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Directorate C - Scientific Opinions
**C3 - Management of scientific committees II; scientific co-operation and networks**

**SCF/CS/ADD/COL 158 Final - correction**

# OPINION

# OF THE SCIENTIFIC COMMITTEE ON FOOD

# ON

# ß-CAROTENE FROM *BLAKESLEA TRISPORA*

## - Correction -

**(Adopted on 22 June 2000, and corrected on 7 September 2000)**

**Terms of Reference**
The Committee is asked to give an opinion on the safety of ß-carotene from a dried biomass source, obtained from a fermentation process with *Blakeslea trispora* for use as a colouring matter for foodstuffs.

**Background**
An application has been received for inclusion of a fermentation-produced ß-carotene, extracted from the biomass produced by co-fermentation of two strains, DS 30627 and DS 30628, of the mould *Blakeslea trispora* as an alternative source of ß-carotene requested for use as a colouring matter for foodstuffs, as a micronutrient, and as a dietary supplement.

**Microbiological considerations**
The source organism, the mould *Blakeslea trispora* is a plant commensal of tropical plants, some strains of which produce increased levels of ß-carotene. The fungus exists in a (+) and (-) mating type, of which the (+) type - DS 30627 - synthesises trisporic acid, a precursor of ß-carotene. On mating the two types in a specific ratio the (-) type - DS 30628 - then synthesises large amounts of ß-carotene (1).

The mould has been shown to be non-pathogenic and non-toxigenic, by a literature search (1), a standard pathogenicity experiment in mice (2, 3, 4) and by analyses of extracts of several fermentation mashes for fungal toxins (9) and of the final product, the ß-carotene crystals, by enzyme immunoassays for 4 mycotoxins (1).

**Technological considerations**
The production process proceeds essentially in two stages. In the initial fermentation process seed cultures are produced from the original strain cultures and subsequently used in an aerobic submerged batch fermentation to produce a biomass rich in ß-carotene. In the second stage, the recovery process, the biomass is isolated and transformed into a form suitable for isolating the ß-carotene. The latter is extracted from the biomass with ethyl acetate, suitably purified and concentrated, and the ß-carotene crystallised from the mother liquor. The final product is either crystalline ß-carotene (purity >96.0%) or is formulated as a 30% micronised suspension in vegetable oil. The production process is controlled by GMP procedures, adequate hygiene control, and adequate control of the raw materials. The biomass and the final crystalline product comply with an adequate chemical and microbiological specification (1) and the final crystalline product also complies with the JECFA and EU specifications as set out in Directive 95/45/EC for colouring matters in food. This has been confirmed by the analytical evidence submitted (1).

**Safety of the fermentation-produced ß-carotene**
HPLC analysis, stability tests and microbiological tests have shown that the ß-carotene obtained by co-fermentation of *Blakeslea trispora* DS 30627 and DS 30628 complies with the EC specification for E 160 aii, also including the proportions of cis and trans isomers, and is free of mycotoxins or other toxic metabolites (1, 5). In-vitro tests for gene mutations and chromosomal aberrations with the ß-carotene produced by the manufacturer in the EU showed it to be free of genotoxic activity (6, 7). In a 28-day feeding study in rats with the ß-carotene manufactured in the EU no adverse findings were noted at a dose of 5% in the diet, the highest dose level used (8).

**Conclusion**

Evaluation of the source organism and the production process yielded no grounds to suppose that the final crystalline product, ß-carotene, differs from the chemically synthesised ß-carotene used as a food colorant. The final crystalline fermentation product has been shown to comply with the specification for ß-carotene E 160 aii listed in Directive 95/45/EC (10).

The committee considers that ß-carotene produced by co-fermentation of *Blakeslea trispora* DS 30627 and DS 30628 is equivalent to the chemically synthesised material used as food colorant and is therefore acceptable for use as a colouring agent for foodstuffs.

**References**


1. Gist-Brocades (1997) dossier EC 174.01 on natural ß-carotene submitted to the EU Commission.
2. Matselyukh, B.P. & Sten'ko, A.S. (1987) Strain characteristics of the carotene-containing biomass production. Document submitted by Gist Brocades to the EU Commission.
3. Resnik, S.R., Sten'ko, A.S., Bezkorovaynaya, N.K. (1987) Study of the pathogenicity of *Blakeslea trispora* strain of the (+) and (-) variants. Document submitted by Gist-Brocades to the EU Commission.
4. Barshtein, Yu.A. & Persidsky, Yu.V. (1988) Pathomorphologic investigation. Document submitted by Gist-Brocades to the EU Commission.
5. Hempenius, R.A. & Coenen, T.M.M. (1995) Screening tests with biomass for ß-carotene. Notox projects 147825, 147835, 147803, 147814 submitted by Gist-Brocades to the EU Commission.
6. van Delft, J.H.M. (1996) Ames test with ß-carotene. Report 15.234 submitted by Gist-Brocades to the EU Commission.
7. De Vogel, N. & van Delft, J.H.M. (1996) Chromosome aberration test with ß-carotene in cultured Chinese hamster ovary cells. Report 15.233 submitted by Gist-Brocades to the EU Commission.
8. van Beek,G., Catsburg, J.P., Wijnans, M.V.M. (1997) Subacute (28 days) oral toxicity study with ß-carotene in rats. Report 15.262 submitted by Gist-Brocades to the EU Commission.
9. Samson, R.A. (1995) Report of the Centraalbureau voor Schimmelcultures, NL, 21.12.1995.
10. Commission Directive 95/45/EC of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities L 226, 22.09.95.