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Ascorbic acid and anthocyanins rich powders based on the encapsulation of the Amazon *Myrciaria dubia* fruit: effect of drying method and whey-based carriers

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Abstract

The goal of this work was to evaluate the influence of the drying method and the type of carriers on the stability of camu-camu (Myrciaria dubia) bioactive compounds. Powders were produced by spray- and freeze-drying methods using three types of whey protein: isolate (WPI), hydrolysate (WPH) and concentrate (WPC). The freeze-

dried powders presented irregular structures while the spray-dried particles showed spherical-like morphology. Powders produced by spray-drying employing WPI as carrier presented better retention of bioactive compounds (~60 g ascorbic acid per 100 g^{-1} of powder and ~75 g anthocyanins content per 100 g^{-1} of powder). Particles produced by freeze-drying method presented better stability of anthocyanins and ascorbic acid. The findings detailed here propose the use of the freeze-drying methodology to encapsulate the M. dubia fruit powder, allowing the preservation of its bioactive compounds, besides antioxidant properties and ascorbic acid retention.

Key words: camu-camu, Amazon fruit, food encapsulation, whey protein, freeze-drying, spray-drying, ascorbic acid.

1. INTRODUCTION

Tropical fruits have been attracting the consumer attention and the food industry due to their bioactive compounds, aromas and natural pigments. Camu-camu (*Myrciaria dubia*) is a native fruit from Amazon rainforest and widely recognized for its high ascorbic acid content (~3,000 mg.100 g^{-1}). Moreover, recent studies have shown that *M. dubia* presents great potential in food industry due to its phenolic compounds (gallic and ellagic acid) and anthocyanins (cyanidin-3-*O*-glicoside) [1–3].

The current consumer market has been requiring for products with high concentration of compounds related to health benefits. In general, foods are very sensitive to storage conditions. In order to increase the shelf-life time of bioactive compounds, several processes have been developed to minimize chemical degradation and microbial growth. The microencapsulation process has been widely applied as an efficient tool to protect bioactive substances within a polymer matrix [5]. Among the main encapsulation processes, spray- and freeze-drying are the most used in the food science area [6,7].

Proteins have been extensively used as carriers in encapsulation processes due to its functional properties such as gel formation, emulsification, solubility and film formation. In addition, they can contribute with natural benefits of amino acid rich foods [8]. Moreover, milk proteins can form complexes interacting with small molecules of bioactive compounds, allowing the protection against degradation [9]. Whey protein isolate (WPI) and whey protein concentrate (WPC) have been widely used in food drying process, and promising results related to the stability of bioactive compounds have been reported [10,11]. On the other hand, whey protein hydrolysate is most frequently used for human nutrition, especially in sports. Few studies have been reporting its use as carriers in powdered foods [12].
In this context, the present work aims at to evaluate the effect of drying process (freeze-and spray-drying) and carriers (WPI, WPC and WPH) on the protection and stability of the encapsulated *M. dubia* bioactive compounds. Furthermore, Moisture Content (MC), Activity Water (AW), Encapsulation Efficiency (EE) and the stability of Total Monomeric Anthocyanins (TMA) and ascorbic acid were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Ripe camu-camu (*M. dubia*) (SISGEN authorization AEEF518) were obtained in Rorainópolis, Brazil (0°23'27.3"S/61°48'22.5"W). The fruits were sanitized, pitted and crushed (pulp and peel) using an industrial metal crusher. The extract was filtered and maintained at -18 °C until the powder production. Whey protein isolate (WPI, Hillmar 9020 – 93.5 g protein.100 g^{-1} of powder and 1.5 g lactose.100 g^{-1} of powder), whey protein hydrolysate (WPH, Hillmar 8390 – 78 g protein.100 g^{-1} of powder and 3 g lactose.100 g^{-1} of powder) and whey protein concentrate (WPC, Hilmar 7000 – 83.4g protein.100 g^{-1} of powder and 5.5g lactose.100 g^{-1} of powder) were donated by Hillmar Ingredients (USA).

2.2. Solution preparation

M. dubia juice and carriers (80:20 w/w) were homogenized using magnetic stirrers for 1 h and stored at 4 °C for 12 h allowing the biopolymer hydration. Then, the solutions were homogenized in a mechanical stirrer (T25 digital UltraTurrax, IKA, Germany) using 10,000 rpm for 5 min at 25 °C and submitted to the drying process.

2.3. Freeze- (FD) and Spray-drying (SD) processes

After homogenization, the solutions were rapidly frozen (ultrafreezer at -70 °C) and subjected to freeze-drying process (Lobov, USA). Samples were dried for 7 days until reach constant weight. After drying, powders were subjected to manual milling until granulometry smaller than 100 mesh. Powders were stored at 4 °C until further analysis.

Solutions were also dried using a spray-dryer equipment (model MSD 1.0, Labmaq, Brazil) with a two-fluid nozzle atomizer. The operational conditions were described in previous report [13]: inlet temperature of 170 °C, outlet temperature of 96 °C, air flow of 1.5 $m^3.min^{-1}$ and feed rate of 8 $mL.min^{-1}$. Powders were stored at 4 °C until further analysis.

2.4. Scanning Electron Microscopy (SEM)

The powders morphology was evaluated using an NA3, TECSCAN, Czech Republic, at 25 °C and 20 keV. Powders were placed on aluminium stubs with carbon tape and coated with a gold thin layer prior to analysis.

2.5. Moisture content (MC) and activity water (AW)

MC was gravimetrically determined using an infrared thermobalance (MOC-120H, Shimadzu, Japan) at 105 °C. AW values were assessed by a Water Activity Meter device (Labtouch, Tecnal, Brazil) at 25°C.

2.6. Particle size and size distribution

Particle diameter and size distribution of powders were determined by light scattering using laser diffraction (Mastersizer 2000 Malvern

Instruments Ltd., Malvern, UK) [14]. Samples were analysed on a wet basis, with dispersion in 99.5% ethanol. Polydisperse index was calculated according Eq. 01.

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \qquad \text{Eq. 01}$$

where d_{90}, d_{50} and d_{10} represent the volume diameters at 90%, 50% and 10% of the cumulative volume, respectively.

2.7. Bioactive compounds quantification

Anthocyanins and ascorbic acid contents were quantified in the *M. dubia* pure extract and encapsulates. The values found in the extract before the encapsulation process were used as control for bioactive compounds retention [11].

2.8. Retention of bioactive compounds after drying process

To evaluate the efficiency of the different drying treatments, the anthocyanins and ascorbic acid contents presented in the *M. dubia* extract were used as parameters before and immediately after the drying process. The bioactive compounds retention (*R,* g bioactive compound.100 g^{-1} of powder) was calculated using the Eq. 02:

$$R = 100 \times \frac{BC_{encap}}{BC_{extract}} \qquad \text{Eq. 02}$$

where, BC_{encap} is the bioactive compound of *M. dubia* encapsulated extracted and $BC_{extract}$ is bioactive compound extracted before encapsulation.

2.9. Total monomeric anthocyanins (TMA)

TMA content of the *M. dubia* extract and powders were determined by the spectrophotometric method and expressed as cyanidin-3-*O*-glucoside, which was identified as the major anthocyanin present in *M. dubia* [15]. The molar absorptivity (E_{molar}) of cyanidin-3-O-glucoside was 34,300 $L.mol^{-1}.cm^{-1}$ at 535 nm in HCl/water/ethanol solution (1/29/70) at 25 °C [16].

2.10. Ascorbic acid (AA) content

The AA content was quantified by high performance liquid chromatography (HPLC). A portion of 15 mL of the extractive solution (3 mL metaphosphoric acid.100mL^{-1} of solution, 8 mL acetic acid.100 mL^{-1} of solution, 0.3 N sulfuric acid and 1 mM EDTA) and 5 mL of ultrapure water (Milli-Q, Millipore, Germany) were mixed with the *M. dubia* powder samples. The solution was homogenized and centrifuged for 30 min (5,000 rpm/4 °C). The supernatant was filtered through a filter with a porosity of 0.45 µm. The filtered solution was injected into an HPLC (Shimadzu, Japan). The chromatograms were obtained using a diode array detector (DAD) with wavelength of 245 nm, C18 column and mobile phase (NaH_2PO_4/EDTA) at pH 3 with a flow rate of 1 mL.min^{-1} and run time of 6 min [17]. The identification of AA was performed by comparing the retention times obtained for the standard and for the samples analysed under the same conditions. The DAA (dehydroascorbic acid) was reduced to AA with 40 mM dithiothreitol (DTT), thus determining the total AA content. The DAA quantification was performed by difference between the total AA content (after conversion from DAA to AA) and content (before the DAA conversion). Good linearity was found with high coefficient of linearity ($R^2 = 0.9972$).

2.11. Color

For the coloration analysis, the L^* (brightness), a^* (greenish to reddish), and b^* (blueish to yellowish) parameters were measured using a colorimeter (UltraScan VIS®, Hunter Lab, USA).

2.12. Accelerated storage stability

Stability of bioactive compounds was determined through the accelerated storage methodology at 60 °C/84% relative humidity [11]. Powder samples (~ 3 g) were stored in a 7 x 7 cm polypropylene plastic bags. Samples were evaluated regarding antioxidant compounds (DPPH test), phenolic compounds, TMA content and AA under intervals of 24 h for 7 days.

2.13. Half-life time

The half-life of the encapsulates was evaluated considering that both bioactive compounds (anthocyanins and ascorbic acid) degrade following the first-order kinetics.

2.14. Statistical analysis

The R software was used for analysis of variance (ANOVA) to evaluate the effects of different carriers and drying processes on the properties of the *M. dubia* powders. Differences between the mean values obtained for each treatment were evaluated at 5 % significance level ($p \leq 0.05$) using the Tukey´s test.

3. RESULTS AND DISCUSSION

3.1. SEM Analysis

Microstructural analysis of encapsulated foods represents an important tool to evaluate the morphology and surfaces after the drying process. **Figure 1** shows the SEM images of the *M. dubia* powders.

Powder morphology was consistent with the different drying methods: for spray-dryer treatments, powders presented spherical morphology with a smooth surface without pores or cracks. In contrast, the powders obtained by the freeze-drying process showed irregular lamellar morphology with surface irregularities such as dents/concavities typical of this drying process. Morphology is a result of the breaking down of the spongy structure formed after the removal of ice crystals through sublimation [20].

Considering the freeze-drying treatments, WPC presented more irregular surface with several fractures. This observation can be explained by the lower concentration of proteins, avoiding the formation of a rigid and regular surface. In the study on different drying methods for *Lactobacillus rhamnosus* encapsulation [21], the authors also observed very irregular structures for WPC treatments. On the other hand, no differences in the surface and morphology of the powder were observed for the spray-drying treatments, even for

different carriers. Furthermore, no difference in the particle structure was observed in encapsulates of linoleic acid and WPI or WPC produced by spray-drying [22].

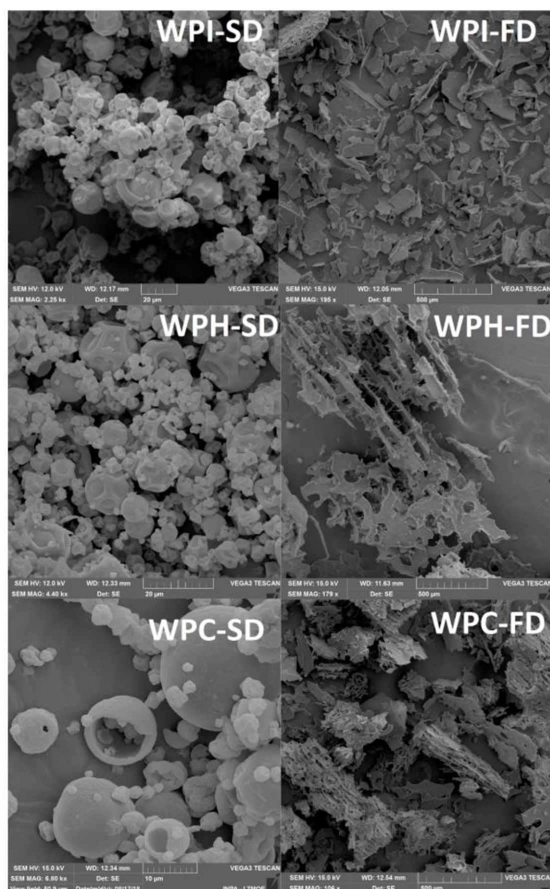

Figure 1. SEM images of camu-camu powders. WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying.

3.2. Moisture content (MC) and activity water (AW)

Foods with high MC and AW values are more likely to microbial growth and degradation of bioactive compounds. **Table 1** shows the MC values for the *M. dubia* powder. In general, the values ranged

from 9.3 to 14.9 g water. 100 g^{-1} dry basis. The different drying methods were significant (p-value < 0.05), and the spray-drying treatments presented lower MC. Similar behaviour was also observed in studies on pulp and residue encapsulation of *M. emarginata* [6], *M. dubia* [23] and blackberry [7]. The different carriers did not present a significant difference (p-value > 0.05), which can be explained by the similar materials composition (all milk-based proteins). The presence of lactose in all milk-derived proteins may be an explanation for the relative humidity values, since these materials are more hygroscopic.

Table 1. Moisture Content (MC), Activity Water (AW), particle size and span of camu-camu powders.

Parameter	WPI-SD	WPH-SD	WPC-SD	WPI-FD	WPH-FD	WPC-FD
Moisture Content (g water.100g^{-1} dry basis)	9.3 ± 0.2^{b}	9.4 ± 0.2^{b}	10.7 ± 0.1^{b}	11.7 ± 0.2^{a}	11.9 ± 0.1^{a}	12.2 ± 0.1^{a}
AW	0.32 ± 0.01^{b}	0.29 ± 0.01^{b}	0.28 ± 0.02^{b}	0.37 ± 0.01^{a}	0.37 ± 0.01^{a}	0.36 ± 0.01^{a}
Particle size (μm)	6.4 ± 0.9^{c}	5 ± 1^{d}	7.6 ± 0.5^{c}	175 ± 22^{a}	125 ± 11^{b}	190 ± 12^{a}
Span	1.5 ± 0.1^{c}	1.8 ± 0.1^{c}	1.9 ± 0.1^{c}	2.4 ± 0.1^{b}	2.6 ± 0.1^{a}	2.9 ± 0.1^{a}

Data are expressed as mean ± DP (n = 3). $^{a\text{-}d}$ Different superscript letters in the same line indicate significant differences (p-value < 0.05) between the microparticles. WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying.

To ensure less degradation and microbial growth, the AW values for food powders should be less than 0.3 [6]. Also, following the MC values, the different carriers were not significant (p-value > 0.05) for the WA values, but the different treatments caused a significant modification (p-value < 0.05), resulting in powders of higher AW values for the freeze-drying treatments. High AW values may favour the degradation of bioactive compounds, evidencing the greater degradation for the freeze-dried *M. dubia* powders. High AW values were also observed for sumac extract powder produced by freeze-drying process when compared to the spray-drying one [24].

3.3. Particle size evaluation

The different treatments showed significant difference (p-value < 0.05) with higher values of average diameter for the samples obtained by freeze-drying process. According to results, powders presented micrometric diameters [14]. WPC presented higher particle size,

which may be related to the high sugar content (lactose) with low emulsifying and stabilizing activity, resulting in larger particles during the homogenization process. The smaller particle size obtained using the WPH treatments may be associated with the lower molecular chains of the hydrolysed proteins, allowing the formation of small droplets sizes. The uniformity of the microparticle size distribution was evaluated by the PDI values (**Table 1**). For all treatments, lower PDI values corresponded to narrower distributions.

3.4. Bioactive compounds retention

The retention of bioactive compounds is the most important parameter in encapsulation processes because it defines the process efficiency. **Figure 2** shows the bioactive compounds retention of the produced powders.

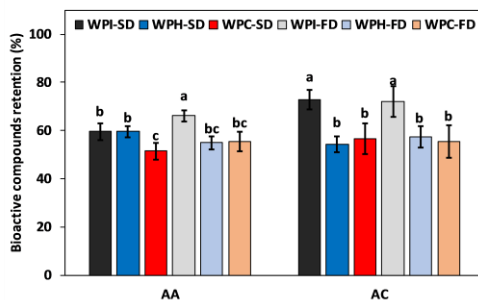

Figure 2. Bioactive compounds retention of powders: ascorbic acid (AA), anthocyanins content (AC). WPI: whey protein isolate; WPH: whey protein hydrolysate. [a,b,c] Same letter in same bioactive compounds group not are significant (p-value > 0.05) (n=3).

Retention values ranged from 38.45 to 73.19 g.100 g^{-1} of powder (ascorbic acid) and 27.32 to 72.88 g.100 g^{-1} of powder (TMA). In general, the spray-drying process and the WPI treatments presented better bioactive compounds retention. Lower anthocyanins retention in may be related to high compounds degradation during the drying process, since the cationic chemical structure of anthocyanins contribute to their thermal sensitivity [25]. For AA, both carriers and drying methods were significant. WPI and WPH presented higher ascorbic acid retention. The different methods only showed significant

difference for WPI treatments. In studies on *M. dubia* microencapsulation [23], the authors observed that the powders produced by freeze-drying presented higher retention of AA than those observed using spray-drying. High temperatures during the spray-drying process can degrade thermolabile compounds. Furthermore, high ascorbic acid retention was also observed for freeze-dried *M. dubia* [1].

3.5. Total Monomeric Anthocyanins (TAM) content

TMA content from *M. dubia* extract was found around 133.45 ± 9.75 mg.100 g^{-1} dry basis. Similar result has been reported [26]. The TMA values based on the cyanidin-3-glucoside concentration during the accelerated stability process is shown in **Figure 3**. The WPI treatments showed high anthocyanin retention, followed by WPH and WPC, independent of the drying process. Carbohydrates present higher water adsorption capacity when compared to proteins. This fact may explain the greater degradation of the bioactive compounds encapsulated in WPC due to the higher concentration of lactose in this carrier [22]. During the accelerated stability test, anthocyanins encapsulated in WPI were rapidly degraded in 24 h, then reducing the degradation rate. This can be explained by the high concentration of anthocyanins in the powder, increasing the possibility of degradation. For WPC and WPH samples, no significant variation was observed in the TMA values.

Considering the different drying methods, the spray-drying treatments resulted in lower anthocyanin retention values than those observed for the freeze-dried samples evaluated immediately after the drying process. This behaviour is explained by the high temperatures used during the spray-drying process. Cyanidin-3-*O*-glucose (major anthocyanins in *M. dubia*) presents good thermal resistance up to 70 °C [27]. Cyanidin-3-*O*-glicose showed higher rate of degradation compared to all other blueberry juice anthocyanins stored for 10 days [28]. The same behaviour was observed in studies on black carrot by freeze- and spray-drying processes [29]. During storage, the powders obtained by freeze-drying process presented slightly greater

protection than those obtained by spray-drying, corroborating with the reports on the anthocyanins stability of *Aristotelia chilensis* powders [30].

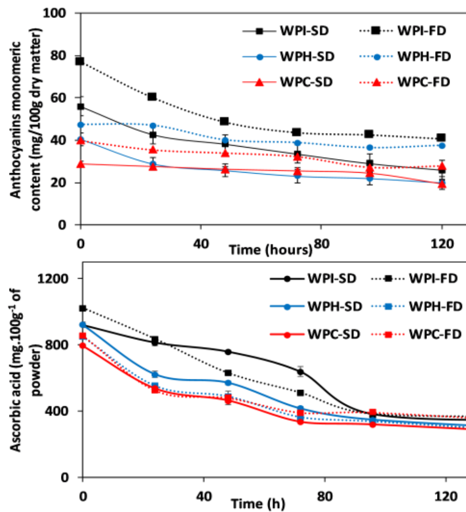

Figure 3. Ascorbic acid (AA) and Total Monomeric Anthocyanins (TMA) contents of powders storage at 60 °C. WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying. Line are only to facilitate the interpretation (n=3).

3.6. Ascorbic acid (AA) content

The AA content from the *M. dubia* extract was found around 1545.89 ± 12.22 mg.100 g^{-1} dry basis. The AA stability of encapsulated *M. dubia* extract (**Figure 3**) also followed the same behaviour as that of anthocyanins. For all treatments, the AA contents reduced rapidly. For low temperature storage (25 – 35 °C) of AA solutions [31], the authors observed rapid degradation. Also, this same heat-labile behaviour was observed in the encapsulation of AA by spray-chilling and storage at temperatures of 7 °C, 24 °C and 35 °C [32]. The particles obtained by freeze-drying and the different treatments using WPC presented greater degradation of AA in comparison to the other treatments. For encapsulated AA [33], the increasing storage

moisture promoted compound degradation. This fact confirms our results for *M. dubia* since the Amazon humidity is greater than 80 %.

3.7. Color

Foods with large amounts of bioactive compounds tend to lose their coloration during storage. **Figure 4** shows the color parameters of the powders during the accelerated stability test.

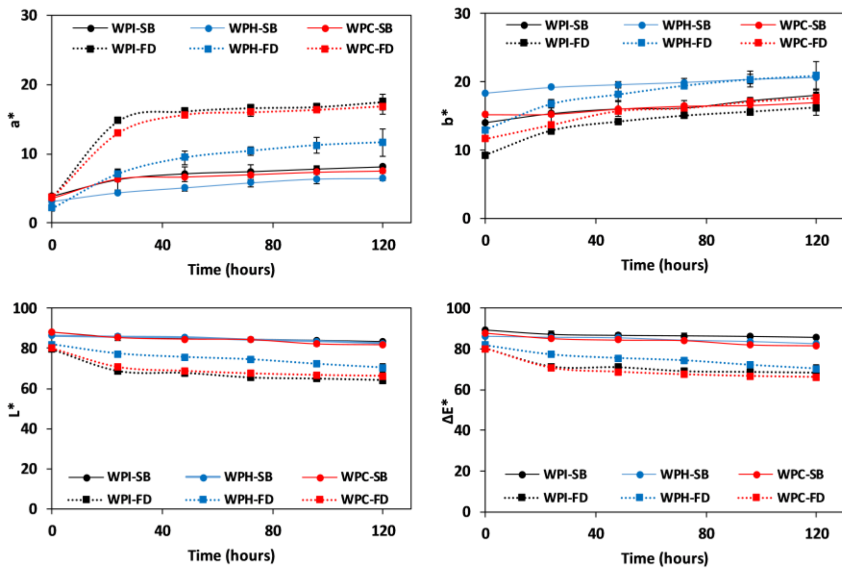

Figure 4. Color parameters (*L*, a*, b* and ΔE**) of powders storage at 60°C. WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying. Line are only to facilitate the interpretation (n=3)

In relation to the luminosity (*L**), all treatments presented reduced values, with darker particles *via* freeze-drying. Studies on the production of powder of acerola pulp [6] and *Vitis labrusca* [34] presented significant variation of *L** when different drying processes were compared, with greater luminosity for the samples obtained by spray-drying. The darker color of the freeze-dried particles may be related to light scattering in voids (large surface pores) formed after sublimation of water [34] and not to the concentration of anthocyanins, since it was lower for freeze-dried samples. For the

different materials, significant variation of L^* was not observed and can be explained by the similar composition of carriers.

The results obtained for a^* and b^* showed that samples were in the first quadrant ($+a^*$ and $+b^*$), indicating a tendency of coloration near yellow and red. Although the dominant color of the camu-camu extract is pink ($+a^*$ and $-b^*$) - mainly due to the composition of anthocyanins in the shell - the results obtained can be explained by the coloration of the carriers (yellow) and the Maillard reactions caused by the proteins and lactose from carriers [35], resulting in brown compounds. The drying processes showed little difference between them. For blueberry powders obtained by spray- and freeze-drying [36], the authors also observed increased yellow color for WPI treatments, confirming the Maillard reaction.

The color of powdered foods may be affected by the type and concentration of carrier, natural color of food and non-enzymatic reactions between proteins and sugars at high drying temperatures [37]. The particles produced by spray-drying presented lower colour variation (ΔE^*) when compared to samples produced by freeze-drying. This can be related to the values of the anthocyanin contents shown in **Figure 3** or the variation of the L^* and b^* parameters.

The spray-drying process produced more rigid and uniform polymer structures due to the denaturation of the proteins caused by the high temperature of the drying process. Thus, bioactive compounds are more protected from non-ideal storage conditions. Also, the light reflection effect resulting from the microstructural changes of the powder particles may contribute more to color changes than to the Maillard reaction [38]. Higher ΔE^* was observed for fish oil, phytosterol esters and limonene particles obtained by freeze-drying when compared to spray-drying [20].

Regarding the different carriers, the treatments using WPC presented greater variation of color in relation to the other processes, corroborating the values of L^*, a^* and b^*. Values of ΔE^* above 5 were visually more notable, which may be related to the consumer acceptance [39]. The appearance of the powders during storage (**Figure 5**) confirms the results of ΔE^*.

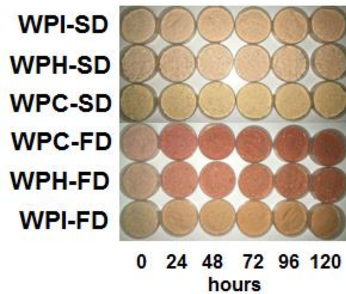

Figure 5. Appearance of powders during the accelerated stability test. WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying. Line are only to facilitate the interpretation

3.8. Degradation kinetic parameters

The stability of TMA and AA of *M. dubia* was investigated in this work since its application in the food industries is limited by its instability under specified conditions. Table 2 shows the degradation kinetic parameters of anthocyanins and AA of the encapsulated *M. dubia* extract. The half-life time of AA ranged from 101 to 443 days. The values of reaction rate constant (*k*) of the WPI samples were higher when compared to other treatments. These results may be in the opposite direction to the results of previous analyses, since the WPI treatments presented better results. This high degradation rate may be associated with the higher concentration of bioactive compounds encapsulated using WPI when compared to WPH and WPC. Other authors also observed that the half-life of berry extract bioactive compounds encapsulated using WPC was higher than WPI [40].

Considering the AA, the treatments using WPH presented higher degradation rate and, consequently, shorter half-life. The half-life time of AA ranged from 99 to 135 days. The higher the amount of water adsorbed, the greater the possibility of AA degradation. When different carriers were compared in the same AW, they presented different degradation rates, suggesting that the type of carrier modulates the AA degradation. The powders produced using WPH and WPI presented the highest degradation rate, which indicated the

effect of the carrier on the retention of these bioactive compounds. This may be caused by the increased adsorption of water to the hydroxyl groups of the proteins that are exposed during the hydrolysis process, producing lower molecular weight peptides [41].

Table 2. Degradation kinetic parameters of anthocyanins and ascorbic acid of the encapsulated *M. dubia* extract.

Bioactive Compound	Parameter	WPI-SD	WPH-SD	WPC-SD	WPI-FD	WPH-FD	WPC-FD
Anthocyanins	k ($10^{-3}.h^{-1}$)	5.4 ± 0.3^b	1.5 ± 0.4^e	2.4 ± 0.1^d	6.8 ± 0.2^a	3.7 ± 0.4^c	2.2 ± 0.3^d
	$t_{1/2}$ (h)	128 ± 2^e	443 ± 3^a	288 ± 5^c	101 ± 2^e	185 ± 3^d	306 ± 3^b
	R^2	0.97	0.87	0.89	0.84	0.82	0.93
Ascorbic Acid	k ($10^{-3}.h^{-1}$)	6.5 ± 0.1^b	6.9 ± 0.3^a	6.5 ± 0.2^b	6.4 ± 0.2^b	6.7 ± 0.0^b	5.1 ± 0.3^c
	$t_{1/2}$ (h)	105.0 ± 0.5^b	99.0 ± 0.3^b	107.0 ± 0.4^b	108.0 ± 0.2^b	103.0 ± 0.1^b	135.0 ± 0.2^a
	R^2	0.91	0.98	0.96	0.97	0.96	0.87

Data are expressed as mean ± DP (n = 3). $^{a-d}$Different superscript letters in the same line indicate significant differences (p-value < 0.05) between the microparticles. Reaction rate constant (k); half-life time ($t_{1/2}$); coefficient of determination (R^2); WPI: whey protein isolate; WPH: whey protein hydrolysate; WPC: whey protein concentrate; SD: spray-drying; FD: freeze-drying.

CONCLUSIONS

The powders produced by freeze-drying process presented higher MC and AW values. The bioactive compound retention values presented no significant variation for different carriers, since their compositions are very similar. For the different drying methods, powder produced by spray-drying presented better bioactive compound retention, while the freeze-dried powders presented better conservation of bioactive compounds during storage. The molecular structure of WPH proteins influenced water adsorption and increased the AA degradation rate. For future work, it is suggested that the powders can be applied in different food matrices aiming at adding nutritional value and diversifying the consumer market.

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