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Effect of high hydrostatic pressure on lycopene stability

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Abstract

The stability and isomerization of lycopene by high hydrostatic pressure (HHP) were evaluated. Lycopene standard in hexane and tomato puree were pressurized at 100, 200, 300, 400, 500 and 600 MPa for 12 min at controlled temperature (20 ± 1 °C). After application of pressure, samples were stored at refrigerator temperature (4 ± 1 °C) and ambient laboratory temperature (24 ± 1 °C) under lightproof conditions. HPLC and spectral analysis were employed to analyze lycopene and its *cis*-isomers in samples after HHP and after 2, 4, 8 and 16 days of storage. High pressure affected the content of total lycopene and the percentage of the presumptive 13-*cis* isomer, both in lycopene solution and tomato puree. Furthermore, the higher the storage temperature, the greater was the loss of total lycopene and the higher the percentage of 13-*cis* isomer. However, the pressure effects were widely different in lycopene solution and tomato puree. 500 and 600 MPa led to a significant ($P < 0.05$) loss of lycopene while 400 MPa retained the maximal stability of lycopene in solution. The highest stability of lycopene was found when tomato puree was pressurized at 500 MPa and stored at 4 ± 1 °C.

Keywords: Lycopene; High hydrostatic pressure (HHP); Stability; Isomerization

1. Introduction

Lycopene is the predominant carotenoid found in tomatoes and responsible for the redness of ripe tomato fruits and tomato products. It is also one of the major carotenoids present in human serum and organs (Stahl & Sie, 1996). Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with decreased risk of chronic disease such as cancer and cardiovascular disease in numerous studies (Clinton, 1998). Therefore, the content and stability of lycopene in food has taken on added importance.

Lycopene is a C_{40} polyisoprenoid compound. It has 11 conjugated carbon–carbon double bonds and can theoretically assume 2048 geometrical configurations. However, because of steric hindrance, only 72 lycopene isomers are structurally favourable (Zechmeister, 1962). Studies show that all-*trans* lycopene and its *cis*-isomers, such as 5-*cis*, 9-*cis*, 13-*cis*, 15-*cis* lycopene, are present in tomatoes and human blood plasma (Schierle, Bretzel, & Buhler, 1997). The chemistry and bioavailability of all-*trans* and *cis*-isomers of lycopene are different. Many researchers suggest that *cis*-isomers of lycopene are better absorbed than the all-*trans* form because of the shorter length of the *cis*-isomers, the greater solubility of *cis*-isomers in mixed micelles, and/or as a result of the low tendency of *cis*-isomers to aggregate. *Cis*-isomers of lycopene make up 50% of the total lycopene found in human serum and tissues (Clinton, Emenhiser, & Schwartz, 1996; Stahl & Sies, 1992) yet, in tomatoes and tomato-based food products, all-*trans* lycopene comprises 79–91% of total lycopene (Clinton et al., 1996).

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High performance liquid chromatography (HPLC) is the conventional separation method of choice for lycopene. To separate lycopene isomers, reversed-phase C_{30} stationary phase is often employed to achieve superior selectivity of lycopene isomers compared to conventional C_{18} reversed-phase and silica normal-phase columns (Emenhiser, Simunvi, & Sander, 1996; Sander, Sharpless, Craft, & Wise, 1994). C_{30} columns not only provide excellent separation of the all-*trans* lycopene from its *cis*-isomers, they also exhibit remarkable selectivity among individual *cis*-isomers themselves (Emenhiser et al., 1996).

High hydrostatic pressure treatment (HHP, 100–1000 MPa) is used as an alternative preservation method to heat treatment (Knorr, 1993). High pressure processing may provide distinct product quality merits over conventional heat processing. It is claimed that this process is clean and energy – efficient compared with many conventional processes. The effect of high pressure on lycopene stability has not been studied. As the isomeric forms of dietary lycopene may possess different biological properties, an understanding of the various factors affecting the formation of geometrical isomers is also important. The aim of this study is to investigate the changes of total lycopene and its *cis* isomers in both lycopene standard solution and tomato puree after the application of different levels of high pressure.

2. Materials and methods

2.1. Chemicals and reagents

Lycopene standard was provided by Hong Sheng Tang Healthy Products Company, Ltd. All solvents used were of HPLC grade and purchased from Tedia Company, INC, USA.

Dark red tomatoes were bought from local supermarket.

2.2. Equipment

NIR: Thermo Nicolet Avatar 360 FTIR ESP Thermo Spetra-Tech; HPLC pump model: Waters 515; Double wavelength detector: Waters 2487; Column: YMC™ Carotenoid S-5 4.6 × 250 mm, Lot No 055913121E 02, Waters; UV-2401PC UV–VIS Recording Spectrophotometer: *Shimadzu.* Balance: *Shimadzu* L-200 SM.

2.3. Preparation and pressurization of samples

Just before HHP, lycopene standard was dissolved in hexane at a concentration of 50 μg/ml and transferred into 10 ml plastic tubes that were then completely filled and sealed. The tubes were packed in polyethylene bags which were sealed under vacuum.

Whole washed tomatoes were chopped into pieces and mixed for 150 s in a laboratory blender (CDEL-23D, multi-functional food blender, Shanghai). Then the puree was homogenized in a homogenier (SHP-60 MPa High pressure homogenier, Shanghai University of Science and Technology) at 14 MPa. The purees (each about 30 g) were-vacuum packed in polyethylene bags (100 × 120 mm).

Samples were pressurized in a laboratory hydraulic reactor, thermostatted at 20 ± 1 °C which was controlled by an external thermostat. Isostatic pressures of 100, 200, 300, 400, 500 and 600 MPa were held for 12 min. In order to minimize adiabatic heating, the rate of pressure increase was approximately 100 MPa per minute, releasing time was just a few seconds. Pressure treatment time did not include pressure build-up and releasing time. After treatment, lycopene solution was transferred into dark volumetric vessel and stored at −28 °C and tomato puree bags were kept at 4 ± 1 °C for further use within 24 h. Then all the samples were stored at either 4 ± 1 °C or 24 ± 1 °C (ambient laboratory temperature). HPLC measurements were carried out immediately after HHP and after 2, 4, 8 and 16 days of storage, as described below.

2.4. Analysis of lycopene and its cis-isomers

2.4.1. Iodine isomerization

For determining the nature of the geometric isomers, stereomutation was induced by iodine-catalysed photoisomerization. Just before HPLC analysis, lycopene standard was dissolved in hexane to which iodine was added at 1.0% of the mass; the solution was then exposed to ambient laboratory light for 20 min.

2.4.2. Extraction and separation of lycopene from tomato puree

Triplicates of each tomato puree sample (5 g) were extracted with 120 ml mixture (hexane:acetone:ethanol = 2:1:1) until the extracts were colourless. 10 ml distilled water were applied to wash the solution. The supernatant was obtained for HPLC analysis.

2.4.3. HPLC

All-*trans* lycopene and its isomers were analyzed by reversed-phase HPLC, using a C_{30} column. The mobile phase consisted of methanol, methyl-*tert*-butyl ether and ethyl acetate (50:40:10) at a flow rate of 1.5 ml/min. Injection volume was 10 μl. Column temperature was ambient laboratory temperature (24 ± 1 °C). All steps were performed under diminished light.

2.5. Statistical analysis

Triplicate analysis was conducted for each HHP. All the data were subjected to analysis of variance and the

Student's *t*-test (Excel 2000). The differences were considered significant when $P < 0.05$.

3. Results and discussion

3.1. UV–vis absorption spectrum of test lycopene standard

Fig. 1 shows the UV–vis absorption spectrum of test lycopene standard in hexane which was similar to the pure lycopene standard sample (90–95% purity, L9879, Sigma) and contained three characteristic bands for lycopene (λ = 443.6, 470.4, 502.1 nm).

3.2. NIR spectrum of test lycopene standard

Fig. 2 shows the near infrared spectrum of the test lycopene standard by KBr press method. The diagram suggested it had the typical conjugated polyethylene structure. 3432 cm^{-1} was the absorption peak of moisture when lycopene was pressed with KBr. 3035 cm^{-1} was the stretching vibration peak of =C–H. 2970 cm^{-1} was the stretching vibration peak of non-symmetric methyl. 2852 cm^{-1} was the stretching vibration peak of symmetric methylene. 1654 cm^{-1} was the stretching vibration peak of C=C. 959 cm^{-1} was the swing vibration peak of $R_1HC{=}CR_2H$ (*trans*). There was no absorption peak within 730–665 cm^{-1} which was the absorption band of $R_1HC{=}CR_2H$ (*cis*). This indicated there were no *cis* isomers in the sample. All-*trans* form was the main type of lycopene in the test lycopene standard.

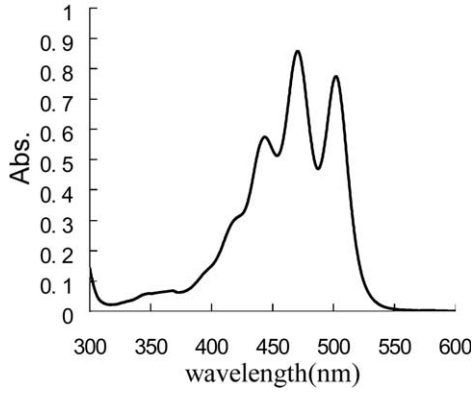

Fig. 1. UV–vis absorption spectrum of lycopene.

3.3. Identification of all-trans and cis-isomers of lycopene

Fig. 3 shows the eluting compositions of lycopene after stereomutation. Certain chromatograhic peaks were tentatively identified by comparison with previous separation on polymeric C_{30} columns (Emenhiser et al., 1996; Ferruzzi, Nguyen, & Sander, 2001; Lee & Chen, 2001; Re, Fraser, & Long, 2001; Schierle et al., 1997) and UV–vis absorption spectra. Therefore, peak 9 (RT 23.5 min) was all-*trans* lycopene, peak 10 (RT 23.4 min) was presumably 5-*cis* lycopene, peak 5 (RT 9.2 min) was presumably 13-*cis* lycopene; peaks 1, 2, 3, 4, 6, 7, 8 were all other *cis*-isomers of lycopene.

3.4. Effect of HHP on the stability and isomerization of lycopene standard

Food processing has been postulated to cause lycopene degradation. Processing conditions such as high temperature, time, light and oxygen have been shown to have effects on lycopene isomerization and oxidation

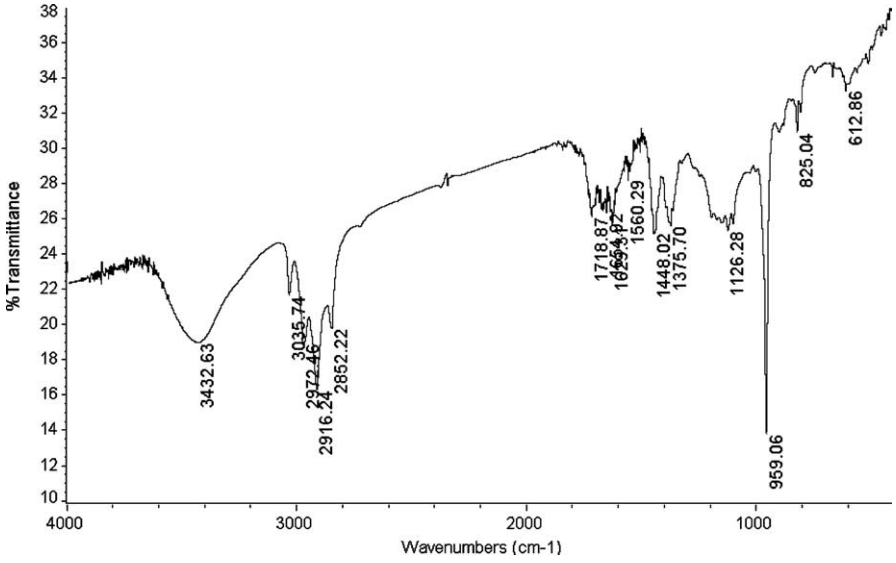

Fig. 2. NIR spectrum of lycopene.

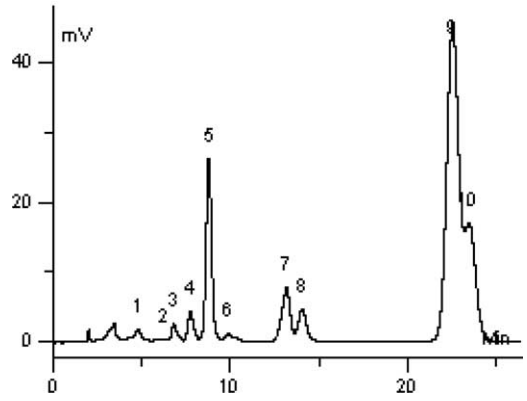

Fig. 3. Isocratic separation of iodine isomerized lycopene by HPLC.

which lead to the degradation of lycopene. As a highly conjugated polyene, lycopene is susceptible to degradation. According to our investigation, during lycopene pressurization and storage, several isomers of lycopene formed, in which the presumptive 13-*cis* isomer changed distinctively. Concentrations of other *cis*-isomers were very low or they changed minutely. It has been reported that the isomerization of β-carotene at the central double bond was lower than in the other position (Zechmeister, 1944). This phenomenon could be applied to lycopene and a large percentage increase was thus found for 13-*cis* isomer, followed by other isomers (Lee & Chen, 2002). That was why this isomer was chosen as an index to show diversification of lycopene isomers. Results reveal high pressures of varied magnitudes had different impacts on the stability of lycopene (Table 1). 500 and 600 MPa for 12 min at 20 ± 1 °C caused statistically significant losses of total lycopene, which were 20.8 ± 1.12% and 56.3 ± 3.02%, respectively. However, 100, 200, 300 and 400 MPa showed no variation compared with the untreated sample. Simultaneously, pressure caused the increase of 13-*cis* isomer (Table 2). The effects were highly pressure-dependent. With the increase of applied pressure, 13-*cis* isomer increased gradually. 500 and 600 MPa brought about a prominent increase of 13-*cis* isomer. It could accordingly be assumed that the decrease of total lycopene was due to isomerization. Isomerization converts all-*trans* isomers to *cis*-isomers, due to additional energy input, and results in an unstable, energy-rich situation (Shi & Le Maguer, 2000). Some all-*trans* lycopene might transform to *cis*-isomers during high pressure processing.

In refrigerated temperature (4 ± 1 °C) storage, with the extending of storage time, the losses of total lycopene increased gradually (Table 1). After 16 days of storage, the loss of total lycopene in untreated sample was significant, varying from 2.10 ± 0.02% to 7.89 ± 0.44%, while samples treated by different HHP lost a small portion. The reduction of total lycopene exhibited by 400 MPa after storage was insignificant. In the meantime, 13-*cis* isomers also increased with the length of storage time (Table 2). After 16 days of storage, the percentage of 13-*cis* isomer in untreated sample changed significantly from 0.55 ± 0.02% to 4.37 ± 0.19%; however, 13-*cis* isomer, in samples treated by HHP, changed a little.

In ambient laboratory temperature (24 ± 1 °C) storage, as storage time increased, the losses of total lycopene increased differently (Table 3). Untreated samples lost significantly more lycopene than samples treated by HHP. After 16 days of storage, untreated sample lost 15.2 ± 0.73% while samples treated by HHP lost less. 100, 200 and 300 MPa lost 9.29 ± 0.31%, 9.42 ± 0.49% and 8.09 ± 0.51%, respectively. However, 400 MPa treatment showed significant difference compared with these three treatments. The total lycopene losses changed to 4.98 ± 0.34%. Meanwhile, with time, the percentage of 13-*cis* isomer in untreated sample increased markedly more than those in samples treated by HHP. After 16 days of storage, the increase of the percentage of 13-*cis* isomer in untreated sample changed significantly, from 0.55 ± 0.02% to 10.8 ± 0.19%. The increase of the percentage of 13-*cis* isomer in samples treated by 100, 200 and 300 MPa was less while, in the sample treated by 400 MPa, it changed from 3.01 ± 0.02% to 4.48 ± 0.28%. 400 MPa was the pressure that could exert a stable effect on lycopene in lycopene standard solution (See Table 4).

3.5. Effect of HHP on the stability and isomerization of lycopene in tomato puree

According to our examination, the concentration of total lycopene of tomato puree was approximately 5.16 ± 0.12 mg/100 g in which all-*trans* lycopene was

Table 1
The percentage of losses of total lycopene in lycopene standard as a function of storage time at 4 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	2.10 ± 0.02	2.10 ± 0.02	2.11 ± 0.02	2.11 ± 0.02	2.13 ± 0.02	20.8 ± 1.12	56.3 ± 3.02
2	3.05 ± 0.23	2.10 ± 0.02	2.11 ± 0.02	2.11 ± 0.02	2.13 ± 0.02	20.8 ± 1.12	56.3 ± 3.02
4	5.22 ± 0.34	2.40 ± 0.05	2.52 ± 0.09	2.34 ± 0.07	2.29 ± 0.09	21.7 ± 1.19	57.4 ± 3.34
8	6.13 ± 0.40	2.49 ± 0.07	2.63 ± 0.09	2.45 ± 0.09	2.39 ± 0.11	22.7 ± 1.21	57.4 ± 3.45
16	7.89 ± 0.44	4.21 ± 0.23	3.29 ± 0.28	3.78 ± 0.22	2.70 ± 0.28	25.7 ± 1.41	60.4 ± 3.76

Values reported are means of triplicate determinations ($n = 3$) ± SD.

Table 2
The percentage of 13-*cis* isomer of lycopene in lycopene standard as a function of storage time at 4 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	0.55 ± 0.02	0.77 ± 0.01	1.31 ± 0.03	1.74 ± 0.04	3.01 ± 0.08	6.99 ± 0.31	7.01 ± 0.34
2	2.0 ± 0.02	0.77 ± 0.01	1.33 ± 0.03	1.74 ± 0.04	3.01 ± 0.08	6.99 ± 0.31	7.01 ± 0.30
4	3.5 ± 0.06	0.79 ± 0.01	1.38 ± 0.04	1.84 ± 0.05	3.22 ± 0.09	7.09 ± 0.32	7.11 ± 0.23
8	3.69 ± 0.08	1.07 ± 0.06	1.51 ± 0.08	1.99 ± 0.04	3.57 ± 0.10	7.21 ± 0.31	7.33 ± 0.24
16	4.37 ± 0.19	1.88 ± 0.08	2.31 ± 0.11	2.45 ± 0.11	3.71 ± 0.11	7.79 ± 0.32	7.87 ± 0.35

Values reported are means of triplicate determinations ($n = 3$) ± SD.

Table 3
The percentage of losses of total lycopene in lycopene standard as a function of storage time at 24 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	2.20 ± 0.02	20.8 ± 1.12	56.3 ± 3.02
2	6.00 ± 0.50	2.90 ± 0.04	2.91 ± 0.05	2.89 ± 0.03	2.97 ± 0.02	20.9 ± 1.11	56.9 ± 3.12
4	8.00 ± 0.54	3.20 ± 0.05	3.51 ± 0.11	3.66 ± 0.10	3.38 ± 0.11	21.9 ± 1.21	57.9 ± 3.34
8	10.9 ± 0.44	4.21 ± 0.11	4.51 ± 0.21	4.66 ± 0.31	4.26 ± 0.22	23.2 ± 1.33	59.9 ± 3.52
16	15.2 ± 0.73	9.29 ± 0.31	9.42 ± 0.49	8.09 ± 0.51	4.98 ± 0.34	26.9 ± 1.43	62.9 ± 3.62

Values reported are means of triplicate determinations ($n = 3$) ± SD.

Table 4
The percentage of 13-*cis* isomer of lycopene in lycopene standard as a function of storage time at 24 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	0.55 ± 0.02	0.77 ± 0.01	1.31 ± 0.03	1.74 ± 0.04	3.01 ± 0.08	6.99 ± 0.31	7.01 ± 0.34
2	2.4 ± 0.02	0.87 ± 0.03	1.43 ± 0.07	1.94 ± 0.10	3.21 ± 0.09	7.09 ± 0.33	7.06 ± 0.31
4	4.21 ± 0.02	1.27 ± 0.05	1.55 ± 0.09	1.99 ± 0.18	3.29 ± 0.11	7.45 ± 0.41	7.26 ± 0.41
8	7.23 ± 0.02	1.99 ± 0.11	1.99 ± 0.14	2.29 ± 0.22	4.09 ± 0.19	7.55 ± 0.46	7.39 ± 0.49
16	10.8 ± 0.19	3.39 ± 0.20	3.55 ± 0.35	4.08 ± 0.29	4.48 ± 0.28	8.79 ± 0.79	8.89 ± 0.66

Values reported are means of triplicate determinations ($n = 3$) ± SD.

the predominant carotenoid, accounting for 89.5%, followed by other *cis*-isomers (7.7%), and 13-*cis* isomer (2.8%). This is in agreement with the reported 35–96% of all-*trans* lycopene and <1–7% 13-*cis* isomer in tomato and other tomato products (Schierle et al., 1997). The average lycopene content in raw samples was approximately 4.7 mg/100 g, which was reported as an average for tomatoes (Shi & Le Maguer, 2000). Results show that the contents of total lycopene in tomato puree increased slightly under all processing conditons, whereas there were no significant differences between samples treated by 100, 200, 300, 400, 600 MPa and untreated (Table 5). 500 MPa was found to be the particular HHP that led to the most of total lycopene content in tomato puree (6.25 ± 0.23 mg/100 g). It is unclear why this effect occurred only at this particular HHP. The phenomenon might be attributed to the fact that high pressure can rupture the tissue of tomato puree and thereafter release more lycopene. Furthermore, lycopene is relatively easier extracted from tomato. It has been reported that tomato tissues became softer as the pressure increases to 400 MPa (Tangwongchai, Leward, & Ames, 2000). Results agree with the study of De Ancos, Gonzalez, and Cano (2000), who found an increase in extractable carotenoids due to high pressure treatment of fruit purees. As lycopene is naturally present in the *trans* form in food products, the formation of *cis* forms of lycopene is probably due to processing or storage (Nguyen & Schwartz, 1998). Based on our experiment, though the *cis*-isomer profile of lycopene in tomato puree was different from those in lycopene standard, the presumtive 13-*cis* isomer was also the type of isomer most susceptible to change. Nevertheless, no significant change of the percentage of 13-*cis* isomer of lycopene in tomato puree was found between HHP samples and untreated sample (Table 6).

At the storage temperature of 4 ± 1 °C, the degradation of total lycopene in tomato puree (whether treated or untreated) was not pronounced within the initial 8 days of storage (Table 5), probably because of the mild storage condition. However, after 16 days of storage, total lycopene content in untreated sample declined more

Table 5
Content of total lycopene in tomato puree as a function of storage time at 4 ± 1 °C, at each HHP condition (mg/100 g)

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	5.16 ± 0.12	5.33 ± 0.13	5.39 ± 0.11	5.48 ± 0.12	5.55 ± 0.12	6.25 ± 0.23	5.10 ± 0.10
2	5.18 ± 0.13	5.39 ± 0.12	5.42 ± 0.12	5.50 ± 0.13	5.50 ± 0.13	6.20 ± 0.21	5.11 ± 0.11
4	5.18 ± 0.13	5.37 ± 0.12	5.43 ± 0.12	5.51 ± 0.13	5.50 ± 0.13	6.21 ± 0.20	5.10 ± 0.12
8	5.17 ± 0.13	5.37 ± 0.13	5.40 ± 0.15	5.51 ± 0.13	5.48 ± 0.14	6.19 ± 0.22	5.08 ± 0.10
16	4.37 ± 0.10	5.17 ± 0.12	5.22 ± 0.16	5.26 ± 0.12	5.18 ± 0.13	6.11 ± 0.23	4.88 ± 0.12

Values reported are means of triplicate determinations ($n = 3$) ± SD.

Table 6
The percentage of 13-*cis* isomer of lycopene in tomato puree as a function of storage time at 4 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	2.80 ± 0.04	2.82 ± 0.04	2.87 ± 0.04	2.89 ± 0.04	2.79 ± 0.04	2.92 ± 0.04	2.88 ± 0.04
2	2.87 ± 0.04	2.91 ± 0.05	2.99 ± 0.03	2.98 ± 0.06	2.89 ± 0.05	2.99 ± 0.04	2.99 ± 0.04
4	3.09 ± 0.05	2.95 ± 0.05	3.06 ± 0.09	3.08 ± 0.07	2.94 ± 0.06	3.08 ± 0.06	3.09 ± 0.08
8	3.89 ± 0.04	2.99 ± 0.04	3.19 ± 0.04	3.11 ± 0.07	2.99 ± 0.05	3.12 ± 0.05	3.14 ± 0.08
16	4.34 ± 0.04	3.52 ± 0.05	3.48 ± 0.06	3.54 ± 0.07	3.59 ± 0.05	3.22 ± 0.06	3.43 ± 0.04

Values reported are means of triplicate determinations ($n = 3$) ± SD.

(15.3% loss) than that in treated (about 3.0% loss) samples. 500 MPa kept the total lycopene content maximum (6.11 ± 0.23 mg/100 g). In the meantime, after a storage of 8 days, the percentage of 13-*cis* isomer in treated samples did not change too much while that in untreated sample changed (38.9% increase) (Table 6). After 16 days of storage, the percentage of 13-*cis* isomer in samples treated by 100, 200, 300, 400 and 600 MPa began to increase slightly while that in untreated sample changed considerably (55.0% increase); the percentage of 13-*cis* isomer in sample treated by 500 MPa continued to remain relatively stable.

At the storage temperature of 24 ± 1 °C, for up to 8 days, contents of total lycopene in untreated sample changed (14.5% loss) more than those treated by 100, 200, 300, 400 and 600 MPa (4.3–6.75% loss) (Table 7). 500 MPa retained the total lycopene content (only 2.72% loss) substantially. Comparatively, after 16 days of storage, the content of lycopene in untreated samples changed (33.1% loss) significantly more than those treated by 100, 200, 300, 400 and 600 MPa (8.4–14.5% loss). Though the loss of total lycopene in sample treated by 500 MPa was 8.0%, its absolute content was still the highest. Meanwhile, with increase of storage time, the percentage of 13-*cis* isomer in HHP samples and untreated sample also showed a different trend (Table 8). Eight days of storage saw significant differences. After 16 days of storage, the percentage of 13-*cis* isomer in untreated sample changed (88.5% increase) more than those treated by 100, 200, 300, 400 and 600 MPa (42.4–77.0% increase). The increase of the percentage of 13-*cis* isomer in the sample treated by 500 MPa continued to be the smallest (15.4%).

According to the results of this study, in view of lycopene stability, application of HHP on pure lycopene in hexane and lycopene in tomato puree were obviously different. 500 and 600 MPa caused a great loss of total lycopene in lycopene solution. However, 500 MPa led to increase of total lycopene in tomato puree. Reasons for this are unclear. HHP might improve lycopene content by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, thus making lycopene more accessible. The presence of some macromolecules in tomato puree may offer protection for lycopene. High pressure may also lead to a release of other antioxidants found in tomato (other carotenoids, ascorbic acid, phenolic compononents). These active components could have a good retention and thus protect lycopene from degradation. The fact that no significant change of the percentage of 13-*cis* isomer of lycopene in tomato puree was found after HHP treatment might explain this. It has been reported that, in tomato products, lycopene was relatively resistant to degradation, including thermally induced *trans–cis* isomerization reactions (Nguyen & Schwartz, 1998).

Significant differences were found between samples (whether treated or untreated) stored at 4 ± 1 °C and 24 ± 1 °C. The lower the storage temperature, the less the loss of total lycopene. 13-*cis* isomer in samples kept at 4 ± 1 °C changed more tardily than those stored at 24 ± 1 °C. 13-*cis* isomer is the product of lycopene isomerization. Therefore, temperature was one of the factors that affected isomerization rate. This is consistent with the study of Lee and Chen (2002). According to our investigation, untreated tomato puree lost its flavour

Table 7
Content of total lycopene in tomato puree as a function of storage time at 24 ± 1 °C, at each HHP condition (mg/100 g)

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	5.16 ± 0.12	5.33 ± 0.13	5.39 ± 0.11	5.48 ± 0.12	5.55 ± 0.12	6.25 ± 0.23	5.10 ± 0.10
2	5.18 ± 0.13	5.39 ± 0.12	5.42 ± 0.12	5.50 ± 0.13	5.50 ± 0.13	6.20 ± 0.21	5.11 ± 0.11
4	5.01 ± 0.13	5.10 ± 0.13	5.22 ± 0.11	5.35 ± 0.15	5.48 ± 0.13	6.06 ± 0.22	5.02 ± 0.10
8	4.39 ± 0.11	5.07 ± 0.11	5.08 ± 0.11	5.11 ± 0.12	5.28 ± 0.14	6.08 ± 0.22	4.88 ± 0.12
16	3.45 ± 0.09	4.88 ± 0.10	4.70 ± 0.12	4.68 ± 0.14	4.99 ± 0.24	5.75 ± 0.23	4.68 ± 0.14

Values reported are means of triplicate determinations ($n = 3$) ± SD.

Table 8
The percentage of 13-*cis* isomer of lycopene in tomato puree as a function of storage time at 24 ± 1 °C, at each HHP condition

Storage time (days)	Untreated (0 MPa)	Pressure applied (MPa)					
		100	200	300	400	500	600
0	2.80 ± 0.04	2.82 ± 0.04	2.87 ± 0.04	2.89 ± 0.04	2.79 ± 0.04	2.92 ± 0.04	2.88 ± 0.04
2	2.92 ± 0.04	2.98 ± 0.05	3.12 ± 0.03	2.99 ± 0.06	2.98 ± 0.05	3.03 ± 0.04	2.99 ± 0.04
4	3.12 ± 0.07	3.08 ± 0.08	3.22 ± 0.05	3.19 ± 0.09	3.19 ± 0.05	3.12 ± 0.06	3.18 ± 0.06
8	3.98 ± 0.07	3.19 ± 0.06	3.49 ± 0.05	3.44 ± 0.08	3.39 ± 0.07	3.26 ± 0.06	3.33 ± 0.08
16	5.28 ± 0.08	4.99 ± 0.08	4.98 ± 0.07	4.83 ± 0.06	4.80 ± 0.04	3.37 ± 0.07	4.44 ± 0.08

Values reported are means of triplicate determinations ($n = 3$) ± SD.

after 10 days of storage. Most likely, lycopene degraded along with the rapid deterioration of the tomato matrix.

Samples after HHP were more appreciated than raw tomato puree, possibly because of the better homgenization. Tomato puree treated by 500 MPa appeared to be stable during storage at 4 ± 1 °C for about 6 months. This can be probably explained by the sterilization effect of HHP (Aleman, 1996; Meyer, 2000). Mildest treatment (500 MPa) already yielded a microbiologically stable product (Porretta, Birzi, Ghizzoni, & Vicini, 1995). This observation can also be linked with the rate of losses of enzyme (lipoxygenase, pectin methylesterase, polygalacturonase) activities. HHP can inactivate these three types of enzymes that lead to the deterioration of tomatoes (Krebbers et al., 2003; Tangwongchai et al., 2000). HHP has different effects on each of these enzymes, and the remaining total enzymatic activity in tomato puree may be difficult to estimate (Krebbers et al., 2003). The degradation of lycopene can be influenced by different parameters, such as temperature, enzymes, oxygen and sugar content. In this study, the effects of HHP mostly mediated enzymatic activity. At high temperature storage, the enzymatic activity is reduced due to denaturation or degradation of the enzyme. The temperature of 24 °C in this study does not have a great effect on enzyme degradation. Therefore, at 24 °C, higher rates of enzyme-mediated losses of lycopene would be anticipated since catalytic activity would be highest. It has been reported that thermal processing of tomatoes into paste can result in decrease of lycopene concentration of 9–28% (Takeoka et al., 2001). It has also been reported that conventional processing results in up to 30% losses of lycopene. Lycopene appeared to be unstable during prolonged intensive heat treatment during conventional sterilization (Takeoka et al., 2001). All the results suggested that proper high pressure treatments could keep lycopene in tomato puree stable for a certain period of time.

4. Conclusion

High performance liquid chromatography using C_{30} column has been confirmed to be a powerful tool for analyzing lycopene and its *cis*-isomers. On the whole, HHP had a great impact on the content of total lycopene and the percentage of the presumptive 13-*cis* isomer, both in lycopene solution and tomato puree. Temperature had a great effect on the stabilization of lycopene. The higher the storage temperature, the lower was the content of total lycopene and the higher the percentage of presumptive 13-*cis* isomer. However, the pressure effects on lycopene were different in lycopene solution and tomato puree. 500 and 600 MPa led to the highest reduction of lycopene while 400 MPa could retain the maximal stability of lycopene in lycopene solution. 500 MPa was found to increase total lycopene content in tomato puree and it retained its stability when tomato puree was stored. The mechanism of loss appeared to be isomerization. Tomato puree treated with 500 MPa for 12 min under 20 ± 1 °C appeared to be stable during storage at 4 ± 1 °C for about 6 months. Results indicated that HHP is an alternative method for producing ambient-stable tomato products in terms of lycopene preservation. However, the applied conditions need to be optimized.

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