Natural sources and analytical tools for the analysis of carotenoids

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ABSTRACT

In recent years, it has been a global trend toward the use of natural-plant food components, such as dietary fibers and phytochemicals like carotenoids. Carotenoids are molecules widely distributed in nature, with over six hundred of known carotenoids molecules classified in two classes, xanthophylls and carotenes. The first analysis of many carotenoids was determined in a qualitative form by thin-layer chromatography, but this technique needs a previous separation, nowadays, there are many powerful analytical tools for qualitative and quantitative analysis of these metabolites. This article shows the analytical characteristics of the mass spectrometry for the identification of molecules (small and large) in situ of high performance analyses. Also are discussed the use of chromatography such as gas chromatography (GC) and High-performance liquid chromatography (HPLC) as a versatile method of carotenoid analysis.

Keywords: Carotenoids, natural sources, mass spectrometry, High-performance liquid chromatography.

INTRODUCTION

Carotenoids are isoprenoid molecules, which are widely distributed in nature as pigments in most of fruits, vegetables, flowers and crustacea. Carotenoid pigments account for natural yellow, orange or red colours of many foods. Therefore, they are related to the perception of quality, since the color affect the consumer’s preferences [1-3].

There are over six hundred known carotenoids; these are classified into two classes, xanthophylls (which contain oxygen) and carotenes (essentially hydrocarbons with absence of oxygen). All carotenoids are tetraterpenoids and are mainly produced from 8 isoprene molecules that contain 40 carbon atoms. Carotenoids absorb light energy for use in photosynthesis in plants and algae, moreover, carotenoids like α-carotene, β-carotene and β-cryptoxanthin have vitamin A activity and can also act as antioxidants in humans. The interest in these compounds has increased considerably because of their involvement in the prevention or protection against serious human health disorders, such as heart disease, cancer and macular degeneration [4-6].

The present interest for natural products such as vegetables and fruits is to know their biological and chemical diversity and its benefit on health, motivating the study of specific components that may be responsible to reduce the risk of diseases. One group of components with important biological activity are carotenoids. Carotenoids are yellow, orange, and red pigments present in many commonly eaten fruits and vegetables.

2. Natural sources of carotenoids

The carotenoids are pigments widely distributed in nature, these are synthesized in a complex process from the isoprenoid pathway in plants, combining C5-isoprenoid units that are extended in a biosynthesis to C20 molecules (geranylgeranyl pyrophosphate GGPP) by the geranylgeranyl pyrophosphate synthase, however, the most common carotenoids have 40 carbon atoms (Figure 1), which is the result of the condensation of two molecules de 20 carbon
atoms mediated by phytoene synthase [7]. The main precursor of carotenoids is the lycopene, that then is enzymatically converted to carotenes as α- and β-carotene [8].

![Carotenoid basic structure and biosynthesis pathway in plants](image)

**Figure 1.** Carotenoid basic structure and biosynthesis pathway in plants [9, 10]

### 2.1. Carotenoids in vegetables

Carotenoids and xanthophylls are natural antioxidants that are considered as an indicator of quality in foods and are present in vegetables as kale, red paprika, leaf of parsley, spinach, Lamb’s lettuce, and carrot [11]. These compounds participate in light harvesting and are essential in the process of photoprotection in photosynthetic tissues, also are natural pigments from the range of yellow to red in many plant species [12]. A source of nutritional carotenoids and antioxidants is the broccoli; has vitamin, carotenoids and is an active dietary source [13], Holden et al., (1999) [14] reported in the Broccoli 0.001, 0.78 and 2.45 mg/100 g of α-carotene, β-carotene and Lutein+zeaxanthin, respectively, and Heinonen et al., (1989) [15] found 1.0 and 1.80 mg/100g of α-carotene, β-carotene respectively. For its part, dos Reis et al., (2015) [16] reported 0.070 mg/100 g of α-carotene, and for β-carotene 1.67 mg/100g in the varieties of Daucus carota (commonly known as carrots) vegetables that accumulate massive amounts of carotenoids and are source of retinoids (including vitamin A), the red carrots provide a substantial amount of lycopene and the yellow carrots accumulate lutein (Table 1) [12].

Other important dietary source of some carotenoid as lutein and β-carotene is the Spinach [17], in this vegetable was found 3.3 to 5.3 mg/100g and 1.9 to 3.1 mg/100 g of Lutein and β-carotene, respectively, these carotenoids are the most abundant in the spinach [18]. Brussels sprouts is a source of carotenoid with antioxidant capacity, for example, ORAC values in fresh weight extracts showed more antioxidant capacity than the broccoli and cauliflower [19], Holden et al., (1999) determined the amount of carotenoids in Brussels sprouts, finding concentrations of 0.006, 0.4 and 1.6 mg/100g of α-carotene, β-carotene and Lutein+zeaxanthin, respectively [14].

### 2.2. Carotenoids in fruits

Fruits and vegetables containing vitamin C, Vitamin E (tocopherols), and carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene) and provide a natural source of antioxidants [20]

Mango is a tropical fruit widely consumed (in fresh or processed form), this fruit contains different antioxidant compounds like polyphenols, vitamins and carotenoids [21]. Sogi et al., (2015), [22] reported total carotenoids values for Mango (*Mangifera indica*) of 3.3 to 5.2 mg/100 g. In other study by Pertuzatti et al., (2015), [23] was reported the identification and carotenoid content of passion fruit (*Passiflora edulis*) from organic and conventional crop (Table 1). The amount of total carotenoids was 13.99 and 25.10 mg/100 g for organic and conventional crop, respectively, being almost twice, the amount of carotenoids in conventional crop. Additionally, were identified carotenoids such as, Licopene, Lutein+Zeaxanthin, β-Criptoxanthin, Licopene and β-Carotene. Also, the fruits of Peach palm are a good source of bioactive compounds, particularly for its high carotenoid content (1.1 to 22.3 mg/100 g), being β-carotene (26.2 to 47.9%), Z-γ-carotene (18.2 to 34.3%) and Z-lycopene (10.2 to 26.8%) the predominant ones [24]. Moreover, Ordoñes-Santos et al., (2015), made an extraction of total carotenoids from peach
palm fruit (*Bactris gasipaes*), which ranged from 112.76 to 163.47 mg/100g of dried peel, employing ultrasonic-assisted extraction [25].

Speek et al., (1988)[26] observed that the total carotenoid concentration in Thai durian cultivar was 3.8 µg per gram of fresh weight and 1.9 µg per gram of fresh weight of β-Carotene. Other study in durian cv. Nyekak from Indonesia showed in two samples a total carotenoid content of 11 and 15 mg/100 g, respectively, and 7.6 and 11 mg/100 g for β-Carotene, thus, β-carotene represented about 70% of the total carotenoids [27].

On the other hand, the effect of temperature on the chemical integrity was studied when orange juice, was heat-pasteurized (90 to 105 °C for 10-30 s), losses the carotenoid content from a ranged of 20% to 46% and 9 to 38% [28], further, Lee et al., (1999), [29] did not find significant changes in the content of β-Carotene and lycopene after thermal pasteurization (91 °C for 10 s) of red grapefruit juice.

Table 1. Carotene, Cryptoxanthin and Lutein+zeaxanthin content of different vegetables and fruits

<table>
<thead>
<tr>
<th>Description</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lutein+zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricots, raw</td>
<td>0</td>
<td>2</td>
<td>254</td>
<td>2</td>
</tr>
<tr>
<td>Avocados, raw, all commercial varieties</td>
<td>12</td>
<td>2</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td>Bananas, raw</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Carrots, raw</td>
<td>4649</td>
<td>1</td>
<td>8836</td>
<td>1</td>
</tr>
<tr>
<td>Corn, sweet, yellow, canned</td>
<td>33</td>
<td>1</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Grape leaves, canned</td>
<td>629</td>
<td>1</td>
<td>2838</td>
<td>1</td>
</tr>
<tr>
<td>Mangos, raw</td>
<td>17</td>
<td>1</td>
<td>445</td>
<td>2</td>
</tr>
<tr>
<td>Melons, cantaloupe, raw</td>
<td>27</td>
<td>3</td>
<td>1595</td>
<td>5</td>
</tr>
<tr>
<td>Orange juice, frozen</td>
<td>2</td>
<td>2</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Papayas, raw</td>
<td>0</td>
<td>1</td>
<td>276</td>
<td>3</td>
</tr>
<tr>
<td>Passion fruit, yellow, raw</td>
<td>35</td>
<td>1</td>
<td>525</td>
<td>1</td>
</tr>
<tr>
<td>Peas, green, raw</td>
<td>19</td>
<td>2</td>
<td>485</td>
<td>2</td>
</tr>
<tr>
<td>Peppers, sweet, red, raw</td>
<td>59</td>
<td>1</td>
<td>2379</td>
<td>3</td>
</tr>
<tr>
<td>Spinach, raw</td>
<td>0</td>
<td>5</td>
<td>5597</td>
<td>8</td>
</tr>
<tr>
<td>Squash, winter, acorn, raw</td>
<td>0</td>
<td>1</td>
<td>220</td>
<td>1</td>
</tr>
<tr>
<td>Tomato juice, canned</td>
<td>112</td>
<td>4</td>
<td>428</td>
<td>2</td>
</tr>
</tbody>
</table>

2.3. Other Natural Sources of Carotenoids

Halophytes are a type of vegetation that includes a large number of species belonging to different families of plants, such as *Aizoaceae, Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Combracetaceae*, among others. There are more than 2500 halophyte species, known to possess some salinity tolerance and among them, several could be suitable candidates to be used in cash crops (edible plants, fodder, fuel, medicine, chemicals and ornamentals).

Because of this salinity tolerance these plants are equipped with a strong antioxidant system, that protects them against the reactive oxygen species (ROS) generated by the salt accumulation in which they live [42-45]. Opuntiaficcus-indica (cactus pear) is an interesting halophyte, well adapted to extreme temperature and edaphic conditions. Bensadón et al., (2010)[46], have determined the nutritional value of by-products obtained from cladodes and fruits from two varieties of Opuntiaficcus-indica and reported a total carotenoid content of the by-product samples of 15.16 to 22.84 β-carotene equivalents (expressed as g/100 g of dry matter).

It is known that some cereals, pulses, vegetables, spices and condiments have appreciable amounts of carotenoids, Kandlakunta et al., (2008)[47], provides new data on the total carotenoids and β-carotene content of these natural products, the separation was realized with High-performance liquid chromatography (HPLC), which showed that β-carotene is the predominant carotenoid in all foods in the range of 20 to 120 mg/100 g of dry matter. Also, the unconventional foods *Gulmohar, Peltophorum, Lucerne* and *Spirulina* were studied for total carotenoids and β-carotene. *Gulmohar* petals contain almost twice of the provitamin A levels, than *Peltophorum* flowers. The flower petals of *Peltophorum ferrugineum* are yellow in colour and was found to have a higher concentration of total
carotenoids (3.4 mg/100 g), than the red coloured petals of *Gulmohar* (2.5 mg/100 g) while the species *Medicago sativa* (Lucerne) and *Spirulina fusiformis* showed levels of total carotenoids of 3.8 mg/100 g and 41.3 mg/100 g, respectively. The author suggests that this non-conventional source needs a toxicological evaluation before they can be used as a dietary source of β-carotene.

3. Characterization and analysis of carotenoids

The general procedure for the analysis of carotenoids consists in sampling, sample preparation, extraction, partition, saponification, washing, concentration or evaporation of the solvent, separation techniques, identification, and quantification [48]. The first analysis of many carotenoids were determined in a qualitative form with thin-layer chromatography (TLC), the problem of this technique is that needs a previous separation [49]. Thus, the analysis of carotenoids using mass detectors have shown great advantages over techniques like High-performance liquid chromatography (HPLC), that takes time for the sample preparation and analysis [50]. Nevertheless, the most common technique used to identify carotenoids is HPLC with UV–Vis detector, however, given that many carotenoids are similar (e.g., β-cryptoxanthin and zeaxanthin), the identification is not possible, therefore, is necessary the use of other complementary detection methods [51] as NMR [52] or RAMAM [53, 54], but both techniques require sample preparation.

3.1. Mass Spectrometry (MS)

Mass spectrometry (MS) is nowadays, a powerful analytical tool for qualitative and quantitative applications, since its discovery has evolved from analyzing small inorganic molecules to biological macromolecules, practically without mass limitations [55]. Some methods that have been reported for the analysis of carotenoids by mass spectrometry are electron impact (EI), fast atom bombardment (FAB), matrix-assisted laser desorption/ionization (MALDI), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and more recently, atmospheric pressure photoionization (APPI) and atmospheric pressure solids analysis probe (ASAP) [56]. The mass spectrometry also has the ability to separate isomers as lycopene and β-carotene, these isomers were detected as partially overlapping peaks through ion mobility mass spectrometry (IM-MS) [57], also can distinguish between the protonated ions of lysine and glutamine at *m/z* 147.1128 and 147.0764, respectively. Today, with the modern high resolution MS with a resolving power of over 10,000, will be easier to distinguish these two ions [58]. Thus, the MS can identify between two carotenoids of similar mass, the APCI, also has been important for the identification and quantification of carotenoids in biological samples, previous studies have described the advantages (Figure 2) over other techniques such as ESI and FAB for the analysis of specific carotenoids [59].

3.2. High-performance liquid chromatography – Mass Spectrometry (HPLC-MS)

The analysis of carotenoids by HPLC, generally is realized in reversed-phase using C18 and C30 columns [60]. To separate the analytes is necessary to know what are the correct stationary and mobile phases; for the analysis of carotenoids the mobile phases most used are water, methanol, acetonitrile, 2-propanol, acetone, ethyl acetate, tetrahydrofuran, t-butyl methyl ether (MTBE), dichloromethane (DCM) and chloroform [61]. In case of the column, in general, between the absorbent polymer (C30 and C18), the C30 provides better separations [62, 63], however, the C18 column is relatively good for the separation of geometric isomers of β-carotene, lutein and zeaxanthin[48, 64].

To identify carotenoids by HPLC is needed a detector and for this technique the UV–Vis detectors are the most common, but this detector is not able to identify carotenoids with a similar spectra (e.g., β-cryptoxanthin and zeaxanthin) [51, 53], in the last years, mass detectors have proven effectiveness for the analysis of carotenoids, as it is possible to achieve the elucidation of their structure on the basis of the molecular mass and their fragmentation pattern [65]. For this reason, the MS and the ionization with APCI associated to chromatography has been the most widely used ionization technique for carotenoids due to the high sensitivity [66, 67], likewise, has the capacity to ionize not only carotenes or xanthophylls, also has the capacity to ionize esters of carotenoids [68, 69]. Other promising technique to ionize non-polar molecules, such as carotenoids is APPI, technique that recently has been introduced as a new ionization method with similar characteristics [67], some examples are showed in table 2.
3.3. Matrix-Assisted Laser Desorption Ionization (MALDI)
In the pursuit of new techniques of detection, the analytical chemists have sought new methods for the direct analysis and determination of molecular mass of proteins, carbohydrates and another molecules [85]. MALDI, is one of the most effective methodologies, is a rapid and reliable technique for analysis of a wide range of biomolecules of high and low molecular weight [86]. This technique, is a process that involves optical and mechanical phenomena, as well as, thermodynamic and physicochemical processes [87]; the analysis of a sample covers several crucial steps: first the sample is prepared in a matrix, the second step is the excitation and disintegration of the condensed phase, generation and separation of charges and ionization of the analyte molecules, finally, extraction and separation according to the mass to-charge ratio of the ions in the mass spectrometer, and detection [87, 88].

3.3.1. Matrix-Assisted Laser Desorption Ionization - Time Of Flight (MALDI-TOF)
Nowadays, there are a considerable effort for the development and optimization of MALDI-TOF procedure, to determining carotenoid mass spectra, due to that MALDI allows the direct detection of Carotenoids present in crude extracts, as for example, in Arabidopsis thaliana (vegetative tissue), Capsicum fruit (red, yellow and green), Brassica oleracea (lorets), ripe tomato fruit and sweet potato tuber (Figure 3) [89]. This technique for the qualitative determination of carotenoids, has proved that is possible to use crude extracts from a variety of plants and
crops, without solvent extraction procedures, is possible to acquire a fruit spectra in five minutes, while per sample is over 20 times quicker than traditional HPLC analysis, also, can be used for in vivo analysis of Carotenoids from isolated cells [89].

Figure 3. (a) Matrix blank, no biological material included in the extraction; (b) non-polar extracts from wild-type (Ailsa Craig) ripe tomato fruit, 536.460 m/z corresponds to lycopene/β-carotene; (c) non-polar extracts from phytoene synthase-1 (Psy-1) antisense (carotenoid devoid) ripe tomato fruit; (d) non-polar extracts from Tangella ripe tomato fruit. [89]
Table 2. Identification of carotenoids by different analytical methods of mass spectrometry

<table>
<thead>
<tr>
<th>Technique</th>
<th>Source</th>
<th>Carotenoid</th>
<th>Mass (m/z)</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-APCI-MS</td>
<td>Moringaoleifera (from India)</td>
<td>all-E-luteoxanthin</td>
<td>[M+H-H₂O]⁺ 583.64; [M+H]⁺ 601.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13-Z-lutein</td>
<td>[M+H]⁺ 569.53</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>all-E-lutein</td>
<td>[M+H-H₂O]⁺ 551.60</td>
<td>[70]</td>
</tr>
<tr>
<td>LC-(ESI) MS</td>
<td>from Amazonian Fruits</td>
<td>Lutein</td>
<td>[M+H]⁺ 569</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-Cryptoxanthin</td>
<td>[M+H]⁺ 553</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y-Carotene</td>
<td>[M+H]⁺ 537</td>
<td>[71]</td>
</tr>
<tr>
<td>HPLC-DAD-APCI-MS</td>
<td>Fruits of Sea Buckthorn (Hippophae rhamnoides L.)</td>
<td>Lutein di-palmitate</td>
<td>(C16:0, C16:0) [M+H]⁺ 597.2</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Astaxanthin di-palmitate</td>
<td>[M+H]⁺ 581.3</td>
<td>[72]</td>
</tr>
<tr>
<td>LC–DAD and LC–MS</td>
<td>skin of red-legged</td>
<td>Lutein</td>
<td>[M+H]⁺ 1045</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Astaxanthin</td>
<td>[M+H]⁺ 581.3</td>
<td>[72]</td>
</tr>
<tr>
<td>LC–PAD, GC–MS and UHPLC–ESI-MS.</td>
<td>Romanian sea buckthorn (Hippophae rhamnoides L.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lutein di-palmitate</td>
<td>[M+H]⁺ 597.2</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lutein di-palmitate</td>
<td>[M+H]⁺ 581.3</td>
<td>[72]</td>
</tr>
<tr>
<td>UPLC-UV-TOF-MS</td>
<td>species in algae</td>
<td>Antheraxanthin</td>
<td>[M+H]⁺ 585.4294</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All-trans-zeaxanthin</td>
<td>[M+H]⁺ 589; [M+H-H₂O]⁺ 551</td>
<td>[76]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9-cis-astaxanthin</td>
<td>[M+H]⁺ 597; [M+H-H₂O]⁺ 579</td>
<td>[76]</td>
</tr>
<tr>
<td>LC–DAD-APCI-MS/MS</td>
<td>Echinoderm</td>
<td>9-cis-violaxanthin</td>
<td>[M+H]⁺ 601; [M+H-H₂O]⁺ 583</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>boiledSolanum tuberosum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC–APCI-MS</td>
<td>orange-colored Chinese cabbage algae species</td>
<td>Lutein</td>
<td>[M+H]⁺ 569.4387</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neochrome</td>
<td>[M+H]⁺ 601</td>
<td>[79]</td>
</tr>
<tr>
<td>LC–APCI-MS</td>
<td></td>
<td>Antheraxanthin</td>
<td>[M+H]⁺ 585</td>
<td>[79]</td>
</tr>
<tr>
<td>LC–ESI-MS</td>
<td>Carica papaya</td>
<td>Lycopene</td>
<td>[M+H]⁺ 537</td>
<td>[80]</td>
</tr>
<tr>
<td>LC–DAD–APCI-MS</td>
<td>Taraxacum formosanum</td>
<td>All-trans-neoxanthin</td>
<td>[M+H]⁺ 601.5; [M+H-H₂O]⁺ 583.4</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antheraxanthin</td>
<td>[M+H]⁺ 585.4</td>
<td>[82]</td>
</tr>
<tr>
<td>LC– APCI - MS</td>
<td>from green algae</td>
<td>Viola xanthin</td>
<td>[M+H]⁺ 593</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lutein</td>
<td>[M+H]⁺ 569</td>
<td>[82]</td>
</tr>
<tr>
<td>LC-DAD-MS-APIC</td>
<td></td>
<td>All-trans-neoxanthin</td>
<td>[M+H]⁺ 601</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All-trans-lutein</td>
<td>[M+H]⁺ 569</td>
<td>[84]</td>
</tr>
</tbody>
</table>

Figure 4. Direct MALDI-TLC analysis of pigments after separation with acetone:n-heptane (1:1) and from crude extract. (a) MALDI spectrum of crude acetone extract prior to TLC separation; (b) MALDI spectrum of TLC spot 1; (c) MALDI spectrum of TLC spot 2; (d) MALDI spectrum of TLC spot 3; (e) MALDI of TLC spot 4.[90]
3.3.2. Matrix-Assisted Laser Desorption Ionization - Thin-Layer Chromatography (MALDI-TLC)

The technique of MALDI have a high sensitivity for carotenoid analysis and associated with TLC allow the direct detection of these pigments, example of the analysis are the Carotenoids obtained from the biosynthesis of bacterioruberin pigments from *Haloferax mediterranei* [90]. The carotenoid pigments extracted were treated with TiO2 and separated by TLC with a mobile phase of acetone/n-heptane 1:1 [91], then, was added a matrix solution (10 µL of a solution of 0.05 M α-Cyano-4-hydroxycinnamic acid in 3:1 Acetonitrile:Water containing 0.1% trifluoroacetic), and were analyzed the spots in the TLC (Figure 4).

The results showed that the compound of the spot 1 (m/z value of about 708.887), was previously identified as bisanhydrobacterioruberin, Spot 2 (m/z 725.315) was identified as Monoanhydrobacterioruberin, the spot 3 (m/z 621.608) 2-isopentenyl-3,4-dehydro rhodopin and the spot 4 (m/z 740.68) bacterioruberin (Figure 5 a-d).

![Figure 5. a) bisanhydrobacterioruberin b) Monoanhydrobacterioruberin c) 2-isopentenyl-3,4-dehydro rhodopsin and d) bacterioruberin](image)

**CONCLUSION**

Carotenoids are playing an important role as colorants, feed supplements, and nutraceuticals in food, medical, and cosmetic industries. Over the last decade, there has been an exponential growth of publications related to the identification of carotenoids by mass spectrometry.

This review could help researchers to identify the most promising methodologies associated with mass spectrometry for identification of carotenoids in different natural products sources. Also, in this work examples and techniques considerations to use this powerful analytical tool for the identification of these metabolites are exposed.

**REFERENCES**


