

Original Research Paper

# Effect of Ultra-High Pressure Technology on Isomerization and Antioxidant Activity of Lycopene in *Solanum Lycopersicum*

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**Abstract:** To evaluate the influence of the Ultra-High Pressure processing (UHP) on the isomerization and antioxidant activity of lycopene in tomatoes (*Solanum lycopersicum*), High Performance Liquid Chromatography (HPLC) and Ultraviolet-Visible Spectroscopy (UV-Vis) were used to separate and identify the isomer of lycopene. The scavenging activities against DPPH· and ·OH present samples with and without UHP treatment were tested with ascorbic acid as positive control. Full wavelength scanning figure showed that a characteristic absorption peak was created at 361 nm, which was caused by trans-lycopene isomerizing into cis isomer. HPLC analysis found that five peaks (Rt = 19.082, 13.307, 9.790, 8.123, 7.207 min) were produced after UHP treatment, which were further identified by UV-Vis and four peaks among them showed a strong absorption peak at 361 nm which was in accordance with the UV-Vis spectroscopy of cis-lycopene. The total antioxidant, capable of scavenging hydroxyl radicals ·OH and DPPH· radical of lycopene treated by UHP, were all higher than that of control and lycopene without UHP treated. Taken together, UHP treatment played the role in cis-trans conversion of lycopene and could also improve the antioxidant activity of lycopene oleoresin with a dose-dependent fashion.

**Keywords:** Ultra-High Pressure Processing, Lycopene, Isomerization, Antioxidant Activity

## Introduction

Lycopene, a functional natural red pigment, is rich in fruits and vegetables like GAC, tomatoes, watermelon, pink guava and red bell pepper and widely used in healthy food, pharmaceuticals and cosmetics due to its antioxidant properties. The lycopene in tomato is considered to possess the highest antioxidant activity. It is well known that 90-98% of lycopene from a natural source existed in the form of trans. A number of studies have suggested that cis-isomers of lycopene could be better absorbed in the human body than the all-trans-isomer due to the change in solubility and physical properties of lycopene by the cis-isomerization. According to researches, lycopene could inhibit the proliferation of breast cancer cells (Murakami *et al.*, 2017), decrease the risk of heart disease (Li *et al.*, 2017) and inflammation (Peng *et al.*, 2017) induced by rhinovirus infection and lipopolysaccharide

(Saedisomelia *et al.*, 2009; Armoza *et al.*, 2012). They also show that lycopene have protective effect on serum cholesterol and blood pressure (Petyaev *et al.*, 2014). Therefore, more and more scientists are devoted to the study of lycopene isomerization. Phan-Thi *et al.* (2014) observed the impact of heating on cis-isomerization of oil-free lycopene in hexane at 50 and 80°C during 240 min and found the initial all-trans-form isomerized to the 13-cis isomer more rapidly at 80°C. Cooperstone *et al.* (2016) found the formation of cis-lycopene in intense thermal pasteurization processing, but high pressure sterilization limited the overall lycopene isomerization compared to the equivalent thermal sterilization process. Takehara *et al.* (2015) reported that (15Z) lycopene was prepared by thermal isomerization of (all-E)-lycopene derived from tomatoes. Heat treatment is widely used in food processing. Heat treatment to tomato productions can make trans-lycopene transform into cis-structure while some other active substances in lycopene, such as VE and

phytosterol, were also oxidized and degraded (Sy *et al.*, 2015). As polyene structure of lycopene makes it very sensitive in food processing (Wang, 2013; Silva *et al.*, 2012), vulnerable to heat, light and pro-oxidants, the main change in its structure is the degradation and isomerization (Ines *et al.*, 2013). Thus, it is meaningful to explore innovative technology in lycopene isomerization.

Cold sterilization technology has recently received greater attention, which not only can kill microorganisms in foods but also can maximally keep the color, flavor and nutritional ingredients of foods. Among them, there is no limitation of the size (Kumar and Sabhapathi, 2014) and geometry of the products (Kwon *et al.*, 2010). Ultra-High Pressure technology (UHP) is a physical cold sterilization method. UHP can minimize damage to food ingredients (Shin *et al.*, 2010; Park *et al.*, 2012; Cho *et al.*, 2013) and meet the high quality (Ferragut *et al.*, 2015) and microbiological safety requirements of food under non-thermal conditions (Yan *et al.*, 2015). Studies have shown that the ultra-high pressure treatment of carotenoids and lycopene can not only play a bactericidal role, but also make the whole trans carotenoids at 9, 13 and 15 sites with low energy to undergo partial cis isomerization or degradation to generate small molecular fragments, which can promote the conversion of lycopene into cis isomerization and bioavailability of active substances. However, there are no studies on the effect of UHP processing on isomerization of lycopene.

Hence, the aims of study were to give insight into the effect of UHP processing on isomerization of lycopene. In this study, UHP is used to treat lycopene, then HPLC is utilized to separate and identify the components of lycopene. Furthermore, antioxidant capability of the lycopene treated with UHP is evaluated.

## Materials and Methods

### Material and Reagents

Ingredients: Lycopene oleoresin (lycopene content 6%, Xinjiang Tecom Biotechnology Co., Ltd., Xinjiang China); Reagents: Lycopene standards (97% purity lycopene, Shanghai to Granville Chemical Co., Ltd., Shanghai China); hexane (AR, Tianjin Davydenko Davydenko chemical reagent Co., Tianjin China); acetonitrile, methanol, methyl tert-butyl ether, triethylamine (HPLC grade American sigma company, American) phenanthroline (AR, Shenyang reagent company, Shenyang China); 2-diphenyl-1-acid bitterness phenylhydrazine (DPPH-) (AR, Tianjin chemical reagent factory, Tianjin China).

### UHP Treatment of Lycopene Sample

2.5 mg lycopene oleoresin was dissolved in 50 mL of n-hexane, configured concentration at 50 µg/mL, sealed with double polyethylene plastic bags and

packaged with vacuum. In order to study the effect of UHP on isomerization and antioxidation of lycopene, high pressure treatment were carried out at different degrees (400 Mpa, 500 Mpa), time (8 min, 10 min) and low temperature (room temperature, 50°C) while the specific operation followed to device instructions.

### Uv-vis Spectra Identification of Lycopene Structure

UV-visible spectrophotometer was used to scan the liquid of lycopene samples treated with UHP, with UHP untreated lycopene as control group. The differences between scanning spectra of the two samples were compared to determine whether there was the cis-lycopene after UHP treatment.

Lycopene isomers could be analyzed and identified according to retention time and spectral characteristics of high performance liquid chromatogram. According to preliminary research, cis-lycopene showed absorption peaks at 472 and 362 nm as well in the HPLC diagram. The ratio of this peak absorbance and the main peak absorbance were called Q value, which is Acis/Atrans. At the same time, the maximum absorption wavelength ( $\lambda_{max}$ ) of main absorption peak of the cis-lycopene isomer moved to short-wavelength, "blue shift", direction 5-10 nm, compared to all-trans and the molar absorption coefficient decreased. Therefore, lycopene samples could be identified according to preliminary research.

### Isolation and Identification of Different Isomers of Lycopene by HPLC

Different isomers of lycopene were isolated and identified by HPLC (Honda *et al.*, 2017). Column: C30YMC CarotenoidS-5 (4.6×250 mm); mobile phase A: Mixture of acetonitrile and methanol (acetonitrile: Methanol volume ratio was 3:1); mobile phase B: MTBE (methyl tert-butyl ether) was added. Triethylamine (0.05%) was added to mobile phase A and B, respectively, eluted with gradient concentrations. Mobile phase B: The 8 min, 0~55% (V/V); 8~30 min: Maintaining 55% (V/V), flow rate was 1 mL/min (Li and Hui, 2010).

The content of cis and trans-isomers in the sample were determined according to the retention time and spectral characteristics of the separated components under the HPLC analysis conditions in terms of injection volume 10 µL, detection wavelength 471 nm.

### Lycopene Standard Curve

10.02 mg lycopene standard (HPLC≥97%) was accurately weighed, dissolved with 5 mL dichloromethane. Then n-hexane was used to set the volume to 50 mL to formulate 200 µg/mL n-hexane solution of lycopene and then this solution was diluted to lycopene standard solution series whose lycopene concentration was 20, 40, 60, 80, 100 µg/mL, respectively. The area of peak of each solution was measured by HPLC, concentration of the lycopene

standard solution was taken on the horizontal axis and peak area on the vertical axis, lycopene standard curve was plotted (Wang, 2013) and linear equation  $Y=81.71x-11.51$  ( $R^2 = 0.9639$ ) was obtained.

### Antioxidant Capability Assay

N-hexane was used as solvent to prepare sample liquor whose concentration is 1mg/mL; the preparation of ascorbic acid liquor was consistent with the sample.

### Total Antioxidant Capability Assay

The operation method was carried out according to the instructions of total antioxidant capacity assay (T-AOC):

$$\text{Total antioxidant capability}(U / mg) = (ODU - ODC) / 0.01 \div 30 \times N \div C_{prot} \quad (1)$$

where,  $ODU$  was the absorption value of sample;  $ODC$  was the absorption value of control;  $N$  represented the diluting times of system (which was to say, the ratio of total volume of reaction and sample volume);  $C_{prot}$  was the concentration of the sample to be measured.

Based on preliminary experiment, such as the linear relationship of standard  $V_C$  concentration of the sample was set to 10, 20, 50, 100, 200, 300  $\mu\text{g/mL}$ , respectively, concentration of the ascorbic acid was consistent with sample.

### Hydroxyl Radical ( $\bullet OH$ ) Assay

Preparation of the sample: The samples were weighed accurately, dissolved in distilled water and then n-hexane was added and used with ultrasonic extract 10 min to make lycopene transferred to the organic phase. It was repeated several times until the water layer became colorless. Organic phases were combined and dried by anhydrous sodium sulfate. Set the volume of organic phase to 50 mL and then diluted to formulate test solution whose concentration was 10, 20, 40, 60, 80, 100  $\mu\text{g/mL}$ , respectively.

Phenanthroline- $\text{Fe}^{2+}$  method was used. Firstly, 4 mL phosphate buffer (pH 7.4) were added to a test tube and mixed with 1.5 mL of 5 mmol/L  $\text{K}_3\text{Fe}(\text{CN})_6$  solution and blended thoroughly. Secondly, 1 mL 7.5 mmol/L ferrous sulfate solution was added, mixed immediately. Thirdly, 1mL of sample was added, mixed immediately, then 1.5

mL n-hexane was added to supplement the volume. Lastly, 1 mL 0.1% hydrogen peroxide solution was added, mixed gently and then incubated for 1 h at 37°C. The absorbance of the final solutions was measured at 536 nm with a UV/VIS spectrophotometer after holding them at 37°C for 1 h.

### DPPH• Radical Assay

200  $\mu\text{g/mL}$  lycopene liquor was diluted to the concentration 10, 20, 40, 60, 80, 100  $\mu\text{g/mL}$ , respectively. 2 mL sample of each concentration was taken, 2 mL  $2 \times 10^{-4}$  mol/L DPPH-ethanol solution was added, vibrated, left in the dark for 30 min as a sample group; DPPH-solution was replaced by an equal volume of n-hexane in blank group; lycopene solution was replaced by an equal volume of ethanol in control group; an equal volume of a mixture of ethanol and n-hexane was taken as a blank zero.  $A_i$ ,  $A_j$  and  $A_0$  were measured at 517 nm and VC was taken as a positive control (Xu and Hang, 1999):

$$\text{DPPH-scavenging ratio}\% = [1 - (A_i - A_j) / A_0] \times 100, \quad (2)$$

Where:  $A_i$ -sample group absorbance;  $A_j$ -blank group absorbance;  $A_0$ -control absorbance values.

### Statistical Analysis

Excel 2007, GraphPad Prism 5.01, Origin 8.5, SPSS19.0 were carried out to analyze experimental data by ANOVA and process the graphics. Significance analysis was performed by using T-test,  $P < 0.05$  was considered statistically significance while  $P < 0.01$  indicated a significant difference.

## Results and Discussion

### Effects of UHP Processing Conditions on Lycopene Isomerization

High pressure treatment carried out at different pressures (400, 500 Mpa), time (8, 10 min) and temperature (Room temperature, 50°C) and degree of cis-isomerization of lycopene and content of lycopene were measured. The results were shown in Table 1.

**Table 1:** Effects of UHP processing conditions on lycopene isomerization

Sample	Cis-lycopene rate (%)	Content of lycopene(mg/g)
Untreated sample	13.25	52.37
400 Mpa, 10 min, Room temperature	32.78	83.95
400 Mpa, 10 min, 50°C	34.76	87.94
400 Mpa, 8 min, 50°C	31.65	82.82
500 Mpa, 10 min, 50°C	45.69	103.24
500 Mpa, 10 min, Room temperature	43.23	98.79
500 Mpa, 8 min, 50°C	39.51	97.67

As shown in Table 1, the proportion of cis-lycopene and content of lycopene under the condition of 500 Mpa, 10 min, 50°C was the highest, the second was under that of 10 min, room temperature and the lowest was under that of 8 min, 50°C while under 400 Mpa the results was similar to 500 Mpa, which may be because that high pressure treatment caused the collide of the Van Der Waals forces between lycopene crystal molecules and  $\pi$  electron orbital fracture, resulted in isomerization of lycopene and increased the proportion of cis-isomer (Jenny *et al.*, 2001; Huo *et al.*, 2011).

#### Identification of Lycopene Isomers by UV-Vis

By comparing the full wavelength scan of lycopene sample before and after UHP treatment, the paper found that the full wavelength scans of lycopene had been obviously changed after UHP treatment, appeared cis-peak at 362 nm as it had been reported in the literature (Xu and Hang, 1999), Fig. 1. There were three maximum absorption wavelength of lycopene at 447, 471, 503 nm respectively in the two full wavelength scans, but the full wavelength scans of lycopene after UHP treatment had a change: There was a characteristic absorption peak at 361 nm, due to the transfer of trans-isomers into cis-isomer, thus it can be concluded that UHP could change lycopene structure.

#### Separation and Identification of Lycopene cis-Isomer by HPLC

The lycopene oleoresin treated with UHP showed 5 more peaks than the standard lycopene (Fig. 2) and then UV-Vis spectra of the 5 peaks mentioned above were obtained after separated and preliminarily identified (Fig. 3). Moreover, the component Rt = 19.082 corresponding to was basically confirmed as

all-trans lycopene due to its response time was consistent to that of all-trans lycopene while the peak pattern in UV-Vis spectra of the other components four peaks (Rt = 13.307, 9.790, 8.123, 7.207 min) corresponding were similar to that of the all-trans lycopene in the 440-500 nm wavelength range, whose peak showed a purple shift of 3-10 nm and a strong absorption peak appeared at 361 nm which was consistent with UV-Vis spectra of cis-lycopene. Taken together, the 5 peaks mentioned above were the same type of substance and lycopene isomers. Furthermore, we preliminarily inferred that these four components (Rt = 13.307, 9.790, 8.123, 7.207 min) were identified as the four kinds of cis-isomers of lycopene and could be separated for further study.

#### Antioxidant Activity

##### Total Antioxidant Capability

As what can be seen in Fig. 4: Total antioxidant activity of ascorbic acid, lycopene oleoresin, UHP lycopene oleoresin were measured and found. Total antioxidant capacity of these three materials were showed in the concentration dependent manner and good linear relationship ( $R = 0.9939, 0.9618, 0.9300$ , respectively.). Within 10-300  $\mu\text{g/mL}$  concentration range, total antioxidant capacities of these three materials were: UHP lycopene oleoresin>lycopene oleoresin>ascorbic acid. There was no significant difference ( $P>0.05$ ) between the total antioxidant capacity of UHP lycopene oleoresin and that of lycopene oleoresin, but there was significantly difference ( $P<0.01$ ) between the total antioxidant capacity of UHP lycopene oleoresin and that of ascorbic acid, while that of ascorbic acid and lycopene oleoresin were significantly different ( $P<0.05$ ).

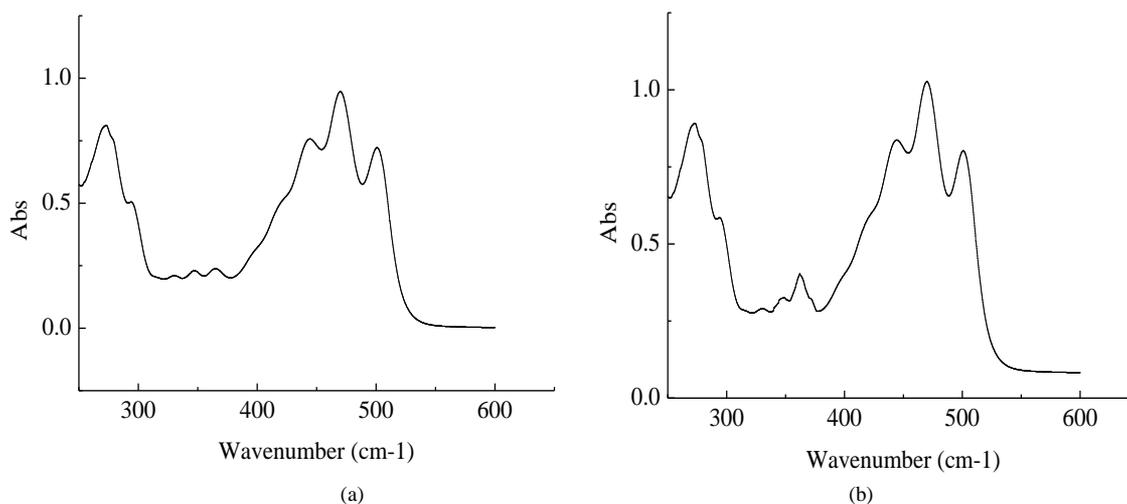
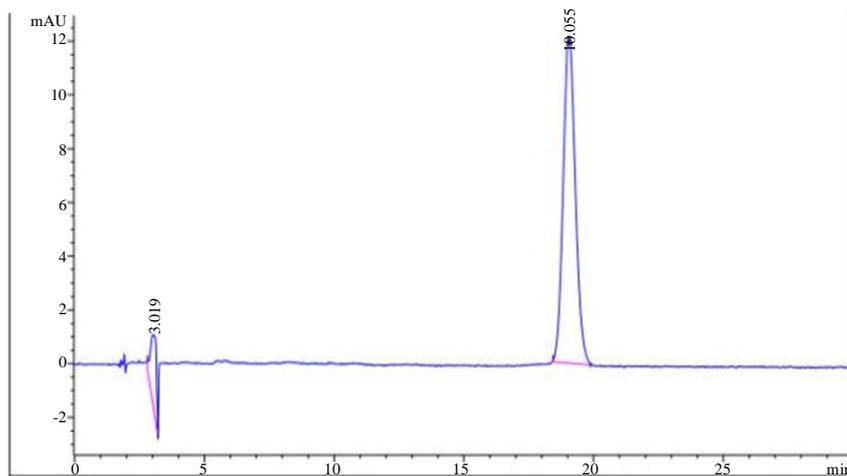
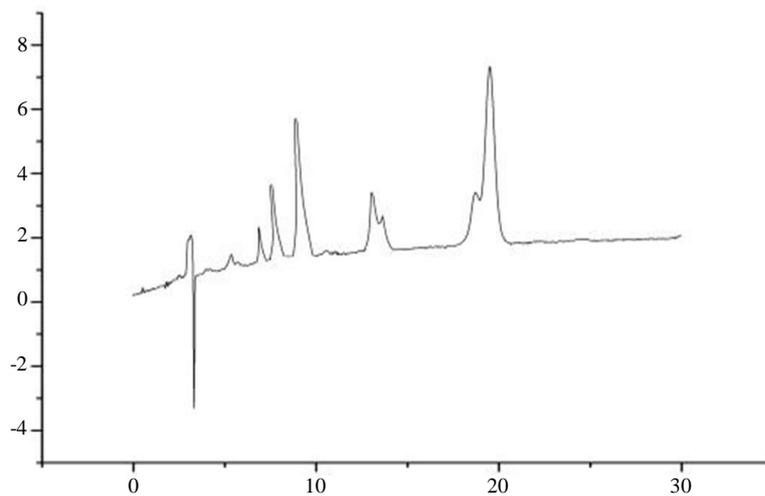


Fig. 1: The full wavelength scans of lycopene; (a) Before UHP; (b) After UHP

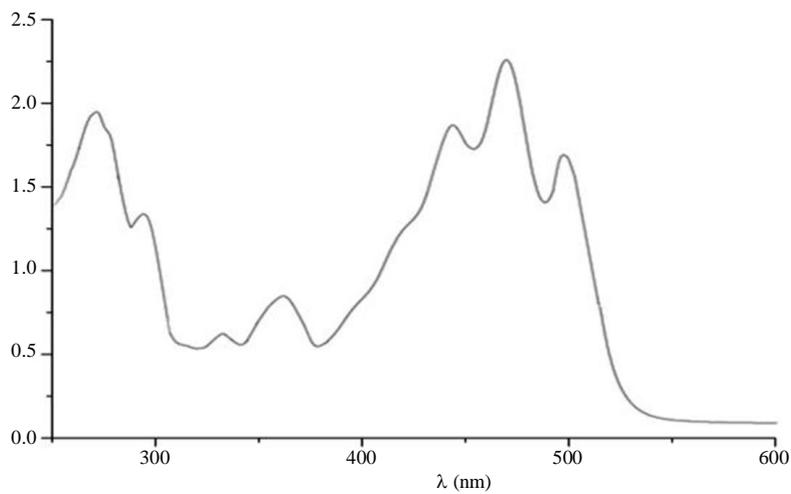


(a)

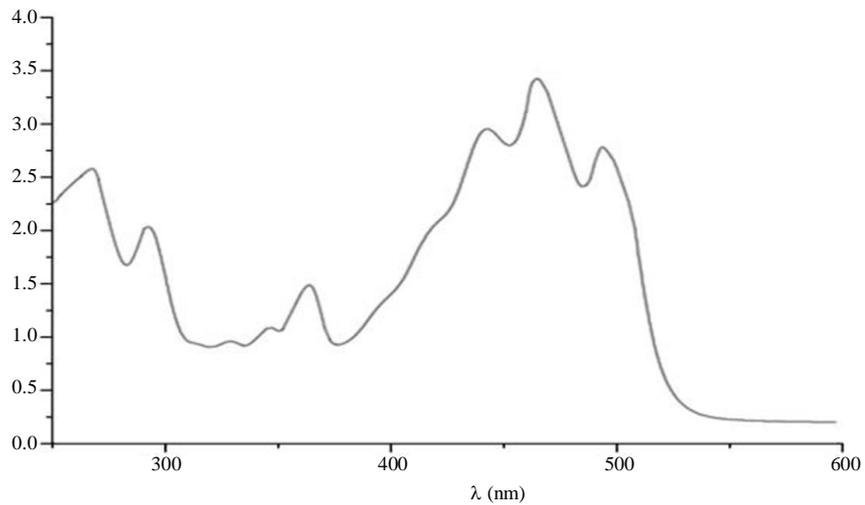


(b)

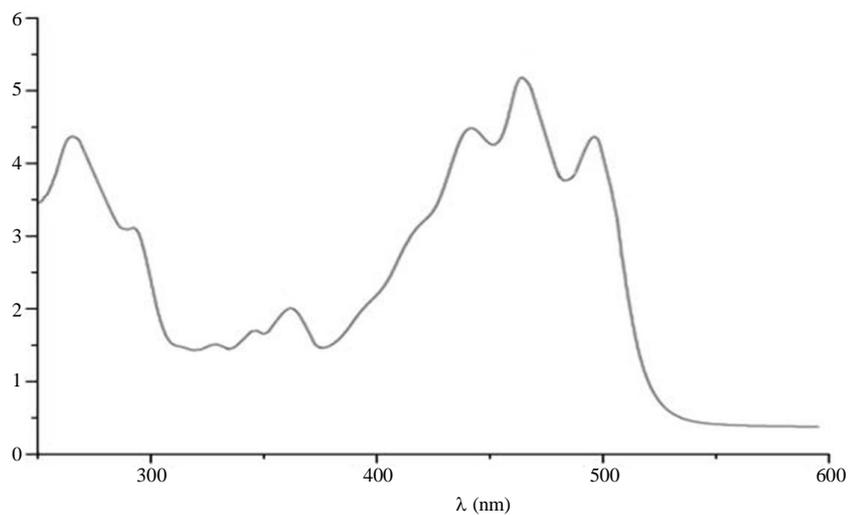
**Fig. 2:** HPLC spectrum; (a) HPLC spectrum of lycopene standard; (b) HPLC spectrum of lycopene oleoresin after UHP  
Note: X for retention time (min), Y for peak high (mAU)



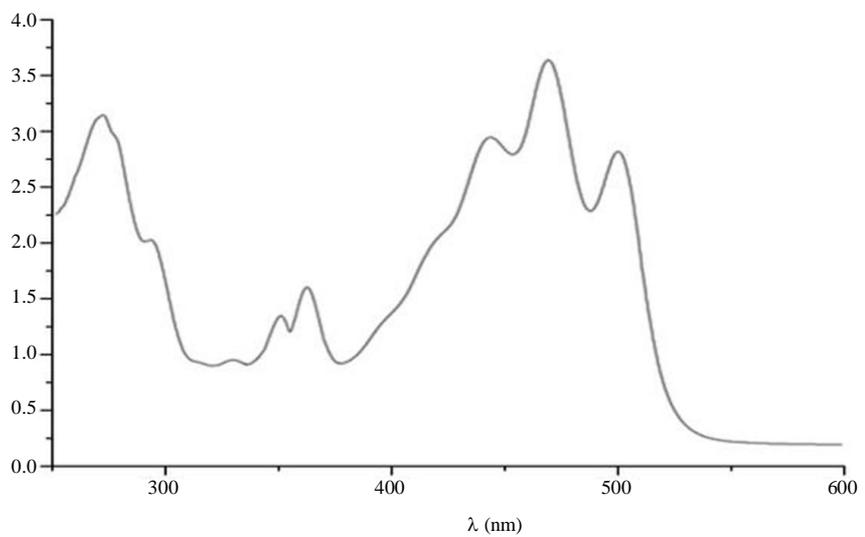
(a)



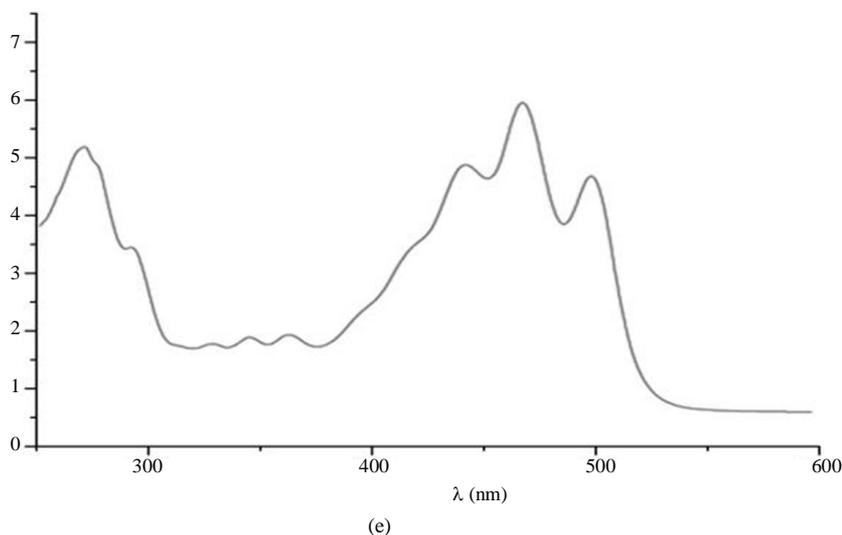
(b)



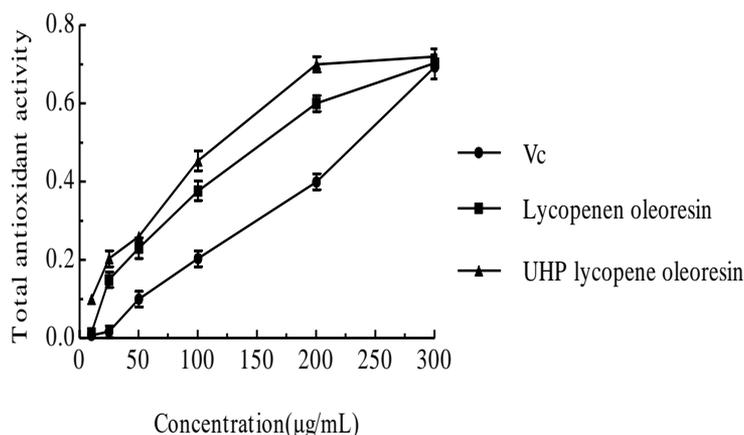
(c)



(d)



**Fig. 3:** The full wavelength scans of each HPLC peaks after UHP; (a) Peak 1; (b) Peak 2; (c) Peak 3; (d) Peak 4; (e) Peak 5  
 Note: X for  $\lambda$ (nm), Y for peak high (mAU)



**Fig. 4:** Total antioxidant capacity of lycopene product

#### Hydroxyl Radical-OH Assay

Hydroxyl radical, produced by the body during the metabolism process, is one of radicals which have strong toxicity to organisms. It contributes to the oxidation of sugars, amino acids, nucleic acids and other substances in body's tissues which results in various diseases and accelerate the aging of the body. Ascorbic acid, the samples of lycopene oleoresin before or after UHP treatment all had a significant scavenging effect on the hydroxyl radicals and showed a certain dose-dependent relationship (Fig. 5) with a good linear relationship ( $R = 0.9726, 0.9412, 0.9340$  respectively).  $IC_{50}$  value of ascorbic acid, lycopene oleoresin, UHP lycopene oleoresin were 45.6298, 40.2601, 24.0639  $\mu\text{g/mL}$ , respectively. Therefore, scavenging hydroxyl radical capabilities of these three substances were: UHP

lycopene oleoresin > lycopene oleoresin > ascorbic acid. There were significant differences ( $P < 0.01$ ) between the scavenging hydroxyl radical capability of the UHP lycopene oleoresin and that of lycopene oleoresin, but no significant difference ( $P > 0.05$ ) with ascorbic acid and there was no significant difference ( $P > 0.05$ ) between ascorbic acid and lycopene oleoresin.

#### DPPH·radical Assay

As it can be seen from Fig. 6 all of the ascorbic acid, lycopene oleoresin, UHP lycopene oleoresin had a strong DPPH·radical scavenging capability. All three of them showed a certain dose-dependent relationship over a range of 20-100  $\mu\text{g/mL}$  with a good linear relationship ( $R = 0.9695, 0.9476, 0.8990$ , respectively).  $IC_{50}$  value of ascorbic acid, lycopene oleoresin, UHP lycopene oleoresin was 47.3698, 52.0821, 39.6950  $\mu\text{g/mL}$ , respectively.

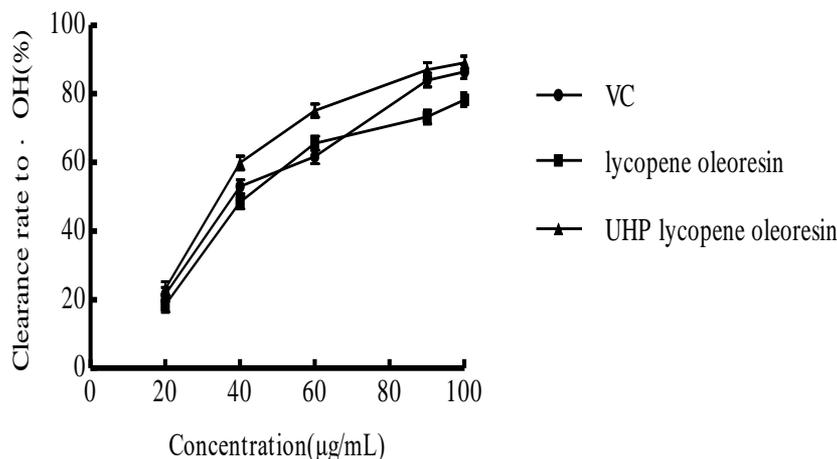


Fig. 5: Ability of lycopene products clear hydroxyl radical ( $\cdot\text{OH}$ )

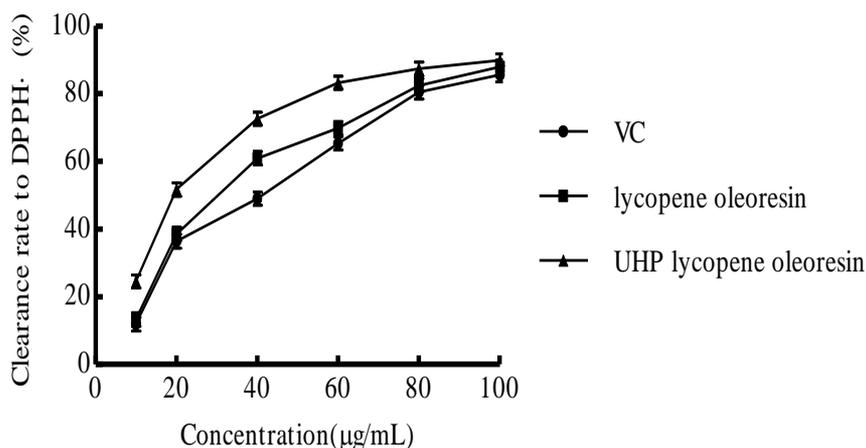


Fig. 6: Ability of lycopene products clear diphenyl picryl hydrazinyl radical ( $\cdot\text{DPPH}$ )

Thus, the capability of these three substances to scavenge DPPH·-radical was: UHP lycopene oleoresin>ascorbic acid>lycopene oleoresin. There were significant difference ( $P<0.01$ ) between the scavenging DPPH·-capability of UHP lycopene oleoresin and that of ascorbic acid and lycopene oleoresin, while there was no significant difference ( $P>0.05$ ) between that of ascorbic acid and lycopene oleoresin.

## Conclusion

In this study, we found that pretreatment with UHP could not only efficiently improve the proportion and content of cis-isomer of lycopene in oleoresin but also strengthen the antioxidant activity of lycopene in vitro. Therefore, we can conclude that UHP treatment was a promising method for converting lycopene into cis-trans isomerism with higher antioxidant activity. However,

further work will be needed to raise the cis-isomer proportion of lycopene to more than 50% by UHP.

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## Author's Contributions

**Siqun Jing:** She designed the research plan and organized the study.

**Hui Shi:** She participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

**Saisai Wang:** She participated to collect the materials related to the experiment.

**Zainixi Lasheng:** She contributed to the writing of the manuscript.

## Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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