Carotenoid profile of a vegetable by-product.

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ABSTRACT – In recent years, bioactive compounds such as carotenoids present in by-products from fruits and vegetables are receiving increased attention because of their potential antioxidant activity which is related to several health benefits. Also, integral exploitation was recognized as a sustainable action that reduces the disposal of waste by food industries. Peels and seeds are the major residue obtained during the processing of fruits and vegetables and for the past few decades have been considered as important sources of bioactive compounds and pigments. The aim of this study was to assess the total carotenoids content of a dry residue obtained from the manufacturing of a natural supplement by HPLC-DAD; and the concentrations of lutein, α-carotene, β-carotene, lycopene, 13-cis-β-carotene and 9-cis-β-carotene. β-carotene was predominant (1317 ± 207 µg.100g⁻¹). Lower amounts of other carotenoids were also observed indicating that this residue could be a valuable source of carotenoids.

PALAVRAS-CHAVE: carotenoids; aproveitamento de resíduo; CLAE-DAD; identificação; quantificação.

KEYWORDS: carotenoids; food residue; HPLC-DAD; identification; quantification.

1. INTRODUCTION
Agro-industrial waste is mainly composed of skins, stems and seeds of fruit and vegetables, and are recognized as sources of bioactive compounds that have important functional properties in human body, and are therefore related to several health benefits (Müller, Bub, Watzl, & Rechkemmer, 1999; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Bellos, 2014; Visioli, Riso, Grande, Galli, & Porrini, 2003).

Over 700 carotenoids have been identified (Dugo et al., 2008), and approximately 50 are characterized by having in its chemical structure at least one β-ionone ring unsubstituted with a minimum of 11 carbons and, hence, presents pro-vitamin A activity. Of these, β-carotene is the most abundant in food and presents the highest activity of pro-vitamin A, because it has in its chemical structure two rings of β-ionone, resulting typically two retinol molecules (vitamin A) (Leuenberger, Engeloch-Jarret, & Woggon, 2001).

Carotenoids are an important group of lipophilic micronutrients, whose functional application is related to their antioxidant capacity and pro-vitamin A (Stahl & Sies, 2003). These compounds are also characterized by giving color to foods and are important sources of natural colorants varying from shades of yellow to red, depending on the predominant carotenoid in food (Mortensen, 2006).

The food industry has been seeking in food waste new sources of bioactive compounds and natural colorants among other applications, in order to increase the development of new functional foods, and propose a sustainable food chain from the farm up to the consumer (Mirabella, Castellani, & Sala, 2014).

Based on these, it is possible to identify the waste generated by agro-industry as potential sources of bioactive compounds, with many applications in industry. Thus, the aim of this study was to identify and quantify through high-performance liquid chromatography (HPLC-DAD) the carotenoids: lutein, α-carotene, β-carotene, lycopene, 13-cis-β-carotene and 9-cis-β-carotene present in a food waste generated after processing 3 fruits and 8 vegetables, used in the preparation of a natural food supplement.

2. MATERIAL AND METHODS

2.1. Sample

The following species of fruits and vegetables were randomly obtained in a local market of Rio de Janeiro, Brazil: Selecta orange (Citrus sinensis), passion fruit (Passiflora edulis) and watermelon (Citrullus lanatus), lettuce (Lactuca sativa), courgette (Cucurbita pepo), carrot (Daucus carota), spinach (Spinacea oleracea), mint (Mentha s.p.), taro (Colocasia esculenta), cucumber (Cucumis sativus) and rocket (Eruca sativa).

Fruits and vegetables were properly washed in flowing water. After they were sanitized for 30 min in a bath containing 200 ppm of sodium hypochlorite (NaClO) before rinsing in flowing water again, then all fruits and vegetables were processed using an industrial juicer, to obtain a concentrated juice. After the production of the concentrated juice, the remaining solid residue (FVR) was immediately dried in a drying oven with air renewal and circulation (Marconi, model MA035, Brazil) at 65°C for 6 h. finally, the dehydrated residue was ground using a food processor for 5 min and dried out for 1 h at 90°C before grinding once more for 1 min (Ferreira et al., 2015).

Flour samples were stored at room temperature (RT) in aluminized aseptic bags until analysis.

2.2. Carotenoids extraction and quantification
To determine the total amount of carotenoids, approximately 10 g of the samples plus 3 g of celite 454 (Tedia, Ohio, USA) 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.

The extract obtained was transferred to a separatory funnel containing 40 mL of petroleum ether. Water was added to the extract to remove the acetone, and the aqueous phase was discarded. This procedure was repeated until no residual solvent remained.

Finally, the extract was transferred to a volumetric flask. The volume was made up by petroleum ether, and the samples were read at 450 nm in a spectrophotometer (Model UV-1800-Shimadzu®), to quantify the total amount of carotenoids.

2.3. Identification and quantification of carotenoids using a C30 column coupled to a PDA and scanned between 350-550 nm

For identification and quantification of lutein, α-carotene, β-carotene, lycopene, 13-cis-β-carotene and 9-cis-β-carotene, 1 mL was removed from the carotenoid extract and dried in an amber flask under nitrogen flow. The sample was diluted in 200 μL of acetone and transferred to an amber flask for HPLC analysis.

Lutein, lycopene, α-carotene, β-carotene and the cis-isomers were analyzed with an HPLC (Waters 2695 — Alliance Model, Milford, USA) 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. When necessary, samples were saponified with a methanolic solution of potassium hydroxide 10% (w/v) in the dark for 16 hours (D. B. Rodriguez-Amaya, 2001). Separation was achieved using a C30 column (YMC Carotenoid S-3; 4.6 x 250mm reversed phase) from Waters, 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. The column temperature was set in 33 °C with a total analysis time of 28 min (Pacheco, 2009). All data was reported as the mean ± standard deviation of duplicate determinations.

3. RESULTS AND DISCUSSION

The analysis methods employed allowed good resolution of the peaks which ensured adequate identification and quantification in the FVR. Data obtained by HPLC-DAD are summarized in Table 1 and illustrated in Figure 1.

Table 1: Total carotenoids, lutein, α-carotene, β-carotene, 9 and 13- β-carotene isomers and Lycopene of FVR (n=2).

<table>
<thead>
<tr>
<th>Carotenoids</th>
<th>FVR (µg.100g⁻¹)</th>
<th>RE value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total content</td>
<td>2643 ± 479</td>
<td>-</td>
</tr>
<tr>
<td>Lutein</td>
<td>169 ± 125</td>
<td>-</td>
</tr>
<tr>
<td>α-carotene</td>
<td>493 ± 74</td>
<td>41.08¹</td>
</tr>
<tr>
<td>β-carotene</td>
<td>1317 ± 207</td>
<td>219.5²</td>
</tr>
<tr>
<td>13-cis β-carotene</td>
<td>44 ± 3.5</td>
<td>-</td>
</tr>
<tr>
<td>9-cis β-carotene</td>
<td>167 ± 30</td>
<td>-</td>
</tr>
<tr>
<td>Lycopene</td>
<td>296 ± 62</td>
<td>-</td>
</tr>
</tbody>
</table>
Standard Deviation and mean of duplicates of FVR.

Figure 1 - Chromatogram of the lutein, α-carotene, β-carotene, cis-β-carotene isomers and lycopene of FVR.

Among the carotenoids identified, β-carotene (1317 ± 207 µg.100g⁻¹) was found to be predominantly present in the FVR, α-carotene (493 ± 74 µg.100g⁻¹) was the second major carotenoid found, followed by lutein and lycopene (296 ± 62 µg.100g⁻¹).

The total carotenoid content found in our study was 26.43 mg.kg⁻¹, higher than the content found in other residues reported in the literature where the average amount was 6mg.kg⁻¹ (De Abreu et al., 2013). Our findings are low compared to other tropical fruits such as papaya (44 – 46 mg.kg⁻¹), similar to mango (18 – 25 mg.kg⁻¹) (Gancel, Alter, Dhuique-Mayer, Ruales Najera, & Vaillant, 2008), and high compared to camu-camu (3.54 – 10.95 mg.kg⁻¹) a native berry from Amazon (Zanatta & Mercadante, 2007) and cagaita (7.7 ± 0.3 mg.kg⁻¹) a native fruit from the Cerrado of Minas Gerais, Brazil (Cardoso, Martino, Moreira, Ribeiro, & Pinheiro-Sant’Ana, 2011).

Also, β-carotene amounts found in FVR were higher than those found in other by-products. Albuquerque and researchers (2016) found 117 ± 21 (µg/100g) in saponified extracts of Annona cherimola Mill peel of Mateus II cultivar.

With respect to lutein, FVR presented a similar concentration compared to dried corn (1.99 mg.kg⁻¹) a recognized cereal rich in lutein, but very low concentration compared to dried onion stalk (8.76 mg.kg⁻¹) and broccoli (11.33 mg.kg⁻¹) (Mamatha, Arunkumar, & Baskaran, 2012).

During food processing, the levels of cis-isomers increase due to the isomerization of the trans-isomers. Trans–cis isomerization of carotenoids leads to a decrease of color intensity and some of these isomers were found to be less effective than all-trans-β-carotene to scavenge reactive oxygen species and are degraded faster that the all-trans isomer (Schieber & Carle, 2005). Thereby, lower amounts of these cis-carotene forms are desired to be found in food, to preserve the functional property of the product, as the observed in the FVR extracts in this study.

Gupta, Sreelakshmi, & Sharma (2015) highlight the need to quantify carotenoids in both foods and biological samples to understand their importance and pathway in body metabolism and health.

The synergistic effect of several carotenoids in different combinations has been reported as a more effective way to obtain their functional property (Hadad & Levy, 2012). In this aspect, the
composition of FVR ensures the presence of several different carotenoids, in concentrations unusually found in nature (Delia B. Rodriguez-Amaya, Kimura, & Amaya-Farfan, 2008), that can act in synergism.

Moreover, FVR has demonstrated an important potential as source of vitamin A (retinol) with lower values found in other fruits and vegetables recognized as source of carotenoid, such as acerola (192 RE), mango ‘Extreme’ (215 RE), melon (184 RE) and hydroponic lettuce (208 RE) (Delia B. Rodriguez-Amaya, Kimura, & Amaya-Farfan, 2008).

4. CONCLUSION

According to these results, FVR has a great potential as a natural source of carotenoid, with a considerable amount of compounds that are known for their antioxidant capacity and health promotion benefits. Further studies on isolation and characterization of individual phenols and flavonoids would be a valuable contribution for the characterization of functional properties of this flour. These results highlight this by-product functional properties and support their employment as added value natural extracts in cosmetic, pharmaceutical, and food processing industries. Furthermore, this work will contribute to promoting the sustainable development and exploitation of these fruits and vegetables in Brazil.

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6. REFERENCES


