

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR LYCOPENE FROM *BLAKESLEA TRISPORA*

Applicant	Vitatene
Responsible person	Dr Rodríguez-Otero
EC Classification	2.2

INTRODUCTION

1. An application was submitted by Vitatene to the UK Competent Authority for authorisation of lycopene derived from the fungus *Blakeslea trispora* for use as a novel food ingredient.
2. Lycopene (C₄₀H₅₆) is an aliphatic branched hydrocarbon with a molecular weight of 536.9 Daltons. It exists predominantly in the trans- form and is a red crystalline powder soluble in fats and organic solvents, but virtually insoluble in water, methanol or ethanol.
3. Solvent extracted lycopene from tomatoes is approved for use as an additive (E160d) and is used in dietary supplements and as an ingredient (food colour) in a range of foods. Synthetic lycopene is also used as a dietary supplement outside the EU, but is not permitted for use as a colour additive. *Blakeslea trispora* is a fungus found on a number of tropical plants, and strains of *B. trispora* are able to synthesise large quantities of carotenoids. Following the publication of a positive opinion from the SCF in 2001 α -carotene from *B. trispora* was approved for food additive use. Although lycopene *per se* has a history of consumption, and is produced using the same biosynthetic pathway as α - carotene, the organism has not hitherto been used for production of lycopene sold in the EU and the product requires authorisation under regulation (EC) 258/97 before it can be marketed.

I. Specification of the novel food

Information on this aspect is provided on pages 1 – 6 of the Application dossier

4. The applicant intends to market lycopene from *B. trispora* as a nutritional food ingredient. The purified, crystalline lycopene is dissolved in high oleic sunflower oil, supplemented with tocopherol to minimise oxidation.

Tocopherol is added at levels consistent with those specified in the relevant food additives directive 95/2/EC. The Novel Food (NF) will be available in this oil suspension form (5% and 20%) only.

5. Detailed compositional analyses of the NF are given in the Application dossier. For these analyses the company has tested both crystalline lycopene and oil suspensions. The Applicant's specification of the novel food states that it should be not less than 95% lycopene of which at least 90% is trans-lycopene. The remainder comprises of a number of low level contaminants, such as the extraction solvent, isobutyl acetate (not greater than 1%), sulphated ash (not greater than 1%) and subsidiary colouring matters (not greater than 5%). This company's specification was exceeded in each of three non-consecutive, representative lots described in the application.

Discussion. *The Committee was satisfied with the specification of the novel food.*

II. Effect of the production process applied to the novel food

Information on this aspect is provided on pages 5, 7-14 of the Application dossier

6. Lycopene from *B. trispora* is obtained by the co-fermentation of 2 sexual mating types of the fungus, obtained using classical strain selection techniques to increase the efficiency of lycopene production. The strains used are the same as those approved for the production of α -carotene. The mating types are stable and are preserved and maintained using GLP methods and are deposited in a culture collection.
7. Fermentation of the fungi to produce lycopene is a two-stage process. Flasks are inoculated with each of the mating types, and grown under controlled conditions. Once vegetative growth is established, the contents of the flasks are individually transferred aseptically to larger growth tanks containing sterile medium. Once sufficient cell mass has accumulated the strains are transferred aseptically into another tank where co-fermentation commences. It is at this point that the fungi start to produce lycopene. The process is further controlled by the addition of imidazole which inhibits the formation of carotene.
8. After completion of the fermentation process, lycopene rich biomass is subject to an initial purification process using isopropyl alcohol, which removes any oils and other lipophilic substances. The residue is evaporated to dryness, milled and extracted with isobutyl acetate. The resulting enriched solvent is separated and concentrated by vacuum distillation. The lycopene is then crystallised. Due to its susceptibility to oxidation the lycopene is crystallised under nitrogen. The crystalline lycopene is dissolved in high oleic acid sunflower oil containing tocopherol (1%) and diluted in accordance with the desired specification. The purification and extraction processes are identical to those used in the production of beta-carotene from *B. trispora*, which have been examined

and cleared by the SCF. Each batch of the final product is assayed to check compliance with the specification Application dossier Section 1.e.

9. The applicant has supplied data indicating that in comparison with lycopene from other sources, lycopene from *B. trispora* is predominantly present in the trans- form (at least 90%). The data also indicates that the purity of the fungal lycopene is comparable with synthetic lycopene (Application dossier Table II c-1).
10. The applicant has demonstrated that the NF (20% oil suspension) is stable for a period of at least two years when stored at 5°C. Other studies demonstrate that lycopene (5% and 20% oil suspension) can be stored in sealed containers for at least 6 months at a range of temperatures (3°C, 25°C and 40°C) with no appreciable deterioration in product quality. In all cases the tests took place in conditions conducive to oxidation as, although the NF was sealed in bottles, the applicant did not sparge with nitrogen.

Discussion. *The Committee was satisfied that the production process is controlled and that the in-process monitoring steps are appropriate to ensure a safe and consistent product, that does not deteriorate during storage. The Committee accepted clarification from the applicant that consumption of the novel food in a dietary supplement form did not raise levels of exposure to the extraction solvent, isobutyl acetate to levels that would be toxicologically significant.*

III. History of the organism used as a source of the novel food

Information on this aspect is provided on pages 15 – 17 of the Application dossier

11. The applicant has based previous dietary exposure to *B. trispora* on its use as a source of β -carotene, noting that the safety of the organism was assessed by the SCF (2000) and the Joint Expert Committee on Food Additives (JECFA) (2001). The SCF concluded that, based on the information supplied, the organism is non-pathogenic and non-toxicogenic. A subsequent 28-day oral feeding study using Wistar rats, Jonker (2000) (see also Section XIII) also demonstrated that the organism was both non-toxicogenic and non-pathogenic.
12. JECFA concluded that β -carotene from *B. trispora* is acceptable for food additive use, providing that it met the specification of its synthetic counterpart. The applicant is of the view that this finding is consistent with their view that the source organism is safe.
13. The applicant also carried out mycotoxin assays on each of three non-consecutive batches to determine whether aflatoxin B1, Mycotoxin T2, ochratoxin and zearalenone were present. The results, for both crystalline and oil suspended lycopene, were all negative.

Discussion *The Committee was reassured that the SCF assessment of the use of the source organism in the production of beta-carotene provided reassurance that there was a history of safe food use. The Committee also*

noted the similarity of the production process for production of the novel food would not give raise to any additional concerns.

IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on pages 21-27 of the Application dossier

14. The applicant intends to use the NF as a nutritional food ingredient. In addition to its use in dietary supplements, the ingredient will be used in a range of foodstuffs, including fat spreads, milk products and confectionery. A full list of the proposed uses is given in the Application dossier (Table IX a-1).
15. In order to predict the intake of the NF the applicant has used the most up to date information available from UK dietary surveys. The applicant has used proposed maximum use levels for **all** foods described above to predict potential intake. In order to compare the data over a 7-day period across a number of different surveys that target different sub-groups of the UK population, the applicant has applied a weighting factor. The UK CA sought the views of experts in the Food Standards Agency who were satisfied with the validity of the methodology.
16. The applicant has used dietary intake data for children (1.5–4.5), young people (4-10), male and female teenagers and male and female adults. Given that the proposed range of foodstuffs is wide, the applicant notes that the percentage of potential users was high amongst all age groups (>98%).
17. The intake estimates are summarised below. The largest consumers of the NF on an absolute basis are predicted to be male adults, whereas children have the highest predicted intakes on a body weight basis. These figures are likely to overestimate actual consumption, as they are based on the assumption that consumers always select foods that are fortified at the maximum level.

Population Group (age)	ESTIMATED DAILY INTAKE			
	Mean (mg)	97 th %tile (mg)	Mean (µg/kg bw)	97 th %tile (µg/kg bw)
Children (1½-4½)	0.22	0.65	15.1	44.9
Young People (6-11)	0.37	0.93	14.6	36.0
Teenager (F) (11-18)	0.40	1.02	7.6	20.6
Teenager (M) (11-18)	0.42	1.18	7.9	23.8
Adult (F) (16-64)	0.46	1.23	7.4	21.0
Adult (M) (16-64)	0.60	1.68	8.1	22.6

18. Based on the available intake data the applicant notes that the highest amount of lycopene from a food source would be obtained by consumption of fortified soups and soup mixes.
19. The applicant also intends to market the NF in supplement form at levels up to 20mg per day. Supplements containing lycopene from other sources are currently on the market in the EU, and it is likely that the NF would replace those already being consumed and overall consumption levels would not increase. In contrast, incorporation of lycopene into foods would result in additional intake.
20. The applicant has used the most recent adult dietary survey data available, however the Food Standards Agency is able to make estimates of intake based on a 2001 survey of British adults, which is not currently in the public domain in a form that the applicant could use to assess consumption of their product. Analyses of these data that confirm the applicant's consumption estimated are similar to those obtained with the newer survey data..

Discussion. *As the proposed levels of incorporation were low the Committee was content that the intended use of the product did not give any cause for concern, based on scientific information currently available.*

X Information from previous human exposure to the novel food or its source

Information on this aspect is provided on pages 28-30 of the Application dossier

21. Lycopene is a normal constituent of the diet in a number of red fruits and vegetables such as tomatoes and watermelon. Levels of lycopene in tomato are dependent both on the species of tomato and the degree of ripening but are generally in the range 3.1-7.7 mg / 100g.
22. The applicant highlighted a 1996 UK study that indicated that consumption of a lycopene-rich diet would lead to consumption of 1.03mg/person/day lycopene. These results are similar to levels seen in Finland (0.70 and 0.87 mg/day for females and males respectively).
23. However the applicant also highlighted other studies that show that intake of lycopene outside the EU shows markedly varied levels of consumption. The applicant has summarised a number of North American dietary surveys that reinforce the European findings that consumption of lycopene is intrinsically varied and dependent on dietary preference. Consumption of lycopene in North America indicates a large variation dependent upon method of data collection, however in all cases mean levels were significantly higher than those seen for UK subjects. A USDA study showed that mean lycopene intake for the general US population was 4.7mg/day however a number of other dietary surveys indicate that consumption could be as high as 25.2mg/person/day.
24. The Applicant also notes that there are no reliable consumption figures available for the current consumption of lycopene in dietary supplement

form despite such products being freely available in Europe and the North America.

Discussion *The Committee was reassured that lycopene has a history of consumption in the EU, albeit from a different source. The Committee noted that, in addition to its presence in fresh fruit and vegetables, dietary supplements containing lycopene extracted from tomatoes at levels in excess of 20mg were widely available in the UK.*

XI. Nutritional information on the novel food

Information on this aspect is provided on pages 31-33 of the Application dossier

25. The applicant is of the view that, although the source of lycopene is novel, the nutritional value of the novel food is unchanged when compared to existing lycopene. Other constituents of the novel food (high oleic acid sunflower oil and tocopherol) will have a negligible impact on the nutritional value of the lycopene oil suspension as they are relatively common in the diet.
26. Lycopene is an effective antioxidant, and these antioxidant properties are perceived to be primarily responsible for the potential health benefits of dietary carotenes.

Discussion *The Committee was reassured that altering the source of the novel food would not affect its nutritional value.*

XII. Microbiological information on the novel food

Information on this aspect is provided on pages 34-35 of the Application dossier

27. Microbiological information supplied by the applicant indicates that, three non-consecutive batches had no detectable moulds, yeast, *Salmonella* or *Escherichia coli*. These findings applied to both the crystalline lycopene, and oil suspension (5% and 20% forms).

Discussion *The Committee was content with the microbiological data supplied, but requested further information from the applicant to demonstrate the absence of the anaerobic spore forming pathogen *Clostridium botulinum*. The applicant was able to supply this information, and the Committee was satisfied that the absence of this organism from the final product could be demonstrated.*

XIII Toxicological Information on the Novel Food

Information on this aspect is provided on pages 36-57 of the Application dossier

28. The applicant presented a number of toxicological studies on both the novel food and the source organism. The applicant has noted that the NF is chemically comparable to others on the market (Application dossier Table 2.c-1) and has therefore included toxicological studies on lycopene products from other manufacturers as supporting data.

Summary of studies

29. The applicant assessed the sub-chronic toxicity of the source of the novel food by testing the lycopene-rich biomass extracted from *B. trispora*.

Supplementary information to demonstrate the safety of the source organism has been supplied from an independent scientist, the SCF and JECFA. A 90-day oral toxicity study has been carried out on the NF (20% oil suspension).

30. The applicant also highlighted details of acute, sub-chronic and chronic, carcinogenicity, mutagenicity and genotoxicity, reproductive toxicity trials and human safety data for lycopene from other sources. Developmental toxicity investigations were carried out on two US lycopene products, whilst human safety data were mostly based on high levels of consumption of commonly available lycopene-rich foods.

Lycopene biomass (Application dossier p37)

31. Lycopene-rich biomass obtained under the fermentation conditions described in section II was used in a sub-chronic toxicity study. Four groups of 40 rats (20/sex) were assigned. The first formed a control group whilst the other three received lycopene biomass at levels of 0.1, 0.3 or 1% of the total diet. These percentages corresponded to daily doses of 90, 272 and 906 mg/kg body weight in males and 87, 260 and 868 mg/kg bodyweight in females respectively. The lycopene-enriched diet was administered for a period of 28 days following which the animals were sacrificed.
32. Clinical observations, neurobehavioural observations, growth, food consumption and food conversion efficiency were assessed throughout the study and haematology, clinical chemistry, organ weights and macroscopic and microscopic examinations were carried out at necropsy.
33. No treatment related differences were found in mean body weights and relative/absolute organ weights between the control and treatment groups. Food consumption and food conversion efficiency were also not adversely affected by the treatment. No treatment related clinical signs or neurotoxic indications were found as a result of the lycopene biomass administration. These were assessed using neurobehavioural observations and motor activity assessments.
34. Haematological measurements showed a statistically significant decrease in mean corpuscular volume and prothrombin time in the high dose male group only. However no significant changes were noted for other red blood cell groups, coagulation variables, white blood cell counts, packed cell volume or haemoglobin concentrations and the authors considered the decrease in mean corpuscular volume as an incidental finding and of no toxicological significance. The decrease in prothrombin times was found to be small (6%) and within the limits of historical controls.
35. No adverse effects were noted in the clinical chemistry variables and macroscopic and microscopic examinations at necropsy revealed no treatment related changes except a statistically significant decreased incidence of increased hyaline droplet nephropathy in the high dose male group. Again, the authors of the study attached no toxicological significance to this finding.

Toxicological assessment of *B. trispora* (Application dossier p40)

36. The two mating strains of *B. trispora* are stable cultures that are preserved under conditions that adhere to good manufacturing practices. The strains are considered to be non-toxicogenic and non-pathogenic on the basis of 28-day oral feeding study described above. The applicant also notes that *B. trispora* is formally classified in Germany as “risk group 1”, organisms that pose no risk for humans and vertebrates
37. The production of lycopene by *B. trispora* is an intermediary of the beta-carotene synthetic pathway and the SCF considered the use of *B. trispora* as a source of beta-carotene as acceptable. The Committee concluded that the “source organisms and the production process yielded no grounds to suppose that the final crystalline product, beta-carotene, differs from the chemically synthesised beta-carotene used as a food colourant” (SCF, 2000)

Final Product (Application dossier p38)

38. A 90-day oral toxicity study was carried out to assess the toxicity of the 20% lycopene oil suspension in male and female Wistar rats. Groups of 20 rats received a diet containing 0, 0.25, 0.5, or 1.0 % lycopene in the form of a sunflower oil suspension. These percentages corresponded to daily doses of 0, 145 291 and 586 mg/kg bodyweight for males and 0, 156, 312 and 616 mg/kg bodyweight for females.
39. The animals were monitored for viability, clinical signs of toxicity, body weights and food consumption. Prior to necropsy, neurobehavioural testing and ophthalmoscopic examinations were performed and blood and urine analyses were obtained. Following necropsy, gross and histopathological examinations of various tissues were performed and organ weights recorded.
40. A pink discolouration of the fur was noted in all animals in the high dose group and many in the mid-dose group. This was attributed to the direct contact of the animals to the red staining lycopene mixture in the diet. No adverse effects were noted from the examinations described above and as a result the no observed effect level (NOAEL) was set at 1% in the diet. This was equivalent to a dose of 601mg / bodyweight per day, averaging the doses received by the male and female groups.
41. The genotoxicity of a 20% cold water dispersal of lycopene from *B. trispora* was assessed using a bacterial mutation test and an *in vitro* chromosome aberration test. As a result of these studies the investigator concluded that lycopene is not genotoxic.

Margin of safety (Application dossier p39)

42. Comparing the NOEL of 601mg lycopene/kg bodyweight/day from the sub-chronic rat study with the anticipated maximum intake from food use of between 1 and 2 mg/day gives a 20000-fold safety margin. Likely intake from food supplement at a level of 20mg/day is associated with a 2000-fold safety margin.

Toxicological assessment of lycopene from sources other than *B. trispora*

43. The applicant has supplied details of additional toxicological studies with lycopene derived from natural tomato extracts, tomato paste and synthetically produced lycopene in a number of forms including cold water dispersible (CWD) and water-soluble (WS) beadlet formulations and dietary supplements.

- Acute toxicity studies (Application dossier p40).
- Sub-chronic and chronic toxicity studies (Application dossier p41).
- Carcinogenicity studies (Application dossier p45).
- Mutagenicity / Genotoxicity studies (Application dossier p46).
- Reproductive toxicity studies (Application dossier p49).
- Human safety data (Application dossier p45).

***Discussion.** The Committee was satisfied with the toxicological data supplied by the applicant. However the Committee requested further information on the relevance of a significant change in the incidence of hyaline droplets in the sub-chronic toxicity study (Application dossier p38). The Committee also requested confirmation that the sub-chronic toxicity study parallel tests done using beta carotene biomass (Application dossier p38) did not raise any additional concerns. The applicant has responded to these comments highlighting that the increase in hyaline droplet nephropathy seen in male rats is not a toxicologically significant finding, noting that the mechanism of action, is of no relevance to humans. The applicant also confirmed that the parallel test with the beta carotene biomass revealed no additional toxicological findings. The Committee was content with the applicant's responses.*

Allergenicity

Information on this aspect is provided on page 58 of the Application dossier

44. The applicant is reported that the primary source of allergenic material, the source organism, is not present in the final products to any significant degree. This is borne out by the microbiological information (See para.30 above). Protein assays carried out on both the novel food (5% and 20% suspensions) and the sunflower oil were negative at the limit of detection (1µg protein/ml or 1µg protein in 400mg lycopene oil suspension). The applicant concludes that this is indicative of the absence of allergenic potential.

***Discussion** The Committee was content that the final product did not give rise to any allergenic potential.*

Labelling

Information on this aspect is provided on page 22 of the Application dossier

45. The applicant proposes that the ingredient would be described on food labels as "lycopene" without identifying the source to the consumer. The applicant confirms that labelling of products containing the NF will comply

with current EU regulations and may include the statement 'contains an additional source of lycopene'.

Discussion *The Committee was of the view that the proposed labelling should be expanded to indicate the source of the lycopene, in order that individuals who do not wish to consume products derived from, or containing fungi are adequately informed.*

OVERALL DISCUSSION

46. The applicant has provided a clear specification of the proposed novel food and indicated, on the basis of analysis from a number of non-consecutive batches, that the specification is achievable. The process is similar to the production of beta-carotene from *Blakeslea trispora*, which was given a positive evaluation by the SCF in 2001.
47. Given that lycopene is present in a large range of fresh fruits and vegetables, and lycopene extracted from tomatoes is widely available no additional nutritional concerns or benefits associated with consumption of the novel food have been identified. Based on scientific information currently available to the applicant there is sufficient reassurance that consumption of the novel food does not give rise to any toxicological concerns.
48. The applicant has demonstrated that the novel food is stable under normal conditions and when subject to mild temperature abuse. The applicant has also demonstrated that the novel food is microbiologically safe.
49. Although the proposed labelling of the product is adequate, the applicant should comply with general food labelling legislation and ensure that the labelling of the products and the source does not mislead the consumer.

Conclusion

50. The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Vitatene that the range of uses for lycopene from *Blakeslea trispora* is acceptable subject to the applicant's adherence to the proposed specification, and the production parameters described above.

April 2004