Stability of spray-dried beetroot extract using oligosaccharides and whey proteins

Eloá Lourenço do Carmoa, Rhana Amanda Ribeiro Teodoro¹, Pedro Henrique Campelo Félixb, Regiane Victória de Barros Fernandesc, Érica Resende de Oliveiraa, Taís Regina Lima Abreu Veigad, Soraia Vilela Borgesa, Diego Alvarenga Botrela,⁎

ABSTRACT

The properties and stability of spray-dried beetroot extract using maltodextrin (MD), inulin (IN), and whey protein isolate (WPI) as carrier agents were evaluated. The values of moisture, betalains content, and retention were 3.33–4.24%, 348.79–385.47 mg/100 g (dry-basis), and 88.45–95.69%, respectively. Higher values of antioxidant activity were observed for the treatments using WPI. The treatment with inulin alone presented higher hygroscopicity in the moisture adsorption isotherms at 25 °C and lower thermal stability when evaluating the thermogravimetric curves. When stored at 60 °C, the use of WPI alone conferred lower stability to the beetroot extract powder. In general, the simultaneous use of IN and WPI as carrier agents resulted in good stability of the beetroot extract powder, representing an opportunity for innovation in food products.

1. Introduction

Beetroot (Beta vulgaris L.) belongs to the family Chenopodiaceae, originating in the temperate regions of Europe and North Africa. The vegetable draws great interest due to its beneficial effects on human health, including stimulation of the immune and hematopoietic systems, and anti-inflammatory, antitumor, and hepatoprotective properties (Martínez et al., 2015). The high concentration of antioxidant substances called betalains provide these benefits (Martínez et al., 2015). The high concentration of antioxidant activity was observed for the treatments using WPI. The treatment with inulin alone presented higher hygroscopicity in the moisture adsorption isotherms at 25 °C and lower thermal stability when evaluating the thermogravimetric curves. When stored at 60 °C, the use of WPI alone conferred lower stability to the beetroot extract powder. In general, the simultaneous use of IN and WPI as carrier agents resulted in good stability of the beetroot extract powder, representing an opportunity for innovation in food products.

containing ingredients from natural sources (Janiszewska, 2014).

However, spray drying of beetroot juice without the addition of carrier agents is difficult due to the presence of components with low molecular weight and low glass transition temperature, such as sucrose, fructose, glucose, and acids. These components can adhere to the walls of the drying chamber during the process, resulting in operational troubles and low yield of the final product (Janiszewska, 2014). These problems are avoided using carrier agents, which have high molecular weight and can contribute to increase the glass transition temperature of the material. In addition, the use of carrier agents averts other problems, such as the formation of agglomerates and crystallization during the processing and storage of the product, which is important especially in the case of sugar-rich products (Toon, Brabet, & Hubinger, 2010).

One way to ensure the quality and stability of the powdered products obtained by spray drying is the appropriate choice of a carrier agent or combination of carrier agents. Several materials can be used for this; therefore, studying the application of new polymer blends, especially those using biopolymers, and interactions between the material components is becoming important because of their direct influence on the characteristics and stability of the obtained product.

Maltodextrin (MD) is a carrier agent widely used for obtaining food dried products. This material exhibits good stabilization of the
components reducing their mobility in the matrix, which results in protection against outward adverse conditions (Estupinian, Schwartz, & Garzón, 2011). Moreover, MD presents low hygroscopicity, which confirms its efficiency as carrier agent (Tonon, Brabet, & Hubinger, 2008). Whey protein isolate (WPI) has a percentage of proteins higher than 90%, consisting mainly of β-lactoglobulin and α-lactalbumin (Khem, Small, & May, 2016), and helps to reduce fat and maintain muscle mass in the human body (Frestedt, Zenk, Kuskowski, Ward, & Bastian, 2008). After contact with hot air during spray drying, WPI immediately forms a film around the particle (Adhikari, Howes, Bhandari, & Langrish, 2009). This property is potentially useful for applications in drying of beetroot juice, since the process yield can increase by avoiding the adhesion of low-molecular-weight compounds present in the beetroot to the walls of the drying chamber. Studies involving the use of inulin (IN) as a carrier agent have been increasing in recent years, which has good technological properties for use in processes such as spray drying (Botrel, Fernandes, Borges, & Yoshida, 2014). In addition, inulin is classified as a prebiotic (Robert, García, Reyes, Chávez, & Santos, 2012), a characteristic of interest that contributes to the innovation and development of functional foods.

Based on all these considerations, this work had the objective of evaluating the influence of different biopolymers and the combination between them as carrier agents on the properties of spray-dried beetroot extract. Moreover, physicochemical and structural changes when submitted to different relative humidities and temperatures, as well as the stability in terms of betanin and vulgaxanthin-I levels were evaluated.

2. Materials and methods

2.1. Preparation of solutions for drying

The beetroots were purchased from a market in Lavras-MG, Brazil. Maltodextrin with dextrose equivalent (DE) 10 (Cassava S.A., Maripá, PR, Brazil), Inulin with a degree of polymerization (DP) greater than 10 (Orafti® GR, BENEO-Orafti, Tienen, Belgium), and Whey Protein Isolate (Hilmar, USA) were used as carrier agents in the spray drying process.

2.2. Spray drying

The beetroots were washed, peeled, and beetroot juice was obtained with a food processing centrifuge (Philips Walita Juicer model RI1858, Royal Philips Electronics, Barueri, Brazil). The juice was filtered first in organza and afterwards under vacuum on qualitative organza and afterwards under vacuum on qualitative filter paper for elimination of suspended solids. Carrier agents (MD, IN, and/or WPI) were added to beetroot juice under stirring at 5000 rpm for 5 min using a homogenizer (Ultra-Turrax IKA T18 basic, Wilmington, USA). The obtained solution was used as feed in the spray drying process.

2.3. Experimental design

The experiment was conducted with three repetitions, and the measurements were performed in triplicate in a completely randomized design. The carrier material content was 15% (w/w) in relation to the final solution, defined from preliminary tests (data not shown). The solids content of the beetroot juice was 8.7% (w/w), which resulted in a ratio beetroot juice solids:carrier agent of 1:2 (w/w). Six treatments were obtained: three solutions containing only one type of carrier agent (MD, IN, or WPI), and three solutions obtained from the addition of carrier agents in combination (MD:IN, MD:WPI, and IN:WPI) in a 1:1 ratio.

2.4. Physicochemical and morphological analyses of particles

2.4.1. Moisture

The gravimetric method was used to determine the moisture content of the powders, according to the Association of Official Analytical Chemists (AOAC, 2007) for food. The percent weight loss was obtained after drying 2.5 g at 105 °C until a constant weight, and moisture content (%) was calculated.

2.4.2. Betalains content and retention

The spectrophotometric method described by Elbe (2001) was used to determine the content of betacyanins (violet pigment) and betaxanthins (yellow pigment), expressed in terms of betanin and vulgaxanthin-I, respectively. The total betalains concentration in the beetroot extract powder was calculated from the sum of betacyanins and betaxanthins, and all results were presented on dry basis (d.b.). The sample (1g) was diluted 500-fold in 0.05 M phosphate buffer (pH 6.5), and using a spectrophotometer (Shimadzu UV–VIS SP 2000, Bel Photonics, Piracicaba, Brazil), the absorbances were measured at 538 nm and 476 nm and used to calculate the concentrations of betanin and vulgaxanthin-I, respectively. In addition, the absorbance of the samples at 600 nm was obtained and used for correction of the absorbance values, due to small amounts of impurities that may be present in the samples. The equations used for the calculation are described below.

\[
C_B = \left( \frac{1.095 \times (A_{538} - A_{600})}{1120} \right) \times \frac{1}{TSC} \quad (1)
\]

\[
C_{V-I} = \left( \frac{A_{476} - 0.258 \times A_{538} - 0.742 \times A_{600}}{750} \right) \times \frac{1}{TSC} \quad (2)
\]

where \( C_B \) is the concentration of betanin, \( C_{V-I} \) is the concentration of vulgaxanthin-I, and \( TSC \) is the total concentration of betalains, all expressed as mg/100 g d.b. The values of 1.095, 0.258, and 0.742 correspond to correction factors, and \( A_{538} \), \( A_{476} \), and \( A_{600} \) correspond to the absorbance of the sample at 476, 538, and 600 nm, respectively. The term \( TSC \) represents the dilution factor, and the conversion between grams and milligrams was included. Furthermore, 1120 and 750 are the absorbivity values (A unit) for betanin and vulgaxanthin-I at wavelengths of 538 and 476 nm, respectively. To obtain the results on a dry basis, the pigment concentration was divided by the total solids content (TSC), calculated for each treatment.

The betalains retention (BR,%) in the microparticles was calculated as follows:

\[
BR(\%) = \frac{C_{T, \text{powder}}}{C_{T, \text{solution}}} \times 100 \quad (4)
\]

where \( C_{T, \text{powder}} \) and \( C_{T, \text{solution}} \) correspond to the total concentration (on dry basis) of betalains in the powder and in the solution before drying, respectively.

2.4.3. Antioxidant activity

The antioxidant activity of the beetroot extract powders was determined by monitoring the consumption of the free radical 2,2-di-phenyl-1-picrylhydrazyl (DPPH), which was performed according to the methodology described by Dima, Cotărlet,Alexe, and Dima (2014), with some modifications. The sample was homogenized in water-ethanol solution (2:1, v/v) using a sonicator (Branson Digital Sonifier®, Model S-450D, Branson Ultrasonics Corporation, Danbury, USA) for
1 min at a power of 200 W, obtaining an extract with a concentration of 1.6 g/L. From this solution, 3 mL was added to 2 mL of ethanolic DPPH solution (0.1 mM), followed by vortexing for 10 s. This mixture was maintained in a dark environment for 1 h. The absorbance at 515 nm of each mixture was obtained and, the antioxidant activity \((AA, \%)\) was obtained according to the following equation:

\[
AA(\%) = 100 - \left( \frac{A_i - A_0}{A_c} \right) \times 100
\]

where \(A_i\), \(A_0\), and \(A_c\) are the absorbances obtained at 515 nm for the sample, blank, and control, respectively. The blank corresponds to a solution containing 3 mL of extract (beetroot extract powder homogenized with a water:ethanol solution) and 2 mL of ethanol, and the control corresponds to 3 mL of ethanol and 2 mL of ethanolic DPPH solution.

2.5.3. Accelerated storage stability

Three procedures were used to evaluate the stability of the beetroot extract powders: adsorption isotherms, thermogravimetric analysis, and accelerated storage stability. In addition, beetroot juice without the addition of carrier agents was dried using a lyophilizer (model LABCONCO FreeZone 2.5, Canada, vacuum of 0.420 mBar and temperature of \(-50 ^\circ C\)). This lyophilized beetroot juice (LBJ) was used as a control treatment to evaluate how the presence of carrier agents influences the stability of the beetroot extract powder. This control treatment was not obtained by spray-drying, because of the several low-temperature glass transition compounds present in the beetroot that can adhere to the dryer walls, resulting in low process yield.

2.5.2. Thermogravimetric analysis

The thermogravimetric curves (TGA/DTG) were obtained by a thermogravimetric analysis of variance (ANOVA) as a way of evaluating the effects of different carrier agents on the properties of the beetroot extract powders obtained by spray drying. Differences between the mean values obtained for each property were evaluated at a 5% significance level (\(p \leq .05\)) using the Duncan test.

The mathematical models of moisture adsorption isotherms were correlated to the experimental data, using a nonlinear Quasi-Newton regression at a 5% level of significance. The most suitable model was considered based on higher coefficient of determination \((R^2)\) and lower relative mean error \((E)\), defined by the following equation:

\[
E = 100 \frac{\sum_{i=1}^{N} n_i - m_{pi}}{m_i}
\]

where \(m_i\) and \(m_{pi}\) are the experimental and predicted values, respectively, and \(N\) is the population of the experimental data.

2.6. Statistical analysis

Statistica software (ver. 8.0, Stat. Soft Inc., Tulsa, USA) was used for analysis of variance (ANOVA) as a way of evaluating the effects of different carrier agents on the properties of the beetroot extract powders obtained by spray drying. Differences between the mean values obtained for each property were evaluated at a 5% significance level (\(p \leq .05\)) using the Duncan test.

The mathematical models of moisture adsorption isotherms were correlated to the experimental data, using a nonlinear Quasi-Newton regression at a 5% level of significance. The most suitable model was considered based on higher coefficient of determination \((R^2)\) and lower relative mean error \((E)\), defined by the following equation:

\[
E = 100 \frac{\sum_{i=1}^{N} n_i - m_{pi}}{m_i}
\]

where \(m_i\) and \(m_{pi}\) are the experimental and predicted values, respectively, and \(N\) is the population of the experimental data.
vulgaxanthin-I contents different between treatments, which was not observed for betanin. The highest value of vulgaxanthin-I observed was for the MD-WPI treatment. A possible explanation is described by Adhikari et al. (2009), who observed that during the spray-drying of sucrose, a WPI film formed around the particles immediately after contacting the drying air, even faster than maltodextrin. Thus, in the present study, the rapid formation of the WPI film may have resulted in a greater protection barrier for vulgaxanthin-I, which prompted higher pigment content in the particle matrix. Moreover, the presence of maltodextrin in this treatment may have contributed to this result, since this material exhibits good stabilization of the components, reducing their mobility in the matrix (Estupiñan et al., 2011).

The betalains content and retention did not show significant difference between treatments (p > .05). Lower values of betalains (from 174 to 190 mg/100 g d.b.) were observed in the study conducted by Janiszewska and Wlodarczyk (2013), in which maltodextrin was used as carrier in the spray drying of beetroot juice. It is important to consider that betalains retention found by these authors (from 26.7 to 29.3%), which were also lower than the present study, is probably due to the different drying conditions used in the studies (total solid content, nozzle atomizer, feed and air flow rates).

The WPI, MD-WPI, and IN-WPI treatments achieved the highest antioxidant activities, while for the beetroot juice was 88 ± 4.36%. The antioxidant activity was not directly dependent on the betanin or vulgaxanthin-I concentration, which may be due to the possible presence or degradation of other substances with antioxidant activity. The high sugar content of beetroot and presence of protein from the carrier agent (WPI) might have caused Maillard reaction, which usually happens during food processing at high temperatures or during product storage for a long period of time, forming compounds with antioxidant activity (Tonon et al., 2010). Thus, the values observed for antioxidant activity of the powders may have been influenced by possible presence of Maillard reaction products, or even the presence or degradation of compounds from beetroot, like other betacyanins and betaxanthins that were not quantified in the present study, as well as phenolic compounds. Tonon et al. (2010) also mentioned the possible occurrence of the Maillard reaction when they observed an increase in antioxidant activity in açai powders obtained by spray drying using maltodextrin, gum arabic, and tapioca starch as carrier agents. Fernandes, Dias, Carvalho, Souza, and Oliveira (2014) found antioxidant activity values between 82.92% and 88.54% in spray dried guava leaves extracts using maltodextrin, colloidal silicon dioxide, β-cyclodextrin, and gum arabic as carrier agents.

The different treatments of beetroot extract powder (Fig. 1) allowed the formation of particles without cracks or fissures, most of them with irregular surfaces except for the IN treatment. Analyzing the particle morphology of this treatment, the initial stages of solubilization and subsequent agglomeration of the particles was observed (Fig. 1B), possibly due to the hygroscopic characteristic of the inulin, which was greater than other carrier agents in this study. This agglomeration is due to the presence of a liquid interface between the particles, owing to the adsorption of water on their surface (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010). Observing images of spray-dried oregano essential oil particles using different concentrations of inulin and inlet air temperatures, Beirão-da-Costa et al. (2013) noticed the same agglomeration behavior of the particles. In order to decrease the agglomeration of the IN treatment particles, a combination of carrier agents with inulin can be used to reduce its hygroscopicity, as shown in the images obtained for the MD-IN and IN-WPI particles (Fig. 1D and F, respectively).

### Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Vulgaxanthin-I (mg/100 g d.b.)</th>
<th>Betanin (mg/100 g d.b.)</th>
<th>Betalains (mg/100 g d.b.)</th>
<th>Betalains Retention (%)</th>
<th>Antioxidant Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>3.37 ± 0.24a</td>
<td>143.74 ± 4.41ab</td>
<td>223.86 ± 7.15a</td>
<td>367.60 ± 11.56a</td>
<td>91.6 ± 2.88a</td>
<td>66.25 ± 1.84a</td>
</tr>
<tr>
<td>IN</td>
<td>3.33 ± 0.07a</td>
<td>136.86 ± 10.88b</td>
<td>211.93 ± 12.56a</td>
<td>348.79 ± 21.43a</td>
<td>88.6 ± 5.45a</td>
<td>60.57 ± 4.65a</td>
</tr>
<tr>
<td>WPI</td>
<td>3.81 ± 0.27c</td>
<td>151.63 ± 8.54b</td>
<td>217.69 ± 13.93b</td>
<td>369.32 ± 22.42</td>
<td>91.34 ± 5.54a</td>
<td>78.19 ± 2.42c</td>
</tr>
<tr>
<td>MD-IN</td>
<td>3.58 ± 0.26cd</td>
<td>138.43 ± 13.11b</td>
<td>213.10 ± 18.04c</td>
<td>351.53 ± 30.91c</td>
<td>88.45 ± 7.78c</td>
<td>72.71 ± 4.34c</td>
</tr>
<tr>
<td>MD-WPI</td>
<td>4.16 ± 0.19b</td>
<td>155.37 ± 3.28b</td>
<td>220.10 ± 0.39bc</td>
<td>385.47 ± 3.03c</td>
<td>95.69 ± 0.82c</td>
<td>78.40 ± 2.37bc</td>
</tr>
<tr>
<td>IN-WPI</td>
<td>4.24 ± 0.10a</td>
<td>149.49 ± 2.50b</td>
<td>218.38 ± 12.06a</td>
<td>367.87 ± 12.76c</td>
<td>92.21 ± 3.2c</td>
<td>85.01 ± 0.47c</td>
</tr>
</tbody>
</table>

a,b,c,d Means followed by the same letters in the same column did not differ significantly (p > .05) by Duncan’s test.

3.2. Stability

3.2.1. Moisture adsorption isotherms

The GAB model was chosen to describe the moisture adsorption isotherms of the beetroot extract powders, because it provided the lowest relative mean error (E) and the highest coefficient of determination (R²) (see Supplementary material, Table S1). From this, the adsorption isotherm curves of beetroot extract powders were also constructed (Fig. 2), as well as for carrier agents (maltodextrin, inulin and whey protein isolated). Thus, it can be observed the influence of beetroot components in the powdered product, which resulted in higher hygroscopicity, i.e., the beetroot extract powders showed higher moisture adsorption compared to pure carrier agents.

The GAB model is interesting since it describes very well the behavior of sorption isotherms in a wide range of water activities (Al-Muhtaseb, McMinn, & Magee, 2002) and provides the monolayer moisture content (Xₘ), an important parameter for dehydrated products. This value corresponds to the amount of water strongly adsorbed at specific sites of the material, considered relevant to ensure food stability (de Souza, Thomazini, Balieiro, & Fávaro-Trindade, 2015). As such, the Xₘ is a critical moisture threshold that provides the longest time in storage with a minimum loss of product quality at a given temperature (Botrel et al., 2017).

The Xₘ values obtained for the different treatments (from 4.84 to 6.60 g/100 g d.b.) showed significant difference between them (p ≤ .05), and the highest value was found for the IN treatment. This indicates greater availability of specific water adsorption sites, related to the large number of hydrophilic groups in inulin (Botrel et al., 2014). Conversely, treatments using two types of carrier agents (MD-IN, MD-WPI, and IN-WPI) had the lowest values of Xₘ (4.88, 4.84, and 5.26 g/100 g d.b., respectively).

According to Gabas, Telis, Sobral, and Telis-Romero (2007), the presence of carrier agents in the drying of fruit pulp to obtain powder juices probably modifies the balance of hydrophilic/hydrophobic sites, resulting in less water adsorbed by the product, which may also occurred in the beetroot extract powder, especially in those treatments that were used two carrier agents. It should be also considered that, in the case of biopolymers, structural changes can occur due to material swelling when exposed to the drying. Then, the conformation and topology of the molecule and the hydrophilic/hydrophobic sites at the polymer matrix is altered (Pérez-Alonso, Beristain, Lobato-Calleros, Rodriguez-Huezo, & Vernon-Carter, 2006). Therefore, the use of different carrier agents in the same treatment may have decreased the availability of hydrophilic sites, and consequently, lower monolayer moisture content was observed. The values of Xₘ found in the present study were within the range found by Souza et al. (2015) (from 4.0 to 10.61 g/100 g d.b.) when evaluating powdered pigment obtained by
spray-drying of the vinification byproducts of bordo grapes (*Vitis labrusca*).

Observing the format of the curves (Fig. 2), the moisture adsorption isotherm of the lyophilized beetroot juice (LBJ) was type III (non-sigmoidal), while the others were type II (sigmoidal) (Al-Muhtaseb et al., 2002). Moisture adsorption isotherms of biological and food materials usually have sigmoidal curves (type II). In contrast, some crystalline materials, such as sugars, may have a relatively low moisture adsorption until the water activity becomes sufficient for solubilization, and the adsorption increases. These isotherms normally show type III behavior (Ross, 1995), as observed in the LBJ curve. Guadarrama-Lezana et al. (2014) observed type II curves when evaluating particles of beetroot juice spray-dried using gum arabic as carrier agent.

In general, the treatments showed a change of state from \( a_w = 0.733 \) (Fig. 2), except for those using maltodextrin and inulin (MD:IN) and inulin (IN) as carrier agents and LBJ. The MD:IN treatment changed phase from \( a_w = 0.576 \), while the IN treatment showed agglomeration of the material at the lowest water activity (\( a_w = 0.112 \)). The LBJ began to change phase at this same water activity. Interestingly, when only inulin was used as carrier agent, the powders changed state when stored at low water activities, although the equilibrium moisture in these conditions was quite close to the other treatments (Fig. 2). Namely, the IN treatment was the least stable when stored in environments with different relative humidities. Thus, it is important to evaluate the effect of moisture on the behavior of each material, since the phase change due to moisture adsorption is characteristic of each biopolymer (Fernandes, Borges, & Botrel, 2014). Samples without the addition of a carrier agent, such as LBJ, present higher hygroscopicity. This characteristic is considered a critical factor for the storage stability of the microparticles, since they tend to adsorb moisture from the environment, which facilitates compound degradation reactions (Vergara et al., 2014). This emphasizes the need to use carrier agents to obtain beetroot extract powder with excellent quality and stability when stored at different relative humidities.

Another interesting observation is the color change of the powders at high water activity (\( a_w = 0.843 \)). The high moisture adsorption may have favored the betalains hydrolysis, resulting in betalamic acid, which has yellow coloration (Herbach, Stintzing, & Carle, 2006). Otálora, Carriazo, Iturriaga, Osorio, and Nasareno (2016) observed that hydrolysis was the main degradation mechanism of encapsulated betalains, leading to the formation of betalamic acid during storage at high relative humidities.

The WPI treatment was the only treatment that showed a color change in the powders at \( a_w = 0.733 \) but did not show the
Agglomeration characteristics among the particles as observed in the other treatments. In addition to the hydrolysis of betalains, the color change in this treatment could have been caused by the Maillard reaction. Specifically, the higher moisture adsorption by the particles results in greater mobility of the components in their matrix, which facilitates the occurrence of physicochemical reactions (Tonon et al., 2010), such as the Maillard reaction. As a result, the higher protein content provided by the WPI treatment compared to the others and high sugar content of the beetroot juice may have favored this reaction, which forms brown compounds.

### 3.2.2. Thermogravimetric analysis

Thermogravimetric analysis is a useful technique to evaluate thermal stability of a material by studying its mass loss with increasing temperature. Thus, it allows to verify food product characteristics when submitted to process with high temperatures, obtaining information, for example, on drying, dehydration, dehydroxylation (Otálora, Carriazo, Iturriaga, & Nazareno, 2015), cooking and pasteurization. The thermogravimetric curves (TGA) of the beetroot extract powders (spray-dried and lyophilized) and the carrier agents used in this study are shown in Fig. 3 [for derivative (DTG) curves, see Supplementary material, Fig. S1]. Importantly, the first stage of mass loss of the TGA curves (between 50 and 110 °C) refers to the loss of moisture from the material, while the second stage (above 110 °C) corresponds to the decomposition processes of the particle constituents (Fritzen-Freire et al., 2012), such as proteins and carbohydrates (Macêdo, de Moura, Souza, & Macêdo, 1997).

In general, lyophilized beetroot juice was stable up to 150 °C, while the spray-dried beetroot extract powders were stable up to 200 °C (Fig. 3A and C). This result was similar to that found by Fritzen-Freire et al. (2012) in the spray-drying of bifidobacteria using inulin and oligofructose as carrier agents.

At a temperature of 225 °C, clear differences in mass loss between treatments could be detected. The highest loss percentages of 27% and 25% were for the IN and IN:WPI treatments, and the two smallest ones of 15% and 17% were for MD and WPI, respectively. At the same temperature, LBJ lost 39% of its mass. Higher mass loss may be related to degradation of some beetroot components, which present low molecular weight and low glass transition temperature, such as sucrose, fructose, glucose, and acids (Janiszewska, 2014). These results demonstrate the importance of using carrier agents in the spray drying of beetroot juice to increase the thermal stability of the powdered extract.
The same behavior was observed by Keawchaoon and Yoksan (2011) when comparing the thermal stability of pure and encapsulated carvacrol using chitosan as carrier agent, with the latter having the highest thermal stability.

### 3.2.3. Accelerated storage stability

The Figs. 4 and 5 represent the contents of the pigments (betanin and vulgaxanthin-I) during storage and the ΔE* values calculated for each treatment, respectively (for the L*, a* and b* parameters, see Supplementary material, Table S2). Among the six treatments for beetroot extract powder obtained by spray-drying, the WPI only treatment was the least stable with the shortest half-life of betanin (t1/2 = 77 weeks) and highest value of ΔE*(10), while the others treatments reached a maximum of 5.5. In addition, at the end of the storage time, the vulgaxanthin-I content was higher than the betanin content (Fig. 4C), indicating a possible conversion of betanin (violet pigment) to vulgaxanthin-I (yellow pigment), which also explains the higher ΔE* value observed for this treatment. This result is in agreement with Herbach et al. (2006), who observed the formation of betaxanthins from betacyanins in food products. When subjected to higher temperatures, degradation of the betacyanins is usually followed by a color change due to the formation of yellow degradation products, which are betalamic acid, neobetacyanins, and betaxanthins (Herbach et al., 2006). Treatments with higher protein contents also provided low preservation of anthocyanins when evaluating the storage stability of spray-dried grape juice using maltodextrin/whey protein concentrate and maltodextrin/soy protein isolate (Moser et al., 2017).

In the other treatments, the oscillation of the ΔE* values up to a certain storage time may be due to the possible occurrence of some reversible reactions in the beetroot pigments in which these pigments are continuously regenerated (Herbach et al., 2006). After a time, the reactions may become irreversible, and an increase in ΔE* values was observed since betacyanin regeneration occurs less frequently when betalamic acid is consumed in betaxanthin formation (Herbach et al., 2006).

Another fact that is important to highlight is the stability of LBJ. The LBJ had higher vulgaxanthin-I content than betanin starting from the fifth week of storage with a half-life for betanin of 15 weeks and ΔE* value reaching a maximum of 16.5. Specifically, a much greater degradation of the LBJ pigments and consequent color change could be observed when compared to the treatments obtained by spray-drying with carrier agent. Interestingly, although the lyophilization process is conducted at a temperature lower than spray drying, the stability of the LBJ was much lower compared to the beetroot extract powder obtained by spray drying. Since LBJ powder was produced without carrier agents, the lower stability of this control treatment is explained. This fact emphasize the importance of using these materials to obtain a beetroot extract powder with good stability. The same behavior was observed in the study conducted by Khazaei, Jafari, Ghorbani, and Kakhki (2014), in which there was greater degradation in powders of anthocyanins from saffron petals without carrier agents than those containing maltodextrin and gum arabic.

### 4. Conclusions

All evaluated carrier agents provided low moisture content, high values of betalains retention and antioxidant activity. The use of inulin alone as carrier agent did not present satisfactory results when evaluating the thermal stability of the powders and storage with adverse conditions of relative humidity, as well as the use of whey protein isolate alone did not provide satisfactory protection of beetroot pigments when evaluating the accelerated storage stability. Nevertheless, the use of whey protein isolate together with inulin achieved high stability and antioxidant activity. In addition, these materials could spur interesting possibilities for innovation in the food industry by applying them in food matrices to create synergy between the functional properties of various products since whey protein isolate is rich in protein and inulin is classified as a prebiotic.
Fig. 4. Betanin, vulgaxanthin-I and betalains contents, half-life ($t_{1/2}$) and first-order reaction rate constant (k) of betanin during storage: (A) MD, (B) IN, (C) WPI, (D) MD:IN, (E) MD:WPI, (F) IN:WPI, and (G) LBJ. (▲) betanin, (○) vulgaxanthin-I and (△) betalains.

Fig. 5. ΔE* values for each treatment during storage of the powders at 60 °C.

Acknowledgments

The authors thank the National Council for Scientific and Technological Development (CNPq) for financial support (Process: 448530/2014-7); the Laboratory of Electronic Microscopy and Ultrastructural Analysis of the Federal University of Lavras, the Financier of Studies and Projects (FINEP), the Minas Gerais State Research Foundation (FAPEMIG), and the Improvement Coordination of Higher Level Personnel (Capes) for the provision of equipment and technical support for experiments involving electronic microscopy.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.12.076.

References


Technology, 286, 527-537.


