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PII: S0308-8146(96)00177-X

Food Chemistry, Vol. 59, No. 3, pp. 459–465, 1997

0308-8146/97 $17.00+0.00
Analytical, Nutritional and Clinical Methods Section

Content and isomeric ratio of lycopene in food and human blood plasma

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(Received 12 December 1995; revised version received 26 March 1996; accepted 26 March 1996)

Lycopene content up to 520 $\mu g\,g^{-1}$ was measured in a number of tomato-based foodstuffs and meals. (all-*E*)-Lycopene was the predominant geometrical isomer but varied from 96% to 35% of total lycopene. (5*Z*)-Lycopene ranged from 4% to 27%. The proportion of (9*Z*)-lycopene fluctuated between < 1% and 14%. (13*Z*)-Lycopene and (15*Z*)-lycopene ranged (together) from < 1% to 7% and the sum of the other (*Z*)-isomers varied between < 1% and 22% of total lycopene. It was shown that, during preparation of meals, lycopene undergoes (*E*/*Z*)-isomerisation, increasing the portion of (*Z*)-isomers.

Compared to food, in human blood plasma the isomeric ratio of lycopene was found to be shifted in favour of the (*Z*)-isomer fraction, with (5*Z*)-lycopene as the predominant non-(all-*E*) component.

INTRODUCTION

Like other carotenoids, lycopene occurs in various geometrical isomers. In most raw foods, the (all-*E*)-isomer is quantitatively the most important, and within the relatively small proportion of (*Z*)-isomers, (5*Z*)-, (9*Z*)- and (13*Z*)-lycopene usually predominate. For example, in red tomato fruit, typically 94–96% (all-*E*)-lycopene, 3–5% (5*Z*)-lycopene, 1% (13*Z*)-lycopene and 0–1% (9*Z*)-lycopene can be found (Schiedt *et al.*, unpublished results).

But there are also fruits in which other geometrical isomers of lycopene are prevalent. In Valaisan apricots (*Prunus armeniaca* L.), for example, the di-*cis*-isomer (7*Z*,9*Z*)-lycopene has been found to contribute to 53% of total lycopene, whereas the (all-*E*)-isomer portion was only 8% (Schiedt *et al.*, unpublished results, 1988). In Tangerine-type tomatoes the tetra-*cis*-isomer (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene (or prolycopene, identified by Englert (1979) and by Clough and Pattenden (1979)), was shown to account for more than 90% of the lycopene content (Hirota *et al.*, 1982; Schiedt *et al.*, unpublished results, 1988). Although demonstrating that the ratio of lycopene isomers in food can vary over a wide range, such proportions can certainly be regarded as exceptions.

Tomatoes are the main source of lycopene in many diets. The present work eludicated the isomeric ratio of lycopene in tomato products, some of which were additionally cooked or otherwise heat treated. Additionally, the (*E*/*Z*)-isomer composition of lycopene in human blood plasma was analysed and compared to that of the foods.

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MATERIALS AND METHODS

Samples

The following tomato-based foods were purchased at local stores: canned peeled tomatoes ('Chris', Roger Sud, Italy); tomato concentrates ('Tomatenmark', Panocchia, Italy; 'Miracoli', Kraft, Germany); ketchups ('Hot Ketchup', Del Monte, Italy; 'Tomato Ketchup', Heinz, Pittsburgh, USA); instant meal ('Eier-Ravioli', Hero, Switzerland); sauces ('Hamburger Relish', Heinz, The Netherlands; 'Sauce Bolognaise', Barilla, Italy). A red Malaysian palm oil was received from Roche Hongkong. The materials were extracted immediately after opening the tins, aluminium foil bags or preserving jars, respectively. The samples 'Miracoli', 'Sauce Bolognaise' and the 'Eier-Ravioli' were additionally cooked for 10 or 15 min and then analysed. From canned peeled 'Chris' tomatoes, two spaghetti sauces were prepared by cooking for approx. 45–60 min at 85–110°C, together with water, salt, olive oil, flour, onions and various fresh and dried spices.

Single human blood plasma samples were received from various Swiss hospitals. Pooled human blood plasma was obtained from the blood donor centre in Basle.

Analytical sample preparation

With the exception of ketchups and tomato concentrates, all tomato-based foods were homogenised using a dispersing instrument (Polytron). For extraction, 1–2 g of the material were combined in a 250 ml beaker with approx. 10 g of magnesium sulphate hydrate and approx. 30 ml of acetone. The mixture was homogenised by the dispersing instrument and filtered under partial vacuum through sintered glass. The residue was scraped off the filter and re-extracted twice with acetone as above. If any colour remained in the final extract, the residue was ground in a mortar with sea sand and approx. 10 ml n-hexane containing 1% acetone, and filtered. This procedure was repeated until the filtrate was colourless. The combined filtrates were evaporated under partial vacuum at 50°C by rotary evaporation. In order to remove the water, approx. 50 ml of ethanol were added in several portions to the concentrate and re-evaporated. The dry residue was dissolved in approx. 10 ml of n-hexane containing 1% acetone and purified by aluminium oxide in an open column. The column was prepared by covering the fritted glass bottom of a chromatography tube (length 25 cm, inner diameter 2 cm, with stopcock) with an approx. 0·5 cm thick layer of sea sand. A suspension of approx. 10 g of deactivated aluminium oxide in approx. 30 ml of n-hexane was slowly poured into the chromatography tube, forming an approx. 3·5 cm layer which was permanently covered with n-hexane. The extract was quantitatively transferred onto the column, rinsing the flask with approx. 20 ml n-hexane. The carotenes (including lycopene) were eluted with 100 ml of n-hexane; the more polar xanthophylls remained on the column. The eluate was evaporated by rotary evaporation at 50°C under reduced pressure, and the residue was dissolved in a defined volume of n-hexane containing 1% acetone for high-performance liquid chromatography (HPLC). The recovery of added lycopene (in the presence and absence of tomato products) subjected to the extraction procedure was 90–94%.

In case of palm oil, approx. 1 g was dissolved in approx. 100 ml of n-hexane, and 10 μl of the solution were injected into the HPLC system.

Deep-frozen human blood plasma was thawed and centrifuged in Eppendorf vials for 10 min at approx. 2500*g*. To 200 μl of clarified plasma, in 4 ml tubes, were added 200 μl of demineralised water and 400 μl of ethanol to precipitate the protein. The suspensions were mixed for approx. 10 s on a Vortex mixer. After adding 800 μl of n-hexane, the tubes were shaken for approx. 10 min on a shaking device and centrifuged at approx. 2500*g* for approx. 10 min. Aliquots (500 μl) of the clear supernatant were pipetted into 2 ml Eppendorf vials and evaporated at approx. 25°C using a Speed-Vac concentrator. After pre-dissolving the residue in 10 μl of dichloromethane, the solution was diluted with 250 μl of n-hexane and analysed by HPLC. Recoveries ranged from 95% to 102% when lycopene was added (in the ethanol) to the plasma samples before extraction.

High-performance liquid chromatography

High-performance liquid chromatography was performed using a modified version of the system described by Hengartner *et al.* (1992), with a Waters autosampler (Model 717) with sample cooling at 5°C, a Severn Analytical HPLC detector (Model SA 6504) adjusted to 470 nm, and a VAX-based chromatography system (VG-Multichrom) for integration of peak areas and calculation. As a mobile phase n-hexane containing 0·15% n-ethyldiisopropylamine was pumped at a rate of 1 ml min^{-1} through a combination of three 250 mm × 4·6 mm columns packed with Nucleosil 300-5 (Macherey-Nagel) yielding a pressure of approx. 100 bar; sample volumes of 20–100 μl were injected. The run time was approx. 35 min. The retention time of (all-*E*)-lycopene was in the range of 22–30 min. Retention times of the other carotenes relative to (all-*E*)-lycopene were: 0·38 for (all-*E*)-ζ-carotene, 0·44 for (13*Z*)-β-carotene, 0·45 for (15*Z*)-β-carotene, 0·47 for (9*Z*)-β-carotene and (all-*E*)-β-carotene, 0·48 for (all-*E*)-α-carotene, 0·67 for (all-*E*)-γ-carotene (co-eluting with (13*Z*)-lycopene), 0·68 for (13*Z*)-lycopene, 0·71 for (15*Z*)-lycopene, 0·87 for (9*Z*)-lycopene, 0·95 for (5,5′*Z*)-lycopene, 1·05 for (5*Z*)-lycopene. Much longer relative retention times were obtained for xanthophylls (e.g. 3·0 for the comparatively apolar ethyl-β-apo-8′-carotenoate).

Peak identification was performed by use of reference substances of (*E*/*Z*)-isomers of β-carotene, (all-*E*)-α-carotene, (all-*E*)-γ-carotene, (all-*E*)-ζ-carotene, (all-*E*)-lycopene, (5*Z*)-lycopene and (5,5′*Z*)-lycopene), or, in the case of (9*Z*)-lycopene, (13*Z*)-lycopene and other (*Z*)-isomers of lycopene (referred to as (*x*Z)-lycopene), by comparing the relative retention times of isomers from iodine-isomerised lycopene solutions with those in the original nuclear magnetic resonance (NMR)-controlled HPLC system (Hengartner *et al.*, 1992). Additional information was obtained from photodiode array spectra recorded with a Hewlett-Packard Instrument Model 1050 or from stop-flow spectra.

For calibration, solutions of (all-*E*)-β-carotene and (all-*E*)-lycopene were prepared by dissolving approx. 3 mg of carotenoid in 20 ml of dichloromethane and making to 1 litre with n-hexane. Immediately afterwards, the exact carotenoid concentrations were measured with a spectrophotometer at λ_{max} and calculated using $E^{1\%}_{1\,cm}$ values of 3450 (at approx. 471 nm) for lycopene and 2590 (at approx. 450 nm) for β-carotene. For food analysis, the spectrometrically measured solutions were used as standard solutions, whereas for plasma

analysis the solutions were further diluted with n-hexane in a ratio of 1:10. Repeat volumes (20 μl) of the standard solutions were injected into the HPLC system; mean values of the relevant peak areas were taken from the chromatograms and divided by the spectrometrically measured carotenoid concentrations of the standard solutions. The resulting response factors of (all-*E*)-lycopene or (all-*E*)-β-carotene were used to calculate the contents of all the according geometrical isomers. Therefore, (*Z*)-isomers with a lower specific absorption than the (all-*E*)-isomer, such as (9*Z*)- or (13*Z*)-lycopene (Giger, unpublished data, 1994; see Table 3) and other (*Z*)-lycopene isomers (see Hengartner *et al.*, 1992) were somewhat underestimated. In contrast, the quantification of (5*Z*)-lycopene can be regarded as exact, since this isomer was shown to behave as (all-*E*)-lycopene with respect to both the maximum and the specific intensity of absorption (Hengartner *et al.*, 1992).

Reagents

Aluminium oxide for chromatography (neutral, activity 1; Camag, Switzerland) was deactivated by shaking 100 g of aluminium oxide with 12 ml of demineralised water in a closed flask, and could be used for up to 3 months. Magnesium sulphate hydrate, dried, 22·5–25% H_2O, ph.h.VI, was from Lohmann, Germany. Standard substances of (all-*E*)-α-carotene, (all-*E*)-β-carotene, (9*Z*)-β-carotene, (13*Z*)-β-carotene, (15*Z*)-β-carotene, (all-*E*)-γ-carotene, (all-*E*)-ζ-carotene, (all-*E*)-lycopene, (5*Z*)-lycopene, (5,5′*Z*)-lycopene and ethyl-β-apo-8′-carotenoate were from Hoffmann-La Roche (Switzerland). All solvents were p.a. grade from Fluka (Switzerland) or Merck (Germany).

RESULTS AND DISCUSSION

Lycopene in food

In canned tomatoes and various tomato-based foods the contents of lycopene were found to range from 6 to 520 $\mu g\,g^{-1}$ (Table 1). The proportion of (all-*E*)-lycopene varied from 96% of total lycopene in preserved tomato paste down to 35% in a long-term cooked spaghetti sauce prepared from canned tomatoes. (5*Z*)-Lycopene ranged from 4% to 27% of total lycopene. The amount

Table 1. Contents of carotene, lycopene and (*E*/*Z*)-isomers of lycopene in foodstuffs and meals

Sample	Total carotene ($\mu g\,g^{-1}$)	Total lycopene ($\mu g\,g^{-1}$)	(*E*/*Z*)-Isomers of lycopene (% of total lycopene)				
			(All-*E*)	(5*Z*)	(9*Z*)	(13*Z*+15*Z*)	(*x*Z)
Tomato paste (Panocchia)	7	520	96	4	<1	<1	<1
'Hamburger Relish' (Heinz)	1	30	93	5	<1	3	<1
'Miracoli' (Kraft)	1	37	91	5	1	2	<1
10 min cooked	1	37	90	6	1	3	<1
'Hot Ketchup' (Del Monte)	3	95	88	7	2	3	1
'Tomato Ketchup' (Heinz)	1	30	77	11	5	7	1
'Eier-Ravioli' (Hero)	1	6	76	8	5	6	5
15 min cooked	1	6	71	10	6	7	6
'Sauce bolognaise' (Barilla)	8	92	67	14	6	5	8
10 min cooked	9	93	60	14	8	7	11
Canned tomatoes[a] (Roger Sud)	2	71	84	5	3	5	3
Prepared as:							
Spaghetti sauce I	1	34	35	27	14	3	22
Spaghetti sauce II[a]	2	62	56	16	9	7	13
Red palm oil (Malaysia)	11 100	450	16	19	6	30	29

[a]Chromatograms are shown in Fig. 1.

Table 2. Changes of lycopene (*E*/*Z*)-isomer ratios caused by analytical sample preparation

Sample	Total lycopene (mg litre^{-1})	(*E*/*Z*)-Isomers of lycopene (% of total lycopene)				
		(All-*E*)	(5*Z*)	(9*Z*)	(13*Z*+15*Z*)	(*x*Z)
Almost pure (all-*E*)-lycopene						
Control	31·3	96·9	2·0	—	—	1·1
After preparation	28·1	94·4	2·5	0·6	1·7	0·8
Mixture of lycopene (*E*/*Z*)-isomers						
Control	25·5	58·9	13·8	1·5	17·2	8·6
After preparation	23·6	65·7	14·9	2·6	11·0	5·9

Solutions of almost pure (all-*E*)-lycopene and of a mixture of various (*E*/*Z*)-isomers were analysed before (controls) and after subjecting them to the sample preparation for tomato-based foods. The isomeric mixture was generated by refluxing reference substances of (all-*E*)-lycopene and (5*Z*)-lycopene in n-hexane at approx. 80°C for 1 h.

of (9Z)-lycopene fluctuated between < 1% and 14%. (13Z)- and (15Z)-lycopene (in some cases not baseline-separated) ranged from < 1% to 7%. Similarly, the sum of the other (Z)-isomers varied from < 1% up to 22% of total lycopene.

In a red palm oil, a well-known source of α- and β-carotene, the comparatively small content of lycopene was found to consist mostly of (Z)-isomers. It cannot be excluded, however, that in palm oil or in some of the other foods analysed small amounts of γ-carotene were present. Since, in the HPLC system used, γ-carotene co-elutes with (13Z)-lycopene, this would somewhat alter the reported isomeric ratios of lycopene.

In order to estimate to what extent spontaneous (E/Z)-isomerisation of lycopene took place during the performed analyses, solutions of almost pure (all-E)-lycopene and of an isomer mixture including a large amount of (Z)-isomers were analysed in the same way as the food samples. As shown in Table 2, the analytical procedure caused the percentage of (all-E)-lycopene to decrease or increase by approx. 2–7%, depending on the initial contribution of this isomer. The proportion of (13Z)-lycopene was found to be shifted to a similar extent, whereas the percentages of (5Z)-lycopene, (9Z)-lycopene and of the other (Z)-isomers were clearly more stable. These results indicate that the measurement of the isomeric ratios was subject to certain errors which varied for the individual isomers and depended on the isomeric status of the samples. It should be noted, however, that this experiment gives only a rough idea as to the extent of (E/Z)-isomerisation in a food matrix.

In the present study, the various (E/Z)-isomers of lycopene were identified by co-chromatography with reference substances or by their retention times in relation to those in the original NMR-controlled HPLC system. In the case of an extract from a long-term cooked spaghetti sauce (chromatogram in Fig. 1;

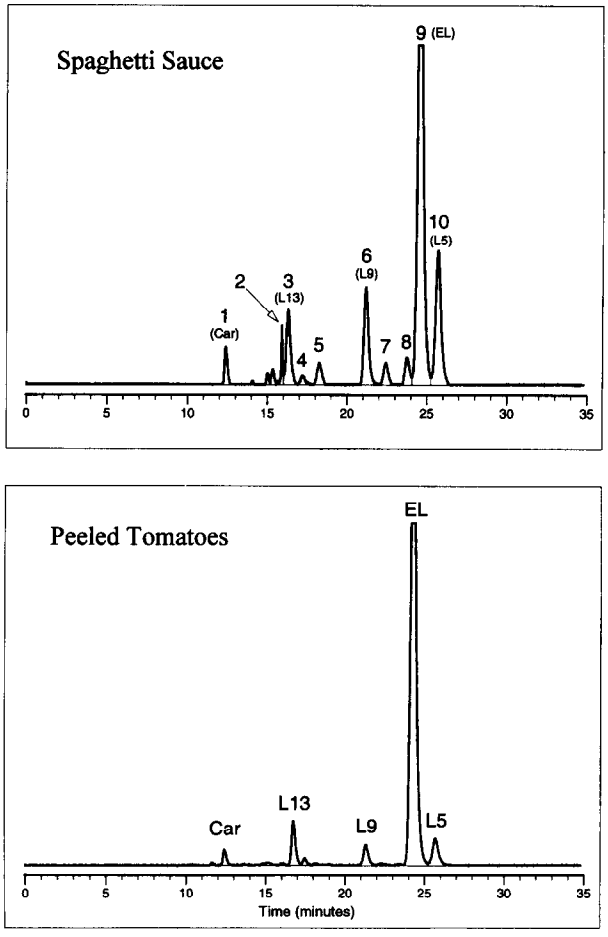

Fig. 1. HPLC chromatograms of a spaghetti sauce and of canned peeled tomatoes used to prepare the sauce. A spaghetti sauce was prepared from preserved peeled tomatoes as described in the methods section. Photodiode array spectra were taken for peaks 1–10 of the chromatogram. Spectral data are listed in Table 3. For carotenoid contents see spaghetti sauce II in Table 1. CAR, carotene; L13, (13Z)-lycopene; L9, (9Z)-lycopene; EL, (all-E)-lycopene; L5, (5Z)-lycopene.

Table 3. Photodiode array data from HPLC peaks of a spaghetti sauce in comparison with UV/Vis data from isolated lycopene isomers

HPLC peak[a]	Carotenoid	Absorption maxima[b] (nm) and [relative absorption of 'cis-peak'][c]									
		Diode array data of HPLC peaks					UV/Vis data of isolated lycopene isomers				
1	Carotene	—	—	—	449	491	—	—	—	—	—
2	(xZ)-Lycopene	[0·38]	360	437	463	494	—	—	—	—	—
3	(13Z)-Lycopene	[0·55]	360	437	463	494	[0·56]	360	437	463	494
4	(xZ)-Lycopene	[0·56]	360	437	466	494	—	—	—	—	—
5	(xZ)-Lycopene	[0·15]	363	438	464	494	—	—	—	—	—
6	(9Z)-Lycopene	[0·12]	360	438	464	494	[0·13]	360	438	464	495
7	(xZ)-Lycopene	[0·12]	360	436	464	494	—	—	—	—	—
8	(5,5'Z)-Lycopene	[0·06]	363	444	470	502	[0·08]	362	443	470	502
9	(all-E)-Lycopene	[0·06]	362	444	470	502	[0·06]	362	443	470	502
10	(5Z)-Lycopene	[0·06]	362	442	470	502	[0·07]	362	443	470	502

[a]Peak numbers as in chromatogram shown in Fig. 1.
[b]Main maxima are underlined.
[c]Relative absorption of 'cis-peak' (shown in square brackets) means absorption at the subsidiary peak (at approx. 360 nm) divided by the absorption at λ_{max}. Comparatively high relative absorptions, as recorded for peaks 2 and 4, indicate lycopene (Z)-isomers with more-central (Z)-double-bond(s) (e.g. (13Z)-lycopene). In contrast, low relative absorptions are characteristic of more-terminal (Z)-double-bonds (see peaks 8 and 10).
UV/Vis data of (all-E)-, (5,5Z)-, (5Z)-lycopene (Hengartner *et al.*, 1992) and of (9Z)- and (13Z)-lycopene (Giger, unpublished data, 1994) were recorded in n-hexane including 2% dichloromethane.

lycopene contents in Table 1), additional peak identification was done by photodiode array detection. As shown in Table 3, the spectra of the predominant HPLC peaks of the chromatogram corresponded well with the UV/Vis data obtained by Hengartner *et al.*, 1992 and by Giger (unpublished data, 1994) for synthesised or isolated lycopene (*E*/*Z*)-isomers with respect to both λ_{max} and the relative absorption of the so-called '*cis*-peak'.

As expected from the effect of heat, which is known to promote (*E*/*Z*)-isomerisation, the percentage of (all-*E*)-lycopene was generally decreased by cooking the food. Correspondingly, the (*Z*)-isomer proportion was increased. The effect of heat on the isomeric ratio was studied more precisely in an experiment in which tomato paste was dispersed in water or oil and heated for more than 3 h at approx. 75°C. As shown in Table 4, in both cases, heat treatment clearly increased the percentage of all the (*Z*)-isomers looked at. However, heating in oil had a stronger effect than heating in water and especially favoured the rise of (9*Z*)-lycopene and (5*Z*)-lycopene.

Even without heating, in case of the oily dispersion, the percentages of (9*Z*)-lycopene and (13*Z*)-lycopene were elevated compared to the aqueous dispersion (Table 4). This may be due to some isomerisation occurring during the analytical sample preparation (e.g. evaporation of solvent at 50°C), which was probably greater in the presence of oil than of water.

It is obvious from these results that food processing and preparation of tomato-based meals increases the proportion of lycopene (*Z*)-isomers. This may be mainly caused by (*E*/*Z*)-isomerisation, but a differential loss of the various isomers could also contribute to the shift in the isomeric ratio. Apparently, not only the duration and temperature of heat treatment, but also food matrix components such as oil or fat, influence the isomeric status of lycopene.

An increase in the (*Z*)-isomers of lycopene has also been observed in foam-mat dried tomato paste (Lovric *et al.*, 1970) and during drying of whole tomatoes (Boskovic, 1979). In both cases, re-isomerisation of (*Z*)-forms to (all-*E*)-lycopene occurred after drying. Similarily, thermal processing of guava juice (Padula & Rodriguez-Amaya, 1987) or papaya puree (Godoy & Rodriguez-Amaya, 1991) led to a 5- and 7-fold increase of the (*Z*)-lycopene content. Heat treatment also increased the (*Z*)-lycopene proportion in an aqueous suspension of lycopene extracted from tomato puree (Kanasawud & Crouzet, 1990).

Isomerisation of β-carotene in fruits and vegetables was demonstrated during processing in a similar way (Sweeney & Marsh, 1970, 1971; Panalaks & Murray, 1970; Tsukida *et al.*, 1981; Bushway, 1985; Chandler & Schwartz, 1987; Quackenbush, 1987).

Lycopene in human blood plasma

As shown in Table 5, in various samples of human blood plasma the level of lycopene ranged from approx. 0·2 to 1·0 μmol litre^{-1}. As in food, (all-*E*)-lycopene was the predominant geometrical isomer. However, compared to foodstuffs, in human plasma the isomeric ratio of lycopene was clearly shifted in favour of the (*Z*)-isomers. The percentage of (all-*E*)-lycopene averaged only 41% of total lycopene, whereas (5*Z*)-lycopene reached 28%, (13*Z*)- and (15*Z*)-lycopene together amounted to 12%, and the other (*Z*)-isomers of lycopene 16% on average. The contribution of (9*Z*)-lycopene was less in human plasma than in most of the analysed foodstuffs.

When a human plasma sample was analysed either by the routine plasma procedure (i.e. extraction with n-hexane) or by a procedure involving exhaustive acetone extraction (as in the method for food), the

Table 4. Effect of heat on the ratio of lycopene (*E*/*Z*)-isomers in aqueous and oily dispersions of tomato paste

Duration of heat treatment (min)	(*E*/*Z*)-Isomers of lycopene (% of total lycopene)				
	(All-*E*)	(5*Z*)	(9*Z*)	(13*Z*+15*Z*)	(*xZ*)
(A) Water					
0	92·6	4·5	0·9	1·6	0·5
15	92·3	4·4	0·9	1·6	0·5
30	88·1	5·1	2·1	2·3	2·5
60	87·1	5·2	2·2	2·7	3·0
120	86·2	5·5	2·7	2·6	3·1
180	83·4	6·1	3·6	3·2	3·8
(B) Olive oil					
0	87·4	4·8	4·3	3·0	0·5
30	85·2	5·8	5·5	2·9	0·5
90	83·5	6·2	5·9	3·3	1·2
120	80·3	7·0	6·9	3·2	2·6
180	76·7	8·1	8·8	3·1	3·3

Tomato paste was dispersed in demineralised water (A) or in cold-pressed olive oil (B) in a ratio of 1 g per 10 ml and heated for 3 h in a water bath (temperature of the suspensions: 74–75°C). After the time intervals indicated, 2 ml samples were taken and analysed as described for tomato-based food samples. Absolute contents of lycopene increased during the heat treatment within 3 h by 48% (A) or 22% (B) due to evaporation of liquids.

lycopene levels and isomeric ratios obtained were in good agreement (Table 6). Thus, the observed differences between food and plasma is not the result of an analytical artifact caused by differences in the extraction procedure.

(5*Z*)-Lycopene was identified by its retention time in relation to (all-*E*)-lycopene and by a stop-flow spectrum taken from the assumed (5*Z*)-lycopene peak of a plasma sample (Fig. 2). The spectrum showed maxima at 443 nm, 470 nm and 502 nm and troughs at 453 nm and 484 nm, and was practically identical with that of (all-*E*)-lycopene. These data correspond well to those of Hengartner *et al.* (1992) for the corresponding synthesised lycopene isomers in n-hexane containing 2% dichloromethane. It is evident from these results that (5*Z*)-lycopene is quantitatively by far the most important (*Z*)-isomer of lycopene in human blood plasma.

In agreement with our results, Sakamoto *et al.* (1994) detected large amounts of (*Z*)-lycopene in human blood plasma, although most of the lycopene in the tomato juice taken up by the test subjects was (all-*E*)-lycopene. These results are consistent with *in vivo* isomerisation of lycopene as well as with discrimination

Table 5. Level and isomeric ratio of lycopene in human blood plasma

Sample	Total lycopene (μmol litre^{-1})	(*E*/*Z*)-Isomers of lycopene (% of total lycopene)				
		(All-*E*)	(5*Z*)	(9*Z*)	(13*Z*+15*Z*)	(*x*Z)
Pool 22[a]	0·36	43	29	2	12	14
Pool 23	0·42	46	31	2	11	11
Pool A	0·82	45	31	2	8	14
Pool B	0·29	40	28	2	13	17
Single 1	0·98	37	30	4	8	20
Single 2	0·19	43	27	2	12	15
Single 3	0·26	32	20	1	19	28
Single 4	0·34	44	29	2	13	12
Mean		41	28	2	12	16
Standard deviation		5	4	1	3	5
Coefficient of variation		11	13	39	29	33

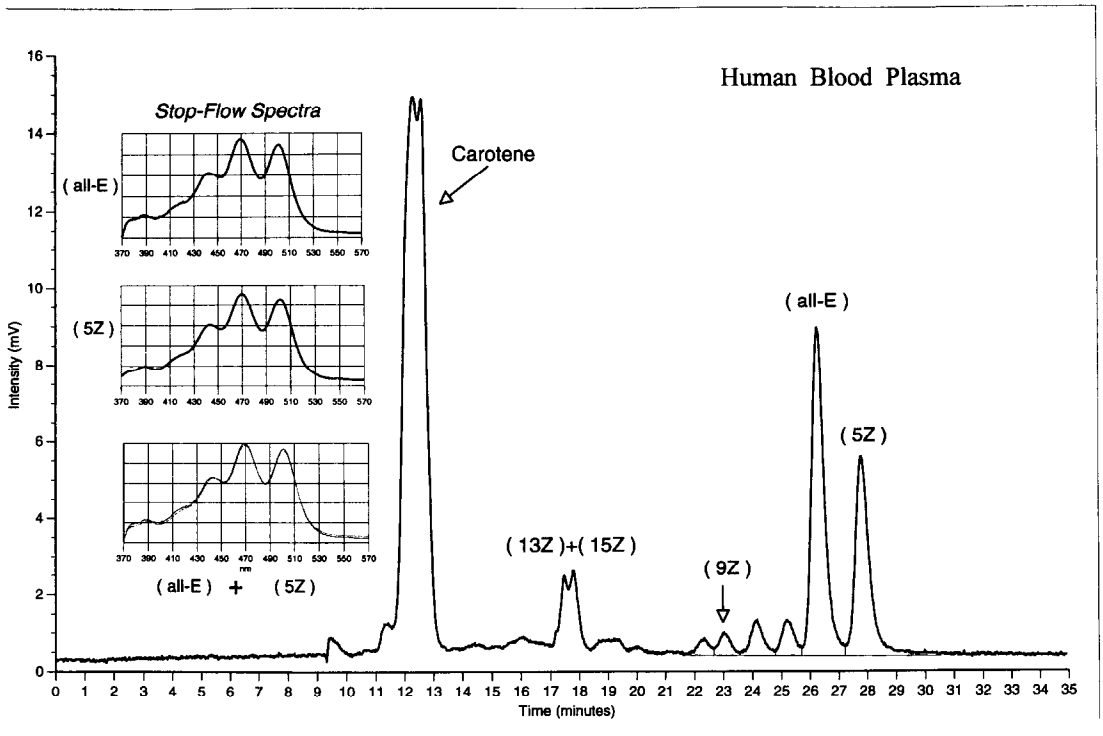

Fig. 2. HPLC chromatogram of human blood plasma and stop-flow spectra taken from the two main peaks of lycopene isomers. For the peaks of (all-*E*)- and (5*Z*)-lycopene maxima were found at 502 nm, 470 nm, 443 nm, and troughs at 484 nm and 453 nm. Configurations of geometrical isomers of lycopene are indicated in parentheses.

Table 6. Comparison of analytical methods for the analysis of lycopene in human blood plasma

Method	Total lycopene (μmol litre^{-1})	(*E*/*Z*)-Isomers of lycopene (% of total lycopene)				
		(All-*E*)	(5*Z*)	(9*Z*)	(13*Z*+15*Z*)	(*x*Z)
Routine plasma method	0·55	38	27	5	8	22
Acetone method	0·54	40	27	4	7	22

A human plasma sample was analysed using the method described for plasma in parallel with acetone extraction followed by evaporation of the extract at 50°C under vacuum and dissolution of the residue in n-hexane containing 1% acetone.

between lycopene isomers in metabolic or transport processes.

High levels of (*Z*)-isomers of lycopene were also found by Stahl *et al.* (1992) in human serum, accounting for more than 50% of total lycopene. In that study, a reversed-phase HPLC system was used which was not able to resolve (5*Z*)- and (9*Z*)-lycopene, so that in some plasma samples (9*Z*)-lycopene seemed to be present in higher amounts than (all-*E*)-lycopene. In a later publication, Stahl *et al.* (1993) deduced from diode-array spectra that the (*Z*)-isomer peak occurring in a reversed-phase HPLC chromatogram directly after (all-*E*)-lycopene might be caused mainly by (5*Z*)- or (7*Z*)-lycopene and not by (9*Z*)-lycopene.

ACKNOWLEDGEMENTS

We would like to thank our colleagues from Hoffmann-La Roche, Basle, for their support; in particular, K. Schiedt and A. Giger for kindly providing unpublished data on the (*E*/*Z*)-isomers of lycopene, E. Glinz for his help in establishing the HPLC system, and K. Bernhard for the supply of standard substances.

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