Isomerisation and degradation of lycopene during heat processing in simulated food system

[1,2]Huawei, Z., [2]Xiaowen, W., [2]Elshareif, O., [2]Hong, L., [2]Qingrui, S. and [1, 2]*Lianfu, Z.

[1]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, 214122, China
[2]School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China

Abstract

The degradation and/or isomerisation of lycopene have an important influence on its bioavailability and bioactivities. Existing studies have shown that the configuration profiles and stability of lycopene will be changed because of heat processing, but the relationship between the structure conversion of lycopene and the intensity of heat treatment is yet to be further studied. To simplify the experiments, Lycopene-Octyl/Decyl Glycerate-TBHQ system was established to simulate real food system, C30-HPLC system was used to determine the amount of different lycopene isomers, from which total lycopene, the percentage of Z-isomers to total lycopene before and after heat processing can be calculated to describe the isomerisation and degradation of lycopene during heat processing. The results showed that the degradation of lycopene in the simulation system follows a first-order kinetic model and the degradation rate constants were 2.4×10^{-3}, 4.3×10^{-3}, 1.52×10^{-2} and 3.32×10^{-2} min^{-1} at 100, 115, 130 and 145°C respectively. The apparent activation energy was 77.24 kJ / mol. The contents of total lycopene, all-E lycopene and its Z-isomers followed fairly good regularity during the processing.

Introduction

Lycopene is a kind of food colorant naturally existing in tomatoes with potent antioxidant activity and exceptional physiological functions (Xu, and Diao, 1998). An appropriate intake of foods containing lycopene may effeciently reduce the incidence of many chronic diseases such as prostate cancer, breast cancer and cardiovascular system diseases (Giovannucci, 1999; FDA, 2005; Story *et al.*, 2010). Lycopene has eleven conjugated double bonds and each of them could be either in an E or Z configuration, so theoretically lycopene has 2^{11}, that is 2048 all-E and Z-isomers. However, the steric effects caused by the methyl on chain greatly reduce the amount of isomers. In fact, there are only 72 Z-isomers can be detected and among which the most common ones are 5-Z, 9-Z, 13-Z and 15-Z.

All-E lycopene is the predominant isomer in plants, representing about 80–97% of total lycopene in tomatoes and related products. In human body fluids and tissues such as plasma, prostate, testis and skin, 25–70% of lycopene is found in various Z forms (Dewanto *et al.*, 2002). The high concentrations of Z-isomers in vivo trigger the hypothesis that they are more bioavailable and/or that all-E lycopene may be transformed into Z-isomers within the human body (Wang *et al.*, 2004). Actually, when we heat tomato pulp to kill microorganisms, inactivate enzymes in order to extend the shelf life of tomato products, lycopene contained in the pulp will go through high-temperature sterilization and this processing will change its content and steric configuration (Shi and Mayer, 2000; Varma *et al.*, 2010).

Currently, there some groups report the degradation and isomerisation of lycopene during the process of tomato oleoresin and tomato ketchup (Sharma and Maguer, 1996; Shi *et al.*, 2003). Wang *et al.* (2004) deduced the isomerisation rate constants at different temperatures by studying the isomerisation of lycopene caused by soybean oil heating. The research conducted by Henry *et al.* (1998) focused on lycopene's degradation kinetics and isomerisation out of safflower oil heating. Ax *et al.* (2003) investigated the stability of lycopene at the micro-emulsion state of the water-dispersible and described the isomerisation phenomena at a qualitative level. Shi *et al.* (2003) drew a conclusion from their research that hot and illumination could cause degradation and isomerisation of lycopene in tomato juice and ketchup.

In this study, we try to establish a simulated food system so that we can simplify the parameters of heat processing and pre-treatment to characterize the spatial configurations of lycopene. Also, we try to analyze the distribution regularities of different lycopene isomers and the degradation rules of lycopene accompanied by the heat processing, looking forward to provide

*Corresponding author.
Email: *lianfu@jiangnan.edu.cn*
Tel: +86 510 85917081; Fax: +86 510 85917081

guidance for tomato products processing.

Materials and Methods

Materials

All-E lycopene (purity is 90%) was purchased from North China Pharmaceutical Group Co., Ltd. The HPLC-grade solvents, including acetonitrile, methyl tert-butyl ether (MTBE), methanol, triethylamine, and ethyl acetate were obtained from Sinopharm Chemical Reagent Co., Ltd. Octyl/Decyl Glycerate and TBHQ were purchased from Hangzhou Jianghui science and technology Co., Ltd.

Instruments

Waters 2695 reversed-phase (RP) -HPLC equipped with a photodiode array detector (Waters Assoc., Milford, Mass., U.S.A.) was used. Separation was achieved using a 5 μm polymeric C30 RP-HPLC column (250 mm × 4.6 mm inner dia) from YMC, Inc. (Wilmington, N.C., U.S.A.). GKC-214 digital temperature oil bath (Shanghai Suda Experiment Instrument Co., Ltd.) was used to heat the samples.

Establishment of simulated food system

Add octyl/decyl glycerate (1 mL) into a ground test tube of 10 mL and take TBHQ (0.02%, m/m) as an antioxidant, then 11.11 mg lycopene powder was added and shook the tube until all lycopene powder was completely dissolved. This mixture was used as simulated lycopene-containing food system: it had similar apparent viscosity, heat conductivity coefficient, lycopene content as tomato pulp but easy to extract and determine the lycopene in it. Notice: in order to minimize the experimental error, we should pre-heat octyl/decyl glycerate to the suitable temperature to make sure that lycopene powder could be dissolved quickly and completely.

Heat processing

Fill the tubes containing simulated lycopene-containing food system with CO_2 and seal it with a tube plug, put them into the oil bath which was pre-heated to 100, 115, 130 and 145°C and kept for different period of time according to the experiments design, shaking the tube and protect it from light while heating. Take samples after different heat processing, extract lycopene from it with ethyl acetate, and determine the quantity and steric configuration of it.

Determination of of lycopene

The extracted, filtered lycopene samples were injected into the HPLC set to determine the quantity and steric configuration.
HPLC Conditions: Column: YMC C30 (4.6×250 mm); mobile phase A: MeCN-MeOH (3:1, V/V), mobile phase B: MTBE,. both mobile phase A and B contain triethylamine at 0.05%, V/V. gradient elution: mobile phase B increased from 0% to 55% within 8 mins and remains at 55% for the next 27 mins; flow rate: 1.0 mL/min; wavelength: 475 nm; PDA spectral absorption range: 260~700 nm; the injection amount: 20 μL.

The steric configuration of lycopene isomers were determined according to their HPLC behaviour and UV-visible spectra, the quantity of each isomers was calculated according to the standard curve.
Standard Curve: Precisely weighing lycopene standard and dissolve it in ethyl acetate to prepare series standard solutions, in which 10.0, 8.0, 6.0, 4.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.2 mg/100 mL of lycopene was contained. An HPLC sytem described above was used to determine lycopene content, the lycopene standard curves can be made by illustrating graphs with the standard solution concentration as the X axis and the peak area as the Y axis. The contents of Z-isomers and all-E lycopene were measured according to this standard curve. Because the molar extinction coefficient of Z-isomers was smaller than that of all-E lycopene, the data we got would be smaller than the actual ones (Li *et al.*, 2007).

Data processing

Each heat processing condition was operated and analysed for 3 times. The final data was the average value of all the 3 testing results. The pseudo-first order kinetics was to be applied to the process of lycopene degradation data. The degradation rate constant could be calculated according to the following formula:

$$K = -Ln(C_A/C_{A0})/t$$

C_A represents the concentration of lycopene after procession; C_{A0} represents the initial concentration of lycopene before procession; t represents heat treatment time.

Results and Discussion

The establishment of the standard curve

In order to compare the lycopene concentration under different conditions during the heating process a linear standard curve was performed. The obtained linear equation of the calibration curves and the corresponding correlation coefficient are :

$$Y = 3.0E+6X+19868 , (R^2 = 0.9991)$$

Identification of lycopene isomers

Heating process converted all-E lycopene to

Table 1. Total amount (mg) changes, content (mg) and percentage (%) changes of lycopene and its Z-isomers during heating at 100°C

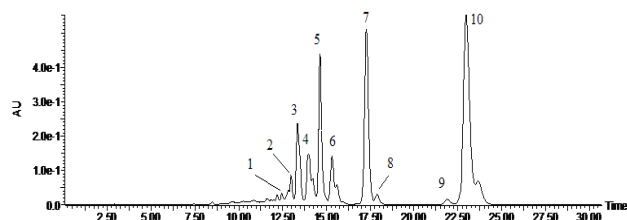| Heating time (min)	lycopene												
	15-Z		5-Z		9-Z		13-Z		All-Z		All-E		Total
	c	%	c	%	c	%	c	%	c	%	c	%	(mg)
0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	10.00	100	10.00
3	0	0.00	0	0.00	0.083	0.88	0.844	8.99	0.927	9.88	8.459	90.12	9.386
6	0	0.00	0	0.00	0.108	1.16	0.971	10.39	1.079	11.55	8.265	88.45	9.344
10	0.052	0.57	0.049	0.54	0.144	1.58	1.011	11.08	1.256	13.77	7.865	86.23	9.121
30	0.109	1.24	0.072	0.82	0.225	2.56	1.206	13.75	1.612	18.38	7.16	81.62	8.772
60	0.163	1.97	0.125	1.51	0.285	3.45	1.203	14.57	1.776	21.51	6.482	78.49	8.258
100	0.165	2.22	0.132	1.78	0.305	4.11	1.091	14.71	1.693	22.83	5.723	77.17	7.416

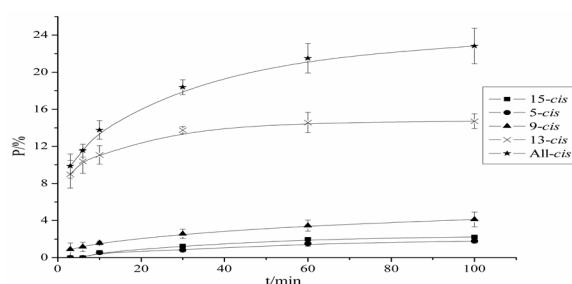Figure 1. HPLC chromatogram of lycopene in simulation system after heating at 130°C for 10 min

Figure 2. The trend of each Z isomers and total amount with the processing time at 100°C

Z-isomers as a result of additional energy input. According to the methods reported earlier, all-E lycopene can be identified by UV-visible optical spectra. All-E lycopene was identified by comparison of retention time of absorbent peak with the standard lycopene peak at the same wave length, quantification was accomplished by determine the peak area and calculate Q value of each Z-lycopene isomer (occurred between 350~370 nm) on the basis of standard curves generated with pure compounds (Shi *et al.*, 2008). whereas Q value is the ratio of isomer peak absorbance to the main peak absorbance, $Q = A_Z / A_{trans}$.

All-E lycopene ultraviolet spectrum was compared with Z-lycopene among the different conditions studied, other studies have found that, the ultraviolet spectrum of Z- isomers move 5~10 nm towards the short wavelength and the molar absorptivity of Z-lycopene have gone down. Therefore, the ultraviolet scans of chromatographic peaks in the liquid chromatography system were performed to finish the tentative identification. For convenience's sake, chromatographic peaks will be named peak 1~peak 10 in order (shown in Figure 1). On the basis of the above description and previous researches (Lee and Chen, 2001; Zhang and Zhang,

2011), it can be clear seen that peak 10 was all-E lycopene; peak 4, 5, 7 and 9 were mono-Z peaks, which were considered to be 15-Z, 13-Z, 9-Z and 5-Z, respectively. However, peak 2, 3, 6 and 8 were di-Z peaks (Zechmeister and Tuzson, 1938; Euglert and Vecchi, 1980); peak 1 might be the intermediate products of lycopene oxidative degradation but its concrete structure remains to be discussed.

The analysis of the lycopene's thermal stability in simulation system

During the heating of lycopene in simulation system at 100°C, the contents of all-E lycopene and Z-isomers were changed as shown in Table 1. Lycopene undergoes geometrical isomerisation with thermal process. Results in Table 1 shows that the effect of heating on lycopene was highly time dependent, thus, the all-E lycopene, Z-isomer concentration (mg) were compared for different heating conditions, a short-term heating of the sample generates isomers. Heating for long time decreased the rate of Z-isomer formation, which is due to the degradation reaction; and the same trend is also reflected in all-E lycopene levels. By compare the lycopene aggregates with the content of Z-isomers, we can find that part of all-E lycopene was degraded, while another part was transformed into its Z-isomers. But the transformation we have mentioned above was just a trend, for the lycopene's E/Z isomerisation was a reversible reaction (Zechmeister and Tuzson, 1938; Shi *et al.*, 2002). According to the trend chart, in which the change of different isomers and total amount of lycopene at 100 among to the time is shown Figure 2, the variation trends of all kinds of homeopathy isomers were showed the same behaviour, firstly were increased and then changed gently. The different relative energies of steric configuration lycopene isomers resulted in their different thermal stabilities, contents, and order of appearance after the lycopene was heated. As can be seen from Figure 2, 13-Z and 9-Z occurs more easily, while 5-Z and 15-Z were more difficult to generate and their levels are relatively low, which was roughly the same. And this was in agreement with that observed by Chasse *et al.* (2001). Among the heating time, the concentration of 13-Z and 9-Z

Table 2. Total amount (mg) changes, content (mg) and percentage (%) changes of lycopene and
its Z-isomers during heating at 115°C

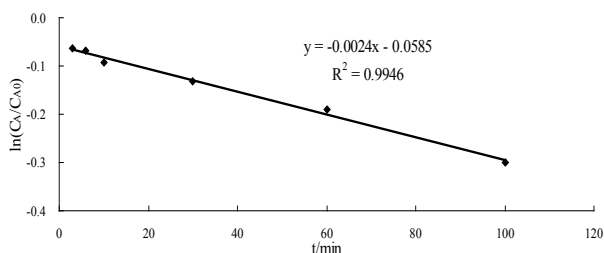| Heating time (min)	lycopene												
	15-Z		5-Z		9-Z		13-Z		All-Z		All-E		Total
	c	%	c	%	c	%	c	%	c	%	c	%	(mg)
0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	10.00	100	10.00
3	0.043	0.47	0.034	0.38	0.068	0.75	1.182	13.04	1.372	15.14	7.738	85.36	9.065
6	0.228	2.65	0.189	2.20	0.22	2.56	2.214	25.75	2.851	33.16	5.747	66.84	8.598
10	0.276	3.40	0.211	2.60	0.369	4.54	2.157	26.54	3.013	37.07	5.115	62.93	8.128
20	0.304	3.92	0.215	2.77	0.616	7.95	1.972	25.44	3.107	40.08	4.645	59.92	7.752
40	0.33	4.60	0.258	3.59	0.871	12.13	1.676	23.34	3.135	43.66	4.046	56.34	7.181
70	0.372	5.68	0.312	4.76	1.141	17.43	1.325	20.24	3.15	48.11	3.398	51.89	6.548

Figure 3. First order plot for the degradation of lycopene
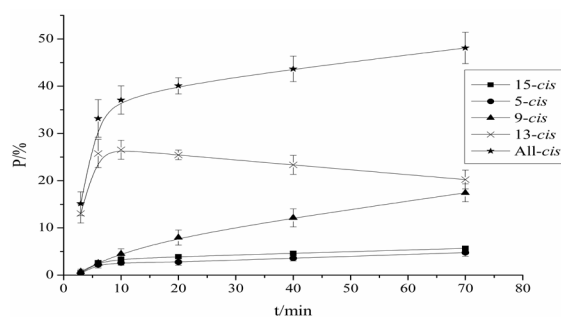in simulation system during heating at 100°C

Figure 4. The trend of each Z-isomers and total amount
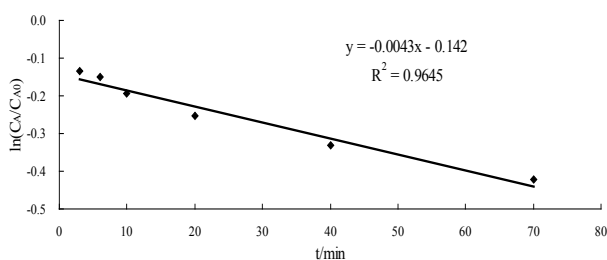with the processing time at 115°C

Figure 5. First order plot for the degradation of lycopene
in simulation system during heating at 115°C

was first increased, and then decreased, possibly as a result of lycopene degradation and transformation between Z- and E-isomers. When the sample was heated at 100 for six minutes to ten minutes, another two isomers were observed, which were relatively low in composition percentage and stable.

Figure 3 shows a curve of lycopene degradation kinetics at 100°C in simulation system, which had a good linearity ($R^2 = 0.99$), reflecting the degradation fits the first-order reaction kinetics model at this temperature, with the degradation rate constant was 2.4×10^{-3} min^{-1}. The result in Table 2 shows that the

lycopene simulation system was heated at 115°C. Compared with the experiment carried out at 100°C, all-E lycopene levels and Z- lycopene contents were roughly the same, while that of isomers was at the other extreme from each other. It can be directly seen from Figure 4, in which the changes of different isomers and total amount of lycopene at 115°C among the time is shown, that the variation trends of 13-Z percentage composition was firstly increased and then decreased with a higher starting point, made a great difference from the experiment carried out at 100°C. This suggested that the 13-Z was easier to convert, and more accessible to the oxydative degradation, as well as to the transformation and generation into other configurational isomers. Among the heating time, the concentrations (mg) and percentage compositions (%) of other configurations of the Z-isomer were decreased.

Figure 5 shows a curve of lycopene degradation kinetics at 115°C in simulation system, which reflected well the degrading trend of lycopene at this temperature. However, we can see that lycopene' degradation during the first one minute was consistent with the first-order reaction kinetics model, which can also be found when the temperature was 100°C. The explanation could be, that as the sample was being heated, the oxygen remained in the liquid and the surrounding carbon dioxide caused the oxidation of lycopene. The degradation rate constant in this case was 4.3×10^{-3} min^{-1}.

Table 3 demonstrates the variation of the lycopene aggregates (mg), the amount of Z- and E- isomers (mg) and percentage composition of them (%), as the sample was heated at 130°C, and the changing trend of different lycopene isomers and its aggregates is shown in Figure 6. It took only one minute to generate 2.327 mg Z-isomers from the starting point, illustrated that high temperature had a great influence on lycopene's configuration. The 13-Z amount was 2.382 mg in about three minutes, and then decreased gradually; the 9-Z kept increasing; the 15-Z and 5-Z both were firstly increased and then decreased. Figure 7 shows the curve of the sample' degradation

Table 3. Total amount (mg) changes, content (mg) and percentage (%) changes of lycopene and its Z-isomers during heating at 130°C

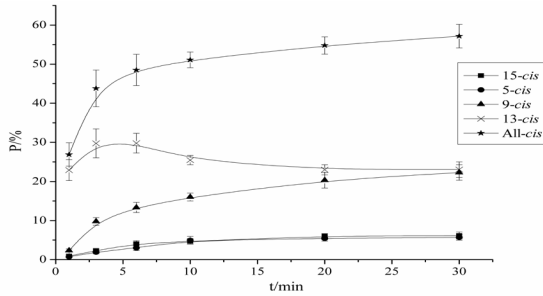| Heating time (min)	lycopene												
	15-Z		5-Z		9-Z		13-Z		All-Z		All-E		Total
	c	%	c	%	c	%	c	%	c	%	c	%	(mg)
0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	10.00	100	10.00
1	0.078	0.90	0.065	0.75	0.201	2.32	1.983	22.90	2.327	26.88	6.331	73.12	8.658
3	0.187	2.33	0.154	1.92	0.783	9.78	2.382	29.74	3.506	43.77	4.504	56.23	8.01
6	0.237	4.04	0.223	3.03	0.98	13.32	2.169	29.78	3.569	48.51	3.789	51.49	7.358
10	0.318	4.91	0.335	5.18	1.083	16.73	1.724	25.46	3.46	51.09	3.312	48.91	6.772
20	0.359	6.04	0.323	5.43	1.207	20.31	1.366	22.98	3.255	54.76	2.689	45.24	5.944
30	0.336	6.18	0.309	5.68	1.212	22.28	1.252	23.01	3.109	57.15	2.331	42.85	5.44

Figure 6. The trend of each Z-isomers and total amount with the processing time at 130°C
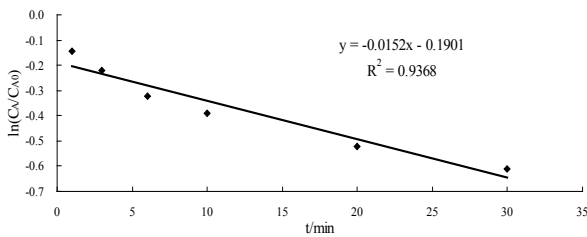

Figure 7. First order plot for the degradation of lycopene in simulation system during heating at 130°C
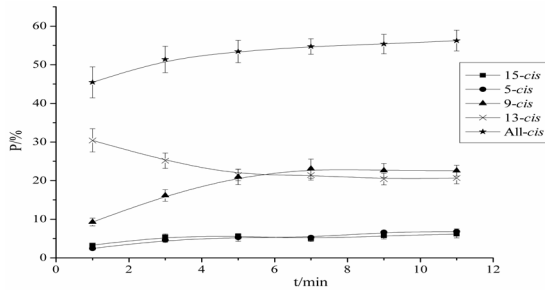

Figure 8. The trend of each Z-isomers and total amount with the processing time at 145°C

Figure 9. First order plot for the degradation of lycopene in simulation system during heating at 145°C

Figure 10. Plot of (lnk) vs.(T^{-1}) for lycopene degradation during heating

kinetics at 130°C, with the degradation rate constant of 1.52×10^{-2} min^{-1}.

Table 4 shows the experimental result, of which the lycopene simulation system was heated at 145°C. To observe the changes trend of different lycopene isomers and its aggregates among the time (Figure 8), we can find that 13-Z reached its maximum in both content and percentage within 1 minute. But as the heating-time prolongs, both of the content and the percentage of 13-Z went down gradually, showed that its feature of instability, while the percentage compositions of the other 3 Z-isomers all tend to
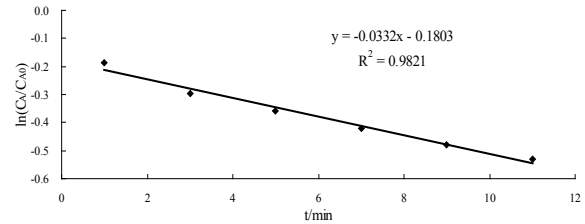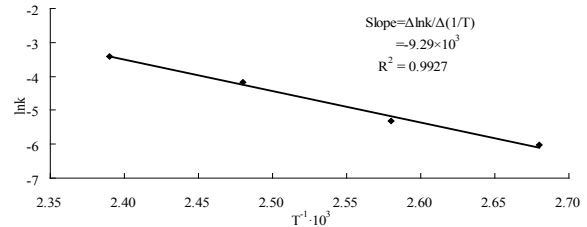
change gently. However, the concentrations of all were decreased, showed the possibilities of them for degradation or transformation into other di-Z isomers. Z isomer took three minutes to reach its maximum of 3.813 mg. Then the aggregates decreased, while the percentage composition climbs steadily, due to the reduction of all-E lycopene. Compared with the experiment carried out at 130°C, findings the trends of lycopene's degradation, and the degradation and transformation of Z- and trans- isomers were roughly the same, while the degradation and transformation rates were faster, which can be concluded from the curve of lycopene' degradation kinetics at this temperature (Figure 9). The degradation of lycopene at 145°C was consistent with the first-order reaction kinetics model, and had a good linearity (R² = 0.98). The degradation rate constant was 3.32×10^{-2} min^{-1}, relatively fast at this temperature.

At 100, 115, 130 and 145°C the degradation rate constant were 2.4×10^{-3}, 4.3×10^{-3}, 1.52×10^{-2} and 3.32×10^{-2} min^{-1}. Obviously, the higher the temperature is, the faster the degradation rate was, which was also applied to the generation or degradation of isomers at different temperatures. If we draw a graph, took the natural logarithm (lnk) of the degradation rate constant

Table 4. Total amount (mg) changes, content (mg) and percentage (%) changes of lycopene
and its Z-isomers during heating at 145°C

Heating time (min)	lycopene												
	15-Z		5-Z		9-Z		13-Z		All-Z		All-E		Total
	c	%	c	%	c	%	c	%	c	%	c	%	(mg)
0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	10.00	100	10.00
1	0.275	3.32	0.202	2.44	0.769	9.28	2.520	30.41	3.766	45.45	4.520	54.55	8.286
3	0.407	5.48	0.342	4.61	1.198	16.14	1.866	25.14	3.813	51.37	3.610	48.63	7.423
5	0.400	5.72	0.369	5.28	1.465	20.96	1.500	21.46	3.734	53.41	3.257	46.59	6.991
7	0.322	4.91	0.347	5.29	1.513	23.08	1.405	21.43	3.587	54.71	2.969	45.29	6.556
9	0.353	5.69	0.411	6.63	1.403	22.63	1.265	20.40	3.432	55.35	2.769	44.65	6.201
11	0.362	6.16	0.401	6.82	1.327	22.58	1.215	20.67	3.305	56.24	2.572	43.76	5.877

as the X axis and the reciprocal of the thermodynamic temperature (1/T) as the Y axis, we can use the slope of the curve to calculate the lycopene' apparent Arrhenius activation energy. As it is shown in Figure. 10, the curve had a good linearity ($R^2 = 0.99$), with the degradation rate constantly -9.29×10^3. According to the Arrhenius equation, the activation energy was calculated to be 77.24 kJ/mol.

$$\ln(k/[k]) = -\frac{Ea}{R}(\frac{1}{T/K}) + \ln(A/[k])$$

where E_a is activation energy (J/mol) and R is the ideal gas constant (8.3148 J/k mol).

Conclusions

It was showed good regularity of the configuration transformation and degradation when lycopene in simulation system was heated. With increasing of temperature, the degradation rate of lycopene demonstrated a linear increased trend; while the simulation system was at constant temperatures of 100, 115, 130 and 145°C, the degradations of lycopene were consistent with the first-order reaction kinetics model, levels of all-E and varieties of Z-isomers were closely tied up with the heating time. In this research, the highest percentage of Z- isomers was up to 57.15%, while the extent of degradation reached 45.6%. Considering the economic benefits, biological values, and physiological functions, we can draw a conclusion that the best thermal processing conditions were supposed to be at 130°C, the time period within six to ten minutes, the Z- isomers was 48 % and the degradation rate was only 27%.

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