

Elderberry juice from Argentine Patagonia encapsulated by freeze-drying and spray-drying using different carriers: characterization and stability against heating and storage

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ABSTRACT

Elderberry is a rich source of bioactive compounds, particularly anthocyanins, making the development of stable ingredients containing these compounds of considerable interest. In this study, elderberry juice was micro-encapsulated using spray-drying and freeze-drying techniques with three carriers: maltodextrin (MD), whey protein concentrate (WPC), and soy protein isolate (SPI). The resultant powders were characterized for several physicochemical properties, including moisture content, water activity, hygroscopicity, water sorption isotherms, glass transition temperature (T_g), caking/visual aggregation, and colorimetric parameters. Although high anthocyanin retention was observed in all powder formulations, notable differences in physicochemical attributes were evident. The drying method significantly influenced water sorption isotherms and caking behavior under conditions of 38°C at 44% or 58% relative humidity (RH). Powders containing WPC and SPI demonstrated greater resistance to caking compared to MD-based powders; however, these differences could not be explained solely by T_g values, which were similar across all carriers at comparable moisture levels (48–53°C). After 9 days of storage at 38°C and 44% or 58% RH, powders formulated with WPC (spray-dried or freeze-dried) remained free-flowing, whereas spray-dried powders formulated with SPI exhibited limited agglomeration. In contrast, MD-based powders showed caking under all tested conditions. During thermal treatment at 85°C, freeze-dried powders exhibited a similar trend, with MD formulations displaying higher anthocyanin retention but lower resistance to caking, indicating that protein-based powders maintained superior physical functionality and anti-caking properties after thermal exposure or storage at elevated RH.

1. Introduction

Elderberry (*Sambucus nigra* L.) is a red fruit of great interest in Argentine Patagonia, and it stands out along with other berries as a source of bioactive compounds associated with the prevention of certain diseases. These compounds include anthocyanins, which are responsible for the characteristic purple-violet color of berries, making derived products or ingredients, such as dehydrated forms, highly potential in the food industry as natural colorants (Busso Casati et al., 2015). Current studies support the antioxidant, anti-inflammatory, anticancer, and other health-beneficial effects of anthocyanins present in a wide variety of berries. Their effects are related to the reduction of free radicals and a possible decrease in oxidative stress and cellular damage (Ahmed et al., 2023; Ashique et al., 2024; Osman et al., 2023; Redha et al., 2022; Sidor

& Gramza-Michałowska, 2015; Tena et al., 2020; Uhl & Mitchell, 2024).

In Argentina, elderberry is mainly consumed as juices, jams, candies, a mix of preserved fruits, ice cream pulses and variegates, dehydrated as part of fruit teas, or in dietary supplements. Products derived from these fruits have multiple applications in the food industry due to their attractive color and high content of bioactive compounds that enrich their nutritional profile. In the case of berries, one of the key factors to consider when preserving products derived from these fruits is that the anthocyanins present are susceptible to chemical and biochemical degradation when exposed to oxygen, light, pH variations, elevated temperatures, enzymes, solvents, and other reactive compounds such as ascorbic acid, due to the unstable structure of the core flavylium cation (Tan et al., 2021).

Dehydration techniques such as spray-drying and freeze-drying

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enable the production of stable products that largely preserve the color and bioactive compounds of fresh fruit without requiring special storage conditions, besides appropriate packaging. Different studies have reviewed the main dehydration techniques, operational conditions, and types of encapsulants used to achieve high efficiency in processing and stability of the final product (Baeza et al., 2021; Buljeta et al., 2022; Deng et al., 2023; Lu et al., 2021). These techniques yield ingredients that encapsulate bioactive compounds within a stable matrix, resistant to oxidation, caking, or collapse, with good conservation during shelf-life (Baeza et al., 2021; Buljeta et al., 2022; Lu et al., 2021). The dehydration method employed influences the type of encapsulant and the physicochemical characteristics of the resulting powder. Although spray drying is more commonly used in industry, freeze-drying is also applied to red fruits as it better preserves color and requires lower proportion of encapsulants (Baeza et al., 2021; Baeza & Chirife, 2021). Several studies have reported high anthocyanin retention in spray-dried systems, with values ranging from 70 to 100% for different fruit matrixes (Buljeta et al., 2022; Liu et al., 2025). Similarly, freeze-dried products also exhibit high retention of bioactive compounds, including anthocyanins and polyphenols, with reported values above 90% in various fruits (Romero-González et al., 2020; Buljeta et al., 2022; Coşkun et al., 2024). In addition to ensuring adequate retention of antioxidant compounds, it is essential to characterize the color of dehydrated systems and to assess their stability against the factors to which they are exposed during shelf life. Anthocyanins and other phenolic compounds are readily oxidized and are therefore susceptible to oxidative degradation during various stages of processing and storage (Patras et al., 2010). Color loss or browning in berry-derived products is often associated with a reduction in bioactive compounds, which negatively affects consumer perception of quality. Furthermore, color stability represents a limiting factor for the use of natural colorants derived from red fruits and can significantly influence their life. Several recent studies and reviews have addressed color stability in encapsulated products obtained from red fruits, employing different encapsulating agents and evaluating stability under conditions such as thermal treatment, pH variation, storage conditions (e.g., relative humidity), and oxygen exposure, among others (Albuquerque et al., 2021; Alifaki et al., 2022; Oancea, 2021; Harsito et al., 2021; Ren et al., 2021; Deng et al., 2023).

Encapsulant type and proportion are critical for producing stable powders without caking and for preserving anthocyanin content and color. Baeza et al. (2021) showed that even low encapsulant levels (0.5:1 encapsulant: elderberry solids) yielded satisfactory products, with anthocyanin retention between 71 and 88%, depending on the encapsulant used. The proportion of encapsulant to fruit solids reported in literature varies widely, with ratios between 2 to 40% encapsulant per 100 g of juice (with Brix levels of 8-14 depending on the fruit). While higher quantities of encapsulant improve drying efficiency and prevent caking, the anthocyanin content per gram of powder significantly decreases (Baeza et al., 2021).

Recent studies emphasize the role of different encapsulants in anthocyanin encapsulation to achieve high efficiency and powder stability. Commonly used encapsulants for both spray drying and freeze-drying include maltodextrins with various DE values, blends with other polysaccharides, starches, and proteins such as whey, soy, pea protein, and gelatin (Baeza & Chirife, 2021; Buljeta et al., 2022; Dahiya et al., 2023). In addition to enabling the stabilization of anthocyanins and preventing caking of the dehydrated matrix, the use of proteins as encapsulating agents can be of interest to enhance the nutritional value of the obtained ingredient. Different protein addition percentages have been reported, with encapsulate-to-solid ratio ranging from 0.1:1 to 1:1 depending on the fruit and the drying method (Baeza et al., 2021; Buljeta et al., 2022; Ravichandran et al., 2023).

Dehydrated ingredients obtained by spray-drying or freeze-drying can be used as sources of fruit, antioxidants, or natural colorants in products that require heating, such as baked goods, bars, and cereals. Therefore, understanding powder behavior under heating and bioactive

retention is essential, with several studies reporting good performance and anthocyanin stability after processing (Buljeta et al., 2022; Cordeiro et al., 2021). Based on all the above, the objective of this study was to investigate the drying and encapsulation of anthocyanins in elderberry pulp from Argentine Patagonia using different encapsulating agents and two dehydration methods: spray-drying and freeze-drying. Three different carriers were used: maltodextrin DE10 (MD), the most commonly used encapsulant, and its performance was compared with two proteins: whey protein concentrate (WPC) and soy protein isolate (SPI). Furthermore, to allow a comparative evaluation of the systems, the same encapsulating agent proportions were used for both drying methods, maintaining a 2:1 encapsulating agent-to-fruit solids ratio. The physicochemical properties of the systems and the retention of the predominant anthocyanins were characterized in the dehydrated samples, as well as during storage at 38°C under different relative humidity conditions (commonly used tests for accelerated shelf-life assessment), and under heating conditions representative of those to which powders may be subjected during the production of snacks or baked goods.

2. Materials and methods

2.1. Materials

Elderberry (*Sambucus nigra*) pulp was provided by an Argentinian producer from El Bolsón, Río Negro, and it was obtained from a same harvest fruits with similar mature degree. The fruit was initially blanched in hot water (80°C, 3 min) and then crushed and peeled in an industrial pulper, packed, pasteurized (85°C, 15 min) for enzyme inactivation and microbial growth inhibition and frozen at -18°C.

The encapsulants used were maltodextrin DE10 (MD) from Productos de Maíz S.A. (Buenos Aires, Argentina), 90 % soy protein isolated (SPI) Supro 500E (IFF) and 81% whey protein concentrate (WPC) from Just Whey, Star Nutrition (Buenos Aires, Argentina).

Ethanol and chlorohydric acid used as solvents for pulp and encapsulated systems extraction were from Biopack, Buenos Aires, Argentina.

Cyanidin-3-glucoside HPLC standard was purchased from Sigma-Aldrich, USA.

All chromatographic solvents were of HPLC grade and the purity of the reagents used was p.a. or similar.

2.2. Dehydration of elderberry juice by freeze-drying and spray-drying

Elderberry pulp (originally with 9 °Brix) was diluted with water in a ratio 1:0.7 (pulp:water), then was passed through a stainless steel mesh strainer (in order to remove fibers or peel fragments that could hinder passage through the spray nozzle), and mixed with the correspondent encapsulant in a 2:1 encapsulant:elderberry solids proportion. Adding water before sieving the pulp is necessary because the high consistency of the original pulp makes it difficult to pass through a sieve. The liquid systems before dehydration contained 5.4 % elderberry solids and 10.7 % encapsulant.

In the freeze drying procedure, liquid systems were poured onto an aluminum tray, frozen at -30°C during 40 hours and then freeze dried at room temperature (22 ± 3°C) in a FIC-LI-I-E300-CRT freeze dryer (Buenos Aires, Argentina) operated with a freezing plate and condenser at -40°C and a vacuum of 100 mm Hg during 40 h. The freeze dried products were milled to obtain a powder that was conserved in hermetic flasks at -18°C until use.

The spray drying encapsulation of liquid systems was performed in a mini spray dryer Buchi B-290 (Büchi Labortechnik AG, Switzerland). The operating conditions were inlet temperature 155°C, outlet temperature 80-81°C and feed flow rate 30 % (8 ml/min). Spray dried powders were preserved in same condition of freeze dried systems.

2.3. Storage of dehydrate powders: freeze-dried and spray-dried systems

were stored in darkness at 38°C for 9 days in open small flasks equilibrated in desiccators with saturated solutions corresponding at a_w 0.43 and 0.58 (relative humidity 43 and 58 %). The main objective was to equilibrate the systems at a relatively high water activity and to evaluate the effects of caking and agglomeration on anthocyanin stability and color. At selected times, two samples were removed to take measurements. A temperature of 38°C was selected because it is usually recommended in accelerated shelf-life studies of foods that are to be marketed at ambient temperature (Labuza, 1982). For the construction of sorption isotherms, samples were equilibrated at 38°C in desiccators containing standard salt solutions providing water activities between 0.33 and 0.75.

2.4. Thermal treatment of freeze-dried powder

Hermetic tubes containing 1 g of freeze-dried powder were heated at 85°C in a water bath and samples were withdrawn in duplicate at selected times to take measurements.

2.5. Physicochemical properties of liquid and dehydrated systems

2.5.1. Alcoholic extract

Freeze-dried and spray-dried powder samples were reconstituted with water to their original solids concentration prior to analysis. 2.5 grams of reconstituted systems were extracted in 10 ml ethanol: HCl 0.1 N (85:15) by shaking and centrifugated 15 minutes at 5000 rpm. The resultant extracts were utilized for total monomeric anthocyanins and HPLC assays (Busso Casati et al., 2019).

Water activity (a_w) was determined using an electronic dew-point water activity meter Aqualab TE (Decagon Devices, Pullman, WA). The equipment was calibrated with saturated salt solutions in the water activity range of interest (Favetto et al., 1983).

Total soluble solids content was evaluated with a manual refractometer Atago N-1E (Tokyo, Japan) and recorded as °Brix.

Moisture content of dehydrated powder was performed on 1.5 g of powder in an oven at 100°C by 3 hours up to constant weight.

Hygroscopicity was determined on the dehydrated powders, both freeze and spray dried, as increase in weight (%) due to water adsorption after 4 day at relative humidity 75 % and 25°C.

Glass transition temperatures (T_g) of each dehydrated system were determined by differential scanning calorimetry (DSC; onset values) using a TA Instrument Q2000 calorimeter (New Castle, England). The instrument was calibrated with indium (156.6°C). All measurements were performed at a heating rate of 10°C/min in a 0-100°C range of temperature. Hermetically sealed 40 µL medium pressure pans were used, (an empty pan served as a reference). Thermograms were evaluated using Q2000 V24.11 program.

Color parameters were analyzed using a Minolta Spectrophotometer CM-600d (Konica Minolta Observer), with D65 illuminant and an observer angle of 2°. The measurement was obtained by placing 0.15 g of powder, in plastic white containers. CIELab parameters (CIE 1976 L^* a^* b^*) were L^* for lightness, a^* for redness and b^* for yellowness. All measurements were made by triplicate. The hue angle (h°) and total color difference (ΔE^*) were calculated by equations (1) and (2) respectively:

$$h^\circ = \arctan (b^*/a^*) \quad (1)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

Total color difference was calculated as comparison of monomeric anthocyanins and color stability and expressed the magnitude of difference between 0 and 9 days of powder storage at 38°C. The instrument was standardized with a white tile ($L^* = 91.10$, $a^* = 1.12$ and $b^* = 1.26$).

Collapse: The collapse of the dehydrated powders was recorded by photographs, after storing for 9 days at 38°C and relative humidities (RH) of 43% and 58%.

2.5.2. Cyanidin-3-glucoside content by HPLC

HPLC analyses were performed on elderberry extracts with an Agilent 1260 series HPLC system according to the method reported by Hager et al. (2008). The injection volume was 20 µl of juice extract. Separation was achieved on a reverse phase C18 Gemini® 150 × 4,6 mm; 5 µm column. Detection was performed using a diode array detector. Two solvents were used during the analysis. Solvent A: distilled water/formic acid (95/5) and solvent B: methanol. A constant flow of 1 ml/min was applied with a linear gradient elution profile. An external calibration curve of cyanidin-3-glucoside (Cy-3G) was used for quantification, in the range 10–100 µg/ml. The content of total anthocyanins (TA) and the major compound (cy-3G) were analyzed by absorption at 515 nm and the results expressed in mg of cyanidin equivalents per liter of berries' pulp (mg Cy-3G/l).

2.5.3. Total monomeric anthocyanin content

Total monomeric anthocyanin content of alcoholic extracts was determined by pH differential method (Giusti & Wrolstad, 2001). Absorbances were read at 510 and 700 nm, and its content was calculated as cyanidin-3-glucoside in mg/100 g of product (MW: 449.2 g mol⁻¹ and ϵ : 26 900 L cm⁻¹mol⁻¹). Total monomeric anthocyanin retention (%) was relative to the initial content considered as 100 %.

To evaluate % retention, a fraction of the freeze-dried or spray-dried sample was placed in hermetic flasks, or open flasks in the case of storage at 38°C. The bottles were removed at different times, and the powder was first rehydrated, then the alcoholic extraction was carried out as indicated in 2.5.1.

2.5.4. Polymeric color

Polymeric color (PC) was determined on alcoholic extracts according to the method reported by (Giusti & Wrolstad, 2001). Samples of the extract were treated with bisulfite or water and the absorbances read at 420, 510, and 700 nm. PC was expressed in percent (PC %).

2.6. Data analysis

Replicates of each sample were analyzed. All physicochemical parameters were determined in triplicate, except for glass transition temperature (T_g), which was measured in duplicate. Results are reported as mean ± standard error. Cy-3-glucoside retention and all other variables were subjected to analysis of variance (ANOVA) using InfoStat v.2017 (Universidad Nacional de Córdoba, Argentina). Mean comparisons were performed using Tukey's test at a 95% confidence level ($p < 0.05$, $n = 3$, except T_g : $n = 2$).

3. Results

3.1. Physicochemical characteristics and anthocyanin retention of powders

Fig. 1 shows the appearance of the powders obtained by spray-drying (SD) and freeze-drying (FD). As observed, all powders were free-flowing and exhibited notable visual differences in color, depending on the type of encapsulant used and drying method. The pulp used for the preparation of the systems exhibited the following characteristics: total solids content of $9.03 \pm 0.15\%$, pH 3.63 ± 0.01 , and AMT content of 42.75 ± 2.55 mg per 100 g of fruit.

The moisture content, water activity (a_w), and hygroscopicity are the key factors to the storage stability of powder product. Table 1 presents the physicochemical properties of elderberry powders obtained via freeze-drying and spray-drying using different encapsulating agents: whey protein concentrate (WPC), maltodextrin (MD), and soy protein

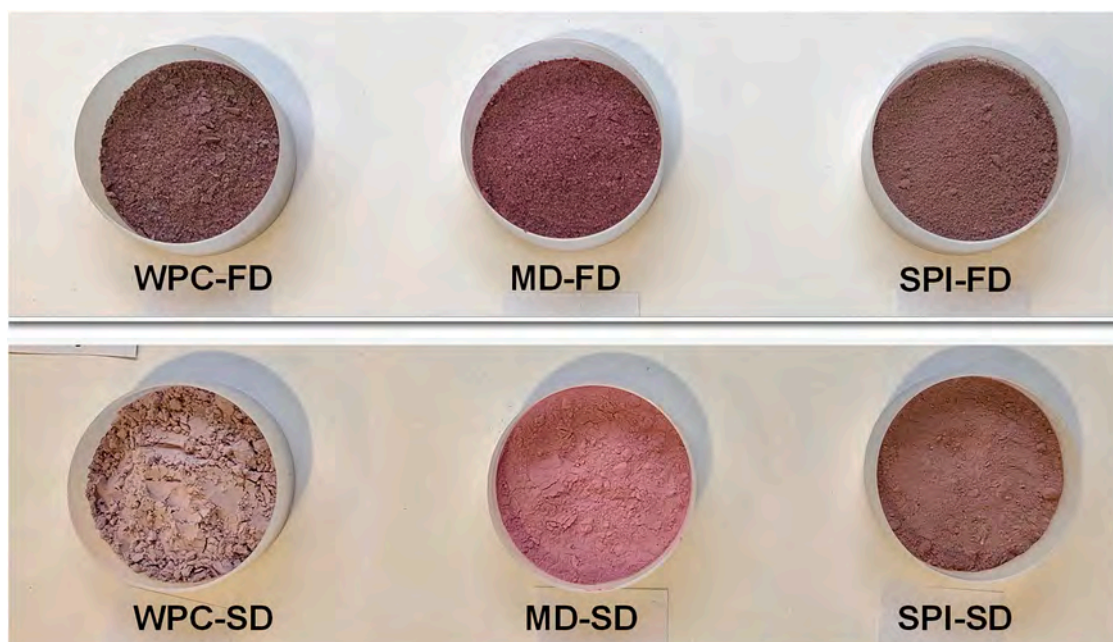


Fig. 1. Appearance of elderberry powders with different encapsulating agents: WPC, MD and SPI, either freeze-dried (FD) or spray-dried (SD).

Table 1

Physicochemical characteristics of freeze-dried (FD) and spray-dried (SP) elderberry systems obtained with different encapsulating agents.

System	Water activity (a_w)	Moisture content (%w/w)	T_g (°C)	Moisture gain (%)	cy-3G content		cy-3G retention (%)
					mg/100 g powder	%	
WPC-FD	0.051±0.001 ^b	3.12±0.12 ^b	50±1.0 ^{a,b}	16.41±0.15 ^c	44.4±2.0 ^{a,b}		70.5±3.5 ^a
MD-FD	0.061±0.001 ^c	2.81±0.06 ^a	47±0.9 ^a	15.95±0.06 ^b	53.6±5.0 ^c		85.2±4.0 ^b
SPI-FD	0.038±0.002 ^a	3.04±0.04 ^b	49±0.5 ^a	17.58±0.09 ^d	46.6±3.0 ^{a,b,c}		74.1±4.4 ^{a,b}
WPC-SP	0.094±0.001 ^d	3.34±0.04 ^c	52.9±0.6 ^b	15.22±0.05 ^a	40.6±2.5 ^a		64.5±3.2 ^a
MD-SP	0.170±0.001 ^e	3.19±0.03 ^b	49.5±0.5 ^a	16.39±0.06 ^c	52.7±3.5 ^{b,c}		83.9±4.5 ^b
SPI-SP	0.192±0.001 ^f	5.26±0.04 ^d	51.2±1.0 ^b	21.08±0.12 ^e	52±1.0 ^{b,c}		82.7±4.1 ^b

Values are means ± standard error. Values in the same column with different letters are significantly different ($p < 0.05$).

isolate (SPI). Spray-dried powders generally exhibited higher water activity than freeze-dried samples. Samples containing protein showed higher moisture levels than those containing MD. Among them, SPI-SP showed the highest water activity (0.192) and moisture content (5.26%), whereas MD-containing samples showed lower moisture values for both MD-SP (3.19%) and MD-FD (2.81%). For freeze-dried powders, SPI-FD showed the lowest a_w (0.038), significantly different from both MD-FD (0.061) and WPC-FD (0.051). The higher moisture content of protein-containing samples compared to MD samples may be attributed to the higher water-holding capacity of proteins in their amorphous state (Correia et al., 2017; Wang et al., 2020). Lower water activity in freeze-dried samples has also been reported by Correia et al. (2017) for encapsulated wild blueberry powders prepared using wheat flour, chickpea flour, coconut flour, and soy protein isolate. In that study, water activity values ranged from 0.060 to 0.095 for freeze-dried samples, whereas spray-dried powders exhibited values around 0.2. The lower a_w values observed in freeze-dried samples may be attributed to the fact that freeze-drying is a long-duration process that removes water through ice sublimation under reduced pressure, thereby effectively minimizing the amount of residual unbound water in the dried product. Nevertheless, several studies have reported that final a_w values may vary depending on the drying conditions employed (e.g., inlet temperature in spray drying) or on the duration of the freeze-drying process (Allameh & Orsat, 2024).

Glass transition temperature (T_g) varied slightly among samples, ranging from 47 to 53°C. These values are substantially lower than those of the pure carriers (SPI, MD, WPC) at the same moisture level, due to

the presence of sugars and other low-molecular-weight compounds present in the elderberry juice. The highest T_g was observed in WPC-SP (52.9°C), significantly different from MD-FD (47°C) and SPI-FD (49°C). The other systems showed intermediate T_g values without clear statistical separation.

The T_g values are considerably higher than those reported for dehydrated systems without added encapsulants. In previous studies such as those published by Khalloufi et al. (2000), values of T_g ranging from 15 to 20°C were reported for various freeze-dried berries equilibrated at a water activity of 0.113. Similarly, Syamaladevi et al. (2009) reported T_g values for freeze-dried raspberry systems between 12 and 22°C for samples with moisture contents of 4.5% to 3.6%, respectively. These low T_g values indicate that the use of encapsulants during spray drying is essential, as typical powder outlet temperatures are above 70°C. In the studied systems, a ratio of 2:1 encapsulant to juice solids was used, which is appropriate for both freeze-dried and spray-dried encapsulation processes. In a previous study (Baeza et al., 2021), systems containing a lower proportion of encapsulant were employed with good results for freeze-dried elderberry systems; however, in this study, the same ratio was used to compare the spray-dried and freeze-dried systems. Gómez-Mattson et al. (2020) reported similar glass transition temperature (T_g) values for elderberry powders formulated with a 2:1 MD:fruit solids ratio (approximately 50°C). The slight increase in T_g observed in the presence of proteins, particularly in spray-dried (SD) systems, may be attributed to the surface-active properties of proteins in solution. Protein migration to the particle surface can rapidly lead to the formation of a very thin protein-rich film during spray drying. This film

may exhibit a relatively higher T_g , allowing it to remain in the glassy state and thereby reducing particle-to-particle interactions and stickiness (Fang & Bhandari, 2012).

Moisture gain (measured at 75% RH) reflected the hygroscopicity of the powders. The moisture gain during storage at 38°C showed significant differences ($p < 0.05$). SPI-SP exhibited the highest moisture gain (21.08%), significantly higher than all other samples, with values approximately 30% greater than the rest of the systems. WPC-SP had the lowest moisture gain (15.22%), followed by MD-FD (15.95%) and MD-SP (16.39%). When expressed as an increase in moisture on a dry basis, the system SPI-SP increased from 3.1% to 24.8% moisture (dry basis). Pan (2007) reported values close to 25% moisture (dry basis) for SPI after 10 days of storage at 25°C. To better understand the behavior of the systems, the hygroscopicity of the encapsulating agents alone and of freeze-dried elderberry alone (without encapsulants) was determined. Hygroscopicity values, expressed as g water per 100 g dry solids at 75% RH, were 15.22% for WPC, 16.6% for SPI, 14.24% for MD, and 28.58% for freeze-dried elderberry. Therefore, the higher moisture uptake observed in systems containing SPI may be attributed to the greater intrinsic hygroscopicity of this ingredient, as previously reported for protein-based systems (Correia et al., 2017).

Table 2 presents the color characteristics (L^* , a^* , and hue angle h°) of elderberry powders and their corresponding alcoholic extracts, obtained through freeze-drying (FD) and spray-drying (SP) using different encapsulating agents. Significant differences ($p < 0.05$) were observed in all color parameters, as determined by Tukey's test at a 95% confidence level. Among the powders, L^* values ranged from 16.96 (WPC-FD) to 35.29 (WPC-SP), indicating that spray-dried powders, particularly WPC-SP and MD-SP, were significantly lighter than their freeze-dried counterparts. SPI-FD showed a significantly higher L^* than both WPC-FD and MD-FD, but was darker than any spray-dried system.

For the a^* parameter, which reflects redness, MD-SP had the highest value (15.57), significantly greater than all other systems. This was followed by MD-FD (11.83), with the lowest values observed in WPC-FD and WPC-SP (6.98 and 6.82, respectively). SPI-based systems showed intermediate redness.

Hue angle (h°) also showed marked variation among powders. MD-SP had the lowest h° (8.61), indicating a shift toward a purer red hue, while SPI-SP exhibited the highest value (23.58), significantly different from all other samples except WPC-SP.

In alcoholic extracts, L^* values followed a similar trend. SPI-FD extract was the brightest (41.46), significantly different from MD-FD and MD-SP, which had the lowest L^* values (~33). Redness (a^*) was highest in extracts from maltodextrin-based systems (MD-FD and MD-SP), while SPI-based systems exhibited significantly lower a^* values.

Table 2

Color parameters of freeze-dried (FD) and spray-dried (SP) elderberry systems obtained with different encapsulating agents. The values for the powder and reconstituted systems (liquids) at 15% w/w are shown.

System	Powder			Extract		
	L^*	a^*	h°	L^*	a^*	h°
WPC-FD	16.96 ±1.61 ^a	6.98 ±0.01 ^a , ±0.01 ^b	21.04 ±0.07 ^c	39.27 ±0.80 ^b	10.26 ±0.44 ^b	30.1 ±0.69 ^c
MD-FD	17.78 ±0.59 ^a	11.83 ±0.02 ^d	10.87 ±0.08 ^b	33.05 ±0.86 ^a	19.49 ±1.03 ^c	16.42 ±0.07 ^a
SPI-FD	22.26 ±0.47 ^b	7.19 ±0.12 ^b	23.58 ±0.12 ^d	41.46 ±0.26 ^c	7.71 ±0.19 ^a	44.21 ±0.35 ^d
WPC-SP	35.29 ±0.41 ^e	6.82 ±0.17 ^a	24.52 ±0.17 ^d	41.30 ±0.25 ^c	9.60 ±0.69 ^b	28.66 ±0.07 ^b
MD-SP	32.80 ±0.39 ^d	15.57 ±0.22 ^e	8.61 ±0.19 ^a	33.23 ±0.88 ^a	19.82 ±1.00 ^c	16.04 ±0.04 ^a
SPI-SP	25.68 ±0.12 ^c	8.61 ±0.02 ^c	23.58 ±0.82 ^d	40.88 ±0.26 ^{b,c}	7.45 ±0.16 ^a	49.79 ±0.15 ^e

Values are means ± standard error. Values in the same column with different letters are significantly different ($p < 0.05$).

The hue angle of the extracts also varied significantly. MD-based extracts had the lowest hue values (~16), consistent with a more red-dominated color, while SPI-SP extract had the highest hue angle (49.79). This indicates that in the case of the systems with proteins (extracts and powders), more brownish tones are observed, both in the presence of WPC and SPI. In recent studies, it has been reported that color shifts in anthocyanin-protein systems can result from non-covalent interactions such as hydrogen bonding and hydrophobic association (Zang et al., 2021, Ren et al., 2021). In the case of systems with SPI, an increase in h° may be accentuated by the color of the carrier itself, also reported by Dumitraşcu et al. (2021) in systems with cornelian cherry juice and SPI as encapsulant. Murugesan & Orsat (2011) reported higher h° values for spray-dried elderberry powder systems containing SPI compared to those formulated with MD.

Fig. 2 (A and B) shows the water adsorption isotherms at 38°C for elderberry powders microencapsulated using the three carriers. The isotherms are not markedly different from each other; however, it is observed that, above a_w 0.58, there is a tendency for the mixtures with maltodextrin (MD) to be below those with whey protein concentrate (WPC) and soy protein isolate (SPI), both for the powders obtained by spray drying and for the freeze-dried samples. A clear observation for any of the carriers is that the isotherms of the spray-dried powders display a plateau between a_w 0.43 and 0.58, a feature that is less evident in the isotherms of the freeze-dried powders. This is clearly visible in Fig. 2B. The plateau region in the isotherm represents an a_w range in which the amount of water adsorbed by the product does not change significantly with increasing a_w . It is generally attributed to the

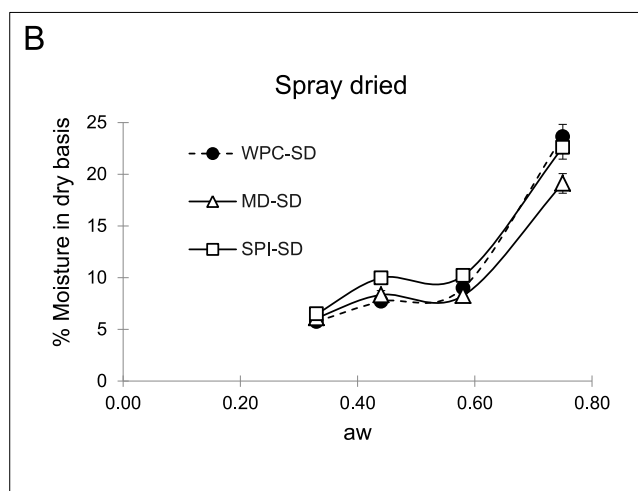
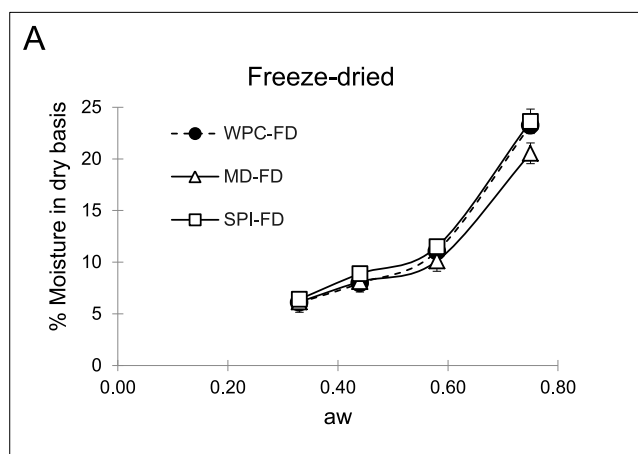


Fig. 2. Water adsorption isotherms at 38°C for encapsulated elderberry powders using WPC, MD, and SPI, for freeze-dried (A) and spray-dried (B) systems.

crystallization of certain amorphous components, which in this case would be sugars present in the elderberry pulp.

Cyanidin-3-glucoside (cy-3G) is one of the two major anthocyanins in elderberry, along with cyanidin 3-sambubioside (Veberic et al., 2009). Several studies have reported that its proportion can range between 45% and 60%, depending on the elderberry variety and geographic origin (Veberic et al., 2009; Jungmin & Finn, 2007). In this work, it was determined that cy-3G accounts for 70% of the monomeric anthocyanins present in elderberry pulp from El Bolsón, Patagonia. Table 1 shows the cy-3G content in the powder and the percentage of retention relative to the system prior to drying. In the liquid system, prior to drying, the cy-3G content was approximately 63 mg/100 g of solids, as determined by HPLC.

Additionally, determinations using the pH differential method were performed on the systems prior to drying; however, interferences were detected with this method in the presence of proteins, so it was not used to analyze differences in the absolute content of TMA between systems. Nevertheless, the retention relative to the initial value was assessed in each case. In the dehydrated systems, the cy-3G content determined by HPLC ranged from 40.6 to 53.6 mg/100 g powder. MD-FD retained the highest concentration of cy-3G (53.6 mg/100 g), significantly higher than WPC-SP (40.6 mg/100 g). The retention of cy-3G (%) was significantly influenced by both the drying method and the encapsulating agent. MD-FD exhibited the highest retention (85.2%), significantly greater than WPC-SP (64.5%) and WPC-FD (70.5%). MD-SP and SPI-SP also maintained high retention levels (83.9% and 82.7%, respectively), with no significant difference from MD-FD. Overall, these results align with previous studies on similar systems with other berries, where maltodextrin or various proteins were used (Liu et al., 2025). By the pH differential method, the retention of total monomeric anthocyanins (TMA) observed ranged from 85% to 100%.

3.2. Stability of powders at 43% and 58% relative humidity

The behavior of elderberry powders during storage is of fundamental importance, as these ingredients could be stored at room temperature as standalone ingredients or as components of stable products at ambient temperature (e.g., in cookies, cereal bars, breakfast cereals, etc.). During the storage of stable products at room temperature, such as dehydrated items, water vapor can pass through the permeability of the packaging over the product's shelf life, which may lead to an increase in the a_w value compared to the initial state. Furthermore, during the product's shelf life, partial moisture absorption can occur due to the frequent opening and closing of the packaging during use, which can increase both the a_w and the initial moisture level. Beyond their physical stability (e.g., their resistance to caking or agglomeration), it is also important to analyze the stability of anthocyanins as bioactive compounds and the overall color of the systems.

The evolution of total monomeric anthocyanins (TMA), cy-3G, and color parameters was evaluated during the storage of spray-dried and freeze-dried powders at 38°C and 43% or 58% relative humidity, corresponding to a_w values higher than the initial ones reported in Table 1. Although the storage period was relatively short compared to the potential shelf life of the product, the main objective was to analyze the behavior of the systems in response to agglomeration and caking, as well as the possible effects on anthocyanin and color stability under conditions of a_w higher than the initial levels. In Fig. 3A, the retention of the compound cy-3G at 43% RH in freeze-dried powders is shown, while Fig. 3B depicts the retention of TMA. Similar results are observed in Fig. 4A and 4B for the spray-dried powders.

In Fig. 3A, it can be seen that for the freeze-dried systems, the retention of cy-3G ranged between 59% and 69%. For TMA, retention was between 71% and 84% after 9 days at 43% RH. The difference in retention between TMA and cy-3G averaged approximately 13%. In the spray-dried powders (Fig. 4A and B), the retention of cy-3G ranged from 53% to 75%, while TMA retention ranged from 82% to 85%. In this case,

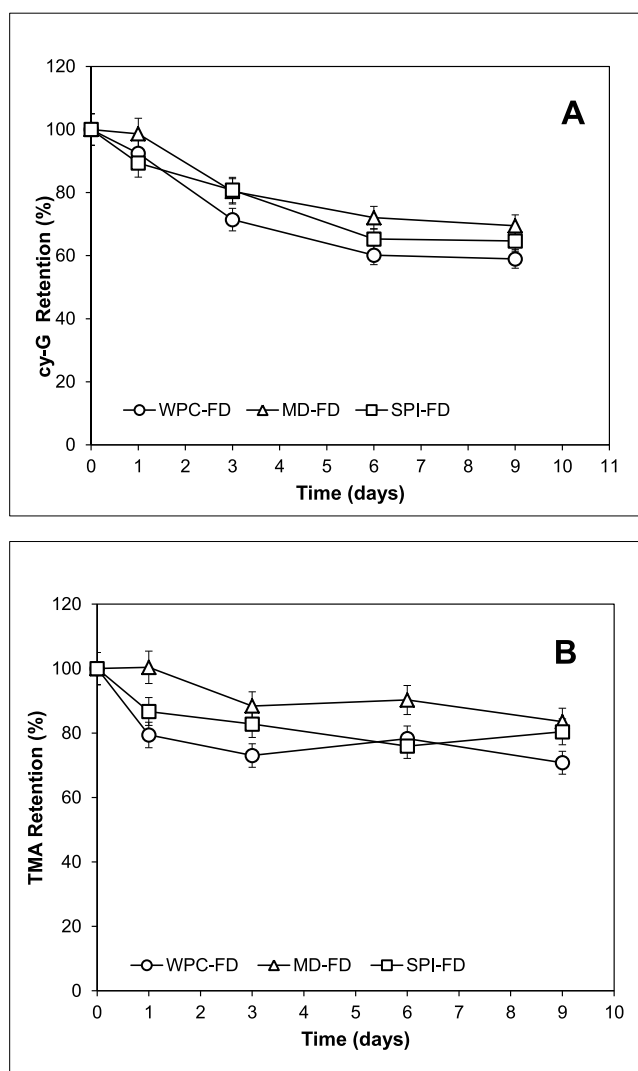


Fig. 3. Retention of cy-3G (A) and TMA (B) in freeze-dried powders during storage at 38°C and 43% RH.

it was also observed that, for each system, the percentage of TMA retention was higher than that of cy-3G, but with larger differences among the encapsulants: 7% for MD, 26% for WPC, and 33% for SPI. The system with MD showed the greatest retention of the monomeric compound in both freeze-dried and spray-dried powders. Conversely, for anthocyanin retention, the results were more similar across the different systems.

Additionally, the retention of anthocyanins during storage at 58% RH for 9 days was assessed for both freeze-dried and spray-dried systems. The retention of cy-3G ranged between 33% and 68%, with the highest value observed in the MD system—68% for freeze-dried and 46% for spray-dried powders. For TMA, retention ranged from 66% to 85%, showing the highest retention values. Similar values of anthocyanin retention levels during storage have been reported in other studies. Selim et al. (2008) evaluated the degradation kinetics of encapsulated Roselle anthocyanins at water activities of 0.43, 0.54, 0.64, and 0.75, observing the lowest degradation rate at a_w 0.43, which increased with higher a_w values, reaching a maximum at a_w 0.64. Rocha-Parra et al. (2016) demonstrated that increasing a_w from 0.11 to 0.58 significantly increased the loss of total anthocyanins in encapsulated freeze-dried red wine powder. Todorović et al. (2022) reported about a 20% loss of TMA in freeze-dried bilberry encapsulated with MD and gum Arabic after 5 days at 40°C. Brownmiller et al. (2008) reported that loss of monomeric

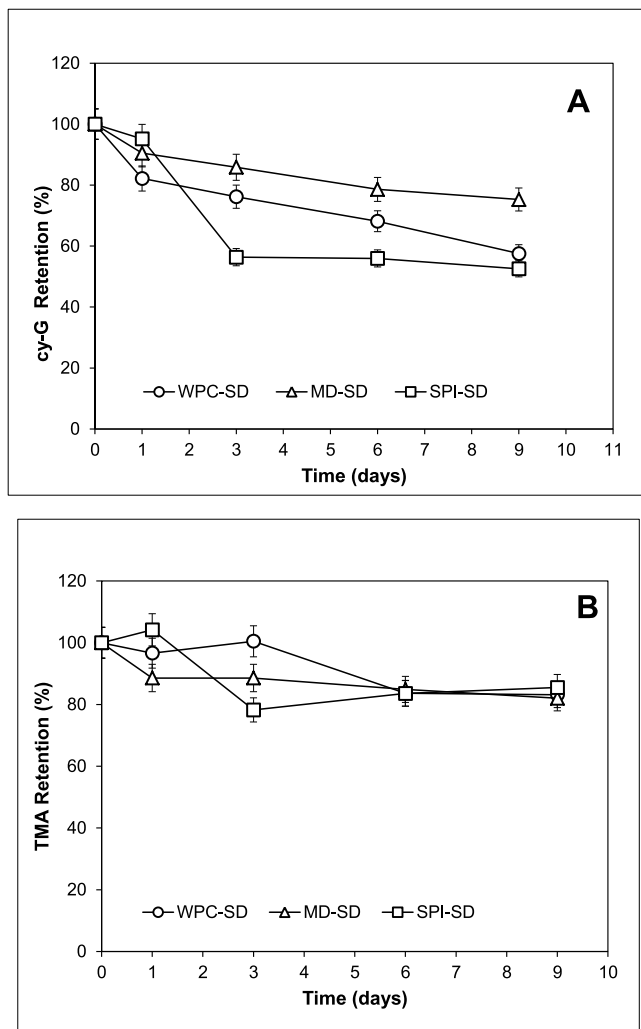


Fig. 4. Retention of cy-3G (A) and TMA (B) in spray-dried powders during storage at 38°C and 43% RH.

anthocyanins may be due to several factors, including residual enzyme activity or condensation reactions of anthocyanins with other phenolics.

The stabilization of anthocyanins can occur through different mechanisms, such as microencapsulation or association reactions, including self-association between anthocyanins, copigmentation, and the association of metal ions with the o-hydroxy groups of anthocyanins (Cavalcanti et al., 2011; Trouillas et al; 2016). Microencapsulation is defined as the entrapment of a substance (core material) within an immiscible substance (wall material), which forms a protective barrier for the core material. Maltodextrins with different dextrose equivalents (DE) are commonly used as wall materials due to their high water solubility, low viscosity, and low sugar content. They are frequently used alone or in combination with other polysaccharides such as gum arabic, pectins, or alginates (Lu et al., 2012; Mahdavi et al., 2016). Numerous studies have reported enhanced stability of microencapsulated anthocyanins when maltodextrin is used as a carrier (Cavalcanti et al., 2011; Baeza & Chirife, 2021). Another stabilization mechanism, copigmentation, has been defined as a molecular interaction between anthocyanins and other compounds that can modify both anthocyanin stability and color. This phenomenon may occur during spray drying or freeze-drying in the presence of copigments such as ferulic acid, rutin, maltodextrin, caffeic acid, catechin, gum arabic, acetic acid, phenolics, gallic acid, proteins, alginate, pectin and metal ions (Harsito et al., 2021; Tan et al., 2021).

In the case of proteins such as whey or soy protein, it has been

proposed that they stabilize anthocyanins through hydrophobic interactions and hydrogen bonding. For example, Zang et al. (2021) studied the effect of whey protein isolate and bovine serum albumin on the stability of blueberry anthocyanins. It has been reported that the anthocyanin-whey protein complex is mainly formed by hydrophobic interactions on the protein surface and is subsequently stabilized by hydrogen bonding and van der Waals forces (Khalifa et al., 2018).

Fig. 5 compares the color parameters at the beginning and after 9 days at 43% RH. In the freeze-dried systems, an increase in the hue angle (h°) is observed for the systems with WPC and SPI. Similarly, in the spray-dried powders, there is an increase in h° and a decrease in the a^* parameter. The overall color changes are illustrated in Fig. 6. It is evident that the most significant color changes occur in the spray-dried systems, with differences within these systems becoming more pronounced in the case of SPI.

As can be seen, the relative reduction of the color parameter a^* is less intense than the loss of content of total monomeric anthocyanins or cy-3G. It is possible that compounds formed in copigmentation reactions would retain the red color despite a decrease in cy-3G molecules and other anthocyanins present, so the phenomena are less visually observable or by color measurement. This may be attributed to interaction between monomeric anthocyanins and colorless copigments that stabilize the colored anthocyanins delaying the color shift or loss (Rein 2005). This color stabilization, observed despite the degradation of monomeric anthocyanins, has been reported in numerous studies. Previous research has noted greater stability of red color despite a more pronounced decrease in monomeric anthocyanins (Baeza et al., 2021; Busso Casati et al., 2015). Similar results were reported by Idham et al. (2012) in encapsulated anthocyanins in hibiscus sabdariffa powders, where monomeric anthocyanin degradation was more pronounced than the deterioration in color. Reactions of anthocyanins do not necessarily lead to colorless compounds, but also pigmented compounds like polymers may be formed which contribute to the color (Weber et al, 2017). Numerous studies have reported color stabilization through copigmentation, particularly in berries (Torruillas et al., 2016; Lee et al., 2020). Additionally, polymeric color determinations were performed using the

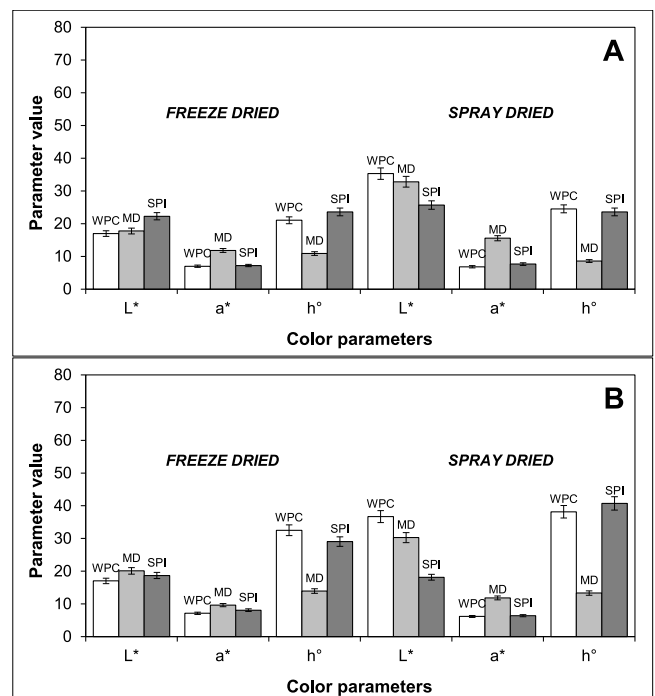


Fig. 5. Color parameters of freeze-dried and spray-dried elderberry using WPC, MD, and SPI as encapsulating agents, at initial time (A) and after storage at 38°C and 43% RH for 9 days (B).

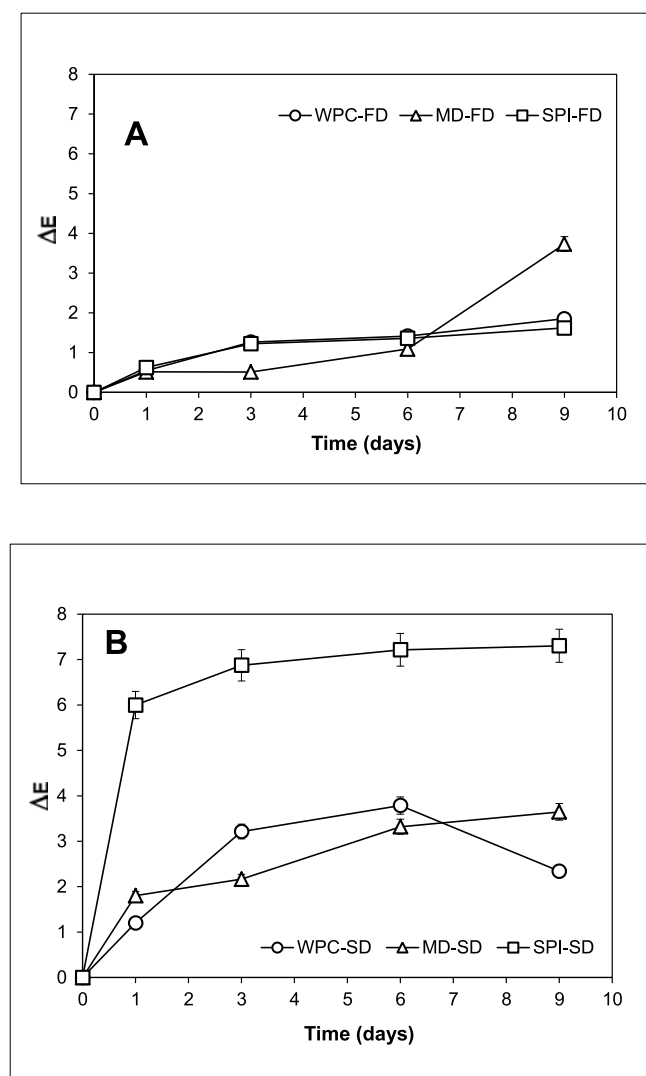


Fig. 6. Color change (DE) in freeze-dried systems (A) and spray-dried systems (B) stored at 38°C and 43% RH.

technique described by Giusti & Wrolstad (2001) on MD systems stored at 43% and 58% RH. MD system presented fewer interferences in the pH differential method and was used to evaluate the degree of polymerization during storage and heating. For the spray-dried milk protein (SP-MD) system, %PC increased from 45.7% ($t=0$) to 46.4% after 9 days at 43% RH, and to 53.3% after 9 days at 58%.

The observed increase in the percentage of polymeric color may indicate the formation of condensation reactions between anthocyanins and other polymeric compounds, such as procyanidins, resulting in the formation of polymerized phenolic compounds. It is notable that the initial values already showed a high degree of anthocyanin polymerization, which could be due to processes related to fruit handling, storage, pasteurization, packaging, etc. Lao & Giusti (2016) reported %PC values between 25% and 56% in processed purple corncocks. In a previous study, an increase in the percentage of polymeric color was observed in commercial elderberry juices after heat treatment or storage at 38°C, with values ranging from 75% to 93%, depending on the treatment (Busso Casati et al., 2015). In comparison, the final %PC values obtained for the powders were lower.

Fig. 7 (A, B, C) shows the structural changes experienced by elderberry powders (spray- or freeze-dried) exposed to 43% RH and 38°C over 9 days. Fig. 7 (E, F, G) displays the powders exposed at 58% RH. Caking and agglomeration are physical deterioration phenomena that

occur in stored food powders. The extent of these phenomena depends mainly on the powder's moisture content, storage temperature, the glass transition temperature (T_g) of the product, and the duration of exposure to those conditions (Roos & Drusch, 2015). It should be remembered that T_g is inversely proportional to system moisture, and even a slight increase in moisture percentage drastically decreases T_g . Although the T_g values at 43% and 58% RH (when the powders equilibrate to water activities of 0.43 and 0.58, respectively) were not determined, it can be assumed that these values are much lower than those reported in Table 1, since the moisture content is around 8-10%. As mentioned earlier, moisture has a strong influence on T_g .

It is observed that powders obtained by freeze-drying show fewer adverse changes (such as agglomeration) compared to spray-dried powders. Additionally, deformation and collapse are more pronounced in powders made with MD compared to those with SPI or WPC, and the degree of collapse is always greater in the powders produced via spray drying than in those produced by freeze-drying. It is observed that in the systems with MD at 43% RH, there is noticeable caking in the SD sample, while at 58% RH, both the FD and SD samples have experienced caking, with the SD sample showing greater compaction and a color change of the powder. Powders with SPI also exhibit the same phenomenon: the freeze-dried powders are more resistant to deformation and collapse than those obtained by spray drying under the same temperature, RH%, and exposure time conditions. In the systems with WPC or SPI stored at 58% RH, a slight agglomeration is observed, but they remain fluid when the vials are inverted. In the systems with WPC, the formation of agglomerates is seen in the volume contained in the vial, but the powder remains fluid when is inverted.

The greater resistance to caking observed in freeze-dried powders compared to spray-dried ones cannot be attributed solely to differences in glass transition temperature, since the values reported in Table 1 do not show major differences among the systems. A significant difference was observed only for spray-dried systems containing proteins compared to those formulated with MD. Furthermore, for MD-based powders, moisture contents were similar to—or even slightly lower than—those of protein-containing systems; nevertheless, they generally exhibited more pronounced agglomeration or caking. Correia et al. (2017) studied spray-dried and freeze-dried wild blueberry systems containing proteins and attributed the stabilizing effect to protein binding and complexation with blueberry polyphenols, as well as to protein migration to the particle surface, which can minimize stickiness between particles and/or droplets. In addition, previous studies have reported that the higher hygroscopicity observed in spray-dried systems may be related to the larger specific surface area of the droplets formed, in contrast to the larger, plate-like structures typically observed in freeze-dried systems (Correia et al., 2017, Wang et al., 2020).

3.3. Stability against thermal treatment

Exposure to high temperatures is one of the main factors causing anthocyanin degradation and may limit the use of these ingredients in baked goods or other foods requiring thermal processing. Numerous studies have reported anthocyanin degradation in berries subjected to different thermal treatments, mainly in liquid systems (Patras et al., 2010; Busso Casati et al., 2015).

In powder systems, anthocyanins are partially protected due to the presence of the encapsulating material and the low molecular mobility of the system. Under these conditions, powders are typically in a glassy state at temperatures below the glass transition temperature (Franceschinis et al., 2014). However, at temperatures above T_g , increased anthocyanin degradation may occur as a result of enhanced molecular mobility and greater exposure to environmental factors such as light, oxygen, and other reactive agents (Todorović et al., 2022).

Anthocyanin degradation and the evolution of colour parameter a^* has been found to follow a first-order reaction model (Busso Casati et al., 2017) with reported values for degradation rate constants (k) and

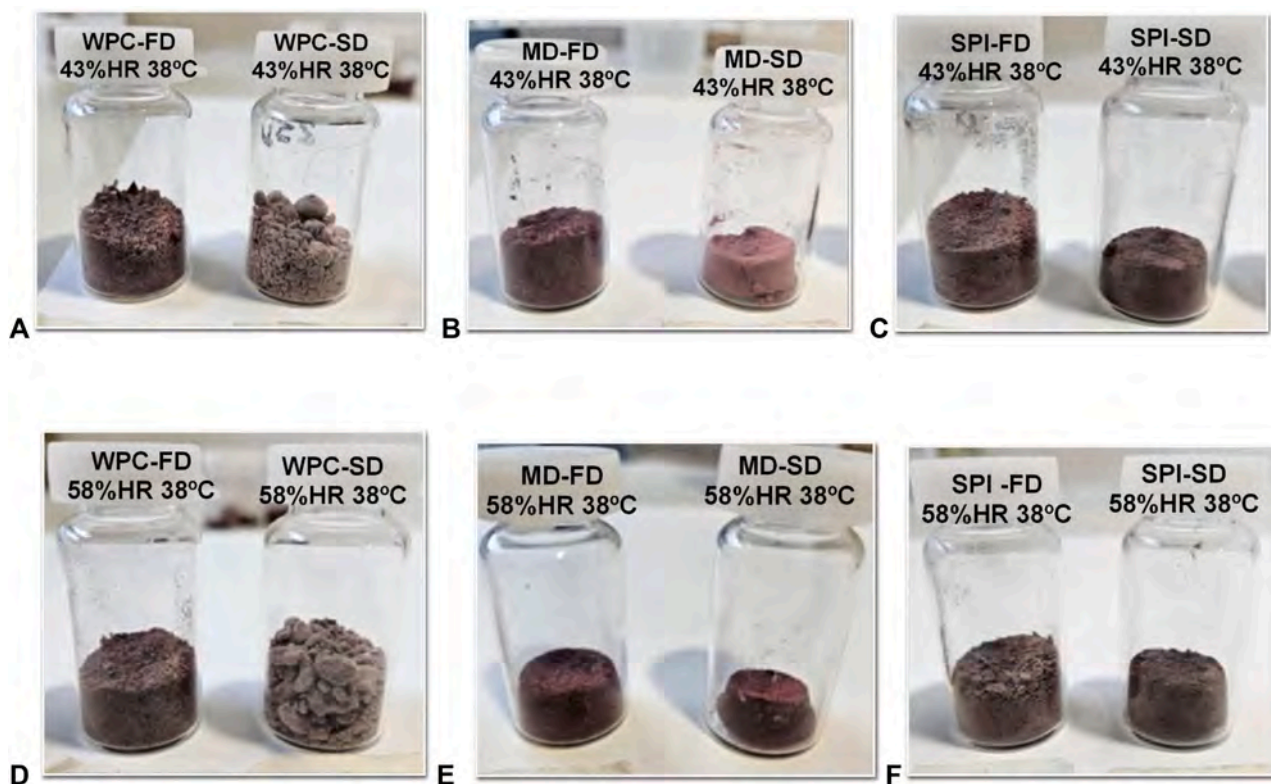


Fig. 7. Elderberry encapsulated using different carriers WPC, MD, and SPI stored at 38°C. A, B, and C: stored for 9 days at 43% RH; D, E, and F: stored for 9 days at 58% RH. In each image, the freeze-dried (FD) and spray-dried (SD) systems can be observed comparatively.

half-life time ($t_{1/2}$), according to the following equations:

$$\ln(C/C_0) = -k \cdot t \quad (3)$$

$$t_{1/2} = -\ln 0.5 \cdot k^{-1}, \quad (4)$$

being C_0 the initial content, and C the content at time t .

In powdered systems, heating can lead to the degradation of monomeric anthocyanins, as well as changes in their physical characteristics, such as caking or agglomeration. A potential use of these powdered ingredients is as colorants or sources of bioactive compounds in foods that require subsequent cooking or exposure to high temperatures, such as in baked goods, snacks, and bakery decorations, either alone or in mixtures with other ingredients. Therefore, it is of interest to study the physical stability of the powders subjected to heating, as well as the retention of color and anthocyanins during heating.

For spray-dried powders, heating tests were conducted, revealing rapid caking and agglomeration phenomena at temperatures above 60°C. This is probably due to their higher water activity at the end of drying, as well as the greater interaction between particle surfaces resulting from their smaller size and larger exposed surface area compared to freeze-dried powders (Wang et al., 2020). These factors suggest that their use in processes requiring heating could be challenging if the goal is to maintain "free-flowing" properties. In contrast, freeze-dried powders remain fluid or pourable after heating, allowing for the assessment of the stability of total monomeric anthocyanins (TMA) and cy-3G content during heating of freeze-dried powders containing 1% and 3% moisture. The powders with 1% moisture were obtained by an additional 24-hour freeze-drying step.

Fig. 8A shows the retention of TMA during heating at 85°C. The powder containing maltodextrin (MD) with 1% moisture exhibited an 89% retention after heating, whereas the powders with WPC and SPI showed values close to 75%. For the powders with 3% moisture (Fig. 8B), lower stability was observed after 6 hours of treatment, with the most pronounced effect in the WPC samples, which showed only

23% retention.

The stability of the major component, cy-3G, was also analyzed in the low-moisture systems (1%), as shown in Fig. 9A significant decrease in the compound was observed in all cases. The retention was higher in the system with MD (65%), whereas in systems with WPC or SPI, retention was 30% and 45%, respectively. As can be seen, the degradation was much more pronounced for the monomeric compound cy-3G than for the total anthocyanins TMA. Giusti & Wrolstad (2019) mentioned that the pH differential method can detect anthocyanins even with partial degradation, possibly due to molecular associations involving cy-3G monomers. Lao & Giusti (2016) reported a good correlation between HPLC and pH differential methods for anthocyanin determination, though it is possible that HPLC detects fewer polymerized anthocyanins, which may be lost during filtration or due to the formation of larger or insoluble compounds. Lee et al. (2016) reported that discrepancies between pH differential and HPLC results can occur depending on the anthocyanin profile and other components in the fruit, with differences of up to 25% in fruits such as blueberry and cranberry, with pH differential measurements generally yielding higher values than HPLC.

Table 3 summarizes the half-life ($t_{1/2}$) values for anthocyanin degradation in freeze-dried systems with 1 and 3% moisture heated at 85°C. In all cases, $t_{1/2}$ was highest for MD-based systems, followed by SPI and WPC. Increasing moisture content to 3% significantly reduced anthocyanin half-life, highlighting the strong effect of moisture on degradation kinetics. Additionally, at 1% moisture, the time required to reduce total monomeric anthocyanins (TMA) by 50% was approximately three times longer than that required for cy-3G. The results of Oancea (2021) report mean degradation times ranging from 2 to 4 hours in various juices, while in dehydrated systems, the half-life extended up to 16 hours. Pure cyanidin-3-O-glucoside, the major anthocyanin found in fruits and vegetables, exhibited a half-life of 6.4 hours when heated at 80°C for 2 hours. In commercial anthocyanin extracts and açai fruit extracts, purified anthocyanins degraded more rapidly compared to those in crude extracts, due to inter- and intramolecular copigmentation

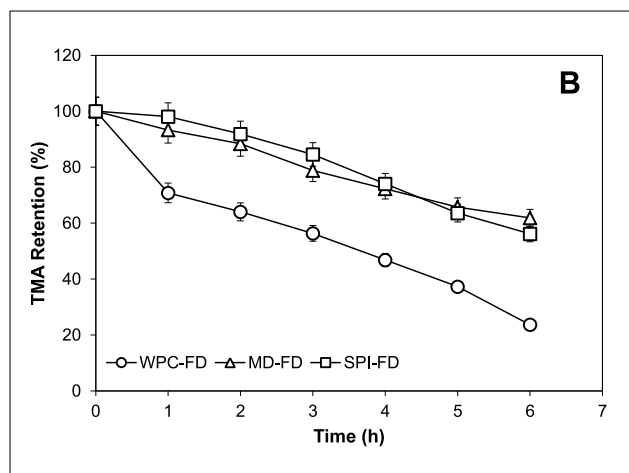
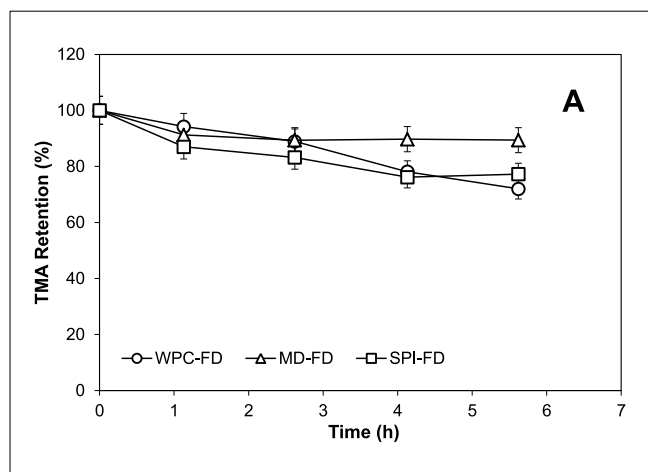


Fig. 8. Retention of total monomeric anthocyanins (TMA) in freeze-dried elderberry systems with different initial moisture contents: 1% (A) and 3% (B), heated during 6 hours at 85 °C.

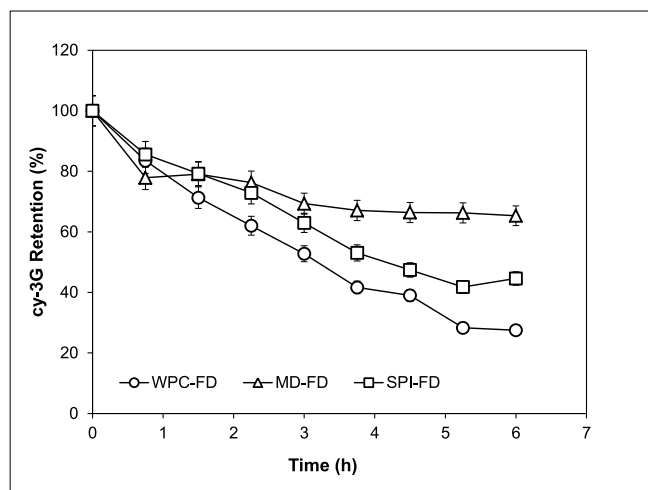


Fig. 9. Retention of cy-3G during heating at 85°C of elderberry encapsulated by freeze-drying using WPC, MD, and SPI in systems with 1% moisture.

reactions. The average half-life of anthocyanin degradation at 60°C is approximately 19.7 hours for fruits and 22.3 hours for vegetables, decreasing significantly with increasing temperature. In a previous study, the average degradation times of elderberry pulp (without

Table 3

Half-life (hours) for the degradation of total monomeric anthocyanins (TMA) and cyanidin-3-glucoside (cy-3G) in freeze-dried systems heated at 85°C.

System	$t_{1/2}$ TMA		$t_{1/2}$ cy-3G
	1% moisture	3% moisture	1% moisture
MD-FD	42.8±1.2	7.7±0.2	11.7±0.3
WPC-FD	11.6±0.3	3.2±0.1	3.1±0.1
SPI-FD	15.5±0.4	6.8±0.2	4.6±0.2

Values are means ± standard error.

additives) were approximately 8 hours at 80°C and 1.6 hours at 90°C, indicating a significant effect of temperature (Busso Casati et al., 2015).

Fig. 10 illustrates the color evolution of the freeze-dried samples (moisture 1%) during heating at 85°C. A high stability of the parameter a^* (related to red color) is observed, although an increase in the hue angle (h°) was noted, indicating a shift to more brownish tones, especially in those containing WPC or SPI (Fig. 10B and 10C). For the powder

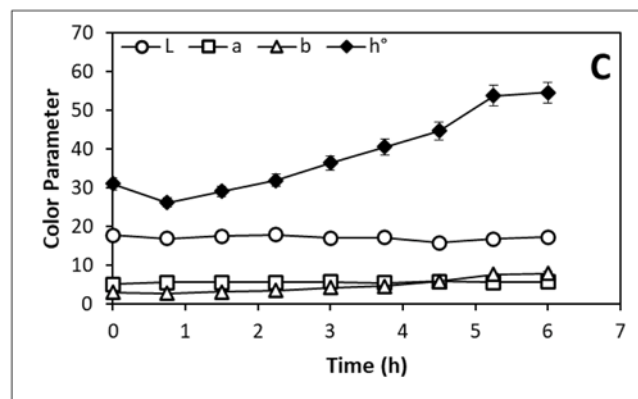
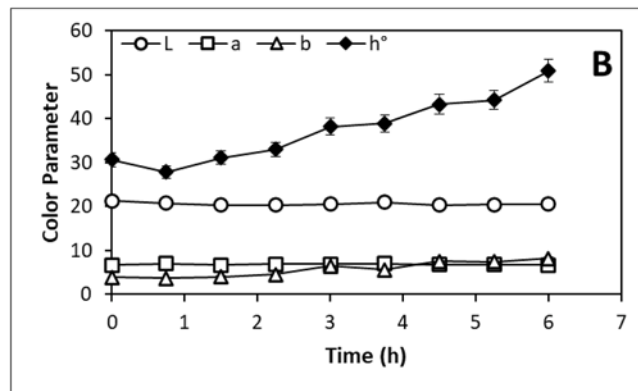
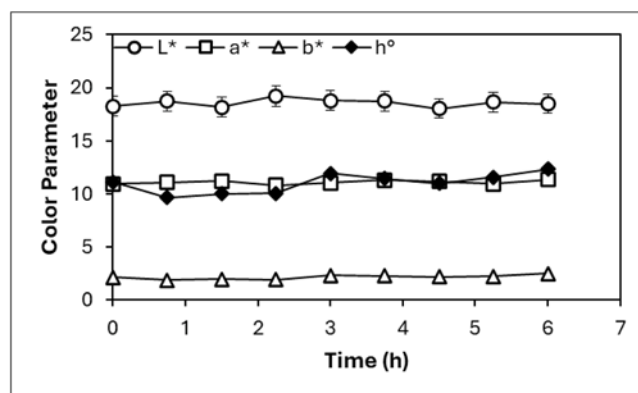


Fig. 10. Evolution of color parameters during heating at 85°C of elderberry freeze-dried powders (1 % moisture) with encapsulating agents: MD (A), SPI (B), WPC (C).

with MD, all parameters were more stable; additionally, higher a^* values and lower b^* values were maintained even after heating. This was also visually evident, with a more intense red color for powders with MD and a more browned appearance in samples obtained with proteins.

An increase in polymeric color was observed in FD-MD powder, with an initial value of 48.7% ($t=0$). The final values for the freeze-dried samples were 56.5% after 8 hours of heating at 85°C, 54.6% after 9 days stored at 44% RH, and 60.3% after 9 days stored at 58% RH. For the spray-dried milk SP-MD system, %PC increased from 45.7% ($t=0$) to 46.4% after 9 days at 44% RH, and to 53.3% after 9 days at 58%. In all cases, a significant increase in polymeric color was observed.

In previous studies it was observed that degradation rate of anthocyanins was significantly higher than that of color (Baeza et al., 2015; Busso Casati et al., 2017). As in previous work, the changes in color during heating (Fig. 10) were less pronounced than the degradation of anthocyanins (Figs. 8 and 9). This may be attributed to color stabilization phenomena arising from different mechanisms, such as copigmentation and microencapsulation, as discussed previously. It is possible that compounds formed in these reactions retain the red color despite a decrease in cy-3G molecules and other anthocyanins present, making these phenomena less visually observable or detectable through color measurements (Baeza et al., 2021; Rein, 2005).

4. Conclusions

The three tested carriers resulted in acceptable retention of anthocyanins (70–85%) after drying. The retention levels observed in spray-dried encapsulates were quite similar to those in freeze-dried samples, confirming that spray drying, when properly conducted, is highly suitable for labile bioactives. Overall, powders encapsulated with MD exhibited higher retention of anthocyanins and monomeric cy-3G during storage or heating compared to those encapsulated with WPC or SPI; however, maintaining a low RH environment is required to preserve their free-flowing state. WPC and SPI encapsulated samples remained predominantly free-flowing, showing only minimal agglomeration under the conditions evaluated. Although the parameter a^* showed high retention across all samples, higher values of hue angle (h°) and the percentage of polymeric color were associated with the polymerized phenolic compounds. Freeze-dried systems showed greater physical stability upon heating compared to spray-dried powders, although the impact of moisture was significant.

Further studies are required to optimize spray-drying processing conditions in order to achieve lower a_w values without compromising anthocyanin retention. Such optimization may enable the production of spray-dried powders with improved stability, capable of withstanding higher temperatures without exhibiting caking over storage periods representative of shelf life. Moreover, the stabilizing effect of proteins against caking, likely associated with their interfacial properties, could be combined with the protective role of maltodextrin as a core material to develop mixed-carrier formulations. These systems may enhance powder flowability, color stability, and anthocyanin retention. Future research should also address the influence of protein characteristics, such as hydrophobicity and solubility, on their performance as encapsulating agents.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to carry out part of the translations of the work (Spanish to English). After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Ethical approval/ patient consent

This manuscript does not contain any studies with human participants or animals performed by any of the authors. Ethics approval or Patient consent was not required for this study.

CRediT authorship contribution statement

Rosa Baeza: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Carolina Busso:** Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Virginia Sánchez:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data availability

The data that has been used is confidential.

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