BIOACTIVE PROFILE OF A SUPPLEMENT FROM FRUITS AND VEGETABLES

M.C.P. dos Santos¹, M.P.Seljan², S. Pacheco³, R.L.de O. Godoy⁴, E.C.B.A. Gonçalves⁵

ABSTRACT – Food supplements are widely used to provide specific amounts of micronutrients and non-nutrients as vitamins, carotenoids, and phenolic compounds. These, are known because of their potential health benefits. Thus, the aim of this study was to characterize the bioactive compounds in a food supplement powder from fruits and vegetables (FVS) and evaluate the structural stability during storage by DPPH and FRAP assays and the structural stability of spray-dried particles using the Scanning Electron Microscope. The results demonstrated that FVS presents mainly phenolic acids and β-carotene, and an important antioxidant activity. The microstructure was stable during storage. It was possible to conclude that FVS is a supplement rich in dietary phenolic compounds and carotenoids with a potential health effect.

PALAVRAS-CHAVE: suplemento alimentar; CLAE-DAD; carotenoides; compostos fenólicos

KEYWORDS: Food supplement; HPLC-DAD; carotenoids; phenolics compounds
1. INTRODUCTION

The particular capacity of phenolics and carotenoids is the result of a cumulative synergistic action of a variety of antioxidant compounds (Hadad and Levy, 2012), such as vitamins, phenolic compounds, carotenoids minerals, and fiber contained in plant-based foods (Agudo and Joint, 2005; Ibge, 2010; Liu, 2013; Murphy et al., 2012).

However, their content and antioxidant activity are directly linked to the variety, the degree of ripening (Palafox-Carlos et al., 2012), storage conditions and processing (Rinaldo et al., 2010). In this sense, the manufacturing of powder supplements from fruits and vegetables is an alternative to preserving main bioactive components of the food matrix and ensure product availability and quality (Jiménez-Aguilar et al., 2011; Santana et al., 2014).

Thus, the aim of this study was to characterize the bioactive compounds in a powder supplement from fruits and vegetables and evaluate the encapsulation stability during storage. The final formulation of the concentrated juice contained 50% vegetables 50% and fruits was previously reported by Ferreira et al. (2015).

2. MATERIAL AND METHODS

2.1. Phenolic compound characterization

Sample: The powder supplement was obtained with the addition of Maltodextrin (1:4; w/w) as an adjuvant to the concentrated juice of fruits and vegetables, setting the final concentration of soluble solids in 32 °Brix. To enhance the drying process all beverage was filtered before atomization. Then, 10.5 Kg (22.82 %) of a supplement of fruits and vegetables (SFV) was placed in packaging type PET, sealed and kept in a clean, dry environment until analysis.

Phenolic compounds extraction: Atomized samples were weighed (10 mg) in centrifuge tubes and extracted with 10 ml of distilled water, at room temperature for 60 min. Then, tubes were at 2000g for 15 minutes and the supernatant was recovered. This extract was used to determine antioxidant activity and extractable polyphenol contents.

Total phenolic content: This analysis was performed according to a modified version of the Folin–Ciocalteu assay described by Singleton et al. (1999). The result was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample.

Antioxidant activity: The free radical scavenging activity of SFV was measured in terms of radical scavenging ability, using 1,1-diphenyl-2-picryl-hydradrazil (DPPH) as described by Brand-Williams et al. (1995) with few modifications. The results were expressed in IC 50 (mg.mL⁻¹). And the ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain (1996), and the results were expressed as micromoles of Fe+2 equivalents per kilogram (µmolFe+2 kg⁻1) of SFV.

Phenolic compound profile using an HPLC-DAD: Samples and standards were analyzed in an HPLC system (Perkin Elmer, Shelton, USA) equipped with a degasser, a column oven and a photodiode array detector (PDA) set at an acquisition data on 260 nm, 280 nm, and 320 nm. Phenolic compounds were eluted from a reversed-phase C18 column (5µm x 150 mm x 4.6 mm) fitted with a security guard column (4 x 3.0 mm) operated at 40°C. The mobile phase consisted of 0.3% (v/v)
formic acid in water (A), methanol (B) and acetonitrile (C), the flow rate was 0.8 ml/min and the elution gradient was performed as describe by Gomes and Torres (2015) with few modifications.

2.2. Carotenoids characterization

Extraction and quantification: To determine the total amount of carotenoids, approximately 10 g of the samples plus 3 g of celite 454 were weighed and successive additions of 25 mL of acetone were made to obtain a paste, which was transferred into a sintered funnel coupled to a 500 mL Buchner flask and filtered under vacuum. This procedure was repeated three times or until the sample became colorless, followed by a filtration step. After filtration, the extract was transferred to a volumetric flask as described by Lima et al. (2009). The volume was made up by petroleum ether, and the samples were read at 450 nm in a spectrophotometer to quantify the total amount of carotenoids.

Identification and quantification of carotenoids by HPLC-DAD: 1 mL of saponified extract was dried in an amber flask under nitrogen flow. Carotenoids were analyzed with an HPLC coupled to a UV/Visible photodiode array detector and scanned between 350 – 550 nm and the compounds were identified by comparison of retention time and UV spectra of the Standards. Separation was achieved using a C30 column, with the temperature set at 33°C, with a gradient elution using methanol and t-butyl methyl ether. The mobile phase flow rate was 0.8 mL/min, and 15 μL of an acetone extract sample was injected (Pacheco, 2009).

2.3. Microstructure assay

Structure (shape and size) of SFV were analyzed by using Scanning Electron Microscope (SEM) coupled with X-ray Energy Dispersive Spectrometer. Each sample was observed at an acceleration voltage in the range of 15-20 kV and EDS signal was recorded at 20 kV acceleration voltage. Analyses were performed in six random sample points.

3. RESULTS AND DISCUSSION

Total phenolic compound observed in the SFV is 1.855 ± 0.03 (mg.g⁻¹), while the antioxidant activity by DPPH and FRAP assay were 1.19 (mg.mL⁻¹) and 27.44 ± 0.35 (µmol Fe²⁺.mL⁻¹).

As observed in Table 1, the major phenolics present in the SFV were p-Coumaric acid (1342.74 mg.Kg⁻¹) and sinapinic acid (1183.85 mg.Kg⁻¹). Free p-coumaric have been described as an important phenolic acid with a good absorption at all sections of the gastrointestinal tract in mice (Zhao and Moghadasian, 2010), which contributes to its biological activities.

p-Coumaric are usually found in lower amounts in cereals, berry fruits, and other vegetables. However, the SFV presented a much higher concentration of this particular phenolic acid. Another important phenolic found in SFV was ferulic and trans-caffeic. These present a smaller bioavailability when compared to p-coumaric, but they also contribute to the antioxidant capacity of fruits and vegetables (Pei et al., 2016).

Table 1. Identification and quantification of phenolic compounds in fruits and vegetables supplement (n=3).

<table>
<thead>
<tr>
<th>Elution order</th>
<th>Assignment</th>
<th>Retention time (min)</th>
<th>Concentration (mg.kg⁻¹) Dry sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4-Dihydroxyphenylacetic acid</td>
<td>7.41**</td>
<td>227.26</td>
</tr>
<tr>
<td>2</td>
<td>Catechin</td>
<td>7.72**</td>
<td>353.80</td>
</tr>
</tbody>
</table>
3 4-Hydroxybenzoic acid 9.07* 398.44
4 Epi catechin 9.63** 210.81
5 Trans-Caffeic Acid 9.87*** 266.27
6 Vanillic acid 10.06* 591.13
7 2,4-Dihydroxybenzoic acid 10.53* 561.15
8 Vanillin 11.3** 222.13
9 p-Coumaric acid 12.02*** 1342.74
10 Ferulic acid 12.28*** 471.63
11 Sinapinic acid 12.38*** 1183.85
12 Rutin 12.84* 330.07
13 Myricetin 13.14*** 79.35
14 Salicylic acid 14.96*** 35.11

Mean obtained from injections triplicate. *260 nm; **280 nm; ***320 nm.

Among the carotenoids identified, β-carotene (848 µg.100g⁻¹) was found to be predominantly present in the SFV, α-carotene (427 µg.100g⁻¹) was the second major carotenoid found, followed by lycopene (402 µg.100g⁻¹), as in Table 2 and Figure 1.

The potential synergistic effect of these compounds present on the same food matrix contributes to the potential health benefits related to this supplement (Hadad and Levy, 2012). The stability of the microencapsulation observed in Figure 2 (shape and size) indicates that some bioactive compounds could be preserved during storage in the SFV.

Table 2: Total carotenoids, lutein, α-carotene, β-carotene, 9 and 13-β-carotene isomers and Lycopene of food supplement powder from fruit and vegetables (n=2).

<table>
<thead>
<tr>
<th>Carotenoids</th>
<th>SFV (µg.100g⁻¹)</th>
<th>RE value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total content</td>
<td>2174</td>
<td>-</td>
</tr>
<tr>
<td>Lutein</td>
<td>161</td>
<td>-</td>
</tr>
<tr>
<td>α-carotene</td>
<td>427</td>
<td>35.58¹</td>
</tr>
<tr>
<td>β-carotene</td>
<td>848</td>
<td>141.33²</td>
</tr>
<tr>
<td>13-cis β-carotene</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>9-cis β-carotene</td>
<td>76</td>
<td>-</td>
</tr>
<tr>
<td>Lycopene</td>
<td>402</td>
<td>-</td>
</tr>
</tbody>
</table>

Standard Deviation and mean of duplicates of SFV. ¹RE = 12 µg of β-carotene; ²RE = 6 µg of β-carotene

Figure 1 - Chromatogram of the lutein, α-carotene, β-carotene, cis-β-carotene isomers and lycopene of SFV before storage.
Figure 3. Scanning electron microstructure of spray-dried fruits and vegetable supplement, before (A, B) and after (C, D) one-year storage.

4. CONCLUSION

The SFV demonstrated a great potential as a dietary source of phenolic compounds and carotenoids, and the particles obtained with the spray-drying process presented a stable shape and size during one-year storage. Further analysis on the stability of these compounds is needed to ensure the bioactivity of this supplement after a long period of storage.

5. ACKNOWLEDGMENT

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6. REFERENCES


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