EFFECT OF TEMPERATURE UNDER THE CO-PRECIPITATION OF THE EXTRACT FROM BLACKBERRY RESIDUE THROUGH SUPERCRITICAL ANTISOLVENT PROCESS

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ABSTRACT

In the present work, extracts of the blackberry residue were encapsulated/co-precipitated with the biodegradable polymer polyvinylpyrrolidone (PVP, MW: 10000) by the supercritical antisolvent process (SAS). Carbon dioxide (CO_2) was used as antisolvent. The ethanolic extract rich in anthocyanins was obtained by ultrasonic assisted extraction. The influence of operating temperature (30-45 °C) was experimentally studied. The decrease in temperature contributed to the production of particles with smaller mean diameter, lower content of residual ethanol and a higher global precipitation yield. Moreover, the increase in temperature contributed to increase theagglomeration of the particles. SEM images showed co-precipitates with shape of irregular agglomerates for the conditions that employed higher temperatures ($T \geq 40$ °C) and spherulite shape for the other conditions ($T \leq 35$ °C). In general, good anthocyanin precipitation yields (≈ 89%) were observed, indicating that the SAS is efficient and selective in the co-precipitation of anthocyanins. The high antioxidant capacity of the precipitates was strongly correlated with their anthocyanin content. The good biodegradability and biocompatibility of the developed particles, as well as the fact that the SAS technique is considered environmentally friendly, makes this biomaterial applicable in nutraceutical and functional products.

Keywords: Co-precipitation, anthocyanins, PVP, SAS, particles.

1. INTRODUCTION

By-products from blackberry processing have been recognized as rich sources of bioactive phenolic compounds [1,2]. These by-products are mainly composed by peel, seeds and stems, which represent around 20% of the whole fruit's mass [3,4]. Flavonoids (such as anthocyanins, kaempferol and quercetin), phenolic acids (gallic, ellagic, caffeic, ferulic and coumaric), ellagitannins and proanthocyanidins are among the main components of blackberry residues, being anthocyanins their main pigments that give this fruits their typical red color [3,5]. The health benefits of polyphenols from blackberry and its products have been investigated due to proven effects against chronic diseases, such as cancer, neurodegeneration, cardiovascular diseases, oxidative stress and block of oxidant signalization paths [6].

Despite the abundance of phenolic compounds in blackberry residues and other vegetable sources, their application is often hampered by their low stability at certain

environmental and process conditions, human digestion and limited solubility in aqueous media [7,8]. In this sense, encapsulation and co-precipitation are attractive techniques to improve the stability, bioavailability and bioaccessibility of such compounds, besides controlling their release rate in the target medium. Many natural or synthetic polymers can be used as encapsulation agents or core materials of bioactive phenolics, once they are biocompatible and biodegradable [9]. Polyvinylpyrrolidone (PVP) stands out for its vast pharmaceutic applicability and for being soluble in water and many organic solvents [10].

In the last two decades, encapsulation, micronization and co-precipitation techniques using pressurized fluids have gained attention, due to their advantages over conventional methods, such as spray-drying, freeze-drying and coacervation [9]. More specifically, the supercritical antisolvent (SAS) process is a promising technique that can be applied to produce particles in nano- and micrometric scale with controlled size and distribution, and without residual solvent [11]. The most used supercritical antisolvent is carbon dioxide (CO_2), because of its particular characteristics [9,12].

Another advantage of SAS for the production of microparticles rich in bioactive compounds is the possibility of improving the process selectivity and yield, since the operation parameters (temperature, pressure, injection nozzle size, antisolvent and solution flow rates) are tunable [13–15]. Although the precipitation of many biomaterials by SAS has been reported [16–18], this technique has not been extensively explored in the processing of chemically complex mixtures, such as vegetal ethanolic extracts + polymer.

Given this context, the present work investigated the effect of temperature on the co-precipitation/encapsulation of an extract rich in anthocyanins obtained from blackberry residues through SAS technique using PVP as encapsulating agent.

2. MATERIALS AND METHODS

2.1 Raw material

The raw material used in this study was blackberry (*Rubus fruticosus*) residue obtained from a pulp processing industry located in Paraibuna, south-eastern Brazil. The residues were freeze-dried, milled, packed in plastic bags and stored under freezing until the extraction.

2.2 Preparation of the extract

Ultrasound assisted extraction (UAE) was used to extract anthocyanins from the blackberry residues. UAE was performed in a bench scale ultrasonic bath (Selecta®, Model 3000513, Barcelona, Spain) at fixed frequency (37 kHz) and power (150 W). For the extraction, 15.0 g of dried and milled blackberry residue were mixed with 200 mL ethanol in 250 mL beakers and sonicated for 10 min at room pressure and temperature. The extracts were filtered under vacuum, collected in a single 10 L vessel and stored at -18 ^{o}C until the SAS process.

2.3 Extract characterization

2.3.1 Total solids

The total solid content (TS) of the extract was determined gravimetrically. Around 6.0 mL of the extract were dried in air circulation stove at 70 °C, until reaching constant mass.

2.3.2 Monomeric anthocyanins

Total monomeric anthocyanins (MA) were determined through the differential pH method described by Giusti and Wrolstad (2001) [19]. MA was expressed as mg cyanidin-3-O-glucoside equivalent (ECy3Gl)/g dry extract (de).

2.3.3 Total phenolic content

The total phenolic content (TP) of the extracts was determined as described by Singleton and Rossi (1965) [20]. The calibration curve was plotted using ethanolic solution of gallic acid with concentrations from 112.5 to 900 mg/L. TP was expressed as mg gallic acid equivalent (GAE)/g de.

2.3.4 Antioxidant capacity - ORAC

The oxygen radical absorbance capacity (ORAC) of the extracts was determined according to Prior et al. (2003) [21] using fluorescein. Trolox was used as standard at concentrations from 12.5 to 200 µM. ORAC was expressed as µmol Trolox equivalent (TE)/g de.

2.4 SAS precipitation

The SAS experiments were designed to analyze the effects of temperature on the co-precipitation of the extract. The influence of temperature was studied in the range 30, 35, 40 and 45 ºC, fixing the other process conditions: pressure (P) = 12.5 MPa; solution flow rate (Q_{sol}) = 2.0 mL/min; antisolvent flow rate (Q_{CO2}) = 2.0 kg/h; PVP concentration in the extract (C_{PVP}) = 0.5%, volume of injected solution (V_{sol}) = 150 mL; and CO_2 drying time to remove residual ethanol from the particles (t_{rem}) = 60 min. The co-precipitation assays were performed in the unit shown in Visentin et al. (2012) [8] and according to the procedure reported in this same work. The produced particles were collected and stored at -18 ^{o}C until being analyzed.

2.5 Characterization of the process and precipitates

2.5.1 Global precipitation yield

The global precipitation yield (GPY) was determined as the mass ratio between the collected particles and the total solids contained in the injected solution.

2.5.2 Particle surface morphology

The surface morphologies of the particles obtained were analyzed through scanning electron microscopy (SEM) in an electronic microscope (Jeol, JSM 820, Burladingen, Germany).

2.5.3 Particle size and distribution

The mean size and distribution of the particles were determined in a laser diffraction unit (Mastersizer 2000, Malvern Instruments Ltd., Model AWMO2002, Malvern, UK).

2.5.4 Residual ethanol

The residual ethanol content (RE) of the particles was measured through the weight loss of a 0.5 g sample kept at 40 ^{o}C for 24 h.

2.5.5 Anthocyanins, total phenolics and antioxidant capacity

MA, TP and ORAC of the precipitates were determined through the methods described in Sections 2.3.2, 2.3.3 and 2.3.4, respectively. The quantifications were performed from around 50 mg of sample diluted in 10 mL of absolute ethanol.

2.5.6 Anthocyanin precipitation yield

The anthocyanin precipitation yield (APY) was calculated as the mass ratio between precipitated anthocyanins and total monomeric anthocyanins injected into the precipitation chamber.

3. RESULTS

3.1 Ethanolic extract

Table 1 shows the characterization of the ethanolic extract obtained by UAE from blackberry residues. As it can be observed, the extract has remarkable MA and TP content, and the ORAC results indicate the high recovery of antioxidant compounds. High TP yields from leaves, grape and blackberry residues were also found by Aybastier et al. (2013) [22], Bonfigli et al. (2017) [23] and Machado et al. (2017) [1], using the same extraction technique.

Table 1 – Characterization of the extract obtained from blackberry residues through UAE: total solids, total phenolics, anthocyanins and antioxidant capacity.

Analyses	Result
TS (%)	1.50
MA (mg Cy3GlE/g de)	6.62 ± 0.69
TP (mg GAE/g de)	53.55 ± 1.55
ORAC (µmol TE/g de)	523.04 ± 9.07

- TS: total solids; MA: monomeric anthocyanins; Cy3GlE: cyanidin-3-*O*-glucoside equivalent; TP: total phenolics; GAE: gallic acid equivalent; TE: trolox equivalent; de: dry extract.
- Results expressed as mean ± standard deviation.

3.2 SAS co-precipitation

SAS at the lowest temperatures (30 and 35 ^{o}C) achieved the highest GPY and the lowest particle diameter and RE, as presented in Table 2 and Fig. 1, and the best distribution of particle size, observed in Fig. 1-E. Moreover, these particles presented spherulite-shaped and lower agglomeration trend than those produced at 40 and 45 ^{o}C.

Temperature has two possible and opposite effects on particle size: the solubility of the solute in ethanol increases with temperature, thus the solvation power of CO_2 is reduced, reducing supersaturation and producing larger particles. On the other hand, higher temperatures enhance mass transfer between solvent and supercritical CO_2, increasing supersaturation and producing smaller particles [24,13]. In this work the first effect prevailed and larger particles were obtained at higher temperatures (Fig. 1).

Table 2 – Global precipitation yield, mean diameter, residual ethanol, monomeric anthocyanins, total phenolics, antioxidant capacity and anthocyanin precipitation yield of the precipitates obtained by SAS process.

SAS condition		GPY (% wt)	$D_{4,3}$ (µm)	RE (% wt)	MA (mg Cy3Gl/g P)	TP (mg GAE/g P)	ORAC (µmol TE/g P)	APY (% wt)
T (°C)	30	64.29 ± 0.54^{A}	39.54 ± 4.12^{A}	2.08 ± 0.30^{A}	8.31 ± 0.72^{A}	58.56 ± 0.51^{A}	2443.66 ± 8.50^{A}	89.62 ± 2.79^{A}
	35	63.70 ± 1.38^{A}	46.75 ± 2.59^{A}	3.23 ± 1.30^{A}	7.95 ± 0.48^{A}	53.11 ± 1.78^{A}	2351.61 ± 9.30^{A}	89.02 ± 3.43^{A}
	40	60.14 ± 0.34^{A}	50.94 ± 5.07^{A}	3.58 ± 0.40^{A}	7.48 ± 0.10^{A}	51.73 ± 0.50^{A}	2303.13 ± 9.88^{A}	87.86 ± 1.16^{A}
	45	58.92 ± 2.83^{A}	54.40 ± 2.18^{A}	4.17 ± 0.08^{A}	7.01 ± 1.41^{A}	51.31 ± 1.81^{A}	1768.16 ± 8.31^{A}	87.16 ± 3.48^{A}

- GPY: global precipitation yield; $D_{4,3}$: mean particle diameter; RE: residual ethanol; MA: monomeric anthocyanin content; TP: total phenolic contents; ORAC: antioxidant capacity; APY: anthocyanin precipitation yield; Cy3Gl: cyanidin-3-*O*-glucoside; GAE: gallic acid equivalent; TE: trolox equivalent; P: particles.
- Results expressed as mean ± standard deviation.
- Equal uppercase letters in the same column indicate no significant difference between the operation conditions, at 5% significance level according to Tukey's test.

Another important effect of temperature concerns particle agglomeration [13], which is related to the reduction of glass transition and melting temperature of the polymer, due to the dissolution of CO_2. At high temperature the particles are first melted and then solidified in the SAS process [7]. In the system PVP+extract+CO_2, temperatures above 40 ^{o}C led to

strongly agglomerated products (Fig. 1-C and 1-D), although some agglomeration is also noted at lower temperatures, where long bridges appear between particles (Fig. 1-A and 1-B). The agglomeration phenomenon can also be due to the plasticizing effect of the compounds of the extract on the polymer.

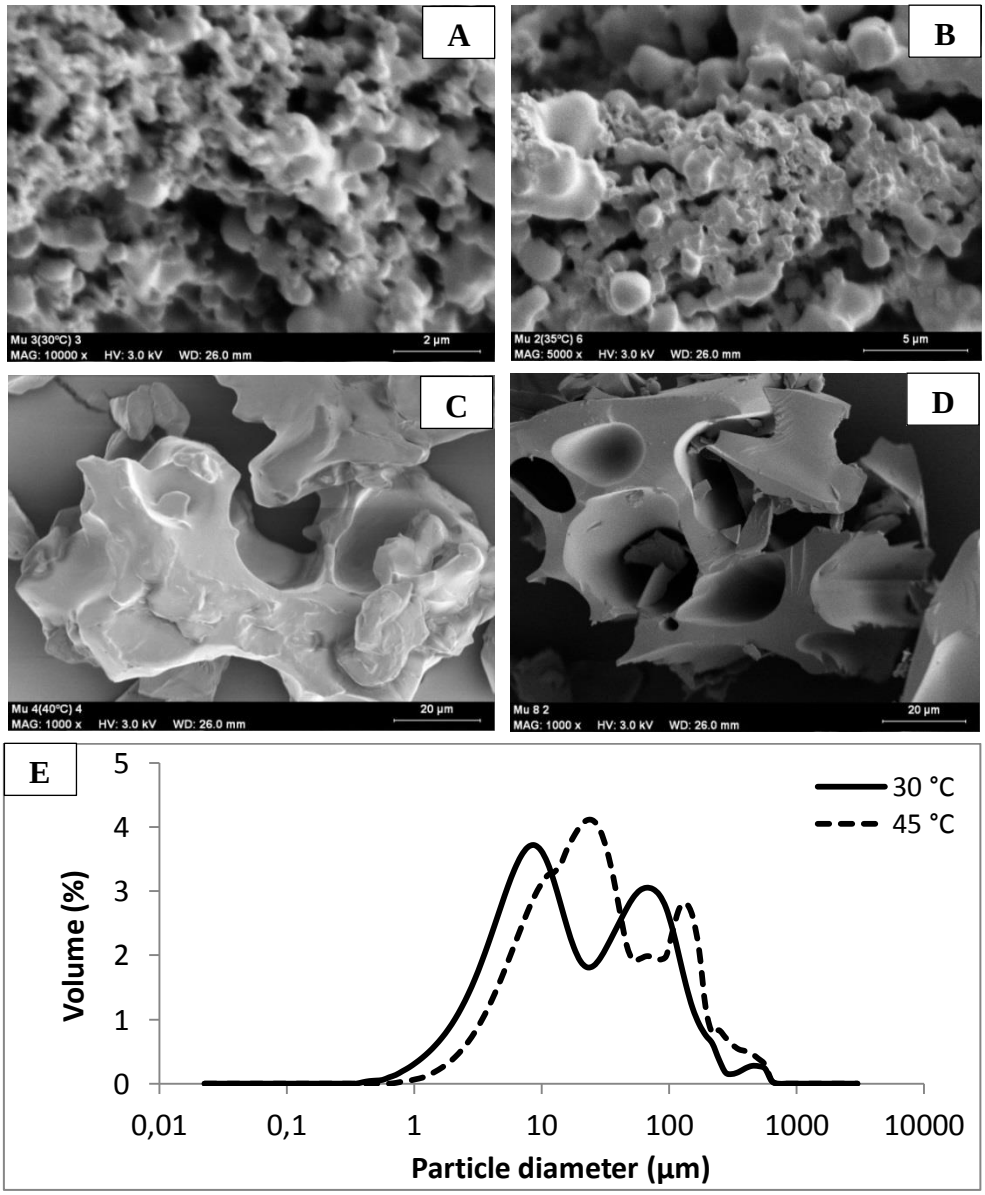

Fig. 1. SEM images of the precipitates obtained by SAS at 30 **(A)**, 35 **(B)**, 40 **(C)** and 45 ºC **(D)** and particle size distribution **(E)**.

It can be noted that the ORAC values are strongly correlated to the TP content (Pearson coefficient r = 0.9508) and MA (r = 0.9391), indicating that the high antioxidant capacity of the precipitates is mainly due to the presence of anthocyanins. A strong correlation was also found between MA and TP (r = 0.9804).

SAS was quite effective and selective in the recovery and encapsulation of anthocyanins, because there is no chemical affinity between anthocyanins and CO_2, and SAS conditions were mild enough to prevent anthocyanin degradation. The efficiency is verified from the high APY reported in Table 2 and the absence of color in the recovered fraction in the separator, which had been removed by CO_2 throughout the process. Thus, SAS processing

allowed a successful fractionation of the extract, enriching it in polyphenols and anthocyanins.

Despite the presented discussions on the possible effects of temperature, statistical analysis shows that the results exposed in Table 2 did not differ significantly at 5% level, according to Tukey's test.

4. CONCLUSION

SAS has been efficiently performed in the co-precipitation of anthocyanin-rich extract obtained from blackberry residues. In the investigated system (PVP + extract + CO_2), the decrease of temperature lead to particles with smaller diameter, better particle size distribution and lower residual ethanol content. Moreover, the increase of such parameters intensified the particles agglomeration. Spherulite-shaped particles were obtained at 30 and 35 °C. Good anthocyanin precipitation yields were achieved, together with a selective removal of oily compounds from the extract, indicating that SAS is an efficient and selective process to co-precipitate anthocyanins from blackberry residues with remarkable antioxidant capacity, resulting in products that could be applied in food and nutraceutical industries.

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