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ORIGINAL PAPER

Microencapsulation of *Malva sylvestris* anthocyanins using maltodextrin and inulin as two alternative coating materials: characterization, stability and release

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Abstract
The aim of this study was to investigate the effect of maltodextrin and inulin as a wall material in five different ratios (MD/IN: 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, 0:1, %w/w) on the stability of encapsulated Malva's anthocyanins. The encapsulation efficiency for the prepared microcapsules was between 84.32% and 98.53%. Analyses of microcapsule properties including moisture content, hygroscopicity, ANCs content (measured by UV and HPLC techniques), antioxidant activity, stability during storage, morphology, and the anthocyanin release in simulated gastrointestinal conditions were considered. Among the five treatments, the F4 (MD/IN: 0.25:0.75) had optimal conditions with the highest ANCs content (1.94 mg g^{-1}), and encapsulation efficiency (98.53%). An accelerated stability test was performed at 5 °C and 40 °C on formulated microparticles for four weeks. F4 indicated superior stability in comparison to other formulations, which retained 95.3% of their load at 40 °C after four weeks. The obtained results of Fourier transform infrared spectroscopy (FT-IR) and DSC analysis proved that the encapsulation was carried out successfully. In addition, antioxidant activity results revealed that the mentioned ratio retained 64.6% of its antioxidant capacity. Scanning electron microscopy (SEM) analysis identified that the size of the microcapsules ranged from 5 to 200 micrometers. Eventually, the in vitro encapsulated ANCs release profile was observed in response to gastric environment with satisfactory controlled release. As a result, MD and IN combinations (ratio 0.25:0.75) microencapsulation can be a suitable method for stabilizing Malva's anthocyanins. It can also have significant potential as a natural food coloring agent and herbal medicine.

Keywords Anthocyanin · Encapsulation efficacy · Antioxidant activity · Release profile · Wall material

Abbreviations

ANC	Anthocyanin
MD	Maltodextrin
IN	Inulin
MSE	*Malva sylvestris* extract
DPPH	2,2-diphenyl-1-picrylhydrazyl

Introduction

Malva sylvestris L is the most important species of Malva [1]. This plant mainly grows in Europe, South-west Asia, North Africa, and throughout the Mediterranean region, whereas some of them grow as invasive weeds all over the world, especially in the United States [2]. In Iran, dried leaves are used to treat cut wounds, dermal infected wounds, eczema, inflammatory disorders, bronchitis, and digestive problems [3]. More recent studies have demonstrated critical therapeutic properties of this plant, such as anticancer, antiulcerogenic, antioxidant, and anti-inflammatory ones [4–6]. Phenolic compounds (Quercetin, Kaempferol, Genistein, Myricetin), anthocyanins (malvin, oenin), mucilages (trehalose, galactose, sucrose, glucose), organic acid (malonate, malate, oxalate, fumarate), sterols (stigmasterol, g-sitosterol), and fatty acids (tricosanoic acid,

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heneicosanoic acid, lignoceric acid, behenic acid and arachidic acid) are effective and bioactive ingredients in *Malva sylvestris* [7–9]. Various studies have been done on this plant in recent years. Almasian et al. expressed that Polyurethane/carboxymethylcellulose nanofibers containing *Malva sylvestris* can heal diabetic wounds [10]. It was also shown that the use of edible coating based on *Malva sylvestris* mucilage and *Cinnamomum zeylani* essential oil is effective in reducing microbial growth in lamb meat during the storage period [11]. Furthermore, in a study conducted in 2022, the development of nanobiocomposite layers of gelatin and inulin was carried out with crystalline nanocellulose (CNC) and *Malva sylvestris* extract (MSE) [12].

ANCs are the most important group of water-soluble pigments that are responsible for red, purple and blue color in many flowers and fruits [13]. ANCs also exhibit different pharmacological properties such as anti-inflammatory, anti-aging, and anti-oxidant [14–17]. Regarding the results of Wang, anthocyanins extracted from *Malva sylvestris* leads to reduction of triglycerides, total cholesterol and plasma [18]. ANCs can be used as color improving and nutraceutical ingredients in food industry due to their high water solubility. However, ANCs are not stable because factors such as temperature, oxygen, and light cause them to be degraded [19, 20]. The bioavailability of the compounds is very low because of rapid metabolism and low absorption in the body. Moreover, the stability of ANCs is reduced in the presence of metal ions such as Fe^{2+}, Cu^{2+}, Zn^{2+} [21]. The general method for overcoming these limitations is to put them into nanocarrier systems to form microcapsules [22–24].

ANCs are hydrophilic compounds and particularly compatible with a water-based gel formulation e.g. maltodextrin (MD), inulin (IN), gum, and starches as wall molecules. These hydrocolloids are suitable for trapping ANCs compounds that are hydrophilic in nature. MD is a polysaccharide that is very common for encapsulation due to its low viscosity, neutral taste, color and high water solubility [25, 26]. However, the low emulsifying capacity has led to its use in combination with other walls. IN is an important natural ingredient used in the food and pharmaceutical industry because of prebiotics properties, flexible structure, and its functional properties [27].

Encapsulation of ANCs by a combination of carrier agents gives them superior physical and chemical properties compared to those obtained with individual wall agents [28–30]. Hamzah et al. obtained the highest amount of encapsulation efficiency (92.83%) and shelf life by using a mixture of MD and gum Arabic in encapsulation of *Clitoria ternatea* L. flower [31]. *Echium amoenum* ANCs encapsulated by modified maize starch and MD exhibited superior stability to heat than encapsulates powder produced with a single carrier agent [32]. Encapsulation of red cabbage ANC extract with a mixture of MD and gum Arabic using drum drying has satisfactory physicochemical properties with the most efficient yields [33, 34]. Therefore, the selection of hydrocolloid mixtures can lead to higher encapsulation efficiency and lower cost than individual biopolymers. This paper aims to develop a new MD/IN combination system loaded with ANC of Malva and study the physico-chemical properties, thermal stability, and storage stability of this system. The proposed combination causes more protection of anthocyanins and secondary metabolites against temperature.

Material and method

Chemicals

IN was provided by World-Way Biotech Inc. (China). MD with DE 16.5–19.5 was purchased from Sigma-Aldrich. Acetonitrile, 2,2-diphenyl-1-picrylhydrazyl (DPPH), citric acid, pancreatin, and pepsin were of analytical grade and were purchased from Merck Company. Malvidin 3,5-diglucoside chloride (malvin chloride) and malvidin 3-glucoside chloride (oenin chloride) were supplied by Carl Roth.

Preparation of Malva extract

Flowers of Malva (*Malva sylvestris*) were collected from natural habitats in Barij Essence farm, 27 miles of Kashan, latitude 51 degrees 3 min, North, and latitude 34 degrees 1 min, East, in May 2021. Then the dried flowers were macerated with 1% citric acid in water/ethanol (3:7), using 1:5 (w/w) plant/solvent to extract ANCs. After a day of maceration, the mixture was filtered through No. 4 Whatman paper filter. The filtrate was concentrated until 60% by weight of dry matter to reach concentrated *Malva sylvestris* extract (MSE).

Preparation of microcapsules by spray dryer

IN and MD with different ratios including F1:10% MD; F2: 7.5% MD and 2.5% IN; F3: 5% MD and 5% IN; F4: 2.5% MD and 7.5% IN; and F5: 10% IN were dissolved in distilled water with stirring at 200 rpm for 30 min and were kept overnight at 4 °C to ensure full saturation and hydration. Then, the MSE was gradually added to the mixture with stirring at 14,000 rpm using Ultra-turrax IKA T18 Basic homogenizer. The homogenized mixtures in the previous step were fed into a laboratory spray-dryer (Dorsa, Iran, DSD02) equipped with 1.2 mm diameter fluid nozzle. Other conditions of the device for preparing microcapsules are as follows: airflow rate: 8 L min^{-1}, input temperature:

120 °C and feed flow rate 0.3 L h^{-1}. The red prepared powders were collected from the receiver vessel.

Moisture content

Moisture content was measured by gravimetric analysis [35]. For this purpose, 0.2 g of each sample, in triplicate, was placed at 70 °C to reach a constant weight. The moisture of each sample was calculated after drying.

Encapsulation efficiency (EE)

The procedure was done according to the method performed by Dima et al. [36]. 100 mg of encapsulated extract was weighed and dispersed in 1 ml of distilled water and was placed in an ultrasonic bath for 15 min, then 9 ml ethanol was added. The solution was centrifuged at 5000 rpm for 10 min (Solution A). In another glass tube, 10 ml of ethanol was added to 200 mg encapsulated extract, shaken for 10 s, and then centrifuged according to the above conditions (Solution B). The percentage of EE was calculated according to Eq. 1, where $A_{\text{solution A}}$ and $A_{\text{solution B}}$ are the absorbance of clear supernatants of solution A and B at 520 nm.

$$EE\% = \frac{(A_{solution\,A} - A_{solution\,B})}{A_{solution\,A}} \times 100 \quad (1)$$

HPLC analysis of ANCs

The analyses were carried out using an Azura system (Knauer, Germany). Chromatographic separation was performed on a Teknokroma (250 × 4 mm, 5 µm) controlled at 28 °C with a column thermostatted system.

The mobile phase consisted of solvent A (water/acetonitrile/formic acid, 87:3:10) and solvent B (water/acetonitrile/formic acid, 40:50:10). The applied gradient program was: from 94 to 80% A in 20 min, from 80 to 60% A in 35 min at a flow rate 1.0 ml min^{-1}. The wavelength of the PDA detector ranged from 200 to 700 nm and the detection was set as 520 nm [37]. The identification of the compounds was made by comparing their ultraviolet spectra and retention times with those of pure standards.

Determination of ANCs

The spectrophotometric method using pH differential was used to measure the total ANCs. Absorption of ANCs extracted from microcapsules in two buffers, hydrochloric acid/potassium chloride buffer 0.025 M (pH = 1.0) and acetic acid/sodium acetate buffer 0.4 M (pH = 4.5) was read using a Lambda EZ210 spectrophotometer. The results were calculated using the Eq. (2):

$$\text{ANC content}\left(\text{mg L}^{-1}\right) = (\text{A} \times \text{MW} \times 1000 \times \text{DF}) / (\epsilon \times \text{L}) \quad (2)$$

Where A is absorption difference of supernatant solution in the corresponding pH; Mw is the molecular weight (449.2166 g mol^{-1}); DF is the dilution factor; ε is the extinction coefficient (26,900 cm^{-1} mol^{-1} L) and L is the path length (cm).

Fourier transform infrared spectroscopy (FT-IR) analysis

Mid-IR spectra of the samples were obtained using an FT-IR spectrometer Nicolet (Magna 550, USA). For this purpose, specimens were mixed with anhydrous KBr (1:100, %w/w) and were compacted into a thin disk-shaped tablet. The data were recorded at room temperature with a wave number resolution of 4 cm^{-1} and 16 scans per sample.

Particle morphology

The surface morphology and the particle shape were investigated with a field emission scanning electron microscope (MIRA3, TESCAN, Czech Republic). For this purpose, a thin layer of the sample was coated with gold alloy using a sputter coater for 150 s at 20 mA. The samples were analyzed under an accelerating voltage of 15 kv.

Thermal analysis

Thermal behavior of microcapsule powders, pure MSE, and matrix were studied by the Thermal Analyzer instrument (DSC 301, Dama Pajouh Arvin Co., Iran). For analysis, approximately 5 mg of sample was heated from 25 to 300 ºC at 10 ºC min^{-1} heating rate under N_2 atmosphere.

Antioxidant activity

The antioxidant activity method was performed using a published method of antioxidant activity assay with some modifications [38]. ANCs extracted from the microcapsules were combined with 2 ml of DPPH methanolic solution (60 µL ml^{-1}). To prepare a control sample, 2 ml of DPPH solution was mixed with 2 ml of methanol. Absorbance was measured at 517 nm, after 70 min incubation in darkness. The percentage of radical scavenging activity (%SA) can be calculated by Eq. 3:

$$\%SA = \frac{A_c - A_s}{A_c} \times 100 \quad (3)$$

A_s is the absorbance of the sample and A_c is the absorbance of the control sample. The sample concentration giving 50% inhibition (IC_{50}) was estimated by plotting inhibition percentages against concentrations of the sample. The samples were analyzed in triplicate.

Stability studies of encapsulated ANCs

Encapsulated pigment powders were stored inside an amber glass airtight container and storage study was conducted at two conditions (5 °C and 40 °C) for a period of 30 days. The effects of storage on antioxidant activity and ANC contents of powder were monitored at every 15-day interval.

In vitro release studies

The release of the total ANC from the MSE and F4 powder was determined in two media, including simulated gastric and intestinal fluid [39]. The gastric environment consisted of pepsin (0.26 g L^{-1}) and 2 g of sodium chloride, with adjusted pH of 2. The simulated intestinal fluid was prepared with pancreatin (10 g L^{-1}) and potassium dihydrogen phosphate to adjust pH to 6.5. Release kinetics of total ANCs from microcapsules under intestinal and gastric conditions were evaluated by adding 0.3 g of each sample in mentioned media, and continuously stirring for 120 min at 50 rpm and 37 ± 1 °C. 1 ml of the solution was taken at specified time intervals (15, 30, 60, and 120 min) and the amount of released ANC was calculated using the UV-Vis method (as described in section determination of ANCs).

Statistical analysis

The obtained data were calculated based on at least three replications and were reported as mean ± standard deviation. A value of $p < 0.05$ was considered to be significant and calculated using Duncan's multiple range tests in SPSS version 17.0. The graphs of mean values and error bars were plotted using Microsoft Excel version 2016.

Results

Moisture

Powder moisture is an important factor due to storage stability, powder flowability, and spoilage control. High moisture can cause adhesion and stickiness to the particles, which leads to the integration, and consequent agglutination of the microcapsules and subsequent oxidation of the encapsulated ANCs. The moisture content of the encapsulated powders ranged from 3.8 to 5.5% (Table 1). Significant differences in the amount of moisture can be related to the type of wall material because the spray drying conditions were the same for all formulas. F1 and F2 exhibited higher moisture content than the other formulations ($p < 0.05$). This result was most likely due to the higher drying rate of microcapsules containing higher amount of MD, which led to the fast formation of a shell that prevented water diffusion and evaporation. These results did not match with the finding of Dima et al. for *Coriandrum sativum* essential oil encapsulation [36], and Fernandes et al. for the preparation of microcapsules loaded with rosemary essential oil [40].

Hygroscopicity

The microcapsules tend to adsorb moisture from the environment, which can lead to the oxidation of ANCs. So, hygroscopicity is considered an important factor for storage stability [41]. As Table 1 shows, the sample produced with pure MD has the least hygroscopic feature, while microcapsules prepared with IN have the highest amount. This may be due to the intrinsic hygroscopic property of IN, because this hydrocolloid has a branched structure, which facilitates the formation of hydrogen bonds and thus making it easier to absorb moisture from the air. Similar results have been observed in the encapsulation of spearmint essential oil with gum Arabic and IN [27] and culinary banana bract with MD [42]. Also, according to Tonon et al. researches, the hygroscopicity amounts were inversely related to moisture content, with less moisture causing more capacity to absorb water [43].

Table 1 Properties of *Malva sylvestris* extract (MSE) and encapsulated powder with different formulation

Formulation	EE%	Total ANCs (mg g^{-1})	Yield w/w%	Hygroscopicity (g/100 g)	Moisture content w/w%
F1	84.32 ± 0.36^{e}	1.69 ± 0.04^{c}	73.48 ± 1.75^{c}	11.03 ± 0.15^{d}	5.3 ± 0.3^{a}
F2	88.56 ± 0.45^{d}	1.78 ± 0.03^{b}	77.40 ± 1.10^{b}	25.93 ± 0.15^{c}	5.5 ± 0.2^{a}
F3	91.56 ± 055^{c}	1.92 ± 0.04^{a}	83.47 ± 1.75^{a}	34.53 ± 0.25^{a}	3.8 ± 0.1^{b}
F4	98.53 ± 055^{a}	1.94 ± 0.03^{a}	84.34 ± 1.53^{a}	30.67 ± 0.55^{b}	4.2 ± 0.2^{b}
F5	95.62 ± 041^{b}	$1.71 \pm 0.04^{b, c}$	$74.34 \pm 1.57^{b, c}$	31.06 ± 0.70^{b}	4.1 ± 0.1^{b}

Results show the mean value ± standard deviation from three samples

Different small letters in the rows represent statically significant difference ($p < 0.05$)

Encapsulation efficiency

The encapsulation efficiency varied from 84.32 to 98.53% with the highest EE (98.53%) achieved for F4. This treatment contained 7.5% IN and 2.5% MD. This result showed that the encapsulation efficiency is completely dependent on the type of wall [44]. The positive effect of interaction of MD with IN on the microencapsulation efficiency showed the synergistic effect of these two components. In addition, this behavior may be related to the relative polarity of these carbohydrates. Statistical analysis showed a significant difference among encapsulation efficiency in the various formulations. In the study by Dima et al. the encapsulation efficiency ranged from 51 to 63%, and was lower when IN and chitosan (50:50) were applied to the wall [36]. In another study for olive extract encapsulation, IN showed lower encapsulation efficiencies than MD [45]. On the other hand, in jussara pulp encapsulation, the mixture of MD and IN showed the highest amount of ANC [46].

Total anthocyanins and individual anthocyanins

The results of total ANC contents, as presented in Table 1, indicated that the highest values of ANC were recorded in F4 (1.94 mg g^{-1}), followed by the F3 (1.92 mg g^{-1}), which means that the least amount of ANC degradation has occurred during spray drying. Since the amount of initial ANC loaded in the microcapsule was 2.3 mg g^{-1}, the amount of degradation is between 15.66% and 26.52%. These results indicated that the MD: IN (0.25:0.75) combination provided a greater capability of encapsulating MSE than only MD and IN, because a single encapsulating wall material may not have all the desired specifications. This result conforms to Gonzalez's research in which the mixture of MD/IN leads to the highest experimental value of total ANC and phenolic content [47].

The color stability is not only related to the total amount of ANCs but also related to the type of ANCs. Therefore, analysis of individual ANCs would be important. Figure 1 shows the chromatogram of MSE extract with two major peaks. The malvidin 3,5-O-diglucoside and malvidin-3-glucoside were identified at retention times of 12 min and 14.6 min, respectively, by comparing their known reference compounds. Our results are in agreement with other literature data that correspond to Malva phytochemical analysis [48, 49].

The UV spectra of peaks 1 and 2 are recorded by the PDA detector (Fig. 2). The UV-Vis spectrum of ANCs shows two basic maxima (λmax), the first one in the UV region (260 nm) and the other one in the visible region (520 nm). These spectra correspond to the general structure of ANCs [50].

Fourier transform infrared spectroscopy

The obtained results of FT-IR analysis of MSE, MD, IN, and F4 are shown in Fig. 3. MD and IN have almost the same spectrum profiles. Their two indicator bands are at 1030 and 3420 cm^{-1}, which are related to C–O–C and O-H bonds, respectively.

The spectra of MSE showed a band at 1725 cm^{-1}, which is related to bending vibration of C-O-C groups, a band at 1632 cm^{-1} related to vibration of the (C = C) bond of benzopyran ring, a band at 1417 cm^{-1} related to (C = C) stretching

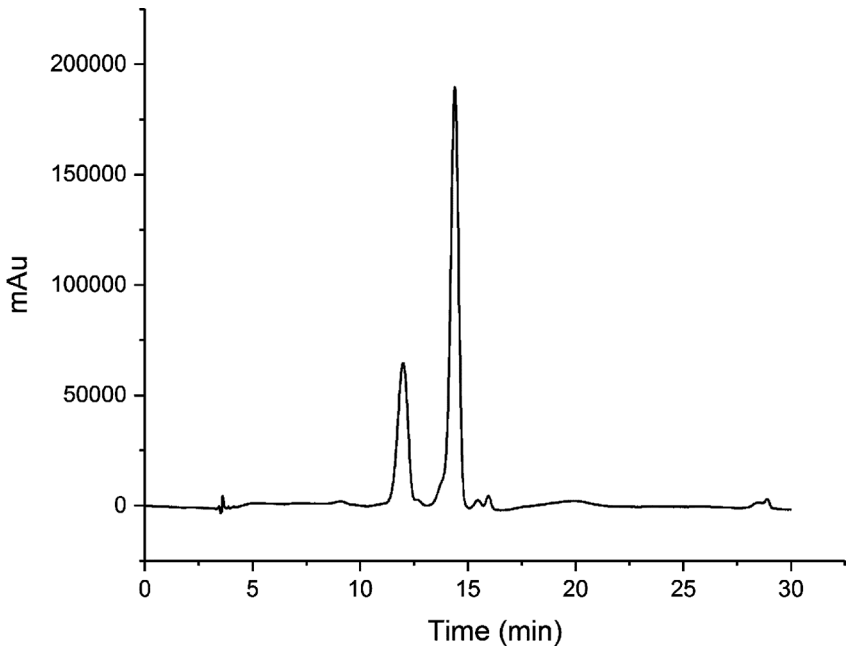

Fig. 1 HPLC chromatogram of ANCs of MSE, (1) malvin, (2) oenin

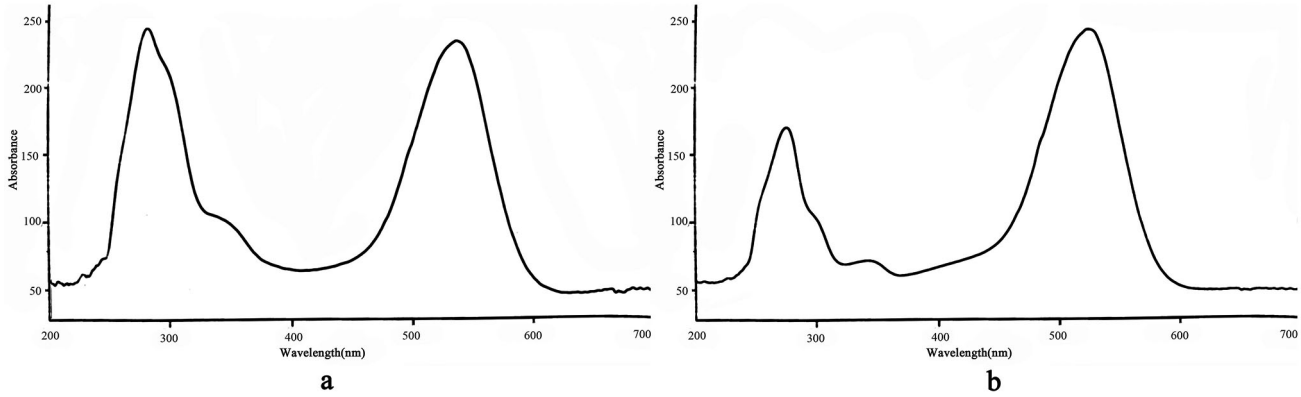

Fig. 2 UV spectra of compounds a (malvin) and b (oenin)

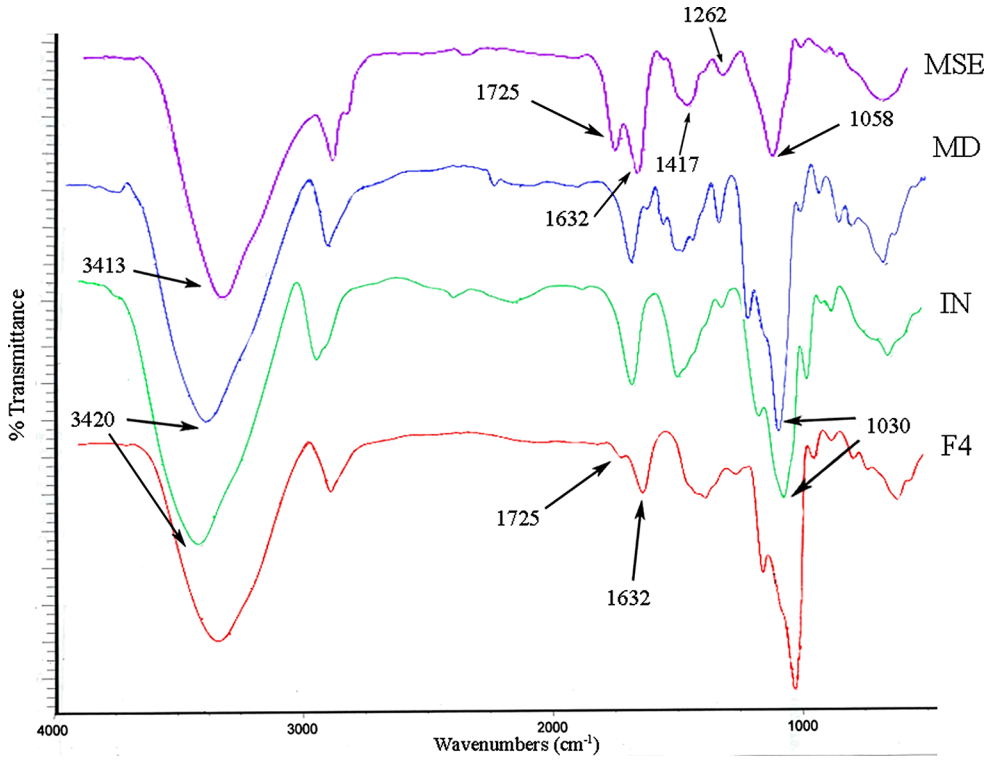

Fig. 3 FTIR spectra of MSE, MD, IN and F4

vibration in the aromatic ring, a band at 1262 cm^{-1} related to stretching of pyran rings in the structure of ANCs, and a peak at 1058 cm^{-1}, assigned to the aromatic C-H in the aromatic ring. The broad band at 3413 cm^{-1} is attributed to hydroxyl group stretching of carbohydrates, carboxylic acids, and residual water in MSE [51]. Most of the peaks related to MSE vanished in the spectra of the microcapsule. This phenomenon can be attributed to the entrapment of MSE in the matrix; in other words, MSE was encapsulated. Nevertheless, two peaks at 1632 cm^{-1} and 1725 cm^{-1} are observed in microcapsule spectra, which correspond to MSE. These tiny peaks may be due to small amounts of unencapsulated extract. However, the microcapsule obtained in this study did not present a characteristic peak of MSE. These results indicate the successful encapsulation of the MSE.

Thermal analysis

Thermal analysis is an effective method for biopolymer analysis for the determination of thermal degradation, oxidative stability, water loss, and thermal events. DSC analysis was carried out on MSE, matrix, and microcapsules produced with the optimal condition. The results obtained from the thermal analysis are depicted in Fig. 4. The DSC curve of the crude extract shows two endothermic events, the first at around 50 °C corresponding to the residual water and the second at 120–170 °C associated with the decomposition

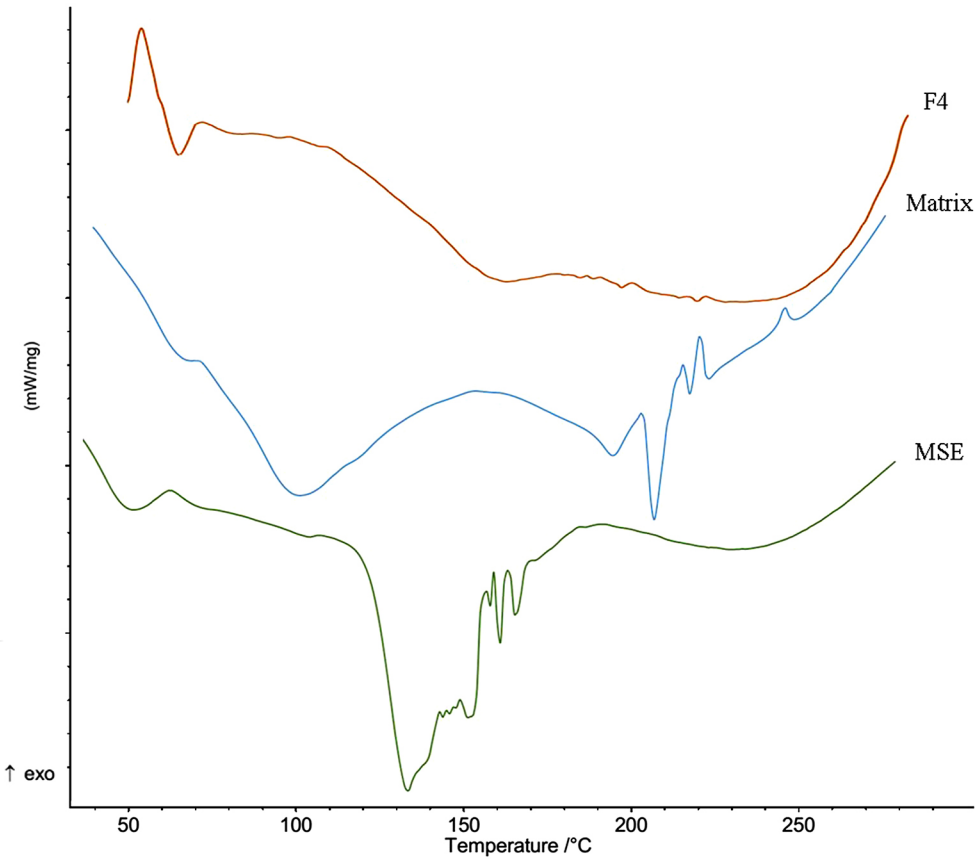

Fig. 4 DSC curves of MSE, matrix and encapsulated extract F4

of pigment. For the matrix, a broad endotherms peak is observed at temperature ranges of 60–124 °C and several endothermic and exothermic peaks are observed within 180–245 °C. In this step, the breakdown of the fructose and glucose chains is predictable resulting in the decomposition of the matrix. Other research has reported similar behavior for IN and MD [52, 53]. In F4, the initial degradation peak has been transferred to 146 °C, which indicates an increase in the stability of ANCs by encapsulation. Moreover, the lack of further peaks in the microcapsules thermogram compared to the matrix demonstrated that there is no interaction between matrix and MSE.

Particle morphology

For all formulations, particle morphology was evaluated by SEM technique (Fig. 5). The microcapsules prepared using the different wall materials presented a relatively spherical shape. Formula 1, in which the highest amount of MD is used in the wall material, has more cracks and fissures of the particles. The presence of cracks and holes leads to decreased encapsulation efficiency since the active compounds are susceptible to volatilization and loss. As the amount of IN increases in the wall, particles become uniform and smoother so that the finest particles can be seen in formulas 3 and 4. Nevertheless, using only IN in F5, the particles agglomerate and form irregular shapes. The hygroscopic characteristic of the IN causes the adsorption of water on their surface [54]. Similar results were noted by Carmo et al. in microcapsules of beetroot extract with IN and whey proteins [41]. Also, Jirayucharoensak et al. pointed out that if the temperature of IN solution is higher than its Tg temperature, the prepared powder will be sticky and adhere together [55]. Besides, de Barros et al. reported the particles containing higher amounts of IN in the wall material formulation showed more cracks in the structure [56].

Antioxidant activity

The trend of changes in antioxidant properties for MSE and prepared microcapsules is shown in Fig. 6. It is clearly observed that DPPH radical scavenging is reduced by MSE compared to the encapsulated powders. Crude Malva extract has lost about 17 times of its antioxidant properties in 30 days, while this reduction is about 3 times for microencapsulated extract. This suggests that wall materials, as a barrier, can protect ANCs from degradation, and thus it does not alter the antioxidant properties much. Among the encapsulated extracts, the highest change in antioxidant properties

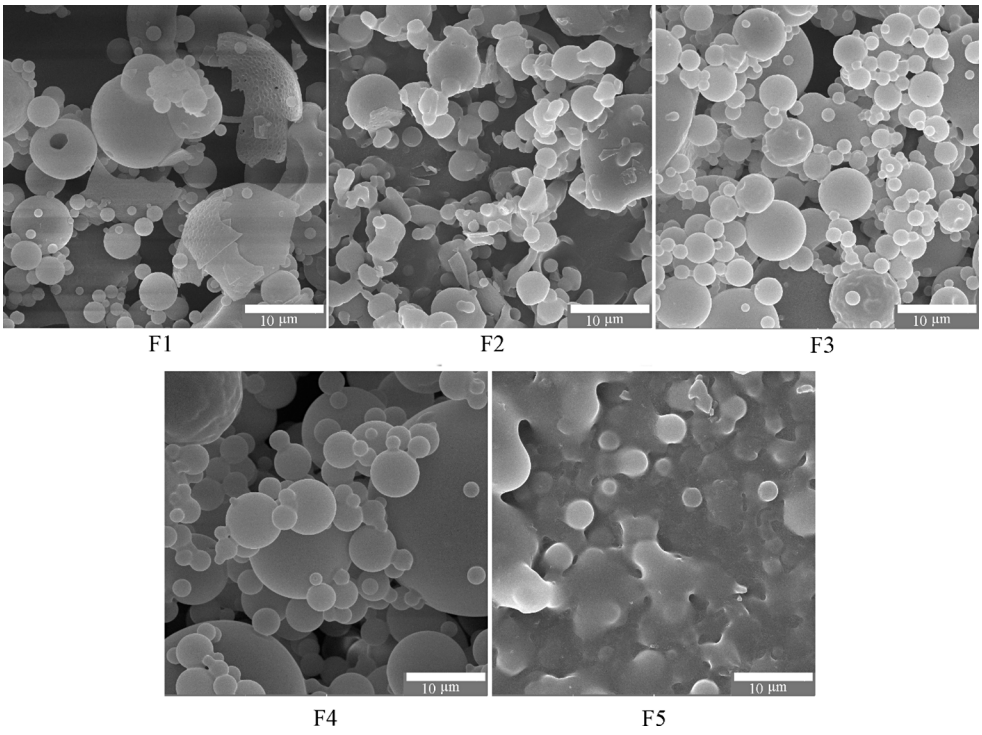

Fig. 5 Scanning electron photomicrographs of spray-dried products (F1, F2, F3, F4, F5)

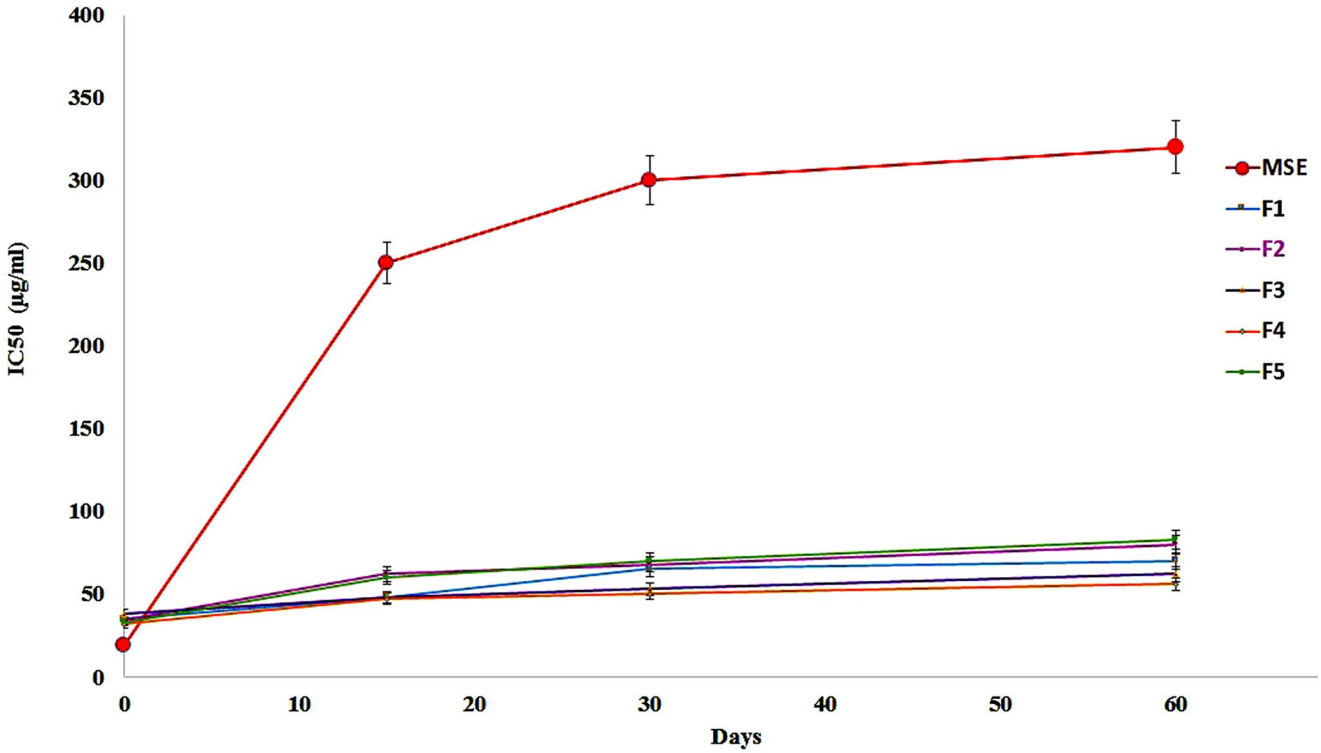

Fig. 6 The trend of antioxidant activity during a month

was in F1 and the least change was in F4. This means that the ANCs were protected appropriately in combination with MD and IN. In Jaboticaba pomace encapsulation, the antioxidant activity of powder containing soy protein isolate and MD as wall material was more than MD-pectin, MD and MD-pectin-soy protein isolate carriers [57]. Lacerda et al. showed that the combination of starch, IN, and MD seemed to improve antioxidant activity from the jussara

pulp [46]. This indicates that the mixture of walls can have a synergistic effect and it can suitably protect the flavylium cation [58, 59].

Stability

Thermal stability is an important factor to characterize how long the ANCs can be kept at the thermal temperature. The result of monitoring the amount of ANCs over 30 days at 25 °C and 40 °C is shown in Fig. 7. As predicted, higher temperatures lead to faster decay of ANCs. The greatest decrease in the amount of ANC in both conditions is related to the crude extract, in which the value of ANC decreased to 12% at 5 °C and 74.9% at 40 °C during 30 days. This result can be due to MSE unencapsulated ANCs, which have exposed them to degradation. Thermal degradation of ANCs may be related to hydrolysis of the glycoside bond and the resulting production of aglycone, or due to the conversion of the flavylium cation into chalcone compound leading to a change in brown color [60].

The percentages of ANCs retained in the microcapsules in different treatments were about 74.8–98.9% and

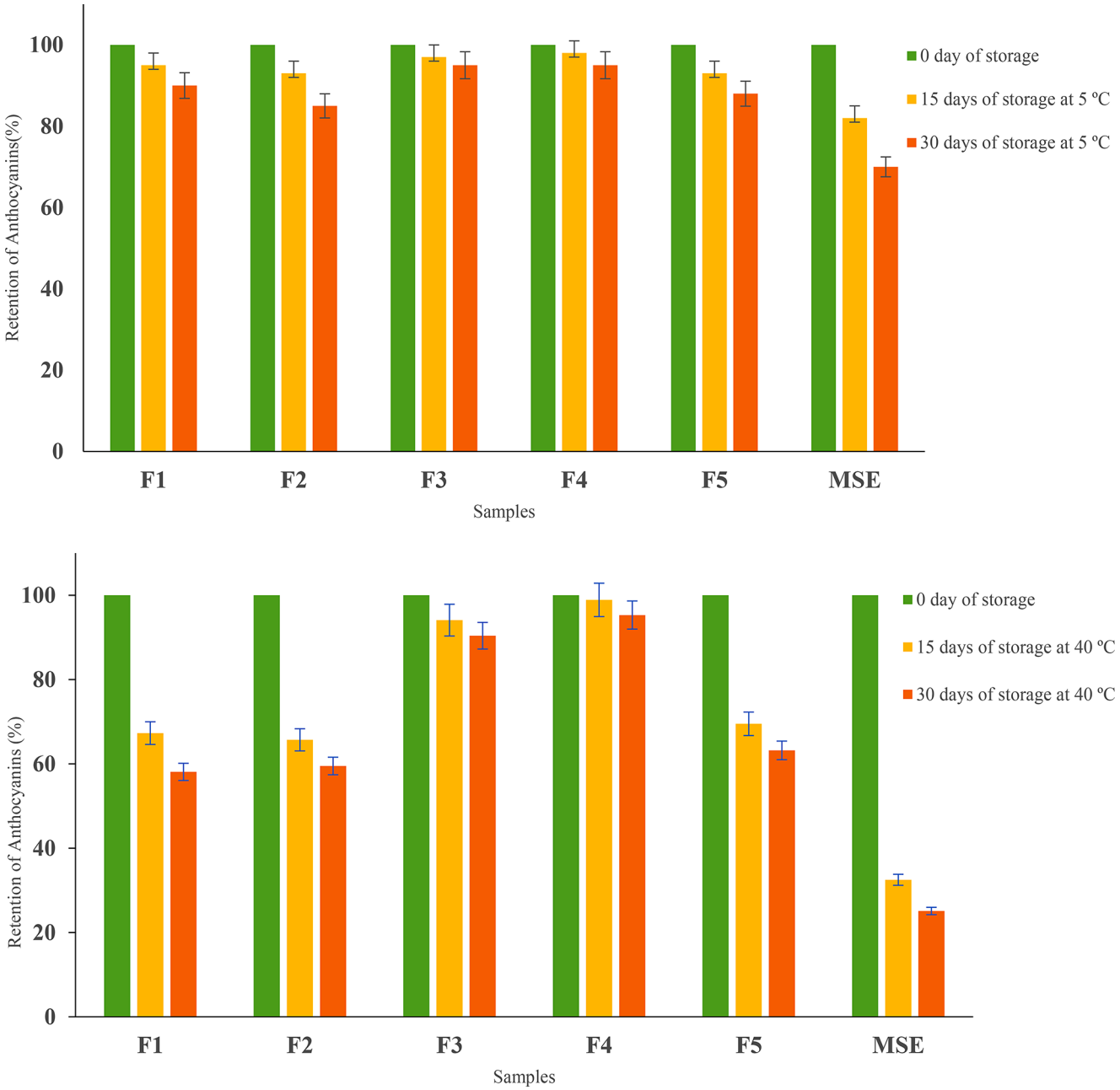

Fig. 7 Storage stability of encapsulated powders during storage at 5 °C and 40 °C for 15 days and 30 days

59.5–95.3% after 15 days and 30 days at 40 °C, respectively. As it is clear in Fig. 7, F4 led to the highest protective effect of the ANCs toward heat treatment. The higher stability of MD/IN mixtures compared to other formulations is due to the synergic interaction of the two polymers in this ratio [61]. This result illustrates that these combinations were more efficient in terms of anthocyanin conservation. Other studies have also shown that the use of a composition of walls leads to greater stability. According to Mahdavi et al., the combination of gum Arabic and MD is the most effective wall material in stabilizing the pigments [62]. Botrel da et al. showed that the mixtures of IN and MD can be excellent wall materials for fish oil [63].

Release

The percentage of the ANCs released from MSE and encapsulated extract F4 using UV-Vis data under simulated gastrointestinal digestion for 2 h is shown in Fig. 8. The released ANCs from the spray-dried microcapsules were significantly lower compared to MSE, which indicates that the release of ANCs is properly controlled. Almost 90% of

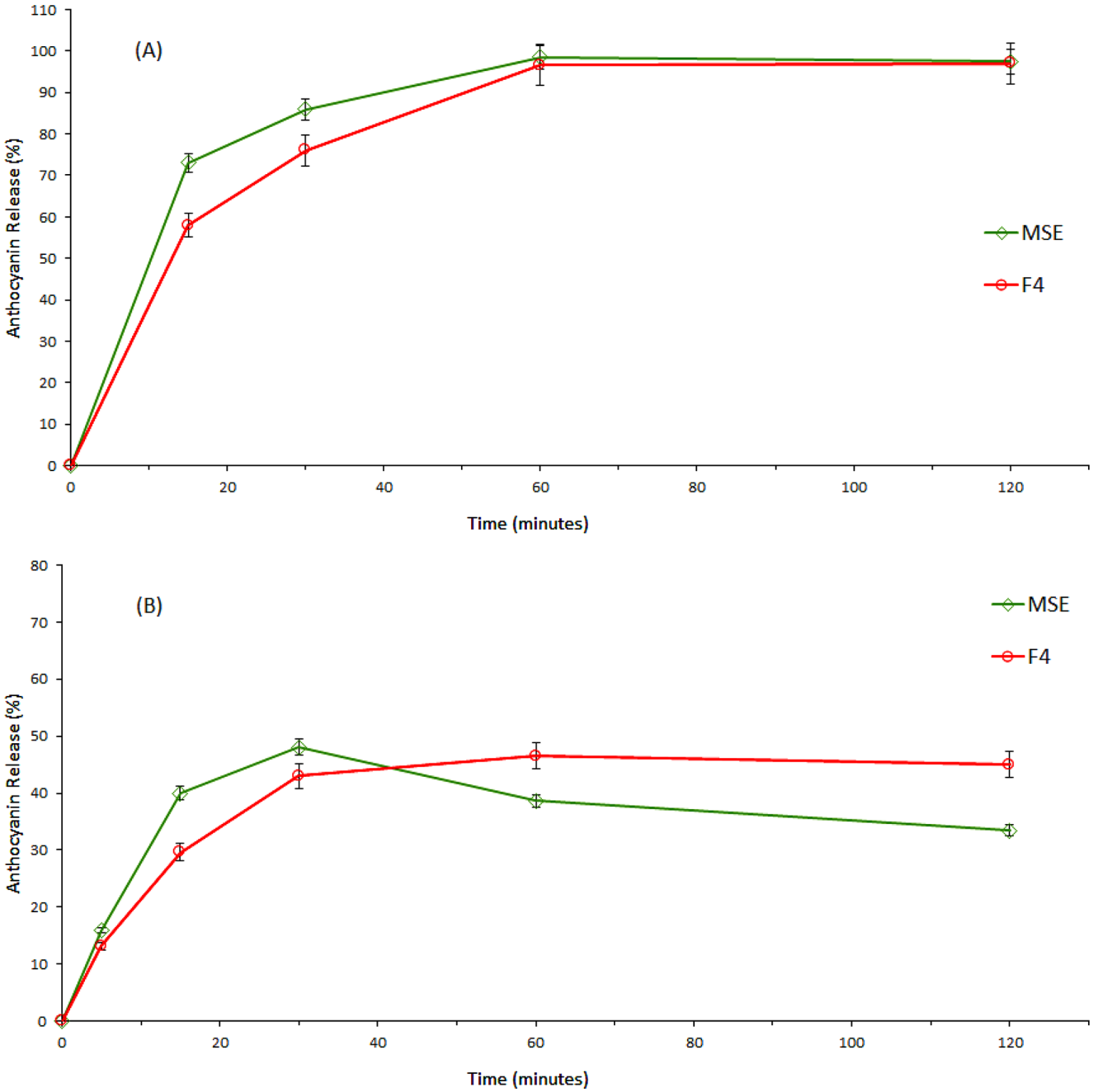

Fig. 8 Percentage of release of ANCs from nanocapsules (**A**) simulated gastric fluids (**B**) simulated intestine fluids

the ANCs were released in the gastric phase at 30 min. The high release rate of the ANCs observed in the microcapsule containing MD and IN (0.25:0.75) is due to its polysaccharide structure. This combination could be digested by the gastric enzyme at the initial stage.

In the intestinal fluid, the digestion solution turns into light green. This color change is due to an increase in the amount of pH. Under neutral pH, the released ANCs can be converted to a carbinol-like form (colorless), and thus destroyed. However, the ANCs are more stable in acidic media due to flavylium cation formation. The percentage of ANCs released from F4 microcapsules in the simulated gastric fluid was 46.5%, compared with 38.6% from MSE at 30 min. These obtained results conform to research by Sun et al. [64]. reporting that almost 75% of the released ANCs were degraded during the simulated intestinal digestion. Also, a similar trend was found in a study carried out by Oidtmann et al., in which the encapsulation of bilberry inhibited the early degradation of ANCs in the intestinal system [65].

Conclusion

Anthocyanins compounds in Malva were effectively microencapsulated using MD and IN with different ratios. The highest encapsulation efficiency was related to the F4 formulation, which contained MD/IN (0.25:0.75) ratio. This optimal formula also had the least degradation (4.7%) of ANCs during one-month storage and the least reduction of antioxidant properties over the same period. Additionally, HPLC analysis revealed that the two compounds of ANCs including malvidin 3,5-O-diglucoside and malvidin-3-glucoside were stable during one month of storage. SEM analysis showed the size of the microcapsules in the obtained powders was smaller than 200 nm. Furthermore, in vitro release studies indicated that more than 90% of ANCs are released in the gastric environment within 60 min and they are more stable in this media. In conclusion, the present research introduces novel functional microcapsules that can be applied as medicine, a natural antioxidant, and red colorants with satisfactory results.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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