LYCOPENE EXTRACT FROM TOMATO
Chemical and Technical Assessment (CTA)

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1. Summary

Lycopene extract from tomato is a lycopene-rich extract prepared from the ripe fruits of tomato (*Lycopersicon esculentum* L.*)*. The product is manufactured by crushing tomatoes, to produce crude tomato juice that is then separated into serum and pulp. The pulp is subsequently extracted using ethyl acetate as a solvent. The final extract consists of tomato oil in which lycopene together with a number of other constituents that occur naturally in tomato, are dissolved and dispersed. These constituents include fatty acids and acylglycerols, unsaponifiable matter, water soluble matter, phosphorous compounds, and phospholipids.

The major colouring principle in tomato extract is all-*trans*-lycopene, however, minor amounts of *cis*-isomers and other carotenoids and related substances including β-carotene, phytofluene, phytoene and tocopherols are also present. The intended use of lycopene extract from tomato is as a food colour in dairy products, non-alcoholic flavoured drinks, cereal and cereal products, bread and baked goods and spreads, to provide colour shades from yellow to red. Lycopene extract from tomato may also be used in food supplements. The use levels of the extract, expressed as lycopene added to food, may vary from 2 mg/l in bottled water to 130 mg/kg in ready-to-eat cereals. Lycopene in the extract was shown to be stable when stored at room temperature and at 4°C for up to 37 months. When used as a food colour, lycopene remained stable in the food matrix under appropriate storage conditions. Lycopene stability depends on the particular food to which it is added, as well as on the production process. This Chemical and Technical Assessment is partly based on data and information submitted by LycoRed Natural Products Industries Ltd[1].

2. Introduction

The major colouring principle of lycopene extract from tomato is all-*trans*-lycopene. Lycopene in tomatoes and tomato products consists predominantly of all-*trans*-lycopene (35-96% of the total lycopene content) and low levels of *cis*-lycopenes (1-22% of the total lycopene content) (Schierle et al., 1997). Lycopene for food use is also manufactured by chemical synthesis or produced by fermentation of *Blakeslea trispora*.

The lycopene content in tomato typically ranges from 70 to 130 mg/kg and depends on the variety, geographic location, technique of cultivation, climatic conditions and degree of ripeness of tomato fruits. The tomato extract described in this application is the ethyl acetate extract of ripe tomato fruits with lycopene content ranging from 150 to 250 mg/kg. The lycopene content of tomato extract ranges from 5% to 15%, depending on the nature of the fruit from which it was extracted, and the amount of tomato seed oil that is included in the extract.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) had previously evaluated lycopene (both natural and synthetic) to be used as a food colour at its eighth, eighteenth, and twenty-first meetings (FAO/WHO, 1965, 1975, 1978), but was not able to establish an Acceptable Daily Intake (ADI) due to the limited information available. At its sixty-seventh meeting JECFA agreed that both synthetic lycopene and lycopene extracted from *Blakeslea trispora* were acceptable as food colours and established a group ADI of 0-0.5 mg/kg bw/day for both preparations (FAO/WHO, 2007).

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3. Description

Lycopene extract from tomato is a dark-red viscous liquid. It is freely soluble in ethyl acetate and *n*-hexane, partially soluble in ethanol and acetone, and insoluble in water. A solution in *n*-hexane shows an absorption maximum at approximately 472 nm.

4. Method of manufacture

Lycopene extract from tomato is produced from a tomato variety with high lycopene content, within the range of 150 to 250 mg/kg. This particular variety is not generally marketed for direct consumption, but is used primarily in the production of this lycopene extract. The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethyl acetate. The final product is obtained after solvent removal by evaporation under vacuum at 40-60°C.

5. Characterization

5.1 Composition

Lycopene extract from tomato contains carotenoids (5-15% w/w) as well as non-carotenoid components. The carotenoid fraction of the tomato extract consists mainly of lycopenes, of which ~86 % is all-*trans*-lycopene, ~6% is 5-*cis*-lycopene, ~2% is 9-*cis*-lycopene and ~2% is 13-*cis*-lycopene, and ~4% are other carotenoids. The major non-carotenoid components of tomato extract include fatty acids and acylglycerols (69-74%), phospholipids (8.9-14%), and waxes (5-8.4%).

The chemical composition of tomato extract as provided to the Committee is detailed in Table 1. The reported values were determined using the analytical methods described in the report of a study that aimed at a full qualitative and quantitative characterization of the extract.

Table 1. Chemical composition of lycopene extract from tomato

Compound	Content [%]	
	Min	Max.
Unsaponifiable matter	13.4	31.4
Lycopene	4.9	15
Phytoene	0.5	1.1
Phytofluene	0.4	0.9
β-Carotene	0.1	0.5
Tocopherols	1.0	3.0
Sterols	1.5	2.5
Others (i.e. waxes)	5.0	8.4
Fatty acids and acylglycerols of which*	69	74
Myristic acid (14:0)	0.5	0.6
Palmitic acid (16:0)	22.5	23.0
Stearic acid (18:0)	5.1	5.4
Oleic acid (18:1)	12.4	13.5
Linoleic acid (18:2)	46.7	48.7
Linolenic acid (18:3)	8.8	10.9
Arachidic acid (20:0)	0.9	1.1
Behenic acid (22:0)	0.5	—
Free fatty acids	5	
Water	0.5	0.9

Compound	Content [%]	
	Min	Max.
Water and soluble matter	2.7	4.8
Lactic acid	0.5	0.7
Other organic acids		0.1
Others	2.2	4.0
Total Phosphorus	0.4	0.5
Organic phosphorus	0.3	0.5
Phospholipids (estimated from phosphorus determined by ICP)	8.9	14
Nitrogen	0.16	0.31
Ash	0.7	0.8

* % of total peak area

All-*trans*-lycopene is an unsaturated acyclic hydrocarbon with chemical formula $C_{40}H_{56}$ and molecular weight of 536.85. Its Chemical Abstract Service (CAS) Number is 502-65-8.

The chemical name of all-*trans*-lycopene is (all-E)-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*-lycopene, and (all-E)-lycopene. The structural formula of all-*trans*-lycopene is shown in Figure 1.

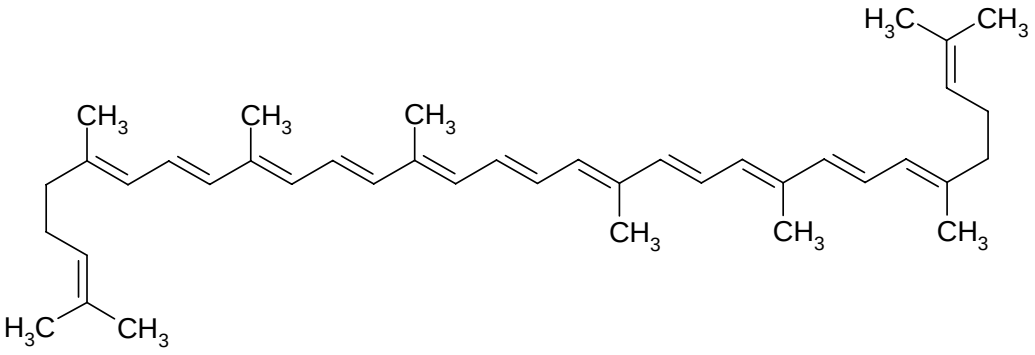

Figure 1. All-*trans*-lycopene

Carotenoids from tomato or tomato extract can be analysed using HPLC (Ishida et al*.,* 2001). A representative chromatogram of the separation of the lycopene isomers of tomato extract is shown in Appendix I.

5.2 Impurities

According to the sponsor, tomato extract may contain residues of ethyl acetate, which is used as solvent in the production process. The Committee established a specification limit for ethyl acetate of not more than of 50 mg/kg. The sponsor also provided information on other potential contaminants including heavy metals and arsenic. Based on this information, the Committee established the specification limits for lead and arsenic (see section 5.4).

5.3. Stability

Lycopene is susceptible to chemical changes such as oxidation followed by degradation or isomerization when exposed to light, heat and oxygen. Lycopene present in tomato extract was shown to be stable under storage at 4^{o}C and room temperature when tested over a time period ranging from 18

to 37 months. Lycopene stability was assessed for nine batches of tomato extract using spectrophotometry and HPLC. The stability data are provided in Appendix II.

5.4 Analytical methods

Analytical methods used to support the specifications for tomato extract are based on general tests in the Combined Compendium of Food Additive Specifications (FAO JECFA Monographs 1, Volume 4, 2006) for identity and purity. The specifications monograph cites specific tests for limits on lead (not more than 1 mg/kg), arsenic (not more than 3 mg/kg), sulfated ash (not more than 0.1%) and loss-on-drying (not more than 2%).The headspace gas chromatography method for residual solvent (ethyl acetate) is described in the new specifications monograph.

The assay is intended to define both the content of total lycopenes and total carotenoids in the extract. The HPLC method of assay provided to the Committee was designed to determine total lycopenes (all-*trans*-lycopene and *cis*-lycopene isomers), while total carotenoids are determined spectrophotometrically using a method compatible with that published in the Combined Compendium of Food Additive Specifications (FAO JECFA Monographs 1, vol. 4, 2006).

5.5 Rationale for proposed specifications

The specifications for tomato extract were developed from considerations proposed by the sponsor, based on the Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on the safety of lycopene oleoresin from tomatoes (EFSA, 2008), as well as on the existing JECFA specifications for synthetic lycopene (FAO/WHO, 2006) and lycopene isolated from *Blakeslea trispora* (FAO/WHO, 2006).

6. Functional uses

Lycopene extract from tomato is intended for use as a food colour. It provides the similar colour shades, ranging from yellow to red, as do the natural and synthetic lycopenes. Lycopene extract from tomato is also used as a food/dietary supplement in products where the presence of lycopene provides a specific value (e.g., antioxidant or other claimed health benefits). The product may also be used as an antioxidant in food supplements.

6.1 Food categories and use levels

Lycopene extract from tomato is intended for use in the following food categories: baked goods, breakfast cereals, dairy products including frozen dairy desserts, dairy product analogues, spreads, bottled water, carbonated beverages, fruit and vegetable juices, soybean beverages, candy, soups, salad dressings, and other foods and beverages.

According to the sponsor, the use levels of tomato extract, expressed as lycopene levels added to food, depend on its intended function and may vary from 2 mg/l in bottled water to 130 mg/kg in ready-to-eat cereals. Food and beverage products will be formulated in such a way that they will provide about 2 mg lycopene per serving. Appendix III contains the proposed uses and use levels of tomato extract (expressed as lycopene levels added to food) arranged according to the Food Category System of the Codex General Standard for Food Additives.

7. Reactions and fate in foods

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) and the subsequent loss of its colouring properties (Xianquan *et al* .; Yang *et al*. 2006); this would render tomato extract ineffective as a food colour.

The Committee received data on lycopene stability in representative foods based on monitoring of the lycopene content in food and the colour of food during 5 days storage under fluorescent light and storage conditions appropriate for each food (room temperature, 4°C, or frozen) . The concentration of lycopene in different food products, to which the commercial product Lyc-O-Mato Oleoresin containing 6% lycopene was added, was in the range of 0.5 to 60 mg/kg (Table 2). Equivalent commercial food products, which were either not coloured or coloured with control colorants such as β-carotene, were used as control samples. Both the test and control samples were analyzed for colour using a Hunter Colorimeter and for lycopene content using HPLC.

Table 2. Lycopene stability in foods prepared with Lyc-O-Mato Oleoresin containing 6% lycopene

Food	**Lycopene level in food (mg/kg)**	**Control colorant level in food (mg/kg)**
Orange gelatine	10-30	Yellow 6/Red (40)
Yellow cake	20-30	β-Carotene (80)
Lemon beverage	3-60	Not coloured
Orange hard candy	5-20	Not coloured
Ice cream	10-20	Not coloured
Salad dressing	20-50	Not coloured
Margarine	0.5-1.0	β-Carotene (2)

Visual inspections and Hunter Colorimetry showed no significant changes in colour after 5 days of storage. The HPLC data showed that ninety-five percent of the added lycopene was recovered at the time of formulation and ninety percent 5 days after formulation. These results demonstrate that tomato extract is stable in a variety of foods under appropriate storage conditions.

Lycopene stability was also assessed in a fruit preparation containing apple and *Aloe vera* formulated with tomato extract. The level of lycopene in the product decreased from approximately 83 mg/kg to 77 mg/kg after four months of storage.

8. References

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Xianquan, S., Shi J., Kakuda, Y., and Yueming J., 2005. Stability of lycopene during food processing and storage. J. Med. Food, 8, 413–422.

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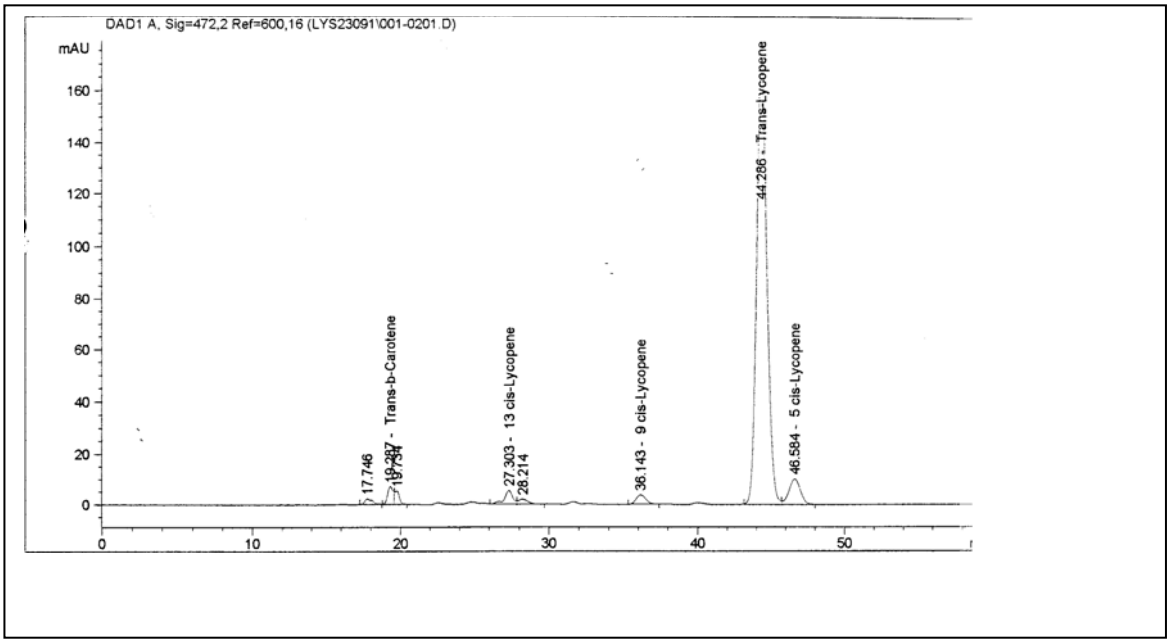
APPENDIX I



Typical chromatogram for tomato extract showing the separation of β-carotene and lycopene isomers, which are eluted in the following order: trans-β-carotene (approximately 19.5 min), 13-*cis*-lycopene (27.3 min), 9-*cis*-lycopene (36.1 min), all-*trans*-lycopene (44.3 min) and 5-*cis*-lycopene (46.6 min).

APPENDIX II. Stability of lycopene in tomato extract under different storage conditions.

Batch no.	Storage conditions	Lycopene concentration (%) *Determined by spectrophotometry*			Lycopene concentration (%) *Determined by HPLC*		
		at t= 0	at t=[months]	Change (%)	at t= 0	at t=[months]	Change (%)
630442	Room temp.	4.95	5.18 [37]	4.4			
630442	4ºC	4.95	4.95[37]	0	4.2	4.24 [37]	0.9
510004	Room temp.	6.82	6.98 [25]	2.3			
510004	4ºC	6.82	6.88 [25]	0.9	6.2	6.32 [25]	1.9
512003	Room temp.	6.73	6.60 [24]	-2			
512003	4ºC	6.73	6.67 [24]	-0.9	6.03	6.12 [24]	2
405043	Room temp.	6.56	6.64 [18]	-1.2			
405043	4ºC	6.56	6.61[18]	0.8	6.05	6.05 [18]	0
#003120	Room temp.	10.81%	10.67[24]	-1.3	10.21	10.08 [24]	-1.3
#003120	4ºC	10.81%	10.73 [24]	-0.7		10.13 [24]	-0.7
902172	Room temp.	10.65	10.45 [24]	-1.9	10.1	10.02[24]	-0.8
902172	4ºC	10.65	10.53 [24]	-1.1		10.06 [24]	-0.4
710023	Room temp.	11.23	11.18 [18]	-0.4	10.64	10.62 [18]	-0.2
710023	4ºC	11.23	11.27 [18]	0.3		10.67 [18]	0.3
705151	Room temp.	16.05	16.01 [18]	-0.3	15.6	15.5 [18]	-0.64
705151	4ºC	16.05	15.95 [18]	-0.6		15.56 [18]	-0.25
703127	Room temp.	15.3	15.38 [18]	0.5	14.91	15.01 [18]	0.67
703127	4ºC	15.3	15.27 [18]	-0.2		14.86 [18]	0.33

APPENDIX III. Proposed uses and use levels of tomato extract (expressed as lycopene level added to food).

Food Category*	**GSFA Food Category and Food-Use**	**Use level (mg/kg)**
Milk Products	0.1.1.2 Flavoured milk and milk drinks	30
	01.2.1 Fermented milk beverages	30
	13.4 Milk-based meal replacements	9-40
Dairy Product Analogues	01.3.3 Imitation milks	30
	01.5 Dry milk	30
	01.5.2 Soy milks	30
	01.7 Yoghurt	20-40
	01.7 Frozen Yoghurt	20-40
Fats and Oils	02.2.1.2 Margarine-like spreads	20
Soft Candy	05.2 Chewy and nougat candy	15
	05.2 Fruit Snacks	15
Hard Candy	05.2 Hard candy	20-70
Chewing Gum	05.3 Chewing gum	15
Breakfast Cereals	06.3 Ready-to-eat cereals	30-130
	06.5 Instant and regular hot cereals	9-20
	07.1.2 Crackers and crisp breads	60
	07.2.1 Cakes, cookies	30
Egg Products	10.4 Egg-based desserts	20
Soups and Soup Mixes	12.5.1 Soups	30
	12.6.1 Salad dressings	30
Gravies and Sauces	12.6.2 Tomato-based sauces	30
	12.9.1.1 Soybean beverage	20-40
Beverages and Beverage Bases	14.1.1.1 Bottled water	2-15
Processed Fruits and Fruit Juices	14.1.2.1 Fruit juice	4-20
	14.1.2.2 Vegetable juice	4-20
	14.1.3 Nectars	4-20
	14.1.4 Energy, sport, and isotonic drinks	4-15
	14.1.4.1 Carbonated beverages	4-20
	14.1.4.2 Fruit-flavoured drinks	9-15
	14.1.5 Tea, ready-to-drink	3-15
Baked Goods and Baking Mixes	15.1 Cereal and energy bars	40-80

* Food category system of the General Standard for Food Additives (GSFA) of the Codex Alimentarius Commission (FAO/WHO, 2009. Food category system. In: General Standards for Food Additives CODEX STAN 192-1995 (Rev. 10-2009), Annex B, pp. 9-48. Food and Agriculture Organization of the United Nations (FAO), Rome, and the World Health Organization (WHO), Geneva). Available at: http://www.codexalimentarius.net/web/standard_list.do?lang=en (Accessed 14 September 2009).