Chapter 3

PRODUCTION OF CAROTENOIDS WITH FUNGI

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1 INTRODUCTION

Humans, like most other animals, need carotenoids but cannot synthesize them. Our main suppliers are the fruits and vegetables, with lesser, mostly indirect contributions from fungi, algae, and bacteria. Carotenoid production is rarely the main objective of a culture, but colourful products rich in carotenoids are obtained from various plants and algae. The oldest example is saffron (Crocus sativus), still planted in La Mancha, Spain, and elsewhere for the stigmas of its flowers. Many fields of central Mexico become bright orange in the summer with the flowers of cempasúchil (Tagetes tenuifolia, a kind of marigold), whose dried petals are fed to chicken for meat and egg yolk colour. Annatto is extracted from the seeds of a tropical tree, Bixa orellana. The most familiar carotenoid-rich product is probably paprika, a powder made by grinding red peppers (Capsicum annum).

The carotenoids may be scarce in the diets of humans and animals for various reasons. Many staple foods and feeds, including most of the cheap sources of carbohydrates and fat, are poor in carotenoids, as witnessed by their lack of colour. The long winters of many countries halt the natural production of carotenoids, and these are relatively unstable and may not survive in stored foods and feeds.

Organic chemists have devised several complete syntheses, which now supply considerable amounts of β-carotene and other purified carotenoids. The current trend towards natural food colours and the discovery of salutary effects of the carotenoids, beyond their important role as provitamin A, stimulate the demand and press towards a biological production.

The fungi can hardly be considered as traditional food colourants, but the relative ease of cultivation and the many possibilities for physiological, genetical, and industrial manipulations make them attractive to biotechnologists. Several species are potential sources of carotenoids, and a large-scale process has been based on Blakeslea trispora.
It seems that the Soviet Union is the only country that uses *Blakeslea trispora* for the industrial production of carotenoids; the yearly production is estimated at about 200 kg purified β-carotene and another 4 Mg β-carotene in the form of mycelia used as an additive to animal feed (A. A. Dmitrovskii, pers. comm., 1988).

The abundant literature on carotenoids has been the subject of many books and reviews. The monumental and indispensable treatise edited by Isler (1971) is complemented by newer reviews (Feofilova, 1974; Goodwin, 1976, 1980, 1988). The practical aspects are explained in great detail by Bauernfeind (1981). Updates are provided by the International Symposia on Carotenoids, published every 3 years as a special issue of *Pure and Applied Chemistry*. There are specialized and recent reviews on the influence of external factors on carotenogenesis in the Mucorales (Lampila *et al.*, 1985a), on *Phycomyces* (Cerdá-Olmedo & Lipson, 1987), on the regulation of carotenogenesis (Bramley & Mackenzie, 1988), on the metabolism and metabolic effects of the carotenoids (Goodwin, 1986), and many other subjects.

2 FUNGAL CAROTENOIDs

Most fungi produce no carotenoids at all and many of the others contain a single major carotenoid. Although the pathway intermediates may be present in amounts widely variable with the strain and the culture conditions, the carotenoid composition of the fungi is usually much simpler than that of the photosynthetic organisms. Here are a few selected examples of fungal carotenoids. β-Carotene predominates in the Mucorales *Blakeslea trispora*, *Phycomyces blakesleeanus* and *Choanephora cucurbitarum*, in the yeast *Rhodotorula aurea*, in *Aspergillus giganteus* (El-Jack *et al.*, 1988) and various species of *Penicillium*; neurosporaxanthin is the end-product of *Fusarium aquaeductuum* (Bindl *et al.*, 1970); neurosporaxanthin and β-carotene, those of *Gibberella fujikuroi* (Avalos & Cerdá-Olmedo, 1987), *Neurospora crassa* and *Verticillium agaricinum* (Valadon & Mummery, 1973); torularhodin and β-carotene, those of the yeasts *Rhodotorula rubra*, *R. minuta*, and *Rhodosporidium diobovatum*; β-carotene, lycopene, and β-zeacarotene are found in different strains of the smut *Ustilago violacea* (Will *et al.*, 1984); canthaxanthin in the mushroom *Cantharellopsis cinnabarina*; astaxanthin, in the yeast *Phaffia rhodozyma*. See Goodwin (1980) for other fungi and additional references.

Figure 1 shows the structure of the carotenoids that have just been mentioned, with their likely biosynthetic pathways. The carotenoids, like all terpenoids, are synthesized from hydroxymethylglutaryl-coenzyme A, which is first converted to mevalonic acid. The specific part of the pathway begins with the condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene, a colourless carotene. Four dehydrogenations transform phytoene into lycopene, the pigment of red tomatoes. Two cyclisations convert lycopene