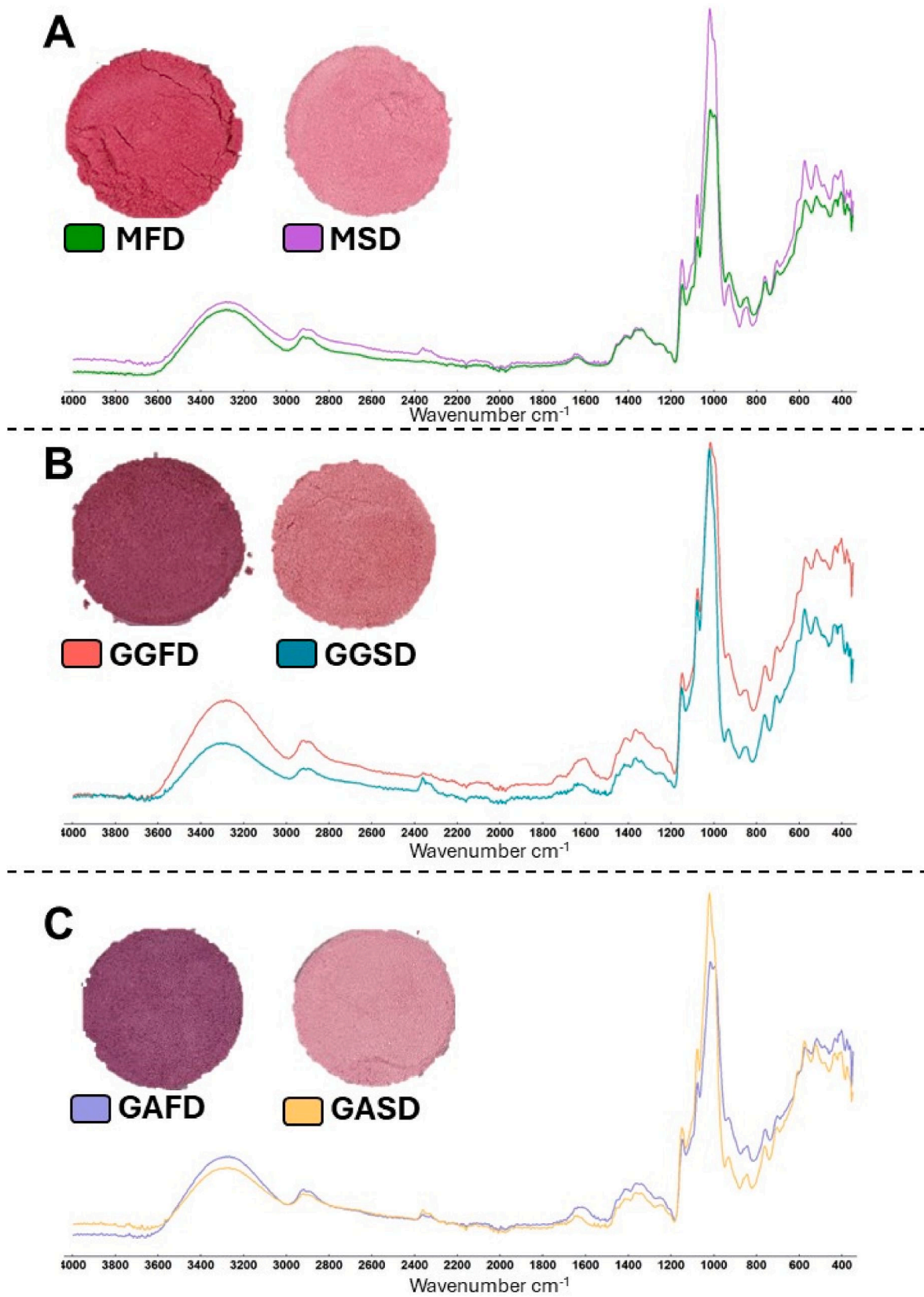
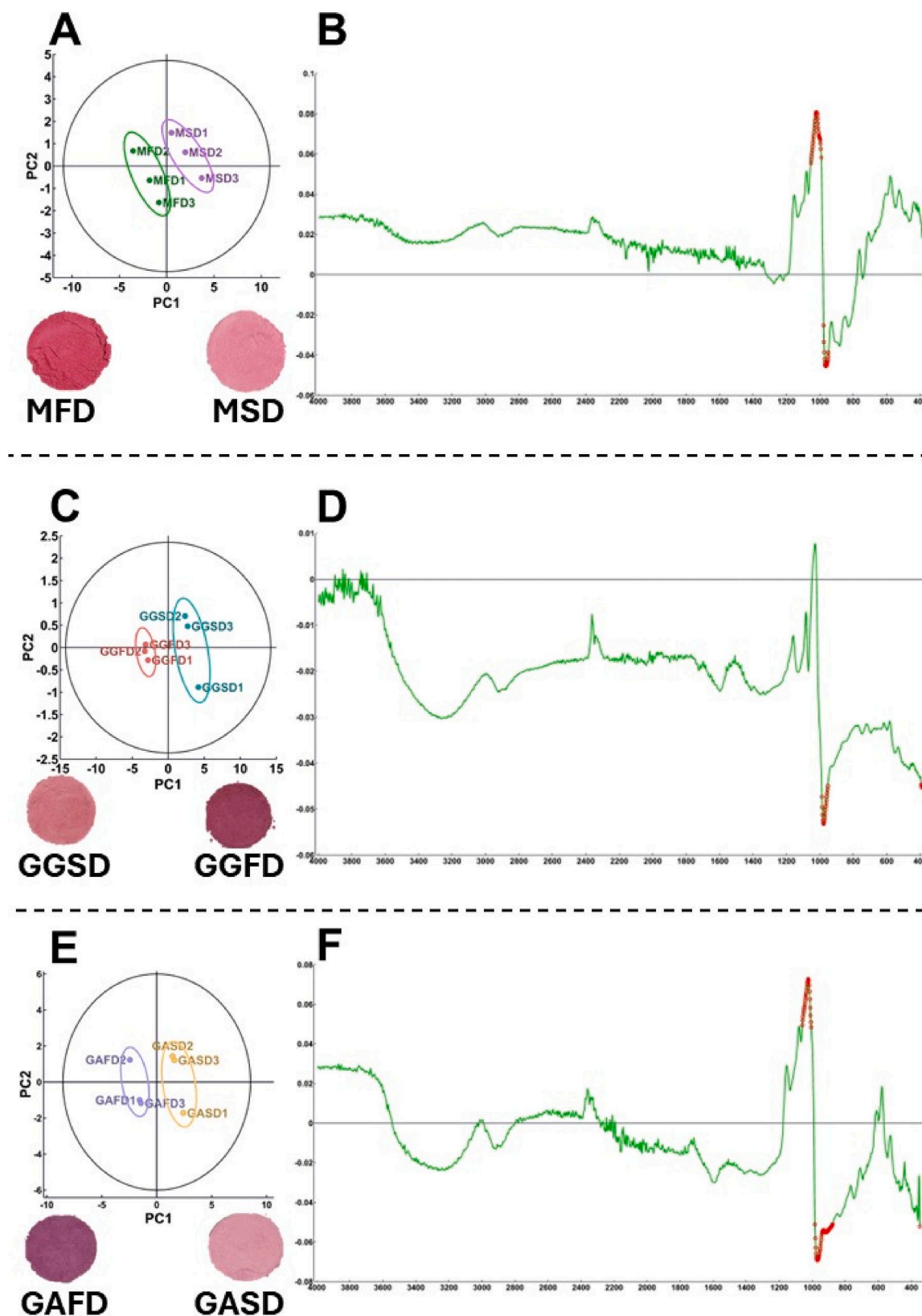


Fig. 5. FT-IR waveform of microencapsulated powders A. Maltodextrin freeze-dried (MFD) and spray-dried (MSD) powders and FTIR spectra. B. Gidyea gum/Maltodextrin freeze-dried (GGFD) and spray-dried (GGSD) powders and FT-IR spectra. C. gum Arabic/Maltodextrin freeze-dried (GAFD) and spray-dried (GASD) powders and FT-IR spectra.



**Fig. 6.** Predictive modelling of anthocyanin co-pigmentation determined by FT-IR spectroscopy and CIELAB colourimetry **A.** PLS-DA scores of MSD and MFD. **B.** PLS-DA loadings of MSD and MFD, where red indicates variable importance projection (VIP) score >2. **C.** PLS-DA scores of GGSD and GGFD. **D.** PLS-DA loadings of GGSD and GGFD, red indicates VIP >2. **E.** PLS-DA scores of GASD and GAFD. **F.** PLS-DA loadings of GASD and GAFD, red indicates VIP >2. All PLS-DA had  $R^2(\text{cum}) > 0.97$  and  $Q^2(\text{cum}) > 0.96$  (shown in [Supplementary Table 1](#)). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 3.7. UV Vis spectroscopy of powders in a beverage model

Co-pigmentation of anthocyanins with other small molecules can be assessed by UV-Vis spectroscopy (Klisurova et al., 2019). However, when anthocyanins are encapsulated within a hydrophilic macromolecule such as maltodextrin, the solubility of the wall material may impair the colour of formulated products because returning to a solution both dissolves the colloidal encapsulation and changes the pH of the solution. A beverage model made by dissolving the powders in water was used to investigate the impact of rehydration on the colour of the powders via UV Vis spectroscopy (Fig. 7). Rehydrated samples had a pH of 3.96–3.99. The visual impact of rehydration is displayed in Fig. 7B. The freeze-dried maltodextrin powder was more saturated than the

spray-dried powder with no obvious change in hue. The gum Arabic powders retained their colour difference observed in the dried powder. The Gidyea gum powders returned to a similar hue and saturation, with the freeze-dried sample retaining a slight purple hue. The difference in hue between each powder processing method of the separate formulations indicates a structural change in the anthocyanins. A bathochromic and hyperchromic shift in the peak region from 490 nm to 550 nm occurred in the rehydrated formulations. Hydroxyl (–OH) groups attaching to the aromatic rings can shift the absorbance to different wavelengths, influencing the exact colour, typically leading to a blue shift (Deineka, Sidorov, Deineka, Kul'chenko, & Blinova, 2020). Specifically, from a change in the flavylium cation state of the anthocyanin structure which dominates at pH 2.0 to 4.0. Additionally, the acylation

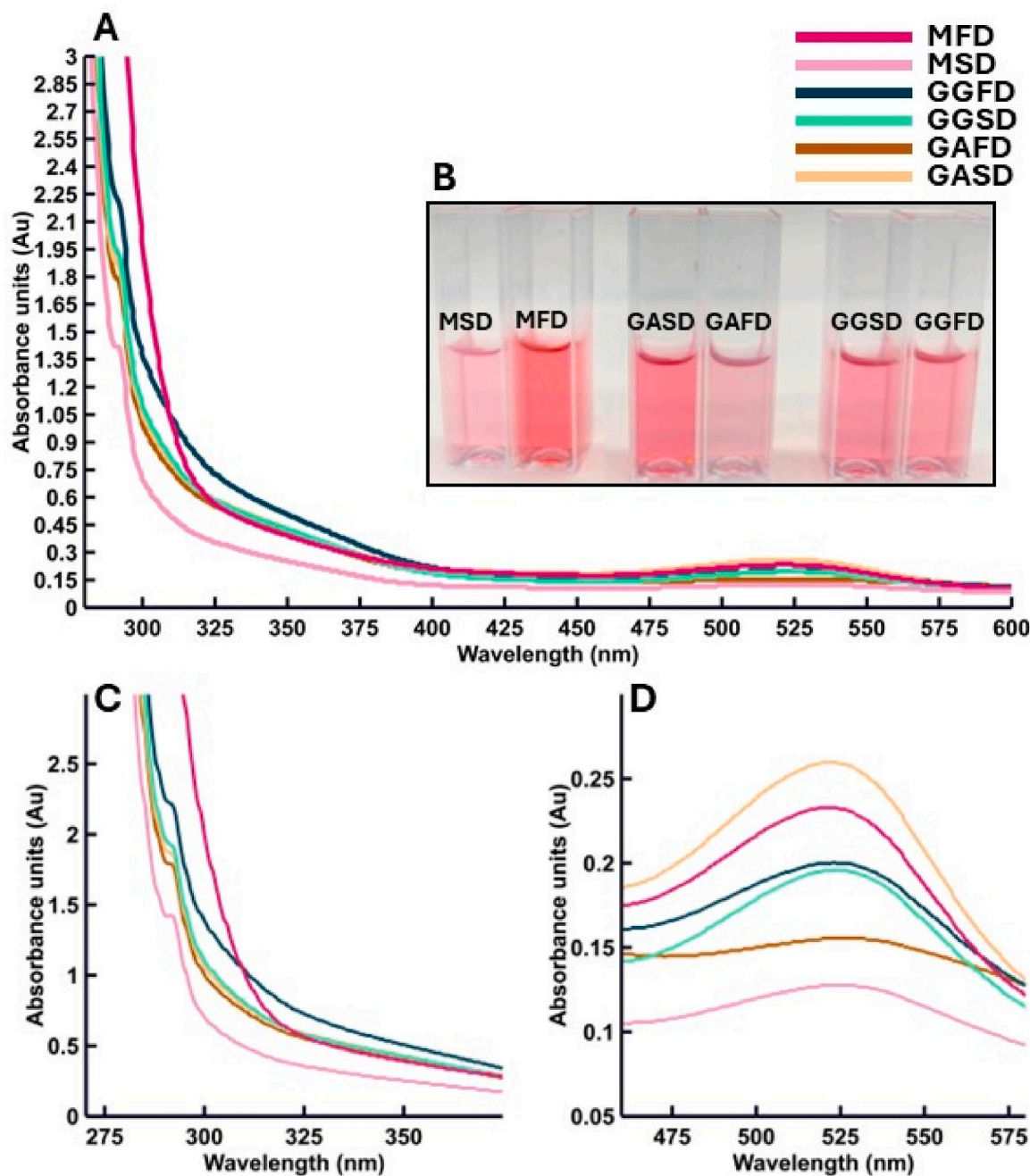


Fig. 7. UV Vis Spectroscopy of microencapsulated powders A. Full UV Vis spectrum of the 6 microencapsulated powders. The spectra shown are the averages ( $n = 3$ ). B. Model water beverage used for the UV Vis spectrometric analysis. C. The 275 nm–380 nm region of UV Vis spectra. D. The 460 nm–580 nm region of UV Vis spectra.

of sugar moieties in anthocyanins stabilises the flavylium cation and enhances colour saturation while increasing the absorption maximum (Morata et al., 2019). Both structural changes can be visualised by the UV–Vis spectra which showed variation from 280 to 350 (Fig. 7C) with a secondary peak occurring at ~300 nm, and hydroxyl group differentiation along the 490 to 550 peak region (Fig. 7D).

#### 4. Conclusion

The comparative analysis of Gidyea gum, gum Arabic, and maltodextrin as wall materials for microencapsulation of anthocyanins has yielded insightful results, highlighting their respective advantages and limitations in preserving colour and stability. Spray-drying proved to be more effective than freeze-drying in maintaining the colour stability of the encapsulated powders, with minimal changes observed over the 30-day storage period. However, an increase in colour vibrancy was observed in the freeze-dried powders. Among the wall materials, maltodextrin combined with either gum Arabic or Gidyea gum showed superior encapsulation efficiency and anthocyanin retention. These results provide preliminary support that Gidyea gum is a functional microencapsulate material. Moreover, the effective colour difference based on the wall material were assessed using spectrometry. FTIR and chemometric analysis determined a co-pigmentation of the anthocyanins present occurred, thereby changing the colour of the powders based on processing means. Further to the colour difference in the powders, rehydration to investigate a water-based beverage showed that the colour saturation was maintained in each wall material, while gum Arabic maintained a visual difference in hue. UV Vis spectrometry identified a bathochromic and hyperchromic change between processing methods, indicating a structural change to the flavylium cation and acylation of the anthocyanins, further endorsing the use of varied materials to facilitate colour differences in end products.

These findings suggest that selecting appropriate wall materials and drying techniques is crucial for optimizing the stability and visual appeal of anthocyanin-rich products. In the food industry, this knowledge can be applied to improve the shelf-life and quality of products such as functional beverages, dietary supplements, and natural colourants. By utilizing Gidyea gum and gum Arabic in combination with spray-drying, manufacturers can enhance the protective effects of anthocyanins, ensuring that consumers receive products with higher nutritional value and improved aesthetic properties. This study paves the way for further exploration of Traditional and novel food gums in microencapsulation, ultimately contributing to advancements in food preservation technologies.

#### CRedit authorship contribution statement

**Thomas Owen Hay:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joseph Robert Nastasi:** Writing – review & editing, Validation, Data curation. **Sangeeta Prakash:** Writing – review & editing, Supervision. **Melissa Anne Fitzgerald:** Writing – review & editing, Supervision, Funding acquisition.

#### Data availability statement

Data is not publicly available due to a non-disclosure agreement with Traditional Owners.

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#### 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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2024.111023>.

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