Carotenoids Extraction from Japanese Persimmon (Hachiya-kaki) Peels by Supercritical CO₂ with Ethanol

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The extraction of carotenoids from Japanese persimmon peels by supercritical fluid extraction (SFE), of which the solvent was CO₂, was performed. In order to enhance the yield and selectivity of the extraction, some portion of ethanol (5–20 mol%) was added as an entrainer. The extraction temperature ranged from 313 to 353 K and the pressure was 30 MPa. The effect of temperature on the extraction yield of carotenoids was investigated at 10 mol% of the ethanol concentration in the extraction solvent, and a suitable temperature was found to be 333 K among the temperatures studied with respect to the carotenoid yield. With increasing the entrainer amount from 0 to 10 mol% at a constant temperature (333 K), the carotenoid yield in the extraction was improved, whereas the selectivity of the extracted carotenoids was drastically depressed. We also conducted qualitative and quantitative analyses for the carotenoid components in the extract by HPLC, and analyzed the extraction behavior of each individual carotenoid (α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin). The selectivity of each carotenoid changed with the elapsed time and its time evolution was dependent on the carotenoid component, indicating that the location profile and the content can be important factors to understand the SFE behavior of each carotenoid in persimmon peels.

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Introduction

Carotenoids, which are natural pigments contained in green-yellow vegetables and edible fruits, are important nutrients for the human body owing to their provitamin A and antioxidant activities. For example, β -carotene is a precursor of vitamin A, which is well known to be capable of preventing serious eye diseases, such as night blindness. Lycopene is a precursor of β -carotene and a strong antioxidant effective for skin, liver, and other types of cancers. Lutein is found to be a preventive agent for various ophthalmopathy. These carotenoid constituent contents in natural plants are quite dependent on the plant species. For example, in a carrot, the content of β -carotene was the largest (60–80%) among the involved carotenoids, followed by α -carotene (10–40%), lutein (1–5%) and the others (0.1–1%).¹ While in an edible part of a persimmon, the content of β -cryptoxanthin was the highest (~50%), followed by lycopene (~10%), β -carotene (~10%), zeaxanthin (~5%), lutein (~5%), and others.²

Typically, carotenoids are generally ingested by eating and drinking. However, ingestion always allows contamination of the other components, such as fibers, sugars, proteins, and fats, leading to high-caloric diets. Another possible ingestion is to take supplemental concentrated carotenoids produced by extraction and separation from the matrix of natural plants. The intake of each carotenoid *via* a supplement is also a good concept for the utilization of waste materials, such as fruit peels, because

removed peels also have active constituents, such as carotenoids.

In Japan, persimmons are consumed directly as fresh fruits and partly as treated products. A typical treated one is “dried persimmon”, produced by stripping the peel of persimmon and drying it in the shade. Fifty tons of stripped peel were produced yearly as a by-product in Miyagi prefecture, one of the main producers of “dried persimmon” in Japan, and was completely burned without effective utilization. However, persimmon peels are known to be rich in carotenoids compared with the edible parts, which indicates that the waste peels would be good materials for carotenoid extraction.

Carotenoids are principally oxidized very easily³ because of their strong antioxidant ability described above. In addition, carotenoids are also sensitive to light and heat. These characteristics of carotenoids reduce the possible range of extraction methodologies; during organic solvent extraction is difficult to prevent the exposure of carotenoid to air and light, and there is also an inevitable solvent residual problem. Hot water can not be a candidate of extraction because of thermal degradation. Supercritical CO₂ extraction (SFE-CO₂) is a promising method to recover carotenoids from natural plants instead of organic solvent and hot water. SFE-CO₂ is a high-pressure technology and is principally operated as a closed process without contacting air and light. The operation temperature is normally around room temperatures due to the critical point of CO₂ (304.2 K, 7.4 MPa). In addition, CO₂ is gaseous under ambient condition and easy to be completely separated from the extracted material only by depressurization. Therefore, many researchers have studied the extraction of carotenoids from various natural products.^{4–15} However, only a

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Fig. 1 Structure of carotenoids in a peel of persimmon.

few carotenoids (β -carotene, lycopene and lutein)^{1,4-9,12-14} have been reported in the literature. In other words, the extraction behaviors of other carotenoids (such as α -carotene, β -cryptoxanthin and zeaxanthin) have not been evaluated in detail.

Sanal *et al.* extracted β -carotene from an apricot pomace by supercritical CO_2 (sc- CO_2) with the addition of ethanol or water as an entrainer,¹⁴ and investigated the effects of the temperature, pressure, and concentration of the entrainer on the solubility of β -carotene. According to their results, 30 MPa was estimated to be optimum for β -carotene extraction at temperatures below 338 K with 15 v/v% of ethanol in their examination. The yield of β -carotene extraction increased nonlinearly with increasing ethanol concentration, and the β -carotene yield was significantly improved between 10 and 15 v/v% ethanol additions. This may be due to a change in the solvent polarity due to the addition of ethanol. On the other hand, water was found to not be effective for the extraction of β -carotene. Sun and Temelli investigated the entrainer effect of water on the extraction of carotenoids from a carrot, and observed that water improved the yield of lutein extraction, whereas the extraction yields of α - and β -carotene were depressed in the presence of water.¹ They discussed that the improvement of lutein extraction was due to the interaction between the OH groups and water molecules, *via* hydrogen bonding. However, this discussion cannot rationalize the above-mentioned β -carotene extraction results on the apricot pomace by Sanal *et al.*, indicating that the entrainer effect may result from complicated factors.

Sanal *et al.* proposed another role of the entrainer: the entrainer molecules adsorb at the active site of the matrix, resulting in loosening the matrix structure and consequently promoting desorption of the solute from the matrix.¹⁴ A similar effect of an entrainer on SFE of carotenoids from a carrot was suggested by Sun and Temelli;¹ they evaluated the effect of a canola oil as entrainer on carotenoids extraction and confirmed that the addition of a canola oil enhanced the extraction yield of carotenoids. Sun and Temelli proposed that the effect of canola oil as the entrainer was due to interactions between the canola oil and the carrot matrix, leading to the release of carotenoids

Fig. 2 Flow diagram of installation for the extraction of carotenoid with supercritical fluid. 1, CO_2 cylinder; 2, 5, 8, valve; 3, pump; 4, cooler; 6, ethanol; 7, solvent pump; 9, preheating part; 10, extractor; 11, needle valve; 12, trap; 13, dry gas meter.

from the matrix, as well as a suggestion by Sanal *et al.*

In this study, we firstly determined the constituents of a peel of Japanese persimmon, "Hachiya-kaki". Then, the extraction of carotenoids from the peel by using sc- CO_2 was performed at temperatures ranging from 314 to 353 K and a pressure of 30 MPa. Ethanol was added at 5–20 mol% as an entrainer. The extraction behavior of each carotenoid constituent (α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin, Fig. 1) was quantitatively analyzed by weighing and HPLC with tuning the column and effluent conditions.¹⁶

Experimental

Material

A peel of "Hachiya-kaki", a Japanese persimmon, was provided by Industrial Technology Institute, Miyagi Prefectural Government. The freeze-dried peel was milled before use. Acetonitrile (> 99.8% of purity), chloroform (> 99.0% of purity), ethanol (> 99.5% of purity), methanol (> 99.8% of purity), pirogarol (> 99% of purity), tetrahydrofuran (THF, > 99.8% of purity), α -tocopherol (> 98% of purity) were purchased from Wako Pure Chemical Co. and used for analyzing the sample as received. CO_2 (99.9%) was distributed by Taiyo Nissan Co. Ltd.

Organic solvent extraction (Folch method)

The Folch method was employed in order to determine the amount of each ingredient (especially carotenoids) in the sample. Since the details of the Folch method can be found elsewhere,¹⁷ the brief procedure of the Folch method is explained. The used solvent was a mixture of chloroform and methanol (the volume ratio of chloroform to methanol was 2). Five grams of the peel, 3 g of pirogarol (an antioxidant for preventing the sample oxidizing), and a 10-mL portion of the extraction solvent were placed together in a mortar and the mixture was ground by a pestle. The ground sample solution was separated into the extracted liquid and the residual solid by an ultracentrifuge. The residual solid was then ground with another 10 mL of the solvent. These treatments were repeated twice. The extracted liquid at each treatment was collected in a vial. The volume of the collected liquid was finally adjusted to 100 mL by adding the extraction solvent, and the diluted liquid sample was defined as "the extract" in the Folch method. A portion of the extract was analyzed by high-performance liquid chromatography (HPLC), of which the condition is mentioned below.

Supercritical CO_2 extraction

Figure 2 shows the setup for the high-pressure supercritical

Table 1 Flow rate of EtOH and CO₂ under various extraction conditions

Concentration of EtOH, mol%	Flow rate of CO ₂ at exit/L min ⁻¹	Flow rate of EtOH at entrance/ml min ⁻¹
0	2	0
5	1.9	0.25
10	1.8	0.5
20	1.6	0.99

CO₂ extraction (SFE-CO₂) system. The volume of the extraction cell was 50 mL. Ten grams of the crushed peel were loaded with a 15-mL portion of glass beads (ϕ 2.1 mm). The glass beads, considered to be an inert material for extraction, were added to enable a homogeneous distribution of the sample in the cell, and also helped to reduce the dead-space volume inside the extraction cell. The extraction cell was placed in an electric oven to keep the temperature constant. CO₂ was provided at a constant flow rate into the extraction cell by a HPLC pump (SCF-get, JASCO), of which the pump-head was cooled by an electric device to liquefy CO₂ from a gas cylinder. In the case of using ethanol as an entrainer, ethanol was supplied by another HPLC pump and mixed with CO₂ before entering the extraction cell. The concentration of ethanol in the CO₂ gas was controlled by changing the flow rate of CO₂ and ethanol. The pressure of the gaseous stream was controlled at 30 MPa by adjusting the backpressure regulator (880-81, JASCO). The backpressure regulator valve was warmed to avoid CO₂ solidification caused by the Joule-Thomson effect. At an interim period, the effluent from the extraction cell was collected in a sample trap that was cooled in an acetone-dry ice bath. After passing the desired elapsed time, the residual in the tubes was washed by ethanol and collected in another sample trap.

The conditions of SFE-CO₂ are listed in Table 1. The extraction process was mainly affected by the solute solubility, which was sensitive to the CO₂ density, the entrainer species and its concentration. In this study, the extraction yield was focused on the effects of the temperature and the entrainer concentration under a constant pressure (30 MPa). Ethanol was chosen as the entrainer because of its safety and its polarity. The flow rate of supercritical CO₂ (sc-CO₂) was set to 2 L (STP)/min in the case without ethanol. In the presence of ethanol, the total mole flow rate of a CO₂-ethanol mixture was set to being constant (0.085 mol/min) by adjusting the flow rates of CO₂ and ethanol.

Analysis

The composition of carotenoids in the extracts by organic solvent extraction or the SFE-CO₂ was analyzed by an HPLC method (Agilent, Model HPLC 1100) with a UV-vis detector, of which the wavelength was 455 nm. The mobile phase was a mixture of acetonitrile/methanol/tetrahydrofuran (55:40:5, v/v/v),¹⁶ which included 50 μ g/ml of α -tocopherol as an antioxidant. The column used in the HPLC system was an ODS column (silica C18M4D, Shodex), of which the size was ϕ 4.6 $\times$ 150 mm. The column oven temperature was set of 40°C.¹⁶

Wingerath *et al.* reported that some carotenoids having OH groups in the molecule formed esters with a fatty acid, and that such esters were transformed to free carotenoids *via* saponification by KOH.¹⁸ Accordingly, we estimated that carotenoids having OH groups in the peel of a persimmon also formed esters, so they were analyzed after saponification in this study.

Fig. 3 Typical HPLC chromatograms (a) before and (b) after saponification. 1, Lycopene; 2, α -carotene; 3, β -carotene; 4, carotenoid ester; 5, lutein + zeaxanthin; 6, β -cryptoxanthin.

Results and Discussion

Figure 3 shows typical HPLC chromatograms before (Fig. 3a) and after saponification (Fig. 3b). In Fig. 3a, large peaks can be found around a retention time of 40 min. On the other hand, after saponification (Fig. 3b), the peak at 40 min disappeared and new peaks around 2 and 5 min were detected. Through an analysis with standard carotenoid samples, we confirmed that the peaks appearing after saponification were lutein, zeaxanthin and β -cryptoxanthin. The assignments of the other carotenoids were done based on the retention time of each standard sample, and quantitative analyses for the assigned carotenoids were conducted by a calibration with a known amount of standard samples. However, it was difficult to directly determine the quantity of lutein and zeaxanthin because their peaks were overlapped. Then, the deconvolution method was used to divide the overlapped peak into two contributions with assuming that each contribution was a Lorentzian curve, and held the retention time from the qualitative analysis of each pure standard sample. Table 2 lists the carotenoid contents in the extracts of the organic solvent extraction (the Folch method). We assumed in this work that the contents in Table 2 represent the whole amount of the carotenoids in the persimmon peel, and that the yield of each carotenoid by the SFE could be evaluated by the following equation:

$$\text{Carotenoid yield}[-] = \frac{\text{Extracted carotenoid } [\mu\text{g}]}{\text{Carotenoid in the peel } [\mu\text{g}]} \quad (1)$$

The selectivity of the carotenoid extraction was defined as:

$$\text{Carotenoid selectivity}[-] = \frac{\text{Extracted carotenoid } [\mu\text{g}]}{\text{Amount of the whole extract } [\mu\text{g}]} \quad (2)$$

Table 2 Carotenoid contents in the extract of the organic solvent extraction

β -Carotene	α -Carotene	Lycopene	β -Cryptoxanthin	Lutein	Zeaxanthin	Total
70.3	5.37	5.74	108	103	100	392

Unit is $\mu\text{g/g}$ sample.

Fig. 4 Temperature effect on the (a) amount of extract, (b) extraction yield of whole carotenoids and (c) selectivity of whole carotenoids at 10 mol% of ethanol concentration. Carotenoid yield $[-] = \text{extracted } [\mu\text{g}] / \text{carotenoid in the peel } [\mu\text{g}]$, carotenoid selectivity $[-] = \text{extracted carotenoid } [\mu\text{g}] / \text{amount of the whole extract } [\mu\text{g}]$.

Effect of temperature: in view of solubility

Figure 4 shows the effect of temperature on the total amount of the extract (Fig. 4a), the extraction yield of whole carotenoids (Fig. 4b), and the selectivity of the carotenoids in the whole extracts (Fig. 4c). The temperature ranged from 313 to 353 K. The ethanol concentration was 10 mol% and the pressure was 30 MPa. For all of the temperatures, the total amount of the extract and the yield of whole carotenoids proportionately increased with increasing the extraction time at an early stage of the extraction (~ 300 min). At a late stage of extraction (>300 min), the carotenoid yield and the selectivity approached constant values. As shown in Fig. 4b, at an early stage, the extraction yield was observed to be insensitive to the temperature, and became sensitive to the temperature at a late stage. The maximum yield (carotenoid yield: 0.8) was observed

at 333 K for 720 min. According to a previous report,⁶ the total extraction yield was generally related to the solubility of the extracts into sc- CO_2 , the higher carotenoid yield at 333 K than at 353 K was possibly due to the balance between an increase in the vapor pressure of the carotenoids and a decrease in the CO_2 density with increasing temperature. It should be noted that the extraction yield was highest at 333 K, and the carotenoid selectivity was lowest at 333 K among the experimental conditions.

Next, for understanding the extraction behavior of each carotenoid, we compared the extraction behavior of the carotenoid constituents in the persimmon peels by focusing on their molecular structures, namely α -carotene, β -carotene, and lycopene have no OH group; β -cryptoxanthin has one OH group; lutein and zeaxanthin have two OH groups. The carotenoids having OH groups (β -cryptoxanthin, lutein and zeaxanthin) were extracted as those esters, and thus the solubility of the esters should be considered. Figures 5 and 6 show the temperature dependence of the six kinds of carotenoids: Fig. 5a is the result of α -carotene, Fig. 5b is β -carotene, and Fig. 5c is lycopene; these are the results of the carotenoids containing no OH group; Fig. 6a is β -cryptoxanthin, Fig. 6b is lutein and Fig. 6c is zeaxanthin, which have OH groups. The yields of α -carotene (Fig. 5a), β -cryptoxanthin (Fig. 6a) and zeaxanthin (Fig. 6c) were affected by the temperature at an early stage of extraction, and were found to be remarkably higher at 313 K than those at 333 and 353 K. However, the extraction yield of each carotenoid was finally insensitive to the temperature at the latter extraction stage. In contrast, lycopene (Fig. 5c) and lutein (Fig. 6b) were obviously affected by the temperature at the late stage of extraction; the final extraction yields of lycopene at 333 and at 353 K were remarkably higher than that at 313 K, and that of lutein at 333 K was higher than those at 313 and 353 K. The extraction behavior of β -carotene (Fig. 5b) was not very sensitive to the temperature in the whole extraction stage. The maximum extraction yields of carotenoids having no OH group (α -carotene, β -carotene, and lycopene) were in the following order: lycopene (0.9 at 333 K) $>$ α -carotene (0.65 at 333 K) $>$ β -carotene (0.5 at 333 K). This may be considered to be due to the temperature dependence of the solubility in these carotenoids, although the mass-transfer phenomena had some effect on the extraction behavior. Generally, it is widely acceptable that similar carotenoids have similar solubility into sc- CO_2 , and lycopene; also, carotenes are very similar in molecular structure. However, the extraction results indicated that their solubility into sc- CO_2 might be different in magnitude, and also temperature dependent, which may originate from the carotenoid-carotenoid interaction, vaporization capability. Unfortunately, vapor-pressure data for supporting the above discussion have not been available in the literature, but limited melting-point data (such as 449 K for lycopene and 453–455 K for β -carotene) may be one informative evidence to support the discussion on the extraction behaviors of carotenoids by SFE- CO_2 . A similar discussion was reported by Cadoni *et al.* through the extraction of β -carotene and lycopene from ripe tomatoes by sc- CO_2 at 313–353 K and 18–28 MPa without

Fig. 5 Temperature effect on the extraction yield of carotenoids having no OH group at 10 mol% ethanol concentration. (a) α -Carotene, (b) β -carotene, (c) lycopene.

Fig. 6 Temperature effect on the extraction yield of carotenoids having an OH group at 10 mol% of the ethanol concentration. (a) β -Cryptoxanthin, (b) lutein, (c) zeaxanthin.

entrainer.⁶ They observed that the temperature dependence of the β -carotene extraction was not significant, whereas lycopene extraction was quite sensitive to the extraction temperature; namely the extraction yield (0.29) of lycopene at 333 K was 5-times higher than that (0.06) at 313 K, and reached 1.0 at 353 K.⁶ They estimated that the possible reason for this temperature dependence was their crystalline shapes.¹⁹

For carotenoids with OH groups (β -cryptoxanthin, lutein, and zeaxanthin), the maximum yields were in the order of β -cryptoxanthin (1.1 at 333 K) > lutein (0.9 at 333 K) > zeaxanthin (0.55 at 313 K). The above order may be closely related to the solubility of these carotenoids, as discussed for those without an OH group, although this is not clear because of a lack of solubility data of carotenoids into sc-CO₂.

Effect of the concentration of ethanol: interaction between the matrix and ethanol

Figure 7 shows the ethanol concentration dependence on the time evolution of the extraction behaviors; Fig. 7a shows the total amount of the extract, Fig. 7b the yield of whole carotenoids and Fig. 7c the selectivity of whole carotenoids. The ethanol concentration ranged from 5 to 20 mol%. The extraction yield without the entrainer is also plotted in these figures. The extraction temperature and pressure were 333 K and 30 MPa, because the temperature (333 K) could provide the

maximum carotenoid yield for sc-CO₂ extraction without the addition of an entrainer, as described in the previous section.

As shown in Figs. 7a, b, and c, the difference in the extraction behavior between 0 and 5 mol% of ethanol was negligibly small, indicating that the low ethanol concentration was not effective for carotenoids extraction. By the addition of 10 mol% of ethanol, the carotenoids yield was significantly enhanced, and an even higher ethanol concentration (20 mol%) was not very effective to improve the extraction compared with the case of 10 mol% ethanol addition. Figure 7c shows the time profile of the selectivity of the carotenoids at the different concentration of the entrainer. It can be clearly observed that the carotenoids selectivity decreased with increasing the extraction time and also the ethanol concentration. As for the selectivity decrease, this may be due to the relative increase of the other extract components in sc-CO₂ with increasing the ethanol concentration (Fig. 7a). These results may indicate that the affinity of the carotenoids for sc-CO₂ was relatively higher than the other ingredients of the persimmon peels (sugars, phospholipids, etc.) and the carotenoids location in the peels was considered to be near the surface from the initial high extraction amount. With increasing ethanol, the polarity of the solvent increased, which promoted dissolution of the polar ingredients, such as sugars and phospholipids, leading to lowering the carotenoid selectivity.

Fig. 7 Concentration effect of ethanol on the (a) amount of extract, (b) extraction yield of whole carotenoids and (c) selectivity of whole carotenoids at 333 K. Carotenoid yield $[-]$ = extracted $[\mu\text{g}]$ /carotenoid in the peel $[\mu\text{g}]$, carotenoid selectivity $[-]$ = extracted carotenoid $[\mu\text{g}]$ /amount of the whole extract $[\mu\text{g}]$.

We then examined the extraction behavior of carotenoids in terms of the structure: as previously mentioned, α -carotene, β -carotene, and lycopene have no OH group; β -cryptoxanthin has one OH group; lutein and zeaxanthin have two OH groups. Figures 8 and 9 show the entrainer concentration dependence of the extraction behaviors of the six carotenoids: Figs. 8a, b, and c are the results of α -carotene, β -carotene, and lycopene, respectively. Figures 9a, b, and c are the results of β -cryptoxanthin, lutein, and zeaxanthin, respectively. As shown in Figs. 8 and 9, the extraction behaviors are strongly dependent on the carotenoid species, and it is difficult to conclude the general entrainer role in the extraction. However, it can be seen that the extraction yields of the carotenoids (α -carotene, β -carotene, β -cryptoxanthin, and lycopene) were increased with increasing the concentration of ethanol. In particular, the extraction of non-polar carotenoids (α -carotene, β -carotene, and lycopene) was remarkably promoted by the addition of 20 mol% of ethanol. In the case of lutein (Fig. 9b), the extraction yield approached a maximum in the case of 10 mol% of ethanol concentration. As shown in Fig. 9c, the entrainer was not effective for zeaxanthin. On these various entrainer effects for each carotenoid, Sanal *et al.* suggested that the entrainer interacted with the matrix, leading to accelerating the desorption

Fig. 8 Concentration effect of ethanol on the extraction yield of carotenoids having no OH group at 333 K. (a) α -Carotene, (b) β -carotene, (c) lycopene.

of carotenoids from the matrix.¹⁴ According to Sanal *et al.*, it can also be possible to estimate the entrainer role as follows. The non-polar carotenoids (α -carotene, β -carotene, and lycopene) were assumed to mainly locate on the absorptive site of ethanol. On the other hand, the location of zeaxanthin was possibly a different site.

To examine the entrainer role in the extraction in more detail, the composition of carotenoids in each trap was plotted against the elapsed time for the extraction sample at 333 K and concentration of ethanol 10 mol% in Fig. 10. The composition of β -cryptoxanthin did not change with the extraction time, and was almost constant composition. The compositions of α -carotene and lycopene were fairly low every minute. On the other hand, the composition of β -carotene gradually decreased with the extraction time. Lutein and zeaxanthin exhibited strongly time-dependent behaviors, but their trends were completely converse. Lutein was extracted at an early stage of extraction, and its composition gradually decreased with time. In contrast, the composition of zeaxanthin increased at a late stage of extraction. This difference in the extraction behavior of these two carotenoids may be attributable to the existence of structure and location in the matrix, as mentioned above. However, we cannot make any conclusive discussion at this moment. To make this issue clearer, further data acquisition should be required with respect to the carotenoid solubility and extraction selectivity

Fig. 9 Concentration effect of ethanol on the extraction yield of carotenoids having an OH group at 333 K. (a) β -Cryptoxanthin, (b) lutein, (c) zeaxanthin.

in SFE- CO_2 . Finally, we would like to mention that a better understanding of the role of an entrainer in SFE- CO_2 can provide the possibility for the selective separation of carotenoids from natural plants, like persimmon peels, in the future.

Conclusion

We performed the SFE- CO_2 of carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin) from Japanese persimmon peels. The SFE- CO_2 with 10 mol% of ethanol showed the best performance with respect to the carotenoid yield in the studied conditions. However, the selectivity of the carotenoids was depressed with increasing the concentration of the entrainer. The extraction behavior of each carotenoid was separately evaluated and the entrainer role was estimated for carotenoid extraction from the viewpoints of solubility, and also the location of the carotenoids in the matrix. However, a limited number of data could not produce a clear elucidation of the entrainer effect on the SFE- CO_2 from natural plants, such as persimmon peels, used in this work. In the future, the solubility of each carotenoid and the interaction of an

Fig. 10 Composition of carotenoids in each trap against the elapsed time for the extraction sample at 333 K and the concentration of ethanol 10 mol%.

entrainer with a matrix should be determined to evaluate the appropriateness of the estimation in this work.

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