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Functional properties and stability of spray-dried pigments from Bordo grape (*Vitis labrusca*) winemaking pomace

Volnei Brito de Souza [a,*], Alice Fujita [b], Marcelo Thomazini [a], Edson Roberto da Silva [a], João Francisco Lucon Jr. [a], Maria Inés Genovese [b], Carmen Sílvia Favaro-Trindade [a]

[a] Universidade de São Paulo (USP), Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Avenida Duque de Caxias Norte, 225, CEP: 13635-900, Pirassununga, São Paulo, Brazil
[b] Universidade de São Paulo (USP), Faculdade de Ciências Farmacêuticas (FCF), Departamento de Alimentos e Nutrição Experimental, Av. Prof. Lineu Prestes, 580 – Bloco 14, Caixa-postal: 66083, Butantã, CEP: 05508-900 São Paulo, SP, Brazil

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ABSTRACT

The stability of anthocyanin and phenolic compounds, the antioxidant capacity, the antimicrobial activity and the capacity to inhibit arginase from *Leishmania* were evaluated in spray-dried powders from Bordo grape winemaking pomace extract. The pigments were produced using maltodextrin as the carrier agent at concentrations varying from 10% to 30% and air entrance temperatures varying from 130 to 170 °C. A sample of freeze-dried extract without the carrier was also evaluated. The anthocyanins in the spray-dried samples showed good stability during storage, better than the freeze-dried and liquid extracts. The samples were capable of inhibiting the growth of *Staphylococcus aureus* and *Listeria monocytogenes* and showed high inhibitory capacity against the enzyme arginase from *Leishmania*. These results provide evidence that Bordo grapes from the winemaking process have the potential to be used as natural pigments with functional properties.

1. Introduction

The grape is one of the most cultivated fruits throughout the world (Xu, Zhang, Cao, & Lu, 2010). In Brazil, the American grape cultivar Bordo (*Vitis labrusca*) is one of the most important grape varieties used in the production of wines and juices, and its processing produces a large amount of byproducts, such as skins, seeds and stalks, among others (Pozzan, Braga, & Salibe, 2012; Rizzon, Miele, & Meneguzzo, 2000). The majority of this material is treated as a low value residue and is used, for example, in animal feed (Rockenbach et al., 2011).

Red grape pomace retains polyphenolic compounds after the production of wines and/or juices; 20–30% of the compounds are retained in the skins and 60–70% in the seeds (Monrad, Howard, King, Srinivas, & Mauromoustakos, 2010). Flavonoids, mainly anthocyanins, are among the principal phenolic compounds found in red grape pomace (Jackson, 2008), and these are recognised as having various activities, such as antioxidant (Rockenbach et al., 2011) and antimicrobial (Oliveira et al., 2013) capacities; the ability to scavenge reactive oxygen species and electrolytes; and the ability to inhibit nitrosation, to chelate metals (such as iron and copper) and to modulate the activities of some enzymes (Ho, Rafi, & Ghai, 2010). Bordo grapes are known to have a high anthocyanin content, higher than varieties such as Cabernet sauvignon, Merlot and Isabel (Rockenbach et al., 2011). The wine from Bordo grapes is used to increase the colour intensity of other wines (Barnabé, Venturini Filho, & Bolini, 2007). This characteristic draws attention to the byproducts of this grape variety as potential sources of natural pigments.

The stability of anthocyanins can be affected by several factors, such as pH, storage temperature, exposure to light and the presence of oxygen, solvents and metal ions. Thus, the stabilisation of these molecules has been the principal focus in recent studies because of their great potential as natural dyes, for example, in foods and cosmetics, and because of their beneficial effects on health (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). The stability of anthocyanins can be evaluated by studying its degradation during storage. Several studies have shown that the degradation of anthocyanins usually follows first order kinetics, i.e., anthocyanin content decreases with time. (Idham, Muhamad, & Sarmidi, 2012; Tonon, Brabet, & Hubinger, 2010). Burin, Rossa, Ferreira-Lima, Hillmann, and Boirdignon-Luiz (2011) studied the anthocyanin degradation of extracts from the grape Cabernet Sauvignon spray-dried with

* Corresponding author. Address: Duque de Caxias Norte, 225, Jardim Elite, Pirassununga, CEP: 13635-900 São Paulo, Brazil. Tel.: +55 19 3565 4282; fax: +55 19 3561 8606.
E-mail address: volnei15@gmail.com (V.B.d. Souza).

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different carriers, but studies of extracts obtained from winemaking byproducts and from the variety Bordo were not found.

The spray-drying process has been used for many years in the microencapsulation of ingredients that are susceptible to degradation by external agents (Ré, 1998; Tonon et al., 2010). Among the polymers used as carrier agents, maltodextrin is one of the most important, mainly because it forms low viscosity solutions in high concentrations – an important characteristic in the spray-drying process (Berg, Bretz, Hubbermann, & Schwarz, 2012; Tonon et al., 2010). Maltodextrin has the additional advantages of low cost and mild taste (Rocha, Trindade, Netto, & Favaro-Trindade 2009).

The antioxidant capacity of anthocyanin pigment powders obtained by spray drying was studied by Tonon et al. (2010) for açai juice and by Peng, Li, Guan, and Zhao (2013) who produced purple sweet potato flours by spray drying. However, no studies on the antioxidant capacity of spray-dried pigments from red grapes have been reported. Just one article, carried out by our research group, presents the antimicrobial activity of spray-dried anthocyanin extracts obtained from the depulping residue of jabuticaba fruits (Brazilian grapes) (Silva et al., 2014). These authors are also the only ones to report the inhibitory activity of powdered pigments containing anthocyanins against arginase of *Leishmania*. This evaluation is interesting in this type of material because other flavonoids have been shown to inhibit this enzyme, which plays a central role in the transmission of leishmaniasis (Da Silva, Maquiaveli, & Magalhães, 2012). However, little information is known about the action of anthocyanins on the activity of this enzyme.

Thus, the objective of the present work was to determine the total phenolic compound content, the antioxidant capacity, the antimicrobial activity, the ability to inhibit the arginase from *Leishmania* and the stability of the pigments obtained by spray drying extracts of winemaking byproducts from Bordo grapes using maltodextrin as the carrier. A sample of the freeze-dried extract with no carrier was used as the control.

2. Material and methods

2.1. Materials

Winemaking byproducts (skins and seeds) from red grapes of the variety Bordo (*V. labrusca*), obtained from the winery 'Vinícola Micheletto' located in the municipality of Louveira, São Paulo, Brazil, were used. The material was composed of approximately 89% skins and 11% seeds. The byproduct was vacuum packed in a low density polyethylene film using a semi-automatic packer (model 200S, Selovac, São Paulo, Brazil) and stored in a freezer at −20 °C. The carrier agent used for spray drying was maltodextrin *MOR-REX® 1910* ($9 \leqslant DE \leqslant 12$, considered here as DE10), donated by Ingredion (Mogi-Guaçu, Brazil).

2.2. Obtaining the pigments

The liquid extract was obtained according to the procedure described by Souza, Thomazini, Balieiro, and Favaro-Trindade (2013) and dried in a pilot spray drier (model MSD 5.0, *Labmaq do Brasil Ltda*, Ribeirão Preto, Brazil), with a 2.0-mm diameter injector nozzle, an air flow of 40 L/min and a feed flow of the mixture of 44 mL/min, performed by a peristaltic pump. The tested entrance air temperatures were 130, 150 and 170 °C, and the carrier concentrations were 10, 20 and 30%. The combination of these conditions in a factorial experiment with a completely randomised design (CRD) gave a total of nine trials; and two repetitions were performed. It was not possible to obtain a dry sample without a carrier in the spray dryer. Thus, for comparative purposes, one sample of the extract was freeze-dried without a carrier. The extract was frozen at −20 °C and freeze-dried in the proper equipment (model LC 1500, Terroni, *São Carlos*, Brazil) for 24 h. The obtained sample was stored at −20 °C.

2.3. Total phenolic content

Total phenolic content analysis was performed according to Singleton, Orthofer, and Lamuela-Raventos (1999) with some modifications. A 0.25-mL aliquot of the sample extract was mixed with 0.25 mL of Folin–Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added, and the mixture was placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (model Libra S-22, Biochrom, Cambridge, UK). Gallic acid was used as the reference standard, and the results were expressed as mg gallic acid equivalents/g of sample on a dry basis.

2.4. Total flavonoid content

The total flavonoid content were determined by the colorimetric method modified by Yang, Martinson, and Liu (2009), using quercetin as a reference standard. The absorbance readings were obtained using a spectrophotometer (model Libra S-22, Biochrom, Cambridge, UK) at 510 nm. The results were expressed in mg quercetin equivalents/g of sample on a dry basis.

2.5. Total anthocyanin content

The total anthocyanin content was determined by the pH differential method (Giusti & Wrolstad, 2001). The absorbance was read at 520 and 700 nm on a spectrophotometer (Model S-22 Pound, Biochrom, Cambridge, UK). The results were expressed as the monomeric pigment concentration in mg equivalent of malvidin-3-glucoside/g of sample on a dry basis. The molar extinction coefficient 28,000 L/cm·mol and the molecular weight 463.3 g/mol were used for malvidin-3-glucoside (Rockenbach et al., 2011).

2.6. Total proanthocyanidin content

Total proanthocyanidin content was determined according to Porter, Hrstich, and Chan (1985). A total of 250 μL of each sample extract and 2.5 mL of the Porter reagent were incubated at 90 °C for 15 min. The blank consisted of 2.5 mL of the reagent and 250 μL of methanol:acetic acid (99.5:0.5). The absorbance was measured at 540 nm using a model U1100 UV/Visible spectrophotometer (Hitachi, Japan). The results were expressed as mg of quebracho tannin/g of sample on a dry basis.

2.7. Anthocyanin stability during storage

The anthocyanin stability was determined by the degradation of the compounds during storage according to the method described by Tonon et al. (2010), with some adaptations. The samples were placed in flasks for maximum surface exposure. After that process, the samples were stored in desiccators containing a $MgCl_2$-saturated solution (32.8% relative humidity). Desiccators were stored in incubators at 25 °C. Samples were stored for 120 days in these conditions and analysed every 30 days for total anthocyanin content. The reaction rate constants (k) and half-life time ($t_{1/2}$) were calculated following the pattern of first-order kinetics as described in Eqs. (1) and (2).

$$-\ln\left(\frac{C_t}{C_0}\right) = kt \quad (1)$$