Determination by RP-HPLC of β-carotene concentration from orange (Citrus sinensis L.) fruits peel extracts

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Abstract
This work presents methods of obtaining some carotenoidic extracts from orange peel and the determination of β-carotene concentration from these, using the reverse phase – high performance liquid chromatography (RP-HPLC). There were analyzed three types of extracts, from both flavedo and albedo orange peel, in three different solvents (ethanol, benzene and petroleum ether). The extracts were obtained using a Soxhlet extraction system and the β-carotene was found only in the ethereal extract. Quantitative determination of β-carotene was achieved using an HPLC system Agilent 1100 with a Zorbax SB-C18 column.

Keywords: β-carotene, orange fruit peel, RP-HPLC analysis, carotenoidic extract.

1. Introduction
Carotenoids are terpenoids synthetized in the plants plastides as hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). These compounds confer to the tissues a yellow, orange or red colour [1]. Carotenoids are notable for their wide distribution, structural diversity and very important biological function. β-Carotene, α-carotene and β-cryptoxanthin are provitamins A. Structurally, vitamin A (retinol) is essentially one-half of the β-carotene molecule. Consequently, β-carotene is the most potent provitamin A; it is also the most widespread. The minimum requirement for a carotenoid to have vitamin A activity is an unsubstituted β-ring with an 11-carbon polyene chain [2]. Carotenoids have been credited with other beneficial effects on human health: enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration. The orange fruits peel represents a rich source of carotenoidic pigments, especially β-carotene, β-cryptoxanthin, violaxanthin and lutein [3,4], that are important A vitamin precursors and brings numerous benefits for human health [5-8]. Orange peel carotenoids concentration and composition varied with plant variety and depends of growth conditions [3,4]. It was established that the β-carotene obtained by extraction from orange peel showed a better bioavailability than the synthetic one and important economically advantages [9]; thus, this natural β-carotene is more and more recommended by the specialists. Recent studies showed an inverse correlation between carotenoids consumption and cancer development [8,10]. Also, it was determined that the antimutagenity is, in the main, associated with hydrocarbon carotenoids fractions (α-, β-carotene, lycopene) and with xanthophylls (lutein, β-cryptoxanthin) [11,12].

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Particularly, β-carotene besides the anticancerous effect, showed a strong antioxidant character, which plays an important role in the prevention and treatment of cardiovascular, ophthalmological, dermatological diseases, prevents the oxidative damages that are specific to ageing phenomena, prevents the immunological disorders [13]. Due to carotenoids great sensitivity to light, heat, oxygen, acids, their isolation from different raw materials must be accomplished choosing the optimal working conditions to gum up their degradation [14].

Today are used both organic solvents carotenoids extraction methods and supercritical fluids extractions (especially supercritical CO₂) in view of their use in different domains (pharmacy, cosmetics, food industry etc.) [1,3, 15-18]. For carotenoidic pigments identification and dosing, in the last years was used more and more the high performance liquid chromatography (HPLC) and HPLC coupled with mass spectrometry (HPLC-MS) [16,19-23].

2. Materials and methods

As raw material for obtaining carotenoidic extracts was used Valencia orange peel. After separation of the external part (flavedo) and the internal part (albedo) of peel, each part was cut into pieces and was submitted, separately, to carotenoids extraction.

For carotenoidic pigments extraction were used the following solvents: ethanol 96%, benzene, petroleum ether, from Chimopar/Reactivul București.

The solvents used for RP-HPLC analysis: acetonitrile and methanol were purchased from Merck&Co., Inc, New Jersey. β-Carotene used as standard was of > 97% purity, from Sigma Chemical Company.

There were used the following apparatus:
- water bath, Precisterm model (Selecta Spain);
- digital analytical balance, AW 320 model (Shimadzu, Japan);
- rotative evaporator, RV-05 basic 1B model (Shimadzu, Japan);
- HPLC chromatograph Agilent 1100 (Agilent, SUA), with Zorbax column SB-C18, 250 x 4,6 mm x mm and 5 µm particles size.

**Extraction with organic solvents.** For flavedo and albedo carotenoids extraction, it was used an Soxhlet extraction system and as organic solvents were used: ethanol 96%, benzene and petroleum ether. Raw material and solvents quantities are presented in table 1. Both flavedo and albedo from orange peel was well grinding and then inserted into an extraction pouch and then into the Soxhlet extractor. It was achieved 4 extraction cycles. The obtained extract was dried on anhydrous calcium chloride, filtered and the solvent was evaporated under vacuum at 45°C.

<table>
<thead>
<tr>
<th>No.</th>
<th>Raw material</th>
<th>m raw material (g)</th>
<th>Solvent</th>
<th>V solvent (ml)</th>
<th>m extract (g)</th>
<th>Extraction yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavedo</td>
<td>25,00</td>
<td>Ethanol 96%</td>
<td>140,0</td>
<td>2,14</td>
<td>8,5</td>
</tr>
<tr>
<td>2</td>
<td>Albedo</td>
<td>32,60</td>
<td>Ethanol 96%</td>
<td>160,0</td>
<td>2,06</td>
<td>6,3</td>
</tr>
<tr>
<td>3</td>
<td>Flavedo</td>
<td>25,00</td>
<td>Petroleum ether</td>
<td>150,0</td>
<td>1,90</td>
<td>7,6</td>
</tr>
<tr>
<td>4</td>
<td>Albedo</td>
<td>25,00</td>
<td>Petroleum ether</td>
<td>215,0</td>
<td>2,10</td>
<td>8,4</td>
</tr>
<tr>
<td>5</td>
<td>Flavedo</td>
<td>25,00</td>
<td>Benzene</td>
<td>140,0</td>
<td>1,90</td>
<td>7,6</td>
</tr>
<tr>
<td>6</td>
<td>Albedo</td>
<td>25,00</td>
<td>Benzene</td>
<td>150,0</td>
<td>1,34</td>
<td>5,4</td>
</tr>
</tbody>
</table>

Carotenoidic extracts RP-HPLC analysis. For β-carotene concentration determination in the obtained extracts (and in albedo and flavedo) it was utilized reverse phase- high performance liquid chromatography analysis (RP-HPLC). The HPLC chromatograph was an Agilent 1100, with Zorbax SB-C18 column, of 250 x 4.6 mm x mm and 5 µm particles size.

As eluant was used a mixture of acetonitrile: methanol 20:80, using a flow capacity of 1 ml/min.; column temperature was of 30°C and the determination was made at 466 nm wave length.

It was injected samples of 20 µl, and concentration determination was made with the aid of β-carotene standard curve.
3. Results and discussion

In view of the β-carotene concentration determination in the obtained extracts, by RP-HPLC, was obtained, for instance, a calibration curve for standard β-carotene (figure 1).

β-Carotene wasn’t identified in the ethanol or benzene extracts (figures 2 and 3) but in the petroleum ether extract (figures 4 and 5), where the determined concentration was of 0,012 g/100 ml for flavedo and 0,010 g / 100 ml for albedo.

From flavedo were obtained 6 ml of extract, β-carotene quantity in the extract being of 0,72 mg; β-carotene concentration in flavedo is of 28,8 µg/g. From albedo were obtained 7 ml of extract, with a β-carotene concentration of 0,70 mg, that represents a β-carotene in albedo of 28,0 µg/g. It could be observed that in flavedo β-carotene concentration is slowly greater than in albedo (difference of 0,8 µg/g).

The obtained values for β-carotene concentration in Valencia orange are comparable with those from the literature data [3, 4].
4. Conclusion

As a result of these studies it could be marked the following conclusions:

- By RP-HPLC analysis of the three types of orange albedo and flavedo carotenoidic extracts, β-carotene was identified only in the petroleum ether extracts. In the ethanol 96%, respectively benzene extracts the β-carotene concentrations were too small to be detected.

- Petroleum ether, a non-polar solvent, could be utilized to β-carotene extraction in good conditions, through the presented method. The fact that the petroleum ether has a low boiling point is very important for carotenoidic pigments extraction because the carotenoids have a very high thermal sensibility.

- The determined β-carotene concentration in the etheric extracts of albedo, respectively flavedo, were very close, respectively of 28,80 µg/g for flavedo and 28,00 µg/g for albedo, values that are comparable to those of literature data.

References


