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Online Extraction-Fractionation of Functional Ingredients from Foods using SFE-SFC System Coupled with A Fraction Collector

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1. Introduction

Lycopene, a carotenoid compound found in tomatoes, has several beneficial effects such as antioxidant effects. Thus, lycopene is attracting attention for its effects of preventing lifestyle-related diseases such as cancer and arteriosclerosis, as well as retarding aging. In some cases, lycopene may be fractionated from samples because commercial standard substances of functional ingredients are not readily available. Additionally, the susceptibility of carotenoids to light, heat, or oxygen exposure should be also considered when developing a method for carotenoid extraction from a sample. The conventional HPLC analysis method requires time for sample pretreatment by a manual method such as reagent addition or centrifugation.
Online supercritical fluid extraction-supercritical fluid chromatography (online SFE-SFC) system is that directly connects the supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). Online SFE-SFC automates the extraction of target analytes from a sample and their subsequent analysis. When a fraction collector is used with online SFE-SFC system, steps involved between the extraction and the fractionation can be all performed on a single platform. This platform will handle a large number of samples and will enhance operational efficiency.

In this study, we report the case of optimization of the SFC method for separating lycopene and other compounds and improvement of the entire sample preparation process such as extraction, purification and fractionation of lycopene in tomatoes by using online SFE-SFC system coupled with a fraction collector.

Nexera™ UC (Online SFE-SFC coupled with the fraction collector)

2. Analytical Equipment

2-1. System Configuration

For online SFE-SFC system, Nexera™ UC system (Shimadzu Corporation, Japan) was used. Figure 1 shows the system configuration.

Lycopene was extracted by delivering the supercritical carbon dioxide into the extraction vessel filled with sample. The entire process, from extraction to data acquisition, is performed by switching flow lines using a valve inside the SFE unit.

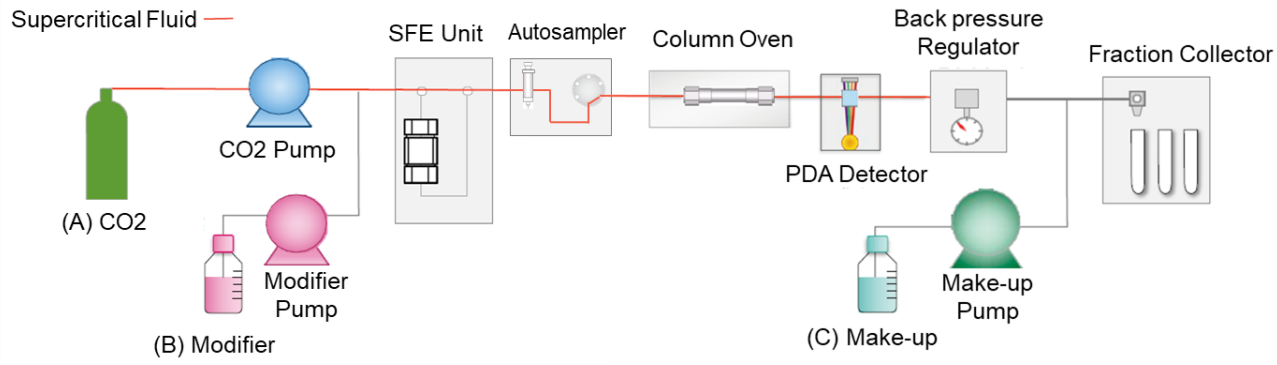


Figure1. System Configuration
(Online SFE-SFC coupled with the fraction collector)

2-2. Principle of Online SFE-SFC

A flow diagram of online SFE-SFC analysis is shown in Figure 2. Two types of extraction operations are involved. After supercritical fluid is introduced to the extraction vessel, static extraction is performed where components are extracted while fluid flow is stopped. Then dynamic extraction is done to extract components while pumping fluid through the extraction vessel. In the case of online SFE-SFC, the sample is transported through the analytical column during dynamic extraction.

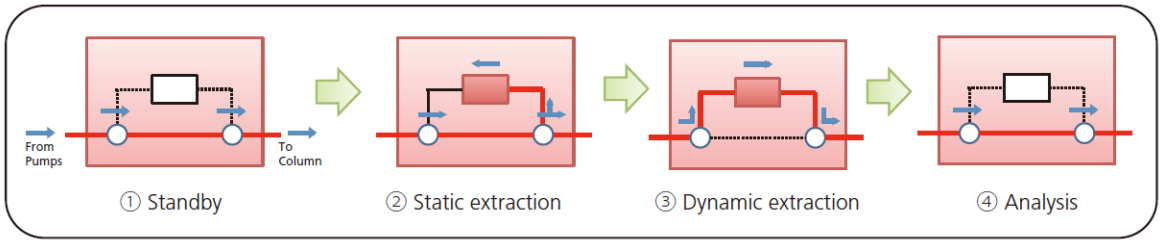


Figure2. Flow Diagrams of Online SFE-SFC

3. Results

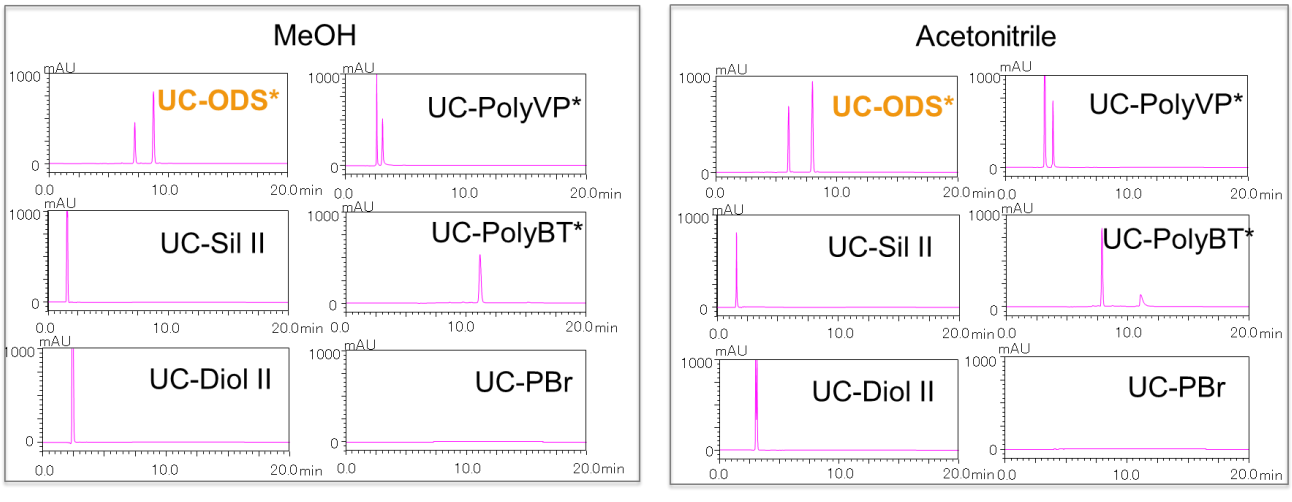
3-1. Column Scouting Evaluation

Since processed tomato foods contain both compounds, it was necessary to optimize the SFC analysis conditions for separating lycopene and β-carotene. Column scouting evaluation was performed using 6 columns, including octadecyl type and diol type etc. Figure 3 shows the chromatogram of each column applied to scouting. As a result of column scouting, the best resolution was obtained under the analytical conditions using the ODS column (Shim-pack UC-ODS). Furthermore, it could be obtained better separation and it could shorten analysis time by replacing methanol with acetonitrile as a modifier. The optimal combination of the column and the modifier which obtained from the column scouting evaluation was applied for extraction and purification.

Table 1 Analytical Condition for Column Scouting

Column	:	Shown in Figure 3 (250 mm L. X 4.6 mm I.D., 5 µm)
Mobile phase	:	A : CO2, B : Methanol , Acetonitrile
Gradient program	:	B. Conc. 5% (0min) -> 40% (10 - 15min) -> 5% (15 – 20min)
Flow rate	:	3.0 mL/min
BPR pressure	:	15 MPa
BPR temperature	:	50 ℃
Column temperature	:	40 ℃
Detection	:	Photo diode array detector (wavelength = 190 – 800 nm) PDA Chromatogram at 460 nm
Sample	:	Lycopene, β –carotene 500 mg/L each (in $CHCl_3$)
Injection	:	3 µL

Figure3. The Results of Column Scouting



3. Results

3-2. Reducing the Time for Sample Pretreatment

Figure 4 shows a comparison of sample preparation workflow between conventional method and online SFE-SFC analysis. In the sample preparation of online SFE- SFC analysis, an extraction vessel with filter paper was filled with a mixture of 0.1 g of processed tomato food and 1 g of dehydrating agent. Then, it was placed in the SFE unit and analysis was executed. The conventional method required about 1 hour for manual sample pretreatment, while the online SFE-SFC analysis reduced the time required for manual pretreatment to 5 minutes.

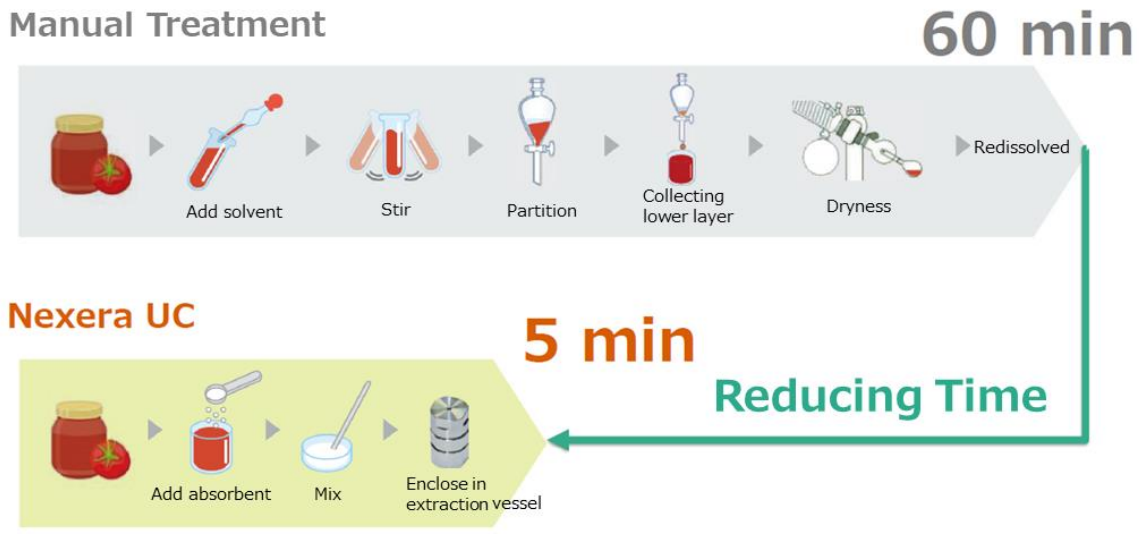


Figure 4. Comparison of sample pretreatment workflow between conventional method and online SFE-SFC analysis

3. Results

3-3. Fraction of Lycopene from Processed Tomato Food

Based on the results of column scouting, the analytical condition for fractionating lycopene in a processed tomato food was optimized by upscaling the column inner diameter and flow rate. Table 2 shows the condition. For the fractionation, we adopted a unique gas-liquid separator that prevents the eluate from scattering and can be expected to obtain a high recovery rate. Figure 5 shows an illustration of the gas-liquid separator, LotusStream.

Lycopene was automatically extracted from 0.1 g of processed tomato food, and then, purified and fractioned under this condition. Figure 6 shows the clipping of fractionation to the sample vial and Figure 7 shows the chromatogram for preparative SFC . Purified lycopene, separated from β-carotene, was automatically extracted from the food and fractionated.

Table 2 Analytical Condition for Fractionation

SFE		
Extraction vessel	:	5 mL
Mobile phase	:	A : CO2, B : Acetonitrile
Flow rate	:	5.0 mL/min
B. Conc.	:	10%
Extraction time	:	Static (0 – 3min) -> Dynamic (3 - 9 min)
BPR Pressure	:	A : 15 MPa , B:40 MPa
SFC		
Column	:	Shim-pack UC-ODS (250 mm L. X 10 mm I.D., 5 µm)
Mobile phase	:	A : CO2, B : Acetonitrile
Gradient program	:	B. Conc. 15% (9min) -> 40% (19 - 30min)
Flow rate	:	5.0 mL/min
BPR pressure	:	A : 15.0 MPa , B : 40.0 MPa
Column temperature	:	40 ℃
Make up solvent	:	2.25 mL/min

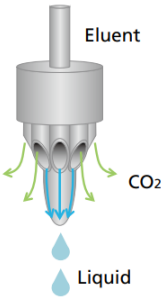


Figure 5. Gas-Liquid Separator

Figure 6. Fractionation to the sample vial

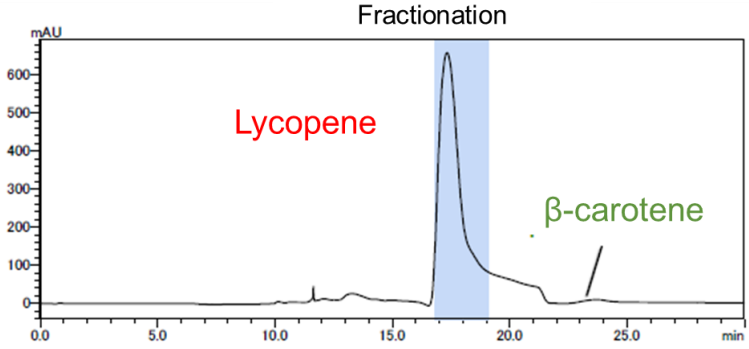


Figure 7. Chromatogram for preparative SFC

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3. Results

3-4. Recovery

The fraction purity of lycopene was measured using PDA detector and LCMS. Figure 8 shows re-analysis chromatogram and Figure 9 shows the MS spectrum. The recovery rate of lycopene with respect to the standard product was 78%, and it was confirmed that the influence of β-carotene, which is an impurity, could be prevented and that lycopene could be recovered with high purity.
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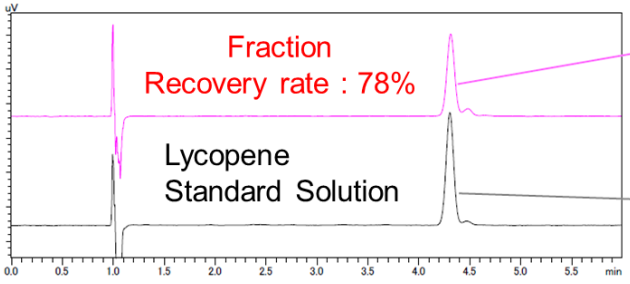

Figure 8. Re-analysis Chromatogram of fraction

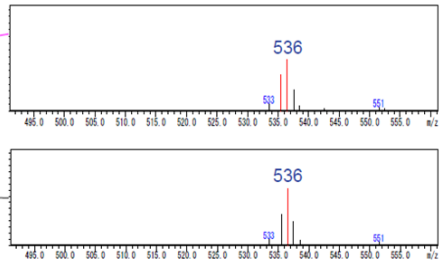

Figure 9. MS Spectrum for fraction

4. Conclusion

This report introduced an example of the fractionation of lycopene, one of the functional ingredients, in the processed tomato food by using online SFE-SFC. High purity lycopene was efficiently recovered by this method. Since the online SFE-SFC system can automatically and seamlessly perform everything from extraction of functional ingredients in foods to analysis and fractionation, significant savings in work time can be expected.

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