INTRODUCTION

Dunaliella is one of the richest sources of natural β-carotene and produces the stereoisomers 9-cis-β-carotene and all-trans-β-carotene in equal amounts depending on culture conditions.

<table>
<thead>
<tr>
<th>Source</th>
<th>Typical Carotene content g kg⁻¹</th>
<th>Carotene composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic</td>
<td>synthetic</td>
<td>All-trans β-carotene 9-cis β-carotene α-carotene Other</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.006 – 0.055</td>
<td>&gt;98                      &lt;2  0  0</td>
</tr>
<tr>
<td>Palm oil</td>
<td>0.63 – 0.7</td>
<td>36                       24  34  6</td>
</tr>
<tr>
<td>Dunaliella (AFDW)</td>
<td>60 – 140</td>
<td>48                       48  3  1</td>
</tr>
</tbody>
</table>

9-cis-β-carotene is implicated in retinal function in people with retinitis pigmentosa, and large quantities are required for clinical trials. 9-cis-β-carotene is not readily synthesised chemically. The use of HPCCC was unable to separate all-trans- and 9-cis-beta carotene isomers due to their similar partition coefficient in the range of solvents systems tested.

Cooling will precipitate all-trans- but not 9-cis-β-carotene


METHODS

Dry algal biomass was extracted with MTBE: MeOH (20:80) (laboratory-scale) to yield a carotenoid-enriched extract. All-trans-β-carotene was isolated from the extract using a solvent-antisolvent method or cooling. Analytical HPLC was performed using a YM30 250 x 4.9mm I.D S-5μ HPLC column with DAD at 25°C; Isocratic elution with 80% methanol: 20% MTBE. Flow 1 mL/min. 450nm profile shown. Preparative HPLC was performed using a C18 HPLC column with DAD at 25°C; Isocratic elution with 95% methanol: 5% acetone.

RESULTS

Crude preparations of carotenoid isomers extracted from Dunaliella powders could not be separated using preparative HPLC, but could be resolved using analytical HPLC.

Analysis of Preparative HPLC Chromatogram obtained for Carotenoid Extract

Carotenoid isomers were poorly resolved using preparative HPLC. Inserts show analysis of fractions 6 and 8, conditions as above

A processing scheme was developed to reduce the concentration of all-trans-β-carotene in preparations before preparative HPLC

CONCLUSIONS

At industrial-scale, highly enriched preparations of 9-cis-β-carotene can be achieved using a combination of solvent extraction, precipitation and chromatography. However preparative HPLC remains limited by low yield, long run times, poor resolution of β-carotene isomers and loading capacity: only 1.8g of sample can be loaded onto a 250mm preparative HPLC column. It is not cost effective at scale.

A cost-effective, sustainable, high-efficiency and low environmental impact commercial process to purify 9-cis-β-carotene from all-trans-β-carotene remains to be developed.