SUPERCRITICAL CO_2 EXTRACTION OF CAROTENOIDS FROM CARROTS AND EVALUATION OF PRODUCTS

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Carotenoids are gaining growing interest in food industries due to their nutritional and colorant properties. The objectives of this study were to extract carotenoids from carrots with supercritical CO_2 (SC-CO_2) and to evaluate the antioxidant activity of the crude carrot oil extract and color of the carrot residue. Crude carrot oil was extracted from freeze-dried carrots with SC-CO_2 at different temperature (40, 55, and 70 °C) and pressure (27.6, 41.3, and 55.1 MPa) conditions for 4 h. Carotenoids in the crude carrot oil were identified and quantified by HPLC analysis. The antioxidant activity of the crude oil was determined using the ferric thiocyanate method. The color parameters ('L', 'a', and 'b') of the carrot residue were determined by HunterLab spectrocolorimeter. Hue, chroma, and the overall color change ΔE between feed material and residue were calculated. The main carotenoids in the crude carrot oil extract were α-carotene, β-carotene, and lutein. The extraction yield of α-carotene was 137.8-330.3 μg/g feed material, β-carotene was 171.7-386.6 μg/g, lutein was 23.5-37.5 μg/g, and total carotenoids was 339.3-745.5 μg/g. The antioxidant activity of the SC-CO_2 extracted carrot crude oil ranged from 24.98 to 43.14, which was approximately half of that of butylated hydroxytoluene BHT (75.04) measured at equal concentrations of crude oil and BHT, which is a commonly used synthetic antioxidant. The intensities of redness 'a' and yellowness 'b' of the carrot particles decreased and the brightness 'L' increased after extraction. Supercritical CO_2 extraction is an efficient technique to obtain natural food colorant with high antioxidant activity.

INTRODUCTION

Due to consumer concerns for food safety and strict government regulations, the consumption of synthetic colorants is decreasing and the demand for natural colorants is growing. Carotenoids are one of the most important natural food colorants. Besides their application as natural colorants, carotenoids also play an important role as food ingredients due to their provitamin A activity and antioxidant function. Theoretically, *trans*-β-carotene possesses 100% vitamin A activity while *trans*-α-carotene possesses 50% vitamin A activity. The antioxidant activity of carotenoids has been associated with reduced risk of lung and colon cancers [1, 2].

The most important sources of carotenoids are fruits and vegetables. Among these, carrots are an excellent source of carotenoids and are commonly consumed. According to Chen *et al.* [3], β-carotene constitutes a large portion (60-80%) of the carotenoids in carrots followed by α-carotene (10-40%) and lutein (1-5%). Carotenoids have been extracted mainly by two extraction methods, namely traditional solvent extraction (TSE) [4-7] and supercritical fluid extraction (SFE) [8-13]. TSE requires multiple steps, consumes a large amount of

organic solvent, and most importantly, extract products have solvent residue. On the other hand, SFE, in most cases supercritical CO_2 (SC-CO_2) extraction, provides an extraction environment with reduced potential for oxidation of carotenoids. The low critical temperature of CO_2 is beneficial for the thermally labile carotenoids. Previous studies [8-13] regarding SFE of carotenoids investigated the effect of extraction conditions on the yield of carotenoids. However, antioxidant activity of carotenoid extracts and color of residue product have not been reported even though these are important characteristics for further product applications. Therefore, the objectives of this study were to extract carotenoids from carrots with supercritical CO_2 at different temperature and pressure conditions and to evaluate the antioxidant activity of the crude carrot oil extract and color of the carrot residue.

MATERIAL AND METHODS

Sample preparation. The carrots (*Apache* variety) were provided by I & S Food Services Ltd., Edmonton, AB. The carrots were washed and chopped into 5 mm cubes at room temperature with a food chopper. The carrot cubes were well mixed and freeze-dried for 5 days to reach a moisture content of ca. 0.8%. The desired particle size distribution (0.5-1 mm) was achieved by grinding the cubes by a Quadro Comil grinder and sieving prior to extraction. All the prepared samples were vacuum packed in several moisture and O_2-barrier bags, then labeled and stored at –18 °C in dark.

TSE. TSE was conducted according to the AOAC Official Method 941.15 [14]. Two grams of freeze-dried carrots were homogenized with a Tissumizer for 5 min at 70 rpm with 100 mL of hexane/acetone (6:4 v/v) containing 0.005% (w/v) BHT. The sample was filtered in vacuo and the residue washed with acetone (2×25 mL) and then with 25 mL of hexane. The combined organic filtrate was washed three times with 100 mL milli-Q water. The organic phase was dried over nitrogen.

SFE. A laboratory scale supercritical fluid extraction system (300 mL cell) was used. The extraction temperature (40, 55, and 70 °C) was monitored by a thermocouple immersed at the centre of the extractor. A stainless steel basket (15 cm × 13 mm I.D.), which fits into the extraction vessel, was fabricated for ease of loading and unloading carrot sample (2 g). CO_2 was compressed to the desired pressure by a diaphragm compressor. The extraction pressure (27.6, 41.3 and 55.1 MPa) was controlled by a back pressure regulator. The flow rate of CO_2 (1 L/min, measured at ambient conditions) passing through the extractor was controlled by manual adjustment of the depressurization valve. The extraction was continued for 4 h. Extracted carrot oil was collected in side-armed glass tubes attached after the depressurization valve, which were held in a refrigerated circulating bath at -20 °C. The extracted oil was transferred into a pre-weighed glass vial by washing the collection tubes with hexane, which was then evaporated under nitrogen flow. The oil samples were weighed and stored at –18 °C until analysis.

HPLC analysis. HPLC analysis of carotenoids was performed using a Shimadzu chromatograph. Supelcosil^{TM} LC-18 column (15 cm x 4.6 cm, 5 µm) was used to separate individual carotenoids at ambient temperature. Samples (50 µL) dissolved in methanol/dichloromethane (1:1 v/v) were injected. Methanol with 10% (v/v) acetonitrile was used as mobile phase. The mobile phase was delivered at a flow rate of 1 mL/min under isocratic conditions. The separated lutein, α-carotene, and β-carotene peaks were monitored by a UV detector at 450 nm. β-Carotene and lutein standards were used for identification and quantification based on a calibration curve. Since it was not possible to obtain α-carotene standard and α-carotene has a response factor similar to that of β-carotene, the concentration of α-carotene in samples was calculated based on the calibration curve of β-carotene.

Antioxidant activity of crude carrot oil extract. The ferric thiocyanate (FTC) method reported by Larrauri *et al.* [15] was used. Mixtures of 0.5 mL of a weighed sample (to provide a final concentration of 0.5 g crude carrot extract or BHT in 1 L assay mixture) in absolute ethanol, 0.5 mL of 2.51% linoleic acid in 99.5% ethanol, 1 mL of 0.05 M sodium phosphate buffer (pH 7.0) and 0.5 mL of distilled water were placed in small vials with screw caps. A control with no test sample addition was also used. The solutions were shaken and an aliquot of 0.1 mL of these solutions was combined with 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixtures, the absorbance was measured against a reagent blank at 500 nm using a spectrophotometer as absorbance at time zero. The remaining portions of the solutions were placed in an oven at 40 °C in dark. The absorbances of sample and control were measured every 24 h until the absorbance of the control reached maximum and the corresponding time, t, was recorded. The oxidation index (OI) and antioxidant activity (AA) were calculated as:

$$OI=100\times(Absorbance_t / Absorbance_{t=0}) \quad (1)$$

$$AA=100-100\times(\text{Carrot extract oxidation index}_t / \text{Control oxidation index}_t) \quad (2)$$

Color determination of carrot residue. A HunterLab sepctrocolorimeter was used to measure 'L', 'a', and 'b' values of carrot extract residues, of which 'L' indicates brightness/darkness, 'a' indicates redness/greenness, and 'b' indicates yellowness/blueness. The sensor was calibrated with a white tile ('L'=91.66, 'a'=-1.0, and 'b'=-0.2). Hue was expressed as a/b. Chroma was expressed as $\sqrt{a^2+b^2}$. The overall color change ΔE of carrot particles before and after extraction was expressed as $\sqrt{(L_1-L_0)^2+(a_1-a_0)^2+(b_1-b_0)^2}$, where L_0, a_0, and b_0 were the 'L', 'a', and 'b' values of the starting material and L_1, a_1, and b_1 were those of carrot residue.

RESULTS AND DISCUSSION

Extraction yield. The yield of crude carrot oil extracted with TSE and SFE under different combinations of temperature and pressure was presented in Figure 1. The crude oil yields of SFE were 1.83-2.69 g/100g dry carrot, which were similar to that obtained with TSE (2.46 g /100g dry carrot). The α- and β-carotene content in the starting material determined with TSE was 524.2 and 611.4 μg/g dry carrot, respectively. Lutein was not recovered by TSE method. The yields of α-carotene, β-carotene, lutein and total carotenoids obtained with SFE under different combinations of temperature and pressure were listed in Table 1. The extraction yield with SFE for α-carotene was 137.8-330.4 μg/g and β-carotene was 171.7-386.6 μg/g feed material, which was approximately half of those in the starting material. However, a substantial amount of lutein (23.5-37.5 μg/g feed material) was extracted by the SFE method at all the conditions studied. The extraction yields of α-carotene, β-carotene and total carotenoids increased dramatically with a temperature increase from 40 to 55 °C, but only slightly with a further increase in temperature from 55 to 70 °C at both 27.6 and 41.3 MPa. However, at 55.1 MPa, the extraction yields of α-carotene, β-carotene, and total carotenoids showed a drop with a temperature increase from 55 to 70 °C following an increase from 40 to 55 °C. The extraction yields of α-carotene, β-carotene and total carotenoids decreased with pressure from 27.6 to 41.3 MPa, but increased with an additional

pressure increase from 41.3 to 55.1 MPa at temperatures of 40 and 55 °C. At 70 °C, however, the extraction yields of α-carotene, β-carotene and total carotenoids showed a steady decrease with pressure from 27.6 to 55.1 MPa. There was no clear trend for the change in the yield of lutein with temperature and pressure.

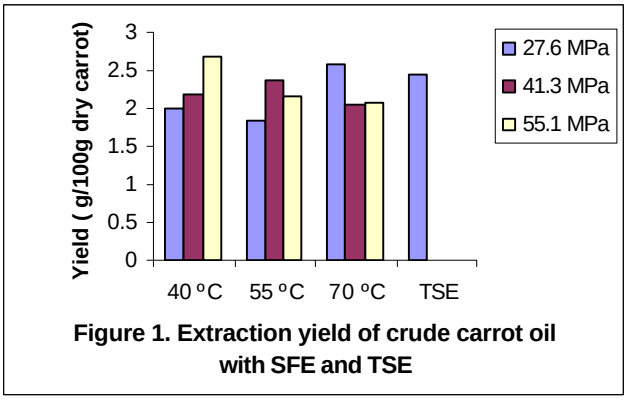

Figure 1. Extraction yield of crude carrot oil with SFE and TSE

Table 1. The yield of carotenoids obtained with SFE

T (°C)	P (MPa)	a-Carotene (µg/g feed)	b-Carotene (µg/g feed)	Lutein (µg/g feed)	Total carotenoids (µg/g feed)
40	27.6	181.6	220.2	30.1	462.1
	41.3	137.8	171.7	29.7	339.3
	55.1	259.9	309.8	37.5	607.3
55	27.6	330.3	378.2	30.3	738.9
	41.3	246.6	299.5	34.5	580.6
	55.1	320.5	367.8	23.5	711.8
70	27.6	328.5	386.6	30.3	745.5
	41.3	269.9	308.5	27.2	605.8
	55.1	250.9	296.5	28.7	576.2

Antioxidant activity of crude carrot oil. The antioxidant activity of SC-CO_2 extracted carrot crude oil at different temperature and pressure conditions ranged from 24.98 to 43.14 (Fig. 2), which was approximately half of that obtained for BHT (75.04) measured at equal concentrations (500 ppm) of crude oil and BHT.

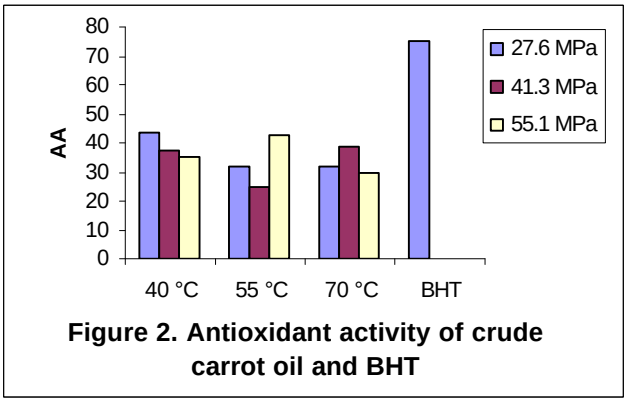

Figure 2. Antioxidant activity of crude carrot oil and BHT

Color of carrot residue. The color parameters of the carrot residue obtained at different combinations of temperature and pressure were presented in Figures 3a to 3f. As expected, the intensities of redness ‘a’ (Fig. 3b) and yellowness ‘b’ (Fig. 3c) of the carrot particles decreased after extraction at all temperature and pressure conditions studied, while the brightness ‘L’ (Fig. 3a) increased after extraction. In general, the higher yield of extracted pigments corresponded to a greater reduction in the redness and yellowness. The brightness increase may be due to the loss of redness and yellowness and therefore the particles becoming whiter in color. The drop in redness ‘a’ increased with temperature, while the drop in yellowness ‘b’ decreased with temperature. This suggests that higher temperature is more favorable for the removal of red tones. Hue and chroma values calculated based on the ‘a’ and ‘b’ values both decreased compared to feed material (Fig. 3d and 3e). The decrease in hue value means that the decrease in redness was greater than that in yellowness, while the decrease in chroma value once again indicated the overall decrease of redness and yellowness. Following the trends in redness ‘a’ and yellowness ‘b’ with an increase in temperature, the hue value decreased with temperature. This further demonstrated the preferential removal of redness at higher temperatures. Figure 3f shows that the overall color change in carrot particles before and after extraction increased with an increase in temperature.

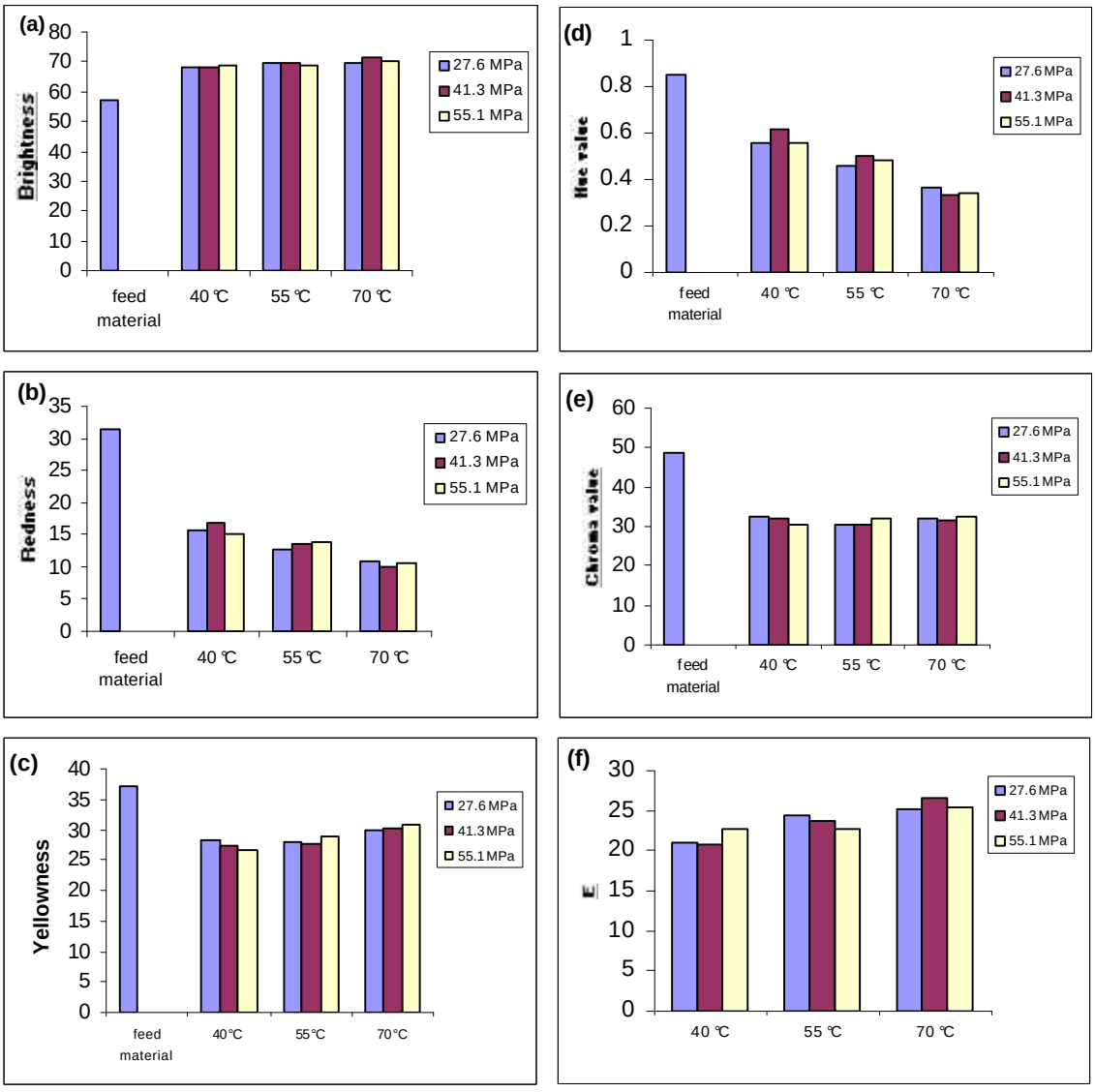

Figure 3. Color of carrot residue after extraction and feed material. (a) brightness ‘L’, (b) redness ‘a’, (c) yellowness ‘b’, (d) hue, (e) chroma, (f) DE value

CONLUSIONS

Carotenoids were extracted from dried carrots using supercritical CO_2. Not only the hydrocarbon compounds such as α- and β-carotene but also the oxygenated carotenoids such as lutein were recovered with supercritical CO_2. The crude carrot oil extracted with SC-CO_2 had high antioxidant activity, which was approximately half of that of a synthetic antioxidant (BHT) measured at equal concentration. Color analysis of carrot particles revealed that redness was removed to a greater extent than yellowness, especially at higher temperatures. In summary, SFE is an efficient extraction technique for the recovery of natural food colorants with high antioxidant activity.

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