

## Supercritical Fluid Extraction of Palm Carotenoids

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**Abstract:** The extraction of carotenoids from crude palm oil was carried out in a dynamic (flow-through) supercritical fluid extraction system. The carotenoids obtained were quantified using off-line UV-visible spectrophotometry. The effects of operating pressure and temperature, flow rate of the supercritical carbon dioxide (SC-CO<sub>2</sub>), sample size of feed used on the solubility of palm carotenoids were investigated. The results showed that the extraction of carotenoids was governed by its solubility in the SC-CO<sub>2</sub> and can be enhanced by increasing pressure at a constant temperature or decreasing temperature at a constant pressure. Increasing the flow rate and decreasing the sample size can reduce the extraction time but do not enhance the solubility. Palm carotenoids have very low solubility in SC-CO<sub>2</sub> in the range of  $1.31 \times 10^{-4} \text{ g kg}^{-1}$  to  $1.58 \times 10^{-3} \text{ g kg}^{-1}$  for the conditions investigated in this study. The experimental data obtained were compared with those published by other workers and correlated by a density-based equation as proposed by Chrastil.

**Keywords:** Carbon dioxide, palm oil, pressure, solubility, temperature

### INTRODUCTION

Carotenoids have been proven to be beneficial to human health apart from having pro-vitamin A which prevents xerophthalmia, a night blindness disease. Carotenoids also play an important role as anti-oxidant by scavenging free radicals<sup>[1]</sup> and as singlet oxygen quencher<sup>[2]</sup>. Carotenoids are also found to be capable of inhibiting growth of certain cancer cells such as the colon cancer<sup>[3]</sup>.

The commercially available carotenoids, mainly  $\beta$ -carotene supplements are produced by synthetic means. Globally, there is a rising trend that people are going for natural products including vitamins and carotenes. The synthetic carotenoids especially  $\beta$ -carotene taken as a supplementation is unlike natural carotenoids which is a mixture of carotenoids compounds, many of which may not be readily metabolized rapidly<sup>[4]</sup>.

Palm oil is the richest plant source of carotenoids in terms of retinol equivalent with 500-700 ppm. A total of 11 types of carotenes have been identified in palm oil<sup>[5]</sup> as shown in Table 1. However, these carotenoids are destroyed by conventional refining. In light of the importance of natural carotenoids, various methods have been investigated to recover the carotenoids from palm oil before refining. These include saponification<sup>[6]</sup>, adsorption<sup>[7]</sup>, solvent extraction<sup>[8]</sup> and transesterification of palm oil followed by molecular distillation<sup>[9]</sup>. However, these processes involve the usage of organic solvents, chemical modifications and

generate waste that has to be treated before it is allowed to be discharged to watercourse.

Table 1: Various types and composition of carotenes in palm oil

Types of carotenes	Composition (%)
Phytoene	1.27
<i>Cis</i> - $\beta$ -Carotene	0.68
Phytofluene	0.06
$\beta$ -Carotene	56.02
$\alpha$ -Carotene	35.16
<i>Cis</i> - $\alpha$ -Carotene	2.49
$\zeta$ -Carotene	0.69
$\gamma$ -Carotene	0.33
$\delta$ -Carotene	0.83
Neurosporene	0.29
$\beta$ -Zeaxanthene	0.74
$\alpha$ -Zeaxanthene	0.23
Lycopene	1.30

Over the last two decades, supercritical fluid extraction (SFE) has been well received as green and promising technology for extraction of natural products<sup>[10]</sup>. Instead of organic solvents, supercritical carbon dioxide (SC-CO<sub>2</sub>) is used. Above its critical pressure and temperature, carbon dioxide behaves both like solvent and gas with higher diffusivity and density but lower viscosity<sup>[11]</sup>. The most significant advantages of using SC-CO<sub>2</sub> are (i) its chemical inertness; (ii) its non-toxic, non-hazardous and non-inflammable characteristics; (iii) it leaves no solvent residue in the products and (iv) the process generates no waste, therefore it is environment benign technology.

The solubility of carotenoids has been well established. However, most of the published data

involved the use of synthetic  $\beta$ -carotene as the starting material<sup>[12-18]</sup>. It has been reported that discrepancies in solubility data measurements can be due to numerous factors including degradation of carotenoids<sup>[19,20]</sup>, purity of starting material<sup>[18]</sup> and method of measurement<sup>[17]</sup>.

Since palm oil is a natural source of carotenoids, the determination of solubility of palm carotenoids in SC-CO<sub>2</sub> is important to study the possibility to extract the carotenoids. Markom *et al.*<sup>[21]</sup> reported the solubility of palm carotenoids in SC-CO<sub>2</sub> at 20 MPa for temperatures of 40, 50 and 60°C. They found that it was difficult to extract  $\beta$ -carotene from palm oil even at high pressure of 20 MPa and temperature had little effect on the solubility of  $\beta$ -carotene in SC-CO<sub>2</sub>. Ooi *et al.*<sup>[22]</sup> reported a counter-current column processing of palm oil. The refined oil produced in the raffinate from the process has higher content of carotene. De Franca *et al.*<sup>[23]</sup> reported the SFE of carotenoids from buriti, an Amazon-region palm fruit under limited extraction conditions of 313K and 328K at pressures of 20 and 30 MPa. They found that a large amount of carotenes were extracted in the diffusion-controlled period due to the reason that carotenes are found in the oleaginous fractions located deep inside the solid particles. Birtigh *et al.*<sup>[24]</sup> and de Franca and Meireles<sup>[25]</sup> reported SFE of carotenoids from palm-pressed fibers, a by-product produced during production of crude palm oil (CPO).

## MATERIALS AND METHODS

CPO was obtained from a local palm oil mill. The carbon dioxide with purity of 99.995% was purchased from Malaysian Oxygen Berhad (Selangor, Malaysia). All other solvents used for analysis were of analytical-grade purchased from Merck (Darmstadt, Germany).

**Apparatus:** The SFE system used in this study is shown in Fig. 1. It consists of a 50ml high pressure extraction vessel placed in a column oven (Model CO-960, Jasco, Japan) that can control the temperature to within  $\pm 0.1^\circ\text{C}$ . The pressure of the system was controlled to within  $\pm 0.1$  MPa by a back pressure regulator (Model 880-81, Jasco, Japan). The carbon dioxide was chilled to  $-5^\circ\text{C}$  before a HPLC pump (Model PU-986, Jasco, Japan) was used to pump the liquified carbon dioxide into the extractor at a constant flow of  $5.0\text{ mL min}^{-1}$ , measured at the pump as compressed carbon dioxide.

**Methods:** CPO (20g) was weighed accurately and loaded into the extraction vessel. The extraction was carried out in a dynamic (flow-through) system. It is assumed that a steady state solute-solvent equilibrium is achieved as the supercritical fluid passes over the solute. Liquid carbon dioxide was pumped continuously into the vessel at the specific extraction conditions. A frit was installed at the outlet of the extraction vessel to

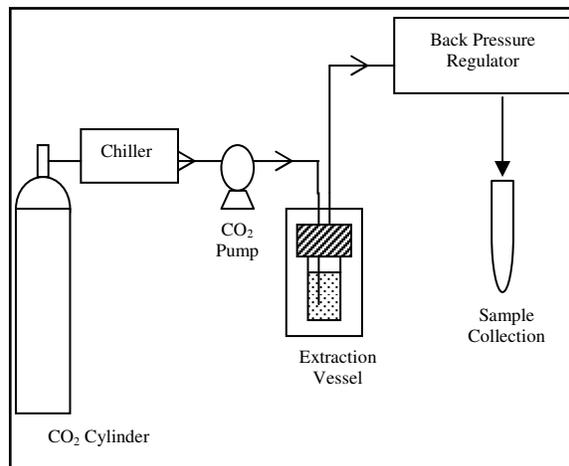


Fig. 1: Flow diagram of supercritical fluid extraction system

prevent any physical carry over of palm oil. The extracts were collected at the outlet of the back pressure regulator at an interval of half hour into container wrapped in aluminium foil. This is to preserve the light-sensitive carotenoids. The extracts were weighed to the nearest  $\pm 0.0001$  g. The samples were blanketed with nitrogen and kept in the dark at  $-10^\circ\text{C}$  prior to analysis to prevent any degradation by heat, air and light.

**Effect of pressure:** The effect of pressure on the extraction of carotenoids from CPO was investigated by conducting the experiments at pressures of 14, 22 and 30 MPa. The flow of carbon dioxide was kept constant at  $5.0\text{ mL min}^{-1}$  throughout the experiment. The low temperature of  $40^\circ\text{C}$  was chosen because of the thermally labile characteristics of carotenoids.

**Effect of temperature:** The effect of temperature on the extraction of carotenoids from CPO was investigated by conducting the experiments at temperatures of 40, 60 and  $80^\circ\text{C}$ . Both the extraction pressure and flow of carbon dioxide were kept constant at 30 MPa and  $5.0\text{ mL min}^{-1}$ , respectively. The temperatures were used due to the similarity of their densities to that of different pressures at  $40^\circ\text{C}$  as shown in Table 2.

**Effect of flow rate:** The effect of flow rate on the extraction of carotenoids from CPO was investigated by conducting the experiments at flow rates of 5.0, 10.0 and  $15.0\text{ mL min}^{-1}$ . The temperature and pressure were the optimum conditions found in previous experiments.

**Effect of sample size:** The effect of sample size of the extraction of carotenoids from CPO was investigated by conducting the experiments at sample sizes of 5 g, 10 g and 20 g. The temperature and pressure were the optimum conditions found in previous experiments.

The flow of carbon dioxide was kept constant at 5.0 mL min<sup>-1</sup>.

**Analysis:** The total carotenoids content was determined using MPOB Test Methods<sup>[26]</sup>. The off-line approach was used to prevent overloading of carotenoids due to increase concentration and larger sample size in the course of extraction. The sample was homogenized and weighed to the nearest  $\pm 0.0001$  g into a 25 ml volumetric flask. The sample was dissolved with *n*-hexane and diluted to the mark. The solution was transferred into a 1 cm quartz cuvette and the absorbance was measured at 446 nm against *n*-hexane using UV-visible spectrophotometry (Spectrophotometer Hitachi U-2000). The total carotenoids content was expressed as ppm of  $\beta$ -carotene. The calculation was as follows:  
Carotenoids content =  $[V \times 383 \times (A_s - A_b)] / (100 \times W)$

Where:

V is the volume used for analysis  
383 is the extinction coefficient for carotenoids  
 $A_s$  is the absorbance of the sample  
 $A_b$  is the cuvette error  
W is the weight of the sample in g

## RESULTS AND DISCUSSION

The initial total carotenoids content in CPO was 650 ppm.

**Effect of pressure and temperature:** The effect of pressure at 40°C on the recovery of palm carotenoids (%) as a function of amount of carbon dioxide (g) is depicted in Fig. 2. It shows that solubility of palm carotenoids increased with pressures at a constant temperature. At a constant temperature, increasing the pressure increases the density of the SC-CO<sub>2</sub>. The solvent strength of SC-CO<sub>2</sub> increases with the density and thus increases the solubility of carotenoids. This suggests that the solubility of palm carotenoids in SC-CO<sub>2</sub> is proportional to the density of SC-CO<sub>2</sub>. However, it was not a linear relationship as shown in Table 2. The solubility of carotenoids increased by >600% from  $1.31 \times 10^{-4}$  to  $9.41 \times 10^{-4}$  g kg<sup>-1</sup> as pressure was increased from 14 to 22 MPa. However, the increment was about 60% from  $9.41 \times 10^{-4}$  to  $1.53 \times 10^{-3}$  g kg<sup>-1</sup> when pressure was increased from 22 to 30 MPa. As discussed by King<sup>[27]</sup>, there is a pressure region where significant increase occurs in the solubility of the solute in supercritical fluid. Thus, from this study it was found that the pressure region for SFE of carotenoids from CPO was in the range of 14-22 MPa.

At a constant pressure, increasing the temperature reduces the density of SC-CO<sub>2</sub>. As carotenoids have no significant vapor pressure, raising the extraction temperature decreases the solubility due to the reduction of solvent density.

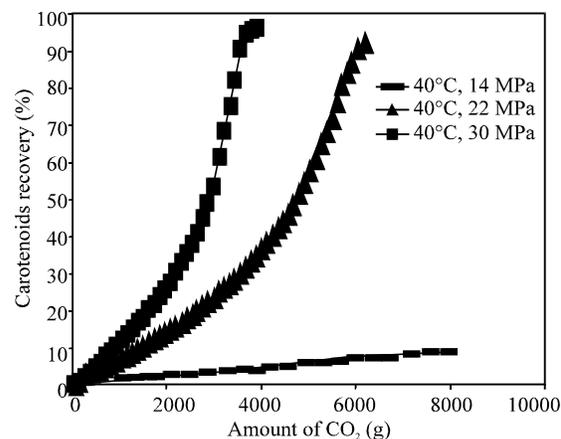


Fig. 2: Effect of pressure at constant temperature of 40°C on the solubility of palm carotenoids in SC-CO<sub>2</sub>

The solubility of palm carotenoids exhibits retrograde behavior as the solubility was inversely proportionate to the temperatures. However, the influence of temperature on the solubility of carotenoids was insignificant as compared to the influence of pressure. These are shown in the solubility of carotenoids for different temperature at 30 MPa with same order of magnitude (Table 2). The solubility of carotenoids reduced from  $1.53 \times 10^{-3}$  to  $1.24 \times 10^{-3}$  g kg<sup>-1</sup> as the temperature was increased from 40 to 80°C. It was also observed that the recovery of carotenoids from CPO was 80% at high temperature of 80°C compared to 40 and 60°C with >90% recovery. This could be contributed to degradation of selected carotenoids as had been reported by several authors<sup>[19,20]</sup>. Our results were conflicting to that reported by Johannsen and Brunner<sup>[14]</sup> as well as Sovová *et al.*<sup>[19]</sup>. Both studies were conducted at temperatures of 40, 60 and 80°C. They reported that increased in temperature resulted in an increased of solubility of  $\beta$ -carotene. The contradiction can be contributed by the presence of other more soluble components in palm oil which are co-extracted with carotenoids at high temperature resulted in lower carotenoids solubility in the present study. Thus, it is noted that solubility of carotenoids in SC-CO<sub>2</sub> from synthetic form and from natural sources are significantly different.

The results showed that at similar densities of the SC-CO<sub>2</sub>, the solubility of carotenoids was significantly different (Table 2). For similar densities of 762.0 kg m<sup>-3</sup> (40°C and 14 MPa) and 739.8 kg m<sup>-3</sup> (80°C and 30 MPa), it was found that the solubility of carotenoids differed by one order of magnitude with  $1.31 \times 10^{-4}$  and  $1.24 \times 10^{-3}$  g kg<sup>-1</sup>, respectively. This suggests the misconception of using density of the supercritical fluid alone in explaining the solubility of solutes. Thus, the combination of temperature and pressure plays a more important role than density alone in manipulation of solubility of solute in supercritical fluids. However, the use of density to describe the solubility of solute is valid at a constant temperature or a constant pressure.

Table 2: Solubility of Carotenoids from Crude Palm Oil in SC-CO<sub>2</sub>

Temperature (°C)	Pressure (MPa)	Density of SC-CO <sub>2</sub> (kg m <sup>-3</sup> )	Feed Size (g)	Flow rate of SC-CO <sub>2</sub> (mL min <sup>-1</sup> )	Solubility of Carotenoids x 10 <sup>-3</sup> (g kg <sup>-1</sup> )
40	14	762.0	20	5	0.131
40	22	861.2	20	5	0.941
40	30	912.4	20	5	1.53
60	30	833.5	20	5	1.33
80	30	739.8	20	5	1.24
40	30	912.4	10	5	1.57
40	30	912.4	5	5	1.58
40	30	912.4	20	10	1.54
40	30	912.4	20	15	1.56

Table 3: Model parameters for carotenoids estimated using Chrastil model at 40°C

References	k	a	b	ΔH (J mol <sup>-1</sup> )
This study	8.08	4217	-19.22	3.5x10 <sup>4</sup>
Sakaki <sup>[12]</sup>	9.3	NA	-34.28*	7.2x10 <sup>4</sup>
Škerget <i>et al.</i> <sup>[13]</sup>	5.47	6287.9	-15.43	5.2x10 <sup>4</sup>
Güçlü-Üstündağ and Temelli <sup>[30]</sup>	7.2	-8844.3	-26.8	NA
Subra <i>et al.</i> <sup>[17]</sup> (37°C)	9.9	NA	NA	NA

$$*b = q - \ln \left( \frac{[1000M_B]^k}{[M_A + kM_B]} \right)$$

NA= not available

**Effect of sample size and flow rate:** The effect of sample size on the recovery of palm carotenoids (%) as a function of time (hour) is shown in Fig. 3. From Fig. 3, reduction of sample size from 20 g to 10 g reduced the extraction time to half from 17 hours to 9 hours. It was found that the sample size of CPO loaded into the extraction vessel was linearly proportional to the rate of carotenoids extraction. The solubility of carotenoids for the three sample sizes were in the range of 1.53 x 10<sup>-3</sup> to 1.58 x 10<sup>-3</sup> g kg<sup>-1</sup> carbon dioxide (Table 2). It was observed that the solubility of carotenoids in SC-CO<sub>2</sub> was independent of the sample size of the CPO loaded into the extraction vessel. The results showed that the removal of carotenoids from the extraction vessel by supercritical fluid is the rate-determining step that depends on the volume of the fluid.

The results are further supported by the effect of flow rate on the recovery of carotenoids (%). The higher flow rate applied resulted in faster extraction rates thus shorter extraction time. Linear correlation was observed for the flow rates and the extraction rates. Double the flow rate from 5 to 10 mL min<sup>-1</sup> reduced the extraction time to half. This confirmed that rapid diffusion of carotenoids from the oil matrix and the extraction is controlled by the ability of SC-CO<sub>2</sub> for solvation. Again the solubility of palm carotenoids were not significantly affected by the flow rate employed with solubility in the range of 1.54 x 10<sup>-3</sup> to 1.58 x 10<sup>-3</sup> g kg<sup>-1</sup> carbon dioxide (Table 2).

The results disagree with those of de Franca *et al.*<sup>[23]</sup> that reported that large amount of carotenes were extracted in the diffusion-controlled period. As the flow rates investigated were in the range of 0.29-0.43 g s<sup>-1</sup> were relatively narrow and thus the effect was insignificant. This can be attributed by the different matrix used in both studies.

**Comparison with literature:** The solubility of carotenoids found in the present study is in the range of 1.31 x 10<sup>-4</sup> to 1.58 x 10<sup>-3</sup> g kg<sup>-1</sup> for pressures investigated (Table 2). The solubility is one order of magnitude lower than those reported by Johannsen and Brunner<sup>[14]</sup> with 1 x 10<sup>-3</sup> to 4 x 10<sup>-2</sup> g kg<sup>-1</sup>). High purity *trans*-β-carotene of 95% was used in their study while our study determined the solubility of carotenoids from CPO. Thus, the competing effect of other more soluble components present in CPO may contribute to lower solubility of carotenoids in SC-CO<sub>2</sub>.

The mole fractions of carotenoids soluble in SC-CO<sub>2</sub> at 40°C (unless otherwise stated) from various studies are shown in Fig. 4. It was found that solubility data reported by Cygnarowicz *et al.*<sup>[15]</sup> was the lowest published data and disagreed with other workers. They used a recirculating loop with an in-line UV detector to investigate the solubility of pure crystalline β-carotene. These are the possible reasons for the substantial difference from our data. The solubility data reported by Hansen *et al.*<sup>[28]</sup> was similar to our results at low pressure but at high pressures, their values are lower than ours. Hansen and co-workers determined the solubility of carotenes from carrots with purity >95% which consists of a mixture of isomers of α- and β-carotenes at a ratio of 1 to 2. As the pressure increases, other carotenoids besides the α- and β-carotenes present in palm oil may be more soluble thus increases the solubility.

Sakaki<sup>[12]</sup>, Subra *et al.*<sup>[17]</sup> and Mendes *et al.*<sup>[18]</sup> have similar solubility data although Subra *et al.* worked at 37°C. Their results were relatively higher than our present study. The major discrepancy can be contributed to the purity of β-carotene used and the presence of other more soluble solutes. The solubility determined in our study was the carotenoids present in CPO with 650 ppm (0.065%) while most authors used

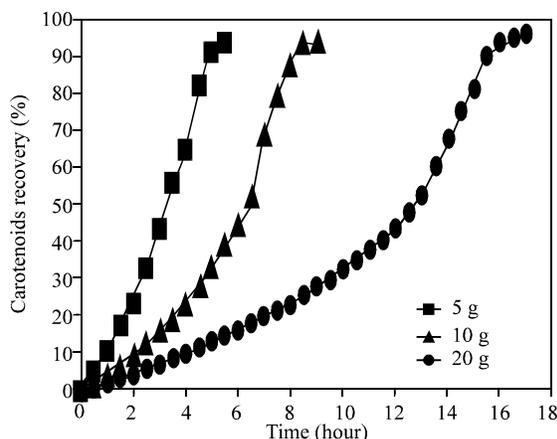


Fig. 3: Effect of sample size on the solubility of palm carotenoids in SC-CO<sub>2</sub>

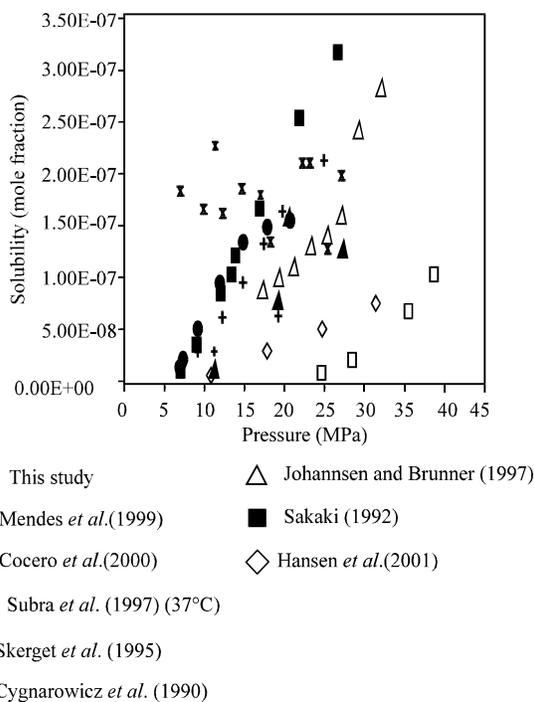


Fig. 4: Solubility of carotenoids in SC-CO<sub>2</sub> at 40°C

synthetic β-carotene with purity of >97%. The results of our study showed that the threshold pressure of SFE of carotenoids from CPO at 40°C was 13 MPa.

**Solubility data and modeling:** The solubility data obtained in the present study was correlated using a density-based equation as proposed by Chrastil<sup>[29]</sup>. This model was based on the direct relationship between solubility and density (concentration) of a solvent. The calculation of the equilibrium concentration was based on the formation of a solvato complex upon the association of a solute with the molecules of the solvent. The equation derived was as follows:  
 $\ln c = k \ln d + a/T + b$

Where:

- C is the concentration of a solute in the solvent (g m<sup>-3</sup>)
- d is the density of the solvent (g m<sup>-3</sup>)
- k is an association number
- a is ΔH/R
- ΔH is total reaction enthalpy
- R is the gas constant
- b is  $\ln (M_A + kM_B) + q - k \ln M_B$
- M<sub>A</sub> and M<sub>B</sub> are the molecular weights of the solute and the solvent, respectively
- q is a constant

Model parameters estimated using the above equation and comparison with other authors using similar models are presented in Table 3. The association number, k from our present study at 40°C is 8.08. This shows that 8.08 molecules of carotenoids were associated with one molecule of CO<sub>2</sub>. The values of our present study fall in the range of reported data.

The total reaction enthalpy, ΔH, found in the present study was in the same order of magnitude with that reported by Sakaki<sup>[12]</sup> and Škerget *et al.*<sup>[13]</sup>. Only a slight deviation in the total reaction enthalpy was observed.

Although some inconsistencies were observed, the variation between literatures can be attributed to (i) purity of samples, (ii) origin of samples, (iii) method for measurement of solubility (i.e. dynamic or static, online or offline), (iv) degradation of carotenoids and (v) isomerisation of carotenoids.

The study shows that carotenoids from CPO has very low solubility in SC-CO<sub>2</sub> at temperatures in the range of 40-80°C and pressures in the range of 14-30 MPa. Its solubility was in the range of 1.31 x 10<sup>-4</sup> to 1.58 x 10<sup>-3</sup> g kg<sup>-1</sup> carbon dioxide. The knowledge of the low solubility of carotenoids provides useful information to possibly exploit SFE for fractionation of valuable components present in CPO.

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