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Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents



Luiza Siede Kuck, Caciano Pelayo Zapata Noreña*

Laboratório de Engenharia de Processos em Alimentos, Instituto de Ciência e Tecnologia de Alimentos (ICTA), Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Prédio 43.212 Campus do Vale, CEP 91501-970 Porto Alegre, RS, Brazil

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ABSTRACT

Bordo grape skin extract was microencapsulated by spray-drying and freeze-drying, using gum arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) as encapsulating agents. Total phenolics and total monomeric anthocyanin, antioxidant activity, color, moisture, water activity (a_w), solubility, hygroscopicity, glass transition temperature (T_g), particle size, and microstructure of the powders were evaluated. The retention of phenolics and anthocyanins ranged from 81.4% to 95.3%, and 80.8% to 99.6%, respectively, while the retention of antioxidant activity ranged from 45.4% to 83.7%. Treatments subjected to spray-drying had lower moisture, a_w , and particle size, and greater solubility, while the freeze-dried samples were less hygroscopic. T_g values ranged from 10.1 to 52.2 °C, and the highest values corresponded to the spray-dried microparticles. The spray-dried particles had spherical shape, while the freeze-dried powders showed irregular structures. The spray drying technique and the use of 5% PHGG and 5% PD has proven to be the best treatment.

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1. Introduction

Grape is one of the main natural sources of phenolic compounds, thus it has been associated to important health benefits (Toaldo et al., 2013). American grape varieties (*Vitis labrusca* L.) are widely cultivated in Brazil, mainly for the production of juices and table wines, and Bordo, Concord and Isabel are the most cultivated varieties. Bordo variety is characterized by high amounts of polyphenols, intense purple–red color, and fruity aroma (Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011).

Although the grape skin accounts for 50% by weight of a grape berry, after production of juices and wines, most peel and seeds are removed, which have been found to contain higher amounts of polyphenols than the pulp (Toaldo et al., 2013).

The polyphenols found in Bordo grape skins are flavonoids, especially anthocyanins, some monomers and dimers of the group of flavan-3-ols, and non-flavonoids such as hydroxycinnamic acids and stilbenes, particularly resveratrol (Lago-Vanzela et al., 2011). These compounds possess antioxidant activity, whose function is

to neutralize and prevent the formation of free radicals (Rice-Evans, Miller, & Paganga, 1997), resulting in health benefits, such as cardioprotective effect, anti-cancer, anti-diabetes, anti-microbial, and anti-inflammatory properties, besides acting against neurodegenerative diseases, kidney disease, and aging (Rodrigo, Miranda, & Vergara, 2011).

In food processing and storage, the polyphenols are unstable under various conditions such as presence of oxidative enzymes, high temperature conditions, pH, moisture, presence of light and oxygen (Fang & Bhandari, 2011). In this context, encapsulation is an alternative process to increase the stability of susceptible compounds, protecting them from adverse environmental conditions.

Although various types of encapsulating agents can be used, some characteristics should be observed, including their ability to form films, biodegradability, resistance to gastrointestinal tract, viscosity, solids content, hygroscopicity, and cost (Silva, Stringheta, Teófilo, & Oliveira, 2013).

The most common wall materials for encapsulating juices and fruit extracts are maltodextrins and gum Arabic (GA) (Tonon, Brabet, Pallet, Brat, & Hubinger, 2009). GA is used mainly due to their high water solubility characteristics, low viscosity, and emulsifying properties (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005). However, despite the good properties, an encapsulating agent may hardly present all the characteristics required. Thus,

* Corresponding author.

E-mail address: czapatan@ufrgs.br (C.P.Z. Noreña).

the use of wall material mixtures is an alternative to further increase the efficiency of the encapsulation process.

The partially hydrolyzed guar gum (PHGG) and polydextrose (PD) are potential encapsulating agents, despite being little studied for encapsulation of food. The PHGG has no taste, color or odor, low viscosity, and is highly soluble in water (Kapoor & Juneja, 2009). PD is a low-calorie polysaccharide, highly soluble in water, odorless, colorless in solution, and does not give food sweet taste, thus not interfering with the application in foods (Mitchell, 1996).

This study aimed to encapsulate the Bordo grape skin phenolic extract by spray-drying and freeze-drying, using different combinations of gum Arabic, partially hydrolyzed guar gum, and polydextrose as encapsulating agents, and to evaluate the physical, chemical and morphological characteristics of the microparticles.

2. Materials and methods

2.1. Materials

The grapes were obtained from a farm located in Cotiporã, Rio Grande do Sul, Brazil, in 2014. The grape clusters were selected, washed in water, packed in polyethylene bags and frozen at $-18\text{ }^{\circ}\text{C}$ until use. The encapsulating agents were: gum Arabic (Instantgum BA, Nexira Brasil Com Ltda., Brasil), Polydextrose (MasterSense Ing Alim Ltda., Brasil), and partially hydrolyzed guar gum (Sunfiber R, R & S Blumos, Com. Prod. Alimentícios Ltda., Brasil). DPPH (2,2-diphenyl-1-picryl-hydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), neocuproine, and gallic acid were purchased from Sigma–Aldrich. All reagents were analytical grade.

2.2. Preparation of the extracts

The grapes were thawed and blanched at $80\text{ }^{\circ}\text{C}$ for 5 min and cooled on ice/water bath for 3 min. After separating the seeds from pulp, the polyphenols were extracted from skins using water acidified with citric acid (2%, w/v). Peels were mixed with solvent at a ratio of 1:3 (w/v), the mixture was ground in a blender, and kept in the dark for 20 h at $22 \pm 1\text{ }^{\circ}\text{C}$. After this period, the extract was filtered with Whatman filter paper No. 1 for separating the solid residue.

2.3. Preparation of microencapsulated powders

Four dispersions were prepared from the extract, as follows: A (5% GA and 5% PD), B (10% GA), C (5% PHGG and 5% PD), and D (10% of PHGG). The dispersions were prepared at 6500 rpm for 5 min using an Ultra-Turrax (T25, IKA). Subsequently, dispersions were encapsulated by spray-drying and freeze-drying, totaling eight treatments.

Encapsulation by spray-drying was performed using a dual fluid atomizer, pneumatic type (Mini Spray Dryer LM MSDI 1.0 LABMAQ, Brasil), with a feed nozzle diameter of 1.0 mm. Feeding was carried out using a peristaltic pump at a flow rate of 0.60 L/h, drying air temperature $140\text{ }^{\circ}\text{C}$, air pressure 3.5 kgf/cm^2 , and air flow rate 40.5 L/h.

For freeze-drying, the dispersions were frozen in ultra-freezer at $-68\text{ }^{\circ}\text{C}$ for 24 h. Then, the samples were placed in a freeze-dryer (LIOTOP L101, Liobras, Brasil) and freeze-dried at $-57\text{ }^{\circ}\text{C}$, at vacuum pressure less than $20\text{ }\mu\text{mHg}$ for 48 h. After freeze-drying, the samples were crushed using a mortar and pestle.

The spray-dried and freeze-dried samples were immediately placed in polyethylene bags, sealed and placed in aluminum pouches. The samples were stored in a desiccator containing silica for further analyses, which were performed in triplicate.

2.4. Spectrophotometric analysis

The extract and the microencapsulated powders were diluted with distilled water in different proportions according to the needs of each analysis. All readings were performed in spectrophotometer Genesys S10, Thermo Scientific.

2.4.1. Total phenolics

The total phenolics were determined using the Folin–Ciocalteu reagent (Singleton & Rossi, 1965). The readings were made in spectrophotometer at 765 nm, and the results were expressed as mg gallic acid (GAE) per g sample on dry basis.

2.4.2. Total monomeric anthocyanins

The total monomeric anthocyanins were determined according to the methodology described by Lee, Durst, and Wrolstad (2005). The extract and the diluted powders were mixed with buffer solutions pH 1.0 and 4.5. The absorbance was measured in spectrophotometer at 520 and 700 nm. The results were expressed as mg malvidin-3,5-diglucoside per g sample on dry basis, using molar absorptivity $37,000\text{ l/cm/mol}$, and molecular weight 724.5 g/mol (Toaldo et al., 2013).

2.4.3. DPPH scavenging capacity

The antioxidant activity was determined by DPPH assay as reported by Brand-Williams, Cuvelier, and Berset (1995). The absorbance was measured in a spectrophotometer at 515 nm after 3 h of reaction. A Trolox standard curve was previously prepared and the results were expressed as μmol Trolox equivalent (TE) per g sample on dry basis.

2.4.4. Cupric-reducing antioxidant capacity (CUPRAC)

The CUPRAC assay was performed according to the methodology described by Apak, Güçlü, Özyürek, and Karademir (2004). The absorbance was measured in a spectrophotometer at 450 nm 60 min after the reaction. A Trolox standard curve was made and the results were expressed as μmol TE per g sample on dry basis.

2.4.5. Hydroxyl radical-scavenging activity (HRSA)

The antioxidant activity by HRSA assay was performed as described by Meng, Fang, Qin, Zhuang, and Zhang (2012). The absorbance was measured in spectrophotometer at 593 nm. The result was expressed as % scavenging, and calculated according to the following equation:

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100\%.$$

2.5. Colorimetric analysis

The color of the microencapsulated powders was measured using a colorimeter (CR400/410, Minolta Co. Ltd., Osaka, Japan), according to the CIELAB (L^* , a^* , b^*) system, where L^* indicates lightness ($0 = \text{black}$ and $100 = \text{white}$), a^* and b^* are coordinates for green ($-a^*$)/red ($+a^*$), and blue ($-b^*$)/yellow ($+b^*$). The instrument was calibrated using white ceramic plate. Hue angle ($H^* = \tan^{-1} b^*/a^*$) was calculated, which indicates the color of the sample (0° or $360^{\circ} = \text{red}$, $90^{\circ} = \text{yellow}$, $180^{\circ} = \text{green}$, and $270^{\circ} = \text{blue}$), while Chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$) indicates color's purity or saturation.

2.6. Determination of the physical properties of the microencapsulated powders

Moisture content of the samples was calculated from the weight loss after heating the sample at $105\text{ }^{\circ}\text{C}$ according to AOAC (1990).

Water activity (A_w) was measured by direct reading in electronic meter (Aqualab 3TE-Decagon, Pullman, USA) according to AOAC (1990).

Solubility was determined according to Cano-Chauca et al. (2005) with some modifications. 1 g sample and 100 mL distilled water were mixed in a beaker and stirred in a magnetic stirrer (Lab Disc, IKA) for 5 min. Then, the solution was placed in a tube and centrifuged at $3000\times g$ (Thermo 16R, Thermo Scientific) for 15 min. An aliquot of 25 mL of the supernatant was transferred to a 50 mL beaker and oven-dried at 105 °C to constant weight. The solubility (%) was calculated by weight difference.

For determination of the hygroscopicity, 1 g of powder sample was weighed and placed in an airtight glass container with saturated NaCl (relative humidity of 75%), and stored in an incubator chamber (411/FDP Ethik Technology, Brasil) at 25 °C (Tonon, Brabet, & Hubinger, 2008). After 1 week, samples were weighed every 24 h until equilibrium was reached. The hygroscopicity was expressed as percentage (%) or 1 g of adsorbed moisture per 100 g dry solids (g/100 g) (Caparino et al., 2012).

The glass transition temperature (T_g) of the samples was determined by differential scanning calorimetry (DSC) (DSC Q2000, TA Instruments, New Castle, DE). Approximately 8 mg sample were placed in aluminum hermetic pans (Tzero, TA Instruments). An empty aluminum pan was used as reference. The purge gas was ultra-pure nitrogen (flow 50 mL/min). The temperature ranged from –80 °C to 120 °C at a heating rate of 40 °C/min. The T_g values were calculated using the software TA Universal Analysis 5.5.3.

2.7. Particle morphology and size distribution

The particle structure was evaluated by scanning electron microscope (JSM 6060, JEOL Ltd., Japan). The samples were fixed on stubs using double-sided tape carbon, metallized with carbon, and further examined in the microscope operating at a voltage of 8 kV and $300\times$ magnification for the freeze-dried powders, and $2000\times$ for spray-dried powders.

The particle size distribution was determined in particle size analyzer by laser diffraction (CILAS 1180, Compagnie Industrielle de Lasers, France). Samples were dispersed in isopropyl alcohol under constant stirring and sonicated using ultrasound equipment for 30 s. The mean diameter ($D[4,3]$) and the equivalent volume diameters at 10%, 50%, and 90% cumulative volume were determined. The particle size distribution in the powder (span) was calculated using the following equation: $\text{Span} = [(d_{90} - d_{10})/d_{50}]$ (Fernandes, Borges, & Botrel, 2014).

2.8. Statistical analysis

Data were subjected to ANOVA, and the treatments to Tukey's multiple comparison tests, using the program SAS 9.3.

3. Results and discussion

3.1. Total phenolics, total monomeric anthocyanins and antioxidant activity

The total phenolics, total monomeric anthocyanins and the antioxidant activity of grape skin aqueous extract and microencapsulated powders are shown in Table 1.

Regarding the total phenolics content, the values were significantly lower for all microencapsulated powders when compared to the original extract. The retention percentages ranged from 95.3% to 81.4% for the spray-dried treatment with 10% GA, and the freeze-dried treatment with 5% PHGG and 5% PD, respectively.

Polyphenols losses during the drying process can occur due to several factors. In the spray-drying process, phenolics losses are related to exposure to oxygen and high temperatures, which may have caused degradation and polymerization thereof. In the lyophilization process, the grinding of the freeze-dried product may lead to degradation compounds, since the product's exposure may induce the occurrence of oxidation reactions. van Golde, van der Westelaken, Bouma, and van de Wiel (2004) freeze-dried wine samples, and found approximately 70% of the original phenolics. The authors suggested that further studies are needed to investigate at which step the losses occur.

Both the spray drying and freeze drying may result in the formation of microspheres, which is a dispersion of the active compounds within a structure made of a continuous phase of one or more polymers (Mathiowitz, Kreitz, & Brannon-Peppas, 1999). In addition, when the microparticles are produced by spray drying, rapid evaporation of water caused by the use of high temperatures may result in formation of fissures and concavities in the surfaces, which cause a premature release of the encapsulated component, and this way its degradation (Ramírez, Giraldo, & Orrego, 2015). In the other hand, freeze drying leads the formation of pores in the microparticle, due to the sublimation of water. In this study, spray dried samples had a reduction of 4.7–18.4%, while that freeze dried samples had a reduction of 6.4–18.6% in the total phenolics. So, there was not a great difference in the losses of polyphenols between the two methods. Although a reduction of total phenolics was observed in this study, a good retention (95.3–81.4%) was obtained, with percentages close to or higher than the values found in literature, as 94.5% retention of phenolics from açai pulp encapsulated with GA (Tonon et al., 2009).

The spray-dried treatment containing 10% GA presented the highest retention of phenolic compounds. The high efficiency of encapsulation with gum Arabic is related to its structure, since it is a highly branched heteropolymer of sugar, having a small amount of protein covalently linked to the carbohydrate chain, acting as an excellent agent film formation, which allows effective encapsulation of molecules (Burin, Rossa, Ferreira-Lima, Hillmann, & Bordignon-Luiz, 2011). However, no significant differences were observed for the freeze-dried treatments with 5% GA and 5% PD, and spray-dried treatments with 5% PHGG and 5% PD, indicating the potential of PHGG and PD for encapsulation of phenolic compounds.

The retention of anthocyanins in the powder was in the range of 99.58–80.75%. The higher retention was found for both spray-dried treatments made with PHGG, with no significant differences when compared to the initial extract. The other treatments did not differ significantly from each other. Ferrari, Germer, Alvim, Vissotto, and Aguirre (2012) found 78.2% retention of anthocyanins in blackberry extract encapsulated with 7% GA, and Souza, Thomazini, Balieiro, and Fávoro-Trindade (2015) found 88.3–93.8% retention of anthocyanins when 10% maltodextrin was used for encapsulating grape skin aqueous extract.

The antioxidant activity of the microencapsulated powders by DPPH assay decreased when compared to the initial extract, and the retention ranged from 45.4% to 59.1%, with the highest values for the freeze-dried samples with 5% GA and 5% PD, followed by the spray-dried samples with 5% PHGG and 5% PD, with no significant differences between them, and the spray-dried sample with 5% GA and 5% PD. The values were according to those found by Souza et al. (2015), 69.7 at 74.9 $\mu\text{mol TE/g}$ dry sample in the encapsulation of Bordo grape skin phenolic extract with 10% maltodextrin. When measured by the CUPRAC assay, the retention of the antioxidant activity was in the range of 73.1 at 83.7% when compared to the initial extract. The highest values were found for the spray-dried treatments with 10% GA, and 5% PHGG and 5% PD. Both the extract as microparticles had lower antioxidant activity by DPPH

Table 1
Total phenolics (TPC), total anthocyanins (ACN), and antioxidant activity (by DPPH, CUPRAC and HRSA) of the grape skin aqueous extract microencapsulated with gum Arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) by spray drying and freeze-drying.

Treatments	TPC*	ACN**	DPPH***	CUPRAC***	HRSA (%)
Extract	26.26 ± 0.12 ^a	21.15 ± 1.06 ^a	128.12 ± 4.47 ^a	181.10 ± 2.70 ^a	66.98 ± 0.18
1	23.59 ± 0.17 ^b	17.18 ± 0.17 ^b	69.47 ± 1.68 ^{b,c}	143.41 ± 3.00 ^b	78.94 ± 0.24 ^a
2	25.03 ± 0.07 ^c	18.41 ± 1.02 ^b	60.13 ± 2.06 ^{d,e}	151.62 ± 1.49 ^c	79.64 ± 0.24 ^a
3	24.57 ± 0.05 ^c	18.38 ± 0.28 ^b	75.72 ± 0.56 ^f	141.75 ± 1.21 ^b	84.67 ± 0.17 ^b
4	22.61 ± 0.07 ^{d,e}	17.27 ± 0.40 ^b	58.20 ± 1.86 ^e	132.93 ± 4.14 ^d	73.50 ± 0.75 ^c
5	23.39 ± 0.16 ^{c,d}	21.05 ± 0.08 ^a	73.42 ± 2.38 ^f	150.73 ± 0.58 ^c	82.86 ± 0.20 ^{d,e}
6	21.43 ± 0.04 ^f	20.84 ± 0.15 ^a	65.48 ± 1.20 ^{c,d}	133.52 ± 0.96 ^d	84.40 ± 0.61 ^b
7	21.37 ± 0.09 ^f	17.07 ± 0.10 ^b	58.26 ± 1.04 ^e	132.43 ± 0.99 ^d	83.94 ± 0.40 ^{b,d}
8	21.89 ± 0.15 ^{e,f}	18.25 ± 0.28 ^b	50.82 ± 1.81 ^g	140.57 ± 0.93 ^b	82.20 ± 0.23 ^e

Different letters within the same column indicate significant differences ($p < 0.05$). * results expressed as mg GAE/g sample on dry basis, ** mg malvidin-3,5-di glucoside/g sample on dry basis, TE *** $\mu\text{mol/g}$ sample on dry basis. The weight of the encapsulating agents was discounted. Treatment 1: spray-dried, with 5% GA and 5% PD; Treatment 2: spray-dried, with 10% GA; Treatment 3: freeze-dried, with 5% GA and 5% PD; Treatment 4: freeze-dried, with 10% GA; Treatment 5: spray-dried, with 5% PHGG and 5% PD; Treatment 6: spray-dried, with 10% PHGG; Treatment 7: freeze-dried, with 5% PHGG and 5% PD; and Treatment 8: freeze-dried, with 10% PHGG.

when compared to CUPRAC assay, which has also been observed by other authors (Meng et al., 2012).

For the antioxidant activity by HRSA, the highest values were found for the freeze-dried treatment with 5% GA and 5% PD, spray-dried treatment with 10% PHGG, and freeze-dried treatment with 5% PHGG and 5% PD, all with values near 84%. Zheng, Ding, Zhang, and Sun (2011) used a similar method to evaluate the antioxidant activity of microencapsulated bayberry polyphenols, and found 83.13% activity when analyzing the diluted powder at a concentration of 6 mg/mL.

3.2. Colorimetric analysis

Table 2 shows the results of the color parameters. A difference in color was evidenced by the L^* values, once the freeze-dried powder was darker, with L^* values between 20 and 22, and the spray-dried powders were clearer with values between 37 and 40. During freeze-drying, the products' color can be related to the pore size on the surface (Aguilera & Lillford, 2008). Freezing leads to the formation of ice crystals, altering the pore structure after water sublimation. Thus, the surface of these particles is darker due to light scattering properties of the empty spaces after sublimation (Aguilera & Stanley, 1999). The kind of wall material used did not influence the results of freeze dried treatments, while that in the spray dried, the use of polydextrose decreased the luminosity values. This may be consequence of the formation of some dark compounds during the drying process.

The results obtained for the values of a^* and b^* show that all data were located in the fourth quadrant ($+a^*$, $-b^*$), indicating a tendency to blue and red, characteristic of anthocyanins. A similar result was found for the Bordo grape skin aqueous extract encapsulated with 10% maltodextrin by spray-drying (Souza et al., 2015). The spray dried samples had a deeper red color, as indicate

the larger positive a^* values, than the freeze dried samples. In relation to b^* values, the difference between spray and freeze dried samples were lower.

The Hue angle values between 348 and 359 confirmed the tendency of the samples to the red hue (Hue = 360), with the highest values for freeze-dried treatments, which were also darker. Chroma was higher for the spray-dried sample, which means that these samples have higher saturation or color purity, which is a desirable characteristic.

3.3. Physical properties of the microencapsulated powders

The parameters moisture content, water activity, and hygroscopicity are essential for powder stability and storage, while solubility is associated with reconstitution of the powder (Tonon et al., 2008).

As can be seen in Fig. 1A, the lowest moisture contents were found for the spray-dried treatments, which did not differ significantly from each other, and ranged from 2.41% to 2.57%. Souza et al. (2015) found 2.96% moisture for grape extract microencapsulated with 10% maltodextrin by spray drying at 170 °C. In contrast, the moisture content of the freeze-dried powders was higher than that the spray-dried powders, ranging from 7.65% to 8.06%, with no significant difference between them. Freezing temperatures lower than -40 °C result in rapid freezing, and hence the pores in the outer layer are smaller, which may hinder mass transfer and act as a barrier against sublimation, resulting in increased moisture retention (Ezhilarasi, Indrani, Jena, & Anandharamkrishnan, 2013).

The water activity of the treatments (Fig. 1B) ranged from 0.160 to 0.360, and all treatments were significantly different from each other, except for the spray-dried samples with 10% GA, and 5% GA and 5% PD. The highest aw values, about 0.3, were found for the

Table 2
Color parameters (L^* , a^* , b^* , Hue angle, and Chroma) of the grape skin aqueous extract microencapsulated with gum Arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) by spray drying and freeze drying.

Treatments	L^*	a^*	b^*	Hue (°)	Chroma
1	38.06 ± 0.60 ^a	46.28 ± 0.23 ^a	-7.42 ± 0.13 ^a	350.89 ± 0.11 ^a	46.87 ± 0.24 ^{a,b}
2	40.17 ± 0.21 ^b	46.29 ± 0.05 ^a	-9.57 ± 0.02 ^b	348.34 ± 0.04 ^b	47.27 ± 0.04 ^a
3	22.46 ± 0.13 ^c	27.11 ± 0.13 ^b	-0.44 ± 0.07 ^c	359.06 ± 0.14 ^c	27.11 ± 0.13 ^c
4	22.28 ± 0.12 ^{c,d}	28.36 ± 0.11 ^c	-2.44 ± 0.02 ^d	355.08 ± 0.02 ^d	28.47 ± 0.11 ^d
5	37.89 ± 1.11 ^a	46.03 ± 0.11 ^a	-5.91 ± 0.02 ^e	352.68 ± 0.02 ^e	46.41 ± 0.11 ^b
6	40.33 ± 0.14 ^b	46.22 ± 0.12 ^a	-6.33 ± 0.06 ^f	352.20 ± 0.06 ^f	46.66 ± 0.12 ^b
7	20.63 ± 0.44 ^d	25.36 ± 0.24 ^d	-1.02 ± 0.02 ^g	357.70 ± 0.03 ^g	25.38 ± 0.24 ^e
8	22.60 ± 0.15 ^{c,d}	29.08 ± 0.24 ^e	-0.46 ± 0.05 ^c	359.10 ± 0.09 ^c	29.08 ± 0.24 ^f

Different letters in the same column indicate significant differences ($p < 0.05$). Treatment 1: spray-dried, with 5% GA and 5% PD; Treatment 2: spray-dried, with 10% GA; Treatment 3: freeze-dried, with 5% GA and 5% PD; Treatment 4: freeze-dried, with 10% GA; Treatment 5: spray-dried, with 5% PHGG and 5% PD; Treatment 6: spray-dried, with 10% PHGG; Treatment 7: freeze-dried, with 5% PHGG and 5% PD; and Treatment 8: freeze-dried, with 10% PHGG.

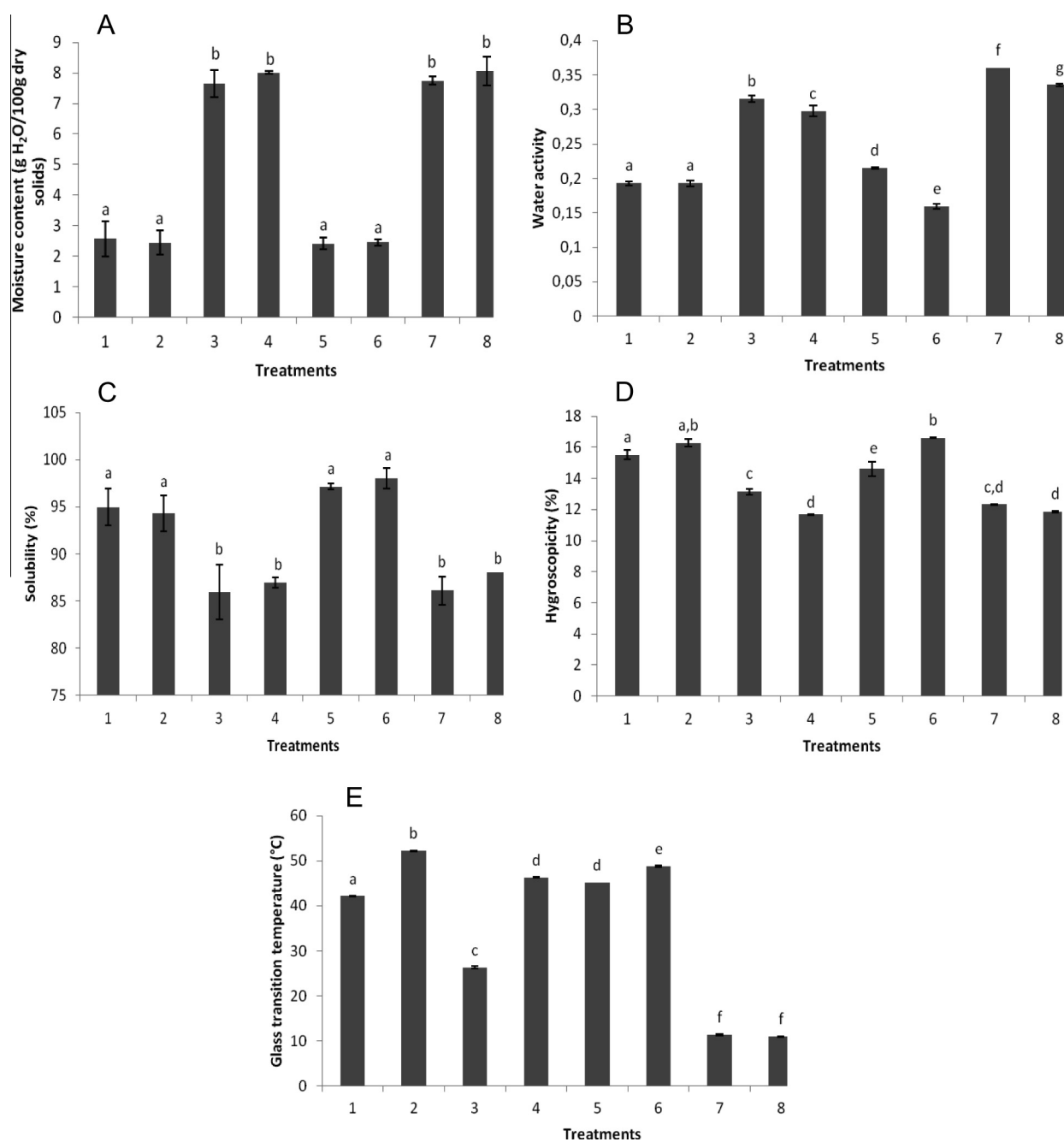


Fig. 1. Physical properties of grape skin aqueous extract microencapsulated with gum Arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) by spray-drying and freeze-drying. Treatment 1: spray-dried, with 5% GA and 5% PD; Treatment 2: spray-dried, with 10% GA; Treatment 3: freeze-dried, with 5% GA and 5% PD; Treatment 4: freeze-dried, with 10% GA; Treatment 5: spray-dried, with 5% PHGG and 5% PD; Treatment 6: spray-dried, with 10% PHGG; Treatment 7: freeze-dried, with 5% PHGG and 5% PD; and Treatment 8: freeze-dried, with 10% PHGG.

freeze-dried samples. Gurak, Cabral, and Rocha-Leão (2013) found aw of 0.43 for grape juice microencapsulated with maltodextrin by freeze-drying, while Tonon et al. (2008) found aw of 0.244 for acai pulp encapsulated with GA by spray-drying.

In this study, the kind of wall material used had no significant effect on the solubility of the powders, while that the applied drying method had a significant influence. The powders obtained by spray-drying exhibited higher solubility than the freeze-dried powders (Fig. 1C), which ranged from 94.3% to 97.99% and 85.96% to 88%, respectively, with no significant differences between the spray-dried or freeze-dried treatments. Souza et al. (2015) found solubility of approximately 91% for the Bordo grape

aqueous extract encapsulated with 10% maltodextrin by spray-drying at 130 °C. The higher solubility may be related with the smaller particle size obtained, because the smaller the particle size the greater the surface area available for hydration.

The hygroscopicity values (Fig. 1D) ranged from 11.67% to 16.61%, values close to those found in the literature for similar encapsulated products. Silva et al. (2013) found hygroscopicity of 17.75% for jaboticaba bark extract encapsulated with a mixture of GA and maltodextrin, while Souza et al. (2015) found hygroscopicity values between 12.4% and 17.4% for Bordo grape pigment encapsulated with maltodextrin. The hygroscopicity of juices and powdered fruit extracts is related to the low molecular weight sug-

ars and organic acids with low Tg, and moisture content, which leads to a high hygroscopicity (Bhusari, Muzaffar, & Kumar, 2014; Ferrari et al., 2012; Tonon et al., 2008).

Among the spray-dried samples, the lowest hygroscopicity was observed in the treatment with 5% PHGG and 5% PD. The freeze-dried treatments showed the lowest hygroscopic values, despite having the highest moisture contents. Khazaei, Jafari, Ghorbani, and Kakhki (2014) found no relationship between moisture and hygroscopicity of saffron petal's anthocyanins microencapsulated with gum Arabic and maltodextrin by freeze-drying. The lower hygroscopicity values found for the freeze-dried powders can be related to the larger particle size when compared to the spray-dried product (Man, Irwandi, & Abdullah, 1999), because the larger the particle size, the lower the exposed surface area and consequently the lower the water absorption is (Tonon et al., 2009).

Tg values are related to the powder stability during storage, thus the higher the Tg value, the greater the powder stability. The Tg of the microencapsulated powders (Fig. 1E) ranged from 10.98 to 52.2 °C. All freeze-dried treatments, except the treatment with 10% GA, had low Tg values (11–26 °C). On the other hand, the spray-dried treatments showed values between 42 and 52 °C. Tg is affected by several factors, such as molecular weight, chemical structure, and moisture content of the material (Fernandes et al., 2014). In juices and fruit extracts the presence of high amounts of low molecular weight sugars and acids results in low Tg (Adhikari, Howes, Bhandari, & Troung, 2004). However, as consequence of adding carrier material, the final Tg is also related to the molecular weight of polymers presents in this material (the Tg increases with increasing molecular weight), as well as water content. Among the three encapsulating agents used in this study, PD has the lowest molecular weight (less than 5000 Da) (Mitchell, 1996), followed by PHGG (average of 20,000 Da) (Kapoor & Juneja, 2009) and GA (average of 350,000 Da) (Al-Assaf, Phillips, & Williams, 2005). The addition of PD reduced Tg, except for the freeze-dried treatment with PHGG (5% PD and 5% PHGG), which did not differ significantly from the treatment with 10% PHGG. The freeze-dried treatments had lower Tg due to their higher moisture contents. However, this was not observed in the freeze-dried treatment with 10% GA, probably due to compensation for the higher GA content, which has the highest molecular weight among the encapsulating agents used.

3.4. Particle morphology and size distribution

The particle size distribution in all treatments was bimodal. Table 3 shows the average size and the particle size distribution of the eight treatments. The freeze-dried microparticles showed

Table 3
Average diameter and particle size distribution (Span) of the grape skin aqueous extract microencapsulated with gum Arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) by spray-drying and freeze-drying.

Treatments	Average diameter [D(4,3)] (μm)	Span
1	8.35	1.91
2	4.82	1.98
3	123.39	3.60
4	104.30	2.79
5	14.25	2.26
6	8.48	1.94
7	684.85	3.32
8	485.12	5.99

Treatment 1: spray-dried, with 5% GA and 5% PD; Treatment 2: spray-dried, with 10% GA; Treatment 3: freeze-dried, with 5% GA and 5% PD; Treatment 4: freeze-dried, with 10% GA; Treatment 5: spray-dried, with 5% PHGG and 5% PD; Treatment 6: spray-dried, with 10% PHGG; Treatment 7: freeze-dried, with 5% PHGG and 5% PD; and Treatment 8: freeze-dried, with 10% PHGG.

the greatest diameter (104.3–684.9 μm) while a smaller diameter (4.8–14.3 μm) was observed for the spray-dried microparticles. According to Man et al. (1999), the particle diameter of the spray-dried product ranges from 1 to 15 μm, which can reach 300 μm in freeze-dried products. The freeze-dried microparticles with PHGG showed particle size above 300 μm, which was higher than the microparticles with GA. The largest particle size of the freeze-dried samples is due to the low process temperature, and the lack of strength to break the frozen drops or to alter the surface during drying (Chen, Chi, & Xu, 2012). In addition, the process used to reduce the particle size, which was manually done with a mortar and pestle may have affected the particle size of the microparticles.

It can also be seen that all treatments with PD had larger particle size when compared to the treatments without PD. For both spray-dried as freeze-dried treatments, the lowest diameter was found for the samples encapsulated only with GA. According to Masters (1991), in spray drying the particle size is associated to the viscosity of the wall material, once the higher the viscosity, the greater the particle size is. Furthermore, the presence of larger particles can be due to the beginning of agglomeration process, since the formation of irreversible bridges leads to formation of larger particles (Tonon et al., 2008). Ezhilarasi et al. (2013) also observed that the use of different wall materials significantly affected the particle size of Garcinia extract encapsulated by freeze-drying.

The span values are related to particle size distribution, with lower values indicating a more homogeneous distribution (Fernandes et al., 2014), which are desirable. The smallest span values were observed for the spray-dried treatment with GA and the spray-dried treatment with 10% PHGG, with span values below 2. Lower diameters were also observed for these treatments.

With respect to the particles morphology, the spray-dried microparticles (Fig. 2A, B, E and F) exhibited spherical structures with different sizes, without fissures or cracks, which are characteristics of microparticles produced by this drying method (Ferrari et al., 2012).

The spray-dried powders with GA presented concavities and rough surface, which was also observed by other authors (Bhusari et al., 2014; Ferrari et al., 2012). The concavities are formed due to the rapid water evaporation in the spray drying process, and consequent contraction or shrinkage of the particles (Rosenberg, Kopelman, & Talmon, 1985). Bhusari et al. (2014) suggest that the concavities in the microparticles with GA are due to the protein fraction in the molecule. The particles produced with PHGG were more spherical, having fewer concavities when compared to those produced with GA.

The attempt to produce powders using only PD as wall material did not show positive results, as no powder was obtained. This may be due to the PD can undergo depolymerization when subjected to high temperatures and low pH, releasing glucose molecules (Craig, Anderson, Holden, & Murray, 1996), which can increase the hygroscopicity and adhesiveness of the powder in the drying chamber, thus PD should be used in combination with other encapsulating agents. On the other hand, a reduction in the number of concavities was observed in the treatments using PD, GA, and PHGG as encapsulating agents. The formation of concavities or shrinkage can also be related to the size of the encapsulating agent molecule, since a larger chain can impede the passage of water molecules (Bhusari et al., 2014). It is known that among the three encapsulating agents used, the PD has the lowest molecular weight, followed by PHGG and GA (Al-Assaf et al., 2005; Kapoor & Juneja, 2009; Mitchell, 1996).

The spray-dried treatments with 5% PHGG and 5% PD showed the best microstructure (Fig. 2E), with more spherical particles, and few concavities and roughness. Microcapsules with very porous surfaces, rough, or cracked have poor flowing properties

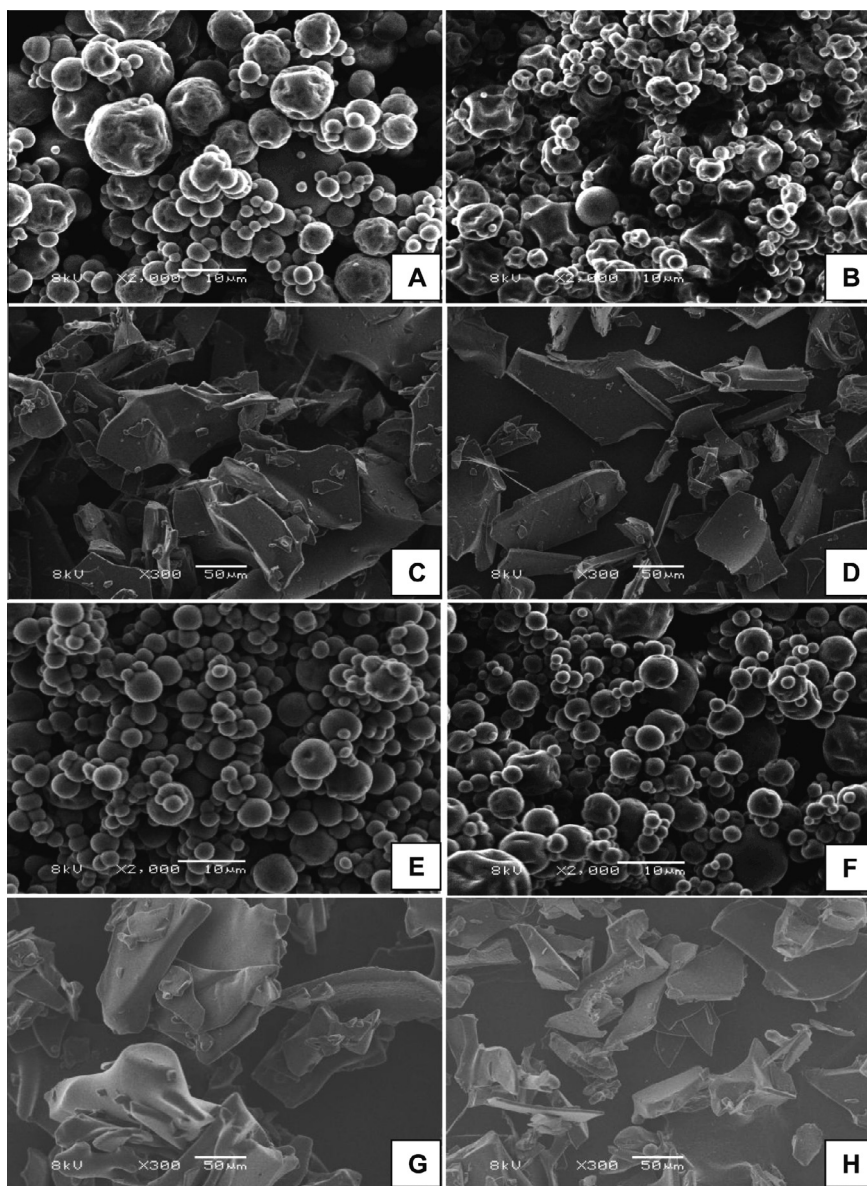


Fig. 2. Micrographs of grape skin aqueous extract microencapsulated with gum Arabic (GA), partially hydrolyzed guar gum (PHGG), and polydextrose (PD) by spray-drying and freeze-drying. A: treatment 1 (spray-dried, with 5% GA and 5% PD); B: Treatment 2 (spray-dried, with 10% GA); C: Treatment 3 (freeze-dried, with 5% GA and 5% PD); D: Treatment 4 (freeze-dried, with 10% GA); E: Treatment 5 (spray-dried, with 5% PHGG and 5% PD); F: Treatment 6 (spray-dried, with 10% PHGG); G: Treatment 7 (freeze-dried, with 5% PHGG and 5% PD) and H: Treatment 8 (freeze-dried, with 10% PHGG).

(Rosenberg et al., 1985), in addition to having greater contact surface, which can make the materials more susceptible to degradation reactions.

The freeze-dried microparticles (Fig. 2C, D, G and H) exhibited a completely different structure when compared with the spray-dried particles. Structural characteristics of freeze-dried materials, irregularly shaped, like broken glass and various sizes were observed (Khazaei et al., 2014; Man et al., 1999). According to Aguilera and Stanley (1999), the structural rigidity provided by the frozen surface where the sublimation takes place, and the lack of water in the liquid state results in a porous structure with no shrinkage, which is the primary quality factor of freeze-dried foods.

An aggregation of the microparticles was observed in the treatment with PD, probably due to the tendency of polydextrose to bind water and form bridges between molecules. This corroborates the results of a_w , since the freeze-dried samples with PD showed

slightly higher a_w values than the samples subjected to the same treatment, but without PD.

4. Conclusions

It was possible to encapsulate Bordo grape phenolic extract using gum Arabic, partially hydrolyzed guar gum, and polydextrose as encapsulating agents, obtaining powders with retention of phenolics and anthocyanins greater than 80%. In addition, high antioxidant activities were also observed, representing a potential dye for use in functional foods. In general, spray-dried powders had better physical characteristics when compared to freeze-dried powders. Considering the set of results, the spray-dried treatment with 5% PHGG and 5% PD presented the best behavior due to the better retention of phenolic compounds, anthocyanins, and antioxidant activity by DPPH and CUPRAC assays. Furthermore, as observed

for the other spray-dried treatments, lower moisture content, aw, and particle size, higher Tg and solubility, and lower hygroscopicity values were observed among the spray-dried treatments. It presented the best morphological characteristics with spherical microparticles, and lower incidence of roughness and concavities. The application of these microparticles in food has been investigated.

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