A SFC ANALYSIS METHOD FOR LYCOPENE IN TOMATO EXTRACTS

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Abstract: Lycopene is a carotinoid naturally present in many fruits and vegetables. It is an important antioxidant providing protection against damage from free radicals, thus reducing risk of various cancers. Extraction of lycopene from tomato by organic solvent or supercritical solvent has been applied to get products with higher lycopene concentration. Accurate and quick measurement of lycopene concentration in the extracts is needed. A method for lycopene analysis in the extract of tomato powders has been established with supercritical fluid chromatography (SFC). With C_{18} as stationary phase, CO_2, CO_2+hexane and CO_2+ethanol as mobile phase, separation lycopene with its degradation product andß -carotene were carried out. The detection of lycopene by high pressure UV detector was set at 472 nm. It was found that separation of lycopene and its degradation product was major task. Therefore measurement of their retention time and separation resolution were studied in various of temperature, 25-50? , inlet pressure, 15.0-20.0MPa, pressure drop along column and modifier types and their concentration within 30v%. Lower temperature, higher inlet pressure and higher temperature were found favor better separation and higher efficiency. But higher modifier concentration over 16v% greatly reduces the resolution. Calibration plot and equation was obtained with optimized conditions in linear range of 0.025 mg/ml to 0.1mg/ml lycopene content. The lycopene content in the extracts by supercritical propane extraction were quantified accurately within 5 minutes.

Keywords: supercritical fluid chromatography, lycopene, retention time, extract, tomato

Introduction

Lycopene has received great attention in recent years because of its beneficial effect in the treatment of diseases such as skin cancer and prostate cancer because its important antioxidant properties to quench free radicals [1-3]. Lycopene can be extracted from the sources it occurs naturally. A number of processes have been proposed and are currently used for the extraction of oleoresins from tomatoes [4]. Our group has carried out lycopene extraction from tomato powders by supercritical propane, which is reported in the proceedings. Accurate analysis for lycopene plays important roles to product processing and further researches. HPLC [5], TLC [6] and UV spectrophotometer are widely used methods. HPLC method is considered the best. But the method by UV spectrophotometer may suffer from interference of ß -carotene and TLC method may be a complicated one and time consuming.

Analysis of ß -carotene and a - carotene by supercritical fluid chromatograph (SFC) have been reported[7,8]. SFC has been shown many advantages especially as separation and preparation tools. This work was to develop a quantitative SFC method for lycopene analysis in its containing products and also provide basics for high purity lycopene preparation by Preparative-SFC.

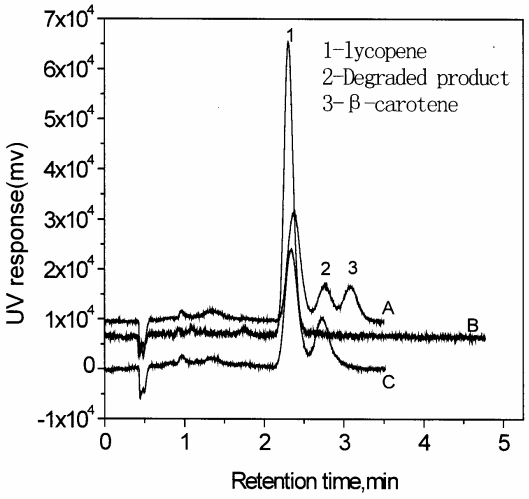
1. Experiments

A self-designed SFC, composed of two ISCO 260DM syringe pumps for CO_2 recycle and one ISCO 100DM for modifier pumping, a high pressure TSP-100 UV-VIS detector, a Rhendyne 7125 injection valve with 20μL sample loop and a Spherisorb C_{18} F 4.6 × 250mm column (10 μm). The UV detection for lycopene was set at wavelength 472nm.

CO_2 with purity >99.99%, ethanol and hexane of HPLC grade were used. Pure lycopene and ß -carotene were used as purchased from Sigma-Aldrich. The extract of lycopene oleoresin was obtained by supercritical fluid extraction from tomato by products. All the samples are solved in hexane for analysis.

2. Results and discussion

Generally speaking, ß -carotene is involved in the extraction and separation of lycopene from tomato and other plants. As well, lycopene is sensitive to heat and light illumination. Thus isomerization and degradation may occur [9]. In this research lycopene solution in hexane was exposed to light for two weeks to get its degradation product. Experiments showed separation between lycopene and its degraded product is difficult than that withß -carotene, as shown in figure 1. Here in the mobile phase is CO_2 plus 12 v% ethanol with flowrate 7.0ml/min, column temperature is 26? and column inlet pressure is 20.0 MPa with pressure drop of 4.0MPa. The influence of temperature, pressure, modifiers and their concentration on separation selectivity between lycopene and its degraded product and their retention were carried



A- Mixture of degraded solution & ß -carotene, B-pure lycopene C-degraded solution

Figure1 Chromatograph for three solutions

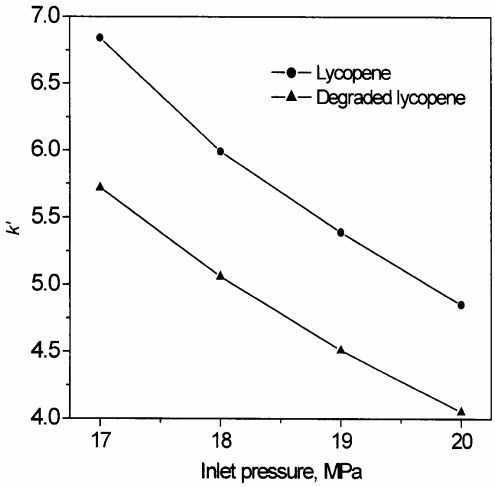


Figure 2 Capacity factor as a function of inlet pressure

out to optimize their separation and efficiency. Figure 2 shows that as pressure increase from 17.0MPa to 20.0MPa , the retention times are reduced significantly when keeping temperature at 27? and with CO_2 plus 8v% ethanol as mobile phase, column outlet pressure of 15.0MPa. The selectivity between lycopene and its degraded product is kept around 1.2. Pressure drop along the column also show important effects on retention and separation, see figure 3. As column inlet pressure was kept 20.0MPa and temperature at 27? when CO_2 plus 12v% ethanol was as mobile phase, the increase of outlet pressure will result retention time decreasing. But the selectivity also decreases. Hence, higher pressure and lower pressure drop along column favor higher efficiency or shorter analysis time while keeping a certain resolution. Temperature is another key factor that influence the separation. Figure 4 and figure 5 show that as temperature increase from 25 to 48? , capacity factor for them will decreases by 35% when mobile phase is CO_2 containing 8v% ethanol, column inlet pressure is 20.0MPa and outlet pressure is18.0 MPa. It means operating at room temperature can give higher selectivity.

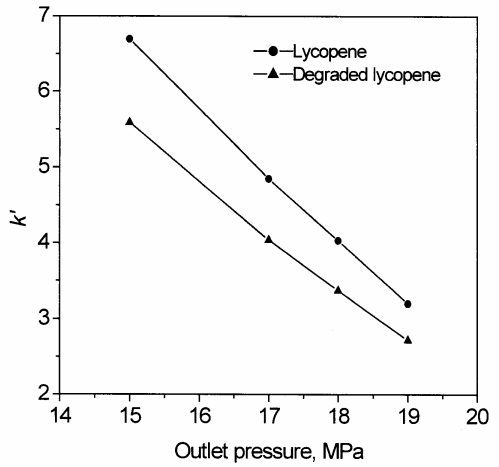


Figure 3 Capacity factor as a function of outlet pressure

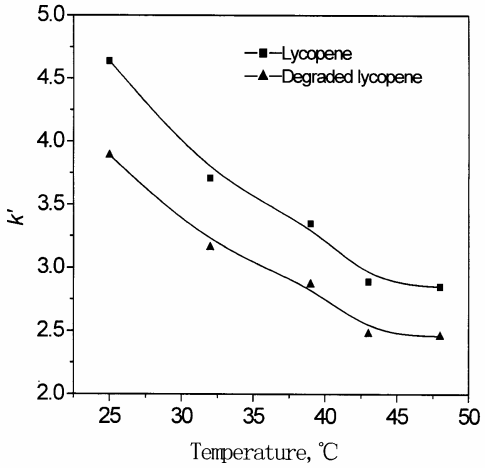


Figure 4 Capacity factor as a function of temperature

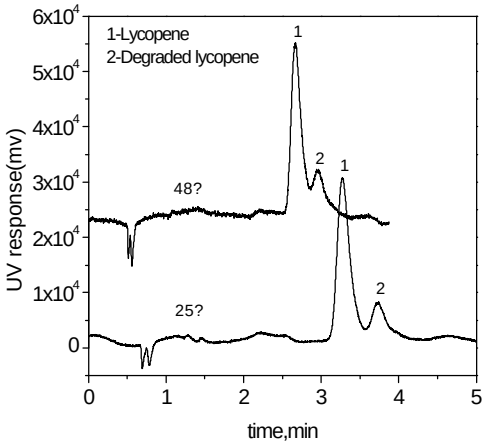


Figure 5 Chromatograph for lycopene and its degradation product under different temperature

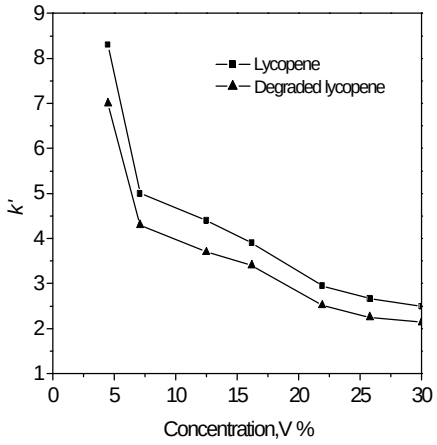


Figure 6. Capacity factor as a function of ethanol concentration

Modifier ethanol in mobile phase up to 30v% will greatly reduce the retention of both lycopene and its degraded product, especially in range of concentration 5 to 8v% when temperature is kept at 27? , inlet pressure is 20.0MPa and outlet pressure is 19.0MPa. However, when concentration is over 16v%, the selectivity decreases significantly. Hence, to keep a short retention time and good separation, optimized ethanol concentration is range of 8-12v%. If taking hexane as modifier, its behavior is similar to ethanol as shown in table 1, but hexane can give shorter retention time. The influence to selectivity between lycopene and its degraded product is similar in two cases. In range of 8-12v%, both modifiers give a number of about 1.2. Ethanol was chosen as modifier in subsequent quantitative analysis since it has been widely used as modifier in other analysis.

Table 1 Retention comparison between two modifiers

Hexane		Ethanol	
Conc., v %	k'	Conc., v %	k'
4.5	5.8	4.5	7
9.7	3.4	9.5	4.2
12.2	1.92	12.5	3.7
17.5	1.55	16.2	3.4
20	1.24	22	2.3

After a series of experiments, influence of operating conditions on separation of lycopene and its degraded product was understood and optimized operating conditions were set up. The conditions are: mobile phase of CO_2 plus 12v% ethanol, temperature of 25? , inlet pressure of 20.0MPa and outlet pressure of 18.0MPa. A number of lycopene solutions with lycopene content ranging from 0.025 mg/ml to 0.1mg/ml were injected and calibration plot was obtained as figure 7. Regression of the data gives a linear equation as the following.

$$C = -0.049 + 7.42\times10^{-7} A, \quad r=0.9990 \tag{1}$$

Where C is lycopene concentration in mg/ml and A is peak area.

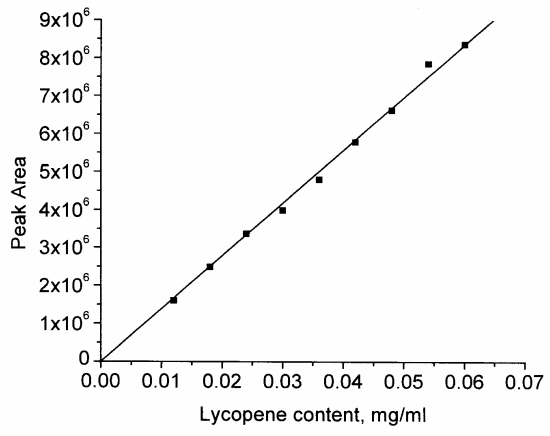


Figure 7. Calibration plot for lycopene

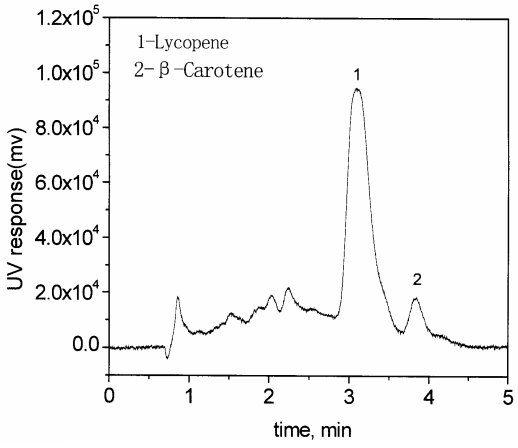


Figure 8 Chromatograph for lycopene oleoresin

Solving lycopene product in hexane and inject the solution could get its SFC chromatograph as shown in figure 8. Lycopene concentration and is content in the product can be directly calculated by equation (1). The content of lycopene in tomato feeds, their products via supercritical propane extraction and extracted residue were analyzed with 4 repeats for each sample. The data listed in table 2 gives an average deviation within 6%. The material balance of lycopene for a laboratory extraction by supercritical propane is listed in table 3. The result is no bad while there is lycopene loss, which may be due to product loss in collection stage.

Table 2. Lycopene analysis results (n=4)

		Content, wt%	RSD(%)
feed	a	0.094	1.36
	b	0.098	3.04
product	a	5.4	2.27
	b	5.5	4.15
Extracted residue	a	0.0040	1.80
	b	0.0042	5.40

Table 3 Material balance for a laboratory extraction by supercritical propane

	Mass g	Lycopene content mg/100g	Lycopene Quantity mg
Feed	300	0.096	288
Product	4.2	5.45	228.9
Residue	295.8	0.0041	12.1

3. Conclusion

A method for lycopene analysis in the extract of tomato powders has been established with supercritical fluid chromatography (SFC). Lower temperature, higher inlet pressure and lower pressure drop were found favor better separation and higher efficiency in range of temperature, 25-50? , inlet pressure,15.0-20.0MPa. Both modifier hexane and ethanol reduce retention time dramatically within 30v%. But modifier concentration over 16% reduces the resolution. Calibration plot and equation was obtained under optimized conditions for lycopene contents in linear range of 0.025 mg/ml to 0.1mg/ml. The lycopene content in the extracts by supercritical propane extraction could be quantified within 6% deviation in less than 5 minutes.

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