

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**LYCOPENE-RICH OLEORESIN FROM TOMATO****ISSUE**

This paper presents further information on this novel food ingredient, addressing the outstanding issues identified at the Committee's March meeting. Members are invited to consider these data and to finalise their opinion on this novel food application.

Background

1. An application has been submitted by LycoRed for the authorisation of an lycopene-rich oleoresin derived from tomato, as a novel food ingredient (NI). This application was accepted by the UK Competent Authority, on 7 September 2004. In accordance with Article 6(3) of the novel foods regulation (EC) 258/97, the UK has 3 months to prepare an initial assessment report, this has been extended in the light of Members request for additional information from the applicant in the November 2004, January and March 2005 meetings.
2. At the March meeting, Members agreed with the expert's opinion provided by LycoRed that the increased lung weights of female rat observed in the semi-chronic toxicity study was not indicative of a target organ toxic effect but related to the animal's body weight increases, caused by treatment. This has been reflected in the draft initial assessment.
3. The Secretariat therefore wrote to the applicant on 5 April 2005 (see Appendix 1), informing the applicant about the above and requesting clarification as the position with regards to the last outstanding question, namely protein analysis. Although not solely applicable to this application, Members also asked whether the applicant had any information on the potential transfer of lycopene to breast-fed infants
4. LycoRed has now provided the results of an additional analysis of the protein level of their oleoresin (see Appendix 2).
5. Regarding the transfer of lycopene to breast-fed children, the applicant has reviewed the available studies (see Appendix 2). The main focus of this document was a study by Allen *et al* (2002) on the relationship between

tomato consumption and lycopene concentrations in breast milk and plasma. A copy of this research paper is attached (Appendix 2).

Committee Action required

6. The Committee is asked whether it is satisfied with the additional data provided by LycoRed showing the absence of protein in the oleoresin. If not, the Secretariat will draft the opinion to reflect Members concerns with regard to allergenicity.
7. The Committee is invited to consider and comment on the draft initial opinion of LycoRed's application provided in Appendix 3 and to indicate whether it is an accurate reflection of their assessment of this NI.
8. The draft initial opinion will be published via the website for a short period of public comment. Once this is completed the initial assessment will be forwarded to the Commission and will form the basis for the UK's formal assessment of this application.

**Secretariat
May 2005**

Appendices attached:

- **Appendix 1:** Letter detailing ACNFP comments
- **Appendix 2:** Responses from LycoRed
- **Appendix 3:** Draft initial opinion for consideration (Restricted)

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

**APPLICATION FOR THE APPROVAL OF LYCOPENE-RICH OLEORESIN
FROM TOMATO AS A NOVEL FOOD INGREDIENT**

LETTER DETAILING ACNFP COMMENTS
(5 April 2005)

Mr Peter Berry Ottaway
Berry Ottaway and Associates Ltd
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Plough Lane
Hereford HR4 0EL

e-mail: boa@berryottaway.co.uk

5 April 2005

Reference: NFU 482

Dear Peter,

ACNFP COMMENTS

On 30 March 2005, our Advisory Committee on Novel Foods and Processes considered the expert advice regarding the significance of the increased lung weights of female rats observed in the semi-chronic study performed on the LycoRed's oleoresin containing 5% lycopene. The Committee also discussed a draft initial opinion on LycoRed's application.

You will be pleased to know that the Committee agreed with the expert's conclusion that this observed increased lung weights was not indicative of a target organ toxic effect but related to the body weight increases for female rats, caused by treatment. This will be reflected in our final initial opinion, and I have attached to this letter the expert advice for information.

Whilst considering the first draft initial opinion, Members noted that it was recognised that carotenoids pass into breast milk. If you have any information on the potential transfer of lycopene to breast-fed infants, could you please forward it to us.

Finally, the Secretariat informed the Committee that LycoRed was currently working on providing new analytical data on protein content for their lycopene rich-oleoresin. I would be grateful if you could give me an update on this.

Please note that the next ACNFP meeting will be held on 19 May 2005. I hope that we can conclude the discussions at this meeting.

Yours sincerely,

(By e-mail only)

Annie-Laure Robin

Novel Foods, Additives and Supplements Division

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

**APPLICATION FOR THE APPROVAL OF LYCOPENE-RICH OLEORESIN
FROM TOMATO AS A NOVEL FOOD INGREDIENT**

RESPONSES FROM LYCORED SENT BY E-MAIL

1. Protein in LycoMato® oleoresin (9/04/05)
2. Transfer of lycopene to breast-fed children (22/04/05)

April, 9 2005

1. Protein in LycoMato® oleoresin

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Results

The “silver stain” is very sensitive method for the detection of proteins. Sensitivity is less than 1 ng/in SDS gel lane (not shown). Unfortunately there is some cross reactivity with lycopene and/or other compounds/carotenoids present in the oleoresin. However, these hydrophobic compounds probably do not move significantly during the electrophoresis procedure and remain mostly in the origin (A) or in the boundary between the stacking and separating gels (B). A faint red color was seen in these locations before performing the silver stain procedure suggesting that this is actually lycopene.

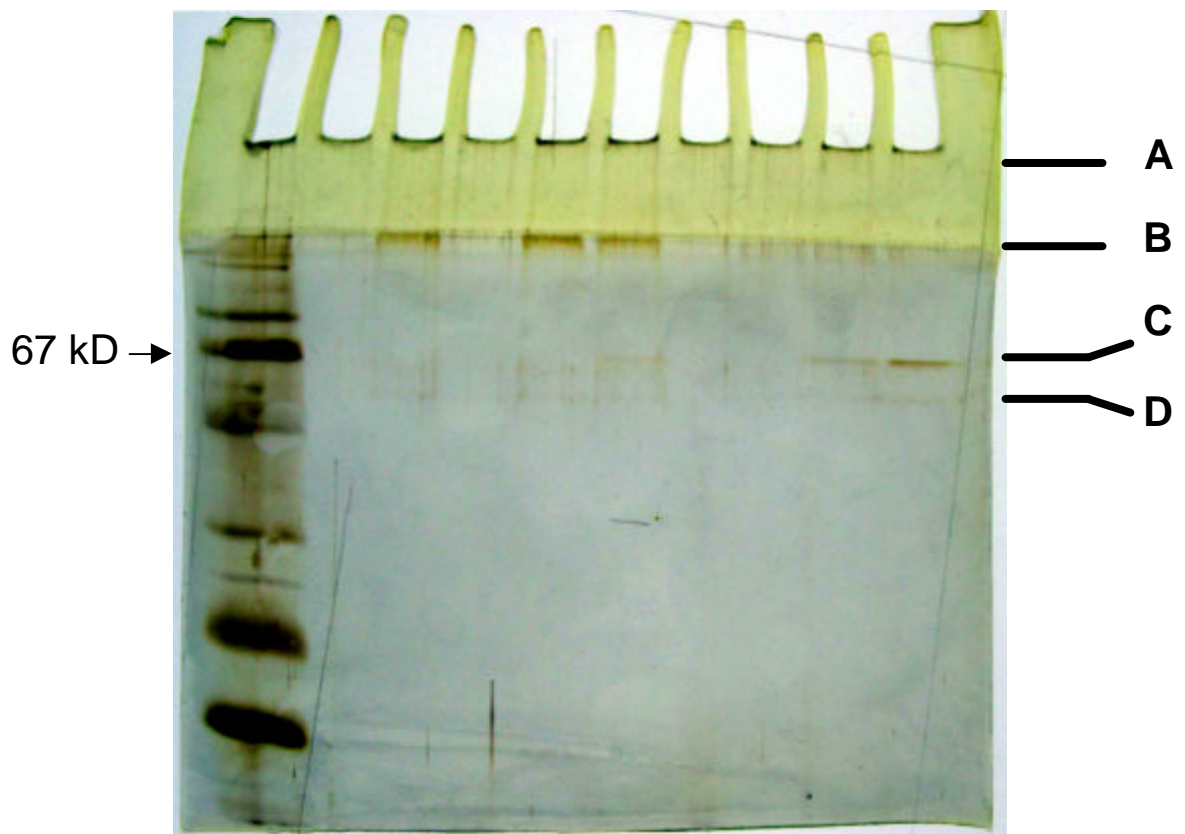


Fig: Absence of proteins in LycoMato® oleoresin preparation (6% lycopene) as assessed by Silver staining

Two contaminant bands (which could not be excluded) are present in all lanes. One, with MW of 67 KDa (C) and second, faster moving band (D) (see MW standards lane 1). These bands are evident (with somewhat different intensities) also in the control lane, containing only buffers (lane 8) and in lanes that were not loaded with any sample (2, 4 and 7). Spiking of the oleoresin samples with 5ng (lane 9) or 20ng of BSA (lane 10) resulted, as expected, in the 67 KDa band (C). LycoMato® 1.2 mg applied in lane 3 does not contain detectable amount of proteins. Spiking with 5 ng of protein (lane 5) is not significant, however spiking with 20 ng results in fortification of the expected 67 KDa band close to the level of 5 ng (lane 6). The reason for the reduction in the intensity of the bands in the oleoresin lanes is not known but it means that there is only partial (about ¼) extraction of the spiked proteins.

Conclusion

We cannot detect any amounts of proteins in LycoMato® oleoresin preparation (6% lycopene) as assessed by Silver staining. The sensitivity of detection is ~ 1:240000 (or ~ 4ppm), since 1.2 mg oleoresin are applied in the lane and the detectable level is around 5 ng as estimated by the spiking experiment.

Oleoresin sample preparation:

Oleoresin was weighed and suspended at 25 mg/ml in saline containing 0.2% SDS to extract any protein that may be present in the oleoresin. The suspension was stirred for 30 min at room temperature to disperse the oleoresin. After that, the suspension was equally distributed to three laboratory beakers and suitable amount of BSA (spiking) was added to two of them in order to achieve 5 and 20 ng/lane. 150 µl from each beaker were centrifuged for 30 min in "AirFuge" ultracentrifuge. 40 µl of supernatant were mixed with 8µl of 6x sample buffer. A sample containing 0.3 µl molecular weight markers (Rainbow, Amersham) was prepared by dilution in saline/sample buffer.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE):

BSA and oleoresin samples prepared as above were heated at 95°C for 5 min, loaded onto 12% polyacrylamide gel along with Rainbow protein molecular weight markers, and subjected to SDS-PAGE at 140 V (40 mA/gel). The gel was then fixed in a solution of 40% methanol and 10% acetic acid.

Silver staining procedure:

Silver staining was carried out using a Bio-Rad Silver Stain Kit (cat# 161-0443) according to the manufacturer's recommended protocol, as follows. Gel was incubated (2 x 30 min) with fixative (10% ethanol/5% acetic acid) followed by exposure to oxidizer (10 min). Gel was then washed with

deionized water (3 x 10 min), incubated with silver reagent (30 min), and washed again with water (2 min). The gel was incubated with developer (3 x 5 min) and finally soaked in 5 % acetic acid. The stained gel was photographed using an ImageMaster VDS-CL video image analyzer (Amersham-Pharmacia).

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2. Lycopene in Breast Milk

A query has been raised by the Advisory Committee on Novel Foods and Processes about the potential transfer of lycopene from breast milk to the infant.

A search of the literature indicates that there have been relatively few studies on lycopene in human breast milk. Almost all the studies on carotenoids in human maternal plasma and breast milk have tended to focus on the provitamin A carotenoids, particularly in the context of the vitamin A status of breast-fed infants.

More than 30 carotenoids, including lycopene and its isomers have been identified in human milk¹.

Canfield *et al* found that concentrations of human milk carotenoids were less than one tenth of their respective levels in the maternal serum^{2,3}. A study reported by Yeum *et al*, investigated the relationship between maternal and cord plasma concentrations of carotenoids in healthy pregnant women. The results indicated that the cord plasma concentration of carotenoids were significantly lower than that of maternal plasma ($p < 0.001$)⁴.

A very relevant study is that of Allen *et al*⁵, which investigated the effect of tomato consumption on lycopene concentrations in plasma and breast milk of lactating women ($n = 24$). The subjects were assigned to three groups ($n = 8$ per group), two treatment groups and a control group. One treatment group was assigned a diet designed to provide 50 ± 2 mg lycopene over three days from fresh tomatoes, whilst the second group received the same intake of lycopene from processed tomatoes (tomato sauce) during the same period. The control group consumed two daily servings of 10 grapes containing no lycopene, in place of the tomato sources. The feeding and sample collection schedules were standardised for the three groups.

Milk lipid concentrations were estimated as a percentage of total volume by the creatinocrit assay and the carotenoid concentrations of the milk were expressed both as nmol/l milk and as nmol/g lipids. The results indicated that the two major isomers of lycopene in both plasma and milk were all-*trans* and 5-*cis* lycopene, and that the ratios of the isomers before and after intervention did not change in any group. The ratios were similar in both milk and blood.

Over the three day period the consumption of 50mg lycopene by the treatment groups showed a 1.8 fold greater plasma lycopene concentration in the group consuming lycopene from processed tomatoes when compared to fresh tomatoes.

As with the plasma, milk total lycopene concentration increased when processed sauce was consumed but did not change for the fresh tomato group. In the control group the milk total and *trans*-lycopene decreased.

However, the statistical analysis showed that the milk total lycopene concentrations were not significantly different from baseline in any group when adjusted for the fat content of the milk.

The authors discuss the limitations of such a study in the context of the very wide range of milk fat concentrations between subjects (16-80 g/l). No correlation was found between milk fat and milk lycopene concentrations, but this was possibly due to the small sample size.

In view of their findings, and until more data are available on carotenoids transfer to milk, the authors suggest that it is important to report milk carotenoids concentrations per volume as well as per gram of fat.

The authors concluded that for dietary recommendations during lactation, consumption of processed tomatoes (i.e. as tomato sauce) can increase milk lycopene concentrations more effectively than consumption of fresh tomatoes.

References

1. Khachik F, Spangler J, Smith JC (1997) 'Identification, quantification and relative concentrations of carotenoids and their metabolites in human milk and serum' *Anal. Chem.* 69, 1873-1881.
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