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A COMPARISON OF SYNTHETIC β-ZEACAROTENE AND SAMPLES OBTAINED FROM *RHODOTORULA GLUTINIS* AND YELLOW CORN

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Abstract—β-Zeacarotene was obtained from *Rhodotorula glutinis* grown at 5° and from frozen yellow corn. These preparations were spectroscopically and chromatographically identical to synthetic β-zeacarotene and probably identical with the β_1-zeacarotene previously isolated from corn gluten and "xanthophyll oil".[1]

INTRODUCTION

Two closely related compounds designated β and β_1-zeacarotene were isolated from extracts of yellow corn gluten and corn endosperm oil by Petzold, Quackenbush and McQuistan.[1] These authors proposed the structure of the former pigment as all *trans*-7′,8′-dihydro-γ-carotene and this was confirmed by Rüegg *et al.*[2] who carried out a total synthesis. However, the absorption characteristics of the synthetic material more closely resembled those reported for β_1-zeacarotene.

Davies *et al.*[3] cited kinetic evidence that β-carotene is formed via β-zeacarotene and not lycopene in *Phycomyces blakesleeanus*. Simpson and co-workers[4,5] found that the level of β-zeacarotene and other intermediates increased in *Rhodotorula glutinis* when the yeast was either cultured at 5° or treated with either β-ionone or methyl heptenone. Since under these conditions lycopene could not be detected, a key role was ascribed to β-zeacarotene in the formation of γ-carotene from neurosporene in *R. glutinis*. In these studies both β and β_1-zeacarotenes were isolated.

It was the purpose of the present study to isolate β-zeacarotene from frozen sweet corn and from *R. glutinis* and to compare it with a synthetic specimen.

RESULTS AND DISCUSSION

Table 1 gives the spectral characteristics of the synthetic β-zeacarotene compared with samples obtained from *R. glutinis* and frozen yellow corn. The absorption curves recorded by a spectrophotometer were superimposable. Samples of the three β-zeacarotene samples were also co-chromatographed on alumina in pairs, and in each case only one band was obtained.

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TABLE 1. SPECTRAL CHARACTERISTICS OF SYNTHETIC β-ZEACAROTENE COMPARED WITH SAMPLES OBTAINED FROM *R. Glutinis* AND YELLOW CORN

Sample	λ_{max} (mμ)*		
Synthetic	406†	427	454
R. glutinis	407†	427	454
Yellow corn	407†	427	454

* In light petroleum.
† Shoulder.

In preliminary experiments with carotenoids from *Rhodotorula* spp. both β- and β_1-zeacarotenes were isolated[4,5] and initial attempts to isolate β-zeacarotene from either corn meal or from frozen corn also resulted in the appearance of both forms of β-zeacarotene. In later work with frozen corn, only the carotenoid with the characteristic longer wavelength band was found. It was observed that the absorption maxima of solutions of β-zeacarotene from *R. glutinis* and yellow corn as well as the synthetic sample tended to shift to a shorter wavelength on standing in the dark at refrigeration temperature. On chromatographing this sample on an alumina column, a major (452, 426 and 404 mμ) and a minor band (445, 420 and 398 mμ) were observed. Iodine catalysis[1] of these fractions as well as a chromatographically pure sample of the synthetic pigment yielded equilibrium mixtures with the same spectral characteristics (450, 425 and 404 mμ). Column chromatography of the iodine-treated samples gave a similar distribution of isomers as was reported by Petzold *et al.*[1] The major band (49·3 per cent) had absorption maxima at 405, 425–426 and 451 mμ.

Thus these results show that the β-zeacarotene with maxima at 407, 427 and 454 mμ (light petroleum) is probably the naturally occurring pigment in both yellow corn and *R. glutinis*. The pigment with absorption at 406, 426 and 452 mμ is probably derived from the former carotenoid by *cis-trans* isomerization (cf. all *trans*-β-zeacarotene Ref. 2 and Table 1). The β_1-zeacarotene of Petzold *et al.*[1] with its main maxima at 427 mμ probably corresponds to the β-zeacarotene that we find in *R. glutinis* and frozen yellow corn and which is identical with the synthetic specimen.

EXPERIMENTAL

Solvents. The solvents used were light petroleum (A.R., b.p. 40–60°), diethyl ether (A.R.) and acetone (A.R.). All were obtained from British Drug Houses Ltd., Poole, Dorset. The light petroleum and acetone were redistilled prior to use. The diethyl ether was freed from peroxides immediately before use by passage through a column of aluminium oxide.

Chromatographic adsorbents. Aluminium oxide (neutral) was obtained from M. Woelm, Eschwege. Hyflo Super-Cel was obtained from L. Light & Co. Ltd., Colnbrook, England. Magnesium oxide of chromatographic adsorption grade was obtained from British Drug Houses Ltd.

Other materials and chemicals. Synthetic β-zeacarotene was a gift from Dr. O. Isler, F. Hoffmann-La Roche Ltd., Basle, Switzerland; it was rechromatographed on aluminium oxide prior to use. All other chemicals used were of A.R. grade and were obtained from British Drug Houses Ltd. The frozen sweet corn on the cob was obtained as a gift from Dr. H. Wilkinson, Unilever, Sharnbrook, Bedford, England, through Birds Eye Foods Ltd.

Organism and cultural conditions. Rhodotorula glutinis (48-23T) was obtained from prof.

H. J. Phaff, Department of Food Technology, University of California, Davis, California; it was cultured on agar slopes in flat bottles.[6]

Extraction and chromatography of R. glutinis *pigments.* The harvested yeast cells were disrupted in a modified French Press[7] and the pigments were extracted with acetone and transferred to light petroleum with the addition of water.[6] Two hundred ml of 10% (w/v) methanolic potassium hydroxide were added to the light petroleum solution. Saponification was completed by heating for 5 min on a water-bath. Light petroleum was added to the cooled alcoholic solution, and the water-soluble materials were removed by several washes with water. The light petroleum solution was dried over anhydrous sodium sulphate.

The pigments were chromatographed on a 2·0 × 30 cm column of magnesium oxide—Hyflo Super-Cel (1:2, w/w)—and developed with light petroleum and 1% (v/v) acetone in light petroleum. After the yellow band which contained β-zeacarotene had separated between the bands of β-carotene and γ-carotene, the column was extruded and the β-zeacarotene zone was removed and eluted with acetone and transferred to light petroleum, and the solvent dried over Na_2SO_4.

The β-zeacarotene fraction was rechromatographed on a 10 g column (1·1 cm in diameter) of alumina (activity grade 1) and developed with light petroleum containing increasing amounts of diethyl ether (20–40 per cent). Three major zones were formed. β-Zeacarotene was adsorbed above β-carotene and below a multi-component band which could only be moved with higher concentrations of diethyl ether (50–100 per cent). The spectrum of the β-zeacarotene was not changed by rechromatography on either alumina or calcium hydroxide columns.

Extraction and chromatography of the corn. The corn was thawed and the kernels were cut from the cob. Excess water was removed from the corn by placing it in canvas bags and subjecting it to pressure in a hydraulic press; the pulp so obtained was refrozen and thawed prior to use. Ten-pound lots of the pulp were macerated in an M.S.E. blender in acetone (10 l.) and filtered under vacuum. The filtrates from repeated extractions were combined and the carotenoids were concentrated in the upper phase as water and light petroleum were added. On occasion saturated solutions of NaCl were used to break emulsions as the light petroleum was washed free from acetone. The light petroleum (7 l.) was concentrated under reduced pressure to about 1 l. Two litres of 10 per cent methanolic KOH were added and saponification was completed by heating for 1 hr at 100°. The pigments were transferred to light petroleum as described before.

The corn carotenoids were chromatographed on a 5 × 45 cm column of magnesium oxide—Hyflo Super-Cel—and developed with the light petroleum acetone mixture. The crude β-zeacarotene zone was removed by sectioning and transferred to light petroleum as described before. The β-zeacarotene band was rechromatographed on a 2·0 cm in diameter column of 25 g of alumina. Increasing concentrations of diethyl ether in light petroleum (2–40 per cent) were used in the development of the column. An additional band (ζ-carotene) was observed on this column compared with the chromatogram of the corresponding extract from *R. glutinis* as ζ-carotene formed a large band above β-zeacarotene. The β-zeacarotene was rechromatographed on alumina.

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