LYCOPENE FROM *BLAKESLEA TRISPORA*
CHEMICAL AND TECHNICAL ASSESSMENT (CTA)

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1. Summary

This Chemical and Technical Assessment summarizes the information about lycopene from the fungus *Blakeslea trispora* submitted to JECFA by Vitatene S.A. in a dossier dated December, 2005. This document also discusses published information pertinent to lycopene in general.

Lycopene belongs to a group of naturally-occurring pigments known as carotenoids. Lycopene is a natural constituent of red fruits and vegetables and of certain algae and fungi. Tomatoes and tomato-based products are the major sources of lycopene in the human diet. In analogy to other carotenoids, lycopene occurs in various geometrical configurations. Lycopene present in fresh tomatoes consists predominantly of all-*trans*-lycopene. Lycopene from *B. trispora* described in this dossier contains at least 90% of all-*trans*-lycopene and minor quantities of 13-*cis*-lycopene and β- and γ-carotene.

Lycopene from *B. trispora* is produced by co-fermentation of two nonpathogenic and nontoxigenic sexual mating types (plus and minus) of *B. trispora*. Lycopene is extracted from the biomass of the fermentation broth and purified by crystallization and filtration. Isopropanol and isobutyl acetate are the only solvents used in the manufacture of lycopene. Pure lycopene crystals are unstable when exposed to oxygen and light. To assure adequate stability, lycopene is stored under nitrogen or other inert gas in light-proof containers. Commercial lycopene preparations intended for use in food are formulated as suspensions in edible oils or as water-dispersible powders (referred to in the dossier as cold water dispersions) and are stabilized with antioxidants.

Lycopene from *B. trispora* is intended for use in certain foods and beverages as a colour. The intended use levels of lycopene range from 10 mg/kg to 50 mg/kg.

2. Description

Lycopene isolated from *B. trispora* occurs as a red crystalline powder. Lycopene is insoluble in water and nearly insoluble in methanol and ethanol, but is freely soluble in chloroform and tetrahydrofuran. Lycopene is sparingly soluble in ether, hexane, and vegetable oils. A 1% solution of lycopene in chloroform is clear and has intensive orange-red colour. A solution in hexane shows an absorption maximum at approximately 470 nm.

Lycopene belongs to a large group of naturally-occurring pigments known as carotenoids, however, it has no provitamin A activity. Lycopene is a natural constituent of red fruits and vegetables and of certain algae and fungi. The major sources of natural lycopene in the human diet are tomatoes and tomato-based products. Other significant sources of lycopene include watermelon, pink grapefruit, pink guava, papaya, and apricots (Nguyen and Schwartz, 1999).

3. Manufacturing

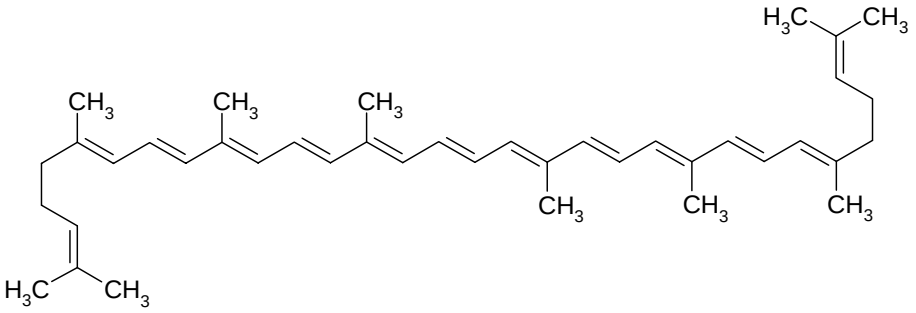
Lycopene from *B. trispora* is manufactured by co-fermentation of two sexual mating types (plus and minus) of the fungus *B. trispora*. Although each strain is capable of producing low levels of carotenoids, the co-cultivation of both strains enhances the synthesis of these compounds. Both strains of *B. trispora* are considered to be nonpathogenic and nontoxigenic based on animal feeding studies and immunoassays for several mycotoxins conducted in relation to the production of β-carotene from *B. trispora*. Lycopene is an intermediate in the biosynthetic pathway of β-carotene. Its production process is nearly identical to that used to manufacture β-carotene from *B. trispora*. The only difference is in the addition of imidazole to the fermentation broth in order to inhibit the formation β- and γ-carotene from lycopene. Beta-carotene from *B. trispora* was evaluated at the 57th JECFA (June 5-14, 2001, Rome) (JECFA, 2001).

Following the fermentation phase, lycopene is extracted from the biomass and purified by crystallization and filtration. The solvents used in these processes are isopropanol and isobutyl acetate. Lycopene is unstable when exposed to oxygen (Lee and Chen, 2002) and light and must be stored under inert gas in light-proof containers. Commercial lycopene preparations intended for use in food are formulated either as suspensions in edible oils or as water-dispersible powders (referred to in the dossier as cold water dispersions or CWDs) and are stabilized with antioxidants such as tocopherol. All processes related to the recovery, formulation, and packaging are carried out under nitrogen to avoid the degradation of lycopene.

4. Chemical characterization

Lycopene is an unsaturated acyclic hydrocarbon. It contains 13 double bonds, of which 11 are conjugated. Lycopene occurs in the all-*trans* and various *cis* configurations. Naturally-occuring lycopene consists predominantly of all-*trans*-lycopene. For example, lycopene present in red tomato fruits typically contains 94-96 % of all-*trans*-lycopene (Schierle et al., 1997). Lycopene from *B. trispora* contains at least 90% of all-*trans*-lycopene and low levels of 13-*cis*-lycopene. In certain literature sources, all-*trans*-lycopene is referred to as (all-E)-lycopene and *cis* isomers are referred to as Z isomers.

The chemical name of lycopene is 2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene. Common names include *Ψ,Ψ*-carotene, all-*trans*-carotene, and (all-E)-lycopene. The chemical formula is $C_{40}H_{56}$.
The structural formula of all-*trans*-lycopene is shown below:



The molecular weight of lycopene is 536.9 and the Chemical Abstract Service (CAS) number is 502-65-8.

Lycopene is unstable when exposed to light, heat, and oxygen. Exposure to light and heat triggers isomerization from the *trans* to *cis* configurations. The c*is* isomers of lycopene have different physical and chemical characteristics than all-*trans*-lycopene (Nguyen and Schwartz, 1999). Some of these differences include lower melting points, lower specific absorption, and a shift in the absorption maximum. Lycopene can also undergo oxidation when exposed to oxygen with the formation of epoxides. To prevent isomerization and oxidation, pure lycopene form *B. trispora* is kept under inert gas in lightproof containers and stored in a cool place.

Lycopene from *B. trispora* consists of at least 95% of total lycopene and up to 5% of other carotenoids (β- and γ-carotene). The extraction solvents, isopropanol and isobutyl acetate may be present in the final product at levels below 0.1% and 1%, respectively. Imidazole used during fermentation may be found in lycopene at levels below 1 mg/kg.

5. Proposed specifications

The proposed specifications are based on the manufacturing process of lycopene from *B. trispora* and are different from the specifications proposed for synthetic lycopene. The assay is intended to define the content of total lycopenes (not less than 95%) and all-*trans*-lycopene (not less than 90%) in the final poduct. Purity specifications include limits on loss on drying (not more than 0.5%), lead (not more than 1 mg/kg), other carotenoids (not more than 5%), and residual solvents used in extraction and purification of lycopene (isopropanol, not more than 0.1%; isobutyl acetate, not more than 1.0%). These limits are intended to ensure that lycopene used in food is equivalent to that evaluated in the toxicological tests.

The HPLC method of assay was designed to determine total lycopenes (all-*trans*-lycopene and *cis*-lycopene isomers), all-*trans*-lycopene, and other carotenoids (β- and γ-carotene). The *cis* isomer routinely detected in lycopene from *B. trispora* is 13-*cis*-lycopene. The method of assay includes spectrophotometric determination of lycopene in the lycopene standard (i.e., determination of standard purity). Representative chromatograms of a high-purity lycopene used as a standard and of a typical lycopene product are shown in Appendix 1.

The analytical methods for other proposed specifications (solubility, spectrophotometry, loss on drying, lead, and residual solvents) are based on general tests for identity and purity published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Volume 4.

6. Functional use

Lycopene from *B. trispora* is intended for use as a colour in foods and beverages including orange to red non-alcoholic flavoured drinks, fine bakery wares such as biscuits, cakes and cookies, dairy products and analogues such as fruit and flavoured yoghurts and dairy desserts, confectionary, fish products, soups and sauces, and certain sweet liquors. The intended use levels of lycopene range from 10 mg/kg to 50 mg/kg. The intended uses and use levels of lycopene are provided in Appendix 2.

7. Reactions and fate in food

The chemical structure of lycopene, particularly the long chain of conjugated carbon-carbon double bonds, predisposes lycopene to isomerization and degradation upon exposure to light, heat, and oxygen (Lee and Chen, 2002). Lycopene stability in commercial formulations containing lycopene from *B. trispora* was evaluated under various conditions for up to 12 months.

The results of these tests consistently demonstrated that lycopene was stable in these products. Furthermore, the stability of the 20% lycopene oil suspension used for a subchronic toxicity study was monitored for a period of 2.5 years and indicated that lycopene was stable when the suspension was refrigerated. Although Vitatene has not studied lycopene stability in food, it is expected that the stability of lycopene from *B. trispora* would be comparable to the stability of lycopene naturally present in food. Food processing conditions may result in partial isomerization of all-*trans*-lycopene to *cis* isomers as well as in oxidation with the formation of epoxides.

Lycopene naturally present in tomatoes may undergo isomerization during processing tomatoes into various tomato products. It has also been shown that human blood plasma contains several lycopene isomers (Schierle at al, 1997). This information is summarized in Table 1 below.

Table 1. Isomer composition of lycopene in tomato products and human blood plasma (as % of total lycopene)

Sample	**All-*trans*-lycopene**	**5-*cis*-lycopene**	**9-*cis*-lycopene**	**13-*cis*- and 15-*cis*-lycopene**	**Other *cis*-isomers of lycopene**
Raw red tomatoes*	94 – 96	3 – 5	0 – 1	1	<1
Cooked tomato-based foods	35 – 96	4 – 27	<1 – 14	<1 – 7	<1 – 22
Human blood plasma**	32 – 46	20 – 31	1 – 4	8 – 19	11 – 28

*Unpublished results cited in Schierle et al., 1997.
**Four pooled samples and four single-donor samples

8. References

Lee M.T. and Chen, B.H., 2002. Stability of lycopene during heating and illumination in a model system. Food Chem., 78, 425-432.

Nguyen, M.L., and Schwartz, S.J., 1999. Lycopene: Chemical and biological properties. Food Technol., 53, 38-45.

Schierle, J., Bretzel, W., Bühler, I., Faccin, N., Hess, D., Steiner, K., and Schüep, W., 1997. Content and isomeric ratio of lycopene in food and human blood plasma. Food Chem. 59, 459-465.

APPENDIX 1

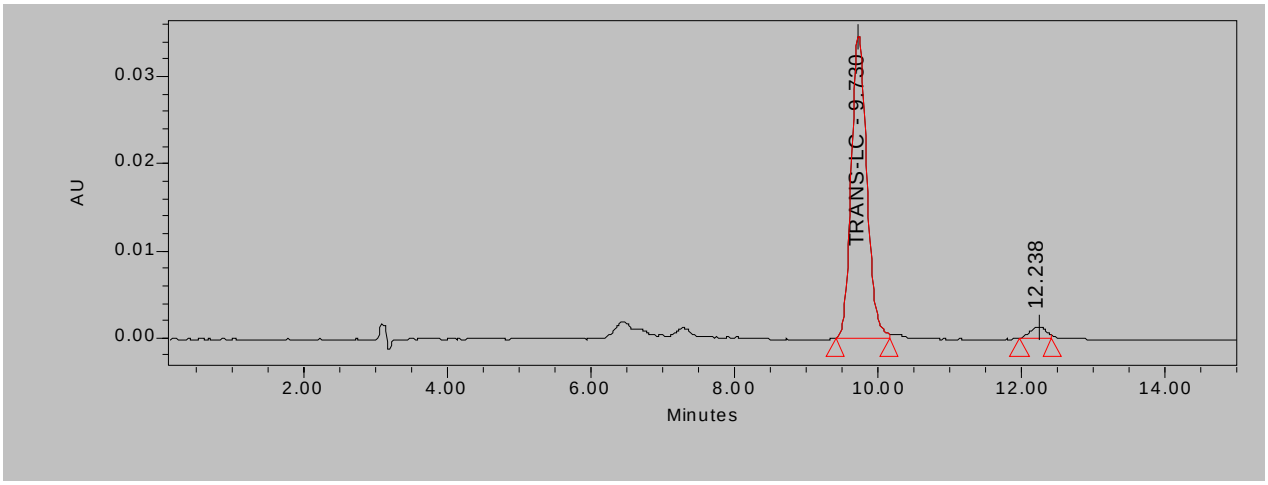


Fig. 1. High-purity lycopene
(9.730 min: all-*trans*-lycopene; 12.238 min: 13-*cis*-lycopene)

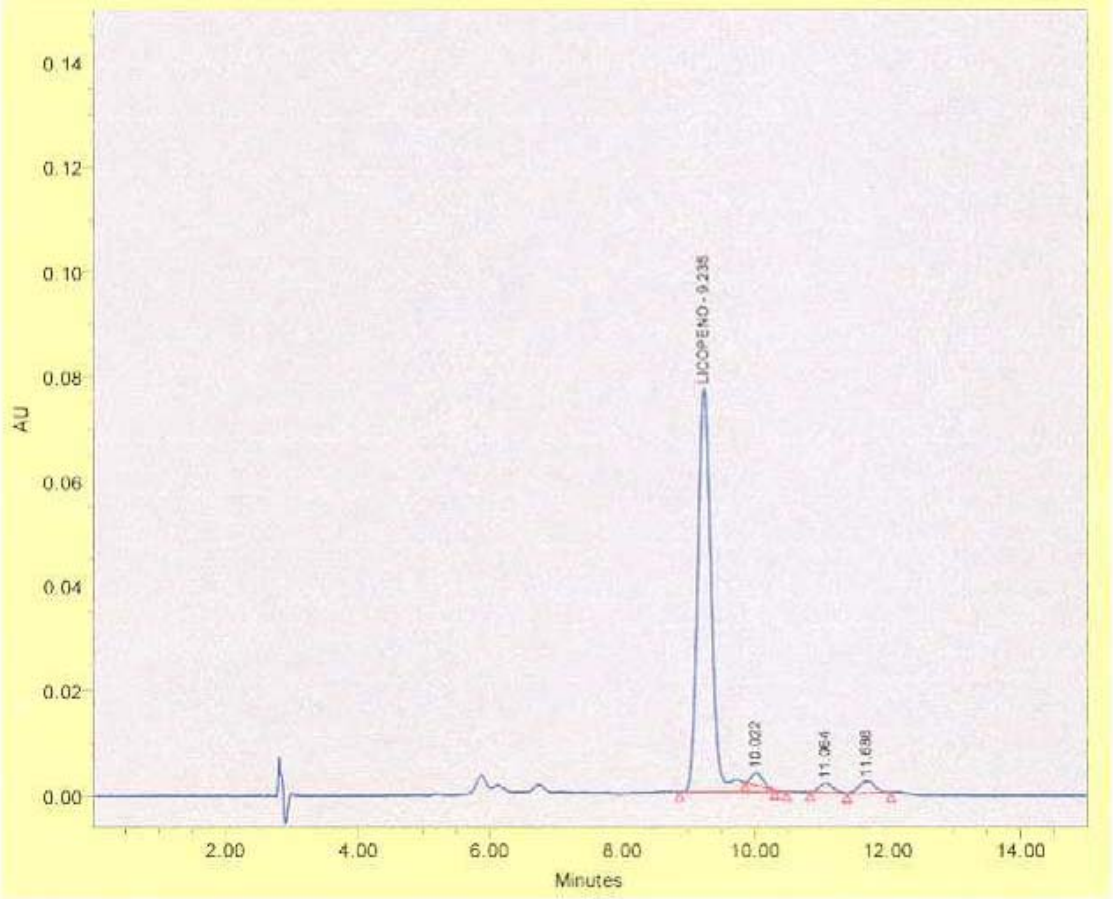


Fig 2. Typical lycopene product
(9.235 min: all-*trans*-lycopene; 10.022 min: γ-carotene; 11.054 min: β-carotene; 11.655 min: 13-*cis*-lycopene)

APPENDIX 2

Proposed uses and use levels of lycopene from ***Blakeslea trispora***

Food Category	Maximum use level mg/kg
Non-alcoholic flavored drinks	
Bitter	30
Apple	10
Peach	20
Orange	30
Others	20
Fine Bakery Wares	
Biscuits, cakes	20
Cookies	20
Confectionery	20
Edible Ices	
Ice cream, sherbet	25
Desserts, including flavoured milk products	
Fruit, flavoured yoghurts	15
Dairy desserts	15
Fish	
Pre-cooked crustaceans	20
Smoked fish	30
Sauces	
Sauces with reddish coloration	50
Soups	
Soups and creams	30
Spirituous Beverages	
Sweet liquors	20