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Improving the stability of lycopene *Z*-isomers in isomerised tomato extracts

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ABSTRACT

Tomato-based foods rich in *Z*-lycopene are potentially more bioavailable and have greater bioefficacy compared to natural tomato products which mainly contain all-*E*-lycopene. To prepare a stable tomato extract with a high level of *Z*-lycopene, geometrical isomerisation of lycopene was studied in organic solvents either alone or in the presence of a tomato extract. Interconversion between the isomers was observed in all systems with 13*Z*-lycopene being the least stable. Heating a tomato extract containing mainly the all-*E*-isomer in ethyl acetate produced successively 13*Z*-, 9*Z*- and 5*Z*-lycopene. An isomerised tomato oleoresin with a minimal content of the most unstable 13*Z*-lycopene could be obtained by refluxing tomato oleoresin in ethyl acetate for 1 week. In this isomerised tomato oleoresin, total lycopene and lycopene isomer profiles were shown to remain constant for 1 year at room temperature. Accordingly, this product is a valid source of stable and potentially highly bioavailable lycopene.

1. Introduction

Lycopene is the predominant carotenoid found in tomatoes and is the pigment responsible for the red colour of ripe tomato fruit and tomato products. *In vitro* studies have demonstrated that lycopene has interesting antioxidant capacities, for example acting as a potent quencher of singlet oxygen and other electronically excited molecules that are produced by photoexcitation or chemiexcitation (Sies & Stahl, 1995). Due to its antioxidant properties lycopene may play an important role in the reduction of oxidative stress linked to certain degenerative diseases. Indeed, dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with decreased risk of chronic disorders such as cancer or cardiovascular disease (Arab & Steck, 2000; Clinton, 1998).

As a result of the 11 conjugated carbon–carbon double bonds in its molecular backbone, lycopene can theoretically assume 2^{11}, *i.e.* 2048 geometrical configurations. However, because of steric hindrance only certain double bond groups of lycopene actually undergo geometrical isomerisation. In fact only about 72 lycopene *Z*-isomers are structurally favourable (Zechmeister, 1962). Among them, 5*Z*-, 9*Z*- and 13*Z*-lycopene usually predominate (Schierle et al., 1997).

The physicochemistry and bioavailability of all-*E*- and *Z*-isomers of lycopene are known to be different. Lycopene *Z*-isomers have been described to be more bioavailable than the naturally occurring all-*E*-isomer (Boileau, Boileau, & Erdman, 2002; Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999; Shi & Le Maguer, 2000; Stahl & Sies, 1992; Unlu et al., 2007). This could be due to differences in physical properties between the all-*E*- and *Z*-isomers such as a greater solubility in mixed micelles, and/or a lower tendency to aggregate (Boileau et al., 1999; Boileau et al., 2002; Shi & Le Maguer, 2000). In terms of bioactivity, it has been pointed out that the bioactive potency of *Z*-lycopene isomers is different from that of the all-*E*-form, because of structural differences (Shi & Le Maguer, 2000). Böhm et al. (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002) found that *Z*-isomers have a stronger antioxidant activity than the all-*E*-isomer. In addition, it has been demonstrated that *Z*-isomers of lycopene make up 50% of the total lycopene in human serum and tissues (Clinton et al, 1996; Ferruzzi, Nguyen, Sander, Rock, & Schwartz, 2001; Schierle et al., 1997; Stahl & Sies, 1992). For these reasons lycopene *Z*-isomers are regarded as having potentially higher health benefits than the all-*E*-isomer.

Lycopene is biosynthesised in plants mainly (above 90%) as the all-*E*-isomer. Most available sources of lycopene maintain the natural isomeric distribution ratio (Schierle et al., 1997; Shi & Le Maguer, 2000). It was shown that during food processing lycopene undergoes geometrical isomerisation, increasing the proportion of *Z*-isomers (Schierle et al., 1997; Shi & Le Maguer, 2000; Xianquan, Shi, Kakuda, & Yueming, 2005). In particular, lycopene may isomerise to *Z*-forms with the presence of heat or oil, or during dehydration (Xianquan et al., 2005). Besides, lycopene underwent further geometrical isomerisation, mainly retro-isomerisation,

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during storage of processed foods (Shi & Le Maguer, 2000; Xianquan et al., 2005). Thus, an ideal source of highly bioavailable lycopene should be a tomato extract containing stable *Z*-isomers which do not undergo retro-isomerisation.

Isomerisation of lycopene has been achieved by different means, *e.g.* using heat, acids, active surfaces, complexation with boron trifluoride and also light in the presence or absence of a photosensitiser (Lee & Chen, 2002; Molnar & Szlabolcs, 1993; Schierle et al., 1997; Shi & Le Maguer, 2000; Shi, Maguer, Bryan, & Kakuda, 2003; Xianquan et al., 2005; Zechmeister, 1962). These isomerisation processes not only induce the formation of various *Z*-isomers but also underline that degradation competes with isomerisation. With long heating times or at temperatures above 50 °C degradation proceeds faster than isomerisation (Lee & Chen, 2002).

To explore the expected additional health benefits of tomato-based foods rich in lycopene *Z*-isomers, we recently prepared various isomerised tomato oleoresins each having a different lycopene isomer profile, *i.e.* containing mainly the 5*Z*-, the 13*Z*- or a mix of 9*Z*- and 13*Z*-isomers (Bortlik, Lambelet, Richelle, & Saucy, 2004). Tomato extract rich in 5*Z*-lycopene was prepared by iodine-catalysed photoisomerisation whilst tomato extracts rich in 13*Z*- and/or 9*Z*-lycopene were produced by heating a tomato extract in ethyl acetate. Lycopene contents and lycopene isomer profiles were quite stable when these tomato extracts were stored at low temperature (specifically −80 °C). However, at higher temperature, although total lycopene contents remained fairly stable, isomer profiles changed markedly. In fact, some lycopene *Z*-isomers underwent significant retro-isomerisation at room temperature, as already described for processed food (Bošković, 1979; Shi & Le Maguer, 2000; Shi, Maguer, & Bryan, 2002).

Aiming at developing a technological process that yields a shelf-stable tomato extract rich in *Z*-lycopene, we first investigated lycopene isomerisation either during its incubation in organic solvents or during heating of a tomato extract rich in lycopene. Based on these results, we then identified experimental conditions of isomerisation to obtain a tomato oleoresin specifically enriched in stable lycopene *Z*-isomers. These experimental conditions were applied to produce an isomerised tomato extract whose stability (both in terms of lycopene isomer profile and total lycopene content) was determined over a 1-year's storage test at room temperature.

2. Materials and methods

2.1. Materials

Two sources of lycopene, *i.e.* pure lycopene and lycopene contained in a tomato matrix were used for geometrical isomerisation trials and stability tests. Pure lycopene was obtained from tomato pomace by extraction with boiling ethanol using a two-step process (Bortlik, Mortezavi, & Saucy, 1999). Lycopene was re-crystallised from a methanol–dichloromethane mixture just before use. All-*E*-lycopene represented more than 95% of total lycopene contained in the product.

Lycopene-rich tomato extract was prepared according to a process already described (Giori, 2006). Briefly, crushed fresh tomatoes were heat concentrated to remove most of the water, then extracted with ethyl acetate. The organic solution was washed with water and concentrated to dryness under reduced pressure to obtain a non-isomerised (raw) tomato extract. Its total lycopene content amounted to 8.8%, of which the all-*E*- and the (5*Z*)-isomers represented 96.6% and 3.4%, respectively. β-Carotene, phytoene and phytofluene were also present in the raw tomato extract, but in lower amounts.

Di-*t*-butyl-hydroxy-toluene (BHT) and *N*-ethyl di-isopropylamine were from Fluka AG. All solvents were HPLC grade and were used without further purification.

2.2. Methods

2.2.1. Preparation of isomerised tomato extracts

Isomerised extracts were prepared either from the raw tomato extract or from fresh tomatoes.

Isomerised extracts were produced by heating the raw tomato extract in ethyl acetate. Ten grams of raw tomato extract were suspended in 100 ml of ethyl acetate and the resulting suspension was refluxed under stirring for a maximum of 7 days. The suspension was protected from light, oxygen and humidity during this isomerisation reaction. After cooling, solvent was evaporated under reduced pressure and the residue dried at 50 °C under vacuum for 18 h to obtain the isomerised extract.

Alternatively, isomerised tomato extracts were obtained from fresh tomatoes by modifying as following the procedure for producing the tomato extract (Giori, 2006): chopped fresh tomatoes were homogenised and most of the water was distilled off. The tomato concentrate was extracted three times with water-saturated ethyl acetate, the tomato residue separated by centrifugation and the extracts pooled. The resulting solution was washed with water and concentrated under reduced pressure to obtain a suspension with 10% w/v dry residue. The suspension was refluxed (76 °C) under stirring for a maximum of 7 days, and then cooled to room temperature. Isomerised tomato extract was obtained by removing ethyl acetate under reduced pressure and gently heating the reaction mixture at 30 °C.

2.2.2. Isolation of pure lycopene isomers

Pure 5*Z*-, 9*Z*-, 13*Z*- and all-*E*-lycopene were isolated from isomerised tomato extract (prepared by refluxing raw tomato extract for 1 h in ethyl acetate), by collecting the fractions containing the corresponding peaks after HPLC separation. Experimental conditions were identical to those used for analytical determination of lycopene except that a combination of semi-preparative columns was used (10 mm internal diameter × 250 mm length) at a flow rate of 3 ml/min. Peaks were collected during two consecutive HPLC runs and the corresponding fractions were pooled.

2.2.3. Lycopene isomerisation tests

Isomerisation tests were performed in organic solvents containing either only lycopene or a tomato extract rich in lycopene.

Geometrical isomerisation of pure lycopene was followed during 33 days incubation of lycopene isomers in *n*-hexane, and during 55 days incubation of a solution (3 mg/l) of lycopene containing about 97% all-*E*- and 3% of 5*Z*-isomer either in dichloromethane, di-ethyl ether, *n*-hexane or *n*-hexane containing 50 ppm BHT. Incubations were achieved at room temperature and in the absence of light. Samples were taken at various time intervals and the lycopene isomer profile analysed by HPLC.

Lycopene geometrical isomerisation in the presence of tomato components was studied during refluxing of the raw tomato extract in ethyl acetate. The tomato extract was suspended in 10 volumes of ethyl acetate and the mixture was refluxed for a maximum of 168 h. Homogeneous samples were taken at various time intervals from the isomerisation reactor and diluted in hexane before analysis of isomer profile by HPLC. Alternatively, isomer profiles were assessed in dried samples. In that case liquid samples taken from the isomerisation reactor were dried by heating under vacuum at 50 °C for 18 h before analysis by HPLC.

2.2.4. Stability tests

Stability of lycopene isomer profiles in two isomerised tomato extracts with different isomer profiles was investigated during storage at room temperature in the absence of light, oxygen and moisture. For a 13*Z*- rich extract (obtained by refluxing tomato

extract for 1 h in ethyl acetate) lycopene isomer profile was checked over 17 days; for a 5*Z*-, 9*Z*- rich extract (prepared by refluxing tomato extract for 7 days in ethyl acetate) lycopene isomer profile was monitored during 1 year.

In addition, the stability of lycopene was determined in the 5*Z*-, 9*Z*- rich tomato extract by measuring its total lycopene content at regular time intervals during storage.

2.2.5. Lycopene analysis

Lycopene isomer profiles were determined by normal phase HPLC according to the method described by Schierle et al. (1997). Dry samples were obtained by evaporating the solvent under nitrogen. These samples were then dissolved in *n*-hexane containing 50 ppm BHT and spun at maximum speed in a Biofuge A Lab centrifuge (Kendro Laboratory Products AG, Zürich, CH). The resulting supernatants were immediately analysed by HPLC. The HPLC system used was a 1100 series Hewlett-Packard model equipped with an ultraviolet–visible photodiode array detector. Data were simultaneously acquired at 470 nm, 464 nm, 346 nm and 294 nm. Samples (10 µl) were separated using a combination of three Nucleosil 300-5 columns (4 mm internal diameter × 250 mm length, Macherey-Nagel AG, Oensingen, CH). Separation was achieved at room temperature under isocratic conditions with a mobile phase consisting of *n*-hexane with 0.15 % *N*-ethyl di-isopropylamine. Flow rate was 0.8 mL/min.

Main lycopene isomers, *i.e.* 5*Z*-, 9*Z*-, 13*Z*- and all-*E*-isomers (see Fig. 1 as example of a HPLC chromatogram) were identified according to the literature (Schierle et al., 1997). Minor compounds eluting between 14 and 23 min (Fig. 1) have previously been identified as lycopene isomers using LC–MS (identification based on same molecular weight as the all-*E*-lycopene) and LC–MS/MS (identification based on generation of same major fragments as the all-*E*-lycopene) analysis (data not shown). The peak areas of these un-identified *Z*-lycopene isomers were added and reported as lycopene x-*Z*- isomers.

Amounts of lycopene isomers were calculated based on surface areas of the HPLC peaks using specific extinction coefficients for the all-*E*-, 5*Z*-, 9*Z*- and 13*Z*-isomers (Hengartner, Bernhard, Meyer, Englert, & Glinz, 1992; Müller, Bernhard, Giger, Moine, & Hengartner, 1997). Other lycopene isomers were quantified using the same extinction coefficient as the all-*E*-lycopene. Therefore, the lycopene concentration in products containing *Z*-isomers is slightly underestimated since it is recognised that the extinction coefficients of most *Z*-isomers are lower than that of the all-*E*-isomer.

Total lycopene contents were obtained by summing all the peaks of the HPLC chromatogram corresponding to lycopene isomers (Fig. 1).

3. Results

3.1. Isomerisation of pure lycopene

Results of the geometrical isomerisation of lycopene isomers during incubation in *n*-hexane at room temperature in the absence of light are reported in Table 1. All isomers, included the all-*E*-isomer, underwent a geometrical isomerisation during incubation. The all-*E*-lycopene was gradually converted (around 40% conversion after 33 days) into a range of *Z*-lycopene isomers, *i.e.* mainly 13- and 5*Z*. The isomerisation pathway and extent of isomerisation were not the same for the 13*Z*-lycopene as for the other *Z*-isomers. Whilst the 13*Z*-isomer was mainly converted into the all-*E*-isomer, the 5*Z*- and 9*Z*-isomers were principally transformed into

Table 1
Geometrical isomerisation of lycopene isomers during incubation in *n*-hexane at room temperature

Incubation time (days)	Lycopene isomer contents (% of total lycopene)				
	All-*E*	5*Z*	9*Z*	13*Z*	x-*Z*
all-E-lycopene					
0	96.5	0.5	0.6	2.3	0.1
1	80.1	1.2	1.4	16.0	1.4
2	70.7	2.4	1.1	23.1	2.7
5	60.7	3.4	2.3	29.1	4.5
12	59.9	5.8	2.2	27.3	4.9
33	52.4	12.2	4.2	23.8	7.5
(5Z)-lycopene					
0	1.1	95.6	n.d.	n.d.	3.4
1	2.2	84.6	n.d.	n.d.	13.2
2	2.4	77.3	n.d.	n.d.	20.3
5	3.8	68.8	n.d.	n.d.	27.3
12	5.5	64.8	0.8	2.8	26.0
33	10.3	52.7	2.7	4.6	29.7
(9Z)-lycopene					
0	3.6	1.5	94.0	0.8	n.d.
1	4.8	1.7	87.9	3.2	2.4
2	5.0	1.3	84.4	4.4	4.9
5	4.6	1.3	79.7	7.3	7.1
12	5.9	2.0	67.2	11.6	13.3
33	8.2	3.5	56.8	14.9	16.5
(13Z)-lycopene					
0	1.4	n.d	n.d	98.0	0.6
1	30.4	n.d.	0.3	69.2	n.d
2	47.0	n.d.	n.d.	51.8	1.2
5	59.0	1.7	1.6	34.1	3.7
12	56.9	4.7	2.7	30.6	5.0
33	50.5	10.6	4.5	25.4	9.0

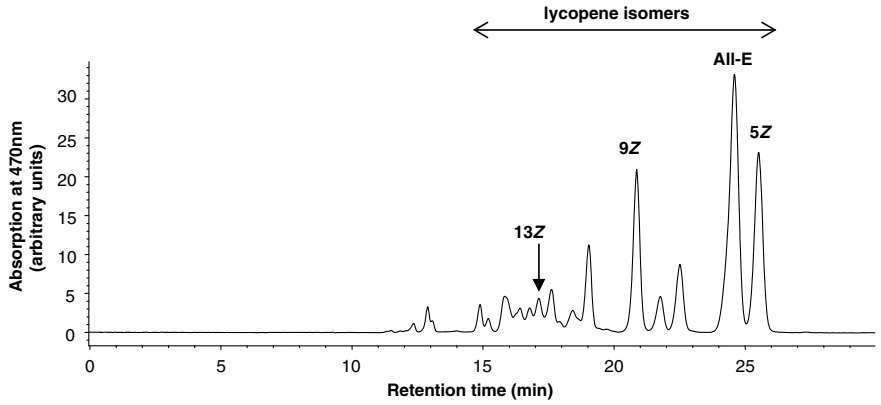

Fig. 1. HPLC chromatogram of an isomerised tomato oleoresin.

Table 2
Geometrical isomerisation of pure lycopene (mainly all-*E*-isomer) during incubation in organic solvents at room temperature

Incubation time (days)	Lycopene isomer contents (% of total lycopene)				
	All-*E*	5*Z*	9*Z*	13*Z*	x-*Z*
A. Dichloromethane					
0	95.0	2.2	n.d.	2.8	n.d.
1	79.8	3.9	0.5	14.8	1.0
6	58.6	9.4	2.1	26.5	2.3
27	35.5	22.1	9.6	16.0	16.9
55	23.1	23.4	12.9	12.1	28.4
B. Diethyl ether					
0	97.2	1.6	n.d.	1.2	n.d.
1	90.0	2.7	n.d.	6.6	0.7
6	72.8	3.2	n.d.	22.4	1.6
27	58.5	7.5	1.7	26.7	5.6
55	55.0	11.9	2.3	24.1	6.7
C. Hexane					
0	98.1	1.6	n.d.	0.3	n.d.
1	91.2	1.8	n.d.	6.4	0.5
6	71.7	2.8	n.d.	23.8	1.7
27	59.0	6.7	1.3	29.2	3.8
55	59.6	11.4	n.d.	26.1	2.9
D. Hexane/BHT					
0	98.4	1.6	n.d.	n.d.	n.d.
1	90.7	1.9	n.d.	6.8	0.5
6	71.5	3.1	n.d.	23.7	1.7
27	59.5	7.5	1.4	26.8	4.9
55	53.1	12.1	2.3	24.8	9.5

unknown *Z*-isomers during incubation in *n*-hexane. Also, whereas around 40% of 5*Z*- and 9*Z*-isomers were isomerised after 33 days of incubation, more than 80% of 13*Z*-lycopene were isomerised during this period of time.

Results of lycopene geometrical isomerisation of pure lycopene (sample containing >95% all *E*-isomer) during incubation in various organic solvents are reported in Table 2. Lycopene isomerisation from all-*E* to *Z*-isomers occurred in all tested solvents. In agreement with previously reported data (Qiu, Jiang, Wang, & Yuan, 2004) lycopene isomerisation was quicker in dichloromethane than in other solvents. All-*E*-Lycopene steadily decreased over time from a maximum of 98.5% (assuming 100% as the sum of the all-*E*-, 5*Z*-, 9*Z*- and 13*Z*-isomers) down to 25%, 66.9% and 61.5% after 55-day incubation at room temperature in dichloromethane, *n*-hexane and diethyl ether, respectively. Whereas amounts of 5*Z*- and 9*Z*-progressively increased, the quantity of 13*Z*-isomers passed through a maximum during the incubation. This maximum occurred after 6 days in dichloromethane and after 27 days in the other solvents. After having reached a maximum the content of 13*Z*-isomer steadily decreased during incubation. The addition of BHT to *n*-hexane had no effect on the stability of the lycopene isomer profile.

3.2. Isomerisation of lycopene in the presence of tomato extract

Results of lycopene isomerisation during heating of the raw tomato extract in ethyl acetate are reported in Table 3A. The 13*Z*-, 9*Z*- and 5*Z*-isomers were successively produced. Thus, a considerable amount of 13*Z*-isomer was formed at an early stage of the reaction, and then its proportion regularly decreased over time. Accordingly, a long reaction time will favour a low concentration of 13*Z*-isomer in the product. The fraction of the 9*Z*-isomer was pretty low at the beginning of the reaction but it rapidly increased within the first 48 h and then slightly diminished over the next 120 h. Only a tiny amount of the 5*Z*-isomer was observed at the beginning of the reaction but its proportion steadily increased as a function of time.

Table 3
Geometrical isomerisation of lycopene during refluxing of raw tomato extract in ethyl acetate

Heating time (h)	Lycopene isomer contents (% of tomato extract)			
	All-*E*	5*Z*	9*Z*	13*Z*
A. Isomer contents in solution				
0	8.5	0.3	n.d.	n.d.
0.5	4.9	0.2	0.3	2.6
1	4.6	0.2	0.4	2.6
2	4.3	0.2	0.6	2.6
4	4.0	0.2	0.9	2.4
8	3.5	0.3	1.2	2.2
24	3.0	0.4	1.7	1.7
48	2.8	0.5	1.7	1.6
72	2.1	0.8	1.4	1.2
96	2.3	0.7	1.5	1.4
168	1.7	1.1	1.1	1.0
B. Isomer contents in the dried extract				
24	4.2	0.5	1.6	0.7
72	3.2	0.7	1.4	0.6
168	2.9	1.3	1.1	0.2

Considering that, for a concrete application of the isomerisation process a drying step is necessary to isolate any isomerised material, three samples were taken during the heating process, *i.e.* after 24 h, 72 h and 168 h heating, and dried by heating at 50 °C under vacuum for 18 h. The resulting dry isomerised extracts were analysed and compared with the starting solutions. As can be seen in Table 3B the drying step had only a slight influence on 9*Z*- and 5*Z*-isomer content, whilst a strong retro-isomerisation occurred for the 13*Z*- with a concomitant large increase of the all-*E*-isomer. Accordingly, a big change in isomer profile occurred during isolation of products with a high content of the 13*Z*-isomer.

3.3. Stability of lycopene in isomerised tomato extracts

Stability of lycopene isomer profiles during storage of the two isomerised tomato extracts, *i.e.* extracts obtained by heating in ethyl acetate for 3 and 7 days are reported in Tables 4 and 5, respectively. As already mentioned, the lower the content of 13*Z*-isomer, the more stable was the isomer profile. Thus, lycopene isomer profile in the tomato extract with a high 13-isomer content was fairly unstable at room temperature: after only 17 days of

Table 4
Room temperature stability of lycopene isomer profile in dried isomerised tomato extract prepared by refluxing raw tomato extract for 3 in ethyl acetate

Storage time (days)	Lycopene isomer contents (% of tomato extract)			
	All-*E*	5*Z*	9*Z*	13*Z*
0	3.2	1.4	1.4	0.6
10	3.6	1.4	1.4	0.4
17	3.5	1.3	1.3	0.2

Table 5
Room temperature stability of lycopene isomer profile in a dried isomerised tomato extract prepared by 7 day refluxing of the ethyl acetate suspension obtained from fresh tomatoes

Storage time (months)	Lycopene isomer contents (% of tomato extract)			
	All-*E*	5*Z*	9*Z*	13*Z*
0	3.2	2.3	1.3	0.2
3	3.5	2.7	1.2	0.1
6	3.3	2.4	1.0	0.1
9	3.2	2.2	1.2	0.1
12	3.2	2.4	1.3	0.1

Table 6
Room temperature stability of total lycopene in a dried isomerised tomato extract prepared by 7 day refluxing of the ethyl acetate suspension obtained from fresh tomatoes

Storage time (months)	Total lycopene (% of tomato extract)
0	8.9
3	9.6
6	9.4
9	9.2
12	9.5

storage at room temperature, the 13*Z*-isomer content was reduced to less than one third of its initial value due to retro-isomerisation into the all-*E*-form (Table 4).

Conversely, if the amount of the 13*Z*-isomer was low, the isomer profile was stable for at least 12 months at room temperature (Table 5). Additionally, the total lycopene content in the low 13*Z*-isomer containing isomerised tomato extract was stable during 1-year storage at room temperature (Table 6).

4. Discussion

It can be inferred from isomerisation results obtained with pure isomers that lycopene based products are very dynamic systems where lycopene isomers can be transformed into other isomers. For example the all-*E*-isomer is transformed into *Z*-isomers, mainly 13*Z* and 5*Z*, but is also formed by retro-isomerisation from *Z*-isomers, principally from 13*Z* (Table 1). These results also show that the 13*Z*-lycopene was much less stable than either the 5*Z*- or the 9*Z*-, or the all-*E*-isomer, which is in agreement with *ab initio* calculations demonstrating that the stability of lycopene isomers decreases in the order: 5*Z* > all-*E* > 9*Z* > 13*Z* >15*Z* > 7*Z* > 11*Z* (Chasse et al, 2001). In addition, the 5*Z*-, all-*E*-, 9*Z*- and 13*Z*-isomers have relative energies within 1 kcal/mol whereas the other isomers have relatively higher energies (Chasse et al., 2001). Accordingly, the equilibrium state will be characterised by substantial amounts of 5*Z*- and all-*E*-isomers, lower amounts of 9*Z*- and minute quantities of the 13*Z*- and the other lycopene isomers. Such an equilibrium mixture was obtained following photoisomerisation of lycopene in CH_2Cl_2 in the presence of iodine as catalyst (Bortlik et al., 2004), a standard procedure known for reaching thermodynamic equilibrium (Zechmeister, 1962).

Since lycopene is biosynthesised in plants mainly as the all-*E*-isomer, it is mainly (above 90%) present in this form in natural tomato products. Although being much different from that of the equilibrium state, the lycopene isomer profile encountered in natural tomato extracts (~95% all-*E*- and ~5% 5*Z*-isomers) is stable. Such an extract can, thus, be stored at room temperature for a long period of time without lycopene isomerisation taking place. This stability comes from the lycopene being predominantly (>99%) in the crystalline form, *i.e.* in a form that cannot participate in isomerisation reactions leading to the equilibrium state. Modification of lycopene isomer profile in a tomato extract requires solubilisation of lycopene. After solubilisation lycopene will isomerise so that the isomer profile will evolve towards that of the equilibrium state. Kinetics were faster in CH_2Cl_2 than in other organic solvents (Table 2) probably because of the presence of acid impurities often found in this type of solvent (Jeevarajan, Wei, & Kispert, 1994). Starting from the all-*E*-form isomerisation is characterised first by the formation and then by the disappearance of the unstable 13*Z*-isomer (Tables 1 and 2). Accordingly, for enhanced stability an isomerised tomato product should contain as little as possible of the 13*Z*-isomer.

Although the all-*E*- and various *Z*-isomers are very close on the free-energy scale isomerisation requires appreciable energy of activation (Chasse et al., 2001). This activation energy is lowered in the presence of a catalyst allowing equilibrium to be reached easily. For example an equilibrium mixture could quickly be obtained by photo isomerisation of tomato oleoresin dissolved in CH_2Cl_2 in the presence of iodine as catalyst (Bortlik et al., 2004). However, if such a process can easily be applied on a lab-scale basis, it is not realistic from an industrial point of view because iodine is quite difficult to remove from the product afterwards.

An isomerised tomato extract with a stable lycopene isomer profile, *i.e.* with a tiny amount of the 13*Z*-isomer, can also be prepared by reaction in organic solvents. Actually, results of reactions of lycopene in various organic solvents at room temperature (Table 2) show that whilst the 13*Z*-isomer is formed predominantly at the beginning of the reaction, its level decreases as the reaction proceeds. The same trend was found during refluxing tomato extract in ethyl acetate for 7 days (Table 3A). For tomato extracts obtained by heating in ethyl acetate for a maximum of 3 days, the drying process had a substantial effect on isomers distribution (Table 3B). In these products the 13*Z*-isomer content was significantly lower in the dried product than in the corresponding solutions. Conversely, in the product heated for 7 days in ethylacetate, the 13*Z*-isomer content was almost 15 times lower than the sum of the more stable 9*Z*- and 5*Z*-isomers. In this product the lycopene isomer profile did not change during drying (Table 4).

From an industrial perspective isomerising lycopene in tomato oleoresin by heating in ethyl acetate is much more convenient. In fact isomerisation rather than degradation of lycopene occurred during heating in ethyl acetate (Calvo, Dado, & Santa-Maria, 2007). This way of isomerisation has the further advantage that ethyl acetate is already used by food ingredient manufacturers for the preparation of tomato extracts. For that reason, extraction and geometrical isomerisation can be achieved in a single step. Besides, when stereoisomeric equilibrium has been reached, removing of the solvent down to the level requested for human consumption can easily be achieved by distillation and drying under reduced pressure without causing retro-isomerisation.

Because appreciable activation energy is required for lycopene isomerisation (Chasse et al., 2001) stereoisomeric equilibrium can only be reached very slowly with non-catalysed reactions. Accordingly, lycopene isomerisation in refluxing ethyl acetate is slow. To prepare an isomerised product with a low level of 13*Z*-isomer from a natural tomato product containing more than 90% all-*E*-isomer would require a long reaction time, at least a week.

Incorporating a long period of heating in ethyl acetate during the preparation of isomerised tomato extracts allows us to obtain a potentially active ingredient with good lycopene stability at room temperature both in terms of total lycopene content and lycopene isomer profile (Tables 5 and 6), which is a mandatory parameter for its further use in food products.

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