Determination of Optimum Conditions for Supercritical Fluid Extraction of Carotenoids from Carrot (*Daucus carota* L.) Tissue

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The extraction and assay of antioxidant vitamins, such as carotenes, by traditional solvent extraction (TSE) require multiple steps, are time-consuming, and consume large amounts of organic solvent. Supercritical fluid extraction (SFE), a newer method for extracting natural products, is cost effective and eliminates toxic organic waste. The objective of this study was to optimize SFE of carotenoids from freeze-dried carrots and to compare this extraction to TSE. To optimize SFE for carotenoids, a factorial experiment was conducted. The factors assessed were the temperature of the sample chamber (30, 40, or 50 °C), the pressure of the extraction fluid (300, 400, or 500 atm), and cosolvent modification (5 or 10% ethanol). The optimum conditions for extraction of α-carotene and β-carotene by SFE were 50 °C, 300 atm, and 10% cosolvent ethanol. Total provitamin A activity (α- plus β-carotene) was greater in SFE than in TSE. The time required for SFE is 1 h as compared to 6 h for TSE. SFE is a reliable and improved method for extraction of carotenoids from carrot tissue.

Keywords: *Supercritical fluid extraction; carotenoids; carrots*

INTRODUCTION

Provitamin A carotenoids, such as α-carotene and β-carotene, are essential vitamins of the human diet. These vitamins, important nutritive components due to their antioxidant and anticancer properties, are abundant in carrots. Epidemiological studies have established an inverse relationship between the risk of laryngeal, lung, and colon cancers and the consumption of foods containing carotenoids (Block et al., 1992; Steinmetz and Potter, 1993). Many studies have shown growth inhibition of cancer cells and tumor regression in animals fed carotenoid-rich diets (Burton and Ingold, 1984; Wattenberg, 1983).

A need exists to improve available methods for the extraction and quantitation of carotenoids from foods (Marsili and Callahan, 1993; Eitenmiller, 1990). Traditional solvent extraction (TSE) for carotenoids is time-consuming, requires multiple steps, and consumes a large amount of organic solvent (AOAC, 1992). In addition, spectrophotometric assay may overestimate carotene content because of plant pigment interference. Due to the problems associated with traditional extraction, improved methods for the extraction and quantitation of carotenoids in foods are being sought (Marsili and Callahan, 1993; Spanos et al., 1993).

Supercritical CO_2 fluid extraction (SFE), a newer method for the extraction of natural products from food and other materials, is a desirable alternative to TSE for many applications. Supercritical fluid has a greater ability to diffuse into the solute than the parent liquid (Thomson and Chesney, 1992). As such, extraction of apolar substances is fast and thorough. Supercritical CO_2 provides an extraction environment with reduced potential for oxidation of the extracted solutes. The low critical temperature of CO_2 is beneficial in extracting thermally labile compounds such as carotenoids. In addition, CO_2 is nontoxic, and its use can eliminate the cost and adverse health effects associated with solvent disposal and long-term exposure to potential toxic vapors (Lee and Markides, 1990).

The objective of our research was to optimize SFE of carotenoids from freeze-dried carrot tissue (an excellent source of β-carotene) by optimizing the factors that impact SFE. The second objective was to compare the results of SFE vs TSE of carotenes with regard to α-carotene and β-carotene yield, time required for extraction, and solvent consumed during extraction.

MATERIALS AND METHODS

Sample Preparation. Carrots were harvested and processed according to established agronomic practice and shipped overnight from Grimway of Salinas, CA, a commercial grower. The carrots were selected for quality based on their overall appearance and absence of injury. Samples were ground at room temperature using a Kitchen-Aid (Model K45SS, Kitchen-Aid Inc., St. Joseph, MI) grinder to uniform size (2 mm) and then freeze-dried in a RePP Model 41 Sub Special freezer (Vertis, Detroit, MI) *in vacuo* (10 mmHg) for 2 weeks. The freeze-dried carrots were macerated with a mortar and pestle in liquid nitrogen and stored at −80 °C prior to carotene analysis by SFE or TSE.

SFE. A Dionex Multi Cell supercritical fluid extractor with cosolvent addition (Model 723, Dionex Corp., Salt Lake City, UT) was used to extract α-carotene and β-carotene, the primary, biologically active carotenes of carrots. Our preliminary work demonstrated that the addition of the ethanol modifier significantly increased the solubility and extractability of these carotenes in supercritical CO_2 as compared to less polar solvents including hexane and methylene chloride. The supercritical fluid extractor, equipped with an external modifier pump, continuously delivered absolute ethanol to the extraction cell at 5 or 10% of the supercritical CO_2 fluid. The restrictors allowed independent control of the flow of the extraction fluid (supercritical CO_2 and EtOH) during extraction up to 500 μL/min. The carrot extract was collected in 10 mL of hexane/acetone (9:1 v/v) containing 0.005% (w/v) BHT.

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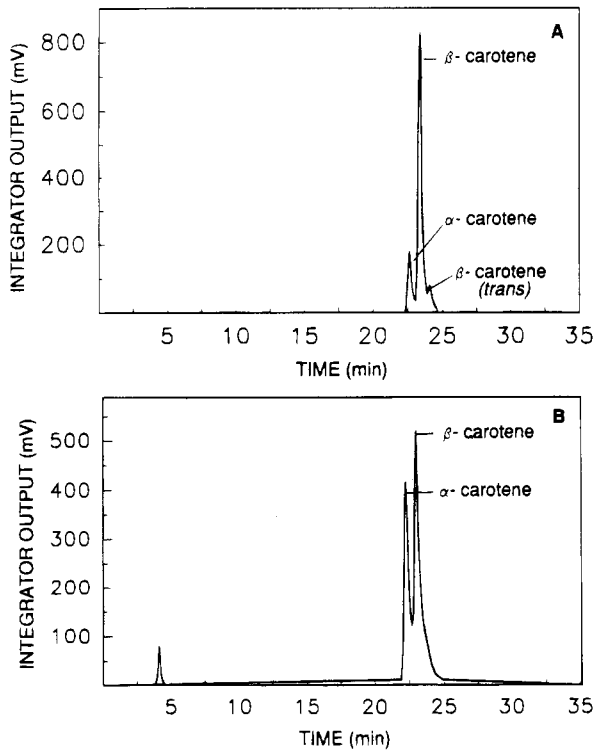

Figure 1. Representative HPLC chromatogram of α-carotene and β-carotene standards (A) and of carrot tissue extracted under optimal conditions (B).

A factorial experiment was conducted, and the factors assessed were as follows: temperature of the extraction chamber (30, 40, or 50 °C); pressure of the extracting fluid (300, 400, or 500 atm); and cosolvent modification (5 or 10% ethanol). Supercritical CO_2 pressurized with He was used for SFE of the plant carotenes.

One gram of acid-washed sea sand (Fisher Scientific, Cincinnati, OH) was placed in the SFE extraction cell (5 mL) to prevent plugging during extraction. Freeze-dried carrot samples (0.5 g) were added, and the remainder of the cell was filled with sea sand. One milliliter of hexane:acetone (9:1 v/v) containing 0.005% (w/v) BHT was added to the freeze-dried carrot sample to enhance carotenoid extraction.

TSE. TSE of α- and β-carotenes from carrot tissue was conducted according to an AOAC (1992) method for fresh plant materials. Two grams of freeze-dried carrots was homogenized using a Tissumizer (Model SDT 1810, Tekmar, Cincinnati, OH) 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 25 mL of hexane. The combined organic filtrates were washed three times with nanopure-processed water. The organic phase was dried over anhydrous Na_2SO_4.

High-Performance Liquid Chromatography. HPLC (Model 421, Beckman, Arlington Heights, IL) (250 μL/sample) was used to determine the carotene concentrations of the SFE and TSE extracts of the freeze-dried carrot tissue. The instrument was equipped with a Beckman Ultrasphere C_{18} reversed-phase column (5 μm particle size, 4.6 mm × 250 mm), Part 235329, and a variable UV–Vis SSI 500 detector at 450 nm. The elution profile employed absolute methanol for 5 min followed by a linear gradient to 100% acetonitrile/methylene chloride/octanol 90/25/0.1 v/v/v) in 20 min and then 10 min at acetonitrile/methylene chloride/octanol (90/25/0.1 v/v/v). Hoffman-La Roche (Nutley, NJ) kindly provided the α- and β-carotene standards (Figure 1A).

Preparation of SFE and TSE Extracts for HPLC. The carotene-containing extracts from SFE were diluted to 10 mL with hexane. One milliliter of SFE extract and 5 mL of traditionally prepared extract were dried under Ar and resolubilized in acetonitrile/methylene chloride/octanol (90/25/0.1 v/v/v). α-Carotene, β-carotene and total vitamin A activity

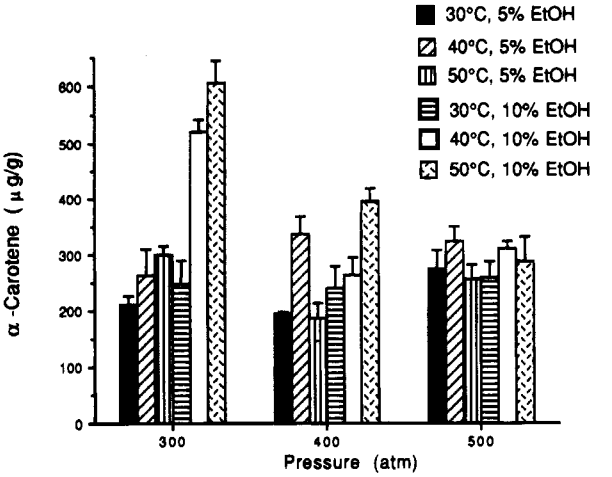

Figure 2. SFE of α-carotene from freeze-dried carrot tissue. Each value represents α-carotene extracted at various pressures (300, 400, or 500 atm), temperatures (30, 40, or 50 °C), and ethanol cosolvent addition (5 or 10%). Each value is the mean ± SEM of three independent determinations.

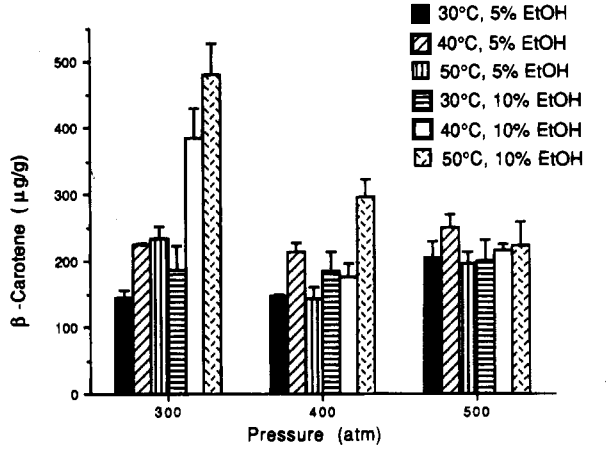

Figure 3. SFE of β-carotene from freeze-dried carrot tissue. See Figure 2 for additional experimental details.

(α- plus β-carotene) were expressed as either milligrams per gram or international units per gram of dry matter.

Data Analysis. Analysis of variance (ANOVA) was used to analyze the extraction yield for both α-carotene and β-carotene. The statistical significance of the effect of temperature, pressure, or cosolvent was determined at the 0.05 level. The data were analyzed using the SAS GLM procedure (SAS, 1988).

RESULTS AND DISCUSSION

The results of SFE of α-carotene and β-carotene in carrot tissue are shown in Figures 2 and 3. The effects of temperature, pressure, and cosolvent addition on the SFE of carrot tissue are similar for both α- and β-carotene content.

Effect of Temperature. At 300 atm, elevated temperature increases the extractability of α-carotene and β-carotene in both 5 and 10% cosolvent addition samples. At 400 atm and 5% cosolvent ethanol, the 40 °C samples exhibit a greater extractability than do 30 or 50 °C samples. A higher temperature resulted in enhanced extractability of carotenes at 400 atm and 10% cosolvent ethanol. At 500 atm, a slightly higher α-carotene yield was observed at 40 °C than at 30 or 50 °C. Overall, the best temperature for extraction, with either 5 or 10% cosolvent and 300 or 400 atm of pressure, was 50 °C. No significant difference was observed for the

Table 1. ANOVA Data (p Values) for the Influence of Temperature Pressure and Cosolvent on the α- and β-Carotene Extracted from Freeze-Dried Carrot Tissue

SFE factor	α-carotene (Pr > F)	β-carotene (Pr > F)
temperature	0.0001	0.0001
EtOH	0.0001	0.0001
temperature/EtOH	0.0001	0.0001
pressure	0.0002	0.0001
temperature/pressure	0.0003	0.0001
EtOH/pressure	0.0001	0.0002
temperature/EtOH/pressure	0.0034	0.0506

effect of temperature on the extraction yield of carotenes at 500 atm at either 5 or 10% cosolvent addition (Figures 2 and 3).

Effect of Pressure. At 50 °C and 10% cosolvent ethanol, the pressure and extractability of carotenes from carrot tissue were inversely related. At 40 °C and 10% cosolvent ethanol or at 50 °C and 5% cosolvent ethanol, the highest carotenoid yield was at 300 atm. Overall, 400 atm minimizes the extraction of carotenoids from carrot tissue. No significant difference in extractability was seen at 30 °C with either 5 or 10% cosolvent ethanol or at 40 °C 5% cosolvent ethanol addition regardless of pressure (Figures 2 and 3).

Effect of Cosolvent Addition. At 300 atm, 10% cosolvent ethanol yields much higher levels of α- and β-carotene from carrot tissue than does 5% cosolvent with respect to temperature. Samples extracted at 400 atm using 10% cosolvent ethanol have more carotenoid at 30 and 50 °C. However, at 400 atm and 40 °C, 5% cosolvent ethanol elicited better extractability of carotenes. The effect of the addition of ethanol on the extractability of α-carotene or β-carotene was diminished at 500 atm (Figures 2 and 3). Our results showed that continuous (dynamic) addition of ethanol with a modifier pump did not significantly improve carotene yield over noncontinuous (static) ethanol addition (Marsili and Callahan, 1993).

Optimum SFE. The data from ANOVA are presented in Table 1 and revealed that the interaction among the factors significantly influenced extraction of carotenes from carrot tissue. The experimental findings of Figures 2 and 3 were rationalized by an increase in the temperature of the extraction fluid, which caused a diminution in the interaction of solvent particles. This decrease in solvent–solvent interaction amplified solvent–solute interaction, thereby affording greater extractability. In contrast, pressure had an inversely proportional effect on solvent–solvent interaction, thereby attenuating solvent–solute interaction. The efficacy of ethanol as a cosolvent could be explained by its ability to enhance the hydrophobicity of the solvent, which increased solubilization of the carotenoids. In light of these principles, the optimum conditions for carotenoid extraction were 50 °C, 300 atm of pressure, and 10% cosolvent ethanol (Figure 1B). Under these conditions, mean α-carotene extraction yield was 605 mg/g and β-carotene extraction yield was 485 mg/g of dry weight.

Carotene Yield. TSE yielded 675 mg/g α-carotene and 420 mg/g β-carotene. SFE resulted in 10% less α-carotene and 15% more β-carotene at the optimum extraction conditions. This result was consistent with the finding of Marsili and Callahan (1993) that α-carotene and β-carotene were best extracted from nine vegetables at 338 atm, 40 °C, and noncontinuous ethanol addition. Although the yield of α-carotene under SFE was slightly lower than TSE levels, the total vitamin A activity (α-carotene plus β-carotene) was still 7% greater in the SFE than in the TSE samples. Our results showed that collection of SFE extracts into the simple solvent trap used in our experiments was as effective as the more complicated technique using sorbent traps as reported by Marsili and Callahan (1993).

Extraction Time and Organic Solvent Consumed. The average times required for the extraction of carotenes from carrot tissue by SFE and TSE were 1 and 6 h, respectively. SFE also benefits from eight simultaneously conducted extractions. The amount of solvent consumed per sample extraction was 40 mL with SFE as compared to 200 mL with TSE.

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