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Anthocyanin Stability in a Mix of Phenolic Extracts Microencapsulated by Maltodextrine, Whey Protein and Gum Arabic

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Abstract The objective of this work was to study the stability of the anthocyanins in a mixture of of jabuticaba, jussara and blueberry phenolic extracts microencapsulated by the spray drying technique during storage under different conditions: light, dark and 40°C. Combinations of maltodextrin, gum arabic and whey protein encapsulating agents were used, totaling 12 assays. The mean values of total anthocyanins among the three storage conditions for the M1 mixture were 1360.86 mg·100 g^{-1} to 1184.15 mg·100 g^{-1}, at 0 and 75 days of storage. The overall color difference values for all samples were less than 5.0, indicating that there were no perceptible color differences between them throughout the stability study. M1 and M3 presented good rehydration capacity and smaller particle size among the samples evaluated. The microencapsulation technique was successfully applied to maintain the stability of the anthocyanins from a blend phenolic extract under different storage conditions.

Keywords: *stability, total anthocyanins, antioxidant capacity, wall materials, microencapsulation*

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1. Introduction

Natural dyes have been gaining popularity in the food ingredients market, as the use of synthetic dyes becomes smaller because of their effects on health, especially the health of children; they can induce behavioral disorders, such as attention deficit and hyperactivity, in addition to allergenic effects [1,2].

Anthocyanins are natural dyes with an intense red colour, which have a broad colour spectrum according to the pH value of the medium. Their main property is their antioxidant capacity, which contributes to beneficial effects in reducing the risk of heart disease, neurodegenerative disease and cancer [3,4,5]. In this sense, consumers are demanding products that contain these bioactive compounds, which, in addition to the function of colouring, can provide some health benefits.

One of the great challenges to the use of anthocyanins as natural dyes is the instability of their physical and chemical changes during storage. These changes lead to a decreased dye power and bioactivity in storage. The physico-chemical stability of anthocyanin is affected by many factors, including pH, oxygen, light, temperature, enzymes and the presence of compounds such as ascorbic acid that contribute to its degradation [4,6].

The technique of microencapsulating bioactive compounds by spray drying is a viable alternative for the conservation and stability of these compounds; the rapid evaporation of the water keeps the temperature of the particles low. Thus, this technique allows for the drying of heat sensitive products without affecting their quality, in addition to the wall material protection of the active material during the storage period. Microencapsulation promotes the stability of anthocyanins, providing greater protection against environmental damages, such as light, temperature and oxygen, in addition to the influence of pH [7,8,9].

The most used encapsulating agents for spray drying are polysaccharides (starch, maltodextrin, corn syrups and gum arabic), lipids (stearic acid, mono and diglycerides) and proteins (gelatin, casein, whey, soybeans and wheat) [5,10]. Maltodextrins have high water solubility, low viscosity, are colourless and have a pleasant taste. They are classified according to their degree of hydrolysis,

expressed as equivalent dextrose (ED) [5,11]. Whey proteins have gelling and emulsifying properties, and an amino acid profile suitable for beverage fortification. They can also be processed into pH-sensitive hydrogels or nanoparticles for the controlled release of bioactive compounds, such as anthocyanins [12]. Finally, gum arabic is a branched heteropolysaccharide and has the ability to produce stable emulsions with most oils in the widest pH range; it also forms a visible film at the oil interface and is widely used in combination with maltodextrin for the preservation of functional compounds [10,13].

The objective of this work was to study the stability of the anthocyanins in the microencapsulated phenolic extracts of jussara, jabuticaba and blueberry, using the spray drying technique, under different conditions (light, dark and temperature) during the storage period.

2. Material and Methods

2.1. Reagents and Material

Jussara (*Euterpe edulis Mart.*) and jabuticaba (*Mryciaria jaboticaba*) fruits were collected in the rural area of the city of Viçosa, Minas Gerais, Brazil. Blueberries (*Vaccinium ashei*) were collected in Barbacena, Minas Gerais, Brazil. All fruits were collected at the harvest point, during the 2014–2015 harvest season. Folin-Ciocalteu (FC) reagent, ABTS (2,2'-azinobis-3-ethyl-benzothiazoline-6-sulphonate), sodium carbonate and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). The following reagents were analytical grade: ethanol and potassium persulphate (Vetec Química Fina Ltd, Rio de Janeiro, Brazil), gallic acid (Analyticals Carlo Erba, France), hydrochloric acid (Synth, São Paulo, Brazil). Maltodextrin 10DE (Gemacon Tech, Brazil), gum arabic (Vetec Química Fina Ltd, Rio de Janeiro, Brazil) and whey protein isolate (WPC) were kindly provided by Inovaleite/UFV.

2.2. Preparation of Extracts

The extracts were obtained according to the methodology described by Rocha et al. [14]. Subsequently, they were vacuum filtered using Whatman # 1 filter paper and then concentrated on a rotary evaporator (IKA RV 10, LabScience, Brazil) at 45 °C until removal of the alcohol. All extracts were concentrated to the total soluble solids content of 6° Brix and packed in an amber bottle under refrigeration (5 ± 1°C). By means of the extended simplex-centroid mixtures design, a previous study was conducted in order to determine the best extract combination that provided a higher content of total anthocyanins and total phenolics. The optimum mixture was 0.2167: 0.5667: 0.2167 (v/v), (jabuticaba: jussara: blueberry).

2.3. Preparation of Microcapsules by Atomization and a Preliminary Study

Solutions of the encapsulating agents (maltodextrin, gum arabic and whey protein isolate) were prepared in 10% (w/v) concentration. The experiment was conducted in the delineation of simplex (3.3), with three factors (maltodextrin, gum arabic and WPC) and three replicates at the central point, totaling 12 assays. The combination of the three wall materials was performed in order to evaluate the potential combinations that could result in a greater microencapsulation efficiency of total anthocyanins and phenolic extracts. Table 1 presents the proportions of wall materials in the mixture, selected by the simplex centroid mixture design, to determine the mixtures with the highest encapsulation efficiency.

Table 1. Experimental design for the optimization of the microcapsule production of the mixtures of jabuticaba, jussara and blueberry extracts by means of three different encapsulating agents, maltodextrin (X1), whey protein isolate (WPC; X2) and gum arabic (X3)

Mixture	Encapsulating agent ratios (w/v)		
	Maltodextrin (X1)	WPC (X2)	Gum arabic (X3)
M1	1	0	0
M2	0	1	0
M3	0	0	1
M4	0.5	0.5	0
M5	0	0.5	0.5
M6	0.5	0	0.5
M7	0.670	0.165	0.165
M8a	0.333	0.333	0.334
M8a	0.333	0.333	0.334
M8a	0.333	0.333	0.334
M9	0.165	0.670	0.165
M10	0.165	0.165	0.670

a: central point.

The extract mixture was added to the wall materials (Table 1) in a ratio of 1:1 (v/v). The resulting mixtures were homogenized in two stages (Tecnohomo Tecnolab homogenizer) at 30 °C with flow and pressure adjusted to 30 $L \bullet h^{-1}$ and 500 bar, respectively. Spray drying was performed in a spray dryer (LabMaq MSD 1.0) following optimal conditions proposed by Silva et al. [15]. The extracts were fed to the atomizer using a peristaltic pump at a set speed of 1.12 $L \cdot h^{-1}$. The drying parameters were: air flow of 1.7 $m^3 \cdot min^{-1}$, air inlet 180 ± 5 °C, air outlet temperature 78.5 ± 3.5 °C. The obtained powders were packed in polyethylene bags containing a layer of laminate and the packages were stored in the dark.

2.4. Stability of Microparticles in Storage: Light, Dark and 40°C

The three mixtures with high anthocyanin encapsulation efficiency were selected for stability evaluation after storage, as well as mixtures that presented moderate and minimum values of encapsulation efficiency, totaling five samples. The microparticles were placed in hermetically sealed transparent polyethylene bags and stored in a light chamber (TrueVue® 2. Data colour), under the influence of two fluorescent lamps (corresponding to daylight). with a monitored temperature of 25 ± 2°C. Another group was packed in laminated polyethylene bags and stored in the dark, with a monitored temperature of 25 ± 2°C. Lastly, another group of samples were placed in laminated

polyethylene bags and conditioned in an oven (Tecnal) with a monitored temperature of 40 ± 2°C. A portion of each powder (approximately 0.2 g) was withdrawn from each sample at each determined time interval and diluted in 10 mL of distilled water; this was called the stock solution used for all stability analyses. Samples were evaluated for a period of 75 days, at intervals of 15 days for colour, antioxidant capacity and total anthocyanins, as described in sections 2.4.1 to 2.4.3.

2.4.1. Colourimetry

The colour of the mixtures was determined in a Colorquest XE Colorimeter (Hunter Lab, Reston, VA, USA), with direct readings of the values of the coordinates L* (luminosity), a* (intensity of red vs. green) and b* (intensity of yellow vs. blue). Hue (h*) and saturation (C*) were calculated from the a* and b* values, according to equations 1 and 2, respectively. The overall colour difference was also calculated between 0 and 75 days for the three storage conditions, according to equation 3.

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$c^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

2.4.2. Antioxidant Capacity

The antioxidant capacity was determined by a TEAC (Trolox equivalent antioxidant capacity) test using the ABTS (2,2'-azinobis-3-ethyl-benzothiazoline-6-sulphonated) radical, according to the method described by Re et al. [16]. The results were expressed in μM Trolox per gram of powder.

2.4.3. Anthocyanin Content

The total anthocyanin content of the microcapsules was determined according to the methodology described by Lees and Francis [17]. The aliquots were conveniently diluted from the stock solution with ethanol solution and 1.5 mol·L^{-1} HCl (85:15, v/v) and transferred to 15 mL falcon tubes. The samples were then centrifuged in a laboratory centrifuge (Excelsa/Model 206 MP, Fanem, Sao Paulo, Brazil) at 25 °C for 10 min at a relative centrifugal force of 2800 x *g* before spectrophotometric readings. A direct reading of the absorbance was taken in a UV/VIS-1601 PC spectrophotometer, (Shimadzu) at 535 nm.

2.5. Particle Morphology

The morphology and particle size of the different powder samples were evaluated by scanning electron microscopy (TM3000, Hitachi Ltd. Tokyo, Japan). The samples without previous preparation were deposited in the support of the microscope and evaluated at several magnifications (300–2000 times).

2.6. Particle Size Analysis

The particle size distribution during the rehydration process of the powders was obtained using a Beckman Coulter LS 13 320 laser diffraction analyser (Beckman Coulter, Miami, FL, USA) coupled to the liquid analysis module (Beckman Coulter, Miami, FL, USA). Sufficient quantities of the samples to generate the turbidity required for the readings were added to the reservoir of the liquid analysis module containing water at room temperature. The addition of the samples was performed slowly to prevent the formation of agglomerates. Recirculation of the mixture was guaranteed by having the liquid analysis module pump operate at 100% capacity, and the data were collected for 100 s. The results were obtained using a refractive index of 1.332 for the dispersing medium (water) and 1.45 for the particles.

2.7. Adsorption Isotherms

The mixtures were submitted to different storage conditions (relative humidity and water activity) in order to determine the adsorption isotherms. The adsorption isotherms were determined in three replicates by the static gravimetric method at room temperature (25°C). Six saturated salt solutions ($LiCl.H_2O$, $MgCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, NaCl, KCl and K_2SO_4) were used in order to obtain equilibrium relative humidity (URe) environments equal to 32.8, 52.9, 75.3, 84.3 and 97.3%, which correspond to A_w values of 0.110, 0.328, 0.529, 0.753, 0.843 and 0.973 [18].

In the isotherm models, the tri-parametric mathematical models of OSWIN and BET (Brunauer-Emmett-Teller) (Equations 4 and 5, respectively) were adjusted. These models are widely applied in food samples [19]. The coefficient of determination (R^2) was used to evaluate the fit of the models to the experimental data. The error (ε) between the experimental and predicted moisture values and the mean of the errors ($|\varepsilon_m|$). Mean error values less than 10% were considered indicators of good adjustments for the adsorption isotherms [20].

$$X_e = C\left[\frac{A_w}{1-A_w}\right]^D \quad (4)$$

$$X_e = \frac{X_M C_{BET} A_w \left[1-(n+1)A_w{}^n{}_{-+} n(A_w)^{n+1}\right]}{(1-A_w)\left[1+(C_{BET}-1)A_w - C_{BET}(A_w)^{n+1}\right]} \quad (5)$$

X_e = equilibrium moisture (g water/g dry solids);
X_M = moisture in the molecular monolayer (g water/g dry solids);
N = number of molecular layers;
$C_{BET.}$ $C_{OSWIN.}$ D = constant.

2.8. Statistical Analysis

The encapsulation efficiency data were evaluated by an analysis of variance (ANOVA), as well as the significance ($p < 0.05$) and adequacy of the model. Data were evaluated using Statistica 7® software (V7.0, Statsoft Copyright. Inc).

Stability data were submitted to a factorial variance analysis to verify the significance of each factor: storage condition (in three levels: light, dark, 40°C), time (in six levels: 0, 15, 30, 45, 60 and 75 days), type of mixture and

the interaction between the factors, when necessary. A Tukey test was used to compare means at a 5% level of significance. The statistical tests were performed in the statistical program SAS (Statistical Analysis System), version 9.1 (2008).

3. Results and Discussion

3.1. Preliminary Study

Linear and quadratic models were tested to describe the influence of wall materials on the encapsulation efficiency. Both models were non-significant ($p > 0.05$), indicating that regardless of the combination of material, there was no significant influence on the encapsulation efficiency of anthocyanins. The M1, M2 and M3 mixtures were selected, which presented the best efficiencies, as well as M8, which contained equal proportions of all wall materials and median efficiency values. The M9 mixture was also evaluated; it presented the minimum values of encapsulation efficiency. The encapsulation efficiency data of anthocyanins are described in Table 2.

Table 2. Encapsulation efficiency values of anthocyanins in 12 preliminary trials

Mixture	Encap.efi.TA*(%)
M1	95.79
M2	93.53
M3	92.54
M4	56.30
M5	79.16
M6	89.28
M7	86.82
M8a	83.91
M8a	79.44
M8a	74.54
M9	69.16
M10	64.26

* TA: total anthocyanins; Encap.efi: encapsulation efficiency.

3.2. Microcapsule Stability Study

A stability study was performed with the mixtures that presented the maximum and minimum values of encapsulation efficiencies for anthocyanins. The best results were obtained with mixtures M1, M2 and M3, while less satisfactory results were obtained with mixtures M8 and M9. In Figure 1, the graphs of anthocyanin content over the storage period are shown.

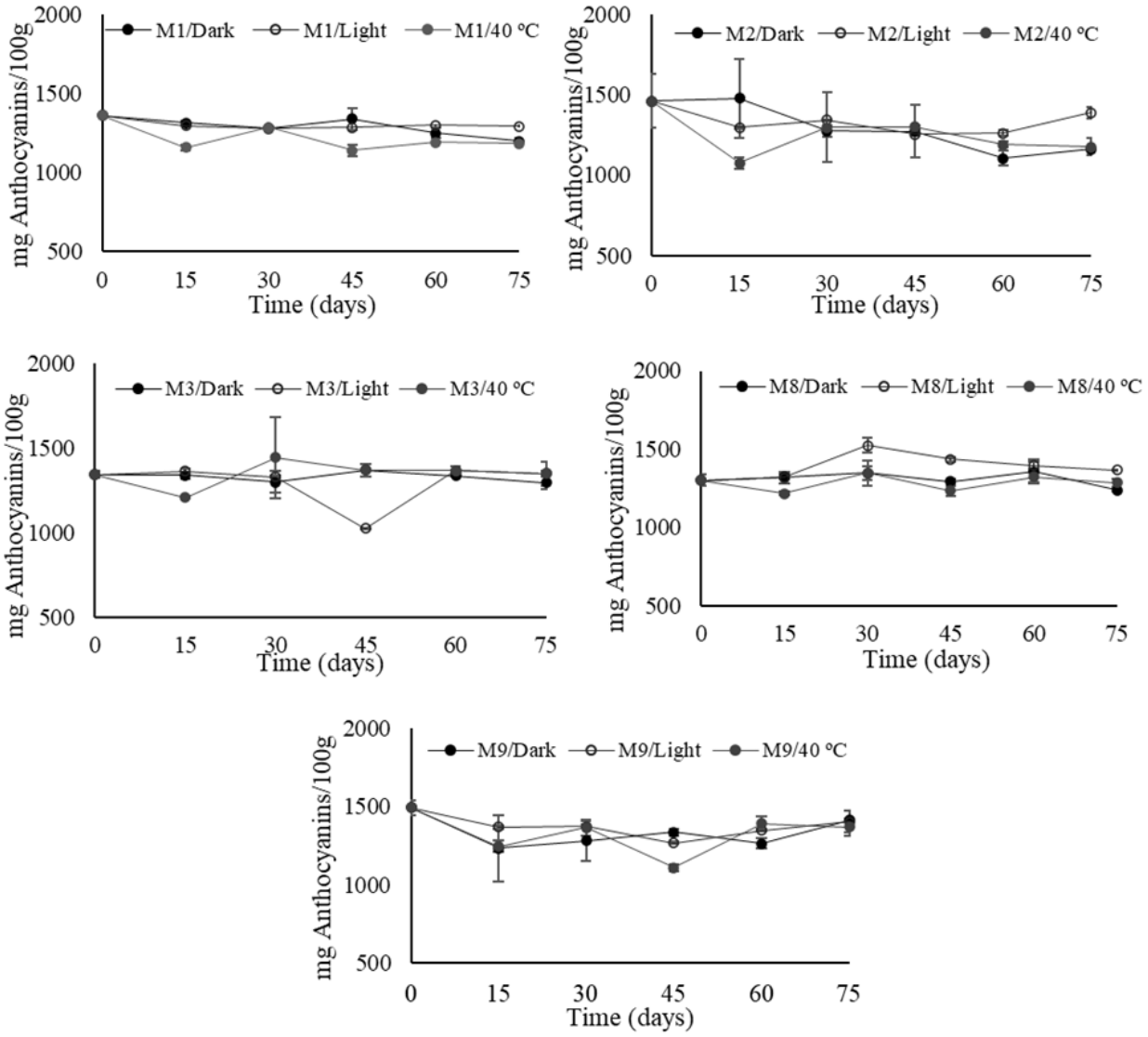


Figure 1. Concentration of total anthocyanins in powders M1, M2, M3, M8 and M9 over 75 days of storage

There was a significant interaction between all studied factors (p> 0.05). The interaction was decomposed as a function of the mixture, and linear and quadratic models were tested. However, it was only possible to adjust the model that explained this variation for M9 (Eq. 6); the other mixtures were not significant. The model was not significant (p> 0.05) for all mixtures. Regardless of the storage conditions that were submitted, the anthocyanin content remained constant throughout the study period. Microencapsulation proved to be efficient in maintaining the stability of anthocyanins over the storage period.

$$Y\left(mg/100\ g\right) = 1471.79 - 10.44.t + 0.13t^2, R^2 = 0.77 \quad (6)$$

Y = total anthocyanins;
t = storage time.

There was no significant effect (p> 0.05) of storage time or of storage conditions between the studied mixtures. The mean total anthocyanin values for M1, for example, ranged from 1360.86 mg·100 g^{-1} to 1184.15 mg·100 g^{-1}. For M3, the mean values of anthocyanins among the three storage conditions ranged from 1345.70 mg·100 g^{-1} to 1296.38 mg·100 g^{-1}. He et al. [7] evaluated the stability of nanocapsules of blueberry anthocyanins using chitosan as an encapsulating material. A comparison between free and encapsulated anthocyanins showed a greater degradation of the free anthocyanins during the 42 days of analysis, demonstrating the importance of nanoencapsulation for the protection of anthocyanins. However, during storage at 40°C for 9 days, there was only 30.8% retention of anthocyanins, in contrast to the present work, in which there was no significant difference (p> 0.05) over the storage period for all the evaluated samples.

Microencapsulation is a technique that helps to increase the stability of anthocyanins and facilitate their application as a natural dye. The positive results of the stability study show the importance of this technique in reducing the degradation of anthocyanins. Hernández-Herrero & Frutos [21] found a low stability of blackberry juice with ascorbic acid added, so in the first days of storage, there was a 90% loss of anthocyanin content. The authors attribute these results to three main hypotheses for the mechanisms involved in this degradation: the condensation of the two molecules, the degradation of anthocyanins for the prevention of ascorbic acid oxidation and the degradation of anthocyanins by hydrogen peroxide, generated from the oxidation of ascorbic acid. Mahdavee Khazaei et al. [22] also found no significant (p <0.01) degradation of anthocyanins in malenodextrin and gum arabic microencapsulated petals evaluated over 10 weeks at 35°C, which was attributed to the protective effect of the wall materials against heat during storage.

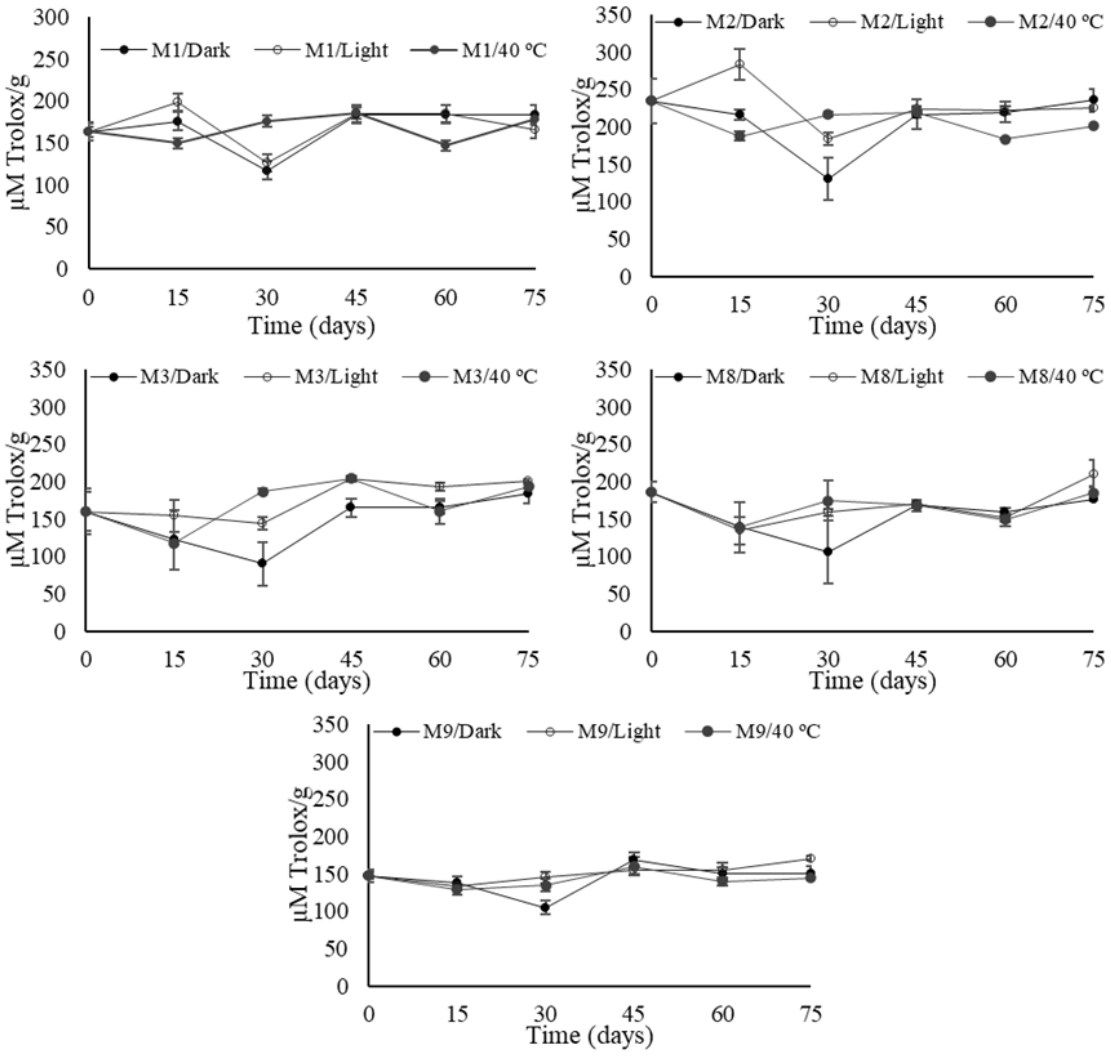


Figure 2. Antioxidant capacity of powders M1, M2, M3, M8 and M9 over 75 days of storage

Figure 2 presents the results of the antioxidant capacity, evaluated over time in the mixtures M1, M2, M3, M8 and M9.

There was a significant interaction between the studied factors ($p < 0.05$), so a study of the interactions was performed, thus, fixing each mixture. Linear and quadratic models were tested for all the mixtures and it was not possible to fit a model that explained the variation of the antioxidant capacity over time; under different storage conditions, the lack of adjustment was significant ($p < 0.05$) and/or the factors of the equation were not significant ($p > 0.05$).

Regardless of the storage condition and the type of mixture used, the antioxidant capacity remained constant throughout the storage period; small variations in values were not significant ($p > 0.05$). In the M9 mixture, for example, the antioxidant capacity of the three storage conditions over the 75 days of storage was constant. One of the most important properties of anthocyanins is their antioxidant capacity, which attributes specific properties to this substance, acting in the prevention of a series of diseases. The anthocyanin content was not significantly influenced by storage time and storage conditions, as was also observed for antioxidant capacity. These results reinforce the positive correlation between anthocyanin content and antioxidant capacity.

To study the effects of storage time separately, the antioxidant capacity at the start of storage was considered to be 100%, thus, excluding any differences between the three treatments. In the M2 sample, for example, the initial antioxidant capacity was 234.84 µM trolox·g^{-1} of powder, while the mean final value was 220.32 µM trolox·g^{-1} of powder, presenting a reduction of 6.18%. For M9, the reduction was even lower, with values varying from 147.63 to 144.33 µM trolox·g^{-1} of powder, with a reduction of 2.23%.

Moser et al. [23] found a reduction in the antioxidant capacity of microencapsulated violet grape juice with the mixture of wall materials: maltodextrin, whey proteins and soy proteins evaluated at different storage temperatures 5, 25 and 35°C. The authors stated that both temperature and time did not significantly influence ($p > 0.05$) antioxidant capacity over the storage period. For treatments with the soybean protein mix and maltodextrin, there was a 25% reduction in antioxidant capacity after 45 days of storage. For treatments with whey protein and maltodextrin, there was no tendency for degradation over time and the reduction in antioxidant capacity was small, ranging from only 6 to 14%.

Lacerda et al. [24] found degradation of the antioxidant capacity of inulin, starch and maltodextrin microencapsulated jussara pulp over 38 days of storage at 50°C. The antioxidant capacity of the microparticles decreased, averaging 16 and 65% when evaluated by FRAP and TEAC (Trolox equivalent antioxidant capacity), respectively. The low stability of these microparticles was attributed to the insufficient formation of a protective layer around the nucleus, as well as its physicochemical attributes (greater hygroscopicity) and morphology (formation of agglomerates). The loss of antioxidant capacity was also justified by the degradation of other non-anthocyanin phenolic compounds present in jussara pulp, such as hydroxybenzoic derivatives, hydroxycinnamic acid and hydroxyphenylacetic acid, since the anthocyanins were not degraded during storage.

Colour is a sensory attribute that is directly related to the consumer's acceptance of a product. Throughout a storage period, anthocyanins can undergo a degradation process, leading to loss of colour (saturation and/or shade change) by the formation of degradation compounds, resulting in a different product colouration than the original. A number of factors can lead to the degradation of the colour of anthocyanins, such as light, temperature, oxygen, pH change and time. The colourimetric coordinates L*, a* and b* were evaluated during the 75 days of storage. The coordinates a* and b* were used to calculate the saturation (C*), hue angle (h*) and overall colour difference (ΔE*).

In food, the analysis of isolated a* and b* coordinates are often not correct, because food is often a mixture of colours rather than pure colours. The evaluation of the hue (h*) and saturation (c*) are more appropriate, because in the process of degradation of anthocyanins, for example, two basic changes related to their colouration can occur: the colour can become gradually less intense, by the loss of saturation, and/or there can be a change of tonality, by the formation of degradation compounds, resulting in different colours from the original [25].

There was a significant interaction ($p < 0.05$) between the studied factors for the coordinates L*, C* and h*. Linear and quadratic models were tested, however, the lack of adjustment was significant ($p < 0.05$), and it was not possible to fit a model that explained the variation. Table 3 presents the L*, C* and h* coordinate data for the three storage conditions at 0 and 75 days, since time and temperature factors were not significant ($p > 0.05$).

Table 3. Colourimetric parameters L*, C* and h* of the microcapsules during the stability study, under three storage conditions: light, dark and 40°C

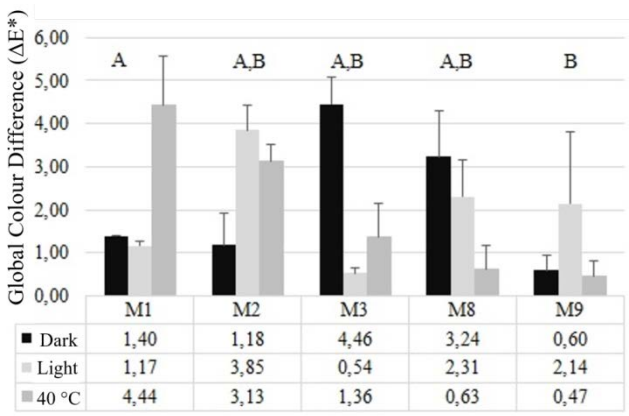
Amostras	Dias	L*	C*	h*
M1- Escuro	0	34,17±0,81	30,50±0,78	21,33±0,21
	75	34,24±0,19	31,32±0,36	23,11±0,44
M1 - Luz	75	34,03±0,20	30,75±0,27	22,60±0,01
M1 - 40ºC	75	32,45±0,16	26,20±1,01	20,44±0,92
M2 - Escuro	0	35,13±1,53	18,47±1,40	13,74±0,79
	75	33,66±0,33	17,41±0,38	10,80±0,34
M2 - Luz	75	34,19±0,09	17,91±0,07	11,84±0,02
M2 - 40ºC	75	36,53±0,21	27,85±0,61	19,49±0,16
M3 - Escuro	0	35,51±0,28	34,73±0,93	23,75±0,00
	75	39,52±0,30	33,07±0,60	23,47±0,24
M3 - Luz	75	35,62±0,07	34,58±0,52	24,27±0,22
M3 - 40ºC	75	34,58±0,21	32,01±0,37	22,86±0,21
M8 - Escuro	0	35,02±0,19	34,99±1,69	24,97±2,10
	75	36,76±0,50	37,48±0,28	25,72±0,40
M8 - Luz	75	37,42±0,40	38,86±0,50	26,41±0,25
M8 - 40ºC	75	36,83±0,09	37,47±0,23	25,62±0,15
M9 - Escuro	0	39,80±0,84	40,37±1,03	24,94±0,24
	75	39,71±0,36	40,09±0,38	24,85±0,00
M9 - Luz	75	41,35±1,17	41,07±0,62	24,28±0,37
M9 - 40ºC	75	41,22±0,48	40,85±0,18	23,96±0,30

For the coordinates L*, C* and h*, a very small degradation of their respective values was verified (Table 3). Even after 75 days of storage and under different external conditions, colour was maintained,

indicating that the microencapsulation was efficient in protecting the anthocyanins from degradation under different storage conditions throughout the storage period.

When evaluating the stability of microencapsulated saffron petals with maltodextrin and gum arabic for 10 weeks at 35°C, Khazaei et al. [22] observed a significant difference ($p < 0.05$) between the different combinations of wall material in terms of the colourimetric coordinates (L*, a*, b*, W*). There were differences over the storage period for all coordinates except h*; these differences, reflected in (ΔE*), were between 3.21 and 17.19, with these variations detectable to the human eye.

Figure 3 shows the overall colour difference data () of the blends for the three storage conditions. There was only a significant difference ($p < 0.05$) between the values for samples M1 and M9; among the others, there was no significant difference ($p > 0.05$). Values ranged from 0.47 to 4.46. These values are considered small and are not detectable to the human eye. According to Obón et al. [26], ΔE* values between 0 and 1.5 indicate colour differences that are indistinguishable by the human eye, and ΔE* values between 1.5 and 5.0 may be distinguishable, whereas for values above 5.0, the colour difference becomes evident. The ΔE* values of the present study were lower than 5.0 and were therefore not detectable to the human eye.



	M1	M2	M3	M8	M9
Dark	1,40	1,18	4,46	3,24	0,60
Light	1,17	3,85	0,54	2,31	2,14
40 °C	4,44	3,13	1,36	0,63	0,47

Figure 3. Global colour differences of M1, M2, M3, M8 and M9 between 0 and 75 days of storage. Means followed by the same letter in a row do not differ at the 5.0% level of significance, according to a Tukey test

The ΔE* values found by Lacerda et al. [24] for jussara pulp microparticles were less than 2.7, indicating that the instrumental colour differences of the microparticles after 38 days of storage at 50°C would be difficult to distinguish by consumers, and that the microencapsulation process was efficient. SILVA et al. [27] evaluated the stability of maltodextrin, gum arabic and jabuticaba bark anthocyanins, and their combinations, during 21 days of storage, observing ΔE* values between 3.61 and 18.96; the samples that used gum arabic separately or in a mixture with maltodextrin presented detectable values above 5.0.

Figure 4 shows the micrographies of some of the evaluated samples during the stability study (M2 and M3). No major evolution of the particle morphology as a function of the different storage conditions was demonstrated. However, for sample M2 (WPC), there was a greater agglomeration of powders stored at 40 °C, which presented a greater association between their particles when compared to the treatment exposed to light and dark, with more individualized particles. For M3, there was no evident change in relation to the storage conditions. The characteristics of the microparticles were spherical, mostly with depressions on the surface, a remarkable feature of spray dried microencapsulated particles.

Lacerda et al. [24] also observed the formation of agglomerates of jussara pulp microparticles encapsulated with inulin, starch and maltodextrin shortly after processing, especially in those containing inulin. Arkhavan Mahdavi et al. [28] also verified the agglomeration of anthocyanin microparticles encapsulated with maltodextrin, gum arabic and gelatin shortly after atomization. The microcapsules obtained from the mixture of gum arabic and maltodextrin were smooth and non-uniform, showing minimal agglomeration, whereas microcapsules of gelatin plus maltodextrin and maltodextrin separately were rougher and formed agglomerates, showing the influence of the wall materials on the morphology of the microparticles.

Figure 5 shows the particle size distribution for the rehydrated powders evaluated in the stability study under different storage conditions. The mixtures M2, M8 and M9 presented a very similar and complex rehydration profile. The three mixtures had the common characteristic of the presence of WPC as a wall material; this constituent directly influences the solubility of these mixtures. Whey proteins are in a dispersed form in solution, due to their hydrophobic bonds. The storage conditions also affected the rehydration profile of M2, to different degrees depending on the storage conditions; in increasing order of rehydration ease, these were: dark > light > 40°C. A 40°C temperature leads to the formation of agglomerates of the microparticles, making rehydration of the powders difficult.

A curious result occurred in the M1 sample, in which the exposure to light negatively affected the rehydration process, which presented major particle populations around 100 μm (well above the other treatments). Compared to the dark-stored sample, the sample stored at 40°C presented slightly larger particles, demonstrating slightly less effective rehydration. The M3 sample presented an excellent rehydration profile under all storage conditions. Two populations (both less than 1 μm) were formed in the sample stored at 40°C. Although there was a slight displacement of the major particle populations as a function of the different storage conditions, the behaviour of the samples was quite similar. For samples M8 and M9, a very similar rehydration profile was observed. There was little difference in the behaviour during rehydration as a function of the different storage conditions in both samples.

Tonon, Brabet and Hubinger [29] found mean values from 0.1 to 41.0 μm for açaí juice particles microencapsulated with maltodextrin and gum arabic. These particles had a bimodal distribution; that is, a distribution with two distinct peaks, each representing a predominant size. This distribution is considered desirable since the "population" of smaller particles can penetrate the spaces between the larger ones, thus occupying less space. These results are in agreement with those presented by samples M1 and M3, which presented particles less

than 1.0 μm for M3 and less than 10.0 μm for M1 (under the dark and 40 °C storage conditions). These authors also observed that the particles encapsulated with maltodextrin 20DE were smaller than those employing maltodextrin 10DE. The size of the particles is related to the size of the molecule of each encapsulating agent, such that the higher the ED value of maltodextrin, the greater the degree of hydrolysis and, therefore, the lower its chains.

According to data from Carvalho et al. [30], the mean particle diameter of jussara extract microencapsulated with maltodextrin 10DE, 20DE and 30DE, and gum arabic ranged from 7.0 to 11.5 μm, with a bimodal distribution of the peaks; these results are similar to the present work. The presence of larger particles (approximately 100 μm) with the formation of a third peak was associated with the presence of agglomerates, as occurred in the microparticles containing maltodextrin 20DE and 30DE and in the mixture of maltodextrin 10DE and gum arabic (25:75). The formation of particle agglomerates may be associated with their higher hygroscopicity.

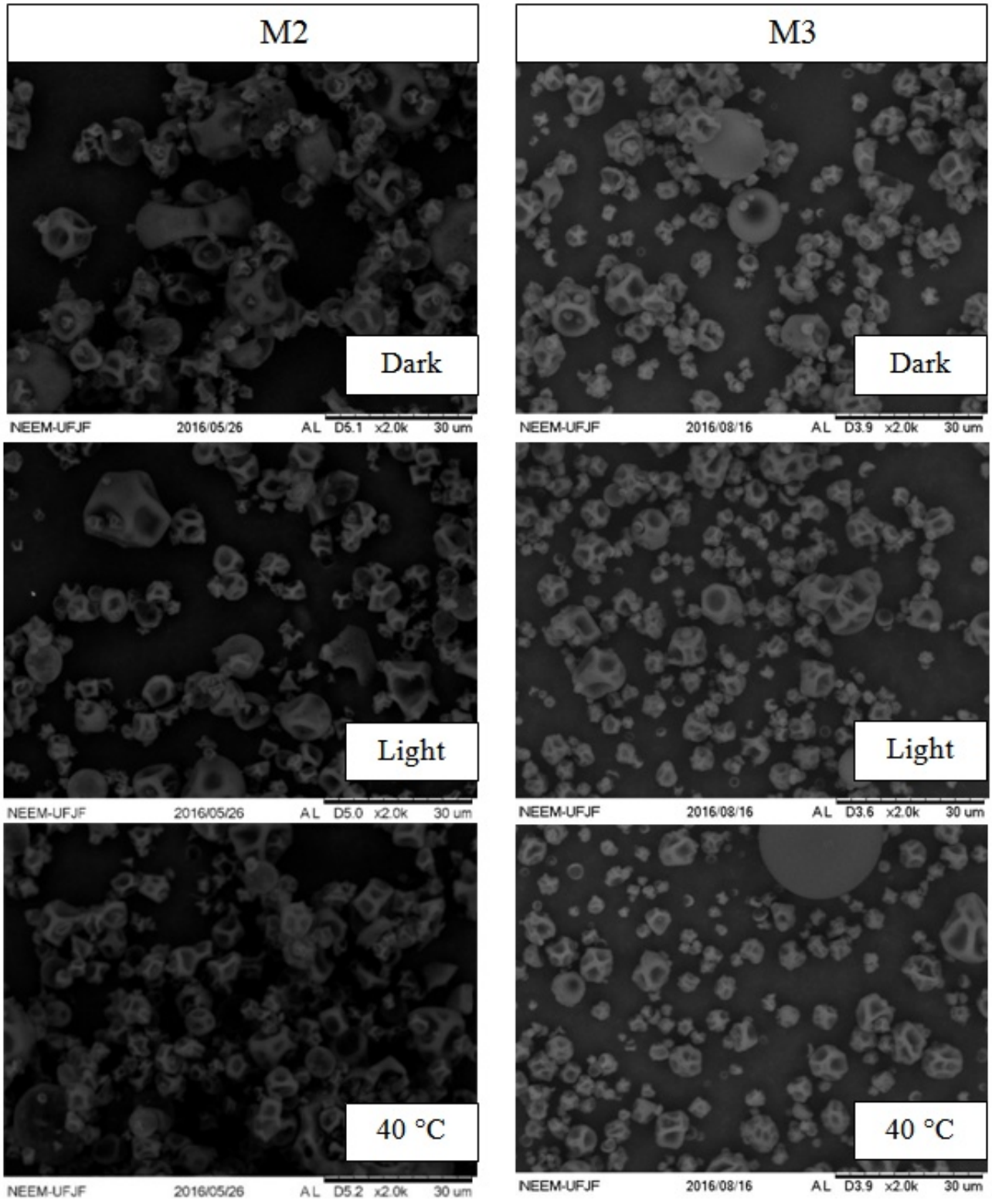


Figure 4. Micrographs of the M2 and M3 mixtures after 75 days of storage under three storage conditions: light, dark and 40°C

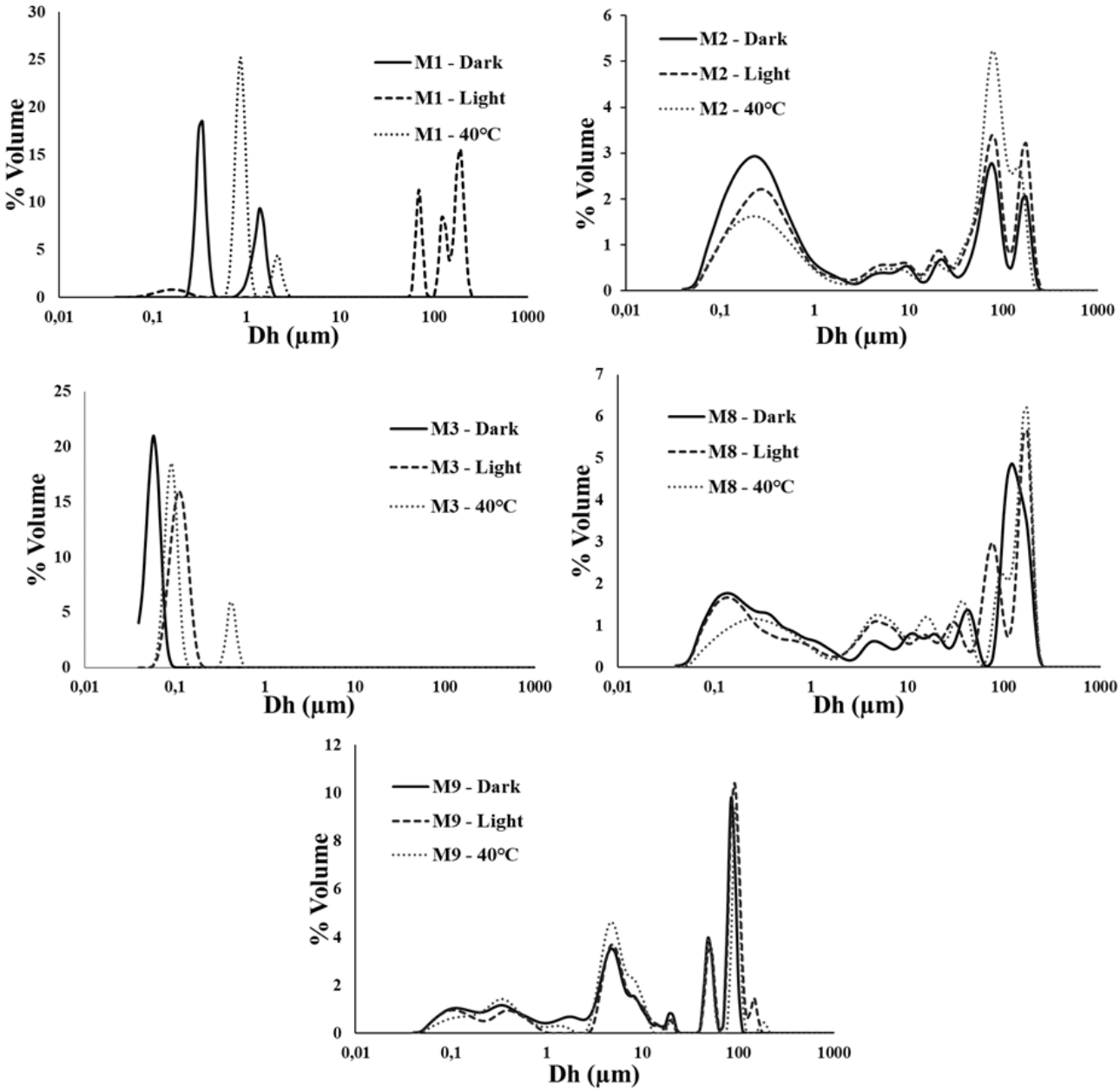


Figure 5. Particle size and solubility of M1, M2, M3 and M8 after 75 days of storage under three storage conditions: light, dark and 40°C

Table 4. Estimation of the adsorption isotherm parameters of powder samples (microparticles) for M1, M2, M3, M8 and M9

Model	Parameters and constants	Microparticles				
		M1	M2	M3	M8	M9
BET	X_m	1,254	1,118	1,713	1,423	1,317
	C_{BET}	8,886	27,101	6,136	8,394	4,698
	N	32,950	34,141	33,723	32,560	32,724
	R^2	0,9988	0,9970	0,9991	0,9982	0,9916
	$\lvert \varepsilon_m \rvert$	0,018	0,056	0,043	0,064	0,045
OSWIN	C	2,849	2,720	3,745	3,240	2,808
	D	0,518	0,507	0,534	0,515	0,534
	R^2	0,9977	0,9992	0,9976	0,9978	0,9959
	$\lvert \varepsilon_m \rvert$	0,143	0,015	0,337	0,095	0,346

The parameters and estimated constants for the models tested in the adjustment of adsorption isotherms are presented in Table 4.

The two models were fitted to the experimental data, with R^2 values close to 1 and $\lvert \varepsilon_m \rvert$ less than 10%. The R^2 values were very similar between the BET models and the

Oswin model. The BET model is one of the most used models in the literature for the construction of sorption isotherms of powdered food products, since it is related to the water sorption process. This model provides the moisture value in the monolayer (X_m), which indicates the amount of water that is strongly adsorbed to the specific sites on the food surface. Knowledge of this parameter is particularly important as it provides a first estimate of the water content and the maximum stability of a dehydrated product [31]. Taking into account the theoretical basis of the BET model, and the R^2 and $|\varepsilon_m|$ values, this model was used to represent the adsorption isotherms of the microcapsules (Figure 6).

The X_m values obtained for M1, M2, M3, M8 and M9 were 1.25, 1.11, 1.71, 1.42 and 1.31%, respectively. As such, the moisture content was considered to be safe to guarantee the stability of these powders during storage. Jiménez-Aguilar et al., [1] found a X_m value equal to 18.9% for blueberry extract powders with mosque gum and TONON et al. [32] found values between 3.1 and 5.8% for acai powder obtained by spray drying, depending on the carrier agent that was used (maltodextrin, gum arabic and tapioca starch). The blends showed very similar monolayer values (X_m), demonstrating that the stability of the mixtures under different relative humidity conditions is very similar.

Adsorption isotherms are a useful tool for understanding the stability issues inherent to food storage and post, to the microcapsules, the isotherms showed a sigmoidal shape, which is characteristic of most powdered food [33]. As can be seen in Figure 6, among the microcapsules, M2 showed a lower adsorption of water, while M8 and M3 were the most hygroscopic. These differences in water adsorption may be explained by differences in the structure and amount of each carrier agent used in the microcapsule. The smaller the particle size, the larger the surface exposed and, consequently, the greater the adsorption of water from the environment [32].

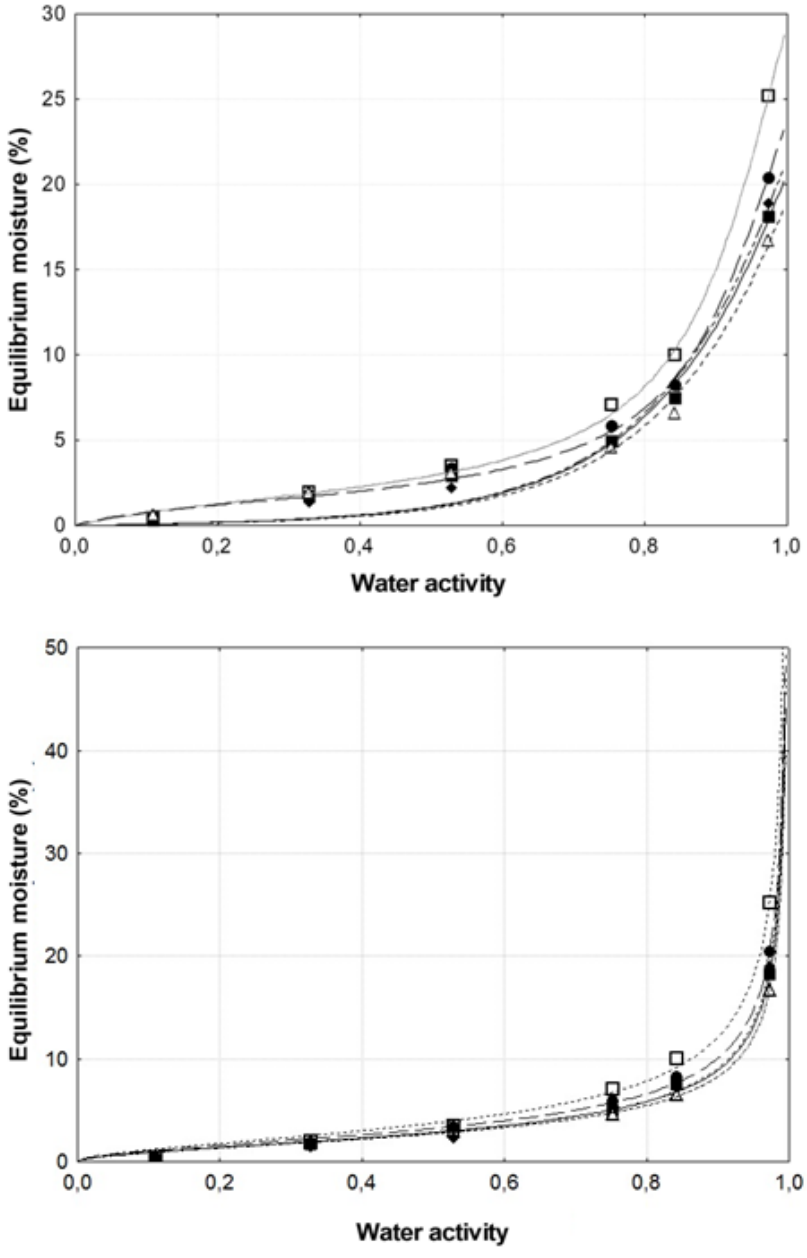


Figure 6. Adsorption isotherms of powder samples M1 (- ■-), M2 (.. Δ..), M3 (- □-). M8 (-- ●--) and M9 (-- ◆--), adjusted by the BET(**A**) and Oswin (**B**) models

Through the adsorption isotherms, it can be observed that the higher the water activity of the hygroscopic behaviour of the samples, the higher the relative humidity of the environment, the more hygroscopic the microcapsule and, consequently, the more susceptible it is to undesirable reactions, thus requiring greater care in handling and storage. This same trend was observed by Tonon et al. [32] in açaí powder juice, by Jiménez-Aguilar et al. [1] in blueberry anthocyanin extract powder and by Mosquera et al. [34] in strawberry powder.

4. Conclusion

The microencapsulation technique was successfully applied to maintain the stability of the anthocyanins from the mixture of jabuticaba, jussara and blueberry phenolic extracts, under different storage conditions. The light, dark and 40°C temperature storage conditions, and a storage time of 75 days, did not significantly influence the antioxidant capacity values and the colourimetric coordinates of the mixtures ($p > 0.05$). The overall colour difference values (ΔE^*) were lower than 5.0, indicating that there was no human detectable degradation of the colours of the samples over the storage period under different storage conditions. The morphology of sample M2 was influenced by the temperature (40°C), which led to the formation of agglomerates. The mixtures M1 and M3 showed good rehydration after 75 days of storage and a smaller diameter of the microparticles, while the mixtures M2, M8 and M9 presented low rehydration and a larger microparticle diameter due to the wall material (WPC).

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Conflicts of Interest

The authors declare no conflict of interest.

Practical Application

This work deals with the study of the stability of microencapsulated anthocyanins with different wall materials under different storage conditions. This is a relevant study, as anthocyanins are molecules widely used in industrial products, but they present great chemical instability when exposed to light and temperatures above 25 °C for a long time. The results show that the treatments used showed excellent stability for these molecules and can be an alternative to industrial use.

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