



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees II; scientific co-operation and networks

Scientific Committee on Food

SCF/CS/ADD/COL/173 Final

21 February 2003

Opinion of the Scientific Committee on Food

**on the safety in use of isopropyl alcohol and isobutyl acetate for
the extraction of beta-carotene from *Blakeslea trispora***

(expressed on 5 March 2003)

**Opinion of the Scientific Committee on Food
on the safety in use of isopropyl alcohol and isobutyl acetate
for the extraction of beta-carotene from *Blakeslea trispora***

Terms of Reference

The Committee is asked to evaluate the safety in use of extraction solvents isopropyl alcohol and isobutyl acetate as described by the company for the production of beta-carotene from *Blakeslea trispora*.

Background

The Committee adopted an opinion on 22 June 2000 (corrected on 7 September 2000) on the safety of beta-carotene obtained by a fermentation process using a mixed culture of the sexual mating types (+) and (-) of natural strains of the fungus *Blakeslea trispora* (SCF 2000). In this fermentation process, the beta-carotene is extracted from the biomass with ethyl acetate. Recently, the Commission services have received a request to use the solvents isopropyl alcohol and isobutyl acetate for the extraction stage of the manufacturing process.

Technological data

According to the applicant, the basic manufacturing process of beta-carotene from *Blakeslea trispora* is the same as described in Directive 2001/50/EC (EC, 2001), except that the beta-carotene is extracted from the biomass using firstly isobutyl acetate and then isopropyl alcohol. The isopropyl alcohol has a purity not lower than 99.7%, while the isobutyl acetate has a purity not lower than 99%. Both solvents are equivalent to food grade materials (Vitatene, 2002).

The levels of these solvents in 5 non-consecutive batches of beta-carotene were reported to be 0.018 – 0.063% isopropyl alcohol and 0.78 – 1.08% isobutyl acetate. On this basis, the applicant proposes residues of not more than 0.1% for isopropyl alcohol and not more than 1% for isobutyl acetate to be included in the chemical specifications for beta-carotene obtained by this manufacturing process. The Committee was informed that the other

chemical and microbial specifications of beta-carotene produced by the new process comply with the Commission Directive 2001/50/EC (EC, 2001).

Exposure

Assuming a daily intake of 2 mg betacarotene and a residue of 0.1% isopropyl alcohol in betacarotene, the intake of isopropyl alcohol from this source would be 0.002 mg/day. In the case of 1% residual isobutyl acetate in beta-carotene, the intake would amount to 0.02 mg/day.

Isobutyl acetate also occurs naturally in many fruits and is used as flavouring substance in non-alcoholic beverages up to levels of 130 mg/kg (Burdock, 1994). The total daily intake from its natural occurrence and its use as flavouring substance has been estimated to be 4.8 mg (Stofberg and Grundschober, 1987; Maarse et al., 1992; Lucas et al., 1999). The mean per capita intake as flavouring substance in Europe was estimated to be only 0.041 mg/day (JECFA 1999).

Conclusion

Isopropyl alcohol was evaluated by the Committee as extraction solvent for food and considered acceptable for this use based on a temporary acceptable daily intake of 0-1.5 mg/kg bw (SCF, 1981) and on the low residues resulting from this use (SCF, 1991a). Therefore, the Committee considers the use of isopropyl alcohol for the extraction of beta-carotene from *Blakeslea trispora* as acceptable.

Isobutyl acetate has not yet been evaluated as extraction solvent. Its use as flavouring substance, however, was considered acceptable by the Committee (SCF, 1991b) on the basis of the evaluation of the Expert Committee on Flavourings of the Council of Europe. This Expert Committee classified isobutyl acetate in category A of substances which may be used in foodstuffs and established practical upper levels of 150 mg/kg in food and 10 mg/kg in beverages (CoE, 1992). JECFA also considered isobutyl acetate as flavouring substance and concluded that the current levels of intake are of no safety concern (JECFA, 1999). The potential daily intake of 0.02 mg isobutyl acetate as solvent residue in beta-carotene is low compared to the potential daily intake from natural and flavoured food estimated to be 4.8 mg. Thus, the Committee considers the use of isobutyl acetate for the extraction of beta-carotene from *Blakeslea trispora* to be acceptable as well.

In all other respects, the product beta-carotene must comply with the current specifications.

References

Burdock, G.A. (Ed.), 1994. Fenaroli's Handbook of Flavor Ingredients (3rd Ed.). CRC Press. Inc.; Boca Raton; Florida, pp 387, 390.

CoE, 1992. Council of Europe. Flavouring substances and natural sources of flavourings. (4th Ed.). Vol I. Chemically defined flavouring substances. Maisonneuve. Strasbourg.

EC (2001). Commission Directive 2001/50/EC of 3 July 2001 amending Directive 95/45/EC laying down specific purity criteria concerning colours for use in foodstuffs. Official Journal of the European Communities L 190, pp.14-17, 12.7.2001

JECFA, 1999a. Evaluation of Certain Food Additives and Contaminants. Forty-Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 884, Geneva 1999.

Lucas, C.D., Putnam, J.M. and Hallagan, J.B., 1999. Flavor and Extract Manufacturers' Association of the United States 1995 Poundage and Technical Effects Update Survey. Flavor and Extract Manufacturers' Association (FEMA). Washington, DC.

Maarse, H., Visscher, C.A., Willemsens, L.C., Boelens, M.H. (Eds.), 1992. Volatile Compounds in Food: Qualitative and Quantitative Data. Supplement 3 and Cumulative Index. TNO Biotechnology and Chemistry Institute, Zeist; The Netherlands.

SCF, 1981. Reports of the Scientific Committee on Food (11th series). Report on extraction solvents. Opinion expressed on 15 January 1981.

SCF, 1991a. Reports of the Scientific Committee on Food (29th series). Second report on extraction solvents. Opinion expressed on 21 June 1991.

SCF, 1991b. Guidelines for the evaluation of flavourings for use in foodstuffs: 1. Chemically defined flavouring substances. Opinion expressed on 10 December 1991.

SCF, 2000. Opinion on Beta-carotene from *Blakeslea Trispora*. Adopted on 22 June 2000 and corrected on 7 September 2000.

Stofberg, J., Grundschober, F., 1987. Consumption ratio and food predominance of flavoring materials. 1987. *Perfum Flavor* 12(3), 27-68.

Vitatene, 2002. Application to amend specifications for beta carotene derived from *Blakeslea trispora*, submitted to the EU Commission April 24, 2002.