Natural food colour from algae

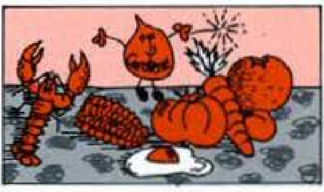
Carotenes give oranges, carrots, tomatoes, apricots, egg yolk, corn, and lobsters their colour. The most common form is *beta*-carotene; food companies pay about $400 a kilogram for synthetic *beta*-carotene, which is used to colour margarine, cheese, fruit juice, soft drinks, and many other products. World-wide, the food industries use about a million kilograms of the chemical annually, and demand is outstripping supply.

When you buy a 500-g container of margarine, you get about 2·5 mg of *beta*-carotene. After you have eaten it, the bacteria in your gut convert the *beta*-carotene to vitamin A. Vitamin A, or its precursor, *beta*-carotene, is essential in the diet for healthy eyes and skin, and to increase resistance against infection. Some scientists have suggested that a large intake of vitamin A may also protect against some forms of cancer.

A new Australian company, Betatene Ltd, plans to supply the international market with *beta*-carotene isolated from the salt-lake-dwelling alga *Dunaliella salina*.

Dunaliella sp. is widely distributed in the sea and inland salt lakes. In saturated salt solution it produces *beta*-carotene (up to 8% of the dry weight of the algae). It is one of the few organisms that can tolerate the harsh salt-lake environment. Salt lakes containing *Dunaliella* have a characteristic pink colour.

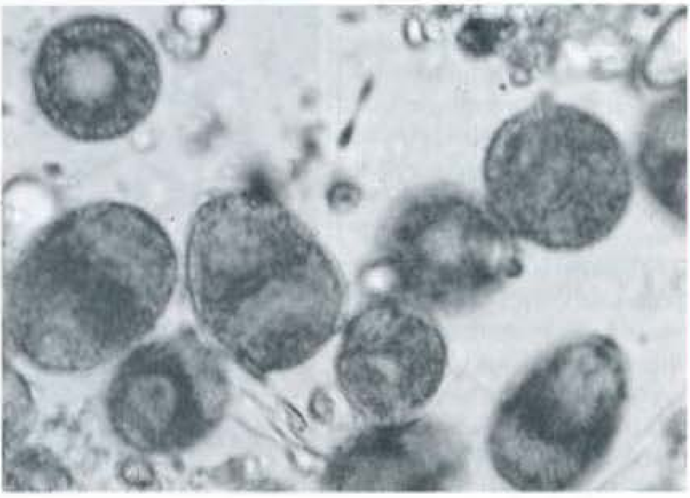
Betatene is operating a pilot plant at Whyalla, South Australia. The lakes there are already used by BHP for salt production, and Betatene's 10-year agreement with BHP to use 950 ha of the lakes for algae harvesting has significantly reduced the new company's start-up costs. Limited production of *beta*-carotene from 9 ha is under way, and a full-scale plant will be commissioned early in 1986.

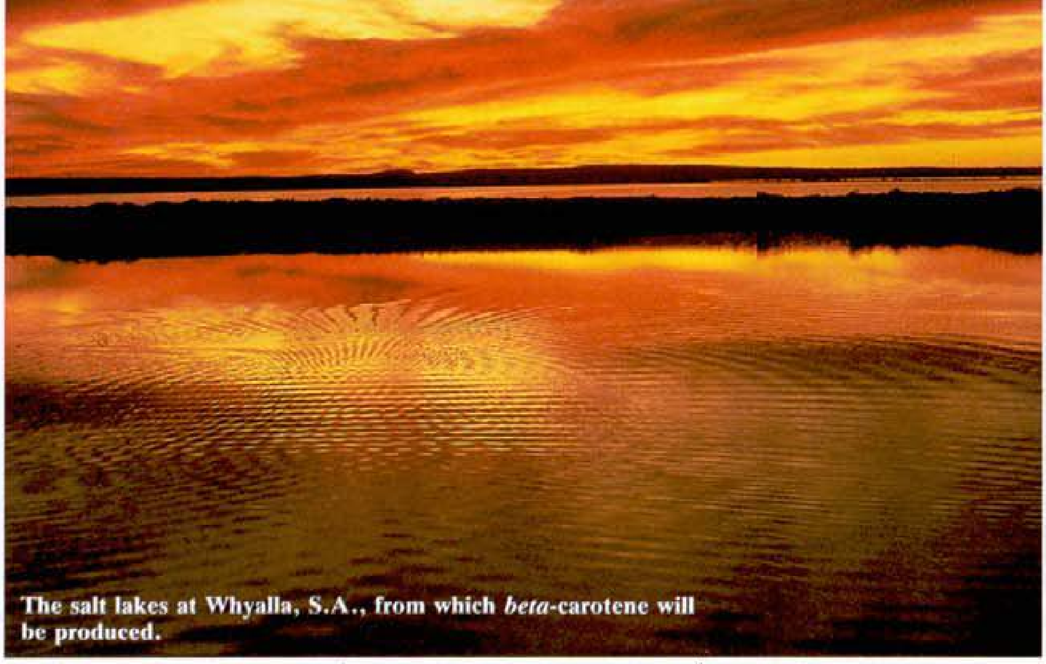


Beta-carotene from *Dunaliella* is a natural product. Betatene Ltd believes that it will achieve rapid market acceptance because it does not contain the residues of solvents or chemical reaction products found in the synthetic product.

The possibility of using *Dunaliella* as a large-scale source of *beta*-carotene was pointed out in 1959 by Dr G. Aisen-Jensen of CSIRO. Since

The alga *Dunaliella salina*, which contains *beta*-carotene. The single-celled organism is only about 10 μm in diameter.





The salt lakes at Whyalla, S.A., from which *beta*-carotene will be produced.

then, many people have tried to harvest *beta*-carotene, but only one large-scale operation has resulted. This operation, in the Ukraine, extracts about 20 tonnes of the substance a year from *D. salina* growing in the evaporation pond of a salt works.

There have been two major obstacles to commercial harvesting of the organism. Firstly, although each cell can contain high concentrations of *beta*-carotene, the concentration of algae in lakes is so low that the *beta*-carotene content rarely exceeds 5 mg per litre of brine. Secondly, the cells are small (8–15 μm across), with a density only slightly greater than that of saturated brine, and therefore the simple separation methods of filtration, sedimentation, and centrifuging don't work very well.

The key to Betatene's commercial operation is an extraction process conceived in 1980 by Dr Cyril Curtain of the CSIRO Division of Chemical and Wood Technology. He and Mr Harvey Snook, a director of the new company, developed the concept in the laboratory. The present commercial process is the result of several years' work by the company, financed by private venture capital and a grant from the Australian Industrial Research and Development Incentives Scheme.

Dr Curtain's concept was based on adsorption techniques similar to those used for the separation of animal cells in the laboratory. These had never before been used for algae, or on a large scale.

Adsorption has the potential for delivering a high-purity product: if the adsorbent surface could be tailored to make it specific for *D. salina*, the bacteria and other contaminants occurring in open-air culture ponds would be left behind.

To pick the right adsorbent, it was first necessary to study the algae's surface properties. Dr Curtain used electron spin resonance (ESR) to do this, attaching 'spin labels' onto the proteins of the cell membranes. The spin label, a stable chemical group, has a characteristic ESR spectrum that changes depending on the spin label's immediate chemical environment.

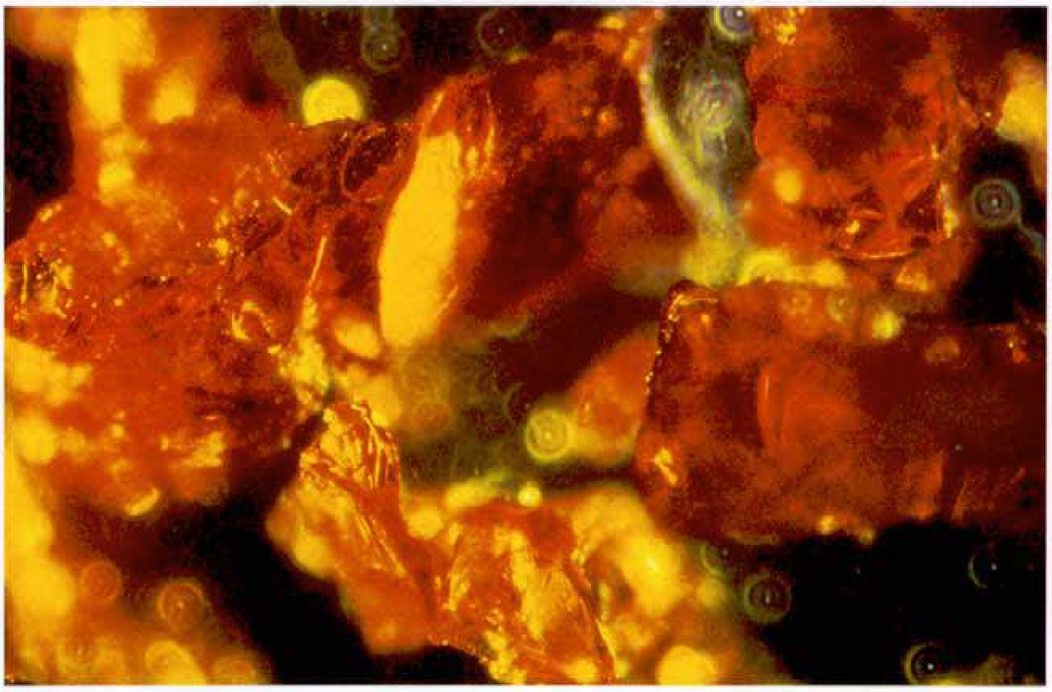
D. salina has an unusual structure. Unlike all other algae, it lacks a rigid cell wall; instead, it's a naked protoplast. The electron spin resonance revealed that about half of the outer membrane of this naked protoplast was protein, and that most of the exposed amino acids were hydrophobic (water-repellent) in concentrated brine. This meant that the cells would adhere to a hydrophobic surface. Indeed, if you grab a handful of the algae, it feels greasy.

Dr Curtain found that 85% of the cells in a concentrated brine solution could be adsorbed onto the surface of glass beads coated with a water-repellent substance. The *beta*-carotene could be recovered by stirring the beads in a suitable solvent. The cell residues still attached to the beads could be washed off in fresh water so that the adsorbent could be used again.

An early pilot plant used a hydrophobically coated magnetite as the adsorbent and liquid carbon dioxide under a pressure of 6 MPa as the solvent. The magnetite can be easily recovered using a magnetic separator, as used with the Sirofloc and Sirotherm processes developed at the Division (see *Ecos* 31 and 45). Liquid carbon dioxide is an excellent food-grade solvent, leaving no unacceptable residues.

Beta-carotene can be readily crystallized from the oily extract remaining after the carbon dioxide has evaporated. The extract contains negligible amounts of

Crystals of *beta*-carotene extracted from *D. salina*, seen through the microscope.



The tinge of colour is what it's all about.

contaminants from bacteria or other foreign material, allowing its use for pharmaceutical- and food-grade *beta*-carotene.

The process has undergone some further proprietary refinements, but remains the same essentially simple yet specific technique.

Experiments in Israel with open brine ponds have shown that sustained yields of 600 mg of *beta*-carotene per sq. m per day are possible. And yet this substance represents only a small fraction of all products that will be extracted from *Dunaliella* sp. in South Australia.

Initially, the major by-product will be an algal meal for stock food, but the quantity of algae needed for 1 kg of *beta*-carotene will also yield 150 kg of glycerol, which is used in making a range of plastics, and in the preparation of some foods and pharmaceutical products. The company claims that this production route should be cheaper than current sources.

Other products will be various terpene oils widely used in the food and plastics industries.

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Production of β-carotene from *Dunaliella salina*. C.C. Curtain. In 'Annual Research Review 1984 — CSIRO Division of Chemical and Wood Technology'. (CSIRO: Melbourne 1985.)

Changes in the ordering of lipids in the membrane of *Dunaliella* in response to osmotic-pressure changes — an e.s.r study. C.C. Curtain, F.D. Looney, D.L. Regan, and N.M. Ivancic. *Biochemical Journal*, 1983, **213**, 131–6.