# An Extreme Source of β-Carotene

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The carotenoids of the green form of the flagellated green algae *Dunaliella salina* consist of α- and β-carotene, lutein, neoxanthin, and violaxanthin.[1,2] The red variety contains mainly β-carotene together with small amounts of xanthophylls.[2,3] Development of the red β-carotene rich form has been shown to be favoured by high light intensity,[4] increased temperature,[4] N- and P-deficient growth conditions,[1] $O_2$-deficiency,[1] and high salinity.[2,5] The total carotenoid content is considerably increased in the red variety.[1,2,4]

Mass cultures of the red variety of *D. salina*, known to occur irregularly and for short periods, are responsible for the pink colour of the Pink Lakes, Linga, Victoria, Australia.[6] A β-carotene content of 40 % of the dry body weight has been stated to be present in *D. salina* of this natural habitat,[6] a carotenoid content that greatly exceeds that found in other sources. Some extreme values of other sources are quoted for comparison: *D. salina* (1—2.5 % of the dry weight [4,7,8]), *Trentepohlia aurea* (0.7 % of the dry weight [7]) and *Chromatium okenii* (0.98 % of the acetone extracted residue [9]), coronas of *Narcissus* (2 % of the dry weight) and of the red fringes of coronas of *Narcissus poeticus recurvus* (16.5 % of the dry matter).[9b]

In the present work the carotenoids of brick red salt samples from Pink Lake have been re-investigated in a quantitative manner. The high salt content complicated the analysis.

The sample investigated was collected from red patches at the shore of the Pink Lakes and consisted of a mixture of crystalline salts and brine, all brick red in colour. After receipt the salt mixture was stored in a light tight container at 2°C until used.

Microscopical examination revealed no live flagellates, few if any bacteria, only highly pigmented debris of sizes and structures consistent with being of algal origin. Small amounts of material were used as inoculum in liquid and solid medium for *Dunaliella* [10] and the yeast extract-tryptone medium for halophilic bacteria [11] with 15—25 % NaCl or Pink Lake salt added to *ca.* 20 %. No growth of algae or bacteria appeared after several months of incubation at assumed favourable conditions.

Portions (40—50 g) of the wet salt mixture were dialyzed against distilled water (3×10 l) at 2°C for 2—3 days. The desalted suspensions were filtered through coarse glass wool to remove particles like bits of insects *etc.* and aliquots of the filtrate were centrifuged at 35 000 g for 20 min and the sediments and supernatants were analysed separately.

Dry weight was determined by drying the dialyzed samples at 105°C. Aliquots of the same samples were exhaustively extracted with acetone and finally with 3 portions of boiling methanol-chloroform (3:1) and the total extracted material named lipids. The ash content was determined by combustion of the extraction residues at 425°C. The dry weight of the sample minus the ash content of the residue after lipid extraction was taken as a measure of total organic matter in the sample analyzed.

The dialyzed sediment and supernatant were easily extracted with acetone at room temperature. Over-saturation of β-carotene in the acetone extract of the sediment was indicated by crystallization on standing at room temperature. The acetone extracts of the sediment were collected by centrifugation or filtration and the carotenoid content determined spectrophotometrically using $E(1\ \%, 1\ \text{cm}) = 2500$ at 452 nm in acetone. The carotenoids of the aqueous acetone extract of the supernatant were transferred to ether and determined spectrophotometrically.

Circular paper chromatography [12,13] revealed that β-carotene comprised more than 95 % of the total carotenoid. Seven minor more strongly adsorbed yellow and orange zones did not exhibit carotenoid-like absorption spectra in visible light and presumably represented decomposition products.

β-Carotene crystallized from the acetone extract in high yield as violet, shiny crystals, m.p. 177–178°C (evacuated tube). With synthetic β-carotene m.p. 179–180°C no depression (m.p. 178–180°C) was observed, and no chromatographic separation was achieved on kieselguhr or aluminium oxide papers.[12,13] The spectral properties further corresponded to those of synthetic β-carotene. Our sample had abs. max. 452 and 477 nm, % III/II $^{14}$=16 in acetone; $\nu_{max}$ (KBr) *3100, 2990, 2900,* 1640, 1570, *1460, 1380,* 1315, 1260, 1180, *975* and 840 $cm^{-1}$ (main bands in italics); τ ($CDCl_3$) 8.97 (12 H *gem.* Me), 8.50 (8 H non-allylic methylene), 8.29 (*ca.* 6 H end-of-chain Me), 8.03 (12 H in-chain Me), 7.9 (4 H allylic methylene) and 3.84–3.0 (14 H olefinic); *m/e* 536 (M), M–92, M–106.

100 g of wet salt sample contained *ca.* 750 mg of organic material, about 9 % of which remained in solution after removal of the salts by dialysis. The residue after extraction of carotenoids and other lipids had an N content of 8.05 % (micro Kjeldahl) or 9.14 % (Dumas), respectively.

| | % of total organic matter |
|---|---|
| β-Carotene, in total sample | 13.8 |
| in sedimentable material | 14.9 |
| Lipids including carotenoids, | |
| in total sample | 41.5 |
| in sediment | 45.0 |
| Protein (6.25 × N-Kjeldahl) | 28.0 |

β-Carotene comprised 13.8 % of the total dry, organic matter of the salty sample or 28 % when calculated on the basis of the organic matter in the dialyzed extraction residue. The value is thus lower than the one reported by Cane [6] but still remarkable. Attempts by other workers to grow cultures of *D. salina* in artificial media with comparable carotene content have not been successful. Milko [1,4] reports a maximum content of 2.5 % β-carotene under optimal conditions for carotenoid synthesis and 0.4 % under optimal conditions for growth.

The material examined here and which is assumed to consist mainly of debris of cells of *D. salina* had a high lipid content (30 % except carotenoids), *cf.* Ref. 15. From the extraction behaviour we assume that β-carotene is not deposited in the cells in the crystalline form but dissolved in the lipids of the cells.


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