Extraction of Lycopene from Tomato Skin and Pomaces with Supercritical CO_2

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ABSTRACT
This work describes the influence of some parameters for the supercritical carbon dioxide ($SCCO_2$) extraction of lycopene from tomato skins and pomaces which are almost all of the waste after tomato processing.
As a first work, $SCCO_2$ extraction of lycopene from waste tomato skins was investigated. The experiments were carried out in 10 mL extraction vessel at pressures and temperatures ranging from 20 to 50 MPa and 313 to 373 K, respectively, without using any modifiers at 2.5 mL/min CO_2 flow rate for 330 min extraction time. Solvent flow rate effect was examined for CO_2 flow rates from 1.5 to 4.5 mL/min. Chromatographic analysis indicated that lycopene was extracted from tomato skin with negligible degradation at the optimum operating conditions; 40 MPa, 373 K, and 2.5 mL of CO_2/min and the amount extracted represented more than 94% of the total carotenoid content of the sample.
Extractions of lycopene using supercritical CO_2 from tomato pomaces were further important study subsequent to previous work. The extraction efficiency of lycopene with 56% recovered from dried tomato pomaces using $SCCO_2$ at the optimum operating conditions in 10 mL extraction vessel increased by using EtOH as co-solvent up to 91.7%.

Keywords: Lycopene, Supercritical CO_2 extraction, Tomato skin and pomace, HPLC

1 INTRODUCTION

Supercritical fluid extraction (SFE) with carbon dioxide as a solvent has provided an excellent alternative to the use of chemical solvents. The utilization of supercritical carbon dioxide ($SCCO_2$) for extraction of various carotenoids from a number of matrices, including tomatoes, has been studied by several authors [1-5]. However, $SCCO_2$ technology has been applied only for the recovery of carotenoids from tomato wastes at lower operating conditions: temperature and pressure and use of organic solvents as modifier, leaving out the purity of lycopene. Most lycopene extracted for nutritional supplements was not pure enough for scientists. Industrially, the separation of components from, for example, solid materials by means of a supercritical fluid as a solvent under high pressure is troublesome and requires high capital costs for high-pressure extraction equipment. Nevertheless, operating costs can be minimized by optimization of operating conditions of lycopene extraction. Nowadays, commercial plants prefer high pressure $SCCO_2$ extractions for natural materials to achieve their desired products considering the economical aspects.
With respect to this, the recovery of lycopene from tomato skin by optimization of the SFE process was studied first. The purpose of this work was to obtain the best extraction conditions by assessing the influence of pressure and temperature at higher levels and CO_2 volume on SFE of lycopene from waste tomato skins without using any modifier. The results of SFE extraction were also compared with results obtained by traditional solid-liquid

extraction. Moreover, extraction of lycopene from tomato pomaces with $SCCO_2$ was examined. Total extractable lycopene amounts were calculated by using Soxhlet extraction. The optimum extraction conditions obtained in the previous work-extraction of lycopene from tomato skin-were further used to verify whether these conditions were also the best extraction conditions for lycopene extraction from tomato pomaces. Lastly, ethanol and acetone were used as a modifier during the $SCCO_2$ extraction. Yields of lycopene obtained from both only $SCCO_2$ extraction and $SCCO_2$ extraction with a modifier were compared with the yield of traditional Soxhlet extraction. Small-scale SFE experiments are useful in early process development and in shortening development work at the pilot stage. In the scope of commercial production, large scale $SCCO_2$ extraction of lycopene from tomato pomaces was also performed in 100 mL and 1 L $SCCO_2$ extraction systems.

2 MATERIALS AND METHODS

Materials

Tomato skins were dried for 24 h in oven at 333 K. Tomato pomaces were dired in a double drum dryer (Johnsonboiler Co., Ltd., Japan) at 393 K. They were stored at 253 K until used and did not undergo any further pretreatment. CO_2 was acquired from Uchimura Sanso with a purity of 99.9%. Ethanol and acetone used as a modifier in $SCCO_2$ extraction and HPLC-grade solvents, including methanol and tetrahydrofuran (THF) were purchased from Wako Chemicals (Japan).

Methods

Extraction

Extraction with $SCCO_2$ was performed in laboratory-scale system. Liquid CO_2 flowing from the cylinder into the extraction vessel (10 and 100 mL vessel, Thar Designs, Inc., USA) was compressed and controlled with a HPLC pump [PU-2080-100 MPa, 5 mL/min, SSQD (slow suction, and quick delivery) pumping system is ideal to ensure the most reliable and pulse free solvent flow, Jasco Co., Japan] which was cooled with a chiller (Sibata Co., Japan) to keep CO_2 in a liquid state. After reaching the extractor, CO_2 was transformed into a supercritical state by a heating chamber (Tabai Espec Co., Japan) that envelops the extractor. Operating pressure was controlled by a back-pressure regulator (max. 60 MPa, Akico Co., Ltd., Japan). The supercritical CO_2 flowing through the fixed bed in the extraction vessel was expanded into a collection tube immersed in an ice bath, where the extracted lycopene and the CO_2 solvent were easily separated. The amount of CO_2 consumed during the extraction period was determined by use of a wet gas meter (Sinagawa Co., Tokyo, Japan). Supercritical CO_2 extraction was conducted on samples of different volumes. Tomato skins were ground immediately prior to extraction and the total volume of the vessel was well filled with tomato skins and glass beads as the fixed bed formation. Similar to tomato skin, tomato pomaces were also prepared and charged into extraction vessel in the same way. It was found that smaller particles of tomato skin, and pomaces as well resulted in improved recovery of lycopene. Filters were placed at the top and bottom of the vessel to provide a continuous flow of CO_2. To minimize the decomposition and oxidation of extracted compounds, all samples were protected from the actions of light and oxygen in the air by use of aluminum foil.

Analysis

The total amount of extractable *trans*-lycopene from tomato samples was determined after three 5-h extractions with chloroform using a Soxhlet extractor. The amount of lycopene was

determined in an HPLC apparatus equipped with a UV-visible detector (UV-970, Jasco, Japan) and a column (STR ODS-II 10 L × 4.6 (S), 250 mm, Shinwa Chemical Ind., Ltd.). A mixture of methanol and THF in a 90:10 ratio was used as the mobile phase, with a flow rate of 1.5 mL/min (6μL injection volume) and a column temperature of 303 K, supplied by a column heater (Sugai U-620, Japan) in 25 min detection time; detection was set at 470 nm for lycopene, which are previously known absorbance values [6].

3 RESULTS & DISCUSSION

The initial lycopene content of tomato dried skins was found to be 1.13 mg of lycopene per gram, as determined by Soxhlet extraction. Extraction of lycopene was carried out by supplying the tomato skins with a continuous flow of $SCCO_2$. To investigate the effects of temperature, pressure (1), and CO_2 volume (2) and thus optimize the operating conditions, $SCCO_2$ extraction was carried out under the following sets of conditions: (1) temperature ranging from 343 to 373 K and pressure from 20 to 50 MPa, at a constant CO_2 flow rate of 2.5 mL/min for 330 min extraction time; (2) CO_2 flow rate ranging from 1.5 to 4.5 mL/min at a constant temperature and pressure of 360 K and 40 MPa, respectively at the same extraction period of 330 min.

Figure 1 illustrates the effect of pressure on extraction yield at constant temperature of 343 K and CO_2 flow rate of 2.5 ml/min. It can be seen that increasing the operating pressure from 20 to 40 MPa at 10 MPa intervals resulted in a gradual increase in the yield of extraction as well as in the recovery of lycopene. It is known that an increase in pressure at constant temperature enhances solvent density, and at higher densities, molecular interactions between the solvent and the solute boosted, resulting in greater dissolution of the solute. In contrast, at a constant temperature of 343 K, an increase in pressure from 40 to 50 MPa did not improve the total amount of lycopene extracted, even though initial solubility (initial slope of the extraction curve) of $2.97*10^{-2}$ g/L at 50 MPa was higher than that of the solubility of $1.99*10^{-2}$ at 40 MPa. The reason for this might be that the increased pressure caused compacting of the sample and channeling of the CO_2 flow, resulting in the restriction of CO_2 movement in to and out of the tomato skin. Compacting or squeezing was observed in residue removed from the extraction column. Similar behavior was seen for $SCCO_2$ extraction at a constant temperature of 353 and 363 K with varying pressure.

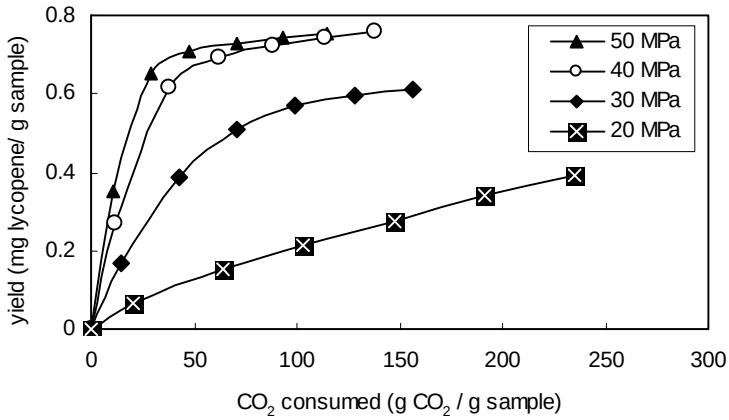

Figure 1. Yield vs. CO_2 consumed at constant T (343 K)

Further, while the pressure was held constant at 40 MPa, the extraction temperature was increased up to 373 K. Figure 2 shows the effect of temperature on the extraction yield ranging from 343 to 373 K. Usually, an isobaric increase in temperature decreases the density of the supercritical solvent and hence decreases solubility due to the density effect. However, the same increase in temperature increases the volatility of the solute, resulting in an increase in solubility due to the volatility effect. A further increase in temperature from 363 to 373 K at a constant pressure of 40 MPa provided almost the same amount of lycopene (1.17 and 1.18 mg of lycopene/g of sample, respectively), as there was some degradation of lycopene at the

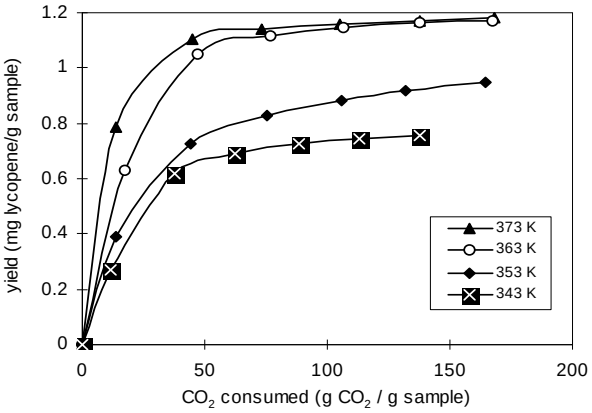

Figure 2. Yield vs. time at constant P (40 MPa) and different temperatures at a flow rate of 2.5 ml/min

elevated temperature.

To investigate the effects of CO_2 volume on the extraction yield, $SCCO_2$ extraction was carried out at a CO_2 flow rate ranging from 1.5 to 4.5 mL/min with a constant temperature and pressure of 363 K and 40 MPa, respectively for 330 min extraction time. It was shown that although the initial rate of extraction is higher at a greater flow rate of 4.5 mL/min, it decreased rapidly soon after the initial extraction time, compared with extraction at a flow rate of 2.5 mL/min. Such a high extraction rate is due to the solubility of the extracted material in supercritical solvent. In other words, this amount of solvent is enough to remove lycopene molecules from the tomato skin cells through the extraction vessel. Increasing the flow rate of CO_2 from 2.5 to 4.5 mL/min resulted in a decrease in the amount of lycopene extracted at later stages of extraction. One of the reasons for this may be the channeling effect, whereby the solvent is forced through the sample at such a high flow rate that it passes around the solid matrix and does not diffuse through the pores within the sample. For a CO_2 flow rate of 1.5 mL/min, although it seemed that the time of contact between solvent and sample was greater, insufficient amounts of CO_2 led to a reduction in the extraction yield. According to results obtained at various conditions, optimum extraction conditions were obtained at 40 MPa and 373 K with a CO_2 flow rate of 2.5 mL/min, providing an extraction yield of 1.18 mg/g sample.

$SCCO_2$ extraction of lycopene as well from tomato pomace was examined as a further study. the Total amount of the extractable lycopene from tomato pomace was determined as 5.8 mg/g sample, after extraction with chloroform using a Soxhlet apparatus for 5 h. $SCCO_2$ extraction of lycopene from tomato pomaces were initially performed at pressure 40 MPa, temperature 363 K, and CO_2 flow rate 2.5 ml/min for 5 h extraction time without using co-solvent (previously found optimum conditions for the extraction of lycopene in tomato skin). Calculating the lycopene content of tomato pomace with respect to HPLC results, $SCCO_2$ extract gave a yield of 3.25 mg lycopene/g sample which means 56% extraction efficiency based on Soxhlet extraction. Unlike tomato skin, $SCCO_2$ was not capable of extracting almost all lycopene from tomato pomace at the optimum conditions. This might be due to the difference in cell configurations or the structure of the matrix. To be able to increase the yield of extraction, and extraction efficiency as well, co-solvents or modifiers were used. Basically, a small amount of a co-solvent increases the ability of supercritical carbon dioxide to dissolve polar compounds. Neat $SCCO_2$ has dissolving properties similar to hexane. This means that, by itself, carbon dioxide is very good for dissolving relatively non-polar materials. The addition of just a small quantity of co-solvent enhances the solubilizing power of the supercritical carbon dioxide making it possible to extract much more polar molecules.

With respect to this, $SCCO_2$ extraction was repeated at the same operating conditions by using acetone, ethanol (EtOH) and water as a modifier (co-solvent) with a flow rate of 0.05 ml/min during 5 h extraction time. Unlike acetone and EtOH, water was co-filled and mixed well with the sample into the extraction vessel in the ratio of 1ml water to 1 g sample. HPLC results showed that adding co-solvent gradually increased the lycopene amount. Calculated amounts

of lycopene for each extraction were tabulated in Table 1. Compared to acetone, $SCCO_2$ extraction with EtOH gave higher yields of lycopene amount. Extraction efficiency of $SCCO_2$ extraction with EtOH was calculated as 91.7%. On the other hand, $SCCO_2$ extraction with water slightly enhanced the yield of extraction compared to others.

Table 1 Calculated amounts of lycopene presents in tomato pomace for each extraction

(mg/ g sample)	Lycopene
Soxhlet Extraction	5.80
$SCCO_2$ Extraction without co-solvent	3.25
$SCCO_2$ Extraction with acetone	4.79
$SCCO_2$ Extraction with EtOH	5.32
$SCCO_2$ Extraction with water	3.34

Large scale $SCCO_2$ extraction was performed in 100 ml extraction vessel filled with 45 g grounded tomato pomace at pressure 40 MPa, temperature 363 K, and CO_2 flow rate of 6 ml/min for 5 h extraction time without using co-solvent. Soxhlet extraction was repeated again and lycopene content was calculated as 2.45 mg/g sample. It was seen that lycopene amount (5.8 mg/g sample illustrated in Table 1) in tomato pomace was decreased by 57.8% due to some degradation and oxidation of lycopene during the storage of tomato pomace in a period of one month. Extraction efficiency of lycopene obtained at 40 MPa, 363 K, and 6 mL/min CO_2 flow rate was found as 69.4%. Since CO_2 flow rate was one of the most effective parameter on the yield of extraction, it was over investigated at 40 MPa and 80 ^oC ranged from 3 to 6 mL/min for 5h extraction time. Figure 3 illustrate the effects of CO_2 flow rate at 5 and 6 mL/min on extraction yields at constant temperature 80 ^oC and constant pressure of 40MPa for lycopene. As shown in figures, increasing the CO_2 flow rate enhanced the yield of extraction for lycopene. $SCCO_2$ extraction of lycopene at 3 and 4 mL/min CO_2 flow rate gave lower yields.

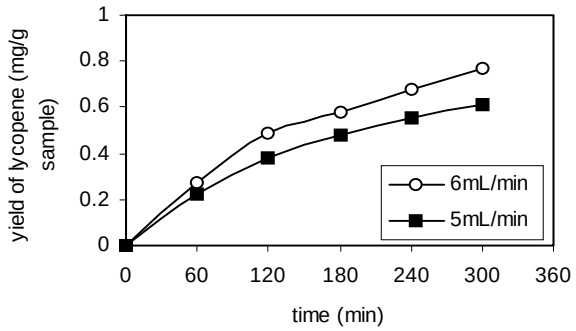

Figure 3. Yield of lycopene vs. time at 40MPa, 80 ^oC at various CO_2 flow rate

Since consumers are more educated and health conscious than ever before, and are demanding higher quality products without toxic materials we further carried out $SCCO_2$ extraction of lycopene at higher operating conditions; pressure 45-46 MPa, temperature 90 ^oC and CO_2 flow rate for the first 2h at 8 mL/min and the rest 3h at 6mL/min. Calculated amount of lycopene presents in tomato pomace at different extraction conditions in 100 mL extraction vessel are listed in Table 2. The results showed that further increasing the operating pressure and CO_2 flow rate enhanced the yield of extraction of lycopene giving an extraction efficiency of 89.4%.

Table2. Calculated amount of lycopene presents in tomato pomace at different extraction conditions in 100 mL extraction vessel

(mg/ g sample)	Soxhlet Extraction	$SCCO_2$ Extraction 40MPa, 90 ^oC	$SCCO_2$ Extraction 45-46MPa, 90 ^oC
Lycopene	2.45	1.70 (69.4 %)	2.19 (89.4 %)

$SCCO_2$ extraction of lycopene from tomato pomace was lastly performed in modified 1L $SCCO_2$ extraction system where the maximum pressure and CO_2 flow rate can be applied at 50 MPa and 20 mL/min, respectively. According to previous results, lycopene extraction was performed at the operating conditions given as; $SCCO_2$ extraction without co-solvent; sample amount 265 g, temperature 90 ^oC, pressure at 40 MPa, CO_2 flow rate at 13 mL/min for 4h extraction time; with EtOH as co-solvent; sample amount 266 g, temperature 90 ^oC, pressure at 40 MPa, CO_2 flow rate at 13 mL/min, EtOH flow rate at 1ml/min for 5h extraction time; and with water as co-solvent; sample amount 145 g, temperature 90 ^oC, pressure at 40 MPa, CO_2 flow rate at 13 mL/min, with 145 mL pure water put into extraction vessel for 5h extraction time. The results are shown in Table 3. It was seen that co-solvent effect on the yield of extraction carried out in 1L extraction system was similar to that obtained in 10 mL extraction vessel. Other than this, extraction efficiency of lycopene obtained 90 ^oC, 40 MPa, and CO_2 flow rate of 13 mL/min without co-solvent was only calculated as 26.3% where optimization of operating conditions are still interesting to study.

Table3. Calculated amounts of carotenoids presents in tomato pomace for each extraction at 1L extraction vessel

(mg/ g sample)	Soxhlet Extraction	$SCCO_2$ Extraction without co-solvent	$SCCO_2$ Extraction with water	$SCCO_2$ Extraction with EtOH
Lycopene	1.71	0.45	0.79	1.10

4 CONCLUSION

The results of this study indicate that operating conditions (temperature, pressure, flow, time, etc.) are crucial points in the supercritical carbon dioxide extraction of lycopene from waste dried tomato skins. Pure high-quality lycopene can easily be extracted and recovered from tomato skin without the use of modifiers by using supercritical fluid technology at the optimum operating conditions. Operating the extraction process at elevated pressures and temperatures increased the solubility of lycopene in $SCCO_2$. This is probably the most challenging point in industrial applications. Extractions of lycopene and other valuable carotenoids using supercritical CO_2 from tomato pomaces were another important study. Total extractable lycopene amount from tomato pomace was calculated as 5.8 mg lycopene/g sample based on the Soxhlet extraction that is an awful lot compared to lycopene amount in tomato skin. A total of 3.25 mg of lycopene/g of sample with low extraction efficiency of 56% was recovered from dried tomato pomaces at the optimum operating conditions, which were obtained in the previous study. Further, extraction efficiency was increased by using EtOH as co-solvent up to 91.7%. Other than this, similar yields and chromatographic fingerprints were obtained for extracts prepared using analytical and large scale (1L) equipment under comparable conditions. A major difference between analytical and pilot scale SFE instruments involves the separation stages.

4 REFERENCES

[1] G. Vasapollo, L. Longo, L. Rescio, L. Ciurlia, J. of Supercritical Fluids., 29, **2004**, p.87

[2] E. Sabio, M. Lozano, V. Montero de Espinosa, R. L. Mendes, A. P. Pereira, A. F. Palavra, J. A. Coelho, Ind. Eng. Chem. Res., 42, **2003**, p.6641

[3] E. Cadoni, M. R. De Giorgi, E. Medda, G. Poma, Dyes and Pigments., 44(1), **1999**, p.27

[4] N. L. Rozzi, R. K. Singh, R. A. Vierling, B. A. Watkins, J. Agric. Food Chem., 50, **2002**, p.2638

[5] U. Topal, M. Sasaki, M. Goto, K. Hayakawa, J. Agric. Food Chem., 54, **2006**, p.5604

[6] K. S. Epler, L. C. Sander, R. G. Ziegler, S. A. Wise, N. E. Craft, J. Chromatogr., 595, **1992**, p.89